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Follicular development and post-implantation loss assessments in non-pregnant and pregnant rats orally exposed to *Polyscias fruticosa* leaf extract

Alex Boye[1,2], Desmond Omane Acheampong[3], Victor Yao Atsu Barku[4], Adamu Yussam[1], Ernest Amponsah Asiamah[3]

[1]Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

[2]Department of Pharmacology, Institute of Natural Medicine, Anhui Medical University, Hefei, China

[3]Department of Biomedical Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

[4]Department of Chemistry, School of Physical Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana

ABSTRACT

Background: Previously, folk claims of *P. fruticosa* were ascertained pharmacologically; and its safety studied, but its effect/safety on female reproductive system remained unknown.

Objective: The study assessed *P. fruticosa* leaf extract (PFE) on follicular development in non-pregnant rats; implantation and post-implantation loss in pregnant rats.

Methods: The study used healthy adult non-pregnant and pregnant female and male Wistar rats (150–200 g). Non-pregnant rats were randomly assigned to five groups: normal saline (5 ml/kg *po*), clomiphene citrate (CL) (50 mg/kg *po*), and PFE (100, 200, and 500 mg/kg *po*) and treated once daily for 21 days. Dams were sacrificed under deep anesthesia on day 22. Enzyme-linked immunosorbent assay kit was used to measure serum estrogen, follicle stimulating hormone (FSH), and luteinizing hormone (LH). Uterus and ovary were histologically assessed. Dams were co-habited with fertile males for 1 week; confirmed day 1 pregnant rats were randomly re-assigned to five groups with misoprostol (200 mg/kg) as reference and treated once daily for 15 days. Implantation and post-implantation loss were assessed (6 and 15 gestations).

Results: PFE and CL increased follicular development at the primordial and primary follicle stages compared to control. PFE improved uterine musculature compared to control. PFE decreased serum FSH, but increased ($P < 0.05$) serum estrogen and LH compared to control. PFE increased gravid uterine weight compared to control. Total implantation sites were comparable across all groups. Misoprostol and PFE (500 mg/kg) produced post-implantation loss compared to control.

Conclusion: PFE (≤100 mg/kg) improved follicular development in non-pregnant rats, but pose risk of post-implantation loss in pregnant rats at ≥500 mg/kg.

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KEYWORDS

Follicular development; *Polyscias fruticosa*; post-implantation loss

Introduction

Use of herbs as medicament to promote human health has become common globally [1,2]. Primary healthcare of most developing countries relies on the use of indigenous healing systems, mostly herbal medicine therapy as first line of treatment for common diseases [3]. In effect, several herbs are used to improve general health and specifically reproductive health [2,4]. Previously, folklore claims of *Polyscias fruticosa* for the treatment of upper respiratory disorders including asthma were ascertained pharmacologically [5–7]. The medicinal importance of *Polyscias fruticosa* in folk medicine has earned it many local names across Afro-Asian

Contact Alex Boye ✉ boye_alex@yahoo.com Department of Medical Laboratory Science, School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana.

regions of the world such as "Taiwan momiji" in Japanese, "cay goi ca" in Vietnamese language, "imba" in Sudanese language, and "ming aralia" in common English. Importantly, it was demonstrated that *Polyscias fruticosa* leaf extract (PFE) at 100 mg/kg improved caudal epididymal sperm count in a testosterone-independent manner [8]; however, its effect on female reproductive system particularly follicular development, implantation, and post-implantation events remained unresolved. The present study investigated PFE on follicular development in non-pregnant Wistar rats, in view of the crucial role of folliculogenesis in ovulation and overall female fertility [9,10]. Also, pregnancy rate, total implantation sites, gravid uterine weight, and post-implantation events were monitored in pregnant rats.

Materials and Methods

Chemicals and drugs

Chemicals and drugs used in this study included: absolute ethanol (PS Park Scientific Limited, Northampton, UK), clomiphene citrate (CL) (Doppel Farmaceutici S.p.a, Via delle Ande 15, 00144 Rome, Italy), normal saline (NS) (Amanta Healthcare Ltd., Gujarat, India), silica gel (VWR International bvba/spr, Haasrode, Belgium, Batch: 09B200018), phosphate buffered saline, distyrene, a plasticizer and Xylene, and chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), 10% Neutral Buffered formalin, 1% Eosin W/V (BDH Chemicals Ltd, England) and sodium hydrogen carbonate (PROLABO®, EC-EMB 45053), misoprostol (Piramal Healthcare UK Limited, Northumber, UK), chloroform (Khimprom JSC, Promyshlennaya STR 101, Russia), xylene (BDH Chemical Ltd, Poole, England), and toluidine blue (Alpha Chemika, India).

Collection, identification, and authentication of **P. fruticosa**

Fresh *P. fruticosa* leaves were obtained from the botanical gardens of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Identification and authentication were done at the Herbal Medicine Department, KNUST. A voucher specimen (KNUST/HM/13/W010) was deposited at herbaria as previously reported [6,7].

Preparation of **Polyscias fruticosa** *leaf extract*

PFE was prepared as previously described [6–8,11] with some modifications. Briefly, leaves of *Polyscias fruticosa* were washed, shade-dried in a well-ventilated area for 3 weeks and then powdered using a mechanical blender. A 1.8 kg quantity of the powder was soaked in 4.8 L of absolute ethanol in a flat bottom flask and shaken manually on daily basis for 7 days. The infusion was filtered five times using a funnel and a cotton wool. Absolute ethanol was retrieved using a rotary evaporator (Buchi Oilbath B-485, Switzerland) with a water bath set at 40°C. The residue obtained was dried with the aid of activated silica gel in a desiccator. The dried residue weighed 62.4 g with a percentage yield of 3.4%. The extract was labeled PFE and stored at 4°C n a refrigerator until use.

Qualitative phytochemical analysis of PFE

PFE was subjected to standard qualitative methods [12,13] to ascertain its phytochemical constituents. Also, thin layer chromatography (TLC) and gas chromatography coupled with mass spectrometry (GC–MS) analysis were conducted on PFE as previously reported [8].

Animal husbandry

Healthy 8–10 weeks old male and female Wistar rats weighing 150–200 g were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana and transported to the animal house of the Department of Biomedical Sciences, University of Cape Coast. The rats were allowed a period of 2 weeks to acclimatize with laboratory conditions before the start of all experiments. The rats were kept in aluminium cages (40 × 35 × 15 cm) with saw dust as bedding. The beddings of rats were regularly changed. Rats were kept under 12 hours light/dark cycle, normal ambient temperature, and humidity. Rats were fed on standard pellet diet (Essaar grower mash, Essaar Agro-West Africa Ltd, Ghana) and had access to water *ad libitum*. Rats were humanely handled and treated in accordance with 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.

Dose selection and route of administration

The selection of doses and the route of administration of drugs were based on previous studies on *Polyscias futicosa* [6,7,11]. Body weights were

measured daily and doses were adjusted to reflect body weight changes.

Experimental design

Non-pregnant adult (12 weeks old) female Wistar rats (150–200 g), with confirmed stage of estrous cycle, were randomly assigned to five groups (control group, NS: 5 ml/kg *po*, *n* = 5; CL: 50 mg/kg *po*, *n* = 5; and PFE, 100, 200, and 500 mg/kg *po*, *n* = 5) and treated daily with the respective drugs by oral gavage for 21 days. On the 22nd day, rats were sacrificed under general anesthesia. After carefully isolating Y-shaped uterus and oviduct, bilateral oophorectomy was carried out as previously described [14] with modification. Briefly, left (L) and right (R) ovaries were carefully removed and preserved in 10% formaldehyde in buffered saline for 72 hours. Isolated tissues were appropriately labeled. Sections were prepared according to standard methods and stained with hematoxylin and eosin (H&E) as previously described [15]. Prepared slides were thoroughly studied microscopically by three independent researchers. Final descriptions of observations were made by consensus. In a separate experiment, after 2 weeks of pre-treatment of female Wistar rats (12 weeks old) with PFE, female rats were co-habited with confirmed fertile males (12 weeks) for a week. After confirmation of pregnancy [16,17], dams of comparable body weights were randomly re-assigned to one of five groups [control group (NS: 5 ml/kg *po*; *n* = 5), model group (misoprostol: 200 mg/kg *po*, *n* = 5), and PFE (100, 200, and 500 mg/kg *po*, *n* = 5)] for implantation, resorption, and post-implantation loss assessments at gestational days 6 and 15, respectively.

Assessment of implantation, number of embryos, and post-implantation loss

Assessment of implantation and post-implantation loss were done by following previously described methods [16,18,19] with some modifications. Briefly, at gestational days 6 and 15, number of implantation sites, number of embryos, and post-implantation loss were, respectively, assessed at the specified gestational days for each treatment group. To assess the number of implantation sites, xylene was applied on the tails of dams to induce dilation of the tail vein making the veins more prominent. Each dam was then injected with toluidine blue (0.1 ml of 1%) through the tail vein. The dye was allowed 30–50 minutes of time to react with the uterine endometrium specifically at implantation sites, where molecules of the toluidine blue biochemically react with the implantation site making it not only prominent but also easy to be visually identified. After a period of 50 minutes, dams were sacrificed by deep anesthesia using chloroform (99.8%). The uteri of sacrificed dams were isolated and wet weight of gravid uteri measured. The Y-shaped uteri were cut opened and the number of implantation sites determined per uteri/four dams/group. The whole experiment continued to gestational day 15 with the remaining dams, but this time the number of implantation and the number of embryos were determined for each treatment group by randomly selecting four dams from each group. Dams were sacrificed and their gravid uteri isolated and weighed. Uteri were cut open and the number of implantation and embryos determined. % post-implantation loss was estimated for each group by using a previously described formula [20] as shown below:

$$\%\ \text{post-implantation loss} = \frac{\text{total number of resorption sites}}{\text{total number of implantation sites}} * 100$$

Measurement of serum sex hormone levels

Serum gonadotropins [follicle stimulating hormone (FSH) and luteinizing hormone (LH)] and ovarian estrogen were determined using rat-specific enzyme-linked immunosorbent assay (ELISA) kits (Sangon Biotech, Shanghai, Co., Ltd, 698 XiangMin Road, SongJiang District, Shanghai, China) by strict adherence to manufacturer's instructions. After preparation of sera, it was stored at −20°C until use. Absorbance of controls (calibrators) and specimens (sera from dams in each treatment group) were determined using an ELISA microplate reader (Multiskan Ascent plate reader, MTX Lab Systems, Inc., Brandenton, FL). The sensitivity of hormone detection was 0.005 ng/ml. All the samples were analyzed in a single assay to avoid inter-assay errors.

Data analysis

Data were analysed by using GraphPad Prism Version 6 software for Windows (Graph Pad Software, San Diego, CA). Data were presented in tables, and line and bar graphs as mean ± standard deviation (SD). Mean comparison between groups was done by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. $P \leq 0.05$ was considered statistically significant in all analyzes.

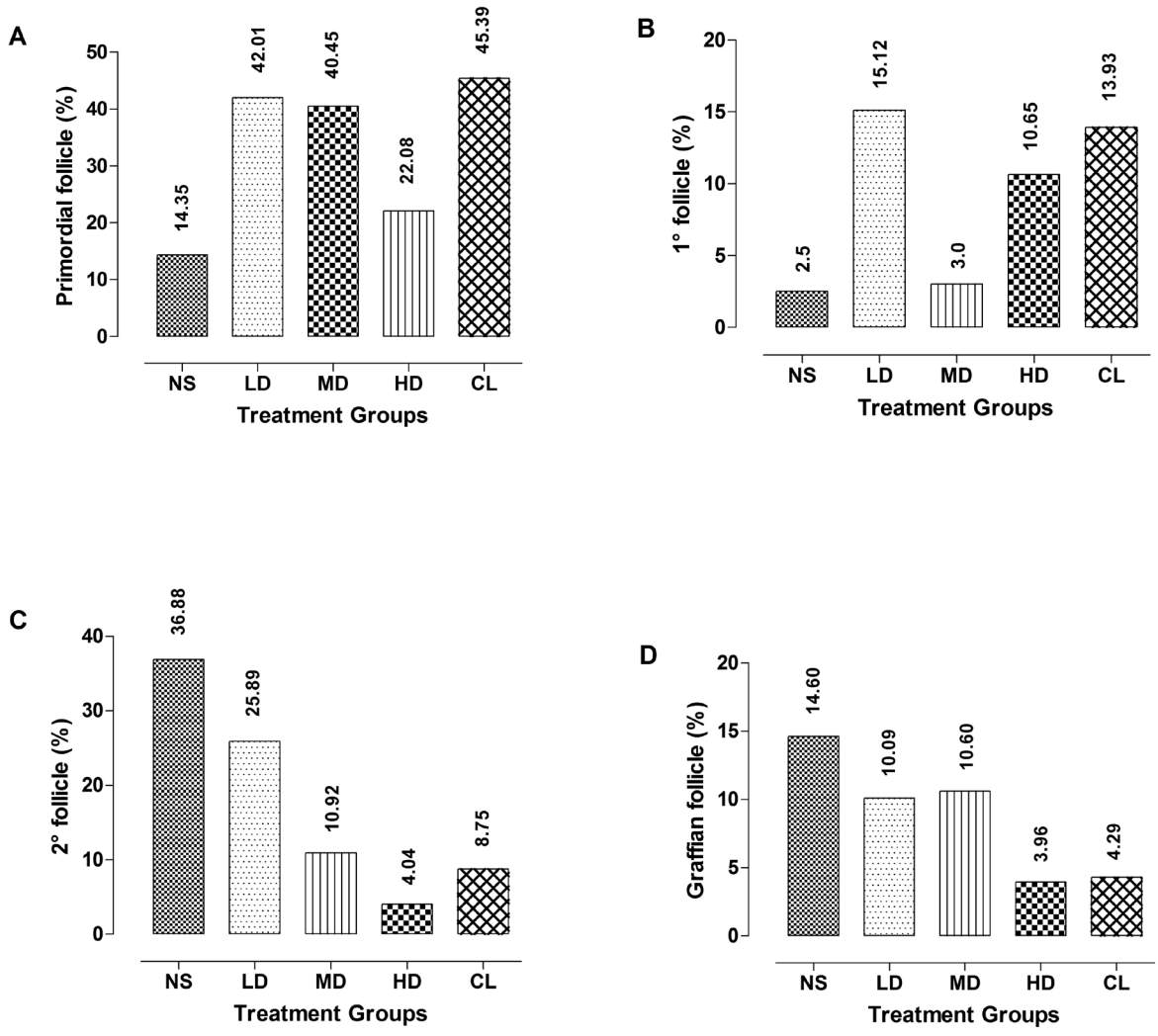

Figure 1. Effect of treatments on follicular development. (A) Primodial follicle, (B) primary follicle, (C) secondary follicle, (D) graffian follicle; NS = normal saline (5 ml/kg), LD = low dose (100 mg/kg) of PFE, MD = medium dose (200 mg/kg) of PFE, HD = high dose (500 mg/kg) of PFE, and CL = clomiphene citrate (50 mg/kg). 1° = primary and 2° = secondary.

Results

Phytochemical analysis on PFE

Qualitative phytochemical analysis on PFE showed the presence of alkaloids, saponins, cyanogenic glycosides, and sterols. TLC analysis showed four spots, while GC–MS analysis produced 12 peaks out of which eight matched library compounds (unpublished data).

PFE produced stage-specific effects on follicular development in non-pregnant rats

The number of primordial follicles of both left and right ovaries combined expressed in percentage increased in PFE-treated rats, particularly in low- and medium-dose PFE (100 and 200 mg/kg) and CL groups relative to control. Average number of primary follicles increased in all treatment groups relative to control, except PFE (200 mg/kg) group. Except PFE (100 mg), all other treatments groups did not improve the average number of secondary follicles compared to control. Average number of Graffian follicles improved in low- and medium-PFE (100 and 200 mg/kg) groups though lower compared to control (Fig. 1).

The number of primordial follicles in left ovary (LO) was higher than that of the right ovary (RO) in low- and medium-dose PFE (100 and 200 mg/kg) group compared to control group, while this trend was also observed in misoprostol group relative to control. For all treatment groups, the number of primary follicles in the left ovary was higher than that of the RO compared to that of control. Although the number of secondary follicles in both left and right ovaries across all treatment groups was lower compared to control, the trend was similar to that

Table 1. Effect of 21 days drug treatments on follicular development in non-pregnant rats.

Group	Primodial follicle (LO) (%)	Primodial follicle (RO) (%)	1° follicle (LO) (%)	1° follicle (RO) (%)	2° follicle (LO) (%)	2° follicle (RO) (%)	Graffian follicle (LO) (%)	Graffian follicle (RO) (%)
NS	15.00	13.69	0.00	5.00	27.50	46.27	7.50	21.71
CL	50.01	40.78	19.84	8.03	9.284	8.21	6.65	1.93
PFE (mg/kg)								
100	56.67	24.24	4.00	2.00	11.50	10.33	7.78	13.42
200	48.07	35.96	18.74	11.49	22.43	29.34	16.97	3.21
500	16.22	27.93	10.89	10.40	1.428	6.64	1.46	6.46

1° = primary, 2° = secondary, NS = normal saline (5 ml/kg); CL = clomiphene citrate (50 mg/kg).

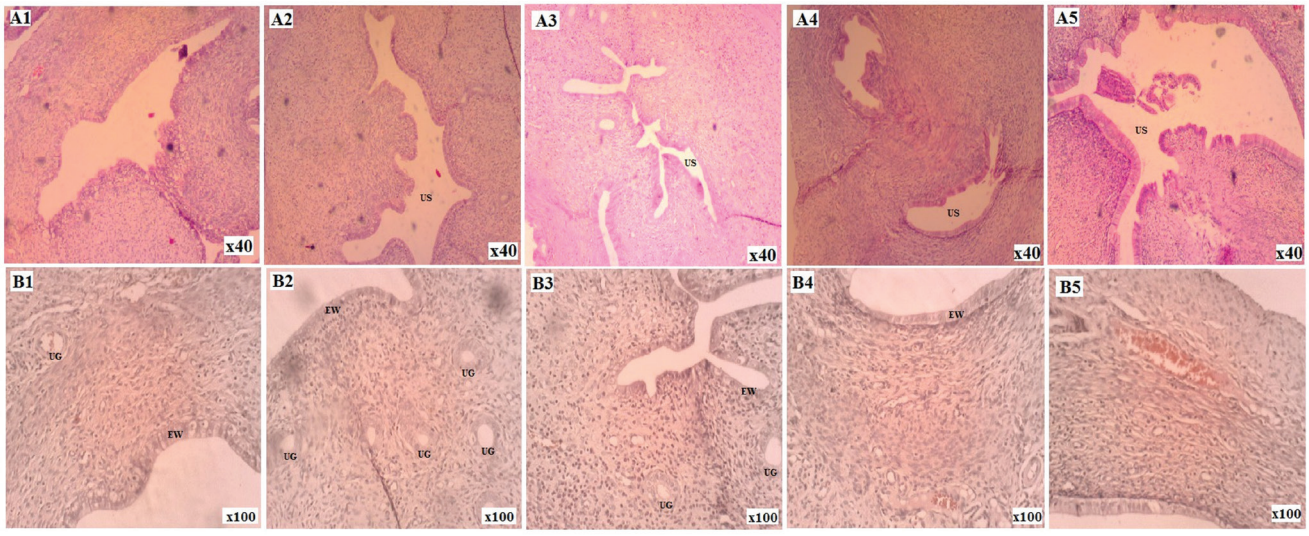

Figure 2. H&E stained sections of rat uterus (A1–A5) and rat uterine endometrial layer (B1–B5) after 21 days of drug treatments. Generally all sections showed columnar epithelium with microvilli. However, there was infiltration of lymphocytes and macrophages in intermediate endometrial layer and collagenous endometrium, especially in B4 and B5. There was presence of uterine glands in B2 and B3 compared to B1. A1 and B1 = NS (5 ml/kg), A2 and B2 = low-dose PFE (100 mg/kg), A3 and B3 = medium-dose PFE (200 mg/kg), A4 and B4 = high-dose PFE (500 mg/kg), and A5 and B5 = clomiphene citrate (50 mg/kg). US = uterine space; UG = uterine gland; EW = endometrial wall.

observed with respect to primary follicles (above) except in medium- and high-dose PFE (200 and 500 mg/kg) group. All but low- and medium-PFE (100 and 200 mg/kg) increased the number of Graffian follicles in LO relative to control. The number of Graffian follicles in RO across all treatment groups was lower compared to that of control (Table 1).

Effect of treatments on uterine musculature and ovarian structure in non-pregnant rats

Generally all sections showed columnar epithelium with microvilli. However, there was infiltration of lymphocytes and macrophages in intermediate endometrial layer and collagenous endometrium, especially in B4 and B5. There was presence of uterine glands in B2 and B3 compared to B1 (Fig. 2). Generally fimbrae with atypical pseudostratified ciliated columnar epithelium, clumped microvilli, and dilated blood vessels (DBVs) were observed in B3, B4, and B5 (Fig. 3). There were marked dilatation of blood vessels near growing follicles and corpus luteum, infiltration of macrophages and lymphocytes in ovarian stroma (ST) and corpus luteum, especially in C, D, and E (Fig. 4).

