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Standardization and phytochemical investigation of fruit extract of *Gymnosporia montana* (Celastraceae)

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Abstract

Standardization the process of developing and implementing technical standards and to maximize compatibility, safety, repeatability and quality of the drugs is one of the important measures in view of the various practical problems encountered from time to time especially in the field of herbal drugs and Ayurveda. Scientific data pertaining to the standardization of the herbal drugs could be of immense value to substantiate efficacy, safety or toxicity of an herb. Hence present study was intended to standardize *Gymnosporia montana* fruit extracts, as it is an important plant which possess medicinal actions like its use in the treatment of dysentery, jaundice, toothache, vermifuge etc. Standardization of fruit of *Gymnosporia montana* were carried out using phytochemical qualitative analysis and quantitative flavonoid analysis to assess the quality and purity of drug. The different extracts of *Gymnosporia montana* fruit were prepared starting with a highly nonpolar solvents viz., Petroleum ether followed by Chloroform, and Ethanol (95%) using Soxhlet apparatus. The present study assessed qualitative phytochemical characteristics of different extracts which revealed presence of alkaloids, flavonoids, glycosides, phenols, anthraquinones, carbohydrates, saponins, phytosterols and triterpenoids. It indicates *G. montana* was a good source of flavonoids. Total flavonoid contents were calculated as quercetin equivalent from calibration curve prepared using quercetin and was found to be 12.15 mg quercetin equivalents/g respectively. Results indicated the authenticity of the herbal drugs used in the study. Study was successful in establishing quality standards for the fruit of *Gymnosporia montana*.

Keywords: Standardization, *Gymnosporia montana*, flavonoid, extract, phytochemicals

Introduction

Gymnosporia montana (Roth) Benth. is a traditional herbaceous plant of Celastraceae family found in different regions of India [1]. In vernacular language it is called as *Vikalo* [2]. It is a large, much branched, spinescent, woody shrub or a small tree. It is a shrub found in Southeast Asia. The stems when young are prickly. Leaves: drying yellowish, thick, and 3 cm–5 cm × 2 cm–3.8 cm. The petiole is 3mm–10 cm long. The blade is elliptic or obovate, round at the apex, entire or crenulate, and tapered at the base. The inflorescences are axillary cymes. The flower pedicel is long and thin, and the bracts are small and lanceolate. The calyx lobes are very small, broadly elliptic-oblong, round at the apex, and ciliate. The petals are 3mm long and elliptic-oblong. The filaments of the stamens are flattened and dilate at the base. The nectary disc is succulent and 10 lobed. The ovary is glabrous and orbicular. The style is fertile flowers and deeply 2-3 lobed. The fruits are 5mm long globose capsules ripening into purple. The seeds are glabrous, chestnut brown, rugose and embedded in an aril.



Fig 1: *Gymnosporia montana* Plant

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Taxonomy of *Gymnosporia montana*

Kingdom: Plant

Division: Spermatophyta

Sub-division: Angiospermae

Class: Dicotyledoneae

Sub Class: Polypetalae

Group: Disciflorae

Order: Celastrales

Family: Celastraceae

Genus: *Gymnosporia* (Wt. & Arn.) Benth & Hook.

Species: Montana

Plant's Name: *Gymnosporia montana* (Roth.) Benth.Synonym: *Maytenus senegalensis***Properties and uses**

In several Ayurvedic literatures like Bhavprakash, Nighantu Adarsh, Shaligram Nighantu, Vanaspati Shrusti, Aryabhishek, Shankar Nighantu, Vanaspati Chandrodaya, the plant has been mentioned for various uses. It is claimed to be useful in jaundice, inflammation and rheumatic pain, corneal opacity, ulcers, gastrointestinal disorders, dysentery, toothache and also as a vermifuge [3-6].

