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Assessment of Antioxidant Activity, Total Phenolic and Flavonoid Content of Methanol Extracts From *Meyna spinosa*

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ABSTRACT

The current study investigates the in vitro antioxidant potential, total polyphenolic contents, total flavonoid contents and qualitative phytoconstituent analysis of the methanol extract of the traditionally employed herb *Meyna spinosa*, by using standard techniques. The results revealed that the methanolic extract of *Meyna spinosa* is enriched with phytoconstituents such as tannins, terpenoids, alkaloids, saponins, flavonoids, and phenolic compounds. It contains no carbohydrates, fixed oils, or fats. The TPCs of the leaf and stem MeOH extracts were determined to be 92.51 ± 1.87 mg GAE/g and 52.47 ± 0.46 mg GAE/g, respectively. On the evaluation of TFC, the value for the TFC was 63.23±1.12 mg QE/g for leaf extract and 36.48±1.38 for the stems. The DPPH radical scavenging assay evaluated the antioxidant ability of the leaf and stem extracts. The IC_{50} values of the leaves were 19.88±0.52 µg/ml, and the stems were 52.46±0.67 µg/ml. These findings indicate that methanol extract of *Meyna spinosa* leaves and stems is a potential source of phytoconstituents which may be helpful to improve health and reduce its adverse effects in food technology, pharmaceutical industries, and ethnopharmacological.

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

I. INTRODUCTION

Since the beginning of human civilisation, humans have looked to the natural world - and plants in particular - as a supplementary source of medicine. Anthropotherapy is a captivating method that relates to the use of the therapeutic properties of well-known and scientifically validated medicinal plants for the treatment of various kinds of discomforts, particularly where modern medicine does not have any effective treatment. The traditional medical systems in India, including Ayurveda and Siddha, have long been using a variety of plant and herbal extracts as they have rich knowledge about natural remedies.

Meyna spinosa is one such respected plant of worship with ethnomedicinal prominence. This wild deciduous shrub or little tree is a member of the Rubiaceae family and is known for its toughness and versatility. *Meyna spinosa* is perhaps best cultivated in India and adjacent countries, but it can also be found in Bangladesh, China, Java, and Myanmar[1]. Its use in indigenous medicine reminds us of the great depth of ethnic knowledge and the vast unexplored potential of natural for healing.

Meyna spinosa, one such plant reported to have multipurpose applications for medicinal and other domestic uses, also has various synonyms such as *Vangueria miqueliana*, *Vangueria spinosa* Roxb., *Vangueria pyrostria* Boerl.,and *Pyrostria spinosa* Miq. [2] Leaves and stems extracts are a rich source of several phytoconstituents with excellent antioxidant and antibacterial properties; therefore, they have various therapeutic applications of this plant.

This plant has a long tradition in natural medicine and has been used in the treatment of many health conditions. It is partially effective in the treatment of diabetes and relief from diphtheritic symptoms and digestive complaints [3,4]. It has also been used to help treat headaches, aid liver function, alleviate indigestion, and alleviate painful urination. Skin diseases, acne, and pimple eruptions have also been cured with its uses[5,6]. *Meyna spinosa* fruits, in particular, are appreciated for their property of resolving fever, inflammation, and for biliary insufficiency and hepatic stasis. They are also used to treat diphtheria and facilitate the healing of broken bones[7,8].

Apart from the well-known uses, the history of use of *Meyna spinosa* in renal problems and skin diseases depicts its varied uses as a medicinal herb. The main objective of this study is to undertake a comprehensive evaluation of the bioactive potential of *Meyna spinosa*, specifically emphasizing its total polyphenol content (TPC), total

flavonoid content (TFC), and antioxidative properties, and thereby offer a qualitative analysis of the phytochemical composition.

