JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 1–7
10.5455/jice.20170909091946

eJManager

ORIGINAL ARTICLE

Open Access

Comparative study of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiacaceae) effects on glycemia in rats

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ABSTRACT

Aim: In order to promote African traditional pharmacopoeia, studies have been undertaken to evaluate the effects of aqueous extracts of *Annona senegalensis* (Annonaceae) (EAAs) and *Hallea ledermannii* (Rubiaceae) (EAHl) in white rats of Wistar strain.
Methods: A phytochemical screening and a toxicological study according to the Organisation for Economic Co-operation and Development guidelines 423 were carried out. Pharmacological effects on blood glucose were evaluated. The different treatments were performed orally.
Results: The aqueous extracts of EAAs and EAHl, respectively, at the maximum doses of 3,000 and 5,000 mg/kg bw, did not cause death in rats. Phytochemical screening revealed the presence of polyphenols, flavonoids, and quinonic compounds in both extracts. This study showed that in addition to the common compounds, the *Annona senegalensis* extract contained sterols, polyterpenes, catechic tannins, and alkaloids, while that of *Hallea ledermannii* showed the existence of saponosides. *Annona senegalensis* (100 mg/kg bw) and *Hallea ledermannii* (200 mg/kg bw), provoked more hypoglycemia, respectively, of 40% and 35.34% in rats. EAAS (27.78% vs. 25.41%) showed better anti-hyperglycemic effect in pretreated rats while EAHl (40.30% vs. 29.37%), provoked more anti-hyperglycemic activity in post-treated animals.
Conclusion: The effects of EAAs and EAHI on blood glucose value may be related to the presence of chemical compounds such as flavonoids and saponosides highlighted in a phytochemical study. These compounds recall those of certain insulin-secreting agents and justify their use in traditional medicine.

ARTICLE HISTORY

Received September 09, 2017
Accepted January 03, 2018
Published January 09, 2018

KEYWORDS

Annona sengalensis; Hallea ledermannii; Glibenclamide; hypoglycemia; anti-hyperglycemia; rat

Introduction

Diabetes is a metabolic disorder due to insufficiency or misuse of insulin characterized by a fasting hyperglycemia, verified twice. This disease affects more and more people in the world and is today a real public health problem. According to Danaei et al. [1], this disease caused, following its complications, the death of nearly 3.4 million people. In Côte d'Ivoire, 3–7% of the population suffers from diabetes [2]. The therapeutic management of this disease is costly given the low purchasing power in most developing countries [3]. The traditional pharmacopoeia offers a solution for the populations with low income. To enable access to health care at lower cost, ethnobotanical studies of medicinal plants have been carried out to develop improved traditional medicines. Thus, extracts of certain plants have been tested for their antidiabetic activity [4–6]. It is in this perspective that we undertook to study the effects of aqueous extracts of *Annona senegalensis* (Annonaceae) (EAAs) and *Hallea ledermannii* (Rubiaceae) (EAHl), two plants of the traditional African pharmacopoeia, known, as antidiabetic plants.

Annona senegalensis, a medicinal plant used to treat numerous infectious pathologies [7], also has antiparasitic activity on a resistant strain of Plasmodium falciparum [8]. In addition,

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antibacterial activities have been reported by More et al. [9]. The stems, leaves, fruits, and roots of *A. senegalensis* have been used in the treatment of skin cancer, cough, and to heal wounds [10,11]. According to Konaté et al. [12], the aqueous extract of bark from the root of this plant would be used in Burkina as a remedy against epilepsy and convulsions.

As for *Hallea ledermannii*, it is used as a local anesthetic, decreases blood pressure, and causes disorders in the lymphatic system of the intestine [13]. This plant also has antimicrobial and antioxidant activity [14].

The aim of this work was to evaluate the effects of EAAs and EAHl on blood glucose levels in rats.

Material et Methods

Plant material

The plant material consists of dry leaves of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae) harvested, respectively, in Bouaflé and yopougon, a neighborhood in Abidjan (Côte d'Ivoire). They were identified at the National Floristic Center of the Félix Houphouët Boigny University (Côte d'Ivoire) by the late Professor Aké Assi. Samples of *Annona senegalensis* (Annonaceae) and *Hallea ledermannii* (Rubiaceae) are kept, respectively, under the herbarium numeros 9,809 Lamto 06/12/1967 and 2,538 Forest of the banco 14/10/1954.

Preparation of extracts

To three hundred (300) grams of dried leaves of *Annona senegalensis* or *Hallea ledermannii*, which were cut into pieces, 1.5 l of distilled water is added and the whole is brought to a boil for 1 hour. The decoctate obtained is filtered several times on hydrophilic cotton. The filtrate is dried in an oven at 60°C. After drying, we obtained, respectively, 18 g (7.2%) and 22.5 g (9%) powder of *Annona senegalensis* and *Hallea ledermannii*.

Animal material

The experiments were carried out on rats of the species Rattus norvegicus of Wistar strain weighing between 90 and 150 g and bred in the animal house of Biosciences of the Felix Houphouët-Boigny University, at the ambient temperature (25°C). These rats had access to food and water *ad libitum*, enjoying 12 hours of light and darkness. The animals were acclimatized to laboratory condition before the start of experiment. They were used for the toxicological study and for the evaluation of pharmacological effects on blood glucose.

Phytochemicals studies of the aqueous extracts of Annona senegalensis and Hallea ledermannii

The aim of this study was to identify the chemical groups contained in the EAAs and EAHl, showing pharmacological interest, namely, sterols, polyterpenes, flavonoids, tannins, quinone compounds, saponosides, and alkaloids. For this, we used a qualitative method based on specific chemical reactions described by Bechro et al. [15], Nene Bi et al. [16], and Abo [17]. These tests are based on visual observation of color change or formation of precipitate after specific reactions.

Toxicity study of Annona senegalensis and Hallea ledermannii

This study was carried out according to the principles of Organisation for Economic Co-operation and Development [18] and covered a total of 20 rats for each extract. The healthy rats, weighing between 90 and 150 g, are divided into four batches of five rats, comprising one control batch and three test batches. After subjecting the animals to an 18-hour fast, each animal received a single dose orally with a gastric tube and different doses of the test substances were administered to the rats of the three test batches. The rats of the control group each received distilled water at a rate of 10 ml/kg. The maximum volume administered for each dose does not exceed 2 ml/100 g body weight. Animals are observed individually during the first 30 minutes after the administration of the substances, then every 8 hours and finally every day for 14 consecutive days in order to observe changes in behavior and possible mortality.

Pharmacologic study

This study consisted of determining blood glucose levels in temporary normoglycemic and hyperglycemic rats using the Accu-Chek glucose meter with reagent strips. The blood glucose value is given in mg/dl [19].

Normoglycemic rats

For this study, a total of 60 rats was used, each of these experiments was made from a total of 30 Wistar rats for each extract. The animals were divided into five batches of six rats and were fasted

for 18 hours. Prior to the administration of the test substances, blood glucose is measured in all animals at a T0 time. The rats of batch 1 (control batch) receive 2 ml of distilled water and those of batches test 2, 3, 4, and 5, respectively, receive doses of 50, 100, 200, and 300 mg/kg bw of *Annona senegalensis* or *Hallea ledermannii*. The effect of *Annona senegalensis* (Annonaceae) and that of *Hallea ledermannii* (Rubiaceae) on blood glucose in normoglycemic animals are followed for 3 hours at regular intervals of 30, 60, 90, 120, 150, and 180 minutes after administration of test substances.

Temporary hyperglycemic rats

For this experiment, 40 normoglycemic rats (20 pretreated rats and 20 post-treated rats) weighing between 90 and 150 g were fasted 18 hours before the start of the experiment. They had four (4) lots of five rats for each type of experiment. Blood glucose was measured at regular time intervals of 0, 30, 60, 90, 120, and 180 minutes.

For pretreated rats, the distribution is as follows:

- Lot 1: (R-T) Control rats treated with 2 ml of distilled water (p.o),
- Lot 2: (R-Glib) treated with glibenclamide (10^{-2} g/kg bw) and then 30 minutes afterwards, with 4 g/kg bw anhydrous glucose (p.o),
- Lot 3: (R-EAAs) treated with 100 mg/kg bw of EAAs and then 30 min afterwards, with 4 g/kg bw of anhydrous glucose (p.o),
- Lot 4: (R-EAHl) treated with 200 mg/kg bw of EAHl, then 30 min after with 4 g/kg bw of anhydrous glucose.

For tests with post-treated rats, groups were formed as above, with the only difference that the animals first receive the glucose and then the test substance.

Statistical analysis

Graph Pad Instat (San Diego, Calif., USA) and Graph Pad Prism 5 (San Diego, Calif., USA) were used for the statistical analysis of the values and the graphical representation of the data. The statistical difference between the results was obtained by using the variances (ANOVA), followed by the Tukey–Kramer multiple comparison test, with a significance level $p < 0.05$.

Resultats

Phytochemical study of Annona senegalensis and Hallea ledermannii

Phytochemical screening of EAAs and EAHl revealed certain types of compounds (Table 1).

This study showed that both extracts contain polyphenols, flavonoids, and quinone compounds.

In addition to these compounds, the *Annona senegalensis* extract contains sterols, polyterpenes,

Table 1. Chemical composition of the aqueous extract of *Annona senegalensis* (EAAs) and *Hallea ledermannii* (EAHl).

Chemical compounds		EAAs	EAHl
Polyphenols		+	+
Sterols and polyterpenes		+	–
Flavonoids		+	+
Saponosides		–	+
Quinonic compounds		+	+
Alkaloids		+	–
Tannins	Catechic	+	–
	Gallic	–	–

Presence (+); Absence (–)

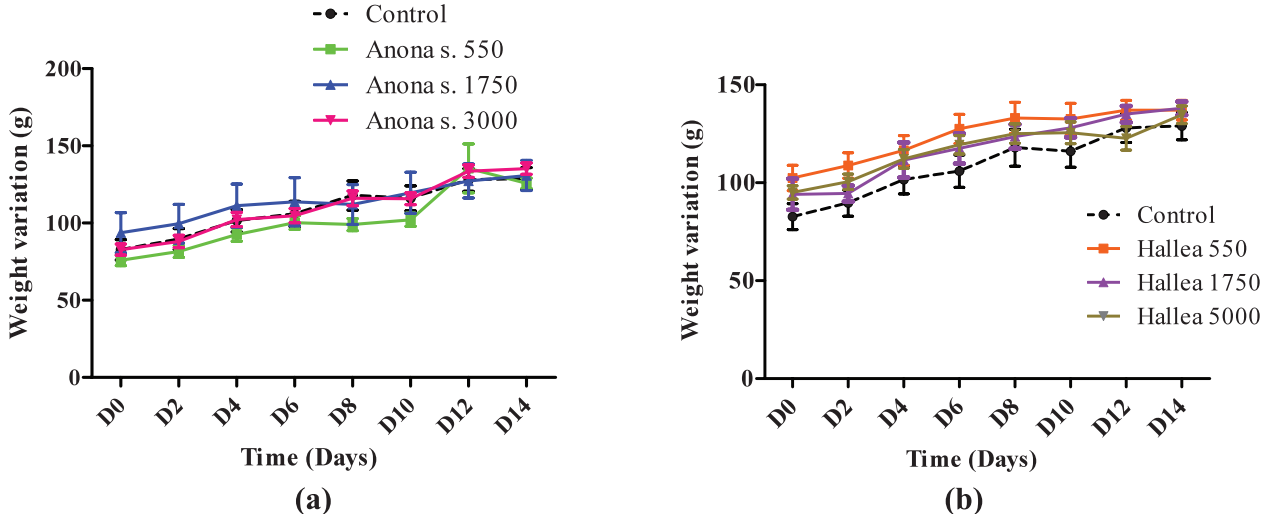

Figure 1. Effect of aqueous extracts of *Anona senegalensis* (a) and *Hallea ledermanii* (b) on weight in rats after 14 days. Values are expressed as mean ± ESM.

alkaloids and catechic tannins, in contrast to the extract of *Hallea ledermannii* which contains saponosides.

Toxicological study of aqueous extracts of Annona senegalensis and Hallea ledermannii

In rats, the extracts of *Annona senegalensis* (550, 1,750, and 3,000 mg/kg bw) and *Hallea ledermannii* (550, 1,750, and 5,000 mg/kg bw) were administered orally by causing a brief decrease of motricity without death in these animals.

Effects of the administration of EAAs and EAHl on the weight of rats

Increasing doses of EAAs (550, 1,750, and 3,000 mg/kg bw) and EAH1 (550, 1,750, and 5,000 mg/kg bw) were administered to the rats of different test batches. After two weeks of the study, no significant variation ($p > 0.05$) of weight was observed in these animals. The experiments were carried out several times ($n = 5$) and the mean values obtained allowed to plot the dose–response curves of different doses of EAAs (Fig. 1(a)) and EAHl (Fig. 1(b)).

Effects of aqueous extracts of Annona senegalensis and Hallea ledermannii on blood glucose in normoglycemic rats

Figure 2 shows the effects of increasing doses (50, 100, 200, and 300 mg/kg bw) of EAAs and EAHl on blood glucose in normoglycemic rats.

EAAs, at the doses of 50, 200, and 300 mg/kg bw, do not significantly ($p > 0.05$) alter blood glucose values after T180 (180 minutes) in treated rats compared to T0 (each T0 represents the control for the concerning group). For the dose of 100 mg/kg bw, there is a significant ($p < 0.05$) effect from 30 to 150 minutes, 60 to 150 minutes, and 90 to 150 minutes, on blood glucose when T30, T60, and T90 are compared to T150. The dose of 100 mg/kg bw reduced glycemia of 40% compared to its T0 (Fig. 2(a)).

Figure 2(b) shows the effect of EAHl on glycemia in rats. At the dose of 200 mg/kg bw, this extract significantly ($p < 0.05$) decreases the blood glucose level, from 30 to 150 minutes and 30 to 180 minutes, when T30 is compared to T150 and T180. EAHl significantly reduced ($p < 0.05$) glycemia of 35.34 % after 180 minutes compared to its T0.

Blood glucose measurement in temporary hyperglycemic rats

Figure 3 shows the effects of EAAs (100 mg/kg bw) and EAHl (200 mg/kg bw) on blood glucose level in pretreated (Fig. 3(a)) and post-treated rats (Fig. 3(b)).

In pretreated rats (Fig. 3(a)), with the test substances and then 30 minutes with glucose solution (4g/kg bw), hyperglycemic peaks were observed at 60 ($p < 0.01$) and 90 ($p < 0.01$) minutes with EAAs. EAAs reduced hyperplycemia compared to the peaks (60 and 90 minutes) of 27.78% and 25.57% after 150 minutes. In EAHl pretreated animals, hyperglycemia was observed at 90 minutes. EAHl induced the decrease of blood glucose value in rats after 120 minutes of 25.41% compared to the peak value.

In post-treated animals (Fig. 3(b)) with EAAs, peaks of hyperglycemia were observed at 30 and 60 minutes and glycemia level decreased after 180 minutes of 27.94% and 29.37% compared to T30 and T60. In animals post-trated with EAHl, hyperglycemia peak was observed at 30 minutes. Compared to the peak glycemia level, EAHl reduced hyperglycemia in rats of 40.30% after 180 minutes. Blood glucose values in all test batches returned to normal and were not statistically

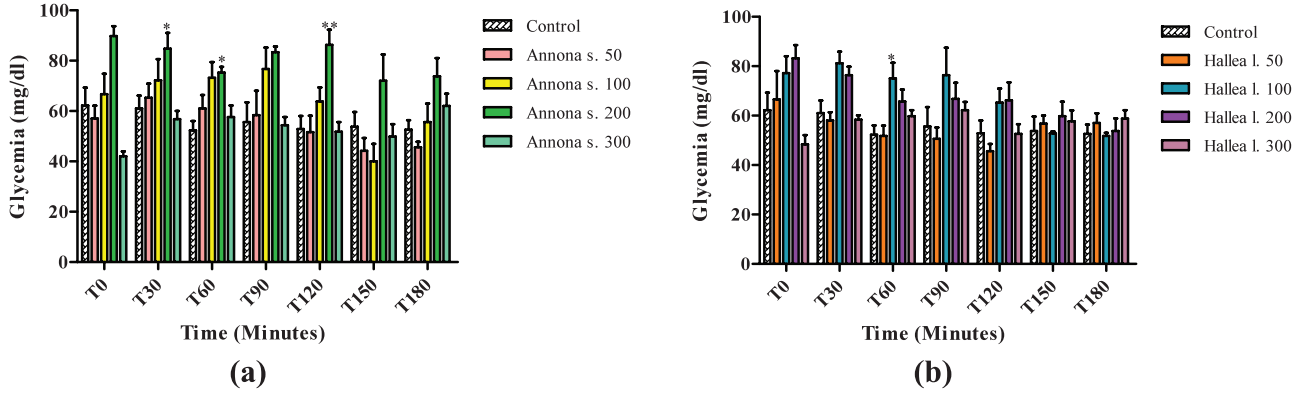

Figure 2. Dose-response effects of *Annona senegalensis* (a) and *Hallea ledermannii* (b) aqueous extracts on blood glucose in normoglycemic rats. Data are presented as mean ± SEM, $n = 6$ (*$p < 0.05$, **$p < 0.01$).

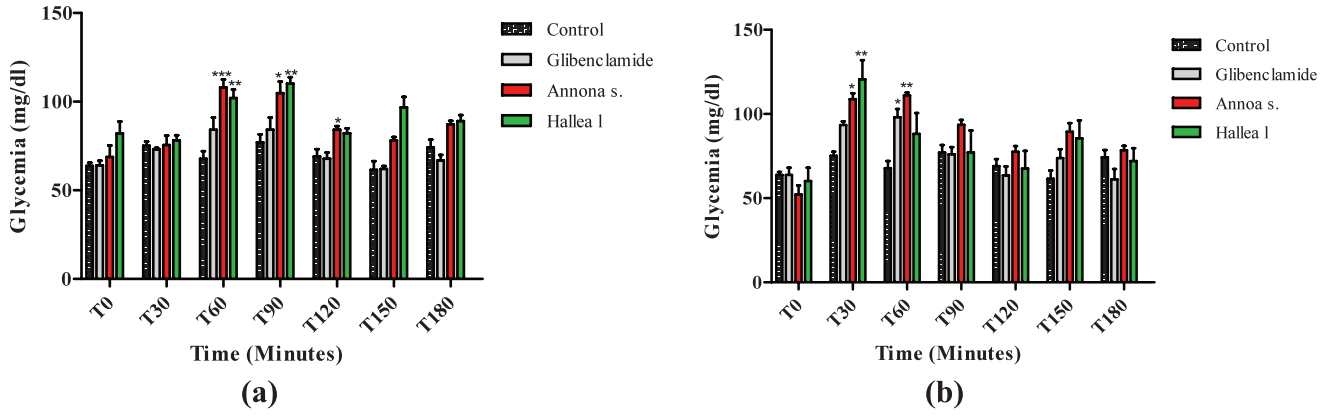

Figure 3. Effects of EAAs and EAHl in pre-treated (a) and post-treated (b) temporary hyperglycemic rats. Results are presented as mean ± SEM, $n = 5$ (*$p < 0.05$, **$p < 0.01$, $p < 0.001$).

different ($p > 0.05$) from the normal control values after 180 minutes.

Discussion

The phytochemical screening carried out with the aqueous extract of *Annona senegalensis* (Annonaceae) revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, quinones, catechic tannins, and alkaloids. This test did not reveal the presence of saponosides. These results are in agreement with those obtained by Konaté et al. [12], with the aqueous extract of the root bark of this plant. On the other hand, studies by Yéo et al. [20] did not reveal the presence of alkaloids and quinone compounds in the ethanolic extract of the leaves of *Annona senegalensis*.

As for the EAHl, this study revealed the presence of polyphenols, flavonoids, quinones, and saponosides. According to our work, this extract would not contain sterols and polyterpenes, tannins, and alkaloids. Previous work by Sofowora [21] with the methanol extract of leaves of this plant, contrary to our results, revealed the presence of alkaloids. This difference could be due to the type of solvent used.

Toxicological studies with the EAAs and EAHl in rats, at the respective doses of 550 mg/kg to 3,000 mg/kg bw and 550 mg/kg to 5,000 mg/kg bw, did not result in deaths. Similar results were obtained with the aqueous extract of the root barks of *Annona senegalensis* by Konaté et al. [12]. These authors showed that the extract of this plant was not toxic in rats at doses lower than or equal to 3,000 mg/kg bw. According to these results, the extracts of *Annona senegalensis* and *Hallea ledermannii* are non-toxic plants.

In addition to the absence of toxicity, these plants do not cause significant variations in the weight in the treated animals.

The results of work on the measurement of blood glucose in normoglycemic rats showed that extracts of *H. ledermannii* and *A. senegalensis* cause hypoglycemia in these animals at the respective doses of 200 mg/kg and 100 mg/kg bw. However, after glucose overload, EAAs exhibited better anti-hyperglycemic effect in the pretreated rat, unlike EAHl. In the post-treated animals, EAHl showed more anti-hyperglycemic effect than EAAS.

Similar properties have been observed in many medicinal plants derived from the African Pharmacopoeia. Indeed, Sy et al. [22–24], studying the activity of the acetonic extract of *Vernonia colorata* leaves on blood glucose levels in rabbits, showed that this extract caused hypoglycemia in these animals. Similar results were obtained with extracts of *Rauvolfia vomitoria* [25], *Boscia senegalensis* [26], *Gnetum africanum* and *Gnetum bulchozzianum* [27], which presented hypoglycemic activities in rats.

Extracts of *Annona senegalensis* and *Hallea ledermannii* showed antihyperglycaemic properties in rats. Similar results were obtained with the F2 fraction of Vernonia coloratapar [28]. These authors demonstrated that the F2 fraction of Vernonia colorata caused a drop in blood glucose in rats with temporary hyperglycemia.

The results of our studies on the phytochemical screening of the extracts of *Annona senegalensis* and *Hallea ledermannii* have made it possible to demonstrate the presence of certain chemical compounds such as flavonoids, tannins, alkaloids, and saponins. Indeed, authors N'Diaye et al. [29] and Olagbende-Dada et al. [30] demonstrated the antihyperglycaemic properties of flavonoids, which act by improving

the body's sensitivity to insulin [31,32]. According to Kambouche et al. [33] the saponins possess an antihyperglycemic effect. Thus, the antihyperglicemic effects of the extract of *Annona senegalensis* and *Hallea ledermannii* in rats are likely due to the presence of chemical compounds such as flavonoids and saponins.

Conclusion

The phytochemical studies of *Annona senegalensis* revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, quinones, catechic tannins, and alkaloids. Those of *Hallea ledermannii* made it possible to demonstrate the presence of polyphenols, flavonoids, quinones, and saponosides.

The EAAs and EAHl, administered orally, are nontoxic in rats at the maximum respective doses of 3,000 mg/kg and 5,000 mg/kg bw. These plants have effects on blood glucose value which are likely due to the presence of chemical compounds such as flavonoids and saponins.

References

[1] Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 2011; 378(9785):31–40.

[2] Djédjé H. Etude prospective pour la réduction et la stabilisation de la glycémie chez le lapin diabétique, par DIACODA, une substance de source végétale. Diplôme d'Etudes Approfondies de Biotechnologie, Université de Cocody-Abidjan, U.F.R. Biosciences, Laboratoire de Biochimie, p 36, 2002.

[3] Mangambu M. Taxonomie, biogéographie et écologie des Ptéridophytes de l'écosystème forestier des montagnes du Parc National de Kahuzi-Biega à l'Est de la R.D. Congo. Thèse de doctorat, Université d'Anvers, Belgique, p 463, 2013.

[4] Tra Bi Fézan H, Irié GM, Kohué CC, N'Gaman, Mohou CHB. Études de quelques plantes thérapeutiques utilisées dans le traitement de l'hypertension artérielle et du diabète : deux maladies émergentes en Côte d'Ivoire. Sci Nat 2008; 5(1):39–48.

[5] Kazemi S, Sedigheh A, Moshtaghian J, Rafieian M, Azadeh A, Shamsi F. Liver-protective effects of hydroalcoholic extract of *allium hirtifolium* boiss. In rats with alloxan-induced diabetes mellitus. ARYA Atheroscler 2011; 6(1):11–5.

[6] Soulimani R. Hypoglycemic and anti-hyperglycemic effects of *Anabasis articulata* (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant. Afr J Biotechnol 2009; 8(20):5589–94.

[7] Okoli CO, Onyeto CA, Akpa BP, Ezike AC, Akah PA, Okoye TC. Neuropharmacological evaluation of *Annona senegalensis* leaves. Afr J Biotechnol 2010; 9(49):8435–44.

[8] Fall D, Badiane M, Ba D, Loiseau P, Bories C, Laurencs A, et al. Activité antiparasitaire *d'Annona* du Sénégal utilisé en medecine traditionnelle. Dakar Méd 2003; 48(2):112–6.

[9] More G, Tshikalange TE, Lall N, Botha F, Meyer JJM. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol 2008; 119:473–7.

[10] Abdullahi MN, Adamu JK, Onu FB. The DNA technological approaches in breeding high plants for genetic viability. J Biotechnol 2012; 4:17–23.

[11] Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of northern Nigeria. J Ethnopharmacol 2007; 111:625–9.

[12] Konaté AWR, Sawadogo F, Dubruc O, Caillard M, Ouedraogo, Guissou IP. Phytochemical and Anticonvulsant Properties of *Annona senegalensis* Pers. (Annonaceae), plant used in Burkina folk medicine to treat epilepsy and convulsions. Br J Pharmacol Toxicol 2012; 3(5):245–50.

[13] Burkill HM. The useful plants of West Tropical Africa. 2nd edition, Vol. 4, Royal Botanical Garden, Kew, Richmond, UK, pp 546–7, 1997.

[14] Adesegun AS, Anyika N, Oluseyi AT, Godwin ES. Antibacterial and antioxidant investigations of *Hallea ledermannii* leaf extract. Indian J Sci Technol 2012; 5(1):1885–7.

[15] Bechro YA, Bechro MJA, Boua BB, Tra Bi FH, Ehilé EE. Etudes ethnobotaniques et screening phytochimiques de *Caesalpinia benthamiana* (Baill.) Herend. Et Zarucchi (Caesalpiniceae). Sci Nat 2007; 4:217–25.

[16] Néné Bi SA, Traore F, Zahoui OS, Soro TY. Composition chimique d'un extrait aqueux de *Bridelia ferruginea, benth*. (euphorbiaceae) et études de ses effets toxicologique et pharmacologique chez les mammifères. Afr Sci 2008; 04(2):287–305.

[17] Abo KJC. De la plante à la molécule: toxicité, effets pharmacologiques et mécanisme d'action de *Justicia secunda* (Acanthaceae), plantes antihypertensive, sur le système cardio-vasculaire de mammifères. Thèse de doctorat d'état ès Sciences Naturelles, Université Félix Houphouët Boigny, Abidjan, Côte d'Ivoire, p 351, 2013.

[18] OECD Test guideline 423. OECD guideline for chemicals, pp 1–14, 2001. Available via https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf

[19] Mansar-Benhamza L, Djerrou Z, Hamdi Pacha Y. Evaluation of anti-hyperglycemic and side effet

of *Erythreaecentaurim* (L.) Pers. in rats. Afr J Biotechnol 2013; 12(05):6980–5.

[20] Yeo D, Rodica D, Houphouet FY, Bianca F, Mirela P, Djaman Allico J, et al. Evaluation de l'activité anti-inflamatoire et screening phytochimique des feuilles de *Annona senegalensis*. Thérapie 2011; 66(1):73–80.

[21] Sofowora AE. Screening plants for bioactive agents. In: Sofowora AE (ed.) Medicinal plants and traditional medicine in Africa. 2nd edition, Spectrum books Ltd, Ibadan, Nigeria, p 289, 1993.

[22] Sy GY, Nongonierma RB, Sarr M, Cissé A, Faye B. Activité antidiabétique de feuilles de *Vernonia colorata* (Willd.) *Drake* (Composeae) sur un modèle de diabète alloxanique chez le rat. Dakar Méd 2004; 49:36–9.

[23] Sy GY, Cissé A, Nongonierma RB, Sarr M, Mbodj NA, Faye B. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan induced diabetic rats. J Ethnopharmacol 2005; 98:171–5.

[24] Sy GY, Nongonierma RB, Cissé A, Dièye AM, Wélé A, Gadiaga NF, et al. Mécanismes d'action des extraits acétonique et hexanique de feuilles de *Vernonia colorata* Willd. (Drake) (Composeae) sur la régulation de la glycémie. Dakar Méd 2006; 51(1):42–6.

[25] Samba Babila C, Diatewa M, Ngoma Mouanda HC, Ampa R, Abena AA. Evaluation des propriétés hypoglycémiantes et antihyperglycémiantes de l'extrait aqueux des feuilles de *Rauvolfia Vomitoria Afzel* chez le Rat. Ann Univ M NGOUABI 2007; 8(4):88–93.

[26] Sakine MNA, Mahmout Y, Gbenou J, Agbodjogbe W, Moudachirou M. Inventaire ethnobotanique des plantes du Tchad utilisées contre le diabète : effet antihyperglycémiant des extraits de *Boscia senegalensis* (Pers.) Lam. ex Poiret et de *Colocynthis*. Phytothérapie 2011; 9:268–73.

[27] Ndomou M, Djidjou Kammegne P, Ayong Ntah M, Gouado I, Tchiegang C. Evaluation de l'activité antidiabetique des extraits de feuilles de *Gnetum africanum* et *Gnetum bulchozzianum* (Gnétacées). Sci Technol Dev 2014; 15:60–5.

[28] Sy Gy, Faye FS, Wele A, Gueye PM, Gueye CD, Cissé A, et al. Activité antihyperglycémiante de la fraction F2 de l'extrait total acétonique des feuilles de *Vernonia colorata* (Composeae). Pharm Méd Trad Afr 2008; 15:6–10.

[29] N'Diaye M, Diatta W, Sy GY, Fall AD, Faye B, Bassene E. Activite antihyperglycemiante de l'extrait éthanolique de Feuilles *d'Icacina senegalensis Juss* (Icacinaceae). Med Black Afr 2008; 55:441–5.

[30] Olagbende-Dada SO, Ogbonnia SO, Coker HAB, Ukpo GE. Blood glucose lowering effect of aqueous extract of *Gratophyllum Pictum (Linn) Griff*on alloxan induced diabetic rats and its acute toxicity in mice. Afr J Biotechnol 2011; 10(6):1039–43.

[31] Lean Me, Moroozu M, Kelly I, Burns J, Taluar D, Sattar N, et al. Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. Diabetes 1999; 48:176–81.

[32] Ford ES, Will JC, Bowman BA, Narayan KM. Diabetes mellitus and serum carotenoids: findings from the third national health and nutrition examination survey. Am J Epidemiol 1999; 149:168–76.

[33] Kambouche N, Merah B, Derdour A, Bellahouel S, Benziane MM, Younos C, et al. Etude de l'effet antidiabetique des saponines extraites d'*Anabasis articulata* (Forssk) Moq, plante utilisée traditionnellement en Algerie. Phytothérapie 2009; 7(4):197–201.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 8–24
10.5455/jice.20171011075112

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Ethnobotanical documentation of traditional knowledge about medicinal plants used by indigenous people in the Talash Valley of Dir Lower, northern Pakistan

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ABSTRACT

Aim/Background: The indigenous communities of the Talash Valley district Dir Lower, in Northwest Khyber Pakhtunkhwa, Pakistan, depend on ethnomedicine for their basic health care. The aim of this survey was to identify, collect, and document significantly distinguishable ethnomedicinal plants and their ethnopharmacological application among the indigenous communities of the Talash Valley, Dir Lower, Pakistan.

Materials and Methods: Open-ended and semi-structured interviews, questionnaires, inquiries, and group discussion were conducted from March 2014 to September 2015 to obtain ethnobotanical data from the local herbalist and elder villagers. Quantitatively, the ethnobotanical data were analyzed by using indices, Use Value, Relative frequency of citation, and Informant Agreement Ratio.

Results: The study identified a total of 50 medicinal plant species belonging to 33 botanical families and 46 genera in the 17 villages. Lamiaceae with 6 species is the dominant family, and herbs (68%) the main sources of herbal formulations. Leaves (41%) are the main parts for ethnomedicine, and 32% of drug orally administrated in the form of decoction.

Conclusion: The Talash Valley is rich in its medicinal plant's flora and the associated traditional knowledge. Ethnomedicine plays an important role in the local healthcare system. The finding of new medicinal uses, recipes; vernacular plant names, using new morphological parts, and harvesting methods in the current study show the importance of the documentation of plant resources and ethnobotanical knowledge. We suggest and recommend that documented plants to be screened for further ethnopharmacological studies.

ARTICLE HISTORY

Received October 11, 2017
Accepted December 21, 2017
Published January 06, 2018

KEYWORDS

Ethnobotany; Medicinal plants; Survey; Talash valley; Indigenous knowledge; Pakistan

Introduction

Despite the increasing development and growth of the pharmaceutical industry, still the world uses much ethnomedicine to treat basic ailments [1]. Nowadays, ethnomedicines have gained popularity in many countries and indigenous people living in different parts of the world use medicinal plants as sources of medicine for the treatment of various human ailments [2,3]. Ethnomedicine plays a very important role in health issues of indigenous communities, and they also address healing practices as well as the healthcare seeking process [4]. From early on, mankind used natural materials and thereby gained a considerable indigenous knowledge base for using ethnomedicinal plants that was built up over time. This knowledge was passed through generations by generations and initially by oral communication while later in a written form by using baked clay tablets, papyri, parchments, scientific literature like manuscripts, and herbals [5,6]. This knowledge was used for treatment in successive civilizations as a fundamental means for health maintenance, disease prevention, and also curing extensive ranges of ailments from the previous time [7].

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The use and reputation of ethnomedicine are very important and are increasing day by day around most parts of the world [8]. Especially in rural areas, and also in many tropical countries, the health facilities are less developed and sometimes not even provided [9]. The estimated 50,000–60,000 *tabbies* (practitioners) and also a huge number of unregistered medicine practitioners are scattered in the rural and mostly remote hilly areas of Pakistan. There is an estimate that about 60% of the human population uses ethnomedicine of the traditional practitioners. Approximately, 80% of the population in Pakistan lives in rural households where medicinal plants are available easily. While a lower income situation and unavailability of the modern health facilities in the remote rural areas limits the access of local inhabitants to modern medicines [10]. In Pakistan, the available modern and synthetic healthcare services are sometimes insufficient, inaccessible and at times unaffordable to the majority of people. Moreover, due to poverty and illiteracy, most of the poor people are dependent on herbal products for curing various diseases [11].

Although the estimated 422,000 angiosperms are found worldwide [12], only 50,000 plants species are used for medicinal purposes [13]. In addition, only about 5,000 plants have been investigated for their phytochemicals. However, the ethnobotany is playing a very crucial role in conserving the natural sources and also medicinal plant diversity [14]. According to one report in Pakistan, a total of 1,572 plant genera and about 5,521 species of angiosperms are identified. Among these, only 400–600 plants are known to be important medicinally. Of these plant species, about 400 species of plants are believed to be endemic to Pakistan [15].

The worldwide market of traditional and ethnomedicine in the last three decades has seen a considerable increase in herbs and their relevant products. People are interested in ethnomedicine for basic traditional systems of health care and so trade and demand of herbal products are rapidly increasing around the globe [7]. The estimated global trade from sales of herbs and herbal products was summed to an amount worth US Dollar 60,000 million by the year 2002 [16]. A survey conducted by Pakistan Forest Institute determines that 75 of crude ethnomedicines are widely exported while more than 200 are traded locally in Pakistan [17]. According to a study by Chaudhary et al. [18], approximately 500 families are linked with a collection of medicinal plants only in the District of Swat (Pakistan) and they are estimated to collect approximated 5,000 tons of medicinal plants in a single year.

A study by Teklehaymanot and Giday [19] indicated that documentation of the traditional uses of the medicinal plants needs immediate attention. It is very important to preserve or document the knowledge since it seems that this knowledge is at the risk of extinction due to many reasons. These include the migration from rural to urban areas, industrialization, loss of biodiversity, loss of natural habitats, and also due to changing lifestyle. At present, the traditional knowledge about medicinal plants and its practices are rapidly disappearing and losing their inherent values at a shocking rate due to many reasons in various countries worldwide which are botanically rich and ethnomedicine uses [20]. The aim of this survey was to identify the collected plants for ethnopharmacological application by the indigenous communities of the Talash valley, located in the Lower Dir of Pakistan and to document the herbal preparation, local names, and uses of these plants. It is hoped that the result of this study will demonstrate the importance of documentation of traditional knowledge as well as local medicinal plants for the development of ethnomedicinal drugs to treat basic human ailments.

Materials and Methods

Study area

The present investigation was carried out in the Talash valley, located in the district of Dir Lower, Khyber Pakhtunkhwa, northern Pakistan. The Talash valley consists of four union councils (UCs or administrative units): Shahi Khel, Bandagai, Nora khel, and Bagh dushkhel (Fig. 1). It is located between 71° 47′ to 71° 58′ E longitudes and from 34° 41′ to 34° 47′ N latitudes in Dir Lower district. The Dir Lower district shares an international boundary with Afghanistan (Kunar province) in the west, by Swat district in the east, Malakand district in the South, while the Upper Dir lies in the North [8]. The population of the Lower Dir district increased by more than double during the last 19 years (1998–2017) from 717,649 people in 1998 to 1,435,917 people in 2017. The average annual growth rate of population in Lower Dir was 3.71 during 1998–2017 [21,22].

The landscape of the investigated area was covered with the plain and hilly region. The most common vegetation in the area includes *Ficus palmate, Morus alba, Morus nigra, Olea ferruginea, Ailanthus*

altissima, Acacia modesta, Dodonaea viscosa, Artemisia spp., Berberis lycium, Calotropis procera, Adhatoda vasica, Celtis australis, Cannabis sativa, Ajuga bracteosa, etc.

Field survey

Regular ethnobotanical surveys were arranged from March 2014 to September 2015 in 17 villages of the Talash valley, with the aim to collect and document ethnobotanical knowledge from the local peoples. Before starting the interview, we informed local participants that it was a student academic project and investigation was only for our research purposes, not for any commercial or other benefits [1]. Before conducting a field work in the study area, permission to conduct our study in each area was obtained from the local government authorities and elders of the study region. We also received formal consent from informants regarding data collection and publication. The International Society of Ethnobiology Code of Ethics was strictly followed during project planning (http://ethnobiol ogy.net/code-of-ethics/).

Informant interviews and ethnobotanical data collection

The ethnobotanical information was mainly obtained through interviews, group discussions, questionnaires, and casual walk. For ethnobotanical investigation, we mostly contacted the local *hakeem* (traditional herbal), farmers, and elder people, who had sufficient knowledge of indigenous medicinal plants. Those informants who voluntarily agreed were further interviewed and invited for group discussions. Meanwhile, the people who have more ethnobotanical information and experience were requested to go with us on casual walks in the field. The precise nature of ethnomedicinal knowledge interviews are given here. Almost 27 *hakeem* were interviewed in their herbal shops. We also conducted several group discussions where among 60 respondents 25 villager elders were interviewed. Thirty respondents were requested to go on a causal walk on woods and hills. Ten respondents, who also participated in the group discussions, also walked. For the 27 women respondents, we divided this group into 2 parts, one group with the age range of 50–70 years old who we directly approached. The second group with the age range of 30–45 years old. According to local cultural and societal norms, it is not acceptable to directly approach and talk with the second group in this age range, so we distributed questionnaires among school students and their relatives to invite these house women to share their knowledge with us.

In the ethnobotanical interviews, the related questions have been asked in the local language Pashto which is spoken throughout the study area. Using the standard methods of Martin [23] and Cotton [24] in ethnobotanical interviews and group discussions with local informants, we asked relevant questions regarding the ethnomedicinal use, parts used, the local name of the plants, herbal formulation methods, diseases treated, administration, and side effects if any.

Furthermore, face-to-face interviews and meetings were arranged with research coordinators from the World Wide Fund for Natura, Pakistan, District Forest Office Lower Dir, Wildlife Department of Lower Dir, and Chairman of the Forest Department to learn about local herbal practices and current conservation strategies.

Plant collection, identification, and deposition in herbarium

During interviews, the informants use the local name of the plants for specific diseases. After confirming plant identity with informants, the plants were collected and photographed. The collected plants were brought to the Herbarium in

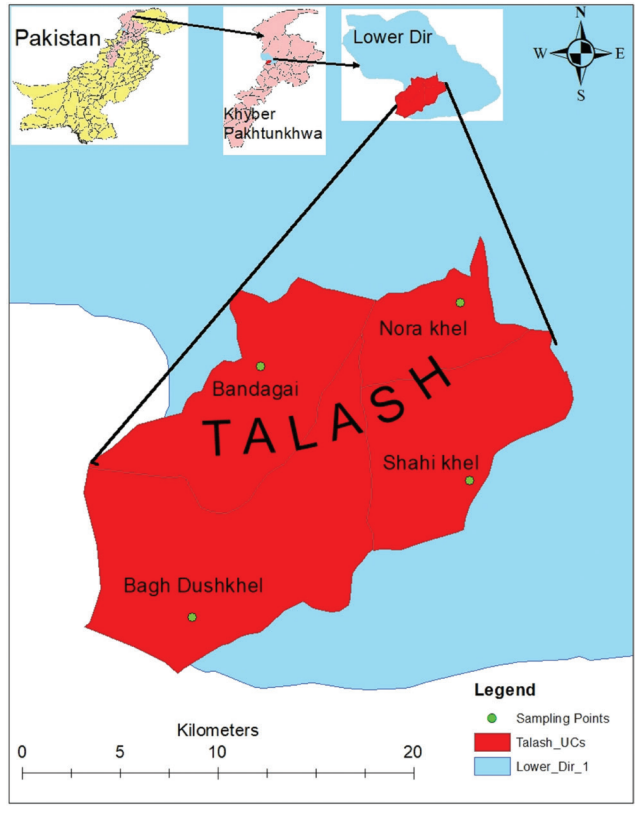

Figure 1. Map of the study area.

the Department of Botany, University of Peshawar, Pakistan. The collected plants were identified with the help of expert plant taxonomists, compared with the specimens of the Herbarium in the Department of Botany, and Flora of Pakistan (Ali and Nasir 1970–2002). For naming the plant species and current taxonomy, we follow The Plant List (www.theplantlist.org) and International Plant Names Index (www.ipni.org).

Data Analysis

Use value

Use value (UV) evaluates the relative importance of each medicinal species based on its relative use among informants [25]. UV was calculated using the following formula:

$$UV = (\sum U_i)/N,$$

where U_i is the number of use reports mentioned by each informant i and N is the total number informants interviewed for a given plant species.

Relative frequency of citation

Relative frequency of citation (RFC) is a quantitative index that gives us the local importance of a species in the ethnobotanical investigation [26]. According to the standard method of Vitalini et al. [25],

RFC is calculated as follows:

$$RFC = (0 < RFC < 1),$$

where FC is the number of informants who mentioned the importance of local species and N is the total number of informants who participated in interviews and group discussions.

Informant Agreement Ratio

To determine variability of the ethnomedicinal plant use, the informant agreement ratio (IAR) was used. According to Trotter and Logan [27], IAR is used to determine the agreement between informants concerning what ethnomedicinal plants to use for specific usage categories. It gives us information about the agreement or uniformity of the informant's indications as to the usage of a certain use-category, e.g., digestive system disorders or skin problem. It is one widely used method for analyzing quantitative data in ethnobotany [27]. This factor ranges from 0 to 1. A high value (close to 1) indicates that relatively few taxa are used by a large proportion of the informants, while a low value indicates that the informants disagree on the taxa's use within a category [28]. The IAR is calculated as

$$IAR = (N_{ur} - N_t)/(N_{ur} - 1),$$

where IAR is the Informant Agreement Ratio, N_{ur} is the number of mentions in each category, and N_t is the number of taxa used in each category.

Result

Socio-demographic data

The information regarding the ethnobotany and medicinal uses of plants was collected from 87 local inhabitants in the study area. Out of these, 60 were men (69%) and 27 were women (31%). Men informants, 27 *Hakeem* (Traditional herbal medicine practitioners), and the remaining were mostly elderly people; 78% of informants were married and 22% unmarried. Furthermore, most informants were illiterate (30%), elementary school (27%), secondary school (20%), high school (15%), and university (8%).

The ethnic composition of Dir Lower mostly Pashtun and the primary local language in the area is Pashto. The study area is characterized by difficult geographical and environmental conditions and limited livelihood opportunities. In the field, most respondents that we interviewed were farmers. People of the valley mainly depend on agriculture. Farming is the most prevalent livelihood activity followed by overseas labors and non-agriculture based labor. However, overseas labor (foreign remittances) is the primary income source for most of the households. Other sources include livestock rearing, mining, small-scale trading, and forestry. Major crop in the area includes vegetables (cash crop), wheat, maize, and mustard. The people of the area also depend for their livelihood on livestock rearing, namely, cow, goat, sheep, and poultry. Paid daily wages for labor are in the range of 600–1,000 Pakistani rupee (PKR) (1 US $ = 101 PKR).

During ethnobotanical interviews, it was reported that 30% of the respondents used the ethnomedicine because of less expensive, 23% easily available, 20% lack of basic health facilities, 17% learn from elder, and 10% because of low side effects. According to the questionnaire results, about 40% respondents were involved in the collection of herbs (2 kg per capita per month), 30% shrubs collection (2 kg per capita per month), 10% tree collection (1 kg per capita per month), 10% grasses, 5% climber and weeds each.

Plants identified by family and growth habit

In our study, a total of 50 medicinal plants species belonging to 34 botanical families and 46 genera were described by the local people. These ethnomedicinal plants used in the 17 villages of the Talash valley, and are presented in Table 1 in order the family have more plant species along with the local name, parts used, and relevant information. Lamiaceae with 6 species is the most common plant families, followed by Asteraceae, Moraceae, and Rosaceae (Fig. 2).

In life form, herbs (68%) were found to be the most used plants followed by shrubs (20%) and trees (12%) (Fig. 3). According to Baydoun et al. [7],

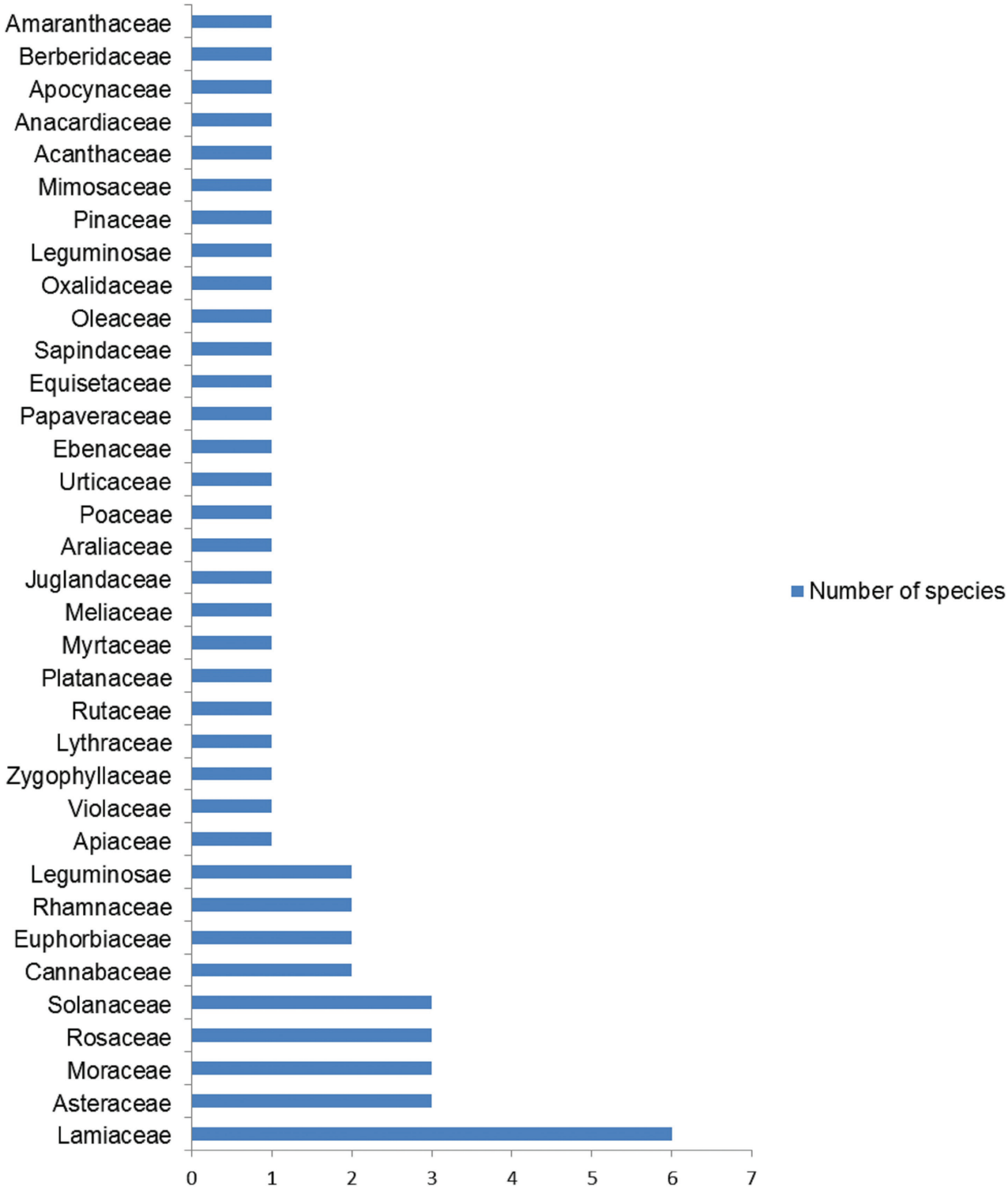

Figure 2. Ethnomedicinal plant species distribution among botanical families of Talash Valley in Dir Lower.

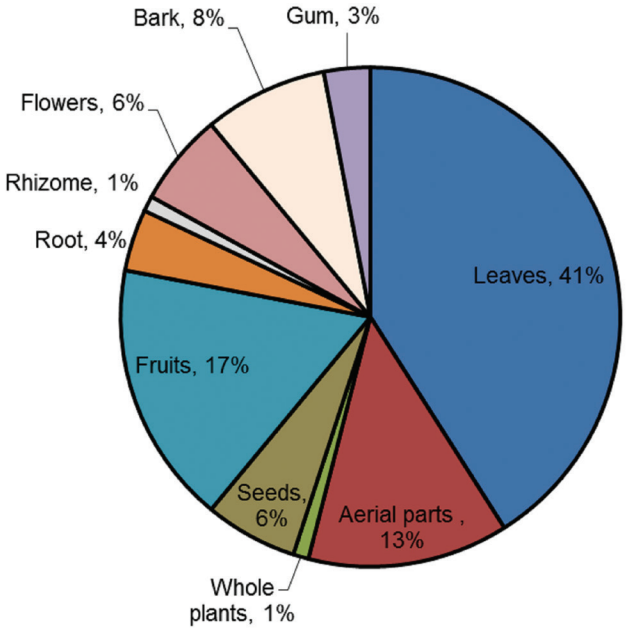

Figure 3. Proportion of different morphological parts used as herbal medicine by local inhabitants.

due to their medicinal properties, herbs were used dominantly in the herbal preparation and serving basic human various ailments and therapeutic indications.

