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 (C. L. et P. J. F.) 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, antihelminthic, 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-arrhythmics, 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], Hartyl (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

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

Chemicals
Ethanol, methanol, chloroform, petroleum ether, distilled water, fehling solution A & B, concentrating sulphuric acid, ammonia solution, HCl, Wagner, Hager, Mayer, Dragendorff 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) Dragendorf’s Test: Filtrates were treated with Dragendorff’s reagent. Formation of red precipitates indicates the presence of alkaloids.

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) Salkowski’s test: 0.5 g of the plant extract was dissolved in 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.

d) 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) Frot 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

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and 1.5 ml of 20 % Na₂CO₃ solution added. Further the volume was made up to 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. 5 ml of the filtrate was then pipetted into a test tube, mixed with 2 ml of 0.1 M FeCl₃ 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% NaNO₂ solution. The mixture was incubated for around 5-6 minutes and 0.2 ml of 10% AlCl₃ 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 gm 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 mg of catechin 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 trichoaguin 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

<table>
<thead>
<tr>
<th>Chemical tests</th>
<th>Lindernia ruellioides (Colsm.)</th>
<th>Dalbergia stipulacea Roxb.</th>
<th>Justicia gendarussa Burm. f.</th>
<th>Trichosanthes bracteata (Lam.) Voigt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer’s reagent</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dregendorff’s reagent</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Wagner’s reagent</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hager’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Salkowski test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Libermann Burchard’s</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Alkaline reagent test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

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

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

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

**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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**References**

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