II. METHODS

2.1 Plant material

Fresh plant material of the *Meyna spinosa* was collected during January-March of 2025 from the regions of Ganga river in Patna district . The plant was authenticated by the Department of Botany, Ganga Devi Mahila College, Patliputra University, India. After washing with running water, plant materials were washed with distilled water. The leaves and stems of the plant were separated, cut into small pieces, air dried to retain the quality, and then pulverized into powder for extraction.

2.2 Preparation of plant extracts

For methanol extraction, approximately 70 grams of powder of leaf and stem was used. The extraction was performed using a Soxhlet apparatus. The extracts were collected and made solvent-free under reduced pressure using a rotary evaporator. They were then stored at a temperature of 5 degrees Celsius until further use. The extraction yield was calculated by the formula ([WE/WD]×100), in which WD represents the dry weight of the plant material and WE the weight of the solvent-free extract.

2.3 Qualitative chemical profiling analysis of extracts

Qualitative Chemical Profiling of Extracts is an essential step, offering a complete picture of the sample features and composition. Standard procedures were employed to evaluate the quantitative preliminary phytochemical screening of *Meyna spinosa* leaf& stem extracts [9, 10]. To check for the presence of alkaloids, tannins, starch, fixed oils, lipids, terpenoids, saponins, phytosterols, and phenolic compounds, nine different qualitative tests were performed.

2.4 Total phenolic content (TPC)

The TPC of the extracts was determined by a slightly modified Folin–Ciocalteu colorimetric method [11–12]. Fifty microliters of stem and leaf extracts were first mixed with 2.5 ml of Folin-Ciocalteu reagent. 2.5 millilitres of a seven percent (w/v) Na2CO3 solution were added, and the mixture was allowed to incubate in the dark for 50 minutes at 40 degrees Celsius. A UV-1901 PC UV-Visible spectrophotometer (Shimadzu, Japan) was applied to obtain the absorbance of the resulting solution at 725 nm. The phenolic content of each sample was expressed as gallic acid equivalents (mg GAE/g DW).

2.5 Total flavonoid content (TFC)

The TFC of the extracts was determined by the aluminium calorimetric method with little modifications[13-15]. To each test tube, 150 microliters of sample/standard quercetin concentrations, 0.2 millilitres of ten per cent aluminium chloride, 0.2 millilitres of 1 M potassium acetate, 1 millilitre of seventy per cent methanol, and 0.80 millilitres of distilled water were added. The tubes were then shaken and mixed well, and each incubated at forty degrees Celsius for fifty minutes. The absorbance was measured at 540 nm, and the quercetin equivalents were expressed as mg quercetin/g of DW.

2.6 In Vitro Antioxidant Activity.

The Brand-Williams et al. (1995) method, with slight modifications, was used for testing the extract as a free radical scavenger using the DPPH radical scavenging test. Specifically, 10 test tubes with three millilitres of DPPH solution and two millilitres of each extract, at different concentrations, were taken. The tubes were after being kept at room temperature in darkness for 30 min, were measured for absorbance at 520 nm with a UV spectrophotometer. A sample of methanol was prepared as a blank for calibration. The inhibition per cent of the DPPH radical scavenging assay was calculated using the following formula, and the IC_{50} of each extract was calculated.t DPPH radical scavenging (%) = [(A control−A sample)/A control] × 100 (11) Where, A control = absorbance of the control; A sample = absorbance of sample.

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

III. RESULTS & DISCUSSION

Table 1: Chemical profiling studies of the leaves and stems of *Meyna spinosa.*

S. No.	Phytochemical	Specific test	Leaf	Stem
1.	Alkaloids	Wagner's test	Present	Present
2.	Fats	Spot test	Absent	Absent
3.	Flavonoids	Alkaline reagent test	Present	Present
4.	Phenolic compounds	Ferric chloride test	Present	Present
5.	Phytosterols	Acetic anhydrate test	Present	Present
6.	Terpenoids	Salkowski's test	Present	Present
7.	Reducing sugars	Fehling's test	Absent	Absent
8.	Tannins	Gelatine test	Present	Present
9.	Saponins	Froth test	Present	Absent

Table 2: Yield and tests of TPC, TFC, and DPPH radical scavenging activity from the leaf & stem extract of *Meyna spinosa*.

