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GC-MS PROFILING OF BIOACTIVE COMPOUNDS IN *ASPARAGUS RACEMOSUS*: IMPLICATIONS FOR PHARMACOLOGICAL PROPERTIES

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Keywords:

Phytoconstituents, *Asparagus racemosus*, Antioxidant activity, Antibacterial activity, Anti-inflammatory activity

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ABSTRACT: Background: Plants for remedial applications are well known, realizing the pharmacological potentials of plants through analytical techniques. *Asparagus racemosus* is a well-known medicinal plant analyzed using biological approach. The present study was carried out to evaluate the antioxidant, ani-inflammatory, antibacterial effects and the possible bioactive compounds present in the chloroform, methanol and aqueous extract of *A. racemosus* plant. **Methods:** The whole plant was subjected to serial solvent extraction using chloroform, methanol, and aqueous. Phytochemical screening, total phenolic and total flavonoid contents were determined using standard methods and was followed by GCMS and FTIR for biological activity of compounds. The plant extracts were assessed for antioxidant activity by DPPH and FRAP assays, anti-inflammatory activity by protein denaturation method, and antibacterial activity by agar well diffusion method. **Results:** The phytochemical tests revealed the presence of phytochemicals like alkaloids, flavonoids, glycosides, triterpenoids, and saponins. The GCMS of whole plant chloroform, methanol and aqueous extract revealed the compounds that are rich in free radical scavenging activity and proven to be the best source for antioxidant activity. The phenolics, flavonoids are linked with its antibacterial property. **Conclusion:** The present study helps to find out the novel bioactive compounds having pharmacological properties to formulate the safer drugs for the treatment of deadly diseases bothering mankind.

INTRODUCTION: Plants represent a prominent source for many pharmaceuticals as the phytochemical compounds or the secondary metabolites present in the plants have been used for treating a number of human ailments. Drugs obtained from medicinal plants comprise 25% of total drugs in developed countries and about 80% in developing countries [1].

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Plant products have been part of phytomedicines since time immemorial and can be derived from barks, leaves, flowers, roots, fruits, seeds. India has a rich cultural history as plants are the primary source of medicine in Ayurveda, Siddha, and Unani systems of medicine [2].

The secondary metabolites or bioactive compounds derived from the plants are the primary components of phytomedicine. They have numerous applications in the treatment of various ailments including, chronic and infectious diseases [3]. Despite this, oxidative stress is the primary cause for the development and progression of several diseases [4]. Antioxidants reduce the harmful effects

of oxidative damage caused by reactive oxygen species (ROS). These antioxidants are found in variety of plant species that can reduce the oxidative damage caused on by ROS [5], resulting in the prevention of cancer, cardiovascular diseases, and other neurological disorders [4]. Therefore, the major focus in modern pharmacology is the exploration of medicinal plants for their bioactive metabolites, such as phenols, alkaloids, terpenoids etc., which have various pharmacological effects like antioxidant, anti-inflammatory, and antibacterial properties [4].

Asparagus racemosus (Family: Asparagaceae), is a tropical and subtropical medicinal plant of India. *A. racemosus* is a woody climber growing to 1-2 m in height. The unique characteristics of this plants are its small, uniform and pine needles like leaves with whitish coloured flowers [6]. It is commonly known as 'Shatavari' and found to have immense pharmacological properties such as antioxidant, anti- HIV, hepato-protective, anti-diarrhoeal, antiulcer, and antibacterial effects [7-9]. *A. racemosus* has been used in Ayurveda as a galactagogue, aphrodisiac, anodyne, diuretic, antispasmodic and nervine tonic since time immemorial [10].

Even though the plant *A. racemosus* has many therapeutic applications, the systematic analysis of this plant is still undetermined in terms of their chemical constituents. The present study was carried out to identify the phytochemical compounds and their pharmacological functions as present in the chloroform, methanol and aqueous extracts of the plant through GCMS and FTIR analysis. The plant extracts were also evaluated for their antioxidant, antibacterial, and anti-inflammatory properties.

MATERIAL AND METHODS:

Collection of Sample: The disease-free plant sample was collected from the Botanical Garden of Karnataka University in Dharwad, Karnataka, India (15.4589ºN, 75.0078ºE). The plant sample was authenticated by the Botanical Survey of India at the Southern Regional Centre in Coimbatore and identified as *Asparagus racemosus*, belonging to the Asparagaceae family **Fig. 1**. The collected sample was wash thoroughly several times with tap aqueous, shade dried, grind to fine powder and stored in tight sealed containers. The nature of the powder is examined physically by the characteristics such as color, odour and texture.

