



IN VITRO ANTIOXIDANT ACTIVITY OF *ASPARAGUS RACEMOSUS* LINN LEAVES: EFFECT OF EXTRACTION SOLVENTS

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ABSTRACT

In vitro antioxidant activity of *Asparagus racemosus* Linn (*A. racemosus* L.) is known in literature. But there is controversy on the use of solvent by which maximum antioxidant activity can be obtained. Aim of the present study was, therefore, to see the effect of different extraction solvents on *in vitro* antioxidant activity of *A. racemosus* L. leaves. *A. racemosus* L. leaves were collected from the medicinal plant garden of the North Bengal University and identified by the taxonomist. Solvent extractions of the leaves were made separately by using chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone. Extracts were separately dried and processed for *in vitro* antioxidant activity through superoxide anion generation by xanthine- xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay. Quercetin was used as standard antioxidant. Results showed that ethanol extract of *A. racemosus* L. leaves had maximum antioxidant activity against the methods used which was comparable to that of quercetin.

KEYWORDS: *Asparagus racemosus* linn. Leaves, solvent extractions, xanthine- xanthine oxidase assay, linoleic acid peroxidation assay, DPPH photometric assay.

1. INTRODUCTION

A. racemosus L. (Family, Asparagaceae) locally known as Shatavari, is distributed throughout Australia, Sri Lanka, Africa, Java, Southern parts of China and India, but it is mainly cultivated in India. The plant is a woody climber growing to 1-2 m in height prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1300-1400 m elevation. Leaves are like pine needles, small and uniform, and shiny green. Flowers are white and have small spikes. Fruits are blackish purple like globular shapes. Roots are silvery white or ash-color externally and white internally. Roots are smooth when fresh but wrinkles upon drying.^[1]

A. racemosus L. has different vernacular names viz. Bengali: *Shatamuli* Gujarati: *Satawari* Hindi: *Satavari*, *Shatawar* or *Satmul* Kannada: *Majjigegadde* or *Aheruballi* Kumaon: *Kairuwa* Madhya Pradesh: *Narbodh* or *atmooli* Malayalam: *Chatavali* Marathi: *Shatavari* or *Shatmul* Nepali: *Kurilo* Rajasthan: *Norkanto* or *Satawar* Sanskrit: *Satavari* Tamil: *Shimaishadavari* or *Inli-chedi* Telegu: *Toala-gaddalu* or *Pilli-gaddalu*^[2]

Classification of *A. racemosus* L. is,
Kingdom: *Plantae* Clade: *Angiosperms* Order:
Asparagales Family: *Asparagaceae*

Sub family: *Asparagoideae* Genus: *Asparagus*
Species: *Asparagus racemosus*.^[2]

A. racemosus L. has many traditional uses. In Ayurveda the plant is used to increase longevity, impart immunity, prevent ageing, improve mental function, vigor and add vitality to the body. It is also used in inflammation, neuropathy, nervous disorders, dyspepsia, tumors, hepatopathy and in cases of threatened abortion. Roots of the plant are used in bronchitis, hyperacidity, diarrhoea, dysentery, cough, and certain infectious diseases. In Unani and Siddha system of medicine *A. racemosus* is used in treatments of chronic fevers, excessive heat, stomach ulcers, epilepsy, kidney disorder and liver cancer. It is used to increase milk secretion in nursing mothers and regulate sexual behaviors. The plant is also used as immune stimulant and antioxidant. The plant has anti-dyspepsia and anti-tussive effects.^[3]

A. racemosus L. contains many bioactive compounds like, Saponins, immunoside, asparagine A, isoflavones-8-methoxy-5, 6, 4-trihydroxy isoflavone-7-O-beta-D-glucopyranoside, undecanyl cetanoate, racemosol, dihydrophenantherene, racemofuran, dihydroxy-2-O (-2 hydroxy isobutyl) benzaldehyde and kaepfrol, essential fatty acids, mucilage, glycosides of quercetin, rutin and hyperoside, sitosterol, 4, 6-

dihydroxy-2-O (-2-hydroxy isobutyl) benzaldehyde and undecanyl cetanoate, kaepfrol, essential fatty acids, gamma linoleic acids, vitamin A, diosgenin, quercetin 3-glucourbnides etc.^[4]

