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Research Article

PHARMACOGNOSTICAL, PHYTOCHEMICAL, ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF *ASPARAGUS RACEMOSUS* WILLD.

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ABSTRACT

This paper describes the preliminary organoleptic examination, extractive values, physical constant values, fluorescence analysis and effects on treatment with different chemicals of *Asparagus racemosus*. The preliminary organoleptic examination, and physicochemical constants like moisture content, ash values such as total ash, acid insoluble ash, water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value, was determined. The benzene extract shows minimum extractive value, whereas methanol shows maximum extractive value. The fluorescence analysis was also carried out to observe the behaviour of powder with different reagents. These help in maintaining pharmacopoeial standards for the drug. The phytochemical investigation of root extracts of *A. racemosus* Willd. plant was done by using the different extracts, obtained through successive solvent extraction with petroleum ether, benzene, chloroform, acetone, alcohols and water. The petroleum ether, benzene, chloroform, acetone, alcohol and aqueous extract were yellow grey, deep brown, brownish black, reddish brown, reddish brown, and light brown color respectively. Phytochemical investigation also shows the presence of Sterols, Saponins, Alkaloids, Flavonoids, Tannins, carbohydrate, and lactones while Amino acid, Resins and Starch are absent. It was observed that maximum numbers of constituents were present in alcoholic extract. Antioxidants and free radical scavenging assay of methanolic extract of *Asparagus racemosus* Willd. shows at lower concentration it is better scavenger for Nitric oxide radicals in comparison to DPPH, Superoxide or ABTS radical whereas at higher concentration it works better for DPPH radical scavenger than Superoxide or Nitric oxide or ABTS radicals. IC_{50} (µg/ml) is maximum in ABTS radical scavenging assay whereas it is minimum for superoxide radical scavenging assay.

Keywords: Aphrodisiac, Carminative, Hepatotoxicity, Dyspepsia, Galactogogue.

INTRODUCTION

Asparagus racemosus Willd. (Common name- Shatavari) is a member of family Asparagaceae. It is an important medicinal plant. It has been specially recommended in cases of threatened abortion and as a galactogogue [1]. Root of asparagus has been referred as bitter-sweet, emollient, cooling, nerve tonic, aphrodisiac, diuretic, rejuvenating, carminative, and stomachic antiseptic and tonic. It is reported to be useful against diarrhoea, dysentery and in general debility. *Asparagus racemosus* is recommended in traditional medicine for the prevention and treatment of gastric ulcers, dyspepsia and nervous disorders. Besides these the plant also has antioxidant [2], immunostimulant, antidyspepsia, antitussive effects [3]. *Asparagus racemosus* have been used for the treatment of the ulcers, depression, inflammation cancer, lithiasis, hepatotoxicity, diabetes [4]. It is also used for dry coughs and gastric ulcers [5]. Recent research indicates Shatavari enhances immune function, increases corticosteroid production, and promotes cell regeneration. This paper describes the preliminary organoleptic examination, extractive values, physical constant values, fluorescence analysis and effects on treatment with different chemicals of *Asparagus racemosus* Willd. Further the present study describes preliminary phytochemical analysis and various radical scavenging assay of *Asparagus racemosus* Willd root extracts.

MATERIALS AND METHODS

The roots of *Asparagus racemosus* Willd were collected in the month of April 2012 from Chidiyatapu of South Andaman district of Andaman and Nicobar Islands and were authenticated by PG Department of Plant Science, J.N.R.M, Port Blair. The herbarium was prepared and kept for future reference. The collected roots were washed; shade dried and pulverized with mechanical pulveriser for size reduction. It was then passed through mesh 40 and the fine powder was collected and used for preparation of extract for the experiment.

Pharmacogonistic Studies & Physico-chemical Parameters

Preliminary organoleptic examination was carried out by simple determination technique. Colour, odour, size, shape; taste; surface and texture are observed [6] (Table 1). Physicochemical parameters such as extractive values, ash values[7] and moisture content were determined by Indian Pharmacopoeia (Table 2,3).For fluorescence studies[8] , powder was sieved through 40 mesh and observations made following Chase, Pratt and Usha kumari *et.al.* (Table 4).Treatment on *Asparagus racemosus* Willd. roots with different chemical reagents and their colour reaction is shown (Table 5).

Phytochemical studies

Successive solvent extraction was carried out by using different solvents according to order of polarity and extracts obtained were tested for different phytoconstituents [9, 10] (Table 6). Different qualitative test were performed for establishing profiles of various extracts for their nature of chemical composition. The extracts obtained were subjected to chemical

tests for identification of various phytoconstituents as per the methods given by Brain and Turner, Kokate and Harborne [11].

Antioxidant properties

Antioxidant properties [12] of *Asparagus racemosus* Willd. were evaluated involving free radical scavenging mechanisms, namely the DPPH[13] (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay, Super oxide scavenging assay, Nitric oxide radical scavenging assay and ABTS (2, 2'-azinobis-3-ethyl-benzothiozoline-6-sulphonic acid) radical scavenging assay (Table 7).

