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Research Article

PHYTOCHEMICAL PROFILING, ASSESSMENT OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF ETHNOMEDICINAL PLANT, *MEYNA SPINOSA* FROM ASSAM

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ABSTRACT

Objective: The present study qualitative phytoconstituents examine the total phenol, total flavonoid content (TFC), and antioxidant efficiencies traditionally used plant, *Meyna spinosa.*

Methods: Chemical profiling, estimation of total phenolic content (TPC), TFC, and antioxidant activity of ethanol extracts of *M. spinosa* have performed by applying standard protocols. Antioxidant activity of leaf and stem was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. TPC and TFC of the plant were assessed using Folin–Ciocalteu colorimetric and aluminum colorimetric assay, respectively.

Results: The findings of the study exhibit that ethanol extract of *M. spinosa* is proved to be the presence of phytoconstituents (7/9) such as alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids, and phenolic compounds, while carbohydrate, fixed oils, and fats are unavailable. In addition, phenolic compositions of ethanol extract of leaf and stem; 93.21±2.93 and 54.33±0.69 mg gallic acid equivalents/g extract, respectively; TFCs of leaf and stem have recorded as 61.55±1.21 and 37.55±1.28 mg quercetin equivalents/g extract, respectively. Antioxidant efficiency of both leaf and stem is tested using DPPH radical scavenging assay as IC_{50} 20.68±0.32 and 50.99±0.56 µg/ml, respectively.

Conclusion: From the above results, it has concluded that the ethanol extract of the *M. spinosa* leaves and stems seizes rich phytoconstituents which can be applied in food technology, drug industries, ethnopharmacological fields, etc., for the development of healthiness and to battle against negative health consequences.

Keywords: *Meyna spinosa*, Qualitative phytochemical profiling, Total polyphenolic, Total flavonoid contents, Antioxidant activity.

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INTRODUCTION

Plants are the prime source of medicine that has been practiced by a large number of ethnic groups since the prehistoric period of time. This is due to their ethnopharmacological availability that makes it medicinal. Rubiaceae family is one of the largest families of the plant kingdom with about 13,150 species including herbs, shrubs, and trees under 611 genera distributed in tropical regions of the earth [1]. Out of such species, 10 species of the genus Meyna are accepted (0.076%) [2].

One of the species of Rubiaceae, *Meyna spinosa* is a significant ethnomedicinal plant, which is a wild deciduous shrub or small tree species. This plant is mainly distributed in India and this plant is mainly distributed in India and its three neighboring nations such as Bangladesh, China, and Myanmar [3]. It has different synonyms such as *Pyrostria spinosa* Miq., *Vangueria miqueliana* Kurz, *Vangueria pyrostria* Boerl., *Vangueria spinosa* Roxb., and *Vangueria stellata* Blanco [4].

The plant has been consumed as folklore medicine to conquer different health-related problems such as diabetes, diphtheria, stomach pain, headache, liver problem, indigestion, throbbing urination, and skin problems such as pimples and acne problems. Hence, the main objective of the present study is to display the appraisal of bioefficiencies such as qualitative phytochemical profiling, antioxidant effectiveness, total polyphenol, and total flavonoid content (TFC).

METHODS

Plant material

Plant materials of *M. spinosa* were collected from Rabha Hasong Autonomous Council Area of Assam during January and May 2019. The plant was identified at the Botany Department, Gauhati University. Plant samples were washed in running water thoroughly and finally by distilled water. Stem and leaf parts of the plant kept separately, cut into pieces and air-dried and prepared powder for extraction.

Preparation of plant extracts

About 70 g of powders of respective plant parts were taken for ethanol extraction. Soxhlet apparatus has used for the procedure. Then, the extracts were collected and make solvent free in rotavapor under reduced pressure and were preserved at 5°C until used.

Yield of the extracts

The yield of the extracts was calculated by the formula ($[W^E/W^D]\times100$), where W^E is the weight of the solvent-free extract and W^D is the dry weight of the plant materials.

