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The inhibitory effect of an ethanol extract of *sida rhombifolia* leaves on key carbohydrate hydrolyzing enzymes

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ABSTRACT

Aim: The study was conducted to screen the ethanol extract of *Sida rhombifolia* (ESR) leaves for its phytochemical constituents and elucidate some of its possible mechanism of action in lowering hyperglycemia.
Methodology: The ethanol ESR was prepared and its effect on carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) both *in vitro* and *in vivo*, and glucose uptake by muscle tissues was investigated. Qualitative phytochemical screening was also conducted.
Results: *Sida rhombifolia* extract contained bioactive phytochemicals and showed significant dose-dependent inhibition of α-amylase and α-glucosidase with IC_{50} values of 831.76 and 1202.3 µg/ml, respectively. It significantly promoted glucose uptake by rat hemidiaphragms and reduced postprandial glycemia in normal rats administered with starch and sucrose.
Conclusion: *Sida rhombifolia* ethanolic extract contains bioactive compounds and displayed strong anti-diabetic properties, therefore, it has potential use as an anti-diabetic agent.

ARTICLE HISTORY

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KEYWORDS

Diabetes; Sida rhombifolia; α-amylase; α-glucosidase.

Introduction

Diabetes mellitus (DM) is a complex metabolic disorder resulting from either impaired synthesis and secretion of insulin by beta cells of the Islets of Langerhans (type 1 DM) or impaired sensitivity of tissues to insulin action (type 2 DM). It is characterized by chronic hyperglycemia that results in diabetic complications such as retinopathy, neuropathy, and nephropathy because of oxidative stress. Oxidative stress is highly increased in the diabetic state because hyperglycemia promotes the generation of free radicals and weakens the ability of the body's natural anti-oxidation defense systems [1,2]. Free radicals have been extensively implicated in the pathogenesis of DM and its associated macro- and micro-vascular complications. These free radicals are produced due to hyperglycemia, lipid peroxidation [3], and elevated concentrations of heavy metals like arsenic which can be found in agricultural produce like vegetables [4]. The clinical management of DM is based on oral anti-hyperglycemic drugs and exogenous insulin. However, despite the availability of the various medications for the management of DM, its global morbidity, mortality, and prevalence is increasing with projections of 366 million cases by 2030 [5]. α-amylase and α-glucosidase inhibitors are a class of promising drugs for management of postprandial hyperglycemia in type 2 DM. However, they are associated with side effects like hypoglycemia, diarrhea, and abdominal pains. Therefore, there is an urgent need for the discovery of drugs which can manage DM with less or no side effects. In the search for such drugs, botanicals have become of more interest due to their multi-pronged effects on the disease. It has been established that some botanicals possess bioactive phytochemicals like phenols, flavonoids, tannins, saponins, and glycosides which confer various mechanisms of action in managing DM [6].

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In the present study, the ethanol extract of *Sida rhombifolia* (ESR) leaves was evaluated for its anti-diabetic properties. *Sida rhombifolia* belongs to the Malvaceae family and is widely distributed across tropical Africa. The plant is characterized by dark green, diamond-shaped leaves with grayish hairs and spiny stipules on the bases of petioles [7]. The plant is used traditionally for the management of a headache, rheumatism, diabetes, and cardiovascular diseases [8]. Previous studies have demonstrated its free radical scavenging ability, hypoglycemic, and hypolipidemic effects on diabetic animals [9,10]. Therefore, this study was conducted to determine the phytochemicals in ESR and to evaluate its *in vitro* and *in vivo* effects of α-amylase and α-glucosidase.

Materials and Methods

Chemicals and reagents

Pancreatic α-amylase from porcine, dinitrosalicylic acid (DNSA), and soluble starch were purchased from Sigma Aldrich (USA). Glucose oxidase kit was purchased from Aggape Diagnostics, India. All other chemicals and reagents used were of analytical grade.

Preparation of plant extract

The plant was collected in Gaborone along the Notwane river on October 2016. The plant was authenticated at the University of Botswana Herbarium and given voucher specimen number UB 019. The leaves were dried at room temperature and ground to fine powder using a laboratory grinder (Brand name: Zhong Xing, Model number: FW80). ESR was prepared by macerating 100 g of the powdered leaves in 70% ethanol for 32 hours. The mixture was filtered using a Whatman 0.45 µm filter paper and the filtrate was evaporated in vacuum via rotor evaporator. The crude extract was dried at room temperature in a fume hood.

Qualitative phytochemical analysis

ESR was screened for bioactive phytochemicals using qualitative methods [11,12].

In vitro analysis of anti-diabetic properties

Measurement of α-amylase inhibitory effects of plant extract/ESR

To determine the effect of the ESR on α-amylase, a method described by Kamtekar et al. [13] was followed with modifications. Briefly, 0.5 ml of distilled water dissolved plant extract (concentrations 200, 400, 600, 800, and 1,000 µg/ml) was incubated with 0.5 ml of porcine pancreatic α-amylase solution (2 units/ml) in 0.02 M sodium phosphate buffer pH 6.9 with 6.7 mM sodium chloride) at 37°C for 10 minutes. Then, 0.5 ml of 1 % starch solution was added and the mixture was further incubated at 37°C for 10 minutes. After incubation, the reaction was stopped by the addition of 1 ml of DNSA reagent and further incubation at 85°C in a water bath for 5 minutes. After 5 minutes, reaction mixture color changed to orange-red and was removed from the water bath and cooled to room temperature. The mixtures were diluted to 5 ml using distilled water and absorbance measured at 540 nm using a Shimadzu UV-Vis spectrophotometer. Control samples were prepared in a similar way except that for each plant extract concentration, the enzyme solution was replaced by a buffer. The experiment was performed in triplicates and α-amylase inhibitory activity was calculated using the following formula:

$$\%\text{inhibition} = \frac{Abs\ (control) - Abs\ (sample)}{Abs\ (control)} \times 100\%.$$

A plot of percentage inhibition against the logarithm of sample concentration was constructed and the concentration inhibiting 50% (IC_{50}) was determined.

Measurement of mode of α-amylase inhibition by ESR

To study the mode of inhibition of α-amylase by ESR, different concentrations of starch (substrate) (0.05, 0.1, 0.15, 0.20, and 0.25 M) were used. They were incubated with α-amylase in the absence of ESR (inhibitor) and with 800 µg/ml ESR at 37°C. The amount of glucose released was quantified using a glucose standard curve. The type of inhibition was determined from the constructed Lineweaver–Burk plot based on K_m and V_{max} values.

Measurement of α-glucosidase (sucrose) inhibitory effects of ESR

For α-glucosidase inhibition assay, the enzyme was isolated from the small intestines of normal Sprague Dawley rats [14]. Normal male rats weighing 150–200 g were sacrificed under diethyl ether anesthetic and dissected. Small intestines were removed and cleaned with cold normal saline. The luminal surface of the intestines was scrapped out using a microscope slide and the epithelial layer collected and homogenized in phosphate buffered saline pH 7.4 containing 1% Triton ×100 and centrifuged at 12,000 rpm for

15 minutes. The pellet was further homogenized in the same buffer with cold butanol added to remove Triton. The sample was partially purified overnight by dialysis method. The protein concentration was estimated by the Lowry method [15] and the sample stored at −20°C until needed for use.

To determine the effect of ESR on sucrose, ESR was diluted to make concentrations; 19.53125–2,500 µg/ml in distilled water. 0.5 ml of ESR solution was incubated with 0.5 ml of sucrose (substrate) solution contained in 50 ml test tubes [16]. The tubes were incubated for 3 minutes at 37°C and after which 0.25 ml of 5 mg/ml crude rat intestinal α-glucosidase was added. After thoroughly mixing the contents, the tubes were at 37°C for 15 minutes. The activity of sucrose was stopped by the addition of 0.5 ml of 2.0 M Tris-HCL buffer (pH 6.9). The amount of glucose liberated was determined using the glucose oxidase kit (Agappe Diagnostics, India) and the percentage inhibition of the enzyme by ESR was calculated from the following equation:

$$\%\text{inhibition} = \frac{Abs\ (control) - Abs\ (sample)}{Abs\ (control)} * 100\%$$

The experiments were carried out in triplicates and results represented as a mean and standard error of means (SEM). A plot of percentage inhibition against the concentration of ESR was used to determine the inhibitory concentration giving 50% inhibition (IC_{50}).

Measurement of mode of α-glucosidase inhibition by ESR

To determine the type of inhibition exhibited by ESR on α-glucosidase, different concentrations of sucrose (substrate) (0.05, 0.1, 0.15, 0.20, and 0.25 M) were incubated with α-glucosidase in the absence of ESR (inhibitor) and with 1.25 mg/ml ESR at 37°C. A Lineweaver–Burk plot was constructed and K_m and V_{max} values were determined from it.

Effect of ESR on glucose absorption by rat hemidiaphragms

The effect of ESR on glucose uptake by rat hemidiaphragms was conducted based on a method described by Ahmed and Urooj [17] with minor changes. Fresh diaphragms were harvested from overnight fasted normal Sprague Dawley rats sacrificed under diethyl ether. The diaphragms were divided into two equal halves, rinsed with normal saline, and placed in well-labeled test tubes containing different media as per the following groups;

Normal control (NC): 2 ml of Tyrode solution with 2% glucose and 2 ml of distilled water.

Positive control (PC): 2 ml of Tyrode solution with 2% glucose and 2 ml of 2 U/ml of human insulin.

ESR 1: 2 ml of Tyrode solution with 2% glucose and 2 ml of 150 mg/ml of ESR.

ESR 2: 2 ml of Tyrode solution with 2% glucose and 2 ml of 300 mg/ml of ESR.

The test tubes were incubated at 37°C on a shaker at 140 cycles/minute for 30 minutes. Then, the amount of glucose in the original Tyrode solution (initial glucose concentration) and from the experiments (final glucose concentration) was determined using the glucose oxidase kit (Agappe Diagnostics). The amount of glucose absorbed per tissue was calculated as the difference between the initial and final concentration of glucose in the medium. The experiment was performed in triplicates.

In vivo effects of ESR on carbohydrate digestion in normal albino rats

Oral starch tolerance test

In this experiment, 15 normal non-diabetic Sprague Dawley rats of mass 200–250 g fasted overnight. Their fasting blood glucose (BG) was determined on a hand-held glucometer (Accu-check Active) after a tail puncture. Animals were randomly divided into three groups:

NC: 1 ml of distilled water

ESR 1: 150 mg/kg bw ESR

ESR 2: 300 mg/kg bw ESR

Animals were orally administered a starch solution (3 g/kg bw) [18] followed by the above treatments. BG level was then determined at periods 30, 60, 120, and 180 minutes to determine the effect of the extract on postprandial glycemia. The results were plotted on a graph and area under the curve (AUC) for each graph was determined based on the following equation.

$$AUC\left(\frac{mmol}{L}\right)\cdot h = \frac{BG0 + BG30 * 0.5}{2} + \frac{BG30 + BG60 * 0.5}{2} + \frac{BG60 + BG120 * 1}{2} + \frac{BG120 + BG180 * 1}{2}$$

Where BG represents blood glucose levels at time intervals 30, 60, 120, and 180 minutes [19].

Oral sucrose tolerance test

For this test, the same method used for starch tolerance test was used. 4 g/kg bw of sucrose was orally administered to rats in the place of starch.

Statistical analysis

All results were represented as mean (n = 3) and SEM. Significance of experimental results was computed using two-way analysis of variance and results were considered significantly different at $p < 0.05$.

Results

Qualitative phytochemical analysis

Percent yield of ESR obtained after 70% ethanol soaking was 8.24%. Phytochemical analysis showed positive results for many bioactive phytochemicals as shown in Table 1.

ESR and α-Amylase inhibition

ESR inhibited the activity of α-amylase with an increase in the concentration of ESR (Figure 1a). The highest percentage of inhibition of 56.7% was recorded at 1,000 µg/ml. The IC_{50} was 831.76 µg/ml. The mode of inhibition of the enzyme using a glucose standard curve (Figure 1b) and Lineweaver–Burk plot (Figure 1c) was determined as non-competitive inhibition. K_m and V_{max} were determined from the Lineweaver–Burk plot and are presented in Table 2.

ESR and α-glucosidase inhibition

ESR displayed α-glucosidase inhibition which increased steadily with an increase in the concentration of ESR, Figure 2a. The IC_{50} determined graphically was found to be 1202.3 µg/ml. From the Lineweaver–Burk plot (Figure 2b) and the kinetics constants (Table 2), the inhibition was concluded to be mixed non-competitive inhibition.

Table 1. Phytochemical composition of ESR.

Tested compound	Result
Phenols	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Cardiac glycosides	+

+ = present, − = absent

ESR and glucose uptake by rat hemidiaphragms

The effect of ESR on glucose uptake by rat hemidiaphragms is shown in Figure 3. Insulin and ESR significantly increased the uptake of glucose by the diaphragms. The ESR showed a significant dose-dependent effect on the uptake of glucose by the isolated rat hemidiaphragms.

Effects of ESR on starch and sucrose tolerance tests

The effect of ESR on the digestion of starch and sucrose *in vivo* was studied in this study using their tolerance tests in normal rats. The results are presented by graphs in Table 3, Figures 4 and 5. Compared to the NC administered distilled water, ESR exerted inhibitory effects on the digestion of both starch and sucrose to release glucose. ESR 1 (150 mg/kg bw) showed minimal inhibition with no significant (P = 0.05) difference in comparison to the normal. However, ESR 2 (300 mg/kg bw) exerted a strong inhibition of both starch and sucrose digestion. ESR significantly (P = 0.05) reduced peak BG levels and the AUCs for both starch and sucrose.

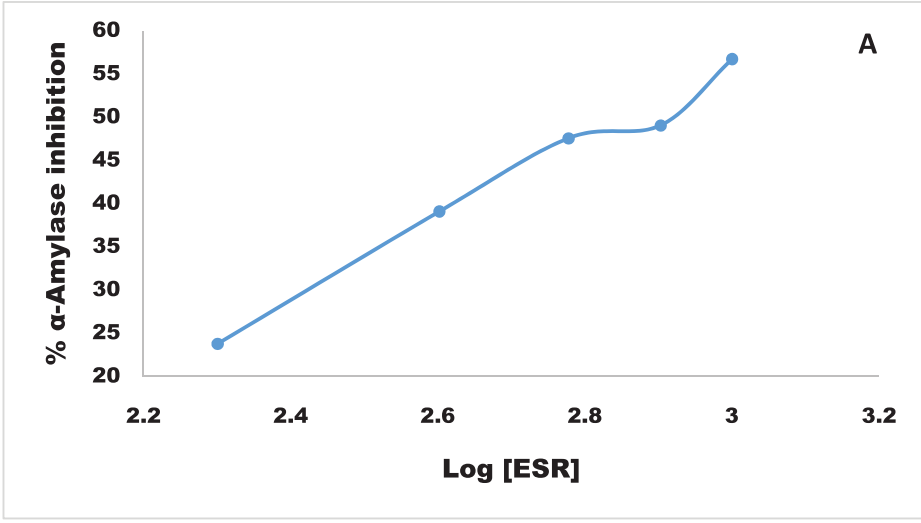
Discussion

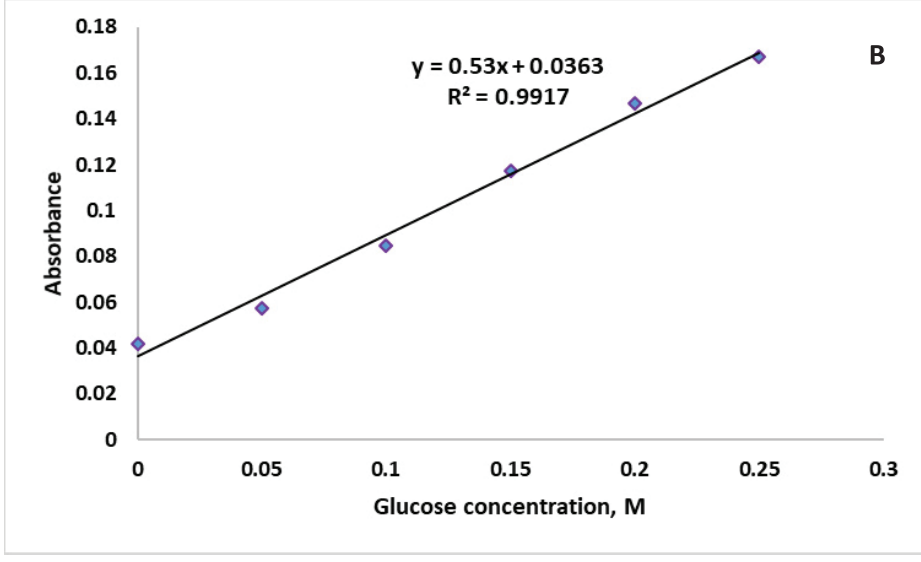
The discovery and development of effective anti-diabetic drugs with less or no adverse side effects remains a challenge globally, hence, there is a much interest in botanicals. Botanicals seem to offer a better management of diabetes due to their holistic approach to the pathophysiology of the disease and few or no side effects. In the present study, the anti-diabetic activity of the ESR was evaluated together with its phytochemistry. Qualitative phytochemical screening showed that ESR contains phytochemical constituents like phenols, flavonoids, glycosides, tannins, saponins, and steroids. The results of the study are in agreement with the findings of Shaheen et al. [19] even though they used the methanol extract. These phytochemicals have been reported to have anti-oxidative and anti-diabetic [20] effects; hence, the traditional use of *Sida rhombifolia* in the management of diabetes complications. Phenols and flavonoids,

Table 2. The kinetic constants of α-amylase and α-glucosidase inhibition.

Enzyme	Control/ESR	K_m (mg/ml)$^{-1}$	V_{max} (mg·ml^{-1}·S^{-1})
α-amylase	Control	0.222	100
	ESR	0.222	58.8
α-glucosidase	Control	0.17	71.4
	ESR	0.2	52.6

ESR = extract of *Sida rhombifolia*





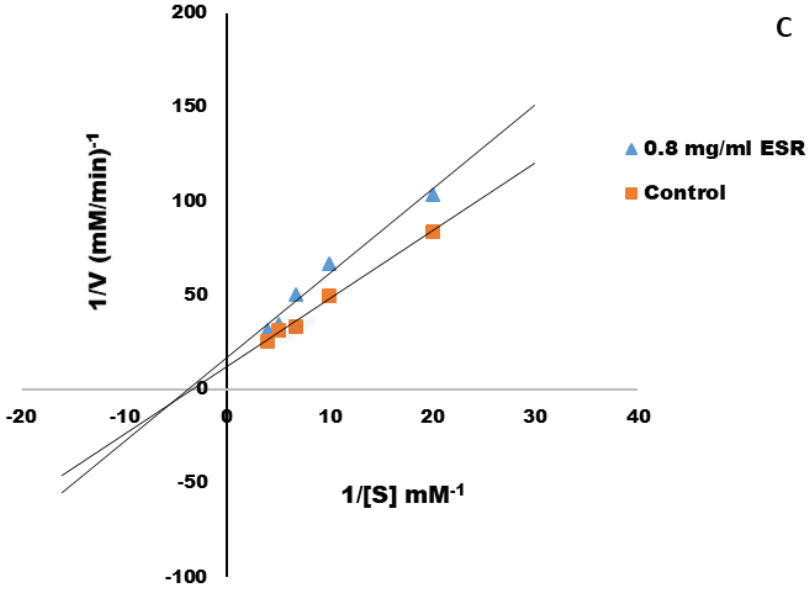


Figure 1. α-Amylase inhibition and mode of inhibition: (a) The percentage inhibition of α-amylase, (b) the glucose standard curve, and (c) the Lineweaver–Burk plot.

