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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS-ETHANOL EXTRACT OF AERVA LANATA (L.) JUSS EX SCHULT (AMARANTHACEAE)

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ABSTRACT

Aerva lanata (L.) root of the family Amaranthaceae is a Indian medicinal plant used traditionally for the management of Raktavikara, Sopha (Sotha), Dusta Vrana. The drug Gorakṣagañja is a good diuretic and the roots are given in dysuria (mūtra krichhra) and calculus (aśmari) (Halliwell, 2008). It is used for alleviating diseases caused by kapha and vāta doṣ. The roots are used (particularly in southern India) as an effective diuretic drug specially for calculus, as substitute or a source plant of Pāṣāṇabhēda. Studies that show the pharmacological basis for some of such uses have been reported. There is, however, no scientific report on its antioxidant activity of aqueous ethanol extract to the best of our knowledge. This study was therefore aimed at investigating the antioxidant Activity of aqueous ethanol extract of *Aerva lanata*. The antioxidant activity of aqueous ethanol extracts of root was evaluated by various in vitro antioxidant assays such as evaluation of reducing power, DPPH radical scavenging activity, nitric oxide scavenging activity, superoxide anion scavenging activity. These results conclude that *A. lanata* root possesses high antioxidant activity and can be used for the development of natural and safe antioxidant compounds.

INTRODUCTION

Antioxidants, molecules with a radical-scavenging capacity, are thought to exert a protective effect against free radical damage (Lindley, 1998). The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several present-day diseases and means to delay aging symptoms (Halliwell, 2008). These biomolecules may contribute to the prevention of many chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, diabetes, asthma, hepatitis and arthritis (Middleton *et al*., 2000). Oxidizing agents may damage a number of biological molecules such as nucleic acids, membrane lipids, enzymes, or synovial fluid polysaccharides. Secondary metabolites from medicinal plants function as small molecular weight antioxidants, but their particular mechanisms of action are variable, and depend both on a structure and environment (Halliwell, 2008).

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"Aerva lanata (Linn) Juss.ex Schult" of Amaranthaceae family is an important medicinal plant, found throughout tropical India as a common weed in fields and waste land[4]. It is commonly identified and known as Gorakshaganja in Ayurveda system of medicine. It is considered as one among the few botanical sources of Pashanabheda. The plant is extensively used in urinary disorders like Ashmari (Urinary calculi), Mootrakrichra (Dysuria), Mootravikara etc by most of the Ayurveda and Siddha practitioners in southern India, in the name of Pashanabheda. As the plant bears almost all the properties similar to that of the original source of Pashanabheda[5]. The plant is diuretic, used in lithiasis. The root is demulcent, diuretic, useful in strangury (slow to be and painful discharge of urine). The roots are used in the treatment of headache. The plant is regarded as a demulcent on the Malabar Coast (Anonymous, 1959). It is called as Gorakshaganja, Satkabhedi, Aadaanpaak in Sanskrit, Kapurijadi or Gorakhabooti in Hindi and Mountain knot grass in English (Nagaratna *et al*., 2014). A. lanata plant has many medicinal properties due to the presence of numerous secondary metabolites. Sitosteryl palmitate, hentriacontane, β-sitosterol and its D-glucoside, α-amyrin and betulin were

isolated from the whole plant (Aiyar *et al*., 1973; Chandra, 1990). The plant is reported for a number of pharmacological activities viz., anthelmintic, demulcent, anti-inflammatory,diuretic, expectorant, hepatoprotective and nephroprotective, anti-diabetic, anti-hyperglycaemic , anti-microbial, cytotoxic, urolithiatic, hypoglycemic, anti-hyperlipidemic, anti-parasitic and anti -helmentic activities[10-20]. In the recent years, the interest is centered on antioxidant derived from Ayurvedic medicines in view of their medicinal benefits. In view of this, we selected Gorakṣaganja (*Areva lanata*) root to assess the *in –vitro* antioxidant study.

MATERIALS AND METHODS

Material collection and identification

Gorakshaganjja (*Aerva lanata* (L.) root was collected from the Ambikapur district, Chhattisgarh. Taxonomic identification of collected material was done in the Raw Materials Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication And Information Resources (CSIR-NISCAIR), Dr. K. S. Krishnan Marg, New Delhi (Ref. No. NISCAIR/RHMD/CONSULT/2015/2885/78).

Instruments

Shimadzu UV-VIS Spectrophotometer (1240) was used for all spectrophotometric studies. Remi R24 centrifuge was used for centrifugation.

