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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF *ASPARAGUS RACEMOSUS* ROOT EXTRACT

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ABSTRACT

Asparagus racemosus is one of the most frequently used herb in Indian traditional medicine is selected to study its medicinally active substances present in ethanol-extract. Preliminary Phytochemical screening of the extracts revealed the presence of triterpenoids, flavonoids, alkaloids, glycosides, fatty acids, phenolic compounds, saponins and tannins. The ethanol extract of the root of *A. racemosus* has showed the spectrum of inhibition on *Staphylococcus aureus, Klebsiella pneumonia, E. coli, Enterococcus faecal,* indicating that the phytocompounds present in it has promising antimicrobial activity. Further it is subjected to identify the number of constituents present in the *A. racemosus* by GC-MS analysis. The results obtained by the above extract have more or less similar phytoconstituents. In total there are16 compounds were identified in GC-MS analysis of ethanolic extracts of *Asparagus racemous* root which are medicinal important to become a potential drug in future perspective.

Keywords: *Asparagus racemosus*, phytochemical, antibacterial activity, secondary metabolites.

INTRODUCTION

Medicinal plants have been playing a vital role on the health and healing of several diseases since the down of human civilization. Several plants are still remained as major sources of drugs in modern as well as traditional medicines, despite of tremendous developments in the field of allopathic medicines in the present 21st century. The plant extracts have been the major source of medicinal agents from centuries. Millions of plant species are known to have medicinal value and used to cure specific ailments worldwide[1]. India is one of the most medico-culturally diverse country in the world where the medicinal plants sector is part of a time honoured tradition that is respected even today. Most of the developing countries have drastically dragged their attention towards herbal medicines for the primary health care in last decade. A survey made by world health organization (WHO) during 2002-2005 had reveal that about 80% of world population dependent on herbal medicines[2]. *Asparagus racemosus is* locally called as 'Shatavari' in Hindi and 'Majjigegadde' in Kannada belongs to the family *Asparagaceae* and genus *Asparagus*. It is an under shrub climbing herbs with a tuberous root. It is widely distributed in tropical and subtropical regions of India. *A. racemous* is spinous under shrub with numerous scucculent roots and grows at an altitude of 1500 m. The rhizome is used in traditional medical applications and also as a food supplement. Its medicinal use has been reported in Indian traditional medicine such as Ayurveda, Unani and Siddha. *A.racemous* is widely used as antioxidant[3,4], gastro protective[5], neuroprotective[6]. The tremendous potential of the plant in health care and trade is proven by various experimental studies; which authenticates the traditional practices of this plant extracts in curing many of the above mentioned diseases. The objective of the present investigation is to identify the phytochemical constituents of ethanolic extract of *A. racemous* root extract and its antibacterial activity.

MATERIAL AND METHOD

Plant Material

The plant of *A. racemosus* was collected from Bidar District, Karnataka, India. It is identified and authenticated by the Department of Botany, Gulbarga University, Kalaburagi.

Preparation of plant root extract

The root sample was thoroughly washed; shade dried and powdered using a mechanical grinder. The powder kept in air tight bottle for further study. Extraction of Phytoconstituents was done by Soxhlet Extraction Method[7].

Qualitative analysis of biomolecules

The presence of carbohydrates, alkaloids, steroids, glyceroids, flavonoids, saponins, tannins, amino acids, proteins, and phenols were detected by the method[8].

Antibacterial activity

The antibacterial activity of ethanolic extract of the root of *A. racemosus* were determined by using the agar well diffusion method[9] to determine the zone of inhibition against, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Enterococcus faecalis.* The Bacterial cultures were procured from Department of Biotechnology, Gulbarga University, Kalaburagi, Karnataka, India. The collected bacteria were maintained on nutrient agar slants at 37 ºC and sub-cultured at regular interval for further studies.

Table- 1. Phytochemical analysis of ethanol roots extract of *A. racemosus*

S.N	**Phytochemicals**	**Inference**
1	Alkaloid	+
2	Carbohydrate	+
3	Steroids	+
4	Glycosides	+
5	Flavonoids	+
6	Saponins	+
7	Tannins	+
8	Terpenoids	+
9	Aminoacids and proteins	+
10	phenols	+

Table-2. Antimicrobial activity of *A. racemosus* on different organism on nutrient agar.

