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Research Article

ASSESSMENT OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF AQUEOUS STEM BARK EXTRACT OF *HOLOPTELEA INTEGRIFOLIA* PLANCH

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ABSTRACT

Objective: The aim of this study was to evaluate the in vitro antioxidant potentials and phenolic content of aqueous extract of *Holoptelea integrifolia* Planch (HAE) stem bark.

Methods: The antioxidant activity of aqueous extract of *Holoptelea integrifolia* was determined by the method of DPPH radical scavenging activity, nitric oxide scavenging activity, superoxide scavenging activity and its reducing power ability assays.

Results: The total phenolic content of aqueous extract of *H. integrifolia* was found to be 61.73±0.23 mg GAE/g. The DPPH radical scavenging activity of aqueous extract (10, 20, 40, 60, 80, 100 µg/ml) was increased in dose dependent manner, which was founded in the range 13.14-55.17% inhibition compared to Ascorbic acid 22.56-93.68 % inhibition. The IC 50 value of plant extract for scavenging free radical were 77.10, 74.95, 86.78 µg/ml DPPH, nitric oxide, superoxide, respectively while IC50 of ascorbic acid were 33.70, 24.96, 48.59 µg/ml respectively. The reducing power activity of aqueous extract of plant increased with increase in concentration.

Conclusions: Based on the results, the present study revealed that the aqueous extract from *H. integrifolia* contained ample amount of phenolic compounds due to which, showed significant stronger degree of antioxidant activity in different invitro test system in a dose dependent manner.

Keywords: Holoptelea integrifolia, Total phenolic content, Free radical, Antioxidant, Superoxide.

INTRODUCTION

Free radicals and reactive oxygen derived species (ROS) produced by cellular metabolism and exogenous agents in the cells, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. Tissue damage resulting from an imbalance between ROS-generating and scavenging systems has been implicated in the pathogenesis of as variety of disorders, including degenerative disorders of the CNS, such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, cataract, AIDS and aging[1]. Antioxidants, which can inhibit or delay the oxidation of an oxidisable substrate including proteins, lipids, DNA and carbohydrates by inhibiting the initiation or propagation of oxidative chain reaction .These systems of antioxidants prevent these reactive species from being formed or remove them before they can damage vital components of the cell, would therefore seem to be very important in the prevention of these diseases [2-8]. Therefore, substance which is containing properties of antioxidants have been used as possible treatments of these disorders [9,10]. Medicinal plants with a high content of bioactive compounds such as phenolic acids, flavonoids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity [11].

Holoptelea integrifolia Planch (Hindi-chilbil, Sanskrit-chirivilva and English-Indian Elm Tree) belongs to the family Ulmaceae. It is a medium sized glabrous, deciduous tree that reaches the height of about 15m to 25m. The stem bark is of whitish or yellowish grey, exfoliating in irregular flakes and with an offensive smell when freshly cut [12]. Leaves are simple, alternate, elliptic-ovate, acuminate, base rounded or subcordate in shape. It bears greenish yellow flower. Fruit a one seeded samara, light brown, obliquely elliptic or orbicular. The flowering time of the tree is January to February, whereas, fruiting is seen in April to May [13]. It is an important pollen allergen of India and sensitizes almost 10% of the atopic population in Delhi [14]. The plant is common roadside tree and native to Asia-Tropical region including India, Nepal, Sri Lanka, Indo-China, Cambodia, Laos, Myanmar, Vietnam, Burma and China [15]. The stem bark contains the triterpenoidal fatty acid esters, holoptelin-A (epi-friedelinol palmitate) and holoptelin- B (epi-friedelinol stearate), friedelin and epi-friedelinol [16]. Recently in some investigations have been reported on this plant in which antiviral activity [17], antioxidant, antimicrobial & wound healing activity [18] and antiemetic activity [19] is important. In Indian traditional medicine, this plant is used for the treatment of inflammation, gastritis, dyspepsia, colic, vomiting, intestinal worms, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism [20]. Its bark and leaves are used as bitter, astringent, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, rheumatism and in urinary astringent [21]. The seeds paste and stem bark is externally used in treatment of ringworm, eczema and cutaneous affections [22]. This plant has been used for the treatment of obesity, edema, bronchitis [23], and also possess ovipositor deterent activity and protease inhibitor activity [24]. The boiled juice of bark is reported to be useful as an external application against rheumatism [25], intestinal tumors [26] and is oxytoxic in pregnancy [27]. In this study, we evaluated the antioxidant activity of HME employing various in vitro assay systems, such as DPPH, superoxide, nitric oxide radical scavenging, reducing power, in order to understand the usefulness of this plant as a foodstuff and in medicine.

