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PHYTOCHEMICAL PROFILE OF MEYNA SPINOSA ROXB. EX LINK.

Botany

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ABSTRACT

Background: Meyna spinosa of family Rubiaceae is a spinous, armed small tree or large shrub. It is a significant ethnomedicinal plant widely distributed in India, Bangladesh, China, and Myanmar. The plant parts of Meyna spinosa has been consumed as traditional or folk medicine to treat 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. **Aim:** To study the phytochemical profile of stem and leaves of M. spinosa **Objectives:** To study the presence or absence of phytochemicals in polar and non polar solvents. **Method:** The different phytochemicals of Meyna spinosa in their stem and leaves have been analysed qualitatively and quantitatively in three non-polar solvents viz., chloroform, petroleum ether and hexane and three polar viz., ethanol, methanol and distilled water in terms of Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and terpenoids, diterpinoids etc. **Results:** The solvent extracts of stem and leaves of M. spinosa showed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, carbohydrates, glycosides, steroids, phlobatannin, terpenoids, diterpenoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins, amino acids, emodins and phytosterol. The stem and leaves of Meyna spinosa contain a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. However, stem contained relatively higher amounts of phytochemicals than leaves. **Conclusions:** The present study contributes valuable information of bioactive compounds in stem and leaves of Meyna spinosa. Methanol, ethanol and aqueous extracts of stem and leaves had all the phytochemicals like flavonoids, glycosides, saponins, phenols, steroids, tannins and terpenoids.

KEYWORDS

Meyna Spinosa, Stem, Leaves, Solvents, Phytochemicals

INTRODUCTION

Meyna spinosa Roxb. ex Link. Syn. *Vangueria spinosa* Hook. F. of family Rubiaceaeis a spinous, armed small tree or large shrub about 5-8 m tall. Brunches are busy, bark dark-colored, nearly smooth. Spines are axillary or supra-axillary, straight, sharp, 5-40 mm. Leaves are membranous, ovate or elliptic-oblong, 4-15 cm × 2.5-5 cm, slightly pubescent above, densely pubescent beneath, apex bluntly or obtusely acuminate, margin entire, base rounded or acute; secondary nerves 7-9 pairs, tertiary nerves prominent below; petiole to 1 cm, densely hairy; stipules short, broad, very obtuse, caducuous. Flowers are crowded into fascicles and have shorter pedicels and petioles. Flowers are small, 4-5 merous, pale green or greenish white with faintly fragrant on short pedunculate axillary cymes, calyx 5 teethed corolla 5 lobes. Fruits are yellowish, sub globose drupe, smooth with persistent calyx lobes. Flowering time is in the month of April and May and while fruits appear in August to December[1,2,3].

Meyna spinosa Roxb is a significant ethno medicinal plant widely distributed in India, Bangladesh, China, and Myanmar.The plant parts of *Meyna spinosa* has been consumed as traditional or folk medicine to treat 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.

Materials and Methods

In the present investigation the different phytochemicals of *Meyna spinosa* in their stem and leaves have been analysed qualitatively and quantitatively in six different solvent extracts viz. chloroform, petroleum ether, ethanol, methanol, hexane and distilled water in terms of Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and terpenoids, diterpinoids etc.

Qualitative determination of Phytochemicals: The fresh stem and leaves of *Meyna spinosa* were collected from earthen pot grown plants and brought to the laboratory, washed with water and air–dried at room temperature for 7 days, and then oven-dried at 40^{0}C to remove the residual moisture. The dried parts were powdered using a mixer grinder and stored in air-tight container for future use. Six different solvents such as Chloroform, Petroleum ether, Ethanol, Methanol, Hexane and Distilled water were used for extraction. About 1 gm of the plant samples were added separately into the test tubes containing 5 ml solvents, and were extracted at room temperature. The extracts in all the six solvents of stem and leaves were tested for the presence of biological compounds following standard methods [4,5,6].

Quantitative determination of phytochemicals: Determination of Alkaloids: Alkaloids content was measured by method suggested by Harborne[6] and expressed as mg/g of sample.

Determination of Flavonoids: The flavonoids content was also determined by Harborne method[1]. The percentage of total flavonoids were calculated from the calibration curve of Quircetin (200-1000µg) plotted by using the same procedure and total flavonoids was expressed Quircetin equivalents (QE) in mg per gm sample.