Morphological parts used and status of medicinal plants

In our ethnobotanical survey, the local people described 10 different parts of the medicinal plants. Leaves were the most dominant plant parts followed by fruits, above-ground plant parts, and bark (Fig. 4). Easy collection of leaves compared to other parts of the plant makes it a favorite for herbal preparation [29]. However, scientifically, leaves are the most active part of the plant in terms of production of metabolites and photosynthesis [30]. Furthermore, easy collection and availability make the leaves and flowering parts common for herbal preparations [7].

In our field survey, almost 90% of plants described by the local inhabitants were wild, and the remaining was cultivated for various purposes.

Methodology of herbal formulation and administration

Almost 65% of ethnomedicines were administrated internally, and in the survey, local informants described seven different methods for herbal drug preparation to treat different kinds of human ailments. The most common was decoction followed by powder, direct eating, and poultice (Fig. 4).

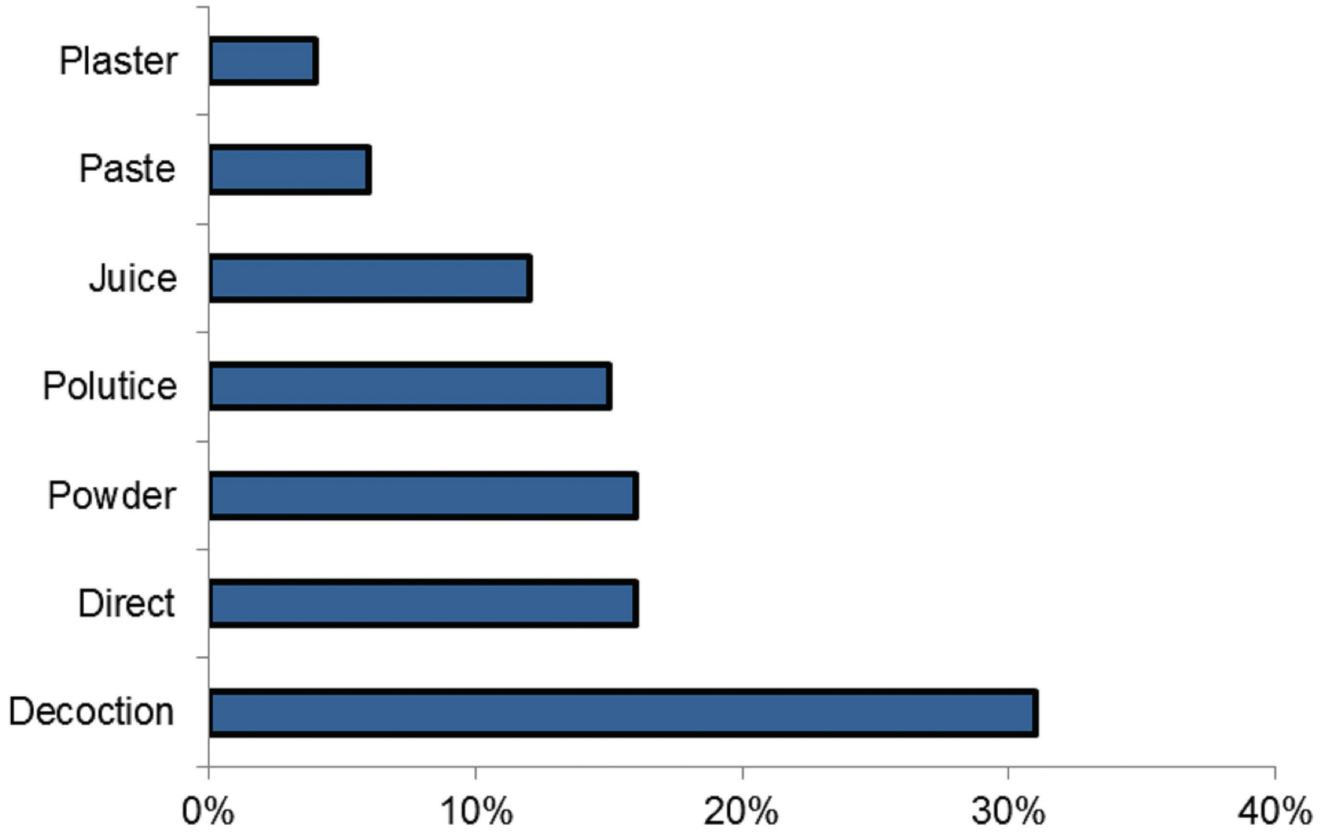

Figure 4. Preparation method of herbal remedies herbal in the management of various human ailments.

Table 1. AMedicinal plant species of the Talash valley, Dir Lower with its use values, relative frequency citation, mode of preparation and administration.

Scientific name/ (Voucher no.)	Local name	Growth form	Status[1*]	Parts used	Mode of preparation	*UV*[3*]	*RFC*[4*]	Medicinal uses	Taking route
Lamiaceae									
Ajuga bracteosa Wall. Ex Benth.	Boote	Herb	W	WP	Decoction	0.85	0.195	Hypertension, jaundice, and fever	Oral
Mentha longifolia (L.) L.	Welanai	Herb	W	AP	Powder	0.73	0.206	Abdominal pain, diarrhea, and emesis.	Oral
					Decoction			Fever and heart problem.	Oral
Mentha spicata L.	Podina	Herb	C,W	AP	Powder	0.53	0.091	Emesis and abdominal discomfort	Oral
					Decoction			Hypertension and mineral deficiency	Oral
Otostegia limbata (Benth.) Boiss.	Spin azghay	Shrub	W	LV	Powder	0.3	0.045	Jaundice	Oral
					Juice			Gum diseases	External
Teucrium royleanum Wall. ex Benth.	Aspa Bootay	Herb	W	AP	Decoction	0.63	0.08	Fever and considered as antiseptic, stimulant Also used as a vermifuge	Oral
Salvia moorcroftiana Wall. ex Benth.	Kherghwag	Herb	W	LV	Poultice	0.33	0.057	External wound	External
Asteraceae									
Artemisia vulgaris L.	Tarkha	Herb	W	LV	Decoction	0.7	0.137	Stomachache, hypertension, and dysentery	Oral
					Poultice			Scorpion sting and snakebites	External
Sonchus asper (L.) Hill	Shodapai	Herb	W	AP	Poultice	0.22	0.022	Curing wound and also used for Boils	External
Calendula arvensis M.Bieb	Khwaga Abai	Herb	W	FL	Juice	0.41	0.08	Toothache	External
				LV	Poultice			Skin diseases and healing wounds	External
Moraceae									
Ficus palmata Forssk.	Ormal	Shrub	W	LV	Direct	0.44	0.091	Curing wasp stings	External
									Oral
Morus alba L.	Spin toot	Tree	W	FR	Direct (Fresh as well as dried)	0.43	0.11	Digestive stimulant, and source of cheap carbohydrates	Oral
Morus nigra L.	Tor toot	Tree	W	FR	Direct	0.45	0.103	Cough, fever, and for sore throat, as cooling agent	Oral
Rosaceae									
Rosa moschata Herrm.	Zangali gulab	Shrub	W	FL	Decoction	0.4	0.12	Stomach disorder	Oral
Rubus fruticosus G.N.Jones	Karwara	Shrub	W	LV	Powder	0.29	0.09	Fever, and diarrhea	Oral
				RT	Decoction			Dysentery	Oral

Continued

Scientific name/ (Voucher no.)	Local name	Growth form	Status[1*]	Parts used	Mode of preparation	*UV*[3*]	*RFC*[4*]	Medicinal uses	Taking route
Spiraea spec.	Krachay	Shrub	W	FL	Decoction	0.25	0.034	Given to the pregnant woman to ease delivery also used for abdominal problems, and stomachache	
				LV	Juice (Fresh)			Cough and fever	
Solanaceae									
Datura innoxia Mill.	Batora	Herb	W	LV	Direct	0.29	0.057	Toothache, headache, and epilepsy	External
				SE	Poultice			Used as anti-septic	External
Solanum nigrum L.	Kachmachu	Herb	W	LV	Juice	0.57	0.091	Liver diseases	Oral
								Skin diseases	External
				FR	Direct			Hypertension.	Oral
Solanum surattense Burm.	Manraghonay	Herb	W	FR	Decoction	0.48	0.14	Stomachache	Oral
					Paste (Honey)			Chronic cough	Oral
				FL	Juice			Used in Opthalmia	Oral
Cannabaceae									
Cannabis sativa L.	Bang	Herb	W	LV, SE	Poultice	0.27	0.06	Boils, tonic, sedative, and anodyne	External
Celtis australis L.	Tagha	Tree	W	FR	Direct	0.16	0.022	Allergy and amenorrhea	Oral
Euphorbiaceae									
Ricinus communis L.	Harhanda	Shrub	W	LV	Plaster	0.21	0.034	External wound and burns	External
				SE	Oil			Skin problem like ringworm	External
Euphorbia helioscopia L.	Mandanoo	Herb	W	AP	Plaster	0.27	0.06	Skin diseases	External
Rhamnaceae									
Ziziphus jujuba Mill.	Bera	Shrub	W	LV	Paste	0.32	0.103	Scabies and boils	External
					Decoction			Diabetes.	Oral
					Smoke			Used for headache	
				FR	Direct			Used as Laxative	Oral
Z. nummularia (Burm.f.) Wight & Arn	Bera	Shrub	W	LV	Paste	0.26	0.04	Ulcer	External
					Decoction				Oral
Leguminosae									
Indigofera heterantha Brandis	Ghorija	Shrub	W	RT	Direct	0.14	0.034	Abdominal pain	Oral
Acacia modesta Wall.	Palosa	Shrub	W	Gum	Paste (honey,	0.41	0.149	Used as tonic to the women after birth.	Oral
					almond, flour)				

Continued

Scientific name/ (Voucher no.)	Local name	Growth form	Status[1*]	Parts used	Mode of preparation	*UV*[3*]	*RFC*[4*]	Medicinal uses	Taking route
Umbelliferae									
Foeniculum vulgare L.	Kagu	Herb	C,W	LV	Decoction	0.68	0.126	Urinary disorders, e.g., dysuria	Oral
Violaceae									
Viola biflora L.	Banafsha	Herb	W	LV	Decoction	0.38	0.091	Jaundice, cough, and body weakness	Oral
				FL	Powder			Sore throat, kidney, and liver problems	
Zygophyllaceae									
Tribulus terrestris L.	Markunday	Herb	W	LV	Juice	0.36	0.8	Chronic cough.	Oral
				FR	Powder			Urinary disorder	Oral
Lythraceae									
Punica granatum L.	Zangali Anar	Shrub	W	FR	Direct	0.42	0.195	Removing intestinal Helminthes	Oral
				LV	Paste, decoction			Skin diseases and dysentery	Oral External
Rutaceae									
Zanthoxylum alatum Roxb.	Dambara	Shrub	W	SE,	Powder	0.59	0.149	Fever	Oral
				BK				Gum diseases, dyspepsia, and Cholera	Oral
				FR				Stomachache and toothache	Oral
Platanaceae									
Platanus orientalis L.	Chinar	Tree	W	LV	Fresh	0.2	0.03	Toothache and external wound	External
Myrtaceae									
Myrtus communis L.	Manro	Shrub	W	LV	Decoction	0.61	0.091	Dysentery and stomach diseases	Oral
				FR	Direct			Diarrhea	Oral
Meliaceae									
Melia azedarach L.	Bakyana	Tree	W	BK	Decoction	0.36	0.137	Fever	Oral
				LV	Decoction			Jaundice	Oral
					Poultice			Skin diseases	External
Juglandaceae									
Juglans regia L.	Ghuz	Tree	C	LV,	Direct	0.28	0.114	Tooth whitening and teeth infection,	External
				BK	Direct (Dried)			bark (locally called *dandasa*) for cleaning an sparkling of teeth	
Araliaceae									
Hedera nepalensis K. Koch	Perwati	Shrub	W	LV	Decoction	0.6	0.103	Diabetes and blood purifier	Oral

Continued

Scientific name/ (Voucher no.)	Local name	Growth form	Status[1*]	Parts used	Mode of preparation	*UV*[3*]	*RFC*[4*]	Medicinal uses	Taking route
Poaceae									
Cynodon dactylon (L.) Pers.	Kabal	Herb	W	AP	Fresh	0.3	0.091	Used to control bleeding from nose Placed on injured place to stop bleeding	External
Urticaceae									
Debregeasia saeneb (Forssk). Hepper & J.R.I.Wood	Alajai	Shrub	W	LV	Poultice	0.17	0.03	Urticaria and other skin problems	External
Ebenaceae				LV					
Diospyros lotus L.	Toor amlook	Tree	W	LV	Powder	0.2	0.002	Used for curing constipation and dysentery	Oral
				FR	Direct			Sore throat	Oral
Papaveraceae									
Fumaria indica (Hausskn.) Pugsley	Papra	Herb	W	AP	Powder	0.64	0.126	Hypertension and common fever	Oral
					Decoction			stop emesis	Oral
Equisetaceae									
Equisetum arvense L.	Bandakay	Herb	W	WP	Juice	0.27	0.057	To expel calculus from kidneys	Oral
					Decoction			Jaundice	Oral
Sapindaceae									
Dodonaea viscosa (L.) Jacq.	Ghwaraskay	Shrub	W	LV	Poultice	0.18	0.057	For the treatment of fungal infection	External
					Juice (Hair oil)			Hair tonic	External
Oleaceae									
Olea ferruginea Wall. ex Aitch.	Khonoa	Tree	W	LV	Decoction	0.61	0.126	Hypertension, sore throat, and fever	Oral
				FR	Direct			Liver disorder and stop teeth decay	Oral
Oxalidaceae									
Oxalis corniculata L.	Trokay	Herb	W	RT	Decoction	0.15	0.022	Used to enhance digestion	Oral
Pinaceae									
Pinus roxburghii Sarg.	Nakhtar	Tree	C,W	Gum	Paste (Honey)	0.39	0.126	Diarrhea and sore throat	Oral
Acanthaceae									
Justicia adhatoda L.	Bekar	Shrub	W	LV	Decoction	0.29	0.091	Tuberculosis and asthma	Oral,
Anacardiaceae									
Pistacia integerrima J. L. Stewart ex Brandis	Shany	Tree	W	BK	Plaster	0.31	0.057	Chronic wound	External
				FR	Powder			Jaundice and liver diseases	Oral
Apocynaceae									
Calotropis procera (Aiton) Dryand.	Spalmay	Shrub	W	LV	Poultice	0.25	0.8	Scorpion sting	External

Continued

Scientific name/ (Voucher no.)	Local name	Growth form	Status[1*]	Parts used	Mode of preparation	*UV*[3*]	*RFC*[4*]	Medicinal uses	Taking route
Berberidaceae									
Berberis lycium Royle	Kwaray	Shrub	W	RH	Powder	0.76	0.183	Jaundice and dysentery	Oral
				BK	Poultice			It is used as tonic and nephrological complaints	External
Amaranthaceae									
Chenopodium murle L.	Kharawa	Herb	W	AP	Juice	0.37	0.114	Abdominal pain	Oral
					Decoction			Jaundice	Oral

1*W: Wild, C: Cultivated; 2*RT: Root, RH: Rhizome, LV: Leaves, SE: Seed, FR; Fruit, FL: Flower, AP: Above-ground plant parts, BK: Bark, WP: Whole plant, B: Bulb; 3*UV: Use Value; 4*RFC = Relative frequency of Citation.

According to Nadembega et al. [31], in traditional herbal drugs, decoction can be considered one of the common forms of herbal formulation because it is very easy to prepare ethnomedicine simply by mixing herbal parts with boiling water. Pakistani indigenous communities mostly prefer decoction as a preparation method [32].

Use values and relative frequency of citation

Using the ethnobotanical indices like *UV* and *RFC*, the traditional knowledge on ethnomedicinal plants used in the treatment of various human ailments were analyzed (Table 1). In the present study, *UV* ranged from 0.14 to 0.85. Of the 50 reported ethnomedicine species, 12 plant species were identified with *UV* greater than 0.55; *A. bracteosa* Wall. Ex Benth., *Mentha longifolia* (L.) L., *Teucrium royleanum* Wall. ex Benth., *Artemisia vulgaris* L., *Solanum nigrum* L., *Foeniculum vulgare* L., *Zanthoxylum alatum* Roxb., *Myrtus communis* L., *Hedera nepalensis* K. Koch, *Fumaria indica* (Hausskn.) Pugsley, *O. ferruginea* Wall. ex Aitch., and *B. lycium* Royle (Table 1). While the lowest *Indigofera heterantha* Brandis, *Oxalis corniculata* L., *D. viscosa* (L.) Jacq., *Debregeasia saeneb* (Forssk.) Hepper & J.R.I., *Platanus orientalis* L., and *C. australis* L. (Table 1). The medicinal plant species with low *UV* are also very important and should not be ignored as failing to declare them to upcoming generations could raise the threat of slowly vanishing of the knowledge. Plant species having high *UV*

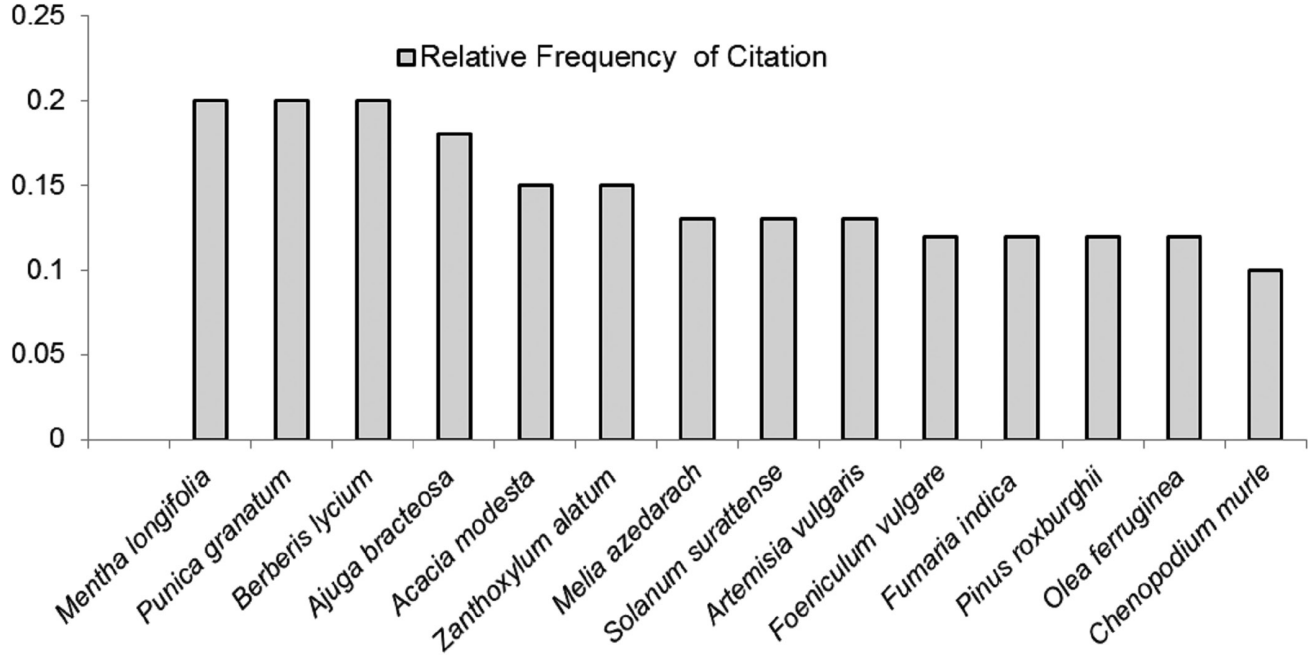

Figure 5. Relative frequency citations for medicinal plant species.

should be further screened in ethnopharmacological studies for active compounds [33].

RFC is used to find the most frequently used species of plants used for various human ailments in the study area. Its value ranged from 0.002 to 0.206. Fifteen plant species reported in this study showed high values *M. longifolia* (L.) L., *A. bracteosa* Wall. Ex Benth., *A. vulgaris* L., *Solanum surattense* Burm., *F. vulgare* L., *Punica granatum* L., *Z. alatum* Roxb., *Melia azedarach* L., *Juglans regia* L., and *F. indica* (Hausskn.) (Fig. 5). The ethnomedicinal plant species with higher values of *RFC* show the fact that these plant species were well known to most of the local people [34]. Those medicinal plant species having high *RFC* must be further assessed for phytochemical analysis and pharmaceutical analysis to identify their active constituents for any drug extraction [25].

Informant Agreement Ratio (IAR)

In this study, we compared the number of times the informant mentioned the use of plants for a specific disease and the number of plant species in each usage category (Table 2). Guiding from Collins et al., we define the different human ailments into specific usage categories [35]. According to the report, the usage categories of the illnesses which received the highest number of mentions are the most prevalent in the communities and also of the greatest importance to people living in the study area [35].

Informant agreement ratio between 0.25 and 0.46 was obtained for the different use categories. In the Talash valley, the most important usage category is digestive system disorders followed by genitourinary system disorders, circulatory system disorders, and skin problem shown in Table 3.

Discussion

The use of medicinal plants of the Talash valley is similar to neighboring districts and other parts of the country. In our study, many of the reported species have already been well-published regarding their ethnomedicinal importance. In Swat, which is a neighboring district, *Berberis lyceum* is used for treatment of diarrhea, jaundice and internal wounds [17]. Similarly, Ahmed et al. [36] and Abbasi et al. [37] reported that the rhizomes of the same plant are used for rheumatism, stomachache, diabetes, and bone fracture. *F. indica* is used as antipyretic, blood purifier, and the aerial part of same plant is also used for hypertension [8,18,37]. The leaves of *S. nigrum* are used for liver problems, diabetes, and diarrhea [33]. In the case of *Viola canescense*, the whole plant is used for cough, cold, and respiratory disorder [36]. *A. bracteosa* of the Lamiaceae is one of the highly-used medicinal plants of the study area. The local people used their fresh leaves for jaundice, sore throat, pimples, and hypertension [8,17]. A *Teucrium stocksianum* mostly found in hilly region of the study area is used for abdominal pain, stomach acidification, and for the management of hypertension [38,39]. Rashid et al. [40] state that whole parts of the same plant showed hypolipidemic, hypoglycaemic, and anti-diabetic activity. Hamayun et al. [17]

Table 2. Usage categories with number of mentions of each ailment in Talash valley.

Digestive system disorders	Diarrhea	5	Emesis	3
	dysentery	2	Stomachache	5
	dyspepsia	2	Intestinal problem	2
	Cholera	1		
Infestations/ infections	Fever	7	Cough	6
	sore throat	3	Headache	4
	allergy	2	Fungal infection	2
	tuberculosis	1		
Circulatory system disorders	Hypertension	7	Heart problem	2
	blood purification	3		
Respiratory system disorder	Asthma	3	Nephrological	2
Venom or stings:	Scorpion sting	2	Snakebites	2
	wasp stings	1		
Skin problems	Skin diseases	4	Scabies	2
	ringworm	4	Urticaria	3
	burns		Boils	2
External injuries and other problems	External wound	3	Stop bleeding	2
	healing wounds	2	Sedative	2
	laxative	1		
Dental problem	Gum diseases	1	Teeth decay	1
	Toothache	3	Tooth whitening	1
	Teeth infection	1		
Nervous system disorder:	Epilepsy	3		
Eyes problem	Opthalmia	2		
Genitourinary system disorders	Disorders dysuria Kidney	4	Urinary disorders	2

Table 3. Usage categories with number of taxa, mentions, and informant agreement ratio values.

Usage category	Various ailments	No. of mentions	Taxa	IAR
1. Digestive system disorders	Diarrhea, emesis, dysentery, stomachache, dyspepsia, and intestinal problem, and cholera	20	15	0.26
2. Infestations/ infections	Fever, cough, sore throat, headache, allergy, fungal infection, and tuberculosis	25	17	0.33
3. Circulatory system disorders	Blood pressure, hypertension, heart problem, and blood purification	12	7	0.45
4. Respiratory system disorder	Asthma and nephrological	5	2	0.75
5. Venom or stings:	Scorpion sting, snakebites, and wasp stings	5	3	0.5
6. Skin problems	Skin diseases, boils, ringworm burns, scabies, boils, and urticaria	15	9	0.42
7. External injuries and other problems	External wound, healing wounds, stop bleeding, sedative, and laxative	10	6	0.44
8. Dental problem	Gum diseases, toothache, teeth decay, tooth whitening, and teeth infection	7	4	0.5
9. Nervous system disorder:	Epilepsy	3	1	1
10. Eyes problem	Opthalmia	2	1	1
11. Genitourinary system disorders	Disorders dysuria kidney, urinary disorders expel calculus from kidneys.	6	4	0.4

reported *M. longifolia* is used for diarrhea and dysentery. According to Ahmed et al. [36], *M. longifolia* is used for digestive stimulant and to stop emesis. According to Ahmad et al. [41], the leaves of *Pistacia integerrima* are used for the treatment of hyperuricemia. The dried fruit powder and other morphological parts of *O. ferruginea* was used previously for diabetes, kidney disorders, skin diseases, toothaches, coughs, colds, and flue [36,42]. The fresh leaves of the same plant in the form of herbal tea are also used for hypertension [8]. The leaves of *Otostegia limbata* is used in Swat for curing of wounds and gum diseases [43]. According to the Haq [44], the leaves and roots of the same plant are used for hypertension and diabetes. Previously, it was reported that *Mentha viridis* was used for dysentery, diarrhea, gastric disorders, and used as a vermifuge herb [34]. The dried powder of leaves and other morphological parts of *H. nepalensis* was used against diabetes, ulcers, fever, and also shows anti-cancer activities [17,36,45].

A comparison of our study with relevance to other researchers in other parts of the world supported many findings. The fruit of *F. vulgare* was used for diabetes, renal diseases, stomach problems, and hypertension [46–48]. Previously, it was reported that *Myrtinus communes* was used for dysentery, rheumatism, hemorrhages, diarrhea, gastric ulcer, and vomiting [49]. The aerial part of highly medicinal herb *F. indica* has antihepatotoxic and hepatoprotective potential [50,51]. Abe and Ohtani [52] in their study reported that *S. nigrum* is used for the management hypertension. Similarly, the ethnomedicinal importance like for breathing problems in children and treating mouth ulcer, the *A. bracteosa* was discussed by Uniyal et al. [53]. The aerial parts of *A. vulgaris*, a member of family Asteraceae is used for the treatment of diabetes [45]. *Tribulus terestris* also has previous ethnopharmacological evidences; it is used for kidney disorder, urinary infections, skin problems, and hypertension [54–56]. It was previously reported that the aerial parts of *M. viridis* are used for diabetes, cold, stomachache, and hypertension [48,55,57]. Herbal formulation of the leaves of *H. nepalensis* leaves was effective in inflammation and cough [58]. In Bangladesh, whole plant of *Cynodon dactylon* is used for the treatment of tuberculosis and diabetes [59]. Zakavi et al. [60] reported that bark and leaves of *J. regia* have anti-microbial potential. In Turkey, it was reported by Gürdal and Kültür [57] that the leaves of *M. nigra* are used for kidney disease. The paste of *P. granatum* flowers are used as a mouthwash for periodontitis [61]. The leaves of *C. procera* are used for headaches [62]. In Congo, a leaf decoction of *M. azedarach* is used for the treatment of malaria [63]. Muthu et al. reported that the leaf juice of *Ricinus communis* is taken orally or washed to increase secretion of milk in women. In the same study, it was reported that the leaves of *Justicia adhatoda* are mixed with the flowers of *Hibiscus rosa-sinensis* and used to treat asthma [64]. In India, the root of *Salvia moorcroftiana* is used for cold and cough [65]. Soleimani et al. [66] established that the methanolic extract of *Equisetum arvense* have anti-diabetic potential. In Morocco, the anti-diabetic activity of *M. communis*

had been well established by Ziyyata et al. [67]. The leaves of *J. adhatoda* and *O. corniculata* in Nepal are used to treat rheumatic pain, dysentery, and stomach disorders [68].

The ethnomedicinal plants of the Talash valley are under huge stress by human related activities such as agricultural land expansion, domestic grazing, deforestation, lack of awareness, and unsustainable collection. The local people depend on plants as there is no alternative source of fuels so they cut the forest severely out of necessity. The people of the Talash valley are poor and they keep a large number of livestock to fulfill their daily requirements. Similarly, women and children mostly collect the medicinal plants in an unsustainably from which the degradation of medicinal plants resources may occur (personal communication, informant interview). According to the questionnaire results, interviews with district forest officers, and other resources, the main threat to the medicinal plants diversity of the study area is deforestation, domestic grazing, and unsuitable collection (Fig. 6).

Major threats to medicinal plants in Talash valley is deforestation. In the study, the winter season is very long and harsh. People need fuel for heating their houses as well as cooking. There are no alternate facilities for heating and cooking. The local people are unaware about the conservation of valuable and indigenous plants in the area [personal communication with District Forest officer, Dir Lower July, 2015]. They go to the nearby forests and collect plants for collecting wood, sometimes they cut whole trees for collecting only branches and twigs. Due to this indiscriminate cutting, not only forests are declining but also valuable medicinal plants species are in danger. According to the questionnaire survey conducted, 40% of medicinal plant resources are degraded due to deforestation. The population is increasing enormously and the people are degrading forests for fuel and shelters [69].

Conclusion

The local people of the Talash valley in Lower Dir, Pakistan, widely used medicinal plants to treat various human ailments. The present study showed that consistent indigenous knowledge on ethnomedicinal plants used in the treatment of basic human healthcare systems existed here. Most of the people live in rural communities in the remote areas and away from the modern healthcare facilities. In the study area, the local residents are heavily dependent on medicinal plants for health issues and so demand of ethnomedicinal plants increases day by day. The importance of biodiversity conservation is therefore fundamental and strategies of sustainable use should be considered for long-term availability of medicinal

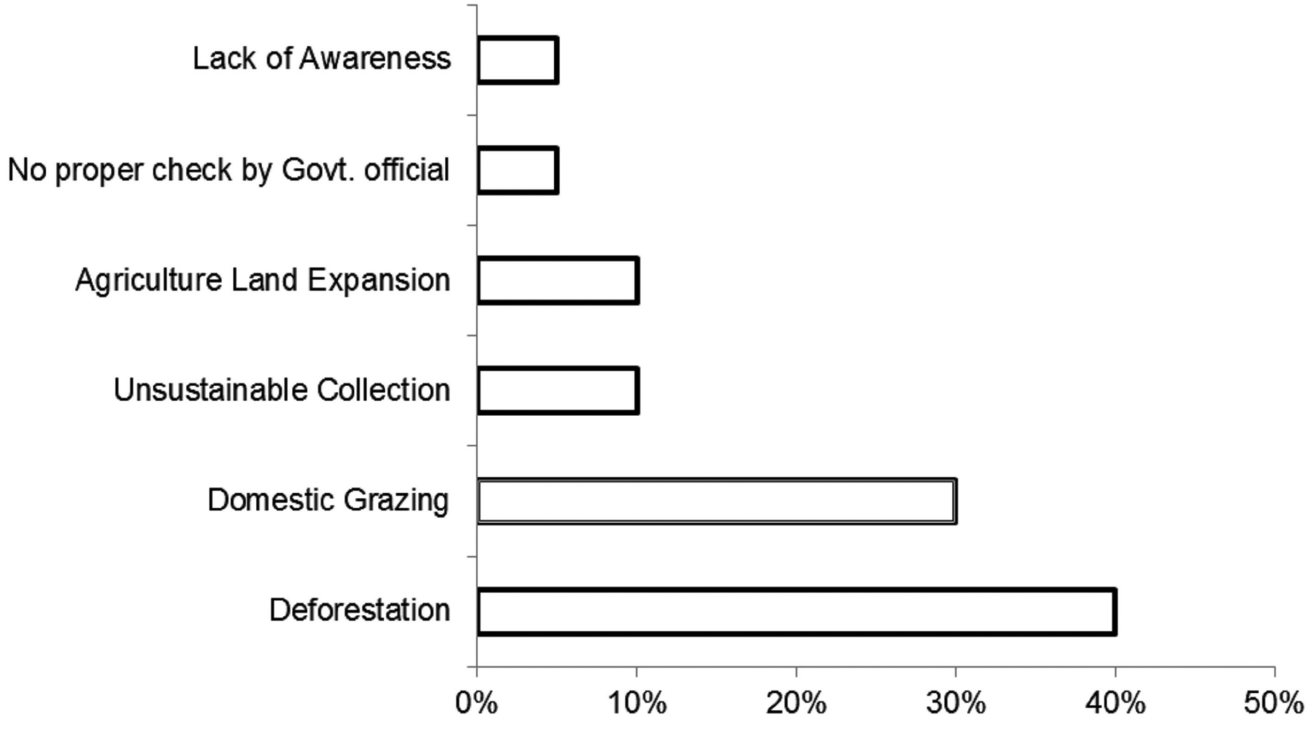

Figure 6. Various Human related activities which threat to the medicinal plants diversity of the study area.

plants here and even in whole country. Possible solutions for the conservation of biodiversity and ethnomedicinal flora of the study area are to strengthen national, regional, and local networking activities regarding conservation and sustainable utilization. There must be cooperation among government, non-government organizations, and local community to help conservation of medicinal plants in the area. Control programs for invasive species should be implemented in the study area. Furthermore, the elder populations of the study area are often unaware about the importance of biodiversity conservation; they also show poor selection of fuel wood species. There is need to re-introduce the indigenous knowledge about the conservation and management of medicinal plants resources. To build the capacity of the local people and develop their interest in growing tree species, medicinal plants demonstration plots may be introduced at UC bases.

Even though there is no available database to deposit the documented traditional knowledge in the study area, elderly people were always pleased when we asked them about medicinal plants and their therapeutic uses. Unfortunately, the younger generations showed a lack of interest in plant related questions. We suggest that the traditional knowledge from the elder people should be documented along with quality photography. In school, awareness session should be arranged for the students and the relevant documents should be made available in school libraries. The results of this study support the ethnomedicinal uses to support previous studies. Future investigations should be carried out in order to ensure safe therapy concerning medicinal plants.

Acknowledgement

The authors thankfully acknowledge the Department of Botany, University of Peshawar for support of this research project and for providing facilities for analyzing data. The authors also thank the local people of the study area for providing relevant information about medicinal plants usage. This study was self-supported and no grants were received from any institution.

References

[1] Güler B, Kümüştekin G, Uğurlu E. Contribution to the traditional uses of medicinal plants of Turgutlu (Manisa–Turkey). J Ethnopharmacol 2015; 176:102–8.

[2] Hill AF. Economic botany. McGraw-Hill Book Company, Tokyo, Japan, 1952.

[3] Matu EN, vanStaden J. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J Ethnopharmacol 2003; 87:35–41.

[4] Pieroni A, Price LL, Vandebroek I. Welcome to Journal of Ethnobiology and Ethnomedicine. J Ethnobiol Ethnomed 2005; 1:1–4.

[5] Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev 2012; 6:1–5.

[6] Teall EK. Medicine and doctoring in ancient mesopotamia. Gd Val J Hist 2013; 3:2.

[7] Baydoun S, Chalak L, Dalleh H, Arnold N. Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. J Ethnopharmacol 2015; 173:139–56.

[8] Ahmad L, Semotiuk A, Zafar M, Ahmad M, Sultana S, Liu QR, et al. Ethnopharmacological documentation of medicinal plants used for hypertension among the local communities of DIR Lower, Pakistan. J Ethnopharmacol 2015; 175:138–46.

[9] Mèdoatinsa SE, Dossa CPA, Yédomonhan H, Ahissou H, Gbohaïda V, Sohounhloué D. Ethnobotanical study of plants used by the lacustrine population of southern Benin in the treatment of malaria. J Pharmacogn Phytochem 2016; 5:430–36.

[10] Kayani S, Ahmad M, Zafar M, Sultana S, Khan MP, Ashraf MA, et al. Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies: abbottabad, Northern Pakistan. J Ethnopharmacol 2014; 156:47–60.

[11] Akhtar N, Rashid A, Murad W, Bergmeier E. Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. J Ethnobiol Ethnomed 2013; 9:25.

[12] Govaerts R. How many species of seed plant are there? Taxon 2001; 50:1085–90.

[13] Schippmann U, Leaman DJ, Cunningham AB. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: Proceedings of the Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. FAO, Rome, Italy, pp 143–67, 2002.

[14] Leonti M, Sticher O, Heinrich M. Antiquity of medicinal plant usage in two Macro-Mayan ethnic groups (Mexico). J Ethnopharmacol 2003; 88:119–24.

[15] Ali SI. Significance of flora with special reference to Pakistan. Pak J Bot 2008; 40:967–71.

[16] Food and Agriculture Organization of the United Nations Rome. Trade in Medicinal Plants. Raw Materials, Tropical and Horticultural Products Service Commodities and Trade Division Economic and Social Department, 2008. https://catbull.com/alamut/Bibliothek/af285e00.pdf.

[17] Hamayun M, Khan SA, Iqbal I, Rehman G, Hayat T, Khan MA. Ethnobotanical profile of Utror and Gabral valleys, district Swat, Pakistan. Ethnobot Leaflets 2005; 1:9.
[18] Chaudhary M, Ahmad S, Ali A, Sher H, Malik S. Technical report. Market study of medicinal herbs in Malakand, Peshawar, Lahore and Karachi. SDC-Inter cooperation, Peshawar, Pakistan, p 7, 2000.
[19] Teklehaymanot T, Giday M. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. J Ethnobiol Ethnomed 2007; 3:1–11.
[20] Jeyaprakash K, Lego YJ, Payum T, Rathinavel S, Jayakumar K. Diversity of medicinal plants used by Adi community in and around area of D'Ering wildlife sanctuary, Arunachal Pradesh, India. World Scientific News 2017; 65:135–59.
[21] Government of Pakistan. District census report of Lower Dir.Statistics Division, Population Census Organization, Islamabad, Pakistan, 1999.
[22] Government of Pakistan. District census report of Lower Dir. Statistics Division, Population Census Organization, Islamabad, Pakistan, 2017.
[23] Martin GJ. Ethnobotany: a method and manual. Champan and Hall, London, UK, 1995.
[24] Cotton CM. Ethnobotany: principles and applications Chichester. John Wiley and Sons, New York, NY, 1996.
[25] Vitalini S, Iriti M, Puricelli C, Ciuchi D, Segale A, Fico G. Traditional knowledge on medicinal and food plants used in Val San Giacomo (Sondrio, Italy): an alpine ethnobotanical study. J Ethnopharmacol 2013; 145:517–29.
[26] Tardio J, Pardo-De-Santayana M. Cultural importance indice: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain). Econ Bot 2008; 62:24–39.
[27] Trotter RT, Logan MH. Informant consensus: a new approach for identifying potentially effective medicinal plants. In: Etkin NL (ed) Plants in indigenous medicine and diet, Redgrave Publishing Company, Bedford Hill, NY, pp 91–112, 1986.
[28] Heinrich M, Inta A, Frei B, Weimann C, Sticher O. Medicinal plants in Mexico: healers' consensus and cultural importance. Soc Sci Med 1998; 47:1859–71.
[29] Giday M, Asfaw Z, Elmqvist T, Woldu Z. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. J Ethnopharmacol 2003; 85:43–52.
[30] Ghorbani A. Studies on pharmaceutical ethnobotany in the region of Turkmen Sahra, North of Iran: (part1): general results. J Ethnopharmacol 2005; 102:58–68.
[31] Nadembega P, Boussim JI, Nikiema JB, Poli F, Antognoni F. Medicinal plants in Baskoure, Kourittenga Province, Burkina Faso: an ethnobotanical study. J Ethnopharmacol 2011; 133:378–95.
[32] Mahmood A, Malik RN, Shinwari ZK, Mahmood A. Ethnobotanical survey of plants from Nelum, Azad Jammu and Kashmir, Pakistan. Pak J Bot 2011; 43:105–10.
[33] Mahmood A, Mahmood A, Malik NR. Indigenous knowledge of medicinal plants from Leepa valley, Azad Jammu and Kashmir, Pakistan. J Ethnopharmacol 2012; 143:338–46.
[34] Kayani S, Ahmad M, Zafar, M, Sultana S, Khan MP, Ashraf MA, et al. Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies - Abbottabad, Northern Pakistan. J Ethnopharmacol 2014; 156:47–60.
[35] Collins S, Martins X, Mitchell A, Teshome A, Arnason JT. Quantitative ethnobotany of two east timorese cultures. Econ Bot 2006; 60:347–61.
[36] Ahmed E, Arshad M, Saboor A, Qureshi R, Mustafa G, Sadiq S, Chaudhari SK. Ethnobotanical appraisal and medicinal use of plants in Patriata, New Murree, evidence from Pakistan. J Ethnobiol Ethnomed 2013; 9:13.
[37] Abbasi AM, Khan MA, Ahmad M, Zafar M, Jahan S, Sultana S. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. J Ethnopharmacol 2010; 128:322–35.
[38] Safa O, Soltanipoor MA, Rastegar S, Kazemi M, Nourbakhsh DK, Ghannadi A. An ethnobotanical survey on hormozgan province, Iran. Avicenna J Phytomed 2012; 3:64–81.
[39] Farooq S, Barki A, Khan MY, Fazal H. Ethnobotanical studies of the flora of tehsil Birmal in South Waziristan Agency, Pakistan. Pak J Weed Sci Res 2012; 18:277–91.
[40] Rashid M, Mushtaq MN, Malik MH, Ghumman SA, Numan M, Khan AQ, et al. Pharmacological evaluation of antidiabetic effect of ethyl acetate extracts of Teucrium stocksianum Boiss in alloxan-induced diabetic rabbits. J Anim Plant Sci 2013; 23:436–39.
[41] Ahmad NS, Farman M, Najmi MH, Mian KB, Hasan A. Pharmacological basis for use of Pistacia integerrima leaves in hyperuricemia and gout. J Ethnopharmacol 2008; 117:478–82.
[42] Ahmad M, Qureshi R, Arshad M, Khan MA, Zafar M. Traditional herbal remedies used for the treatment of diabetes from district Attock (Pakistan). Pak J Bot 2009; 41:2777–82.
[43] Iqbal I, Hamayun M. Studies on the traditional uses of plants of Malam Jabba valley, District Swat, Pakistan. Ethnobot Leaflets 2004; 1:15.
[44] Haq F. The ethno botanical uses of medicinal plants of Allai Valley, Western Himalaya Pakistan. Inter J Plant Res 2012; 2:21–34.
[45] Qureshi RA, Ghufran MA, Gilani SA, Sultana K, Ashraf M. Ethnobotanical studies of selected medicinal plants of Sudhan Gali and Ganga Chotti hills, district Bagh, Azad Kashmir. Pak J Bot 2007; 39:2275–83.

[46] Jouad H, Haloui M, Rhiouani H, Hilaly J, Eddouks M. Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). J Ethnopharmacol 2001; 77:175–82.
[47] Tene V, Malagon O, Finzi PV, Vidari G, Armijos C, Zaragoza T. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J Ethnopharmacol 2007; 111:63–81.
[48] Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). J Ethnopharmacol 2007; 110:105–17.
[49] Sumbul S, Ahmed MA, Asif M, Akhtar M. Myrtus communis Linn.—a review. Indian J Nat Prod Resour 2011; 2:395–402.
[50] Rao KS, Mishra SH. Antihepatotoxic activity of monomethyl fumarate isolated from Fumaria indica. J Ethnopharmacol 1998; 60:207–13.
[51] Rathi A, Srivastava AK, Shirwaikar A, Singh AK, Mehrotra S. Hepatoprotective potential of Fumaria indica Pugsley whole plant extracts, fractions and an isolated alkaloid protopine. Phytomedicine 2008; 15:470–77.
[52] Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J Ethnopharmacol 2013; 145:554–65.
[53] Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed 2006; 2:14.
[54] Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, et al. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Plant Res 2010; 4:1089–101.
[55] Yasari E, Vahedi A. Study of Iranian biospherical reservation areas for medicinal plants diversity. World Acad Sci Eng Tech, Int J Biomed Biol Eng 2011; 5:53–56.
[56] Talha J, Priyanka M, Akanksha A. Hypertension and herbal plants. Int Res J Pharmacol 2011; 2:26–30.
[57] Gürdal B, Kültür Ş. An ethnobotanical study of medicinal plants in Marmaris (Muğla-Turkey). J Ethnopharmacol 2013; 146:113–26.
[58] Leto C, Tuttolomondo T, La Bella S, Licata M. Ethnobotanical study in the Madonie Regional Park (Central Sicily, Italy): medicinal use of wild shrub and herbaceous plant species. J Ethnopharmacol 2013; 146:90–112.
[59] Dulla O, Jahan FI. Ethnopharmacological survey on traditional medicinal plants a Kalaroa Upazila, Satkhira District, Khulna Division, Bangladesh. J Intercult Ethnopharmacol 2017; 6:316–25.
[60] Zakavi F, Golpasand Hagh L, Daraeighadikolaei A, Farajzadeh Sheikh A, Daraeighadikolaei A, Leilavi Shooshtari Z. Antibacterial effect of Juglans regia bark against oral pathologic bacteria. Int J Dent 2013; 2013:854765; doi:10.1155/2013/854765.
[61] Ahuja S, Dodwad V, Kukreja BJ, Mehra P, Kukreja P. A comparative evaluation of efficacy of Punica granatum and chlorhexidine on plaque and gingivitis. J Int Clin Dent Res Organ 2011; 3:29–32.
[62] Yetein MH, Houessou LG, Lougbegnon TO, Teka O, Tente B. Ethnobotanical study of medicinal plants used for the treatment of malaria in plateau of Allada, Benin (West Africa). J Ethnopharmacol 2013; 146:154–63.
[63] Kasali FM, Mahano AO, Nyakabwa DS, Kadima NJ, Misakabu FM, Tshibangu DST, et al. Ethnopharmacological survey of medicinal plants used against Malaria in Bukavu City (D.R. Congo). European J Med Plants 2014; 4:29–44.
[64] Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram district of Tamil Nadu, India. J Ethnobiol Ethnomed 2006; 2:43.
[65] Kumar M, Paul Y, Anand VK. An Ethnobotanical study of medicinal plants used by the locals in Kishtwar, Jammu and Kashmir, India. Ethnobot Leaflets 2009; 13:1240–56.
[66] Soleimani S, Azerbaijani FF, Nejati V. The effect of Equisetum arvense L. (Equisetaceae) in histological changes of pancreatic β-cells in streptozotocin-induced diabetic in rats. Pak J Biol Sci 2007; 10:4236–40.
[67] Ziyyata A, Legssyera A, Mekhfia H, Dassoulia A, Serhrouchnia M, Benjellounb W. Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol 1997; 58:45–54.
[68] Joshia AR, Joshi K. Indigenous knowledge and uses of medicinal plants by local communities of the Kali Gandaki Watershed Area, Nepal. J Ethnopharmacol 2000; 73:175–83.
[69] Tariq M, Rashid M, Rashid W. Causes of deforestation and climatic changes in Dir Kohistan. J Pharm Alt Med 2014; 3:28–37.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 25–44
10.5455/jice.20171202114930

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A survey of plants used for family planning in Bayelsa State, southern Nigeria

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ABSTRACT

Aim: The medicinal plants employed in the ethnomedicine of Bayelsa State of Nigeria for family planning are studied. Bayelsa State is largely populated with a low literacy level stimulating a high poverty rate aggravated by dwindling global oil prices. Thus, the need to keep small family size is now embraced by the people of the State. The survey aims to identify and document the plants used amongst the indigenous people of Bayelsa State for planning.

Materials and methods: Using semi-structured questionnaires, information was gathered through personal interviews with 39 traditional birth attendants (TBAs) and 53 community elders.

Results: A total of 35 medicinal plant species representing 33 genera and 26 families were employed by the TBAs and elders for contraception, labor induction, and abortion among the people of Bayelsa State, Nigeria. Among these, only three plant species were mentioned for male contraception purpose.

Conclusion: The survey provides a veritable source of information for TBAs and medicinal plant researchers. These medicinal plants may be incorporated into the healthcare delivery system of the country.

ARTICLE HISTORY

Received December 02, 2017
Accepted December 25, 2017
Published January 06, 2018

KEYWORDS

Traditional birth attendant; spermicidal; labor inducing; contraception; abortifacient

Introduction

Family planning has been defined as a way of living that is adopted voluntarily upon the basis of knowledge, attitude, and responsible decision making by individuals or couples in order to pin the number, timing, and spacing of the children that they want so as to promote the health and welfare of the family and contribute to the advancement of society [1,2]. Family size control helps to reduce maternal mortality rate by allowing mothers to recover and have enough time to recover before the next birth [3–5]. Generally, some Africans believe that procreation purely originates from God and that man should do nothing to limit the number of children provided by God [1,6]. In most rural settings, a vast number of children are given birth to in order to use the male children for farm labor. Unfortunately, these acts or any other acts of uncontrollable birth lead to overpopulation of a country [7]. However, due to the attendant problems of overpopulation, Government of Nigeria has continually made frantic efforts to control birth through methods such as the use of oral contraceptive pills, intrauterine device, sterilization, safe periods, and injectables. In addition to the foregoing generally regarded as modern methods, public enlightenment campaign is also employed [8]. Traditional methods are also available which are usually adopted by individuals in a society. They include the use of medicinal plants, prolonged breast feeding, ornaments, and spiritual invocations [1,6,7,9]. However, the use of medicinal plants preponderates over other traditional means because plants are viewed as an integral part of the culture and are easier and safer to use [10,11]. The use of medicinal plants for family planning has been grouped into different categories to clearly define

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their role viz-a-viz the type of family planning to which they are used. Plants regarded as spermicides are those that are capable of destroying viable sperms [12], for instance, *Azadirachta indica* A. Juss (Meliaceae), *Bambusa vulgaris* L. (Poaceae), and *Carica papaya* L. (Caricaceae) [13–16]. Those regarded as contraceptives are those capable of preventing pregnancy by interfering with the normal process of ovulation, fertilization, and implantation of a fertilized egg [17], for e.g., *Allium sativum* L. (Alliaceae), *Aloe barbadensis* Mill. (Asphodelaceae), and *Abrus precatorius* L. (Fabaceae) [18–22]. The plants referred to as abortifacients are those that disturb embryo already implanted in the uterine lining and cause premature termination of pregnancy [17], for instance, *Ananas cosmosus* (L.) Merril (Bromeliaceae), *Butea monosperma* (Lam.) Kuntze (Bombacaceae), and *Hibiscus rosa sinensis* L. (Malvaceae) [20,22–24]. Last, plants with labor inducing (LI) (oxytocic) properties are *Ricinus communis* L. Euphorbiaceae [17], *Agapanthus africanthus* (L.) Hoffmanns (Amaryllidaceae), and *Clivia miniata* (Lindl.) Verschaff (Amaryllidaceae) [25].

