Phytochemical analysis of Gmelina asiatica L. leaves

Florence AR and Regini Balasingh GS

Abstract
Objective: The present study was undertaken to investigate the bioactive components present in the leaf extracts of Gmelina asiatica.
Methods: Aqueous, petroleum ether, chloroform, ethanol and acetone extracts were prepared by adding 100 g of leaf powder to 1000 ml of these solvents and subjected to soxhlet extraction. The extracts were concentrated by using vacuum evaporator and dried at 60 °C. Preliminary phytochemical screening was performed by Harborne method. Total tannin and flavonoid content was determined by using Folin Ciocalteau reagent and Aluminium Chloride method respectively.
Results: Different extracts showed the bioactive components such as alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Total tannins content in the ethanolic leaf extract was estimated as 0.042µg/µl and flavonoids content was estimated as 0.045µg/µl.
Conclusion: The findings of the study revealed that various chemical constituents present in the leaf extracts of G. asiatica is rich in phytopharmaceutical importance.

Keywords: Gmelina asiatica L., leaf extract, phytoconstituents, solvents, Verbenaceae

1. Introduction
In developing countries medicinal plants and their components produce a diverse assortment of secondary metabolites of therapeutic importance and widely used in human therapy, veterinary, agriculture, scientific research and other countless areas [1-4]. Generally plants are the potential and inexhaustible sources of secondary metabolites which are able to synthesize a variety of chemical substances such as proteins, aminoacids, alkaloids, terpenes, flavonoids, glycosides, resins, saponins, volatile oils, gums, tannins, etc. which are responsible for medicinal activity [5-8]. Therefore, the most of the ongoing search for biologically active secondary metabolites and dietary supplement derived from plants have increased [9]. Among the secondary metabolites flavonoids and tannins are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants [10]. Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity [11]. Flavonoids from medicinal plants are safe and bioactive, dietary flavonoids are recognized for their antioxidant [12,13], anti-proliferative [14], antimicrobial [15-18], anti-allergic, anti-inflammatory, antihypertoxic, antiulcer, antiviral and antispasmodic effects [19-22] which may protect the body from various diseases and anticancer activities [23]. Tannins exhibit diverse biological activities such as antisympathetic, antidiarrheal, antimicrobial, antioxidant [24,25], anthelmintic, antiviral [26], cytotoxic and antineoplastic agents [27].

Gmelina asiatica L. (Syn: Gmelina parvifolia Roxb.), is a deciduous large sized bush or shrub belonging to the family Verbenaceae which comprises about 35 species and 2 subspecies spread over in tropical and temperate regions of Asia. It is commonly called “Asiatic Bush Beech” and “Nilakumizh” in Tamil. The whole plant is medicinally important and well documented as a source of bioactive components with medicinal properties such as antimicrobial [28-32], anti-inflammatory [33], antioxidant [33, 34], antihypertensive and hypoglycemic [35], hepatoprotective [33], antipyretic [36], nematicidal [37] and anticancer activity [38-40]. The aerial parts and roots are used in traditional medicine for the treatment of jaundice, rheumatism, syphilis, gonorhea, burning sensation of eyes, fever, dysuria, wounds, dandruff, diabetes, hepatic diseases, catarrh of the bladder, blood purifier and also to reduce body heat [29, 30, 41-44]. The present study provides valuable information about secondary metabolites present in Gmelina asiatica leaf extracts.
2. Materials and Methods

2.1 Collection and extraction of plant materials
Fresh leaves of *Gmelina asiatica* were collected from Scott Christian College Campus, Nagercoil, Kanyakumari District, South Tamil Nadu, India and identified by using taxonomic keys [45]. The collected leaves were shade dried at room temperature for about two weeks to dry. They were made into powder with the help of a mechanical grinder and sieved. The powdered leaves were used for extraction. The dried powdered sample (100 g) of *G. asiatica* was extracted with 1000 mL of solvents such as aqueous, petroleum ether, chloroform, ethanol by a Soxhlet apparatus separately. The resultant filtrate was concentrated in powdered form by evaporation of the solvents using Rotary evaporator which was stored in a refrigerator at 4 °C used for phytochemical analysis as per the standard methods of Harborne [46].

2.2 Estimation of Flavonoids
Total flavonoid content in the extract was determined by the aluminium chloride calorimetric method [47] using gallic acid as the standard; 1mL of test sample and 4 mL of water were added to a volumetric flask (10 mL volume). After 5 min, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminium chloride were added. After 6 min of incubation at room temperature, 2 mL of 1 M sodium hydroxide (NaOH) was added to the reaction mixture. Immediately the final volume was made up to 10 mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as gallic acid equivalents in mg gallic acid/g dried extract.

2.3 Estimation of Tannins
Tannin content of the leaf extract was estimated by Folin Ciocalteau spectrophotometrically method [48]. One millilitre of the extract was mixed with 5 mL of vanillin hydrochloride reagent (a mixture of equal volumes of 8% HCl in methanol and 4% vanillin in methanol). The mixture was allowed to stand for 20 min and the absorbance measured at 500 nm against reagent blank. The standard graph was plotted for working standard catechin solution (0 to 250 µg/µL).