FIG. 1: HABITAT OF THE PLANT *ASPARAGUS RACEMOSUS*

Solvent Extraction: The serial solvent extraction of the coarsely powdered sample was done using Soxhlet apparatus with (1:10) ratio with chloroform, methanol, and aqueous as a solvent. After extraction the solvent was evaporated and the residue was stored in airtight containers for further use.

Preliminary Phytochemical Screening: The presence of various phytochemicals including alkaloids, flavonoids, glycosides, phenols, tannins, steroids, saponins, terpenoids, carbohydrates and proteins were analysed in the crude extracts of *Asparagus racemosus* plant using standard procedures [11, 12, 13].

Estimation of Total Phenolic Content (TPC): The total phenolic content of the *A. racemosus* plant extract was determined by Folin-Ciocalteu method. The plant extract was oxidized using 1.5 mL of Folin-Ciocalteu (FC) reagent and 7.5% Sodium carbonate (Na_2CO_3) solution. After 1 hour incubation at room temperature, the absorbance was read at 750nm. The amount was calculated using gallic acid calibration curve and expressed as Gallic acid equivalent (GAE) mg/g of sample [14]. The experiment was performed in triplicates and results were expressed as mean ± standard deviation.

Estimation of Total Flavonoid Content (TFC): The total flavonoid content of *A. racemosus* plant extract was determined using a spectrophotometric method. The plant extract was mixed with 10%

Aluminum chloride ($AlCl_3$) and 1M sodium acetate. The absorbance was measured at 415nm after the incubation in the dark for 45min.The experiment was performed in triplicates and results were expressed as mean ± standard deviation. The amount was calculated using Quarcetin calibration curve and the results were expressed as quercetin equivalent (QE) mg/g of sample [15].

GCMS Analysis: GC-MS analysis of the different extracts of *A. racemosus* was carried out by using the GC-MS instrument (Model GCMS-QP 2010 plus, Shimadzu). The instrument was operated in electron impact mode at ionization voltage (70 eV), injector temperature (250ºC) and detector temperature (280ºC). About 1µL of the sample was injected into mobile phase consisting of helium (99.9% purity) at a flow rate of 1mL/min. The oven temperature was initially programmed at 60ºC (isothermal for 2 min) before being raised to 100 ºC and finally to 280ºC at a rate of 5 ºC/min for 9 min.

The Gas Chromatogramran for 34 minutes in total and the relative percentage amount of each component was calculated by comparing its average peak area to the total area. The comparison was made between the spectra of unknown component and the spectrum of the known components with the help of the National institute of Standards and Technology-5 (NIST-5) library, and the compounds were identified including the compound's name, their molecular formula, molecular weight and their structure.

FTIR Spectroscopic Analysis: FTIR analysis of *A. racemosus* plant was performed using Perkin Elmer Spectrum system (version 10.7.2), which was used to detect the characteristic peaks ranging from 500-4000 cm^{-1} and their functional groups. The peak values of the FTIR were recorded.

Pharmacological Activity of *Asparagus racemosus:*

***In-vitro* Antioxidant Activity of *A. racemosus* Plant Extract:**

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging Assay: Free radical scavenging activity of the plant extracts of *A. racemosus* was evaluated according to Vardhini *et al.* method [16]. 1mL of DPPH solution was mixed with different concentrations of the plant extracts (50, 100, 150, 200, and 250 µg). The mixture was incubated for 30 min in the dark at room temperature and the absorbance was measured at 517 nm. Ascorbic acid was used as standard reference. The capacity of radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A_C - A_S)/ A_C \times 100$$

Where, A_C is the absorbance of the control reaction and A_S is the absorbance of the sample. The experiment was performed in triplicates and the IC_{50} value was calculated for all the samples.

Ferric Ion Reducing Power Assay: Ferric ion reducing power (FRAP) assay was measured according to Asraoui method [17] with slight modifications. The different concentration of *A. racemosus* plant extracts (50, 100, 150, 200, and 250 µg) were added to the mixture containing 2.5 mL of 0.2 M phosphate buffer and 2.5mL of 1% potassium ferrocyanide. This mixture was incubated at 50ºC for 30 min. After cooling, 2.5 mL of 10% trichloroacetic acid and 0.5 mL of 0.1% ferric chloride was added. The mixture was left at room temperature for 10 min to form bluish green colour complex. The absorbance was measured at 700 nm and Ascorbic acid was used as a standard.