Sr. no.	**Test Microorganism**	**Zone diameter (mm)**			
		25µg/ml	**50µg/m**	**75µg/ml**	**Standard Streptomycin 50µg/ml**
1	*Staphylococcus aureus*	12	14	16	18
2	*Klebsiella pneumoniae*	10	12	12	16
3	*E. coli*	8	10	11	16
4	*Enterococcus faecalis*	14	16	18	20

Table 3: Phyto- components identified in the *A. racemosus* root extract by GC-MS analysis

SN	**RT**	**Compound Name**	**Molecular Formula**	**Area %**	**Nature of compound**
1	6.13	à-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl), cyclic methylboronate	C14H31BO6Si2	0.79	carbohydrate
2	7.71	Dodecanoic acid, tricosafluoro-	C12H24O2	0.71	Lauric acid
3	8.44	1H-2,8a Methanocyclopenta[a] cyclopropa[e]cyclodecen-11-one, 5,6-bis-(acetyloxy)-4-[(acetyloxy)methyl] 1a,2,5,5a,6,9,10,10a- octahydro-5a-hydroxy-1,1,7,9-tetramethyl, [1aR(1aà,2à,5á,5aá,6á,8aà,9à,10aà)]-	C26H34O8	3.74	Myristic acid
4	10.25	Oleic acid, eicosyl ester	C38H74O2	1.96	Fatty acid
5	11.28	Anobin	C15H20O5	3.24	Sesquiterpene
6	11.47	1H-Indol-2(3H)-one,3-hydroxy-3-(3- indolyl)-	C16H12N2O2	1.08	alkaloids
7	12.31	à-D-Glucofuranose,6-O-(trimethylsilyl)- ,cyclic1,2:3,5-bis (butylboronate)	C17H34B2O6Si	1.17	carbohydrates
8	13.60	d-Mannose	C6H12O6	9.85	Aldohexose
9	15.61	9-Octadecenoic acid (Z)	C32H62O2	0.54	unsaturated fatty acid
10	15.95	Digitoxin	C41H64O13	1.40	Steroid
11	17.70	9,10-Secocholesta- 5,7,10(19)- triene1-1, 3-diol, 25- [(trimethylsilyl)oxy]-,(3á,5Z,7E)	C30H52O3Si	0.84	Steroid
12	19.22	2,5-Furandione, Dihydro-3-isooctadecyl-	C22H40O3	1.40	Alkoloid
13	20.16	Butanoic acid, 4chloro, 1, 1a, 1b, 4, 4a, 5, 7a, 7b, 8, 9-decahydro-4a, 7b-dihydroxy- 3-(hydroxymethyl)- 1, 1, 6, 8-tetramethyl-5-oxo-9aH- cyclopropa [3,4] benz[1,2-e]a zulene-9, 9a-diyl ester, [1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-	C28H38Cl2O8	2.83	Carboxylic acid
14	24.03	2-Cyclopenten-1-one, 3,4-dihydroxy-5-(3-methyl-2-butenyl)-2-(3-methyl-1-oxobutyl)-4-(4-methyl-1-oxo-3-pentenyl)-	C21H30O5	0.81	hydrocarbon
15	25.88	13- Docosenamide, (Z)-	C22H43NO	3.00	Amide group
16	31.94	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	C36H69NO6Si3	0.22	Amino acid

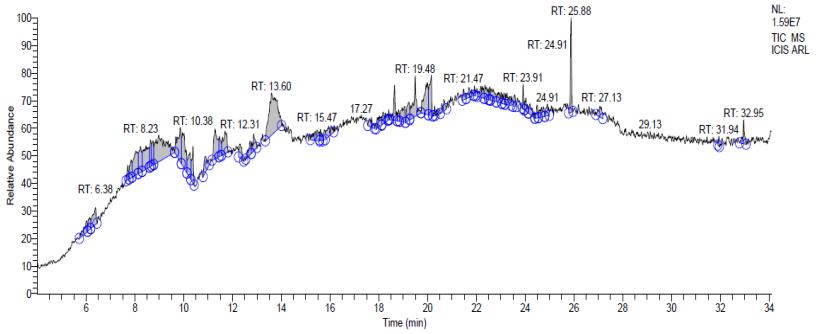

Figure 1 GC-MS analysis of ethanol extract of *A. racemosus.*

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The phytochemical investigation of ethanol extract of *A. racemosus* was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo TSQ 8000 Gas Chromatograph - Mass Spectrometer. The MS part consists of Triple Quadrupole, This mass spectrometer comes paired with the Thermo GC-TRACE 1300. Experimental conditions of GC-MS system are as follows: DB 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40 °C raised to 290 °C at 5 °C/min and injection volume was 1.0 µl. A scan interval of 0.5 seconds with scan range of 40-600 m/z. Total GC running time was 30.09min and the results were compared by using NIST library search programme[10].

