INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

THE STUDY OF PHENOLIC COMPOUNDS AND ANTIOXIDANT POTENTIAL OF CRUDE EXTRACT AND FRACTIONS OF *MIMOSA HAMATA*

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Article Received on: 21/05/17 Approved for publication: 19/06/17

DOI: 10.7897/2230-8407.086101

ABSTRACT

The present study aimed to evaluate the phenolic compounds and *in vitro* antioxidant properties of ethanolic extract and selected fraction of *Mimosa hamata* whole plant. Phytochemical analysis of the extract of *M. hamata* indicated the presence of phenols and flavonoids in plant. The highest total phenolic and flavonoid content was observed in the IG fraction of *M. hamata* (654.33 ± 0.008 mg/g and 689.66 ± 0.032 mg/g respectively) in comparison to other fractions. The present investigation showed that ethanolic extract and fraction of *M. hamata* at various concentrations have good antioxidant capacity. Therefore, the overall results of the present studies were indicated that these bioactive compounds have been of interest for health benefits, the present analytical study proved a potential application to identify and quantify the phenolic compounds in plant extract and fractions.

Keywords: *Mimosa hamata*, phenolic compounds, antioxidant activity, 2,2-diphenyl-1 picryhydrazyl (DPPH).

INTRODUCTION

Presence of oxygen is the main source of life on earth which gives us energy by oxidation of food i.e. essential for living. During this progression highly reactive and harmful oxygen species are also generated which can cause damage to living organisms[1]. Free radicals cause oxidative damage to diverse molecules, e.g. lipids, proteins and nucleic acids and thus are concerned in the beginning phase of some degenerative diseases[1]. Research has revealed that free radical mediated oxidative stress is among the main causal factors in induction of several chronic and degenerative diseases as well as atherosclerosis, diabetes mellitus, ischemic heart disease, ageing, cancer, immunosuppression, neurodegenerative diseases and others[2,3,4]. An antioxidant may be defined as: any substance which present in low concentrations compared to that of an oxidisable substrate considerably delays or inhibits the oxidation of molecules, by inhibiting the initiation or propagation of oxidizing chain reactions[5,6]. Medicinal plants are most significant sources of biologically active antioxidants. Several phytoconstituents that are antioxidants, have been isolated from extracts of different parts of plants, such as roots, stems, leaves, seeds, fruits and flower[7,8]. Natural, stable, non-toxic and multifunctional natural compounds from plants which are pharmacologically valuable or with low or no side effects are preferred for use in defensive medicine and also used in food industry[9,10]. Therefore, this study was conducted to investigate the comparative antioxidant activities of extract and fractionates of plant *Mimosa hamata* whole plant parts (Leaves, stems, seeds and flower) for finding new sources of natural antioxidants and also evaluate the phenolic compounds. *M. hamata* commonly known as Jinjani belongs to family Mimosaceae (Touch me not) which is a much branched, armed shrub, commonly distributed along the open sandy places, often gregarious and abundant throughout the arid zone of Rajasthan, Punjab, Central and South India. [11-16]. *M. hamata* is one of the indispensable medicinal plants used in the traditional system of medicine for the treatment of assorted diseases such as jaundice, diarrhea, coagulant, fever, dysentery, blood-purifier, wounds, tonic in urinary complaints, piles. Paste of leaves is applied to burn, over glandular swelling and also used in dressing for sinus, sores and piles[15, 16]. However, for the foreseeable future, long- term tolerance studies are needed the isolation of natural bioactive compounds from plants for human health. Pharmaceutical industry further may also utilize and formulate actual content to achieve one more step against these chronic diseases.

MATERIALS 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- 21155 respectively.

Preparation of extract and fractions

M. hamata whole plant powder (35 g) was filled in the thimble and extracted successively with 95 % ethanol (ethanol: distilled water; 95: 5) solvents in soxhlet extraction unit for 48 hours[17]. Fractionation of bioactive compounds from extract of *M. hamata* using column chromatographic technique was carried out with a glass column of internal diameter 2.0 cm and length 75 cm (Borosil). Solvent system ethyl acetate and di- ethyl ether (1:1) were selected for isolation of phenolic compounds according to the method of Meena and Patni, (2008) with slight modification[18].