Anti-inflammatory Activity: Anti-inflammatory activity of different extracts of *A. racemosus* plant was evaluated by protein denaturation assay using Aspirin as a standard drug [18]. To the different concentrations (50, 100, 150, 200, 250µg) of the plant extracts, 1 mL of phosphate buffered saline and 50 µL of bovine serum albumin (BSA) was added, and incubated for 15 min at room temperature followed by incubation at 70 ºC for 30 min. After cooling, the absorbance was read at 660 nm. The percent of inhibition of protein denaturation was calculated using the following formula:

$$\text{\% Protein denaturation activity} = (A_C - A_T)/A_C \times 100$$

Where, A_C is the absorbance of the control reaction and A_T is the absorbance of the sample, and IC_{50} value was calculated for all the samples.

Antibacterial Activity: Antimicrobial activity of *Asparagus racemosus* plant extracts were studied by agar well diffusion method [19] using *Pseudomonas aeruginosa*, *Escherichia coli*, *Xanthomonas* cultures as the test organisms. 100µL of the saline suspension was swabbed uniformly

over the sterile agar plates. The different concentrations of the plant extracts (30, 60, 90 and 120µg) along with standard drug Ciprofloxacin (30 µL) were added to the medium. The plates were incubated at 37 ºC for 24 hours and the diameter of zone of inhibition formed around the well after incubation was measured in millimetres.

Statistical Analysis: The experiments were performed in triplicates and the results were expressed as mean ± standard error. The significance was analyzed through two-way ANOVA analysis with significant *p* value (0.01 and 0.05).

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening: Plants are a substantial source of potentially useful bioactive components for the development of novel chemotherapeutic medicines due to their rich phytochemicals [20]. Medicinal plants are renewable source of drugs, offering safety and efficacy with minimal side effects compared to synthetic drugs, and have been used since time immemorial and their utility is increasing day by day in the present world [21]. The medicinal plant *A. racemosus* is a boon to mankind as its rich phytochemicals are used to treat numerous diseases. The qualitative phytochemical screening of different extracts of *A. racemosus* showed the presence of alkaloids in methanol and aqueous extracts; sterols in chloroform and aqueous extracts; flavonoids, glycosides, phenols, tannins and saponins were detected in all the three extracts i.e., chloroform, methanol and aqueous extracts whereas terpenoids, carbohydrates and proteins were not detected in any of the extracts. The result of the phytochemical analysis is presented in **Table 1**.

Total Phenolic and Flavonoid Content: Several reports tend to show that secondary metabolites that are phenolic nature including flavonoids are responsible for the variety of pharmacological activities [22, 23]. Because of this, the total phenolic and total flavonoid contents of *A. racemosus* plant extracts were determined. In case of total phenolic content, the aqueous extract had the highest amount (73.79 ± 0.009 mg/g of GAE), followed by methanol extract (31.23 ± 0.003 mg/g of GAE), and chloroform extract being the least (10.43 ± 0.006 mg/g of GAE) respectively. While in case of total flavonoid content the aqueous extract of *A. racemosus* plant showed the maximum flavonoid content (97.43 ± 0.003 mg/g of QE), followed by methanol (84.54 ± 0.005 mg/g of QE) and then the chloroform extract (34.40 ± 0.004 mg/g of QE). A wide range of variation was observed in total phenolic and total flavonoid content in each extract **Table 2**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE WHOLE PLANT EXTRACTS OF *ASPARAGUS RACEMOSUS*

Tests	Inference		
	Chloroform	Methanol	Aqueous
Alkaloids	-	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Sterols	+	-	+
Saponins	+	+	+
Terpenoids	-	-	-
Carbohydrates	-	-	-
Proteins	-	-	-

'+' indicates Present; '-' indicates absent

TABLE 2: TOTAL PHENOL AND TOTAL FLAVONOID CONTENT FROM *ASPARAGUS RACEMOSUS* WHOLE PLANT EXTRACTS

Extract	Total Phenolic content (mg/g of GAE)	Total Flavonoid content (mg/g of QE)
Chloroform extract	10.43 ± 0.006	34.40 ± 0.004
Methanol extract	31.23 ± 0.003	84.54 ± 0.005
Aqueous extract	73.79 ± 0.009	97.43 ± 0.003