Traditional birth attendants (TBAs) are referred to as the community or family members who are normally females of old age groups that are a product of tradition and assist mothers during home delivery [26,27]. They often acquire initial skills by delivering babies themselves or through an apprenticeship to other TBAs. Since the introduction of Safe Motherhood Program, training of TBAs has been recognized as one of the interventions intended to improve reproductive and child health [28,29]. Despite the existence of modern health facilities in Nigeria, over 58% of deliveries still take place at home under the supervision of TBAs. An estimate of between 60% and 80% of all deliveries in developing countries occur outside modern healthcare facilities with a significant proportion attended to by TBAs [30]. For instance, out of 93 of rural women registered for antenatal care in the Eastern part of Nigeria, 49% delivered at home under TBAs' supervision [31]. In another report, 65% of mothers had delivery by TBAs in a Southwestern town of Nigeria and 73% had at one time or the other sought help from them [30]. TBAs also play a significant role in family planning through the use of herbs and counseling [30,32]. For family control, search for plants with antifertility activities through an ethnobotanical survey is on the increase due to the perceived safety of medicinal plants [33]. However, fewer studies are recorded for male contraception [34]. It is now generally accepted that current modern fertility control methods are inadequate to meet the varied and changing personal needs of couples at different times in their reproductive lives, and in the widely differing geographical, cultural, and religious settings that exist around the world [16]. In recent years, the use of ethnobotanical information in medicinal plant research has gained considerable attention in some segments of the scientific community [16,35], employing medicinal plants and their products to solve many health problems including regulation of fertility in many countries [16,36,37]. Ethnobotanical survey information on medicinal plant research has also gained prominence in recent years [35,38]. Drugs of plant origin are on the intense search as plants show promises as a source of newer alternatives with lesser side effects. Plants have served as a natural source of antifertility substances and women dwelling in rural areas have used plants before coitus for prevention of pregnancy [39]. There has been a steady accumulation of information regarding the screening of plants having antifertility efficacy [2]. Bayelsa is a small but highly populated state with an economy solely dependent on Federal revenue which in turn is largely derived from oil explored in the southern Nigeria where Bayelsa is situated. The poverty rate (92.9%) of Bayelsa State is high with per capital of 2,484 USD and a gross domestic product of 4,337 occupying the 26th position of the 36 states [40]. The level of literacy is also low with education index of 0.67%, the lowest in the south region. Due to the economic hardship, occasioned by huge population and non-industrialization of the state, the indigenous people of Bayelsa state are now making conscious effort to control family size. Traditional medicine is more widely used for this control because it is integrated in their culture and also because of paucity of modern health facilities available to them. The survey, therefore, aims to identify and document the plants and other materials used amongst indigenes of Bayelsa State for family planning.

Materials and Methods

Study area

Bayelsa is one of the nine states that make up the Niger Delta region of Nigeria with her headquarters situated at Yenagoa. It is bounded in the North, South, East, and West by Rivers, Delta, Lagos State as well as the Atlantic sea, respectively (Fig. 1). It is an oil rich State comprising eight local government areas with a population of 1,703,358. The State is characterized by an almost all year round

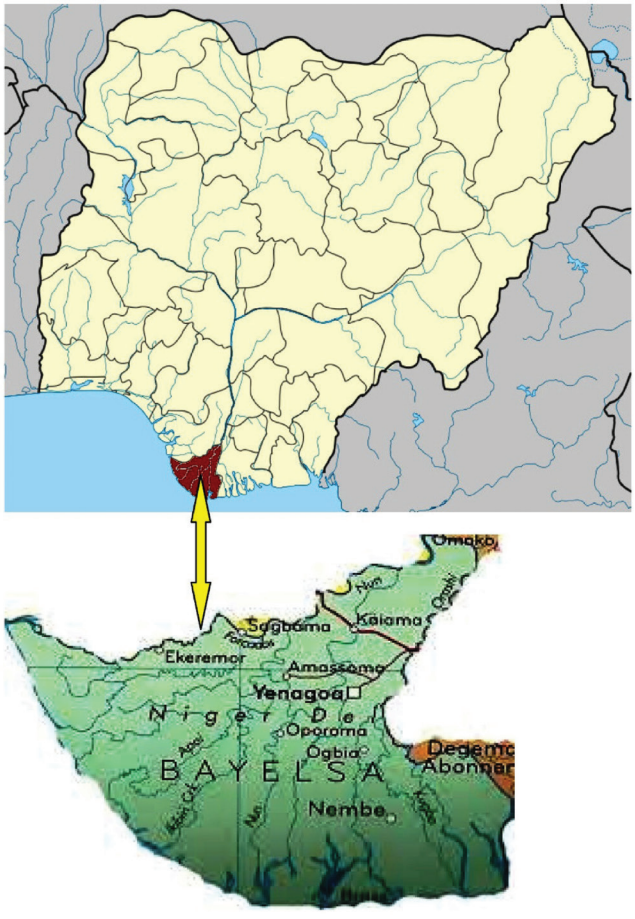

Figure 1. Map of Nigeria showing Bayelsa State with the eight Local Government Areas.

rainfall of 3,000–4,000 mm annually and maximum and minimum relative humidity of 90%–100% and 21%–23%, respectively [41], thereby making the vegetation an evergreen one. Basically, the State is made up of mangrove and lowland rainforests. The indigenous people are majorly occupied in fishing and farming. The study was carried out in three towns in each of the eight local government areas where cooperation was offered by the TBAs. The pre-ethnobotanical survey visits to all the local government areas indicated that three communities in Yenagoa (Okolobiri, Ovom, and Agbia), Sagbama (Sagbama, Toru beni, and Ogobiri), and Southern Ijaw (Amassoma, Amatolo, and Ebeni), Kolokuma/Opokuma (Odi, Sampou, and Trofani), and Ogbia (Ogbia, Imiringi, and Otuoke). Local government areas had the highest concentration of TBAs and elders who were conversant with the issues of family planning. However, study could only be carried out in one town in Nembe (Nembe town), Brass (Brass town), and Ekeremor (Ekeremor town) because human population is scant in the other areas due to the presence of large water body.

Data collection

Regular field tours were made between January and October, 2015, to the study area. The ethnobotanical survey was carried out with the aim of documentation of plants and other materials used for family planning purpose; plants used for family planning were divided into four main areas such as spermicides, LI (or oxytocic), and contraceptive plants as well as those that can cause abortion (abortifacient) which may be used for emergency contraception. Informants were 39 renowned TBAs and 53 elders. The methods used included interviews with these informants guided by a semi-structured questionnaire and observation/informal conversation on site. An experienced guide who understood the culture and language of the people was also involved. Meetings were regularly held with the community people to confirm the information gathered. Informed consent was obtained orally from all participants before the administration of the questionnaires and commencement of interview. Detailed information on the local names and plant parts was used and methods of preparation and use amongst others were documented. Collection of all plants was made as soon as the plants were indicated. They were identified and authenticated by Prof. K.K. Ajibesin and Dr. A.T. Oladele of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Nigeria. Voucher numbers were also issued for the plants and the herbarium samples were deposited at the herbarium of the same institution. In addition, the taxonomy of the plants was further confirmed by using "The Plant List, 2013" [42]. The study went through the Department of Pharmacognosy and Herbal Medicine Research Committee, Niger Delta University before the commencement of the study and the approval number "Pharmacog Res. 2015/001" granted.

Data analysis

The following ethnobotanical analytic tools were employed:

Reported use value (per plant part)

Reported use value (per plant part) was used to analyze the data. It was calculated for the number of use categories cited for each plant part, mathematically expressed as $\Sigma\ RU_{(plant\ part)}$ where RU indicates the total use categories for each plant part [43]. This was used to determine the most used parts of the plant.

Familial use value

The importance of a family is measured by its familial use value [44] and it was determined by totaling the number of species mentioned under each family across all the categories of uses.

Informant consensus

The importance of each species for each specified category of uses was determined by calculating its informant consensus [43], and it was obtained directly from the number of informants who mentioned the species.

Informant consensus factor (ICF) was determined by the formula

$$\text{Nur} - \text{Nt} / \text{Nur} - 1,$$

where Nur is the number of use reports from informants for a particular plant use category and Nt is the number of species that are used for that plant use category for all informants. This is an indication of agreement of informants for a plant species for a particular condition. The values range between 0 and 1 where 1 indicates the highest level of informant consensus [45].

Consensus between authors on cited species and families

Consensus between authors on cited species [46] was obtained from the formula:

$$\text{Number of species} \times 100/\text{Number of authors}.$$

The lower the value, the better the consensus and vice versa.

Uses totaled

Uses totaled is a simple sum of all known use categories for each [43] species. It was modified that the three categories of use in female family planning are contraceptive, abortifacient, and LI. A plant species mentioned for one use out of the three categories is scored 1/3 (0.33), 2/3 (0.67) for two, and 3/3 (1.00) for all the three categories.

Results

The survey revealed the indigenous knowledge of plants used as contraceptives, abortifacients, labor inducers, and spermicides in Bayelsa State of Nigeria. A total of thirty five (35) plant species distributed among 26 families were reported as plants used in female and male family planning by the TBAs and the elders of various communities of Bayelsa State of Nigeria (Table 1). Plants used for family planning included plants used as contraceptives, abortifacients, and LI in females and spermicides in males. A total of 92 informants were surveyed and interviewed. Thirty-four plants were cited for female family planning while only three plants were mentioned for male family planning (Table 1). Seven groups of two to three plants were mentioned for female family planning purpose (Table 1). *Aframomum melegueta* had the highest frequency of citation in the multi-herbal preparation. It is followed by *Xylopia aethiopica* and *Aspilia africana*. However, 22 plants were mentioned separately as single component preparation for female family planning, while only three also used singly were mentioned for male family planning (Table 1).

Spondias mombin and *Nymphae lotus* were mentioned as plants that are useful in family planning in both male and female (Table 1). They were mentioned as sperm reducing as well as abortifacient (Table 1). Abortion is not a common practice in the study area as revealed from these categories (Fig. 2).

The most popular method of herbal preparation employed in this survey was infusion (42.3%) followed by pounding/crushing (19.2%) and juice extraction or squeezing (17.3%) (Fig. 3), while the main mode of administration was oral (53%) followed by insertion into the vagina (33%) (Table 1). The media of extraction included water, beer, fermented palm wine, and illicit gin (Table 1), and the commonest was however the medium that contained alcohol (beer, fermented palm wine, and illicit gin). The excipients in the preparation of the herbal potions included native chalk and salt (Table 1). Also, salt is sometimes added for preservative purpose [73]. World Health Organization (WHO) [74] affirmed that excipients like inorganic ingredients are permissible inclusions in traditional medicines.

Leaf (78.5%) was the most commonly utilized plant part in female family planning; this was followed by root (10.0%) and seed (6.1%). Others were scarcely used. In male family planning, leaf (100%) was recorded as the only plant part used (Tables 1 and 2).

Pilo ovate (Water snail) and *Dasyatis garouaensis* (String ray fish) were mentioned by informants for family planning purpose (Table 3).

Asteraceae was the most important family having the highest familial use value of 4 and [a consensus between authors on cited species and

Table 1. Medicinal plants used in family planning.

Family	Botanical name	Frequency (informant consensus)	Local name	Common name	Voucher number	Part	Method of preparation	Administration	Activity	Reported ethnomedicinal uses for family planning
Acanthaceae	*Acanthus montanus* (Ness) T. Anderson	6	Konowei-emimi, idule-emimi	Leopard's claw	NDUP 110	L	Chewing	Chewing , regularly	Contraception	None, but pharmacologically validated [47,48]
Alliaceae	*Allium sativum* L	2	Nkarika	Garlic	NDUP 111	B	Chewing	To be chewed regularly and immediately after sex	Contraception	Philadelphia [49]
Anacardiaceae	*Spondias mombin* L.	2	Iginiyan	Hog plum, yellow mombin	NDUP 112	L	Chewing, Pound leaf with limestone Chewing	To be chewed regularly after sex To be chewed regularly	Abortifacient Spermicidal	Nigeria [50–52]; Ghana [53] Nigeria [54]
Annonaceae	*Xylopia aethiopica* (Dunal) A. Rich	8	Ikani	Ethiopian pepper	NDUP 113	Fr	Fruits pounded with *Ocimum gratissimum* leaves and *Piper guineense*	Insertion into the vagina 3–4 times daily during the days of intercourse.	Contraception	Nigeria [55,56]
Araceae	*Colocasia esculenta* (L.) Schott.	2	Odu	Cocoyam, Mama koko	NDUP 114	S	Extract juice	Insertion into vagina during the days of intercourse	Contraception	None
Asteraceae	*Aspilia africana* (Pers.) C.D Adams	8	Younkore	Hemorrhage plant	NDUP 115	L / R	Root or leaf pounded with native chalk	Daily insertion into the vagina for 3 days	Abortifacient	Nigeria [57]
	Ageratum conyzoides L.	10	Furotuoru	Goatweed	NDUP 116	L	Leaves pounded with *Aframomum melegueta seeds*	Insertion into the vagina during the days of intercourse	Contraception	Trinidad Tobago, [58]; Nigeria [59,60]
	Acmella caulirhiza Delile	2	Kala awou igina	Brazil cress	NDUP 117	L/F	A little more flower than leaf in quantity, pound together to extract juice	Juice inserted into the vagina during the days of intercourse	Contraception	None
	Vernonia amygdalina Delile	16	Diri esen	Bitter leaf	NDUP 118	L/R	Pound with the leaves of *Chloris pilosa* Extract the juice in water and filter	To be drunk regularly	Labor inducer, abortifacient	Ethiopia [61] South Africa [62]
Bignoniaceae	*Newbouldia laevis* P. Beauv.	2	Iginiga Obrizzii	*Newbouldia*	NDUP 119	L	Extract the juice in little quantity of water	To be drunk regularly	Labor inducer, abortifacient	[63]
Caricaceae	*Carica papaya* L.	18	Indu	Pawpaw	NDUP 120	L/S/R	Leaves/stem/root with *Physalis angulata* leaves extracted into illicit gin, Extract leaf in beer and dilute with water, Extract root/stem in illicit gin	Chew leaf regularly To be drunk regularly	Contraception labor inducer, abortifacient	Nigeria [56,59], India [17,23], Ghana [53]

(Continued)

Table 1. Medicinal plants used in family planning. (Continued)

Family	Botanical name	Frequency (informant consensus)	Local name	Common name	Voucher number	Part	Method of preparation	Administration	Activity	Reported ethnomedicinal uses for family planning
Cleomaceae	*Cleome rutidosperma* D.C.	2	Agbalala	Spider plant	NDUP 121	L	Extract leaf in water	To be taken once in large quantity	Abortifacient	None
Commelinaceae	*Commelina diffusa* N.L. Burman	2	Owei-ikpisekpise	Dayflower, small blue flower	NDUP 122	L	Decoction of leaf	To be drunk twice daily	Contraception	None
Crassulaceae	*Bryophyllum pinnatum* (Lam.) Oken	2	Ombukoromo-diri	Never die	NDUP 123	L	Chewing	Chewing, regularly	Contraception	Mexico [64]
Euphorbiaceae	*Manihot esculenta* Crantz.	4	Ebia buru	Native Cassava	NDUP 124	R	Dried peeled powdered *cassava* root is mixed with potash	Chewing during the days of intercourse and immediately conception is established	Abortifacient Contraception	Ghana [53]
Lamiaceae	*Plectranthus monostachys* (P. Beauv.) B.J. Pollard	2	Okobu-toru	Catnip	NDUP 126	L	Chewing, marceration in illicit gin, steamed with food	Orally, thrice daily	Contraception	Cameroun [65] Cote d' Ivoire [66], Nigeria [67]
	O. gratissimum L.	6	Furu kana Karan	Tea bush, scent leaf, mosquito plant	NDUP 127	L	Leaves pounded with *X. aethiopica* fruit	Once in 2 days insertion into the vagina 3-4 times.	Contraception	None but pharmacologically validated [68]
	Hyptis lanceolata Poir.	2	Obrigae, Poifurukana	Lesser round weed	NDUP 128	L	Extract juice by squeezing	To be taken immediately pregnancy is established. To be drunk regularly	abortifacient Induce labor, Contraception	None
Leguminosae	*Baphia nitida* Lodd.	4	Abode	*Cam wood*	NDUP 133	L	Pound leaves with *Aspilia africana* leaves and 7 seeds of *A. melegueta*, pound with *Ageratum conyzoides* leaf in a local pot with rubber chain	Daily insertion into vagina during the days of sexual intercourse	Contraception	Nigeria [38]
	Senna alata (L.) Roxb.	4	Ekiri-kuma-tin	Ringworm shrub	NDUP 125	Rb	Pieces of bark with heads of *A. melegueta* soaked in illicit gin.	Drink regularly	Contraception	India [23]
Musaceae	*Musa x paradisiaca* L.	2	Beriba Abanga-tin	Plantain	NDUP 129	Fr	Extract the juice of fresh fruit	Orally, frequently	Contraception	Ghana [53], India [69,70]
Myrtaceae	*Psidium guajava* L.	2	Guava-tin	Guava tree	NDUP 130	S	Extract the stembark in illicit gin	Orally, frequently	Abortifacient	Indonesia [71]

(Continued)

Table 1. Medicinal plants used in family planning. (Continued)

Family	Botanical name	Frequency (informant consensus)	Local name	Common name	Voucher number	Part	Method of preparation	Administration	Activity	Reported ethnomedicinal uses for family planning
Nymphaceae	*Nymphaea lotus* L.	4	Osungbo digi	Water lilly	NDUP 131	L	Extract leaf in water Extract leaf in water	To be drunk regularly To be drunk regularly	Abortifacient Contraception Spermicide	None None
	Nymphaea odorata Aiton	4	Beni asisa	Water lilly	NDUP 132	L/F	Flower is squeezed, Raw leaves placed on the floor	Insertion into vagina	Contraception	None
Piperaceae	*Piper guineense* Schumach. & Thonn.	2	Aziza	Ethiopian pepper African pepper	NDUP 134	Se	Pounded with *X. aethiopica* fruit and extracted with illicit gin	To be drunk regularly	Contraception	Ghana [53]
	Peperomia pellucida (L.) Kunth.	6	Ofonibuo	Shiny bush Silver bush	NDUP 135	L	Pound with the leaves of *C. pilosa* and *V. amygdalina*. Extract the juice in water and filter	To be drunk regularly	Labor inducer, abortifacient	None
Poaceae	*C. pilosa* Schumach. & Thonn.	12	Amakubu osuga	Wire grass	NDUP 136	L	Pound with the leaves of *P. pellucida* and *V. amygdalina*. Extract the juice in water and filter	To be drunk regularly	Contraception, abortifacient	Nigeria [72]
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms.	4	Lakwa	Water hyacinth	NDUP 137	L	Leaves pounded with Leaf of *Portulaca oleoracea* in ratio 1:2 with one aerial part of *A. melegueta* to obtain a paste, palm oil added	Paste inserted into the vagina with a clean cloth to hold it in place for 24 hr. before and after intercourse	Contraception	None
Portulacaceae	*Portulaca oleracea* L.	4	Eke beri	Purslane	NDUP 138	L	Leaves pounded with Leaf of *Eichhornia crassipes* in ratio 1:2 with one aerial part of *A. melegueta* to obtain a paste, palm oil added	Paste inserted into the vagina with a clean cloth to hold it in place for 24 hours. before intercourse and immediately after it.	Contraception	Ghana [53] *P. quadrfola*
Rutaceae	*Citrus x limon* (L.) Osbeck	4	Alalanda	Lemon	NDUP 139	R/Fr	Chewing root, Extract juice of fruit	Chewing regularly, Juice to be drunk regularly	Contraception	India [35], Nigeria [55]
Solanaceae	*Physalis angulata* L.	18		Ground cherry	NDUP 140	L	pounded leaves mixed with Beer and water Leaves with *C. papaya* leaves extracted into illicit gin	To be drunk regularly	Contraception	None
Thelypteridaceae	Cyclosorus afer (Christ.) Ching	2	Obribunumo	Wood fern, Parasitic tri-vein fern	NDUP 141	L	Leaf extracted in illicit gin	Orally, frequently	Spermicide	None

(Continued)

Table 1. Medicinal plants used in family planning. (Continued)

Family	Botanical name	Frequency (informant consensus)	Local name	Common name	Voucher number	Part	Method of preparation	Administration	Activity	Reported ethnomedicinal uses for family planning
Vitaceae	*Cissus aralioides* (Welw. ex Baker) Planch.	2	Egbulokpo	Five fingers	NDUP 142	L	Extract juice	Juice drunk immediately after sex, and subsequently regularly	Contraception	None
Zingiberaceae	*Aframomum danielli* (Hook.f.) K. Schum.	2	Ikanla	Grains of paradise	NDUP 143	R	Pounded root in illicit gin	Orally, frequently	Contraception	None
	A. melegueta K. Schum.	42	Ange Fisani	Grains of paradise	NDUP 144	Se/L	Pounded leaves with seven seeds extracted in palm wine, filtered and allowed to stand for 1 hour. Seeds pounded with leaves of *Ageratum conyzoides*. Seeds pounded with *A. africana* leaves and *Baphia nitida* leaves. Seeds pounded with *Eichhorinia crassipes* leaves and *Portulaca oleoracea* leaves to obtain a paste and palm oil Seeds with pieces of root bark of *Senna alata soaked in illicit gin*	Daily inserted into the vagina for 3 days during sexual intercourse. To be drunk regularly To be drunk regularly	Contraception	Nigeria [55,56] Ghana [53]

NDUP: Niger Delta University Pharmacognosy

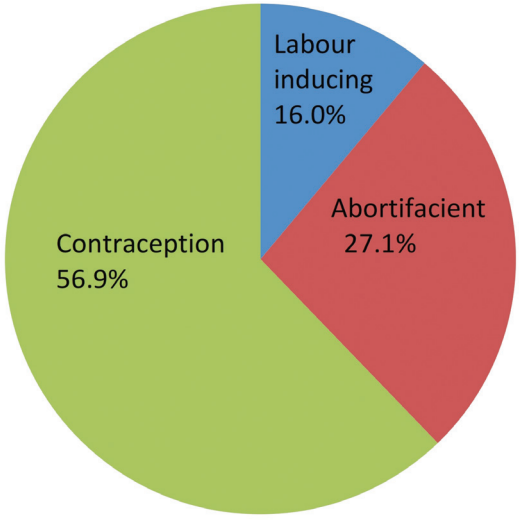

Figure 2. Female planning categories.

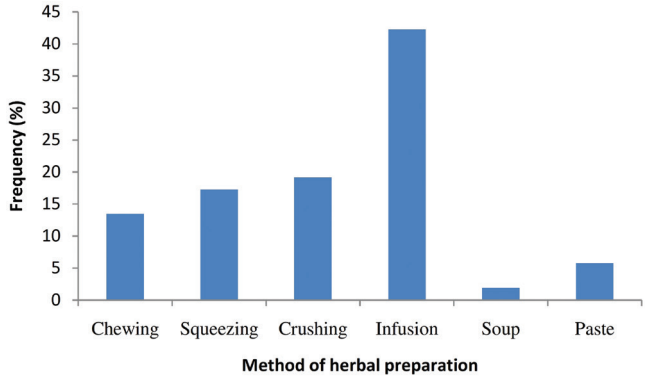

Figure 3. Methods of preparation of plants used for family planning.

Table 2. Reported use value (per plant part) of cited plants for female family planning purpose.

	Contraception (C)	Abortifacient (A)	Labor inducing (LI)	Total usage (C + A + LI)	Reported use value (ΣRU)	Categorized reported use value in fraction
Plant Part						
Seed	22	—	—	22 (6.1%)	1	0.33
Fruit	10	—	—	10 (2.8%)	1	0.33
Flower	6	—	—	6 (1.7%)	1	0.33
Leaf	176	70	38	284 (78.5%)	3	1.00
Stem	4	4	2	10 (2.8%)	3	1.00
Root	14	18	4	36 (10.0%)	3	1.00
Bulb	2	—	—	2 (0.6%)	1	0.33

Table 3. Animals cited for use in family planning.

Zoological name	Family	Common name	Local name	Part	Method of preparation	Administration	Activity
Pila ovata (Olivier)	Ampullariidae	Water snail	Gbegbe	Whole flesh	Pounded with black soap	To be inserted into the vagina before intercourse	Contraception
Dasyatis garouaensis Stauch & Blanc	Dasyatidae	String ray fish	Sika	Whole fish	As food	To be taken regularly	Labor induction, abortifacient

family of 11.1 (Table 4)], given its number of taxa used in family planning, followed by Lamiaceae with a familial use value of 3. Other important families included Leguminosae, Nymphaceae, Piperaceae, and Zingiberaceae with a familial use value of 2 (Table 4).

Although *A. melegueta* was the most mentioned plant species by the informants for female family planning, it is only used as a contraceptive (Tables 1 and 5). This implies that it is not versatile in family planning. It belongs to the family Zingiberaceae which had a consensus between authors on cited species and family of 4.5 (Table 4). *C. papaya* was however mentioned for the three categories in female family planning (contraceptive, labor inducer, and abortifacient) (Tables 1 and 5). Others like *Vernonia amygdalina*, *Newbouldia laevis*, *Manihot esculenta*, *Hyptis lanceolata*, *Nymphaea lotus*, *Peperomia pellucida,* and *Chloris pilosa* were separately mentioned for two categories while each of the remaining plant species fell into only one category (Table 5). The frequency of use also showed *C. papaya* as the most important plant species coupled with a use totaled of 1.00.

Table 4. Familial use value of the cited plants.

Family	Species	Familial use value	Consensus between authors on cited species and family
Acanthaceae	1	1	16.7
Alliaceae	1	1	50.0
Anacardiaceae	1	1	50.0
Annonaceae	1	1	12.5
Araceae	1	1	50.0
Asteraceae	4	4	11.1
Bignoniaceae	1	1	50.0
Caricaceae	1	1	5.6
Cleomaceae	1	1	50.0
Commelinaceae	1	1	50.0
Crassulaceae	1	1	50.0
Euphorbiaceae	1	1	25.0
Lamiaceae	3	3	30.0
Leguminosae	2	2	25.0
Musaceae	1	1	50.0
Myrtaceae	1	1	50.0
Nymphaceae	2	2	25.0
Piperaceae	2	2	25.0
Poaceae	1	1	8.3
Pontederiaceae	1	1	25.0
Portulacaceae	1	1	25.0
Rutaceae	1	1	25.0
Solanaceae	1	1	5.6
Thelypteridacea	1	1	50.0
Vitaceae	1	1	50.0
Zingiberaceae	2	2	4.5

Contraception (ICF, 0.9) was on top of the list of categories in family planning, and therefore, the most important category as recorded in the survey. Twenty-five taxa (71.4%) were cited for it, followed by 12 taxa (34.3%) for abortion and labor induction with five taxa (11%) while the least was spermicidal with three taxa (8.6%). The least important category was those used for male family planning (Table 6).

Discussion

Several reports of ethnobotanical survey in different geographical zones of Nigeria and in other parts of the world revealed that leaves are more commonly utilized than other plant parts [72,109–112]. This practice has proved to be helpful in reduction rate of threat on plant species because harvesting leaves in a sustainable manner ensures the continuity of the plant, while harvesting bark and roots may lead to extinction of plant species [113]. It is also a common knowledge that leaves are known to be the main site of synthesis of a variety of secondary metabolites [73]. Another possible reason may be the fact that leaves are not as seasonal as in the case of parts like seeds, fruits and flowers; they are mostly available for harvesting throughout the year.

Table 5. Use categories and chemical constituents of the plants mentioned for female family planning.

Family	Botanical name	% citation	Activity	Uses totaled	Chemical constituents
Acanthaceae	*Acanthus montanus* (Ness) T. Anderson	2.7	Contraception	0.33	Saponins, gammaceranes-acanthusol and its 3-0-β-D-glucopyranoside [75]
Alliaceae	*Allium sativum* L	0.9	Contraception	0.33	Allicin, Alliin, (Z)-Ajoene, (E)-Ajoene, Allixin, 1,2-Vinyldiithin, methy allyl disulphide [76]
Anacardiaceae	*Spondias mombin* L.	1.9	Abortifacient	0.33	6-alkenyl salicylic acid, coumaroyl, quercetin, gallic acid [77].
Annonaceae	*Xylopia aethiopica* (Dunal) A. Rich	3.7	Contraception	0.33	Xylopic acid, diterpene acid [78]
Araceae	*Colocasia esculenta* (L.) Schott.	0.9	Contraception	0.33	Flavonoids, alkaloids [79]
Astearaceae	*Aspilia africana* (Pers.) C.D Adams	3.7	Abortifacient	0.33	3β-O-[α-rhamnopyranosyl-(1→6)-β-glucopyransyl-(1→3)-ursan-12-ene, 3β-Hydroxyolean-12-ene and 3β-acetoxyolean-12-ene [80].
	Ageratum conyzoides L.	4.6	Contraception	0.33	5,6,7,8,3', 4', 5'-heptamethoxyflavone, 5,6,7,8,3'-pentamethoxy-4', 5'-methylenedioxyflavone and coumarin, 5,6,7,8,3'-Pentamethoxy-4', 5'-methylenedioxyflavone [81]

(Continued)

Table 5. Use categories and chemical constituents of the plants mentioned for female family planning. (Continued)

Family	Botanical name	% citation	Activity	Uses totaled	Chemical constituents
	Acmella caulirhiza Delile	0.9	Contraception	0.33	Spilanthol, N-isobutylnona-2E,4E dien-8-ynamide, N-isobutyi-2E,4E,8E,10Z-dodecatera 2,4,8–10 amide [82]
	Vernonia amygdalina Delile	7.4	Labor inducer, abortifacient	0.67	4- caffeoylquinic, 3- caffeoylquinic, chlorogenic acid, 1,3- caffeoylquinic, 1,4 caffeoylquinic, 3,4-caffeoylquinic, 3,5- caffeoylquinic, 4,5- caffeoylquinic [83]
Bignoniaceae	*Newbouldia laevis* P. Beauv.	0.9	Labor inducer, abortifacient	0.67	Coumarins, flavonoids, tannins [84]
Caricaceae	*Carica papaya* L.	8.3	Contraception, labor inducer, abortifacient	1.00	β- crytoxanthin, β-carotene-5,6-epoxide, lycopene, zeta- carotene, β-cryptoxanthin [85], papain, chymopapain [86]
Cleomaceae	*Cleome rutidosperma* D.C.	0.9	Abortifacient	0.33	alkaloids, steroids, tannins, flavonoids, and cardiac glycosides [87]
Commelinaceae	*Commelina diffusa* N.L. Burman	0.9	Contraception	0.33	
Crassulaceae	*Bryophyllum pinnatum* (Lam.) Oken	0.9	Contraception	0.33	Syringic acid, caffeic acid, 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, p-hydroxycinnamic acid, ferulic acid, epigallocatechin-3-o-syringate, luteolin, rutin, kaempferol quercetin, quercetin-¬3L-rhamonsido-L-arabino furanoside, β-amyrin, β-amyrinacetate, bryophollenone , bryophollone [88]
Euphorbiaceae	*Manihot esculenta* Crantz.	1.9	Abortifacient contraception	0.67	Manieseulentins A and B, yucalexin p-21, calliterpenone, cleistanthenes type sonderianol [89]
Lamiaceae	*Plectranthus monostachys* (P. Beauv.) B.J. Pollard	0.9	Contraception	0.33	beta-pinene, oct-1-en-3-ol, β-caryophyllene, octan-3-ol (E,E)-α-farnesene [58]
	Ocimum gratissimum L.	2.8	Contraception	0.33	Eugenol, methyl eugenol, cis-ocimene, trans-ocimene, pinene, camphor, germacrene- D, trans-carypohyllene, farnesene and l-bisabolene [90], anthraquinones and flavonoids [91]
	Hyptis lanceolata Poir.	0.9	Induce labor, abortifacient	0.67	germacrene D β-caryophyllene β-elemene [92]
Leguminosae	*Baphia nitida* Lodd.	1.9	Contraception	0.33	Baphianoside [93]
	Senna alata (L.) Roxb.	1.9	Contraception	0.33	chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranosyl-(1->6)-β-D-glucopyranoside 17-hydrotetratriacontane, n-dotriacontanol, n-triacontanol [94]
Musaceae	*Musa paradisiaca* L.	0.9	Contraception	0.33	Alkaloids, flavonoids [95]
Myrtaceae	*Psidium guajava* L.	0.9	Abortifacient	0.33	ursolic acid, 2α-hydroxyursolic acid, 2α-hydroxyoleanolic acid, morin-3-O-α-L-arabopyranoside, quercetin, hyperin (6), myricetin-3-O-β-D-glucoside, quercetin-3-O-β-D-glucuronopyranoside, 1-O-galloyl-β-D-glucose [96]

(Continued)

Table 5. Use categories and chemical constituents of the plants mentioned for female family planning. (Continued)

Family	Botanical name	% citation	Activity	Uses totaled	Chemical constituents
Nymphaceae	*Nymphaea lotus* L.	0.9	Abortifacient contraception	0.67	alanine, tyrosine, phenyl alanine, valine, threonine, arginine, leucine, D and L-isoleucine and aspartic acid), 2 alkanoic acids in form of butanoic acids and its ã- hydroxyl isomer, a dipeptide (serine-arginine) as well as a rare compound named 2-amino-7-methyl octanoic acid [97]
	Nymphaea odorata Aiton	1.9	Contraception	0.33	Tannin, gallic acid [98]
Piperaceae	*Piper guineense* Schumach & Thonn.	0.9	Contraception	0.33	N-pyrrolidyl-2,4-octadecadienamide and N-piperidyl-2,4-octadecadienamide [99], essential oils [100]
	Peperomia pellucida (L.) Kunth.	2.8	Labor inducer, abortifacient	0.67	eperomins A, B, C, patuloside A, dihydronaphthalenone, sesamin, isoswertisin, tetrahydrofuran lignans [101]
Poaceae	*Chloris pilosa* Schumach & Thonn.	5.6	Contraception, abortifacient	0.67	Alkaloid [102]
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms.	1.9	Contraception	0.33	alkaloid, phthalate derivatives, propanoid and phenyl derivatives [103]
Portulacaceae	*Portulaca oleraecea* L.	1.9	Contraception	0.33	cyclo (Phe-Ile), cycle (Tyr-Ala), adenine, friedelin, isoselachoceric acid [94]
Rutaceae	*Citrus x limon* (L.) Osbeck	1.9	Contraception	0.33	Limonene, Neral, neryl acetate, p-menth-1-en-7-al, β-pinene, and nerol, γ-terpinene, β-pinene, geranial, neral [104]
Solanaceae	*Physalis angulata* L.	8.3	Contraception	0.33	Physalins A-K, physagulin A-D, withanolides, whitaminimin, whitangulatin [105]
Vitaceae	*Cissus aralioides* (Welw. ex Baker) Planch.	0.9	Contraception	0.33	phytol, 1,2,4-trimethylbenzene, ethylene glycol mono-tert butyl ether hexahydrofarnesyl acetone [106]
Zingiberaceae	*Aframomum danielii* (Hook. F.) K. Schum.	0.9	Contraception	0.33	1,8-cineole β- pinene, α-terpineol, α-pinene α-terpinyl acetate [107]
	Aframomum melegueta K. Schum.	19.4	Contraception	0.33	paradol,shogaol gingerdione, α-humulene, gingerol [108]

Keys:
0.33: used for 1 out of the 3 uses category.
0.67: used for 2 out of the uses category.
1.00: used for the entire 3 uses category.
Categories (contraception, abortifacient, LI)

Table 6. Importance of the use categories.

Category of use	Number of use report for disease category	Number of species (Nt)	Informant consensus factor
Contraception	164 (55.4%)	25 (71.4%)	0.9
Abortifacient	78 (26.4%)	12 (34.3%)	0.9
Labor inducing	46 (15.5%)	5 (14.3%)	0.9
Spermicidal	8 (2.7%)	3 (8.6%)	0.7

The use of leaves is found commonly in reports of survey of ethnobotanical studies [73,109,114,115].

The use of infusion in herbal preparation agrees with the general pattern of medicinal plant preparation in Africa [109]. The use of infusion is not only limited to Africa, it is also indicated as a frequently used method of herbal preparation in some other parts of the world [50,116–118]. Oral administration of drug is a less cumbersome form which can be self-administered with little or no supervision

by TBAs and with minimal or no side effects. A great majority of drugs are taken this way [119]. In this region, many remedies are prepared in crude form devoid of quality control and standardization of dosage. Alcohol is commonly used for their additional preservative potential apart from being a good medium of extraction.

Astearaceae was mentioned to be among the families mostly used in the Niger Delta ethnomedicine [109] as well as in different tribes of Bangladesh [120]. It is one of the most targeted families in drug discovery as a result of their rich content of secondary metabolites such as flavonoids, alkaloids, and saponins [109]. The seed of *A. melegueta* together with the fruits of *X. aethiopica*, leaf of *Sorgum bicolor,* and *Citrus x limon* juice soaked together in illicit gin and a glass taken in the morning and at bed time in Lagos, Nigeria, was reported as a contraceptive [55]. *N. laevis*, *H. lanceolata*, *N. lotus,* and *P. pellucida* have not been reported for family planning in the literature but are indicated for treating diseases such as elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, pile, stomach ulcers, fungal diseases, tumor, and wound [121–124]. On the other hand, *V. amygdalina*, *M. esculenta,* and *C. pilosa* with fraction uses totaled of 0.67 have been reported for family planning in Nigeria and other parts of Africa. *C. papaya*, in the family Caricaceae is known to be the only species in the genus *Carica* and family and it originated from the tropics of the America, likely from Southern Mexico and Central America, its neighbor [125]. From this study, *C. papaya* showed a consensus between authors on the cited species and family value of 5.6 (Table 4). It was the usual practice for the women in Bangladesh, India, Pakistan, and Sri Lanka to use green *C. papaya* as herbal potion for contraception and abortion purposes. Female slaves were also reported to eat it in order to prevent pregnancies so as to forestall birth into slavery [125]. *V. amygdalina*, in the family Asteraceae, is a common shrub in West Africa whose leaf and root decoction is administered orally for contraception in Keffi Local Government of Nasarawa State in the Northern part of Nigeria [63]. The decoction of the leaf is also employed in Ghana and Uganda in West Africa as abortifacient [126]. TBAs in Malawi also use the leaves to induce uterine motility [127]. The powdered leaf of *C. pilosa* is taken with milk for contraception in Sokoto State, in the Northern part of Nigeria as contraceptive in females [72]. Likewise, *M. esculenta* is employed as contraceptive in Ghana [53].

Some of the experimental validations of important species are highlighted.

The abortifacient effect of *C. papaya* seed has been validated pharmacologically in Sprague-Dawley rats. It was reported that 1 mg/kg p.o. exhibited 100% abortifacient effect from the second trimester of pregnancy and resulted into zero fetus at the end of the third trimester [125]. In a different report, administration of the pulp extract of the seedless fruits orally at 1 g/kg to female rats for 60 days showed an arrest of the development of follicles of the ovaries histologically, showing follicular atresia and degenerative changes of the granulosa cells. In addition, there was a reduced lumen and folds of the epithelium. Uterine glands also reduced in number and myometrial thickness decreased as well. All these confirmed the antifertility effect of *C. papaya* [128]. Similarly, when aqueous extracts of green and ripe papaya epicarp were separately administered orally (1 mg/g body weight/day) to pregnant mice from day 10 and onwards after conception, a group of seven mice and another group of six administered with green epicarp extract and misoprostol, an abortive drug, respectively, experienced embryonic resorption, while this effect was observed in none of the mice given ripe epicarp extract and water. The average body weight of live pups delivered by mice given green epicarp extract (1.12 ± 0.04 g) was significantly lower than those delivered by mice given water (1.38 ± 0.02 g) [129]. Also, administration of crude papaya latex induced spasmodic contraction of the uterine muscles similar to oxytocin and prostaglandin F(2α). The response of the isolated rat uterine smooth muscles to 0.2 mg crude papaya latex/ml was comparable to 0.23 μ prostaglandin F(2α) and 32 mU oxytocin/ml. On the 18th and 19th day of pregnancy, tetanic spasms were experienced by the rats [130].

In a 30-day study in male albino rats, there were significant ($p < 0.05$) reductions in sperm motility and concentration with oral administration of 50, 100, and 150 mg/kg doses of extract of *C. papaya* in a dose-dependent manner [131].

Aqueous extract of *V. amygdalina* at a dose of 0.3 mg/ml/kg i.p. caused an increased uterine contraction of rats which was sustained for thirty minutes with elevated concentrations [132,133], and has therefore been proposed to be a potent oxytocic [134]. Another report on guinea pig showed that dams given 100 mg/kg aqueous extract produced the largest quantity of milk (14.33 ± 1.53 g), while those given 5 mg/kg of the extract produced the least quantity of milk (9.00 ± 2.00 g). Milk production

was taken as an indication of contraception. At 100 mg/ml, the uterine contraction amplitude matched that of ergometrine (9.58 ± 0.39 mm). However, the mammary gland contraction amplitude for *V. amygdalina* at 100 mg/kg was lower than that of ergometrine [135]. This supports the oxytocic property and justifies its use by TBAs.

C. x limon is a native of North West region of India. Petroleum ether, alcoholic, and aqueous seed extracts administered at 200 and 500 mg/kg p.o. separately to female albino mice for seven days after insemination showed that the alcohol extract that exhibited the highest activity had a significant 80% reduction of pregnancy at both 200 and 500 mg/kg, while the number of implants and pulps delivered was 20% [136].

Oral administration of 1,000 mg/kg/day aqueous extract of *Acanthus montanus* to pregnant rats on days 1–6 (preimplantation) or 6–15 (postimplantation) of gestation, respectively, caused appreciable preimplantation losses of 36.8% ± 6.5% ($P < 0.05$), but there was no postimplantation losses. The extract also caused delayed fetal growth [48].

Oral administration of *Ocimum gratissimum* acetone extract of stem to female albino rats at a dose of 100, 200, and 500 mg/kg per day to mated rats on days 1–5 of pregnancy resulted in a decline in fertility index, number of uterine implants, and live fetuses in a dose-dependent manner as was confirmed by laparotomy on day 15 of pregnancy [137]. In a similar study, oral administration of methanol (100 and 200 mg/kg) extract of the leaf to female rats for 21 days exhibited a dose-dependent significant decrease ($p < 0.05$) in serum testosterone level (30.46 ± 0.5), (34.29 ± 3.32) compared to the control (35.18 ± 2.31), leutinizing hormone decreased nonsignificantly while the increases recorded for follicle stimulating hormone, progesterone, and prolactin were not significant ($p < 0.05$) [138]. Also, oral administration of 11, 22, 44, and 88 mg/kg leaf extract to male mice for 1, 2, 3, and 4 weeks showed reduction in sperm count and motility. Similarly, percentage of abnormal sperm cells and primordial cells increased dose and time dependently [68].

Effects of oral administration of *A. africana* leaf extract (500 and 1,000 mg/kg) on oestrous cycle and ovulation have been studied in cyclic female rats which were divided into two study groups: the oestrous study and ovulation study group. It resulted in alteration of oestrous cycle; an indication of a prolonged proestrous and a reduced dioestrous and oestrous in a dose-dependent manner. The extract reduced the ovulation which was marked by reduced number of ova observed in the oviduct. Inflammation of the fallopian tube, degeneration in the ovarian cortex in the stroma cells of the ovary and disruption of the endometrium of the uterus were observed. It had multiple effects on uterus, fallopian tube and ovary [139].

Oral administration of chloroform extract of *Portulaca oleracea* given to female albino rats at a dose level of 500 mg/kg body weight daily for 14 days showed anti-ovulatory activity of 3.8 ± 1.28 [140].

Oral administration of 800 mg/kg aqueous leaf extract of *S. mombin* to female rats post coitus 1–4 days resulted in 60% significant anticonceptive activity ($p < 0.05$) attributed to a direct action of the extract on the uterus [52]. Histology of the testis of male rats administered with ethanol extract of *S. mombin* (250 and 500 mg/kg) for six weeks caused a dose dependent distortion in the arrangement of seminiferous tubules, loose germinal epithelium, low number of germ cells and Sertoli cells. Also, tubular sizes of epididymis were reduced with vacuolation and decreased sperm [54].

Oral administration of aqueous extract of *Senna alata* (250, 500, and 1,000 mg/kg) to pregnant rats on days 10–18 post coitum, resulted in 100% abortion in the rats administered with 500 and 1,000 mg/kg and 40% in those fed with 250 mg/kg, post implantation loss of 100% was also recorded at 500 and 1,000 mg/kg and 88% at 250 mg/kg [141].

Among the chemical constituents in the plants used for family planning are flavonoids, alkaloids, terpenes, and tannins (Table 5). All these classes of secondary metabolites have been implicated in antifertility activities. For instance, alkaloids have been reported as a likely class of compounds responsible for altering the reproductive systems in animals and human, for instance, *Areca catechu*, *Claviceps purpurea*, *Rauwolfia serpentine*, *Solanum marginatum,* and *Cissampelos sympodialis* are alkaloid-containing plants with antifertility activity tested on various animal models [95]. Steroids are also common components of contraceptives [142]. Also, Plants containing flavonoids, tannins, terpenes, quinones, and diterpenoid lactones have been reported to induce male infertility effects by different mechanisms [98,143,144].

The inclusion of animals in family planning program has some basis in the definition of traditional medicine by the WHO which includes the incorporation of animals [74]. This is also similar to a report in which crayfish and lizard were used as components of herbal medicine [73]. In Africa,

animals and mineral substances are common components of traditional medicine [145].

Scientific literatures were surveyed for validation of the uses of the medicinal plants cited by informants in this survey (Table 1). This assists to reveal their level of significance in the traditional medicines of other countries. Twenty plant species (57%) used in Bayelsa communities are also used in other States of Nigeria as well as in other parts of the World for similar family planning purpose (Table 1). It is possible to have the same plant active for both female and male family planning [21,23–25,136,146–150].

None of the male contraceptive plants mentioned in this study has a similar use in any other parts of the world (Table 1).

This study agrees with the report that the majority of the currently available family planning options are for females and that participation of the male counterpart is still poor [151].

Conclusion

This study revealed that abortion is not a common practice in the study area but contraception which was the most embraced of the family planning options available. Some of the plants mentioned by respondents are also used elsewhere around the world while some are only known for family planning in the study area. Such plants need to be verified scientifically for the acclaimed ethnomedicinal uses.

Acknowledgment

The authors are grateful to the following for assistance in the field: Mr. I. Yereyere, Mr. G. Yousuo, Department of Pharmacognosy & Herbal Medicine, Mrs. K. Ajibola, Proprietor of Fortunate Group of Schools, Amassoma, Bayelsa State, and all TBAs and elders of communities that were surveyed.

References

[1] Aninyei IR, Onyesom I, Ukuhor HO, Uzuegbu UE, Ofili MI, Anyanwu EB. Knowledge attitude to modern family planning methods in Abraka communities, Delta State, Nigeria. East Afr J Public Health 2008; 5:10–2.

[2] Park KP. Park's textbook of preventive and social medicine. 17th edition, Banarsidas Bhanot Publishers, Jabalpur, India, 2009.

[3] Allag, IS, Rangari K. Extragenomic action of steroids on spermatozoa prospect for regulation of fertility. Health popul 2002; 25:38–44.

[4] Chipeta EK, Chimwaza W, Kalilani-Phin L. Contraceptive knowledge, beliefs and attitudes in rural Malawi: misinformation, misbeliefs and misperceptions. Malawi Med J 2010; 22(2):38–41.

[5] Cates Jr W, Stanback J, Maggwa B. Global family planning metrics-time for new definitions? Contraception 2014; 90(5):472–5.

[6] Obisesan KA, Adeyemo AA, Ohaeri JU, Aramide FA, Okafor SI. The family planning aspects of the practice of traditional healers in Ibadan, Nigeria. West Afr J Med 1997; 16:184–90.

[7] Garba A, Kullima A, Kolo MMA, Babagana. The practice of traditional family planning among rural Kanuri communities of Northeastern Nigeria. Int J Biol Med Res 2012; 3:1277–80.

[8] The Borgen project: Nigeria's struggle with overpopulation [Internet]. 2015 [Cited 2017 Aug 17]. Available via https://borgenproject.org/nigerias-struggle-overpopulation/

[9] Delano G. Guide to family planning. New Edition, Spectrum Books publishers Ltd, Ibadan, Nigeria, 1990.

[10] Ebuehi OM, Akintujoye IA. Perception and utilization of traditional birth attendants by pregnant women attending primary health care clinics in a rural Local Government Area in Ogun State, Nigeria. Int J Womens Health 2012; 4:25–34.

[11] The Nigerian observer: traditional method of birth control: desired or not, good or bad [Internet]. 2015 [Cited 2017 Mar 20]. Available via http://www.nigerianobservernews.com/2015/05/traditional-method-of-birth-control-desired-or-not-good-or-bad/

[12] Mariam-Webster Dictionary: spermicide [Internet]. 1828 [Cited 2017 Mar 20]. Available via http://www.merriam-webster.com/dictionary/spermicide

[13] Udoh P, Kehinde A. Studies on antifertility effect of pawpaw seeds (*Carica papaya*) on the gonads of male albino rats. Phytother Res 1999; 13:226–28.

[14] Lohiya NK, Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, et al. Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in languor monkey. Asian J Androl 2002; 4:17–26.

[15] Khillare B, Shrivastav TG. Spermicidal activity of *Azadirachta indica* (Neem) leaf extract. Contraception 2003; 6:225–9.

[16] Sathiyaraj K, Sivaraj A, Thirumalai T, Senthilkumar B. Ethnobotanical study of antifertility medicinal plants used by the local people in Kathiyavadi village, Vellore District, Tamilnadu, India. Asian Pac J Trop Biomed 2012; 2(3):S1285–8.

[17] Namulindwa A, Nkwangu D, Oloro J. Determination of abortifacient activity of the aqueous extract of *Phytolacca dodecandra* (L'Her) leaf in Wistar rats. Afr J Pharm Pharmacol 2015; 9(3):43–7.

