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Original Article

QUALITATIVE AND QUANTITATIVE ESTIMATION OF BIOACTIVE COMPOUNDS IN MIMOSA HAMATA

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ABSTRACT

Objective: The present study aimed to investigate the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Mimosa hamata* in its ethanolic and methanolic extract of whole parts of plant.

Methods: Studies were carried out in terms of methanolic and ethanolic extraction, total extractive values, qualitative and quantitative estimation of phytochemicals.

Results: The percentage value of yield extraction in ethanolic extract was 34.74% and methanolic extract was 24.2%. The preliminary phytochemical analysis showed the presence of alkaloids, phytosterols, glycosides, tannins & phenolic compounds, flavonoids, saponins, carbohydrates, protein and amino acids, fixed oils and fat test. The total phenolic content ranged from 280.5 to 287.5 mg/g of dry weight of extract, expressed as Gallic acid equivalents. The total flavonoid concentrations varied from 264.4 to 267.3 mg/g, expressed as Quercetin equivalents.

Conclusions: It signifies that results revealed the presence of various bioactive constituents which could be exploited for their potential applications for medicinal purposes.

Keywords: *Mimosa hamata*, Extraction, Phytochemicals.

INTRODUCTION

India possesses a rich biodiversity of the medicinal plants that were still not explored completely. Medicinal plants have been a valuable source of natural products for maintaining human health. Nowadays, the need for natural products for pharmaceutical purposes from the plant has attained a great interest in the present research world due to the cost and the higher side effects that are associated with the chemically manufactured drugs [1, 2, 3, 4]. According to WHO (World Health Organization), 80% of the people rely primarily on traditional health care system and mostly on herbal medicines [5]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosoids, and volatile oils [6, 7]. It is necessary to identify that bioactive constituent of medicinal plants usually employed by herbalists in the treatment of infectious diseases. *M. hamata* (Mimosaceae), commonly known as Jinjani and hooked *Mimosa,* and mostly found in open sandy places, the arid zones of Rajasthan, Punjab, Delhi, Central and South India. Due to their notable pharmacological effects, *M. hamata* are widely used in traditional and modern medicine for preparation of tonics against general weakness, treatment of urinary complaints, applied to burn, over glandular swelling and also used in dressing for sinus, sores and piles [8]. The aim of present study was to identify the qualitative and quantitative estimation of phytoconstituents present in methanolic and ethanolic extracts of *M. hamata.* Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, anti-inflammatory antibiotic, hypoglycemic and anti-carcinogenic [9, 10, 11]. Phenols and polyphenols are major secondary metabolites present in the plant kingdom. They have been reported to have multiple biological effects, including antioxidant activity and antimicrobial activity. They are imperative source in plant for normal growth development and defense against infection and injury. Isolation and identification of the bioactive compounds of plants have always been a challenging task for researchers [6, 12, 13]. The Preliminary qualitative phytochemical analysis were used to evaluate the presence of the biomolecules such as anthroquinone, alkaloids, catachol, flavonoids, phenolic compounds, saponins, steroids, tannins and triterpenoids respectively. The quantitative estimation of total phenolic and total flavonoid contents from the whole parts of *M. hamata* were also analyzed for the estimation of amount of total phenol and flavonoids by spectrophotometric method.

MATERIAL AND METHODS

Collection and identification of plant

M. hamata plant was collected from Sariska National Park, Alwar during the month of September 2012. Further plant material was identified and voucher specimens were submitted in 'Herbarium' Department of Botany, University of Rajasthan, Jaipur and registration number allotted to *M. hamata* were Reg. No. RUBL – 21111 respectively. The plant material was dried under shade at room temperature for about 15 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 40-100 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of extracts

M. hamata dried and powdered plant materials (45 g) was also filled in the thimble and extracted successively with 80 % methanol (methanol: distilled water; 80: 20) and 95 % ethanol (ethanol: distilled water; 95:5) solvents in soxhlet extraction unit for 48 hours [14]. The plant extracts were filtered and then concentrated using rotary evaporator at 40 ºC and each extract were transferred to glass vials and kept at 4º C before use.

Yield of the extract obtained was calculated by formula as mentioned below:

$$\text{Extractive yield value} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant dried powder}} \times 100$$

Qualitative phytochemical analysis of plant extract

The different qualitative chemical tests were performed for establishing profile of given extracts for its chemical composition. The extracts (methanolic and ethanolic extracts) were reported the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, tannins and flavonoids. All tests were done as per the procedure given in the standard book [15].

Quantitative Phytochemical Analysis

Total Phenols Determination

The amounts of total phenolic contents of extracts of *M. hamata* were determined by the spectrophotometric method of Kim *et al.*, [16] with slight medication. A diluted plant extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample. The solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (Fig. 1). The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic Content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample (Table 3).

