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Antioxidant, anti-inflammatory, and adaptogenic activity of *Asparagus acutifolius* extract

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SUMMARY

Although many species of Asparagus have been studied scientifically and shoots are used in the diet of Sardinians, there is very little literature available on the medicinal uses of *Asparagus acutifolius* Linn. The acetone-ethanol (1:1) extract was screened for antioxidant, anti-inflammatory and adaptogenic activities. The extract showed good anti-oxidant activity in DPPH, hydroxyl radical, and nitric oxide radical assays. The extract also exhibited anti-inflammatory activity in the carrageenan-induced rat paw edema and adaptogenic activity in the milk induced leucocytosis assay in rats. The results of the present study suggest need to investigate other pharmacological activities of *Asparagus acutifolius*.

Key words: *Asparagus acutifolius*; antioxidant; antiinflammatory; adaptogenic; DPPH

INTRODUCTION

Several plants have been shown to possess anti-oxidant activity. There exists a causal link between oxidative stress and various intractable diseases such as diabetes, aging, cancer, heart diseases, inflammatory diseases, etc. Although oxidative stress produces several free radicals exerting useful actions for the body in the immune system (Vuillaume, 1987), excess of these free radicals react with biological components and induce oxidative disorders, leading to heart diseases, diabetes, and cancer (Slater, 1987). Various investigations have shown that antioxidant substances are useful in treatment of these diseases (Block, 1992; Noda *et al*., 1997). Excess supply of oxygen generates endogenous reactive oxygen species (ROS) imbalance with formation of hydroxyl and superoxide radicals (Halliwell and Gutterdge, 1998). Plants contain many substances like phenolics and steroidal principles that possess antioxidant property (Hochstein and Atallah, 2002). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also have metal chelation potential (Rice *et al*., 1996).

In addition to the ROS, the excess nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS) is also implicated in the development of a number of diseases. Due to the absence of any natural specific enzymatic defence system *in vivo*, the consumption of certain foods which exhibit selective suppressive ability as regards NO overproduction might boost the host's protective effects against

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NO-mediated toxicity. Spices, rich in phenolics, are speculated conceivably to act as potential NO-scavengers or iNOS suppressors.

Crude extracts of fruits, herbs, vegetables and other plant materials rich in phenolics are increasingly of interest in the nutrition industry because they retard oxidative degradation of lipids and thereby improve nutritional value of food (Lolinger, 1991). The antioxidants have been found useful in treatment of many conditions. Bhattacharya *et al*. (1997) have suggested that the antioxidant effect of active principles of *W. somnifera* may explain, at least in part, the reported antistress, immunomodulatory, cognition-facilitating, anti-inflammatory and anti-aging effects produced by them in experimental animals, and in clinical situations. Excessive stress or nervous debility aggravates symptoms of many diseases and this can be reduced by use of anti-stress herbs, which facilitate adaptation to stress. The reversal of milk-induced leucocytosis has been regarded as a suitable animal model to evaluate the adaptogenic (antistress) activity (Brekhman and Dardymove, 1969).

The preliminary phytochemical studies indicated presence of triterpenes, flavonoids and phenolics in the shoots of *Asparagus acutifolius* L. (Liliaceae), a plant which is commonly used in the diet of Sardinian people. The flavonoids have been identified as rutin, isorhamnetin-3-D glucoside and luteolin (Panova *et al*., 1984). Recently, Lacaille-Dubois *et al*. (2007) have reported two new steroidal saponins from *Asparagus acutifolius*. Very little information is available on the medicinal uses of this plant. The infusion of shoots is useful as a diuretic (Pieroni *et al*., 2004). We therefore evaluated the free radical scavenging activity, adaptogenic activity, and anti-inflammatory activity of acetone-ethanol extract of Asparagus acutifolius.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh young shoots of *Asparagus acutifolius* L. (Family: Liliaceae) were obtained from the market and were authenticated at the Department of Botanical Sciences, University of Cagliari, Italy. The specimen was preserved in the Herbarium section of the department (Voucher No. 1409). Two hundred grams of fresh young shoots were macerated with acetone: ethanol (1:1) for one week and the extract was concentrated in dark under reduced pressure. The extract (12.2 g) was dried in dark and stored in amber coloured bottle in refrigerator.

Animals

Male Albino rats and mice were purchased from National Toxicology Centre, Pune, India and were housed under standard conditions of temperature and light. Animals had free access to food and water. They were deprived of food 6 hour before and during the experiments. The Institutional Animal Ethics Committee approved the protocol of the study.

Chemicals

All the chemicals required for analysis of free radicals were of analytical grade obtained from Hi-Media and Qualigens, Ltd. India. Gallic acid was a generous gift by Prof. S. C. Pal, MVP's College of Pharmacy, Nashik, India.

Phytochemical Studies

The extract was subjected to the identification of phytoconstituents as suggested by Harborne (1974).

Determination of Total Phenolics

Total phenolics content was determined colorimetrically using phosphomolybdic-phosphotungstic acid reagents as described by Singleton and Rossi (1965). The experiment was repeated thrice.

