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Short Communication

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Antioxidant Activity and Total Phenolic Content of Various Extracts from *Mimosa hamata* Willd., Mimosaceae

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Abstract

Jinjani (*Mimosa hamata* Willd., Mimosaceae) is widely distributed in India and Pakistan, which contains several interesting bioactive constituents and possesses health promoting properties. In this study, the antioxidant activity with the total phenolic content of different successive extracts (pet. ether, chloroform, n-butanol and water) from leaves, stem, root and seeds of *M. hamata* were assessed in an effort to compare and validate the antioxidant potential of the particular part of the plant. The antioxidant activity was determined by 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) assay and total phenolic content was estimated by using Folin-Ciocalteu's reagent. All tested extracts possessed appreciable antioxidant potential but n-butanol extract of roots was significantly higher in total phenolic content (73.16±0.19 mg GAE/g extract) and in antioxidant assay (IC_{50} = 5µg/ml). It is also noteworthy that in n-butanol extract of roots higher yield (85.60%) was also observed. This study validates the antioxidant potential of the roots in *M. hamata* and the positive relationship between total phenolic content and antioxidant activity.

Keywords: Mimosa hamata Willd., total phenolic content, antioxidant activity, folin-Ciocalteu's reagent, 2,2-diphenyl-1-picryhydrazyl (DPPH), ascorbic acid.

Introduction

Mimosa hamata Willd., a much-branched straggling shrub belonging to family *Mimosaceae* occurs in tropical areas and widely distributed in India and Pakistan[1]. Traditionally the rural people use this plant to cure urinary complaints and make a tonic against sexual weakness in males[2]. A paste of leaves is applied over glandular swellings and is used in dressing for sinus, sores and piles. Its roots possess contraceptive efficacy while seeds are used as a blood purifier[3-4]. On phytochemical study, 4-ethylgallic acid[5] from flowers, a triterpenic saponin B (3-O-L-arabinosyl-D-glucosyl morolic acid) from roots[6], ethyl gallate and gallic acid from leaves[1] and mimonoside A, B, C, and saponin A (3-O-D-glucosyl-L-rhamnosyl morolic acid) from its roots[7] have been reported. Various bioefficacies viz. antifungal activity of deproteinized leaf extract[8-9], antibacterial activity of alcoholic extract of aerial parts[1], and antiviral activity of the methanolic extract of roots[10-11] have been studied. However, little information is available concerning the antioxidant potential of *M. hamata*[12-13]. In particular, polyphenols in this plant have not been well characterized. Phenolic compounds are found in food (vegetable, fruits, chocolate, tea, coffee, wine, grape juice, vinegar) at different concentrations[14]. Epidemiological evidence indicates an inverse relationship between the intake of food rich in phenolic compounds (i.e. flavonoids) and the reduction of certain chronic diseases and coronary heart disease mortality[15-17]. Although polyphenols have been shown to exhibit different biological activities, such as antioxidant, to decrease platelet aggregation and endothelial adhesion, to mediate nitric oxide production, to suppress cancer cell growth, and to reduce oxidative stress[18-22], the exact nature of their protective effect remains to be established. In the present study, the influence of some critical extraction variables on the phenolic yield and antioxidant activity is researched. An experimental design was performed in order to find the relevant optimal values of these variables. The aim of this study was also to examine the total phenolic content and antioxidant activity in the resultant extracts under different solvents. Antioxidant potential has been determined as the free radical scavenging ability using a stable radical 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) and also ascertained by estimating total phenolic content by Folin-Ciocalteu's reagent.

Materials and Methods

Chemicals

Pet. ether, chloroform, n-butanol, Folin and Ciocalteu's reagent, were obtained from Merck (India). 2, 2-Diphenyl-1-picryhydrazyl (DPPH) and Ascorbic acid were obtained from Sigma-Aldrich

Chemical Co. (USA). Double distilled water was used for all assays.

