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Research Article

PHENOLIC CONTENT AND *IN VITRO* ANTIOXIDANT ACTIVITY OF *MILLINGTONIA HORTENSIS* LINN

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ABSTRACT
The present study was designed to evaluate the antioxidant potential of *Millingtonia hortensis* Linn. (*M. hortensis*) is an important medicinal tree in Indian traditional system of medicine. In this study, the various extract of stems of *M. hortensis* was investigated by qualitative preliminary phytochemical test and designed to screen antioxidant potential of various extracts of *M. hortensis* stems by determining Nitric oxide (NO) radical, Superoxide (SO) radical, Hydrogen peroxide (OH) radical scavenging and anti-lipid peroxidation activity. Ascorbic acid was used as a standard drug. Present study revealed that *M. hortensis* stems have significant antioxidant potential when compared with standard. Our finding suggested that among comparative significance of various extracts, the methanolic extract of *M. hortensis* stems having better efficacy and significant activity. The present study enlighten the antioxidant potential of this plant and also helps to support the traditional medicinal claim and believes of this plant in therapeutics and highlighted profound potential of *M. hortensis* stems to be investigated for bioactive compounds responsible for antioxidant effect for their antioxidant activity.

Keywords: *Millingtonia hortensis* Linn, Total phenolic content, Free radical scavenging potential.

INTRODUCTION

A very tall tree, *Millingtonia hortensis* Linn (*M. hortensis*) belongs to family "*Bignoniaceae*" commonly known as 'Akas Nim' has been a great medicinal value in Southern Asia, ranging from India, Burma, Thailand and Southern China. In folklore medicine, the stems of *M. hortensis* are used as antipyretic, sinusitis, cholagogue and tonic. Dried flower is a good lung tonic and used in the cough diseases. Bark is used to produce yellow dye.[1, 2]. Literature reports suggested that stems contains hispidulin, scarotene, dinatin, rutinoside. Bark having bitter substances and tannins. Flowers showed presence of hispidulin[3], scutellarein, scutellarein-5-galactoside[4], hortensin[5], Cornoside, recimic rengyolone, rengyoside B, rengyol, rengyoside A and iso rengyol[6], Millingtonine[7]. Plant has reported for pharmacological actions like apoptosis and antipoliferation activity[8, 9], mutaginicity and antimutaginicity[10], antifungal[11] and anticonvulsant activity[12]. Flowers & leaves showed antiasthmatic and larvicidal activity respectively[13, 14]. Flowers and leaves both reported for antimicrobial activity[15, 16]. Although the plant has great medicinal value in the treatment of various diseases, still preliminary reports was found on this plant. Therefore, present investigation was planned to find out scientific evidences and data for evaluation of phenolic content and antioxidant potential of stems of *M. hortensis.*

MATERIALS AND METHODS

Plant materials

The stems of the plant, *M. hortensis* were collected at Rajapur area, Sangamner, Maharashtra in May 2015. The plant was authenticated and herbarium deposited at the Department of Botany, S. N. Arts, D. J. M. Commerce & B. N. S. Science College, Sangamner, Ahmednagar, Maharashtra, India under voucher specimen number HRS/143. The stems of the plant were dried, powdered and passed through

40 mesh sieve and stored in an airtight container for further use.

Extraction & Preliminary phytochemical analysis

The air-dried stems of *M. hortensis* were made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried[17]. The preliminary phytochemical screening of various extracts of *M. hortensis* stems was carried out by performing qualitative chemical test[18].

Estimation of phenolic content

100 mg of extract was added into 40 ml ethanol and then was mixed and sonicated for about 30 min and shaked about 10 min. The volume was made up to 100 ml with HPLC grade water. Mixed well, this solution was filtered with No.1 Whatman paper. An aliquot of this solution was mixed with 0.5 ml of Folin-Ciocalteu Phenol reagent. After 5 min, 1.5 ml of 20% sodium carbonate solution was added and the volume was made up to 10 ml with HPLC grade water. After 2 h, the solution was filtered with No.1 Whatman paper and the absorbance at 760 nm was recorded. The same solution without the extract solution was used as blank solution. The blank was similarly prepared without using any extract. The standard solutions were prepared and analyzed by the same manner using 20 mg of accurately weighted Gallic acid. The same solution without Gallic acid was used as the blank solution. Calculation of content of total phenols in percent was based on Gallic acid standard. Total Phenols % = Absorbance (sample) X Weight (standard) X 100/Absorbance (standard) X Weight (sample).[19]

***In- vitro* Antioxidant activity**

Nitric oxide radical scavenging activity

The reaction mixture (3ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the drug in different concentrations (10-100 µg/ml) was incubated at 25^0C for 150 minutes. At intervals samples (0.5 ml) of incubation solution were removed and 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test compounds, whereas ascorbic acid was taken as standard. The % of inhibition was calculated by using equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] x 100.[20, 21]