Evaluation of Free Radical Scavenging Activity

DPPH assay

1 mg extract powder was dissolved in 1 ml of 50% ethanol solution to obtain 1000 μg/ml sample solution. The solution was diluted with 50% ethanol to

contain 25 μg, 50 μg, 100 μg, 200 μg, 500 μg, and 750 μg in 0.05 ml. In each reaction, the solutions were mixed with 1 ml of 0.1 μM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 μM Tris-HCl buffer (pH 7.4), and 0.05 ml samples at room temperature and kept aside for 30 min. 50% ethanol solution was used as control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. L-ascorbic acid was used as positive control. The inhibition ratio (percent) was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of test sample)/absorbance of control] x 100%. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve as described by Yokozawa *et al*. (1998).

Scavenging of nitric oxide

The ethanolic extract was dissolved in PBS in different concentrations and sodium nitroprusside was added (5 μM) in each tube and tubes were incubated at 25 °C for 5 h. Control experiments without test compounds were carried out with identical conditions. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess Reagent. The absorbance was taken at 546 nm. Experiment was repeated for three times. (Sreejayan and Rao, 1997).

Scavenging of hydroxyl radical

The hydroxyl scavenging activity was determined using the method of Lopes *et al*. (1999). Briefly, reactions were started by addition of Fe^{2+} (6 μM final concentration) to solutions containing 5 mM^{2-} deoxyribose, 100 mM H_2O_2 and 20 mM phosphate buffer (pH 7.2). Mannitol (50 mM) was used as antioxidant standard. To measure hydroxyl radical scavenging activity, different concentrations of extract were added to the system before addition of Fe^{2+}. Reactions were carried out for 15 min at room temperature and were stopped by addition of 4% phosphoric acid (v/v) followed by 1% thiobarbituric acid (TBA, w/v, in 50 nM NaOH). Solutions were boiled for 15 min at 95 °C, then cooled at room temperature. The absorbance was measured at 532 nm and results were expressed at IC_{50}. The experiment was repeated thrice.

Evaluation of anti-inflammatory activity

Male albino rats weighing 125 - 150 g were divided in 4 groups, each containing 5 animals. The rats received orally, vehicle (Distilled water), diclofenac sodium (10 mg/kg i.p.), *Asparagus acutifolius* extract, 100 mg/kg and 200 mg/kg, 60 min prior to the subplantar injection of 0.1 ml of 1% carrageenan in the right paw. The paw volume was recorded at 0, 1, 2, and 3 h after carrageenan using plethismometer (UGO BASILE, Italy). The inhibition of edema was measured at 3h after carrageenan (Winter *et al*., 1962).

Evaluation of adaptogenic activity: Milk-induced leucocytosis

Mice were divided into four groups each containing five animals. Blood samples were collected from retroorbital plexus. Total leukocyte count was carried out for each group using Neubauer's Chamber. Animals were treated with vehicle, diazepam (1 mg/kg i.p.), the Asparagus acutifolius extract (100 and 200 mg/kg i.p.), followed by subcutaneous injection of milk (boiled and cooled, 4 ml/kg). After 24 h the leukocyte count was repeated as described by Brekhman and Dardymove (1969).

RESULTS

Phytochemical studies

The chemical tests indicated that the *Asparagus acutifolius* extract contains phenolics, triterpenes, and flavonoids.

Determination of Total Phenolic Content

The acetone: ethanol extract contained 110 ± 2.6 mg/gm of the phenolic compounds.

Free radical Scavenging activity

DPPH Assay

The IC_{50} values of *Asparagus acutifolius* extract was found to be 550 µg/ml. The IC_{50} values of ascorbic acid was found to be 50 µg/ml.

Scavenging of nitric oxide

The IC_{50} values of *Asparagus acutifolius* extract was found to be 1 mg/ml. The IC_{50} value of ascorbic acid was found to be 0.16 mg/ml.

Scavenging of hydroxyl radical

The IC_{50} of *Asparagus acutifolius* extract was found to be 125 µg/ml whereas that of mannitol was 182 µg/ml.

Evaluation of anti-inflammatory activity

In the vehicle treated rats the volume of edema was 1.45 ml at the third hour. Diclofenac sodium reduced the paw edema by 60%, whereas the extract of *Asparagus acutifolius* (100 and 200 mg/kg) reduced the edema by 25% and 38% respectively. The observations are given in Table 1.

Evaluation of adaptogenic activity: Milk induced leucocytosis

The leukocyte count in the milk treated mice was 6750 ± 227.76/cubic mm. Diazepam significantly decreased the leukocyte count from to 2533 ± 48.23 cubic mm, whereas, *Asparagus acutifolius* extract (100 and 200 mg/kg) reduced the leukocyte count to 4922.5 ± 102.73, 4332.5 ± 102.73 cubic mm respectively ($P < 0.05$).