MATERIALS AND METHODS

Chemicals

Nitroblue tetrazolium (NBT), ferrozine and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (Steinheim, Germany). Ascorbic acid, o-phosphoric acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, was purchased from Merck, Mumbai, India. Gallic acid, potassium ferricyanide, sodium carbonate, sodium nitroprusside, trichloroacetic acid (TCA), and ferric chloride were purchased from SD fine chemicals, India. All other reagents were of analytical grade.

Collection of plant material-

The bark of *H. integrifolia* were collected in May 2011 District, Varanasi, India and identified by Prof. Dr. K.N. Dwivedi. A voucher specimen was deposited in the herbarium of the Faculty of Dravyagun, faculty of medicinal science B.H.U. Varanasi; herbarium code number: KND/JS09.

Preparation of the plant extract-

The air-dried bark of *H. integrifolia* Planch (100 g) were powdered and then extracted with 500 ml of aqueous of distilled water by

maceration process. The crude extract was filtered through Whatman No. 1 filter paper, and evaporated under reduced pressure to give a viscous dark mass with a percentage yield of 17.96% (w/w). The extract was stored at 4ºC for further use. This crude extract was dissolved in water or solvent and used for the assessment of antioxidant activity.

Determination of total phenolic content

Determination of total phenolic content was performed according to the method of using Folin-Ciocalteu reagent [28]. Briefly, 0.5 ml of each extract was mixed with 5 ml of Folin-Ciocalteu reagent (1:10 with distilled water), and then 4 ml of 1 M Na_2CO_3 was added to the mixture and the mixture was allowed to stand for 15 min at room temperature. The absorbance of the mixtures was measured at 765 nm. A standard curve was prepared by using gallic acid in various concentrations (50, 100, 150, 200, and 250 mg/ml). All measurements were carried out in triplicate and the results were expressed as gallic acid equivalents per gram of dry weight (mg GAE/g dry weight). Results represented as mean ± standard deviation.

DPPH radical scavenging activity assay

DPPH free radicals scavenging activity was measured according to the procedure described by Blios [29]. The HAE at different concentration (10-100µg/ml) were added with 3ml of .1mM methanolic solution of DPPH. The mixture was shaken and allowed to react in dark at 37 ˚C for 30 minute. After 30 min. incubation, reduction of DPPH was determined at 517 nm using spectrophotometer. The experiment was carried out in triplicate. Methanol is served as blank and ascorbic acid as standard. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound. The DPPH radical scavenging activity was calculated by using the following formula

% inhibition = (Ac - As / Ac) ×100

Where Ac was the absorbance of the control (blank, without extract) and As was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Nitric oxide scavenging activity assay

The procedure is based on the method, where 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 Greiss reagent [30]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of aqueous extract of *H. integrifolia* dissolved in methanol and incubated at room temperature for150 min. After the incubation period, 0.5ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the blue chromophore formed was read at 546 nm.

% inhibition = (Ac - As / Ac) ×100

Where Ac was the absorbance of the control (blank, without extract) and As was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Superoxide anion ($O_2^{\cdot-}$) radical scavenging activity

Measurement of superoxide anion scavenging activity of ethanol and water extracts was based on the method described by Liu [31]. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the radiation of NBT. In this experiment, the superoxide radicals were generated in 3ml of Tris-HCl buffer (100mM, pH 7.4) containing 0.75ml of NBT (300µM) solution, 0.75ml NADH (936µM) solution and 0.3ml of different concentration (10-100µg/ml) of sample extract. The reaction was started by adding 0.75ml of PMS solution (120µM) to the mixture. The reaction mixture was incubated at 25˚C for 5min and the absorbance was measured at 560nm against blank sample. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated by using the following formula.

% inhibition = (Ac - As / Ac) ×100

Where Ac was the absorbance of the control (blank, without extract) and As was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu [32]. 1 ml of the different concentration (100-1000µg/ml) extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 30 min. After cooling the mixture, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the upper layer was pipette out and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%) was added. The absorbance was measured at 700 nm. These reducing powers of extract compare with standard ascorbic acid. The intensity of reducing power is directly proportional to the absorbance of the reaction mixture.