The results are expressed as mg/g equivalent ± standard deviation

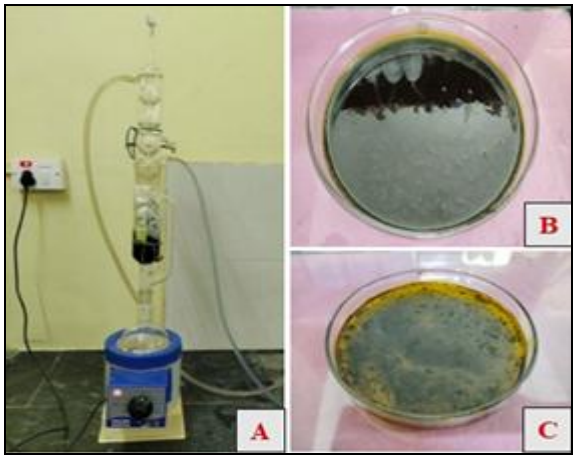

FIG. 2: EXTRACTION OF THE WHOLE PLANT *ASPARAGUS RACEMOSUS*; A. SOXHLET EXTRACTION OF THE PLANT; B. EXTRACTED SOLVENT; C. CRUDE EXTRACT

GC-MS Analysis: The GCMS spectra of chloroform, methanol and aqueous extract of *A. racemosus* plant showed the presence of 19, 64 and 54 compounds, respectively. Based on the peak area, Phytol, Tetratetracontane, Eicosane and Octadecanal are the major compounds found in chloroform extract. Methanol and aqueous extracts had Sucrose as major compounds while 1,2-Dithiolane-3-carboxylic acid, and Dotriacontane major compounds found in aqueous extract. The compounds identified had a diverse pharmacological propertysuch as Stigmasterol was known to have anti-inflammatory, antioxidant, antimicrobial, anticancer, antiarthritic and anti-asthama activity [24, 25]. Phytol was known to have antimicrobial, anti-inflammatory, antiallergic, anticancer, diuretic, antidiabetic, cytotoxicity, antiproliferative, cancer preventive properties [24, 26]. The GCMS spectra, compound name, retention time, peak area, molecular weight, molecular formula and their uses are depicted **Table 3-5** and **Fig. 3-5**).

