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Invitro Antioxidant Activity of *Argemone mexicana* Roots

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ABSTRACT : AIM: The aim of this study was to investigate the antioxidant effect of Argemone mexicana Linn roots. The antioxidant activity was evaluated by various antioxidant assays, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and hydrogen peroxide scavenging method. The antioxidant activities were compared to standard antioxidant ascorbic acid.
Argemone mexicana root extract showed a significant antioxidant activity in DPPH, ABTS and H2O2 scavenging methods. The findings of the present study suggest that Argemone mexicana Linn root could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.
KEY WORDS: *Argemone mexicana*, root, antioxidant activity.

INTRODUCTION

Oxygen derived free radicals, such as the superoxide anion and hydroxyl radicals are cytotoxic and promote tissue injury. Antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals. Furthermore although medicinal plants are used as 'antioxidants' in traditional medicine, their claimed therapeutic properties could be due, in part, to their capacity for scavenging oxygen free radicals.

Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals, hydroxyl radicals, singlet oxygen and non-free-radical species such as hydrogen peroxide are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors. ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn on the attention of many experimental works. In living organism, various ROS can form by different ways. Normal aerobic respiration stimulates polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides [1, 2, 3]. ROS can cause lipid peroxidation in foods, which leads to the deterioration of the food [4, 5]. In addition, it is well known that ROS induce some oxidative damage to biomolecules like lipids, nucleic acids, proteins, amines, deoxyribonucleic acid and carbohydrates. Its damage causes ageing, cancer, and other many diseases [6, 7]. As a result of this, ROS have been implicated in more than hundred diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer. ROS are continuously produced during normal physiologic events, and removed by antioxidant defence mechanisms [8]. There is a balance between generation of ROS and antioxidant system in organisms. In pathological condition, ROS are

overproduced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in cellular membrane or intracellular molecules [9]. Various endogenous antioxidant defence mechanisms play an important role in the elimination of ROS and lipid peroxides to protect the cells against toxic effects of ROS and lipid peroxides [10]. Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers [11, 12]. Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenecity. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods [13].

Argemone mexicana (Linn), Papaveraceae is commonly known as Mexican poppy or prickly poppy. The plant is pantropic in distribution and it is a weed in waste places. It is distributed throughout the hotter parts of India. The whole plant, roots, leaves, stem, flowers are extensively used in traditional system of medicine for various ailments like leprosy, malaria, jaundice, rheumatism, pain, inflammation, skin diseases, fever, piles, warts, dysentery, tumors and worm infestations [14,15,16]. The plant is known to possess antimalarial [17], antimicrobial [18], antibacterial [19] and antifungal [20] activities. Hence in the present study the ethanolic extract of roots were examined for its antioxidant activity.

EXPERIMENTAL

MATERIALS AND METHODS

Plant material

Roots of *Argemone mexicana* were collected in July, 2008, from the waste lands in the villages of Coimbatore, Tamil Nadu, India. The identification was done by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. Voucher specimen (No.BSI/SC/5/23/08-09/Tech) was deposited in the Herbarium of the Department of Pharmaceutical Chemistry, J. K. K. Nataraja College of Pharmacy, Komarapalayam, Tamil Nadu, India.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma Aldrich Co, St Louis, USA. Ascorbic acid and rutin were obtained from SD Fine Chemicals Ltd., Mumbai, India. Methanol and dimethyl sulphoxide were obtained from Ranbaxy Laboratories Ltd., Punjab, India. Hydrogen peroxide (30%) was obtained from Qualigen Fine Chemicals, Mumbai, India. All chemicals used were of analytical grade.

Preparation of the extract

The roots were cleaned, dried under shade and ground into powder. The coarse powder material was extracted with ethanol 95% using soxhlet extractor by hot continuous extraction method. The extract was concentrated to dryness under reduced pressure and controlled temperature (40 °C – 50 °C). The dried extract was used for further studies.

Phytochemical studies

Freshly prepared Argemone mexicana root extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol.

DPPH radical scavenging activity

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of various concentrations of the extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ºC for 30 min. Absorbance was measured at 490 nm using ELISA reader. IC_{50} value is the concentration of the sample required to scavenge, 50 % DPPH free radical [21]. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

ABTS radical cation decolourisation assay

ABTS (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm. The assay was performed in triplicate [22, 23].

Scavenging of hydrogen peroxide

It can be generated through a dismutation reaction from superoxide anion by superoxide

dismutase. It can generate the hydroxyl radical in the presence of metal ions and superoxide anion [24].

$$O_2 + H_2O_2 \rightarrow OH^- + OH^+ + O_2$$

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide [25].

RESULTS

The preliminary phytochemical investigation revealed the presence of carbohydrates, flavonoids, alkaloids, tannins and steroids.

The antioxidant activity of AM root was evaluated by DPPH, ABTS and Hydrogen peroxide radical scavenging methods. AM root showed a dose dependent scavenging activity and free radical inhibition of DPPH, ABTS and H_2O_2 comparable to free radical scavenging activity of ascorbic acid.