Qualitative chemical profiling analysis of extracts

The qualitative phytochemical screening of stem and leaf extracts of *M. spinosa* was estimated using standard protocols [5,6]. Nine different qualitative tests were taken to resolve the occurrence of alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids and phenolic compounds, starch, fixed oils, and fats.

Total phenolic content (TPC)

TPC of the extracts was evaluated using the Folin–Ciocalteu colorimetric method [7,8] including some modifications. A 50 µL of stem or leaf extract was added with 2.5 mL of Folin–Ciocalteu phenol reagent. After 5–10 min, 2.5 ml (7%, w/v) Na_2CO_3 was combined and the solution was kept at 40°C for 50 min in the dark for incubation. The absorbance of the combination has observed at 725 nm by ultraviolet (UV)–visible

spectrophotometer (UV-1601 PC, Shimadzu, Japan). The results of phenolic contents (Table 1) of all the samples (mg GAE/g DW) were calculated as milligrams of gallic acid equivalent per gram of dry weight.

TFC

The TFC of the extracts was tested following the aluminum colorimetric assay [9-11] with few modifications. 150 µL of sample and 150 µl standard quercetin solutions were put in different test tubes and 10% aluminum chloride (0.2 ml), 1 M potassium acetate (0.2 ml), 70% methanol (1 ml), and distilled water (0.80 ml) were poured and mixed. All tubes were kept at 40°C for 50 min. The absorbance was observed at 540 nm. The results of flavonoid contents (Table 1) of all the samples (mg quick time event/g DW) were calculated as milligrams of quercetin equivalent per gram of dry weight.

Antioxidant activities

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the extract was evaluated using the standard protocol of Brand-Williams *et al*. [12] with some modifications. 2 mL of extracts were added in 10 test tubes at 10 different concentrations, with 3 mL of DPPH solution in each. Then, these were placed in a dark site at room temperature for 30 min, and then, the absorbance was measured at 520 nm on UV spectrophotometer. Here, a blank ethanol taken for calibration. The percentage of inhibition for DPPH radical scavenging assay by the following formula was calculated and then IC_{50} value for all the extracts has measured.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Ethanol solvent was taken for the preparation of extract for two plant parts, leaf and stem of *M. spinosa*. The leaf part showed the yield as maximum 3.2% followed by stem part 2.4%.

Secondary metabolites are biological product found in a variety of plant extracts that hold diversed medicinal properties [13]; in this study, qualitative chemical profiling of ethanol extracts of *M. spinosa* was performed and exhibited the occurrence of such phytoconstituents such as alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids, and phenolic compounds and absence of carbohydrate and fixed oils and fats. However, different plant parts produce different secondary metabolites in diverse quantities [14]. The leaf part of *M. spinosa* showed the presence of total seven phytochemicals, alkaloids, terpenoids, saponins, tannins, phytosterols, flavonoids, and phenolic compounds, whereas in stem part, total six phytochemicals alkaloids, terpenoids, tannins, phytosterols, flavonoids, and phenolic compounds were observed (Table 2).

Phenolic compounds have been reported for its therapeutic properties such as antimicrobial activity, antioxidant property, curing of heart disease, hepatoprotective, anti-inflammatory, and anticancer properties [15]. The TPC and TFC contents of both plant parts of *M. spinosa* were showing significant availability. Consequently, in the TPC, the study was estimated by Folin–Ciocalteu's method using gallic acid as standard (Fig. 1). Leaf part was showing more TPC value, 93.21±2.93 mg gallic acid equivalents/g than stem part, 54.33±0.69 mg gallic acid equivalents/g extract, whereas the TFC of the plant estimated by the aluminum chloride colorimetric assay using quercetin as standard (Fig. 2). TFC value also showed higher in leaf part 61.55±1.21 mg quercetin equivalents/g than stem part 37.55±1.28 mg quercetin equivalents/g extract of the plant (Table 2).

The DPPH radical scavenging activities of both leaf and stem of *M. spinosa* were evaluated by applying in a stable free radical, DPPH. IC_{50} value measures to estimate the scavenging capacity of the plant. Higher antioxidant efficiency shows lower IC_{50} value [16,17]. The leaf part showed less IC_{50} value 20.68±0.32 µg/ml than the stem part 50.99±0.56 µg/ml (Table 1).

