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Original Article

In vitro antioxidant activities of root extract of *Asparagus racemosus* Linn.

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ABSTRACT

Objective: The purpose of the study is to investigate potential of antioxidant property of ethanolic root extract of *Asparagus racemosus* Linn (EEAR).
Methods: *In vitro* evaluation antioxidant property of EEAR was done using various methods like DPPH scavenging activity, hydroxyl radical scavenging activity, and nitric oxide scavenging activity. HPTLC fingerprint analysis was performed for qualitative determination of possible number of components from the ethanolic extract. Acute toxicity study was performed in Wistar rat and an OECD guideline 423 was followed.
Results: The yield value was found 0.96% from EEAR. A concentration of 468.57 ± 3.002 μg/ml of probable antioxidant material from EEAR was required to scavenge 50% of DPPH. The IC_{50} value of EEAR were found to be 508.17 ± 7.37 μg and 416.57 ± 5.08 μg when determined by hydroxyl radical and nitric oxide scavenging assay respectively. The reducing powers of EEAR was 0.295 ± 0.0037 at 125 μg/ml and increased to 0.934 ± 0.0005 at 500 μg/ml. HPTLC fingerprint data supports several basic informations like isolation, purification, quality evaluation and standardization. No sign of toxicity was observed after treated with 2000 mg/kg of EEAR.
Conclusion: The obtained data highlight the potential role of EEAR as a source of natural antioxidants.

1. Introduction

Free radicals can defined as some free entities having one or more unpaired electrons which play a vital role in the development of various human diseases including aging.[1] They can generate as a by-product from various endogenous (like normal cellular metabolism) or may be exogenous (like irradiation) processes. Free radicals can easily react with reactive oxygen species (ROS) and make themselves active radical. ROS are different activated forms of oxygen and of two distinct types, free activated oxygen radicals (like superoxide anion radical O_2^-, hydroxyl radical OH^-) and non-free activated oxygen radicals (like hydrogen peroxide H_2O_2, singlet oxygen $1O_2$). Human cells are exposed 10,000 oxidative hits every second by these activated ROS.[2]

Free radicals are well known for degrading food products, resulting in off taste and reduced shelf-life. Antioxidants are able to manage the degradation of food products by deactivating active free radicals thus act as preservative.[3] To minimize the deterioration, dietary products contain a pervasive amount of flavonoids, a class of polyphenols having strong antioxidant activity.[4]

Like Trolox Equivalent Antioxidant Capacity (TEAC) assay, several methods are established to identify most potent antioxidants by comparing antioxidant capacity of enormous compounds.

In chemistry, oxidation is a chemical reaction in which an electron is transfer to an oxidizing agent from any substance. An antioxidant (counter part of oxidant) is chemical substance which reduces the rate of a particular oxidation reaction in a definite extent, hence preventing oxidative damage to the cells and biochemicals. They can prevent uncontrolled production of ROS,

Abbreviations: AHRF, Asthagiri Herbal Research Foundation; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HPTLC, high performance thin layer chromatography; OECD, The Organisation for Economic Co-operation and Development.

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protein damage and DNA stand disruption and ensuring good health.[5,6] Development of diseases is highly co-related with oxidative damage by free radicals. In this regard, association of LDL oxidation in cardiovascular disease can be taken as example. This LDL oxidation by free radical acts as a precursor in various life threatening diseases like atherosclerosis, cardiovascular disease etc by triggering various chemical or enzymatic pathways.

According to scientists, antioxidant rich foods or dietary supplements can able to reduce the cell damage by free radicals. A regular diet of antioxidants from plants is very essential to maintain proper health as plants are rich source of organic antioxidant chemicals.[7] Certain diseases like aging can be slow down or prevent with an adequate diet of antioxidant rich food.

Keeping the advantage and importance of antioxidant in daily life, many nutraceutical companies have launched various forms of antioxidant (like carotene, vitamin C, vitamin E, selenium, resveratrol etc) as dietary supplement.

The principle aim of this research work is to find out antioxidant potential of EEAR i.e. ethanolic root extract of *Asparagus racemosus* Linn.

1.1. Plant introduction[8]

A. racemosus is growing at low altitude in shade and available throughout Asia, Africa, and Australia. In India, *A. racemosus* is used as traditional medicine by tribal people in various parts of the country. It is belongs to Asparagaceae family. Basically *A. racemosus* is a woody climber of average 1–2 m in height. Small, uniform and pine needles leaves are the unique characteristics of this plant with whitish coloured flowers. Shatawari, satamuli, vrishya are few of the names in Indian languages which is used to describe this miracle plant. The term “shatawari” means “curer of a hundred diseases”.