DISCUSSION

The results obtained in the present study clearly reveal the antioxidant activity of acetone: ethanol extract of *Asparagus acutifolius* extract. This effect of extract could be due to the phenolics, as observed by several other researchers (Ninfali *et al*., 2005; Shah *et al*., 2005). As compared to the reference standards, the free radical scavenging activity of the *Asparagus acutifolius* was very low. The anti-inflammatory activity at the dose of 200 mg/kg was only 38%. The adaptogenic (anti-stress) activity of the extract was very low compared to that of diazepam.

Phenolics have been reported to have a capacity to scavenge free radicals. They are ubiquitously present in edible and non-edible plants and have multiple biological effects (Kahkonen *et al*., 1999). The *Asparagus acutifolius* extract contained 110.0 ± 2.6 mg/gm phenolics. The results of the present study suggest that the effectiveness of the antioxidant activity of *Asparagus acutifolius* extract is probably related to their phenolic contents and the observed antioxidant activities of the extract may be due to the hydroxyl groups in phenolics. The antioxidant activity of the *Asparagus acutifolius* extract was found to be commensurate with that of other extracts having higher amount of phenolics, when compared with the antioxidant activity of vitamin C (Hsu, 2006). Although a study on 92 plant extracts containing phenolics clearly indicated that the antioxidant activity cannot be predicted on the basis of its total phenolic content as there is a lack

Table 1. Effect of *Asparagus acutifolius* on carrageenan-induced rat paw edema

Treatment of edema at (mg/kg)	Volume of edema in ml (mean ± S.E.M.)				% inhibition
	0 h	1 h	2 h	3 h	3 h
Vehicle	0.9 ± 0.08	1.5 ± 0.07	1.85 ± 0.07	2.35 ± 0.09	--
Diclo (10)	0.9 ± 0.08	0.95 ± 0.09*	1.15 ± 0.07*	1.48 ± 0.07*	60.0
Aa (100)	0.88 ± 0.08	1.63 ± 0.09	1.85 ± 0.09	1.98 ± 0.09*	25.0
Aa (200)	0.9 ± 0.09	1.49 ± 0.09	1.56 ± 0.07*	1.77 ± 0.08*	38.6

n = 5, *$P < 0.05$ compared to vehicle treated group.
Aa = *Asparagus acutifolius*; Diclo = Diclofenac sodium.

of correlation between the phenolic contents and antioxidant potency (Kahkonen *et al*., 1999), Kim *et al*. (2003) have shown a good correlation between total phenolics and vitamin C equivalent antioxidant capacity at the high level of $P < 0.001$.

DPPH is a stable radical that has been widely utilized to appraise the antioxidant activity of various natural products (Yakozawa *et al*., 1998; Hsu, 2006). In this study, *Asparagus acutifolius* extract exhibited DPPH scavenging activity. In cellular oxidation reactions, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents (Liu *et al*., 2000). The cellular damage resulting from hydroxyl radicals is strongest among free radicals. Hydroxyl radicals can be generated by biochemical reaction. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of transition metal ions such as iron and copper or by ultraviolet photolysis. Hydroxyl radicals can attack DNA to cause strand scission (Liu and Ng, 2000; Hsu, 2006). The scavenging of hydroxyl radicals by *Asparagus acutifolius* extract suggests that it may inhibit DNA damage.

Excess NO produced by iNOS is implicated in the development of a number of diseases. Due to the absence of any natural specific enzymatic defence system *in vivo*, the consumption of certain foods which exhibit selective suppressive ability as regards NO overproduction might boost the host's protective effects against NO-mediated toxicity. Spices, rich in phenolics, are speculated conceivably to act as potential NO-scavengers or iNOS suppressors (Tsai *et al*., 2007). Nitric oxide plays an important role in regulation of vascular permeability and carcinogenesis. It has been shown that nitric oxide scavengers inhibit vascular permeability in solid tumors (Maeda *et al*., 1994). Thus the nitric oxide scavenging activity of *Asparagus acutifolius* extract may be useful in preventing diseases secondary to excessive nitric oxide formation.

Jiang and Dusting (2003) have reported that phenolic compounds have potential role in inflammatory conditions. Satya Prasad *et al*. (2004) have further shown that phenolics inhibit polymorphonuclear lipoxygenase, an enzyme involved in inflammatory conditions. Menon and Sudheer (2007) have reported anti-inflammatory activity of curcumin. The phyotochemical analysis of *Asparagus acutifolius* indicated presence of triterpenes. It is reported that triterpenes possess anti-inflammatory activity (Liu, 1995). This justifies the anti-inflammatory activity of *Asparagus acutifolius* extract in the carrageenan-induced rat paw edema. *Asparagus acutifolius* extract significantly reduced milk-induced leucocytosis, complying with the requirement of adaptogenic activity. It is worthwhile to note that several herbs having nitric oxide scavenging activity have shown adaptogenic and anti-inflammatory potential (Jagetia *et al*., 2004).

Kiefer *et al*. (2004) have shown that supplementation with mixed fruit and vegetable juice concentrates increased serum antioxidant and folate in healthy adults. Taken together, this study indicates that *Asparagus acutifolius* extract clearly has anti-inflammatory, adaptogenic, and antioxidant effects. Combination of *Asparagus acutifolius* with other vegetables and fruits may further enhance these activities.

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