Total Phenolic and flavonoids Determination

The amounts of total phenolic contents of whole parts (Leaves, stems, seeds and flower) extract and fractions of *M. hamata* were determined by the spectrophotometric method as described earlier by Kim *et al.,* (2003) with slight modification[19]. Calorimetric method with aluminum chloride was also used for flavonoids determination according to Katasani (2011)[20]. Total phenolic and flavonoids content were determined from extrapolation of the calibration curve, which was made by preparing various concentrations of Gallic acid and quercetin solution. The estimation of the phenolic and flavonoids compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried sample and flavonoids content was also expressed as milligrams of quercetin equivalents per gram of dried sample.

In vitro* antioxidant screening of *M. hamata
DPPH radical scavenging activity

The scavenging activity of the plant extract and selected fractions against DPPH (2,2-Diphenyl-1-picrylhydrazyl) was done according to the Liyana – Pathiranan and Shahidi, (2005) with slight modification[21]. One militer of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of various concentrations (0.05- 0.25 mg / ml) of extract and fractions of selected plant (*M. hamata*); ascorbic acid, Butylated hydroxytoluene (BHT), quercetin. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Data were processed using EXCEL and concentration that 50% reduction in absorbance (IC_{50}) was calculated.

Hydrogen peroxide scavenging activity

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide according to Jayaprakasha *et al.,* (2004)[22]. A solution of H_2O_2 (20 mM) was prepared in phosphate buffer saline (PBS at pH – 7.4). Various concentrations of extracts and standard in methanol (1ml) were added to 2 ml of H_2O_2 solution in PBS. Then finally the absorbance was measured at 230 nm after 10 minutes. Ascorbic acid and Butylated hydroxy toluene (BHT) was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 230 nm using a spectrophotometer.

RESULTS AND DISCUSSION

In the present study, the ethanolic extract of *M. hamata* was subjected to column chromatography using ethyl acetate and di ethyl ether solvents in 1:1 ratios to yield several sub-fractions. These fractions were coded as IA to IH (IA, IB, IC, ID, IE, IF, IG, IH). The result of total phenol and flavonoid content of ethanolic extract and various fractions of *M. hamata* were summarized in Table 1.1. The total phenolic and Flavonoids content in the ethanolic extract of *M. hamata* was 288 mg GAE /g and 256.33 ± 0.12 mg QE/g of dry weight of extract respectively. The highest total phenolic and flavonoid content was observed in the IG fraction of *M. hamata* (654.33 ± 0.008 and 689.66 ± 0.03 mg/g respectively). In this study, among the ethanolic extract and selected fraction (IG fraction) of *M. hamata* (IG fraction) was exhibited potent antioxidant activity in DPPH assays respectively (Figure 1.1). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. The value of percentage inhibition of IG fraction was found to be highest as compare to ascorbic acid standard (93.52 ± 0.12 %). In the current study, ethanolic extract and IG fraction of *M. hamata* also demonstrated the noteworthy H_2O_2 scavenging ability that was 67.81 ± 0.22 % and 88.43 ± 0.10 % at 100 µg/ml concentrations respectively (Figure 1.3). The values of percentage inhibition of IG fraction were found to be highest in comparison to standard ascorbic acid (86.87 ± 0.10 %) correspondingly. With regard to IC_{50} values of hydrogen peroxide scavenging ability, IG fraction of *M. hamata* (25.84 µg/ml of IC_{50} value) had the highest radical scavenging ability than standard ascorbic acid (28.60 µg/ml).

The results of present study were also in agreement with the study of Zhang *et al.,* (2011) and Azmi *et al.,* (2011), who reported that whole plant, stems, leaves, and seeds of *Mimosa pudica* had highest amount of polyphenolics compounds[23, 24]. Similar results also reported by de Queiroz Siqueira *et al.,* (2012) who examined that *Mimosa tenuiflora* (Willd.) Poir., popularly known as jurema-preta, exhibited the highest tannin content (12.58%)[25]. The finding of the present study was also matched with Singh *et al.,* (2012) who reported that 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 amount of polyphenolics compounds of the particular part of the plant and highest total phenolic content was observed in n-butanol extract of roots of *Mimosa hamata* was highest among the all tested extracts[26]. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow coloured α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant[27]. Furthermore, hydrogen peroxide is a weak oxidizing agent and it is not very reactive, can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus removing H_2O_2 is very important for the protection of living systems[28]. The present finding were also endorsed by earlier workers Lau *et al.,* (2004) and David *et al.,* (2007) who reported that the several species of *Mimosa* had significant antioxidant property[29,30]. Correspondingly, Genest *et al.,* (2008) also demonstrated that the n-hexane, dichloromethane (DCM) and methanol (MeOH) extracts of *Mimosa pudica* and *Mimosa rubicaulis*, two Bangladeshi medicinal plants, were also had free radical scavenging activity[31]. The current results were also coincided with Das *et al.,* (2014) who reported that IC_{50} values of the methanolic extracts of *M. pudica* leaves were found to be 126.71 µg/ml in DPPH scavenging assay[32].