Chemicals and reagents

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Company, U.S.A. Folin-Ciocalteau reagent and NBT were obtained from Sisco Research Laboratories, Mumbai. Ascorbic acid and BHT were obtained from Himedia Lab. Ltd., Mumbai, India. All other chemicals were of Analytical Grade.

Preparation of the extract

The powdered plant material was extracted with Aqueous Ethanol (80%) using Soxhlet apparatus. The solvents were then removed under reduced pressure which obtained sticky residues. All the dried extracts were dissolved in 99% Ethanol and distilled water, respectively for various studies.

Estimation of Total Phenolic Compounds

The method of Naczk (Shirwaikar, 2014) was followed. 0.1ml of 10mg/ml aqueous solution of the extract was diluted with 46 ml of distilled water in an Erlen meyer flask. Afterwards 1 ml of Folin–Ciocalteu Reactive (FCR) was added into this mixture followed by addition of 3 ml of Na_2CO_3 (2%) after 3 min. Subsequently mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. The concentration of total phenolic compounds was determined as micrograms of pyrocatechol equivalent by using the equation that was obtained from the standard pyrocatechol graph.

Absorbance = 0.001 X Pyrocatechol (μg) + 0.0033

Evaluation of reducing power

The reducing power of the extract was determined according to the method of Oyanzu (Vetrichelvan, 2002).

The plant extract (50-500μg) in 1 ml of distilled water was mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% Potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. Following incubation 2.5 ml of 10% Trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated by the method of Nagai[17]. The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol and 0.3 ml of extract solution of concentrations varying from 50 μg to 500 μg/ml. The solutions were rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (1 mM) was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. The percent (%) radical scavenging was calculated by the following equation.

% DPPH radical-scavenging = $[(A_c - A_s) / A_c] * 1oo$.

Where Ac = Absorbance of control at 517 nm and
As = Absorbance of sample at 517 nm.

Nitric oxide Scavenging Activity

The method of Sreejayan (Dulaly, 2002) was followed. For the experiment Sodium nitropruside (10 mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in Methanol and incubated at room temperature for 2½ h. The same reaction without the sample but equivalent amount of Methanol served as control. After incubation period 0.5 ml of Griess reagent was added. Absorbance of the chromophore formed was measured at 546nm. Ascorbic acid was used as positive control. The procedure is based on the principle that Sodium nitropruside solution spontaneously generates Nitric oxides which reacts with oxygen to produce nitric ions that can be estimated using Greiss reagent. Scavengers of nitric oxides compete with oxygen leading to reduce production of nitric ions.

Superoxide anion scavenging activity

The assay for super oxide ion scavenging activity was performed as per standard procedure Beuchamp[19] was followed. The reaction mixture contained 50 mM phosphate buffer (pH 7.6), 20 μg riboflavin, 12 mM EDTA and 0.1mg/ml of NBT (nitro blue tetrazolium) all added in chronological sequence. Reaction was started by illuminating the reaction mixture containing different concentrations of the sample extract for 90 sec and then measuring the absorbance at 590 nm. Ascorbic acid was taken as the positive standard.

RESULTS

Amount of total phenolic compounds:-As shown in Table-1 the plant extract have good amount of phenolic compounds.

***Reducing power**:-The reducing powers determined by the present assay depend on the redox potentials of the compounds present in the extract of the plant characterized by the complexity of their constituents. As shown in Table-2, reducing power of the extract was shown. BHT was taken as a positive control. All the results are comparable with the standard.*

***DPPH radical scavenging activity**:-As shown in Table-3, DPPH decolorization was increased by the plant extract in a concentration dependent manner. The extract at a concentration of 500 µg was able to scavenge 50.66 % of DPPH free radical. All the results are comparable with Ascorbic acid used as standard.*

***Nitric oxide Scavenging Activity**:-The percent inhibition of NO-scavenging as shown inTable- 4, proves the extract of the plant inhibits the reaction in a concentration dependent manner. At a concentration of 50µg/ml the extract was able to produce 53.33 % inhibition. All the results are comparable with Ascorbic acid which was able to scavenge 77.33% NO-free radical at the same level of concentration.*

***Assay for Super oxide ion Scavenging Activity**:-We studied the extract of the plant for their ability to scavenge super oxide ion. The extract was able to scavenge the super oxide ion up to 50 % at the concentration of 37.5µg/ml. The Ascorbic acid was used as standard which was able to scavenge up to 58% at the same concentration. All the results are comparable with the standard.*

Table 1. Total phenolic content of Aqueous Ethanol extract of *Aerva lanata* (linn.) juss root

Extracts	Pyrocatechole Equivalents
Aqueous Ethanol extract	28.7±3.15

Table-2. - Reducing activity of Aqueous Ethanol extracts of *Aerva lanata* (linn.) juss (root)

Concentration (µg/ml)	Absorbance	
	Extract	BHT
50	0.117±0.003	0.264±0.019
100	0.249±0.0029	0.340±0.019
250	0.282±0.0027	0.621±0.022
500	0.316±0.0029	0.717±0.010

n=3, values are Mean ± S.E.M

Table-3. DPPH-Radical scavenging activity of Aqueous Ethanol extracts of *Aerva lanata* (linn.) juss root.