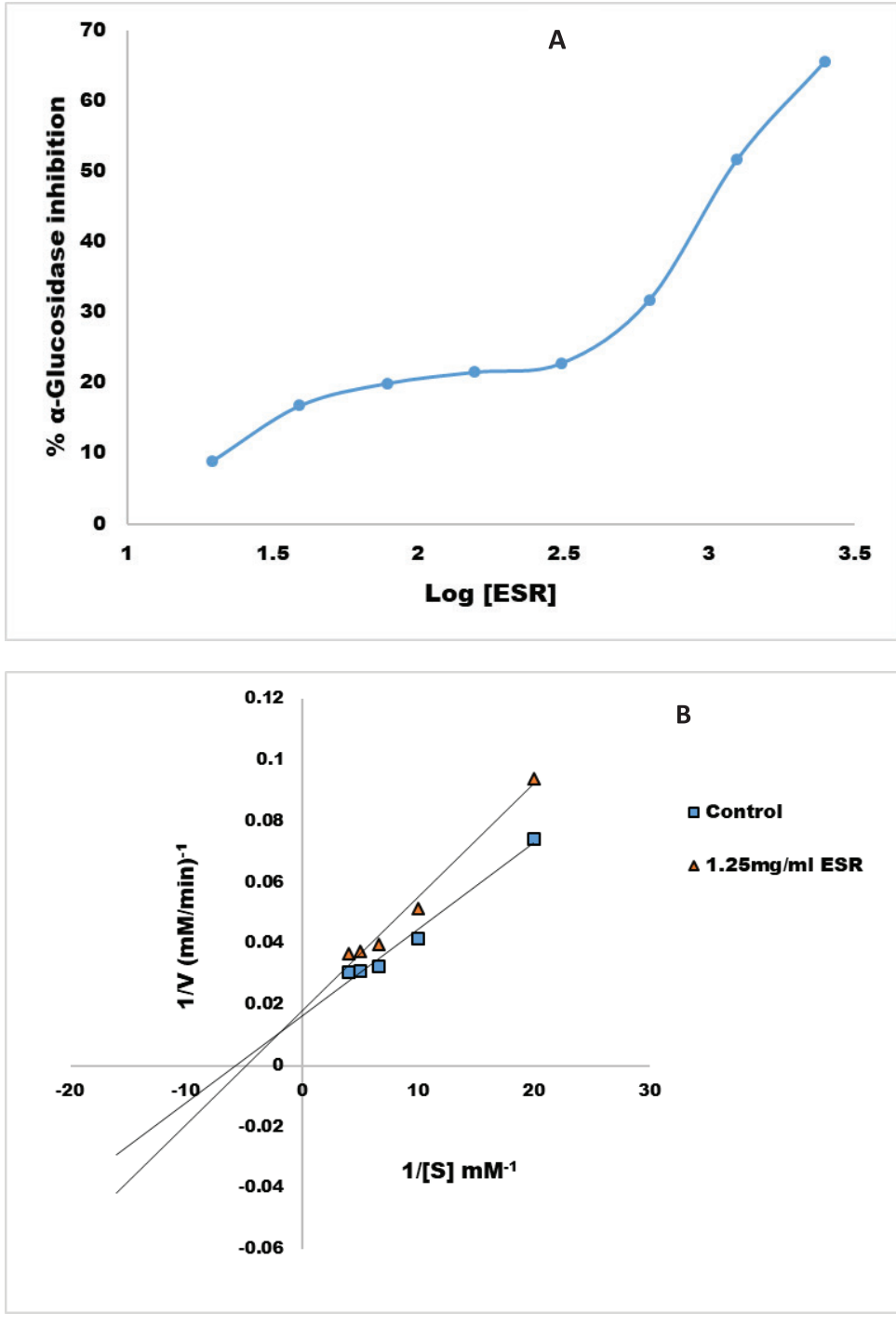


Figure 2. α-Glucosidase inhibition and mode of inhibition: (a) dose–response curve and (b) Lineweaver–Burk plot.

Table 3. Effect of ESR on AUC after starch and sucrose loading on normal rats.

Groups	Starch		Sucrose	
	AUC (mmol/l.h)	% reduction of AUC	AUC (mmol/l.h)	% reduction of AUC
NC	18.63 ± 0.06	–	21.15 ± 0.02	–
ESR 1	17.89 ± 0.32	3.97	20.21 ± 0.22	4.44
ESR 2	15.95 ± 0.25*	14.4	16.94 ± 0.02*	19.9

*P = 0.05 in comparison to NC

NC = normal control, *ESR 1* = 150 mg/kg bw extract, *ESR 2* = 300 mg/kg bw extract

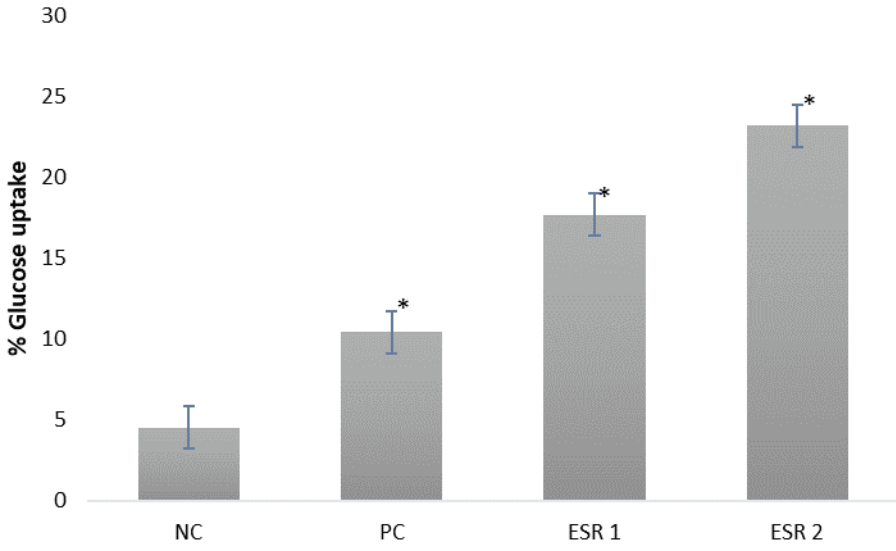


Figure 3. Effects of ESR on glucose uptake by rat hemidiaphragms. *P = 0.05 in comparison to NC. *NC* = normal control, *PC* = positive control (insulin), *ESR 1* = 150 mg/kg bw extract, *ESR 2* = 300 mg/kg bw extract.

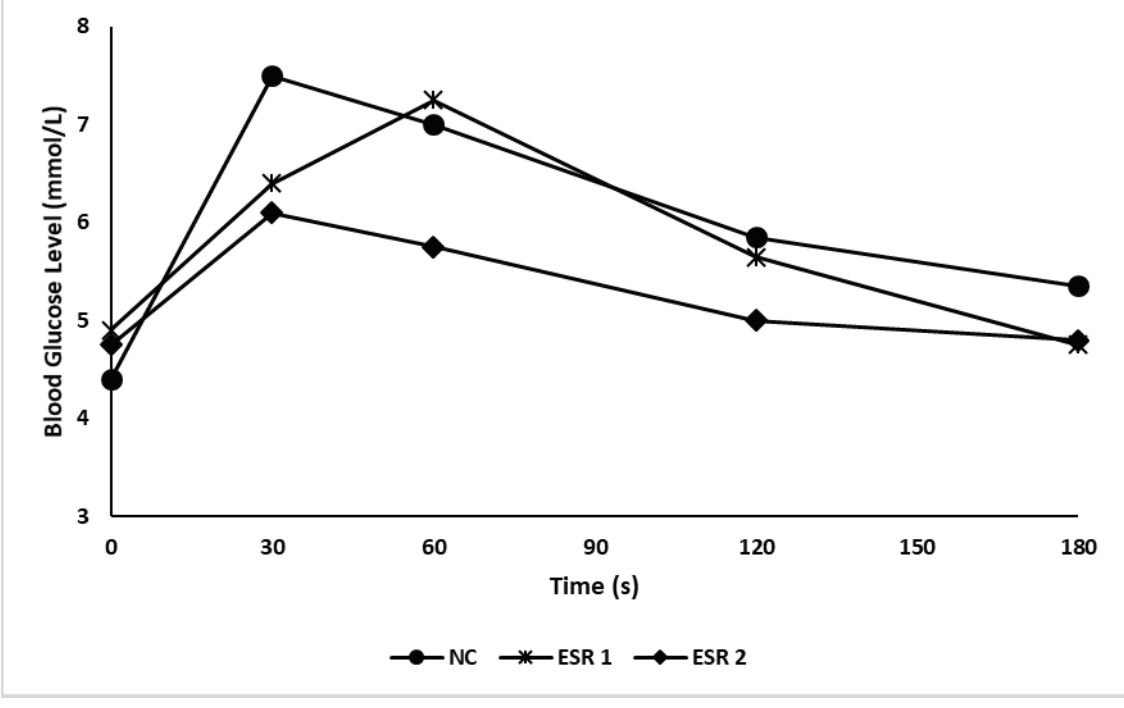


Figure 4. Effect of ESR on starch tolerance test in normal rats. *NC* = —Normal control, *ESR 1* = —150 mg/kg bw extract, *ESR 2* = —300 mg/kg bw extract.

which are secondary metabolites, possess pharmacological properties like free radical scavenging activity, strong antioxidant activity, anti-inflammatory action, inhibition of hydrolyte, and oxidative enzymes [21]. Flavonoids inhibit α-glucosidase and aldose reductase thereby reducing postprandial BG level [21]. Tannins inhibit digestive enzymes like lipases, proteases, and glucosidases [18], the same mechanism of action used by other clinical synthetic drugs such as xenical and acarbose. Alkaloids and some saponins have BG reduction effect and antioxidant properties [22]. The phenolic and flavonoid richness of the extract may be responsible for the hypoglycemic activity of ESR.

The anti-diabetic effects of ESR were investigated by *in vitro* studies of α-amylase, α-glucosidase, and

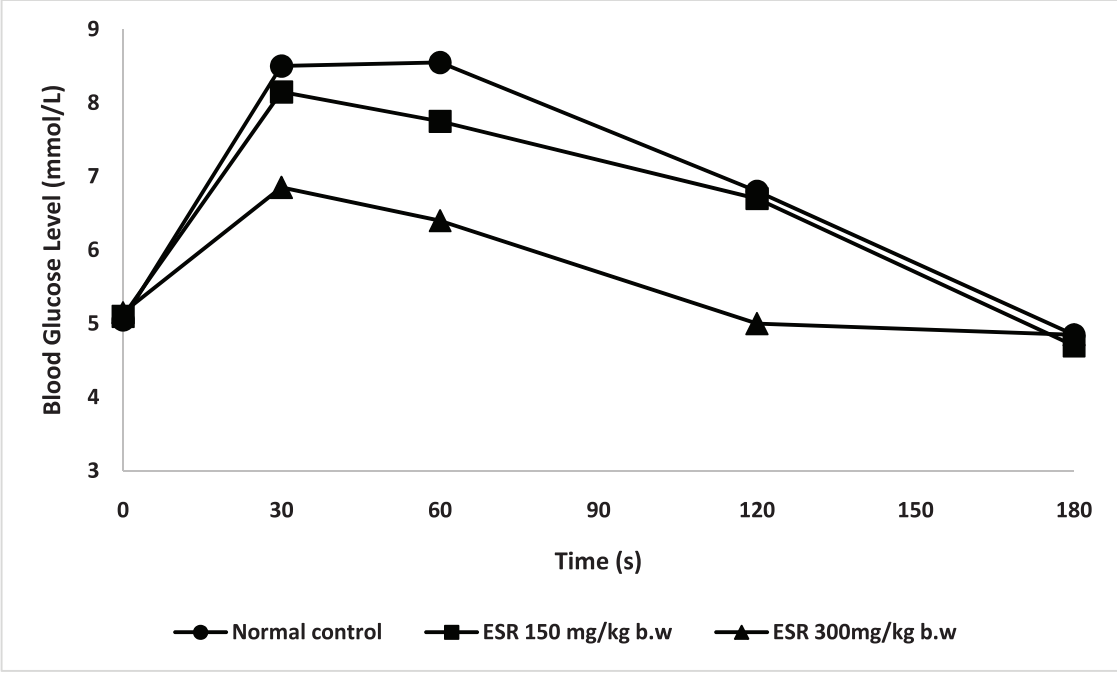


Figure 5. Effect of ESR on sucrose tolerance test. *NC* = —normal control, *ESR 1* = —150 mg/kg bw extract, ESR 2 = —300 mg/kg bw extract.

rat hemidiaphragms. α-amylase is produced and released by the salivary glands and the pancreas to break starch into maltose and sucrose [22]. On the other hand, α-glucosidase found on the luminal surface of the small intestines breaks down disaccharides into the monosaccharide glucose for absorption into the bloodstream [23]. ESR significantly inhibited the catalytic activity of both α-amylase and α-glucosidase with IC_{50} values of 831.76 and 1202.3 µg/ml, respectively. The inhibition of these enzymes by ESR was concentration dependent. This, therefore, shows that ESR plays a significant role in the management of postprandial hyperglycemia by slowing down the digestion of carbohydrates and their absorption into the bloodstream. The inhibition of the mammalian α-glucosidase by *Sida rhombifolia* was also reported by Arciniegas et al. [24] with the acetone extract having the highest percentage inhibition than methanol and hexane. The inhibition of α-amylase and α-glucosidase by ESR may be linked to the presence of phenolic compounds such as flavonoids and tannins present in it [25].

The mode of inhibition of α-amylase and α-glucosidase by ESR was also investigated and determined on Lineweaver–Burk plots. It was concluded that ESR inhibited α-amylase in a non-competitive manner and α-glucosidase in a mixed non-competitive fashion as supported by K_m and V_{max}. This implies that compounds in ESR do not bind to the substrate active sites of the enzymes; hence, inhibition cannot be overcome by increasing substrate concentration [26] an advantage over competitive inhibitors of same enzymes such as acarbose. The non-competitive inhibition of the carbohydrate hydrolyzing enzymes by ESR is like that of other reported plants extracts [27].

In vivo studies were conducted as confirmatory to *in vitro* results. It was found out that ESR at a dose of 300 mg/kg bw strongly reduced the peak BG concentrations and AUC under both starch and sucrose tolerance tests. This implies that after a carbohydrate meal where BG levels normally rise, ESR reduces them and maintains a steady glucose homeostasis. Henceforth, ESR is potentially suitable for the management of postprandial glycemia in type 2 diabetic patients. The reduction of postprandial glycemia implies that ESR inhibits α-amylase and α-glucosidase from digesting starch to maltose and sucrose, and sucrose to glucose, respectively [23]. This reduction confirms the inhibitory effects of ESR shown by the *in vitro* studies. Therefore, ESR can be attributed to have a similar mechanism of action to acarbose and miglitol, clinically used drugs for the management of postprandial diabetes [27]. Additionally, the ability of ESR to cause non-competitive inhibition of α-amylase and α-glucosidase

potentially makes it a better treatment option than the current standard of care for DM.

The effect of ESR on glucose uptake by muscle tissues was investigated using rat hemidiaphragms. ESR significantly promoted the uptake of glucose in a dose-dependent manner. ESR contains phytochemicals which may stimulate the expression of glucose transporters 4four muscle tissues [28] which aid in the efficient absorption of glucose. ESR may possess insulin-like properties which enhances uptake of glucose by respiring cells. ESR may also increase the endogenous production of insulin from pancreatic beta cells resulting in enhanced glucose uptake [29]. Because insulin sensitivity is impaired in type 2 DM, ESR may alleviate the sensitivity of the adipose and muscle tissues to the action of the circulating insulin. The increased peripheral uptake of glucose may contribute greatly to controlling postprandial hyperglycemia in type 2 DM.

Conclusion

It was concluded that ESR contains bioactive phytochemical constituents which may be responsible for its hypoglycemic activity. The anti-diabetic effects of ESR may be due to the inhibition of α-amylase and α-glucosidase, and increased uptake of peripheral glucose by muscle tissues. Therefore, ESR needs further research for potential use as an anti-diabetic drug.

Acknowledgments

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Conflict of Interest

We declare no conflict of interest.

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Qualitative determination of toxic pyrrolizidine alkaloids in *Trichodesma indicum*: A prevalent ethnomedicine of Northern Pakistan

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ABSTRACT

Aim/Background: *Trichodesma indicum* (L.) Lehm. is a prominent medicinal plant in Pakistan. Various indigenous Pakistani communities use this plant orally to treat various human ailments. The present study is carried out with the aim to investigate the leaves of *T. indicum* for the presence or absence of toxic pyrrolizidine alkaloids (PAs). If these alkaloids are found, then the plant should be used with more precaution.

Materials and Methods: For this, the dried leaf samples of *T. indicum* were collected from Northern Pakistan and brought to Beijing Normal University for further experimentation. Furthermore, to collect literature on the medicinal uses of *T. indicum*, we searched the scientific databases of PubMed, Google Scholar, and Scopus. In the laboratory, *T. indicum* was investigated for PAs by using high-performance liquid chromatography (HPLC)–ultraviolet.

Results: The literature survey shows that the species is used for medicinal purposes throughout Pakistan. The result of HPLC fingerprint analysis showed that leaves were PA positive and out of five PA standards, four were detected, namely, (1) supinine, (2) europine, (3) heliotrine, and (5) echimidine.

Conclusion: To the best of our knowledge, the present study reports, for the first time, the presence of unsaturated PAs in the leaves of *T. indicum.* Given this, we suggest that the plant should be used with more caution and should follow the rule defined by the German Federal Institute for Risk Assessment which requires that the exposure to unsaturated PAs from food should be as low as possible which is a daily intake of 0.007 µg PAs kg^{-1} for human's body weight.

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Introduction

Plants are the basis of both herbal remedies and discovery of modern drugs. Currently, throughout the world, approximately 40,000–70,000 plant species are used as medicines [1]. Around the world, ethnomedicine is used to treat various human ailments and to improve basic health care. According to a recent report, in Chile (70%), Colombia (40%), and throughout the continent of Africa (80%), much of the population relies on herbal medicine [2]. According to recent reports, the estimated value of herbal markets reached about US $100 billion by 2015 [3] and US $107 billion by 2017 [4]. According to a report, approximately 20% of all plants found in the world are used for therapeutic purposes to treat various human ailments [5]. Often, people in developing countries rely more on the traditional medicine possibly due to limited access to modern health services [6]. Indigenous people around the world depend on plants for basic health care and economic value. These benefits are identified based on the experience, need, and observation of elder and native people [7]. Utilization of local medicinal flora by traditional healers or practitioners is often cheap and easily available to people with a low income. The ethnomedicinal uses of plants by the indigenous communities are very important and noteworthy. Ethnomedicine is not only the study of

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herbal medicine as used by local communities but also it provides a basis for drug discovery from new sources [8,9].