RESULTS AND DISCUSSION

Total phenolic content

Recently there has been increase much interest in the potential of Plant bioactive components as antioxidants useful for preventing various oxidation stress related loss. Phenolic compounds is one of the largest and most ubiquitous group of plant metabolites have ability to scavenge free radical due to presence of an aromatic ring bearing one or more hydroxyl constituents. Phenolic compounds widely found in the secondary products of medicinal plants, as well as in many edible plants. many reports showed that plants which bearing more phenolic content show the good antioxidant activity by which there is direct correlation between total phenol content and antioxidant activity [33,34].The mean of total phenol content per mg of the *H. integrifolia* was founded to be 61.73 ±0.23 mg gallic acid equivalent of phenols

DPPH scavenging activity assay

The DPPH scavenging activity of aqueous extract of *H. integrifolia* (HAE) stem bark was compared with ascorbic acid. The HAE significantly exhibited dose dependent inhibition of DPPH activity and the scavenging activity of the extract and antioxidants increase with increase concentration. The aqueous extract of stem bark at different concentration (10-100µg/ml) showed antioxidant activity in a concentration dependent manner (13.14, 25.63, 38.95, 45.55, 52.25, 55.17% inhibition) respectively in DPPH radical scavenging activity (Fig.1). The percentage inhibition of HAE and standard ascorbic acid at higher concentration (100µg/ml) was 55.17and 93.68µg/ml % inhibition) respectively. The IC 50 value of HAE and standard in DPPH radical scavenging assay exhibited 77.10µg/ml and 33.67µg/ml respectively (Table.1). The results were found to be statistically significant ($P < 0.05$) by using PRISM software.

Nitric oxide scavenging activity assay

The HAE exhibited dose dependent inhibition of nitric oxide with IC50 value 74.95µg/ml. Ascorbic acid as standard 24.96µg/ml (Table.1). HAE at different concentration (10-100µg/ml) showed nitric oxide inhibition in concentration dependent manner 18.21, 29.79, 35.67, 44.21, 52.90, 59.40% inhibition) respectively (Fig. 2). Plant bark extract shows lesser nitric oxide scavenging activity than standard. The percentage inhibition of HAE and ascorbic acid at higher concentration (100µg/ml) 59.40 and 91.25 µg/ml. Nitric oxide was generated from sodium nitroprusside and measured by Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide [35] which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of

nitric oxide compete with oxygen leading to reduce the production of nitric oxide. NO is short lived free radical essential as bioregulatory molecule. It plays an important role as a pleiotropic mediator of physiological processes like smooth muscle platelet aggregation and regulation of cell mediated toxicity [36] but when present in high concentration they mediate toxic effect like DNA fragmentation, cell damage and neural cell death [37]. The results were found to be statistically significant ($P < 0.05$) by using PRISM software.

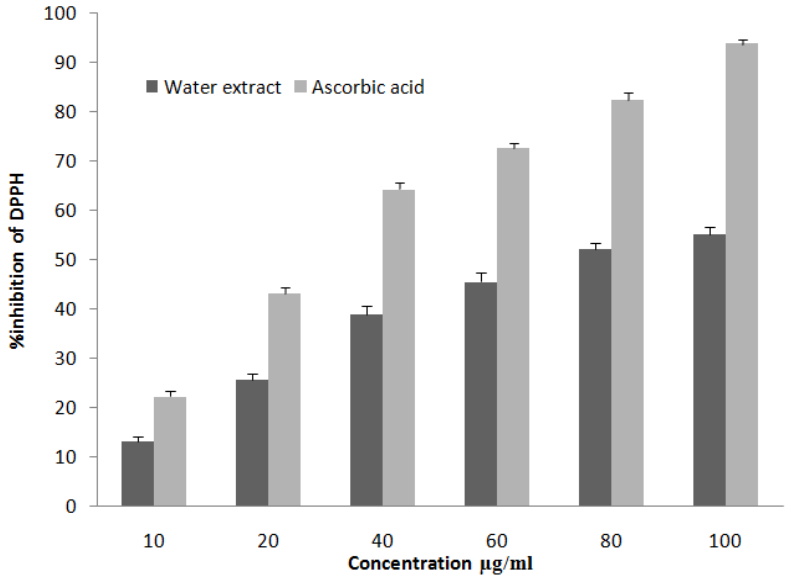

Fig. 1: DPPH radical scavenging activities of the aqueous stem bark extract of *H. integrifolia* (HAE). Ascorbic acid was used as the positive control.