1.2. Traditional uses of A. racemosus Linn.

A. racemosus having anti-ulcer,[9] antitussive activity,[10] antineoplastic activity,[11] antistress effects,[12] immunomodulatory activities,[9] hypoglycemic, hypolipidemic,[13] antidiarrhoeal, antiseptic, bronchitis and hyperacidity,[14] immunoadjuvant and antilithiatic affect,[9] wound healing property.[15]

1.3. Phytochemical evaluation study

From literature survey it was proved that *A. racemosus* Linn. possesses shatvarin I to VI (Steroidal saponins),[16] asparanin A,[17] racemosol,[18] racemofuran,[19] flavonoids (kaempferol, quercetin, and rutin),[20] sitosterol,[21] diosgenin and quercetin 3-glucourbnides etc.[22,23]

2. Materials and methods

2.1. Collection of plant

The plant materials were collected from Kulasekharam of Kanyakumari district and authenticated by Prof. P. Jeyaraman Chief Botanist PARC, Chennai, India.

2.2. Preparation of plant extracts[24]

Air-dried roots (1.5 kg) were extracted (hot maceration) with absolute ethanol (3 L) at a temperature not exceeding 55 °C for 4 days and filtered. This filtrate was concentrated by rotary evaporator. This concentrated material was used for further study.

2.3. Source of chemicals

All the chemicals were of analytical reagent grade and purchased from Loba chemie, ACROS Organics, Merck lab, S.D. Fine chemicals, Fluke.

2.4. In vitro antioxidant evaluation

2.4.1. DPPH method

Free radical scavenging assay of ethanolic extract of *A. racemosus* (EEAR) was measured by DPPH method.[25]

DPPH percentage inhibition (%) = Abs (control) − Abs (sample)/Abs (control) × 100

2.4.2. Hydroxyl radical scavenging assay

The free radical scavenging capacity of EEAR was measured by hydroxyl radical scavenging method.[26]

Hydroxyl scavenging activity (%) = Abs (control) − Abs (sample)/Abs (control) × 100.

2.4.3. Nitric oxide scavenging assay

Nitric oxide scavenging method was used to determine the antioxidant activity of EEAR.[27]

Nitric oxide scavenging assay (%) = Abs (control) − Abs (sample)/Abs (control) × 100.

2.4.4. Total reducing ability

The reducing power of the root extract was determined by different concentrations of the ethanolic extract, phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%), trichloroacetic acid (10%), ferric chloride (1%).[28]

2.5. HPTLC analysis

HPTLC of EEAR samples were identified by AHRF method. The sample applicator used for the procedure was CAMAG Linomat IV. The applied sample was scanned by CAMAG TLC Scanner II.

Volume of sample loaded: 10 μl
Mobile phase: Chloroform:Acetic acid:MethanolWater (5:3.5:1.5:1)
λ_{max}: 254 nm
Lamp: Deuterium

2.6. Determination of toxicity

2.6.1. Experimental animals

Adult male Wistar rats weighing 150–180 g were used for the study. The animals were fed a standard laboratory diet and tap water *ad libitum*. The care and use of the animals were strictly in accordance to the guidance of the Institutional Ethical Committee (constituted under the guidelines Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Reg. No. – 1237/PO/C/2008/CPCSEA). After one week of habituation, animals were subjected to the experiments. Each animal was tested only once. All efforts were made to minimize animal suffering.

2.6.2. Acute oral toxicity study ($OECD^{423}$)[29–31]

For carrying out oral toxicity study OECD guidelines 423 was followed. It is a stepwise procedure with three animals per step. Depending on the mortality and/or morbidity of the animals a few

steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data. The method uses defined doses (2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system. The concentrated extract was dissolved in distilled water for oral administration. The starting dose of the plants extract was 2000 mg/kg bodyweight (p.o). The dose was administered to the mice which were fasted overnight with water *ad libitum* and observed for signs of toxicity symptoms like change in skin colour, salivation, diarrhoea, sleep, tremors, convulsions and also respiratory, autonomic and central nervous system effects.

