International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2018; 6(2): xx-xx
Received: 10-01-2018
Accepted: 12-02-2018

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Determination of the phytochemical ingredients and antioxidative potential of hydromethanolic extract of *Asparagus racemosus* Willd

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Abstract
Asparagus racemosus is considered as an important medicinal plant of India. In the present study fifty percent hydromethanolic extract of roots of *Asparagus racemosus* (ARE) was prepared and analyzed for presence of various phytoconstituents in ARE employing different biochemical qualitative tests and GC-MS analysis. It was further explored for its antioxidative potential through hydrogen peroxide scavenging assay. Biochemical tests confirmed presence of various phytochemicals *viz*., resins, tannins, Saponins, flavonoids, alkaloids, glycosides, etc. GC-MS analysis showed presence of thirty compounds. Major phyto-constituent predicted in *Asparagus racemosus* is Di-n-octyl phthalate (89.58%) upon comparison with NIST and WELLY library. The present study indicated anti-oxidative property of ARE. The percent H_2O_2 scavenging activity of ARE was found to be 25.41%. Thus it could be concluded that ARE displayed presence of various phytoconstituents which could be responsible for its antioxidative potential.

Keywords: *Asparagus racemosus*, GC-MS analysis, antioxidative, hydrogen peroxide scavenging assay

1. Introduction
Medicinal usage of *Asparagus racemosus* (*A. racemosus*) has been reported in the Indian and British Pharmacopoeias and is recommended in traditional Indian medicine (Ayurveda) for nervous disorders, antihepatotoxic, immunomodulatory, effects etc [1-3]. The genus *Asparagus* includes about 300 species around the world. The plant is a spinous under shrub, with tuberous, short rootstock bearing numerous succulent tuberous roots (30–100 cm long and 1–2 cm thick) that are silvery white or ash coloured externally and white internally [4]. The genus is considered to be medicinally important because of the presence of steroidal saponins and sapogenins in various parts of the plant. The roots part of the plant is used in various medicinal preparations. Out of the 22 species of *Asparagus* recorded in India, *A. racemosus* is one of the most commonly used in traditional medicine [5]. *A. racemosus* belongs to family Liliaceae and commonly known as 'Satawar'. The plant grows at an altitude of 1500 m throughout India, Nepal, Sri Lanka and Himalayas [6]. It was preferentially studied for its medicinal properties as it possess medicinally valuable phyto-constituents and their derivatives which could be used for treatment of some most dreaded diseases like AIDS and cancers [7, 8].
Alcoholic extract yields asparagin- an anticancer agent. It also contains a number of antioxytocic saponins, viz. Shatavarisn- I to IV [9]. It is useful in nervous disorders, dyspepsia, diarrhoea, tumours, inflammations, vitiated conditions of vata and pitta, burning sensation, hyperdipsia, ophthalmopathy, nephropathy, hepatopathy, strangury, scalding of urine, throat infections, tuberculosis, cough, bronchitis, gleet, gonorrhoea, leucorrhoea, leprosy, epilepsy, fatigue, hyperacidity, colic haemorrhoids, hypertension, abortion, agalactia, cardiac and general debility [4].
Root of *A. racemosus* has been referred as bitter to sweet, emollient, cooling, nervine tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, anti-septic and as tonic. Beneficial effects of the root of *A. recemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentry, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious disease [1, 6]. Major phytocontituents in of *A. racemosus* are avonoid compounds, rutin and its aglycone quercetin, were reported to have beneficial biological effects, such as antagonizing the increase of capillary fragility associated with hemorrhagic disease, reducing hypertension and anti-carcinogenic activity [5, 10]. Keeping in view the mentioned facts, study was planned to explore phytochemicals in fifty percent hydro-methanolic root extract of *A. racemosus* (ARE) through

biochemical and GC-MS analyses. The antioxidative potential was assesses through hydrogen peroxide scavenging assay.

2. Materials and Methods

2.1 Plant material

The authentic plant material *i.e.*, roots of *A. racemosus* were obtained from Medicinal Plant Research and Development Centre (MRDC), Pantnagar, Uttrakhand, India.