Superoxide anion radical scavenging activity

Superoxide radical (O_2^-) was generated from auto-oxidation of hematoxylin and was detected by an increase in absorbance at 560 nm in a spectrophotometer. The reaction mixture contains 0.1 M of phosphate buffer (pH-7.4), EDTA (0.1mM), hematoxylin (50µM) and incubated at 25ºC for different time periods. Inhibitions of auto-oxidation of hematoxylin by crude, boiled extracts over the control were measured. The absorbance at 560 nm is measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] x 100.[22, 23]

Hydrogen peroxide radical scavenging activity

All solutions were prepared freshly. A Solution of Hydrogen Peroxide (20 mM) was prepared in Phosphate buffer Saline (PBS) (pH 7.4).Various concentration of the extract or standard in methanol (1 ml) were added to 2 ml of Hydrogen Peroxide Solution in PBS. After 10 min the absorbance was measured at 230nm. After cooling, the absorbance was read at 230 nm against a blank (containing only buffer and deoxyribose). The absorbance read at the end of the experiment was used for the calculation of the % inhibition. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] x 100.[24]

Thiobarbituric acid-reactive substance (TBARS) assay

The peroxide formation was measured in the reaction mixture contained rat liver homogenate (0.1 ml, 25%, w/v) in Tris-HCl buffer (20 mM, pH 7.0), KCl (150 mM), ferrous ammonium sulphate (0.8 mM), ascorbic acid (0.3 mM) , was incubated for 1 hr at 37 0C (14; 15). The incubated reaction mixture (0.4 ml) was treated with 0.2 ml of 8% sodium dodecyl sulphate (SDS), thiobarbituric acid (1.15 ml, 8%) and acetic acid (1.5 ml, 20%, pH 3.5). The total volume was then made upto 4 ml by adding

distilled water and kept in a water bath at 100 0C for 1 hr. After cooling, 1ml of distilled water and 5ml of a mixture of n-butanol: pyridine (15:1 v/v) was added and shaken vigorously. The absorbance of the organic layer was measured at 560 nm using UV-Visible spectrophotometer after centrifugation. The % inhibition of lipid peroxide formation was determined by comparing the results of the extract and control samples. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] x 100.[25, 26]

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. The values are expressed as mean ± SEM and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Present study includes extraction of stems of *M. hortensis* showed presence of extractive values of petroleum ether, methanolic, ethyl acetate soluble, ethyl acetate insoluble and aqueous extract were found to be 3.75%, 8.0%, 3.8%, 4.2%, 8.44% w/w respectively. Preliminary Phytochemical examination of various extracts of stems of *M. hortensis* reveals the presence of carbohydrates, glycosides, flavonoids tannins and phenolic compounds in methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract. Aqueous extracts also showed presence of proteins. Petroleum ether extract shows positive result for the phytosterol.

Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom.[27] Polyphenols are the products of plant metabolism and can range from simple molecules to highly polymerized compounds. Phenolics display a vast variety of structures; here only flavonoids, tannins and phenolic acids are reviewed. Flavonoids, a subclass of polyphenols, are the most common polyphenolic compounds found in nature and are further divided into several subclasses including flavones, flavonols, isoflavones, anthocyanins, flavanols, and proanthocyanidins. Flavonoids and other plant phenolics are especially common in stems, flowering tissues and woody parts such as the stem and bark.[28]

Phenolic content of petroleum ether, methanolic, ethyl acetate soluble fraction of methanolic extract, ethyl acetate insoluble fraction of methanolic extract and aqueous extracts were found to be 16.00%, 24.00%, 28.00%, 28.00%, 38.00%, 32.00% respectively (Table 1). The calibration curve (Concentration Vs Absorbance) of total phenolic content of standard gallic acid is indicated. (Figure 1)

In the present investigation, the various extracts of stems of *M. hortensis* were evaluated for its antioxidant potential. Antioxidant screening showing nitric oxide radical scavenging activity is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.[21]

The various extracts of *M. hortensis* stems showing hydroxyl radical scavenging potential. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen upon heating with thiobarbituric acid (TBA) at low pH. When the test extracts were added to the reaction mixture, they removed hydroxyl radicals from the sugar and prevented their degradation.[29] TBARS were determined by an indicator phospholipid peroxdation and a measure of the extent of DNA and deoxribo damage.[30] Measuring the color of thiobarbituric acid reactive substances (TBARS) formed at the end of the reaction. Malonaldehyde (MDA) which is formed as end product in lipid peroxidation react with thiobarbituric acid (TBA) to give TBARS which is pink in color.[31]

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH which convert active oxygen molecules into non-toxic compounds. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation.[32]

The various extracts of *M. hortensis* stems showed significant inhibition and free radical scavenging potential when compared with standard ascorbic acid and simultaneously reduced lipid peroxidation which revealed that significant decrease in MDA level in extracts groups. Among the various extracts of *M. hortensis* stems, methanolic extract showed significant inhibition and better anti-lipid peroxidation property. Hence the results are

comes to suggested that among the various extracts of *M. hortensis* stems, methanolic extract is better efficacious and having significant antioxidant potential (Table 2 & 3).
Thus, it can be concluded that, present study gives some scientific evidences on effect of extraction solvents was made to find out the therapeutically better efficacious extract. Therefore, after screening among comparative significance of various extracts, the methanolic extract of *M. hortensis* stems having better efficacy and significant antioxidant activity. The present study also support the traditional believes of this medicinal plant and highlighted profound potential of *M. hortensis* stems to be investigated for bioactive compounds responsible for antioxidant effect.