Chemical Constituents

Several sesquiterpene pyridine alkaloids like emarginatine A, B, E, F, G and a sesquiterpene ester, celahin B, have been reported from the family Celastraceae. Tingenone, 3-O-acetyloleanolic acid, hexacosane, hexacosanol, n-triacontanol, betulin, β -amyron, β -amyrin, δ -amyrin, β -sitosterol, celacinnine and kaempferol have been isolated from the leaves of *G. Montana*. Joshi *et al.* have reported isolation of iguesterin, pristimerin, tingenone, β -amyrin, β -sitosterol and maytenonic acid from the stem. Iguesterin, pristimerin, tingenone, β -amyrin, and β -sitosterol have been isolated [7]. The isolation of dukidol and β -amyrin has been reported and presence of (-) epigallocatechin, Emarginatine A33 and Emarginatine G22, two other sesquiterpene pyridine alkaloids have also been isolated from this plant. In spite of this, very less details are available on the contents of the fruits [8-10].

Fruit of *G. montana*

Fruits are two or three valved, globuse capsule with 10 to 20 mm long and 8-9 mm diameter, purplish or black in colour when ripped. 3-Celled. Seeds are brownish white with green and fleshy cotyledons. The fruits of *G. montana* (About 5 kg) were dried in shade under normal environmental condition and homogenized to coarse powder and stored in opaque screw tight jars until use.

Materials and Method**Materials**

For present study the fruit of *Gymnosporia montana* (Roth) Benth. Was collected and authenticated following which they were successively extracted by using different solvents. The extracts were than tested for the different physico-chemical test. The solvents and reagents used were purchased from Rankem, India and Qualigens Fine Ltd., India. All other chemicals were of analytical grade.

Methods for plant extraction**Extraction**

About 1 kilogram of fruit was thoroughly washed under running water to remove adherent soil and impurities. The cut chips were shade dried by making into chips and finally powdered to mesh 60#. The air dried powder was exhaustively extracted by hot percolation method (soxhalation) with different solvents of increasing order of polarity, starting with a highly nonpolar solvents viz., Petroleum ether followed by Chloroform, and Ethanol (95%). Initially about 200 g of powder was extracted with 600 ml of Pet. Ether. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and the solvent was distilled off using rotary vacuum flash evaporator. The obtained residue was dried in a desiccator over anhydrous sodium sulphate. The average yield, colour, odour and constituency were recorded. The left over mark was air dried at room temperature and was similarly extracted with chloroform and Ethanol respectively. All the extracts were stored in a refrigerator for preliminary phytochemical investigation, acute toxicity studies and pharmacological screening. Powdered drug was charged into soxhlet apparatus and extraction was carried out with following solvents successively; 1) Petroleum ether (40-60 °C), 2) Chloroform, 3) Ethanol. Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50 °C under vacuum and dried residue was collected in an opaque glass bottles for further studies. Percentage practical yield of petroleum ether (40-60 °C), chloroform, and ethanolic extracts were found to be 3.85, 2.33, 2.39 % w/w respectively.

Qualitative Phytochemical Screening

Various phytochemicals tests for detection of alkaloids, flavonoids, glycosides, tannins, phenols, anthraquinone saponins, steroid, terpenoids, carbohydrates and proteins were performed using different solvents in the extracts according to the procedures followed by Debela [11].

Table 1: Estimation of Phytochemical analysis of different extract of *Gymnosporia montana*

| Sr. No | Chemical test | | Pet. Ether Extract | Chloroform Extract | Ethanolic Extract |
|--------|-----------------------------------|----------------------------|--------------------|--------------------|-------------------|
| 1 | Test for Alkaloids | Hager's Test | -ve | -ve | -ve |
| | | Mayer's Test | -ve | -ve | -ve |
| | | Dragendorff's Test | -ve | -ve | +ve |
| | | Wagner's Test | -ve | -ve | -ve |
| 2 | Test for carbohydrates | Molisch's Test | +ve | +ve | -ve |
| | | Fehling's Test | -ve | -ve | +ve |
| | | Barford's Test | -ve | -ve | -ve |
| | | Benedict's Test | -ve | -ve | +ve |
| 3 | Test for cardiac glycosides | Baljet test | -ve | -ve | +ve |
| | | Legal test | -ve | -ve | +ve |
| | Test for Anthraquinone glycosides | Modified Borntrager's test | -ve | -ve | -ve |
| | | Borntrager's test | -ve | -ve | -ve |
| | Test for saponins glycosides | Foam test | -ve | -ve | +ve |
| | | Hemolytic test | -ve | -ve | -ve |
| 4 | Test for fixed oil | Stain Test | +ve | +ve | -ve |