2.2 Preparation of Extract of *A. racemosus*

Roots were washed properly, shed dried and ground into a fine powder and stored in sterile containers in a cool dry place till further use. Extraction of plant was carried out by using solvents with different polarities. Hydromethanolic extract was prepared as described by Ukwuani *et al*. [11]. In brief 50 gm of the powder was allowed to soak in 500ml 50% methanol (v/v) for 48 hours under continuous agitation in a shaking incubator. The mixture was first filtered through muslin cloth, then through Whatmann filter paper No 1. The filtrate was then kept in the rotatory evaporator (45 ºC). Finally the extract was obtained by drying the filtrate under hot circulating air at 40 ºC followed by lyophilization. The percent yield was calculated by dividing quantity of plant extracts obtained by 50 gm of dry powder. The percent yield was calculated. The prepared extracts were kept at the -20 ^{0}C till further use.

2.3 Phytochemical Analyses of plant extracts

Qualitative phytochemical tests for the identification of carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides were carried out for ARE as per the methods described by Trease and Evans [12], Harborne [13] and Sazada *et al*.[14].

2.3.1 Test for Proteins: Few drops of nitric acid were added by the sides of the test tube very gently to 1 ml methanol extract. Formation of yellow colour indicated the presence of protein in the sample (Xenthoprotein test).

2.3.2 Test for carbohydrates: 1 ml each of Fehling A and Fehling B were added in diluted extract and heated for 30 minutes and observed for the formation of brick red colour.

2.3.3 Test for Resins: Five milliliter of distilled water was added to the methanol extract and observed for turbidity.

2.3.4 Test for Tannins: 5 ml of 45% ethanol was added to 2 g of the ground sample and boiled for 5 min. The mixture was cooled and filtered. Then 3 drops of lead sub acetate solution was added to 1 ml of the filtrate. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added to 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins.

2.3.5 Test for Saponins: 0.5 g of methanol extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a persistent froth. The frothing was mixed with 3 drops of Olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.3.6 Test for Flavonoids: 0.5 g of the medicinal plant extract of was introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixture was then filtered. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids.

2.3.7 Test for Alkaloids: 5 gm of ground material was extracted with 10 ml Ammonical Chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M Sulphuric acid. Creemish white precipitate was observed for the presence of Alkaloids.

2.3.8 Tests for Steroids: 2 ml of acetic anhydride was added to 0.5 g of methanol extract and 2 ml of Sulphuric acid was added by the sides of the test tube and observed for the colour change from violet or blue-green.

2.3.9 Test for Phenols: Methanol extract was taken in a test tube and mixed with distilled water and warmed. To this 2 ml of Ferric chloride solution was added and observed for the formation of green or blue colour.

2.3.10 Test for Glycosides: About 0.5 ml of methanol extract was taken in a test tube and added 1 ml glacial acetic acid containing traces of ferric chloride. To this solution 1 ml concentration Sulphuric acid was added and observe for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

2.4 Characterization of medicinal plant extracts by GC-MS analysis

The samples were analyzed at commercial facility of GC-MS analysis available at RITL, JNU, New Delhi, with the following parameters.

2.4.1 Sample preparation

200 mg of the medicinal plant extract was dissolved in 2 ml of the methanol and then filtered through syringe filter (0.22µ). Finally prepared sample of each extract was loaded in GC-MS column.

2.4.2 GC-MS analysis

GC MS analysis was carried out by splitless injection of 1µl of sample onto Shimadzu QP2010 GC-MS assembly was fitted with a column, coupled with mass detector. Following GS parameters were used during analysis of extract of medicinal plants. Column Oven Temperature was set at 100.0 °C, pressure was 175.1 kPa with total Flow of 16.3 ml/min, column flow was 1.21 ml/min, linear velocity was 28.9 cm/sec and purge flow was 3.0 ml/min. Mass detector was set with start time 6.00 min and end time 40.49min. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and WELLY library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.5 Hydrogen peroxide scavenging assay

Antioxidative potential of ARE was determined by *in vitro* hydrogen peroxide scavenging assay. The ability of the ARE to scavenge hydrogen peroxide was assessed as per the method described by Ruch *et al.* [15]. The extent of H_2O_2 scavenging by the plant extracts was calculated as per the following formula-

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1) \times 100}{A_0}$$

A_0 - Absorbance of control
A_1 - Absorbance in the presence of plant extract

3. Result

3.1 Percent Yield of ARE

7.26 gram of the hydromethanolic extract was prepared from 50 gram of roots of *Asparagus racemosus* with percent yield of 14.52%.

