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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL SCREENING OF ROOTS OF *ASPARAGUS RACEMOSUS* WILLD.

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ABSTRACT

Present study deals with qualitative and quantitative phytochemical evaluation of bioactive phytochemicals of roots of *Asparagus racemosus*, a traditionally acclaimed medicinal plant and antioxidant and antimicrobial assays of root extracts. The physicochemical and phytochemical analysis including quantitative estimation of total steroidal saponins, total alkaloid, flavonoids and TLC profiling of various extracts of *A. racemosus* roots were done by prescribed methods. The antioxidant activity of methanol extract was performed as reducing power assay and antimicrobial activity of water extract was assayed against *Staphylococcus* inoculums. Physicochemical studies resulted in standard value of certain quality ascertaining parameters. Phytochemical screening indicated the presence of various phytochemicals. Quantitative evaluation of major bioactive constituents revealed considerably high content of total steroidal saponins in roots. Methanol extract of roots recorded a high amount of total phenolics and resultant good reducing activity whereas water extract of roots found to exhibit antibacterial activity against *Staphylococcus.* Standard values for key quality parameters will be helpful in authentication of *A. racemosus* roots. Notable antioxidant and antimicrobial activities of root extracts provide scientific validation of traditional medicinal claims of the plant.

KEYWORDS: *Asparagus racemosus,* Roots, Phytochemical screening, Quantification, Antioxidant & Antimicrobial assay.

INTRODUCTION

Practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine. Our ancestors started to learn

from nature by testing and using what was available. It is well known that old civilizations have flourished in the Middle East and used the natural plants for various daily needs, such as food, shelter, clothes and medicine. The use of traditional medicine is increasing day by day due to the high cost of the allopathic medicines and their potential side effects.[1,2] During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population rely heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.[3-5]

In the quest for new medicines to treat old and emergent diseases such as malaria, cardiovascular, cancer and AIDS, attention is now being given to discovering active ingredients from medicinal plants used routinely and traditionally. Plants, especially used in Ayurveda can provide biologically active molecules for the development of modified derivatives with enhanced activity and /or reduced toxicity.

Asparagus racemosus Willd. (Liliaceae) selected for the present study is an important medicinal plant regarded as 'Rasayana' (plant drugs promoting general well being by increasing cellular vitality and resistance) in the traditional system of medicine. It is a tall climbing under-shrub, found all over India and commonly known as Satmuli (Bengali) and Shatavari (Hindi and Sanskrit). All parts of the plant in general and roots in particular are used by different traditional systems of medicine for the treatment of various human ailments.[6, 7] The plant is well reputed for its diverse chemical constituents and versatile therapeutic application. Review of literature indicated their large scale consumption of the plant in drug production by a number of herbal drug industries has led to its increased demand in herbal market. Therefore, there is a chance of adulteration in the raw materials procured from various sources. Further, concentration of active chemical constituents of plants reported to vary with respect to origin, growing conditions, season, developmental stage, agroclimatic and phytogeographic situations.[8-11] This has made it incumbent to establish standards so as to bring about some sort of uniformity in the manufacture of herbal medicines. Standardization of medicinal plants is one of the major challenges confronted by herbal drug industry. Present study was aimed at evolving standard physicochemical and

phytochemical values for authentication, quantitatively assessing the major bioactive phytochemicals and evaluating antioxidant and antimicrobial activity of the roots of A*sparagus racemosus* willd.

MATERIALS AND METHODS

Plant material

The roots of *Asparagus racemosus* used for the study were obtained from the Herbal Garden of Non-Wood Forest Products Division, Forest Research Institute (FRI), Dehradun (India) and were identified and authenticated by Department of Botany, FRI. Voucher specimen of the collected material is kept in the Chemistry Division for future reference. The collected roots were cleaned properly and dried in shade. Dried roots were chopped and ground to coarse powder. Powdered root was then subjected to further studies.

Chemical reagents

All the chemicals and reagents used for the analytical works were of laboratory grade and refer to s d fine-chem limited. TLC plates were prepared with silica gel G (s d fine-chem limited) and the spots were visualized by spraying with specified detecting reagents like Dragendorff's Reagent, Ferric chloride, Liebermann Burchard Reagent, Ehrlich Reagent, Ninhydrine etc. (for qualitative analysis) depending upon the nature and class of chemical constituents. Some of the reagents were prepared during the experiments while some of the readymade available regents were used for qualitative detection. Anhydrous sodium sulphate (Na_2SO_4) was normally used for drying the organic solvents. Analytical samples were routinely dried over P_2O_5 for 24 h in vacuum.

