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Phytochemical study of selected medicinal plants used by the maring tribe of Chandel district, Manipur, India

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Abstract
The present investigation is carried out phytochemical analysis of four different medicinal plants Viz. *Dalbergia stipulacea* Roxb., *Justicia gendarussa* Burm. f., *Lindernia ruellioides* (Colsm.) Pennell and *Trichosanthes bracteata* (Lam.) Voigt used by the Maring tribe of Chandel district, Manipur, India. This paper aims to identify the presence of phytochemical constituents useful in curing various human ailments and to quantified their amount in the selected medicinal plants. The presence of such highly important phytochemical constituents in these plants will greatly help in developing new drugs.

Keywords: Phytochemical analysis, Maring, Chandel, Manipur, drugs

Introduction
The traditional system of herbal medicines are prepared from a single plant or combination of two or more plants, the efficacy of which depends on the use of proper plant parts and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolites in a raw drug [1, 2]. Important active bio-constituents include saponin, phenol, tannin, alkaloids, flavonoid, terpenoids and glycosides. These active compounds are derived from any part of the plant like bark, leaves, flowers, seed or even the whole plants. In many parts of the world, people still rely on traditional medicines which are cheap and safe unlike the conventional synthetic drugs. This reason alone can attribute to the surge in interest among the scientific community in search of new drugs based on ethnobotanical findings. The rapid advancement of modern medicine today is the result of strong foundation based on ancient wisdom and therefore will remain as one important source of future medicine as well as therapeutics [3]. The plant kingdom has been exploited for the treatment of diseases by different ethnic societies in different parts of the world. Ethno - pharmacological information based on phytochemical research is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [4]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [5]. Terpenes mostly have insecticidal properties and their pharmacological properties include antibacterial, antifungal, antihelmintic, antimalarial [6] while phenolic compounds have a wide range of pharmaceutical activities such as anti-inflammatory, analgesic, antitumour, anti-ulcerogenic [7, 8]. Flavonoids and alkaloids have attracted great interest recently due to their pharmacological significance which includes analgesic, antimalarial, anti-arrythmic, and antispasmodic, in the treatment of cough, pain and gout [9-11].

The interest in the chemical constituents present in different plant species was ignited by the likes of Kapoor *et al*., (1969, 1971-72, 1975) [12-15], Cox (1990, 1994) [16, 17], George *et al*., (1985) [18], Hartly (1973) [19], Sultanbawa *et al*., (1978) [20], Chen *et al*., (2001) [21], Zhang *et al*., (2001) [22]. Other workers include Fransworth and Bingel, (1977) [23], Zhang *et al*., (2004) [24], Khan *et al*., (2011) [25], Gogoi and Islam (2012) [26], Muhammad and Yakubu (2013) [27], Madhu *et al*., (2016) [28]. In-spite of reports on phytochemical works carried by various workers from all over the country, the study on the biochemical evaluation of medicinal plants of north-east state including Manipur was somewhat left out and lacking. Except for a few research papers from workers like Das (2006) [29], Sandhayarani (2010) [30], Devi *et al*., (2016) [31], the region lag behind in this field.

Materials and Methods

Plant materials

A total of four (4) plant species Viz. *Dalbergia stipulacea* Roxb., *Justicia gendarussa* Burm. f., *Lindernia ruellioides* (Colsm.) Pennell and *Trichosanthes bracteata* (Lam.) Voigt was taken.

Sample collection

The selected plant species were documented and collected from Maring inhabited villages of Chandel district (Manipur, India) and further identified botanically with help of available floristic literature and comparing with herbarium specimens at the Ethnobotany and Medicinal Plant Conservation Laboratory, Assam University (Table 1).

Table 1: Plants selected for phytochemical analysis

Name of the plant	Family	Part used	Ailments
Dalbergia stipulacea Roxb.	Leguminosae	Bark	Protection from dental cavities.
Justicia gendarussa Burm. f.	Acanthaceae	Leaves	Body ache/Body pain
Lindernia ruellioides (Colsm.) Pennell	Linderniaceae	Whole plant	Dysuria/calculus complaints
Trichosanthes bracteata (Lam.) Voigt	Cucurbitaceae	Fruit	Dermal tumor/cyst

Chemicals

Ethanol, methanol, chloroform, petroleum ether, distilled water, fehling solution A & B, concentrating sulphuric acid, ammonia solution, HCl, Wagner, Hager, Mayer, Dragendroff reagent, ferric chloride, Folin – Ciocalteu reagent, Potassium ferrocyanide, Aluminium chloride.