As the usage of herbal medicine gained popularity over the past decades, reports of suspected toxicity and adverse events were also described. A meta-analysis of 69 prospective and retrospective studies from different parts of the world involving around 419,000 patients found that nearly 6.7% of all hospitalizations resulted from adverse drug reactions [10]. PAs are also leading plant toxins associated with disease in humans [11]. PAs are widely distributed in plants. In the world, about 3% of all flowering plants contain PAs [12]. Until now, more than 660 PAs have been identified from over 6,000 plants, mainly in Boraginaceae, Asteraceae, and Fabaceae families [13]. In plants, the most naturally occurring unsaturated PAs are known to be hepatotoxic and tumorigenic in humans [14,15]. When human poisoning with PAs occurs, it causes a characteristic disease called hepatic sinusoidal obstruction syndrome (HSOS) which may lead to liver failure [16]. In the past few years, reports on lower levels of dietary exposure via common herbal products and foods such as black, herbal or green teas, PA-producing plant parts, or honey collected from PA-producing plants have caused concerns, especially regarding the potential of carcinogenicity and genotoxicity of unsaturated PAs. These PA-producing plant food products have elevated PA concentrations which pose safety risks [17–19].

Previously, it was well established that intoxication cases due to unsaturated PAs occurred throughout developed as well as underdeveloped countries. Nearly, 10,000 PA-poisoning cases have been documented in many countries, including Great Britain, Germany, Afghanistan, United States, Switzerland, India, China, South Africa, and Jamaica [20]. In 1968 in South Africa, out of 15 children affected, 10 died because of PAs from *Crotalaria* spp. [21]. In India in 1973, 486 people had the veno-occlusive disease (VOD) because of cereals contaminated with *Crotalaria* spp. [22]. In Afghanistan between 1970 and 1972, around 7,200 people were diagnosed with VOD because of wheat contaminated with *Heliotropium popovii* ssp. *gillianum* [23]. In 1993, 3,906 people suffered from ascites, hepatomegaly, abdominal pain, and alteration of consciousness because of *H. lasiocarpum* in Tajikistan [24].

In the world, unsaturated PAs are one of the most widespread natural toxins. However, PAs are protoxins as they require metabolic conversion in the liver to show toxic activity [25]. So, the use of Boraginaceae plants or any PA-containing products as a poultice for wounds or external use does not cause any risk [26]. In plants, unsaturated PAs mainly occur as their N-oxides and so these cannot be directly converted to the hydroxy-PAs, but whenever it is ingested orally, bioactivation takes place in the liver by cytochrome P450 monooxygenases and they are reduced to free bases [25,27]. In humans, domestic animals, and wildlife, PAs may cause acute fatal intoxications. Low-level and intermittent ingestion of unsaturated PAs is more difficult to associate with adverse health effects, especially when they become apparent over years after consumption [28]. These low-level exposures of food or herbal products to 1,2-unsaturated PAs can initiate chronic disease and may cause a wide range of cancers, congenital anomalies, cirrhosis, and pulmonary arterial hypertension [29].

Trichodesma is comprised of about 40–45 species [30,31] mainly found in Asia, Africa, and western and central Australia [32]. *Trichodesma indicum* (L.) Lehm. (Boraginaceae) is an annual herb with densely hairy branches and pale blue flowers [33]. The present study is focused on a literature survey of ethnomedicine *T. indicum* which is utilized for medicinal purposes in Pakistan. The aim of this study is to investigate the leaves of *T. indicum* for the presence or absence of toxic pyrrolizidine alkaloids (PAs). If these alkaloids are found, then the plant should be used with more precautions.

Materials and Methods

Literature review of ethnomedicinal uses of T. indicum

We conducted a systematic search of the literature regarding medicinal uses of *T. indicum* in Pakistan. For this, we searched the scientific databases of PubMed, Google Scholar, Web of Science, and Scopus for the search terms: "*Trichodesma indicum* medicinal uses in Pakistan," "Boraginaceae ethnobotany in Pakistan," and "Ethnobotany of Pakistan." All pertinent articles up to April 2018 were reviewed (Table 1).

Collecting sites of Trichodesma indicum*—Northern Pakistan*

Northern Pakistan (Pan-Himalayan regions) lies between 71°E–78°E longitude and 32°N–37°N latitude and occupies a unique bio-geographic position. The Pakistani Pan-Himalayan regions include Azad Kashmir, Chitral, Swat, Dir, Hazara Division, and Gilgit-Baltistan (Fig. 1). The Himalayas are spread

Table 1. Searched databases with accompanying range of years and search terms.

Database	Search term	Range of years	Total articles	Selected articles
Web of Science	"Ethnobotany of Pakistan"	2000–2018	195	09
	"Boraginaceae and ethnobotany in Pakistan"	2000–2018	08	02
	"*Trichodesma indicum* medicinal uses in Pakistan"	2000–2018	05	01
Google Scholar	"*Trichodesma indicum* medicinal uses in Pakistan"	2000–2018	245	33
Scopus	"Ethnobotany of Pakistan"	2008–2018	126	15
PubMed	"Ethnobotany of Pakistan"	2000–2018	59	5
	"Boraginaceae and ethnobotany in Pakistan"	2000–2018	06	01
	"*Trichodesma indicum* medicinal uses in Pakistan"	2000–2018	02	00

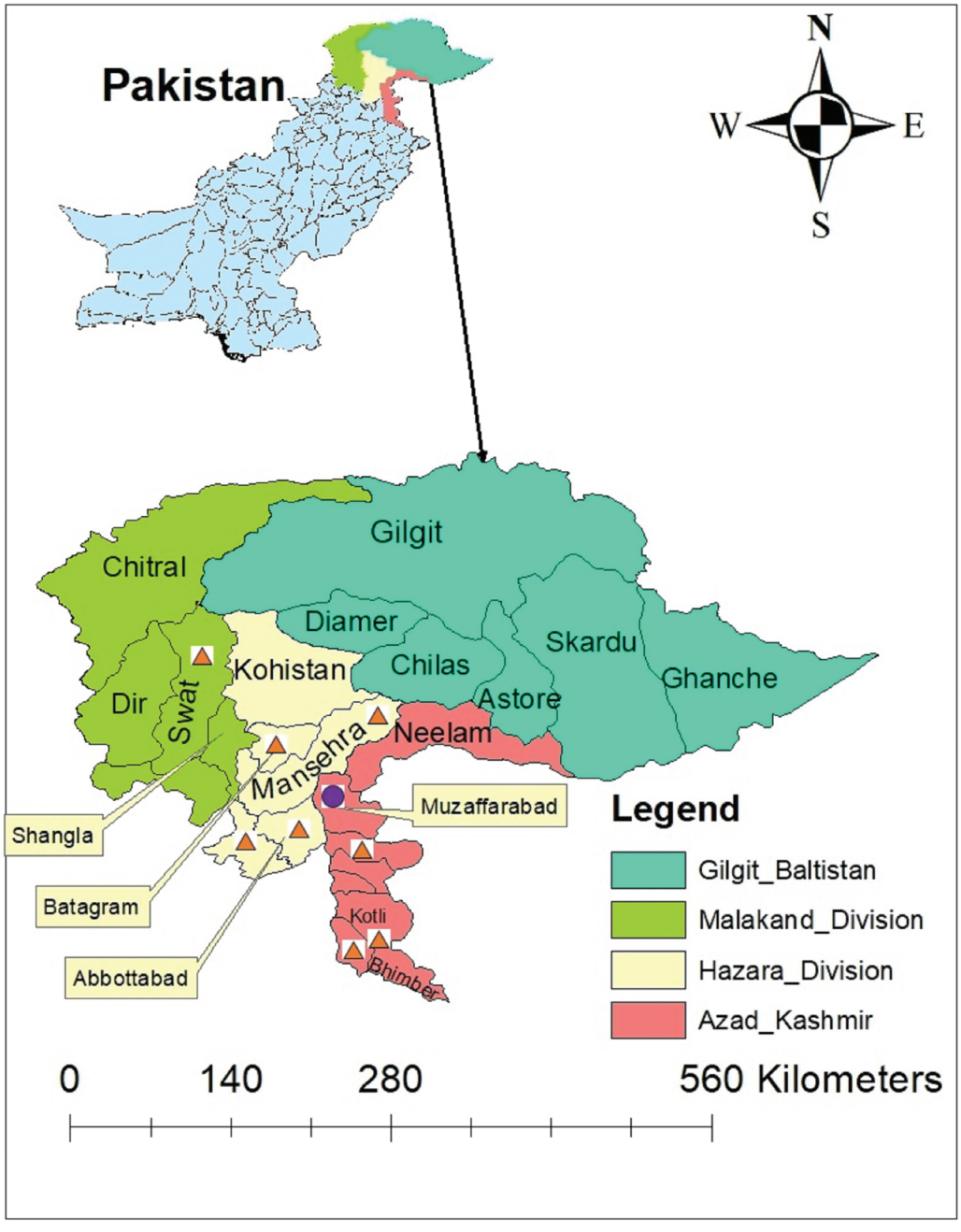

Figure 1. *Trichodesma indicum* collecting sites, Northern Pakistan.

across three of Pakistan's provinces. Northern Pakistan encompasses its surrounding valleys, the Nanga Parbat massif, Azad Jammu, and Kashmir [34].

Plant collection and deposition in herbarium

As part of the preparation of the revision of family Boraginaceae for the *Flora of Pan-Himalayas*, we carried out comprehensive field investigations in Northern Pakistan, from July 2016 to September 2017 to collect samples of Boraginaceae. In the field, whole plants were pressed in the newspaper for herbarium specimens and for phytochemical analysis, fresh plant material was collected in silica gel. *Trichodesma indicum* was collected and identified at Beijing Normal University. All specimens were deposited at the Beijing Normal University Herbarium with appropriate voucher numbers (Fig. 2).

Experimental work

Authentic standards, chemicals, and reagents

Five authentic standards of PAs, i.e., (1) supinine, (2) europine, (3) heliotrine, (4) lycopsamine, and (5) echimidine were obtained from ChemFaces (Wuhan, Hubei 430056, P.R. China) and had a purity >98% (see Fig. 3). All reagents were of high-performance liquid chromatography (HPLC) grade. Methanol and acetonitrile were purchased from DikmaPure (USA). Formic acid was the product of Aladdin Industrial Corporation (Shanghai, China). Throughout the experimental process, ultra-pure water from a Milli-Q water purification system was used (Millipore Corporation, Billerica, MA).

Plant sample preparation for HPLC

For the HPLC fingerprint analysis, the leaves of *T. indicum* were prepared according to the established methodology [35]. Briefly, 500 mg dried leaves of mature *T. indicum* were weighed and crushed into powder with the help of a FastPrep-24™ Instrument homogenizer (M.P Biomedical, Irvine, CA). The samples were sonicated for 35 minutes with 2.5 ml of methanol immediately followed by centrifugation for 15 minutes at 4,000 × *g*. The resulting supernatant was transferred to a new tube and the procedure was repeated for a total of three times.

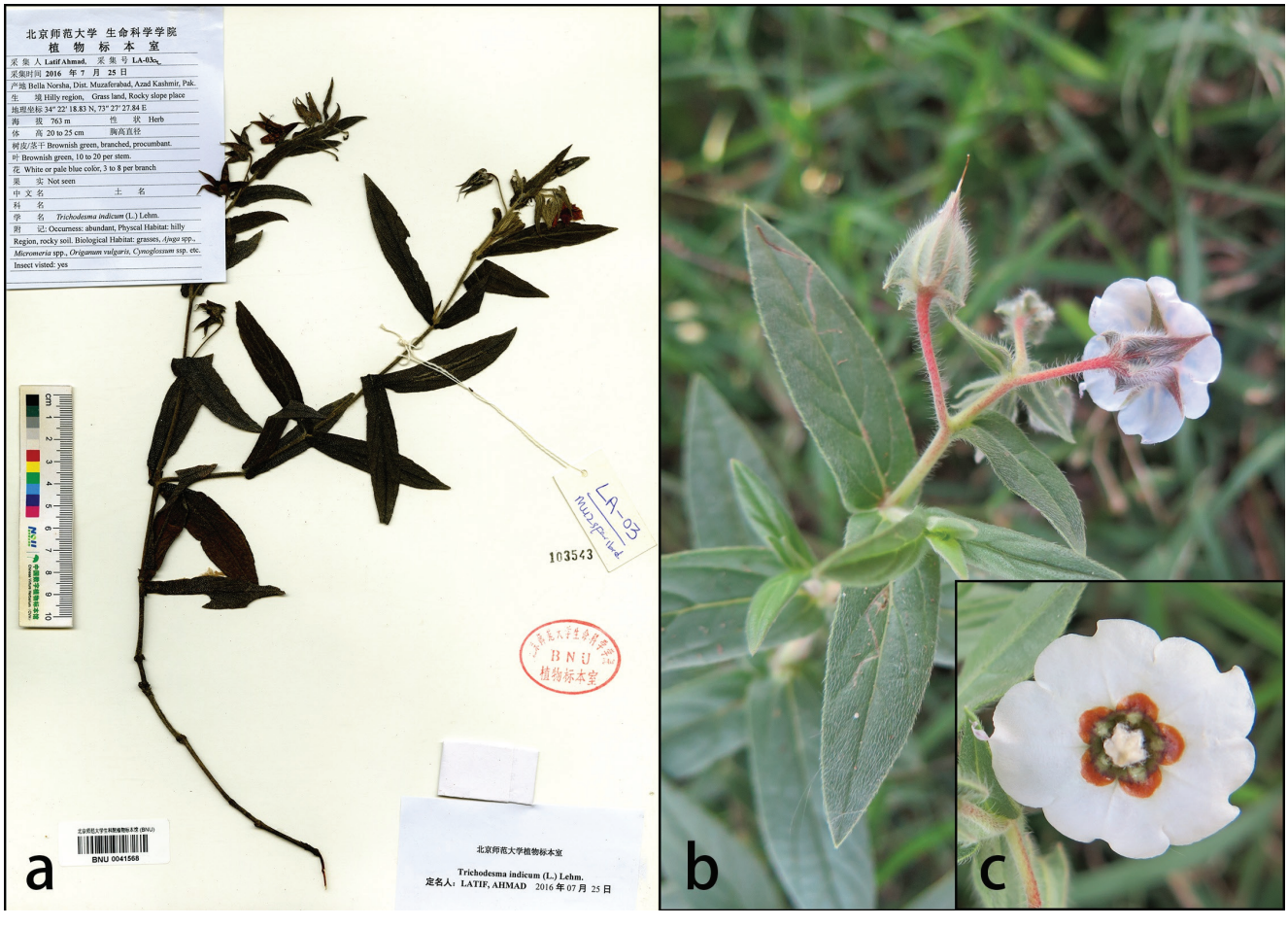

Figure 2. *Trichodesma indicum* (a) Herbarium specimens; (b and c) field photo.

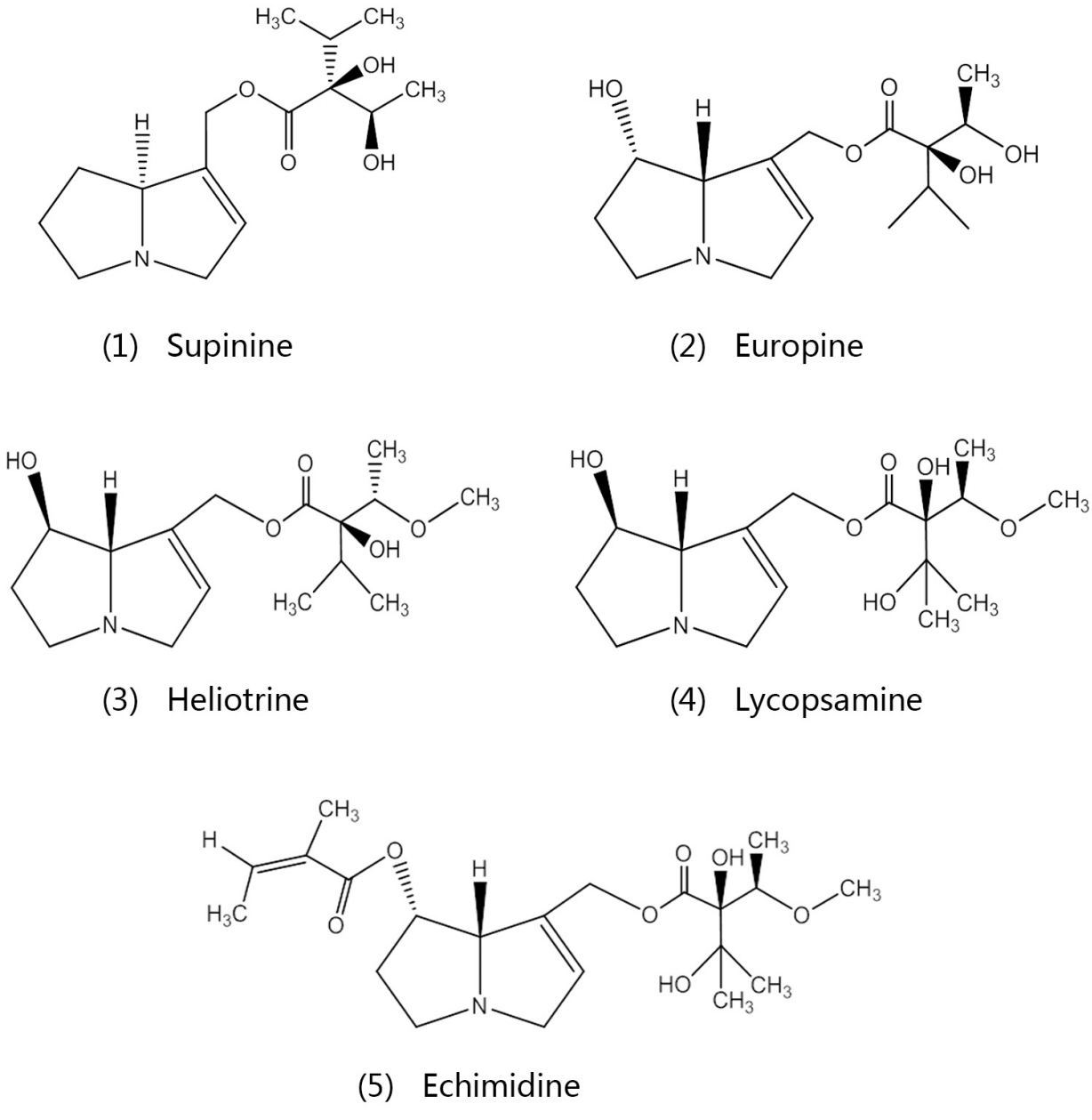

Figure 3. *Chemical structures of the authentic standards.*

Supernatants were pooled and the volume was adjusted to 10 ml with methanol. Samples were mixed thoroughly and 350 µl of sample were filtered with a 13 mm × 0.45 µm FitMax Syringe Filter membrane Nylon (Dikma, USA) before injection.