[18] Sinha R. Post-tecticular antifertility effects of *Abrus precatorius* seed extract in albino rats. J Ethnophamarcol 1990; 28:173–81.
[19] Chakrabarti K, Pal S, Bhattacharryya AK. Sperm immobilization activity of *Allium sativum* L. and other plant extracts. Asian J Androl 2003; 5:131–6.
[20] Gupta RS, Sharma R. A review on medicinal plants exhibiting antifertility activity in males. Nat Prod Rad 2006; 5:389–410.
[21] Sharma P, Sharma A, Agarwal M, Joshi SC. A review on antifertility efficacy of plants in males. Int J Pharm Biol Sci 2013; 4(4):413–28.
[22] Das B, Talukdar AD, Choudhury MD. A few traditional medicinal plants used as antifertility agents by ethnic people of Tripura, India. Int J Pharm Pharm Sci 2014; 6:47–53.
[23] Oyewopo AO, Oremosu AA, Akang EN, Noronha CC, Okanlawon AO. Effects of *Aloe vera* (*Aloe barbadensis*) aqueous leaf extract on testicular weight, sperm count and motility of adult male Sprague-Dawley rats. J Am Sci 2011; 7:31–4.
[24] Nwauzoma AB, Dappa MS. Ethnobotanical studies of Port Harcourt metropolis, Nigeria. ISRN Bot 2013; 2013:829424.
[25] Veale DJH, Oliver DW, Havlik I. The effects of herbal oxytocics on the isolated "stripped" myometrium model. Life Sci 2000; 67:1381–8.
[26] Kamal IT. Traditional birth attendant: a reality and a challenge. Int J Gynaecol Obstet 1998; 63:S43–52.
[27] Mahiti GR, Mkoka DA, Kiwara AD, Mbekenga CK, Hurtig AK, Goicolea I. Women's perceptions of antenatal, delivery, and postpartum services in rural Tanzania. Glob Health Action 2015; 8:28567; doi:10.3402/gha.v8.28567.
[28] Sibley L, Sipe TA, Koblinsky M. Does traditional birth attendant training improve referral of women with obstetric complications: a review of the evidence? Soc Sci Med 2004; 59:1757–68.
[29] Sarmento DR. Traditional Birth Attendance (TBA) in a health system: what are the roles, benefits and challenges: a case study of incorporated TBA in Timor-Leste. Asia Pac Fam Med 2014; 13:12; doi:10.1186/s12930-014-0012-1.
[30] Oshonowoh FE, Nwankwo GC, Ekiyor CP. Traditional birth attendants and women's health practices: a case study of Patani in Southern Nigeria. J Public Health Epidemiol 2014; 6:252–61.
[31] Imogie AO. The practice of Traditional Birth Attendants and women's health in Nigeria. 25th Congress of Medical Women International Association, 2000. Available via http//www.regional.org.au/au/mwia/papers/full/28.imogie.html
[32] Ampofo DA, Nicholas DD, Amonoo-Acquab MB, Ofosu-Amiah S, Nuemann AK. The training of Traditional Birth Attendants in Ghana: experience of the Danfa Rural Health project. Trop Geogr Med 1977; 29:197–203.
[33] Cambie R, Alexandra B. Anti-fertility plants of the Pacific. Csiro Publishing, New Zealand, Melbourne, Australia, 1997.
[34] Dhore M, Dabhadkar D, Zade V, Dhore M. Documentation of fertility regulatory ethnomedicinal plants used by tribal's of Yavatmal District, Maharashtra, India. Int J Sci Res Pub 2012; 2(3):2240–3153.
[35] Heinrich M. Ethnobotany and its role in drug development. Phytotherapy 2000; 14:479–88.
[36] Kaur R, Sharma A, Kumar R, Kharb R. Rising trends towards herbal contraceptives. J Nat Prod Plant Resour 2011; 1:5–12.
[37] Ashidi JS, Olaosho EA, Ayodele AE. Ethnobotanical survey of plants used in the management of fertility and preliminary phytochemical evaluation of *Abelmoschus esculentus* (L.) Moench. J. Pharmacognosy Phytother 2013; 5(9):164–9.
[38] El-Shafey AAM, Seliem MMB, El-Mahrouky F, Gabr WM, Kandil RA. Some physiological and biochemical effects of Oshar extract and abam ectin biocide on male albino rats. J Am Sci 2011; 7(12):254–61.
[39] Patil SA, Patil SB. Spermicidal activity of *Melia azedarach*: *In vitro* and *in vivo* studies. Int J Pharm Res 2012; 4:34–6.
[40] Nigeria Bureau of Statistics. Annual abstract of statistics 2012. Federal Government Press, Abuja, Nigeria, pp 108, 2012.
[41] James GK. Assessment of environmental change and its socio economic impacts in the Mangrove Ecological zone of the Niger Delta, Nigeria. Ph.D Thesis submitted to the Faculty of the University of Missouri-Kansas City, Kansas City, MO, pp 27–36, 2008.
[42] The Plant List: a working list of all plant species Version 1.1 [Internet]. 2013 [Cited 2017 Aug 12]. Available via http://www.theplantlist.org/
[43] Hoffman B, Gallaher G. Importance indices in ethnobotany. ERA 2007; 5:201–18.
[44] Letsela T, Witkowski ETF, Balkwill K. Plant resources used for subsistence in Tsehlanyane and Bokong in Lesotho. Econ Bot 2003; 57:619–39.
[45] Uddin MM, Hassan MA. Determination of informant consensus factor of ethnomedicinal plants used in Kalenga forest, Bangladesh. Bangl J Plant Taxon 2014; 21:83–91.
[46] Molares S, Ladio AH. Ethnobotanical review of the Mapuche medicinal flora: use patterns on a regional scale. J Ethnopharmacol 2009; 31:1–42.
[47] Kemper KJ. Garlic (*Allium sativum*). The Longwood Herbal Task Force, 2000. Available via http://www.longwoodherbal.org/garlic/garlic.pdf
[48] Asongalem EA, Nana P, Dimo T, Kamtchouing P. Antifertility activities of *Acanthus montanus* on female rats with possible mechanism(s) of action. Meth Find Exp Clin Pharmacol 2008; 30:521–8.

[49] Parvez M, Zaman R, Sayeed MA, Haque MA, Rashid MA. Antifertility activity of methanol bulb extract of *Allium sativum* on Swiss Albino male mice and teratogenic effect on neonates of female mice. Glob J Pharmacology 2015; 9(3):272–7.

[50] Taylor L. The healing power of rainforest herbs: a guide to understanding and using herbal medicinals. Square One Publishers Incorporated, New York, NY, 2004.

[51] Uchendu CN, Nwankwo IO. Spasmogenic activity of butanolic leaf extract of *Spondias mombin* in isolated uterine muscle of the rat: role of calcium. J Nat Remedies 2005; 5:7–14.

[52] Uchendu CN, Isek T. Antifertility activity of aqueous ethanolic leaf extract of *Spondias mombin* (Anacardiaceae) in rats. Afr Health Sci 2008; 8:163–7.

[53] Diame GLA. Ethnobotany and ecological studies of plants used for reproductive health: a case study at Bia biosphere reserve in the western region of Ghana. Department of Environmental Science, University of Cape Coast, Ghana (UNESCO YOUNG SCIENTISTS AWARD, 2008), 2010.

[54] Asuquo OR, Ekanem TB, Udoh, PB, Eluwa MA. Histomorphological study of the anti-fertility effect of *Spondias mombin* L. in adult male rats. J Pharma Biol Sci 2012; 3:29–34.

[55] Babalola KA. An examination of the usage of herbal contraceptives and abortifacients in Lagos State, Nigeria. Ethnobot Leaflets 2009; 13:140–46.

[56] Adebisi IM, Bello SO. An ethnobotanical survey of herbal male contraceptives used in south-west Nigeria. Afr J Pharm Pharmacol 2011; 5:289–91.

[57] Gill LS. Ethnomedical uses of plants in Nigeria. Uniben Press, Benin, Nigeria, p ix, 1992.

[58] PROTA: Plant Resources of Tropical Africa. Protabase [Internet]. 2016 [Cited 2017 Mar 20]. Available via http://www.prota.org

[59] Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. J Ethnopharmacol 2008; 115:387–408.

[60] Fasola TR. An ethnobotanical survey of plants used in the management and treatment of female reproductive health problems in Ibadan, Southwestern Nigeria. J Biol Agric Health 2015; 5:7–11.

[61] Desta B. Ethiopian traditional herbal drugs. Part III: anti-fertility activity of 70 medicinal plants. J Ethnopharmacol 1994; 44:199–209.

[62] Steen-Kamp V. Traditional herbal remedies used by South African women for gynaecological complaints. J Ethnopharmacol 2003; 87:97–108.

[63] Mustapha AA. Ethno-medicinal field study of anti-fertility medicinal plants used by the local people in Keffi Local Government, Nasarawa State, Nigeria. Int J Med Plants Res 2013; 2:215–18.

[64] Akpantah AO, Obeten KE, Edung ES, Eluwa MA. The effect of ethanolic extract of *Bryophyllum pinnatum* on the micro anatomy of the testes of adult males Wistar rats. Eur J Biol Med Sci Res 2014; 2:37–44.

[65] Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, et al. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. Int J Med Med Sci 2010; 2(3):60–79.

[66] Malan DF, Neuba DF. Traditional practices and medicinal plants use during pregnancy by Anyi-Ndenye women (Eastern Côte d'Ivoire). Afr J Reprod Health 2011; 15:85–93.

[67] Idu M, Erhabor JO, Ovuakporie-Uvo O. Ethnomedicinal plants used by the Idoma people-Benue State, Nigeria. Am J Ethnomed 2014; 1:72–88.

[68] Obianime AW, Aprioku JS, Esomonu CTO. Antifertility effects of aqueous crude extract of *Ocimum gratissimum* L. leaves in male mice. J Med Res 2010; 4:809–16.

[69] Soni P, Siddiqui AA, Dwivedi J, Soni V. Antiovulatory and estrogenic activity of stem of *Musa paradisiaca* in female albino rats. J Appl Pharm Sci 2013; 3:102–6.

[70] Maurya R, Srivastava S, Kulshreshta DK, Gupta CM. Traditional remedies for fertility regulation. Curr Med Chem 2004; 11:1431–50.

[71] Sri Retno DA, Endang S, Elfi VHS, Setiyani S. Activity test of guava (*Psidium guajava* L.) leaf methanol extract as contraception antifertility to white mice (*Rattus norvegicus*). Indian J Chem 2008; 8:23–6.

[72] Adebisi IM, Alebiosu OC. A survey of herbal abortificients and contraceptives in Sokoto, North-West Nigeria. Int J Curr Res Chem Pharma Sci 2014;1:81–7.

[73] Alade GO, Okpako E, Ajibesin KK, Omobuwajo OR. Indigenous knowledge of herbal medicines among adolescents in Amassoma, Bayelsa State, Nigeria. Glob J Health Sci 2016; 8:217–37.

[74] World Health Organization. Final report of the seminar on the use of medicinal plants in health care. World Health Organization, WPRO Publication, Tokyo, Japan, 1996.

[75] Okoli CO, Akah PA, Onuoha NJ, Okoye TC, Nwoye AC, Nworu CS. *Acanthus montanus*: an experimental evaluation of antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. BMC Complement Altern Med 2008; 8(27):1–11.

[76] Martins N, Petropoulos S, Frreira ICFR. Chemical composition and bioactive compounds of garlic (*Allium sanctum* L.) as affected by pre- and post-harvest conditions: a review. Food Chem 2016; 211:41–50.

[77] Ayoka AO, Adeloye Bell DL, Camacho EJ, Velasquez AB. Male access to emergency contraception in pharmacies: a mystery shopper survey. Contraception 2014; 90(4):413–5.

[78] Ekong DE, Ogan AU. Chemistry of the constituents of *Xylopia aethiopica*: the structure of xylopic acid, a new diterpene acid. J Chem Soc C: Organic 1968; 8:311–2.
[79] Awa E, Eleazu C. Bioactive constituents and antioxidant activities of raw and processed cocoyam (*Colocassia esculenta*), chemical composition of raw and processed cocoyam. Naturafoods 2015; 14:133–40.
[80] Faleye FJ. Terpenoid constituents of *Aspillia africana* (Pers.) C.D. Adams leaves. Int J Pharm Sci Rev Res 2012; 13:138–42.
[81] Moreira MD, Picanco ML, Barbosa LC, Guedes RN, Barros EC, Campos MR. Compounds from *Ageratum conyzoides*: isolation, structural elucidation and insecticidal activity. Pest Manag Sci 2007; 63:615–21.
[82] Crouch NR, Langlois A, Mulholland DA, Nair JJ. A novel alkylamide from the leaves of *Acmella caulirhiza* (Asteraceae), a traditional surface analgesic. S Afr J Bot 2005; 71:228–30.
[83] Johnson CE, Lin L, Harnly JN, Oladehinde FO, Kinyua AM, Michelin R, et al. Identification of phenolic components of *Vernonia amygdalina* and *Russelia equisetiformis*. J Nat Prod 2011; 4:57–64.
[84] Fifa TD, Bothon FTD, Moustapha M, SophieBogninou G, Dossa CPA, Yehouenou B, et al. Chemical characterization and biological activities of *Newbouldia laevis* and *Pterocarpus Santalinoides* leaves. Bull Environ Pharmacol Life Sci 2014; 3:9–15.
[85] Nwofia GE, Ojimelukwe P, Eji C. Chemical composition of leaves, fruit pulp in some *Carica papaya* (L.) morphotypes. Int J Med Aromat Plants 2012; 2:200–6.
[86] Ujas SJ, Khatri TT, Ram VR, Dave PN, Joshi HS. Biochemical constituents in leaf of *Carica papaya*: ethnomedicinal plant of Kachchi region. Int Lett Nat Sci 2014; 12:16–20.
[87] Ojiako OA, Igwe CU. Nutritional and anti-nutritional compositions of *Cleome rutidosperma*, *Lagenaria siceraria*, and *Cucurbita maxima* seeds from Nigeria. J Med Food 2007; 10:735–8.
[88] Kamboj A, Saluja AK. *Bryophyllum pinnatum (Lam.) Kurz*: phytochemical and pharmacological profile: a review. Pharmacogn Rev 2009; 3:364–74.
[89] Pan Y, Zhang Y, Wang X, Chen H, Li S, Di Y, et al. Chemical constituents from the stems of *Manihot esculenta*. Nat Prod Bioprospect 2015; 5:55–9.
[90] Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A. *Ocimum gratissimum*: a review of its chemical, pharmacological and ethnomedicinal properties. Open Complement Med J 2009; 1:1–15.
[91] Umadevi M, Kumar PKS, Bhowmik D, Duraivel S. Medicinal plants with potential antifertility fertility activity. J Med Plants Stud 2013; 1:26–33.
[92] Bhuiyan MNI, Begum J. Chemical component studies on the leaf and inflorescence essential oil of *Hyptis brevipes* (Poit.). J Med Plants Res 2010; 4:2128–31.
[93] Onyekwere BC, Echeme JO, Uchegbu RI. Isolation and characterization of Baphianoside from the leaves of *Baphia nitida*. J Nat Sci Res 2014; 4:138–43.
[94] Liu A, Xu L, Zou Z, Yang S. Studies on chemical constituents from leaves of *Cassia alata*. Zhongguo Zhong Yao Za Zhi 2009; 34:861–3.
[95] Bala K, Arya M, Katare DP. Herbal contraceptive: an overview. World J Pharm Pharm Sci 2014; 3:1305–26.
[96] Fu H, Luo Y, Zhang D. Studies on chemical constituents of leaves of *Psidium guajava*. Zhongguo Zhong Yao Za Zhi 2009; 34:577–9.
[97] Sowemimo AA, Omobuwajo OR, Adesanya SA. Constituents of *Nymphaea lotus* Linn. Nig J Nat Prod Med 2007; 11:83–4.
[98] Millspaugh CF. An illustrated and descriptive guide to plants indigenous and naturalized in the United States which are used for medicine. Dover Publications Inc., New York, NY. 1974.
[99] Adesina SK, Adebayo AS, Gröning R. New constituents of *Piper guineense* fruit and leaf. Pharmazie 2003; 58:423–5.
[100] Owolabi MS, Lawal OA, Ogunwande IA, Hauser RM, Setzer WN. Aroma chemical composition of *Piper guineense* Schumach. & Thonn. From Lagos, Nigeria: a new chemotype. Am J Essential Oils Nat Prod 2013; 1:37–40.
[101] Majumder P, Abraham P, Satva, V. Ethnomedicinal, phytochemical and pharmacological review of an amazing medicinal herb *Peperomia pellucida* (L.). Res J Pharm Biol Chem Sci 2011; 2:358–64.
[102] Burkill HM. The useful plants of west tropical Africa. Vol. 2, Royal Botanic Gardens, Kew, Richmond, UK, p 203, 1985.
[103] Aboul-Enein AM, Al-Abd AM, Shalaby E, Abul-Ela F, Nasr-Allah AA, Mahmoud AM, et al. *Eichhornia crassipes* (Mart) solms: from water parasite to potential medicinal remedy. Plant Signal Behav 2011; 6:834–6.
[104] Hamdan D, Ashour ML, Mulyaningsih S, El-Shazly A, Wink M. Chemical composition of the essential oils of variegated pink-fleshed lemon (*Citrus x limon* L. Burm. f.) and their anti-inflammatory and antimicrobial activities. Z Naturforsch C 2013; 68(7–8):275–84.
[105] Rengifo-Salgado E, Vargas-Arana G. *Physalis angulata*. L (Bolsa Mullaca): a review of its traditional uses, chemistry and pharmacology. B Latinoam Caribe Pl 2013; 12:431–45.
[106] Balogun OS, Oladosu IA, Liu Z. Chemical compositions and radical scavenging potentials of essential oils from *Tragia benthamii* (Baker) and *Cissus aralioides* (WELW). J Biol Active Prod Nat 2016; 6:59–64.

[107] Adegoke GO, Jagan Mohan Rao L, Shankaracharya NB. A comparison of the essential oils of *Aframomum daniellii* (Hook. F.) K. Schum. and *Amomum subulatum* Roxb. Flavour Frag J 1998; 13:349–52.
[108] Fernandez X, Pintaric C, Lizzani-Cuvelier L, Loiseau AM, Morello A, Pellerin P. Chemical composition of absolute and supercritical carbon dioxide extract of *Aframomum melegueta*. Flavour Frag J 2006; 21:162–5.
[109] Ajibesin KK, Bala DN, Umoh UF. The use of medicinal plants to treat sexually transmitted diseases in Nigeria: ethnomedicinal survey of Niger Delta Region. Int J Green Pharm 2011; 5:181–91.
[110] Oladele AT, Alade GO, Omobuwajo OR. Medicinal plants conservation and cultivation by traditional medicine practitioners (TMPs) in Aiyedaade Local Government Area of Osun State, Nigeria. Agric Biol J North Am 2011; 2:476–87.
[111] Oladunmoye MK, Kehinde FY. Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of southwestern Nigeria. Afr J Microbiol Res 2011; 5:2991–3004.
[112] Elufioye TO, Oladele AT, Cyril-Olutayo CM, Agbedahunsi JM, Adesanya SA. Ethnomedicinal study and screening of plants used for memory enhancement and antiaging in Sagamu, Nigeria. Eur J Med Plants 2012; 2:262.
[113] Giday M, Asfaw, Z, Elmqvist T, Woldu Z. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. J Ethnopharmacol 2003; 85:43–52.
[114] Idowu OA, Soniran OT, Ajana O, Aworinde DO. Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. Afr J Pharm Pharmacol 2010; 4:55–60.
[115] Katemo M, Mpiana PT, Mbala BM, Mihigo SO, Ngbolua KN, Tshibangu DST, et al. Ethnopharmacological survey of plants used against diabetes in Kisangani City (DR Congo). J Ethnopharmacol 2012; 144:39–43.
[116] Ch, MI, Khan MA. An ethnomedicinal inventory of plants used for family planning and sexdiseases in Samahni valley, Pakistan. Indian J Tradit Know 2008; 7:277–83.
[117] Rahmatullah M, Rahman ME, Hasan E, Ahmed R, Jamal F, Jahan R, et al. A survey of medicinal plants used by the folk medicinal practitioners of Shetabganj village in Dinajpur district, Bangladesh. Am Eurasian J Sustain Agric 2010; 4:196–203.
[118] Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, et al. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Plants Res 2010; 4:1089–91.
[119] Verma P, Thakur AS, Deshmukh K, Jha AK, Verma S. Routes of drug administration. Int J Pharm Sci Res 2010; 1:54–9.
[120] Hossan S, Agarwala B, Sarwar S, Karim M, Jahan R, Rahmatullah M. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. ERA 2010; 8:61–74.
[121] Zollo PHA, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet, P. Aromatic plants of tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flav Fragr J 1998; 13:107–14.
[122] Awang Chee DN. Phytochemical studies and biological activities of the methanolic extract of *Peperomia pellucida*. A B.Sc. thesis submitted to the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Malaysia, 2008.
[123] Usman H, Yaro AH, Garba MM. Analgesic and anti-inflammatory screening of *Newbouldia laevis* in rodents. Trends Med Res 2008; 3:10–5.
[124] Akinjogunla OJ, Adegoke AA, Udokang IP, Adebayo-Tyo BC. Antimicrobial potential of *Nymphaea lotus* (Nymphaeceae) against wound pathogens. J Med plant Res 2009; 3:138–41.
[125] Ekhator CN, Shelu JO. An experimental study on the abortifacient potentials of unripe seed extract of *Carica papaya* on adult female Wistar rats. Open Sci J Pharm Pharmacol 2015; 3:61–5.
[126] Yeap SK, Ho WY, Beh BK, San Liang W, Ky H, Yousr AHN, et al. *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities. J Med Plants Res 2010; 4:2787–812.
[127] Bullough CHW, Leary WP. Herbal medicines used by Traditional Birth Attendants in Malawi. Trop Geogr Med 1982; 34:81–5.
[128] Punitha N, Shettu N, Saravanan R. Effect of semiripe *Carica papaya* fruit extracts on the reproductive structures in female albino rats: an histological study. Int J Curr Res Life Sci 2015; 4:241–4.
[129] Anuar NS, Zahari SS, Taib IA, Rahman MT. Effect of green and ripe *Carica papaya* epicarp extracts on wound healing and during pregnancy. Food Chem Toxicol 2008; 46:2384–9.
[130] Adebiyi A, Adaikan PG, Prasad RNV. Papaya (*Carica papaya*) consumption is unsafe in pregnancy: fact or fable? scientific evaluation of a common belief in some parts of Asia using a rat model. Br J Nutri 2002; 88:199–203.
[131] Oyedeji KO, Bolarinwa AF, Azeez AA. Effect of methanol extract of *Vernonia amygdalina* on reproductive parameters in male albino rats. Asian J Pharm Clin Res 2013; 6:234–6.

[132] Kamatenesi-Mugisha M. Medicinal plants used in reproductive health care in western Uganda: documentation, phytochemical and bioactivity evaluation. Ph.D. Thesis, Department of Botany, Makerere University, Kampala, Uganda, 2004.
[133] Kamatenesi-Mugisha M, Oryem-Origa H. Medicinal plants used to induce labor during childbirth in western Uganda. J Ethnopharmacol 2007; 109(1):1–9.
[134] Ijeh II, Igwe KK, Ejike CECC. Effect of leaf aqueous extracts of *Vernonia amygdalina* del on contraction of mammary gland and uterus of guinea pig dams. Int J Trop Med Public Health 2011; 1:107–16.
[135] Ijeh II, Igwe KK, Ejike CE. Effect of administration of aqueous extracts of *Vernonia amygdalina* del leaves to guinea pig dams on milk production and contraction of the mammary gland and uterus. Afr J Tradit Complement Altern Med 2009; 6:439–40.
[136] Kulkarni TR, Mateenuddin M, Bodhnankar SL, Saharabudhe ER. Reversible anti-fertility effect of lemon seeds (*Citrus limonum*) in male Albino rats. Int J Res Pharm Biomed Sci 2012; 3:545–50.
[137] Sripriya S, Yuvaraj G, Nema RR, Kumar VM, Deecaraman M. Evaluation of antifertility activity from stem part of *Ocimum gratissimum* in acetone extracts. Int J Pharm Clin Res 2011; 3:41–4.
[138] Egba SI, Omodamiro OD, Obike JC, Ali ES. Influence on some female fertility hormonal response in Wistar albino rats: possible contraceptive role for methanol leaf extract of *Ocimum gratissimum*? J Chem Pharm Res 2015; 7:889–98.
[139] Oyesola TO, Oyesola OA, Okoye CS. Effects of aqueous extract of *Aspilia africana* on reproductive functions of female Wistar rats. Pak J Biol Sci 2010; 13(3): 126–31.
[140] Nayaka HB, Londonkar R, Andumesh MK. Evaluation of *Portulaca oleracea* L. for antifertility effect in female albino rats. Int J Pharm Pharm Sci 2014; 6:86–9.
[141] Yakubu MT, Adeshina AO, Oladiji AT, Akanji MA, Oloyede OB, Jimoh GA, et al. Abortifacient potential of aqueous extract of *Senna alata* leaves in rats. J Reprod Contraception 2010; 21:163–77.
[142] Encyclopaedia Britannica. Steroid chemical compound [Internet]. 2017 [Cited 2017 Mar 20]. Available via https://www.britannica.com/science/steroid
[143] Lourens JD, Zaneveld DVM. Sperm enzyme inhibitors for vaginal and other contraception. Res Front Fertil Regul 1982; 2:1–14.
[144] Meerwal P, Jain GC. Male fertility regulation with plant products: a review. Int J Pharm Chem Biol Sci 2015; 5:146–62.
[145] Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria, 1993.
[146] Sandhyakumary K, Bobby RG, Indira M. Antifertility effects of *Ricinus communis* (Linn) on rats. Phytother Res 2003; 17:508–11.
[147] Ashok P, Meenakshi B. Contraceptive effect of *Curcuma longa* (L.) in male albino rat. Asian J Androl 2004; 6:71–4.
[148] Jain GC, Ali SM. Effect of ethanolic extract of *Cassia alata* L. flowers on reproductive functions of male albino rats. J Exp Zool 2007; 10(1):129–32.
[149] Chauhan A, Agarwal M. Reversible changes in the antifertility induced by *Aegle marmerlos* in albino rats. Syst Biol Reprod Med 2008; 54:240–6.
[150] Gambo IM, Galam NZ, Anyaka LO, Bello MM, Bello M, Rabu AM, Odeh SO. The effect of aqueous leaves extract of *Nicotana tabacum* (tobacco) on some reproductive parameters and micro-anatomical Architecture of the testes n male albino Wistar rats. J Nat Sci Res 2013; 3:137–44.
[151] Bell DL, Camacho EJ, Velasquez AB. Male access to emergency contraception in pharmacies: a majority shopper survey. Contraception 2014; 90:413–5.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 45–56
10.5455/jice.20170701035248

eJManager

ORIGINAL ARTICLE

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Assessment of *polyscias fruticosa* (L.) Harm (Araliaceae) leaf extract on male fertility in rats

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ABSTRACT

Background: *Polyscias fruticosa* is used widely as food, remedy for diseases, and as an ornamental across Afro-Asian countries. For instance, *P. fruticosa* is used traditionally as an anti-asthma, anti-tussive, and a muco-suppressant herbal remedy for asthmatics in Ghana. Although many studies have investigated the pharmacological basis of the ethnobotanical uses of *P. fruticosa*, however, its effect on the reproductive system remains completely unknown.

Aim of study: This study assessed effects of *P. fruticosa* leaf extract (PFE) on male fertility and toxicity in adult male Wistar rats.

Materials and methods: After crude preparation of PFE, it was subjected to qualitative phytochemical, thin layer chromatography and gas chromatography mass spectrometry analyses. The effect of PFE was assessed on male fertility and toxicity by using healthy adult male Wistar rats. Rats were randomly assigned to normal saline (5 ml/kg *po*, *n* = 5), Clomiphene Citrate (CL; 50 mg/kg *po*; *n* = 5), and PFE (100, 200, and 500 mg/kg *po*; *n* = 5, respectively) groups and treated for 21 days. On day 22, rats were sacrificed and male fertility parameters (left testis weight, relative testis weight, caudal epididymal weight, caudal epididymal sperm count, sperm motility, sperm morphology, and assessment of male sex hormones and testicular histology) were assessed.

Results: There were no significant changes in bodyweight, weight of left testis, weights of right and left caudal epididymides between treatment groups (PFE and CL) and control. Caudal epididymal sperm count increased in PFE (100 and 500 mg/kg)-treated rats relative to control. Sperm motility relatively increased in PFE-treated rats compared to control. Sperm abnormality decreased in PFE-treated rats, especially in PFE (100 mg/kg) group compared to control. Serum testosterone levels decreased inversely with serum luteinizing hormone levels in PFE-treated rats compared to control. There were minimal-to-no-alterations in histological sections of testis, except vacuolations at primary spermatocyte stage. Glycosides, saponins, cyanogenic glycosides, sterols, and alkaloids were detected in PFE.

Conclusion: PFE improved caudal epididymal sperm count and may be useful as male fertility enhancer but exhaustive safety studies on key male sex organs needs to be established.

ARTICLE HISTORY

Received July 01, 2017
Accepted July 27, 2017
Published January 06, 2018

KEYWORDS

Luteinizing hormone; Male fertility; *Polyscias Fruticosa*; Testicular toxicity; Testosterone

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Introduction

Herbal medicine is not only popular but also its use has increased among populations entrapped in economically challenged tropical regions of the world [1,2] and recently among populations in developed countries. In the USA, it is reported that one out of every four adult Americans admitted to using herbal products in one way or the other in the past years for medical reasons [3,4]. Increase in herbal product usage is not paralleled by the requisite scientific ascertainment of safety, efficacy, and other pharmacological properties relevant for informed and evidence-based herbal therapy. For instance, many herbal medicines have not been scientifically screened for their safety and efficacy for specific indications, yet they are still in common use including those with known or unknown effects on reproduction [2,5-7]. Pharmacologically, many herbs have been confirmed for their activity against specific indications but lack scientific information on their effects on the reproductive system, despite their systemic exposure. One of such herbs with extensive ethnobotanical use but having no scientific ascertainment of its effects on the reproductive system is *Polyscias fruticosa* (L.) Harms. [syn. *Panax fruticosum* L., *Notho panax fruticosum* Miq.] (Family: Araliaceae).

Ecologically, *P. fruticosa* is distributed across Eastern Asia, tropical islands of the pacific region [8] as well as in Africa, particularly in Ghana and Togo [9,10]. Traditionally, crude forms of *P. fruticosa* are used in Asia as tonic, anti-inflammatory, anti-toxin, anti-microbial as well as a spice and a digestive agent [8]. A crude root extract of *P. fruticosa* is documented as a diuretic, febrifuge, treatment for dysentery, neuralgia and rheumatic aches [11]. In Ghana, the extracts of the aerial parts of *P. fruticosa* were shown to be effective against asthma and other symptoms of upper respiratory disease [9,10,12]. Aside from its use as food and disease remedy, it is also used as an ornamental herb [8]. Phytochemical analysis has shown that *P. fruticosa* contains oleanoic acid saponins and polyacetylenes isolated from leaves [13,14] and roots [8,15]. Previously, we demonstrated anti-inflammatory, anti-tussive, muco-suppressant, and anti-asthmatic effects of *P. fruticosa* in guinea pigs and rats [9,10,12]. Quite recently, we demonstrated reproductive toxicity of *P. fruticosa* in pregnant rats (manuscript under review). However, it remains to be established the effect of *P. fruticosa* on reproduction, specifically male fertility in view of the fact that it is orally administered and therefore systemically exposed. This study investigated effects of *P. fruticosa* leaf extract (PFE) on male fertility in rats by specifically assessing the following: sperm count, sperm motility, sperm morphology, testicular male sex hormones [testosterone and luteinizing hormone (LH)], and testicular histology. This has become necessary in view of the utility of *P. fruticosa* as an herbal medicine and also as food among many populations across Afro-Asian regions of the world.

Materials and Methods

Chemicals and drugs

The chemicals and drugs secured for the study were absolute ethanol (PS-Park Scientific Limited, Northampton, UK), clomiphene citrate (CL; DOPPEL FARMACEUTICI S.p.a, Via delle Ande, 15 – 00144-ROME, Italy), dihydrated aluminium potassium sulphate (E. Merck, Darmstadt. Mol.-Gew.474,39), normal saline (Amanta Healthcare Ltd., Gujarat, India), silica gel (VWR International bvba/spr, Haasrode, Belgium, Batch: 09B200018), phosphate buffered saline, DPX, chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), 10% neutral buffered formalin, 1% Eosin W/V (BDH Chemicals LTD, England), and sodium hydrogen carbonate (PROLABO^{R}, EC-EMB 45053).

Collection, identification and authentication of P. fruticosa

P. fruticosa leaves were sourced from the botanical gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Identification and authentication of *P. fruticosa* leaves were done at the Herbal Medicine Department by a pharmacognosist. A voucher specimen (KNUST/HM/13/W010) was deposited at KNUST herbarium as previously reported [9,10].

Preparation of PFE

PFE was prepared by following a previously described method [9,10,12] with some modifications. Briefly, fresh *P. fruticosa* leaves (2.6 kg) were washed, shade-dried completely, and pulverised into powder (1.8 kg). The powder was soaked in absolute ethanol (4.8 L) for 72 hours, and then filtered. A rotary evaporator (B'U'CHI Olibath B – 485) was used to retrieve the ethanol from a dark-green filtrate at 50°C. The residue was completely dried in a desiccator containing activated silica gel. The crude extract yielded 63.0 g, given

a percentage yield of 3.4%. The whole extraction procedure was repeated until enough extract was obtained for the study. The final crude extract obtained was named PFE. PFE was stored in a refrigerator at 4°C until use.

Qualitative phytochemical screening of PFE

Phytochemical composition of PFE was ascertained by using previously described standard qualitative methods [16,17]. Subsequently, thin layer chromatography (TLC) and gas chromatography mass spectrometry (GC-MS) analysis were conducted on PFE as reported earlier (manuscript under review).

Dose selection

PFE (100, 200 and 500 mg/kg *po*) was selected based on our previous studies [9,12,18]. Dose of CL was based on clinical doses (50 mg/kg *po*) [19]. Bodyweight of rats were measured daily and doses were accordingly adjusted to reflect bodyweight changes.

Acquisition of experimental animals and husbandry

Eight weeks old healthy male Wistar rats weighing 120–200 g were obtained from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana. The rats were housed in aluminum cages (40 × 35 × 15 cm) with a base dressed with saw dust as bedding. Rats were maintained under 12 hours light/dark cycle, ambient temperature and normal humidity. Rats were fed standard pellet diet (Essaar grower mash, Essaar Agro-West Africa Ltd, Ghana) and had access to water *ad libitum*. Rats were allowed to acclimatize with laboratory conditions for two weeks before the start of experimentation. Rats were handled and treated humanely in strict adherence to standard guidelines as enshrined in the "Principles of laboratory animal care" (NIH publication No.85–23, revised 1985) as well as specific national and institutional requirements regarding the use of animals in scientific studies.

Measurement of body and organ weights

Bodyweights of rats were measured daily. Subsequently, bodyweights were measured prior to induction of anesthesia in rats using pentobarbital (60 mg/kg ip) on day 22. Testis and epididymis were surgically removed, trimmed of connective tissues and weighed (Sartorius LP 1200) as previously described [20] with some modifications. Relative organ weight (ROW) for testis and epididymis were determined as previously described [18] with some modifications. The formula used in calculating ROW is shown below:

ROW=Absolute organ weight (g)Body weight gon day 22

Measurement of epididymal sperm count

Epididymal sperm count was carried out according to previous methods [21,22] with some modifications. Briefly, the left and right caudal epididymides from a rat were removed into two sterile petri dishes. They were minced with sharp scissors and the ripped epididymides were transferred to two test tubes labeled as L (left) and R (right) containing 4 ml phosphate buffered saline (PBS) at 37°C. The sperms were then allowed enough time (5–10 min) to disperse. Approximately 10 µl of the diluted sperm suspension was transferred to each counting chamber of an improved Neubauer counting chamber (Haemocytometer) using a fine bore Pasteur pipette to charge the chamber and was allowed to stand for 4 minutes. The chamber was then placed under a binocular light microscope using an adjustable light source. The ruled part of the chamber was then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was calculated by multiplying the number of counted spermatozoa by five and expressed as shown below:

Sperm count = 5 x 106ml,

where (*X*) is the number of spermatozoa counted in five 16-celled squares.

Assessment of sperm motility

Epididymal sperm motility was carried out as described previously [4,23,24]. Briefly, a cut was made at the cauda of the contralateral epididymis. Resulting fluid from the cut was diluted in PBS. A drop of diluted sperm suspension was dropped on a pre-warmed slide, covered with a cover slip and observed under a microscope. Sperm motility was estimated in six separate randomly selected fields through a light microscope at a magnification of 40×. The mean of the six (6) estimations was used as the final motility score. Sperm motility was estimated in three (3) categories as previously described [25]. These categories were defined as progressive (where the spermatozoa are exhibiting a linear motion), nonprogressive (where the spermatozoa are exhibiting a local motion but not to the front or backward) or immotile (where the spermatozoa are not in motion at all). These three parameters

were recorded and expressed in percentages (%) as shown below:

% *P* or *N* or *I* motility

=[*P* or *N* or *I*][Sum of the three Categories for one field] 100; *P*: Progressive motility, *N*: Non progressive, *I*: Immotile

Assessment of sperm morphology

Sperm morphology was evaluated according to a previous method [26] with some modifications. Briefly, a 1 ml sperm suspension from rats in each group was pipetted into an appropriately labeled test tube. Subsequently, 5 drops of 1% Eosin yellow was added and gently mixed manually. The resultant suspension was incubated for 45 minutes at room temperature to facilitate staining. By using a pipette, the suspension was gently agitated. A drop of each suspension was transferred to a clean slide. Smears were prepared by simply spreading the drop in a circular pattern until its size was just big enough (1.8–2.0 cm^2) to facilitate early air-drying. Dried smear was preserved by mounting with cover slips using DPX mountant. Well over 100 sperms per rat from each experimental group were examined as previously described [23].

Measurement of serum levels of testosterone and luteinizing hormone

Blood samples were collected by cardiac puncture using a needle (23 g) and a syringe (3 ml). Collected blood was emptied into blood labeled tubes. Blood samples were centrifuged at 3,500 rpm for 20 minutes few minutes after collection. The serum obtained was separated from the cells into Eppendorf tubes and stored at −20°C until use. Serum levels of LH and testosterone were determined by using an ELISA assay kit (Human Gesellschaft fur Biochemica und Diagnostica mbH Max-Planck-Ring 21 65205, Wiesbaden, Germany) by strictly following manufacturer's instructions. The sensitivity of hormone detection was 0.005 ng/ml. To prevent inter-assay errors all samples were analyzed in a single assay.

Histological assessment of testis

Left testis of each rat in each group was isolated, fixed, sectioned, and stained with hematoxylin and eosin (H&E) according to a previous method [27] with some modifications. Briefly, isolated left testis was fixed in 10% formalin, embedded in paraffin, cut at 3 μm sections, de-waxed, rehydrated, and stained with H&E. Sections were first stained with hematoxylin, washed in running water until appearance of blue color. Subsequently, sections were stained with eosin and viewed under light microscope at a magnification of 40× by three independent persons. Representative photomicrographs were generated for rats in each group.

Statistical analysis

Data are expressed as mean ± SD. Normality analysis was conducted to determine the nature of data distribution before selection of statistical test. Mean comparisons between groups was done by One Way ANOVA using GraphPad Prism for Windows Version 6.01 (GraphPad Software, San Diego, CA, USA) followed by Tukey's *post hoc* multiple comparison. $P < 0.05$ was considered statistically significant in all analysis.

Results

Phytochemical analyses of PFE

Phytochemical screening showed the presence of glycosides, cyanogenic glycosides, saponins, alkaloids, and sterols in PFE as observed earlier (manuscript under review). Subsequently, a TLC analysis showed four (4) spots. Subsequently, a GC-MS profiling of PFE showed twelve (12) peaks out which eight (8) marched library compounds.

Body weight and relative organ weight assessments

Although bodyweights of treatment groups compared to control on day 22 was not significantly ($P \geq 0.05$) different from that of control. PFE (500 mg/kg) was lower compared to control and all other groups. Weight of left testis was comparable among all groups even though it was lower in the case of PFE (500 mg/kg) group. Except PFE (100 mg/kg), the weight of left caudal epididymis was comparable among treatment and control groups. Mean weight of left caudal epididymis of PFE (500 mg/kg) and CL groups was significantly different compared to PFE (100 mg/kg). Except CL group, weight of right caudal epididymis was comparable among all groups (Table 1). There was a dose-dependent increase in mean caudal epididymal weight and relative caudal epididymal ratio in PFE (100, 200, and 500 mg/kg) groups compared to control and CL groups (Figs. 1 and 2).

Epididymal sperm count, sperm motility and morphology

Except PFE (200 mg/kg) and CL groups, the mean left and right caudal epididymal sperm counts of PFE

Table 1. Assessment of body and testicular weights in male Wistar rats after PFE and CL treatments.

Groups	Wt of rats (g)	Wt of left testis (g)	Wt of Left testis/ Wt of Rat	Wt of left caudal epididymis (g)	Wt of Right caudal epididymis (g)
NS	202.52 ± 8.50	1.32 ± 0.15	0.0065 ± 0.001	0.20 ± 0.01	0.20 ± 0.01
LD	189.55 ± 33.04	1.20 ± 0.06	0.0064 ± 0.001	0.14 ± 0.02*	0.15 ± 0.02
MD	198.70 ± 8.68	1.33 ± 0.03	0.0067 ± 0.000	0.17 ± 0.02	0.17 ± 0.02
HD	191.29 ± 13.82	1.06 ± 0.37	0.0055 ± 0.001	0.21 ± 0.04#	0.22 ± 0.06
CL	196.98 ± 7.24	1.26 ± 0.12	0.0064 ± 0.000	0.23 ± 0.05#	0.28 ± 0.08#†
F-value	0.289	1.0028	0.629	3.375	3.717
P-value	0.878	0.439	0.653	**0.041**	**0.042**
N	3	3	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS: Normal saline group, †significantly different in comparison with MD: Medium dose (200 mg/kg) of PFE, CL: Clomiphene citrate (50 mg/kg); Wt: weight; PFE: *Polyscias fruticosa* leaf extract.

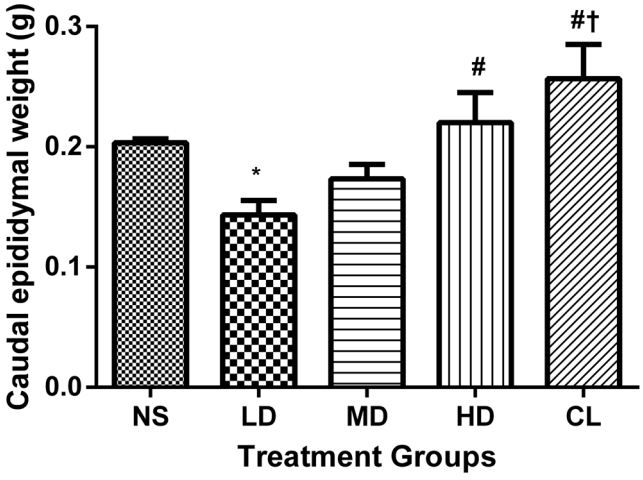

Figure 1. Average changes in weight of caudal epididymis in male rats after 21-day oral treatments. *Significantly different in comparison with NS: Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group; CL: clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

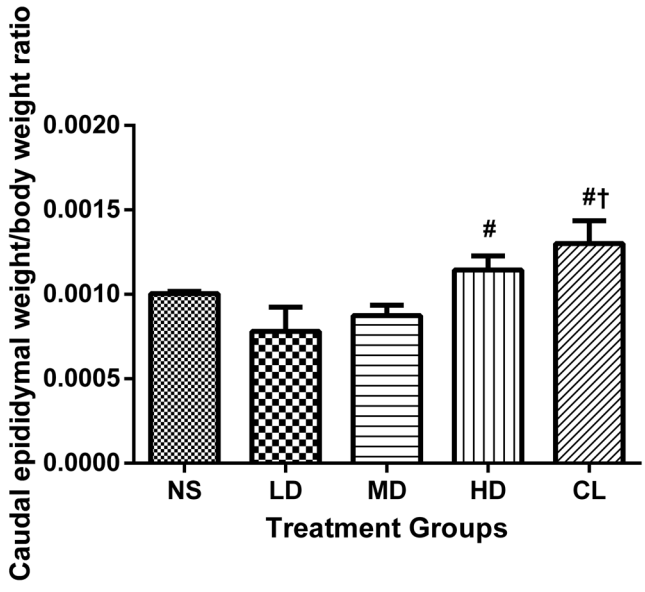

Figure 2. Changes in relative caudal epididymal weight to body ratio of male rats after 21-day oral treatments. *Significantly different in comparison with NS: Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group; CL: clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

(100 and 500 mg/kg) increased significantly compared to control (Fig. 3 and Table 2). There were no significant differences between mean relative left and right caudal epididymal weights between treatments and control except in the case of PFE (500 mg/kg) and CL groups (Table 2). Average caudal epididymal sperm count significantly ($P \leq 0.05$) increased in PFE (100 and 500 mg/kg) groups compared to control (Fig. 3 and 4). Sperm motility with respect to progressive and immotile was comparable among treatment groups (PFE and CL) and control (Table 3). Sperm motility with respect to non-progressive was significantly reduced in treatment groups (PFE and CL) compared to control (Table 3). Except PFE (100 mg/kg) group which showed low sperm abnormality, the remaining treatment groups and the control had comparable sperm abnormality and morphology (Fig. 5 and Table 4).

Evaluation of serum testosterone and luteinizing hormones

Mean serum testosterone levels decreased in PFE (100, 200, and 500 mg/kg) and CL groups inversely with serum LH levels compared to control (Table 5) but the mean difference was statistically insignificant ($P \leq 0.05$). Mean serum LH levels decreased in PFE (100 and 500 mg/kg) and CL groups compared to control, while that of PFE (200 mg/kg) was comparable to that of control (Table 5).

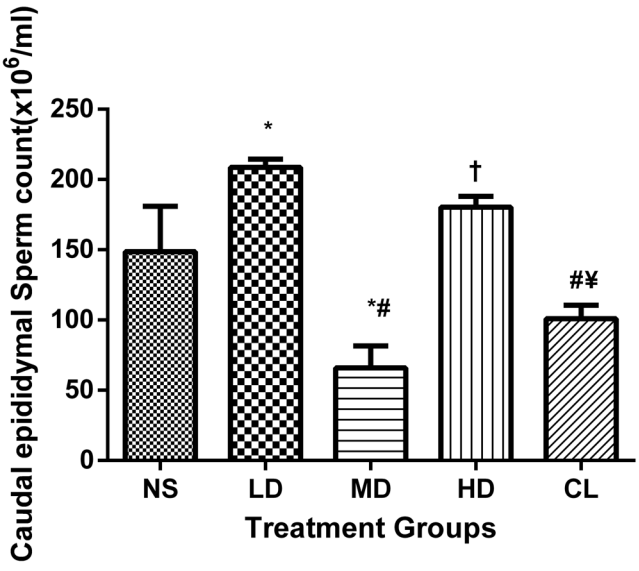

Figure 3. Spermatozoa spread from caudal epididymal spermatozoa suspensions of male rats after 21-day treatment with drugs observed under a microscope. (A) NS: Normal saline group, (B) LD: Low dose (100 mg/kg) group, (C) MD: Medium dose (200 mg/kg) group, (D) HD: High dose (500 mg/kg) group, and (E) CL: clomiphene citrate (50 mg/kg).

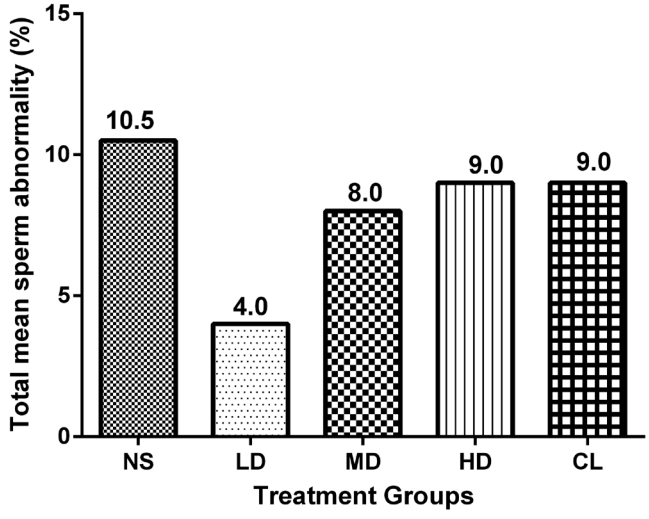

Figure 4. Average caudal epididymal sperm count of male rats after 21 day oral treatments. *Significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD: Low dose (100 mg/kg) group, †significantly different in comparison with MD: Medium dose (200 mg/kg) group, ¥significantly different in comparison with HD: High dose (500 mg/kg) group and CL: Clomiphene citrate (50 mg/kg). $P < 0.005$ was considered significant in all analysis.

Table 2. Assessment of caudal epididymal sperm count in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Groups	Left caudal epididymal sperm count (×10⁶/ml)	Right caudal epididymal sperm count(×10⁶/ml)	Left Caudal epididymal wt/body wt ratio	Right Caudal epididymal wt/body wt ratio
NS	151.67 ± 50.08	145.00 ± 62.65	0.001 ± 0.000	0.001 ± 0.000
LD	240.00 ± 5.00*	176.67 ± 23.63	0.001 ± 0.000	0.001 ± 0.000
MD	66.67 ± 44.81*#	65.00 ± 10.00*#	0.001 ± 0.000	0.001 ± 0.000
HD	191.67 ± 17.56†	168.33 ± 30.14†	0.001 ± 0.000#	0.001 ± 0.000
CL	70.00 ± 26.46*#¥	101.67 ± 10.41#¥	0.001 ± 0.000#	0.001 ± 0.000#†
F-value	3.717	15.543	2.766	3.170
P-value	**0.042**	**<0.0001**	0.087	0.063
N	3	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD - Low dose (100 mg/kg) of PFE, †significantly different in comparison with MD - Medium dose (200 mg/kg) of PFE, ¥significantly different in comparison with HD - High dose (500 mg/kg) of PFE; CL-Clomiphene citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract.

Table 3. Assessment of sperm motility parameters after 21-day PFE and Clomiphene Citrate treatments in male Wistar rats.

Groups	Progressive (%)	Nonprogressive (%)	Immotile (%)
NS	41.50 ± 10.83	41.27 ± 11.11	31.10 ± 12.88
LD	53.13 ± 4.60	20.83 ± 4.35*	24.67 ± 7.85
MD	49.97 ± 3.57	15.07 ± 7.35*	29.70 ± 12.14
HD	44.97 ± 11.01	26.90 ± 5.28*	28.10 ± 11.42
CL	40.87 ± 8.33	15.97 ± 2.36*	39.60 ± 8.11
F-value	1.253	7.515	0.811
P-value	0.350	0.005	**0.546**
N	3	3	3

ANOVA column comparison at $P < 0.005$: *significantly different in comparison with NS-Normal saline group, #significantly different in comparison with LD - Low dose (100 mg/kg) of PFE, †significantly different in comparison with MD - Medium dose (200 mg/kg) of PFE, ¥significantly different in comparison with HD - High dose (500 mg/kg) of PFE; CL - Clomiphene Citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract. Progressive (Many spermatozoa exhibit linear motion); Non-progressive (Many spermatozoa exhibits local motion but not to the front or backward); Immotile (Many spermatozoa were not in motion at all).