Determination of Tannins: The quantitative estimation of tannins was performed by the method of Swain[8]. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

Determination of Saponins: Saponin analysis was performed according to the method described by Brunner[9]. The absorbance of the samples was measured at 380 nm and calculated in mg/g.

Determination of total phenols: The phenolics was determined following the methods of Ododoni and Ochuko by measuring the absorbance of the sample at 505 nm wavelength[10]. The amount of total phenolics was determined by the Folin-Ciocalteu method[11]. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract.
The results obtained have been presented in Table-6-9.

Table-6: Qualitative phytochemical analysis of stem of *Meyna spinosa*

Tests		Extracts used					
		Chloroform	Petroleum ether	Ethanol	Methanol	Hexane	Distilled water
Carbohydrates	Fehling's test	-	-	-	-	-	+
	Benedict's test	+	+	+	+	+	+
	Iodine test	+	-	-	-	+	-

Phenols and Tannins	Ferric chloride test	+	-	+	+	+	-
Falavonoids test	Alkaline reagent test	-	-	-	-	-	-
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	-	-	-	-	-	-
	Salkowski test	+	+	+	+	+	+
	Killer- Killani test	+	+	+	+	+	+
Phenolic compounds		+	-	+	+	+	+
Steroids		+	+	+	+	+	+
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+
	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table- 7: Qualitative phytochemical analysis of leaves of *Meyna spinosa*

Tests		**Extracts used**					
		Chloroform	**Petroleum ether**	**Ethanol**	**Methanol**	**Hexane**	**Distilled water**
Carbohydrates	Fehling's test	+	+	+	+	-	-
	Benedict's test	+	-	+	+	+	+
	Iodine test	-	+	+	+	+	+
Phenols and Tannins	Ferric chloride test	-	+	+	+	+	-
Falavonoids test	Alkaline reagent test	-	+	+	+	+	+
Saponin test	Froth Foam test	-	-	+	+	+	+
Glycosides	Libermann's test	+	-	+	+	-	+
	Salkowski test	-	-	+	+	+	+
	Killer- Killani test	+	-	+	+	-	-
Phenolic compounds		+	+	+	+	-	-
Steroids		+	+	+	+	-	-
Tests for some other phytochemicals	Phlobatannin	+	+	+	+	+	+
	Terpinoid	+	+	+	+	+	+
	Diterpinoid	+	+	+	+	+	+
	Emodins	+	-	+	+	+	+
	Anthraquinones	+	+	+	+	+	+
	Carotenoids	+	+	+	+	+	+
	Anthocyanin	+	+	+	+	+	+
	Coumarin	+	+	+	+	+	+
	Phytosterol	+	+	-	-	+	+
	Fatty acids	+	+	+	+	+	+
	Proteins	+	+	+	+	+	+
	Amino acids	+	+	+	+	+	+

Table-8: Quantitative analysis of phytochemicals in aqueous extract of stem and leaves of *Meyna spinosa*

Phytochemicals	**Stem**	**Leaves**
Alkaloids (mg/g)	26.85 ±0.35	23.45 ±0.56
Flavonoids (QE)	55.75 ±0.37	48.65 ±0.45
Phenols (GAE)	32.85 ±0.45	28.65 ±0.25
Saponins (mg/g)	22.55 ±0.35	24.75 ±0.45
Tannins (mg/g)	20.15 ±0.25	18.65 ±0.35

Table-9: Comparative analysis of total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts of stem and leaves of *Meyna spinosa*

Solvents used	**Extract types**	**Total alkaloids (mg/g)**	**Total flavonoids (QE)**	**Total phenols (GAE)**	**Total saponins (mg/g)**	**Total tannins (mg/g)**
Chloroform	Stem	17.45 ±0.25	5.35 ±0.21	45.35 ±0.32	12.75 ±0.12	17.55 ±0.14
	Leaves	15.65 ±0.34	7.65 ±0.17	40.25 ±0.31	10.45 ±0.14	15.65 ±0.17
Petroleum ether	Stem	13.35 ±0.85	4.45 ±0.15	38.26 ±0.35	11.75 ±0.16	13.75 ±0.15
	Leaves	11.45 ±0.75	3.75 ±0.16	33.35 ±0.21	9.45 ±0.18	11.40 ±0.10
Ethanol	Stem	46.45 ±0.16	7.25 ±0.21	125.25 ±0.35	72.25 ±0.13	64.75 ±0.11
	Leaves	38.35 ±0.18	5.15 ±0.23	105.36 ±0.34	64.35 ±0.15	55.65 ±0.13
Methanol	Stem	47.75 ±0.18	7.35 ±0.12	127.27 ±0.36	73.45 ±0.17	58.45 ±0.23
	Leaves	41.45 ±0.21	5.25 ±0.14	132.28 ±0.27	65.45 ±0.18	60.55 ±0.21
Hexane	Stem	32.65 ±0.35	4.25 ±0.17	35.25 ±0.20	64.75 ±0.21	14.75 ±0.20
	Leaves	28.55 ±0.25	3.87 ±0.17	120.75 ±0.21	15.35 ±0.15	13.65 ±0.18
Distilled water	Stem	51.78 ±0.26	8.25 ±0.16	122.25 ±0.23	75.45 ±0.21	65.85 ±0.23
	Leaves	47.35 ±0.21	7.35 ±0.17	151.35 ±0.17	68.76 ±0.32	56.35 ±0.25

