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Phytochemical screening and quantification of phytoconstituents in *Gmelina arborea* fruits extracts

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Abstract

The aims of present investigation are to carried out phytochemical screening and quantify phytoconstituents of *Gmelina arborea* (*Verbenaceae*) fruits extracts. The ethyl acetate, methanolic and water extract of *G. arborea* were screened and phytochemical analysis showed the presence of carbohydrates, saponin, alkaloid, flavonoids, phenolic compound, steroid and triterpenes. The quantification of total phenolic and flavonoids were carried out using Folin–Ciocalteu method and aluminium chloride method respectively while saponin content determined using vanillin sulphuric acid in *G. arborea* fruits extracts. Crude alkaloid content was determined in *G. arborea* fruit powder. TLC (Thin layer chromatography) is also performed on methanol extract of fruits of *G. arborea*. The Results revealed that ethyl acetate extract showed highest amount of phenolic and flavonoids content whereas the highest saponin content was found in methanolic extract of fruits.

Keywords: *Gmelina arborea*, fruit, phenolic, quantification

Introduction

Herbal medicines are a crucial element of the expansion of modern civilization. The WHO estimates 80% of the world population currently utilize herbal drug for some aspect of primary health care. Presently, major pharmaceutical companies are conducting huge research on plant materials for their effective therapeutic value. It is estimated that at least 25% of all modern medicines are derived, either directly or indirectly from plants ^[1, 2] Plant contain various diversified phytoconstituents. The plant is utilized the phytoconstituents for their growth viz. lipid, proteins and carbohydrates are known as primary metabolite. Plant synthesized some constituents from primary metabolite, having complex structure like phenolic, flavonoids, alkaloid, resin, glycoside, tannins and terpenoid are known as secondary metabolite. Plants secondary metabolites are distinctive reserve of biological active medicament.



Fig 1: *Gmelina arborea* plant

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Gmelina arborea Roxb. (Verbenaceae.) Commonly known as *Gamphari* in Ayurveda. It is deciduous tree with smooth whitish grey bark. Fruits are drupe, 1.8-2.5cm long, obovoid, bear the enlarged calyx; yellow when ripe. Endocarp is bony and 2-celled. Seeds are reddish in colour, lenticular and exalbuminous [3]. Bark, wood, leaf, root and fruits are used in treatment of various ailments in folk medicine [4, 5] Ripe fruits are dried and cooked with cow's milk, for urticarial [6]. Fruits are used in shortness of breath [7], as cooling agent [8] diuretic, nutritive, used in tuberculosis; promote hair growth, menorrhagia and burning sensation. The fruits are edible [9] Decoction of fruits used to treat swelling of body, fever and bilious disorders. Fruits powder is used with milk or ghee in pregnancy for settlement of foetus [10] *G. arborea* fruits have reported diuretic [11], antiepileptic [12], analgesic [13], antipyretic [14], antibacterial, antioxidant, antidiabetic [15] and hepatoprotective activity [16]. The study of quantification of various phytoconstituents is planned on *G. arborea* fruits because as not reported.

Material and Methods

Plant Material

Fresh plant of *Gmelina arborea* was collected from Waghodia road, Baroda in the month of May 2011. Plant was identified and authenticated by Dr. P. S. Nagar at Botany Department of The M. S. University, Vadodara Voucher specimens of *G. arborea* (DC-GM-1) was stored in the herbarium of Pioneer Degree Pharmacy College, Vadodara.

Reagent and chemicals

All the chemicals and reagents used were of analytical grade and procured from E. Merck (Darmstadt, Germany), Hi-Media Laboratories Ltd., Mumbai, India and Sigma (Chemical Co, St. Louis, MO, USA). All UV-Vis measurements were recorded on a Shimadzu UV-1800 (Japan).

Preparation of extracts [17]

Accurately weighed 20g powdered fruit of *G. arborea* was extracted with water, ethyl acetate and alcohol for 24hrs separately. The extracts were filtered, dried and percentage yield were recorded.