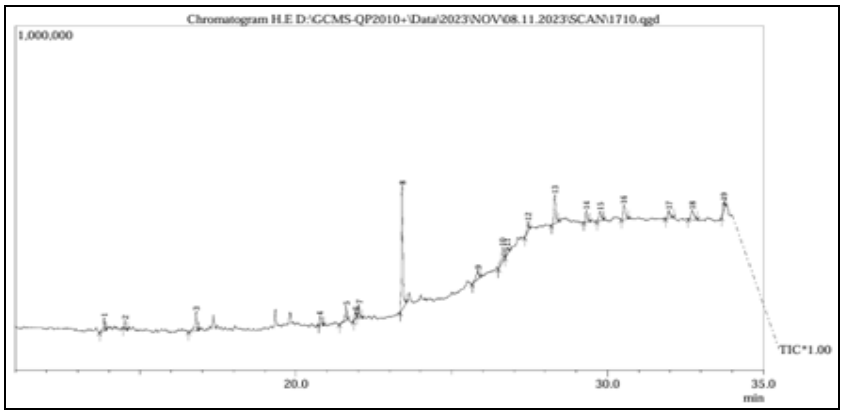

FIG. 3: GC-MS CHROMATOGRAM OF CHLOROFORM EXTRACT OF *A. RACEMOSUS* PLANT

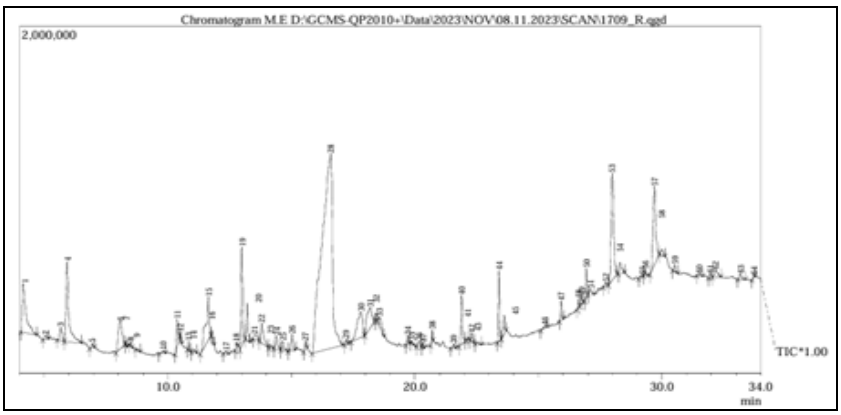

FIG. 4: GC-MS CHROMATOGRAM OF METHANOL EXTRACT OF *A. RACEMOSUS* PLANT

FIG. 5: GC-MS CHROMATOGRAM OF AQUEOUS EXTRACT OF A. RACEMOSUS PLANT

TABLE 3: COMPOUND IDENTIFIED FROM CHLOROFORM EXTRACT OF *A. RACEMOSUS* AND ITS USES

Sl. no.	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time	Peak area %	Compound nature	Uses/Activity
1.	Hexadecane	$C_{16}H_{34}$	226.44	13.852	3.56	Saturated hydrocarbon	Antibacterial, antioxidant property
2.	Dodecane,2,6,11 -trimethyl-	$C_{15}H_{32}$	212.41	14.518	1.84	Aliphatic hydrocarbon	Antimicrobial
3.	Eicosane	$C_{20}H_{42}$	282.5	16.804	6.71	Saturated hydrocarbon	Antioxidant property
4.	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.5	20.778	2.29	Keto group	Hypocholesterolemic, antimicrobial, antioxidant, and lubrication
5.	Eicosane	$C_{20}H_{42}$	282.5	21.604	4.16	Saturated hydrocarbon	Antioxidant property
6.	l-(+)-Ascorbicacid2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.9	21.902	1.90	Lipophilic ester	Antioxidant and anti-inflammatory properties
7.	Tetracosane	$C_{24}H_{50}$	338.7	22.020	2.88	Saturated hydrocarbon	Anticancer
8.	Phytol	$C_{20}H_{40}O$	296.5	23.409	30.46	Diterpene	Antimicrobial, Anti-inflammatory, antiallergic, Anticancer, Diuretic, antidiabetic, Cytotoxicity,
9.	Hexadecane,2,6, 10,14-tetramethyl-	$C_{20}H_{42}$	282.5	25.836	3.82	Saturated hydrocarbon	Antifungal, antibacterial, antitumor, and cytotoxic effects
10.	Hexatriacontane	$C_{36}H_{74}$	507.0	26.640	5.42	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
11.	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl) ethylester	$C_{19}H_{38}O_4$	330.5	26.768	2.46	Amino compound	Antimicrobial
12.	Hexatriacontane	$C_{36}H_{74}$	507.0	27.435	3.07	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
13.	Tetratetra-contane	$C_{44}H_{90}$	619.20	28.308	8.22	Saturated alkane	antioxidant property
14.	Nonacosane	$C_{29}H_{60}$	408.8	29.321	3.49	Saturated hydrocarbon	Antimicrobial property

15.	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}O$	426.7	29.769	3.70	Polysaturated alcohol	Antimicrobial, anti-inflammatory, antioxidant property
16.	Tetracontane	$C_{40}H_{82}$	563.1	30.523	5.64	Alkane	Anti-inflammatory
17.	Hexatriacontane	$C_{36}H_{74}$	507.0	31.965	3.10	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
18.	Octadecanal	$C_{18}H_{36}O$	268.5	32.709	4.69	Saturated aldehyde	Antimicrobial, anti-inflammatory property
19.	Tetracontane	$C_{40}H_{82}$	563.1	33.728	2.59	Alkane	Anti-inflammatory

TABLE 4: COMPOUND IDENTIFIED FROM METHANOLIC EXTRACT OF *A. RACEMOSUS* AND ITS USES

Sl. no.	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time	Peak area %	Compound nature	Uses/activity
1	dl-Glyceraldehyde	$C_3H_6O_3$	90.08	4.148	4.11	Monosaccharide	-
2	3-Furanmethanol	$C_5H_6O_2$	98.10	5.052	0.21	Furan derivative	-
3	Propanoicacid,2-oxo-,methylester	$C_4H_6O_3$	102.09	5.674	0.72	-	Antioxidant, anti-inflammatory potential, flavoring agents
4	dl-Glyceraldehydedimer	$C_6H_{12}O_6$	180.16	5.926	5.21	Aldehyde	Sugar moiety and Preservative
5	1,2-Cyclopentanedione	$C_5H_6O_2$	98.11	6.955	0.16	Diketone	Antioxidant, antimicrobial property
6	Glycerin	$C_3H_8O_3$	92.09	8.082	2.37	Polyphenols	Antimicrobial, Anti-inflammatory
7	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12	8.309	0.11	Cyclic ester	Antioxidant, antimicrobial and anti-inflammatory properties
8	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	8.495	0.10	Cyclic ester	-
9	2-Pentenoicacid,methylester,(E)-	$C_6H_{10}O_2$	114.14	8.751	0.09	Unsaturated ester	Antioxidant, antimicrobial properties
10	Pentanoicacid,4-oxo-	$C_5H_8O_3$	116.11	9.824	0.16	-	-
11	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12	10.383	0.99	Amine	-
12	1-Butene,4-iodo-	C_4H_7I	182.0	10.522	0.15	Unsaturated hydrocarbon	Antimicrobial property
13	Undecane	$C_{11}H_{24}$	156.31	10.848	0.23	Alkane	Lubricants and lubricant additives
14	2-Furanone,3,4-dihydroxytetrahydro	$C_6H_8O_3$	118.09	11.065	0.12	-	Antioxidant, anti-inflammatory, antimicrobial properties
15	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	11.645	3.37	Polyphenol	Antimicrobial, anti-inflammatory
16	2(3H)-Furanone,dihydro-4-hydroxy-	$C_5H_6O_3$	102.09	11.801	0.21	Furanone derivative	Antioxidant, anti-inflammatory, antimicrobial, neuroprotective properties
17	1,2,6-Hexanetriol	$C_6H_{14}O_3$	134.17	12.362	0.12	Alcohol	Antioxidant property
18	2(3H)-Furanone, 5-butyldihydro-	$C_8H_{14}O_2$	142.20	12.788	0.14	Furanone derivative	Antimicrobial, antioxidant and anticancer properties
19	2-	$C_6H_6O_3$	126.11	12.998	3.18	Heterocyclic	-