PFE decreased serum sex hormones in non-pregnant rats

Serum level of FSH decreased in PFE (100, 200, and 500 mg/kg)-treated rats compared to control. Except PFE (100 mg/kg)-treated rats, which had increased serum estrogen compared to control, all other treatment (PFE, 200 and 500 mg/kg and CL, 50 mg/kg) groups had significant decrease in serum estrogen levels compared to control. Serum levels of LH increased significantly in PFE

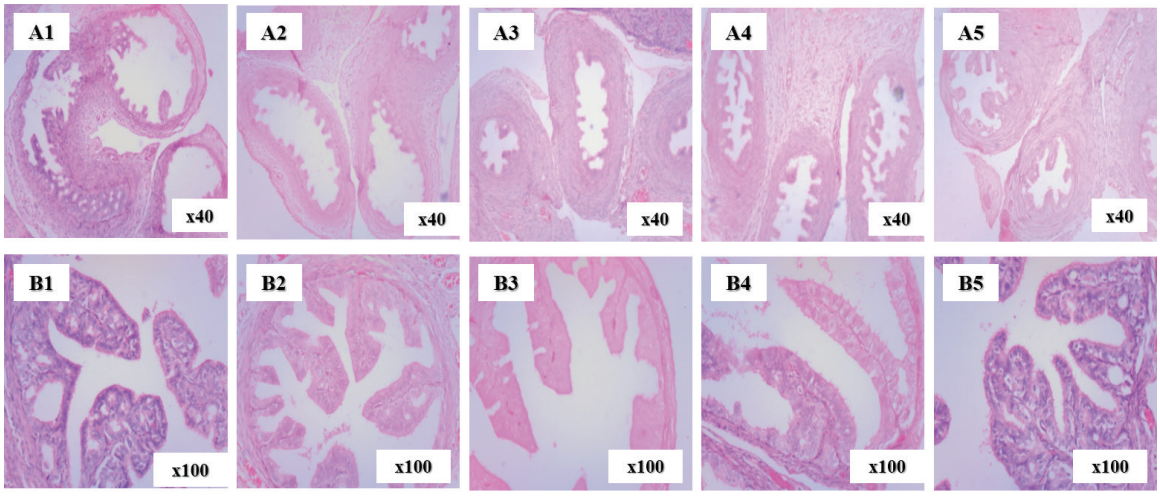

Figure 3. H&E stained sections of oviduct (A1–A5) and fimbriae (B1–B5) after 21 days of drug treatments. Generally fimbrae with atypical pseudostratified ciliated columnar epithelium, clumped microvilli, and DBVs in B3, B4, and B5. A1 and B1 = NS (5 ml/kg), A2 and B2 = low-dose PFE (100 mg/kg), A3 and B3 = medium-dose PFE (200 mg/kg), A4 and B4 = high-dose PFE (500 mg/kg), and A5 and B5 = clomiphene citrate (50 mg/kg).

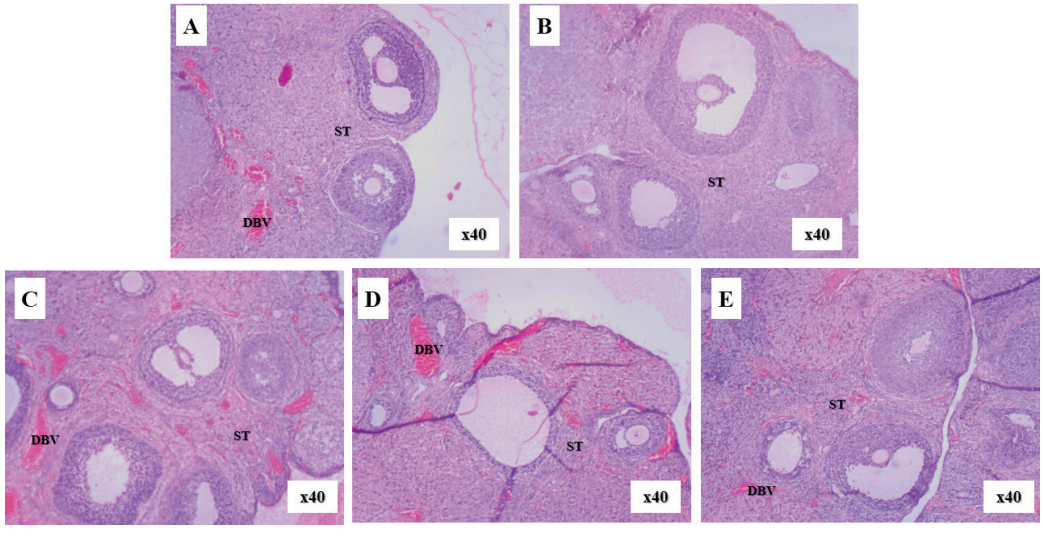

Figure 4. H&E stained sections of ovary after 21 days of drug treatments. There were marked dilatation of blood vessels near growing follicles and corpus luteum, infiltration of macrophages and lymphocytes in ovarian ST and corpus luteum, especially in C, D, and E. Normal saline (5 ml/kg) (A), low-dose PFE (100 mg/kg) (B), medium-dose PFE (200 mg/kg) (C), high-dose PFE (500 mg/kg) (D), and clomiphene citrate (50 mg/kg). ST = stroma; DBV = dilated blood vessel.

Table 2. Effect of 21-day drug treatments on serum levels of female sex hormones in non-pregnant rats.

Groups	FSH (IU/L)	Estrogen (pg/ml)	LH (IU/L)	FSH/LH ratio
NS	0.55 ± 0.50	10.63 ± 0.87	1.67 ± 0.53	0.29 ± 0.20
CL	0.59 ± 0.55	4.56 ± 0.78	0.34 ± 0.09*	1.88 ± 0.80*
PFE (mg/kg)				
100	0.41 ± 0.05	14.63 ± 0.42	48.99 ± 1.10*#	0.11 ± 0.01#
200	0.15 ± 0.03	0.08 ± 0.01*#	0.65 ± 0.09*	0.10 ± 0.04#
500	0.16 ± 0.02	2.31 ± 0.87*#	0.28 ± 0.08*	0.65 ± 0.02#

Each value is the mean ± SD, $N = 3$; $P < 0.005$ was considered statistically significant in all analysis; *compared with NS = normal saline (5 ml/kg), #compared with CL = clomiphene citrate (50 mg/kg).

Table 3. Effect of drug treatments on uterine weight and implantation at gestational day 6.

Treatment groups	No. of pregnant rats	Pregnancy rate[a]	Weight of uterus (g)[b]	Total implantation sites[b]
NS (5 ml/kg)	2	20	0.430 ± 0.042	9.50 ± 0.121
Mis (200 mg/kg)	3	30	0.267 ± 0.047[ns]	0.67 ± 0.077*
PFE (mg/kg)				
100	4	40	0.553 ± 0.048[ns]	4.25 ± 0.708*
200	3	30	0.833 ± 0.021[ns]	6.33 ± 0.055[ns]
500	4	40	0.295 ± 0.054[ns]	0.75 ± 0.057*

[a]Number of pregnant rats/10 * 100/2 weeks pre-treatment; [b]determined from only the pregnant rats in each group. Each value is expressed as mean ± SD (*n* = number of pregnant rats in the respective groups). The level of significance was determined using one-way ANOVA followed by Bonferroni's multiple comparison tests. $P \leq 0.05$ was considered statistically significant in all analyses; ns = not significant; * compared with NS; Mis = Misoprostol (Cytotec).

Table 4. Effect of drug treatments on uterine weight, implantation, and post-implantation loss at gestational day 15.

Treatment groups	No. of pregnant rats	Wet weight of uterus (g)	Total implantation sites	No of embryos	Resorption[a]	% Post-implantation loss[b]
NS (5 ml/kg)	4	13.56 ± 0.16	8.50 ± 0.03	8.50 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Mis (200 mg/kg)	2	9.72 ± 0.11[ns]	6.50 ± 0.71[ns]	4.50 ± 0.71*	2.00 ± 0.71*	30.76 ± 1.42*
PFE (mg/kg)						
100	4	15.76 ± 0.67[ns]	7.75 ± 0.26[ns]	7.75 ± 0.26[ns]	0.00 ± 0.00[ns]	00 ± 0.00[ns]
200	3	19.64 ± 0.17[ns]	7.33 ± 0.06[ns]	7.33 ± 0.06[ns]	0.00 ± 0.00[ns]	0.00 ± 0.00[ns]
500	2	8.85 ± 0.72[ns]	6.50 ± 0.56[ns]	1.50 ± 0.05*	5.00 ± 0.83[a]	76.9 ± 0.07*

Values are expressed as mean ± SD (*n* = 4). The level of significance was determined using one-way ANOVA followed by Bonferroni's multiple comparison test. $P \leq 0.05$ was considered statistically significant in all analyzes; ns = not statistically significant ($P \geq 0.05$) when compared to NS group (control); *$P \leq 0.05$ Treatments *vs*. NS; [a]Total implantation sites—No of embryos; [b]resorption/total implantation sites * 100; NS = normal saline (control) and Mis = misoprostol (Cytotec).

(100 mg/kg)-treated rats compared to control. PFE (200 and 500 mg/kg) and CL groups had low serum LH levels compared to control. Low-dose PFE (100 mg/kg) had significant increase in LH compared to control (Table 2).

PFE produced dose-related effects on pregnancy rate, implantation, and post-implantation loss

Two weeks pre-treatment of rats with PFE and misoprostol showed that PFE (100 and 500 mg/kg) improved pregnancy rate compared to control. Subsequently, post-pregnancy treatment for the first 6 days produced significant ($P < 0.05$) decrease in the number of implantation sites particularly in PFE (500 mg/kg)-treated dams relative to control. Mean wet uterine weight was comparable across groups although there were differences. PFE (100 and 200 mg/kg)-treated dams had a comparable though lower number of implantation sites relative to control but a significantly ($P < 0.05$) higher number of implantation sites compared to misoprostol-treated dams (Table 3). After 15 days of gestational exposure, PFE (500 mg/kg) and misoprostol (200 mg/kg) had significantly ($P < 0.05$) lower mean number of embryos but higher resorption and % post-implantation loss relative to control group (Table 4). However, low-dose PFE (100 and 200 mg/kg) produced a decrease in both resorption and % post-implantation loss comparable to control group. There were no significant ($P > 0.05$) differences in mean gravid uterine weight amongst groups relative to control group (Table 4).

Discussion

This study investigated follicular development in non-pregnant rats, and implantation and post-implantation loss in pregnant rats after exposure of animals to three increasing dose levels of PFE. Quite recently, PFE (100 mg/kg) was shown to improve caudal sperm count in male rats [8], and also, no observed adverse effect level in rodents was established to be over 1,000 mg/kg [6,7]. Conclusions from those two studies were that it should be avoided in pregnancy, since its long term effect in pregnancy cannot be predicted easily. Presently, we showed that PFE (100 mg/kg) significantly ($P < 0.05$) improved follicular development, specifically at the primordial and primary follicle stages and that observation correlated with increase in

the levels of estrogen and LH (Tables 1 and 2) even more than CL, a known ovulation inducer [21]. This observation indicates that PFE at 100 mg/kg may promote female sexual development. The hypothalamic–pituitary–gonadal axis of the endocrine system plays crucial role in sexual reproduction [22]. LH, one of the sex hormones crucial in this axis, physiologically acts on the gonads (testis and ovary) to enhance their development and function [23]. Serum levels of LH fluctuate to reflect the phase (follicular phase, mid-cycle, luteal phase, and post-menopausal phase) of normal menstrual cycle [24]. In particular, during the first phase of the menstrual cycle, LH stimulates the morphological transformation of follicles to Graaf's follicle [25]. Importantly, estrogen is produced in both males and females [26]. In females, it is produced by follicles upon stimulation by FSH. Estrogen is highly bound when in circulation, therefore, its physiological effect is attributed to the free portion which is normally about 2% [27]. In non-pregnant animals, it is secreted in a biphasic manner, for example, high levels are detected prior to ovulation in humans [28,29]. In this study, it was observed that PFE improved primordial and primary follicular development but not secondary and Graaf's follicles. For instance, the number of primordial and primary follicles in both right and left ovaries increased in low-dose PFE (100 mg/kg) group and correlated with increased estrogen and LH levels suggesting that the surge in LH and estrogen levels may have played a crucial role, and that at low doses (≤100 mg/kg), PFE may improve follicular development in non-pregnant mammals. Although the number of primordial follicles increased in CL and medium-dose PFE (200 mg/kg) groups; however, these observations negatively correlated with estrogen and LH levels, perhaps indicating that follicular development, specifically at the primordial stage may not be entirely under hormonal regulation but could also involve other unknown factors including non-hormone factors. Although PFE (100 mg/kg) treatment in non-pregnant rats improved uterine musculature, particularly thickness of the endometrial layer, increased uterine glands, and also improved ovarian microstructures such as the fimbriae, high-dose PFE (200 and 500 mg/kg) produced not only collagenous endometrial layer but also DBVs in the vicinity of developing follicles relative to control but these changes have no toxicological relevance.

To ascertain whether PFE may improve pregnancy rate, implantation, and post-implantation events after gestational exposure for 15 days, it was observed that at gestational day 6, PFE (100 mg) improved pregnancy rate which correlated positively with weight of gravid uterus but negatively correlated with the number of implantation sites. This observation perhaps corroborate an earlier observation where PFE (100 mg/kg) improved folliculogenesis, a crucial step for ovulation [10]. At gestational day 15, PFE (100 and 200 mg/kg) improved post-implantation loss, but the high-dose PFE (500 mg) group was associated with significant ($P < 0.05$) post-implantation loss compared to control and misoprostol, a known abortifacient agent [30,31]. The present study could have benefited from investigating the effect of PFE on pregnant rats up to delivery and post-delivery events such as risk of congenital malformations (teratogenicity) in pubs and post-partum maternal toxicity, notwithstanding our results provide the rationale for further reproductive studies on PFE in view of the fact that it is been used in folk medicine for many indications [5–7]. The observed effects of PFE in non-pregnant and pregnant rats are attributable to the phytochemical composition of PFE. Interestingly, alkaloids, saponins, cyanogenic glycosides, and sterols were detected in PFE corroborating an earlier study on *P. fruticosa* [8]. As indicated earlier, the observed effects of PFE are attributable to its phyto-constituents as reported elsewhere [8].

Conclusion

Put together, PFE at 100 mg/kg improved follicular development at the primordial and primary follicle stages in non-pregnant rats, improved post-implantation loss in pregnant rats, but gestational exposure of PFE > 100 mg/kg should be avoided in pregnancy despite the need to pursue translational application of PFE.

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Conflict of Interest

The authors declare no conflict of interest.

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MINI REVIEW

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Ethnopharmacognosy of *Echinops spinosus* L. in North Africa: a mini review

Amel Bouzabata[1], Fawzi Mahomoodally[2], Carlo Tuberoso[3]

[1]Faculty of Medicine, Badji-Mokhtar University, Annaba, Algeria

[2]Department of Health Sciences, Faculty of Science, University of Mauritius, Moka, Mauritius

[3]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy

ABSTRACT

Background: The genus *Echinops* (Asteraceae family, Echinopeae class) consists of ca. 120 species and is native to Africa, the Middle East, Europe, and Asia. In Algeria, this genus is represented by the very common species *Echinops spinosus* L. also known as "*tesskra,"* which is used as a diuretic, hypoglycemic, for stomachic effects, liver disorders, and post-partum care.

Objective: The aim of this presentation is to provide an overview of the ethnopharmacognosy studies conducted on *E. spinosus* in North Africa. Data on ethnomedicinal uses, chemical constituents, and pharmacological activity were systematically compiled.

Methods: Several popular search databases, including PubMed, ScienceDirect, Scopus, Web of Science, and Stanford libraries were scrutinised to extract relevant information. The research focused only on English-written papers published between 1980 and 2017.

Results: *Echinops spinosus* L. is traditionally used in North Africa, and it was found that the most ethnomedicinal use reports were from Morocco and Algeria. Promising results have been reported regarding its phytochemistry and pharmacological activity. Forty-three compounds were isolated from different parts of this species. No studies have been conducted to highlight the toxicity and clinical safety of this species.

Conclusion: This review highlights the therapeutic potential of *E. spinosus* used in traditional medicine. Furthermore, clinical trials on standardized preparations are necessary to explore the full safety and efficacy of *E. spinosus* in North Africa.

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Echinops species; Algeria; *E. spinosus*; ethnomedical properties; pharmacology

Introduction

The genus *Echinops*, belongs to the family Asteraceae (formerly Compositae) and comprises ca. 120 species distributed throughout the Mediterranean region, in central Asia, and in tropical Africa [1]. In Algeria, this genus is represented by the very common species *Echinops spinosus* L. According the African Plant Database, as well as the Plant List database, this name is synonymous with *E. spinosissimus* Turra [2–4]. It thrives in arid desert conditions with an annual rainfall varying between 20 and 100 mm, and has a wide ecological range for soil, including coastal, calcareous dunes, sandy, and gravelly to rocky surfaces [5]. Botanical classifications have subdivided *Echinops spinosus* L. into two subspecies [6,7]:

E. spinosus ssp. eu. *spinosus* Maire (var. *chaetocephalus* Pomel) and *E. spinosus* ssp. *bovei* (Boiss.) Maire (var. *pallens* Maire.), which is also known as *E. bovei* Boiss [8]. Recent data provided by synonymic survey of the Cardueae (Compositae) genera database, validated the scientific name of *E. spinosissimus* subsp. *bovei* (Boiss.) Greuter ≡ *E. bovei* Boiss [9].

Three other species have been reported in Algeria, but appear to be not very common: a) *E. ritro* L., known under the name of "*oursin bleu*" or "echinops" in French, has a southern European distribution, and occurs in southern Europe, western Asia, and even Siberia; b) *E. sphaerocephalus* L. is a mountainous species; and c) *E. strigosus* L.

Contact Amel Bouzabata ✉ amelbouz2009@gmail.com Faculty of Medicine, Badji-Mokhtar University, Annaba, Algeria.

is distributed in the Iberian and North Africa area, especially in southern Spain and Algeria, and is widespread in the most western part of the Tell, from Tenes to the Moroccan border [6]. The literature reveals that 24 species of the genus *Echinops* have been subjected to varying degrees of scientific investigation [10]. Conversely, very little is known about *E. spinosus* [11,12]. Therefore, the objective of this review is to provide a detailed comparison of the chemical composition and pharmacological properties displayed by *E. spinosus* in North Africa with the widely studied species. Several search databases, including PubMed, ScienceDirect, Scopus, and Web of Science, were probed to extract information between 1980 and 2017.

Figure 1. Morphological aspect of *Echinops spinosus*

Vernacular names

In Algeria, *E. spinosus* L. is known in the Berber language under the names *"Taskra," "Teskera," "Taskra" Ameskelit T,* and *Sarsor,* and in Arabic by the names: *"fouga el djemel," "chouk el djemel," "suk ej-jmal," Kachir, Ikchir, Chouk el Hamir, Chicaou,* and *Sorr* [13–15]. In the Tamahaq language, this species is called Téfariast [16].

The Arabic name of "*qounfoudzia*" (de hérisson), is the transcription in Greek of "*Ekhinos.*" The vernacular name of "*ri ayi el-ibil*" has the meaning "Camel Pasture" [14]. In Morocco, this species is known under the names of "*tasekra, asekra, teskra, chouk el hamir, suk al-himar,* and *tîmat*" [17].

Botanical description and habitat

E. spinosus is a perennial herb growing to 1 m and more, with erect brownish to reddish stems, few long leaves from 10 to 15 cm, hairy, arachnoids, and with very long spines. The inflorescence is often a single hemispherical globe up to 5 cm in diameter during the flowering period. It is surrounded with numerous long spines (Figure 1). The small hermaphroditic flowers that compose the dense head are tubular, turning from green to white and yellowish when in full bloom. The fruits are small achenes topped by membranous scales to ease dispersion [18].

In Algeria, two very polymorphous subspecies have been described: 1) **ssp. *bovei* (Boiss.) Maire**: stems pubescent, not glandular. The achenes are composed into distinct pieces at the base. The leaves are whitish and woolly on both sides. *E. bovei* is a Southern Mediterranean-Saharan taxon, and is widespread in Algeria [19,20]; and 2) **ssp. eu. *spinosus* Maire**: annual plant, upright and firm stems, from 40 to 60 cm. The distribution of *E.* eu *spinosus* is limited to the pre-desert regions in septentrional and central Sahara and is considered as a Saharo-Sindian taxon [6,7].

Phytochemistry

The genus *Echinops* is one of the taxa with characterized alkaloids within the Asteraceae family [21]. An overview of the literature on *E. spinosus* showed that reports concerning the phytochemistry of the Algerian species are very limited, with only two studies undertaken [11,12]. Preliminary qualitative phytochemical screening of various secondary metabolites by specific chemical tests was carried out on extracts of the aerial parts and roots, which indicated that the aqueous extract contained alkaloids, tannins, flavonoids, quinones, reducing sugars, and starch [11].

Phytochemical investigations of *E. spinosus* from North Africa in Morocco, Algeria, Tunisia, and Egypt led to the isolation and identification of 42 metabolites belonging to the phytochemical classes of quinoline alkaloids (S_1–S_2) [1], sesquiterpenoids (S_3) [22], flavonoids (S_4–S_{26}) [12,23], and sterols (S_{27}–S_{39}) [24]. The names of the isolated compounds and their sources are provided in Table 1 and the chemical structures are depicted in Table 2.

In 2009, the isolation of two sesquiterpenoids with a novel carbon framework was reported and named echinopine A and echinopine B [22]. In 2016, Bouattour et al. [24] identified 13 sterols in *E. spinosus* from Tunisia. The two most abundant compounds were ß-sitosterol (44.97%) followed by stigmasterol (34.95%) [24]. In the same year, the occurrence of flavonoids was reported in the aerial parts of *E. spinosus* from Algeria and Egypt.

Table 1. Phytochemical constituents of *Echinops spinosus*.