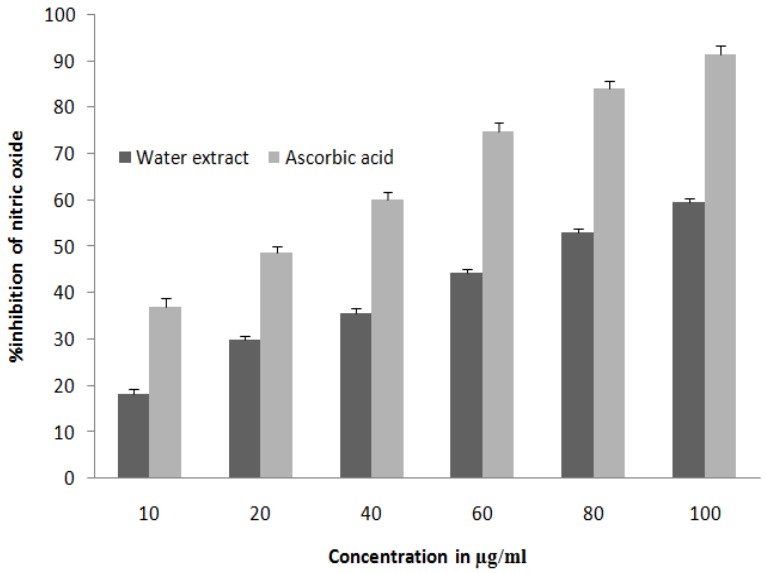

Fig. 2: Nirtic oxide radical scavenging activities of the aqueous stem bark extract of *H. integrifolia* (HAE). Ascorbic acid was used as the positive control.

Superoxide

The superoxide anion radical is one of the strongest reactive oxygen species. It ,play an important role in formation of the other reactive oxygen species such as singlet oxygen, hydrogen peroxide, hydroxyl radical, which can cause damage to the DNA, lipids, proteins leading to various disease [38,39]. The extract of HAE was assayed by The PMS-NADH system. At different concentration, HAE (10-100µg/ml) exhibited stronger superoxide inhibition activity (8.005, 16.74, 26.55, 38.96, 51.23, 56.74% inhibition) respectively. The superoxide scavenging activity of HAE and ascorbic acid increased in dose dependent manner. At the highest concentration of HAE and ascorbic acid (100µg/ml), percentage inhibition of superoxide radical observed was 56.74µg/ml and 80.92µg/ml, respectively. The superoxide scavenging activity of HAE is low than Ascorbic acid. The half inhibition concentration (IC50) value of HAE and Ascorbic acid are 86.78µg/ml and 48.59µg/ml respectively (Table .1). These results suggested that HAE extract showed significant effect on scavenging capacity towards the superoxide anion radical. The results were found to be statistically significant ($P < 0.05$) by using PRISM software.

Reducing power activity

The reducing power capacity HAE showed in figure (Fig. 4). Reducing power increased with increase in concentration. The result suggest that HAE showed the lesser reducing power than the standard ascorbic acid. The reducing capacity of compound is generally depends of reductones [40], which causes breaking of the free radical chain by donating a hydrogen atom and exhibited

antioxidant potential [41]. Reductones are also reported for preventing peroxide formation by react with certain precursor of peroxide [42]. In reducing power assay the substances (antioxidant), which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocynide (Fe^{2+}) then react with ferric chloride to form the ferric ferrous complex through electron transfer ability and with it change in color form yellow to bluish that has an absorption maximum at 700nm.The intensity of color depend on potential of the compounds (antioxidants) present in the absorption; consequently will be the antioxidant activity [43]. The reducing power of the HAE and ascorbic acid at the higher concentration (1000µg/ml) are 0.43 and 1.48 respectively. Therefore results suggest that reducing power of HAE is lower than standard ascorbic acid.