DPPH radical scavenging assay

DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple color, the intensity of which is measured at 510 nm spectrophotometrically. Antioxidants [14] reduce DPPH to 1, 1-diphenyl-2-picryl hydrazine, a colorless compound.

Reagents / chemicals used: DPPH (2, 2-diphenyl-1-picryl hydrazyl) [RM 2798, Himedia, India], Methanol (HPLC grade) [43602, Qualigens], Positive control (Gallic acid) [G7384, Sigma, USA].

Procedure: Various concentrations (200 µl) of test solution and 50 µl of DPPH (0.659 mM) solution are incubated at 25°C for 20 min. Following which the absorbance is read at 510 nm. A control reaction was carried out without the test sample. Linear graph of concentration vs. percentage inhibition was prepared and IC_{50} values were calculated. The % inhibition was calculated according to the following equation-

% Inhibition = $(A_0-A_t) / A_0 \times 100$ Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract.

Superoxide radical scavenging activity (PMS-NADH System): Superoxide anions were generated using PMS / NADH system. The superoxide anions are subsequently made to reduce nitroblue tetrazolium which yields a chromogenic product, which is measured at 560 nm.

Reagents/chemicals used: Reduced nicotinamide adenine dinucleotide Sodium salt (NADH) [RM 393Himedia, India], Phenazine methosulphate (PMS) [5165, Loba Chemie, India], Nitroblue tetrazolium (NBT) [94060, S.d. fine Chemicals, India], Positive control: Gallic acid G7384,[Sigma,USA].

Procedure: Test solution (0.1 mL) in 0.1M phosphate buffer pH 7.4, 62.5 µl of 468 µM NADH solution, 62.5 µl of 150 µM NBT solution and 62.5 µl of 60 µM PMS solution were added to a micro well plate and incubated at room temperature for 5 min. The absorbance was read at 560 nm. Linear graph of concentration vs. percentage inhibition was prepared and IC_{50} values were calculated.

Nitric oxide radical scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be measured at 546 nm spectrophtometrically in the presence of Griess reagent.

Reagents/chemicals used: Sodium nitroprusside [40190, S.d.fine Chemicals, India], Sulphanilamide [3164, NR Chem., India], Orthophosphoric acid [39416, S.d.fine Chemicals, India], N-(1-naphthyl) ethylenediamine [N5889, Sigma, USA], Positive control: Curcuminoids (33533, Synthite, Kochin, India).

Procedure: Test solution of various concentrations (50 µl) and 50 µl of 10 mM sodium nitroprusside are illuminated (using fluorescence light/18W CDL 6500K) at room temperature (25-30°C) for 15 min. Following incubation, 125 µl of Griess reagent was added and incubated for 10 min at room temperature. The color developed was measured at 546 nm. Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated.

ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in absorption which can be followed spectrophotometrically. The relatively stable ABTS radical has a green color and is quantified spectrophotometrically at 734nm.

Reagents/chemicals used: Ammonium persulphate (APS) [Rankem, India], ABTS (2, 2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid) [Sigma, USA], Positive control: Gallic acid (3, 4, 5- Trihydroxy benzoic acid) [Sigma, USA]

Procedure: ABTS radical cations were produced by reacting ABTS and APS and incubating the mixture at room temperature in dark for 16 hours. Add 20 µl of various concentrations of 10 mM PBS pH 7.4 test solutions and 230 µl of ABTS radical solution (0.238 mM). The absorbance is measured immediately at 734 nm. A control reaction was carried out without the test sample. Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated.

Table 1: Preliminary organoleptic characters of *Asparagus racemosus* Willd. Roots

Characteristics	Observation
Colour	The fresh roots are white to buff in colour, dried roots are white to grayish white in colour, internally slight yellowish.
Odour	Characteristic odour
Size	About 5 to 60 cm in length and 1 to 2.5 cm in thickness
Shape	The roots are fleshy, tuberous, tapering towards both end, it swells considerably when soaked in water
Taste	Slightly bitter
Surface	Rough, sign of shrinkage after drying
Texture	Short and fibrous

Table 2: Extractive values of *Asparagus racemosus* Willd. Roots

Solvent	Colour of extractives	Extractive value (%)
Petroleum ether (60-80 ^{0}C)	Yellowish gray	0.419
Benzene	Deep brown	0.195
Acetone	Brownish black	0.609
Chloroform	Reddish brown	10.840
Methanol	Reddish brown	27.697

Table 3: Physical constant values of *Asparagus racemosus* Willd . root

Parameter evaluated	Result (in % w/w)*
Total ash	6.461
Acid insoluble ash	1.297
Acid soluble ash	6.646
Water soluble ash	1.412
Water soluble extractive	30.146
Alcohol soluble extractive	27.100
Loss on drying	10.917

*Each value is an average of three determinations.