Concentration (µg/ml)	Absorbance	
	Ethanol Extract	Ascorbic acid
50	9±1.15	64.33±3.53
100	14.33±1.45	78.33±1.76
250	28.33±2.40	89.33±2.60
500	50.66±1.20	95.66±1.20

n=3, values are Mean ± S.E.M

Table 4. Nitric oxide scavenging activity of Aqueous Ethanol extracts of *Aerva lanata* (linn.) juss (root). n=3, values are Mean±SEM

Concentration (µg/ml)	%of inhibition	
	Ethanol Extract	Ascorbic acid
4.17	15.66±2.40	27.66±1.765
8.33	24.66±1.76	40.33±1.454
16.67	36.32±2.41	51.33±2.029
33.33	43±2.51	66.33±2.188
50	53.33±2.40	77.33±1.858

Table- 5: Superoxide radical scavenging activity of Aqueous Ethanol extracts of *Aerva lanata* (linn.) juss (root). n=3, values are Mean±SEM

Concentration (µg/ml)	%of inhibition	
	Ethanol Extract	Ascorbic acid
12.5	14.66±1.20	21.66±2.029
18.75	18.33±1.45	26.33±0.882
25	27.33±1.76	32±1.734
37.5	50±3.21	58±1.734

n=3, values are Mean±SEM

DISCUSSION

In the present study the extract of the plant showed antioxidant activity. In spite oxygen is essential for life its transformation to reactive oxygen species (ROS) may provoke uncontrolled reactions. The free radicals are involved in the etiology of several degenerative diseases and various inflammatory diseases (Ames, 1993). Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and some other mechanism (Gillman, 1977).The extract which is showing significant antioxidant activity might be helpful in slowing the progress of various oxidative stress related diseases. Our experimentation further support to the local use of the plant as anti-inflammatory and in the treatment of rheumatoid arthritis because the involvement of ROS as mediators of tissue damage in patients with rheumatoid arthritis (Cimen *et al*., 2000; Thabrew *et al*., 2001; Taysi *et al*., 2002). Phenols are very important plant constituents because of their free radical scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans (Tsao *et al*., 2005). In the extract a very good amount of pyrocatechol equivalent was detected. The reducing property of the extract, compared with BHT is shown. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of the samples increased with increased amount of concentration. All of the samples showed higher activity than control and these differences were statistically very significant. DPPH scavenging assay is an important assay to determine the antioxidant activity of the plant extracts in in vitro model. DPPH is free radical which is reacting rapidly with the antioxidant compounds. The antioxidative compounds can donate a hydrogen atom to DPPH and change the color. The intensity of color is measured calorimetrically. The increasing intensity of color is directly proportional to the inhibition of DPPH. The present study shows the increasing concentration of the extract inhibit the activity of DPPH.

Nitric Oxide is a free radical which is formed from sodium nitroprusside and it reacts with oxygen to form nitrite. The antioxidant activity was measured by the inhibition of the nitrite formation, this was done by the plant extracts which directly reacts with oxygen, nitric oxide and other nitrogen compounds. The present study proves that the increasing concentration of the extract have a maximum inhibitory activity against the nitric oxide (Marcocci *et al*., 1994). Hydrogen peroxide is an important reactive oxygen species because it may be toxic if it is converted into hydroxyl radicals in the cells. The antioxidant compounds which donates the electrons to H_2O_2, and neutralize it into water molecule. The present study proves the inhibition of hydroxyl radicals from H_2O_2 in the concentration dependent manner2 (Gulcin *et al*.,

2003; Mathew, 2006). Superoxide dismutase is an important enzyme in an antioxidant defense system. SOD converts the superoxide anion into hydrogen peroxide and thus reduces the toxic effect. The percentage of inhibition of superoxide by SOD may reduce the cellular damages. The present study proves that the increasing concentration of the extract have a maximum inhibitory activity of SOD (Curtis, 1972). The probable mechanism of scavenging the super oxide anions may be due to the inhibitory effect of the sample towards generation of super oxide in the in vitro reaction mixture. Therefore the herbal formulations based on the plant extract can be used for the prevention and treatment of oxidative stress related disorders such as cancer, vascular disease and rheumatism.

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