3.2 Phytochemiclas analyses of ARE

As per the biochemical tests conducted, ARE shown presence of all the tested phytochemicals, viz. carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols and glycosides (Table 1).

Table 1: Phytochemicals present in ARE

S. No.	Phytoconstituents	ARE
1.	Protein	+
2.	Carbohydrates	+
3.	Resins	+
4.	Tannins	+
5.	Saponins	+
6.	Flavonoids	+
7.	Alkaloids	+
8.	Steroids	+
9.	Phenols	+
10	Glycosides	+

3.3 GC-MS analysis of ARE

The major phyto-constituents predicted in *Asparagus racemosus* are Di-n-octyl phthalate (89.58%), Pentadecanoic acid, 14-methyl-, methyl ester (1.45%) 1,2-Benzenedicarboxylic acid, butyl octyl ester (1.56%) *etc.* upon comparison with NIST and WELLY library. The GC- MS analysis of ARE showed presence of fatty acids (Fig. 1; Table 2).

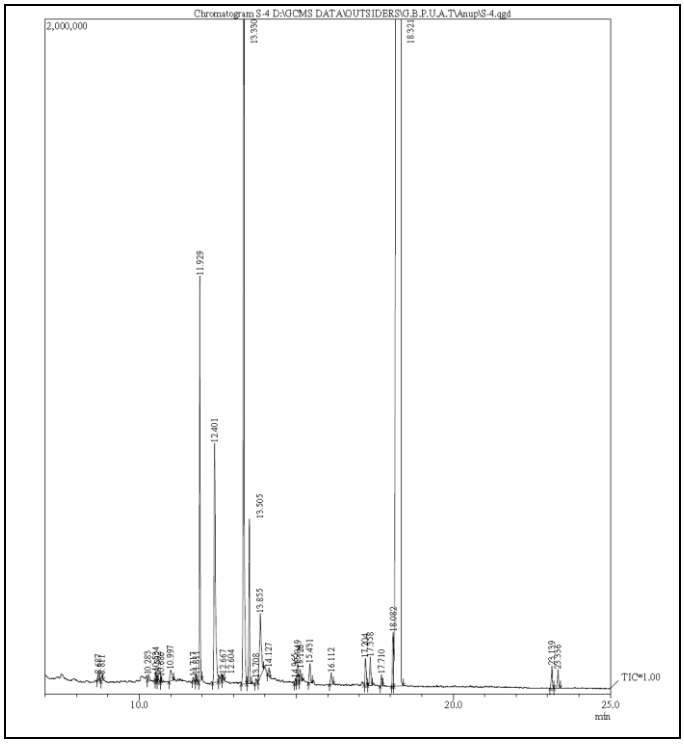

Fig 1: Chromatogram showing peaks for phyto-constituents in *Asparagus racemosus*

Table 2: Phyto-constituents present in *Asparagus racemosus* after determining the retention time peaks with NYST AND WELLEY Library