Extract	Compound	Structure	Reference
E[a]	Echinopsine	S_1	[1]
	Echinorine	S_2	
E[b]	Echinopine A	S_3	[22]
	Echinopine B		
E[c]	Apigenin	S_4	[12]
	Apigenin-7-*O*-ß-glucopyranoside	S_5	
	Apigenin-7-β-D-*O*-(6”-*O*-*E*-*p*-coumaroyl-glucopyranoside	S_6	
E[d]	Luteolin-6-arabinose-8-glucoside	S_7	[23]
	Luteolin-6-glucose-8-arabinoside	S_8	
	Apigenin-6-arabinose-8-galactoside	S_9	
	Apigenin-6-arabinose-8-glucoside	S_{10}	
	Apigenin-6-glucose-8-rhamnoside	S_{11}	
	Luteolin-7-glucoside	S_{12}	
	Narengin	S_{13}	
	Rutin	S_{14}	
	Hesperidin	S_{15}	
	Quercetin-3-*O*-glucoside	S_{16}	
	Rosmarinic acid	S_{17}	
	Apigenin-7-*O*-neohespiroside	S_{18}	
	Kaempferol-3,7-dirhamnoside	S_{19}	
	Apigenin-7-glucoside	S_5	
	Quercetrin	S_{20}	
	Quercetin	S_{21}	
	Naringenin	S_{22}	
	Hespiritin	S_{23}	
	Kaempferol	S_{24}	
	Rhamnetin	S_{25}	
	Apigenin	S_4	
	Acacetin	S_{26}	
E[e]	β-Sitosterol	S_{27}	[24]
	Stigmasterol	S_{28}	
	Campesterol	S_{29}	
	Brassicasterol	S_{30}	
	Campestanol	S_{31}	
	Δ7-Campesterol	S_{32}	
	Δ5,23-Stigmastadienol	S_{33}	
	Cholesterol	S_{34}	
	Sitostanol	S_{35}	
	Δ5-Avenasterol	S_{36}	
	Δ5,24-Stigmastadienol	S_{37}	
	Δ7-Stigmastenol	S_{38}	
	Δ7-Avenasterol	S_{39}	
E[f]	2,2-Ddimethyl-4 [5’-(prop-1-ynyl)-2,2’-biothiphen-5-yl]-1,3-dioxalane	S_{40}	[25]
E[g]	11-Hydroxyisocom-2-en-5-one	S_{41}	[26]
E[h]	A-neooleana-3(5),12-diene	S_{42}	[27]

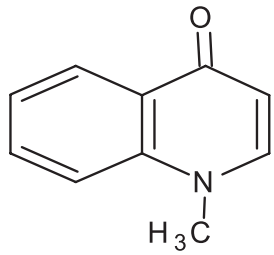
E[a] = chloroform extract prepared from ripe fruits collected in Deltaic coast Egypt, E[b] = methanol extract prepared from root collected in Morocco (2003), E[c] = ethyl acetate extract prepared from aerial parts collected in North Eastern Algeria (April 2009), E[d] = aqueous ethanolic extract prepared from aerial parts collected in Egypt, E[e] = Hexane extract prepared from flower heads in Tunisia, E[f] = dichloromethane extract prepared from roots collected in Morocco, E[g] = dichloromethane extract prepared from roots collected in Morocco, E[h] = crude methanol and ethyl acetate extracts from flowers collected from Sfax, South Tunisia (June 2011).

Twenty-three flavonoids were isolated [12, 23]. One year later, Bouattour et al*.,* isolated a new derivative of apigenin named apigenin-7-*O*-ß-D-glucoside-(4”-*O*-*trans*-*p*-coumaroyl) [27].

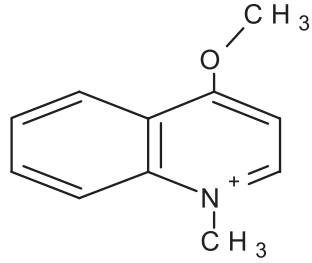
The occurrence of simple quinoline alkaloids in the aerial and/or underground parts was reported in *E. ritro*, *E. echinatus* Roxb [28,29], *E. albicaulis* [30]*,* and *E. niveus* [31]. Furthermore, members of the genus *Echinops* are also reported to contain flavonoids, triterpenoids, and thiophene acetylenes [21,29,30,32–34].

As far as information provided in the literature, thiophenes are a class of heterocyclic compounds which are characteristic secondary

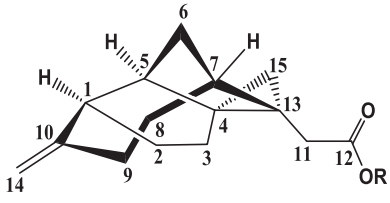
Table 2. Chemical structures of isolated compound extracted from *Echinops spinosus* L.



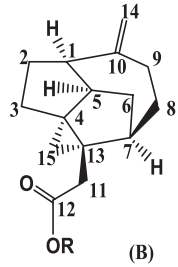
S_1



S_2



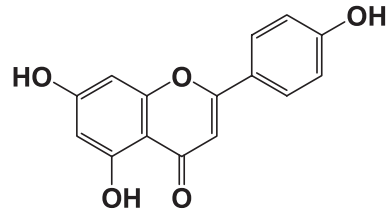
(A)



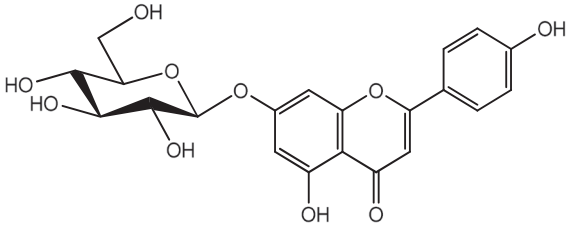
(B)

(A) R = H
(B) R = CH_3

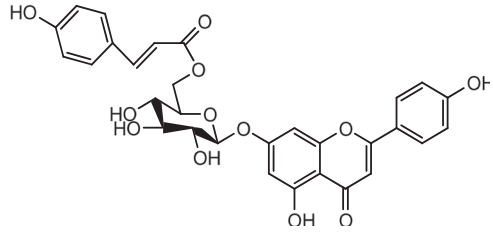
S_3



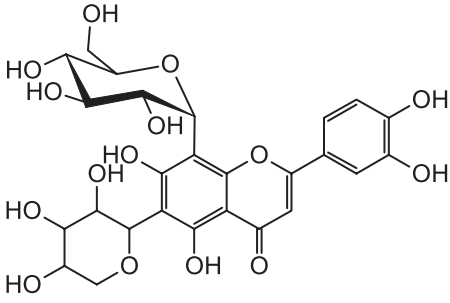
S_4



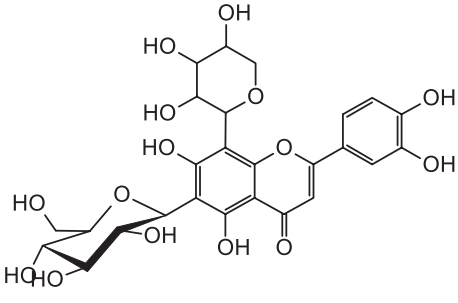
S_5



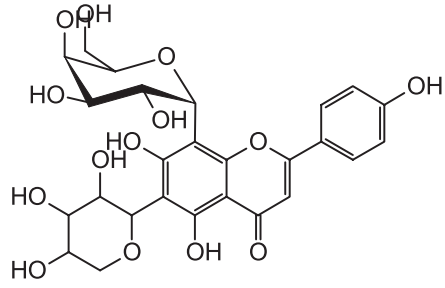
S_6



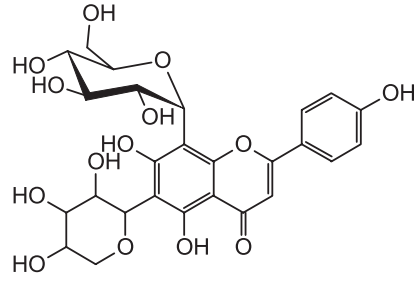
S_7



S_8

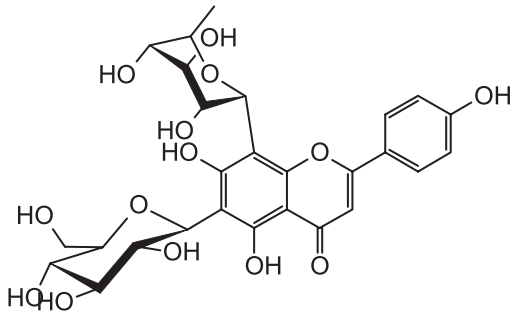


S_9

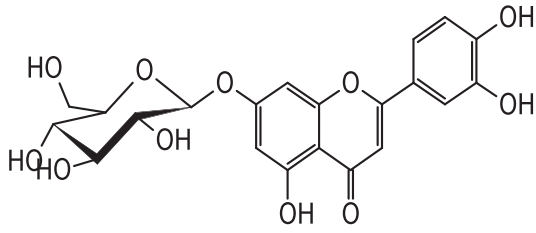


S_{10}

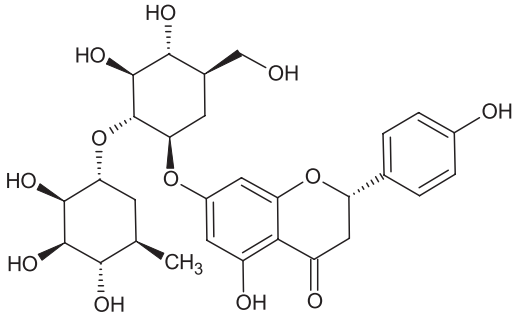
Table 2. Chemical structures of isolated compound extracted from *Echinops spinosus* L. (Continued)



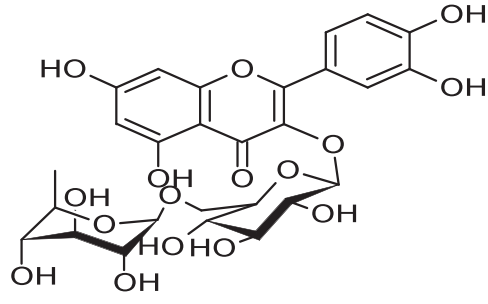
S_{11}



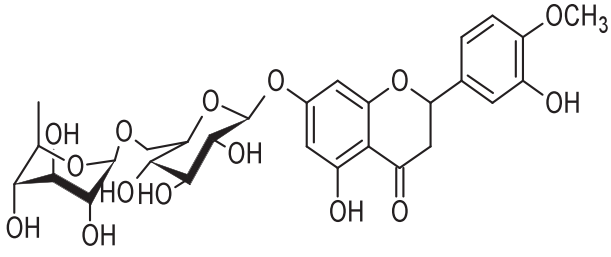
S_{12}



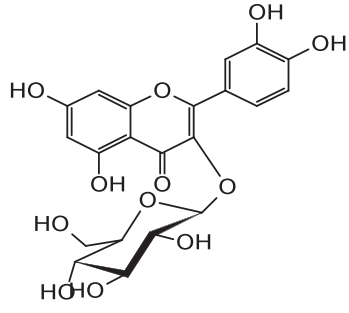
S_{13}



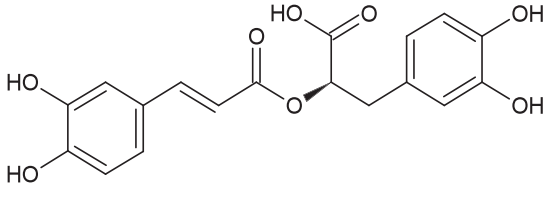
S_{14}



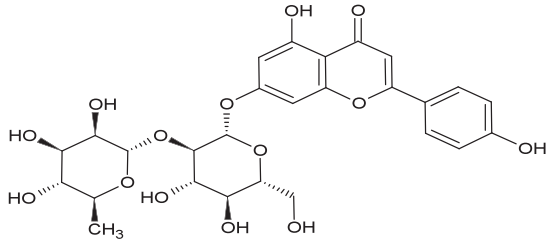
S_{15}



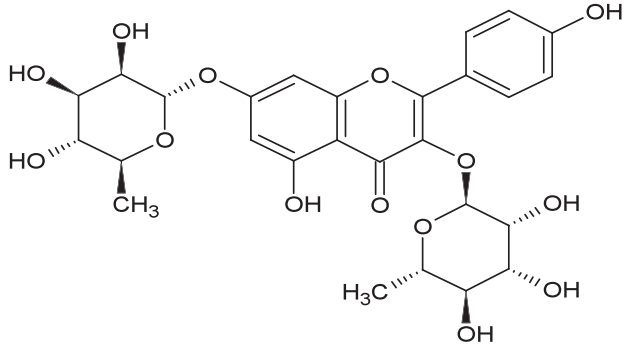
S_{16}



S_{17}

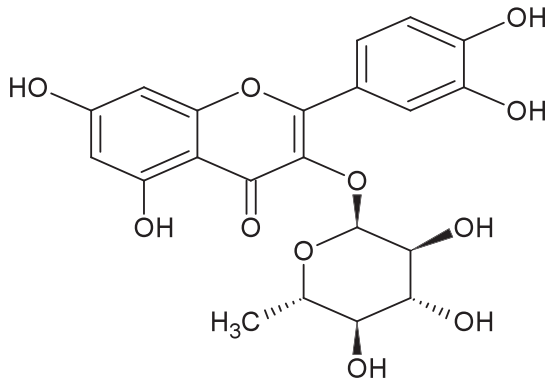


S_{18}

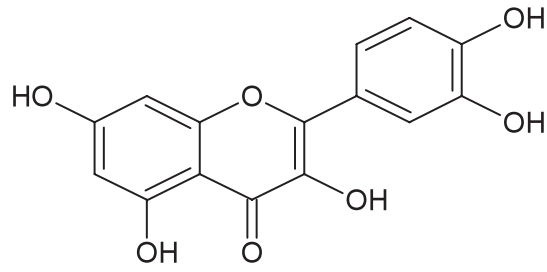


S_{19}

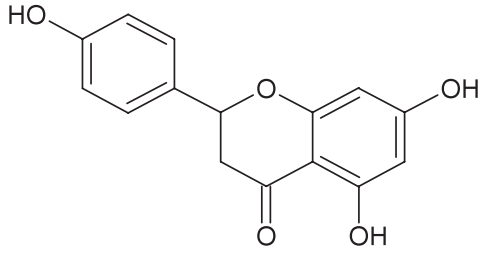
Table 2. Chemical structures of isolated compound extracted from *Echinops spinosus* L. (Continued)



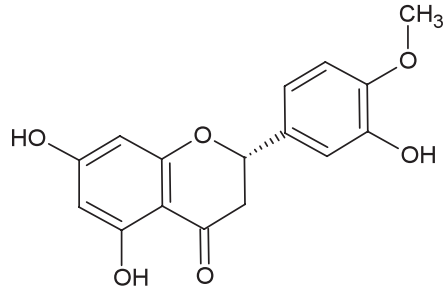
S_{20}



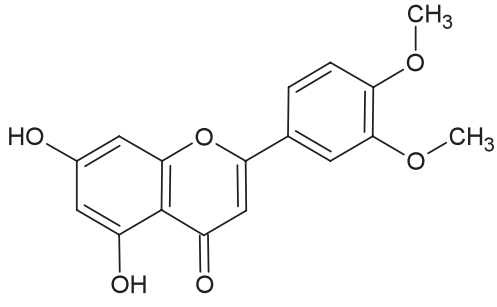
S_{21}



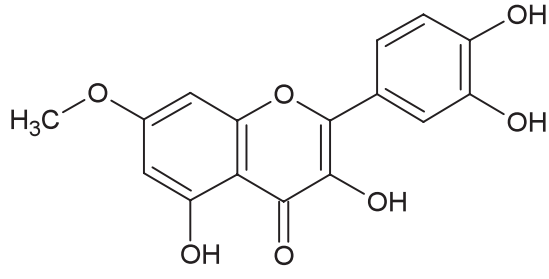
S_{22}



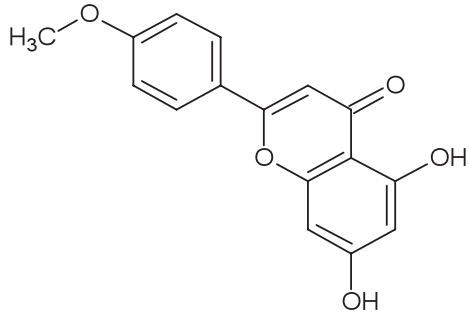
S_{23}



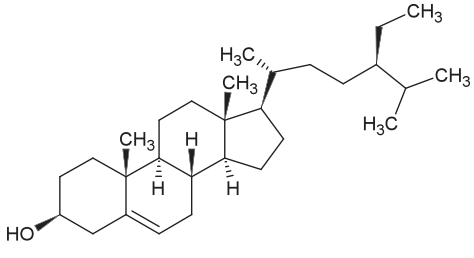
S_{24}



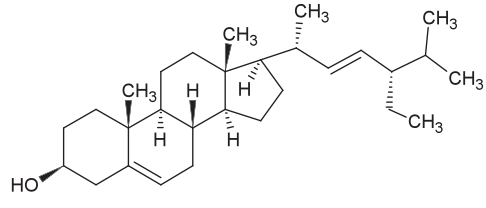
S_{25}



S_{26}

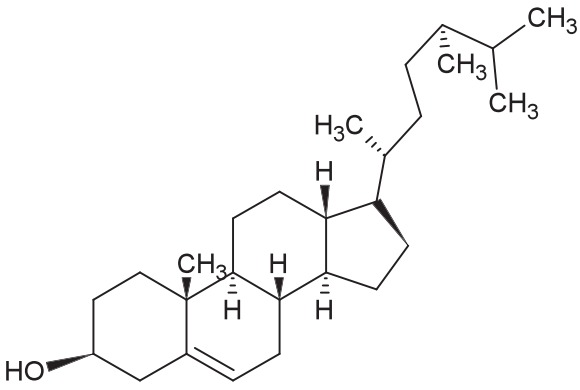


S_{27}

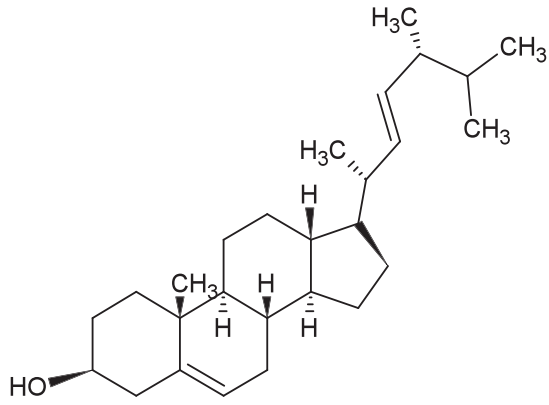


S_{28}

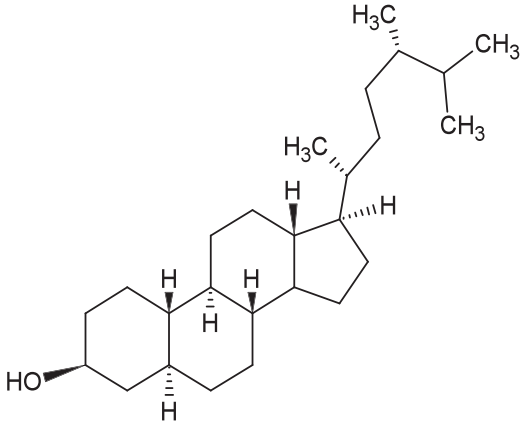
Table 2. Chemical structures of isolated compound extracted from *Echinops spinosus* L. (Continued)



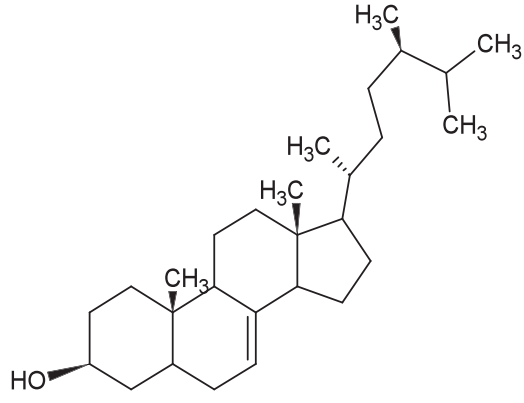
S_{29}



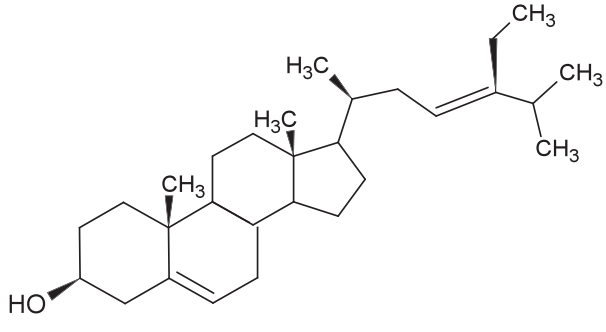
S_{30}



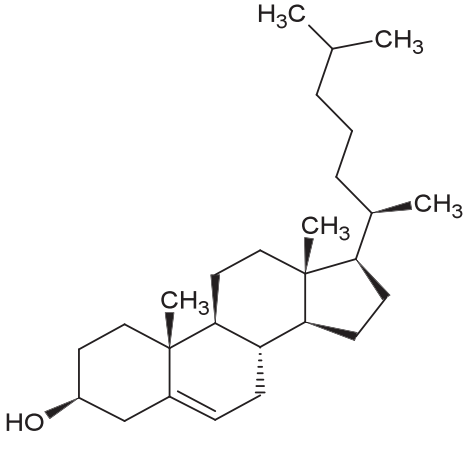
S_{31}



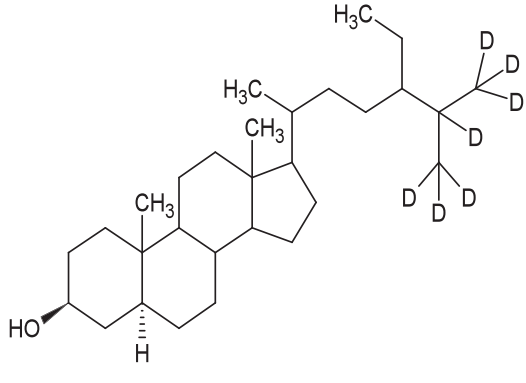
S_{32}



S_{33}

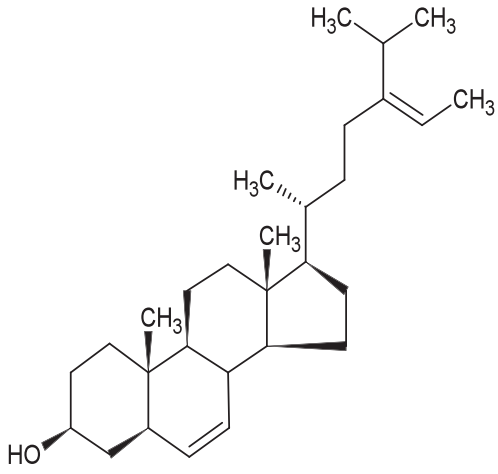


S_{34}

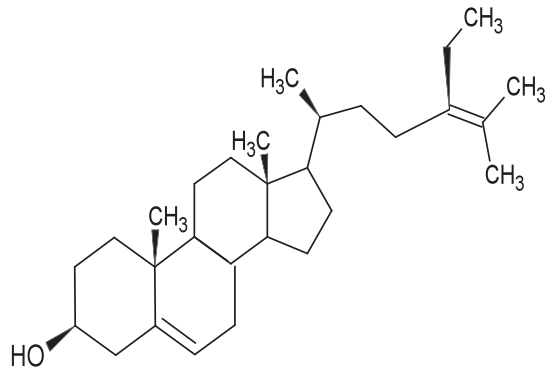


S_{35}

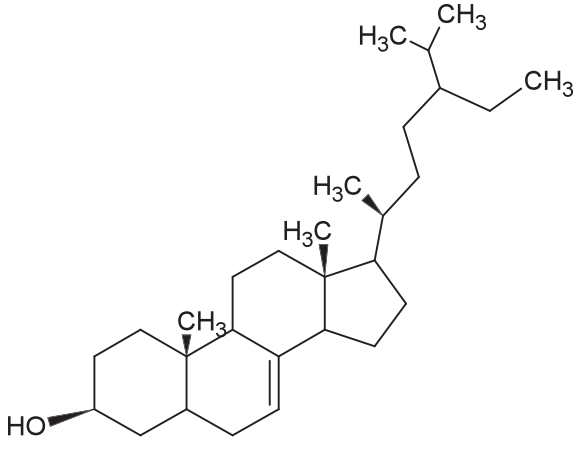
Table 2. Chemical structures of isolated compound extracted from *Echinops spinosus* L. (Continued)