Preparation of plant extract

The plant material was shade dried for few days. The dried sample was then coarse powdered and stored in air tight container. The subsequent extraction of the sample was done by using three different solvent viz. methanol, chloroform and petroleum ether. The extraction process was done at room temperature for 24 hours and filtered. The filtrate was obtained and stored at refrigerator for further use.

Qualitative Screening

Phytochemical test are carried out for all the extracts as per the standard methods of Harborne (1973) [32] and Trease and Evans (2002) [33].

Detection of alkaloids

a) Wagner's Test: Filtrates were treated with Wagner's reagent Formation of brown/reddish precipitate indicates the presence of alkaloids.

b) Hager's Test: Filtrates were treated with Hager's reagent. Presence of alkaloids confirmed by the formation of yellow colored precipitate.

c) Mayer's Test: Filtrates were treated with Mayer's reagent. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

d) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent. Formation of red precipitates indicates the presence of alkaloids.

i) Detection of glycosides: Filtrates were treated with 1ml glacial acetic acid and 3 drops of ferric chloride solution. 1 ml sulphuric acid was poured slowly by the side of the test tube. Appearance of brown ring at the junction of two layer and the upper layer turns bluish-green indicates the presence of glycosides.

ii) Detection of flavonoid:

a) Alkaline Reagent test: Filtrates were treated with 5 drops of NaOH which gives intense yellow colour. The intense yellow coloration becomes colorless on addition of dilute acid indicates the presence of flavonoid.

b) H_2SO_4 test: A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

iii) Detection of phenol and tannin

a) Ferric chloride test: Extract was treated with few drops of ferric chloride solution. Formation of white precipitates indicated the presence of tannin.

b) Gelatin test: To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitates indicated the presence of tannin.

c) About 0.5 g of the extract dissolved in 5 ml of distilled water, filtered and mixed with ferric chloride reagent. Formation of a blue-green precipitate showed the presence of phenolic and tannin compound.

v) Detection of terpenoid: Salkowski's test: 0.5 g of the plant extract was mixed with 2 ml of chloroform and 3 ml of conc. Sulphuric acid was carefully added to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

vi) Detection of phytosterol

a) Salkowski's test: Extracts were treated with chloroform and filtered. The filtrate was treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes.

b) ***Libermann Burchard's test:*** Extracts were treated with chloroform and filtered. The filtrate were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicated the presence of phytosterol.

vii) Detection of saponin

a) Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicated the presence of saponin.

b) Froth test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponin.

Quantitative Phytochemical Analysis

Total phenol content, total tannin content and total flavonoid content was quantified using standard procedures of Hagerman *et al*., (2000) [34] and Kumaran and Karunakaran (2006) [35].

i. Determination of total phenol content: 10 mg of the sample was dissolved in 50 ml of triple distilled water (TDW). Around 1ml of the solution was pipetted and transferred to a test tube, 0.5 ml 2N of Folin-Ciocalteu reagent

and 1.5 ml of 20 % Na_2CO_3 solution added. Further the volume was made upto 8 ml with triple distilled water followed by vigorous shaking and allowed to stand for 2 hours. Absorbance was measured at 650 nm and total phenolic content was estimated using standard calibration curve of gallic acid.

ii. Determination of total tannin content: 50 ml of distilled water was added to 50 mg of the sample, shaken for around 1 hour and filtered in a volumetric flask where the volume was made up - to the mark. 5ml of the filtrate was then pipette into a test tube, mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was measured at 120 nm.

iii. Determination of total flavonoid content: 0.5 ml of the sample solution was mixed with 2 ml of distilled water and subsequently with 5% $NaNO_2$ solution. The mixture was incubated for around 5-6 minutes and 0.2 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes. Another 2 ml of 4% NaOH solution was added with water making the final volume to 5 ml, thoroughly mixed and allowed to stand for another 15 minutes. Absorbance was determined at 510 nm and total flavonoid content was expressed in mg of catechin equivalent per gram of extract.

Results & Discussion

The qualitative phytochemical analysis conducted on *Dalbergia stipulacea* Roxb., *Justicia gendarussa* Burm. f., *Lindernia ruellioides* (Colsm.) Pennell and *Trichosanthes bracteata* (Lam.) Voigt exhibited the presence of some bioactive compounds responsible for the medicinal properties in plants. The presence or absence of an active compound is represented by the symbols viz., '+++' present in high concentration, '++' present in moderate concentration, '+' present in small concentration, '-'absent.