2.7. Statistical analysis

Results were shown as mean ± S.D. for each group (where, number of each *in-vitro* antioxidant experiment, n = 3; number of experimental animal, n = 6). SPSS 9.0 for Windows (Chicago, IL, USA) software was used for statistical analysis. For multiple comparisons, one-way analysis of variance (ANOVA) was performed. In cases where ANOVA showed significant differences, Tukey test was performed. $P < 0.01$ was considered to be statistically significant.

3. Results

3.1. Percentage yield study

The percentage yield of the EEAR was found to be 0.96%

3.2. DPPH radical scavenging assay

Fig. 1 shows the antiradical activity of the root extract of EEAR. The scavenging activity was increased with the increasing concentrations (25–800 μg). IC_{50} value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of EEAR was found to be 468.57 ± 3.002 μg/ml. On the other hand ascorbic acid possesses IC_{50} value 45.27 ± 0.28 μg/ml.

3.3. Hydroxyl radical scavenging assay

Fig. 2 shows the antioxidant capacity of EEAR by scavenging the hydroxyl radical. The antioxidant activity was increased from 8.76 ± 0.59% at 25 μg/ml to 65.63 ± 0.61 at 800 μg/ml concentration. The IC_{50} value of EEAR was found to be 508.17 ± 7.37 μg/ml. By the same time ascorbic acid having 12.3 ± 0.73 μg/ml.

3.4. Nitric oxide scavenging assay

Fig. 3 shows the appreciable antioxidant activity by scavenging the nitric oxide. The value of the antioxidant assay increased from 9.54 ± 0.41% at 25 μg/ml to 66.57 ± 0.43% at 800 μg/ml. The IC_{50} value was found to be 416.57 ± 5.08 μg/ml. In this experiment, ascorbic acid showed IC_{50} value of 10.82 ± 1.02 μg/ml.

3.5. Total reducing ability

The result shows that the EEAR possesses antioxidant properties which could react with free radicals to stabilize and terminate radical chain reactions. The reducing powers of EEAR were 0.295 ± 0.0037 at 125 μg/ml and increased to 0.934 ± 0.0005 at 500 μg/ml (Fig. 4). The standard drug ascorbic acid shows the reducing power 1.63 at 500 μg/ml.

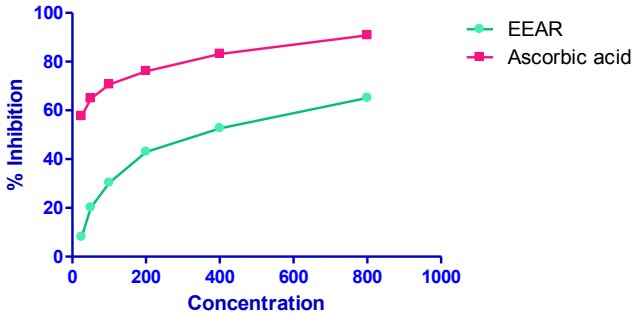

Fig. 1. Free radical scavenging activity of EEAR by DPPH method.

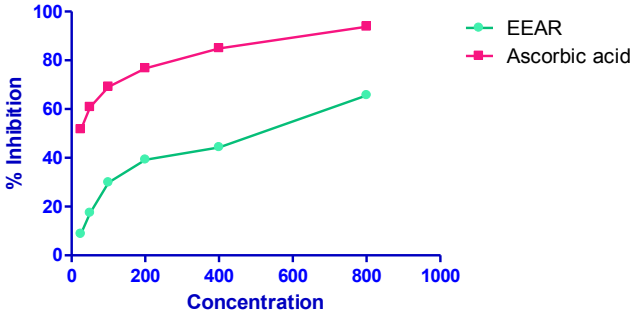

Fig. 2. Free radical scavenging activity of EEAR by hydroxyl radical scavenging method.

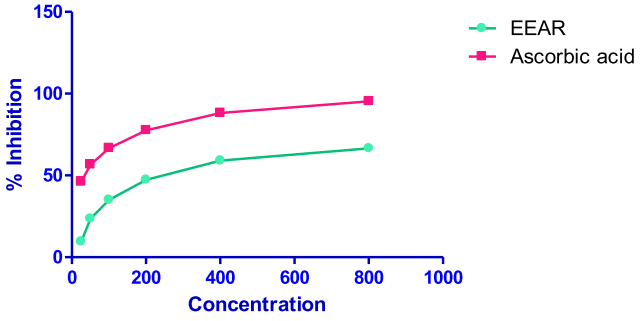

Fig. 3. Nitric oxide scavenging activity of EEAR.