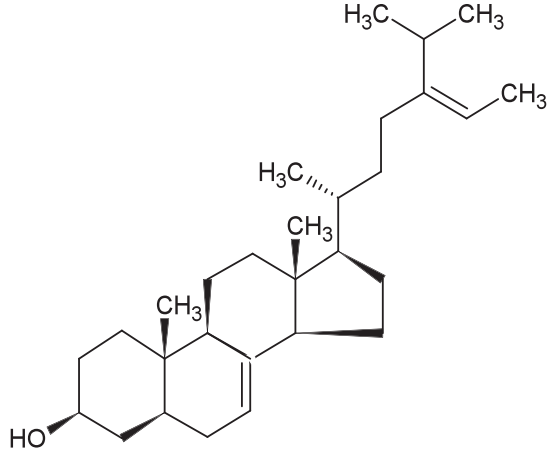
S_{36}



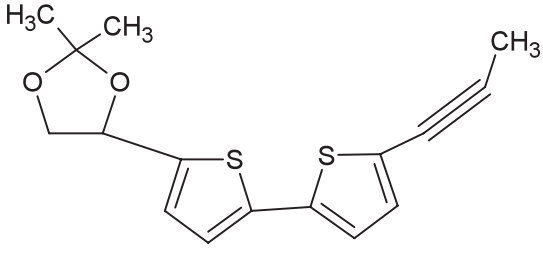
S_{37}



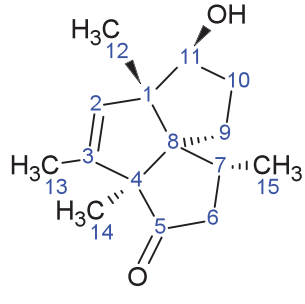
S_{38}



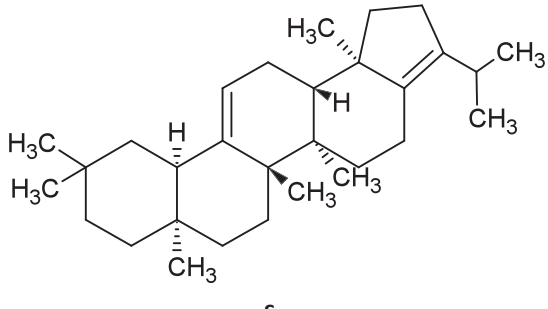
S_{39}



S_{40}



S_{41}



S_{42}

Table 3. Traditional uses and ethnobotanical information on *Echinops spinosus*.

Country	Plant part	Popular name	Geographical distribution	Medicinal uses	Reference
Morocco	Root	*Tassekra*	Spontaneous	Diuretic, hypoglycemic, stomachic, for liver disorders, and post-partum care	[17]
Morocco	Root	*Tassekra, Kherchouf, Chouk al-Himar*	Not indicated	Hypoglycemic, diuretic, post-partum care, liver disorders, appetizing, depurative, and abortive	[38]
Morocco	Not indicated	Not indicated	Not indicated	Against cold, and pain, renal colic, and disinfectant. Warming plant	[39]
Morocco	Root	*Tassekra*	Spontaneous	Obstetric Decoction of roots is administered to women after delivery to expel the placenta and for blood flow	[40]
Morocco	Root and aerial parts Roots and branches Flowers	*Taskra, Bongar*	Hemicryptophyte	Decoction used for colds, kidney, stones disinfectant, diuretic, and hypoglycemic. Decoction: abortifacient, labor pains Tisane: neuralgia, fatigue	[41]
Morocco	Seeds	*Taskra*	Not indicated	Infusion is used as an antidiabetic	[42]
Morocco	Rhizomes	*Taskra*	Not indicated	Decoction: stomachic disorders	[43]
Algeria Mascara	Roots	*Tassekra*	Not indicated	Genital infections (after an abortion), urinary tract infections, inflammation of the kidneys, and blood circulation	[44]
AlgeriaTiaret	Not indicated	*Taskra*	Not indicated	Hygienic agent employed for gynecological reasons	[45]
AlgeriaSetif	Roots and Fruits	Not indicated	Therophyte, southern Mediterranean Sahara	Labor pains, abortion, and neuralgia	[19]
AlgeriaTassili des N'jjers	Aerial Part	*Tefaryast Tassegra*	Saharo-arabic	Infusion in internal use: Eye complaints, trachoma, sore inflammation, digestive diseases, spasms, colic, and fever	[20]

metabolites derived from plants belonging to the family Asteraceae such as *Echinops*. Based on this, the distribution of thiophenes in different species of the genus *Echinops* has been examined in six species, including: *E. grijissii* Hance, *E. pappii* Chiov, *E. hispidus* Fresen, *E. transiliensis* Golosh, *E. latifolius* Taush, and *E. ritro* L. [34]. Twenty thiophenes have been reported in nine Ethiopian species: *E. amplexicaulis*, *E. papii*, *E. ellenbeckii*, *E. hispidus*, *E. hoehnelii*, *E.kebericho*, *E. longisetus*, *E. macrochaetus*, and *E. giganteus* [10]. In parallel, it should be noted that *E. spinosus* has not been screened for thiophene composition; only one chemical report described the structure of a new thiophene in roots collected from Morocco, which is known as the acetylene 2,2-dimethyl-4 [5'-(prop-1-ynyl)-2,2'-bithiophen-5-yl]-1,3-dioxalane (S_{40}) [26]. Furthermore, a new sesquiterpenoid 11-hydroxyisocom-2-en-5-one (S_{41}) was described for the first time in the dichloromethane extract of the roots of *E. spinosissimus* subsp. *spinosus* Greuter from Morocco [22]. In 2017, Bouattour et al., isolated a C_{30}-pentacyclic triterpadiene A-neooleana-3(5),12-diene (S_{42}), which might be a marker of identification of *E. spinosus* from other species of *Echinops* [27].

Ethnobotanical aspects

Traditional preparations of *E. spinosus* are frequently used in folk medicine as an abortifacient, as a diuretic, and for blood circulation, diabetes, gastric pain, indigestion, and spasmolytic problems [35].

In traditional medicine practices, *E. spinosus* is known in the Chinese [36] and North African traditions [37]; the latter reporting the ethnomedicinal use of the stems, leaves, and roots as a diuretic drug.

In Algeria, the roots or flower heads of *E. spinosus* have been used in the treatment of prostatism and dysmenorrhea. This botanical remedy has also been used as a peripheral vasoconstrictor in the treatment of hemorrhoids, varicose veins, and varicocele, in various venous hemorrhages and in metrorrhagia. It is considered as a hypertensive drug [13,14]. Table 3 presents the diverse ethnomedicinal uses of various parts of *E. spinosus* in North Africa.

Table 4. *In vivo* anti-inflammatory properties of *E. spinosus* and *E. echinatus*.

Species	Extract, Plant Part	Dose: dmi, dma	Inhibition	Inflammation model	Route of administration	Control-dose % of inhibition	Reference
E. spinosus	Aqueous, RH	100 mg/kg	59.5 %*	Carrageenan-induced rat sub-plantar edema	**i.p.**	IMC3 mg/kg	[47]
	Ethanol, RH	100 mg/kg	21.3%*				
	Chloroform, RH	100 mg/kg	67.4%*				
E. spinosus	Aqueous, RH	3 mg/ear	NI	Arachidonic acid-induced mouse ear edema	**i.p.**	IMC1 mg/kg	[47]
	Ethanol, RH	3 mg/ear	51.0%				
	Chloroform, RH	3 mg/ear	56.0%				
E. echinatus	Ethanol, WP	100 mg/kg	i.p.: 38.9%** i.o.: 13.3%**	Carrageenan-induced rat sub-plantar edema	**i.p.** **i.o.**	PBZ5 mg/kg	[51]
		800 mg/kg	i.p.: 67.5%** i.o.: 51.8%**				
E. echinatus	Ethanol, WP	25 mg/kg	a.c: 18.2%*** a.ch: 32.3%**	Formaldehyde-induced acute and chronic reactions in rats	**a.c:** + 4 hours reaction. **a.ch:** + 10 days	PBZ50 mg/kg	[51]
		200 mg/kg	a.c: 50.3%*** a.ch: 54.3%**				
E. echinatus	Ethanol, WP	25 mg/kg	a.c: 23.6%** a.ch: 44.1%**	Adjuvant-induced acute and chronic reactions in rats	**a.c:** + 18 hours reaction. **a.ch:** + 21 days	PBZ50 mg/kg	[51]
		200 mg/kg	a.c: 75.4%** a.ch: 75.6%**				
E. echinatus	TA	12.5 mg/kg	13.79%**	Carrageenan-induced rat sub-plantar edema		PBZ50 mg/kg 46.23%**	[52]
		200 mg/kg	63.25%**				
E. echinatus	TA	10 mg/kg	a.c: 17.72%*** a.ch: 29.70%**	Formaldehyde-induced inflammation	**a.c:** + 4 hours reaction. **a.ch:** + 10 days	PBZ50 mg/kg a.c: 22.69%** a.ch: 56.15%**	[52]
		80 mg/kg	a.c: 50.70%** a.ch: 70.64%**				
E. echinatus	TA	10 mg/kg	a.c: 29.50%** a.ch: 39.62%**	Adjuvant-induced inflammation	**a.c:** + 18 hours reaction. **a.ch:** + 21 days	PBZ50 mg/kg a.c: 34.16%** a.ch: 64.21%**	[52]
		80 mg/kg	a.c: 57.91%** a.ch: 67.47%**				
E. echinatus	Flavanone A	45 mg/kg	34.21%	Carrageenan-induced paw oedema	**i.p.**	ASA30 mg/kg 52.63%	[53]

dmi = minimal dose, dma = maximal dose, RH = rhizome, WP = whole plant, NI = not investigated, i.p. = intraperitoneal administration, i.o. = oral administration, a.c. = acute reaction, a.ch. = chronic reaction,
TA = taraxasterol acetate, Flavanone A = 5,7-dihydroxy-8,4′-dimethoxy-flavanone-5-O-α-L-rhamnopyranosyl-7-O-β-D-arabinopyranosyl-(1→4)-O-β-D-glucopyranoside, IMC = indomethacin, ASA = acetyl salicylic acid.
*$p < 0.02$, **$p < 0.01$, ***$p < 0.05$.

Pharmacological properties

Anti-inflammatory activity

Over a long period of time, many medicinal plants have been used for the treatment and management of various forms of inflammatory conditions by African traditional healers and herbalists. However, most of these plants are not documented as compared to the Chinese or Indian traditional medicines. About 5,000 plant species have used for centuries for the treatment of various diseases, including anti-inflammatory diseases. A few African medicinal plants with demonstrated anti-inflammatory and analgesic properties have been documented in the last two decades [18].

The genus *Echinops* is used traditionally in North Africa for its anti-inflammatory actions [46]. In 1999, Rimbau et al., assessed the anti-inflammatory activities of the aqueous, ethanol, and chloroform extracts from the rhizome of *E. spinosus* [47]. Two experimental methods were used: a) carrageenan-induced rats sub-plantar edema inflammatory

models [48,49] with administered extracts at a dose of 100 mg/kg and the reference group was treated with indomethacin (intraperitoneal administration: i.p., 3 mg/kg), and b) the arachidonic acid-induced mouse ear edema [49,50], where extracts were studied at a dose of 3 mg/ear, and the reference group was treated with indomethacin (1 mg/ear). All of the tested extracts showed significant anti-inflammatory activities in both experimental models. However, the chloroform extract showed higher anti-inflammatory activity for the carrageenan experimental model inhibition in rats, with a mean of percentage inhibition of 67.4%, compared with 32.4% for the reference group. In the experimental model in mice, the percentage inhibition for the chloroform extract was 56.1%, compared with 34% for the reference group. Table 4 summarizes the model of inflammation, the extracts used in the study, and the positive control used.

In parallel, a wide range of anti-inflammatory activity has been shown for *E. echinatus* used in the Indian System of Medicine for the treatment of fever and inflammatory diseases. In 1989, *E. echinatus* L. was extensively studied for its acute anti-inflammatory induced in rats by carrageenan, formaldehyde-induced acute and chronic arthritis, and adjuvant-induced acute and chronic arthritis. Taking in to account the methods described to assess the anti-inflammatory activity of *E. spinosus* [48], it was found that the ethanol extract of the whole plant of *E. echinatus* at a dose of 100 mg/kg was less effective than *E. spinosus*. It is noticeable that the percentage of inhibition in the acute carrageenan paw edema was higher in intraperitoneal (i.p.) than oral (p.o.) dosing, with a percentage of inhibition 38.9% ± 2.5%, *versus* 13.3% ± 5.3%, compared with the reference group treated with Phenylbutazone (PBZ) (5 mg/kg) of 61.3% ± 2.8% (i.p.), *versus* 44.6% ± 4.3% for i.o. administration [51].

Another study isolated a triterpenoid taraxasterol acetate from *E. echinatus* and was reported to have an anti-inflammatory effect in albino rats for carrageenan, formaldehyde, and adjuvant-induced inflammation. The effects were dose dependent, and its efficacy was approximately 0.25–2 times that of the reference drug PBZ administrated per i.p. [52].

Interestingly, a new anti-inflammatory agent has been isolated from the methanolic extract from leaves of *E. echinatus*, 5,7-dihydroxy-8,4'-dimethoxy-flavanone-5-*O*-α-L-rhamnopyranosyl-7-*O*-β-D-arabinopyranosyl-(1⟶4)-*O*-β-D-glucopyranoside carried out with non-immunological carragenan-induced hind paw oedema method, which showed an in i.p. administration an inhibitory effect 0.67 times that of the reference drug (Table 4) [53].

Antioxidant activity

The antioxidant activity of flavonoids and tannin extracts from the aerial part (stems and leaves) and roots of *E. spinosus* from Tlemcen was assessed by two methods: the reduction of ferric reducing antioxidant power (FRAP) and the trapping of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). This study showed that the tannin-containing ethyl acetate extract of the aerial arts of *E. spinosus* had higher capacity of reducing iron and also free radical scavenging activity in the DPPH test than extracts of the roots. The inhibitory concentration (IC_{50}) in the DPPH method was 8.25 µg/ml for the aerial parts (*vs*. 23 µg/ml for roots), while the FRAP method confirmed the high reduction capacity at a concentration of 2.5 mg/ml (optical density = 2.85) of tannin-containing ethyl acetate extract of aerial parts compared with the root extracts [11]. Similarly, Khedher et al. (2014) showed that the ethanol extract of *E. spinosus* had the greatest ability to reduce DPPH radicals, with an IC_{50} value of 147 µg/ml. As expected, it was reported for the roots of *E. spinosus* that there is a positive correlation between the condensed tannin content and activity in the DPPH assay [35].

Comparatively, high scavenging DPPH activities were shown for the methanolic extract of seeds and leaves of *E. orientalis* [54]. It was shown that the aqueous extract of *E. ritro* is a source of phenolic compounds based on gallic acid, measured by Folin–Ciocalteau methods (92.24 Gallic Acid Equivalents (GAE) mg/100 g), and exhibited higher DPPH scavenging activity compared with a synthetic antioxidant Butylated hydroxytoluene (BHT) [55].

Antimicrobial activity

The antibacterial and the antifungal activities of the unsaponifiable matter, and a fraction isolated from the hexane extract of *E. spinosus*, were evaluated for their antimicrobial potential against eight Gram-positive and Gram-negative bacteria by measuring the diameter of the inhibition zone around the well, and the determination of their minimal inhibitory concentration (MIC) and minimum bactericidal concentration. The activity tests were conducted using the diffusion disc and broth microdilution assays. Very weak antibacterial activity, with MIC values of 125.0 µg/ml against *Staphylococcus aureus*, *Bacillus cereus*, and *Micrococcus luteus* (MIC > 125.0 µg/ml) was shown by this extract. No significant antifungal activity was observed [22].

Conclusions

The present paper summarizes the limited information on *E. spinosus* and highlights the therapeutic potential, which is used mainly as an anti-inflammatory drug in Algeria, as well as in Morocco. To the best of our knowledge, no study has been conducted to describe the toxicological effects of this species. Therefore, further clinical studies, based on standardized extracts from a sustainable source, must be designed to ensure the safety and efficacy of the extracts of this species which is widely used in traditional medicine in North Africa as an abortifacient drug.

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ORIGINAL RESEARCH

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The *in silico* study of phytoestrogenic activity of soy in substitution of estrogen function

Siti Mudrikatin[1]
[1]Midwifery Program, Husada College of Health, Jombang, East Java, Indonesia

ABSTRACT

Backgroud: Isoflavone compounds in soy are known as phytoestrogen compounds. These compounds are expected to have important roles in replacing estrogen role in menopausal women.
Aim: The purposes of this study were to investigate the estrogen receptor (ER) modulation by soy isoflavone compounds and how the estrogen compound takes a role in gene synthesis related to proliferation and apoptosis.
Methods: The analysis was performed *in silico* manner in which docking as the most important method was carried out using Hex 8.0 software and HADDOCK webserver. Interaction analysis was then done to observe the interactions between soy isoflavone compound and several related proteins using the softwares of Discovery Studio, LigPlus, and NUCPLOT.
Results: The results of this study indicated that soy isoflavone compounds have the ability to bind to ERs.
Conclusions: It can be concluded that soy isoflavone compounds can serve as phytoestrogen that can activate ER.

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Daidzein; estrogen; estrogen receptor; genistein; glycitein; *in silico*

Introduction

Estrogen regulates the differentiation and maintains reproductive tissue, muscle, and other tissues by activating its receptor [1]. The structural study indicates that estrogen and antagonist estrogen compound can induce conformational change in different estrogen receptor alpha (ERα), in which this conformational change determines the recruitment of coactivator or corepressor, leading to diverse biological effects [2]. The most conserved domain of ER is DNA-binding domain which is involved in recognition of DNA and binding to DNA, whereas the ligand binding occurs at ligand-binding domain in COOH-terminal region [3]. ERα and ERβ have high sequence homology level. However, their NH2-terminal domains have similar affinity level to estrogen, and bind to the same DNA response element [4].

Some plants produce compound that has estrogenic activity, so they are called as phytoestrogen compounds. These compounds have similar structure to estrogen. They also have phenolic ring, which is needed for the binding process with ER [5]. Phytoestrogen is contained in food in the forms of aglycone and glucoside. The currently recognized main phytoestrogens are soy isoflavone compounds, such as genistein, daidzein, and glycitein, and the glycoside (genistin, daidzin, and glycitin). Many studies have reported that these phytoestrogen compounds have larger affinity level on ERβ compared with ERα [5]. The genistein has 1,000 times more potential in inducing transcriptional activity in ERβ [6]. Thus, genistein has preference in cells which mainly express ERβ compared with cells that mainly express ERα.

Many studies have reported that the estrogenic effect of soy isoflavones is relatively small

Contact Siti Mudrikatin ✉ mudrisiti@ymail.com Midwifery Program, Husada College of Health, Jombang, East Java, Indonesia.

(1/1,000–1/100,000 compared with estradiol activity), these compounds supposedly have agonistic effect on estrogen. When the estrogen is in abundant amount, the soy isoflavones are assumed to be able to act as anti-estrogen by competing to bind to ER in cells. However, when the estrogen is in very small quantity (menopause), soy isoflavones supposedly have estrogenic effect by replacing the estrogen hormone functions and some of them acts to reduce the osteoporosis symptom, reducing the risks of cardiovascular disease and osteoporosis [7]. This study aimed to focus on the ER modulation by soy isoflavone compounds.

Materials and Methods

Nucleotide sequence and protein structure retrieval

The structures of the components of active compound in soy were obtained from PubChem, an open chemistry database. Three active compounds were analyzed, including daidzein (CID 5281708), genistein (CID 5280961), and glycitein (CID 5317750). Protein sequences of ERα (GI: 262117988) and ERβ (GI: 6978817) were obtained from the sequences database of National Center for Biotechnology Information, the United States National Library of Medicine, and National Institute of Health (http://www.ncbi.nlm.nih.gov).