| | | | | | |
|---|---|-------------------------------|-----|-----|-----|
| 5 | Test for Proteins and Amino acids | Millons's Test | -ve | -ve | -ve |
| | | Biuret Test | -ve | -ve | -ve |
| | | Ninhydrin Test | -ve | -ve | -ve |
| 6 | Test for Phytosterols and triterpenoids | Liebermann-Burchard Test | -ve | -ve | +ve |
| | | Salkowski Test | -ve | -ve | -ve |
| 7 | Test for flavonoids | Shinoda test | -ve | -ve | +ve |
| 8 | Test for tannin | Lead acetate solution | -ve | -ve | -ve |
| | | 5% FeCl ₃ solution | -ve | -ve | -ve |

Quantitative test for flavonoids

The total flavonoid content which is present in ethanolic extract was determined by aluminum chloride colorimetric method [12]. In brief, 0.5 ml of ethanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer (Shimadzu UV-1601, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Total flavonoid contents were calculated as quercetin equivalent from calibration curve prepared using quercetin. The results reveal that total flavonoid content in the ethanolic extract was found to be 12.15 mg quercetin equivalents/g respectively.

Thin Layer Chromatography

All the extracts of *G. montana* were subjected to thin layer chromatographic studies, to determine the probable number of compounds present. Preparation of the plates: The precoated TLC plates (Merk, Germany) made up of silica gel G as an adsorbent, was activated in an oven for 30 minutes at 110 °C. Test samples (1mg/ml of all extracts in respective solvents) were applied in the form of bands using Linomat IV applicator. Development of solvent system A number of solvent systems were tried 8, 9, in order to get maximum separation on plate. After development of plates, they were air-dried and numbers of spots were noted & R_f values were calculated. Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract.