Plant parts	Yield of the methanol extract (%)	TPC (mg GAE/g)	TFC(mg QE/g)	DPPH radical scavenging activity IC_{50} (µg/ml)
Leaf	2.8	92.51±1.87	63.23±1.12	19.88±0.52
Stem	2.6	52.47±0.46	36.48±1.38	52.46±0.67

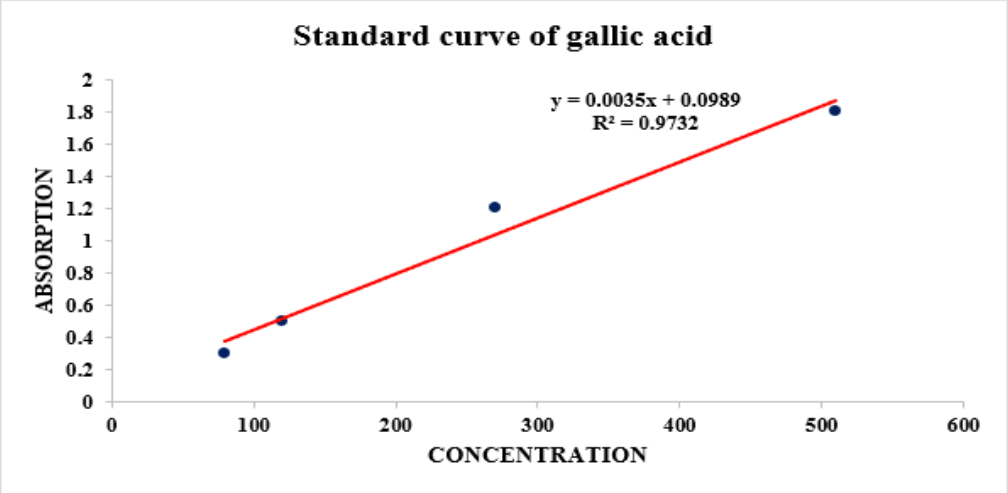

Figure. 1: The standard curve for gallic acid in mg/ml serves as a valuable tool for accurately estimating the total phenol content.

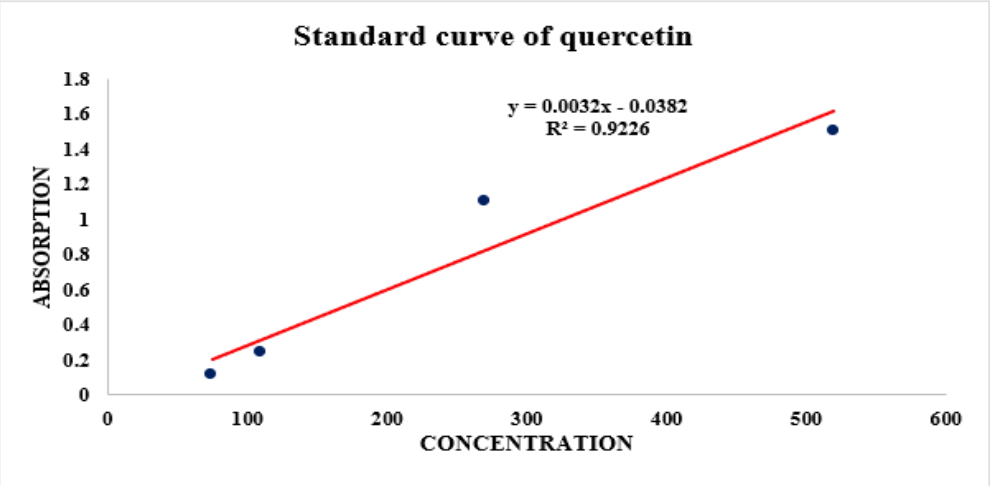

Figure. 2: The standard curve for quercetin in mg/ml serves as a valuable tool for accurately estimating the total flavonoid content.