3D-structural modeling of DNA, protein, and bioactive component

3D-structural modeling of ERα and ERβ was predicted using SWISS-MODEL webserver [8,9] by homology modeling method. 3D structure of protein was then validated using Ramachandran plot analysis. 3D-structural modeling of Hsp70 and Bcl-xL gene promoters was carried out using 3D-DART webserver. Conversion of *.sdf file to *.pdb file of the soy active component was performed using OpenBabel software [10].

Computational docking

Docking simulation was done using HEX 8.0 software [11]. Docking protocol consists of three visualization stages: rigid-body energy minimization, semi-flexible repair, and finishing refinement in explicit solvent. After the execution of each stage, the docking confirmation was then scored and sorted based on scoring function to facilitate the selection of best conformation that will be used at the next stage.

Inter-protein interaction analysis

The docking analysis results are then visualized using Discovery Studio 4.1, LigPlot+ [12], and LigandScout 3.1 softwares [13], while the visualization and interaction analysis between protein and DNA were executed using NUCPLOT software. Interaction analysis was done to observe the formed bonds, such as hydrogen, hydrophobic, and van der Waals bonds. Pharmacophore analysis was also conducted to detect the residues that were directly involved in interaction process, and energy minimization analysis was done to repair the molecular structure and shape at the time of interaction.

Results

Docking analysis aimed to find out the possible interaction between soy active compounds including daidzein, genistein, and glycitein to bind to ER (ERα and ERβ). The analysis of possible interaction was done because one of the compounds in soy is phytoestrogen compound. Of the three soy active compounds analyzed here, glycitein is the most easily interacted compound with ER. Glycitein required an energy of −254.81 kJ/mol to interact

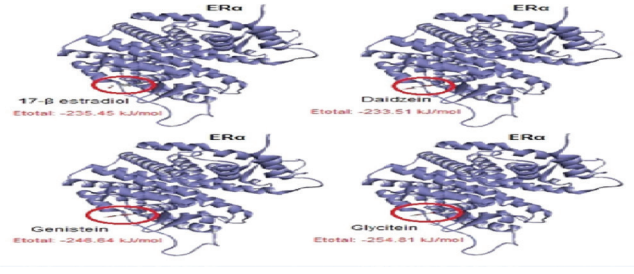

Figure 1. The interaction between estrogen and soy isoflavone compound with ERα. Glycitein required an energy of −254.81 kJ to interact with ERα, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−235.45 kJ/mol).

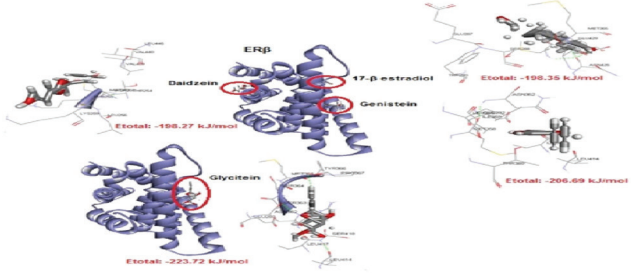

Figure 2. The interaction between estrogen and soy isoflavone compound with ERβ. Glycitein required an energy of −223.72 kJ/mol to interact with ERβ, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−198.35 kJ/mol).

Table 1. Possible interactions between soy active compounds and ERα.

Molecules	Point interaction	Category	Distance (Å)	Binding energy (kJ/mol)
ERα—17β estradiol	17β estradiol: O—ILE391: O	Hydrogen bond	2,406	−235.45
ERα—Daidzein	Daidzein: O—ILE391: O	Hydrogen bond	2,823	−233.51
ERα—Genistein	Genistein: O—ILE391: O	Hydrogen bond	3,134	−246.64
ERα—Glycitein	Glycitein: O—ILE391: O	Hydrogen bond	1,979	−254.81

Table 2. Possible interactions between soy active compounds and ERβ.

Molecules	Point interaction	Category	Distance (Å)	Binding energy (kJ/mol)
ERβ—estradiol 17β	17β estradiol: O—ASN425: O	Hydrogen bond	3,121	−198.35
ERβ—Daidzein	Daidzein: O—Met251: O	Hydrophobic bond	3,167	−198.27
	Daidzein: O—Thr254: O	Hydrophobic bond	2,221	
ERβ—Genistein	Genistein: O—Met358	Hydrophobic bond	3,347	−206.69
	Genistein: O—Leu385	Hydrophobic bond	3,293	
ERβ—Glycitein	Glycitein: O—TYR366: O	Hydrogen bond	2,747	−223.72

with ERα, in which this energy was smaller than the energy required by estrogen (17β estradiol) to bind (−235.45 kJ/mol) (Table 1 and Fig. 1). Glycitein also required less energy (−223.72 kJ/mol) to bind to ERβ compared with estrogen or other active compounds (Table 2 and Fig. 2).

Discussion

The study focused on three main ingredients (daidzein, genistein, and glycitein) based on bioavailability and estrogenic activity of these three materials were higher than the isoflavone-glycoside conjugate form (daidzin, genistin, and glycitin) [14]. Isoflavone concentrations in soy vary depending on a variety of environmental, genetic, harvesting, and processing conditions, but the ratio deidzein, genistein, and glycitein is 1:1:0.1 [15].

Soy glycitein compound is expected to have the highest affinity to ERα and ERβ compared with other soy isoflavone compound. Besides the glycitein, genistein also has fairly high affinity to ERβ. This bond supposedly can replace the estrogen role when a woman experiences estrogen deficiency. Phytoestrogen compound in soy is bound by ER. The bond between phytoestrogen compound and ER activates the intracellular signaling pathway that starts from the activation of phospholipase-C enzyme. This enzyme changes the phosphatidylinositol bisphosphate (PI-2p) to phosphatidylinositol triphosphate (PI-3p). The bond between PI-3p and its receptor located on the surface of endoplasmic reticulum results in the opening of calcium gate; thus, the intracellular calcium ion increases. The calcium ion binds to calcineurin in cytosol. The existence of this calcineurin complex will inhibit the activity of kappa beta (Iκ-β) inhibitor, so the nuclear factor kappa beta (NF-κβ) then translocates to cell nucleus and trigger the transcription of target genes [16,17].

Classic action of estrogen and phytoestrogen isoflavones is mediated through the transcription activation of the genes which are responsive to estrogen, including the intracellular ER [16]. The hormone-receptor complex binds to the estrogen responsive element in promoter region of target gene, thereby inducing the transcription process of such genes. However, the activation of target gene by estrogen may also be mediated by other transcription factor protein, including activating protein 1 and NF-κβ [16].

Based on the results of this study, it can be concluded that soy isoflavone compounds can serve as phytoestrogen that can activate ER.

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Original Research

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Knowledge and uses of common traditional natural products (*Nigella sativa* seed and honey): A comparative study in Mauritius

Muhammad Zakariyyah Aumeeruddy[1], Hudaa Neetoo[2], Mohamad Fawzi Mahomoodally[1]

[1]Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius

[2]Department of Agricultural and Food Science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius

ABSTRACT

Aim: This study was designed to evaluate the knowledge, consumption pattern, and medicinal uses of *Nigella sativa* seed (NSS) and honey among Mauritians in relation to their general attitude towards natural medicines.

Methods: A semi-structured questionnaire was distributed to 90 Mauritians, using equal-quota sampling method, among i) three age groups [(young adults (18–30 years), middle-aged adults (31–55 years), and old adults (above 55 years)] and ii) rural and urban residents.

Results: Mauritians displayed better knowledge, consumption, and medicinal use of honey compared to NSS. Young adults and urban residents showed significantly greater knowledge of NSS compared to old adults and rural residents, respectively ($p < 0.05$). No significant difference ($p > 0.05$) was observed among the three age groups regarding the consumption and medicinal use of NSS and honey. However, a significantly higher score was observed for the consumption and medicinal use of honey among rural population compared to urban population ($p < 0.05$). Furthermore, no significant difference ($p > 0.05$) was observed among age groups concerning the usage and faith in the curative capability of natural medicines, although a slightly higher score was observed among older adults. On the other hand, participants from rural areas showed significantly greater faith in the curative capability and usage of natural medicines compared to urban residents ($p < 0.05$).

Conclusion: Data amassed from this study may be of particular interest for health professionals to propose future therapeutic interventions to maintain the medical importance of NSS and honey.

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KEYWORDS

Nigella sativa seed; honey; consumption pattern; medicinal use; natural medicine; conventional medicine; Black seed

Introduction

Over the past 100 years, the industrial revolution and the development and mass production of synthetic chemical drugs have modernized health care in most parts of the world [1]. However, there has been a recent re-growing interest in the use of natural products for therapeutic purposes due to their low cost, and because of the association of side effects to synthetic drugs. In fact, the use of natural products for therapeutic purposes is as ancient as human civilization [2]. Mauritius is a tropical multicultural island located in the southwestern Indian Ocean, 800 km east of Madagascar. The Mauritian population has a long-standing tradition in the use of natural remedies. Commercially, extracts from several exotic, endemic, and indigenous plants are sold as "tisane" or decoction across the island [3]. Nonetheless, there is still a dearth of documented information on the knowledge and uses of specific natural products available in Mauritius.

Nigella sativa L. (NS) belongs to the Ranunculaceae family and is cultivated in various regions such as Southern Europe, North Africa, Middle Eastern and Mediterranean, and the Southern regions of Asia

Contact Mohamad Fawzi Mahomoodally ✉ f.mahomoodally@uom.ac.mu Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius.

Table 1. Traditional uses of NS and honey (in combination) across the world.

Country	Method of preparation	Ailment/Medicinal use	References
Iran	Mix NSS with honey, infusion	Aphrodisiac gestational diabetes and hypertension, menstruation additive, abortion, and parturition uterus pain	[13]
Algeria	Mix NSS powder with honey	Anemia, respiratory infection, flatulence, anxiety, skin care, and allergy	[14]
Morocco	NSS are mixed with seeds of *Pimpinella anisum* and *Allium cepa* L., resin of *Pistacia lentiscus* and honey. Take the mixture orally	Asthma	[15]
	Mix NSS powder with honey. Take orally	Tonic	
Palestine	Add salt to 1/2 cup of *Olea europaea* L. oil. Add 250 mg of grinded seeds of NS with 250 mg of olive oil to 500 mg of honey. Take two teaspoons daily until recovery	Cancer	[16]
	Mix 1 kg of honey with two large teaspoons of NS, *Sesamum indicum* L. seeds, and nuts. Take large spoon before breakfast daily		
	Prepare a decoction from the aerial parts of *Teucrium capitatum* L. by adding one teaspoon of the plant to boiled water. Mix 1 kg of honey with 100 mg of Ginseng and NS. Take one teaspoon three times daily		
Jordan	Mix NSS powder with honey and take 2–3 times a day	Sexual impotence, arthritis, cough, skin disease, and general weakness	[17]
	Mix NSS with honey 1:1 or 2:1. Eat 1 tsp/day	Diabetes, and antihypertensive	[18]
Iraq	Mix NSS 1:2 with honey, eat one tsp in morning and one at night	Cancer, pneumonia, tonsilitis, hyperlipidemia, and blood circulation	
	Mix NSS 1:1 with one tsp honey, eat 1 time/day		
	Mix 5 g NSS with honey, eat in morning before breakfast	Immune system stimulant	
Turkey	Eat NSS alone or mix with honey and/or garlic	Heart disorders, emmenagogue menstrual regulator, enhancing breast milk production, diuretic, antiedemic, and sore throat	[19]

including Syria, Turkey, India, Pakistan, and Saudi Arabia [4,5]. To the best of our knowledge, NS plant is not cultivated in Mauritius but the seeds and oil are imported from countries such as India, Pakistan, and Saudi Arabia. NS plant grows to 20–90 cm tall, with linear lanceolate leaves and flowers of white, pink, yellow, pale blue, or pale purple color [4]. Inside of the fruit are numerous seeds commonly known as black seeds (English), habbat al-sauda (Arabic), and kalonji in South Asia [6]. The seeds are black colored, funnel shaped, flattened, angular, 0.2 cm long, and 0.1 cm wide [7].

Honey is a natural product made by bees from nectar through a process of regurgitation and evaporation which is subsequently stored in wax honeycombs as a primary food source for the bees inside the beehive [8]. Honey is regarded as the world's oldest sweetener which was replaced by industrial sugar production after 1800 [9]. In Mauritius and its neighbouring Island, Rodrigues Island, total honey production is about 75 tons with about 400 beekeepers and 2,000 hives altogether [10]. The main melliferous plants in Mauritius are longan, tamarind, wild pepper, campeche, litchi, and eucalyptus [11]. However, due to loss of interest by apiarist, Mauritius is not self-sufficient in the production of honey and hence, imports honey from different countries [12].

Nigella sativa seed (NSS) and honey have been used traditionally in combination by several ethnic groups in the management of several ailments (see Table 1). Nevertheless, to the best of our knowledge, information about their knowledge and uses in Mauritius has not been reported so far. The present study therefore endeavors to document the knowledge, consumption pattern, and medicinal uses of NSS and honey among Mauritians in relation to their attitudes towards natural medicines, aiming to identify any association of age and place of residence. Indeed, failure to record such knowledge may lead to losses in their traditional uses in the treatment and/or management of diseases. The research questions of this study were:

1. Do Mauritians tend to be more aware of honey, which is locally produced, compared to NSS, which is imported from other countries?
2. Are older populations more knowledgeable on natural remedies, including honey and

NSS, taking into consideration the recent expansion of pharmaceutical products?

3. Do populations residing in rural regions, which are less industrialized and modernized compared to urban, tend to be more concerned on the use of traditional remedies including NSS and honey?
4. Is there any association between the use of NSS and honey and the general attitudes of the population towards natural medicines?

Methodology

Study area

Mauritius is a subtropical island located in the southwest of the Indian Ocean, 800 km east of Madagascar, with latitude and longitude 20.1625°S, 58.2903°E. The island is 61 km long, 47 km wide, and has a total surface area of 1,865 km^2, with a population of 1,221,975 according to latest estimates in 2017 [3,20,21]. The population comprises of Indo-Mauritians, people of mixed European and African origin, and Sino-Mauritians. Mauritius consists of nine districts namely: Port Louis, Pamplemousses, Riviere du Rempart, Flacq, Grand Port, Savanne, Black River, Plaines Wilhems, and Moka (Fig. 1).

Data collection

This project was approved by the Department of Agricultural and Food Science, Faculty of Agriculture, University of Mauritius, Mauritius. Data were gathered from 90 Mauritians via face-to-face interviews using a semi-structured questionnaire during the period in 2017. Face-to-face interview was performed since it is most convenient to obtain better response [22]. Guidelines for conducting and reporting field studies were followed [23–25]. Participants were approached on the road, home, office, and shops. They were provided with information on the objective of the survey and were assured that their responses would be treated with confidentiality. Vernacular language (Mauritian Creole) was employed to collect accurate data from the participants. Equal-quota sampling method was conducted for i) age group; information was sought from 30 people of the three age groups [young adults (18–30 years), middle-aged adults (31–55 years), and old adults (above 55 years)], and ii) place of residence; information was obtained from 45 people living in rural and urban regions, respectively. The investigation sites were urban areas including Port Louis, and cities of the Plaine Wilhems district such as Quatre Bornes, Vacoas, Reduit (see red dots on Figure 1), and rural regions (see blue dots on Figure 1) including Long Mountain, Calebasse, Riviere du Rempart, Brisee Verdiere, Sebastopol, La Flora, and Surinam.

Questionnaire design

A pilot test was conducted with 20 people to ensure that all the questions were well formulated and easily understood by the respondents. Any criticism obtained from the participants was considered. These 20 participants were excluded from the results. The questionnaire comprised of both open and closed questions, consisting of five sections. Section A included demographic data such as age category, gender, residence, and highest level of education. Section B enquired about the knowledge of NSS and honey, whether participants have ever heard of them and the source of knowledge. Section C investigated into the consumption pattern of NSS and honey, involving questions on whether participants consume them, the frequency, and reasons behind their consumption. Section D dealt specifically with the medicinal use of NSS and honey, enquiring about the ailments for which the products were used, method of preparation, frequency and duration of use, outcome of the treatment, whether used as single therapy or in combination with conventional drugs, whether any side effect was experienced, and the efficacy of NSS and honey compared to the conventional medicine. Finally, Section E was based on the attitudes of participants towards natural medicines, involving questions on their faith in the curative capability of natural medicine, whether they use natural medicine or conventional medicine more often and the reasons behind it, which was partly adapted from the study of Mahomoodally and Ramalingum [26]. Natural medicine was defined as any product from terrestrial or marine sources with medicinal properties, including plants, animals (e.g., milk, bee products), and microorganisms (e.g., yogurt, vinegar). Conventional medicine was defined as any pharmaceutical medications which involve man-made synthesis in the laboratory even if it contains isolated compounds of natural origin.

Data analysis

All data presented in this study were analyzed using Microsoft Excel 2010, Minitab version 16, and SPSS Package version 16.0. Descriptive statistics were used to calculate frequency counts and

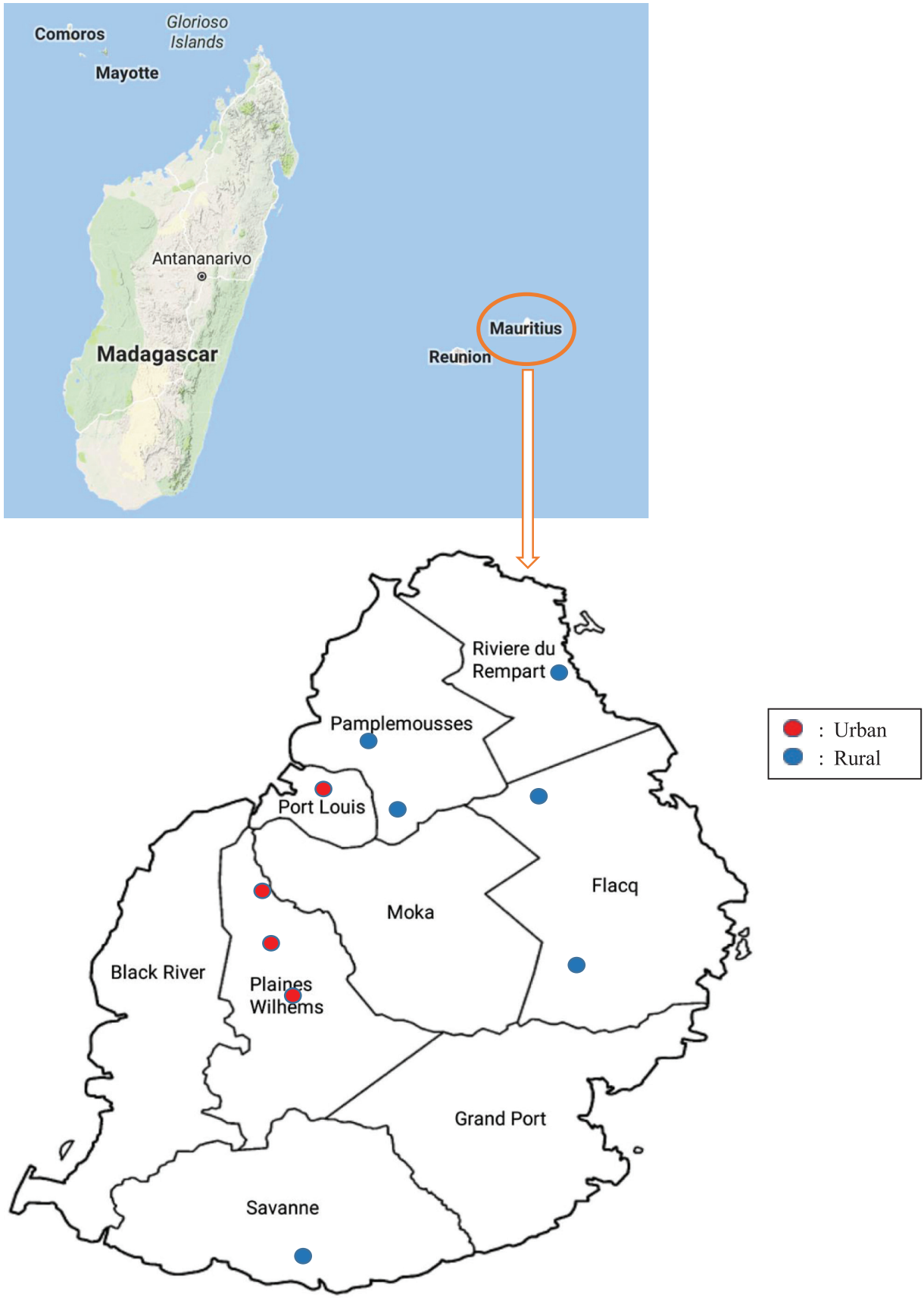

Figure 1. Investigation sites in Mauritius (Red dots indicate urban regions, blue dots indicate rural regions).

Table 2. Demographic profile of respondents.

Demographics	Category	Frequency (%)
Age (Year)	18–30 (young adults)	30 (33.3)
	31–55 (Middle-aged adults)	30 (33.3)
	>55 (old adults)	30 (33.3)
Gender	Male	52 (57.8)
	Female	38 (42.2)
Residence	Urban	45 (50)
	Rural	45 (50)
Highest level of education	No formal education	0
	Primary	1 (1.1)
	Secondary	36 (40)
	Tertiary	53 (58.9)

percentages. One way analysis of variance (Tukey's test) was used for evaluation of significant differences between the variables. $P < 0.05$ was considered as statistically significant.

Result

Demographic profile

The demographic information (gender, age, place of residence, and education level) of the participants are illustrated in Table 2. Out of the 90 Mauritians who participated in this study, males (57.8%) had higher participation rates compared to females (42.2%). In addition, individuals having tertiary education (58.9%) as highest level of education were mostly involved in the study followed by secondary education (40%). Since equal-quota sampling method was used for age group and place of residence, the frequency of participants among their parameters was equal.