Total Flavonoids Determination

The total flavonoids assay was conducted according to Katasani Damodar [17]. Total flavonoids content was determined by using aluminium chloride colorimetric method. Each plant extracts (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 2 to 8 µg/ ml in methanol (Fig. 2). The Total flavonoids Content was expressed as milligrams of quercetin equivalents per gram of dried sample (Table 3).

Table 1: The percentage yield by soxhlet extraction method of crude extract of *M. hamata*

Solvent	Weight of Powdered Plant Material	Volume of Solvent	Weight of Extract	% of yield of Extraction
95% Ethanol	35 gm	200 ml	12.16 gm	34.74%
80% Methanol	35 gm	200 ml	08.47 gm	24.2 %

Table 2: Preliminary phytochemical test of extract of *M. hamata*

S. No.	Phytochemical tests		Observation of plant extract	
	Chemical Constituents	Test	Methanolic extract	Ethanolic extract
1.	Alkaloids	Mayer's Test		
		Wagner's test	Positive	Positive
		Dragendroff's reagent		
		Hager's test		
2.	Phytosterols	Salkowski's test		
		Libermann Burchard's test	Positive	Positive
3.	Glycosides	Borntrager's test	Positive	Positive
		Legal's test		
4.	Tannins & Phenolic compounds	Ferric Chloride test		
		Gelatin – salt Test	Positive	Positive
		Iodine Test		
		Nitric acid Test		
5.	Flavonoids	Alkaline reagent test		
		Lead acetate test	Positive	Positive
		With H_2So_4,		
		Zinc test		
6.	Saponins	Froth Test		
		Foam Test	Positive	Positive
7.	Carbohydrates	Fehling's test		
		Benedict's test	Positive	Positive
		Molisch's test		
8.	Protein and amino acids	Biuret test		
		Ninhydrin test	Negative	Negative
		Xanthoproteic test		
9.	fixed oils and fat test	Spot test	Negative	Negative

Table 3: Quantitative Estimation of Total phenolic and flavonoids content of different extracts of *Mimosa hamata*

Mimosa hamata	Total phenolic (mg/g)	Total flavonoids (mg/g)
Methanolic extract	280.5±0.76	264.4±0.80
Ethanolic extract	287.5±0.76	267.3±0.76

Values are means of three independent determinations ± standard deviation (SD).

RESULTS AND DISCUSSION

The methanolic and ethanolic extracts of *M. hamata* (leaves, flowers, stem and seeds) were subjected to routine qualitative and quantitative chemical analysis to identify the nature of phytochemical constituents present in them. The whole parts (leaves, flowers, stem, and seeds) of plants were extracted using solvent 80% methanol and 95% ethanol by the earlier mentioned method. In the extractive values were found 34.74% of ethanolic extract and 24.2% of methanolic extract (Table 1). *M. hamata* plant extracts and their active ingredients such as alkaloids, flavonoids, tannins, phenols, saponins were used to serve as antioxidants, antibacterial, antifungal and antiviral [18]. Various pharmacological uses such as antifungal activity of deproteinized leaf extract [19, 20], antibacterial activity of ethanolic extract of aerial parts, antiviral activity of the methanolic extract of roots [21] and antioxidant activity have also been reported [22, 23]. Various bioactive compounds that is, 4-ethyl gallic acid [24] from fresh flowers of *M.*

hamata, ethyl gallate, gallic acid from leaves of *M. hamata* and mimonoside A, B, C and saponins A (3-O- D-glucosyl- L- rhamnosyl morolic acid) from its roots have been reported [25]. In the present study, qualitative analysis of both extracts (leaves, flowers, stem and seeds) of *M. hamata* revealed the presence of phytochemical constituents such as alkaloids, phytosterols, glycosides, tannins and phenolic compounds, flavonoids, saponins, carbohydrates. Protein, amino acids, fixed oils and fat were absent in the methanolic and ethanolic extracts of *M. hamata* (Table 2). Maheswari et al. (2012) reported that whole plant of *M. hamata* is used in the treatment of skin disease [26]. The amount of total phenol was determined with Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.12x + 0.102, R^2= 0.995 (Figure 1); where y is absorbance at 750 nm and x is total phenolic content in the extracts of *M. hamata* expressed in mg/gm. The total phenolic content was found in the methanolic and ethanolic extract of *M. hamata* (280.5 and 287.5 mg/gm) (Table 3). The amount of total flavonoid was determined with aluminum chloride reagent. Quercetin was used as a standard compound and the total flavonoid was expressed as mg/g quercetin equivalent using the standard curve equation: y = 0.104x + 0.015 R^2 = 0.993 (Figure 2); where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *M. hamata* expressed in mg/gm. The total flavonoid was 264.4 and 267.3 mg/g in the methanolic and ethanolic extracts, respectively (Table 3). Phenols and flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health [27]. Flavonoids are essential in human diet and are present in plant extracts that have been used for medicinal purpose [28]. Limasset et al. (1993) reported that antioxidant properties, reactive oxygen species scavenging and cell function modulation of flavonoids could account for the large part of their pharmacological activity [29]. The results obtained from the present study showed that the extract of *M. hamata* contain highest amount of phenolic and flavonoid compounds. Further work is going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive principles with the help of advanced technologies to develop plant derived drugs.