Table 4: Fluorescence analysis of root powder of *Asparagus racemosus* Willd

Reagents UV	Short light (255nm)	UV Long light (366nm)	Visible light
Powder as such	White	Light white	Grayish white
Powder with (IN) NaOH	Greenish brown	Green	Brownish green
Powder with picric acid	Grey	Light grey	Yellowish grey
Powder with acetic acid	Light brown	Light brown	Light grey
Powder with (IN) HCl solution	Dark red	Light red	Reddish
Powder with 5% FeCl3 solution	Blackish brown	Light brown	Reddish brown
Powder with HNO_3 & NH_3 solution	Dark brown	Light brown	Coffee brown
Powder with IN NaOH in methanol	Brownish yellow	Light brown	Brown
Powder with methanol	Deep brown	Blackish brown	Brown
Powder with 50% HNO3 solution.	Brownish	Light brown	Light brown

Table 5: Treatment on *Asparagus racemosus* Willd. root with different reagents

Reagents	Observation
Powder as such	Greyish white
Powder with acetic acid	Greyish
Powder with conc. sulphuric acid	Brownish black
Powder with conc. nitric acid	Reddish
Powder with conc. hydrochloric acid	Light white
Powder with ferric chloride solution	Brownish black
Powder with 5% iodine solution	Reddish
Powder with aqueous sodium hydroxide solution (1 N)	Yellowish
Powder with picric acid solution	Greenish yellow

Table 6: Preliminary Phytochemical Screening of Root of *Asparagus racemosus*

Preliminary phytochemical screening	*Asparagus racemosus* Willd						
	Petroleum Ether extract	Benzene extract	$CHCl_3$ extract	Acetone	Methanol	Ethyl Alcohol extract	Aqueous extract
Sterols/Triterpenoids	+	+	+	+	+	+	-
Saponins	-	-	-	+	+	+	+
Alkaloids	-	-	+	-	+	+	-
Tannins	-	-	-	-	+	+	-
Carbohydrates	-	-	-	+	+	+	+
Flavonoids	-	-	-	-	+	+	-
Lactones	+	-	+	-	+	+	-
Amino acid/ Protein	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-

+ denotes the presence & - denotes the absence of the respective group of compounds.

Table 7: Effect (% inhibition) of root extracts of *Asparagus racemosus* Willd. on different radical scavenging assay

Concentration (µg/ml)	DPPH Radical Scavenging Assay	Superoxide Radical Scavenging Assay	Nitric oxide Radical Scavenging Assay	ABTS Radical Scavenging Assay
50	11.01	12.03	18.08	9.10
100	14.83	14.12	20.12	12.88
200	28.11	28.17	33.28	18.86
300	56.08	56.16	50.16	34.14
400	63.86	61.18	58.11	51.07
500	78.11	67.27	61.00	56.65
IC_{50} (µg/ml)	267.47	267.09	299.04	391.61

RESULT AND DISCUSSION

The preliminary organoleptic examination, and physicochemical constants like moisture content, ash values such as total ash, acid insoluble ash, water soluble ash, extractive values such as water soluble extractive value and alcohol soluble extractive value, was determined. The benzene extract shows minimum extractive value, whereas methanol shows maximum extractive value. The fluorescence analysis was also carried out to observe the behaviour of powder with different reagents. These help in maintaining pharmacopoeial standards for the drug.

The phytochemical investigation of root extracts of A. racemosus Willd. plant was done by using the different extracts, obtained through successive solvent extraction with petroleum ether, benzene, chloroform, acetone, alcohols and water. The petroleum ether, benzene, chloroform, acetone, alcohol and aqueous extract were yellow grey, deep brown, brownish black, reddish brown, reddish brown, and light brown color respectively. Phytochemical investigation also shows the presence of Sterols, Saponins, Alkaloids, Flavonoids, Tannins, carbohydrate, and lactones while Amino acid, Resins and Starch are absent. It was observed that maximum numbers of constituents were present in alcoholic extract.

Antioxidants and free radical scavenging assay of methanolic extract of *Asparagus racemosus* Willd. shows at lower concentration it is better scavenger for Nitric oxide radicals in comparison to DPPH, Superoxide or ABTS radical whereas at higher concentration it works better for DPPH radical scavenger than Superoxide or Nitric oxide or ABTS radicals. IC_{50} (µg/ml) is maximum in ABTS radical scavenging assay whereas it is minimum for superoxide radical scavenging assay.

CONCLUSION

The macroscopic and pharmacogonistical characters help in the identification of drug and also in laying down pharmacopeial standards. The result obtained from the phytochemical screening showed the presence of several useful phytochemical compounds which might be responsible for antioxidant and other medicinal properties of the plant. Further chromatographic and spectral analysis is required to authenticate and find out bioactive compound from *Asparagus racemosus*.

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