Mean ±SD of five measurements
Phenols are expressed as Gallic Acid Equivalent (GAE) and Flavonoids as Quercetin equivalent (QE) in mg/100gm

RESULTS AND DISCUSSION

Phytochemical analysis conducted on the stem and leaves of *Meyna spinosa* revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical screening was done with chloroform, petroleum ether, hexane ethanol, methanol and distilled water. Among the six solvent extracts studied the carbohydrate was present in aqueous extract of stem as evidenced by positive Fehling's test. All the six solvent extracts of stem showed positive Benedict's test for carbohydrate. Only the chloroform and hexane extracts of stem showed positive Iodine test for carbohydrate (Table-6). The phenols and tannins were detected only in chloroform, ethanol, methanol and hexane extracts but not in petroleum ether and aqueous extracts. All the six solvent extracts of stem showed negative alkaline reagent test which indicated the absence of flavonoids. Saponin was detected in four solvent extracts of stem viz. ethanol, methanol, hexane and distilled water as evidenced by positive froth foam test. Glycosides were not detected in any of the six solvent extracts of stem in Libermann's test, but in Salkowski and Killer- Kilani tests all the six solvent extracts showed the presence of glycosides. Phenolics were detected in all extracts except petroleum ether. The steroids were present in all the six solvent extracts of stem. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of stem. Similarly, the phytosterols were present in all extracts except ethanol and methanol extracts of stem of *Meyna spinosa* (Table-6).

In the leaves of *Meyna spinosa* carbohydrates were detected in all the six solvent extracts. Fehling's test was positive in chloroform, petroleum ether, ethanol and methanol extract but negative in hexane and aqueous extracts (distilled water extract). Benedict's test was positive in all except petroleum ether extract. Similarly, Iodine test for carbohydrates was positive in all except chloroform extract (Table-7). Phenols and tannins were detected in all except chloroform and aqueous extracts. Flavonoid was detected in all except chloroform, and saponins in all except chloroform and petroleum ether extracts. Petroleum ether extract showed negative results for the presence of glycosides with all the three tests viz. Libermann's, Salkowski and Killer- Kilani. In petroleum ether and hexane extract of leaves glycosides were not detected. Similarly, glycosides were also not detected in chloroform and petroleum ether extracts as Salkowski test was found to be negative. Killer- Kilani test for glycosides was positive with chloroform, ethanol and methanol extracts but negative with petroleum ether, hexane and distilled water extracts. Phenolic compounds and steroids were detected in all the solvent extracts of leaves except hexane and distilled water extracts. Among other phytochemicals phlobatannin, terpenoid, diterpinoid, anthraquinones, carotenoids, anthocyanin, coumarin, fatty acids, proteins and amino acids were detected in all the six solvent extracts. Emodins were detected in all except petroleum ether extract of leaves. Similarly, the phytosterols were present in all extracts except ethanol and methanol extracts of leaves of *Meyna spinosa* (Table-7).

The solvents viz. chloroform petroleum ether, ethanol, methanol and hexane dissolve many hydrophilic and lipophilic components of plants. Distilled water is universal solvents for a large number of phytochemicals and is largely used for bioassay because of its low toxicity. The higher activity of the ethanolic extracts as compared to the distilled water extract can be attributed to the presence of higher amounts of polyphenols. This means that they are more efficient in cell walls degradation which have non polar character and cause polyphenols to be released from cells. The higher concentrations of flavonoids were detected with ethanol due to its higher polarity. Ethanol is easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material[13].