Knowledge of NSS and honey

The knowledge of NSS and honey and the sources from which respondents obtained their knowledge are shown in Figures 2 and 3, respectively. All participants (100%) had knowledge of honey in contrast to NSS, where only 24.4% had knowledge of NSS (Fig. 2). The main sources of knowledge of NSS were from friends (68.2%), followed by Islamic teachings (45.5%). On the other hand, participants gained knowledge of honey mainly from their parents (100%) and grandparents (85.6%) (Fig. 3).

Consumption pattern of NSS and honey

Table 3 shows the participants' consumption pattern of NSS and honey. The consumption of honey was higher (100%) compared to that of NSS (10%). The most common reason for consuming NSS was for disease prevention and honey was mainly taken because of its taste and as a prophylactic agent. In addition, NSS was mainly taken in its raw state, while few participants consumed it cooked with other food or in juice. Similarly, all participants consumed

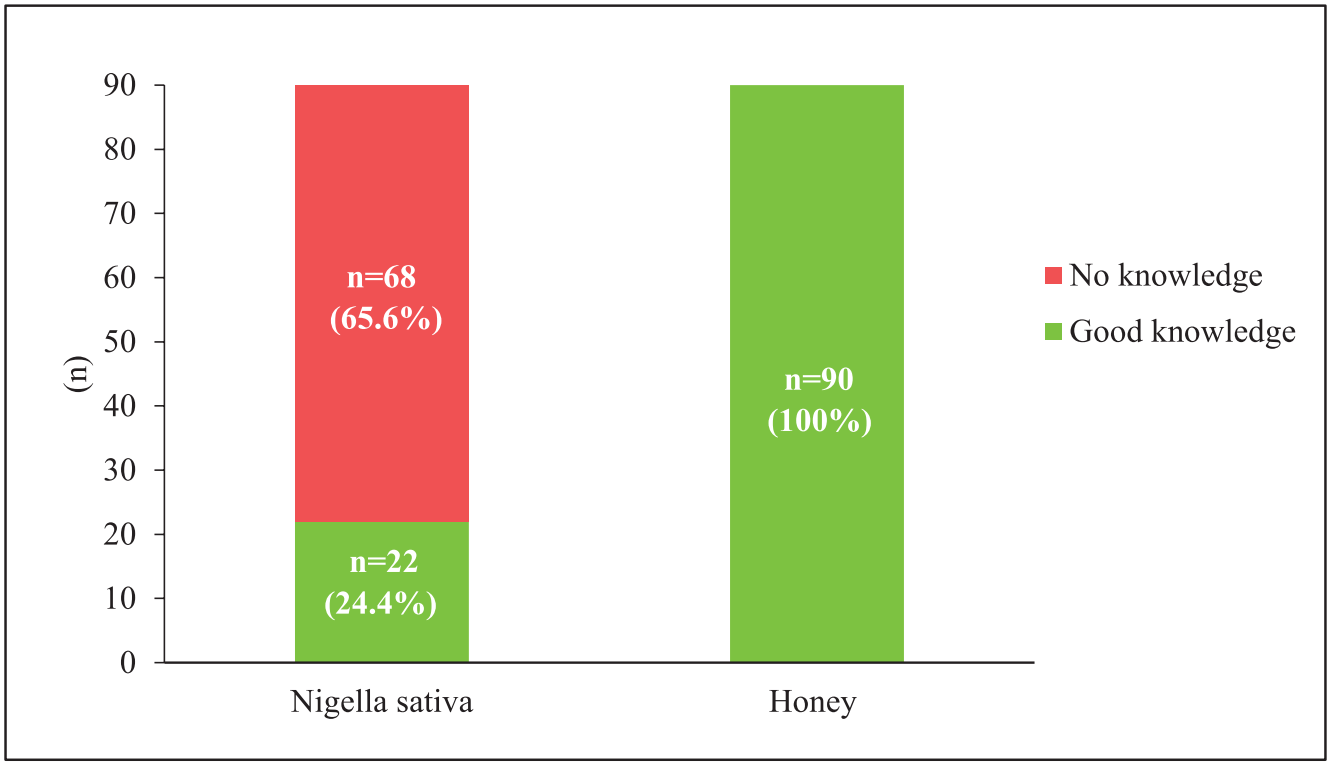

Figure 2. Participants' knowledge of NSS and honey.

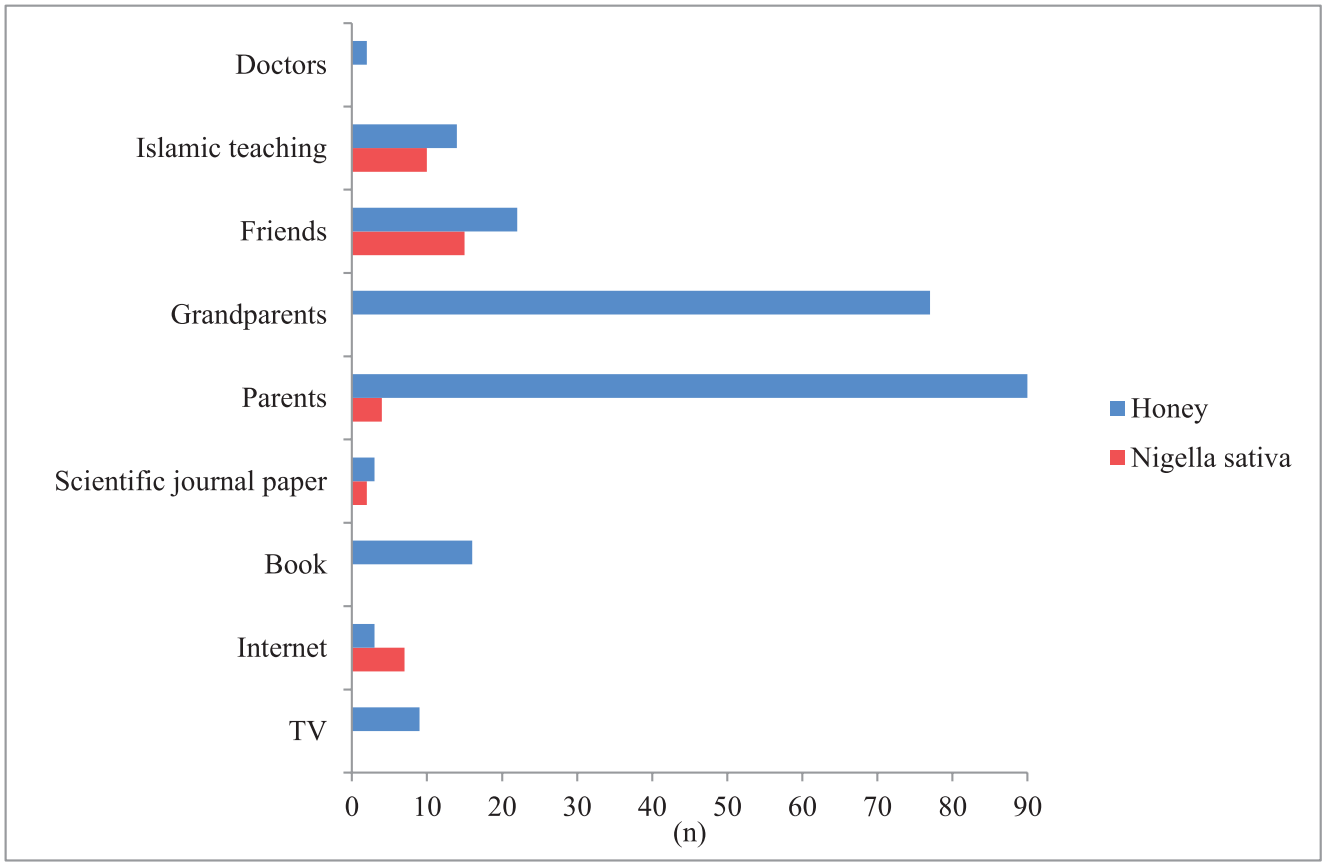

Figure 3. Participants' source of knowledge.

honey in its raw state, although few reported the consumption of honey in cooking (10%), tea (4.4%), juice (3.3%), dessert (1.1%), and milk (1.1%). Regarding the frequency of consumption, NSS was taken rarely, 1–2 times a year (66.7%), in comparison to honey which was consumed more often at least once in 2 weeks (27.8%) or 3 weeks (26.7%).

Table 3. Consumption pattern of NSS and honey.

Consume	NSS *n* (%)	Honey *n* (%)
	9 (10)	90 (100)
Reason for consumption		
like the taste	0	90 (100)
as food flavoring	2 (22)	9 (10)
for disease prevention (prophylactic)	9 (100)	84 (93.3)
as a substitute for sugar	0	8 (8.9)
Method of consumption		
Raw	9 (100)	90 (100)
Cooked with other food	2 (22.2)	9 (10)
In dessert	0	1 (1.1)
In juice	1 (11.1)	3 (3.3)
In tea	0	4 (4.4)
In milk	0	1 (1.1)
Frequency		
Daily	1 (11.1)	7 (7.8)
At least once a week	1 (11.1)	10 (11.1)
At least once in 2 weeks	0	25 (27.8)
At least once in 3 weeks	0	23 (26.7)
At least once a month	1 (11.1)	30 (33.3)
1–2 times a year	6 (66.7)	5 (5.6)

Medicinal uses of NSS and honey

Regarding the medicinal uses of NSS and honey, out of the 90 respondents, only three (3.3%) were recorded to take NSS (Fig. 4). The ailments for which it was used included asthma, runny nose, and constipation (Table 4). The main method of treatment was by taking the seeds raw although other specific methods was used for each disease mentioned. The frequency of usage was mostly once or twice a day, with the duration being 1–3 days for all respondents. On the other hand, a much higher percentage of respondents (91.1%) used honey as medicine, mainly for the treatment of cough and sore throat (95%) and also for asthma. The main method of preparation for treating cough and sore throat was by mixing honey with lemon or ginger while all participants used honey alone for treating asthma. The frequency of usage was mostly once or twice a day, with the duration being 1–3 days for some respondents while 4–7 days for others. However, the frequency and duration of usage

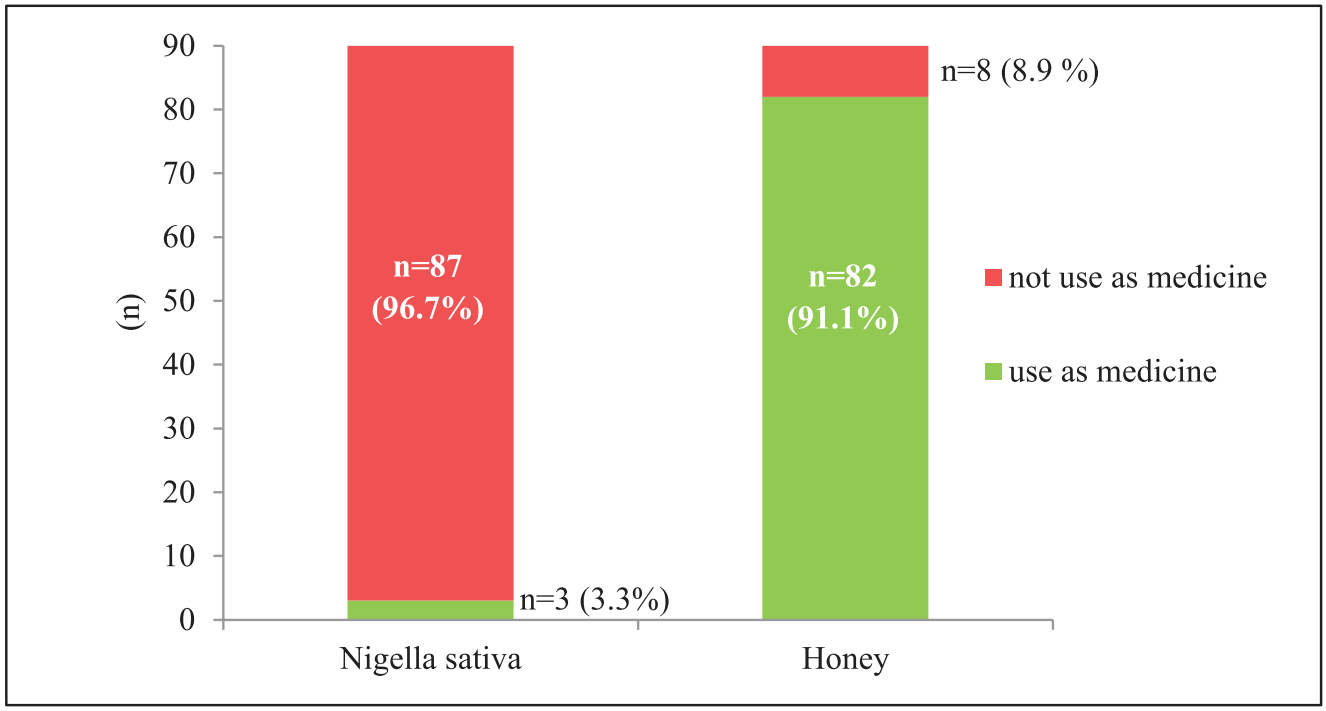

Figure 4. Participant's medicinal use of NSS and honey.

were not specified for a significant number of participants. Regarding the outcome of the treatments, all three participants felt improvement in their health after taking NSS and found it to be more effective than conventional medicines. On the other hand, of the 82 individuals taking honey as medicine, 86.6% found a positive outcome in their health. No experience of side effects was observed among participants. Comparison of the efficacy of honey with conventional medicines revealed that most participants (46.3%) found honey to be more effective, although others found it to be less

Table 4. Medicinal use of NSS and honey.

Product	Ailments/ Diseases	*n*	Method of usage	*n*	Frequency (per day)	Duration (no. of days)	*n*
NSS	Asthma	2	Take seeds raw	2	Once	1–3 days	1
					Twice	1–3 days	1
			Add drops of oil in boiling water and inhale	1	Once	1–3 days	1
	Runny nose	1	Add drops of oil in tissue paper, put under nostrils and inhale	1	Twice	1–3 days	1
	Constipation	1	Take seeds raw	1	Once	1–3 days	1
Honey	Cough and sore throat	78	Mix with lemon	39	Twice	1–3 days	7
					Twice	4–7 days	3
					Twice	Not specified	12
					Not specified	Not specified	17
			Mix with ginger	30	Once	1–3 days	5
					Once	4–7 days	1
					Once	Not specified	9
					Twice	1–3 days	3
					Twice	4–7 days	2
					Twice	Not specified	3
					Not specified	Not specified	7
			Mix with lemon, citronella, and ginger	4	Once	1–3 days	2
					Twice	1–3 days	2
			Mix in warm milk and turmeric	9	Once	1–3 days	9
	Asthma	5	Take raw	5	Once	1–3 days	4
					Twice	1–3 days	1

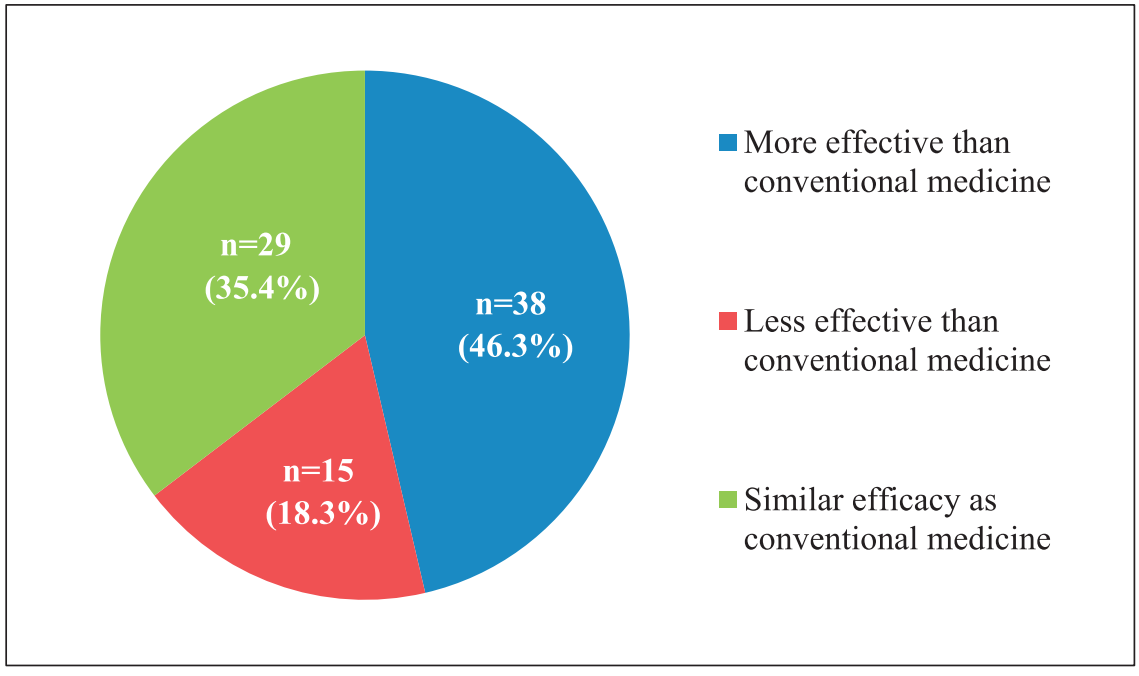

Figure 5. Participants' response on the efficacy of honey in comparison with conventional medicine.

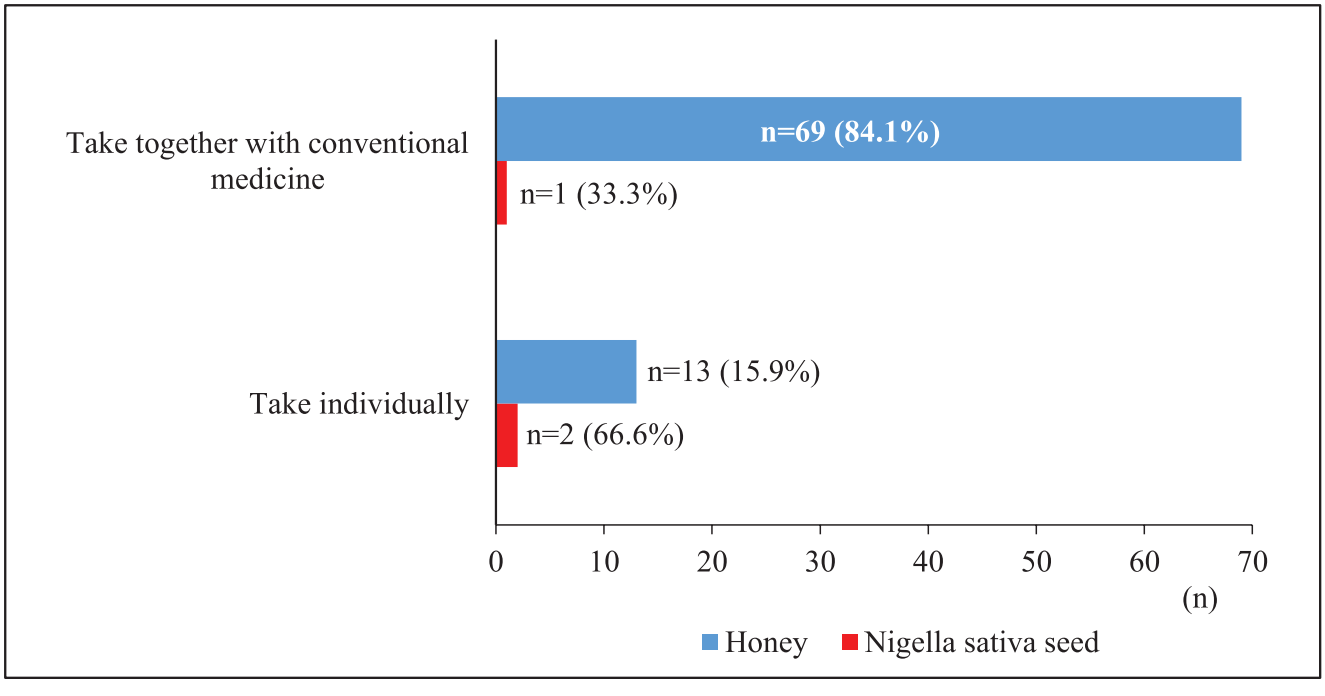

Figure 6. Participants' response on whether NSS and honey were taken individually or together with conventional medicine for medicinal purpose.

effective or having similar efficacy as conventional medicines (Fig. 5). Overall, most respondents used NSS and honey as adjunct to conventional medicines rather than taking the natural products individually (Fig. 6).

Attitudes towards natural medicines

The attitudes of the respondents towards natural medicines are displayed in Table 5. Most participants had low faith in the curative capability of natural medicines (70%). In fact, the use of natural

Table 5. Attitudes towards natural medicine.

	Frequency	(%)
Faith in curative capability of natural medicine		
High faith	27	30
Low faith	63	70
No faith	0	0
Use more often		
Natural medicine	21	23.3
Conventional medicine	69	76.7
Reasons for using more often		
(i) Natural medicine		
No side effect	21	100
More effective	2	9.5
Cheaper	9	42.9
More easily available	8	38.1
Grandparents' remedies	21	100
(ii) Conventional medicine		
No preparation needed/time saving	22	31.9
Available in exact doses for specific ailments	69	100
Scientifically tested before marketing	54	78.3
Prefer doctors' drug prescription	69	100
Lack of knowledge about health benefits of natural remedies	69	100
More effective	69	100
More easily available	69	100

medicines was found to be very low (23.3%) compared to conventional medicines (76.7%). The main reasons for using natural medicines more often were due to the belief of no association of side effects, and following of grandparents' remedies.

Association of age groups and place of residence

The influence of age groups and place of residence on the knowledge, consumption, and medicinal use of NSS and honey and on the faith and usage of natural medicines are illustrated in Table 6. Among the three age groups, young adults had significantly better knowledge of NSS (40%) compared to old adults (13.3%) ($p < 0.05$). However, no significant difference was observed among the three age groups regarding the consumption and medicinal use of NSS. With regards to honey, all participants of the three age groups had knowledge of it. On the contrary, concerning its consumption and medicinal use, middle-aged and old adults used honey more than young adults although statistically no significant difference was observed among the three age groups ($p > 0.05$). Similarly, no significant difference ($p > 0.05$) was observed among age groups concerning the usage and strong faith in the curative capability of natural medicines, although slightly higher score was observed among old adults.