Methanol extract exhibited the maximum positive reaction in the qualitative phytochemical analysis and therefore all further tests are carried out in this extract. Total phenol is determined by the Folin-Ciocalteu method, reported as gallic acid equivalents (mg GAE/gm) by reference to standard curve. Total phenol content was significantly highest in methanolic extract of *Dalbergia stipulacea* Roxb. with 201.1± 0.31 mg GAE/gm followed by *Justicia gendarussa* Burm. f. with 32.2 ± 0.35 mg GAE/gm, *Lindernia ruellioides* (Colsm.) Pennell with 30.26 ± 0.03 mg GAE/gm and *Trichosanthes bracteata* (Lam.) Voigt 15.1 ± 0.01 mg GAE/gm. Total tannin content was highest in *Dalbergia stipulacea* Roxb. with 190.53 ± 0.28 mg followed by *Justicia gendarussa* Burm. f. with 25.1 ± 0.12 mg, *Lindernia ruellioides* (Colsm.) Pennell with 19.03 ± 0.17 and *Trichosanthes bracteata* (Lam.) Voigt with 10.13 ± 0.03. Total flavonoid content is determined by Colorimetric method reported as mg of catechin equivalent per gm (mg CE/gm) of extract, by reference to standard curve. The highest flavonoid content expressed in catechin equivalent was observed in PE extract of *Lindernia ruellioides* (Colsm.) Pennell with 71.3 ± 0.40 mg CE/gm followed by *Dalbergia stipulacea* Roxb. with 43.73 ± 1.43 mg CE/gm, *Justicia gendarussa* Burm. f. with 13.06 ± 0.29 mg CE/gm and *Trichosanthes bracteata* (Lam.) Voigt 9.13 ± 0.14 mg CE/gm.

Studies on plant species employed for teeth blackening suggest the significant content of phenolic compounds and also its actual contribution in teeth blackening. *In vitro* animal studies also showed the interaction of polyphenols with known etiological agents of caries disease (*Streptococcus mutans* Clarke) thereby decreasing dental plaque accumulation and the virulence of this bacterium [36]. This highly support the fact that Dalbergia *stipulacea* Roxb. recorded for the highest total phenolic content among the four plants. Almost all the species of *Trichosanthes* have been reported to contained compounds like trichobetacin and trichoanguin commonly used in the treatment of intestinal disorders, cough and tumors [37]. The leaves extract of *Justicia gendarussa* Burm. f. exhibits the presence of flavonoid compounds vitexin and apigenin further reporting the flavonoid vitexin as a potent anti-inflammatory agent stalling the expression of several inflammatory factors [38-40]. Therefore, the presence of these compounds in high amount in the selected plants strongly proves the medicinal potentiality as claim by the tribal community.

Table 2: Preliminary screening of phyto – constituents of the selected plants

	***Lindernia ruellioides* (Colsm.) Pennell**			***Dalbergia stipulacea* Roxb.**			***Justicia gendarrussa* Burm. f.**			***Trichosanthes bracteata* (Lam.) Voight**		
Chemical tests	**M**	**Ch.**	**P. E.**	**M**	**Ch.**	**P.E.**	**M**	**Ch.**	**P.E**	**M**	**Ch.**	**P.E.**
Mayer's reagent	++	+	++	+	+	+	-	+	+	+	+	+
Dragendroff's reagent	+	+	++	+	+	++	+	-	-	+	+	+
Wagner's reagent	+	++	+	+	+	+	+	+	+	+	+	+
Hager's reagent	+	+	+	+	+	+	-	-	-	+	+	+
Saponin												
Froth test	-	-		-	-	-	+	-	+	-	-	+
Phytosterol												
Salkowaski test	+	-	-	+	-	+	+	+	+	+	+	+
Libermann Burchard's	-	-	+	+	-	+	-	-	-	-	-	-
Phenol												
Ferric chloride test	+	+	-	-	-	-	+	+	+	-	-	+
Gelatin test	-	-	+	+	+	+	+	+	+	+	-	-
Tannin												
Ferric chloride test	+	+	-	+	-	+	+	+	+	-	-	+
Gelatin test	-	-	+	-	-	-	+	+	+	+	-	-
Flavonoid												
Alkaline reagent test	-	-	+	-	+	-	++	+	+	-	-	-

Sulphuric acid test	+	+	+	+	-	+	-	+	+	+	-	+
Terpenoid												
Salkowski's test	++	+	+	+	+	+	-	+	+	+	+	+
Glycosides												
Glacial acetic acid test	++	+	++	++	-	++	-	+	+	+	-	+

*M = Methanol; Ch.= Chloroform; P.E.= Petroleum Ether.

Table 3: Total phenolic, tannin and flavonoid content of the selected plants (Mean ±SD, n=3).