Methanol was the solvent of choice in the extraction of leaves and stems of *Meyna spinosa*. A strikingly high extraction of 3.2% was obtained from the leaves, making it the most efficient portion, with the stem having a slightly lower extraction yield of 2.4% (w/w). This investigation confirms the presence of a variety of secondary metabolites (pharmacologically active compounds with therapeutic value) in the extracts of the plants [19].

Qualitative chemical profiling of methanol extract of *Meyna spinosa* reveals that the fats, carbohydrates, and fixed oils were not present, which clarifies that the plant is not only rich in bioactive components but also in the active constituents. Extracts have high contents of beneficial organics, such as alkaloids, saponins, phytosterols, tannins, terpenoids, flavonoids, and phenolics. Moreover, it should be mentioned that the concentration of these secondary metabolites can diverge substantially between different plant parts [20].

The leaf extract of *Meyna spinosa* with the diversity of seven phytochemicals, emphasises its potential medicinal value. These included pharmacologically active alkaloids, aroma, and biological activity-from building terpenoids, foam-promoting and emulsification-capable saponins, astringent tannins, heart health-supporting phytosterols, antioxidants-flavonoids, and health-accentuating phenolic compounds. The stem of *Meyna spinosa* had a variety of six particular phytochemicals, reaffirms its value in medicinal uses and its rich phytochemical .

Phenolics compound profile have been reported to provide a variety of healthful effects, among others, including protection against heart disease, antioxidant activity, prevention of liver damage, reduction of inflammation, and have the potential to cure many types of cancer [21]. The leaf and stem parts of *Meyna spinosa* were investigated in a study and were observed to have significant TPC and TFC, making it a potential candidate for the natural source of therapeutic agent

TPC was determined using the Folin-Ciocalteu method with gallic acid as the standard (Fig. 1). The analysis showed significantly greater TPC in leaf extract of the *M. spinosa* plant (92.51±1.87 mg GAE/g) compared with the stem extract (52.47±0.46 mg GAE/g).

Additionally, the TFC of *Meyna spinosa* was also determined employing an aluminium chloride colorimetric method with quercetin as the standard (Fig. 2) A high concentration of either TPC or TFC in *Meyna spinosa* indicates that this plant could be interesting from a natural medicinal point of view. The TFC of *Meyna spinosa* leaf extract was 63.23±1.12 mg of QE/g, which is significantly higher than the TFC of the stem extract (36.48±1.38 mg of QE/g) (Table 2). The DPPH assay for the radical scavenging capacity was used to determine the antioxidant activity of M. spinosa. The IC_{50} level is an important index for evaluating the antioxidant activity of this plant. The stem extract, however, is significantly less inhibitory (IC_{50} = 52.46±0.67 µg/ml) compared with the leaf extract, with a lower IC_{50} of 19.88±0.52 µg/ml (Table 2). The relatively high content of TPC and TFC in *Meyna spinosa* implies that this plant could have significant potential for natural therapeutic uses. The current study on antioxidant activity, as well as total flavonoid and phenolic contents of methanol

extracts of *Meyna spinosa*, could highlight the valuable health benefits of these natural products. It is remarkable to think about how these phytochemicals can benefit our health and wellness."

IV. CONCLUSION

The validation of folklore claims about *Meyna spinosa* has been reinforced due to the presence of bioactive compounds, including flavonoids and phenols, which is also remarkable. Nature has given mankind with variety of naturally-occurring phytochemicals which have many biological activities such as phenols, alkaloids, steroids, saponins , and terpenoids. In the current study, the plant *Meyna spinosa* has proved to have a great antioxidant activity as well as high contents of flavonoid and phenolic content.

These results indicate that extracts from this plant may have a potential role in finding new antibiotic drugs from natural sources. As a result, the application of *Meyna spinosa* extends to pharmaceutical industries and food industry, and would contribute to future developments in plant-based drug discovery and nutritional sciences.

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