Study of organoleptic characteristics

Organoleptic evaluation stands for conclusions drawn from studies resulted due to impressions on organs of senses.[12] Morphological studies of roots were carried out by using simple observation technique, shape, size, color and odor were determined. The powdered root samples of the plant *A. racemosus* was placed on watch glasses and observed carefully for apparent characteristics like general appearance, colour, odour and taste.[13] The observations were recorded as accurately as possible that provided information on obvious physical properties of the plant part under investigation. Observations on organoleptic characteristics were replicated thrice.

Physicochemical Studies

Physicochemical characteristics viz. moisture content, ash values (total ash, acid insoluble ash, water soluble ash, sulphated ash) and extractive values both under exhaustive and sequential extraction were studied according to the reported method.[12]

Phytochemical analysis

Qualitative phytochemical screening

Powdered samples of *A. racemosus* roots were subjected to qualitative phytochemical screening for the presence and/or absence of different categories of chemical constituents, such as, alkaloids, flavonoids, phenolics, tannins, steroids, saponins, carbohydrates, glycosides, fixed oils and fats, proteins and free amino acids (FAA) following prescribed methods.[14-18]

Quantitative evaluation of total steroidal saponins

Total steroidal saponins were isolated from the officinal parts i.e. roots of *A. racemosus* according to Tschesche and Pandey (1978).[19] The dried and powdered roots of the plant were extracted with methanol at room temperature. The methanolic extract was distilled and evaporated to dryness. The dried mass was taken in water, defatted with benzene (C_6H_6) and then extracted with butanol (BuOH). For removal of phenolic constituents butanol extract washed with ether thrice. The defatted butanol extract after removal of phenolic compounds was distilled under reduced pressure and evaporated to dryness. The dried extract appeared as light brown mass and positively responded to Ehrlich Reagent suggesting it to be a steroidal saponin of furostanol type.[20] The content of steroidal saponin so obtained was calculated with respect to the initial weight of the root powder.

Quantitative evaluation of total alkaloid content

The total alkaloids were isolated from the roots of *A. racemosus* and content was determined according to Higuchi and Bodin.[21] Powdered roots were extracted exhaustively with methanol. The methanol extract was concentrated through distillation and the slurry so obtained were stirred with 7% citric acid mechanically and then filtered to get aqueous acidic extracts. Aqueous acidic extracts were basified with liquid ammonia up to pH 8 and the liberated total alkaloids were recovered through extraction with chloroform in a separating funnel. The chloroform fraction was concentrated on water bath and dried completely that furnished total alkaloids. The percentage yields of total alkaloids were calculated with reference to the dry weight of powdered root initially taken.

Quantitative evaluation of total flavonoids

Total flavonoids were isolated from the roots of *A. racemosus.*[22] The root powder of the plant were macerated with water for 2 h and filtered. To the filtrate lead acetate solution (prepared by dissolving 9.5gm of lead acetate in 100ml of boiling water) was added till precipitation of available protein and tannins which were removed by filtration. The filtrate was then refluxed with 0.1N HCl for three h when the sugar free flavonoids were liberated which were extracted with ether. The ether extract was evaporated and dried to obtain total flavonoids. The identification of flavonoid was confirmed by positive Shinoda Test.[23] The content of total flavonoid obtained was calculated with respect to the weight of dried root material taken for extraction.

Chromatographical estimation

Preparation of Extracts

Powdered root sample (10g) was exhaustively extracted with solvents (50ml each) of varying polarity; benzene (low polarity), chloroform (medium polarity) and methanol (high polarity). The extracts so obtained were concentrated and then subjected to their TLC analysis.