Table 1.1: Quantitative Estimation of Total Phenolic and Flavonoids Content of Crude Extract and Different Fractions of *M. hamata*

M. hamata	Total phenolic content (mg/gm*)	Total flavonoids content (mg/gm*)
Ethanolic extract	288 ± 0.012	256.33 ± 0.12
IA Fraction	21.9 ± 0.008	19.73 ± 0.032
IB Fraction	160.67 ± 0.014	210.66 ± 0.042
IC Fraction	224.66 ± 0.015	217.66 ± 0.044
ID Fraction	225.66 ± 0.006	246.33 ± 0.056
IE Fraction	588 ± 0.008	574.66 ± 0.075
IF Fraction	244.66 ± 0.014	236.66 ± 0.042
IG Fraction	654.33 ± 0.008	689. 66 ± 0.032
IH Fraction	248.66 ± 0.014	245.5 ± 0.056

*Values are means of three independent determinations ± Standard Error Mean (SEM)

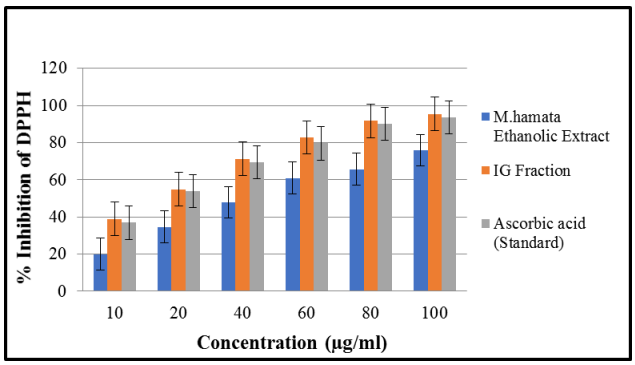

Figure 1.1: Comparison of DPPH Radical Scavenging Activity of Ethanolic Extract and IG Fraction of *M. hamata* (whole plant) with Standard Drug

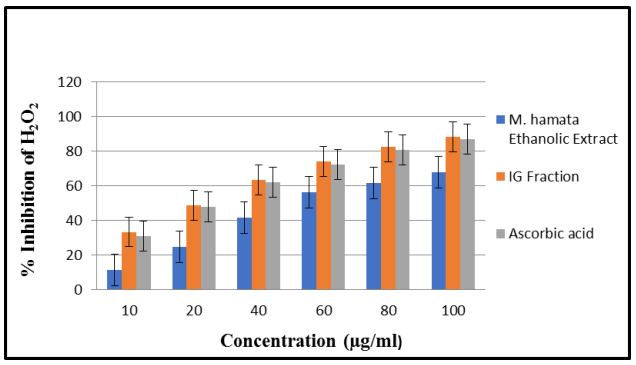

Figure 1.2: Comparison of H_2O_2 Radical Scavenging Activity of Ethanolic Extract and IG Fraction of *M. hamata* (whole plant) with Standard Drug

CONCLUSION

The present study concluded that the crude ethanolic extract and its fraction obtained from the whole plant parts i.e. leaves, stems, seeds and flower of *Mimosa hamata* exhibit interesting antioxidant capacity. The obtained results show that the ethyl acetate and di ethyl ether fraction (IG) contained the highest amount of phenolics compounds and exhibited great antioxidant activities. It can also be concluded that *Mimosa hamata* extract can be used as a good source of natural antioxidant as well as in pharmaceutical applications.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan), for providing the laboratory facilities used in this study.

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Cite this article as:

Richa Saxena *et al*. The study of phenolic compounds and antioxidant potential of crude extract and fractions of *Mimosa hamata*. Int. Res. J. Pharm. 2017;8(6):83-86 http://dx.doi.org/10.7897/2230-8407.086101

Source of support: Nil, Conflict of interest: None Declared