Peak#	R.Time	Area	Area%	Name	FORMULA	CAS No	Mol. Wt.
1	8.687	29112	0.02	DECANE, 1-CHLORO-	$C_{10}H_{21}Cl$	1002-69-3	176
2	8.811	39896	0.03	1-UNDECANOL	$C_{11}H_{24}O$	112-42-5	172
3	10.283	35734	0.03	2-TRIDECENE, 2-CHLORO-1,1,1-TRIFLUORO-, (Z)	$C_{13}H_{22}ClF3$	108400-10-8	270
4	10.534	43760	0.03	DODECANOIC ACID, METHYL ESTER	$C_{13}H_{26}O_2$	111-82-0	214
5	10.592	44425	0.03	(MESO)-3,4-DIHYDROXYMETHYL-3,4-DIMETHYLHEXANE	$C_{10}H_{22}O_2$	122305-34-4	174
6	10.686	25443	0.02	2-TRIDECENE, 2-CHLORO-1,1,1-TRIFLUORO-, (Z)-	$C_{13}H_{22}ClF_3$	108400-10-8	270
7	10.997	143891	0.11	NAPHTHALENE, 1,6-DIMETHYL-4-(1-METHYLETHYL)	$C_{15}H_{18}$	483-78-3	198
8	11.717	18542	0.01	PHTHALIC ACID, 4-CYANOPHENYL NONYL ESTER	$C_{24}H_{27}NO_4$		393
9	11.811	32130	0.02	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	$C_{17}H_{32}O_2$	25779-85-5	268
10	11.929	1949472	1.45	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	$C_{17}H_{34}O_2$	5129-60-2	270
11	12.401	2105322	1.56	1,2-BENZENEDICARBOXYLIC ACID, BUTYL OCTYL ESTER	$C_{20}H_{30}O_4$	84-78-6	334
12	12.604	83539	0.06	DIETHYLENE GLYCOL MONODODECYL ETHER	$C_{16}H_{34}O_3$	3055-93-4	274
13	12.667	38174	0.03	HEPTACOSANOIC ACID, METHYL ESTER	$C_{28}H_{56}O_2$	55682-91-2	424
14	13.33	5995268	4.45	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	$C_{19}H_{36}O_2$	112-62-9	296
15	13.505	910414	0.68	HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER	$C_{19}H_{38}O_2$	5129-61-3	298
16	13.708	33066	0.02	1-TRIDECYNE	$C_{13}H_{24}$	26186-02-7	1311
17	13.855	786795	0.58	9-OCTADECENOIC ACID (Z) IJMAP	$C_{18}H_{34}O_2$	112-80-1	282
18	14.127	44219	0.03	3-METHYL-1-CYCLOOCTENE	C_9H_{16}	13152-05-1	124
19	14.965	30027	0.02	N,N-DIMETHYLDECANAMIDE	$C_{12}H_{25}NO$	14433-76-2	1415
20	15.049	108905	0.08	PHTHALIC ACID, BUTYL UNDECYL ESTER	$C_{23}H_{36}O_4$		376
21	15.126	95986	0.07	DECANAL	$C_{10}H_{20}O$	112-31-2	156
22	15.431	154962	0.12	OXIRANE, [(DODECYLOXY)METHYL]-	$C_{15}H_{30}O_2$	2461-18-9	242
23	16.112	100872	0.07	DODECANOIC ACID, 8-NITRO-11-OXO-, METHYL ESTER	$C_{13}H_{23}NO_5$	87298-03-1	273
24	17.204	199352	0.15	PHENOL, 2,4-BIS(1-PHENYLETHYL)	$C_{22}H_{22}O$	2769-94-0	302
25	17.358	224304	0.17	METHANONE, [1,4-DIMETHYL-7 -(1-METHYLETHYL)-2-AZULENYL]PHENYL	$C_{22}H_{22}O$	39665-56-0	302
26	17.71	64715	0.05	1,2-BENZENEDICARBOXYLIC ACID, DINONYL ESTER	$C_{26}H_{42}O_4$	84-76-4	418
27	18.082	403843	0.3	METHANONE, [1,4-DIMETHYL-7-(1-METHYLETHYL)-2-AZULENYL]PHENYL	$C_{22}H_{22}O$	39665-56-0	2371
28	18.321	1.21E+08	89.56	DI-N-OCTYL PHTHALATE	$C_{24}H_{38}O_4$	117-84-0	2832
29	23.139	165470	0.12	4,5-2H-OXAZOLE-5-ONE, 4- [3,5-DI-T-BUTYL-4-METHOXYPHENYL] METHYLENE-2-PHENYL-	$C_{25}H_{29}NO_3$		3109
30	23.336	148540	0.11	2,4,6-TRIS-(1-PHENYLETHYL)-PHENOL	$C_{30}H_{30}O$		406

3.4 Hydrogen peroxide scavenging assay

The percent H_2O_2 scavenging activity of ARE was found to be 25.41% as depicted in Table 3.