TLC profiling: The clean glass plates were coated with the slurry of TLC grade silica gel G by spreading techniques, dried and activated, the solvent system used was benzene: chloroform: methanol in 6:4:1. Visualization of spots in case of various extracts was done by spraying the plates by Liebermann Burchard Reagent (L.B. reagent) and subsequent heating the plates in an electric oven at 105°C for 5 minutes. In case of therapeutic ingredients, the spots were visualized by spraying the specified detecting reagents.[17, 24-31]

Estimation of Antioxidant and Antimicrobial activity

Extraction of plant material

The powdered roots of *A. racemosus* were extracted with methanol using a soxhlet extractor at 50°C. The extract so obtained was distilled over rotary evaporator and finally evaporated to dryness in vacuum desiccators. The methanolic extract was used for the determination of total phenolics and reducing activity.

Estimation of total Phenolics

The total phenolics of extract were quantified colorimetrically using Folin-Ciocalteu reagent and phenol (crystalline) as standard.[32] Five ml of Folin-Ciocalteu (diluted tenfold in distilled water), 2 ml of sodium bicarbonate (200 g/l) and 2 ml of distilled water were added to 1 ml of

extract and incubated for 15 min at room temperature, the absorbance was read at 730 nm using a spectrophotometer. The results are expressed as phenol equivalents.

Protein Content

The protein content of *A. racemosus* root powder was determined as per Lowry's procedure.[33]

Reducing Capacity Assessment

The reducing capacity (RP) of the extract was assessed as described by Oyaizu (1986). Two milliliters of extracts were added to potassium ferricyanide (2.5 ml, 10 g/l) and the mixture incubated at 50ºC for 20 min. Trichloroacetic acid (2.5 ml, 100 g/l) was added to the mixture, which was then centrifuged for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 1 g/l). The absorbance was read at 700 nm. Higher absorbance indicated greater reducing capacity.

Determination of Antimicrobial activity

The Antimicrobial activity was studied by paper disc method. The paper discs were dipped in the methanolic extract of roots of *A. racemosus* and placed over the nutrient agar medium swabbed with *Staphylococcus* inoculums and incubated at 37±10°C. The zone of inhibition was measured after 16 h. A treatment without extract was maintained as control.

RESULTS AND DISCUSSION

Organoleptic Characteristics

Morphological studies of *A. racemosus* roots were carried out by using simple observation technique, the shape, size, color and odor was evaluated and the observations are given in table 1.

Table 1: Morphological features of *A. racemosus* roots

Parameters	Features
Shape	The roots are fleshy, tuberous, tapering towards both ends, swells when soaked in water.
Size	10-60 cm in length, 1-2.5 cm in thickness
Colour	Fresh roots are white to buff in colour, dried roots are white to grayish white in colour
Surface	Rough, sign of shrinkage after drying
Texture	Short and Fibrous

Physicochemical Evaluation

The root powders of the *A. racemosus* was subjected to physicochemical evaluation for the traits like moisture content, ash values including total ash, water soluble ash, acid insoluble ash, extractive values in different organic solvents of varying polarity. The observation and results of the physicochemical studies are presented in Table 2.

Table -2: Physical evaluation of A. *Rrcemosus* roots

Physical Parameters		**Result (% w/w)**
Moisture Content		9.59
Ash Value		
Total ash		7.83
Acid soluble ash		6.10
Acid insoluble ash		1.73
Water soluble ash		2.67
Water insoluble ash		5.16
Sulphated ash		0.47
Extractives Values		
Solvents	**Exhaustive**	**Sequential**
Benzene	0.55	0.55
Chloroform	1.28	1.23
Ethyl acetate	2.25	2.92
Methanol	6.36	6.15

Moisture content of the sample was found 9.59% which suggest that it would not encourage bacterial, fungal or yeast growth, however it is necessary to prevent the contamination by proper storage. The total ash is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards. Total ash, acid soluble ash, acid insoluble ash, water soluble ash and water insoluble ash of root powder of *A racemosus* was found to be 7.83%, 6.10%, 1.73%, 2.67% and 5.16% respectively. The calculated values are within the permissible limit.

The yield of the extracts prepared was calculated and is shown in Table 2. Exhaustive and sequential extracts were prepared in order to eliminate the border line constituents that may be extracted into the solvents while carrying out the exhaustive extraction. Root powder exhaustively extracted with organic solvents, benzene, chloroform, ethyl acetate and

methanol yielded 0.55%, 1.28%, 2.25% and 6.36% of the of total extract. On the other hand, when extracted sequentially with the same solvents in order of increasing polarity with, 0.55 % of benzene, 1.33% of chloroform, 2.92% of ethyl acetate and 6.15% of methanol extracts were obtained.