Methods

Plant material

M. hamata was collected in the flowering and fruiting stage from the fields of Ajmer, Rajasthan, India in July-August, 2006 and whole plant was dried according to the botanical procedure and stored. A voucher specimen (RUBL 9565) was submitted to Department of Botany, University of Rajasthan, Jaipur-55, Rajasthan, India.

Preparation of extracts

Dried stem, leaves, root and seeds of *M. hamata* were ground in a mechanical grinder (Typhoon Mixer & Grinder, Bajaj, India). The powder was passed through a sieve in order to maintain particle size unity (300µm). Four solvents (pet. ether, chloroform, n-butanol and water) with distinct polarity (dielectric constants 4.3, 4.81, 18, and 80 respectively) were employed sequentially for the preparation of plant extracts. 100 g of powdered plant material was mixed with 500 ml of pet. ether and extracted in a Soxhlet apparatus for 10-12 hrs. The resulting solution was decanted off and freeze-dried (Heto Power Dry PL 3000, India). The plant residue obtained from pet. ether extraction was again sequentially extracted with chloroform, n-butanol and water following the same procedure. All extracts were stored at 4ºC in airtight vials for immediate use. Samples that were not to be used within 24 hr were transferred to a freezer and stored at -20ºC. The extraction yield was calculated by the following equation[23]:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the freeze-dried extract (g)}}{\text{Weight of the original sample (g)}} \times 100$$

DPPH scavenging assay

A concentration of 8mg/10ml extract of each test sample was prepared to obtain different concentrations (80, 60, 40, 20 and 10 µg/ml). From each, 2.5 ml solution was mixed with 2,2-diphenyl-1-picryhydrazyl (DPPH; 2.5 ml; 20mg/10ml), kept in the dark for 15 min at room temperature and the decrease in absorption was measured at 517 nm using UV-VIS spectrophotometer (Varian type Cary PCB 150 Water Peltier System with standard cuvettes). Optical absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that 50% reduction in absorbance (IC_{50}) was calculated[24]. The same procedure was also followed for the standard quercetin and ascorbic acid. Inhibition of DPPH in percentage was calculated by following equation[25]-

$$\% \text{ Inhibition} = 1 - \frac{OD_{\text{test sample}}}{OD_{\text{control}}} \times 100$$

Determination of total phenolic content

Total phenolic content (TPC) were quantified in each test sample, following the protocol of Bray and Thorpe[26], which included the preparation of a regression curve of standard phenol (Gallic acid). Samples were diluted with distill water to give concentration of 0.4mg/ml. A 0.5 ml of each sample was added with 0.5 ml of Folin-Ciocalteu reagent and 1.0 ml of distill water. After a period of 2-5 minutes, the tubes were added with 0.5 ml of 10% Na_2CO_3. After 1 hr incubation at room temperature the absorbance was measured on a spectrophotometer UV-VIS spectrophotometer (Varian type Cary PCB 150 Water Peltier System with standard cuvettes) at 760 nm using distill water as a blank. Gallic acid (0-100 mg/L) dissolved in distill water was used to prepare standard curve concentration and values were expressed as microgram of gallic acid equivalents (mg Gallic acid/g extract). Three replicates were taken for each concentration and the average optical absorbance was plotted against the respective concentration to compute a regression curve which followed the Beer's law.

Results and Discussion

In *M. hamata,* different plant parts, nature of extract, yield (%), total phenolic content (mg GAE/g extract) and IC_{50} (µg/ml) value also shown in Table 1. As seen in Table 1, in different successive extracts highest total phenolic content was observed in n-butanol extract of leaf (56.50±0.58 mg GAE/g extract), chloroform extract of stem (61.80±0.67 mg GAE/g extract), n-butanol extract of roots (73.16±0.19 mg GAE/g extract) and in n-butanol extract of seeds (46.50±0.50 mg GAE/g extract). Antioxidant activity by means of IC_{50} (µg/ml) was appreciable of n-butanol and water extracts of leaf (8 µg/ml), n-butanol extract of stem (5 µg/ml), n-butanol extract of roots (5 µg/ml) and chloroform extract of seeds (7 µg/ml). It is noteworthy that among all the tested extracts, the extract of roots was found to be most effective (IC_{50} = 5 µg/ml; Figure. 1 and 2) having total phenolic content 73.16 ± 0.19 mg GAE/g extract, which was comparable to the standard used (ascorbic acid; IC_{50} = 5 µg/ml).