Table 4. Assessment of sperm morphology in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Group	Pinhead (%)	Flattened head (%)	Tailless head (%)	Headless sperm (%)	Bent neck (%)	Broken tail (%)	Coiled tail (%)	Bent tail (%)
NS	0	1	3.5	2	2.5	0	0.5	1
LD	0	0	1	1	1	0	1	0
MD	0	0.5	1.5	1.5	1.5	0	1.5	1.5
HD	0	1	2	1.5	2	0	1	1.5
CL	0	0	2.5	2	1.5	0	1	2

NS – normal saline (5 ml/kg); LD – Low dose (100 mg/kg); MD – Medium dose (200 mg/kg); HD - High dose (500 mg/kg); CL – Clomiphene citrate (50 mg/kg); PFE – *Polyscias fruticosa* leaf extract

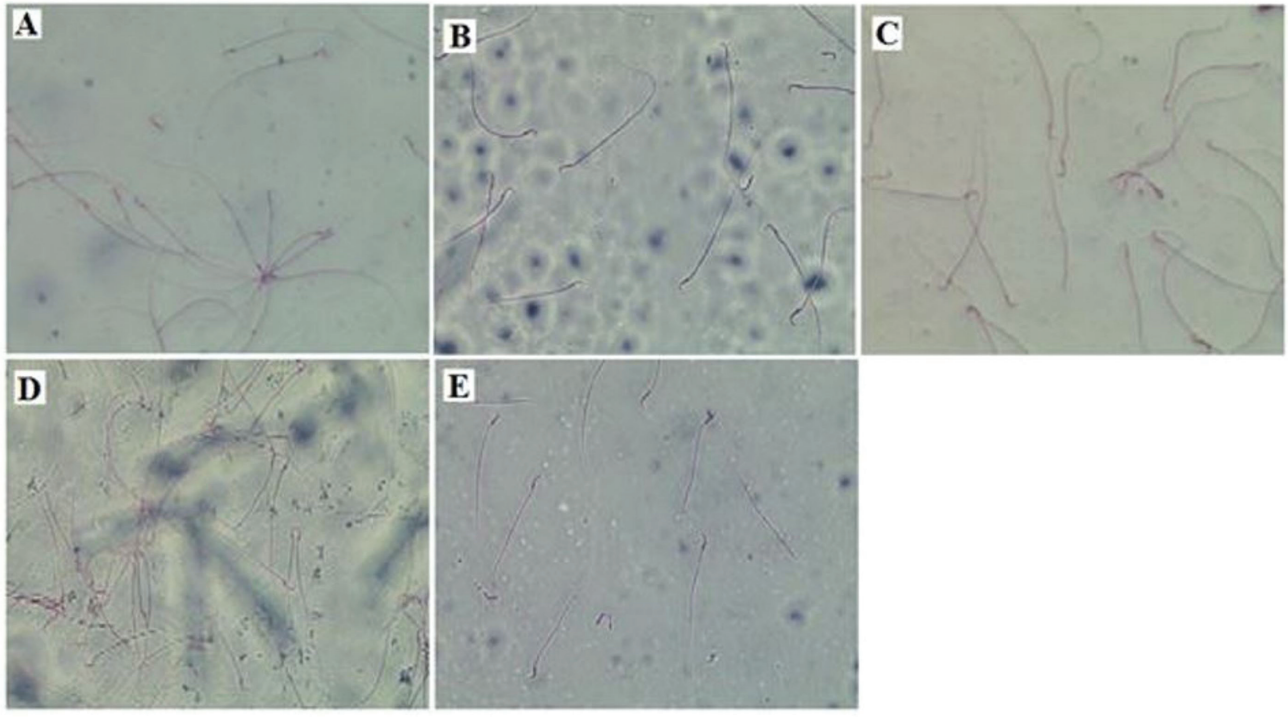

Figure 5. Total mean sperm abnormality of male rats after 21 day oral treatments. NS: normal saline (5 ml/kg), LD: low dose (100 mg/kg), MD: medium dose (200 mg/kg), HD: high dose (500 mg/kg), CL: clomiphene citrate (50 mg/kg).

Table 5. Assessment of serum levels of male sex hormones in male Wistar rats after 21-day PFE and Clomiphene Citrate treatments.

Groups	Testosterone (ng/ml)	LH (IU/L)	Testosterone /LH ratio	LH/Testosterone ratio
NS	3.11 ± 2.42	0.39 ± 0.09	8.57 ± 6.62	0.53 ± 0.35
LD	2.38 ± 1.73	0.11 ± 0.13	58.74 ± 31.36	0.22 ± 0.21
MD	0.30 ± 0.27	0.40 ± 0.59	8.77 ± 7.59	4.78 ± 4.55
HD	0.59 ± 0.35	0.18 ± 0.04	3.86 ± 2.53	3.54 ± 3.31
CL	0.12 ± 0.11	0.07 ± 0.05	82.51 ± 81.74	1.19 ± 0.81
F-value	1.307	0.92	0.828	0.619
P-value	0.332	0.489	0.537	0.659
N	3	3	3	3

ANOVA column comparison at $P < 0.005$, NS – normal saline (5 ml/kg), LD – Low dose (100 mg/kg) of PFE, MD – medium dose (200 mg/kg) of PFE, HD – high dose (500 mg/kg) of PFE, CL – clomiphene citrate (50 mg/kg), IU – Internal unit and LH – Luteinizing hormone, PFE – *Polyscias fruticosa* leaf extract.

Evaluation of testicular histology

The histological sections of left testis of PFE (200 mg/kg and 500 mg/kg)-treated rats compared to control rats were fairly of normal architecture with oval outline, normal seminiferous epithelium, and numerous spermatozoa within the lumen as well as intact interstitium (Fig. 6). However, vacuolation was common across all groups.

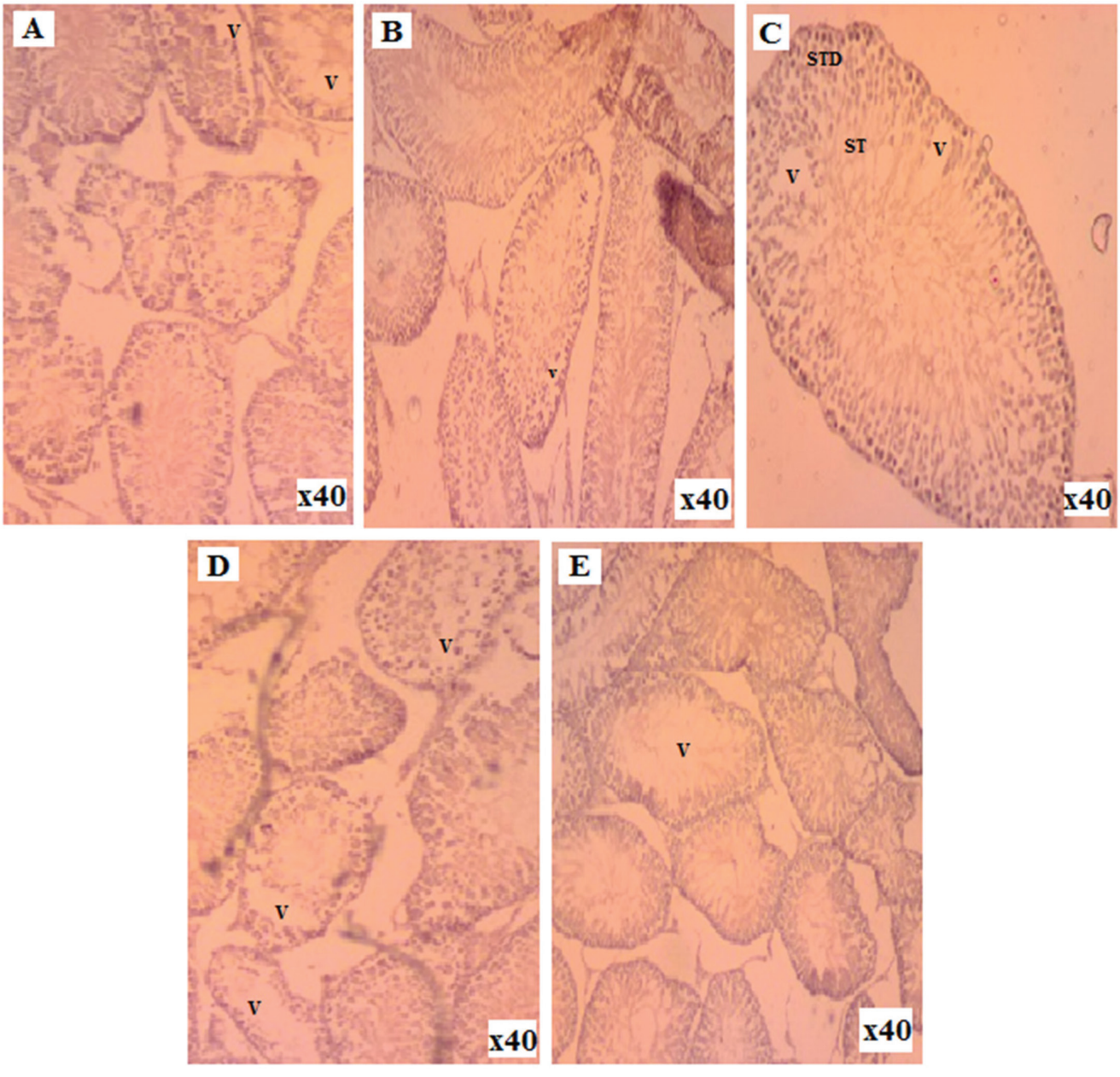

Figure 6. Histomicrographs showing effects of 21-day drug treatments on left testicular microstructure of control (A), LD (B), MD (C), HD (D), and CL (E). NS: normal saline (5 ml/kg), LD: Low dose (100 mg/kg), MD: medium dose (200 mg/kg), HD: high dose (500 mg/kg), CL: CL (50 mg/kg), ST: Sperm tail, STD: Spermatids and V: Vacuolation.

Discussion

Most herbal medicines are orally administered for the treatment of specific diseases and more often their pharmacological assessment is limited to efficacy with respect to the claimed ethnobotanical and folk uses. In view of the systemic exposure of herbal medicines, they certainly produce unknown physiological and pharmacological effects on non-target organ systems of the body including the reproductive system. Previously, folk uses of *P. fruticosa* in Ghana such as anti-inflammatory, anti-tussive, muco-suppressant, and anti-asthma effects were studied [9,10,12] but how *P. fruticosa* affects male fertility and any possible toxic effects on male reproductive organs in view of its systemic exposure to both men and women remain unknown. Presently, we demonstrated that 21-day oral administration of PFE to male rats improved epididymal sperm count (Fig. 3), sperm motility (Table 3), caudal epididymal weight, decreased sperm abnormality (Fig. 4 and Table 4), especially in PFE (100 mg/kg) group and decreased serum levels of testosterone inversely with LH without producing significant changes in bodyweight, weight of male reproductive structures

(testis, left and right caudal epididymides) and testicular structure.

Improvement in weight and size of reproductive structures such as testis and seminiferous tubules are directly related to normal male sexual development [28]. In effect, increase in weight of reproductive organs secondary to drug treatments indicates that perhaps such agents may improve reproductive processes including spermatogenesis. In this study, although there were changes in bodyweight, left testis and caudal epididymal weights between treatment (Low dose, medium dose, high dose and CL) and control groups, these changes were not statistically significant, indicating that PFE may promote normal development of male reproductive organs since it did not decrease the weight of the tested male reproductive structures. Of note reproductive organs are hormone-sensitive, especially to sex hormones [29,30]. As a result, normal development of reproductive organs can be improved with natural products having hormone-like effects mediated through the hypothalamo-pituitary-gonadal axis which plays a crucial role in sexual reproduction [31]. The hypothalamus via a system of releasing hormones regulates the actions of the pituitary gland which secretes many tropic hormones including LH; LH in turn stimulates Leydig cells to secrete testosterone. Free circulating testosterone promotes spermatogenesis through its stimulatory effects on Sertoli cells in the seminiferous tubules [28,32,33]. In effect, increase in circulating levels of testosterone is shown to be correlated with increased epididymal sperm count while LH only promotes testicular development [34]. Also, male reproductive structures such as seminal vesicles and epididymis are highly androgen-sensitive [30,35,36]; therefore, improvement in sperm output and viability is a direct manifestation of the actions of androgens, specifically testosterone. Interestingly from the present study, it was observed that epididymal sperm count and sperm motility in PFE-treated male rats particularly those in PFE (100 and 500 mg/kg) group increased relative to control while their corresponding serum testosterone levels decreased inversely with LH even though increased testosterone level is crucial for spermatogenesis which normally manifest as increased sperm count and sperm motility [6,34]. The inverse serum levels of testosterone and LH as observed in this study was expected in view of the inhibitory effect of testosterone on LH [29] which serves as part of a negative feedback mechanism to ensure homeostatic regulation. Although this observation is the first to be observed in male rats treated with *P. fruticosa* leaf extract, it however mirrors a previous observation in adult men treated with Maca (*Lepidium meyenii*), where the sperm count and sperm motility improved quiet independently of testosterone, LH and follicle stimulating hormone [37] indicating the possibility of herbal medicines such as PFE having an unknown mechanism of improving sperm count and sperm motility in an androgen-independent manner. Contrastingly, the present observation is at variance with that of other herbs which demonstrated in male rodents a direct relationship between testosterone levels and sperm count as in *Cichorium intybus* [38], *Taraxacum officinale* [6], and *Achillea millefolium* [39], in which it was demonstrated that decreased caudal epididymal sperm count correlated with decreased androgen levels particularly testosterone levels. Comparing the present observation with others [6, 38, 39] with respect to the relationship between testosterone levels and sperm count, it is possible that herbal medicines such as PFE may have multiple mechanisms of improving sperm quality. Although the present result is insufficient to completely explain, it is possible that PFE may have improved epididymal sperm count, sperm motility and decreased sperm abnormality in a testosterone-independent manner through an unknown mechanism.

Although PFE treatment improved important male fertility parameters including sperm count, sperm motility, sperm abnormality, left testicular weight, and caudal epididymal weight, it produced minimal-to-no alteration in histological sections of left testis, mostly dominated by vacuolation (Fig. 6) which is commonly mistaken to be a sign of testicular toxicity [40].

Biological and pharmacological activities of herbs are attributable not only to their diverse phytochemical composition but also the complex phytochemical-to-phytochemical interactions [1,41]. In this study, saponins, cyanogenic glycosides, glycosides, sterols, and alkaloids were identified in PFE confirming our previous report [9,10,12]. Although this study did not assay individual phytochemicals in PFE, but given that all the identified phytochemicals were in PFE and could have interacted chemically in unknown ways, it is not unreasonable to attribute the observed spermatogenic effects of PFE to its phytochemical constituents. Saponins from *Albizia lebbeck* and *Cissus populnea* were shown to have no spermatogenic effects in men after 72 hours exposure and also decreased testis, epididymis,

and seminal vesicle weights [42,43]. Similarly sterols have been shown to decrease sperm quality [44,45] just as some alkaloids extracted from herbs [46,47], which deductively indicate that the spermatogenic effect of PFE may not be related to individual phytochemicals but could be attributed to a possible synergistic interactions between all the constituent phytochemicals. This study could have benefited from determining caput and corpus epididymal sperm counts as well as the spermatogenic mechanism of PFE particularly how it may modulate androgen receptors. Nonetheless, the present results provides not only preliminary evidence for possible utility of *P. fruticosa* leaf as a male fertility enhancer but also provides a compelling reason for further studies on *P. fruticosa*, particularly its safety on key male sex organs.

Conclusion

P. fruticosa leaf extract has increased caudal epididymal sperm count in 8 weeks old male Wistar rats quiet independently of testosterone. PFE may be relevant for improving male fertility parameters but its safety on key male sex organs needs to be established.

Author Contributions

A Boye conceived the idea, designed the experiments, wrote the draft manuscript, and critically revised the final manuscript for an important intellectual content. **VYA Barku** carried out phytochemical screening and TLC analysis. **GA Koffuor** read and edited the final manuscript for important intellectual content together with **A Boye**. **AKO Owusu** and **EA Asiamah** performed experiments, data analysis, and literature searches.

Acknowledgements

We are grateful to the following: Dr. Samuel Essien-Baidoo (Department of Medical Laboratory Science, University of Cape Coast, Cape Coast, Ghana), Dr. Elvis Ofori-Ameyaw (Department of Biomedical Science, University of Cape Coast, Cape Coast, Ghana), Mr. Justice Addo (Department of Chemistry, School of Physical Sciences, University of Cape Coast, Cape Coast, Ghana) and Mr. Edem Hope Agbodzakey (Parliament Clinic, MLS, Accra, Ghana) for their technical support. Also, we thank the laboratory staff of the Ghana Standards Authority, Accra, Ghana for helping us with the GC-MS analyses.

Conflict of Interest

Authors declare no conflict of interest.

References

[1] Boye A, Boampong VA, Takyi N, Martey O. Assessment of an aqueous seed extract of Parkia clappertoniana on reproductive performance and toxicity in rodents. J Ethnopharmacol 2016; 185:155–61.

[2] El Hilaly J, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of Ajuga iva in experimental animals. J Ethnopharmacol 2004; 91(1):43–50.

[3] Bent S, Ko R. Commonly used herbal medicines in the United States: a review. Am J Med 2004; 116(7):478–85.

[4] Chung F, Rubio J, Gonzales C, Gasco M, Gonzales GF. Dose-response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats. J Ethnopharmacol 2005; 98(1–2):143–47.

[5] Sharma N, Jocob D. Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of *Mentha arvensis* L. in male albino mice. J Ethnopharmacol 2001; 75(1):5–12.

[6] Tahtamouni LH, Alqurna NM, Al-Hudhud MY, Al-Hajj HA: Dandelion (*Taraxacum officinale*) decreases male rat fertility *in vivo*. J Ethnopharmacol 2011; 135(1):102–09.

[7] Chaudhury RR. The quest for a herbal contraceptive. Natl Med J India 1993; 6(5):199–201.

[8] Huan VD, Yamamura S, Ohtani K, Kasai R, Yamasaki K, Nham NT, et al. Oleanane saponins from *Polyscias fruticosa*. Phytochemistry 1998; 47(3):451–57.

[9] Koffuor GA, Boye A, Ofori-Amoah J, Kyei S, Abokyi S, Nyarko RA, et al. Antiinflammatory and safety assessment of *Polyscias fruticosa* (L.) Harms (Araliaceae) leaf extract in ovalbumin-induced asthma. JPHYTO 2014; 3(5):337–42.

[10] Koffuor GA, Boye A, Ofori-Amoah J, Kyei S, Nouoma CK, Debrah AP. Evaluating muco-suppressant, anti-tussive and safety profile of *Polyscias fruticosa* (L.) Harms (Araliaceae) in asthma management. Br J Med Med Res 2015; 10:1–11.

[11] Quisumbing E. Medical plants of the Phillipines. JMC Press INC Bureau of Printing, 1978.

[12] Asumeng Koffuor G, Boye A, Kyei S, Ofori-Amoah J, Akomanin Asiamah E, Barku A, et al. Anti-asthmatic property and possible mode of activity of an ethanol leaf extract of Polyscias fruticosa. Pharm Biol 2016; 54:1354–63.

[13] Chaboud A, Rougny A, Proliac A, Raynaud J, Cabalion P. A new triterpenoid saponin from *Polyscias fruticosa*. Pharmazie 1995; 50(5).

[14] Proliac A, Chaboud A, Rougny A, Gopalsamy N, Raynaud J, Cabalion P. A oleanolic saponin from *Polyscias fruticosa* Harms var. yellow leaves. Pharmazie 1996; 51(8):611–12.

[15] Lutomski J, Luan TC, Hoa TT. Polyacetylenes in the Araliaceae family. Part IV. Herba Pol 1992; 38(3):137–40.
[16] Cheruth AJ, Al Baloushi SA, Karthishwaran K, Maqsood S, Kurup SS, Sakkir S. Medicinally active principles analysis of Tephrosia apollinea (Delile) DC. growing in the United Arab Emirates. BMC Res Notes 2017; 10(1):61.
[17] Kumar S, Yadav A, Yadav M, Yadav JP. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) Burm.f. BMC Res Notes 2017; 10(1):60.
[18] Koffuor GA, Boye A, Ofori-Amoah J, Kyei S, Nouoma CK, Debrah AP. Evaluating muco-suppressant, anti-tussive and safety profile of *Polyscias fruticosa* (L.). Harms (Araliaceae) in asthma management. Br J Med Med Res 2015; 10:1–11.
[19] Vandekerckhove FW, De Croo I, Gerris J, Vanden Abbeel E, De Sutter P. Sperm chromatin dispersion test before sperm preparation is predictive of clinical pregnancy in cases of unexplained infertility treated with intrauterine insemination and induction with clomiphene citrate. Front Med 2016; 3:63.
[20] Shekarforoush S, Ebrahimi Z, Hoseini M. Sodium metabisulfite-induced changes on testes, spermatogenesis and epididymal morphometric values in adult rats. Int J Reprod Biomed (Yazd) 2015; 13(12):765–70.
[21] Del Val GM, Robledano PM. In vivo serial sampling of epididymal sperm in mice. Lab Anim 2013; 47(3):168–74.
[22] Karnam SS, Ghosh RC, Mondal S, Mondal M. Evaluation of subacute bisphenol—a toxicity on male reproductive system. Vet World 2015; 8(6):738–44.
[23] Karnam S, Ghosh R, Mondal S, Mondal M. Evaluation of subacute bisphenol—a toxicity on male reproductive system. Vet World 2015; 8(6):738–44.
[24] Gonzales C, Rubio J, Gasco M, Nieto J, Yucra S, Gonzales GF. Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (MACA) on spermatogenesis in rats. J Ethnopharmacol 2006; 103(3):448–54.
[25] Shekarforoush S, Ebrahimi Z, Hoseini M. Sodium metabisulfite-induced changes on testes, spermatogenesis and epididymal morphometric values in adult rats. Int J Reprod Biomed 2015; 13(12):765–70.
[26] Lüke L, Vicens A, Tourmente M, Roldan ER. Evolution of protamine genes and changes in sperm head phenotype in rodents. Biol Reprod 2014; 90(3):67.
[27] Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc 2008; 2008(5):pdb. prot4986.
[28] Choi JH, Lee SH, Bae JH. Effect of sleep deprivation on the male reproductive system in rats. J Korean Med Sci 2016; 31(10):1624–30.
[29] Dohle GR, Smit M, Weber RF. Androgens and male fertility. World J Urol 2003; 21(5):341–45.
[30] Lund TD, Munson DJ, Haldy ME, Setchell KD, Lephart ED, Handa RJ. Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. Biol Reprod 2004; 70(4):1188–95.
[31] Gao X, Zhang K, Song M, Li X, Luo L, Tian Y, et al. Role of Nesfatin-1 in the reproductive axis of male rat. Sci Rep 2016; 6:32877.
[32] Russell LD, Ren HP, Sinha Hikim I, Schulze W, Sinha Hikim AP. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. Am J Anat 1990; 188(1):21–30.
[33] Gao HB, Tong MH, Hu YQ, Guo QS, Ge R, Hardy MP. Glucocorticoid induces apoptosis in rat leydig cells. Endocrinol 2002; 143(1):130–38.
[34] Mali PC, Ansari AS, Chaturvedi M. Antifertility effect of chronically administered Martynia annua root extract on male rats. J Ethnopharmacol 2002; 82(2–3):61–7.
[35] Stroheker T, Cabaton N, Berges R, Lamothe V, Lhuguenot JC, Chagnon MC. Influence of dietary soy isoflavones on the accessory sex organs of the Wistar rat. Food Chem Toxicol 2003; 41(8):1175–83.
[36] Gonzales G. Response of the epididymis, seminal vesicles and prostate to the endogenous androgen stimulation in men attending an infertility service. Andrologia 2002; 34:308–16.
[37] Gonzales G, Córdova A, Gonzales C, Chung A, Vega K, Villena A. Improved sperm count after administration of *Lepidium meyenii* (Maca) in adult men. Asian J Androl 2001; 3:301–4.
[38] Roy-Choudhury A, Venkatakrishna-Bhatt H. Spermatogenic inhibition by *Cichorium intybus* L. aqueous root suspension in mice. Naturwissenschaften 1983; 70(7):365–6.
[39] Montanari T, de Carvalho JE, Dolder H. Antispermatogenic effect of *Achillea millefolium* L. in mice. Contraception 1998; 58(5):309–13.
[40] De Grava Kempinas W, Klinefelter GR. Interpreting histopathology in the epididymis. Spermatogenesis 2014; 4(2):e979114.
[41] Majid M, Khan MR, Shah NA, Haq IU, Farooq MA, Ullah S, et al. Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides. BMC Complement Altern Med 2015; 15(1):349.
[42] Ojekale AB, Lawal OA, Lasisi AK, Adeleke TI. Phytochemisty and spermatogenic potentials of aqueous extract of *Cissus populnea* (Guill. and Per) stem bark. ScientificWorldJournal 2006; 6:2140–6.
[43] Gupta RS, Chaudhary R, Yadav RK, Verma SK, Dobhal MP. Effect of Saponins of *Albizia lebbeck* (L.) Benth bark on the reproductive system of male albino rats. J Ethnopharmacol 2005; 96(1–2):31–6.

[44] Saez Lancellotti TE, Boarelli PV, Romero AA, Funes AK, Cid-Barria M, Cabrillana ME, et al. Semen quality and sperm function loss by hypercholesterolemic diet was recovered by addition of olive oil to diet in rabbit. PLoS ONE 2013; 8(1):e52386.

[45] Chavarro JE, Minguez-Alarcon L, Mendiola J, Cutillas-Tolin A, Lopez-Espin JJ, Torres-Cantero AM. Trans fatty acid intake is inversely related to total sperm count in young healthy men. Hum Reprod 2014; 29(3):429–40.

[46] Olayemi FO, Raji Y. Quinolizidine alkaloids: the bioactive principles in Cnestis ferruginea (de Candolle) with male antifertility activities. Afr J Med Med Sci 2011; 40(3):253–63.

[47] Yakubu MT. Effect of a 60-day oral gavage of a crude alkaloid extract from *Chromolaena odorata* leaves on hormonal and spermatogenic indices of male rats. J Androl 2012; 33(6):1199–207.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 57–65
10.5455/jice.20170926111217

eJManager

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Antioxidant content and *in vitro* 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of selected medicinal plants

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ABSTRACT

Background/Aim: The medicinal plants and their derivatives have long been recognized as important sources of antioxidants in the prevention and treatment of various diseases. This study investigated phytochemicals, antioxidant content, and *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities of methanolic and aqueous leaves extracts of *Anogeissus leiocarpus, Ipomoea asarifolia, Bauhinia rufescens, Guiera. Senegalensis*, and *Moringa oleifera*.

Materials and Methods: The extracts were subjected to qualitative phytochemical analysis to identify the bioactive constituents in each plant. Total phenolics and proanthocyanidin contents were determined using Folin–Ciocalteu and vanillin-methanol assays while antioxidant free scavenging activity was estimated using DPPH assay.

Results: The phytochemical screening revealed the presence of alkaloids, flavonoids, and tannins in all the five plants investigated. The total phenolic and proanthocyanidin contents of methanolic extracts were significantly higher ($p < 0.05$) as compared with aqueous extracts. DPPH-free radical scavenging activity of methanolic extracts of *A. leiocarpus* and *M. oleifera* was similar to vitamin C. *In vitro* DPPH radical scavenging activity of methanolic extracts of *A. leiocarpus* and *M. oleifera* were better as compared with *B. refescens, I. asarifolia, G. senegalensis*, and aqueous extracts. The antioxidant activities of both extracts were in a dose-dependent manner. Furthermore, the lower IC_{50} of methanolic extracts of *A. leiocarpus, B. refescens*, and *M. oleifera* correlated with strong scavenging activities of these plants.

Conclusion: This study demonstrated the antioxidant content and DPPH-free radical scavenging activities of five medicinal plants. Thus, the study underscored these plants as potential sources of natural antioxidants that can be explored for the treatment of oxidant-related diseases.

ARTICLE HISTORY

Received September 26, 2017
Accepted December 11, 2017
Published January 06, 2018

KEYWORDS

Antioxidants; DPPH; free radicals; medicinal plants; phytochemicals

Introduction

Medicinal plants contain bioactive ingredients that have potential therapeutic effects against various diseases. Many bioactive compounds with antioxidant activities in plants have been suggested to play a protective role against oxidative stress-related diseases [1,2]. The consumption of these plants has been reported to lower the risk of atherosclerosis, cancer, hypertension, stroke, and hepatic diseases [3–6]. The antioxidant activities of medicinal plants have been attributed to the presence of polyphenols such as flavonoids, phenolic acids, tannins, anthocyanin [5–7], and β-carotene, vitamins C and E [7] which has the ability to scavenge free radicals that are generated in the living system. Oxidative stress occurs when there is an imbalance between oxidant and antioxidant molecules in favor of the oxidant which results in excess production of reactive oxygen species (ROS). The ROS generated are capable of destroying the internal redox balance that may cause tissue damage or premature aging [8–11]. The medicinal plants also serve as a

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source of supplement or functional foods that can safeguard the health of the individual. Several natural antioxidants have been isolated from various plant sources such as cereals, algae, leafy vegetables, fruits, and seeds [12]. The free radical scavenging activities of antioxidant polyphenols in plants and plant products have been attributed to their ability to donate protons to ROS. Phytochemicals such as flavonoids, essential oils, and anthocyanin have received attention as sources of natural antioxidant in health promotion as well as cosmetics because they are safer than synthetic antioxidant [13]. The use of herbs have less adverse effects as they are commonly direct toward aiding the body's own healing process rather than addressing symptoms caused by specific diseases as in the case of synthetic drugs [14].

Anogeissus leiocarpus belongs to the family Combretaceae and is widely used in African traditional medicine for the treatment of various diseases [15]. In Nigeria, rural populations used it as a chewing stick for oral hygiene. The plant has been shown to possess antibacterial activity [15]. The leaves and stem bark of the plant have also been reported for the treatment of jaundice, cough, and fever [16]. *Ipomoea asarifolia* is a hairless, succulent perennial weed of the family, Convolvulaceae. The plant has been reported to have anti-inflammatory activity by decreasing the levels of interleukin 1β, interleukin 6, and tumor necrosis factor α in a murine model of peritonitis [17]. *Bauhinia rufescens* is a shrub that belongs to the family Fabaceae. The plant has been shown to possess therapeutic effects against fibrosis, dysentery, and jaundice [18]. *Guiera Senegalensis* is a semi-evergreen shrub that can grow up to 3 m high and belongs to the family Combretaceae. The leaves have a high reputation as a "cure-all" in Africa, where those are taken in decoctions or mixed with foods for the treatment of a wide range of disease conditions [19]. It is known as being active against diseases such as cough, fever, diarrhea, dysentery, rheumatism, leprosy, and is given to women to promote the flow of milk after childbirth [19]. The plant has also been reported to possess hypoglycaemic effect in type 2 diabetic patients [20]. *Moringa oleifera* is a member of Moringacea. The leaves contain nutrients, especially essential amino acids, vitamins, and β-carotene [21]. Apart from nutritional benefits, the hypoglycemic and hypolipidemic effects of leaf extract of *M. oleifera* have been reported [22]. The leaf extract has also been shown to enhance hepatic glutathione restoration [23]. Ethnobotanical surveys indicated that stem bark, leaves, and root extracts of these medicinal plants have been used for the treatments of various diseases in Nigeria [16,24–26].

Therefore, this study was designed to compare the antioxidant contents and DPPH free scavenging activities of aqueous and methanolic extracts of *A. leiocarpus I. asarifolia, B. rufescens, G. senegalensis*, and *M. oleifera* used in the treatment of various diseases.

Materials and Methods

Plant materials

All the plants except *M. oleifera* were collected from Arkila area of Sokoto, Nigeria. Fresh *M. oleifera* was bought from Sokoto central market, Nigeria. The leaves of all the five plants were collected in October 2015 and air dried. The plants were identified and authenticated at the Botany Unit, Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. The voucher specimens were deposited at the herbarium of the same institution with the following voucher numbers: *M. oleifera* (UDUH/ANS/0225), *A. leiocarpus* (UDUH/ANS/0180), *I. asarifolia* (UDUS/ANS/0140), *B. rufescens* (UDUH/ANS/0210), and *G. senegalensis* (UDUH/ANS/0144).

Preparation of plant extracts

Total of 10 g of each of the plant leaves was washed with water, air-dried, and ground to a powder with mortar and pestle. The powdered leaves were exhaustively extracted with water and methanol separately at room temperature for 48 h with stirring at interval and later filtered with a muslin cloth. The aqueous and methanolic extracts obtained were concentrated to dryness at 40°C, using a rotary evaporator under reduced pressure. The dried extracts were weighed and percentage yield (data not provided) of all the extracts calculated and recorded, then stored for subsequent analysis.

Phytochemical screening

Methanolic and aqueous extracts of each of the plants were subjected to qualitative phytochemical analysis to identify the bioactive constituents of each plant.

Test for alkaloids

Total of 2 ml of test solution was mixed with 2N HCl. The aqueous layer formed was decanted and few drops of Mayer's reagent were added. The formation of cream colored precipitate indicates the presence of alkaloids.

Test for saponins

The leaf extract (0.5 g) was stirred with 10 ml of distilled water in a test tube. The formation of frothing which persists on warming in a water bath for 5 minutes indicates the presence of saponins.

Test for tannins

The leaf extract (0.5 g) was stirred with 10 ml distilled water and then filtered. Then ferric chloride solution (5%) was added drop by drop to the extract and the colored produced was noted. The presence of tannins was observed by the formation of dark-green color.

Test for cardiac glycosides

A quantity (2.5 ml) of 50% H_2SO_4 was added to 5 ml of the extract in a test tube. The mixture was heated in a water bath for 15 minutes, cooled, and neutralized with 10% NaOH. Then 5 ml of Fehling solution was added and the mixture was boiled. A brick-red precipitate indicates the presence of cardiac glycosides.

Test for flavonoids

A few (three) drops of 1% ammonia solution were added to 10 ml of the plant extracts in a test tube. A yellow coloration observed indicates the presence of flavonoids.

Determination of total phenolic contents

The Folin–Ciocalteau reagent method was employed for the estimation of total phenolic contents of each of the extracts according to Lister and Wilson [27]. A solution of crude extracts of the plants was prepared and then 100 µl of the supernatant was taken from each prepared extract and mixed with 0.5 ml (1/10 dilution) of Folin–Ciocalteau reagent. Then the mixture was incubated at room temperature for 1 minute. Thereafter, 1.5 ml of 2% Na_2CO_3 (w/v) solution was added. The final mixture was shaken and incubated in the dark at room temperature for 1.5 hours. The absorbance of the sample was measured at 765 nm using spectrophotometer. Standard calibration curve for gallic acid was prepared in the range of 20–100 µg/ml in the same manner. The results were expressed as milligram gallic acid equivalent per gram of extract.

Determination of proanthocyanidin contents

The total proanthocyanidin of the extracts was determined using the procedure reported by Asowata-Ayodele et al. [28]. A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3.0 ml of 4% vanillin-methanol solution and 1.5 ml of hydrochloric acid and then vortexed. The mixture was allowed to stand for 15 minutes at room temperature, followed by the measurement of the absorbance of the extract at 500 nm. Total proanthocyanidin contents were expressed as catechin (mg/g) from the standard curve.

Determination of DPPH radical scavenging activity

The DPPH-free radical scavenging activity of both aqueous and methanolic extracts was determined as described by Chew et al. [29] with slight modification. Total of 1 ml of diluted extracts (20, 40, 60, 80, and 100 µg/ml in ethanol) was added to 1 ml of DPPH (0.15 mm in methanol) and control consisting of 1 ml each of DPPH and ethanol. The reaction mixture was mixed thoroughly and then incubated in the dark at room temperature for 30 minutes and the absorbance was measured at 517 nm by a spectrophotometer. The ascorbic acid was used as a positive control while ethanol was used as a blank. The DPPH scavenging ability of the plant extracts was calculated using the following equation:

$$\% \text{ scavenging activity} = \frac{AC - AS}{AC} \cdot 100$$

where AC is absorbance of control (DPPH + ethanol) and AS is absorbance of sample/extract.

Data Analysis

The data are expressed as mean ± standard deviation (n = 3). Curve Expert (version 1.4) was used for the determination of IC_{50}. Microsoft Excel 2010 was used for bar chart and line graphs. Statistical package for the social sciences (version 15) was used for boxplot. Kruskal Wallis test followed by Dunn's *Post hoc* test was used for the comparison between multiple groups while unpaired *t*-test was applied for comparison between two independent groups using GraphPad InStat (version 3). $p < 0.05$ was considered to be statistically significant.

Results

The phytochemical screening of plant extracts (Table 1) indicates the presence of alkaloids, flavonoids, and tannins in both aqueous and methanolic extracts of all the five plants. However, saponins were not detected in *A. leiocarpus, B. refescens*, and *I. asarifolia* in both aqueous and methanolic extracts. Cardiac glycoside was below detection limit in *I. asarifolia* and *M. oleifera* of both extracts.

Table 1. Phytochemical screening of aqueous and methanolic leaves extracts of medicinal plants.

Samples	Solvent	Alkaloids	Cardiac glycosides	Flavonoids	Saponins	Tannins
A. leiocarpus	Aqueous extract	+	+	+	–	+
	MeOH extract	+	+	+	–	+
B. refescens	Aqueous extract	+	+	+	–	+
	MeOH extract	+	+	+	–	+
G. senegalensis	Aqueous extract	+	+	+	+	+
	MeOH extract	+	+	+	+	+
I. asarifolia	Aqueous extract	+	–	+	–	+
	MeOH extract	+	–	+	–	+
M. oleifera	Aqueous extract	+	–	+	–	+
	MeOH extract	+	–	+	+	+

+ = detected, – = not detected, MeOH = methanolic.

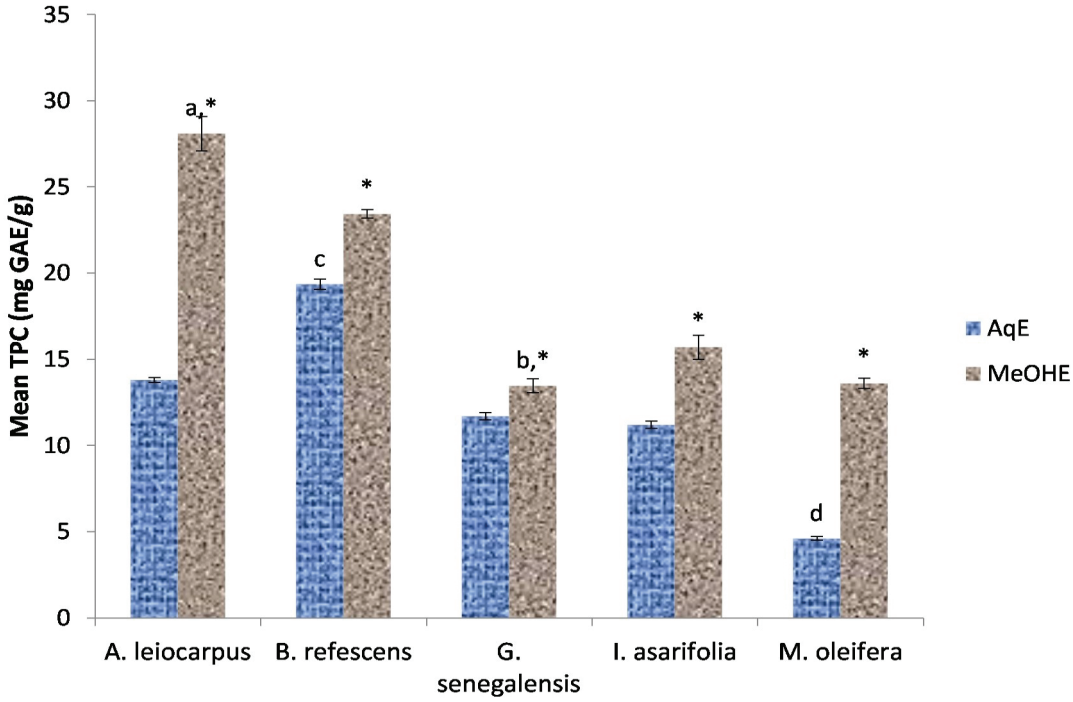

Figure 1. Total phenolic content of aqueous and methanolic extracts of medicinal plants. Values are mean ± SD, $n = 3$, TPC = total phenolic content, AqE = aqueous extract, MeOHE = methanolic extract. *$p < 0.05$ when compared between aqueous and methanolic extracts using student t test, bars with letters a & b are significantly different when compared between methanolic extracts while bars with letter c & d are significantly different when compared between aqueous extracts using Kruskal Wallis test with Dunn's multiple comparison test.

Figures 1 and 2 show the result of the total phenolic and proanthocyanidin contents of aqueous and methanolic extracts. The result indicated that total phenolic contents of methanolic extracts were significantly ($p < 0.05$) higher than the corresponding aqueous extracts. The total phenolic content of aqueous extract of *B. refescens* was significantly ($p < 0.05$) higher as compared with *M. oleifera*. Furthermore, the total phenolic content of methanolic extract of *A. leiocarpus* was significantly ($p < 0.05$) higher as compared with *G. senegalensis*. The proanthocyanidin content of aqueous extract of all the five plants were significantly ($p < 0.05$) lower as compared with their corresponding methanolic extract. Proanthocyanidin content of methanolic extract of *B. refescens* was significantly ($p < 0.05$) higher as compared with *G. senegalensis*. There was no significant difference in the proanthocyanidin content of aqueous extracts of the five medicinal plants.

The mean percentage inhibitions of aqueous and methanolic extracts against DPPH are presented in Figures 3 and 4, respectively. Figures 5 and 6 showed the results of half maximal inhibitory concentration (IC_{50}) of aqueous and methanolic extracts of the five

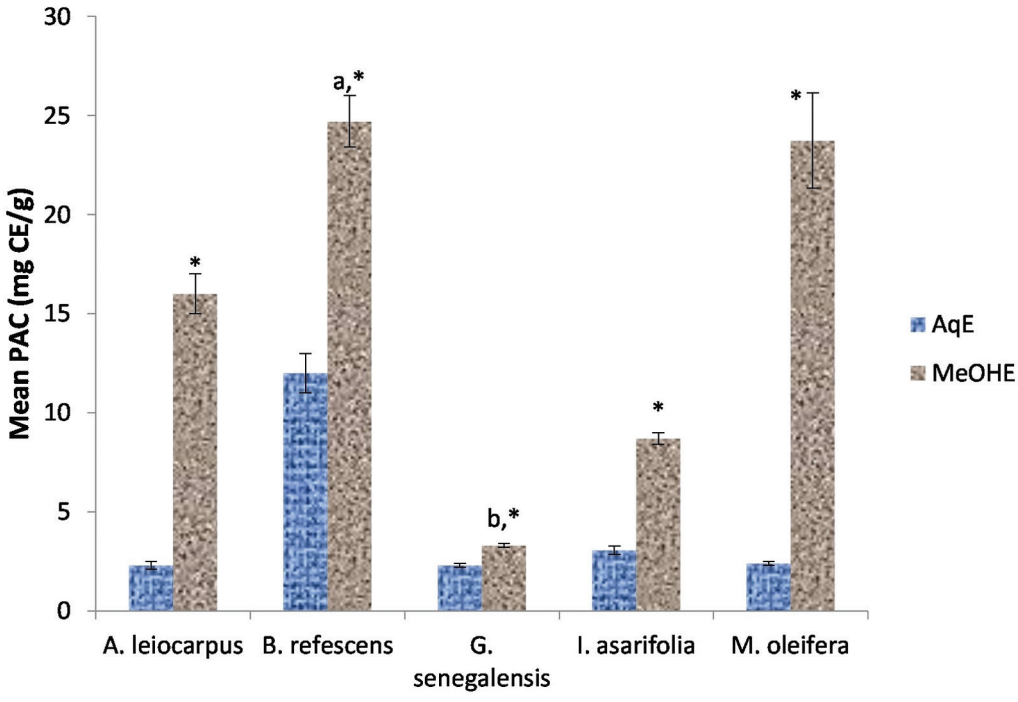

Figure 2. Proanthocyanidin content of aqueous and methanolic extracts of medicinal plants. Values are mean ± SD, $n = 3$, PAC = proanthocyanidin, AqE = aqueous extract, MeOHE = methanolic extract. *$p < 0.05$ when compared between aqueous and methanolic extracts using student t test, bars with letters a & b are significantly different when compared between methanolic extracts using Kruskal Wallis test with Dunn's multiple comparison test.

medicinal plants. The mean percentage inhibition of methanolic extract of *A. leiocarpus* and *M. oleifera* was comparable to vitamin C and had shown better inhibition of DPPH as compared with *B. refescens, G. senegalensis*, and *I. asarifolia*. Also, the methanolic extracts had shown better free radical scavenging activity against DPPH than the corresponding aqueous extracts. The mean percentage inhibitions of these medicinal plants were in dose-dependent manner. The IC_{50} of aqueous and methanolic extracts of the medicinal plants were higher as compared to that of vitamin C, but the methanolic extract of *A. leiocarpus, B. refescens*, and *M. oleifera* has shown comparable results.

Discussion

Phytochemicals are natural bioactive compounds in plants that have been recognized for their biological role as antioxidants that are capable of scavenging free radicals associated with oxidative assault [30,31]. These phytochemicals are compounds such as flavonoids, tannins, polyphenols, proanthocyanidins, and alkaloids that are considered important in the prevention and treatment of chronic diseases caused by oxidative stress [32]. In this study, phytochemical screening, antioxidant content, and *in vitro* DPPH-free radical scavenging activity of aqueous and methanolic extracts of *A. leiocarpus, B. refescens, G. senegalensis, I. asarifolia*, and *M. oleifera* were investigated. The preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins in all the five medicinal plants while cardiac glycosides and saponins were below detection limit in some of the plants. The results indicated that these plants are rich sources of various natural antioxidants that can be isolated for the treatment of oxidative stress-related diseases. Cardiac glycosides were below detection limit in both aqueous and methanolic extracts of *I. asarifolia* in this study which corroborated the findings of Jegede et al. [33]. On the contrary, flavonoids were detected by this study in both methanolic and aqueous extracts of *I. asarifolia* which was not detected in their study. These variations may be attributed to the differences in the season or the location where the plant was obtained. Phenolic compounds are capable of acting as reducing agents, donors of hydrogen, metal ion chelators, or quenchers of singlet oxygen which can be attributed to their redox potentials [34].

The result showed a considerable amount of total phenolics and proanthocyanidins in the methanolic extract as compared with the aqueous extract.

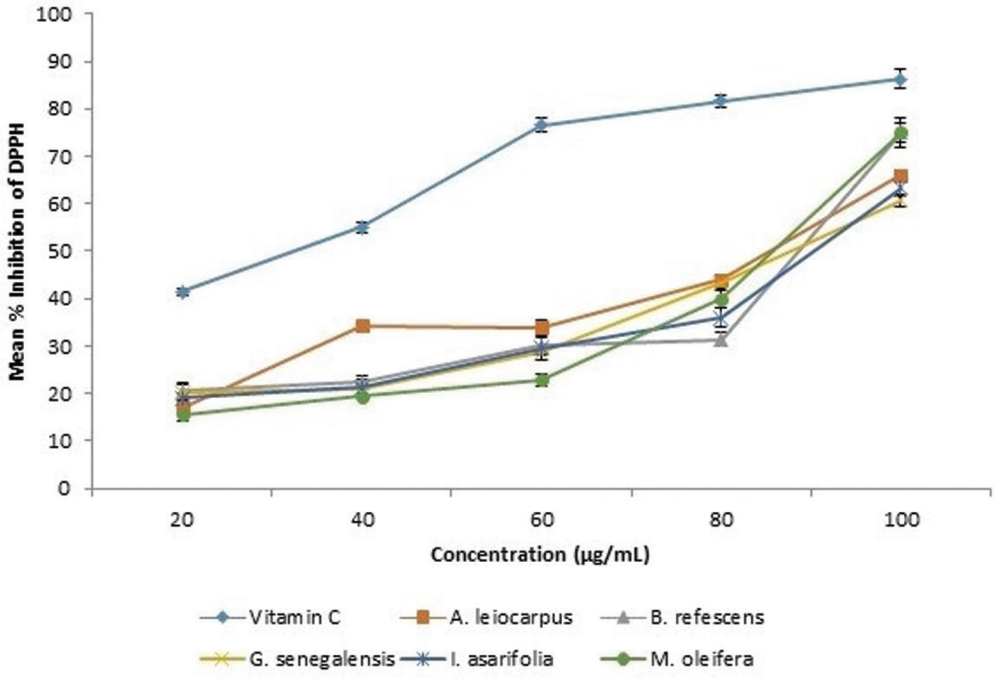

Figure 3. DPPH Radical scavenging activities of aqueous extract of medicinal plants. Values are mean ± SD, *n* = 3.

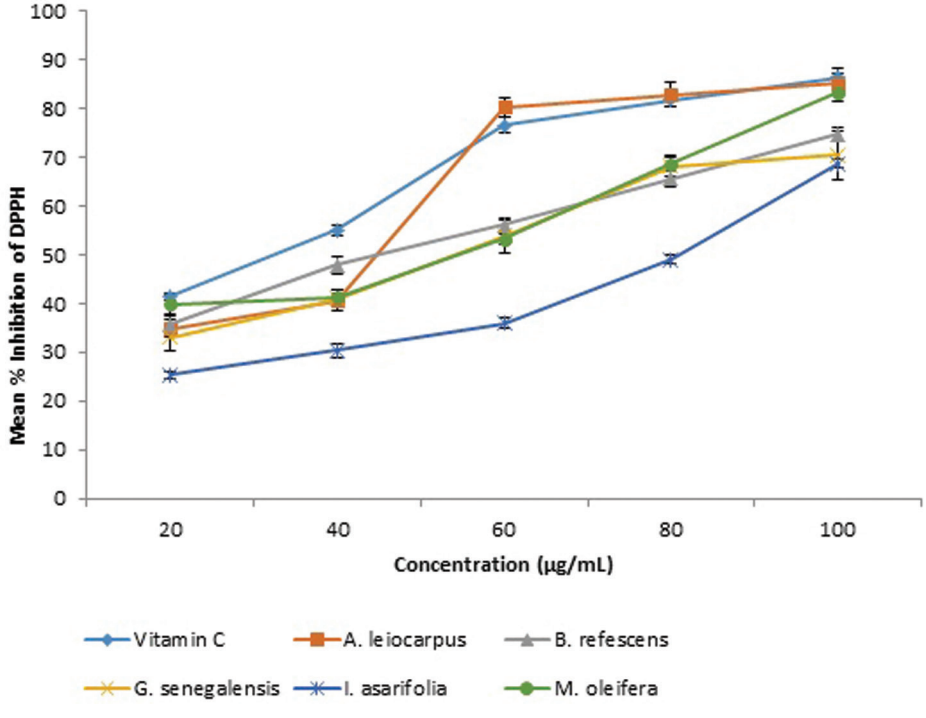

Figure 4. DPPH radical scavenging activities of methanolic extract of medicinal plants. Values are mean ± SD, *n* = 3.

This indicates that solvent system has significant influence on the extraction of bioactive components from medicinal plants. Studies have revealed that total phenolic and flavonoid contents of methanolic extract of *P. californicum* [35], *M oleifera* [36], and antioxidant activity of buckwheat methanolic extract [37] was higher than other solvent systems. The highest amount of total phenolics was observed in methanolic extract of *A. leiocarpus* and *B. refescens* while methanolic extract of *B. refescens* and *M. oleifera* demonstrated the highest amount of proanthocyanidins as compared with the rest of

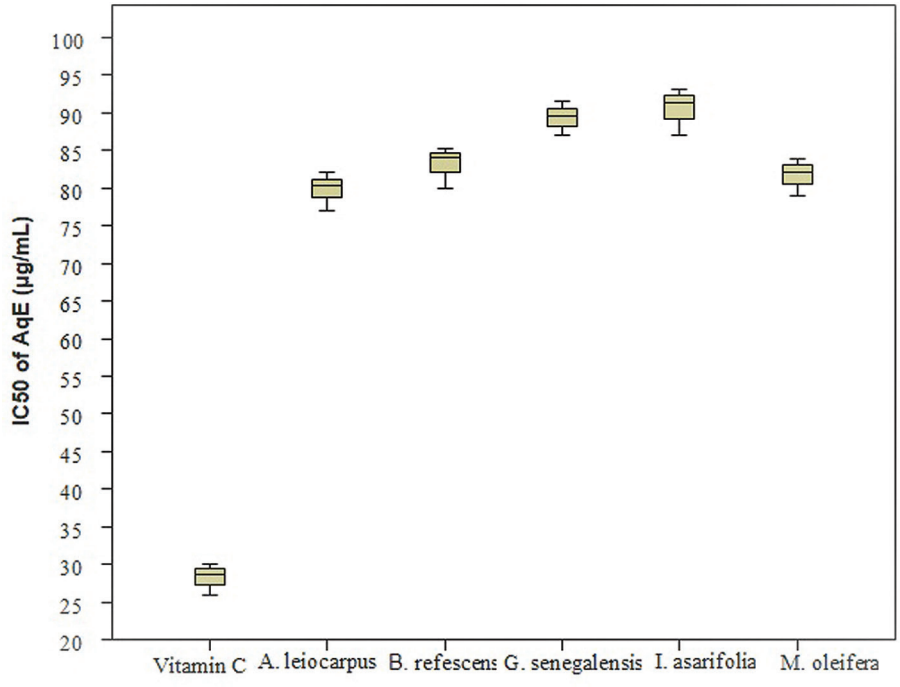

Figure 5. Mean IC_{50} of aqueous extract of medicinal plants. Values are mean ± SD, n = 3, AqE = aqueous extract.