Authentic standard preparation

A stock solution of each PA (1.0 mg/ml) was prepared by weighing and subsequently dissolved in methanol [35,36]. Mixed stock solutions containing heliotrine, supinine, europine, echimidine, and lycopsamine were also prepared at a concentration of 1.0 mg/ml with methanol. Before subjection to HPLC analysis, the solutions of all standards were stored in a refrigerator at 4°C. The standard solutions (300 µl of each) were filtered by a FitMax Syringe Filter membrane Nylon, and an aliquot (10 µl) of each filtrate was subjected to HPLC analysis.

HPLC–UV analysis

Instrumentation for unsaturated PAs analysis from the leaves of *T. indicum* consisted of a Waters Alliance 2695 liquid chromatography (LC) System equipped with a 2487 Dual Wavelength ultraviolet (UV) Detector (Milford, MA). The HPLC system was comprised of the following modular components: a

built-in quaternary pump, auto-injector, four-channel degasser, and auto-sampler with 120 vials (2 ml). The chromatographic analysis was performed on a Zorbax SB-Aq (4.6 × 250 mm, 5 µm particles) column (Agilent, USA) for the separation of the PAs from the leaves of *T. indicum*.

The LC method for the qualitative analysis of unsaturated PAs was developed for the investigation of PAs in the leaves of *T. indicum* from Pan-Himalayan regions in Pakistan. We separated unsaturated PAs using the mobile phase which consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The flow rate was set at 0.5 ml/minute with the following solvent gradient elution: 0–15 minutes: 13% A; 15–17 minutes: 50% A; 17–35 minutes: 100% A; 35–40 minutes: 100% A; 40–55 minutes: 13% A. Analysis runtime was 55 minutes with a 15-minute methanol wash in between each sample. The temperature of the column was maintained at room temperature, the wavelength detection was set at 280 nm, and a 20 µl volume of sample was injected each run.

Results

Quantitative review of medicinal uses of T. indicum

To find the medicinal uses of orally ingested *T. indicum* to treat various human ailments, we searched the literature found on Google Scholar, Web of Science, PubMed, and Scopus up to 2018. The databases were queried with the terms "*Trichodesma indicum* medicinal uses in Pakistan," "Boraginaceae ethnobotany in Pakistan," and "Ethnobotany of Pakistan" in the title, which resulted in 646 studies in the past 18 years (Table 1). Our aim was focused on orally administered *T. indicum* remedies to treat various human ailments in Pakistan. Therefore, published studies outside of our aim were omitted.

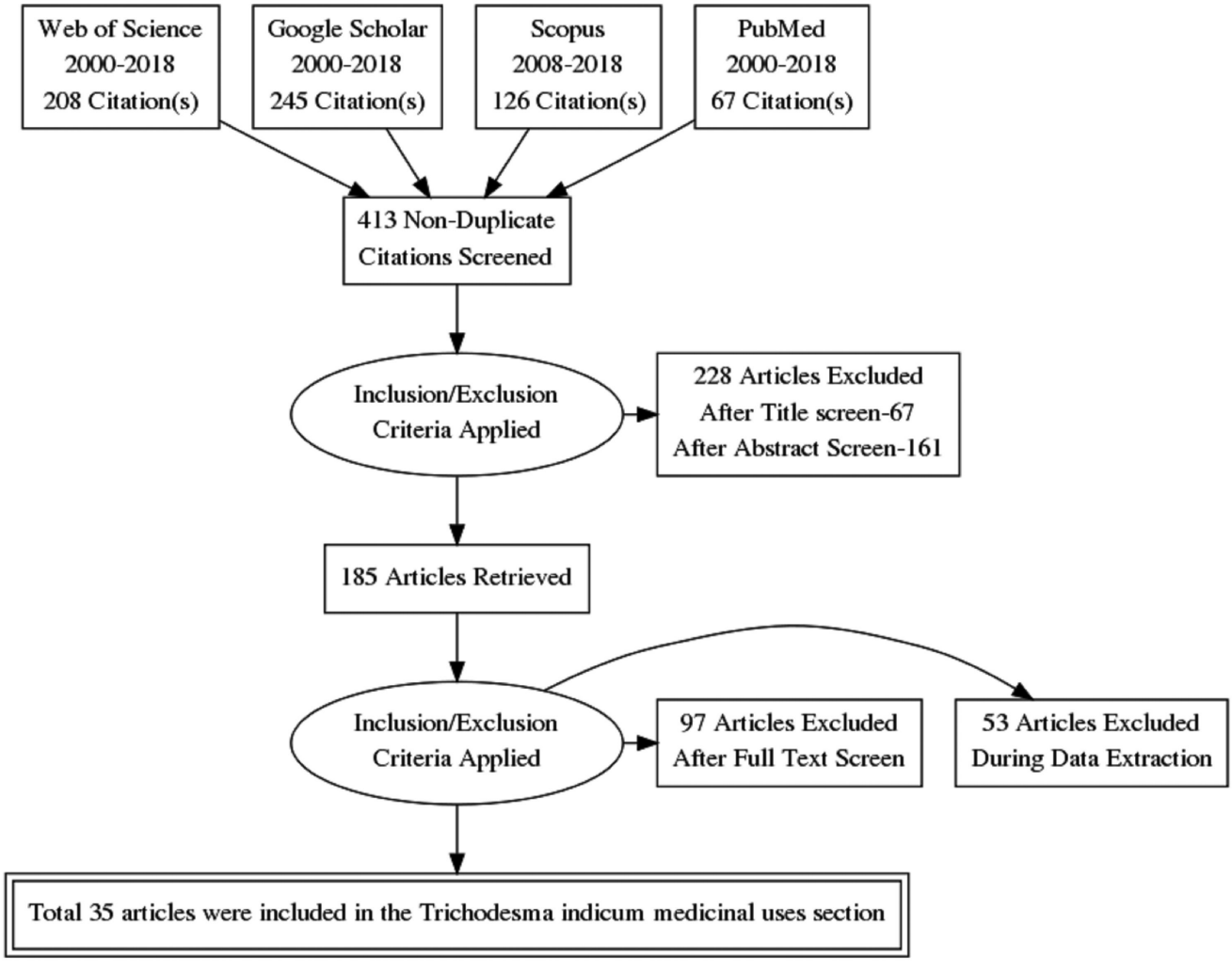

Figure 4. A flow chart of ethnomedicinal information of *T. indicum* derived from different phases of our literature review.

After reading the title, abstracts, keywords, or a full part of the published articles; we focused on articles pertaining to ethnomedicinal uses of *T. indicum* to treat various human ailments. This reduced the sampled articles to 35 publications. The literature search strategy design for the ethnomedicinal uses of *T. indicum* for our review is shown in Figure 4.

Ethnopharmacological use of T. indicum

Trichodesma indicum (L.) Lehm. locally known as Gaaozeban, Handusi booti, Kallri Booti or Handusi is an annual much-branched spreading and densely hairy herb, up to 10–45 cm long, its flowering and fruiting were from March to September. For the investigation of toxic PAs, the samples of *T. indicum* were collected from Muzaffarabad; Azad Kashmir, with an altitude of 763 m. According to the literature surveyed, this species is used for various human ailments. Table 2 presents the literature surveyed of the ethnomedicinal uses of *T. indicum* in Northern Pakistan.

Separation and determination of PAs in T. indicum *extracts*

The structure of PAs varies in plants, but some chemical properties remain constant. We use these properties to try to achieve equal extraction efficiency of each PA. PAs are alkaloids with the characteristic basic nitrogen. This lends them to the classical methanol extraction method for alkaloids. We find

Table 2. Ethnomedicinal uses of *T. indicum* in Pakistan.

Part used	Medicinal uses	Modes of preparation	Literature
Leaves	Flu and cough	Decoction	[37]
	Cough and influenza	Decoction	[38]
	Chest problem	Infusion	[39]
	Dysentery and fever	As a cold drink	[40]
	Fever, diarrhea, and dysentery	Decoction	[41]
	Diarrhea and dysentery	Paste and decoction	[42]
	Fever and dysentery	Decoction	[43]
	Stomach disorder and intestinal worms	Paste	[44]
	Antidote to snakebites	Not mentioned	[45]
	Fever and dysentery	Decoction	[46]
	Medicinal uses	Not mentioned	[47]
	Snakebite	Not mentioned	[48]
	For children dysentery	Cold infusion	[49]
	Diarrhea and dysentery	Paste	[50]
Whole plant	Urinary disease and dysentery	Not mentioned	[51]
	Fever and diarrhea	Not mentioned	[52]
	Snake bite	Decoction	[53]
	Medicinal uses	Not mentioned	[54]
	Influenza and cough	Decoction	[55]
	Urinary disease and snakebites	Powder	[56]
	Blood purifier and fever	Decoction	[57]
	Abdominal pain, backache, and kidney stone	Paste and decoction	[58]
	Medicinal uses	Not mentioned	[59]
	Dysentery, stomach inflammation, and disorder	Decoction	[60]
	Diarrhea and dysentery	Not mentioned	[61]
	Tumor, snake bite, and urinary disease	Not mentioned	[62]
	Fever, blood purifier, diarrhea, dysentery, and urinary problems	Not mentioned	[63]
	Anodyne, diuretic, and anti-rheumatic	Not mentioned	[64]
	Fever and blood purifier	Decoction	[65]
	Anti-venom	Not mentioned	[66]
	Anti-snake venom	Not mentioned	[67]
Leaves and flowers	Cough and flu	Decoction	[68]
	Brain tonic, diuretic, emollient, depurative, and snake bite	Decoction	[69]
	Antispasmodic and lipoxygenase inhibitory activity	Not mentioned	[70]
	Vomiting, joint pain, cough, and fever	Decoction	[71]

that sonication with methanol gives the best result for PA extraction from botanical samples [35].

To obtain the standardized HPLC fingerprint as mobile phases, we used water and acetonitrile with 1% formic acid. For maximum sensitivity, the wavelength was set at 280 nm for detection. In this phytochemical investigation of *T. indicum* from Northern Pakistan, the PAs present were determined and characterized by HPLC–UV. Five PAs namely: (1) supinine, (2) europine, (3) heliotrine, (4) lycopsamine, and (5) echimidine served as an authentic reference. These were used to identify the corresponding PAs in leaves of the *T. indicum*. Using the HPLC conditions discussed in the materials and methods section, the five standards of supinine, europine, heliotrine, lycopsamine, and echimidine were separated with retention times of 4.95, 10.21, 10.87, 37.16, and 46.34 minutes, respectively (Table 3). After identification of the retention time of standards, 20 µl of plant sample was injected and the retention times were compared to those of reference standards. The result of HPLC fingerprint analysis showed that the leaves of *T. indicum* were positive for the PAs supinine, europine, heliotrine, and echimidine. The result of the HPLC chromatogram of *T. indicum* was represented in Figure 5.

Table 3. HPLC results of the PAs.

No	Reference standard	Retention time (minute)	Formula	Molecular weight
1	Supinine	4.95	$C_{15}H_{25}NO_4$	283.368
2	Europine	10.21	$C_{16}H_{27}NO_6$	329.183
3	Heliotrine	10.87	$C_{16}H_{27}NO_5$	314.196
4	Lycopsamine	37.16	$C_{15}H_{25}NO_5$	299.173
5	Echimidine	46.34	$C_{20}H_{31}NO_7$	398.217

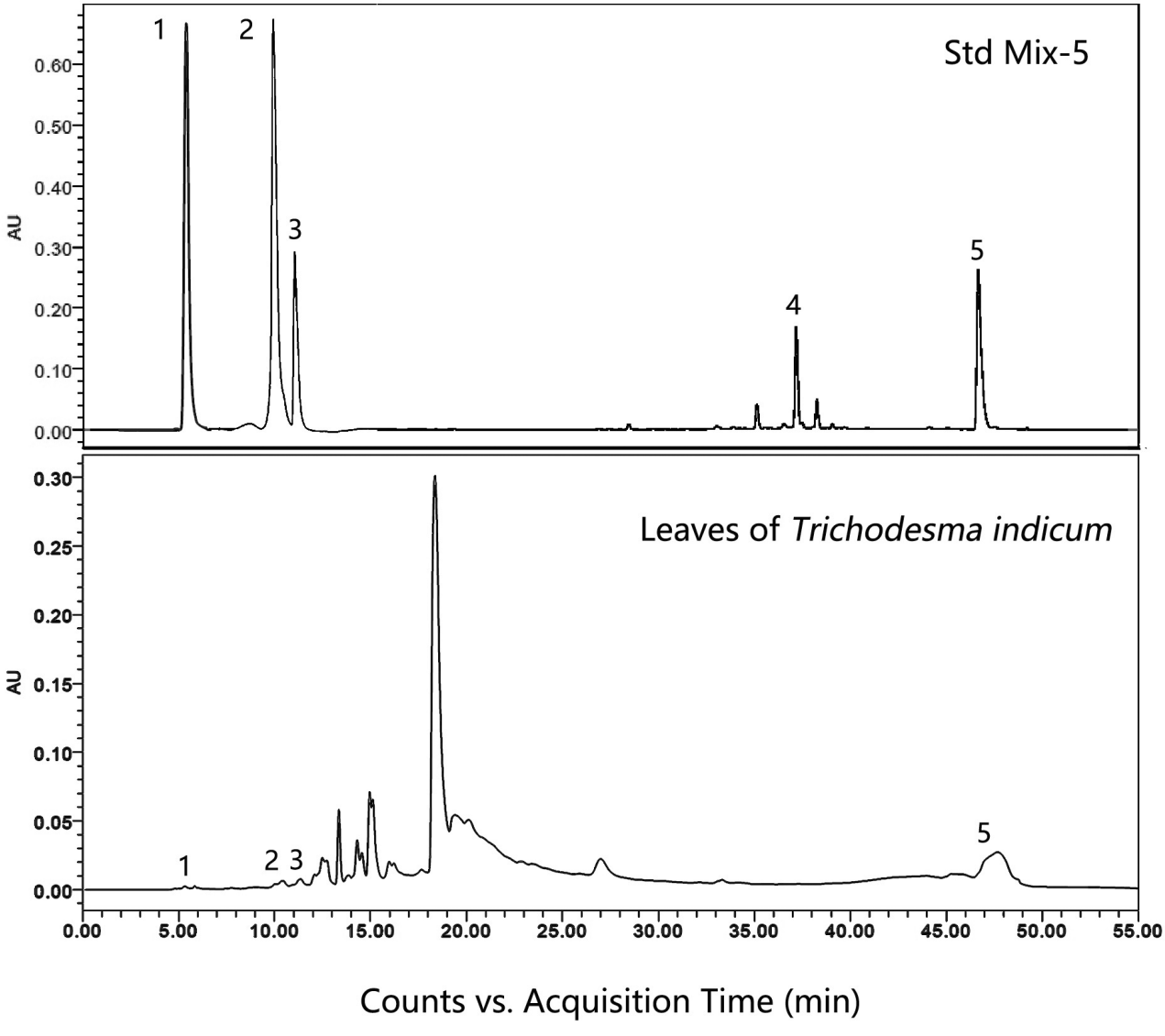

Figure 5. HPLC–UV chromatograms of *T. indicum* and standard mix.

Discussion

Trichodesma indicum is a prominent ethnomedicine of Pakistan. To the best of our knowledge, up to now, no comprehensive study has yet been reported regarding PA determination in *T. indicum.* Our study shows, for the first time reported in the literature, investigation of PAs in *T. indicum*. However, previously it was well established that unsaturated PAs are present in some other *Trichodesma* species such as intermedine, lycopsamine, retronecine, viridiflorine, europine, and trichodesmine in *T. africanum* [72–74], supinine and senkirkine from *T. ehrenbergii* [75], trichodesmine and inanine from *T. incanum* [76], and supinine from *T. zeylanicum* [77]. Unfortunately, the literature survey of *T. indicum* in our study shows oral ingestion in the form of powder or decoction. According to Mei et al. [78], the ingestion of echimidine consumed as herbal tea, as a medicine or a vegetable has led to several cases of HSOS of the liver in human beings. Moreover, heliotrine, as a heliotridine-type PA, induces mutagenesis, chromosome damage, and is carcinogenic to the liver [79–81].

Exposure to unsaturated PAs has been regarded as one of the major causes responsible for the development of HSOS [82]. Individuals who consume PA-containing herbal extracts can experience intrinsic liver injury [83]. Patients with HSOS present typical symptoms of abdominal distension and ascites, pain, jaundice, malaise, hepatomegaly, and body weight increase due to ascites and edema caused by fluid accumulation [84]. Currently, misidentified herbal products are a major clinical challenge as they may harm the health of consumers. In China alone, up until 2008, 41 cases with HSOS were reported [85]. Recently in China, a 54-year-old female was diagnosed with HSOS because of the consumption of the traditional herb *Gynura segetum* Merr., which contains hepatotoxic unsaturated PAs senecionine and seneciphylline [86,87].

Potential poisonings by unsaturated PA may have occurred as isolated incidences in the past, but with increased trade and popularity of herbal medicine in industrialized countries, the potential for intoxication increases. This is perpetuated even further by the "green wave" of people seeking natural remedies [79]. Given the risks and disease reports of unsaturated PA consumption by humans and livestock around the globe, the European Medicines Agency has implemented a limit of 1 µg/day for herbal medicine products with the objective of transitioning down to 0.007 µg of 1, two-unsaturated PA/kg body weight (i.e., 0.14 µg PA per day for 20 kg children and 0.35 µg PA per day for 50 kg adult) [88,89]. The German Federal Institute for Risk Assessment (BfR) also suggests a maximum daily intake of 0.007 µg PA per kg body weight based on a 10,000 margin of exposure (MOE) [88]. This MOE corresponds to the limit of a maximum intake of 0.024 µg PA/PA N-oxides per kg body weight per day. While in Germany it is specified that for a long-term exposure to PA over 6 weeks, one should be very cautious [90,91]. In all, the Federal Institute of Risk Assessment (Bundesinstitut für Riskobewertung, BfR, Germany) and UK Committee on Toxicity policies and statements demonstrate that the exposure to PAs from consumable products should be as low as possible with limits at 0.007 µg of PAs/kg of body weight per day [92,93].

From the literature survey, it is confirmed that *T. indicum* is a prominent ethnomedicine in Pakistan. To the best of our knowledge, this study identified PAs in the leaves of *T. indicum* from the Pan-Himalayan region in Pakistan for the first time. The selected plants were found to be positive for hepatotoxic PAs like supinine, europine, heliotrine, and echimidine. According to the International Program on Chemical Safety, unsaturated PA toxicity depends not only on dose and exposure time but also on age and gender. Males seem to react more than females and children and fetuses are even more sensitive [94]. Therefore, we recommend that this species should be used with more precaution, especially when one considers herbal remedies for children. Previously, it was well established that PAs are an intrinsic component of Boraginaceae and its medicinal use might increase the risk of liver toxicity. Moreover, the information currently available on the use of *T. indicum* is not sufficient to know the risks or any associated chronic health problems caused by unsaturated PAs in this plant. However, recently, the increasing evidence of unsaturated PAs in milk, honey, meat, herbal drugs, grain, and green tea warrants monitoring for PAs to ensure that the intake level does not exceed safety limits.