RESULTS AND DISCUSSION

The results obtained in the present investigation are summarized in table 1 and 2. The Phytochemical analysis of root extract of *A. racemosus* revealed the presence of important bioactive secondary metabolites such as steroid, saponins, alkaloid, glycosides, tannins, flavonoids, amino acids and proteins. Our results align with the constituents obtained for *A. racemosus* by Selvam et al., (2014). The antimicrobial activity of ethanol extract of *A. racemous* roots has showed a significant effect against *Enterococcus faecalis* and *Staphylococcus aureus.* However, the moderate effect against *Klebsiella pneumoniae* and *E. coli* was observed. Sinha and Biswas (2011) reported antimicrobial activities against *Staphylococcus aureus* and *E. coli* which has shown 24 mm and 20 mm, respectively, of zone of inhibition for 100 µg/ml of ethanol extract of *A. racemosus.* In our study the effect has not only against *Staphylococcus aureus* and *E. coli* but also against *Klebsiella pneumoniae* and *Enterococcus faecalis*, indicating the potent antimicrobial activity.

The fragmentation patterns of the mass spectra were compared with standard compounds in National Institute of Standards and Technology (NIST). The GC-MS analysis has shown total 16 active components present in the extract which are identified based on peak area, molecular weight and molecular formula. The group of compounds detected were carbohydrates, lauric acid, myristic acid, fatty acid, sesquiterpene, alkaloids, steroid, alkoloid, carboxylic acid, hydrocarbon, amide group, and amino acids (Table 3). Similar observations were reported by Selvam et al., (2014) in *A. racemosus* root ethanol extract except à-D-Galactopyranoside,methyl 2,6-bis-O-(trimethylsilyl),cyclic methylboronate; 1H-2,8a Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,5,6-bis-(acetyloxy)-4-[(acetyloxy)methyl]1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-1,1,7,9-tetramethyl, [1aR(1aà,2à,5á,5aá,6á,8aà,9à,10aà)]-; Oleic acid, eicosyl ester; Anobin; 1H-Indol-2(3H)-one,3-hydroxy-3-(3- indolyl)-; à-D-Glucofuranose,6-O-(trimethylsilyl)-,cyclic1,2:3,5-bis (butylboronate); d-Mannose; Digitoxin; 9,10-Secocholesta-5,7,10(19)- triene1-1, 3-diol, 25- [(trimethylsilyl)oxy]-,(3á,5Z,7E); 2,5-Furandione,Dihydro-3-isooctadecyl-; Butanoic acid, 4chloro,1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy- 3-(hydroxymethyl)- 1, 1, 6, 8- tetramethyl-5-oxo-9aH- cyclopropa [3,4] benz[1,2-e]a zulene-9, 9a-diylester,[1ar-(1aà,1bá,4aá,7aà,7bà,8à,9á,9aà)]-; 2-Cyclopenten-1-one,3,4-dihydroxy-5-(3-methyl-2-butenyl)-2-(3-methyl-1-oxobutyl)-4-(4-methyl-1-oxo-3-pentenyl)-; and 13- Docosenamide, (Z)-; Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-,methyl. However, further study demands the identification and evaluation of biological activity of above observed class of compounds of *A. racemous.* The phytochemicals such as Valdetamide, Dodecanoicacid, Tetradecanoicacid, Oleicacid, Anobin, 5áCholestane3à, D-Glucofuranose, D-Mannose, 9-Octadecenoic acid (Z), Digitoxin, 9,10-Secocholesta, 2,5-Furandione, Butanoic acid, 2-Cyclopenten1one,13-Docosenamide and Glycine contributes the activities like antimicrobial[11], antioxidant[12], anticancer , hypercholesterolemic, antiulcerogenic, lubricant, nematicide, anti inflammatory, antiandrogenic and other activities[13,14].

CONCLUSION

The present study demonstrates the phytochemical constituents of ethanol extract of *A. racemous* roots have a significant effect of antibacterial activities against *Staphylococcus aureus* and *Enterococcus faecalis* and the moderate antimicrobial activity is observed against *Klebsiella pneumoniae* and *E. coli*. These results implicates the medicinal value of the plant.

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