Qualitative chemical test [18, 19, 20]

The fruits extracts obtained by solvent extraction from *G. arborea* subjected for qualitative chemical tests for phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, and phytosterols.

TLC profile of the extracts [21, 22]

The fruits extracts of *G. arborea* were subjected to thin layer chromatographic (TLC) studies. R_f values were noted for observed compounds.

Quantification of phytoconstituents

Total phenolics content [23, 24]

Total Phenolic content was determined in methanol extract, ethyl acetate extract, water extracts of fruits *G. arborea* by Singleton and Rossi (1965). Total phenolic was determined with Folin-Ciocalteu reagent using Gallic acid as a standard phenolic compound. The calibration curve of Gallic acid was taken using methanol. The 1.0 ml extract solution containing 5mg extract was diluted with 10 ml of distilled water. To this, 1.5 ml of Folin-Ciocalteu reagent was added. The above

mixture was kept for 5 min. and then 4 ml of 20% sodium carbonate solution was added and made the volume up to 25 ml with the distilled water. This mixture was kept for 30 min and the absorbance of the blue color developed was measured at 765 nm, using Shimadzu 1800 spectrophotometer. The percentage of total phenolics was expressed as % Gallic acid.

Flavonoid content [25]

Flavonoids with various biological activities are considered as the key compounds in the plants. Flavonoids content was determined in methanol extract, ethyl acetate extract, water extract of fruits on *G. arborea* using aluminium chloride method. Quercetin was used as a standard phenolic compound. The solution of plant extract and quercetin were prepared using methanol. The 1.0 ml extract solution containing 5mg extract was mixed with 1.5ml 95% methanol, 0.1ml 10% aluminium chloride, and 0.1ml 1M potassium acetate and 2.8ml distilled water. After incubation at room temperature for 30min, the absorbance of reaction mixture was measured at 415nm using colorimeter. Blank solution was prepared substituting the amount of 10% aluminium chloride by the same amount of distilled water. Calibration curve was prepared using concentration of quercetin verses absorbance. The percentage of total flavonoids was expressed as percentage of quercetin.

Total alkaloids [26]

A 10 g powdered drug was treated with 100ml 10% acetic acid in methanol and kept for 4hrs. Mixture was filtered and concentrated on a water bath to 1/3rd of the original volume. Ammonium hydroxide was added drop wise until the complete precipitation. The solution was centrifuged, the precipitates were collected and washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed and total alkaloids content were express as percentage on dry weight basis.

Total saponins content [27]

Saponin content was determined in aqueous and methanol extract in fruits of *G. arborea* using diosgenin as standard saponin.

Stock solution of diosgenin (1mg/ml) was prepared by dissolving 10mg diosgenin in 8ml methanol and 2ml distilled water. From this solution 0, 80,100, 180, 250 μ g/ml was taken in volumetric flask, 0.5ml the vanillin reagent and 5ml of 72% (v/v) H₂SO₄ was added slowly on the inner side of the wall then volumetric flask was transferred on water bath adjusted at 60 °C for 10min, cool the volumetric flask in ice-cold water for 3-4min, and measured absorbance at 544nm against the reagent blank (0 μ l of the diosgenin standard solution). Calibration curve was plotted using concentration of Diosgenin verses absorbance. A known amount of extract was dissolved in methanol. Similarly 0.4ml test solution of the extracts was processed and the percentage of total saponins was expressed as percentage of diosgenin.

Result and Discussion

Extraction methods involve the separation of medicinally active portions of plant chemical from the inactive tissue by using selective solvents. During extraction, solvents enter into the solid plant material and solubilise compounds with similar polarity. Preliminary screening is helpful to identify largest chemical constitute of plant extracted in particular solvent and useful for selection of solvent. It gives idea about nature of phytoconstituents e.g. if the plant contains non-polar

phytoconstituents then they get extracted in non polar solvent like petroleum ether, chloroform. Their % yield, colour and consistency are recorded in Table-1.