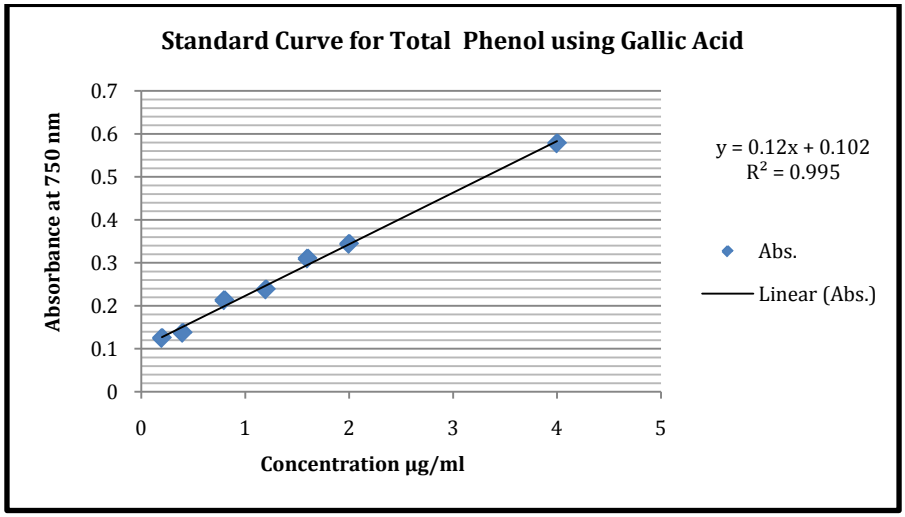

Fig. 1: Standard Curve of Gallic Acid

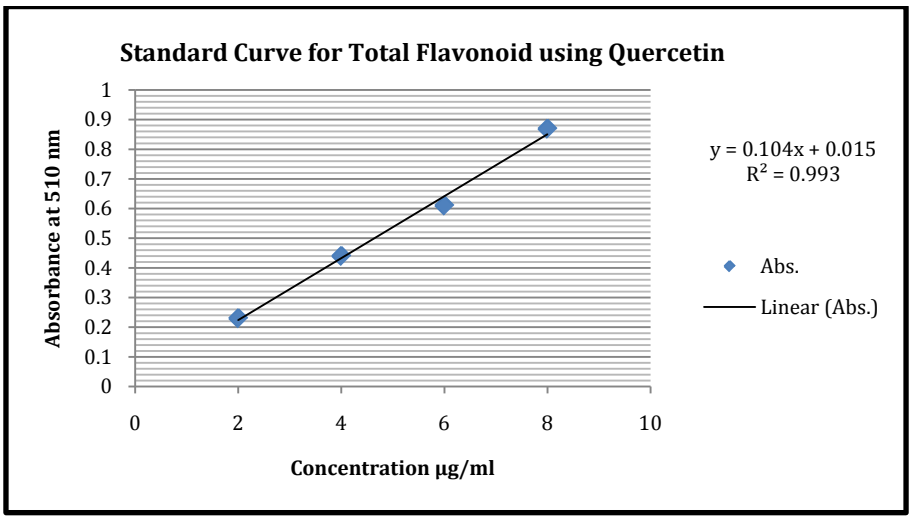

Fig. 2: Standard Curve of Quercetin

CONCLUSION

The results revealed the presence of various bioactive constituents in the whole parts of *M. hamata*. The objective of this study was to provide the information about the significant amount of phenolic and flavonoids in methanolic and ethanolic extract of *M. hamata*. Further work of this study is to correlate relationship of these active constituents for possible biological activities and evaluate *M. hamata*

as a potential source of natural bioactive chemicals against pathogenic microorganism. Further studies are going on concerning this plant in order to study the structure of bioactive compounds by various techniques such as high performance liquid chromatography (HPLC), fourier transform infrared (FTIR) spectroscopy and Nuclear magnetic resonance (NMR) etc.