	Furancarboxaldehyde,5-(hydroxymethyl)-					compound	
20	1,2,3-Propanetriol,1-acetate	$C_5H_{10}O_4$	134.13	13.217	1.22	Glycerol ester	Antimicrobial, antioxidant and anti-inflammatory properties
21	1,2,3,4-Butanetetrol	$C_4H_{10}O_4$	122.12	13.528	0.41	Polyol	Antioxidant, antimicrobial, cryoprotectant properties
22	2-Butenoicacid,2-(acetylamino)-	$C_6H_9NO_3$	143.14	13.813	1.25	Amide derivative	-
23	2-Aminooxy-4-methylvalericacid,methylester	$C_7H_{15}NO_3$	161.20	14.148	0.15	Ester	Neuroactive, antioxidant, antimicrobial and anti-inflammatory activity
24	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	14.397	0.67	Phenolic	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination
25	IsosorbideDinitrate	$C_6H_8N_2O_8$	236.14	14.653	0.43	Ester	Vasodilation, treatment of heart failure
26	Oxalicacid, cyclohexylmethyloctyl ester	$C_{17}H_{30}O_4$	298.4	15.032	0.67	Ester	-
27	Quinoline,8-hydrazino-	$C_9H_9N_3$	159.19	15.563	0.29	Quinoline derivative	Antimalarial, antimicrobial, anticancer property
28	Sucrose	$C_{12}H_{22}O_{11}$	342.30	16.606	45.17	Disaccharide sugar	Energy source, blood sugar regulation, insulin secretion and metabolic effects
29	4-Octanone,5-hydroxy-2,7-dimethyl-	$C_{10}H_{20}O_2$	172.26	17.231	0.17	Aliphatic ketone	-
30	L-Arabinitol	$C_5H_{12}O_5$	152.15	17.810	2.98	Polyol (sugar alcohol)	Antioxidant, antimicrobial, neuroprotective and anti-inflammatory properties
31	3-Deoxy-d-mannoiclactone	$C_6H_{10}O_5$	162.14	18.201	2.46	Cyclic ester	Antibacterial, antimetabolite, anticancer property
32	.alpha.-Methyl-l-sorboside	$C_7H_{14}O_6$	194.18	18.440	0.21	Monosaccharide	Antioxidant property
33	3-Deoxy-d-mannoiclactone	$C_6H_{10}O_5$	162.14	18.551	0.63	Cyclic ester	Antibacterial, antimetabolite, anticancer property
34	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	19.709	0.25	Phenols	Antioxidant, anti-inflammatory, antimicrobial and anticancer properties
35	1,1'-Methylene-bis(di-2-propenylamine)	$C_{13}H_{22}N_2$	206.33	19.884	0.10	Aliphatic amine	-
36	Cyclohexane, 2-butyl-1,1,3-trimethyl-	$C_{13}H_{26}$	182.35	20.168	0.28	Cyclohexane derivative	-
37	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222.28	20.307	0.13	-	-