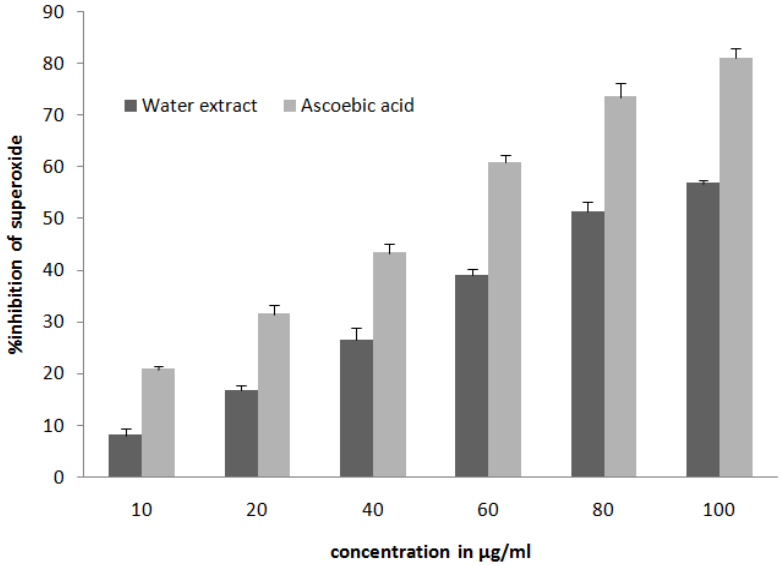

Fig. 3: Superoxide anion scavenging activities of aqueous stem bark extract of *H. integrifolia* (HAE). Ascorbic acid was used as the positive control.

Table 1: Effects of aqueous extract of stem bark of *Holoptelea integrifolia* (HAE) on different radical scavenging activity.

	DPPH radical oxide scavenging activity	AA	Nitric radical Scavenging activity	AA	Superoxide radical scavenging activity	AA
IC50 values of HAE	77.10	33.67	74.95	24.94	86.78	48.59

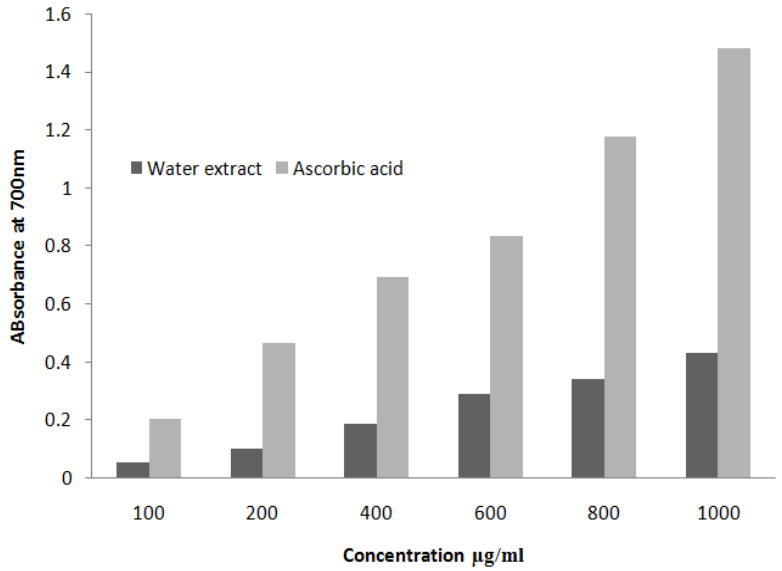

Fig. 4: Reducing power activities of the aqueous stem bark extract of *H. integrifolia* (HAE). Ascorbic acid was used as the positive control.

CONCLUSION

The worldwide increasing much interest in finding antioxidant from medicinal plant, because it is becoming increasingly apparent that antioxidants are important in health and disease prevention. Based on the results, the resent study demonstrated that the aqueous extract from *H. integrifolia* contained high amount of phenolic compounds due to which, showed significant stronger degree of antioxidant activity in different invitro test system in a dose dependent manner, which might be helpful in preparing or slowing the progress of various oxidative-

stress induced diseases by inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as a reducing agent. The antioxidant activity was linearly correlated with the amount of total phenolic compounds present in the respective extract in each assay. Therefore the observation suggests that natural bioactive constituents of *H. integrifolia* might be a potential antioxidant supplement for application in food and as a drink. These findings may help in the further research in the investigation of individual compound in invivo antioxidant activity and the different antioxidant mechanisms and also develop their application in food and pharmaceutical industries.

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