



IDENTIFICATION OF BIOACTIVE CONSTITUENTS OF *ASPARAGUS RACEMOSUS* ROOT EXTRACT USING UV AND HPLC

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ABSTRACT

Herbal medicines and their preparations have been widely used traditionally, for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae *A. racemosus* is frequently used in ayurvedic drug preparations as it is known to treat conditions such as ageing, to boost immunity, improve longevity, vigor, mental function. *A. racemosus* also finds its application in curing neurological disorders, hepatopathy, tumors and dyspepsia. This study identified bioactive constituents of *Asparagus racemosus*. Results showed that the % Yield of roots extract of *Asparagus racemosus* was 42.76%. The R_f value of hydroalcoholic extract of *Asparagus racemosus* was found 0.31, 0.6, 0.7 indicates the presence of alkaloids. Data shows that amount of total flavonoids and alkaloid in hydroalcoholic extract was found 0.543 and 0.897 mg/100mg of dried extract. Quantitative estimation of alkaloid in hydroalcoholic extract of *Asparagus racemosus* was found 0.984% using HPLC.

Key words: *Asparagus racemosus*, bioactive constituents, Herbal medicines, HPLC, UV.

INTRODUCTION:

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis,

osteoarthritis, diabetes, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Kamboj,2000; Pratap *et al.*, 2012).

The uses of traditional medicines are widely spread and plants represent a large source of natural chemicals that might serve as leads for the development of the novel drugs (Gautam *et al.*, 2013) Scientists have devised different ways of alienating the problem and one of the easy and cheapest options is herbal medicines. Herbs have been in use since long time to treat various diseases. Almost one fourth of pharmaceutical drugs are derived from botanicals (Patil *et al.*, 2012; Brown *et al.*, 1959).

Asparagus racemosus (Shatavari) is a widely occurring medicinal plant belonging to the family of Liliaceae. This species is found abundantly in subtropical and tropical zones such as India, Asia, Australia and Africa. *A. racemosus* is frequently used in ayurvedic drug preparations as it is known to treat conditions such as ageing, to boost immunity, improve longevity, vigor, mental function. *A. racemosus* also finds its application in curing neurological disorders, hepatopathy, tumors and dyspepsia. Various therapeutic property of root of *A. racemosus* is well documented in ancient ayurvedic literature. The therapeutic property is owing to the presence of various pharmacological properties such as antioxidant property, anti inflammatory property antiseptic and antimicrobial property (Garde *et al.*, 1970)

We should make all these easily marketed ayurvedic, and other herbal medicines FDA approved and increase public awareness about pros and cons of their uses. The common belief that anything natural is safe is not correct. Herbal Medicines are readily available in the market from health food stores without prescriptions and are widely used in India, China, USA and all over the world. According to recent survey the majority of people who use herbal medicines do not inform their physicians about their consumptions that can cause abnormal test results and confusion in proper diagnosis. However, natural medicines seem to be barely able to provide convincing alternatives to conventional western medicine for global health-care (Adami *et al.*, 1964).

Materials and Methods

Collection of Plant material

Roots of *Asparagus racemosus* were collected from local area of Bhopal in the month of September, 2022.

Reagents and chemicals

Colchicine was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water was used of HPLC grade water from Merck Ltd, New Delhi, India.

Methods

Extraction by maceration process

20 gram of dried roots powdered of *Asparagus racemosus* has been extracted with hydroalcoholic solvent (methanol: water, 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007; Kokate, 1994)

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the extract of *Asparagus racemosus*, were subjected to the phytochemical tests as per standard methods.

Qualitative chromatographic analysis

Thin layer chromatography

Thin layer chromatography: T.L.C. is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method³⁵. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25 µg/ml were prepared in methanol. 10 mg of dried extract was

dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Olufunmiso, *et al.*, 2011)

Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Fazel *et al.*, 2008)

Identification of marker compound (Alkaloids) by HPLC

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical

column with a mobile phase composed of Acetonitrile: Sodium phosphate dibasic [pH-3] (17.5:32.5v/v) and was isocratically eluted at a flow rate of 1 mL min⁻¹. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 254 nm.

Results and discussion

The % Yield of roots extract of *Asparagus racemosus* was found 42.76%. Table 2 showed

the presence of phytochemicals in *Asparagus racemosus* namely alkaloids, flavonoids, proteins, diterpenes and saponins. The Rf value of hydroalcoholic extract of *Asparagus racemosus* was found 0.31, 0.6, 0.7 indicates the presence of alkaloids. Data shows that amount of total flavonoids and alkaloid in hydroalcoholic extract was found 0.543 and 0.897 mg/100mg of dried extract. Quantitative estimation of alkaloid in hydroalcoholic extract of *Asparagus racemosus* was found 0.984% using HPLC

Table 1: % Yield of *Asparagus racemosus*

S. No.	Solvent	% Yield
1.	Hydroalcoholic	42.76%

Table 2: Phytochemical screening of extract of *Asparagus racemosus*

S. No.	Constituents	Hydroalcoholic extract
		Roots extract
1.	Alkaloids	
	Dragendroff’s test	-ve
	Hager’s test	+ve
2.	Glycosides	
	Legal’s test	-ve
3.	Flavonoids	
	Lead acetate	+ve
	Alkaline test	+ve
4.	Phenol	

	Ferric chloride test	-ve
5.	Proteins Xanthoproteic test	+ve
6.	Carbohydrates Fehling's test	-ve
7.	Saponins Foam test	+ve
8.	Diterpenes Copper acetate test	+ve
9.	Tannins Gelatin Test	-ve

Table 3: Calculation of R_f Value (Alkaloid)

Hydroalcoholic extract of <i>Asparagus racemosus</i>		
S. No.	Mobile phase Ethyl acetate: methanol (10:1.3)	R_f value
1.	(Colchicine) Dis. travel by mobile phase= 5.0cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	Long- 0.3 Short- 0.3 Normal- 0.3
2.	(Hydroalcoholic extract) Dis. travel by mobile phase= 5.0cm No. of spot at long UV = 3 No. of spot at short UV = 3 No. of spot at normal light= 0	Long- 0.31, 0.6, 0.7 Short- 0.31, 0.6,0.7 Normal- 0

Table 4: Estimation of total flavonoids and alkaloid content of *Asparagus racemosus* extract

S. No.	Hydroalcoholic Extract	Total alkaloid content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Roots	0.897	0.543

Table 5: Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range (µg/ml)	Correlation co-efficient	Slope	Intercept
Colchicine	5-25	0.998	53.98	16.0.1

Table 6: Quantitative estimation of hydroalcoholic extract of *Asparagus racemosus*

S. No.	Extract	RT	Area	% Assay
1.	Hydroalcoholic extract	3.514	547.68	0.984

CONCLUSION

As *A. racemosus* is recognised to treat conditions like ageing, to enhance immunity, to improve longevity, vitality, and brain function, it is commonly utilised in ayurvedic medicinal compositions. Additionally, *A. racemosus* is used to treat dyspepsia, tumours, hepatopathy, and neurological conditions. This study identified *Asparagus racemosus's*

bioactive ingredients The phytoconstituents like alkaloids, flavonoids, proteins, diterpenes and saponins were present. The HPLC analysis revealed that Total alkaloid & flavonoid content is present in *A. racemosus* roots in quite appreciable amount which plays role in different mechanisms for curing diseases.

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