A. racemosus L. has several pharmacological activities such as, antimicrobial, antidiabetic, anticancer, antidepressant, antiurolithiatic, antioxidant, antiinflammatory, anti HIV, antidyspepsia, antiepileptic, antiulcer, antistress, antidiarrhoeal, antiplasmodial, antitussive, analgesic, adaptogenic, galactagogue, diuretic, lipid lowering, cardioprotective, hepatoprotective, immunostimulant, immunomodulatory etc.^[5,7]

Antioxidant activity of *A. racemosus* L. is reported in literature but there are different opinions on the use of extraction solvent by which maximum activity may be obtained.^[8,14] Therefore, aim of the present work was to see effect of extraction solvents on in vitro antioxidant activity of *A. racemosus* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

A. racemosus L. leaves were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Siliguri (26°41'30.9984" N, 88°27'4.5756" E, elevation, 410 ft), Dist. Darjeeling, West Bengal, sometimes in the month of July – August 2016. Leaves were authenticated by the taxonomist of the department of Botany of the said University. A voucher specimen (No. SM-MB-014/19) was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.



Asparagus racemosus Linn.

2.2 Preparation of plant materials

Leaves of *A. racemosus* L. were washed thoroughly, shed dried and powdered. The powder, used as test drug, was stored desiccated at 4 °C until further use.

2.3 Solvent extraction

Test drug (100 g) was extracted separately with 500 ml of using chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone in soxhlet at 37°C for 15

minutes. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 °C. This was applied separately for all extracts. Brown masses obtained were used for *in vitro* antioxidant assay.

2.4 Antioxidant assays

In vitro anti oxidant activity of different extracts of *A. racemosus* L. leaves was assayed through superoxide anion generation by xanthine-/xanthine oxidase assay,^[15] linoleic acid peroxidation assay,^[16] and by DPPH photometric assay.^[17]

2.5 Chemicals

Chemicals required for the study were purchased from Meerck, Loba Chem. Lab and Himedia Lab.

2.6 Statistical analysis

The statistical significance between antioxidant activity values of the powdered leaves of *A. racemosus* L. was evaluated with a Duncan's multiple range test (DMRT). 5 % was considered to be statistically significant.^[18]

3. RESULTS

In vitro antioxidant activity of powders obtained from different extracts of *A. racemosus* L. through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay were given in Table. 1

It appears from the table that powder obtained from different solvent extractions of *A. racemosus* L. leaves had in vitro antioxidant activity but maximum activity was found in the ethanol extract followed by ethyl acetate extract. Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH in ethanol extract were found 93.6%, 90.5% and 94.3% respectively. Results were comparable with quercetin where inhibitions in xanthine oxidase, linoleic acid and DPPH came 100%, 85.4% and 96.4% respectively.

Table 1: Inhibitory activity of xanthine oxidation and linoleic acid peroxidation and scavenging capacity of DPPH by the powder obtained from different solvent extracts of *A. racemosus* L. leaves.