Table 6. Influence of age groups and place of residence.

	Age			Residence	
	18–30 (n = 30)	31–55 (n = 30)	>55 (n = 30)	Urban (n = 45)	Rural (n = 45)
NSS					
Knowledge	12 (40)^{a}	6 (20)^{ab}	4 (13.3)^{b}	17 (37.8)^{A}	5 (11.1)^{B}
Consumption	5 (16.7)^{a}	2 (6.7)^{a}	2 (6.7)^{a}	8 (17.8)^{A}	1 (2.2)^{B}
Medicinal use	2 (6.7)^{a}	1 (3.3)^{a}	0 (0)^{a}	2 (4.4)^{A}	1 (2.2)^{A}
Honey					
Knowledge	30 (100)^{a}	30 (100)^{a}	30 (100)^{a}	45 (100)^{A}	45 (100)^{A}
Consumption	30 (100)^{a}	30 (100)^{a}	30 (100)^{a}	45 (100)^{A}	45 (100)^{A}
Medicinal use	24 (80)^{a}	29 (96.7)^{a}	29 (96.7)^{a}	38 (84.4)^{B}	44 (97.8)^{A}
Faith in curative capability of natural medicine					
High	8 (26.7)^{a}	7 (23.3)^{a}	12 (40)^{a}	9 (20)^{B}	18 (40)^{A}
Low	22 (73.3)^{a}	23 (76.7)^{a}	18 (60)^{a}	36 (80)^{A}	27 (60)^{B}
No	0 (0)^{a}	0 (0)^{a}	0 (0)^{a}	0 (0)^{A}	0 (0)^{A}
Use more often					
Natural medicine	5 (16.7)^{a}	6 (20)^{a}	10 (33.3)^{a}	6 (13.3)^{B}	15 (33.3)^{A}
Conventional medicine	25 (83.3)^{a}	24 (80)^{a}	20 (66.7)^{a}	39 (86.7)^{A}	30 (66.7)^{B}

Different letter superscript (lowercase (a,b) for age group and uppercase (A,B) for place of residence) between columns means significantly different ($p < 0.05$). Values outside brackets indicate frequency while those within brackets refer to percentage.

With regards to the influence of place of residence, participants from urban regions displayed significantly ($p < 0.05$) better knowledge and consumption of NSS compared to those from rural regions, although no significant difference was observed regarding its medicinal usage ($p > 0.05$). Concerning honey, all participants living in urban and rural areas had knowledge of it. However, regarding its consumption and medicinal use, significant difference was observed such that respondents from rural regions showed greater usage ($p < 0.05$). Similarly, participants from rural areas showed significantly greater faith in the curative capability and usage of natural medicines compared to urban residents.

Discussion

This study is the first of its kind to document the knowledge, consumption pattern, and medicinal uses of NSS and honey in Mauritius. The present investigation indicates a deficiency in knowledge of NSS among Mauritians which might be due to the fact that NS plant is not cultivated in Mauritius. Instead, the seeds and oil are imported from countries such as India, Pakistan, and Saudi Arabia. Therefore, the higher knowledge of NSS among young adults compared to old adults might suggest that the commercialization of NSS in Mauritius was recent. Moreover, the higher knowledge and consumption of NSS among participants in urban regions might indicate less accessibility of commercial NSS to rural regions. To the best of our knowledge, there are still few importers of NSS in Mauritius. On the other hand, all participants had knowledge of honey, suggesting that information has been passed on and preserved from ancestors to the current generations, which is confirmed by the greater acquisition of knowledge of honey from parents and grandparents compared to other sources mentioned. Regarding the sources from which participants gained knowledge of NSS, the main sources were from friends, Islamic teachings, and Internet, hence, indicating a possible association of Islamic faith and the use of NSS. In fact, it is reported in the literature that Prophet Muhammad (Peace and blessings of Allah be upon him) stated that there is healing in black seed (NSS) for all diseases except death [27]. Similarly, this relationship was also observed for honey.

Furthermore, we observed that participants tend to consume NSS and honey for disease prevention which signifies a high knowledge of their medicinal properties among their users. However, the consumption of NSS and honey was not often, indicating a lack of concern for their usage or lack of accessibility as mentioned previously. In addition, although the main method of consuming honey was by eating it in its natural state, honey was also used as an additive in juice, tea, milk, dessert, and cooked food. In fact, honey is known as the world's oldest sweetener which was replaced by industrial sugar production after 1800 [9]. Indeed, in the present study, few participants have reported the usage of honey as a substitute for sugar which may be a good alternative for diabetics. Interestingly, honey has been found to be a potential antidiabetic agent provided that genuine, unadulterated, and natural honey, which normally contain higher amount of fructose than glucose, is administered at appropriate therapeutic doses [28].

Regarding the use of NSS for medicinal purpose, a low frequency of participants was observed. On the other hand, the medicinal use of honey among Mauritians tends to be higher. In fact, a number of previous studies have shown that honey was used as an additive in the preparation of herbal medicines in Mauritius [21,29–31]. Moreover, the slightly higher usage of honey among middle-aged and old adults compared to young adults may be explained by the on-going development and modernization of Mauritius resulting in a shift in the mindset of the younger generations. Indeed, societies are moving towards a greater technological advancement with greater emphasis on modern medicine [32]. In fact, the study of Samoisy and Mahomoodally [33] revealed that elder populations make use of traditional remedies more often as they tend to be more aware and skilled concerning their usage. In addition, the greater medicinal use of honey among rural populations as opposed to urban residents might be explained by the fact that most apicultural sites in Mauritius are found in rural regions (Fig. 7), indicating a greater preservation of the value of honey among rural populations. In fact, the low area of apicultural sites across Mauritius indicates a loss of interest for beekeeping among Mauritians. Indeed, Mauritius is not self-sufficient in the production of honey and hence, import honey from different countries [12]. Additionally, the wide range of method of preparations adopted by Mauritians for the medicinal use of NSS and honey might indicate the preservation of traditional systems with the passage of time although this may not imply for NSS because of its possible recent importation to Mauritius. Besides, it should be noted that

both NSS and honey have been used in several other traditional ways across the world (see Table 1).

Regarding the outcome of the medicinal uses of NSS and honey, an improvement in health and a greater efficacy compared to conventional medicines was observed according to most participants. However, this greater efficacy cannot be generalized due to the fact that most participants made use of conventional medicines together with NSS or honey rather than using the natural products individually. In fact, the faith in the curative capability of natural medicines was found to be low among Mauritians and they tend to use conventional medicine more often as observed in the current study. The most common reasons for the greater usage of conventional medicines were i) because of its higher effectiveness, which is in accordance with the low faith in natural medicines as observed in our study, ii) due to the preference for doctors' prescription and lack of knowledge on the health benefits of natural remedies, which is probably due to the low number of traditional healers in Mauritius, and finally iii) because of easy availability, due to the vast number of pharmacies across Mauritius. On the other hand, the belief of no association of side effects was common among participants using natural medicines more often. Nevertheless, over dosage, presence of

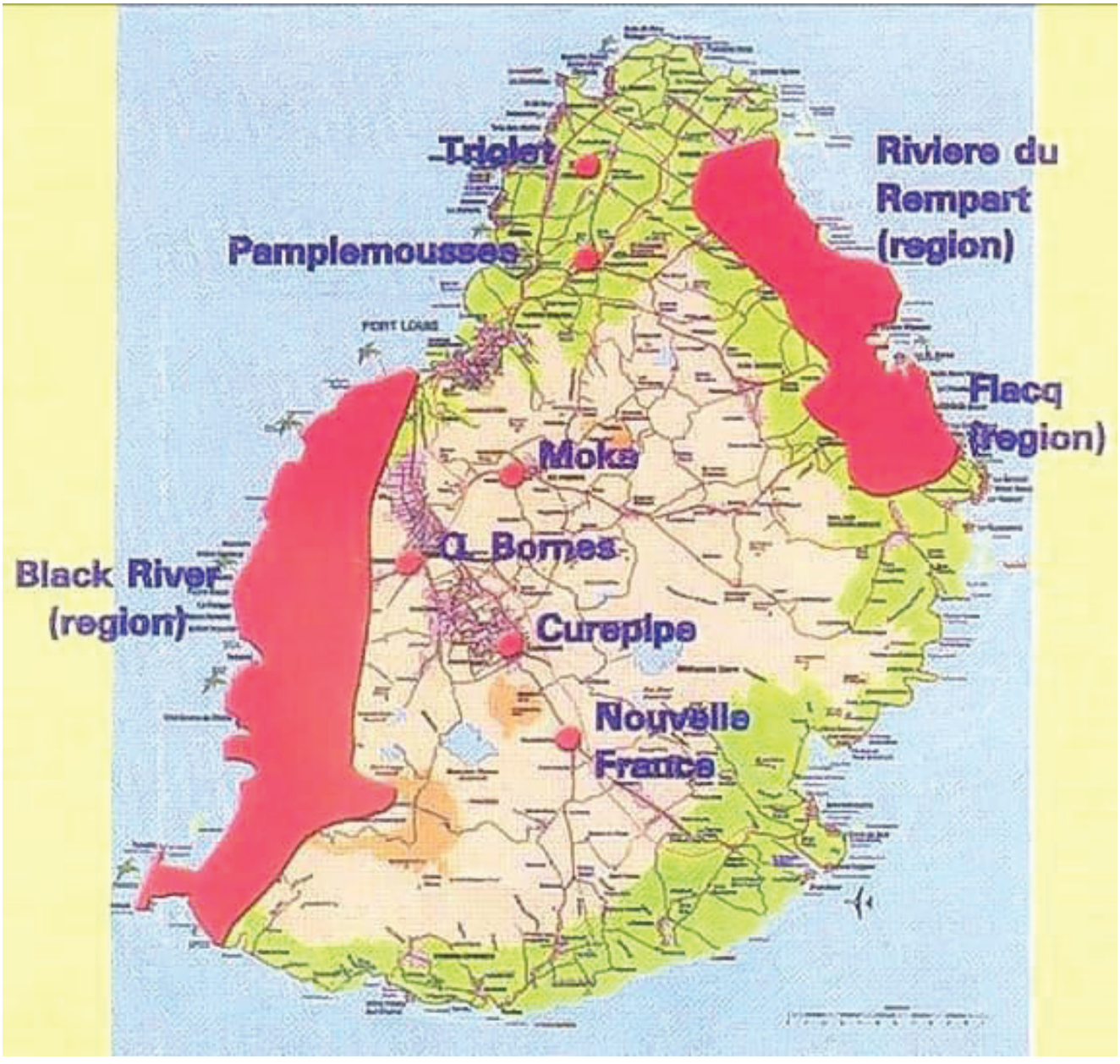

Figure 7. Apicultural sites in Mauritius (shown in red). Adapted from Ministry of Agro Industry and Food Security [11].

toxic compounds, or the interaction of herbs with drugs can result in adverse effects as observed in various studies [34–36]. However, in the study of Picking et al. [37], no experience of side effects was observed among participants when taking herbs and drugs together, which is in agreement with the present study. In fact, the use of herbs or any natural products together with conventional drugs may increase or decrease the desired effect of the drug due to interaction leading to alterations in the absorption and bioavailability of the medication [38].

Conclusion

This study is the first attempt to gather data on the knowledge, consumption pattern, and medicinal use of NSS and honey in Mauritius. From the present investigation, although a deficiency in knowledge, consumption, and medicinal use of NSS was observed, it was found that Mauritians still relies to a great extent on the use of honey which need to be preserved for future generations. Data amassed from this study may be of particular interests for health professionals, including dieticians and nutritionists, to propose future interventions to maintain the medical importance of NSS and honey as well as creating their awareness through education, social activities, and media.

Acknowledgments

Authors wish to thank informants for participation in this study.

Conflict of Interest

The authors declare no conflict of interest.

Abbreviations

NS = Nigella sativa; NSS = Nigella sativa seed.

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LETTER TO THE EDITOR

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Interventions to prevent emergency department visit related to complementary and alternative medicines use

Abubakar Ibrahim Jatau[1], Abubakar Isa[2], Baraka Ado[3], Abubakar Sha'aban[4], Ahmed Awaisu[5]

[1]Division of Pharmacy, School of Medicine, University of Tasmania, Tasmania, Australia

[2]HIV Prevention, Care and Treatment Department, Family Health International Nigeria, Borno State, Nigeria

[3]Pharmacy Department, Staff Clinic, National Board for Technical Education, Kaduna State, Nigeria.

[4]Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia

[5]College of Pharmacy, Qatar University, Doha, Qatar

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Background

Our letter recommends targeted intervention regarding the findings of a systematic review published by the *Journal of Complementary Medicines Research* (formerly, *Journal of Intercultural Ethnopharmacology*) on the use and toxicity of complementary and alternative medicines (CAMs) among patients seeking medical care at an emergency department (ED) [1]. The study summarizes previous studies on CAM use among patients attending ED; definitions of CAM, the prevalence of CAM use among patients at ED, the source of information of CAM use, frequently used CAM, ED visits related to CAM use, and disclosure of CAM use to ED physicians. This letter further recommends some interventions that could be targeted at the identified knowledge gaps, including the role of clinical pharmacists, ED physicians, nurses, and the healthcare policy makers in a bid to reduce burden contributed by CAM use at ED.

The widespread popularity of CAM use in the public may be related to the perception that CAM is "safer" and cheaper than conventional medication, its availability, healthcare-seeking behavior, and philosophical beliefs [2]. CAM is widely used among all age categories in the community for the purpose of medical interventions and well-being [1]. Some of these CAMs, however, have the potentials to cause toxicities, interact with orthodox medications and underlying disease condition leading to hospitalization and unplanned visits to ED. ED (also known as accident and emergency unit), is a clinical setting of a hospital that specializes in the care of patients with acute conditions, which may be life threatening in some cases. ED receives many categories of patients with unplanned visits. Previous studies have shown that up to 25.0% of patients attending an ED were using some forms of CAM at the time of their visit [3]. Moreover, some of the CAMs consumed by the patients have the potential to interact with medications prescribed to the patients at the ED leading to CAM–drug interaction [4]. In addition, some of the CAM users stopped taking their prescribed medication in preference to CAM use [5], while in some cases the ED visit is related to CAM toxicity [1] (Figure 1).

A CAM-related ED visit is, therefore, any visit to an ED with chief presenting complaints associated with CAM use. In some studies conducted in Malaysia and China, one out of eight CAM users at ED presented with chief complaints related to CAM toxicity, with herbal medicines mainly implicated [6,7]. In New Zealand, 4.8% of ED patients using herbal party pills visited the ED due to complaints related to the herbal party use [8]. Most of the reported complaints associated with CAM-related

Contact Abubakar Ibrahim Jatau ✉ pharmjt@gmail.com Division of Pharmacy, School of Medicine, University of Tasmania, Tasmania, Australia.

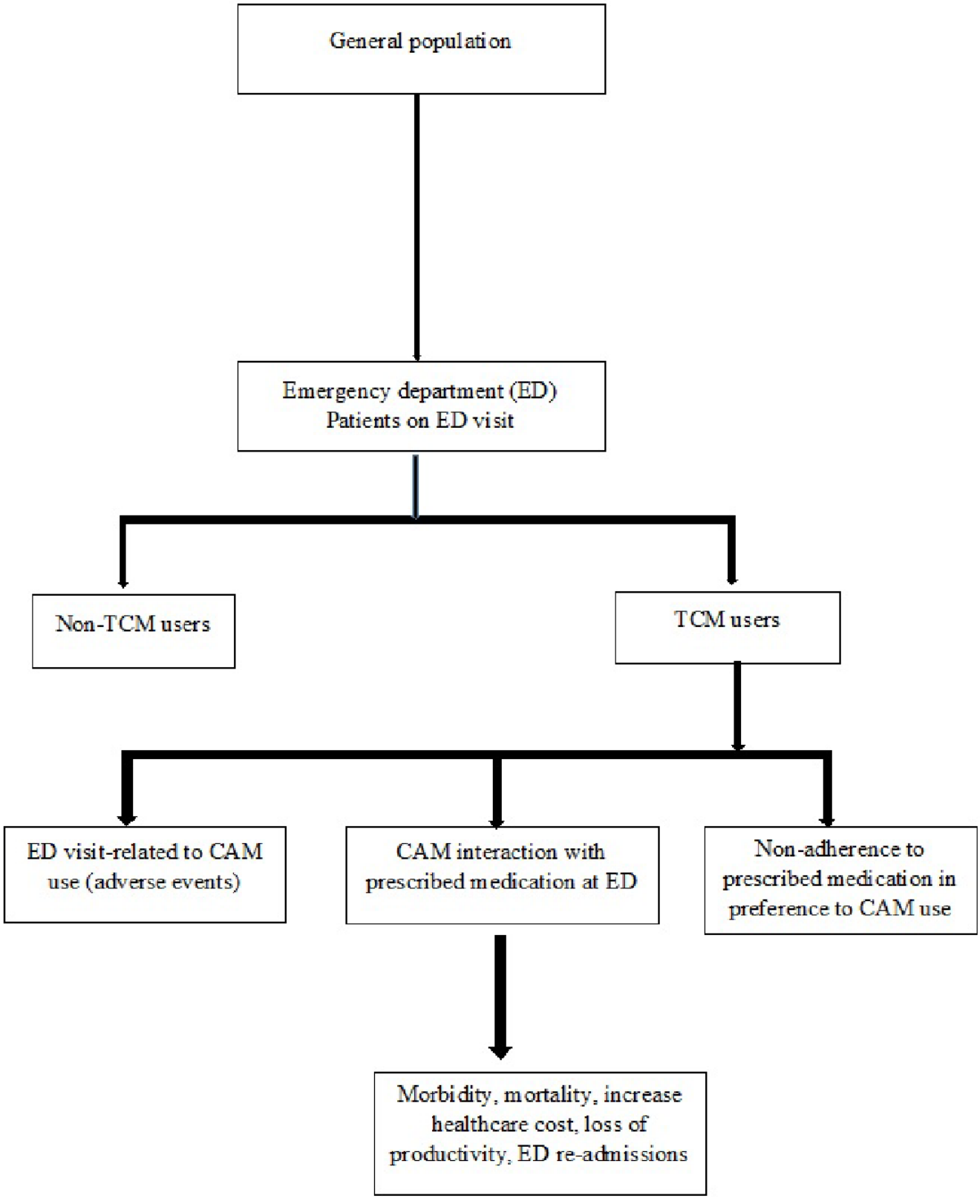

Figure 1. A flowchart for a better understanding of CAM use among patients at ED setting

ED visits were adverse reactions (topical and systemic allergy), toxicity due to an overdose of herbal mixtures, miscarriage, lethargy, thrombocytopenia, acute anticholinergic symptoms, extreme alertness, agitation, palpitation, dizziness, and pruritus [6,8,9]. Importantly, this burden can be prevented through interventional measures. Fundamental steps in the prevention are the identification of the

gaps that require the intervention. Some of these gaps have already been identified in the published systematic review [1].

Identified Gaps Related to CAM-Related ED Visits

Paucity of studies on CAM among ED patients

The utilization of CAM among the general population in developing countries is up to 70% [10], and the use of CAM persists in those countries due to inadequate access and affordability to conventional medicines [11]. Despite this high prevalence, only a few studies were conducted to determine the use and toxicities related to CAM use at an ED setting of healthcare facilities in those countries.

Barriers related to healthcare provider-patients communication

Despite their wide usage and the potentials of CAM to cause toxicities, up to 80.0% of the ED patients do not disclose to the ED physician the CAM that they are currently using at the time of the visit [12]. In a similar manner, the ED physician also does not always ask the patients as to whether they are using any form of CAM or not. There is a paucity of information regarding barriers to open communication and shared decision between healthcare professionals and patients particularly regarding CAM use.

Barriers to reporting CAM use to a healthcare provider

There is dearth of interventions to reduce barriers to reporting CAM use to healthcare professionals at ED. One of the major barriers that prevents most patients from reporting their CAM use to healthcare professionals at ED is the fear of negative reactions from the healthcare provider and the perception that it is not important to disclose [1]. There is a rareness of published strategies to improve reporting of CAM use to healthcare providers at ED.

Inadequate information on the role of healthcare providers regarding CAM use among ED patients

Information is also limited regarding the role of a clinical pharmacist in an ED setting to avert possible CAM interactions with prescribed orthodox medication, or to enquire about the type of CAM the patients are using at home. There is also lack of published studies on the role of ED physicians and other clinicians on CAM surveillance in ED as well as the knowledge, attitude, and perception of CAM use.

Lack of validated screening tool for ADEs related to CAM use

Currently, there is a lack of a validated tool for the screening of CAM-related Adverse drug events (ADEs) among patients at ED. A validated screening (electronic or paper-based) that can quickly detect adverse events related to CAM is absolutely an indispensable tool.

Recommendations for Targeted Interventions

More studies on CAM use among ED patients

More clinical studies are needed, particularly, in developing countries to identify the contributions of CAM use in ED settings including CAM-related adverse events occurring in the ED setting. The studies should focus more on the ED visits related to CAM toxicity. More studies are also needed to investigate the knowledge, attitude, and perception among healthcare practitioners regarding CAM use among patients on ED visit.

Healthcare professionals at ED

There should be an intervention to sensitize the healthcare professionals regarding CAM knowledge, including adverse events related to CAM use. The strategies may include the inclusion of CAM in the curriculum of continuing education programs. Patient education should be improved at all levels of patient care in the ED. Healthcare providers at ED should at all-time ask the patients about CAM use. This is to: (i) ascertain whether the visits could be related to CAM, (ii) to avoid prescribing a conventional medication that may interact with the CAM, (iii) advice the patient on the proper use of CAM, and (iv) risk of non-adherence to conventional medication in preference to the CAM use. This strategy may improve healthcare provider–patient communication.