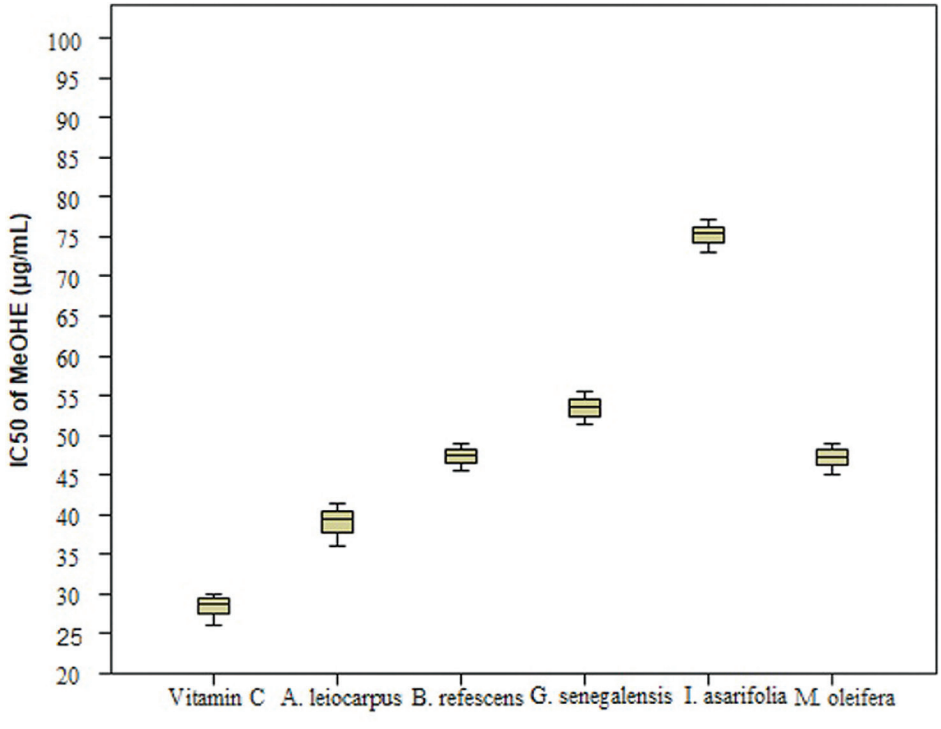

Figure 6. Mean IC_{50} of methanolic extract of medicinal plants. Values are mean ± SD, n = 3, MeOHE = methanolic extract.

the plants. Therefore, it is evident from this study that solvent system plays a critical role in the extraction of antioxidant bioactive components from plants and as such for effective extraction and isolation of antioxidants, it is important to carefully select the solvent system as well as extraction method to achieve better results.

DPPH is a stable nitrogen-containing free radical that produces deep purple color in methanol solution. The assay is based on the reduction of purple colored DPPH in the solution of methanol

to form yellow colored, diphenylpricyl hydrazine in the presence of hydrogen donating antioxidants. The decrease in absorbance is proportional to the antioxidant activity of the plant extract. This study shows that the antioxidant free radical scavenging activities of the extracts were in dose-dependent manner. The highest inhibition of DPPH corresponds with lower IC_{50}. This shows that methanolic extract of *A. leiocarpus, B. refescens*, and *M. oleifera* with the highest reduction of DPPH and lower IC_{50} performed better than the methanolic of *G. senegalensis* and *I. asarifolia* and the corresponding aqueous extract of all the medicinal plants. The high antioxidant activity of methanolic extract of these three plants further buttresses the results of total phenolic and proanthocyanidins. This study provided the evidence of DPPH-free radical scavenging activity of these plants and could be a reflection of the total activities of various components rather than individual component.

Conclusion

This present study demonstrated various antioxidant contents and DPPH free radical scavenging activity of five medicinal plants used traditionally in the treatment of different ailments in Nigeria. The extracting solvents significantly influence the antioxidant contents and DPPH-free radical scavenging activity of these plants. Methanolic extracts have shown better DPPH-free radical scavenging activity than aqueous extracts. The results of this study indicated the potential of these plants as natural sources of antioxidants. Further studies are needed to possible isolate and characterize these bioactive components with a suitable solvent system for the treatment of free radical-induced diseases.

Conflict of Interest

The authors declare no potential conflict of interest.

References

[1] Milner JA. Functional foods and health promotion. J Nutr 1999; 129(7):1395S–97S.
[2] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009; 2:270–8.
[3] Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem 2003; 51(3):609–14.
[4] Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J Food Compost Anal 2005; 18(7):625–33.
[5] Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem 2016; 97(4):705–11.
[6] Muanda F, Koné D, Dicko A, Soulimani R, Younos C. Phytochemical composition and antioxidant capacity of three malian medicinal plant parts. Evid Based Complement Alternat Med 2011; 2011:620862.
[7] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 2010; 4(8):118–26.
[8] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed Res Int 2014; 2014:761264.
[9] Davalli P, Mitic T, Caporalli A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev 2016; 2016:3565127.
[10] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 2014; 94:329–54.
[11] Gupta DK, Pena LB, Romero-Puertas MC, Hernández A, Inouhe M, Sandalio LM. NADPH oxidases differentially regulate ROS metabolism and nutrient uptake under cadmium toxicity. Plant Cell Environ 2017; 40(4):509–26.
[12] Pokorný J. Natural antioxidants for food use. Trends Food Sci Technol 1991; 2:223–27.
[13] Zibbu G, Batra A. *In vitro* and *in vivo* determination of phenolic contents and antioxidant activity of desert plants of Apocynaceae family. Asian J Pharm Clin Res 2012; 5:76–83.
[14] Karimi A, Majlesi M, Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. J Nephropharmacol 2015; 4:27–30.
[15] Elsiddig IME, Muddather AK, Abdel H, Ali R, Ayoub SMH. A comparative study of antimicrobial activity of the extracts from root, leaf and stem of Anogeissus leiocarpous growing in Sudan. J Pharmacogn Phytochem 2015; 4(4):107–13.
[16] Abubakar US, Yusuf KM, Abdu GT, Saidu SR, Jamila GA, Fatima A. Ethnopharmacological survey of medicinal plants used for the management of pediatric ailments in Kano State, Nigeria. Res J Pharmacogn 2017; 4(3):29–39.
[17] Furtado AA, Torres-Rêgo M, Lima MC M, Bitencourt A, Estrela AB, da Silva, et al. Aqueous extract from Ipomoea asarifolia (Convolvulaceae) leaves and its phenolic compounds have anti-inflammatory activity in murine models of edema, peritonitis and air-pouch inflammation. J Ethnopharmacol 2016; 192:225–35.
[18] Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. J Ethnopharmacol 2007; 111(3):625–29.

[19] Burkil HM. The useful plants of West Tropical Africa. Vol. 1, Kew: Royal Botanic Gardens, Richmond, UK, 960 p, 2004.
[20] Gaber KE, Singhal U, Daowd O. Hypoglycemic and hypolipidaemic effects of some common plants extract in type 2 diabetic patients at Eldabba area (North Sudan). J Pharm Biol Sci 2013; 8(6):38–43.
[21] Sharma N, Gupta PC, Rao CV. Nutrient content, mineral, content and antioxidant activity of Amaranthus viridis and Moringa oleifera leaves. Res J Med Plants 2012; 6(3):253–59.
[22] Mbikay M. Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Front Pharmacol 2012; 3:24.
[23] Fakurazi S, Hairuszah I, Nanthini U. Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chem Toxicol 2008; 46(8):2611–15.
[24] Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. J Ethnopharmacol 2014; 155(2):857–924.
[25] Salihu ST, Bello L, Hassan SW, Ali S. An ethnobotanical survey of antidiabetic plants used by Hausa-Fulani tribes in Sokoto, Northwest Nigeria. J Ethnopharmacol 2015; 172:91–9.
[26] Kankara SS, Ibrahim MH, Mustafa M, Go R. Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. S Afr J Bot 2015; 97:165–75.
[27] Lister E, Wilson P. Measurement of total phenolics and ABTS assay for antioxidant activity. Personal communication, Crop Research Institute, Lincoln, New Zealand, pp 235–39, 2001.
[28] Asowata-Ayodele AM, Otunola GA, Afolayan AJ. Assessment of the polyphenolic content, free radical scavenging, anti-inflammatory, and antimicrobial activities of acetone and aqueous extracts of Lippia javanica (Burm.F.) spreng. Pharmacogn Mag 2016; 12(Suppl 3):353–62.
[29] Chew YL, Goh JK, Lim YY. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem 2009; 116(1):13–18.
[30] Ferguson LR, Philpott M, Karunasinghe N. Oxidative DNA damage and repair: significance and biomarkers. J Nutr 2006; 136(10):2687S–89S.
[31] Lee CG, Koo JH, Kim SG. Phytochemical regulation of Fyn and AMPK signaling circuitry. Arch Pharm Res 2015; 38(12):2093–105.
[32] Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules 2015; 20(12):21138–156.
[33] Jegede IA, Nwinyi FC, Ibrahim J, Ugbabe G, Dzarma S, Kunle OF. Investigation of phytochemical, anti-inflammatory and anti-nociceptive properties of Ipomoea asarifolia leaves. J Med Plants Res 2009; 3(3):160–65.
[34] Banerjee SK, Bonde CG. Total phenolic content and antioxidant activity of extracts of Bridelia retusa Spreng Bark: impact of dielectric constant and geographical location. J Med Plants Res 2011; 5(5):817–22.
[35] Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, et al. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of Bucida buceras L. and Phoradendron californicum. BMC Res Notes 2015; 8:396.
[36] El Sohaimy SA, Hamad GM, Mohamed SE, Amar MH, Al-Hindi RR. Biochemical and functional properties of Moringa oleifera leaves and their potential as a functional food. Glo Adv Res J Agric Sci 2015; 4(4):188–99.
[37] Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem 2005; 90(4):743–49.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2017
VOL 1, NO. 1, PAGES 66–75
10.5455/jice.20171013045424

eJManager

ORIGINAL ARTICLE

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Chemical profiling and biological activity analysis of cone, bark, and needle of *Pinus roxburghii* collected from Nepal

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ABSTRACT

Aim: The present study aims to investigate chemical composition and biological activities of *Pinus roxburghii* collected from Kavre district of Nepal.

Material and Methods: Phytochemical screening, antibacterial activities, and antioxidant activities were measured. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the spectrophotometric analysis. Chemical composition was carried out using GC-MS **analysis.**

Results: Phytochemical analysis reveals the presence of interesting metabolites such as cardiac glycosides, saponin, protein, quinone, sterols, tannin and terpenoids. Highest TPC and TFC were observed in a bark crude methanol extract. The result further revealed that bark methanol extract showed the highest antioxidant activity. Furthermore, methanol and acetone extracts of cone, bark, and needle showed a range of *in-vitro* antibacterial activity against Gram positive and Gram negative pathogens. Gas chromatography mass spectroscopy analysis of crude acetone extract of bark revealed the presence of 14 different compounds.

Conclusions: This study showed that needle, cone, and bark of Pinus roxburghii are a source of biologically active metabolites. Furthermore, bark extract revealed the presence of diverse chemical constituent.

ARTICLE HISTORY

Received October 13, 2017
Accepted November 30, 2017
Published January 09, 2018

KEYWORDS

Pinus roxburghii; phytochemical screening; total phenol content; total flavonoid content; antioxidant activities and GC-MS

Introduction

Pinus roxburghii is widely distributed in Himalayan region from Nepal, India and Pakistan and belong to the family Pinaceae [1]. In Nepal, it inhibits in the altitude range from 1,200 to 2,100 m in height. It was reported to have several medicinal importances, such as intestinal antiseptic, antidyslipidemic, and spasmolytic [2]. The wood, resin, gum, oil, seeds, needle, and bark from *P. roxburghii* have been used for the treatment of several diseases in many parts of the world [3]. Furthermore, it is the rich source of terpenoids, flavonoids, tannins, and xanthones [4]. The bark and needle were reported to have diverse chemical constituents. It includes taxifolin, quercetin, catechin, kaempferol, rhamnetin, sterols, and pinosylvin [5]. Furthermore, Pinus bark extract has been reported to act as an anti-proliferation effect on human breast cancer cells and shows strong 2, 2-diphenyl 1-picryl hydrazyl (DPPH) radical scavenging activity, analgesic and anti-Inflammatory activity [6]. Owing to the adverse effect of synthetic antioxidants and antimicrobial, much scientific effort is ongoing to find out the less toxic and cost-effective antioxidant and antimicrobial from natural sources.

Phenolic compounds are secondary metabolites produced by many plant species and played a vital role in defense response in the plant. Together with that many polyphenolic compounds derived from the plant has shown to be a potent antioxidant, antibacterial activities, and analgesic and anti-inflammatory activity [7]. Several scientific reports suggested that plant phenolic compounds such as phenolic acids and flavonoids reduce the risk of

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metabolic syndrome and its associated complication such as type 2 diabetes as well. However, different polyphenols have a different function. Aside from antioxidants activity, these molecules provide beneficial effects against virus, cancer, inflammation, and allergy [8].

The essential oil composition of *P. roxburghii* has been studied in detail in many parts of the world and revealed the presence of several sesquiterpenes as well as monoterpene alcohols. However, lacks the detailed investigation of total phenolic and flavonoid content as well as the comparisons of antioxidant and antibacterial activities of cone, needle, and bark of *P. roxburghii*. Especially, lacks enough scientific data of *P. roxburghii* from Kavre district of Nepal. Furthermore, plants grown in diverse climatic condition varies in the chemical constituents as well as antioxidant and antibacterial activities. Hence, these studies are carried out to compare the chemical constituents, antioxidant and antibacterial activities of cone, needle and bark of *P. roxburghii* collected from Kavre district of Nepal.

Material and Methods

Collection of plant materials

The plant materials were collected from Dhulikhel Latitude and Longitude of 27.6167 and 85.55, respectively. Dhulikhel is located in sub-locality, Dhulikhel locality, Bagmati District, Central Region State of Nepal Country 30.5 km away from the capital. The plant materials were identified by Mrs. Tirtha Maiya Shrestha, Assistant Professor, Department of Pharmacy, Kathmandu University.

Extraction

The shade-dried needle, bark, and cone were grinded in coarse powder form and 20 gm of each were successively extracted at room temperature using 200 ml of solvent. All extracts were filtered separately with Whatman No 1 filter paper and evaporated by Vacuum Evaporator (Hanil P201502902-1) to get dry extracts. After drying, crude extracts were weighed and stored in stock vials and kept in the refrigerator (0–4°C) for further use.

Phytochemical analysis and determination of Total Phenolic Content(TPC)

The phytochemical analysis of alkaloids, flavonoids, phenolic content, saponin, protein, quinone, sterols, Cardic glycoside, Tannin, Terpenoid, and reducing compound was performed following the standard protocol [9]. Total phenolic content (TPC) estimation was measured using Folin Ciocalteu's methods using gallic acid as a standard [10]. The 1 ml of test solution was placed into the separate test tubes followed by addition of 0.5 ml of Folin Ciocalteu's reagent, and 4.5 ml of distilled water was mixed and shaken well, after 5 minutes 4 ml of 7% sodium carbonate was added. Then the blue color mixture was shaken and incubated at 40°C in a water bath. UV-Vis Spectrophotometers was used to measure absorbance at 760 nm. The experiments were performed in triplicates. Results were expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW).

Determination of Total Flavonoid Content

The aluminium chloride colorimetric assay was used for total flavonoid content (TFC) using quercetin as a standard [11]. The 1 ml aliquots of test solution was added into separate test tubes and followed by the addition of 0.3 ml of 5% sodium nitrite solution, 4 ml of distilled water, and shortly after 5 minutes, 0.3 ml of 10% aluminum chloride was added, and followed by the addition of 2 ml of 1 M sodium hydroxide was added. The final volume was adjusted to 10 ml with distilled water and mixed well until the yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using the UV-visible instrument. The experiments were carried out in triplicates. The standard quercetin was used to plot calibration curve. The total flavonoids were expressed as mg of quercetin equivalents per gram of dry weight (mg QE/g DW).

Free radical scavenging activity

DPPH radical was used to determine the free radical scavenging capacity of the extracts using standard protocol [12]. The reaction mixture contained 3.7 ml of 0.004% freshly prepared DPPH methanol solution and 0.3 ml of test sample (final concentration was adjusted to 20–100 µg/ml, respectively). The mixture was vigorously shaken and left for 30 minutes in the dark (until stable absorption values were obtained). The range of reduction of the DPPH radical was dogged by determining the absorption at 517 nm. For reference standard, ascorbic acid was used and DPPH solution was used as the control.

Reducing power assay

Total reducing power of selected medicinal plants was analyzed following the standard method with

some modifications [13]. The 1 ml of test sample (final concentration 200–1,000 µg/ml) was mixed with 2.5 ml of sodium phosphate buffer (pH 6.6, 0.2 M) which was then followed by the addition of 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 20 minutes. The mixture was then supplemented with trichloroacetic acid (10%, 2.5 ml) and centrifuged at 1,000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and ferric chloride solution (0.1%, 0.5 ml) and absorbance was measured at 700 nm, higher absorbance indicates higher reducing power. The above assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The results were expressed as effective concentration (EC_{50}) when the absorbance is 0.5 at 700 nm and ascorbic acid was used as a standard.

Antibacterial activity

The extracts *in vitro* antibacterial screening were carried out against four pathogenic strains, viz., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Enterococcus spp.* by the disk-diffusion method [14,15]. The Mueller–Hinton agar plate dried surface was inoculated over the entire sterile agar surface by streaking the swab. The 20 µl of the plant extract was loaded in sterile filter paper disks of 6 mm diameter. Methanol was used as negative control and Ampicillin was used as positive control. The experiment was performed in triplicates under aseptic conditions. Plates were incubated for 18 hours at 37°C. The antibacterial activity was evaluated by measuring the zones of inhibition. The mean value of the diameter of the inhibition zone of the triplicates sets was taken as the final value.

GC-MS analysis

The analysis of the essential oil was performed using Shimadzu GCMSQP2010 plus. For the analysis, Rtx5 MS (30 m length × 0.25 mm diameter × 0.25 micrometer thickness) was used. The carrier gas was helium at 1.3 ml/minutes in a constant flow mode. The injector temperature was 220°C, the injection volume 1 µl, and the split ratio 1:30. The initial oven temperature of 40°C was held for 3 minutes, then increased at a rate of 12°C/minutes up to 180°C, kept fat 180°C for 5 minutes, and finally ramped at a rate of 12°C min^{-1} to 240°C kept at this temperature for 5 minutes.

Results

Phytochemical screening, total phenolic content and total flavonoid content

Phytochemical screening of *P. roxburghii* was carried out using the standard protocol described in material and methods. The needle, cone and bark metabolites were extracted using four solvent of different polarity index. Results revealed that methanol extract contains the higher amounts of alkoloids, saponin, xanthoprotein, quinone, sterol and reducing sugar, followed by acetone extract. The least phytonutrients were observed in aqueous and hexane extract. It is mainly because of the extraction efficiency of many phytochemicals by these solvents. The phytonutrients present in the needle, cone, and bark is summarized in Table 1.

Quantitative determination of total flavonoids and phenolic content was determined as described in material and methods. The TPC was

Table 1. Phytochemical screening of *Pinus roxburghii* crude extract. WhereThe signs +, ++ and +++ represents the relative higher activity towards the phytonutrients and (–) not detected.

Plant parts	Solvent	Alkaloid	Saponin	Xantho protein	Quinone	Sterol	Cardiac glycoside	Tannin	Terpenoid	Reducing sugar
Needle	Water	+	+	+	+	+	–	+	–	+
	Methanol	++	+	++	++	+	++	++	++	+
	Hexane	–	–	–	+	+	–	+	–	–
	Acetone	+	+	+	+	+	+	++	+	+
Cone	Water	+	++	+	+	+	–	+	+	+
	Methanol	+++	++	++	++	+	+++	+++	+++	++
	Hexane	–	–	–	+	+	–	++	–	–
	Acetone	++	+	+	++	+	++	+++	+	+
Bark	Water	–	–	+	+	+	–	+	–	–
	Methanol	++	+	+	++	+	+	++	++	+
	Hexane	–	–	–	+	+	–	++	–	–
	Acetone	+	–	+	+	+	+	++	+	+

expressed as mg gallic acid equivalent per gram dry weight of the sample (mg GAE equivalent/g DW) and summarized in Table 2. In comparison of four different solvent extracts (water, methanol, acetone, and hexane), the methanol extract of bark showed the highest amount of phenolic content (69.23 ± 0.04 mg GAE equivalent/g DW), followed by needle and cone. Furthermore, the TFC of medicinal plants was expressed as mg quercetin equivalent per gram dry weight of the sample (mg QE equivalent/g DW) and is presented in Table 2. Among all solvent extracts compared, the methanol extract of bark showed highest flavonoids content (62.4 ± 0.03 mg QE equivalent/g DW), followed needle and cone. Results revealed the lowest amount of polyphenol and flavonoids in water and hexane extracts, this might be due to the poor extraction efficiency of the polyphenolic compounds. When compared with needle and cone extracts, bark extract revealed higher amount of TPC and TFC.

Antioxidant Activity

The DPPH radical scavenging activity and IC_{50} values of different medicinal extracts are summarized in Table 3. Generally, the higher % RSA and lower IC_{50} values indicate a higher antioxidant activity. The DPPH radical scavenging properties were found to be concentration dependent. The *P. roxburghii* bark was found to have 36.57%, 38.94%, 52.30%, 57.60%, and 70.64% inhibition at 20, 40, 60, 80, and 100 mg/ml of crude methanol extract. The percentage inhibition of this radical was found to increase with the increase in the concentration of extract. At 20 µg/ml, the inhibition of methanol extract of *P. roxburghii* was 36.57%, whereas ascorbic acid was 38.94% (Fig. 1). In comparison of needle, cone, and bark crude extracts in four different solvents, methanolic extract of bark revealed the highest antioxidant activity (57.2 ± 0.23 µg/ml), whereas the methanolic extract of needle showed the least antioxidant activity with IC_{50} value of 157.35 ± 1.60 µg/ml. The data were compared with ascorbic acid (IC_{50} = 35.05 ± 0.11 mg/ml), as a

Table 2. Total flavonoid content (TFC) and total phenolic content (TPC) of *Pinus roxburghii* crude extract. All experiments were performed in triplicate. TPC was expressed as mg of gallic acid equivalent per gram dry weight (mg GAE/g DW) and TFC was expressed as mg of quercetin equivalents per gram of dry weight (mg QE/g DW).

Solvent	Plant parts	TFC (mg QE equivalent/g DW)	TPC (mg GAE equivalent/g DW)
Water	Needle	4.08 ± 0.05	5.39 ± 0.05
	Cone	2.9 ± 0.03	4.45 ± 0.07
	Bark	9.37 ± 0.04	10.75 ± 0.04
Methanol	Needle	50.98 ± 0.03	57.34 ± 0.05
	Cone	52.71 ± 0.04	54.19 ± 0.06
	Bark	62.4 ± 0.03	69.23 ± 0.04
Hexane	Needle	2.64 ± 0.05	3.18 ± 0.07
	Cone	4.59 ± 0.05	5.23 ± 0.06
	Bark	7.44 ± 0.04	7.68 ± 0.04
Acetone	Needle	41.23 ± 0.05	52.59 ± 0.07
	Cone	44.35 ± 0.03	54.1 ± 0.4
	Bark	48.17 ± 0.05	52.12 ± 0.04

Table 3. Antioxidant activity of *Pinus roxburghii* crude extract. All experiments were performed in triplicate and results were expressed as mean ± SD.

Solvent	Plant parts	Reducing activity EC50 (mg/ml)	DPPH activity IC50 (mg/ml)
Water	Needle	847.86 ± 1.62	100.92 ± 0.50
	Cone	661.2 ± 0.51	152.12 ± 2.05
	Bark	489.25 ± 0.78	134.61 ± 1.09
Methanol	Needle	673.5 ± 0.95	157.35 ± 1.60
	Cone	590.35 ± 1.26	80.19 ± 0.160
	Bark	410.1 ± 0.42	57.2 ± 0.23
Acetone	Needle	743.7 ± 2.19	75.18 ± 0.30
	Cone	624.05 ± 1.12	92.65 ± 0.57
	Bark	459.27 ± 1.36	63.32 ± 0.33
Hexane	Needle	970.9 ± 1.2	104.58 ± 0.54
	Cone	624.64 ± 0.29	145.3 ± 0.59
	Bark	552.7 ± 0.09	102.44 ± 0.18
Reference	Ascorbic acid	255.38 ± 1.04	35.05 ± 0.11

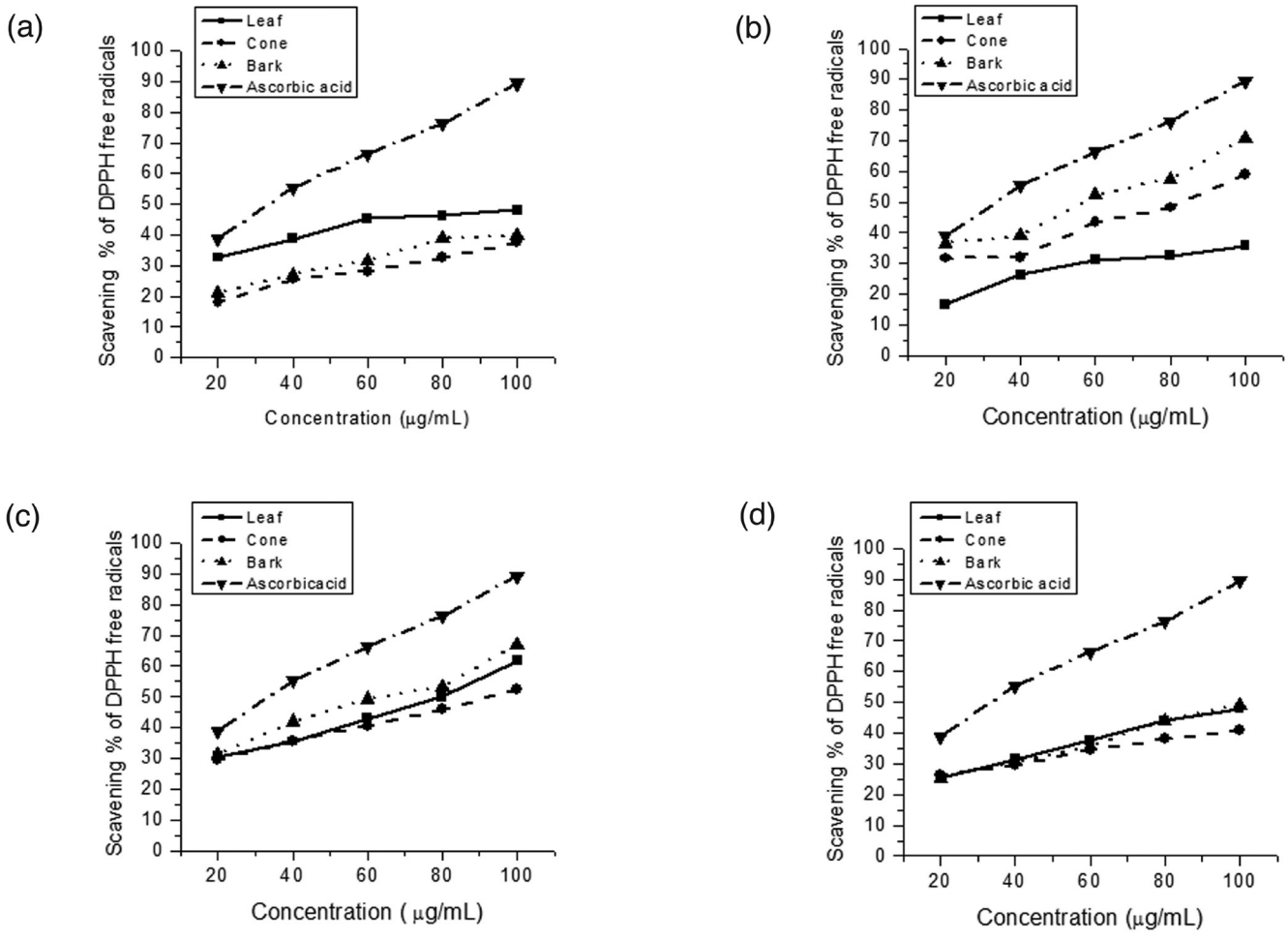

Figure 1. DPPH radical scavenging activity of *Pinus roxburghii*. (a) Water extract, (b) Methanol extract, (c) Acetone extract, and (d) Hexane extract.

standard. However, the lowest antioxidant activities were observed in the case of aqueous and hexane extract.

Similarly, total reducing power of medicinal extracts and their EC_{50} values (effective concentration when absorbance is 0.5) are summarized in Figure. 2 and Table 3. In general, lower the EC_{50} values higher the reducing ability. It was observed that the bark crude methanol extract revealed the highest antioxidant activity with EC_{50} 410.1 ± 0.42 µg/ml. On the other hand, hexane crude needle extract showed lowest EC_{50} value 970.25 ± 1.2 µg/ml. This higher reducing power of methanol extract is attributed to the higher extraction efficiency of bioactive phytonutrients.

Antimicrobial Activity

Antimicrobial activity of *P. roxburghii* extracts was tested against four strains both gram positive and gram negative and the results are summarized in Table 4. The extracts showed a zone of inhibition ranging from 9 to 12.5 mm and compared with standard ampicillin and kanamycin antibiotics. It can be expected that these crude extracts have unique phytochemicals which are responsible for the inhibition of microbial metabolism. Comparison of the antibacterial activity of cone, needle, and bark in four different solvent extracts, it was observed that methanol and acetone crude extracts revealed good antimicrobial activity with a clear zone of inhibition. The cone extracts revealed higher antibacterial activities against *Bacillus substilis* in comparison to needle and bark. On the other hand, needle extract showed relatively lower antibacterial activities in all solvent extracts. Furthermore, hexane and water extracts showed the least activity. It shows that polarity of solvent and compound to be extracted plays a vital role in the extraction of high biologically important compounds. The higher antibacterial activity of methanol and acetone extracts could possibly due to the higher extraction efficiency of

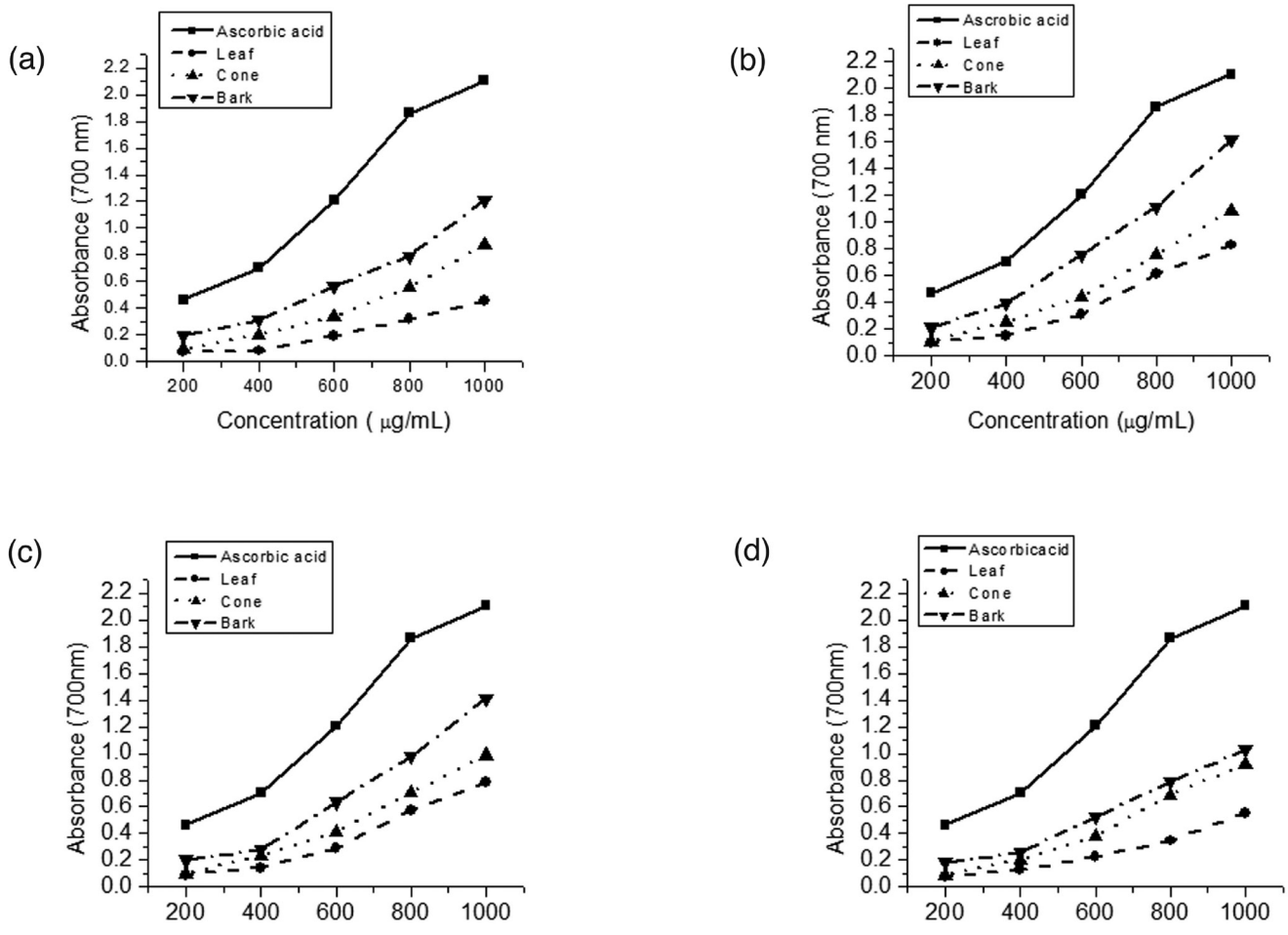

Figure 2. Reducing power assay of *Pinus roxburghii*. (a) Water extract, (b) Methanol extract; (c) Acetone extract, and (d) Hexane extract.

polyphenolic and flavonoids compounds. It is well established that polyphenol and flavonoids possess higher antibacterial activities.

GC-MS Profiling of Chemical Constituents

Gas chromatography-mass spectrometry (GC-MS) analysis of acetone crude extract of *P. roxburghii* bark revealed the presence of 14 different compounds (Fig. 3). The compounds were identified based on the mass fragmentation pattern and the comparing the peak area and retention time of the NIST database. The chemical composition of the crude acetone extract is summarized in Table 5. The most of the compounds are monoterpene and hydrocarbons such as 1,8 cineole, linalool, beta-thujone, chrysantheone, camphor, terpinen-4-ol, *n*-dodecane, *n*-pentadecane, *n*-tetradecance, and *n*-hexadecance. Furthermore, longifolene, diethyl phathalate, and 2-ethylhexanoic acid were also identified through the GC-MS analysis.

Discussion

Plants produce diverse phytochemicals known as secondary metabolites. It is well known that plants produce these metabolites to protect themselves from pathogenic attacks. These secondary metabolites possess several biological activities such as antimicrobial, antifungal, anticancer, and anti-inflammatory activities [16]. Owing to the biological activities of the plant-derived metabolites, it is of great scientific interest. The different parts of the plants revealed different quantities of these metabolites. The phytochemical analysis of bark, needle, and cone extracted with four different solvents (water, methanol, acetone, and hexane) displayed promising phytonutrients such as flavonoids, phenol, alkaloid, saponin, xanthoprotein, quinone, sterol, and cardiac glycoside. The most of these phytochemicals were extracted with methanol, acetone, and water; however, least were observed with

Table 4. Antibacterial activities of *Pinus roxburghii* crude extract. Ampicillin and Kanamycin were used as standard antibiotics, where ND = not detected.

Solvent	Organism	Zone of bacterial growth inhibition (mm)				
		Antibiotics		Pinus roxburghii		
		Ampicillin	Kanamycin	Cone	Bark	Needle
Methanol	*Staphylococcus aureus*	12.0	11.5	9.0	11.5	10.0
	Bacillus subtilis	13.0	12.5	12.5	12.0	11.5
	Klebsiella pneumoniae	14.0	13.5	9.5	11.5	10.0
	Enterococcus spp.	12.5	12.5	9.0	11.5	10.0
Water	*Staphylococcus aureus*	12.0	11.5	9.5	10.0	ND
	Bacillus subtilis	13.0	12.5	11.0	9.5	ND
	Klebsiella pneumoniae	14.0	13.5	ND	ND	ND
	Enterococcus spp.	12.5	12.5	10.0	11.5	10.0
Acetone	*Staphylococcus aureus*	12.0	11.5	10.0	11.0	9.5
	Bacillus subtilis	13.0	12.5	10.5	11.5	10.0
	Klebsiella pneumoniae	14.0	13.5	10.0	11.5	9.5
	Enterococcus spp.	12.5	12.5	9.5	11	10.0
Hexane	*Staphylococcus aureus*	12.0	11.5	ND	ND	ND
	Bacillus subtilis	13.0	12.5	ND	ND	ND
	Klebsiella pneumoniae	14.0	13.5	9.5	10.0	9.0
	Enterococcus spp.	12.5	12.5	ND	10.5	ND

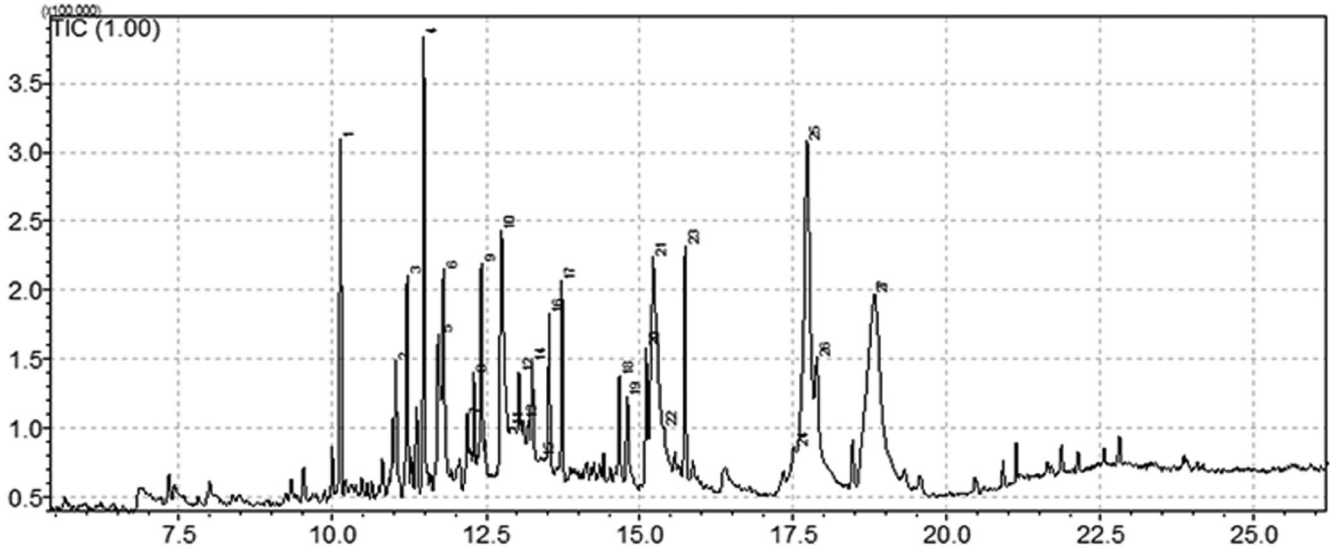

Figure 3. GC-chromatogram of the crude acetone extract from *Pinus roxburghii* bark.

hexane crude extracts. Phenolic and flavonoids are the largest category of phytochemicals and the most widely distributed in plants. It has been reported that polyphenol and flavonoid molecules displayed a high radical scavenging activity as well as anti-inflammatory activities [17]. The higher TPC and TFC contents were observed in the bark crude extract followed by cone and needle. Least amounts of TPC and TFC were observed in the needle crude extract. Among the four different solvent extracts, methanol revealed higher amount of TPC and TFC, followed by acetone and water extracts. On the other hand, least amounts of TPC and TFC were observed with hexane crude. This might be due to the poor extraction efficiency of polyphenolic compounds by hexane. Major flavonoid compounds reported from the bark of *P. roxburghii* were quercetin, catechin, kaempferol, rhamnetin, and gallocatechin [18]. Hence, it is justifiable that bark contains the higher amount of total flavonoids.

Reactive oxygen species (ROS) are essential for life of aerobic metabolism. In normal cells, these ROS are neutralized due to the presence of natural defense mechanism in the human body. However, under certain conditions, ROS production exceeds

Table 5. GC-MS profiling of chemical constituents of the *Pinus roxburghii* acetone crude extract.

Chemical compounds	Molecular weight (g/mol)	Retention time (min)	Chemical formula
1,8-cineole	154.249	10.125	$C_{10}H_{18}O$
Linalool	154.250	11.025	$C_{10}H_{18}O$
beta- thujone	152.237	11.208	$C_{10}H_{16}O$
Chrysanthenone	150.220	11.483	$C_{10}H_{14}O$
Camphor	152.230	11.800	$C_{10}H_{16}O$
Terpinen-4-ol	154.250	12.192	$C_{10}H_{18}O$
n- dodecane	170.340	12.300	$C_{12}H_{26}$
(Z)-3-hexenyl tiglate	182.263	12.892	$C_{11}H_{18}O_2$
n - pentadecane	212.420	13.092	$C_{15}H_{32}$
n- tetradecane	198.390	13.525	$C_{14}H_{30}$
n - hexadecane	226.450	14.667	$C_{16}H_{34}$
Longifolene	204.360	15.108	$C_{15}H_{24}$
Diethyl phthalate	222.240	17.517	$C_{12}H_{14}O_4$
2-ethyl hexanoic acid	144.210	18.817	$C_8H_{16}O_2$

the natural ability of cells to eliminate them from the organism; hence, leads to oxidative stress and causes several diseases such as cancer [19]. To prevent such deleterious action of ROS, antioxidants come in action which scavenges these radicals. DPPH radical scavenging model is a widely used method to determine the antioxidant activity of plants natural products. Our study revealed the highest antioxidant activity of bark extracted with methanol. This might be due to the presence of high polyphenolic and flavonoid contents in *P. roxburghii* bark crude extract [20]. This higher radical scavenging activity reveals *P. roxburghii* as promising natural source of antioxidants and opens new insight for exploitation of its secondary metabolites for medication purposes. Furthermore, antioxidant activity of the extract was confirmed through the reducing power assay. Reducing agent causes the reduction of the Fe^{3++}/ferricyanide complex to the ferrous with color changing to green and blue indicating the reducing ability of the extract. Reducing ability of bark crude methanol extract was found to be highest in comparison with the acetone, hexane, and water extracts. This higher value of reducing power indicates its higher antioxidant activity. Both DPPH and reducing assay revealed bark methanol extract as potent antioxidant agents.

One of the objectives of our research was to investigate the chemical constituents of *P. roxburghii*. Since bark extract revealed the higher antioxidant activity hence we investigated the volatile components of the bark acetone extracts using GC-MS. GC-MS chromatogram revealed the presence of different chemical constituents constituents that is eluted as a function of retention time. Although chromatogram revealed 28 different peaks, we were able to identify 14 different compounds through the careful analysis of the mass fragmentation patterns and NIST library data analysis. The compounds 1,8 cineole, linalool, beta-thujone, chrysanthenone, camphor along with *n*-dodecane, *n*-pentadecane, *n*-tetradecane, *n*-hexadecane, and longifolene have been identified in the bark crude extract. The identified compound terpinen-4-ol were were reported to have antibacterial and antifungal activities. Although essential oils from *P. roxburghii* have been researched elsewhere in the world, there are little information available in the chemical constitutens extracted with different solvents. Our results shed light that bark extract contained several biologically important compounds.

Antimicrobial activites of various plants extracts are being researched in many parts of the world in search of nartural compounds as a potential source of antimicrobial agents. In this study, needle, cone, and bark acetone and methanol crude extracts revealed higher antimicrobial activities in comparision to water and hexane indicating that most of the bioactive constituents are extracted with methanol and acetone as a extracting solvent. The results were compared with standard antibiotic ampicillin and kanamycin. The presecne of bioactive flavonoid, phenolic compounds as well as terpenoid may be responsible for the biological activities.

Conclusion

The present study revealed that *P. roxburghii* needle, cone, and bark are the potential source of diverse bioactive phytonutrients. This is supported by the promising antioxidant activity and antimicrobial activity of the crude extract. Our results also showed that *P. roxburghii* bark, cone, and needle contain a significant amount of flavonoids and phenolic contents. Our analysis further revealed that the bark methanolic extract contained the highest amount of TFC, TPC contributing to greater antioxidant and reducing power activity compared to cone and needles. This encourages the use of bark as a potential source of various phenols and flavonoids for medical application, food industry as well as to the cosmetic product. Furthermore, GC-MS profiling of the bark extract revealed the presence 14 different compounds consisting of monoterpene, hydrocarbon, and ester compounds. Our findings suggest that *P. roxburghii* is the huge source of bioactive compounds. Plenty of rooms left to investigate the potential bioactive flavonoids and phenolic compounds and its impact on human health.

Acknowledgement

The authors are grateful to Department of Chemical Science and Engineering, Kathmandu University for the support to carry out this research. We also like to extend our gratitude to Department of Microbiology, School of Medical Science, Kathmandu University. Furthermore, we also like to acknowledge Mrs. Jyoti Joshi, Deputy Director General and Mr. Kesav Paudel, Department of Plant Resource, Government of Nepal for research support.

Competing Interests

The authors declare that they have no competing interest.

References

[1] Bhat SS, Singh NB, Sankhyan HP, Sharma KR. Variability studies for needle and wood traits of different half sib progenies of *Pinus roxburghii* Sargent. Physiol Mol Biol Plants 2016; 22(2):231–9.

[2] Sharma A, Goya R, Sharma L. Potential biological efficacy of Pinus plant species against oxidative, inflammatory and microbial disorders. BMC Complement Altern Med 2016; 16:35.

[3] Shuaib M, Ali A, Ali M, Panda BP, Ahmad MI. Antibacterial activity of resin rich plant extracts. J Pharm Bioallied Sci 2013; 5(4):265–9.

[4] Kaushik P, Lal S, Rana AC, Kaushik D. GC-MS analysis of bioactive constituents of *Pinus roxburghii* Sarg. (Pinaceae) from Northern India. Res J Phytochem 2014; 8(2):42–6.

[5] Naeem I, Taskeen A, Mubeen H, Maimoona A. Characterization of flavonoids present in barks and needles of *Pinus wallichiana* and *Pinus roxburghii*. Asian J Chem 2010; 22(1):41–4.

[6] Xu R-B, Yang X, Wang J, Zhao H-T, Lu W-H, Cui J, et al. Chemical composition and antioxidant activities of three polysaccharide fractions from pine cones. Int J Mol Sci 2012; 13(11):14262–77.

[7] Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of *Pinus roxburghii* Sarg. Adv Pharmacol Sci 2012; 2012:245431.

[8] Shuaib M, Ali M, Ahamad J, Naquvi KJ, Ahmad MI. Pharmacognosy of *Pinus roxburghii*: a review. J Pharmacogn Phytochem 2013; 2(1):262–8.

[9] Sharma A, Goyal R, Sharma L. Potential biological efficacy of Pinus plant species against oxidative, inflammatory and microbial disorders. BMC Complement Altern Med 2016; 16:35.

[10] Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem 2003; 81(1):321–6.

[11] Eghdami A, Sadeghi F. Determination of total phenolic and flavonoid content in methanolic and aqueous extract of Achillea millefolium. Org Chem J 2010; 2:81–4.

[12] Park YS, Jung ST, Kang SG, Heo BK. Antioxidants and proteins in ethylene-treated kiwifruits. Food Chem 2008; 107(2):640–8.

[13] Benslama A, Harrar A. Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. Int J Herb Med 2016; 4(6):158–61.

[14] Ericsson BH, Tunevall G, Wickman K. The paper disc method for determination of bacterial sensitivity to antibiotics: relationship between the diameter of the zone of inhibition and the minimum inhibitory concentration. Scand J Clin Lab Invest 1960; 12(4):414–22.

[15] Boyan B, James H, Judicael P. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. J Antimicrob Chemother 2008; 61(6):1295–301.

[16] Salido AAG, Assanga SBI, Luján LML, Ángulo DF, Espinoza CLL, Silva ALA, et al. Composition of secondary metabolites in mexican plant extracts and their antiproliferative activity towards cancer cell lines. Int J Sci 2016; 5:63–77.

[17] Bors W, Saran M. Radical scavenging by flavonoid antioxidants. Free Radic Res Commun 1987; 2(4–6):289–94.

[18] Naeem I, Taskeen A, Mubeen H, Maimoona A. Characterization of flavonols present in barks and needles of *Pinus wallichiana* and *Pinus roxburghii*. Asian J Chem 2010; 22(1):41–44.

[19] Lushchak V. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 2014; 224(5):164–175.

[20] Frida SR, Kurnia K, Vania A, Jeremy RI. Total phenol, flavanoid and antioxidant activity of *Physalis angulata* leaves extract by subcritical water extraction. Mod Appl Sci 2015; 9(7):190.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 76–81
10.5455/jice.20171011073247

eJManager

SHORT COMMUNICATION

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Detection of caffeic and chlorogenic acids from methanolic extract of *Annona squamosa* bark by LC-ESI-MS/MS

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ABSTRACT

Aim: The aim of the present study was to determine the metabolite profile of *Annona squamosa* bark using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

Methods: The plant material of *A. squamosa* bark was collected and processed during the month of February. The bark material was extracted by Soxhlet apparatus using methanol as a solvent. The metabolite profile of the plant extract was determined by using LC-ESI-MS/MS.

Results: Caffeic and chlorogenic acids were detected from the methanolic extract of *A. squamosa* bark. To the best of our knowledge, this is the first study to report the presence of caffeic and chlorogenic acids in *A. squamosa* bark.

Conclusion: The study suggested further investigations to be carried out to evaluate these compounds *in vitro* and *in vivo* to develop the pharmaceutical products.

ARTICLE HISTORY

Received October 11, 2017
Accepted December 21, 2017
Published January 11, 2018

KEYWORDS

Plant extract; anticariogenic; metabolite profile; pharmaceutical; pharmacology

Introduction

Annona squamosa belongs to the family Annoanaceae, cultivated in India and other tropical countries. Commonly known as Custard apple in English, Sharifa in Hindi, Seema Atha in Tamil, Seetha Phala in Kannada, and Seetha Pandu in Telugu [1,2].