Table 1: The colour, consistency and % yield of fruit extracts.

Extracts	Colour and consistency	Yield (%w/w)
Ethyl acetate	Dark brownish, sticky	3.5
Methanol	Dark Reddish brown, solid, sticky	28.33
Water	Dark brownish red, solid, sticky	39.2

Table 2: Preliminary phytochemical screening of *G. arborea* fruits extracts

Chemical constituent	Ethyl acetate	Methanol	Water
Carbohydrates	+	+	+
Proteins	—	—	—
Saponins	—	+	+
Alkaloids	+	+	—
Flavonoids	+	+	+
Tannin & phenolics	+	+	+
Steroids & triterpens	—	—	—

TLC profile of the extracts

The quality of raw material is basic requirement for quality of final product. Thin layer chromatography is an important means for evaluation of quality of plant materials. The fruits extract of *G. arborea* were subjected to Thin Layer Chromatography (TLC) to detect and confirm the presence of

phytoconstituents. Silica gel G was used as an adsorbent. TLC profile like solvent system for saponin, steroid & triterpenoids and phenolics; reagent used for detection and R_f value of spots for fruits extracts of *G. arborea* are summarized in Table 3. The TLC Chromatogram for methanol extract of triterpenoids and steroid is given in Fig.2.

Table 3: TLC profile of *G. arborea* fruits extracts

Chemical	Saponin	Steroids & Triterpenoid	Phenolic Compound
Mobile phase	CHCl ₃ :Methanol: water (7:3:0.4)	CHCl ₃ :Tol:EA (6:3:1)	EA: Methanol: FA (9:1:0.4)
Detection	AS reagent	AS reagent	Alcoholic FeCl ₃
Ethyl acetate	0.15, 0.35 (Brownish) 0.68 (yellowish) 0.87 (pink)	0.23, 0.47, 0.65 0.96 (Purple), 0.82 (Pink),	0.9,0.11 (Black)
Methanol	0.16, 0.30 0.61 (yellowish) 0.87 0.93, 0.97, 0.98 (Purple)	0.23, 0.47, 0.65, 0.84, 0.97 (Purple)	0.9, 0.11 (Black)
Water	0.19, 0.78, 0.98 (Purple)	----	0.12, 0.32 (Black)

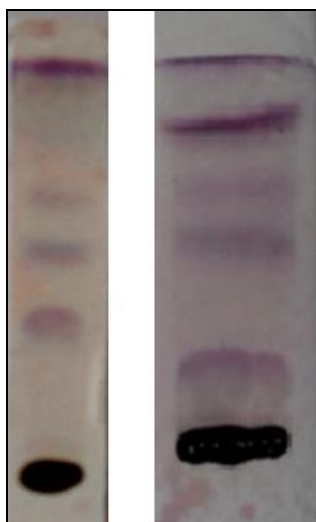


Fig 1: TLC of methanolic extract of fruits

Estimation of secondary metabolites

The estimation of total phenolic, flavonoids, and saponin were carried out in various extract of fruits whereas alkaloid content carried out on *G. arborea* fruit powder.

Qualitative chemical test

The extracts obtained from solvent extraction were then subjected to various qualitative chemical tests for the identification of various plant constituents like steroids, carbohydrates, alkaloids, glycosides, phenolics and tannins. It gives primary idea about type of primary and secondary metabolite present in plants material. The results of qualitative chemical test of *G. arborea* a fruits extracts are reported in Table-2.

Total phenolics content

Folin-Ciocalteu reagent is mixture of phosphomolybdate and phosphotungstate. Phenolic compound reduce phosphotungstate-phosphomolybdate complex to blue reaction products in alkaline conditions. The amount of the substance (phenolics) need to inhibit the oxidation of the reagent was estimated by measurement of the absorbance of coloured complex at 765nm. Gallic acid was use as reference standard. Total phenolic content in various extracts are compiled in Table 4.