Powder obtained from extract of <i>A. racemosus</i> L. leaves	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Petroleum ether	12.1 ± 0.4	17.1 ± 0.8	16.1 ± 0.4
Methanol	25.7 ± 0.2	28.7 ± 0.1	27.5 ± 0.6
Ethyl acetate	43.1 ± 1.0	36.2 ± 1.0	37.3 ± 0.7
Ethanol	93.6 ± 1.0*	90.5 ± 1.1*	94.3 ± 1.0 *
Acetone	37.3 ± 1.1	33.1 ± 0.2	34.1 ± 0.9
Chloroform	32.5 ± 0.4	28.2 ± 0.3	28.8 ± 0.7
Quercetin	100 ± 0.01	85.9 ± 0.3	95.9 ± 0.4

Concentration used: 100 µg / ml. Dose was as per our earlier report^[19]. Results were a mean of triplicate experiments ± SD.*Significant

4. DISCUSSION

Plants possessing anti oxidant activity are known in literature. It is also known that anti oxidant activity of a plant may vary with nature of extraction solvents^[20]. Dent *et al.* used ethanol, methanol and acetone as extraction solvents of dry sage leaves and observed that ethanol extract had maximum antioxidant activity.^[21] Quasim *et al.* studied effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes and noted that aqueous methanol extracts of coastal halophytes had comparatively higher antioxidant activity.^[22] Using methanol, ethyl acetate, acetone, ethanol, and hexane as extraction solvents for walnut green husk, it was observed that acetone, ethanol, and methanol extracts exhibited stronger antioxidant activities, followed by ethyl-acetate and the lowest was for hexane extract.^[23] Thouri *et al.* carried out effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). Result showed that the polar solvent exhibited the highest amount of bioactive compounds specially polyphenols which are responsible for the antioxidant activity.^[24]

While studying antioxidant activity of *A. racemosus* L. different workers had different observations. Dohare *et al.* observed that methanol extract of *A. racemosus* L. had maximum antioxidant activity.^[10] Taepongsorat and Rattana noted that ethanol extract of *A. racemosus* L. had maximum antioxidant activity.^[11]

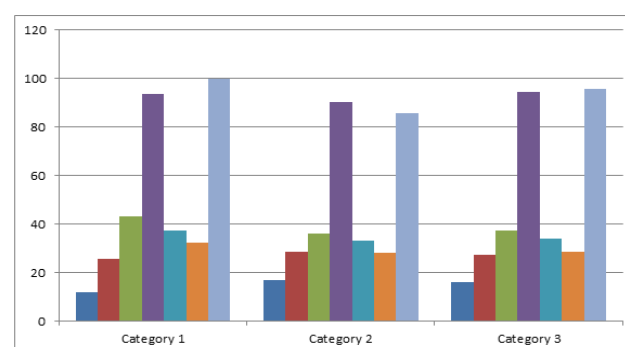
In the present study solvents like chloroform, petroleum ether, methanol, ethanol, ethyl acetate and acetone were used as extraction solvents of *A. racemosus* L. Ethanol extract showed maximum in vitro antioxidant activity as revealed by maximum inhibition of xanthine oxidation and linoleic acid peroxidation as well as scavenging capacity of DPPH in comparison to that of other solvent extracts (Figure – 1). This study, therefore, is in agreement with the observation of Taepongsorat and Rattana.^[11]

Secondary metabolites of plants are usually responsible for their biological activities. It is known that season has significant effect on synthesis of secondary metabolite in

plants thereby changing their biologic activity.^[25,27] It is, therefore, worth to investigate the seasonal effect on in vitro antioxidant activity of *A. racemosus* L. leaves. Work in this direction is now in progress in our laboratory.

CONCLUSION

Toxicity of synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene is known and considered a matter of concern. They may even develop cancer. Therefore, there are high demands for naturally occurring antioxidants. As in present study methanol extract of *A. racemosus* L. leaves powder showed maximum in vitro antioxidant activity, *A. racemosus* L. leaves may be further investigated for search of natural antioxidant compounds.



Category 1 : Xanthine oxidase (% inhibition) Category 2 : Linoleic acid peroxidation (% inhibition) Category 3: DPPH (% inhibition)

■ Petroleum ether ■ Methanol ■ Ethyl acetate ■ Ethanol ■ Acetone ■ Chloroform ■ Quercetin

Figure 1: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by different solvent extract of *A. racemosus* L. leaves.

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Conflict of interest : Nil.

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