A. squamosa was traditionally used in medicine to treat epilepsy, constipation, diarrhea, hemorrhage, fever, dryness, and ulcers [3]. The extract of different parts of *A. squamosa* was reported to have anticancer, antioxidant, anti-inflammatory, and antimicrobial activity [4–7].

In the previous study, we tested the antibacterial activity of the methanolic extracts of *A. squamosa* and *A. reticulata* (leaves and bark) against *Streptococcus mutans* and *Streptococcus sobrinus*, and among the tested plant materials, *A. squamosa* bark showed antibacterial activity [8]. In this background, the present study was aimed to study the metabolite profile of *A. squamosa* bark using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

Materials and Methods

Collection and processing of test plant material

The bark material of *A. squamosa* L was collected and authenticated by Dr. Vasundhara M, professor, Horticulture Department, UAS University, GKVK Bangalore, India. The bark was collected and processed during the month of February 2015. The plant material was cleaned and rinsed at least three times in sterile distilled water and dried in the hot air oven. The dried plant material was squashed by blender and then stored in an airtight bottle for further uses.

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Preparation of plant extract

The extraction of *A. squamosa* bark was performed by soxhlet apparatus. The soxhlet apparatus was filled with 600 ml of 100% v/v methanol (high-performance liquid chromatography [HPLC] grade) and 40 g of the dried material of *A. squamosa* bark. The extraction was carried out at a temperature of 25°C for 30 h. The extract was filtered using two sheets of Whatman paper. The rotary vacuum evaporator was used to concentrate the extract and the yield was transferred to a screw cap bottle and stored at 4°C for further uses [8].

LC-ESI/MS and data analysis

The photochemical from the methanolic extract of *A. squamosa* bark were analyzed by direct injection to auto-sampler in LC-ESI/MS. The LC-ESI/MS was performed using a Perkin Elmer Sciex API-3000 triple quadruple mass spectrometer equipped with an Agilent 1100 series HPLC. A 150 × 3.9 mm C18, symmetry column (Waters, India) was used at a flow rate of 0.5 ml/min. Separation based on gradient chromatography was performed for the solvent extract of the samples by a mobile phase of solvent A: 0.01% formic acid in acetonitrile and solvent B: 0.01% of MilliQ water with a constant flow rate of 0.5 ml/min. The gradient program started with 100% A: 5 min, followed by 85% A: 10 min, 80% A: 20 min, 75% A: 25 min, 73% A: 27 min, 60% A: 30 min, 50% A: 35 min, 10% A: 40 min, 10% A, then returned to 100% A for 50 min and maintained for 60 min at 100% A. HPLC of the extract was measured by MS/MS. All the analyses were performed using the electrospray ionization in both positive ion and negative ion modes with the following settings: ion spray voltage 4200 V for positive, and −4200 V for negative; nebulizer gas (N_2) 7 units, curtain gas (N_2) 12 units, collision gas (N_2) 6 units, declustering potential (DP) between 50 and 80 V for positive and between −50and −80 V for negative, focusing potential 300 V for positive and −300 V for negative, entrance potential 10 V for positive and −10 V for negative, collision energy (CE) 50 V for positive and −50 V for negative, and collision cell exit potential (CXP) 5 V for positive and −5 V for negative. The drying gas (N_2) was heated to 550°C and established at a flow rate of 6,000 cm^3/min. The full scan data acquirement was executed by scanning from *m*/*z* 100 to 1,500 in profile mode with a cycle time of 2 second with a step size of *m*/*z* 0.1 and an interscan pause of 2 μs. The metabolites were identified by comparing the precursor and fragment ions *m*/*z* with METLIN database.

Results

In order to obtain metabolite profile of the methanolic extract of *A. squamosa* bark, an analytical method based on LC-ESI-MS/MS was used. The LC-ESI-MS/MS profile highlighted the existence of a large group of compounds related to the protonated molecular ions of various polyphenols. The *m/z* obtained in both positive mode (Fig. 1) and negative mode (Fig. 2) was subjected to METLIN metabolite search. The search for the respective positive or negative charges was depended on the ionization with an accuracy of 50 ppm tolerance to find the possible metabolites. Among the possible metabolites, caffeic acid and chlorogenic acid were found to be present in the methanolic extract of *A. squamosa* bark. The caffeic acid and chlorogenic acid were subjected to further fragmentation. The metabolites were confirmed by direct infusion method of MS/MS. LC/ESI-MS/MS spectra of caffeic acid (*m/z* 179.0) and chlorogenic acid (*m/z* 353.0) are shown in Figs. 3 and 4, respectively.

Discussion

Among the metabolites present in the methanolic extract of *A. squamosa* bark, caffeic acid (Fig. 3) and chlorogenic acid (Fig. 4) were detected. To the best of our knowledge, this report is the first to detect caffeic acid and chlorogenic acid in *A. squamosa* bark.

Caffeic acid with precursor ion of 179.0 was subjected to fragmentation in positive mode by varying the DP from −101 to −1 volt, fixed potential (FP) from −350 to −50 volts, CE from −130 to −5.0, CXP from −55 to 0. The MS/MS fragments of caffeic acid included 135.0, 134.0, 106.0, and 65.0 which was as reported earlier [9]. The chlorogenic acid was subjected to fragmentation in negative mode (precursor ion of 353.0) by varying the DP from −101 to −1 volt, FP from −350 to −50 volts, CE from −130 to −5.0, CXP from −55 to 0. The MS/MS fragments of chlorogenic acid included 191.5, 161.0, and 111.0 which was as previously reported [10–12].

A. squamosa bark was reported to contain anticariogenic activity against *S. mutans* and *S. sobrinus* [8]. Chlorogenic acid is a polyphenolic

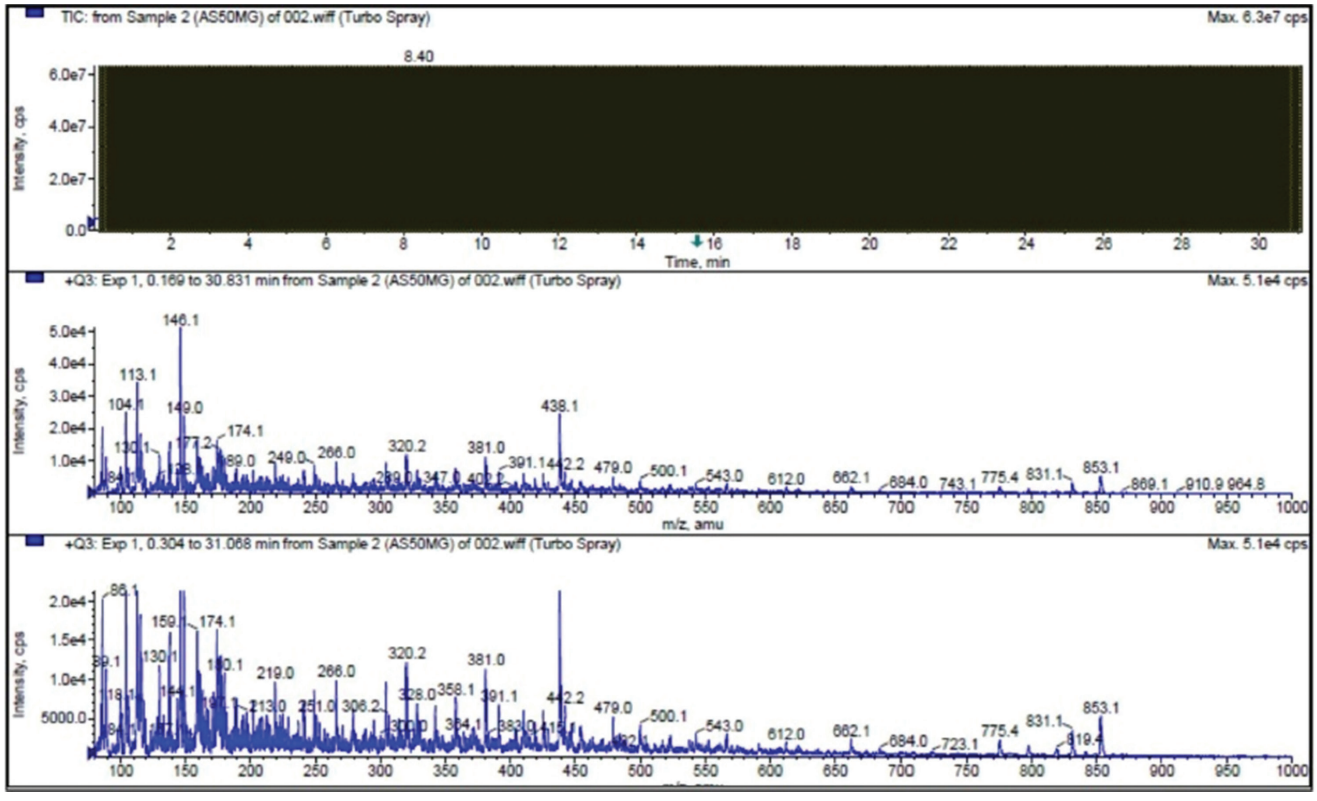

Figure 1. LC/ESI-MS/MS spectra of positive mode of *A. squamosa* bark.

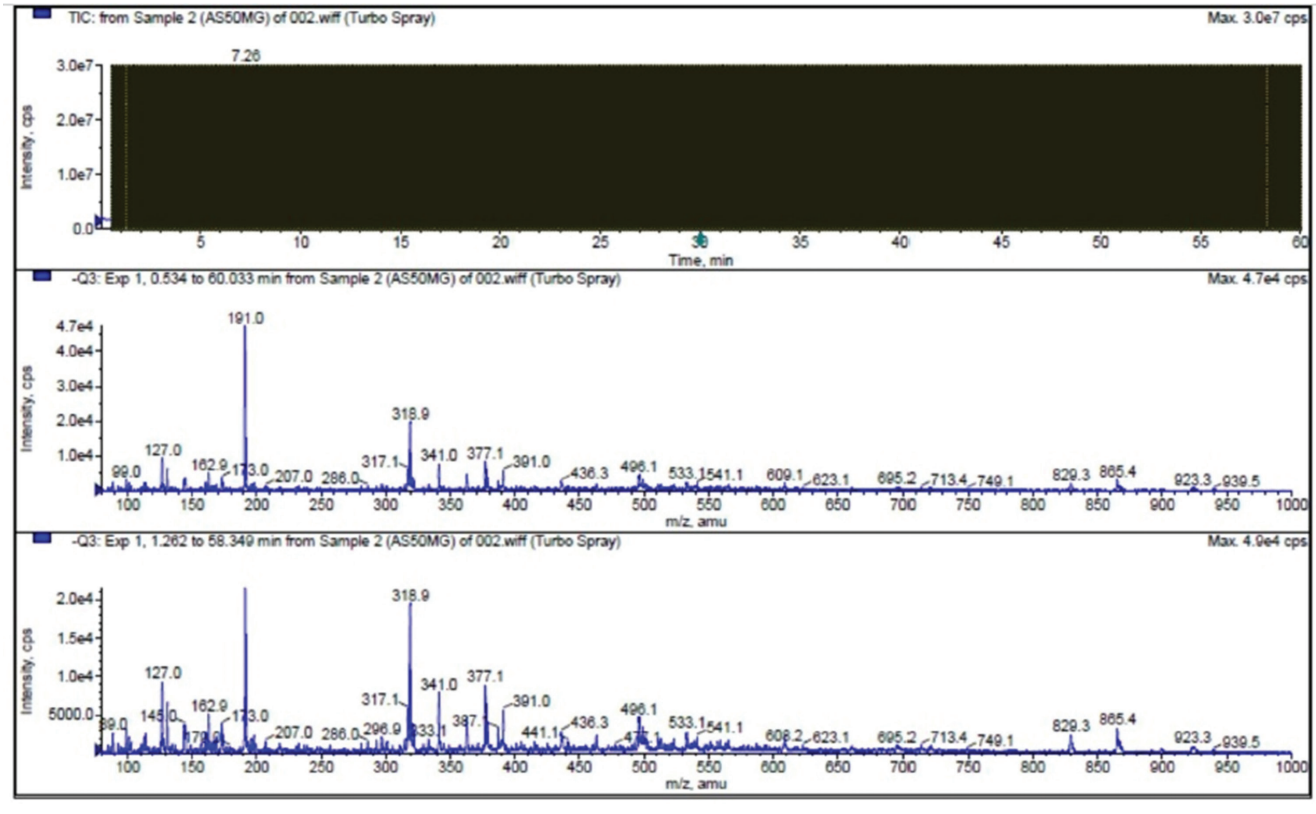

Figure 2. LC/ESI-MS/MS spectra of negative mode of *A. squamosa* bark.

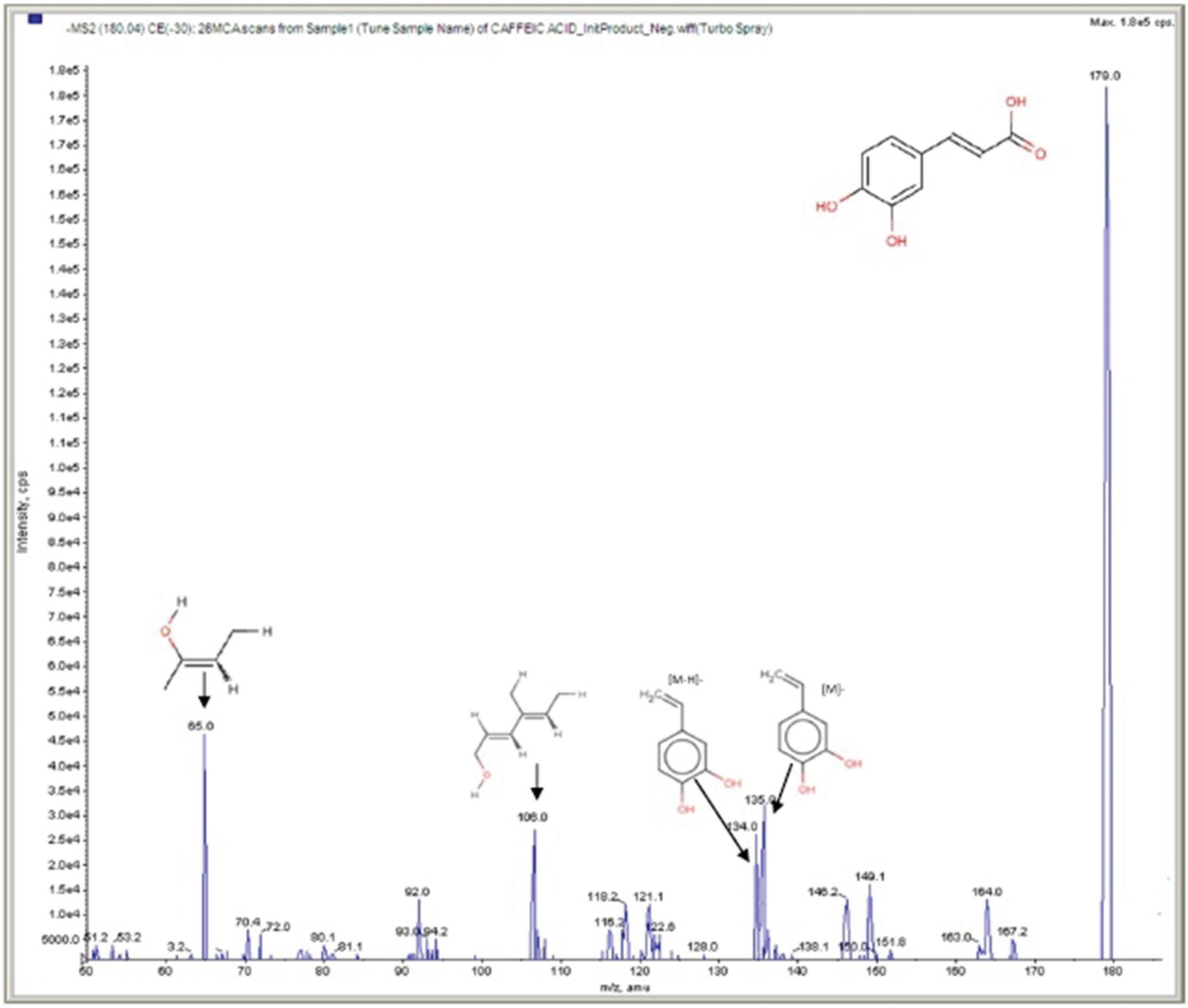

Figure 3. LC/ESI-MS/MS spectra of caffeic acid (*m/z* 179.0) fraction of *A. squamosa* bark.

compound that forms an ester with caffeic acid with the 3-hydroxyl group of a quinic acid. It was demonstrated to have various health benefits including antiviral, antioxidant, antibacterial, antifungal, and other biological activities [13,14]. Furthermore, caffeic acid and chlorogenic acid were also found to be anticariogenic compounds [15,16]. Chlorogenic acid and caffeic acid are nonvolatile organic acids present in coffee, investigators reported the effectiveness of coffee extracts to reduce the adherence of *S. mutans* on the glass surface [17]. Caffeic acid was approved to inhibit the growth of *S. mutans* and *S. sobrinus* [18].

Moreover, Caffeic and chlorogenic acids reduce the ability to inhibit α-amylase and α-glucosidase activities and therefore lead to anti-diabetic effects [19]. Researchers also considered caffeic and chlorogenic acids as promising agents for treating human breast cancer, head and neck squamous, lung and cervical carcinoma cells [20–23].

Conclusion

The study concluded the presence of caffeic acid and chlorogenic acid in *A. squamosa* bark. The current investigation suggested that the combination of caffeic acid and chlorogenic acid may potentially increase the antibacterial activity of *A. squamosa* bark. Further investigations are warranted to understand the possibility of incorporation of these compounds into pharmaceuticals.

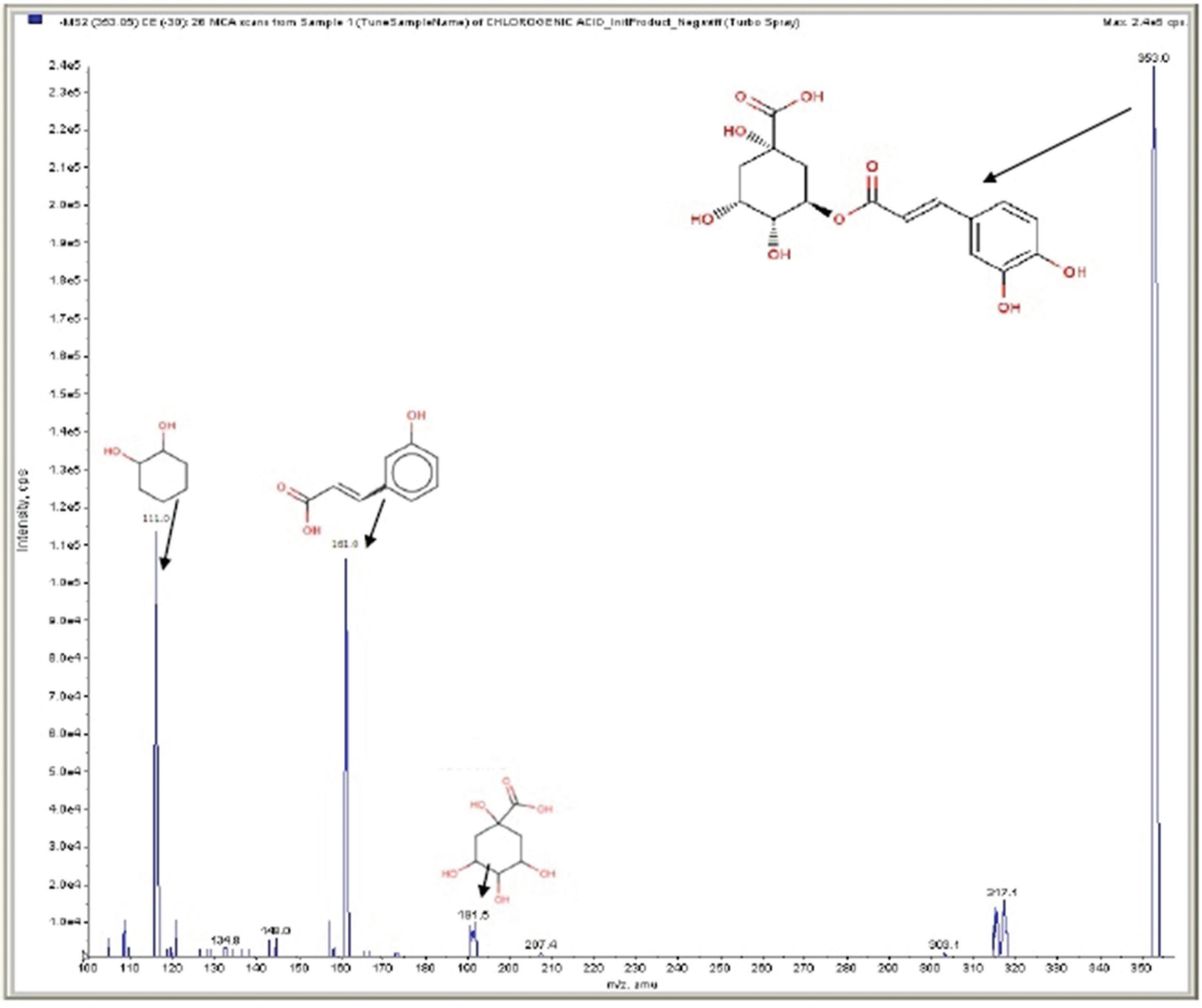

Figure 4. LC/ESI-MS/MS spectra of chlorogenic acid (*m/z* 353.0) fraction of *A. squamosa.*

References

[1] Morton JF. Sugar apple. In: Morton JF (ed) Fruits of Warm Climate. Miami: JF Morton; 1987.

[2] Bhardwaj A, Satpathy G, Gupta RK. Preliminary screening of nutraceutical potential of *Annona squamosa*, an underutilized exotic fruit of India and its use as a valuable source in functional foods. J Pharmacogn Phytochem 2014; 3:172–80.

[3] Vohora SB, Kumar I, Naqvi SA. Phytochemical, pharmacological, antibacterial and anti-ovulatory studies on *Annona squamosa*. Planta Med 1975; 28:97–100.

[4] Pardhasaradhi BV, Reddy M, Ali AM, Kumari AL, Khar A. Differential cytotoxic effects of Annona squamosa seed extracts on human tumour cell lines: role of reactive oxygen species and glutathione. J Biosci 2005; 30:237–44.

[5] Shirwaikar A, Rajendran K, Kumar CD. *In vitro* antioxidant studies of *Annona squamosa* Linn. leaves. Indian J Exp Biol 2004; 42:803–7.

[6] Rahman SM, Rashedul MI, Rahman S, Mosaiab T, Ahmed R, Khatun F, et al. Antihyperglycemic studies with methanol extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) leaves in Swiss albino mice. Adv Nat Appl Sci 2011; 5:218–22.

[7] Kachhawa JB, Sharma N, Tyagi S, Sharma KK. Screening of stem bark methanol extract of *Annona squamosa* for antibacterial activity. Int J Curr Pharm Res 2012; 4:48–50.

[8] Salman HA, Senthilkumar R. Antibacterial activity of *Annona squamosa* L. and *Annona reticulata* L. against clinical isolates of mutans streptococci the causative agents of dental caries. Asian J Pharm Clin Res 2015; 8:152–5.

[9] Li W, Sun Y, Liang W, Fitzloff JF, van Breemen RB. Identification of caffeic acid derivatives in Actea racemosa (*Cimicifuga racemosa*, black cohosh) by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2003; 17:978–82.

[10] Clifford MN, Johnston KL, Knight S, Kuhnert N. Hierarchical scheme for LC-MSn identification of chlorogenic acids. J Agric Food Chem 2003; 51:2900–11.

[11] Clifford MN, Knight S, Surucu B, Kuhnert N. Characterization by LC-MS(n) of four new classes of chlorogenic acids in green coffee beans: dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. J Agric Food Chem 2006; 54:1957–69.

[12] Clifford MN, Marks S, Knight S, Kuhnert N. Characterization by LC-MS(n) of four new classes of p-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. J Agric Food Chem 2006; 54:4095–101.

[13] Jassim SA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol 2003; 95:412–27.

[14] Karunanidhi A, Thomas R, van Belkum A, Neela V. *In vitro* antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia* including the trimethoprim/sulfamethoxazole resistant strain. Biomed Res Int 2013; 2013:392058.

[15] Brandao F, Oliveira L, Landucci L, Koga-Ito C. Antimicrobial activity of coffee-based solutions and their effects on *Streptococcus mutans* adherence. Braz J Oral Sci 2007; 6:1274–7.

[16] Almeida AA, Naghetini CC, Santos VR, Antonio AG, Farah A, Gloria MB. Influence of natural coffee compounds, coffee extracts and increased levels of caffeine on the inhibition of *Streptococcus mutans*. Food Res Int 2012; 49:459–61.

[17] Sharma R, Reddy VL, Prashant GM, Ojha V, Kumar NP. Antimicrobial and anti-adherence activity of various combinations of coffee-chicory solutions on *Streptococcus mutans*: an in-vitro study. J Oral Maxillofac Pathol 2014; 18:201–6.

[18] Barrientos L, Herrera CL, Montenegro G, Ortega X, Veloz J, Alvear M, et al. Chemical and botanical characterization of Chilean propolis and biological activity on cariogenic bacteria *Streptococcus mutans* and *Streptococcus sobrinus*. Braz J Microbiol 2013; 44:577–85.

[19] Oboh G, Agunloye OM, Adefegha SA, Akinyemi AJ, Ademiluyi AO. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (*in vitro*): a comparative study. J Basic Clin Physiol Pharmacol 2015; 26:165–70.

[20] Kabała-Dzik A, Rzepecka-Stojko A, Kubina R, Jastrzębska-Stojko Ż, Stojko R, Wojtyczka RD, et al. Migration rate inhibition of breast cancer cells treated by caffeic acid and caffeic acid phenethyl ester: an *In vitro* comparison study. Nutrients 2017; 9:1144.

[21] Naso LG, Valcarcel M, Roura-Ferrer M, Kortazar D, Salado C, Lezama L, et al. Promising antioxidant and anticancer (human breast cancer) oxidovanadium (IV) complex of chlorogenic acid. Synthesis, characterization and spectroscopic examination on the transport mechanism with bovine serum albumin. J Inorg Biochem 2014; 135:86–99.

[22] Tyszka-Czochara M, Bukowska-Strakova K, Majka M. Metformin and caffeic acid regulate metabolic reprogramming in human cervical carcinoma SiHa/HTB-35 cells and augment anticancer activity of Cisplatin via cell cycle regulation. Food Chem Toxicol 2017; 106:260–72.

[23] Li W, Liu X, Zhang G, Zhang L. Mechanism of chlorogenic acid in apoptotic regulation through notch1 pathway in non-small cell lung carcinoma in animal level. Zhongguo Fei Ai Za Zhi 2017; 20:555–61.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 82–94
10.5455/jice.20170907104442

eJM eJManager

REVIEW ARTICLE

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Expanding the insights into the usefulness of *Brachystegia eurycoma* Harms: A review of its nutritional and medicinal values

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ABSTRACT

Brachystegia eurycoma is a leguminous plant that is popular amongst the people of the Southern part of Nigeria for its ethnomedicinal and nutritional values. However, this legume has been grossly underutilized despite the promise that it holds for food and drug development. Hence, this review sheds light on the past and present states of research as well as the way to go regarding future research on the nutritional and medicinal values of *Brachystegia eurycoma* (*B. eurycoma*) with a view to inciting research interests that may lead to food and drug development from the plant. This review is based on a literature search of scientific journals and books from the library and electronic sources, which revealed that the seeds possess most of the nutritional and medicinal values of the plant. Extracts and purely isolated compounds from the plant have been reported to have analgesic, anti-inflammatory, anti-microbial, wound healing, anti-oxidant, anti-cancer, and blood glucose lowering activities as well as lipid profile, liver enzyme, and gastrointestinal motility modulation activities. Toxicological evaluation of extracts from this plant did not show any significant acute and sub-acute toxicities in rodents. Evaluation of the gums from the seeds of the plant has proven their application as food and pharmaceutical adjutants. Taken together, the findings from this review have unveiled the need for further scientific exploration of the constituents of *B. eurycoma* as potential sources of new food/nutritional adjuvant and medicines.

ARTICLE HISTORY

Received September 07, 2017
Accepted November 22, 2017
Published January 05, 2018

KEYWORDS

Nutritional; chemical constituents; pharmacology; *Brachystegia eurycoma*

Introduction

Plants are a cheap and reliable source of food and medicine. Certain nutrients like proteins and vitamins, which are important to make a balanced meal or diet, are cheaper to get from plants than from animals [1,2]. In the same way, plants used as medicines in folkloric medicine are cheaper than modern medicines in orthodox medicine. Given that resources are very scarce in developing countries, cheaper options of meal and medicines are often preferred by a majority of the population in those countries. Hence, it is amazing that plants have remained an important source of food and medicine in the developing world. Wild legumes are among the plants which have been commonly used in the developing world as a cheap and reliable source of nutrition and medicine. Notable among these nutritious and medicinal legumes is *B. eurycoma* [2].

B. eurycoma is an economic tree that belongs to the family Caesalpiniaceae. It is a dicotyledonous legume that grows in the swaps or rain forests and well-drained soil of South-Eastern Nigeria and Western Cameroun. It is a huge tree which has twisted and spreading branches with a bark that often exudes a buttery gum [3–5]. Its flowers spring forth between April and May and the fruits ripen between September and January. The fruits occur as broad leathery dark purplish brown pods containing four to six brown shiny flat disk-like brown seeds with a hard hull [6,7]. *B. eurycoma* is called Achi in Igbo, Ekalado or Eku in Yoruba, Okweri in Edo, Akpakpa or Taura in Hausa, Apaupan in Ijaw, and Odukpa in Ibibio [8] (Fig. 1).

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Figure 1. Brachystegia eurycoma: seeds and fruits.

The seed flour, which is a good source of carbohydrate and fiber, is used as flavoring and thickening agents for soups in Eastern Nigeria [9,10]. The seeds are used in folkloric medicine to maintain body temperature, soften stool, and protect against colon and rectal cancer [11]. A range of proximate, phytochemical, and pharmacological screening has been carried out in different parts of the plant following anecdotal account of its nutritious and medicinal value by local residents and traditional medical practitioners, respectively, in the localities where the plant predominately grows. Phytochemical screening has shown that *B. eurycoma* contains diverse bioactive compounds including flavonoids, phenolic compounds, alkaloids, saponins, and tannins. Nutritious compounds present in the plant include carbohydrate, proteins, lipids, and minerals [12]. Other than its nutritional value, different parts of the plant have been demonstrated to possess biologic/pharmacologic activities, namely, analgesic, anti-inflammatory, anti-microbial, wound healing, anti-oxidant, anti-cancer, and blood glucose lowering activities as well as lipid profile and liver enzyme modulation activities.

Given its nutritional and medicinal value and the assurance it holds for the development of food/nutritional products, nutraceuticals, and medicines, the need to do a comprehensive literature search on the plant with a view to updating the current state of knowledge and stimulating research interests has become imperative, as such an update will provide a one stop research resource which will assist researchers to carry out research to further explore the plant as a potential source of new food/nutritional adjuvant and medicine. To this end, this review focuses on the nutritional and medicinal values of *B. eurycoma*.

Nutritional Value

The *B. eurycoma* seeds have been shown to be composed of certain nutrients, minerals, vitamins, and food-like chemicals which are essential to human nutrition [13]. Anti-nutrients such as cyanides, phytate, and tannins may be toxic and result in poor palatability and bioavailability, and hence, deficiency of certain nutrients, e.g., proteins were found to be significantly low in the seeds of *B. eurycoma* and the levels of these anti-nutrients (Table 1) detected were below the lethal dosage approved by regulatory body like National Agency for Food Drug and Control in Nigeria [14]. Additionally, it has been proven that different processing methods (Table 2) the seeds are subjected to in the process of using it as food or medicine or for analysis could successfully reduce, remove, or deactivate these anti-nutrients [2]. This evidence indicates that, overall, the seeds of *B. eurycoma* are composed of nutrients, essential minerals, and vitamins and the presence of anti-nutrients is not an obstacle to its use as food or medicine.

Table 1. Anti-nutrient values of Brachystegia eurycoma harm seeds [14].

Anti-nutrients	Value (%)
Cyanide	0.84
Phytate	0.296
Tanins	0.039

Table 2. Processing methods for the seeds of *Brachystegia eurycoma* harm.

Description of processing methods	References
The traditional method of processing involves roasting the seeds for 10–15minutes followed by soaking in water to expose the cotyledons. The cotyledons are then soaked in water overnight after which the water is drained off and the cotyledons sun dried and ground into fine powder.	[10]
1 kg of clean and wholesome seeds is soaked in water for 24 hours to loosen the seed coat or hull. The loosened hulls are washed off with water and the de-hulled seeds are then air-dried and milled mechanically.	[10]
The seeds were dehulled by gentle roasting for 5 minutes and soaking in tap water for 3 hours. The de-hulled seeds are then boiled in distilled water for 45 minutes at 100°C. The boiled seeds are drained using a perforated basket, dried in the oven at 50°C, and grounded into fine powder with a food blender.	[15]
The seeds were dehulled by gentle roasting for 5 minutes and soaking in tap water for 3 hours. The dehulled seeds were then wrapped in blanced banana leaves and allowed to ferment for 3 days after which they were dried in an oven at 50°C and grounded into fine powder with a food blender.	[15]
The seeds were dehulled by gentle roasting for 5 minutes and soaking in tap water for 3 hours. The dehulled seeds were then roasted in a hot iron pan until the seed turned from green to brown followed by drying to a constant weight at 50°C.	[15]

Nutritive/proximate composition

The plant, *B. eurycoma*, is an affordable source of proteins, carbohydrates, and calories. While these nutrients are all essential to human nutrition, their composition in the plant differs [13]. Proximate analysis of the seeds has revealed a value of 7.2% protein, 14.0% fat, 59% carbohydrate, <3% crude fiber, and >5% ash [10]. In another report, the proximate analysis of the seeds showed the values of proteins, fats, carbohydrates, crude fibers, and ash to be 8.75%, 4.49%, 53.57%, 17.2%, and 5%, respectively [14]. Yet another report revealed protein, carbohydrate, lipid, and fiber contents of the seeds to be 7%, 71.74%, 4.20%, and 3.76%, respectively [12]. The seeds have also been demonstrated to yield a total oil content of 5.87 g and analysis of this oil revealed the presence of fatty acids such as linoleic, palmitic, oleic, and stearic acids. This composition was similar to that of sunflower and groundnut seed oil, thus indicating the seeds as an alternative source of edible oil [16–18]. Similarly, fatty acids such as oleic, linoleic, palmitic, and stearic acids in addition to phospholipids such as phosphatidic, phosphatidylinositol, phospatidyserine, and phosphatidyethanolamine have also been found to be present in the seed flour [18]. On the other hand, Ikegwu et al. [19] reported the crude protein, crude fat, crude fiber, moisture, total ash, and starch content, of the seed flour to be 12.77%, 10.52%, 2.2%, 10.25%, 1.48%, and 58.77%, respectively. The differences in these nutrients composition of the seeds highlighted in these reports may be several factors such as environmental factors, the age of the plant, time of collection as well as differences in the method of processing the seeds for proximate analysis. This is more so as variations in proximate and nutritive compositions of the seeds of *B. eurycoma* have been demonstrated to be due to different processing methods [15,18]. Both essential and non-essential amino acids have also been detected in the raw and processed seeds (Table 3). The amino acid content of the processed seeds showed a slight deviation from one another and from that of the raw seeds which again suggests that the amino acid content of the seeds is dependent on the processing method the seeds were subjected to [14,15]. Closely related to these nutrients and equally relevant to human and animal nutrition are minerals and vitamins which the seeds of the plant have also been shown to be composed of [2].

Mineral composition

Analysis has revealed the presence of essential minerals (macro and microelements) in the seeds of *B eurycoma*. The macro and microelements that have been shown to occur in the *B. eurycoma* seeds are sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), copper (Cu), and zinc (Zn), respectively. Harmful heavy metals or microelements such as lead (Pb), cobalt (Co), chromium, arsenic, and cadmium were not found following analysis of the seeds [12,15]. The percentage occurrence of these minerals was different in these reports. This was probably due to the different methods used in preparing or processing the seeds for analysis as shown by Aremu et al. [15].

Vitamin composition

Nutritionally valuable water soluble vitamins necessary for different processes and functions in the human body have also been detected in the seeds of *B. eurycoma*. These include vitamins C (ascorbic acid), niacin (nicotinic acid), riboflavin (vitamin B2), and thiamine (vitamin B1) [12]. The realization of the vitamin content, as well as the mineral and nutrients contents, have continued to make the seeds

Table 3. Amino acid composition (g/100g) of Brachystegia eurycoma seeds.

Amino acids	Raw seeds [15]	Boiled seeds [15]	Fermented seeds [15]	Roasted seeds [15]	Processed seeds [10]
Lysine	3.46	3.24	3.40	3.03	2.24
Histidine	2.27	2.18	2.37	2.02	1.73
Arginine	4.40	4.14	4.57	3.88	5.09
Aspartic acid	12.16	11.15	11.91	10.52	3.94
Threonine	3.12	3.39	3.20	2.87	1.66
Serine	2.60	2.31	2.63	2.09	0.91
Glutamic acid	9.70	9.39	10.07	8.33	4.60
Proline	3.46	3.15	3.46	2.65	1.83
Glycine	3.39	3.41	3.39	3.00	1.44
Alanine	4.09	3.99	4.18	3.80	2.43
Cystine	0.86	0.79	0.86	0.66	0.66
Valine	3.42	3.24	3.45	2.75	2.83
Methionine	0.91	0.91	0.89	0.65	0.73
Isoleucine	3.01	2.79	3.52	2.70	2.32
Leucine	8.25	8.00	9.26	7.70	5.16
Tyrosine	3.18	2.86	3.65	2.86	2.05
Phenylalanine	3.80	3.37	4.13	3.03	2.66
Tryptophan	ND	ND	ND	ND	ND

ND: Not Determined.

attractive for culinary applications in the preparation of food, food products, and nutraceuticals.

Culinary Application

The seed flour of *B. eurycoma* is used in local culinary practices as food additives in soup making in South Eastern Nigeria. It serves as condiment, flavoring agent, and thickening agent in soups [11]. It also serves the purpose of stabilization and emulsification in soups commonly consumed in south eastern Nigeria [10,19]. The soups in which it is normally used as a food additive include egusi (melon), ofe onugbu (bitter leaf), oha (made from oha leaves), and ofe nsala. Additionally, it has been used in bakery products and meat-based products as a functional agent due to its absorption capacity [19]. Other than the flavor it imparts in soups, it also imparts a gummy texture when used in soups and this is a desirable characteristic for the eating fufu, garri, pounded yam, and other staple food normally eaten with soups [20]. The observation that hydrocolloids-starch extracted from the seed flour has good pasting, temperature characteristics and swelling power properties [20–22] prompted its trial as a stabilizing agent in watermelon fruit juice, and it was found to favorably compete with established hydrocolloids or food gums such as gum Arabic and guar gum [13]. Additionally, its application as a stabilizer in yogurt has also been investigated. The sensory scores of yogurt made with *B. eurycoma* seeds were generally accepted by a 20-man judging panellists and the yogurt contained higher protein, fat, ash, and carbohydrate levels and lower moisture level versus control. The microbial count from the yogurt was within acceptable range and no mould growth was observed. Overall, the evidence from this investigation showed that stabilizer from *B. eurycoma* improved the proximate, organoleptic, and physicochemical properties of stirred yogurt [23]. This evidence indicates the promise of *B. eurycoma* as a potential food gum for routine use in the making of fruit juice in the fruit juice industry. Besides its use as food based on its nutritional value, its use as food based on its medicinal value (nutraceutical value) has also been explored. The dietary inclusion of the seeds has been evaluated in the prevention of colon carcinogenesis in rats [24]. Reports in this regard are very rare and the only one motioned in this review is still inconclusive. However, it is worthy of mention given that with time research may establish its eligibility as a useful neutraceutical. While the evidence reviewed so far highlights its consumption as food due to its nutritional and medicinal values, other evidence which is highlighted in next paragraph shows that it is also consumed solely as medicine because if its medicinal value.

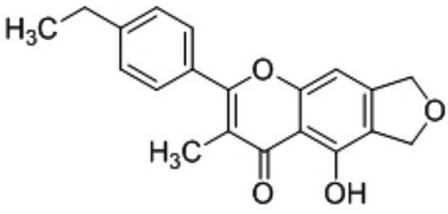
Ethnomedicinal Value

The seeds have been used in folkloric medicine to soften stool, and protect against colon and rectal cancer [8,11]. The plant has been used for treating microbial infections such as syphilis, dysentery, and sore throat [25]. It has also been used as a herbal remedy to control body temperature because it maintains heat within the body system [26]. Furthermore, in traditional medical practice, it is used for the management of guinea worm infections,

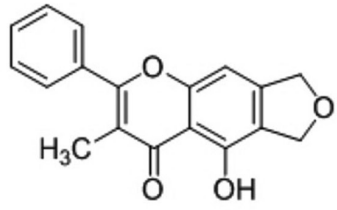
scabies, bronchitis, asthma, and tuberculosis [27]. The various reports on the efficacy of *B. eurycoma* in ethno medicinal management of different medical conditions have prompted several types of research into the phytochemical screening of extract of different parts of the plant as well as the pharmacological screening of the extracts and phytochemical isolates from parts of the plant. On the other hand, insights from the physicochemical properties of the seed flour have prompted the exploitation of its potential as a pharmaceutical excipient for application in the manufacture of pharmaceuticals.

Phytochemical Constituents

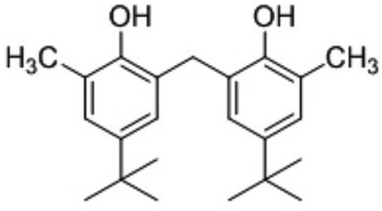
Phytochemical screening has revealed the presence of diverse secondary plant metabolites like flavonoids, alkaloids, phenolic compounds, saponin and tannins in stem bark, and seeds of the plant. The presence of flavonoids, alkaloids, saponin, and tannin has been detected in the seed flour of the plant [12]. Steroids, in addition to alkaloids, saponin, and tannins, have been also detected in the cold and hot aqueous extract and the ethanol extract of the stem bark [9]. Ethanol extracts of the seed flour and stem bark have also shown to contain alkaloids, tannins, saponins, flavonoid, and phenols [6]. Reports on the isolation and characterization of compounds from the plant are scarce. However, few compounds have been isolated from the plant (Fig. 2) and the plant parts from which they were isolated are detailed in Table 4. A new compound isolated from the seeds by column and thin layer



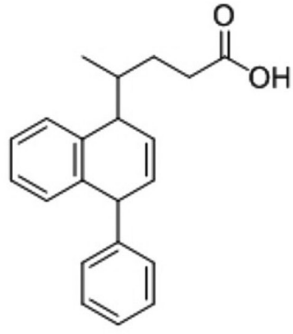
2-(-4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one



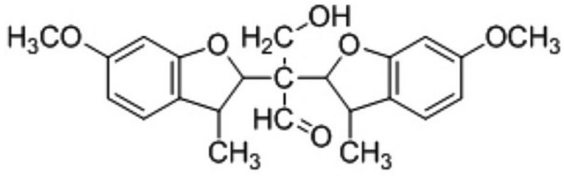
5-hydroxy-3-methyl-2-phenyl-6,7-dihydrofuro-chromen-4-one



6,6'-methylenebis (4-tert-butyl-2-methylphenol)



4-(4-phenyl-1,4-dihydronaphthalen-1-yl)pentanoic acid



3-hydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl) propanal

Figure 2. Structures of compounds isolated from Brachystegia eurycoma [28, 29, 30, 31, 32].

Table 4. Compounds isolates of *Brachystegia eurycoma* harm.

Compound	Type	Plant part	References
2-(-4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one	Furo-chrome-4-one	Seeds	[28]
5-hydroxy-3-methyl-2-phenyl-6,7-dihydrofuro-chrome-4-one	Furo-chrome-4-one	Seeds	[29]
bis-6,6-methylenebis (4-tert-butyl-2-methylphenol)	Polyphenol tertiary butyl	Stem exudates	[30]
3-hydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzoic furan-2-yl	Propanal	Stem exudates	[31]
4-(4-phenyl-1,4-dihydronaphthalen-1-yl)	Naphthalene pentenoic acid	Stem bark	[32]

chromatographic methods has been characterized and named 2-(-4-ethyl phenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one [28]. Another furo-chromen-4-one, isolated by column and thin layer chromatographic techniques from the seeds and characterized as 5-hydroxy-3-methyl-2-phenyl-6,7-dihydrofuro-chrome-4-one, has also been reported [29]. Furthermore, another new polyphenol tertiary butyl compound named bis-6,6-methylenebis (4-tert-butyl-2-methylphenol) has been isolated from the stem exudates of the plant [30]. A propanal identified as 3-hydroxy-2,2-bis(6-methoxy-3-methyl-2,3-dihydrobenzoic furan-2-yl and a new naphthalene pentenoic acid identified as 4-(4-phenyl-1,4-dihydronaphthalen-1-yl) have been isolated from the stem exudates and ethanol extract of the stem bark, respectively [31,32]. Several compounds have been isolated from the volatile oil extracted from the leaves of the plant. These compounds include oxygenated monoterpenoids (35.9%), sesquiterpenoid hydrocarbons (30.7%), 1,8-cineole (23.1%), acorenone (10%), β-caryophyllene (5.6%), and geranyl acetone (4.5%) [33]. Other compounds that have been isolated include starch carbon and hydrocarbon [19,27,34]. The detection of several bioactive secondary plant metabolites and compounds in the plant has partly prompted researchers to screen extracts and isolate from parts of the plant for different pharmacological activities.

Ethno-Pharmacology

The need to validate the claim of its therapeutic value in folkloric medicine and develop therapeutic agents from bioactive phytochemical isolates detected in the plant have led scientists to screen the plant especially the seeds and stem bark for various biologic/pharmacological activities. Several studies have shown that this plant exhibited various biological activities such as analgesic, anti-inflammatory, antimicrobial, wound healing, antioxidant, blood glucose lowering activities, lipid profile, and liver enzyme modulation activities as well as growth inhibitory and cytotoxic activities. Additionally, the need to address safety concerns has also led to the toxicological screening of the plant.

Analgesic activity

The methanol extract of the stem bark following oral administration significantly ($p < 0.05$, $p < 0.01$) reduced acetic acid-induced writhes in Swiss albino mice at the doses (100, 200, and 400 mg/kg) tested. On the other hand, the methanol extract only at a dose of 100 mg/kg significantly ($p < 0.05$) prolong reaction latency in the hot plate test in Swiss albino mice compared to control [35].

Anti- inflammatory activity

Again, the methanol extract at a dose of 100 mg/kg following oral administration, exhibited a significant ($p < 0.01$) inhibition of carrageenan-induced paw edema within the second and fourth hour of the experiment in Wistar rats as well as dextran-induced paw edema within the first and third hour of the experiment in Wistar rats [35]. In another report, the ethanol extract of the seeds produced significant ($p < 0.05$) inhibition of carrageenan-induced acute inflammation in albino rats by 46.66% and 61.92% at a dose of 50 mg/kg and 100 mg/kg, respectively, administered orally. Also, the ethanol extract of the seeds produced significant ($p < 0.05$) inhibition of formalin-induced chronic inflammation in albino rats by 32.82% and 49.84% at a dose of 50 and 100 mg/kg, respectively. On the other hand, the methanol extract of the stem bark caused 54.27% and 66.34% inhibition of carrageenan-induced paw edema at a dose of 50 and100 mg/kg, respectively, as well as 44.82% and 55.45% inhibition of formalin-induced chronic inflammation at 50 and 100 mg/kg, respectively [6]. Contrary to this significant inhibition of formalin-induced chronic inflammation caused by ethanol extracts of the seeds and stem bark, the aqueous extract of the leaves at the doses tested did not produce any significant anti-inflammatory effect [36]. However, the aqueous leaf extract caused a significant inhibition of carrageenan-induced paw edema at a dose of 100, 200, and 400 mg/kg as well as significant ($p < 0.05$) inhibition of dextran-induced paw edema and xylene-induced ear edema

Table 5. Antimicrobial activity of extracts and pure compound isolates from *B. eurycoma.*

Type of extract or pure isolate	Antimicrobial activity	Activity spectrum	References
Cold aqueous extract of the stem bark	Bacteriostatic and bactericidal	*E. coli*	[9]
Aqueous extract of wood samples	Bacteristatic	*B. subtilis*	[37]
Propanal from stem exudates	Bacteristatic	*P. aeruginosa, S. faecalis, and B. cereus*	[31]
Naphtalene petenoic acid from stem bark	Bacteristatic	*S. aureus, E. coli, P. aeruginosa, and S. faecalis*	[32]
Ethanol and water extract from stem bark	Fungistatic	*A. flavors, A. fumigatus, A. niger, Candida albicans, E. floccosium, F. solani, M. mucedo, M. audonii, and T. verrucasum*	[38]
Furo-chromen-4-one from the seeds	Fungistatic	*A. niger, P. notatum, and F. oxysporium*	[29]

at a dose of 100 mg/kg [36]. These results indicate that the extracts of *B. eurycoma* possess considerable anti-inflammatory activities.

Anti- microbial and wound healing activity

Empirical evidences affirming the antimicrobial activity of extracts and pure compounds isolated from *B. eurycoma* are abound (Table 5). Using the agar diffusion and broth dilution methods, cold and hot aqueous and ethanol extracts of the stem bark of *B. eurycoma* were screened for anti-bacterial activity against four pathogenic bacteria strains—*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus vulgaris*. Only the cold aqueous extract showed a mild zone of inhibition (3 mm) against *E. coli* with a minimum inhibitory concentration (MIC) of 12 mg/ml and minimum bactericidal concentration of 25 mg/ml [9]. Similarly, the aqueous extract of the wood sample has been demonstrated to inhibit the growth and cellulolytic activity of *Bacillus subtilis* [37]. Propanal isolated from the stem exudates have been demonstrated to have marked antibacterial activity against *P. aeruginosa, Streptococcus faecalis*, and *Bacillus cereus* with an MIC of 25%, 50%, and 50%, respectively [31]. A new naphthalene petenoic acid isolated from the ethanol extract of the stem bark has also been shown to have anti-bacterial activity against *S. aureus, E. coli, P. aeruginosa* and *S. faecalis* with a MIC of 25% against all four organisms. The order of antibacterial activity of the naphthalene petenoic acid was *E. Coli* > *P. aeruginosa* > *S. faecalis* > *S. aureus* [32]. Other report shows the anti-fungal activity of the plant. Ethanol and water extracts of the stem bark at a concentration of 2 mg/ml inhibited the growth of Aspergillus flavours, *Aspergillus fumigatus, Aspergillus niger, Candida albicans, Epidermophyton floccosium, Fuscarium solani, Mucor mucedo, Microsporum audonii, and Tricophyton verrucasum* following 43 hours of incubation [38]. Furthermore, a new furo-chromen-4-one identified as 5-hydroxy-3-methyl-2-phenyl-6, 7-dihydrofuro-chrome-4-one has been reported to possess *in vitro* antifungal activity. Using the disc diffusion method, the new furo-chromen-4-one was shown to inhibit the growth of three fungal species—*A. niger, Penicillium notatum*, and *Fusarium oxysporium* with a MIC of 50% [29]. Mucin and honey combine in a gel made from *B. eurycoma* gum has been demonstrated to heal wound made by excision model in rats faster than mucin alone. It was also observed that *B. eurycoma* gel alone promoted wound healing faster when used alone. This is not surprising given the anti-microbial properties of *B. eurycoma* which tackles microbial contamination of wounds, a phenomenon that often prolongs the healing time of wounds [39].