Limitations and Conclusions

In the laboratory, HPLC fingerprint analysis, besides the four known peaks of PAs, obtained data shows that there were several unknown unsaturated PAs or other compounds between

13–25 and 35 retention times but with no authentic standard availability, we cannot determine their identity. Several other authentic standards of PAs like intermedine, echinatine, lasiocarpine, and monocrotaline could not be detected well, using the aforementioned HPLC conditions. The present study has revealed new findings of significance to local people regarding *T. indicum*. The literature survey shows that the plant is often used for medicinal purposes throughout Pakistan. The HPLC fingerprint analysis shows that besides ethnomedicinal uses it is also a source of toxic PAs heliotrine and echimidine. The study shows the need to at least find the qualitative determination of these alkaloids for recognition of their potential health risks. Secondly, survey results revealed that nearly all local people either use or collect this species for their traditional therapeutic value. Given this, we suggest that the plant should be used with more precaution and especially kept away from children, and also should not be used in large quantities and not for consecutive days.

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Conflicts of Interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

Review of *Croton membranaceus* Müll. Arg.: Phytochemical, pharmacological, and toxicological perspective

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ABSTRACT

Background: *Croton membranaceus* is used as herbal medicine against benign prostate hyperplasia, cough, diabetes, diarrhea, fever, flatulence, measles, nausea, and urinary retention problems.

Objective: The aim of this study was to review the medicinal, phytochemical, pharmacological, and toxicological properties of *C. membranaceus,* a potentially useful medicinal plant species in West Africa.

Methods: Literature focusing on the botany, phytochemical, pharmacological, and toxicological properties of *C. membranaceus* were obtained from scientific databases such as SCOPUS, ScienceDirect, PubMed, SciFinder, Medline, and Google Scholar. Pre-electronic literature such as books, book chapters, journal articles, conference papers, and other scientific documents was obtained from the university library.

Results: Literature search revealed that the phytochemical compounds isolated from *C. membranaceus* include coumarin, diterpene, glutarimide alkaloid, glutarimide peptide, labdane diterpene, and phytosterol. Pharmacological studies of *C. membranaceus* extracts and compounds isolated from the species revealed antibacterial, antifungal, antihyperglycemic, antioxidant, antiplasmodial, antiproliferative, cytotoxicity, hypoglycemic, and prostate growth inhibitory effects of the species.

Conclusion: The phytochemical, pharmacological, and toxicological studies done so far support the uses of *C. membranaceus* in traditional medicine. Therefore, future research on the species should focus on detailed *in vitro*, *in vivo,* and clinical studies using compounds isolated from the species as well as crude extracts.

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Introduction

Medicines derived from plants and their products are used throughout the world as alternatives to conventional drugs. Plants are important sources of natural products and about 25% of the pharmaceutical drugs in clinical use today come from plants and were considered as basic and essential by the World Health Organization (WHO) [1]. In West Africa, *Croton membranaceus* Müll. Arg. is one of the herbal medicines used as a day-today medicine and its leaf and root extracts sold in informal and commercial sectors of the economy. According to Kyei et al. [2], various herbal concoctions, formulas, or prescriptions of the leaf and root extracts of *C. membranaceus* are in clinical use in Ghana, usually prepared as decoctions or infusions and sold in informal markets, medicinal herbal markets, supermarkets, and pharmacies. Such formulas or prescriptions include uro 500® and prostat 60® sold in capsule form while UR-quick mixture® and prostacure® are liquid herbal concoctions widely used for the management of prostate diseases in Ghana [2]. The root and leaf extracts of *C. membranaceus* have been dispensed to benign prostatic hyperplasia (BPH) patients at the Center for Plant Medicine Research

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in Ghana (a WHO research center) for the past 30 years [3]. In Brazil, Ecuador, Mexico, Peru, and Venezuela, several related *Croton* species are used as herbal medicines, with a herbal concoction known as sangre de grado or dragon's blood as a household remedy in the region [4]. The sangre de grado concoction is derived from several *Croton* species indigenous to the Amazon River Basin region, which include *C. lechleri* Müll. Arg., *C. gossypifolium* Vahl, *C. draconoides* Müll. Arg., *C. urucurana* Baill., *C. draco* Schlect & Cham., *C. xalapensis* Kunth, *C. palanostigma* Klotzsch, and *C. erythrochilus* Müll. Arg. [4]. Traditionally, sangre de grado is applied topically as an herbal medicine for cuts, insect bites, snake or scorpion stings, dermatitis, burns and wounds, and also taken orally for gastrointestinal distress, including ulcers, hemorrhoids, piles, cholera, and diarrhea [5]. Some of these species have pharmacological properties such as analgesic, antibacterial, antidiarrheal, antifungal, antiviral, antihemorrhagic, anti-inflammatory, antioxidative, antitumor and cytotoxic activities, antiulcer, immunomodulatory, wound healing, mutagenic, and antimutagenic activities [4,5]. It is within this background that this study was undertaken aimed at providing an overview of medicinal, phytochemical, pharmacological, and toxicological evidence that may or may not support clinical and medicinal uses of *C. membranaceus* throughout its distributional range. *Croton membranaceus* was selected on the basis that the species is an important medicinal plant in the *materia medica* of West Africa and also listed in the book "Plant Resources of Tropical Africa: Medicinal Plant 1" [6], providing an overview of important medicinal plants in tropical Africa.

Botanical Profile and Taxonomy of *Croton membranaceus*

Croton membranaceus is a species of the *Croton* L. genus, belonging to the spurge or Euphorbiaceae family. The name of the genus, "Croton" was derived from "kroton," a Greek word which translates "thick," as the majority of *Croton* species have thick and smooth seeds [7]. *Croton* is the second largest genus of the Euphorbiaceae family with approximately 1,300 plant species which are usually trees, shrubs, or herbs, occupying different habitats in the tropics and subtropics throughout the world [8]. *Croton membranaceus* has been recorded in Côte d'Ivoire, Ghana, and Nigeria and the species occurs at low altitude and moist bush vegetation and tropical savannah [6].

Croton membranaceus is a monoecious undershrub or herb up to 2 m in height with slender and densely stellate hairy branches [9]. The leaves are opposite or alternate, simple, entire with tiny stipules. The leaf blade is ovate in shape, rounded at the base with acuminate apex, basal glands absent, with sparsely stellate hairs above and densely stellate hairs beneath. The inflorescence is an axillary or terminal raceme with few white flowers, with male flowers at the end and female flowers at the base. The flowers are regular, unisexual, male flowers with elliptical sepals and obovate petals and woolly hairy margins. The female flowers have narrowly lanceolate sepals, petals rudimentary or absent, ovary superior, rounded, and densely hairy [9].

Medicinal Uses of *Croton membranaceus*

In Ghana, the root and leaf extracts of *C. membranaceus* are taken as a remedy for BPH and to treat urinary retention problems caused by an enlarged prostate [2,10,11]. The root and leaf extracts of *C. membranaceus* are also used as remedies for diabetes and measles [12,13]. The essential oils from the stem bark of *C. membranaceus* are taken as a remedy for a cough, diarrhea, fever, flatulence, and nausea [14]. In Nigeria, the root and leaf extracts of *C. membranaceus* are used as a tonic and remedy to improve digestion [14].

Phytochemical Compounds and Their Biological Activities

Aboagye et al. [10] identified a glutarimide alkaloid, julocrotine from root extracts of *C. membranaceus* (Table 1). Lambert et al. [15] isolated a coumarin compound, scopoletin from the root bark extract of *C. membranaceus.* Phytochemical investigations into the ethyl acetate extracts of the root extract of *C. membranaceus* revealed the presence of six compounds including julocrotine, crotomembranafuran, DL-threitol, β-sitosterol, gomojoside H, and β-sitosterol-3-D-glucoside [12,16]. Furthermore, a glutarimide peptide, N[N-(2-methylbutanoyl) glutaminoyl]-2-phenylethylamine was identified from the root extract of *C. membranaceus* [17]. Bayor et al. [12] assessed antibacterial effects of the compounds crotomembranafuran, β-sitosterol, julocrotine, β-sitosterol-3-D-glucoside, DL-threitol, and gomojoside H identified from *C. membranaceus* against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* using the agar diffusion and broth dilution techniques

Table 1. Chemical compounds isolated and characterized from *Croton membranaceus*.

Chemical compound	Method of characterization	Plant part
Coumarin		
Scopoletin [15]	HPLC, SPE, and NMR spectroscopy	Root bark
Diterpene		
Crotomembranafuran [12,16]	ESIMS, EIMS	Roots
Glutarimide alkaloid		
Julocrotine [10,12,16]	ESIMS, EIMS, HMBC, and NMR	Roots
Glutarimide peptide		
N[N-(2-methylbutanoyl) glutaminoyl] 2-phenylethylamine [17]	ESIMS, HSQC, HMBC, and NMR	Roots
Labdane diterpene		
DL-threitol and gomojoside H [12,16]	EIMS and ESIMS	Roots
Phytosterol		
β-sitosterol and β-sitosterol-3-D-glucoside [12,16]	EIMS and ESIMS	Roots

EIMS = electron impact mass spectrometry; ESIMS = electrospray ionization mass spectrometry; HMBC = heteronuclear multiple bond correlation; HPLC = high performance liquid chromatography; HSQC = heteronuclear single quantum coherence; NMR = nuclear magnetic resonance; SPE = solid phase extraction.

with gentamicin as a positive control. Out of the six chemical compounds tested, only gomojoside H exhibited some activities against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* with minimum inhibitory concentration (MIC) values within the range from 3.5 ± 0.6 µg/ml to 18.0 ± 0.8 µg/ml which was comparable to MIC value of 18.3 ± 0.2 µg/ml to 29.1 ± 0.3 µg/ml that was exhibited by the control [12]. The antibacterial activities demonstrated by gomojoside H corroborate traditional uses of essential oils isolated from the bark of *C. membranaceus* against diarrhea in Ghana [14] and secondary bacterial infections in clinical conditions such as measles in Ghana [12].

Bayor [18] and Bayor et al. [16] evaluated cytotoxicity and growth inhibitory activities of six compounds isolated from *C. membranaceus* including julocrotine, crotomembranafuran, gomojoside H, β-sitosterol, DL-threitol, and β-sitosterol-3-D-glucoside against the three human cancer cell lines, namely MCF-7, Ml4, and DLD-1 using the 3-(4,5-dimethyl-2-thiazol-yl)-2,5-diphenyl2H-tetrazolium bromide (MTT) assay. Only three compounds, namely, DL-threitol, β-sitosterol-3-D-glucoside, and crotomembranafuran demonstrated activities against the human prostate cancer (PC-3) cells, with half maximal inhibitory concentration (IC_{50}) values within the range from 4.1 to 10.6 µg/ml. These results support the traditional use of root and leaf extracts of *C. membranaceus* in the management of prostate cancer in Ghana [16,18].

Bayor [18] evaluated *in vitro* antiplasmodial activities of six compounds isolated from *C. membranaceus* including DL-threitol, julocrotine, β-sitosterol-3-D-glucoside, crotomembranafuran, gomojoside H, and β-sitosterol on *Plasmodium falciparum* strain 3D7. The compounds β-sitosterol, DL-threitol, and β-sitosterol-3-D-glucoside demonstrated little or no antiplasmodial activities on *Plasmodium falciparum* exhibiting IC_{50} values exceeding 100 µg/ml. The compounds, gomojoside H, julocrotine, and crotomembranafuran demonstrated weak activities exhibiting IC_{50} values which ranged from 43.6 to 46.1 µg/ml [18]. Although no literature reports were found showing the utilization of *C. membranaceus* against malaria, these findings from a study by Bayor [18] imply that the species has potential as an antimalarial remedy.

Pharmacological Activities

Antibacterial activities

Bayor et al. [12] assessed antibacterial effects of the root methanolic extracts of *C. membranaceus* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* using broth dilution techniques and the agar diffusion with gentamycin (10 µg/ml) as a positive control. The extract showed inhibitory properties against the tested pathogens with a zone of inhibition values which ranged from 1.0 ± 0.6 mm to 11.0 ± 1.6 mm and the zone of inhibition values of the control was 24.1 ± 1.1 mm to 25.6 ± 0.5 mm. The MIC values against the tested pathogens ranged from 0.53 to 1.43 mg/ml [12]. Similarly, Gbedema et al. [19] assessed the antibacterial effects of 50% methanol root extract of *C. membranaceus* against *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* using agar diffusion assay with amoxicillin as the positive

control. The extracts were active with MIC values ranging from 13.0 to 20.0 mg/ml and the amoxicillin exhibited MIC values ranging from 0.3 to 640.0 µg/ml [19]. Gbedema et al. [19] also assessed the influence of *C. membranaceus* extracts on antibacterial effects of amoxicillin against *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* using agar diffusion assay. The *C. membranaceus* extracts enhanced antibacterial effects of amoxicillin against *Escherichia coli* and *Staphylococcus aureus* which exhibited MIC values of 0.6 and 0.2 µg/ml, respectively [19]. The antibacterial effects demonstrated by the root extracts of the species corroborate traditional uses of essential oils isolated from the bark of *C. membranaceus* against diarrhea in Ghana [14] and secondary bacterial infections in clinical conditions such as measles in Ghana [12].

Antifungal activities

Bayor et al. [12] assessed antifungal effects of the root methanolic extracts of *C. membranaceus* against the pathogens *Aspergillus niger* and *Candida albicans* using the broth dilution technique and the agar diffusion method with ketoconazole (10 µg/ml) as the positive control. The extract showed inhibitory properties against *Aspergillus niger* and *Candida albicans* with a zone of inhibition values ranging from 3.7 ± 1.0 mm to 10.0 ± 1.5 mm and 2.5 ± 0.8 mm to 7.5 ± 2.2 mm, respectively, and the zone of inhibition values of the control was 18.6 ± 0.4 mm to 29.2 ± 0.3 mm. The MIC values against *Aspergillus niger* and *Candida albicans* were 0.86 and 0.82 mg/ml [12]. The antifungal activities demonstrated by the extracts corroborate the traditional uses of the root and leaf extracts of *C. membranaceus* against microbial infections in clinical conditions such as measles in Ghana [12].

Antioxidant activities

Sarkodie et al. [13] assessed the antioxidant properties of root ethanol extract of *C. membranaceus* using the spectrophotometric 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method with gallic acid as the positive control. The extract exhibited concentration-dependent DPPH free radical scavenging activities exhibiting an IC_{50} value of 100.24 mg/l and the gallic acid also demonstrated concentration-dependent DPPH free radical scavenging activities exhibiting an IC_{50} value of 3.4 mg/ml [13]. The antioxidant action of *C. membranaceus* extract is significant compared with the antioxidant action of gallic acid, the positive control which is a pure compound.

Antihyperglycemic and hypoglycemic activities

Sarkodie et al. [13] evaluated the antihyperglycemic activities of three different doses, 100, 300, and 600 mg/kg body weight of the ethanol root extracts of *C. membranaceus* in streptozotocin-induced diabetic rats. The species extracts exhibited a dose-dependent reduction of glucose levels over both short-term and long-term treatment periods. The extract at 100, 300, and 600 mg/kg body weight doses and insulin demonstrated significant antihyperglycemic properties when compared with the diabetic control group over the 6 hours and 28 days treatment period, and the extract continuously lowered glucose level in diabetic rats during the entire study period. Similarly, Sarkodie et al. [17] assessed hypoglycemic effects of ethanol and water root extract of *C. membranaceus* with doses of 100 and 600 mg/kg in streptozotocin-induced diabetic rats. The glucose of diabetic rats given water extract (100 and 600 mg/kg) decreased by 39.7% ± 2.1% and 32.6% ±2.4%, respectively after six hours and those treated with ethanol extract (100 and 600 mg/kg body weight) lowered their glucose level by 47.5% ± 2.1% and 34.8% ± 2.4%, respectively. The glucose level of the vehicle (normal control) after 6 hours increased by 20.5% ± 1.0% while insulin, the standard drug lowered glucose level by 86.6% ± 0.1%. On the 28th day, aqueous extract at 100 and 600 mg/kg decreased glucose level by 78.7% ± 0.1% and 77.3% ± 0.1%, respectively while the ethanol extract at 100 and 600 mg/kg decreased glucose level by 61.4% ± 2.3% and 58.5% ± 1.1%, respectively. The vehicle, diabetic control increased by 19.0% ± 0.9% in blood sugar level while insulin lowered glucose by 87.5% ± 0.1%. The aqueous and ethanol extracts and the control exhibited statistically significant hypoglycemic activities in comparison to the vehicle control group [17].

Asare et al. [20] evaluated the effect of *C. membranaceus* aqueous root extract on cardiovascular diseases diabetes in genetic animal models. The spontaneously hypertensive rats were categorized into low, intermediate, and high dose groups which were given 25, 50, and 100 mg/kg body weight of extract for 60 days and control group was given distilled water. The glucose level was also assessed. The *db*/*db* rats were divided into three groups alongside *db*/+ mice as a negative control. The groups 1 and 2 were given 250 mg/kg body weight of extract and metformin, respectively and group 3,

the positive control and *db/+* rats, the negative control was given distilled water and rats monitored for 15 hours. The hypotriglyceridemic activities were assessed, high and low-density lipoprotein cholesterol demonstrated significant increases and decreases, respectively. Blood glucose levels were reduced in mice given metformin and extract after 3 hours [20]. These findings demonstrate the potential of *C. membranaceus* extracts in the management of type 1 diabetes mellitus and diseases in which oxidants or free radicals are implicated [13].

Antiproliferative and cytotoxicity activities

Bayor [18] and Bayor et al. [21] assessed the cytotoxicity properties of root methanolic extracts of *C. membranaceus* against the three human cancer cell lines, namely DLD-1 (colon), MCF-7 (breast), and M14 (melanoma), using the MTT assay technique. The root extracts exhibited some activity with IC_{50} values ranging from 16 to 33.5 μg/ml for the three human cancer cell lines. Afriyie et al. [22] evaluated antiproliferative activities of water root extracts of *C. membranaceus* on the human BPH-1 cancer cells. The mitochondria-dependent apoptogenic activities were determined by the effect of 0, 1, 3, and 5 mg/ml extract for 24, 48, and 72 hours on the morphology and survival BPH-1 cancer cells using the phase-contrast microscopy and the MTT assay technique, respectively. The extract demonstrated significant dose-dependent inhibition properties in the proliferation of BPH-1 cancer cells and changes in the morphology and reduced density of BPH-1 cancer cells. The extract caused dose-dependent staining of the nuclear chromatin, significant DNA rupture with G_0/G_1 sub-diploid cells, and loss of mitochondrial membrane in the treated BPH-1 cancer cells when compared with the control after 48 hours. The extract caused significant upregulation of mRNA and protein levels of Bax and those of Bcl_2 did not change significantly. Based on these findings, induction of mitochondria-dependent apoptosis of BPH-1 cancer cells may be used to explain the mechanism of action demonstrated by the *C. membranaceus* extracts [22].