Table 1: Yield and assays of total phenol, total flavonoid, and DPPH radical scavenging activity of *Meyna spinosa* leaf and stem extract

Plant parts	Yield of the ethanol extract (%)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH radical scavenging activity IC_{50} (µg/mL)
Leaf	3.2	93.21±2.93	61.55±1.21	20.68±0.32
Stem	2.4	54.33±0.69	37.55±1.28	50.99±0.56

DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 2: Qualitative chemical profiling analyses of leaf and stem part of *Meyna spinosa*

S. No.	Name of the phytoche mical	Specific test followed	Leaf	Stem
1.	Alkaloids	Wagner's test	Present	Present
2.	Terpenoids	Salkowski's test	Present	Present
3.	Saponins	Froth test	Present	Absent
4.	Tannins	Gelatin test	Present	Present
5.	Phytosterols	Liebermann–Burchard's or acetic anhydrate test	Present	Present
6.	Flavonoids	Alkaline reagent test	Present	Present
7.	Phenolic compounds	Ferric chloride test	Present	Present
8.	Reducing sugars	Fehling's test	Absent	Absent
9.	Fixed oils and fats	Spot test	Absent	Absent

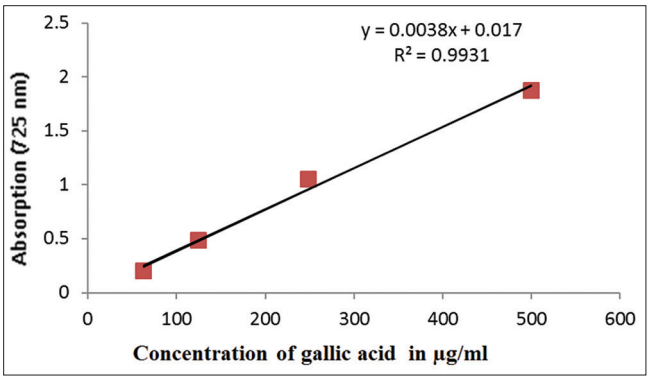

Fig. 1: Standard curve of gallic acid for total phenol content estimation

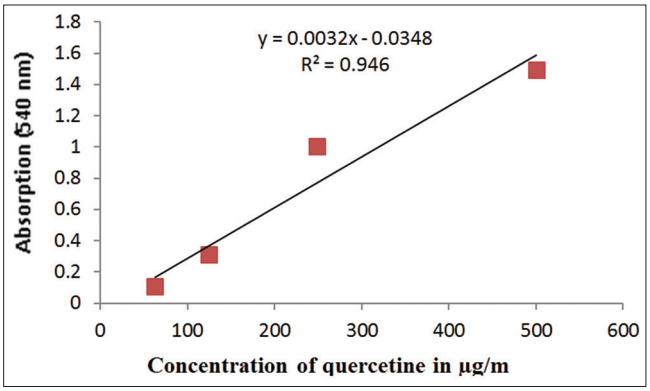

Fig. 2: Standard curve of quercetin for total flavonoid estimation

CONCLUSION

The manifestations of the occurrence of phenols and flavonoids have performed an important function to validate folk claims of *M. spinosa*. Phenolics, alkaloids, steroids, saponins, terpenoids, etc., are the large group of naturally produced phytochemicals which have diverse bioactivity produce abundantly in nature [18]. The present research has displayed the plant *M. spinosa* which have a significant amount of phenolic and flavonoid content and has also exhibited remarkable antioxidant properties. The study also recommends the isolating, purifying, and characterizing the active chemical constituents accountable for the therapeutic properties of *M. spinosa*. Hence, the plant product can be used in pharmaceutical research and food and drug industry in near future for great societal demand.

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AUTHORS' CONTRIBUTIONS

Conception and design of the study: Bora R, Dutta T. Interpretation of data and revising the manuscript: Bora R, Khakhalary S, and Dutta T.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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