Phytochemical analysis

Qualitative phytochemical screening

The qualitative analysis of the extracts from the root of *Asparagus racemosus* showed the status of phytochemical constituents in different extracts which is shown in Table 3.

Table 3: Qualitative phytochemical analysis of A. *racemosus* root extracts

Phytochemicals	Benzene Extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Carbohydrate	--	---	++	++
Alkaloids	--	++	++	++
Flavonoids	--	++	++	++
Sterols	++	++	++	++
Terpenoids	--	--	--	--
Glycoside	--	--	--	++
Protein and Amino Acid	--	--	--	++
Saponin	--	++	++	++
Tannin	--	--	++	--

++ indicates the presence of constituents and – indicates the absence of constituents

It is evident from the table that the methanol extract recorded the maximum number of chemical constituents including carbohydrate, alkaloids, flavonoids, sterols, glycosides, amino acid and saponins. Presence of steroidal compounds is of importance in pharmaceutical application as these compounds are responsible for several biological functions in the human body. This may be the reason that it is used in facilitating the secretion of milk and also in reducing the distension of mammary glands. The presence of flavonoids, which are considered to be good free-radical scavengers, indicates that this plant may have antioxidant properties. Saponins are linked to antibacterial activity and glycosides are associated in lowering blood pressure.

Quantitative Analysis

The roots of *A. racemosus* were quantitatively evaluated for total steroidal saponin, total alkaloids and total flavonoids and the results are presented in Table 4. The quantities of these

major phytochemicals were measured in milligrams obtained from per gram of the root powder.

Table 4: Quantitative determination of major phytochemicals

Phytochemicals	Content (mg/gm dw of roots)
Total steroidal saponins	5.74
Total alkaloids	2.93
Total flavonoids	0.56

Quantitative determination of total steroidal saponins, total alkaloids and total flavonoids in the roots of the plant recorded the contents of steroidal saponins (5.74 mg/gm), alkaloids 2.93 mg/gm) and flavonoids (0.56 mg/gm). The content of total steroidal saponins was found significantly higher as compare to total alkaloids and flavonoids (Fig 1)

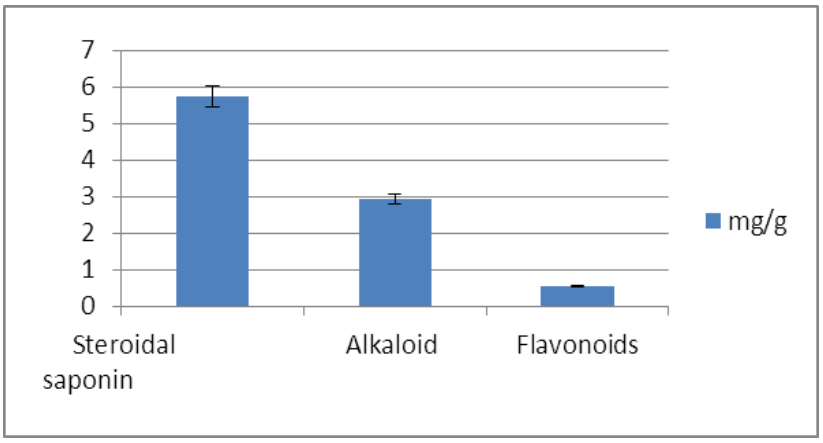

Figure 1 Quantitative values of the major phytochemicals

Higher concentration steroidal saponin, alkaoids and flavonoids may be responsible for versatile pharmacological efficacy of the roots of *Asparagus racemosus* as claimed in traditional medicine systems and observed in various modern pharmacological investigations.

Chromatographical estimation

TLC Profiling

TLC Profiling is helpful as it shows that in which solvent system the various extracts show maximum number of compounds at a reasonably different R_f value. Thin Layer Chromatographic (TLC) studies of benzene, chloroform and methanol extracts of roots of *A. racemosus* was carried out to standardize parameters for best resolution of spots and evolve a standard chemical profile for authentication. Results of TLC examination of the three extracts under the solvent system providing optimum resolution are presented in Table 5.