Asare et al. [23] assessed the cytotoxic effects of water root extract of *C. membranaceus* against the human benign prostate hyperplasia-1 cancer cells (BPH-1 cells) using the colony formation assay. The authors observed an increasing antiproliferative activity in BPH-1 cells as the concentration of the root extracts was increased from 0 to 5 mg/ml. The quantitative analysis of the effect of various concentrations produced a dose-dependent inhibition of growth of BPH-1 cells, with the highest dose tested (5 mg/ml) producing a 100% inhibition and statistically significant differences were also noticed at 3 mg/ml [23]. These authors also assessed the genotoxic effects of the water root extract of *C. membranaceus* using the rodent bone marrow assay. The rats in the treatment, negative, and positive control groups were given 3,000 mg/kg extract, N-nitroso-N-methylurea, and saline, respectively. The bone marrow assay technique demonstrated significant differences between animals given the extract and the negative control regarding the polychromatic erythrocyte-normochromatic erythrocyte ratios. A positive correlation was observed between animals that were given the extract and the positive control regarding their micronucleated polychromatic erythrocytes, thus revealing similar genotoxic potential at very high root extract doses and positive control [23].

In another study, Asare et al. [24] assessed the activities of *C. membranaceus* ethanolic root extracts on BPH management by observing 33 patients before and after a 3-month intake of 20 mg three times a day orally. The international index of erectile function and the international prostate symptom score questionnaires were used, and total/free prostate-specific antigen (PSA) (tPSA, fPSA), liver and renal function, lipid tests and ultrasonographic imaging were determined. Thirty (66 ± 11 years) patients participated in all activities associated with the study and the IPSS findings revealed that 37% demonstrated severe, 40% demonstrated moderate, and 23% demonstrated mild symptoms before taking the extracts. A total of 57% and 43% demonstrated moderate and mild symptoms, respectively, after taking the extracts. The IIED results revealed that 30% of the patients had severe, 40% had moderate, 24% had mild to moderate, 3% had mild, and 3% had no erectile dysfunction before taking the extracts and 20% had severe, 43% had moderate, and 37% had mild to moderate dysfunction, after taking the extracts. The average tPSA decreased from 27.9 ± 19.0 ng/ml to 16.2 ± 11.8 ng/ml, fPSA decreased from 6.1 ± 4.8 ng/ml to 3.9 ± 2.9 ng/ml and prostate volume decreased from 101.8 ± 41.3 cm^3 to 54.5 ± 24.8 cm^3 [23]. These findings corroborate the traditional use of the species in the treatment of cancer in Ghana [2,10,11,18,20].

Prostate growth inhibitory activities

Afriyie et al. [25] assessed the effects of the administration of water root extracts of *C. membranaceus* on particular organs in male Sprague-Dawley rats.

The male rats were divided into four groups with the first group being the control and given distilled water. Groups 2, 3, and 4 were given an oral gavage of 30, 150, and 300 mg/kg body weight of the root extract respectively. The rats were fed for 90 days on the chow diet ad libitum and upon dissection, organs were histologically assessed and serum PSA biochemically assessed as well. Based on this assessment, only prostate was abnormal, and H and E staining showed that the thickness and infoldings of epithelial cells were shrinking with increasing the dose. The 30 mg/kg group exhibited low columnar or flattened epithelium cells while the columnar epithelium infoldings of the 150 mg/kg body weight and 300 mg/kg body weight groups were non-existent. The extract appears to target the prostate showing significant PSA reduction [25]. Afriyie et al. [26] evaluated the curative effectiveness of the water root extract of *C. membranaceus* in a testosterone-induced BPH model in castrated mice. The authors used 50 adult male Sprague-Dawley rats which were divided into five groups. The first group served as the control and was given normal saline p.o. while groups 2–5 were castrated and injected with 5 mg/kg body weight testosterone propionate subcutaneously for 28 days. The second group 2, the model group had no further treatment while the third group was simultaneously treated with 0.5 mg/kg body weight finasteride p.o. throughout the study period. The fourth and fifth groups were given 30 mg/kg body weight (low dose) and 300 mg/kg body weight (high dose) extract, respectively, for 28 days. Rats were dissected at the end of the study period and all prostate organs harvested and prostatic index, wet weights, volumes, dihydrotestosterone levels, and serum PSA were assessed. Based on their histology, the model group demonstrated the massive growth of columnar stromal and epithelial cells. The extract and finasteride caused this growth resulting in a thin layer of stromal and epithelial cells similar to the control and PSA levels were significantly lower in the treatment groups. Therefore, *C. membranaceus* causes a reduction in stromal and epithelial cell growth, causing the enlarged prostate to shrink and these findings offer credence to the traditional medicinal use of the leaf and root extracts of the species in the treatment of BPH in Ghana.

Asare et al. [27] assessed if the apoptotic activities demonstrated by *C. membranaceus* extracts occur through the ceramide pathway by observing 30 patients with BPH who were taking the ethanolic extract of the species at 20 mg three times a day orally for 3 months. The sphingosine lyase (SPL), ceramide/sphingophospho-kinase 1 (SphK1) and 2 (SphK2), lipid profile plus apo lipoprotein A and B, malondialdehyde (MDA), total and free PSA, and the cytotoxic adducts of oxidative stress 4-hydroxy-2-nonenal (4HNE) were assessed. The authors found that total and free PSA and Apo lipoprotein A were significantly different after treatment. The SphK1/SphK2 ratio reduced significantly while SPL, ceramide, and MDA increased significantly after taking the extracts. These results imply that the extracts of the species use the ceramide pathway by modulating the SphK1/SphK2 ratio and increasing SPL to generate oxidative stress resulting in apoptosis [27]. Similarly, Asare et al. [28] evaluated the Ca/Mg balance in 30 BPH patients who were taking the ethanolic root extract of *C. membranaceus* at 60 mg/day for 3 months through determination of parathyroid hormone (PTH), serum Ca, Mg, PSA levels, phosphate, vitamin D, and renal function tests (RFT) before and after taking the extract. The RFT, PTH, and vitamin D before treatment, after treatment, and control were normal. The mean PSA of the control, before treatment and after treatment was 1.0 ± 0.6 ng/ml, 27.9 ± 19.0 ng/ml, and 16.2 ± 11.8 ng/ml, respectively. The Mg and Ca/Mg ratio before treatment, after treatment, and control were significantly different. After taking the extract, the Mg and Ca/Mg ratio were similar to the values of the controls. The overall prevalence of Ca/Mg imbalance was 80% before treatment, 13.3% after treatment, and 3.3% for the control group. Calcium (Ca)–magnesium (Mg) imbalance is implicated in prostate cancer and the Ca/Mg ratio values decreases or increases with apoptosis or proliferation, respectively [28].

Toxicity activities

Asare et al. [14] evaluated acute toxicity properties of *C. membranaceus* on male Sprague-Dawley mice. The low dose and high dose experimental groups were given 1,500 and 3,000 mg/kg body weight water root extract of the species, respectively, and observed over a period of 14 days. Clinical signs of toxidromes and mortality were assessed while dosing and after 0.5, 1, 3, and 6 hours of administering the extract, and thereafter, twice daily assessments were done until the 14th day. There were no physical signs of toxicity as revealed by abnormal breathing and movement. Assessment of treated animals over the study period of 14 days revealed no adverse effects of the extracts. The oral median lethal dose (LD_{50}) for the ethanolic plant extract is

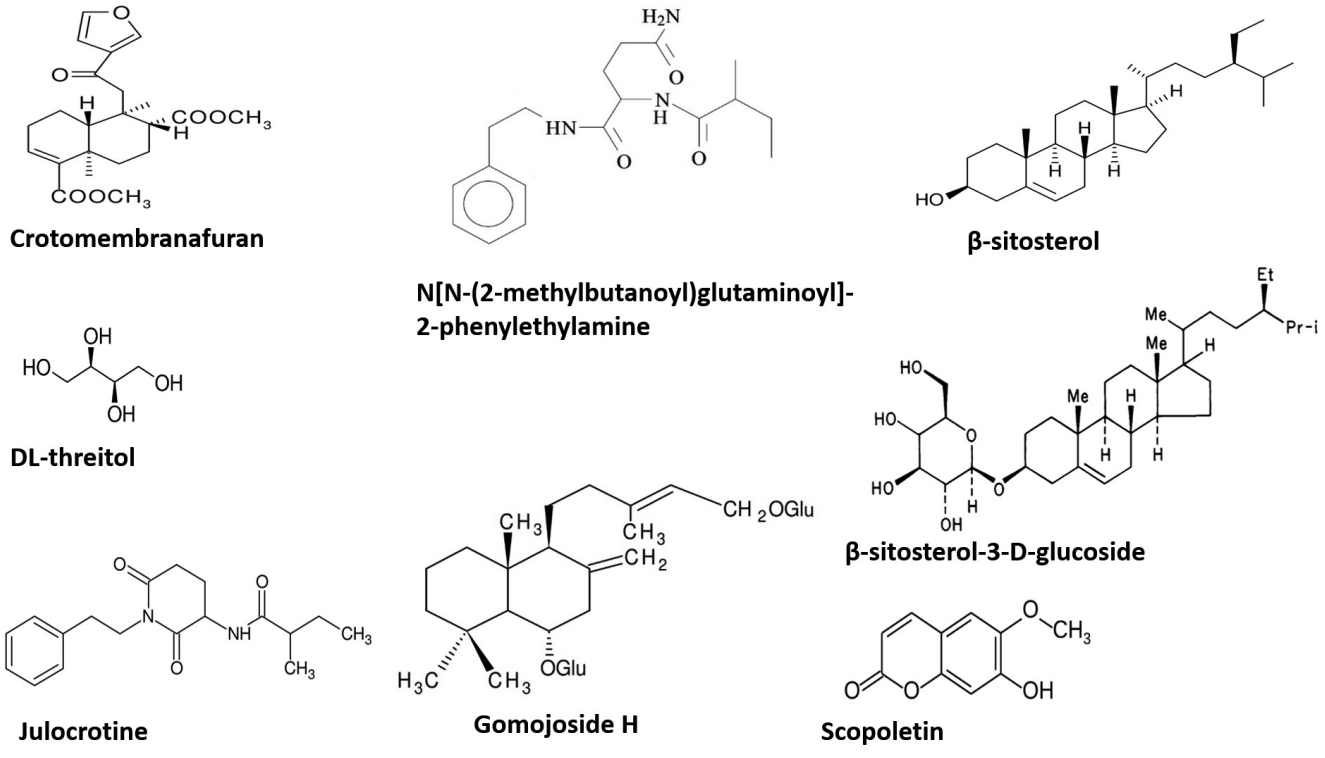

Figure 1. Chemical structures of compounds isolated from *Croton membranaceus*.

higher than 3,000 mg/kg [14]. Similarly, Afriyie et al. [11] evaluated sub-chronic toxicity activities of *C. membranaceus* through a 90-day oral intake of a low dose (30 mg/kg body weight), medium dose intake of 150 mg/kg body weight, and high dose intake of 300 mg/kg body weight of water root extract of the species to the three of male Sprague-Dawley rats including the control group. The authors performed hepato-renal function tests, cardiac enzymes, urinalysis, lipid profile, and routine hematology tests. Macroscopic assessment of the kidney, liver, and heart of the treated groups compared to the control group did not show signs of damage or abnormality, which could be attributed to the extract of the plant. The triglyceride levels and lipoprotein density were significantly reduced, cardiac enzymes-creatinine kinase and lactate dehydrogenase decreased significantly. These results showed that *C. membranaceus* water root extract is non-toxic but has anti-ischemic and anti-atherogenic properties [11].

Sarkodie et al. [13] evaluated the toxic effects of ethanol root extracts *C. membranaceus* by giving a single oral dose of the plant extracts to two groups of six adult Sprague male Dawley rats. The two groups of six mice were given a single oral dose of the extract suspended in distilled water after an overnight fast with an oral gavage needle. The first and second groups received 2,500 and 5,000 mg/kg body weight of extract, respectively. The mortality and general behavior of the rats were assessed over a 48 hour period and surviving rats were assessed for another 12 days for toxic symptoms such as pilo-erection and defects in lachrymatory, locomotory, and respiratory activities. The effect of the single oral dose of 5,000 mg/kg body weight of the freeze-dried extracts of *C. membranaceus* root administered to six rats showed no deaths within 48 hours. This suggests that the oral LD_{50} is higher than 5,000 mg/kg. Observations of animals over 12 days revealed no adverse effects of *C. membranaceus* extracts on the rats. There was no physical damage or signs of toxicity such as abnormal respiratory, locomotory, and lachrymatory activities and the absence of pilo-erection [13].

Conclusion and Perspectives

The phytochemical compounds such as crotomembranafuran, DL-threitol, gomojoside H, β-sitosterol, β-sitosterol-3-D-glucoside, and scopoletin that have been identified from *C. membranaceus* can be correlated to the pharmacological activities associated with the species such as antibacterial, antifungal, antihyperglycemic, antioxidant, antiproliferative, cytotoxicity, hypoglycemic, and prostate growth inhibitory effects (Fig. 1). The compound

gomojoside H isolated from *C. membranaceus* exhibited antibacterial activities [12] while scopoletin that has been isolated from several plant species is known to have antibacterial and antifungal properties [29]. Tanaka et al. [30] argued that the accumulation of scopoletin has been correlated with resistance to microbial attack and other stress-related ailments such as dehydration and mechanical injury. The antihyperglycemic and antioxidant activities exhibited by *C. membranaceus* extracts are probably due to β-sitosterol and scopoletin, both compounds known to exhibit antihyperglycemic and antioxidant activities [31–33]. According to Obasi et al. [34], the compound scopoletin has the ability to increase the bleeding time and lipid levels, suggesting the possible effects on some disorders of blood clotting and lipid metabolism. The compounds β-sitosterol and scopoletin are also known to have anti-inflammatory activities [35–37]. The antiproliferative, cytotoxicity, and prostate growth inhibitory effects demonstrated by *C. membranaceus* extracts are probably due to the compounds crotomembranafuran, DL-threitol, β-sitosterol, β-sitosterol-3-D-glucoside, and scopoletin that have been identified from the species. The compounds crotomembranafuran, DL-threitol, β-sitosterol, and β-sitosterol-3-D-glucoside exhibited antimutagenic, chemopreventive, and cytotoxic effects [16,38–43]. Research by Cometa et al. [44] and Ojewale and Adesina [45] revealed that the compound scopoletin has potent hypotensive and non-specific spasmolytic and neuromuscular blocking effects in laboratory rats. Research by Obidoa et al. [46] revealed that scopoletin has the ability to induce testicular failure at the level of sperm maintenance. Therefore, future research on *C. membranaceus* should focus on the possible biochemical mechanisms of the identified phytochemical compounds.

Based on the literature review that was undertaken, the phytochemical analyses, pharmacological, and toxicological properties supported the ethnomedicinal applications of *C. membranaceus* in the management of BPH and other related ailments. *Croton membranaceus* has been used in Ghana over several decades for the management of and has the ability to shrink the prostate in both animal and human experimental models. The effectiveness of crude extracts and purified ingredients of *C. membranaceus* for the therapy of BPH and related diseases will provide the possibilities of discovering lead compounds needed for pharmaceutical drugs and health products required for the clinical application of the species in modern medicine. Future research should focus on conducting more *in vitro*, *in vivo,* and clinical studies to corroborate other traditional medical applications of the species.

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Conflict of Interest

There are no conflicts of interest.

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ORIGINAL RESEARCH

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Ethnobotanical uses of wild medicinal plants by the local community in the Asi Ganga sub-basin, Western Himalaya

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ABSTRACT

Background: Himalayan region is rich in biological diversity and the communities residing in the region largely depend on it for food, healthcare, and other livelihood practices. Use of various floral species in healthcare remedies plays a significant role in the life of the Himalayan peoples.
Objective: The present study was conducted with aim to document traditional healthcare practices and understand the transfer of knowledge to younger generation in the Asi Ganga sub-basin.
Materials and Methods: The present interview-based study involved a total of 60 respondents (31 males and 29 females) from the seven selected villages of the study area.
Results: We documented the use of 76 wild medicinal plants in primary healthcare remedies by the local community. Maximum species (nine species) represented to the Rosaceae family followed by the family Lamiaceae (four species), Ranunculaceae (three species), Berberidaceae (three species), Pinaceae (three species), and Ericaceae (three species), respectively. The study observed that the traditional healthcare system was still prevalent among the people and mostly preferred before allopathic treatment. However, the knowledge on the medicinal uses of plant species was restricted to elderly members of the community and the younger ones were unaware or knew very less about such practices. Transfer of traditional knowledge system to the new generation was restricted and seems to be declining, could be the key reason behind limited knowledge among the younger members. Apart from the use in healthcare, many of these medicinal plants were important for the livelihood of the community residing in the Asi Ganga sub-basin, as it was contributing about 35%–40% of average household income. However, the current utilization pattern for the economic benefit was perceived to be very critical for the sustainability of these valuable resources and associated traditional practices.

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Introduction

The Himalayan region is endowed with a rich variety of floral and faunal species. The region shows great diversity in vegetation with 21 forest types [1,2] and more than 18,440 species of plants [3]. Among these plant species, 1,748 species are medicinal plants [4] including 118 species of essential oil yielding medicinal plants [5]. It also contributes to a large percentage of crude drugs in the Indian and global market [6–10] and thus, to the economy. The ethnobotanical species also comprise economical, nutritional, aesthetic, cultural as well the sacred value and are an important part of life and livelihood of the indigenous Himalayan communities [4,11]. Uttarakhand state in the Indian Himalayan region supports the highest number of plant species known for medicinal properties [12–14] due to its unique geography, varied topography, and diverse climatic conditions.

Although there are significant advances in modern healthcare facilities, medicinal plants are recognized for their significant therapeutic value in the treatment of a wide range of ailments. Medicinal plants have been the base for the development of new drugs to support human survival [15–17]. Due to the fact, about 80% of the world's

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Table 1. Sample respondents selection in Asi Ganga sub-basin, Western Himalaya.

Villages	*Population*				*Sample population*				
	HH	M	F	Total	HH	M	F	Total	%
Agora	92	228	229	457	12	6	6	12	13.04
Dandalaka	21	48	57	105	4	2	2	4	19.04
Dasda	38	95	79	174	5	3	2	5	13.15
Bhankoli	87	210	211	421	10	5	5	10	11.50
Naugaon	99	233	198	431	12	6	6	12	12.12
Gajoli	94	303	280	583	10	6	4	10	10.63
Seku	61	147	161	308	7	3	4	7	11.48
Total	492	1,264	1,215	2,479	60	31	29	60	12.20

Note: HH = households, M = male, F = female, % = percentage of households.

people use medicinal plants for their primary healthcare needs [18]. The traditional knowledge system (TKS) on various medicinal plant species is indigenous to different ethnic communities of the Himalaya [12,19]. In developing countries like India, the population in rural and remote hilly areas still largely depend on traditional medicine, especially based on plant resources [20] for prevention, diagnosis, and treatment of various physical and mental ailments.