38	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	20.705	0.30	Terpene alcohol	Catechol-O-Methyl-Transfearse inhibitor, antimicrobial, anti-inflammatory
39	Hexadecanoicacid,methylester	$C_{17}H_{34}O_2$	270.5	21.571	0.15	Ester	-
40	Pentadecanoicacid	$C_{15}H_{30}O_2$	242.40	21.899	1.56	Saturated fatty acid	Anti-inflammatory, cardiovascular, antimicrobial, neuroprotective, cancer prevention properties
41	2-Cyclohexen-1-one,3-(3-hydroxybutyl)-2,4,4-trimethyl-	$C_{13}H_{18}O_3$	222.28	22.158	0.08	Cyclohexane derivative	Antioxidant, anti-inflammatory, antimicrobial and neuroprotective effects
42	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10A-	$C_{20}H_{28}O_5$	348.4	22.315	0.29	Polycyclic compound	Antioxidant, anti-inflammatory properties
43	Ethyliso-allocholate	$C_{26}H_{44}O_5$	436.6	22.527	0.10	Ester	Lipid metabolism, anti-inflammatory properties
44	Phytol	$C_{20}H_{40}O$	296.5	23.405	1.91	Diterpene	Antimicrobial, Anti-inflammatory, antiallergic, Anticancer, Diuretic, antidiabetic, Cytotoxicity, antiproliferative, cancer preventive
45	9,12-Octadecadienoicacid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	23.621	0.85	Polyunsaturated fatty acid	Anti-inflammatory, antioxidant, cardiovascular and neuroprotective effects
46	9-Eicosyne	$C_{20}H_{38}$	278.5	25.279	0.15	Unsaturated fatty acid	-
47	Phenol,4,4'-(3-ethenyl-1-propene-1,3-diyl)bis-,(E)-	$C_{17}H_{16}O_2$	252.31	25.925	0.53	Phenol	Antioxidant, anti-inflammatory, anticancer, antimicrobial, neuroprotective and estrogenic activity
48	Triacontane, 1-bromo-	$C_{30}H_{61}Br$	501.7	26.633	0.23	Alkyl bromide	-
49	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethylester	$C_{19}H_{38}O_4$	330.5	26.760	0.21	Amino compound	Antimicrobial
50	Pent-3-ene-2-one,3-phenyl-,oxime	$C_{11}H_{13}NO$	175.23	26.940	0.79	Oxime	-
51	Ergost-5-en-3-ol,(3.beta.)-	$C_{28}H_{48}O$	400.7	27.103	0.30	Steroid	Antioxidant, anti-inflammatory, antifungal properties
52	Cholest-4-en-3-one	$C_{27}H_{44}O$	384.6	27.732	0.18	Keto-steroid	Anticancer, anti-inflammatory and neuroprotective effects
53	Stigmasterol	$C_{29}H_{48}O$	412.7	27.999	5.82	Steroid	Anti-inflammatory, antioxidant, antimicrobial, anticancer, diuretic properties
54	Triacontane, 1-	$C_{30}H_{61}Br$	501.7	28.306	0.56	Alkyl bromide	-

	bromo-						
55	Pseduosarsasapogenin-5-enmethylether	$C_{29}H_{48}O_4$	460.7	29.215	0.08	Steroid	Anti-inflammatory, antioxidant, antimicrobial, anticancer properties
56	Dotriacontane	$C_{32}H_{66}$	450.9	29.316	0.19	Hydrocarbon	Antimicrobial, antioxidant, antispasmodic, antibacterial and antiviral
57	Cholest-5-en-3-ol(3.beta.)-	$C_{27}H_{46}O$	386.65	29.700	4.83	Sterol	Cardiovascular, neurosteroid, antioxidant and immunomodulatory effects
58	8,9,9,10,10,11-Hexafluoro-4,4-dimethyl-3,5-dioxatetracyclo[5.4.1.0(2,6).0(8,11)]d	$C_{10}H_6F_6O_2$	272.14	29.986	0.37	Polycyclic compound	Anticancer, antimicrobial and drug delivery
59	Tetracontane	$C_{40}H_{82}$	563.1	30.512	0.21	Alkane	Anti-inflammatory
60	1,5,9-Cyclododecatriene,1,5,9-trimethyl-	$C_{15}H_{24}$	204.35	31.514	0.10	Trimethylated cycloalkene	Antimicrobial, anti-inflammatory effects
61	Hexatriacontane	$C_{36}H_{74}$	507.0	31.958	0.14	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
62	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	410.7	32.176	0.52	Steroid	Antioxidant, anti-inflammatory, anticancer, antimicrobial effects
63	7-Dehydrodiosgenin3-acetate	$C_{29}H_{42}O_4$	454.6	33.179	0.27	Steroid	Antioxidant, anti-inflammatory, anticancer, antimicrobial, lipid lowering effects
64	Tetratetracontane	$C_{44}H_{90}$	619.20	33.722	0.09	Saturated alkane	-