Test samples	Total phenol (mg /g)	Total tannin (mg /g)	Total flavonoid (mg/g)
Dalbergia stipulacea Roxb.	201.1± 0.31	190.53 ± 0.28	43.73 ± 1.43
Justicia gendarussa Burm. f.	32.2 ± 0.35	25.1 ± 0.12	13.06 ± 0.29
Lindernia ruellioides (Colsm.) Pennell	30.26 ± 0.03	19.03 ± 0.17	71.3 ± 0.40
Trichosanthes bracteata (Lam.) Voigt	15.1 ± 0.01	10.13 ± 0.03	9.13 ± 0.14

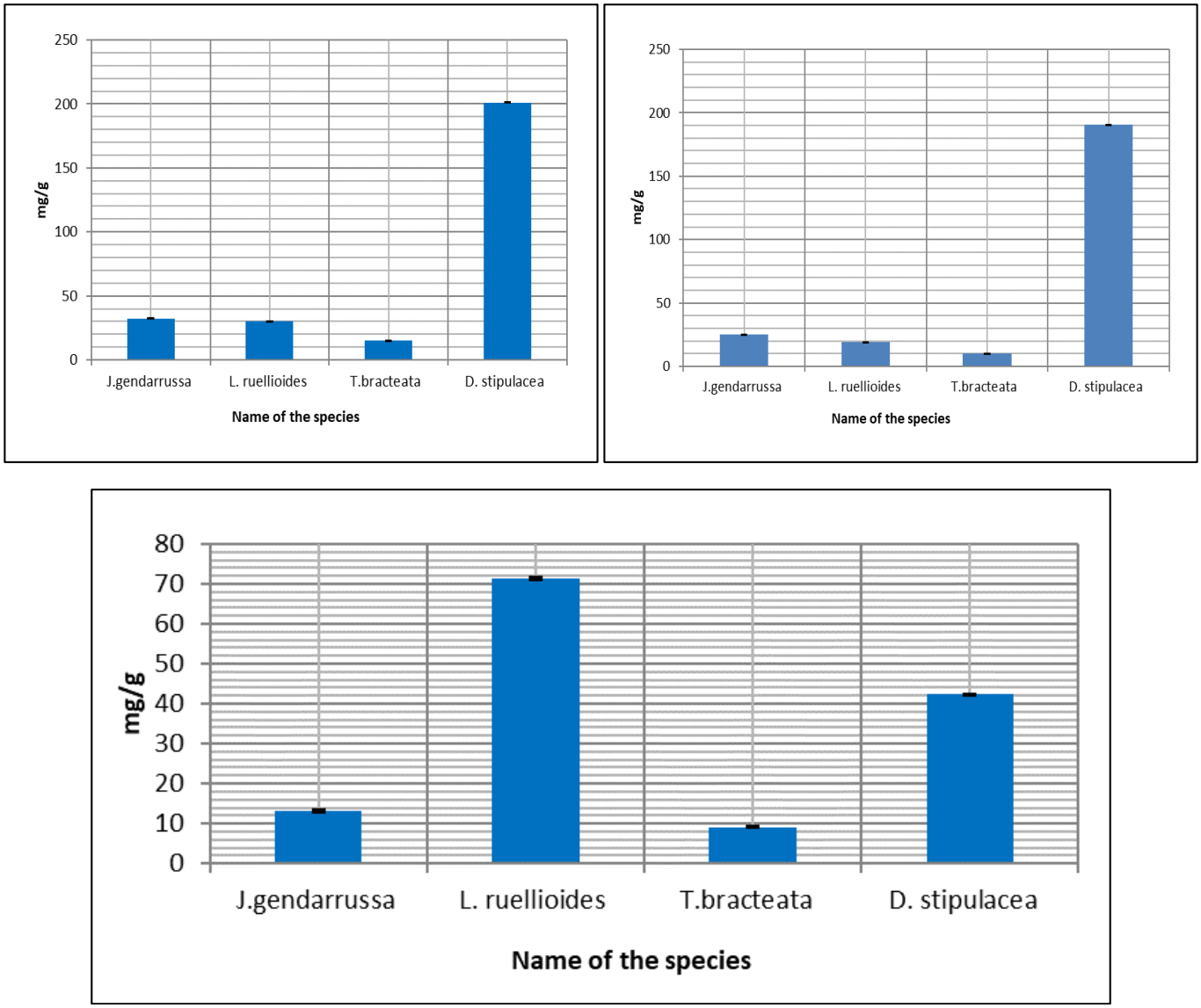

Fig 1: Comparison of total phenol, tannin and flavonoid content of the four medicinal plants

Conclusion

The preliminary qualitative and quantitative estimation of the four medicinally important plants in different solvents concluded that it possess significant amount of potentially health benefitting phytochemicals leading ways for further studies and hence can be employed in pharmaceuticals industry.

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