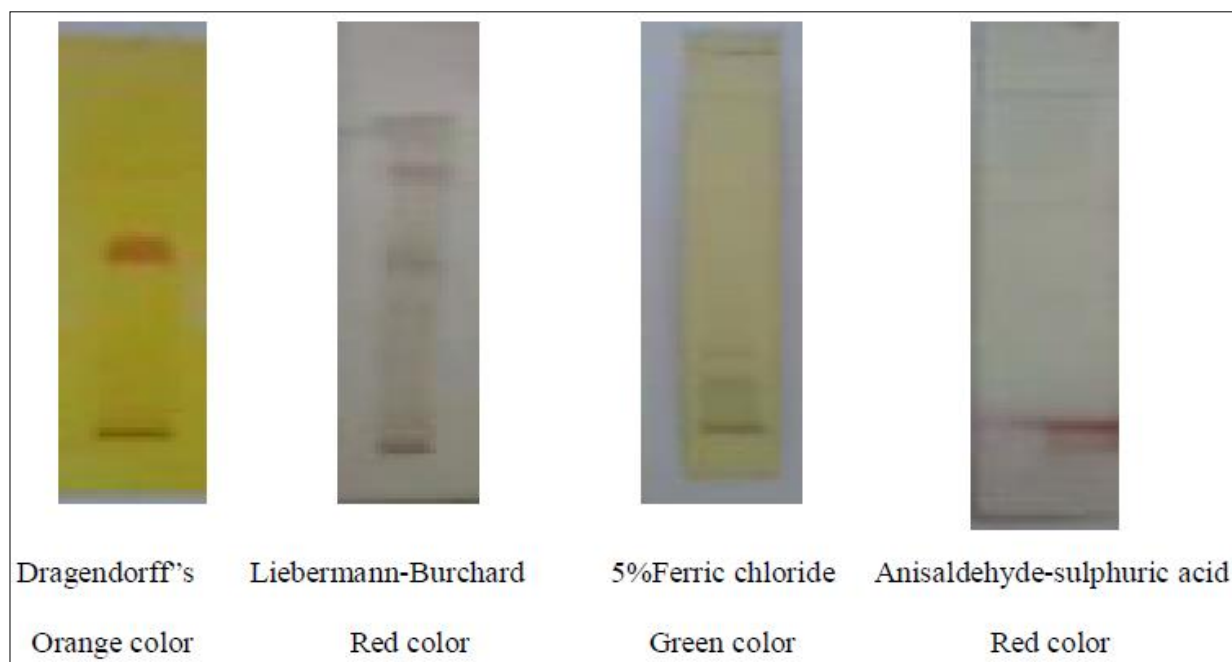


Fig 2: Images of Thin Layer Chromatography Plates

HPLC analysis of Epigallocatechin

The chromatographic conditions were used to to characterize the flavonoid present in the extract and were adapted from the earlier methodology described by Manns and Mansfield (2012). The detection of compounds was performed at 220 nm for catechin (IS), epigallocatechin. The column used was a Zorbax Eclipse Plus RP-C18 (100 × 4.6 mm, 3.5 μm) (rapid resolution column) and the pre-column was a Zorbax C18 (12.6 × 4.6 mm, 5 μm), both manufactured by Zorbax (USA). The oven temperature was maintained at 35 °C, the injection

volume was 20 μL flow rate was 0.8 mL/min. The gradient used in the separation was 0–5 min: 5% B; 5–14 min., where solvent A was 0.1 M phosphoric acid solution (pH 2.0) and solvent B was methanol acidified with H₃PO₄ 0.5%.

The chromatography studies revealed the presence of epigallocatechin gallate in the extract. Epigallocatechin gallate (EGCG), also known as epigallocatechin-3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin.

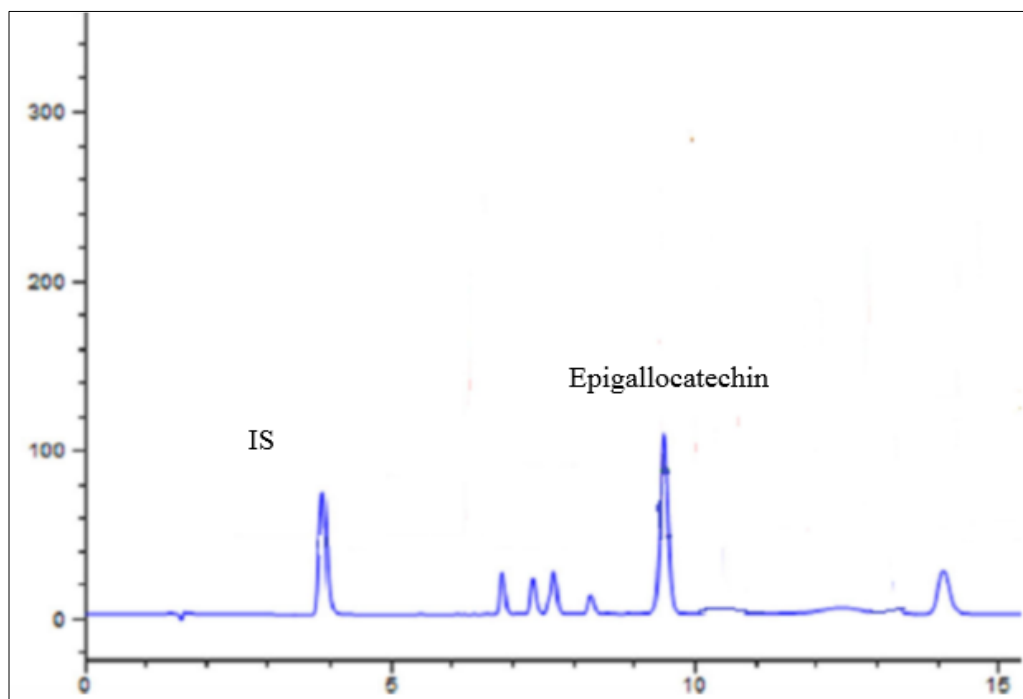


Fig 3: Chromatogram of ethanolic extract of *G. Montana*

Conclusion:

Study was successful in establishing quality standards for the *G. montana* fruit. Results indicated that *G. montana* was a good source of flavonoids. Total flavonoid contents were calculated as quercetin equivalent from calibration curve prepared using quercetin and was found to be 12.15 mg quercetin equivalents/g respectively. Also the chromatography studies revealed the presence of epigallocatechin gallate in the extract. The phytochemical constituents present in the fruit were generally moderate or in high concentration. These preliminary studies may offer great help in initial procurement and assessment of quality of the crude drugs when these are being used as raw materials for preparations of herbal formulations as having both nutritional and pharmacological benefits.

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