Ether is selective for the extraction of coumarins and fatty acids[14]. The extracts mainly revealed the presence of carbohydrates, flavonoids, tannins and saponins. The present observations are supported by Rekha Bora *et al*[12] and Senguttuvan*et al*[15] who have screened a more or less similar phytochemicals from *Meyna spinosa*.

Phenolics, alkaloids, steroids, saponins and terpenoids are the large group of naturally produced phytochemicals which have diverse bioactivity[15]. The present investigation confirms that *M. spinosa* has a significant amount of alkaloids, phenolics and flavonoids which are known to exhibit remarkable antioxidant properties. The plant products of *Meyna spinosa* might be usefull in pharmaceutical research and drug industry in near future for great societal demand.

The stem and leaves of *Meyna spinosa* contained a significant amount of phytochemicals viz. alkaloid, flavonoids, phenolic, saponins and tannin. The stem contained relatively higher amounts of phytochemicals than leaves. The amount of flavonoids in stem was maximum (55.75 ±0.34 QE in mg/100gm) followed by phenols (33.85 ±0.45 GAE in mg/100gm), alkaloids (26.85 ±0.35mg/gm), saponins (22.25 ±0.35mg/gm) and tannins (16.35mg/gm). The amount of phytochemicals in leaves also showed a similar trend i.e. the amount of flavonoids was maximum (48.65 ±0.45 QE in mg/100gm), followed by phenol (28.65 ±0.25 GAE in mg/100gm), saponins (24.75 ±0.45 mg/gm), alkaloids (23.45 ±0.56 mg/gm) and tannins (18.65 ±0.35mg/gm) (Table- 8).

The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts of stem and leaves of *Meyna spinosa* has been presented in Table- 9. In all the six solvent extracts it was found that the stem of *Meyna spinosa* contained higher amount of phytochemicals in comparison to leaves. The concentration of total alkaloids in stem was maximum in distilled water extract (42.65mg/gm), followed by ethanol extract (38.45mg/gm), methanol extract (47.75 ±0.18mg/gm), hexane extract (32.65 ±0.35 mg/gm), chloroform extract (17.45 ±0.25 mg/gm), petroleum ether extract (13.35 ±0.85 mg/gm). Similarly, the concentration of total alkaloids in leaves also showed a similar trend, being maximum in distilled water extract (47.35 ±0.21 mg/gm), followed by methanol extract (41.45 ±0.21mg/gm), ethanol extract (38.35 ±0.18 mg/gm), hexane extract (28.55 ±0.25 mg/gm), chloroform extract (15.65 ±0.34 mg/gm) and petroleum ether extract (11.45 ±0.75 mg/gm). In all the six solvent extracts it was found that the stem contained relatively less amount of total alkaloids in comparison to leaves. The amount of total flavonoids was maximum in distilled water extract (8.25 ±0.16 QE in stem and 7.35 ±0.17 QE in leaves), followed by ethanol extract (7.25 ±0.21 QE in stem and 5.15 ±0.23 QE in leaves), chloroform extract (5.35 ±0.21 QE in stem and 7.65 ±0.17 QE in leaves) and methanol extract (7.35 ±0.12 QE in stem and 5.25 ±0.14QE in leaves). Other extracts contained relatively low amount of flavonoids. The amount of total phenols was highest in ethanol, methanol and distilled water extracts of both stem and leaves. The distilled water extracts of stem and leaves contained 122.25 ±0.23GAE in mg/100gm and 151.35 ±0.17 GAE in mg/100gm respectively; of ethanol extracts contained 125.25 ±0.35 GAE in mg/100gm and 105.36 ±0.34 GAE in mg/100gm respectively and of methanol extracts 127.27 ±0.36 GAE in mg/100gm and 132.28 ±0.27 GAE in mg/100gm respectively of total phenols. The chloroform extracts contained 40.35±0.32 and 40.25 ±0.31 GAE in mg/100g of total phenols in stem and leaves respectively. The amount total saponin and total tannins was maximum in ethanol, methanol and distilled water extracts of both bark and leaves in comparison to non-polar solvents viz. chloroform, petroleum ether and hexane which contained less amount of total saponins and tannins in stem and leaves. The total saponin concentration in stem and leaves was maximum in distilled water extract (75.45 ± 0.21 mg/gm and 68.76 ±0.31 mg/gm respectively), followed by methanol extract (73.45 ±0.17 mg/gm and 65.45 ±0.18 mg/gm respectively) and ethanol extract (72.25 ±0.13mg/gm and 64.35 ±0.15 mg/gm respectively). Other extracts contained relatively very low amount of total saponins in both stem and leaves. A more or less similar pattern for total tannins was recorded in all the six solvent extracts, being maximum in distilled water, ethanol and methanol extracts and lesser in chloroform, petroleum ether and hexane extracts.