Table 1. DPPH Scavenging activity of ethanolic extract of Argemone *mexicana* roots

Sample	Concentration mcg/mL	Percentage Inhibition	IC_{50}
Ethanolic Extract	20	29.21 ± 2.14	45.21
	40	47.53 ± 1.85	
	60	59.21 ± 2.46	
	80	74.72 ± 2.68	
	100	87.17 ± 1.45	
Ascorbic acid			18.53

n=3, values are mean ± S.D

Table 2. ABTS Scavenging activity of *Argemone mexicana* roots

Sample	Concentration mcg/mL	Percentage Inhibition	IC_{50}
Ethanolic Extract	20	16.12 ± 2.14	56.24
	40	29.15 ± 1.31	
	60	55.21 ± 2.42	
	80	69.15 ± 2.21	
	100	75.27 ± 1.91	
Ascorbic acid			34.24

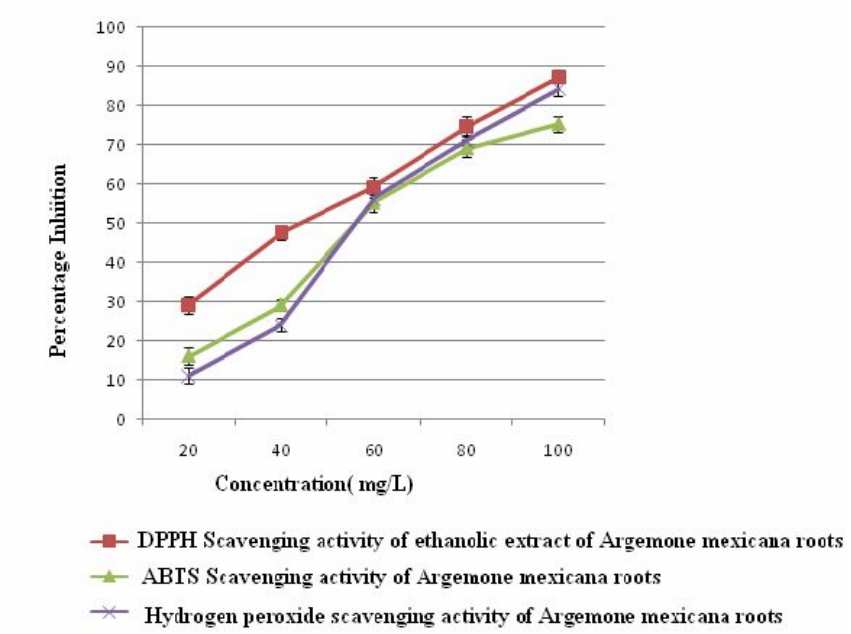
n=3, values are mean ± S.D

Table 3. Hydrogen peroxide scavenging activity of *Argemone mexicana* roots

Sample	Concentration mcg/mL	Percentage Inhiition	IC_{50}
Ethanolic Extract	20	11.16 ± 2.12	56.02
	40	24.12 ± 1.62	
	60	56.25 ± 1.12	
	80	71.15 ± 1.24	
	100	84.25 ± 1.62	
Ascorbic acid			21.41

n=3, values are mean ± S.D

Figure 1. Free radical scavenging activity of Argemone mexicana root extract at different concentrations by DPPH Scavenging activity, ABTS Scavenging activity and H_2O_2 Scavenging activity.



DISCUSSION

Reactive oxygen species are continuously formed in cells as consequence of oxidative biochemical reactions and external factors. However they become harmful when they are produced in excess under certain abnormal conditions such as inflammation, ischemia and in presence of iron ions. Under these conditions the endogenous antioxidants may be unable to counteract ROS formation. Reactive oxygen species formed may cause cellular damage and this damage may involve in etiology of diverse human diseases. Exogenous antioxidant supplement is helpful to overcome this severe problem of free radicals, which may scavenge these free radicals.

The free radical scavenging activity of natural compounds can be evaluated through their ability to quench the synthetic free radicals, in which the absorbance of the reaction mixture is taken in visible range to know whether the compound is having antioxidant activity.

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. DPPH is relatively stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing

agent as a result of which electron become paired off forming the corresponding hydrazine. The solution therefore looses color stoichometrically depending on the number of electrons consumed which is measured spectrometrically at 517 nm [26]. From the results it may be postulated that the root extract of Argemone mexicana have hydrogen donors, thus scavenge the free radical DPPH.

ABTS assay is relatively recent one, which involves a more drastic radical, chemically produced and, is often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The solubility in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS radical for the estimation of the antioxidant activity. The principle behind the technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation ($ABTS^{+}$) a blue green chromogen. In the presence of antioxidant reductant, the colored radical is converted back to colorless ABTS, the absorbance of which is measured at 734nm. The root extract of Argemone mexicana possessed an antioxidant activity with IC50 value being 56.24 mcg/ml, suggest the free radical scavenging activity of Argemone mexicana root extract.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H2O2 can probably react with $Fe^{2+}$and possibly $Cu^{2+}$to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H2O2 by root extract of Argemone mexicana may result from its antioxidant and free radical scavenging activity.

The results obtained in the present study indicate that Argemone mexicana root extract exhibits free radical scavenging activity. The overall antioxidant activity of Argemone mexicana root extract might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that Argemone mexicana Linn root could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

CONCLUSION

The results obtained in the present study indicate that Argemone mexicana root extract exhibits free radical scavenging activity. The overall antioxidant activity of Argemone mexicana root extract might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that Argemone mexicana Linn root could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

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