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Table 1: Total phenolic content of various extracts of *M. hortensis* stems

Sr. No.	Extract	Total Phenolic Content
1	Petroleum ether	16.00%
2	Ethyl acetate soluble	24.00%
3	Ethyl acetate insoluble	28.00%
4	Aqueous	32.00%
5	Methanol	38.00%

Table 2: *In Vitro* Antioxidant activity of *M. hortensis* stems

Group	Treatment (Conc. μg/ml)	% Inhibition			
		NO radical scavenging	SO radical scavenging	Hydrogen peroxide radical scavenging	Anti-lipid peroxidation
I	Petroleum ether extract	0.3515 ± 0.009**	0.3255 ± 0.012**	0.2575 ± 0.009ns	0.4066 ± 0.008*
II	Methanol extract	0.3563 ±0.010**	0.3992 ± 0.015**	0.2618 ± 0.012**	0.3989 ±0.011**
III	Ethyl acetate soluble extract	0.5285 ±0.016**	0.5051 ± 0.018***	0.5324 ± 0.016**	0.5017 ±0.015ns
IV	Ethyl acetate insoluble extract	0.4113 ±0.02**	0.3478 ± 0.017**	0.4245 ± 0.02**	0.4491 ±0.02ns
V	Aqueous extract	0.3568 ±0.024**	0.2756 ± 0.021*	0.3562 ± 0.024**	0.3964 ±0.023ns
VI	Standard Ascorbic acid	0.2562 ±0.029***	0.2050 ± 0.024***	0.2520 ± 0.028**	0.1760 ±0.028**

Values are expressed as mean ± SEM, n=6
When Group (VI) compared with Group (I, II, III, IV and V)
*P<0.05, **P<0.01, ***P<0.001, ns- non significant

Table 3: Data showing IC 50 Values of various extracts of *M. hortensis* stems

Group	Treatment (Conc. μg/ml)	IC 50 Values			
		NO radical scavenging	SO radical scavenging	Hydrogen Peroxide	Anti-Lipid peroxidation
I	Petroleum ether extract	65	121	187	60
II	Methanol extract	65	121	187	60
III	Ethyl acetate soluble extract	65	121	187	60
IV	Ethyl acetate insoluble extract	65	121	187	60
V	Aqueous extract	65	121	187	60
VI	Standard Ascorbic acid	65	121	187	60

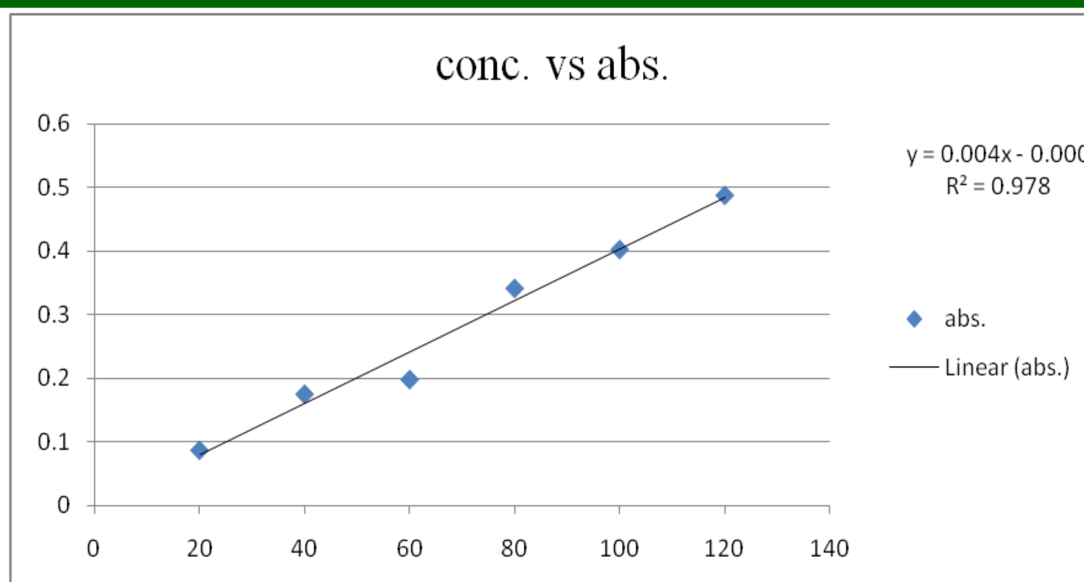

Fig. 1: Calibration curve of total phenolic content of Gallic acid

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