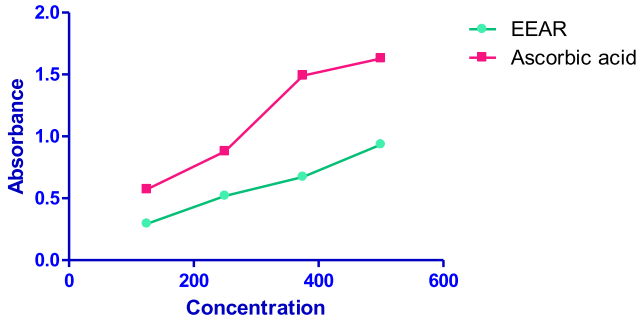

Fig. 4. Determination of reducing power of EEAR.

3.6. HPTLC analysis

The chromatographic profiles of the EEAR were performed on silica gel 60F254. The plate using Chloroform:Acetic acid:-Methanol:Water (5:3.5:1.5:1) as mobile phase was given in Fig. 5. Thin layer chromatography profiles revealed 2 distinct spots under

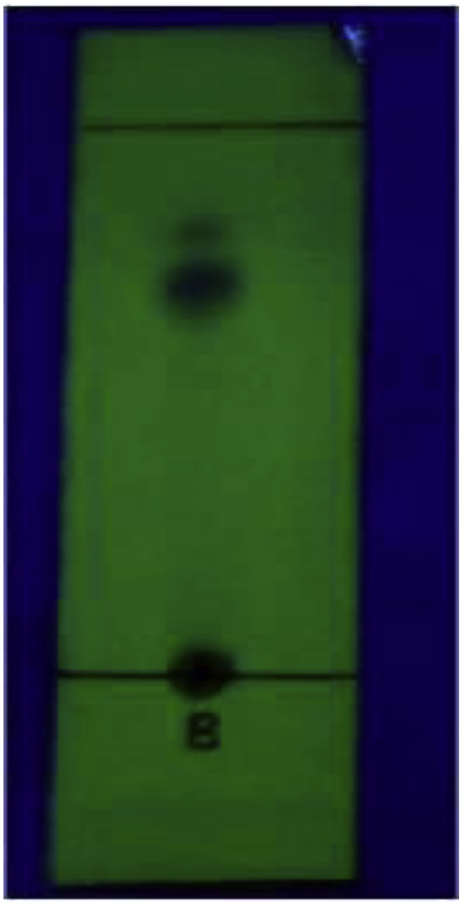

Fig. 5. TLC chromatogram of EEAR.

UV (254 nm). This was again confirmed in HPTLC chromatogram (Fig. 6) where The *Rf* values and the peak area percentage were observed and given in Table 1.

4. Discussion

Three different radical scavenging assay has been performed to find antioxidant potential of EEAR.

Table 1
HPTLC fingerprint of ethanolic extract of EEAR.

Peak	*Rf*	λ_{max}	Height	Area in mV
4	0.7	254	37.3	1552.5
5	0.9	254	19.6	640.2

DPPH becomes diamagnetic molecule after gets reduced into its hydrazine form by electron donation by the antioxidants. λ_{max} of DPPH, a stable free radical is at 517 nm. Decrease in absorption at 517 nm was the characteristic marker of DPPH reduction. Here the result was determined as a ratio between percentage of absorbance decrease of DPPH radical in presence of extract to the absorbance of DPPH radical alone at 517 nm. Based on this research work it may be presumed that DPPH gets reduced to its corresponding hydrazine form by EEAR. This reduction leads to colour change of DPPH from purple to yellow depending on number of electron taken up.[32,33]

In biochemical system, superoxide radical and H_2O_2 react together and form different forms of ROS viz. hydroxyl radical and singlet oxygen. They are responsible for various life threading diseases like carcinogenesis, cytotoxicity and aging by altering or damaging DNA. From the experimental data it may be speculated that EEAR can prevent the damaging properties of ROS at cellular level as it may able to quench hydroxyl radical and scavenge ROS. So, hydroxyl radical scavenging activity of this extract is directly conferring its antioxidant potential.

Nitric oxide is a lipophilic molecule. At physiological pH it reacts with oxygen and produce nitrite ion. Nitric oxide is very essential for controlling of vasodilation; signal transmission, inflammatory response etc.[34] Scavengers of nitric oxide compete with oxygen and inhibit the production of nitric oxide.[35] This research shows that EEAR may be act as antioxidant by inhibiting the production of nitric oxide.