3. Results
The qualitative phytochemical analysis of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *G. asiatica* leaf revealed the presence of alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Aqueous extract showed the presence of carbohydrates, flavonoids, tannins, terpenoids, steroids, phytosterols and alkaloids. The petroleum ether extract showed the presence of glycosides, quinones, coumarins and phytosterols. Chloroform extract revealed the presence of carbohydrates, glycosides, proteins, coumarins and phytosterols. The ethanol extract showed the presence of glycosides, flavonoids, tannins, saponins, terpenoids, steroids, proteins, coumarins and phytosterols. The acetone extract revealed the presence of carbohydrates, glycosides, tannins, saponins, proteins, coumarins and phytosterols. The maximum phytocompounds present in ethanolic extract showed the presence of maximum number of (9/12) compounds and the minimum phytoconstituents were noticed in petroleum ether extract (Table 1; Fig. 1).

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Aqueous</th>
<th>Pet. ether</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Abbreviation:** (-) Absent; (+) Low; (++) Average; (+++) High

The quantitative phytochemical analysis of total tannin and flavonoid content of *G. asiatica* leaves in ethanol extract was studied. The total tannin and flavonoid contents are expressed as µg catechin equivalents/µl of extract and µg gallic acid equivalents/µl of dry weight of the extract respectively. Tannins content in the ethanolic leaf extract was estimated as 0.042µg/µl and flavonoids content was estimated as 0.045µg/µl.
4. Discussion
Plants are the potential sources of medicinal compounds in human life, as the major source of food, maintenance and improvement of health by elimination of the diseases causing microbes [49, 51]. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Over 50% of all modern clinical drugs are herbal in origin and natural products play an important role in drug development programs [52]. With the development of pharmaceutical industries, much more interest has been created on plants and their products [53, 54].

Qualitative phytochemical analysis of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *G. asiatica* leaf indicated the presence of alkaloids, carbohydrates, glycosides, coumarins, quinones, saponins, steroids, terpenoids, proteins, phytosterols, tannins and flavonoids. Among the tested extracts, the ethanolic extract showed the presence of maximum number of (9/12) compounds. This is because ethanol is much polar than chloroform and acetone, hence extracting many of the active ingredients from the plant parts [55]. Several workers indicated that maximum separation of phytochemicals in the ethanolic extract than other solvents [31, 34]. The above result indicates that the plant showed more amount of glycosides in all extracts but absent only in aqueous extract. Observations by Savithramma et al. [8] and Rajesh et al. [56] confirmed the absence of glycosides in aqueous extracts which is also proved by the present study. The presence of glycosides indicates that *G. asiatica* may be a potent in curing cardiac insufficiency, cough and circulatory problems and may acts as good sedatives and have antispasmodic properties [57], whereas alkaloids were absent in all extracts except aqueous extract. Previous works also supported the absence of alkaloids in *G. asiatica* leaf [29, 31, 58] but another work reported the presence of alkaloids in aqueous extract of *G. asiatica* leaf [29, 8]. Phytosterols were found in all the extracts studied. Phytosterols indirectly and directly inhibit the growth and metastasis of prostate cancer (PC-3) cells [59]. The earlier findings in different parts of *G. asiatica* extracts were reported in leaf [29, 31]. When compared to root and stem, leaf extracts contributed wide range of secondary metabolites, because nutrients are abundant in leaves during photosynthesis. It is evident from the present study that the leaf of *G. asiatica* produces different types of secondary metabolites.

In quantitative phytochemical analysis, the total tannins and flavonoids in the ethanolic extract of *G. asiatica* leaves was 0.042 and 0.045µg/µl respectively. According to Salminen *et al.* [60] several environmental factors may be related to the low tannin content of the present study, such as season, area of collection site, effect of air pollution, nutrient restriction of soil and also depend on the type of solvent used, its concentration, the reaction time, temperature and concentration of catechin. In addition, the presence of phenolics and flavonoids are depended on plant parts, maturity at harvest, growing conditions, soil conditions and post-harvest treatment [61, 63]. Accurate estimation of flavonoids is difficult, because of the wide varieties of flavonoids available and the extensive distribution in various plants [64]. The estimated amount of total tannins and flavonoids from *G. asiatica* leaves in the present study is lower than those reported previously from *G. asiatica* stem [34]. The quantification of total tannins and flavonoids in the methanolic extract of the present study is more or less similar with *Verbena tenara, V. venosa* and *V. rigida* [65]. Earlier studies have confirmed the amount and composition of phenols and flavonoid compounds diversified at the sub-cellular level and within plant tissues as well [66, 67].

5. Conclusion
From the finding of this research, the plant *G. asiatica* leaf under investigation showed various phytoconstituents in the tested extract and their medicinal potential can be used as valuable drugs with antimicrobial, antioxidant, anti-diabetic, anti-inflammatory and anticancer properties which also proves its effectiveness in curing various diseases.

6. References