The level of phenolic compound in n-butanol extract of roots was highest among the all tested extracts. Polyphenolics compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds[27]. The activity is believed to be mainly due to their redox properties, which plays an important role in adsorbing and neutralizing free radicals,

Table1Yield, total phenolic content and IC_{50} (µg/ml) value of *M. hamata* extracts[a]

Plant part (% w/w)	Extracts	Extractives (mg/GAE/g extract)[b]	Total phenolic content (µg/ml)	IC_{50}	
Leaf	Petroleum ether	12.51	51.30±0.33	9	
Chloroform	23.22	NT	9		
Butanol	15.44	56.50±0.58	8		
Water	48.81	53.16±0.45	8		
Stem	Petroleum ether	1.29	54.50±0.29	25	
Chloroform	20.78	61.80±0.67	35		
Butanol	39.58	60.16±0.33	5		
Water	38.33	51.80±1.00	22		
Root	Petroleum ether	0.42	26.33±0.17	13	
Chloroform	0.31	72.50±1.26	9		
Butanol	85.60	73.16±0.19	5		
Water	2.59	69.60±1.20	7		
Seed	Petroleum ether	2.00	38.10±0.44	8	
Chloroform	0.62	16.60±0.92	7		
Butanol	1.77	46.50±0.50	8		
Water	25.31	51.16±0.72	10		
Ascorbic acid	-	-	-	5	

[a] Values are expressed as means ± S. E. of three parallel measurements.
[b] GAE, Gallic acid equivalents. NT: not tested