Antioxidant activity of polyphenols can be examined by reducing power. The reducing power is governed by presence of reductones, which exhibit antioxidant property by donating hydrogen atom and breaking free radical chain reaction. Higher the electron transfer ability, more reducing power activity and more antioxidant activity.

In the present study, ferric ion is gets reduced into ferrous ion by antioxidants present in the ethanolic extract which leads to a colour change, depending upon their reducing power capacity.[36]

Proper identification and quality control of specific plant species can be done by HPTLC fingerprint analysis. This chromatographic

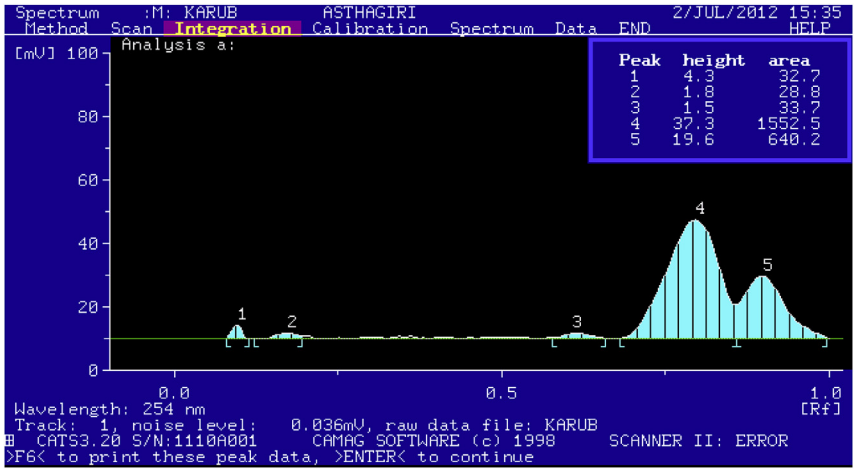

Fig. 6. HPTLC chromatogram of EEAR.

Table 2
Acute toxicity dose 2000 mg/kg (P.O). Initial observation (Day-I) (n = 3).

Parameters observed	I h	II h	III h	IV h
Piloerection	–	–	–	–
Oedema	–	–	–	–
Urine stains	–	–	–	–
Alopecia	–	–	–	–
Loss of writing reflex	–	–	–	–
Circling	–	–	–	–
Nasal sniffing	–	+	+	+
Lacrimation	–	–	–	–
Seizures	–	–	+	+
Righting reflex	+	–	+	+
Grip strength	+	–	–	–
Eye closure at touch	+	+	+	+
Rearing	+	–	–	–
Straub tail	–	–	–	–

+: Presence.
–: Absence.

Table 3
Acute toxicity-daily observation (n = 3).

Parameters observed	Day-1	Day-2	Day-3	Day-4
Piloerection	–	–	–	–
Oedema	–	–	–	–
Urine stains	–	–	–	–
Alopecia	–	–	–	–
Loss of writing reflex	–	–	–	–
Circling	–	–	–	–
Nasal sniffing	–	+	+	+
Lacrimation	–	–	–	–
Seizures	–	–	+	+
Righting reflex	+	–	+	+
Grip strength	+	–	–	–
Eye closure at touch	+	+	+	+
Rearing	+	–	–	–
Straub tail	–	–	–	–

+: Presence.
–: Absence.

data can provide various basic informations like chemical compounds for identification, isolation and purification of a particular plant species.

In HPTLC first 3 peaks was observed due to solvent. Presence of active constituents in EEAR was confirmed by peak 4 and 5. Although further study is required to identify those compounds. This HPTLC fingerprint analysis profile of EEAR may be used for quality evaluation and standardization.

For acute toxicity studies mortality was not observed in the groups treated with EEAR at the dose of 2000 mg/kg (Tables 2 and 3).

5. Conclusion

EEAR showed relevant antioxidant property. This study provides experimental support for the traditional medicinal plants.

To ensure therapeutic efficacy and quality control of the drug along with its identification, this HPTLC data will serve as reference standard for scientists engaged. So along with antioxidant property of this plant, HPTLC fingerprint data of root extract of EEAR can be used as diagnostic tool for the correct identification of the plant and also useful to estimate genetic variability in their population.

Based on the toxicity study, EEAR was found to be non-toxic at a dose of 2000 mg/kg.

Still molecular studies of the above mentioned plant may open up new hope in drug discovery and research.

Conflict of interest statement

The authors declare no conflicts of interest.

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