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REFERENCES

1. Srivastava J, Lambert J, and Vietmeyer N. Medicinal plants:an expanding role in development. World Bank technical;1996. p. 320.
2. Raja A, Gajalakshmi P and Raja MMM. Drugs from the natural bio sources for human disease. Int J Pharmacology. 2010;6:360-63.
3. Jasuja ND, Choudhary J, Sharma P, Sharma N and Joshi SC. A review on bioactive compounds and medicinal uses of *Commiphora mukul*. J Plant Sci. 2012a;7:113-37.
4. Jasuja ND, Saxena R, Chandra S and Sharma R. Pharmacological Characterization and Beneficial uses of *Punica granatum*. Asian J Plant Sci. 2012b;11:251-67.
5. Shanley P and Luz L. The impacts of forest degradation on medicinal plant use and implication for health care in Eastern Amazonia. BioSci. 2003;53:573 – 84.
6. Edeoga1 HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech. 2005;4:685-688.
7. Vyas A, Shukla SS, Pandey R, Jain V, Joshi V and Gidwani B. Chervil:A Multifunctional Miraculous Nutritional Herb. Asian J Plant Sci. 2012;11:163-71.
8. Nadkarni AK and Nadkarni KM. Indian Materia Medica. Popular Book Depot, Bombay;1954.
9. Katalinic V, Miloš M, Kuliši T, Juki M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2004;94:550-57.
10. Mulabagal V and Tsay H. Plant cell cultures-an alternative and efficient source for the production of biologically Important secondary metabolites. Int J Appl Sci Eng Tech. 2004;2:29-48.
11. Borneo R, Le_n EA, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their *in vitro* testing in model food system. Food Chem. 2008;112:664-70.
12. Kähkönen MP, Hopia AI, Vuorela JH, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47**:**3954-62.
13. Bairwa GL, Jasuja ND and Joshi SC. Lipid lowering and antioxidant effects of *Ammomum subulatum* seeds (Family Zingiberaceae) in cholesterol fed rabbits. Arech Phytopathol Plant Prot. 2011;44:1425-31.
14. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK and Staden JV. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol. 1999;68:267-274.
15. Kokate CK. Practical Pharmacognosy. Published by Nirali Prakashan New Delhi;2004. p. 29-107.
16. Kim DO, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 2003;81:321–326.
17. Katasani D. Phytochemical screening, quantitative estimation of total phenolic, flavanoids and antimicrobial evaluation of *Trachyspermum ammi*. J Atoms and Molecules. 2011;1:1–8.
18. Singh R, Jasrai YT. *Mimosa hamata* (Willd.), a plant with antipathogenic properties. Int J Med Arom Plants. 2012;2:677-682.
19. Mukadam DS, Balkhande LD, Umalkar GV. Antifungal activities in deproteinized leaf extract of weeds and nonweeds. Indian J Microbiol. 1976;16:78-79.
20. Umalkar GV, Mukadam DS, Nehemiah KMA. Fungistatic properties of some deproteinized leaf extracts. Sci Cult. 1977;43:437-439.
21. Jain R, Arora R, Alam S, Jain SC. Pharmacological evaluation of *Mimosa hamata* roots. Fitoterapia. 1997a;68:377-378.
22. Jain SC, Jain R, Singh R. Arid zone plants as natural antioxidant agents. J Indian Chem Society. 2009a;86:199-201.
23. Singh R, Jain SC, Jain R. Antioxidant activity of some medicinally important arid zone plants. Asian J Exp Sci. 2009;23:215-21.
24. Mehta BK, Savita S, Avinash D. 4-Ethylgallic acid from two *Mimosa* species. Phytochemistry. 1988;27:3004–05.
25. Jain R, Arora R, Jain SC. Saponins from the roots of *Mimosa hamata* Willd. Indian J Chem. 1997b;36B:61-64.
26. Maheswari UP, Madhusudhana Reddy A, Ramababa M and Basha SKM. Traditional Medicinal Flora Habituated in Various regions of YSR (Kadapa) District, Andhra Pradesh, India. Indian J Fundamental and Applied Life Sci. 2012;2:162-75.
27. Jasuja ND, Sharma SK, Saxena R, Choudhary J, Sharma R and Joshi SC. Antibacterial, antioxidant and phytochemical investigation of *Thuja orientalis* leaves. J Med Plant Res. 2013;7:1886-93.
28. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoids:old and new aspects of a class of natural therapeutic drugs. Life Sci. 1993;64:337-53.
29. Limasset B, Doucen CL, Dore JC, Ojasoo T, Damon M, Paulet AC (1993). Effects of flavonoids on the release of reactive oxygen species by stimulated Limasset B, Doucen CL, Dore JC, Ojasoo T, Damon M, Paulet AC. Effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils. Biochem Pharmacol. 1993;46:1257–71.