Over the period, the growing demand for medicinal plants has laid pressure on these valuable resources and their habitat (19). As per an estimate, more than 90% of plants in trade being collected from the wild (21), the majority of that are collected using unsustainable practices [8,21–23], leading to the extinction of several globally significant species [24].

The documentation of ethnobotanical knowledge on medicinal plants is important to preserve the ancient knowledge system [13,25–27] and initiate management action to conserve valuable biological resource [28]. Documentation of the TKS in remote hilly areas in Indian Himalayan region is still limited [19,29] and local traditional healthcare practices are important to be documented. The present study was conducted in the Asi Ganga sub-basin with aimed to document TKS and an attempt to fill the information gap on medicinal plant and their utilization pattern in the western Himalaya.

Material and Methods

Study area

The present study was conducted in the Asi Ganga sub-basin of Bhatwari block of Uttarkashi district in the western Himalaya during the years 2013–2016. The basin consists of nine major villages with an elevation range between 1,210 m above sea level (masl) and 2,190 masl. The present study was focused on the seven villages, namely Agora, Dasda, Dandalaka, Bhankoli, Gajoli, Naugaon, and Seku which are located in 4–7 km distance from the nearest road head. As per the census 2011, these villages were comprised of about 492 households with a population of approximately 2,479 individuals including male and female (Table 1). The study villages are located between 30°50'N to 30°51'N and 78°28'E to 78°27'E, within the Barahat range of Uttarkashi forest division (Fig. 1). The area is characterized by undulating and rugged terrain with steep slopes, precipitous ridges with interspaced deep gorges, narrow, and covered with very dense forests (falling in the category of sub-tropical, Himalayan moist temperate forest, alpine scrub, and meadows) which support a variety of flora and fauna [30]. Temperature varies with about −4°C–20°C in winters and 10°C–30°C in summers and average annual rainfall was between 1,650 and 2,400 mm. A river originates from the sub-basin called Asi Ganga (Asi or Assi means eighty, Ganga means rivulets) and joins river Bhagirathi, a tributary of river Ganga at a place known as Gangori. During the monsoon in the years 2012 and 2013, the basin witnessed devastating cloudburst events followed by flash floods and other related disastrous incidents. These flood events have severely disturbed the area and damaged basic infrastructure including schools, bridges, roads, and primary healthcare services as well. The basic infrastructure has not been properly re-established as a result of flood events in the consecutive years since 2013. The people in these villages trek a distance of 4–7 km followed by 14 km travel through vehicles on an uneven and *Kachha* road for their primary healthcare need. In such a situation, people prefer traditional medical practices for various diseases and visit hospitals only on serious health cases. The villages are surrounded with sub-tropical and

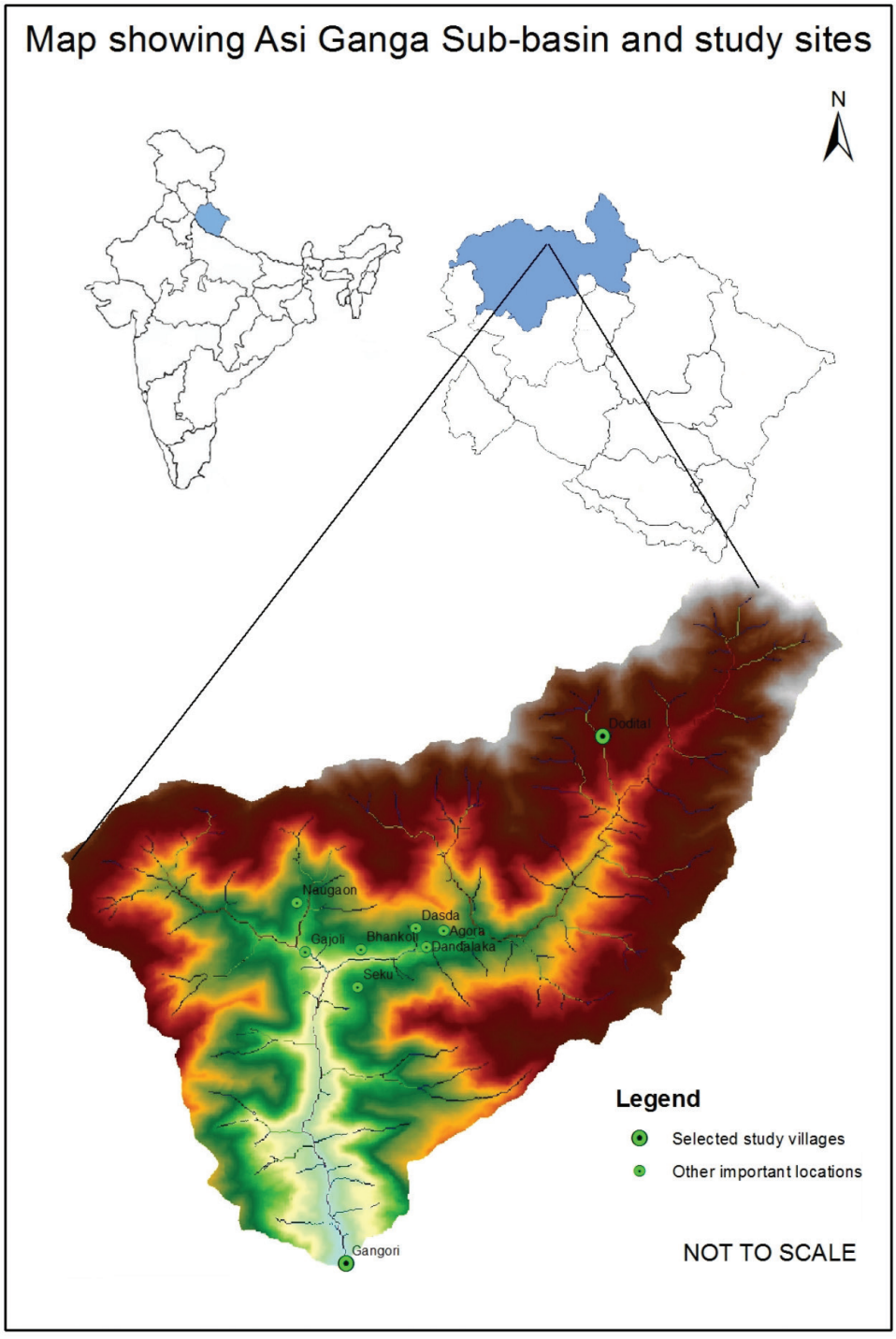

Figure 1: Map showing the Asi Ganga Sub-basin and study villages

mixed temperate forest, from where local people collect a variety of medicinal plants from these forest areas including the alpine meadows (locally known as *Bugyals*).

Designing of questionnaire and data collection

We collected data through structured questionnaire [29,31] in the selected seven villages of the Asi Ganga sub-basin. Earlier to the present study, questionnaire-based research studies on socio-economic issues, seasonal grazing, eco-tourism, and flash flood disaster were conducted in three villages of the study during the year 2012–2013 by the first author. Respondents of these studies have reported the collection of non-timber forest produce (NTFP) and use of plants in healthcare and other livelihood practices. Moreover, it was also observed that NTFP-based

Table 2. Background characteristics of selected respondents.

Variables	Sub-category	Count	Percentage
Sex	Male	31	51.60
	Female	29	48.40
Age	18–40	17	28.33
	41–65	22	36.67
	>66 years	21	35.00
Educational status	Illiterate	17	28.33
	1–5 (primary)	13	21.67
	5–8 (upper primary)	15	25.00
	9–10 (high school)	7	11.67
	11–12 (intermediate)	6	10.00
	Graduate	2	3.00
Marital status	Married	49	81.67
	Unmarried	11	18.33

income was significantly contributing to average household income in these villages. The questionnaire used in the present study was designed to better understand the plant-based traditional healthcare practices and community dependency for their livelihood. The first part (A) of the questionnaire captured data on respondent's profile (name, age, gender, educational status, and profession) and the second part (B) enquired on socio-economic status of households (demography, educational status, livelihood practices, average income generated through each sources, average landholding, and crop productivity). The third section (C) of the questionnaire was focused on queries related to the local name of plant, collected from, part used, a method of use, and preference of plants in the treatment of a particular disease.

The information was gathered through direct interaction or interview with the community members and *Vaidyas* (local healers who provide medicines to the patient) on the local name of the plant. The plants were identified through field observation with local people, and some available with them at home. Photographs were taken of all the unidentified plants and identification was completed with the help of taxonomic experts from Doon University, Wildlife Institute of India, and other organizations working in the study area. Accepted name of each plant species was confirmed through the Plant List portal. A total of 60 respondents (12.20% of total households) were considered for this study (Table 1), including male and females of different age group, to understand the transfer of knowledge among the new generation. Information on the availability of medicinal plants in the meadows and forest was also gathered from the people, especially the shepherds of the selected villages. The fidelity level (FL), the percentage of respondents claiming a particular plant used for the same purpose, was calculated as:

$$FL\ (\%) = (N_p/N) \times 100$$

Where, N_p is the number of respondents that claim the use of a particular species in the treatment of a particular disease and N is the number of respondents that use the species to treat any given ailment [32].

Results

The gender distribution among the respondents was 31 (51.6%) and 29 (48.4%) for males and females, respectively. Most of the respondents were above 40 years (71.67%) and only 17 individuals (28.33%) were with the age of between 18 and 40 years (Table 2). A total of 76 wild plant species belonging to 48 families (Table 3) were documented to be used in treating various ailments of human (73 species) and livestock (three species). Maximum species belong to the family Rosaceae (nine species), followed by the family Lamiaceae (four species), Ranunculaceae (three species), Berberidaceae (three species), Pinaceae (three species), and Ericaceae (three species) while the 10 families represented by two plants species in each (Table 4) and the remaining 41 families were represented by one species in each. Traditional healers and community members used leaves, flower, roots, rhizome, bark, seed, and fruits in different herbal preparations (Table 3) and preparations from these plants are given to the patient through oral (72.37%) followed by dermal and nasal routes. A total of three plant species are used in the treatment of more than four diseases, followed by six species in four diseases, nine species in three different diseases, 33 species in two, and the remaining 25 species are used by communities for only one ailment (Table 3). In terms of plant species used for a particular ailment, maximum of 19 recorded species are used in fever, followed by skin diseases (13 species), cuts and wounds (13 species), and gastrointestinal disorders (12 species), respectively (Table 5). The FL was found maximum for *Picrorhiza kurroa* (98.33%), followed by *Angelica glauca* (95.0%), *Allium humile* (91.67%), and *Aconitum heterophyllum* (83.33%), respectively (Table 6), while minimum for *Alnus nepalensis* and *Quercus semicarpifolia* (3.33% each).

Table 3. Medicinal plants used in the treatment of different ailments.

Botanical name	Local name	Family	Part used	Used in diseases	Mode of use
Aconitum heterophyllum Wall. ex Royle	Atis	Ranunculaceae	Tuber	Fever, diarrhea, and body ache	Paste of tuber with warm water is given orally
Aconitum balfourii Staf.	Mitha, Bish	Ranunculaceae	Tuber	Rheumatism, leprosy, wounds, swelling, and musculoskeletal disorder	Paste of tuber is applied on the skin
Ajuga bracteosa Wall. ex. Benth	Neelkanthi	Lamiaceae	Leaf	Acidity and indigestion	Leaves paste is given orally
Allium humile Kunth	Pharan, Ladu	Amaryllidaceae	Leaf and bulb	Asthma and pectoral complaints	Dried leaves and bulbs are used as spices and taken orally
Alnus ***nepalensis*** D. Don	Utish, Usth	Betulaceae	Latex and Bark	Urination with bleeding in livestock	Extract is given orally
Angelica glauca Edgew.	Chora	Apiaceae	Roots	Cold, cough, stomach pain, and choke	Roots powder is given orally with warm water, tea, vegetable and
Arisaema intermedium Blume	Nagdaman	Araceae	Roots, stem	Dehydration, fever, intestinal pain, body ache, and skin infection	Roots and stem powder is given orally
Arisaema ***tortuosum*** (Wall.) Schott.	Nagdaman	Araceae	Tuber	Rheumatism and breathing disorder	Roots and stem powder is given orally
Arnebia benthamii Wall. ex. G. Don	Balchhadi	Boraginaceae	Roots	Asthma, fever, and hair fall	Roots paste is given orally and dried roots with mustard oil applied on hair
Bauhinia variegata Linn.	Ghural, Kural	Fabaceae	Leaf, bark, flower, and fruit	Skin disease and internal disorder	Paste of leaves and bark is applied on skin, flowers and fruit or pods are taken as a vegetable
Berberis lycium Royle	Kingod	Berberidaceae	Roots and fruits	Indigestion and fever	Juice or extract was given orally
Berberis aristata DC	Kingod	Berberidaceae	Roots and fruits	Indigestion, tiredness, and eye flu	Juice and extract are given orally
Bergenia ***ciliata*** (Haw.) Sternb.	Silpadu	Saxifragaceae	Leaf and roots	Kidney stone and ache	Extract and paste are given orally with warm water
Bergenia ligulata (Wall). Engl.	Bhotiya Chai	Saxifragaceae	Leaves and seeds	Tiredness	Dried leaves and seeds are taken with tea
Betula utilis D. Don	Bhojyuda, Bhojpatra	Betulaceae	Bark and stem outgrowth	Fever, body pain, and wound	Paste is applied on wound and muscles
Cannabis sativa Linn.	Bhang	Cannabaceae	Seed and leaf	Fever, bronchitis, indigestion, and impotency	Roasted seeds are used with foods and leaves are consumed with smoke
***Cedrus deodara* (Roxb. ex D. Don) G. Don**	Devdar	Pinaceae	Fruit, Seed, and hardwood essential oil	Joint pain, fungal infection, and skin disease	Essential oil is applied on skin and joints
***Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm**	Dalchini, Tejpatta	Lauraceae	Leaf and bark	Indigestion	Leaves and bark are used as spices
Cuscuta europaea L.	Akasbail	Convolvulaceae	Climber	Joint pain	Extract is applied on muscles and joints
Dactylorhiza ***hatagirea*** **(D. Don)** *Soó*	Hath Panja, Hath Jadi	Orchidaceae	Tuber	Diarrhea and external wounds	Powder with warm water or milk is given orally and paste is applied on the wound

Continued

Botanical name	Local name	Family	Part used	Used in diseases	Mode of use
Delphinium denudatum **Wall. ex Hook.** f. & Thomson	Nirbisi	Ranunculaceae	Leaf and tuber	Intestinal pain and poisoning	Extract is given orally
Dioscorea bulbifera L**.**	Genthi	Dioscoreaceae	Fruit and bulb	Diabetes and skin disease	Powder is given with warm water
Diplocyclos palmatus (L.) Jeffrey	Shivlingi	Cucurbitaceae	Fruit	Fever	Juice or extract is given orally
Ficus palmata Forssk.	Bedu	Moraceae	Fruit and Latex	Wounds treatment and intestinal disorder	Fruit is consumed and latex is applied on the wound
Fragaria vesca Linn.	Bhumla	Rosaceae	Fruit	Gastrointestinal disorder and dehydration	Fruits are consumed
Fumaria indica (Hausskn.) Pugsley	Pitphapara	Fumariaceae	Leaf	Headache, fever	Extract is given orally
Gentiana kurroo Royle	Kaudi	Gentianaceae	Rhizome	Indigestion and stomach pain	Paste or powder is given with warm water
Girardinia ***diversifolia*** (Link) Friis	Badi Kandali	Urticaceae	Root	Applied on cuts	Paste is applied on cuts or wound
Hedychium spicatum Sm.	Van Haldi	Zingiberaceae	Tuber	Bronchitis, asthma, and pain	Extract is given orally
Hippophae salicifolia D. Don	Amal	Elaeagnaceae	Fruit	Diarrhea and blood purification	Fruit juice is given orally
Impatiens balsamina L. Ed.	Halu	Balsaminaceae	Leaf and seed	Headache, burn, and joint pain	Paste of leaves and essential oils from seed are applied over the skin and joints
Jatropha curcas Linn.	Arandi	Euphorbiaceae	Leaf and seed	Fever and joint pain	Leaves and paste are applied on the skin
Juglans regia Linn.	Akhor/Akhrot	Juglandaceae	Dry fruit and leaf	Pyorrhea and weakness	Leaves paste is applied on gums and dry fruits are given with milk
Lyonia ***ovalifolia*** **(Wall.) Drude**	Ayaar	Ericaceae	Leaf	Skin disease	Extract or paste is applied on the skin
Mallotus philippensis (Lam.) Muell. Arg	Rweni	Euphorbiaceae	Leaf and fruit	Skin disease and external cuts	Paste is applied on the skin
Meconopsis betonicifolia Franch.	Jangali poth	Papaveraceae	Latex and seed	Pain and fever	Latex and paste of seeds are given with warm water
Megacarpaea polyandra Benth. ex Madden	Barmol	Brassicaceae	Roots	Fever, pain, dysentery, and asthma	Paste is given orally
Mentha longifolia (L.) Huds.	Jangali Pudina	Lamiaceae	Leaf	Dehydration and cough	Extract is given orally
Morina longifolia Wall. ex DC	Bishkandaru	Caprifoliaceae	Roots	Snakebite and wound	Paste is applied on the skin
Myrica esculenta Buch. Ham. ex D.Don	Kafal	Myricaceae	Fruit	Gastral disorders and indigestion	Fruit juice and whole fruit are consumed
Nardostachys jatamansi (D. Don) DC.	Masi	Caprifoliaceae	Roots and leaves	Internal pain	Powder is given with warm water
Origanum vulgare L.	Ban tulsi	Lamiaceae	Leaf and seed	Rheumatism, headache, and fever	Leaf paste is applied on skin and extract is given with warm water
Oxalis corniculata L.	Khatibuti	Oxalidaceae	Leaf	Wasp bite	Leaf extract is applied on wasp bite
Paris ***polyphylla*** Sm**.**	Bada Satuwa	Melanthiaceae	Tuber and roots	Fever, headache, burns, wounds, and poisoning	Tuber paste is given orally and applied on the skin
Perilla frutescens (L.) Britton	Bhangzeera	Lamiaceae	Seed	Digestive disorder	Seeds paste is given with warm water