The inclusion of CAM in patients' information sheet

Including CAM in the patient's information sheet for investigating medication history during routine consultation with patients at ED will assist in enquiring about CAM use and improvement in detecting CAM-related ADE [11]. This will further guide the healthcare providers in finding out the etiology of the presenting complaints as well as to

avoid prescribing conventional medications that may interact with the CAM.

Provision of clinical pharmacists in the ED

The services of a clinical pharmacist are needed in ED settings for adequate surveillance of CAM use and related toxicities in the ED. A separate pharmacy department/unit is necessary for an ED for optimum provision of pharmaceutical care. A clinical pharmacist in an ED will assist in detecting and preventing CAM-related adverse events, improve patient counseling on CAM use, and adherence to conventional medication.

Provision of a screening tool to detect CAM-related adverse events

A validated screening tool for detecting CAM-related adverse events among patients at ED should be developed for adequate and easier identification of adverse events associated with CAM use. The tool will increase the detection rate of CAM-related toxicities thereby reducing morbidity and healthcare cost. In addition, this will also contribute to a reduction in ED overcrowding and enhance optimum time utilization in the future.

The inclusion of CAM in public health programs

Awareness regarding adverse events related to CAM use should be incorporated into other public health promotional programs. This will further increase the public awareness to potential adverse events related to CAM use, including interaction with conventional medicines and dangers of stopping prescribed medications in preference to CAM.

Conclusion

CAM use is common among ED patients, and an ED visit may be related to CAM toxicity. Information regarding the burden of CAM in ED is limited despite the growing use among the ED patients. Targeted interventions to determine the contribution of CAM in clinical practice at ED should be targeted at performing more studies to identify the contribution of CAM use in ED, healthcare providers and patients at ED, and the ED settings.

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Original Research

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Survey of zoological materials used in traditional medicine in Sabon Gari and Zaria Local Government Areas, Kaduna State, Nigeria

Salome K. Timothy, Danmalam U. Habib, Ayeni E. Ayodeji
Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

Background/Aim: Animals and their derivative products constitute essential ingredients in the preparation of drugs used in traditional medicine. Their utilization and practices continue to receive high patronage from all works of lives among developing countries with little or no comprehensive health assurance coverage. The aim of this study is to document some zoological materials used as medicine in Sabon Gari and Zaria local government areas, Kaduna State, Nigeria.
Methods: In-depth survey was used to document some ethnozoological materials in the study areas. Seventy-one herbal and traditional medicinal practitioners were interviewed in Hausa language using semi-structured and key respondent interview approach.
Results: The findings showed that the respondents were above 18 years of age and claimed that the ethnozoological usages of some animals and derived parts such as giant African snail shell, Iguana lizard, rock python, peacock, African elephant etc. were inherited from their parent and grandparent. Twenty animal species with their ethnomedicinal uses, Hausa names, parts used, and the photographs of readily available animal parts have been documented. The results showed that some animal parts or products are used in the treatments of snake bites, hypertension, ulcer, pain, aches, cold, and respiratory diseases. Their ethnomedical practices as protection from nightmare, ability to tame wayward women, worn for shield and strength, and tendencies to prevent promiscuity are some significant findings in the study.
Conclusion: The findings showed the rich ethnozoological materials usages of Sabon Gari and Zaria local government areas (Kaduna State) for indigenous medicinal purposes towards meeting and complementing their primary health care system and with the hope that further work will be conducted to evaluate their safety and therapeutic profiles.

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Zootherapy; ethnomedicine; herbal and traditional practitioners; Kaduna, Nigeria

Introduction

Animal and animal derivative products are natural products used in the treatment of various human diseases and health conditions among many cultures [1–4]. Animal-based medicines have played a significant role in the healing practices, magic rituals, and religions of societies all over the world too [5–8]. Many animal products are also used during ceremonial and religious practices as well as fetishes and charms purposes [8]. Many human tribes and communities with structured medicinal systems utilize animals as medicines [1,3,4,9]. Traditional medicine as practiced today continues to receive high patronage from all works of lives including the rich and the poor especially in developing countries where the traditional medical practitioners even made new discoveries, which have cured major ailments in the society [10,11]. Such discoveries from the consistent efforts of traditional healers to eradicate dangerous diseases which have plagued the society including epilepsy, cancer, convulsion, paralysis, snake bites, mental illness, and even other ailments having hereditary origins are being cured by traditional medicine [2,12,13]. Different animal body parts

Contact Ayeni E. Ayodeji ayeniemmanuel91@yahoo.com Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

have also been reported as sources of food medicines [14,15]. World Health Organization estimated that out of the 252 essential chemicals discovered from natural products about 9% came from animals [9]. Also, with the fact that over 70% of many developing nations depend solely on traditional medicines to meet their basic and primary health care need [16,17]. Zootherapy, also known as animal therapy, is a process of healing human ailments by using medicines prepared from different animals or animal derivative products [18]. It constitutes a significant substitute among many other known therapies practiced worldwide [19]. For example, In Latin America, 584 animal species (belonging to 13 taxonomic categories) were recorded with traditional therapeutic value, while 283 species were reported to be used for the treatment of various ailments in Brazil [20,21]. In traditional Chinese medicine, more than 1,500 animal species have been reported to be of some medicinal importance [22]. Lev and Amar [23] documented the use of 20 animal species as traditional drugs in Israel. Alves et al. [24] stated that the global traditional uses of reptiles revealed that at least 165 reptile species, belonging to 30 families and 104 genera, are used in traditional folk medicine around the world. Zoological materials have served as medicinal foods since ancient times and have played a significant role in healing practices especially in European and African cultures, where many of products used as food often also serve as medicine [16,25]. Some studies have also reported ethnozoological materials used as medicines [26,27]. In Nigeria, Soewu documented some wild animals used in ethnozoological practices in Southwestern part [2]. This author also posits its implication on biodiversity conservation. However, the knowledge on the use of different animal materials in traditional medicine by different ethnic communities is largely passed orally from one generation to another and this knowledge is undocumented and sometimes lost with the death of the elderly knowledgeable person or lack of interest by the children to continue in the folkloric claims. Therefore, the study documented some ethnozoological materials used among the people of Sabon Gari and Zaria local government areas (Kaduna State, Nigeria) as medicine towards meeting their primary health demand.

Methodology

The study area

The survey focused on two major herbal markets in Sabon Gari and Zaria local government areas of Kaduna State called "Bayan Gidan Iya" (Sabon Gari) with a coordinate of 13°46′83″ N 5.0183° E and "Kasuwan Armaru" (Zaria city) with a coordinates of 11°04′00″ N 7°42′ E [28].

Geographical locations

Zaria herbal market is popularly known as *Armaru* market located in Zaria city with about 2 km away from the Emir's of *Zazzau* palace. It is one of the major herbal markets well known in Zaria for traditional medicinal materials trading, including animal products. It usually opens once in a week (every Mondays). The market populations cover all ages including nursing mothers, aged ones, educated and uneducated, and even young ladies who may be seeking for natural medication and treatments. *Bayan Gidan Iya* is also a popularly known herbal market in *Sabon Gari*. This market operates on a daily basis as traditional healers reside within the settlement and own the shops in which the business is operated.

Materials and some instrument used

Tape recorder, GPS camcorder, pen, recording sheets, semi-structured questionnaire tools, cabinet, and tags for labeling purposes.

Data collection and sample population

The study was conducted in December 2016–March 2017. Seventy-one key respondents were interviewed using semi-structured questionnaires during the study. The ethnozoological materials (local names of animals, body parts used, and ethnomedicinal uses) were noted and documented.

Consent approval

The respondents consented willingly to participate in the study. The respondents were interviewed in Hausa language at their convenient times and interviewers were ensured that their sales were not interrupted. Some agreed to be interviewed in their houses without any hesitation.

Collections and identification of samples

The animal parts readily available were collected, labeled, and enclosed in a sample cabinet. They were transported and identified in the Department of Zoology, Ahmadu Bello University, Zaria, Nigeria for future references.

Table 1. Socio-demographic characteristics of the respondents (n = 71).

Variables	Sabon Gari (Bayan gida Iya)	Zaria (Armaru)
Gender		
Male	21 (58.30)	23 (65.70)
Female	15 (41.70)	12 (34.30)
Total	36 (100.00)	35 (100.00)
Age (years)		
18–30	10 (27.80)	3 (8.60)
30–45	15 (41.70)	11 (31.40)
45–60	7 (22.20)	17 (48.60)
60 and above	4 (8.30)	4 (11.40)
Total	36 (100.00)	35 (100.00)
Nature of business		
Herb seller	23 (63.90)	17 (48.60)
Traditional practitioner	13 (36.10)	18 (51.40)
Total	36 (100.00)	35 (100.00)

Results and Discussion

The socio-demographical details of the respondents (Table 1) showed more male practitioners (65.70%) in Zaria than in Bayan gida Iya in Sabon Gari local government area (58.30%). But there was more female practitioners (41.70%) in Baya gidan Iya (Sabon Gari) when compared with (34.30%) female practitioners in Zaria (Armaru market). This high indication of male practitioners could be as a result of dominancy of male [15]. Also, Hausa community in Nigeria believes that male children protect cultural heritage than female. This result is in lined with [29,30] who reported similar trends in their findings in India. The respondents (48.60%) were within the ages of 45–60 years in Zaria (Armaru market) while 41.70% were between 30 and 45 years. These aged groups of the society were observed to be more knowledgeable about traditional medicinal uses than that of younger generation. This trend was very similar to the observations in other region of Assam in India made by Verma et al. [30] and also indicated that the aged people were more experienced in zoo-therapeutical practices which were passed to them by their elders. Jansen [31] also confirmed that traditional healing is known as a knowledge of professional secret that should be known by elderly ones mainly for male practitioners [32,33]. The reason of less traditional medicinal knowledge among the younger generation could be due to urbanization and assimilation of alien culture [15]. These also include some disapproval among western beliefs (super impose extra-terrestrial religions front-runners) to young generation. There was high percentage of herbal sellers (63.90%) in *Bayan gida Iya* (Sabon Gari) when compared with (48.60%) herb sellers in Zaria local government area. This high proportion may be due to the closeness of herbal sellers to the popularly known Sabon Gari (Baya gida Iya) market. Similarly, traditional practitioner (51.60%) in Zaria showed higher proportion when compared with practitioner (36.10%) in *Bayan gida Iya* market (Sabon Gari). This is probably due to the fact that the knowledge based traditional practitioners may be self-importance to people in the Zaria to guide the cultural heritage and ensure correct diagnosis and treatment using different zoo therapeutics.

Furthermore, the study documented 20 ethnozoological materials alongside with their different local names, parts used in Hausa language as well as their diseases treated and ethnomedicinal uses (Table 2).

Generally, many of the zoological materials survey are used as food. The meat from giant African snail *Archachatina marginata* is used as herbal remedy when mixed with food and the shell is being used in the treatment of ulcer, hypertension, rheumatism, and other internal diseases.

The oral application of Honey bee *Apis mellifera* is used to dress wound, treat snake bites, burns, hair loss, hemorrhoid, and treatment for arthritis. When honey is mixed with cinnamon powder and lime water, it is used to treat cough.

The shell of the tortoise *Aldabrachelys gigantean*, besides being used as decoration and ornament, is prepared as charm to tame a wayward woman. It is ingested orally after grinding to a powdered form, which is added to food.

The head of *Agama agama* (Red lizard) when prepared as herbal remedy is used for the treatment for chronic cough and the tail when prepared is used orally for the treatment of severe chest pain. Also, *Bucorus abyssinicus* (Ground hornbill) feather is used to enhance beauty when applied topically as a powder on the face.

Apart from the *Crocodylus niloticus* (Nile crocodile) being used as source of food, leather bags, coats, shoes, and wrist watches making, the skin and bones are being used in treating stiffness of joints and muscular disorders. The bones are grounded and soaked in water before drinking and the tooth is usually worn as charm during battle as amulet around waist. It is seen as a symbol for strength and stamina; hence, it is prepared as herbal remedy to give strength (Fig. 1b).

Crocuta crocuta (Spotted hyena) skin is prepared as charm which is usually burnt with wood ash at

Table 2. Animal species, derivative products, and parts used in traditional medicines in Sabon Gari and Zaria local government areas (Kaduna State, Nigeria).

Scientific names (English common names)	Hausa names	Parts or product used	Diseases treated/Ethnomedical uses
Archachatina marginata Swainson (1821) (Giant African snail)	*Dodon kodi*	Shell	Treatment for ulcer, hypertension; Rheumatism and internal diseases.
Apis mellifera Linn (1758) (Honey bee)	*Kudan zuma*	Honey/bee wax	Treatment of snake bites; Heal burns, hair loss, and hemorrhoid; Treatment for arthritis; To treat coughs
Crocodylus niloticus Laurenti (1768) (Nile crocodile)	*Kada*	Skin/Bones	Treatment against stiffness of joints and bone dislocation;
		Tooth	Worn during battle as amulet around waist.
Naja nigricollis Hallowell (1857) (Black cobra)	*Bakin maciji*	Skin	Concoction preparation
		Venom	To treat rheumatism.
		Oil	The oil is used to treat high blood pressure, skin rashes, eczema, arthritis, hypertension, and rheumatoid.
		Teeth	Worn as amulet on neck, waist, and wrist to stop nightmares.
Python sebae Gmelin (1788) (Rock python)	*Mesa/Mai hadiya*	Skin/oil	Treatment of backache; Spinal cord disorders
		Meat	Eating as food; Added as ingredient for herbal preparation and eaten orally as charm
		Tooth	Worn as amulet to scare away snakes
		Venom	Used to treat snake bites/poisoning
Typhlops trinitatus Richmond (1965) (Trinidad burrowing snake)	*Tandara/shanono*	Venom	An antidote to snake bites;
		Oil	Used as a treatment to stop nightmares It is applied in food or water and ingested orally.
Crotalus basilicus Cope, (1864) (Viper)	*Kassa*	Skin	Prepared as charm for strength;
		Venom	Treat insomnia (sleeplessness) Applied on snake bite
Iguana iguana Linn (1758) (Iguana lizard)	*Damo*	Head/Skin/Tail	Epithet applied to a long suffering patient
			Applied topically and also ingested orally
Varanus niloticus Linn (1758) (Bosch monitor lizard)	*Patan tsari*	Skin/head	Used to strengthen teeth after it is soaked in water before bathing.
			Used to treat teething sickness in children.
		Tooth	Prepared as charm and worn as amulet on the waist and neck.
Agama agama Linn (1758) (Red lizard)	*Jan gwada*	Head	Treatment for chronic cough
		Tail	Treatment of severe chest pain
Aldabrachelys gigantean Schweigger (1812) (Tortoise)	*Kunkuru*	Shell	To tame a wayward woman
		Head/Shell	Decoration and ornaments.
Bucorus abyssinicus Boddaaert (1783) (Ground hornbill)	*Burtu (mai bawa)*	Feather	Used to enhance beauty.
Pavo cristatus Linn (1758) (Peacock)	*Dawisu* (*Tsun-tsun Makka*)	Head	Wards off evil people It is ingested orally after mixing with honey and wood ash.
		Meat	Serve as a source of food
		Feather	For decoration at home and museums for tourist attraction.
Stephanoeatus coronatus Brown (1968) (African crowned Eagle)	*Mikiya*	Head/Feather	Prepared as charm for renewal of strength/ endurance; Worn as talisman
		Egg	For renewal of skin Superstitious belief for regeneration

(Continued)

Table 2. Animal species, derivative products, and parts used in traditional medicines in Sabon Gari and Zaria local government areas (Kaduna State, Nigeria). (Continued)

Scientific names (English common names)	Hausa names	Parts or product used	Diseases treated/Ethnomedical uses
Tyto alba Scopoli (1769) (Barn owl/ Bird of wisdom)	Mujiya	Feather	Used for beautification
		Tooth	Prepared as charm to ward off evil attacks
		Beak	Hung on the wall for decoration Worn on the neck as a symbol of wisdom
Upupa epops Linn (1758) (Cameroon hoopoe)	*Alhuda-huda*	Feather	Prepared as part of herbal remedy to increase wisdom and enhance intelligence in a person. It is grounded to powder and mixed with honey and talisman (huntu) and ingested orally.
Loxodonta Africana Linn (1758) (African Elephant)	*Giwa* (*katon Nama*)	Dung (feces)	Remedy for nose bleeding Repellant for mosquitoes
		Tusk	Treatment for head ache and tooth ache.
		Skin/Meat Bones	For strength and stamina
Crocuta crocuta Erxleben (1777) (Spotted hyena)	*Kurah*	Skin; Hair; Tooth	Used to cure skin rashes; Prepared as charm for self-control against adultery and promiscuity; Worn as amulet on wrist and waist.
Felis pardus Linn (1758) (Leopard)	*Damisa*	Skin	Anti-snake venom; Treatment for convulsion; General body weakness
Atelerix albiventris (Wagner, 1841) (Four-toed hedgehog)	*Bushiya*	Hair; Thorn; Head	Wards off evil spirit; Treat skin rashes; Treatment for respiratory problems and cold.

night to cure skin rashes while the spotted hyena hair is used as charm for self-control against adultery and promiscuity, and the tooth worn as amulet on wrist and around waist for strength and endurance (Fig. 1e).

Crotalus basilicus (Viper) skin is used as charm for endurance in people when ingested and the venom treat insomnia (sleeplessness) and act as antidote that cure snake bites when mixed with wood ash (Fig. 1j).

Felis pardus (Leopard) skin is being used in treatment of convulsion, general body weakness, worn as talisman around the neck for protection, the venom as antidote for snake bite, and when mixed with some herbs as body ointment.

The head, skin, and tail parts of *Iguana iguana* (Iguana lizard) when applied topically or ingested orally are being used to ease long suffering patient.

The *Loxodonta Africana* L. (African elephant) is used as food, the meat and bones when consumed are considered as symbol of stamina and strength. The feces (dungs) are used for nose bleeding and as mosquito repellant when burnt, while the tusk is being used for the treatment for head and tooth aches.

Naja nigricollis (Black cobra) skin is used in concoction preparations and the venom when applied topically as herbal remedy to treat rheumatism. The oil is used to treat high blood pressure, skin rashes, eczema, arthritis, hypertension, and rheumatoid. The teeth when worn on neck, waist, and wrist help to prevent nightmares (Fig. 1h).

The hair of *Atelerix albiventris* (Four-toed hedgehog) is used to ward off evil spirit around people and environment. The powder form of the thorn is used to treat skin rashes and the head is used in the treatment for respiratory problems and cold when prepared.

Pavo cristatus (Peacock) feathers are used for decoration at home and museums for tourist attraction. The head when mixing with honey and wood ash and ingested orally wards off evil people and the meat serve as a source of food.

The *Python sebae* (Rock python) skin and oil are used in the treatment of back ache as well as spinal cord disorders. The meat is added as an ingredient for herbal preparation and eaten orally for charm. Teeth are worn as amulet to scare away snakes and the venom as antidote to bites and poisoning (Fig. 1d).

Stephanoeatus coronatus (African crowned eagle) the head and feather is being prepared as charm for renewal of strength and endurance and worn as talisman. The egg when swallowed raw has

Figure 1. Examples of animals and derivative products used in the ethno-medicine of Sabon Gari and Zaria local government areas (Kaduna State, Nigeria). (a) Shell of the giant African snail (*Archachatina marginata*). (b) Skin of Nile Crocodile. (c) Skin of Trinidad burrowing snake. (d) Skin of rock python. (e) Skin of spotted hyena. (f) Feather of Cameroon hoope. (g) Skin of Bosch monitor lizard. (h) Skin of black cobra. (i) Feather of barn owl. (j) Skin of carpet viper.

superstitious belief for regeneration and egg when rubbed on skin renew the skin.

Typhlops trinitatus (Trinidad burrowing snake) skin is being used for leather coats, bags, shoes, watches etc. The venom is also used as an antidote to snake bites when applied topically. Ethnomedically, the snake oil is used as a treatment to stop nightmares when mixed with food or as drop in water and ingested orally (Fig. 1c).

Tyto alba (Barn owl/Bird of wisdom) feather and the beak is used for beautification and decoration, tooth is prepared as charm to ward off evil attacks and worn on the neck as a symbol of wisdom (Fig. 1i).

Upupa epops (Cameroon hoopoe) feather is used during preparation part of herbal remedy to increase wisdom and enhance intelligence in a person. It is grounded to powder and mixed with honey and talisman (huntu) and ingested orally (Fig. 1f).

Varanus niloticus (Bosch monitor lizard) skin and head parts are used to strengthen human teeth after soaked in water before bathing and the tooth is used to treat teething sickness in children, prepared as charm, and worn as amulet on the waist and neck (Fig. 1g).

However, these animal parts or products if subjected to extraction for possible validation of their ethnomedicinal uses may lead to drug discovery and development. Since World Health Organization reported that some essential chemicals were also from animals and animal products origin [9,15]. Some readily available pictures of the zoological materials used were also documented (Fig. 1) that could guide further identification and future studies. This ethnozoological materials survey documented in the two local government areas of Kaduna State has similar findings to different studies carried out in different part of the world [2–4,15,34,35].

Conclusion

This study is the first effort to document the zootherapeutic resources used in traditional medicines among the indigenous people surrounding Sabon Gari and Zaria local government areas (Kaduna State, Nigeria) to the best of our knowledge. The high implication of some animal parts or products are used in the treatments of snake bites, hypertension, ulcer, pain relief, aches etc. are major findings in the study and their roles in treating human illnesses and diseases. Some ethnomedical usages and belief such as protection from night mare, ability to tame wayward women, prevent adultery and besides, is mysterious utilization when worn for protection, staminal, strength, and stopping promiscuous tendencies are fact findings in the study. The therapeutic knowledge of medicinal plants is complemented with animals and animal derivative products for indigenous medicinal purposes in ensuring meeting their primary health care system of Africans and Nigerians with little or no comprehensive health provision. This study also provides the bases for further scientific validation of the therapeutic efficacy of various zootherapeutic materials by these people.

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Conflict of Interests

The authors declare no conflict of interest.

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