Table 5: TLC profile of different extracts of *A. racemosus* roots

Extracts	Solvent System	No. of Spots	Rf Values
Benzene	Benzene–Chloroform–Methanol (2:6:1)	5	0. 64, 0.56, 0.42, 0.37, 0.23
Chloroform	Chloroform–Methanol (8:2)	7	0.84, 0.62, 0.55, 0.48, 0.41, 0.25, 0.11
Methanol	Benzene–Chloroform–Methanol (3:6:1)	5	0.68, 0.62, 0.52, 0.39, 0.23

Thin layer chromatographic studies of *A. racemosus* roots (Table 5) led to the standardization of solvent systems Benzene: Chloroform: Methanol at 2:6:1 for benzene extract; Chloroform: Methanol – 8:2 for chloroform extract and Benzene: Chloroform: Methanol – 3:6:1for methanol extract based on the best resolution of spots. The chromatoplates revealed five spots (R_f values; 0. 64, 0.56, 0.42, 0.37, 0.23) for benzene extract; seven spots (R_f values; 0.84, 0.62, 0.55, 0.48, 0.41, 0.25, 0.11) for chloroform extract, five spots (R_f values; 0.68, 0.62, 0.52, 0.39, 0.23) for methanol extract.

Estimation of Antioxidant and Antimicrobial activity

The methanol extract of *A. racemosus* roots were examined for the total phenolic and protein content. Methanol extract of roots were assessed for reducing property whereas water extract was evaluated for antimicrobial efficacy against the microbe *Staphylococcus* inoculums. All the results are presented in table 6.

Table 6: Total phenolics and protein content, reducing and antimicrobial properties of A. *racemosus* leaves

Parameters	Content /Value
Phenolic content, mg/100g	586
Protein Content, mg/100g	42.15
Reducing Property (%)	2.2
Antimicrobial Properties (Inhibition zone dia. in mm)	14.75

The studies showed 586 mg/100g of total phenolics and reducing capacity of 2.2 percent as per values presented in Table 6. The results were clearly indicative of the high concentration of phenolics and resultant good reducing property in methanol extract of *A. racemosus* roots. On the account of the observations, an important point can be noted that the increased amount of phenolics and reducing property exhibited by methanol extracts of *A. racemosus* roots can help in decreasing the oxidative damage caused by free radicals. The protein content of *A. racemosus* roots was found to be 42.15 mg/100 g dry weight (Table 6). Further, studies on

antimicrobial activity of water extract showed clear inhibition of *Staphylococcus* thus reflecting its antibacterial property.

The study revealed the antimicrobial and antioxidant properties of methanolic extract of the roots of *A. racemosus.* The amount of phenolics and reducing property exhibited by methanol extracts of *A. racemosus* roots can help in decreasing the oxidative damage caused by free radicals. The protein content of *A. racemosus* roots was determined. The water extract showed clear inhibition of *Staphylococcus* which clearly reflects its antibacterial property. Antioxidants have gained interest in recent years due to their ability to neutralize the actions of free radicals.[35] Free radicals are potentially harmful products generated during a number of natural processes in the body and associated with ageing of cells and tissues. Failure to remove active oxygen compounds, over a long term, can lead to cardiovascular disease, cancer, diabetes, arthritis and various neurodegenerative disorders.[36] Hence the recent research on development of healthy foods focuses on antioxidant properties. Though there is wide range of potential antimicrobials available, only few are suitable for use. In the available literatures, the compounds other than phenolics are also suspected to be the antioxidant and antimicrobial principle. The plant studied can be seen as a source of useful drugs. The present work laying down the standard could be useful to detect the authenticity of this medicinal plant material procured from various sources.

CONCLUSION

Standard values of some important quality ascertaining parameters derived from physicochemical and phytochemical studies will be helpful in authentication of *A. racemosus* roots. Phytochemical screening indicated the presence of various phytochemicals. Quantitative evaluation of major bioactive constituents revealed considerably high content of total steroidal saponins followed by total alkaloids in roots. Methanol extract of roots recorded a high amount total phenolics and resultant good reducing activity whereas water extract of roots found to exhibit antibacterial activity against *Staphylococcus.* Notable antioxidant and antimicrobial activities of root extracts, which may be due to high concentration of certain phytochemicals validate the traditional medicinal claim of the plant in term of its chemical make-up and offer the scope of further studies toward confirmation and optimization of the active ingredients for and establishing the plant as potential source of antioxidant and antibacterial drug candidates.

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