It was found that the extraction of various phytochemicals was more effectively done in polar solvents viz. ethanol, methanol and distilled water in comparison to non-polar solvents like chloroform, petroleum ether and hexane. The distilled water, ethanol and methanol extracts of stem and leaves showed presence of most of the tested phytochemicals. Hence, it can be reported that alcoholic and aqueous extracts were the best solvents for extracting the active principle than other solvents. Flavonoids are water-soluble polyphenolic compounds which are extremely common and widespread in the plant kingdom as their glycosides. The flavonoids are known to act through scavenging or chelating process. The present investigation gains support from the

work of Rekha bora *et al*[12], Senguttuvan *et al*[15], Wang[13] and Teixeira *et al*[16] in leaves of *Meyna spinosa* who also found more or less similar results.

CONCLUSIONS:

The present study contributes valuable information of bioactive compounds in stem and leaves of *Meyna spinosa.* Qualitative analysis of phytochemicals in stem and leaves was carried out for Alkaloids, Flavonoids, Glycosides, Saponins, Phenols, Steroids, Tannins and terpenoids, diterpinoids etc. Methanol, ethanol and aqueous extract of stem and leaves had all the phytochemicals like flavonoids, glycosides, saponins, phenols, steroids, tannins and terpenoids.

REFERENCES

[1]. Bora PJ, Kumar Y. (2003): Floristic diversity of Assam: Study of Pabitora Wild Life Sanctuary. New Delhi: Daya Publishing House.
[2]. Pullaiah T. (1997): Flora of Andhra Pradesh. Vol. 1. Jodhpur: Scientific Publisher.
[3]. Sen S. (2014): Phytochemical and Pharmacological Screening of Certain Indigenous Plants of Tripura, India. Ph.D Thesis. Anantapuram: Jawaharlal Nehru Technological University Anantapur.
[4]. Sofowara AE (1993): Medicinal plants and traditional medicine in Africa. Vol. 2. Spectrum Books Ltd, Ibadan, p. 288.
[5]. Trease GE, Evans WC (1989): Pharmacognosy 2nd Edn. Braille Tiridel and Macmillan Publishers.
[6]. Harborne JB.(1973). Phytochemicals Methods. Chapman and Hall Ltd, London, 49-188.
[7]. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analaysis,* 10: 178-182.
[8]. Swain, T. (1979): Tannins and Lignins. In: Resenthal, G. A. Jansen, D. H. (ed.). Herbovores, their interaction with secondary plant metabolites, Academic Press, New York, p. 657- 682
[9]. Brunner, J. H. (1984): Direct Spectrophotometer Determination of Saponin, Analytical Chemistry, Scientific Research, *An Academic Publisher*, 34, 1314- 1326.
[10]. Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global J. Pure Appl. Sci*., 8: 203- 208.
[11]. Folin*, O.* and Ciocalteu*,* V. *(*1927*)*: On Tyrosine and Tryptophane Determinations in Proteins. *Journal of Biological Chemistry*, 73, 627-650.
[12]. Rekha Bora, Seema Khakhalary, Tapan Dutta (2019): Phytochemical profiling, assessment of total phenolic content, total flavonoid content and antioxidant activity of ethnomedicinal plant, *Meyna spinosa* from Assam, *Asian J Pharm Clin Res,* Vol 12, Issue 11*, 61-63*
[13]. Wang GX,(2010). In vivo anthelmintic activity of five alkaloids from Macleaya microcarpa (Maxim) Fedde against Dactylogyrus intermedius in Carassius auratus. Veterinary Parasitology.; 171:305-313.
[14]. Cowan MM.(1999).Plant products as antimicrobial agents. Clinical microbiology reviews. 12(4)**:**564-582.
[15]. Senguttuvan J, Paulsamy S, Karthika K. (2014): Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaerisradicata*L. For *in vitro* antioxidant activities. *Asian Pac J Trop Biomed*, 4: S359-67.
[16}. Teixeira A, Baenas N, Dominguez-Perles R, Barros A, Rosa E, Moreno DA, *et al*., (2014) : Natural bioactive compounds from winery by-products as health promoters: A review. *Int J Mol Sci*,15:15638-7