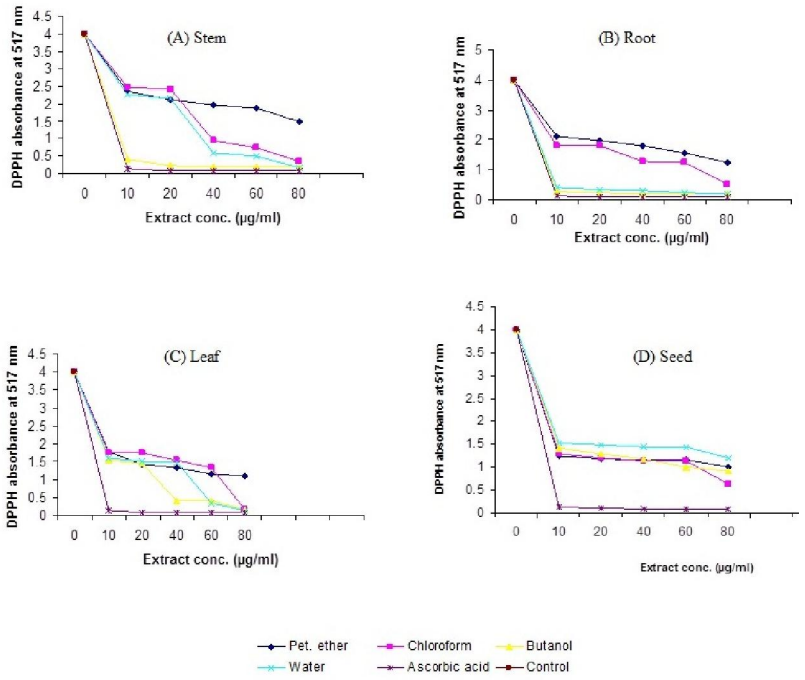

Fig. 1 Antioxidant activity of various parts of *M. hamata*

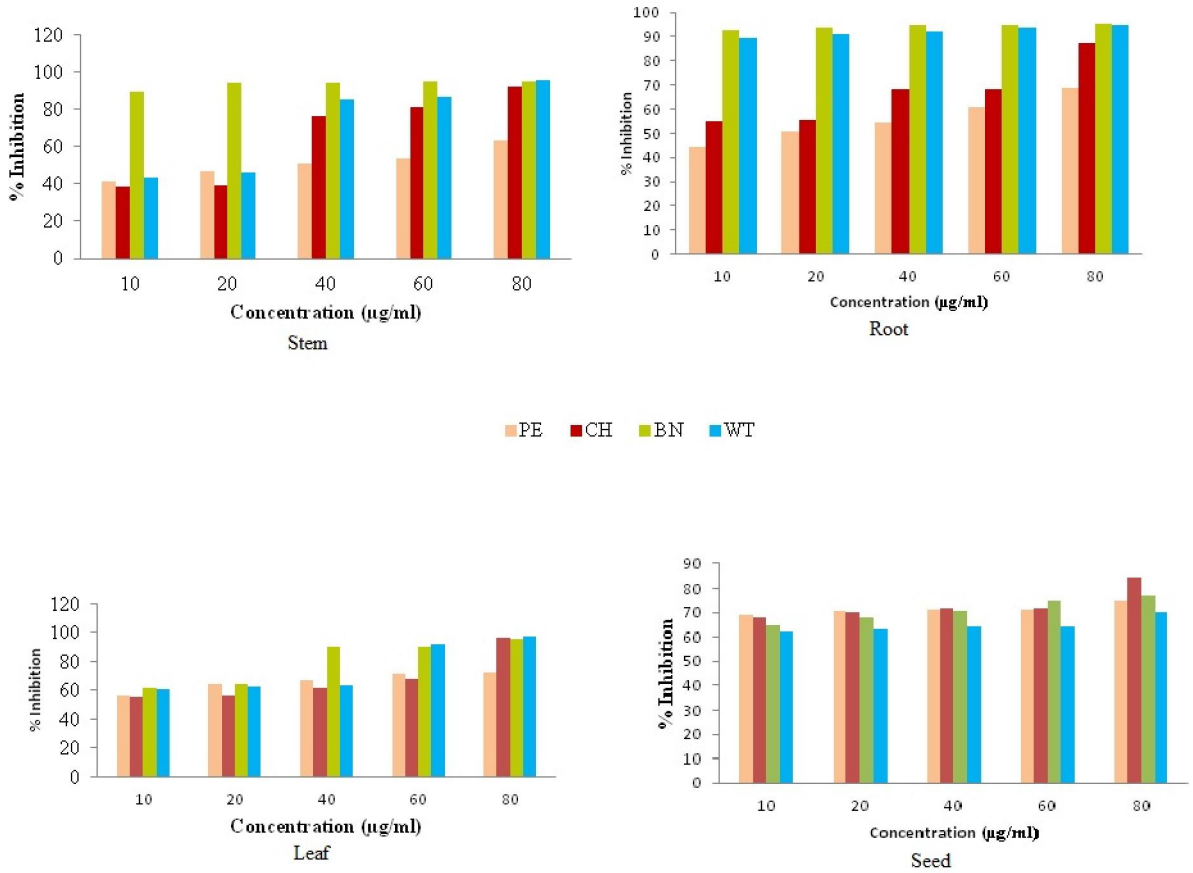

Fig. 2 % Inhibition of various extracts from *M. hamata*

quenching singlet and triplet oxygen, or decomposing peroxides.[28-29]

Mimosaceae members have been well characterized by impressive phytochemical diversity. Polyphenols especially flavonoids and tannins are the common ones[30]. Polyphenols have been known for their high polarity, high solubility in polar solvents and strong antioxidant activity as well[31-32]. This polyphenols could be responsible for the free radical scavenging activity of n-butanol extracts of roots from *M. hamata*.

Conclusions

This study showed that the 16 extracts of *M. hamata* contained phenolic compounds in good quantity. n-Butanol extract of roots, with the highest total phenolic content, had the most active radical scavenger activity among all tested extracts. Further investigations are needed to investigate the active compound/s responsible for such a great antioxidant potential. It is indicated by the result of this work that *M. hamata*, on the condition the proper solvent and extraction processes are established, could serve as a source of natural antioxidants or nutraceuticals.

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