Table 4: Total phenolic content in *G. arborea* fruits extracts

Extract	%w/w
Aqueous fruit extract	2.304±0.264
Methanol fruit extract	1.876±0.55
Ethyl acetate fruit extract	2.44±0.08

Flavonoid content

Flavonoid content was determined in aqueous, methanol and ethyl acetate extract of fruits of *G. arborea* aluminium chloride method at 415nm. Flavonoids content was expressed as % quercetin using calibration curve of quercetin. Flavonoid content content in various extracts are compiled in Table. 5.

Table 5: Flavonoid content in *G. arborea* fruits extracts

Extracts	%W/W
Aqueous fruit extract	0.044 ±0.021
Methanol fruit extract	0.209 ±0.006
Ethyl acetate fruit extract	0.34±0.004

Total alkaloid content: Alkaloids are basic compound and found in salt form with plant acid. Alkaloid is soluble in acidic methanol. On addition of concentrated ammonia, alkaloids get precipitate. The precipitate was dried and weighed to obtain % yields. The alkaloid content was found to 0.05% w/w.

Total saponin content

Saponin content was determined in aqueous, methanol and ethyl acetate extract of fruits of *G. arborea* Total saponin was determined using calibration curve of diosgenin and expressed as %w/w of diosgenin. Saponins content in various extracts are compiled in Table 6.

Table 6: Saponin content in *G. arborea* fruits extracts

Extracts	%W/W
Methanol fruit extract	7.82 ±0.26
Aqueous fruit extract	7.22 ±0.72
Ethyl acetate fruit extract	1.93±0.98

Plant considered as biosynthetic factory contain primary and secondary metabolites like alkaloid, glycoside, tannins, phenolic and triterpenoids. Phenolic compounds such as flavonoids, phenolic acids, and tannins having the antioxidant potential. Phenolic compound various possess anti-inflammatory, anti-atherosclerotic and anticarcinogenic activities [28]. Flavonoids are polyphenolic compounds are found in plants, possess various including antimutagenic and anticancer potential [29, 30]. Alkaloids are secondary metabolites derived from plant, having basic nature, contain nitrogen atoms in a heterocyclic ring and a significant physiological action. Plants produce a larger number of chemical defense compounds. Alkaloids are greatly found in the plant kingdom, among angiosperms and less commonly found in gymnosperms. A single alkaloid can exhibit more than one pharmacological action like reducing blood pressure, relieving pain and spasms, stimulating circulation and respiration, or killing tumor cells [31, 32]. Saponins are naturally occurring surface-active glycosides. They are mainly produced by plant, lower marine animals [33, 34]. Saponin derive their name from their ability to form stable, soap-like foams in aqueous solutions, are high-molecular-weight in which sugars are linked to a triterpene or steroidal aglycone moiety. Saponins on hydrolysis yield an aglycone known as sapogenin and glycone known as sugar [35, 36]. Saponins possess a variety of biological activities such as antioxidant, immunostimulant, antihepatotoxic, anticarcinogenic, antidiarrheal, antiulcerogenic, antioxytoxic, hypocholesterolemic, anticoagulant, hepatoprotective, hypoglycemic, neuroprotective, antiinflammatory, inhibition of dental caries and platelet aggregation [37, 38, 39].

Secondary metabolite contributes major pharmacological action depending on the nature of phytoconstituents.

The experimental parameters have been reported as mean ±SD for three determination (n=3). The variation in a set of data has been estimated with the help of using Graph Pad Prism version 6.00 and MS excel 2007.

Conclusion

Phytochemical study showed that the presence of secondary metabolites like alkaloid, phenolic compound and saponin. The highest concentration of phenolic and Flavonoid content was found 2.44% and 0.34% in ethyl acetate extract respectively while highest saponin content 7.82 % in methanol fruit extract of *G. arborea* fruits. Phenolic, flavonoid saponin and alkaloid having various pharmacological activity. The present research could be useful for isolation and identification of specific phytoconstitute responsible for particular pharmacological activity.

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Nil.

Conflict Of Interest Statement

We declare that we have no conflict of interest.

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