Continued

Botanical name	Local name	Family	Part used	Used in diseases	Mode of use
Picrorhiza kurroa Royle ex. Benth.	Kutki, Kaudai	Plantaginaceae	Roots	Fever, jaundice, pain, and dysentery	Roots extract or powder is given orally
Pinus roxburghii Sarg.	Chir	Pinaceae	Latex	Cuts and wound	Applied on skin
Pinus wallichiana A.B. Jacks.	Kail	Pinaceae	Fruit and latex	Cuts and wound	Applied on skin
Podophyllum hexandrum Royle	Ban Kakdi	Berberidaceae	Tuber and fruit	Skin disease and wounds	Paste is applied on the skin
Polygonatum verticillatum (L.) All.	Salam Mishri	Liliaceae	Tuber and leaf	Weakness	Extract or paste is given orally
Potentilla fulgens Wall. ex Hook	Bajradanti	Rosaceae	Whole plant	Skin disease, gastrointestinal disorders	Leaf paste is applied on skin and plant extract is given orally
Prinsepia utilis Royle	Bhekal	Rosaceae	Fruit and seed	Joint pain	Fruit extract and essential oil from seeds is applied on joints
*Prunus **armenica*** Linn.	Chullu	Rosaceae	Fruit and seed	Nematosis, skin disease, and pain	Fruit or fruit juice is given orally in pain and nematosis, and essential oils from seeds applied on the skin
Pyracantha crenulata (D. Don) M. Roem.	Ghingaru	Rosaceae	Fruit	Blood purification	Fruits or fruit juice is given orally
Pyrus pashia Buch. -Ham. ex. D. Don	Mol, Mehal	Rosaceae	Fruit	Blood purification and eye infection	Fruits and juice are given orally
Quercus leucotrichophora A. Camus	Ban	Fagaceae	Fruit	Cough	Fruit paste is given with warm water
Quercus semicarpifolia Smith.	Kharsu	Fagaceae	Fruit	Gastrointestinal disorder in livestock	Roasted fruits paste with buttermilk
Rheum emodi Wall.	Archu	Polygonaceae	Tuber and root	Skin disease, wound, pain, and dysentery	Paste is applied on the skin and given orally
Rhododendron arboreum Sm.	Buransh	Ericaceae	Flower	Fever, stomach ache, and heart problems	Juice is given orally
*Rhododendron **campanulatum*** D.Don	Simaru	Ericaceae	Flower	Gastrointestinal disorders	Flower extract is given orally
Rosa moschata Herrm.	Kujeen	Rosaceae	Fruit	Gastrointestinal disorders, Indigestion	Fruit paste is given with warm water
Rubus ellipticus Smith.	Hisar	Rosaceae	Beri and root	Skin disease and dehydration	Beri is consumed orally and roots paste is applied on the skin
Rubus niveus Thunb.	Kala Hisar	Rosaceae	Beri	Skin disease	Beri is eaten and the extract is applied on the skin
Rumex hastatus Don.	Almodu, Almor	Polygonaceae	Leaf, flower	Fly or Wasp bite	Leaves or flower extract is applied on the skin
Saussurea costus (Falc.) Lipsch	Kuth	Asteraceae	Tuber	Pain, fever, asthma, and cough	Tuber paste is given orally
Saussurea obvallata (DC.) Edgew.	Brahmkamal	Asteraceae	Flower	Mental disorder	Dried flowers are burned to create a fume
Selinum vaginatum C.B Clarke	Bhutkesh	Apiaceae	Root	Skin disease and swelling muscles	Roots paste is applied on the skin
Solanum nigrum Linn.	Gewai	Solanaceae	Leaf and fruit	Headache and fever	Leaf paste is applied on the forehead and extract bath is taken, and fruit juice is given orally
*Solanum **virginianum*** Linn.	Kanteli	Solanaceae	Fruit	Expelling leech from livestock	Fruit juice is given through nasal route

Continued

Botanical name	Local name	Family	Part used	Used in diseases	Mode of use
Taxus wallichiana Zucc.	Thuner	Taxaceae	Bark and seed	Internal wound	Bark and seeds extract with warm water is given orally
Trillidium govanianum (Wall. ex D.Don) Kunth	Chhota Satuwa, Nag Chhatri	Melanthiaceae	Tuber	Rheumatism, fever, and sexual disorder	Powder or paste is given with warm water
Urtica dioica Linn.	Kandali	Urticaceae	Leaf	Allergy and muscular pain	Leaves are touched on skin
Verbascum thapsus Linn.	Akelabeer	Scrophulariaceae	Roots, flower	Pain and fever	Roots and flower extract are given orally
*Viola **pilosa*** Blume	Banfsa	Violaceae	Flower	Cough and fever	Flower extract or paste is given
Woodfordia fruticosa (L.) Kurz	Dhaula	Lythraceae	Flower	Dysentery and cough	Flower extract is given
Zanthoxylum armatum DC.	Timru	Rutaceae	Leaf, twigs, and seed	Pyorrhea and wound filling	Paste is applied in tooth-ache and on the wound

Out of total male respondents, 35.48% hold traditional knowledge (TK) of healthcare on more than 20 species, 35.48% of 10–19 species and 29.03% hold less than 10 species. While among the women respondents, 37.95% knew more than 20 plant species, 41.38% knew about 10–19 species, and 20.69% knew less than 10 species. In term of illiterate respondents, 52.94% and 41.30% knew TK on more than 20 species and 10–19 species, respectively (Fig. 2). Only 30.23% of literate respondents knew about the use of more than 20 plant species in various ailments. The married respondent (38.78% or 19 individuals) had TK on more than 20 species and 36.73% (18 individuals) had on 10–19 species while only 27.27% (three individuals) unmarried respondents (age above 40 years) knew uses of more than 20 species of medicinal plants. This is because that the most of the married respondents (93.88%) were of more than 40 years of age group. In terms of TK among the age groups, about 5.88% respondent of 18–40 years of age, 27.27% of 41–65 years of age, and 71.43% of more than 65 years of age that knew uses of more than 20 wild medicinal plants for healthcare purposes.

Table 4. Dominant families of medicinal plants used by local communities in Asi Ganga sub-basin.

Family	*Species used in traditional healthcare practices*
Araceae	2
Asteriaceae	2
Berberidaceae	3
Betulaceae	2
Caprifoliaceae	2
Ericaceae	3
Euphorbiaceae	2
Fagaceae	2
Lamiaceae	4
Pinaceae	3
Polygonaceae	2
Ranunculaceae	3
Rosaceae	9
Saxifragaceae	2
Solanaceae	2
Urticaceae	2

Discussion

Plants are one of the important entities to support the life, livelihood, and survival of human being on the planet earth. Their uses in traditional and modern healthcare remedies play a vital role in human well-being. As per an estimate about 80% of the population, depend upon traditional healthcare practices throughout the world [18] while in India, more than 65% people are dependent on the medicinal plant-based healthcare system [16,33]. Moreover, the dependency on medicinal plants for healthcare is much higher among rural and tribal population [19,34]. The indigenous health

Table 5. Major ailments cured by local community using plant-based remedies.

Ailments	*Number of plants used*
Body ache	6
Cold and cough	5
Cut and wounds	13
Dysentery and diarrhea	7
Fever	19
Gastrointestinal disorder	12
Headache	5
Indigestion	8
Joint pain	5
Respiratory diseases	6
Skin diseases	13

Table 6. FL of some most commonly used medicinal plants in Asi Ganga sub-basin.

Species	Local name	Used in disease	Fidelity level (%)
Picrorhiza kurroa Royle ex. Benth.	Kutki, Kaudai	Fever, jaundice, pain, and dysentery	98.33
Angelica glauca Edgew.	Chora	Cold, cough, stomach pain, and choke	95.00
Allium humile Kunth	Pharan, Ladu	Asthma and pectoral complaints	91.67
Aconitum heterophyllum Wall. ex Royle	Atis	Fever, diarrhea, and body ache	88.33
Paris polyphylla Sm.	Bada Satuwa	Fever, headache, burns, wounds, and poisoning	85.00
Arnebia benthamii Wall. ex. G. Don	Balchhadi	Asthma, fever, and hair fall	81.67
Aconitum balfourii Staf.	Mitha, Bish	Rheumatism, wounds, and swelling	80.00
Rhododendron arboreum Sm.	Buransh	Fever, stomach ache, and heart problems	80.00
Dactylorhiza hatagirea (D.Don) Soó	Panja, Hatha jadi	Diarrhea and external wounds	78.33
Rosa moschata Herrm.	Kujeen	Gastrointestinal disorders	78.33
Trillidium govanianum (Wall. ex D.Don) Kunth	Chhota Satuwa, Nag Chhatri	Rheumatism, fever, and sexual disorder	75.00
Prunus armenica Linn.	Chullu	Nematosis, skin disease, and pain	73.33
Oxalis corniculata L.	Khatibuti	Wasp bite	65.00
Delphinium denudatum Wall. ex Hook.f. & Thomson	Nirbisi	Intestinal pain and poisoning	63.33
Juglans regia Linn.	Akhor/Akhrot	Pyorrhea and weakness	63.33
Berberis lycium Royle	Kingod	Indigestion and fever	61.67
Morina longifolia Wall. ex DC	Bishkandaru	Snakebite and wound	61.67
Zanthoxylum armatum DC.	Timru	Pyorrhea and wound filling	61.67
Pyrus pashia Buch. -Ham. ex. D. Don	Mol, Mehal	Blood purification	60.00
Taxus wallichiana Zucc.	Thuner	Internal wound	60.00
Prinsepia utilis Royle	Bhekal	Joints pain	58.33
Berberis aristata DC	Kingod	Indigestion, tiredness, and eye flu	56.67
Rumex hastatus Don.	Almodu, Almor	Fly or wasp bite	55.00
Mentha longifolia (L.) Huds.	Jangali Pudina	Dehydration and cough	53.33
Perilla frutescens (L.) Britton	Bhangzeera	Digestive disorder	53.33
Nardostachys jatamansi (D. Don) DC.	Masi	Internal pain	51.67
Polygonatum verticillatum (L.) All.	Salam Mishri	Weakness	51.67
Pyracantha crenulata (D. Don) M. Roem.	Ghingaru	Blood purification	51.67

practitioners are considered the best information sources on medicinal plants [13], however, it has always remained difficult to obtain their TK or the professional secret [19,35].

The present study revealed that the local people are still dependent on the traditional healthcare practice. The reason behind their dependency on plant-based remedies is because of their faith on the ancient medicine system and unavailability of modern healthcare services at the village level like in other parts of the Himalaya [8,30]. The documentation of 76 wild medicinal plant species used by local people is a contribution to existing knowledge on locally available plants. Three plant species, namely *Alnus nepalensis*, *Quercus semicarpifolia*, and *Solanum virginanum* were used in ethnoveterinary purposes, among which the use of *A. nepalensis* in the treatment of urination with bleeding in livestock was unique in the region. Most of the traditional remedies were given through oral routes followed by dermal routes which permit rapid physiological reaction with pathogens [17] and increases the curative power. Elderly people (both male and female) from these villages having more information on the traditional uses of wild medicinal plants while the younger generation is having limited knowledge. In fact, 48.84% (n = 43) respondents of age over 40 years knew the use of more than 20 species while only 5.88% (n = 17) respondents below 40 years knew more than 20 species of medicinal plants. Most of the elderly people in the study area preferred traditional healthcare and rarely use modern medical consultation. Moreover, the literate (mostly the younger individuals) respondents showed limited knowledge on medicinal plants and only 30.23% (n = 43, literate respondents) or 13 individuals knew about more than 20 species and 37.21% knew about the medicinal use of 10–19 species. However, 52.94% (nine individuals) and 41.18% (eight individuals) of illiterate respondents knew the use of more than 20 species and 10–19 species, respectively. The illiterate traditional healers were keen to share the information and transferring the TK on the medicinal plant to the newer generation [16]. It was also revealed that female respondent knew more about the use of medicinal plant than the male respondents. Although young women hold less indigenous

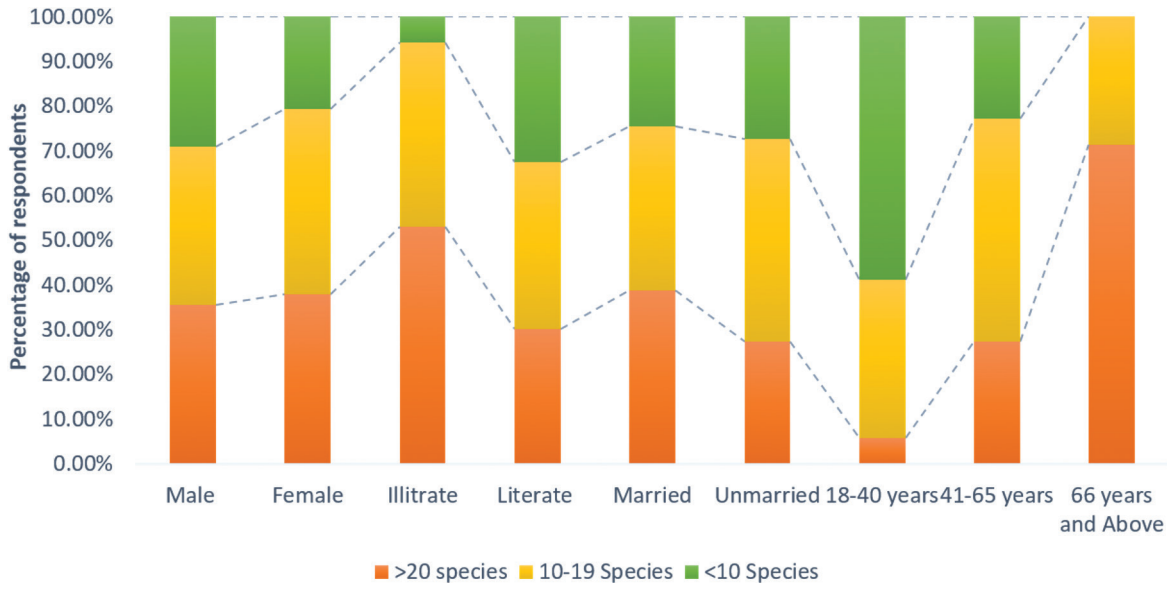

Figure 2: Traditional knowledge on wild medicinal plants with respect to respondent category

knowledge of the medicinal plant, they were more sensitive to conserve the biological resource [16]. The study reveals that with the increasing education and modern healthcare facilities the transfer of TKS to a new generation has naturally been restricted.

Over the time, people in the region became familiar with the economic properties of these medicinal plants and also collecting them for selling purposes. These practices contribute about 35%–40% in average annual income of the households. Species like *Aconitum heterophyllum*, *Aconitum balfourii*, *Allium humile*, *Angelica glauca*, *Arnebia benthamii*, *Bergenia ciliata*, *Dactylorhiza hatagirea*, *Gentiana kurroo*, *Girardinia diversifolia*, *Hedychium spicatum*, *Juglans regia*, *Meconopsis betonicifolia*, *Nardostachys jatamansi*, *Paris polyphylla*, *Picrorhiza kurroa*, *Podophyllum hexandrum*, *Polygonatum verticillatum*, *Prinsepia utilis, Rheum emodi*, *Saussurea costus*, *Taxus wallichiana*, *Trillidium govanianum*, and *Zanthoxylum armatum* are among the important medicinal plant that the community also collects for trade purpose. However, *T. govanianum*, *P. polyphylla*, *D. hatagirea*, *P. kurooa*, and *A. balfourii* are among the most tradable species in the area and contribute to a significant part of average annual income. Moreover, the medicinal plant based income generation activities were observed as the most important measure to cope up with flash flood disaster impacts in the Asi Ganga sub-basin since the year 2012 and 2013. Various studies [4–8,10–14,19,20,24–27] in the Himalayan region have also reported that the medicinal and aromatic plant (MAP) collection and their trade now became an important income generation source along with their use in traditional medicine.

All the identified wild plant species are collected from the forest, high altitude meadows, and wasteland nearby the villages in different seasons; however, summer and monsoon are the best seasons for collection. The most commonly used plant parts for herbal preparation in the Asi Ganga sub-basin were leaves and roots which has supported by previous studies in the Himalayan region [12,13,16,19]. Collection of roots, rhizomes, bulbs, bark, and stem have serious effects on the survival of mother plants and their regeneration pattern [17,20] and the unsustainable collection threatened the mother plants and led the species toward extinction [19]. Similar practices were reported by the local people including the traditional healers and many species have now become rare in the area. The declination of valuable resource could be a result of unawareness, low educational status, and poor socio-economic condition of the people which always neglect the ecological sustainability for more economic benefits [19].

About 300 medicinal plant species are documented from Uttarakhand state [12,24], out of which 35 were considered as rare and endangered species [12]. Most of these species (80%) were found to be restricted to the alpine region of

Uttarakhand Himalayan [12,19]. Moreover, other anthropogenic activities like unregulated grazing and unsustainable tourism practices including the religious rituals based on these medicinal plants depleting the valuable biological resource in the Himalayan region [19,30]. The issues have raised the attention of scientists towards medicinal plant sustainability [13,14,19,27,35,36–41], as most of these important plant species have been severely threatened in their natural habitat. In fact, 121 plant species from Himalaya have been listed under various threatened categories by International Union for Conservation of Nature (IUCN) and Botanical Survey of India has identified 214 plant species as endangered in the Himalayan region [36]. Most of these species are of ethnobotanical and significant economic importance and need to be conserved through appropriate conservation and management planning [8,19,24]. Many researchers working in the western Himalaya and other region have also recommended that local practitioners must be encouraged to perform their TK practices. Also, ex-situ cultivation of MAPs with evaluation of their efficacy and possible toxicity would be important for their long-term conservation in the Indian Himalayan Region. In the region, limited access for collecting the wild MAPs for household use has been provided to the local people by the governmental authorities, and trade activities are prohibited. However, it has always been challenging to regulate the prohibited and unsustainable collection practices over the hilly terrain in the Himalaya including the Asi Ganga sub-basin. The government of Uttarakhand has established Herbal Research and Development Institute to promote large-scale ex-situ cultivation of MAPs for income generation while State Medicinal Plants Board for in-situ conservation of wild medicinal plants [24]. State Biodiversity Board has also been working on conservation of biological resources, sustainable utilization, and equitable sharing of benefits arising from the use of biological resources. The research and conservation activities are also being initiated under the National Mission on Himalayan Studies in all Himalayan states of India including the Uttarakhand state. However, all initiatives are in their initial stage and the community in the study area is yet to be get benefitted from the governmental scheme. In terms of conservation of TKS, the government of India has constituted a task force on TKS under the National Mission on Sustaining Himalayan Ecosystem [42].

Conclusion

The people of Asi Ganga sub-basin inherit a rich TK to support their survival. A number of plant species are used in home-based remedies to treat various ailments as well as support livelihood of the resident communities. TKS of healthcare is unique in the area and the community still relies on traditional preparation as primary healthcare option, due to the remoteness of the area with limited healthcare facilities. Knowledge about traditional uses of plant species among the younger generation is a subject of concern which indicating towards the social barriers in the transfer of such valuable knowledge from one generation to other. The modern economic development has led the overexploitation of the resources is also an issue of concern to be addressed timely. Thus, the present documentation would be helpful to preserve the local TK on medicinal plants and could be promoted through linking with ecotourism in the region. A participatory management and conservation planning can be initiated in the Asi Ganga sub-basin, to conserve the valuable biological resource and betterment of the local people.

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Authors' contribution

Khima Nand and Suneet Naithani design the study. Khima Nand collected the data, analyzed, and prepared the draft of the manuscript. Suneet Naithani revised the manuscript and added a valuable suggestion for its improvement. Both the authors read and approved the final manuscript.

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