Anti-oxidant activity

In vitro antioxidant activity of *B. eurycoma* has been investigated using the ferric thiocyanate method. A new compound named 2-(-4-ethylphenyl)-5-hydroxy-3-methyl-6,7-dihydrofuro-chromen-4-one isolated from the seeds of the plant has been demonstrated to have free radical scavenging activity of 21.65% and 71.22% at a minimum and maximum concentration of 100 and 500 µg/ml, respectively [28]. The ethanol extract of the seeds had been demonstrated to possess free radical scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [40]. Similarly, the methanol extract of the seed flour had been shown to exhibit free radical scavenging against DPPH and reducing power against $FeCl_3$ solution [41].

Anti-ulcer activity

The *B. eurycoma* extract caused a significant ($p < 0.05$) anti-ulcerogenic activity and inhibited ethanol-induced gastric lesions with 44.30%, 79.96%, and 52.74% protection at 50, 100, and 200 mg/kg, respectively. The extract also decreased ulcer index in a dose-dependent manner with the highest dose

(200 mg/kg) producing a statistically significant protection index [42].

Effect on blood glucose, lipid profile, and liver enzyme

Treatment with aqueous extract of the seeds of *B. eurycoma* alone or in combination with aqueous extracts of *Detarium microcarpu* and *Mucuna pruriens* at a single dose of 200 mg/kg for 7 or 14 days had been reported to significantly ($p < 0.05$) lower blood glucose levels compared to vehicle treated female Wistar rats [43]. Similarly, the methanol extract of the seed powder has been demonstrated to have inhibitory effects on key enzymes such as α-amylase, α-glucosidase and aldose reductase linked to the pathology and complications of type 2 diabetes [41]. Aqueous extract of the seeds at a dose of 200 mg/kg had also been reported to cause a significant ($p < 0.05$) increase in the levels of total cholesterol, low-density lipoproteins, high-density lipoproteins and triglycerides in female Wistar rats. Similarly, the aqueous extract of the seeds at a single dose of 200 mg/kg also caused a significant ($p < 0.05$) increase in the levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase liver enzymes. Its combination of the aqueous extracts of *D. microcarpu* and *M. pruriens* did not cause a significant change in the levels of these enzymes [43].

Growth inhibitory and cytotoxic activity

The methanol extract and aqueous fractions at a concentration of 20 mg/ml were demonstrated to completely inhibit the germination of guinea corn seeds in 24 hours. The methanol extract and aqueous fraction produced a significant ($p < 0.05$) inhibition of the length of radicles with an IC_{50} of 5 mg/ml and 1.61 mg/ml, respectively. Investigation of the cytotoxic activity revealed that the methanol extract produced 96.67% mortality at 200 µg/ml with an LC_{50} of 62.5 µg/ml while the aqueous fractions produced 100% mortality at a concentration of 100 µg/ml with LC_{50} of 30 µg/ml [44]. However, other reports show that the ethanol extract of the seeds is devoid of any anti-cancer activity against the tested cell lines [40].

Effect on gastrointestinal motility

Normal intestinal transit, castor oil induced diarrhea as well as intestinal fluid retension test in rodents and spontaneous, acetylcholine, and high KCl induced intestinal contractions have been used to investigate the *in vivo* and *in vitro* acitivities, respectively, of the methanol extract of *B. eurycoma* stem bark on gastrointestinal motility. On the other hand, the crude methanol extract of *B. eurycoma* (100, 300, and 700 mg) caused a modest reduction in normal intestinal transit time, it caused a significant ($p < 0.05$) reduction of propulsive movement in castor oil induced diarrhea versus control. Additionally, the methanol extract caused a dose related, significant delay in the onset of diarrhea as well as a reduction in diarrhea score, number, and weight of wet stools. The extract (300 mg/kg) also produced antidiarrhea index of 37.1% which was lower than that produced by 5 mg/kg loperamide (57.74%). On the other hand, the *in vitro* analysis showed that the crude methanol extract, aqueous, and chloroform fractions caused an attenuation of spontaneous, acetylcholine, and KCl induced contractions of isolated duodenum in a concentration-dependent fashion [8].

Toxicity

Acute toxicity evaluation of the methanol extract of the stem bark revealed neither mortality nor signs of toxicity in male Swiss albino mice [35]. *In vivo* sub-acute toxicity study of the aqueous stem bark extract at doses 100–800 mg/kg administered orally for 14 days, revealed no significant difference in the pattern of weight gain in both sexes of Wistar rats used when compared to control rats. Similarly, sub-acute treatment with the same dose range of the methanol extract did not cause any significant difference in the organ weight index of the heart, kidney, liver, and spleen. However, sub-acute treatment with 100 mg/kg caused a significant ($p < 0.05$) increase in the weight of the lungs compared to control. On the other hand, while sub-acute treatment did not cause any significant change in the serum concentration of biochemical parameters such as alanine transaminase, aspartate transaminase, alkaline phosphatise, total bilirubin, and conjugated bilirubin versus control, it caused an elevation in haematological parameters such as white blood cells and lymphocytes, although there was no significant change in other hematological parameters evaluated. Additionally, the sub-acute treatment caused vascular congestion, mild periportal infiltration of chronic inflammatory cells, and kupfer cell activation in the liver [45]. These pharmacological and toxicological evaluations are important aspects of the process of drug development. Equally important to the process of drug development is the evaluation of pharmaceutical excipients necessary for pharmaceutical dosage forms formulation.

Pharmaceutical Application

B. eurycoma seeds have been severally evaluated as an excipient for application in food and pharmaceutical formulations due to the reported relevance of some of its technological properties such as physicochemical, functional, rheological, and pasting properties of its flour and starch [19,27,46,47]. Also, some of its physical and mechanical properties relevant to the design and building of processing equipment for *B. eurycoma* seeds have been investigated [27,48–50]. These investigations have the potential of paving the way for the production of a processing equipment that will optimize the the phytochemical content, physical and rheological properties of the seed flour during processing as well as optimize post-harvest handling procedures for *B. eurycoma*. A report of the preliminary evaluation of its seed mucilage as a pharmaceutical binder revealed that tablets formulated with *B. eurycoma* seeds mucilage were softer than those formulated with gelatin and had good uniformity of weight as well as rapid dissolution rate and good disintegration within the official specified time for uncoated tablets. These evidence suggest the efficacy and applicability of *B. eurycoma* mucilage as a binder in situations where the fast release of a drug is desired [51]. On the other hand, the mixture of *B. eurycoma* gum and egg albumin were investigated and found to be useful in producing metronidazole tablets with slow release properties [52]. The results from these investigations indicate that *B. eurycoma* has the potential for producing tablets with either fast or slow release profile. Apart from beign studied as a binder in tablets, *B. eurycoma* gum have also been evaluated as a stabilizer in drugs and foods such as ice creams. In ice creams, the gum was comparable to established stabilizers such as sodium carboxylmethylcellulose and sodium alginate [53]. The evaluation of the suspending properties of *B. eurycoma* seed gum in sulphamethoxazole suspension revealed it had better suspending properties at concentrations of 0.2%, 0.5%, 1%, 2%, and 3% than standard suspending agents like acacia and tragacanth gums. The gum extract displayed high viscosity at high concentrations which make it a desirable candidate as a stabilizer and thickener where high viscosity is desired [54]. Similarly, the evaluation of the suspending properties of *B. eurycoma* seed gum extract in metronidazole suspension had also been reported. The metronidazole suspension formulated with *B. eurycoma* at a concentration of 2.5%, 5%, 7.5%, and 10% w/w had high sedimentation rate compared to compound tragacanth at the same concentration. However, *B. eurycoma* gum formed a suspension with better esthetic than the suspension formulated with tragacanth gum [55]. Additionally, evidence from the investigation of the foaming and emulsification properties of the seed flour suggests it can function as a stabilizing or emulsifying agent in formulations [56] The effectiveness of *B. eurycoma* gum as a binder in the production of metronidazole tablets with low brittle fracture tendency had also been reported. In this report, the brittle fracture index (BFI) of tablets formulated with *B. eurycoma* gum was not statistically different from BFI of tablets formulated with standard gum acacia [57].

Significance of Current Research and Strategy for Future Research on *B. eurycoma*

The research reports highlighted in this review show that the seeds of *B. eurycoma* possess most of the nutritional and medicinal value of the plant. Little wonders the seeds have been used as food additives and medicines in ethnomedical practice as well as received more attention for exploration as a potential source of modern medicines, food, and pharmaceutical adjuvant. However, the leaves and stem bark are other parts of the plant that have also been explored as a potential source of modern medicines. Consequently, extracts have been isolated from the seeds, stem bark, and leaves as well as very few bioactive compounds from majorly the seeds. The extracts and isolated compounds from the seeds, stem bark and leaves have been screened pharmacologically and have been shown to possess biologic activities including analgesic, anti-inflammatory, antimicrobial, wound healing, antioxidant, antiulcer, blood glucose lowering, liver enzyme, lipid profile, and gastrointestinal modulating as well as growth inhibitory and cytotoxic activities. The results from the antimicrobial screening of the plant so far are promising, given that the plant has demonstrated the broad antimicrobial spectrum of activity in being inhibitory to the growth of both bacteria and fungi. Hence, the plant should be given more attention as a potential source of broad spectrum antimicrobial agents. The antimicrobial potential of the plant is very significant because it is being observed at a time when there is the urgent need to look at medicinal plants as a potential source of antimicrobial agents. This urgent need is in the light of a major threat facing mankind in the form of antimicrobial resistance [58–60]. The evidence of the

pharmacological activities exhibited by parts of the plant is a further testimony, set apart from the ethnomedicinal claims, that the plant parts contain bioactive compounds capable of modulating biologic activities. However, given that most of the pharmacological studies were investigated with crude extracts of the plant, it has become imperative for the medicinal properties of the plant to be properly harnessed by isolating more pure compounds from the bioactive parts of the plant and screening such compounds for biologic/pharmacologic activities. The apparent safety of the plant in an ethnomedicinal practice was in some way confirmed by the scientific evidence reviewed, which suggest an overall safety of the plant in animals. Nevertheless, it will be useful to do an extensive chronic toxicological screening of the crude extracts and pure isolates from the plant to obtain a better toxicological profile of the plant. This is especially important more so as the preliminary pharmacological screening reviewed showed that the plant has the potential for the development of medicines for chronic conditions like pain, ulcer, and diabetes [36,41–43]. Additionally, the quest to source modern food and medicines from the plant has also led to the isolation of gum from the seeds which had shown great potential for application as suspending agents, emulsifiers, and binders in food and pharmaceutical formulations [61,62]. The gum being natural are less expensive, non-toxic, ecofriendly, and biodegradable [63–65]. These attributes are lacking in synthetic gums which make natural gums superior alternative [66]. The promise the plant holds for the development of quality food and active pharmaceutical ingredients (API) adjuvants should motivate intensive research efforts geared toward the production of safe and affordable API adjuvants for routine manufacturing processes in food and pharmaceutical industries. Thus, if achieved will go a long way to reducing the cost of local production of food and medicines. Given that, cost is a major obstacle limiting access to quality medicines in developing countries [67,68], reduction in the cost of medicines on account of the use of locally sourced APIs and pharmaceutical adjuvants will expand access to quality medicines. In a nutshell, the significance of the nutritional and medicinal usefulness or importance of *B. eurycoma* underscores the need not only for its usefulness to be optimally harnessed through further research but also for its usefulness to be properly preserved. Preservation of the usefulness, in turn, depends on the conservation of the rain forests or vegetation harboring the plant. Hence, it has become urgent to put adequate measures in place to conserve this plant through prevention of deforestation of the natural habitat of the plant more so as there are alarming reports of forest resources depletion in South Eastern Nigeria which harbors the natural habitat of the plant [69,70].

Conclusion

In summary, the significance of the evidences presented in this review has provided an expansion of the insights into the usefulness and potential of *B. eurycoma* as nutrition and medicine. These insights have in turn shed lights on the need for the preservation of the plant through prevention of the deforestation of the natural habitat of the plant. Furthermore, these insights have also revealed the need for further research on the plant with a view to discovering lead compounds that may give rise to new therapeutic agents, food, and pharmaceutical adjuvants. Thus, this review will serve as a useful resource in the quest for the development of food and medicines from *B. eurycoma*.

References

[1] Saikia P, Sarkar CR, Borua I. Chemical composition, antinutritional factors and effect of cooking on nutritional quality of rice bean. Food Chem 1999; 67(4):347–52.

[2] Bhat R, Karim AA. Exploring the nutritional potential of wild and underutilized legumes. Comprehensive reviews in food Science and food safety. Compr Rev Food Sci F 2009; 8(4):305–31.

[3] Giami SY, Wachuku OC. Composition and functional properties of unprocessed and locally processed seeds from three underutilized food sources in Nigeria. Plant Foods Hum Nutr 1997; 50(1):27–36.

[4] Olajide O, Udo ES, Out DO. Diversity and population of timber tree species producing valuable non-timber products in two tropical rainforests in Cross River State, Nigeria. J Agric Soc Sci (Pak) 2008; 4:65–8.

[5] Uzomah A, Ahiligwo RN. Studies on the rheological properties and functional potentials of achi (Brachystegea eurycoma) and ogbono (Irvingia gabonesis) seed gums. Food Chem 1999; 67(3):217–22.

[6] Igwe OU, Okwu DE. Phytochemical composition and anti-inflammatory activities of Brachystegia eurycoma seeds and stem bark. Der Pharma Chemica 2013; 5(1):224–8.

[7] Enwere MJ. Foods of plant origin processing and utilization with recipes and technology profiles. Afro orbis publication ltd, Nsukka, Nigeria, pp 24–228, 1998.

[8] Bafor EE, Chiekwe O, Ofeimun J, Amaechina F, Ayinde B. Brachystegia eurycoma harms (Fabaceae) stem bark extract modulates gastrointestinal motility in animal models. Afr J Biomed Res 2017; 20(3):309–16.
[9] Okoli RI, Turay AA, Mensah JK. The phytochemical analysis and antibacterial effects of stem bark extracts of Brachystegia eurycoma harms. Int J Herb Pharmacol Res 2015; 4(2):10–6.
[10] Uhuegbu FO, Onwuchekwa CC, Iweala EE, Kanu I. Effect of processing methods on nutritive and antinutritive properties of seeds of Brachystegia eurycoma and detarium microcarpum from Nigeria. Pak J Nutr 2009; 8(4):316–20.
[11] Ndukwu MC. Determination of selected physical properties of Brachystegia eurycoma seeds. Res Agr En 2009; 55(4):165–9.
[12] Okwu DE, Okoro E. Phytochemical composition of Brachystegia eurycoma and Mucuna flagellipes, seeds. Med Arom Plant Sci Biotech 2006; 26:1–4.
[13] Nwakaudu AA, Nwakaudu MS, Owuamanam CI, Alagbaoso SO, Njoku NE, Agunwah IM, et al. Extraction and evaluation of hydrocolloids from "Achi" (Brachystegia eurycoma) and its application on a water melon fruit juice. Eur J Food Sci Technol 2017; 5(1):22–8.
[14] Bolanle AO, Funmilola AS, Adedayo A. Proximate analysis, mineral contents, amino acid composition, anti-nutrients and phytochemical screening of Brachystegia eurycoma Harms and Pipper guineense schum and thonn. Am J Food Nutr 2014; 2(1):11–7.
[15] Aremu MO, Ohale IM, Magomya AM, Longbap DB, Ushie OA. Compositional evaluation of raw and processed harms (Brachystegia Eurycoma) seed flour. Appl Food Biotechnol 2014; 2(1):9–18.
[16] Ajayi IA, Oderinde RA, Kajogbola DO, Uponi JI. Oil content and fatty acid composition of some underutilized legumes from Nigeria. Food Chem 2006; 99(1):115–20.
[17] Ajayi IA. Comparative study of the fatty acid composition of some seed oils from Nigeria. Afr J Plant Sci Biotech 2009; 3:59–62.
[18] Ajayi F, Aremu M, Mohammed Y, Madu P, Atolaiye B, Audu S, et al. Effect of processing on fatty acid and phospholipid compositions of harms (Brachystegia eurycoma) seed grown in Nigeria. Chem Process Eng Res 2014; 22:18–25.
[19] Ikegwu OJ, Okechukwu PE, Ekumankana EO. Physico-chemical and pasting characteristics of flour and starch from achi Brachystegia eurycoma seed. J Food Technol 2010; 8(2):58–66.
[20] Nwosu JN, Ogueke CC, Owuamanam CI, Onuegbu N. The effect of storage conditions on the proximate and rheological properties of soup thickener Brachystegia enrycoma (Achi). Rep Opin 2011; 3(5):52–8.
[21] Uzomah A, Odusanya OS. Mucuna sloanei, detarium microcarpum and brachystegia eurycoma seeds: preliminary study of their starch-hydrocolloid system. Afr J Food Sci 2011; 5(13):733–40.
[22] Nwokocha LM, Williams PA. Solution properties of Brachystegia eurycoma seed polysaccharide. In: Williams P, Phillips G (eds) Gums and Stabilisers for the Food Industry 17, RSC Publishing, Cambridge, UK, pp 123–38, 2014.
[23] Mbaeyi-Nwaoha IE, Nnagbo CL, Obodoechi CM, Nweze BC, Okonkwo TM. Production and evaluation of yoghurt contained local stabilizers-Brachystegia eurycoma ("Achi") and Detarium microcarpuim ("Ofo"). Int J Biotech Food Sci 2017; 5(2):23–31.
[24] Atawodi S, Iliemene UJ. P-259Evaluation of brachystegia eurycoma (harms) seed dietary inclusion in the prevention of colon carcinogenesis. Ann Oncol 2017; 28(Suppl 3):91.
[25] Irondi EA, Oboh G, Akindahunsi AA. Methanol extracts of Brachystegia eurycoma and Detarium microcarpum seeds flours inhibit some key enzymes linked to the pathology and complications of type 2 diabetes *in vitro*. Food Sci Hum Well 2015; 4(4):162–8.
[26] Onimawo A, Egbekun MK. Comprehensive food science and nutrition. Revised edition, Ambik Publishers, Benin City, Nigeria, 1998.
[27] Ndukwu C, Igbozulike AO, Nwakuba NR, Dirioha C, Inekwe G, Otaru AE. Impact of postharvest processes on the functional and physical properties of Brachystegia eurycoma: a review. NIAE Conf Proceed 2017; 38:108–13.
[28] Igwe OU, Okwu DE. Isolation, characterization and antioxidant activity of a furo-chromen-4-one from the seeds of Brachystegia eurycoma harms. Int J Chem Sci 2013; 11(1):121–28.
[29] Igwe OU, Echeme JO. Isolation, characterization and antifungal activity of 5-hydroxy-3-methyl-2-phenyl-6, 7-dihydrofuro-chromen-4-one from the seeds of Brachystegia eurycoma harms. Int Res J Pharmaceut Appl Sci 2013; 3(6):47–50.
[30] Igwe OU, Okwu DE. Isolation and characterization of polyphenolic tertiary butyl from the exudate of Brachystegia eurycoma (HARMS). Asian J Plant Sci Res 2013; 3(2):42–6.
[31] Igwe OU, Okwu DE. Isolation, characterization and antibacterial activity of 3-hydroxy-2, 2-bis (6-methoxy-3-methyl-2, 3-dihydrobenzofuran-2-yl) propanal from the stem exudates of Brachystegia eurycoma harms. Der Pharma Chemica 2013; 5(2):39–44.
[32] Igwe OU, Echeme JO. Isolation, characterization and antibacterial activity of 4-(4-phenyl-1, 4-dihydronaphthalen-1-yl) pentenoic acid from the stem bark of Brachystegia eurycoma harms. Int J Drug Dev Res 2013; 5(2):335–40.

[33] Ogunbinu AO, Ogunwande IA, Walker TM, Setzer WN. Identification of the volatile constituents of Brachystegia eurycoma Harms. Int J Aromather 2006; 16(3):155–8.
[34] Ibeto CN, Okoye CO, Ofoefule AU. Comparative study of the physicochemical characterization of some oils as potential feedstock for biodiesel production. ISRN Renew Energ 2012; 2012:1–5.
[35] Igbe I, Ayinde BA, Izuchukwu A. Anti-inflammatory and analgesic effects of the methanol stem bark extract of Brachystegia eurycoma harms (Fabaceae). Eur J Med Plant 2012; 2(4):356–56.
[36] Igbe I, Inarumen GO. The effect of leaf aqueous extract of Brachystegia eurycoma harms (Fabaceae) in acute and chronic inflammatory animal models. Brit J Pharmaceut Res 2013; 3(3):391–400.
[37] Femi-Ola TO, Aderibigbe EY. Studies on the effect of some wood extracts on growth and cellulase production by strains of Bacillus subtilis. Asian J Plant Sci 2008; 7(4):421–3.
[38] Adekunle AA. Antifungal property of the crude extracts of Brachystegia eurycoma and Richardia brasiliensis. Niger J Nat Prod Med 2000; 4(1):70–2.
[39] Adikwu MU, Enebeke TC. Evaluation of snail mucin dispersed in Brachystegia gum gel as a wound healing agent. Anim Res Int 2017; 4(2):685–90.
[40] Iweala EE. Anti-cancer and free radical scavenging activity of some Nigerian food plants in vitro. Int J Canc Res 2015; 11(1):41–51.
[41] Irondi EA, Oboh G, Akindahunsi AA, Boligon AA, Athayde ML. Phenolic composition and inhibitory activity of Mangifera indica and Mucuna urens seeds extracts against key enzymes linked to the pathology and complications of type 2 diabetes. Asian Pac J Trop Biomed 2014; 4(11):903–10.
[42] Sofidiya MO, Osesusi L, Olowe J, Familoni OB. Antiulcer and in vitro antioxidant activities of Brachystegia eurycoma. Planta Medica 2012; 78(11):PD133.
[43] Onyeso GI, Nkpaa KW, Wigwe UP. Ethno-medicinal studies of Brachystegia eurycoma Harms, Detarium microcarpum Guill. & Perr and Mucuna pruriens (L.) DC on blood glucose levels, liver enzymes, and lipid profile of female wistar rats. J Mol Pathophys 2016; 5(4):66–72.
[44] Ayinde BA, Abada NB. Growth inhibitory and cytotoxic effects of the methanol extract of Brachystegia eurycoma Harms (Fabaceae) leaves. J Pharm Bioresource 2010; 7(2):139–45.
[45] Igbe I, Ayinde BA, Innih S, Ezeadiugwu NA, Okhuarobo A. Sub-Acute toxicological evaluation of the aqueous stem bark extract of Brachystegia eurycoma (harms). Bayero J Pure Appl Sci 2016; 9(2):107–13.
[46] Nwosu JN. The rheological and proximate properties of some food thickeners ("Ukpo","Achi" and "Ofo") as affected by processing. Int J Basic Appl Sci 2012; 1(4):313–21.
[47] Ikegwu OJ, Okechukwu PE, Ekumankama EO, Okorie PA, Odo MO. Modelling the effect of toasting time on the functional properties of Brachystegia eurycoma flour. Niger Food J 2013 Dec 31; 31(1):108–14.
[48] Aviara NA, Edward MY, Ojediran JO. Effect of moisture content and processing parameters on the strength properties of Brachystegia eurycoma seed. Glob J Eng Design Technol 2013; 2(1):8–20.
[49] Aviara NA, Ibrahim EB, Onuoha LN. Physical properties of Brachystegia Eurycoma seeds as affected by moisture content. Int J Agric Bio Eng 2014; 7(1):84.
[50] Oji I, Asoegwuand SN, Okereke CD. Effect of moisture content variation on some physical properties of Achi seeds. Agric Eng Int: CIGR J 2016; 18(3):266–72.
[51] Olayemi O, Jacob O. Preliminary evaluation of Brachystegia eurycoma seed mucilage as tablet binder. Int J Pharmaceut Res Innov 2011; 3:1–6.
[52] Uzondu AL, Nwadike KN, Okoye EI. Application of Brachystegia eurycoma gum-egg albumen mixture in the formulation of modified release metronidazole tablets. Int J Pharm Sci Drug Res 2014; 6(2):109–13.
[53] Okore VC. African tropical plant gums: grossly unexploited carriers or adjuncts in drug delivery systems. Extraction 2009; 3:4.
[54] Agukwe O, Muazu J. Characterization, formulation and evaluation of the suspending properties of achi (Brachystegia eurycoma) seed gum in sulphamethoxazole suspension. Am J Pharm Health Res 2014; 2(3):25–34.
[55] Uhumwangho MU, Ileje IL. Preliminary evaluation of the suspending properties of Brachystegia eurycoma gum on metronidazole suspension. Am J Pharm Health Res 2014; 3(11):328–30.
[56] Madubuike FN, Ojimelukwe PC, Ajah PO. Proximate composition, energy content and physiochemical properties of Afzelia africana and Brachystegia eurycoma seeds. Plant Food Hum Nutr 1994; 46(4):339–44.
[57] Osazuwa EQ, Uhumwangho MU. Potential of Brachystegia eurycoma gum in ameliorating brittle fracture during tableting. Niger J Sci Env 2013; 12(1):98–102.
[58] Anyanwu MU, Okoye RC. Antimicrobial activity of Nigerian medicinal plants. J Intercult Ethnopharmacol 2017; 6(2):240–59.
[59] Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant Salmonella typhi. Phytother Res 2004; 18(8):670–3.
[60] O'Mahony R, Al-Khtheeri H, Weerasekera D, Fernando N, Vaira D, Holton J, et al. Bactericidal and anti-adhesive properties of culinary and medicinal plants against Helicobacter pylori. World J Gastroenterol 2005; 11(47):7499.

[61] Williams PA, Phillips GO (eds). Gums and stabilisers for the food industry 17: the changing face of food manufacture: the role of hydrocolloids. Royal Society of Chemistry, London, UK, 2014.
[62] Igwe OU, Nwokocha LM. Gum isolation from the seeds of Brachystegia eurycoma Harms and its synergistic studies with cassava and maize starches. Int J Chem Pharmaceut Sci 2014; 2(2):597–602.
[63] Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Control Release 2007 May 14; 119(1):5–24.
[64] Guo JH, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring water-soluble polymers. Pharm Sci Technolo Today 1998 Sep 1; 1(6):254–61.
[65] Prajapati VD, Jani GK, Moradiya NG, Randeria NP. Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydr Polym 2013 Feb 15; 92(2):1685–99.
[66] Bhosale RR, Osmani RA, Moin A. Natural gums and mucilages: a review on multifaceted excipients in pharmaceutical science and research. Int J Pharmacogn Phytochem Res 2014–15; 6(4):901–12.
[67] Kaplan W; Laing R. 2005. Local Production of Pharmaceuticals: Industrial Policy and Access to Medicines, An Overview of Key Concepts, Issues and Opportunities for Future Research. Health, Nutrition and Population (HNP) discussion paper;. World Bank, Washington, DC.
[68] Kaplan WA, Ritz LS, Vitello M. Local production of medical technologies and its effect on access in low and middle income countries: a systematic review of the literature. South Med Rev 2011; 4(2):51–61.
[69] Okoji MA. Depletion of forest resources in south eastern Nigeria: who loses?. The Environmentalist 2001; 21(3):197–203.
[70] Anyanwu JC, Amaku GE, Izunobi LC, Egbuawa IO, Onwuagba SM. Impact of deforestation on biodiversity in Anambra State, Nigeria. Int J Ecol Ecosolution 2016; 3(3):40–4.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 95–96
10.5455/jice.20171129094221

LETTER TO THE EDITOR

Novel natural products are the perfect assassination tools

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ARTICLE HISTORY

Received November 29, 2017
Accepted December 11, 2017
Published January 11, 2018

Recently, I was being filmed for a documentary while collecting an endemic New Zealand nettle in an effort to identify the active neurotoxic compound (Fig. 1). While performing the field extraction, I realized that if I accidently squirted a drop of the liquid onto a mucosal surface, or potentially my skin, I would likely die from perturbations of the autonomic nervous system [1]. While the extract has been shown to contain histamine, 5-hydroxytrptamine, and acetylcholine, there is an unidentified neurotoxic compound [2]. This neurotoxic compound is potentially very potent as the known compounds do not explain the weakness, confusion, profuse sweating, salivating, labored breathing, and "writhing in agony from cramps" that are experienced when exposed to this plant [2]. Exposure to the concentrated extract could potentially be more severe than the typical clinical presentation. While accidental exposure to this concentrated extract could make the film very exciting, this realization of risk was particularly concerning since the active compound in this extract is unknown [1–4], thus treating this exposure would be difficult.

This awareness caused me to reflect on how an uncharacterized natural product is the perfect assassination weapon.

Everyone in ethnopharmacology has worked with individuals that have historic pharmacologic knowledge [5]. This project was no different. Except that during the interviews regarding this plant, the film producer requested that we go into deep detail regarding the motivation, goals, and vision of the indigenous New Zealand Māori. During that interview, our Māori colleague made a specific comment around how it would be unacceptable to have the shared indigenous knowledge used for "…things like bioweapons." I have previously published on neurotoxins [6–8], but I had never before considered the potential moral gravity of this knowledge as our Māori colleague did.

Analyzing the exhumed body of Yasser Arafat established that he was likely killed by polonium [9], and the ex-KGB officer Alexander Litvinenko was certainly assassinated with polonium [10,11]. Even for Mr. Litvinenko who was hospitalized for nearly three weeks, his polonium poisoning was only identified shortly before he died—this challenge identifying the poisoning agent was because of the rarity to test a patient's polonium levels. Nonetheless, at least it is possible to test for polonium.

Uncharacterized natural products do not need to be synthesized, are relatively stable, and potentially entirely novel [12]. These characteristics make the compounds easy to get, easy to transport, and potentially hard to detect. These are desirable properties if you wanted to develop a tool for assassinations.

Those who are working in drug discovery can be the first people to translate powerful information on novel compounds into an easy-to-disseminate form that can be shared globally. However, with that ability to share knowledge comes a concomitant responsibility to consider the non-altruistic utility of this information as well, something I had forgotten but was at the forefront of our Māori collaborator's mind.

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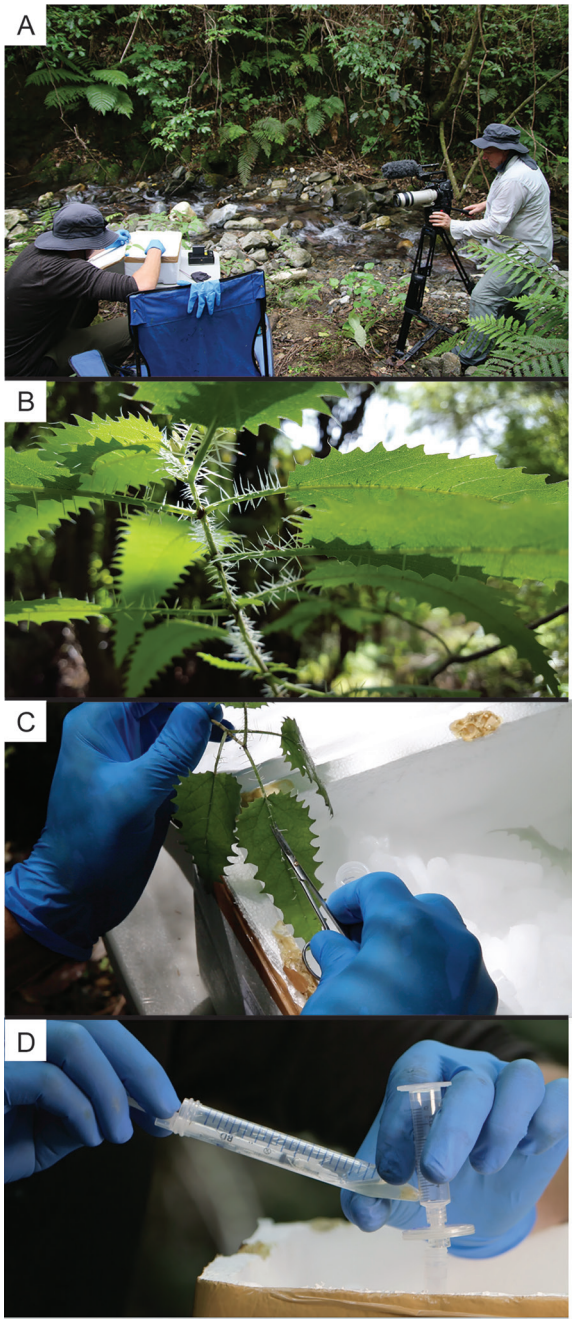

Figure 1. Making a documentary (A) of identifying the potential neurotoxic compounds in the New Zealand endemic nettle *Urtica ferox* which involved harvesting the white hair-like trichomes (B and C) and performing and extraction of the active compound (D) highlighted the risk of working with deadly, uncharacterized, toxic compounds. Photographs provided by Klaasz Breukel, Nelson Marlborough Institute of Technology, New Zealand, and John Irwin, Wild Sweet Productions, New Zealand.

References

[1] Hammond-Tooke GD, Taylor P, Punchihewa S, Beasley M. Urtica ferox neuropathy. Muscle Nerve 2007; 35(6):804–7.

[2] Clark FP. Tree nettle (Urtica ferox) poisoning. N Z Med J 1993; 106(957):234.

[3] Pilgrim R. Some properties of the sting of the New Zealand nettle, Urtica ferox. Proc R Soc Lond B Biol Sci 1959; 151(942):48–56.

[4] Kanzaki M, Tsuchihara T, McMorran D, Taylor P, Hammond-Tooke GD. A rat model of Urtica ferox neuropathy. Neurotoxicology 2010; 31(6):709–14.

[5] Buenz EJ, Verpoorte R, Bauer BA. The ethnopharmacologic contribution to bioprospecting natural products. Annu Rev Pharmacol Toxicol 2018; 58:509–530; doi:10.1146/annurev-pharmtox-010617-052703

[6] Howe CL, LaFrance-Corey RG, Mirchia K, Sauer BM, McGovern RM, Reid JM, et al. Neuroprotection mediated by inhibition of calpain during acute viral encephalitis. Sci Rep 2016; 6:28699.

[7] Buenz EJ. Lead exposure through eating wild game. Am J Med 2016; 129(5):457–8.

[8] Buenz EJ, Howe CL. Beta-methylamino-alanine (BMAA) injures hippocampal neurons in vivo. Neurotoxicology 2007; 28(3):702–4.

[9] Froidevaux P, Bochud F, Baechler S, Castella V, Augsburger M, Bailat C, et al. (210)Po poisoning as possible cause of death: forensic investigations and toxicological analysis of the remains of Yasser Arafat. Forensic Sci Int 2016; 259:1–9.

[10] Rubin GJ, Page L, Morgan O, Pinder RJ, Riley P, Hatch S, et al. Public information needs after the poisoning of Alexander Litvinenko with polonium-210 in London: cross sectional telephone survey and qualitative analysis. Br Med J 2007; 335(7630):1143.

[11] Stather JW. The polonium-210 poisoning in London. J Radiol Prot 2007; 27(1):1–3.

[12] Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 2015; 14(2):111–29.

JOURNAL OF INTERCULTURAL ETHNOPHARMACOLOGY, 2018
VOL 7, NO. 1, PAGES 97–100
10.5455/jice.20171015041941

LETTER TO THE EDITOR

The potential use of flavonoids as venoactive drugs and the role of citrus fruits

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ARTICLE HISTORY

Received October 15, 2017
Accepted November 19, 2017
Published November 24, 2017

Sir,

This letter addresses the reported phlebotonic activity of some flavonoids (Fls) recommended in venous conditions such as chronic venous insufficiency (CVI) and varicose veins, both having considerable socioeconomic impact [1]. Supplementation of Fl as drugs or nutritional supplements versus enhanced consumption of citrus fruit is also discussed in this letter. It is known that substances with unproven efficiency are sometimes promoted as evidence-based medications. Besides, certain pharmaceuticals can be replaced by a diet modification [2,3].

Fls constitute the largest part of dietary polyphenols; they can be found in many fruits, vegetables, and cereals [4,5]. Some supposedly venoactive Fls (rutin, escin, quercetin) have been extracted from medicinal plants. Today, the micronized purified Fl fraction (MPFF) is broadly used, consisting of 90% diosmin and 10% hesperidin. Diosmin is synthesized from hesperidin, which is extracted from a type of small immature oranges [6]. Other preparations which have been associated with the treatment of venous disease are hydroxyethylrutosides, also known as troxerutin, and pycnogenol [7,8]. In the U.S., Fl preparations are classified as dietary supplements, and in some European countries as drugs, which does not necessarily mean extensive use. So, in Scandinavia drugs are hardly ever prescribed for chronic venous disease [9]. In Spain, for certain phlebotonics the indication for CVI has been withdrawn, and for several other countries—such as diosmine, hidrosmine, escin, and some rutosides—the use during exacerbations of CVI has been limited to two or three months [10].

The following effects of venoactive Fls have been discussed: phlebotonic, anti-edematous, anti-inflammatory, and anti-oxidative. The action mechanisms are not well established [6,10,11] and not clearly understandable theoretically. The smooth muscles (SM) of large and medium-sized veins studied *in vitro* had no tone and did not relax under the impact of vasodilators [12]. The lumen of collapsed veins is slit-like, the circular SM layer is thin, bunches of SM alternating with connective tissue. In post-thrombotic syndrome and varicose veins, where Fls are generally recommended, venous walls are partly distended, SM being atrophic and replaced by fibrous tissue [1,13]. All these are against any significant phlebotonic effect of Fls; in particular, its durability is doubtful. This pertains to potentiation of norepinephrine action under the influence of Fls discussed in the literature [6,13–15]. The vasoconstrictor effect of norepinephrine is short-term, its blood concentration fluctuates in stress, etc., whereas Fls have been proposed for the treatment of chronic conditions such as CVI and varicose veins. At the same time, there were reports on the inhibition by quercetin of vascular contraction induced by norepinephrine [16]. A significant phlebotonic effect seems to be improbable without any impact on arterial SM and the blood pressure [11]. Should Fls considerably enhance the action of norepinephrine or otherwise cause vasoconstriction, it would probably elevate blood pressure in arterial hypertension. Although some degree of venous tone does exist *in vivo* [17,18], there is no convincing evidence that it can be significantly influenced by Fls. If vasoconstriction is indeed favorable in venous

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diseases, known vasoconstrictor agents could be used instead of the substances with unproven efficiency.

There are objective assessment methods of drug effects in the vascular bed, e.g., using isolated veins [18]. For example, dihydroquercetin did not modify the basal tone of isolated rat veins [19]. On the basis of experiments with rat femoral veins, it was reported that diosmin increases the sensitivity of SM to calcium, which could explain the phlebotonic action [6,20]. On the contrary, hesperetin (aglycone form of hesperidin) induced vasodilatation in humans and hypertensive rats [21,22]. The vasorelaxing property was demonstrated also for eriodictyol, an Fl from lemon [21]. Further independent research could estimate the magnitude and duration of the phlebotonic effect, if any, for different Fls.

Overall, the quality of reporting on this topic is regarded to be poor and loaded with biases, and beneficial effects tending to be exaggerated [7,11]. For example, the most rigorously conducted trial did not show any additional benefit from Fls in the treatment of venous leg ulcers [7]. Among positive results, subjective improvements (quality of life, pains, cramps, sensation of swelling, heavy legs) are often reported [6,13,23,24], which may be caused by a placebo effect. Admittedly, an improvement under the influence of MPFF of venous hemodynamics assessed by strain gauge plethysmography and supramaleolar circumference in patients with CVI has been reported [25–27]. The data of foot volumetry have been unconvincing [9], also in the recent study [28].

Mechanisms of supposed anti-inflammatory and anti-edematous effects of Fls are hardly comprehensible. It can be asked in this connection why, instead of the substances with unproven efficiency, known anti-inflammatory, or diuretic drugs cannot be used. However, these medications are not generally applied in edema due to venous disease [24]. Furthermore, the anti-oxidative capacity of Fl has been discussed [4]. In general, antioxidants are regarded to be far from scientifically founded clinical application [29]. It largely remains unclear whether, when, and how much antioxidants should be taken [29–31], the more so as some antioxidants may act as pro-oxidants [32]. In any case, it is unclear why antioxidants should be used in conditions associated with tissue hypoxia, such as CVI.

Finally, Fls are not free from adverse effects reported to be mild to moderate across studies. The most common events were skin changes (including eczema), gastrointestinal disturbances, and hypertension [7,10]. Without going into details of Fl biology, their role as repellents should be noted, protecting plants from herbivores. Certain Fls were reported to be toxic for insects or other organisms [5,33,34]. Presumably, Fls may act as mild toxin stimulating endogenous defense mechanisms [35], so that their abundant intake is not *a priori* beneficial especially for individuals with compromised defense mechanisms, e.g., in chronic diseases or advanced age [2].

Despite the arguments presented above, there are many publications reporting favorable effects of Fl in vein diseases. Some of such studies were sponsored by manufacturers. Obviously, verification in large-scale independent experiments is needed. Should the useful properties of Fl be confirmed, the question will arise whether pharmaceuticals could be replaced by enhanced consumption of citrus fruits known to be among the most common phenolic-rich dietary sources [4]. Taking into account the maximum concentration of total Fl in grapefruit juice (up to 84.28 mg/100 ml) [36], the average for commercial grapefruit juice (65 mg/100 ml) [4] and the higher content of Fl in whole fruits than in juice [21,37], consumption of 1–2 grapefruits means the intake of about 500 mg of Fls (naringin, narirutin, hesperidin, etc.) Naringin and naringenin are bitter and occur predominantly in grapefruits [4,5]. Relatively high concentration of hesperidin (a component of MPFF) was found in orange and mandarin juices (25–40 mg/100 ml), being the highest in *C. clementina* (39.9 mg/100 ml on average) [4]. The concentration of diosmin (another component of MPFF) is relatively high in commercial sweet orange juice (3.46 mg/100 ml) [4]. Lemon is also an important source of hesperidin and diosmin [4,6]. Detailed information on different Fls in citrus juices is available from the review by Prof. Giuseppe Gattuso and co-workers [4]. Remarkably, some commercial citrus juices contain more Fls than hand-pressed ones [4], which may be caused by forceful pressing or use of the pulp. However, there are local differences: some commercial products labeled as citrus juices in the former Soviet Union have been apparently diluted, contained added sugar and artificial flavors. The concentration of Fls in drugs and nutritional supplements is higher than in a typical diet. Excessive amounts of polyphenols reaching the colon may inhibit the growth of beneficial microbiota potentially leading to dysbiosis [38]. Considering the above, and also vitamins and microelements in fruits, consumption of citrus fruits and juices might be preferable to the supplementation of Fls by drugs and dietary supplements.

In conclusion, the data in favor of the phlebotonic action of Fl are inconsistent, while clinically significant effects are hardly comprehensible theoretically. The effectiveness of venoactive drugs needs verification in large-scale studies protected from conflicts of interest, using objective methods such as measurements of supramaleolar circumference, plethysmography, water volumetry, and optoelectronic methods building three-dimensional models of legs [1,39]. Potential difficulties in performing water volumetry are described in [39]. Finally, considering limited bioavailability and rapid metabolism of some dietary polyphenols [5,40,41], further research and review of pharmacokinetics of different Fls is needed.

References

[1] Nicolaides AN, Cardiovascular Disease Educational and Research Trust, European Society of Vascular Surgery, The International Angiology Scientific Activity Congress Organization, International Union of Angiology, Union Internationale de Phlebologie at the Abbaye des Vaux de Cernay. Investigation of chronic venous insufficiency: a consensus statement (France, March 5–9, 1997). Circulation 2000; 102(20):E126–63.

[2] Jargin SV. Hormesis and homeopathy: The artificial twins. J Intercult Ethnopharmacol 2015; 4:74–7.

[3] Jargin SV. Supplementation of glycosaminoglycans and their precursors in osteoarthritis versus diet modification. Int J Rheum Dis 2012; 15(3):e45–6.

[4] Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid composition of Citrus juices. Molecules 2007; 12(8):1641–73.

[5] Brglez Mojzer E, Knez Hrnčičč M, SŠkerget M, Knez ZŽ, Bren U. Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules 2016; 21(7):901.

[6] Katsenis K. Micronized purified flavonoid fraction (MPFF): a review of its pharmacological effects, therapeutic efficacy and benefits in the management of chronic venous insufficiency. Curr Vasc Pharmacol 2005; 3(1):1–9.

[7] Scallon C, Bell-Syer SE, Aziz Z. Flavonoids for treating venous leg ulcers. Cochrane Database Syst Rev 2013; (5):CD006477.

[8] Schoonees A, Visser J, Musekiwa A, Volmink J. Pycnogenol® (extract of French maritime pine bark) for the treatment of chronic disorders. Cochrane Database Syst Rev 2012; (4):CD008294.

[9] Danielsson G, Jungbeck C, Peterson K, Norgren L. A randomised controlled trial of micronised purified flavonoid fraction vs placebo in patients with chronic venous disease. Eur J Vasc Endovasc Surg 2002; 23:73–6.

[10] Martinez-Zapata MJ, Vernooij RW, Uriona Tuma SM, Stein AT, Moreno RM, Vargas E, et al. Phlebotonics for venous insufficiency. Cochrane Database Syst Rev 2016; (4):CD003229.

[11] Kharkevich DA. The principles of action and use of phlebotropic agents. Klin Med (Mosk) 2004; 82(11):4–10.

[12] Bevan JA. Some bases of differences in vascular response to sympathetic activity. Circ Res 1979; 45:161–71.

[13] Perrin M, Ramelet AA. Pharmacological treatment of primary chronic venous disease: rationale, results and unanswered questions. Eur J Vasc Endovasc Surg 2011; 41(1):117–25.

[14] Iablokov EG, Bogachev VIu, Domoradskaia AI. The drug therapy of chronic venous insufficiency (a trial of the clinical use of the preparation Detralex). Ter Arkh 1996; 68(10):80–1.

[15] Juteau N, Bakri F, Pomies JP, Foulon C, Rigaudy P, Pillion G, et al. The human saphenous vein in pharmacology: effect of a new micronized flavonoidic fraction (Daflon 500 mg) on norepinephrine induced contraction. Int Angiol 1995; 14(3 Suppl 1):8–13.

[16] Duarte J, Peérez-Vizcaiíno F, Zarzuelo A, Jimeénez J, Tamargo J. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. Eur J Pharmacol 1993; 239(1–3):1–7.

[17] Schmitt M, Blackman DJ, Middleton GW, Cockcroft JR, Frenneaux MP. Assessment of venous capacitance. Radionuclide plethysmography: methodology and research applications. Br J Clin Pharmacol 2002; 54(6):565–76.

[18] Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins - 2. Br J Clin Pharmacol 1994; 38:289–305.

[19] Ivanov IS, Sidekhmenova AV, Nosarev AV, Tyukavkina NA, Plotnikov MB. Effect of dihydroquercetin on the tone of isolated rat veins. Bull Exp Biol Med 2013; 155(1):65–6.

[20] Savineau JP, Marthan R. Diosmin-induced increase in sensitivity to Ca2+ of the smooth muscle contractile apparatus in the rat isolated femoral vein. Br J Pharmacol 1994; 111:978–80.

[21] Testai L, Calderone V. Nutraceutical value of citrus flavanones and their implications in cardiovascular disease. Nutrients 2017; 9(5):502.

[22] Takumi H, Nakamura H, Simizu T, Harada R, Kometani T, Nadamoto T, et al. Bioavailability of orally administered water-dispersible hesperetin and its effect on peripheral vasodilatation in human subjects: implication of endothelial functions of plasma conjugated metabolites. Food Funct 2012; 3(4):389–98.

[23] Aziz Z, Tang WL, Chong NJ, Tho LY. A systematic review of the efficacy and tolerability of hydroxyethylrutosides for improvement of the signs and symptoms of chronic venous insufficiency. J Clin Pharm Ther 2015; 40(2):177–85.

[24] Cheatle TR, Scurr JH, Smith PD. Drug treatment of chronic venous insufficiency and venous ulceration: a review. J R Soc Med 1991; 84(6):354–8.
[25] Geroulakos G, Nicolaides AN. Controlled studies of Daflon 500 mg in chronic venous insufficiency. Angiology 1994; 45(6 Pt 2):549–53.
[26] Duchene Marullaz P, Amiel M, Barbe R. Evaluation of the clinical pharmacological activity of a phlebotonic agent. Application to the study of Daflon 500 mg. Int Angiol 1988; 7(2 Suppl):25–32.
[27] Allaert FA. Meta-analysis of the impact of the principal venoactive drugs agents on malleolar venous edema. Int Angiol 2012; 31(4):310–5.
[28] Rabe E, Agus GB, Roztocil K. Analysis of the effects of micronized purified flavonoid fraction versus placebo on symptoms and quality of life in patients suffering from chronic venous disease: from a prospective randomized trial. Int Angiol 2015; 34:428–36.
[29] Giorgio M. Oxidative stress and the unfulfilled promises of antioxidant agents. Ecancermedicalscience 2015; 9:556.
[30] Edeas M. Anti-oxidants, controversies and perspectives: how can the failure of clinical studies using anti-oxidants be explained? J Soc Biol 2009; 203:271–80.
[31] Denisov EV, Afanas'ev IB. Oxidation and antioxidants in organic chemistry and biology, Taylor & Francis, Boca Raton, FL, 2005.
[32] Papas AM. Antioxidant status, diet, nutrition, and health, CRC Press, Boca Raton, FL, 1999.
[33] Abu Raihan SM. Effect of plant flavonoids on mosquito larvae. Nat Univ J Sci 2014; 1:27–30.
[34] Lorent K, Gong W, Koo KA, Waisbourd-Zinman O, Karjoo S, Zhao X, et al. Identification of a plant isoflavonoid that causes biliary atresia. Sci Transl Med 2015; 7(286):286ra67.
[35] Goszcz K, Deakin SJ, Duthie GG, Stewart D, Leslie SJ, Megson IL. Antioxidants in cardiovascular therapy: panacea or false hope? Front Cardiovasc Med 2015; 2:29.
[36] Ross SA, Ziska DS, Zhao K, ElSohly MA. Variance of common flavonoids by brand of grapefruit juice. Fitoterapia 2000; 71(2):154–61.
[37] Jourdan PS, McIntosh CA, Mansell RL. Naringin levels in citrus tissues: II. Quantitative distribution of naringin in citrus paradisi MacFad. Plant Physiol 1985; 77(4):903–8.
[38] Duda-Chodak A. The inhibitory effect of polyphenols on human gut microbiota. J Physiol Pharmacol 2012; 63(5):497–503.
[39] Rabe E, Stucker M, Ottillinger B. Water displacement leg volumetry in clinical studies: a discussion of error sources. BMC Med Res Methodol 2010; 10:5.
[40] Scholz S, Williamson G. Interactions affecting the bioavailability of dietary polyphenols in vivo. Int J Vitam Nutr Res 2007; 77(3):224–35.
[41] Pandareesh MD, Mythri RB, Srinivas Bharath MM. Bioavailability of dietary polyphenols: factors contributing to their clinical application in CNS diseases. Neurochem Int 2015; 89:198–208.