Table 3: H_2O_2 scavenging activity of ARE

Treatments	Optical Density (O.D.) at 230 nm				Mean O.D.±S.E.	% H_2O_2 scavenging activity
Control	0.484	0.489	0.52	0.46	0.488±0.0123	_
ARE	0.325	0.365	0.41	0.357	0.364±0.0175	25.41

4. Discussion

A phytochemical is a natural bioactive compound found in plant foods that works with nutrients and dietary fiber to protect against disease. Phytochemical studies of the plant preparations are necessary for standardization, which helps in understanding the significance of phytoconstituents in terms of their observed activities. Phytochemistry also helps in standardizing the herbal preparations so as to get the optimal concentrations of known active constituents and in preserving their activities. The main active constituents of *Asparagus racemosus* are steroidal saponins (Shatavarins I–IV) that are present in the roots. Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose [16]. A new isoflavone, 82methoxy25, 6, 4′2trihydroxyisoflavone 2,7,2,O-2-β-2-d-2glucopyranoside was also reported from *A. racemosus*. The isolation and characterization of polycyclic alkaloid called Asparagamine, a new 9, 10 dihydrophenanthrene derivative named Racemosol and Kaempferol were also isolated from the ethanolic root extract of *A. racemosus* [17]. Other primary chemical constituents of Asparagus are essential oils, asparagine, arginine, tyrosine and resin [18]. Aqueous extract of *A. racemosus* was fractionated and screened for the polysaccharide fraction. The characterization was done by enzymatic, Size Exclusion, gas chromatography with flame ionization detector (GC-FID), high pressure anion exchange chromatography (HPAEC) and thin layer chromatographic analyses. Phyto-chemical evaluation confirmed the presence of 26.7% of 2→1linked fructo-oligosaccharides (FOS) [19].

GC-MS is a technique that can be used to separate volatile organic compounds (VOCs). The Gas Chromatography-Mass Spectrometry (GC-MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It was found that medicinal plant extracts contain maximum amount of fatty acid and their derivatives which could be responsible for their therapeutic properties. Comparative profile of VOCs showed presence of aldehydic and alcoholic groups in ARE. One of the properties of aldehyde volatile oils is their insect repellent activity due to very strong scent. Similarly, ketone dominating species showed lipolytic, mucolytic, sedative, analgesic, anti-coagulant, anti-inflammatory, digestive, expectorant and stimulating properties. This would be due to moderate electronegativity and strong polarity of ketones. Some ketones also are known to be neurotoxic [20].

Methanolic extract of *A. racemosus* given orally at the dose of 100 mg/kg body weight/day for 15 days significantly increased in antioxidant defense, that is, enzymes superoxide dismutase, catalase, and ascorbic acid where as a significant decrease in lipid peroxidation was observed [21]. Dartsch [22] evaluated potential of asparagus to inactivate reactive oxygen radicals in combination with parsley leaves and found it effective. Kamat *et al*. [23] concluded from their study that extracts from *A. racemosus* exhibited potent antioxidant properties *in vitro* in mitochondrial membranes of rat liver. The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant [24]. Karmakar *et al*. [25] explored antibacterial and antioxidative potential of *A. racemosus*. Thus it could be inferred from the study that the presence of various phytochemicals as revealed through biochemical and GC-MS analyses may be responsible for antioxidative potential of *A. racemosus* root extract. However, advance analysis is required to further harness the medicinal potential of *A. racemosus.*

5. Acknowledgement

Authors are thankful to the Director, MRDC, G.B.P.U.A. &T., Pantnagar, for providing the plant material. The facilities provided by Director Experiment Station; Dean, Veterinary & Animal Sciences, GBPUA&T, Pantnagar; to carry out present study, are duly acknowledged.

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