TABLE 5: COMPOUND IDENTIFIED FROM AQUEOUS EXTRACT OF *A. RACEMOSUS*PLANT AND ITS USES

Sl. no.	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time	Peak area %	Compound nature	Uses/Activity
1	dl-Glyceraldehyde	$C_3H_6O_3$	90.08	4.155	3.62	Monosaccharide	-
2	2-Furanmethanol	$C_5H_6O_2$	98.10	5.032	0.36	Furfuryl alcohol derivative	Antioxidant, antimicrobial and neuroprotective properties
3	Propanoicacid,2-oxo-,methylester	$C_4H_6O_3$	102.09	5.660	1.53	-	Antioxidant, anti-inflammatory potential, flavoring agents
4	dl-Glyceraldehydedimer	$C_6H_{12}O_6$	180.16	5.912	7.16	Aldehyde	Sugar moiety and Preservative
5	Butyrolactone	$C_4H_6O_2$	86.09	6.655	0.30	Cyclic ester	-
6	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	98.10	6.953	2.80	-	Antioxidant, anti-inflammatory, antimicrobial, anticancer properties
7	Glycerin	$C_3H_8O_3$	92.09	8.021	1.69	Polyphenols	Antimicrobial, Anti-inflammatory
8	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12	8.311	0.35	Cyclic ester	Antioxidant, antimicrobial and anti-inflammatory properties
9	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	8.503	0.25	Cyclic ester	-
10	2H-Pyran-2,6(3H)-	$C_5H_4O_3$	112.08	8.650	0.46	Heterocyclic	Antioxidant,

	dione						antimicrobial and antitumor properties
11	Pantolactone	$C_6H_{10}O_3$	130.14	9.538	0.81	Cyclic lactone	-
12	Pentanoicacid,4-oxo-	$C_5H_8O_3$	116.11	9.824	0.31	-	-
13	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12	10.378	1.29	Amine	-
14	3-Nitro-2-methyl propene	$C_4H_7NO_2$	101.10	10.527	0.27	Unsaturated hydrocarbon	-
15	Dodecane	$C_{12}H_{26}$	170.33	10.851	0.20	Saturated hydrocarbon	Food additives, antifungal and antibacterial activity.
16	1-Hexanethiol	$C_6H_{14}S$	118.24	11.050	0.60	Aliphatic alcohol derivative	-
17	Aceticacid,hexylester	$C_8H_{16}O_2$	144.21	11.462	1.27	Ester	Antimicrobial property
18	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	11.644	2.69	Polyphenol	Antimicrobial, anti-inflammatory
19	2(3H)-Furanone,dihydro-4-hydroxy-	$C_5H_6O_3$	102.09	11.799	0.62	Furanone derivative	Antioxidant, anti-inflammatory, antimicrobial, neuroprotective properties
20	1-Methoxy-4-(methylthio)but-2-ene	$C_6H_{12}OS$	132.23	12.125	0.28	Alkene derivative	-
21	2(3H)-Furanone, 5-heptyldihydro-	$C_{11}H_{20}O_2$	184.27	12.365	0.20	Heterocyclic compound	-
22	2,2'-Bi-2H-pyran,octahydro-	$C_{10}H_{18}O_2$	170.25	12.777	0.45	Heterocyclic compound	-
23	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126.11	13.011	1.23	Heterocyclic compound	-
24	1,2,3-Propanetriol,1-acetate	$C_5H_{10}O_4$	134.13	13.214	2.86	Glycerol ester	Antimicrobial, antioxidant and anti-inflammatory properties
25	1,2-Ethanediol,monoacetate	$C_4H_8O_3$	104.10	13.407	0.19	Ester	-
26	2-Butenoicacid,2-(acetylamino)-	$C_6H_9NO_3$	143.14	13.813	2.50	Amide derivative	-
27	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	14.397	0.83	Phenolic	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination
28	IsosorbideDinitrate	$C_6H_8N_2O_8$	236.14	14.650	0.62	Ester	Vasodilation, treatment of heart failure
29	2-Formyl-9-[.beta.-d-ribofuranosyl]hypoxanthine	$C_{11}H_{12}N_4O_6$	296.24	15.651	1.10	Purine derivative	Antiviral, anticancer, purine synthesis enzyme inhibitor, immunosuppressive properties
30	1,2-Dithiolane-3-carboxylicacid	$C_4H_6O_2S_2$	150.2	15.947	6.85	Heterocyclic	Antioxidant, antimicrobial, enzyme modulating activity
31	Sucrose	$C_{12}H_{22}O_{11}$	342.30	16.355	35.29	Disaccharide	Energy source, blood sugar regulation,

							insulin secretion and metabolic effects
32	D-Allose	$C_6H_{12}O_6$	180.16	16.636	0.80	Monosaccharide	Antidiabetic, antioxidant, anticancer and prebiotic effects
33	[3,3'-Bi-1H-1,2,4-triazole]-5,5'-diamine	$C_4H_6N_8$	166.15	17.513	0.31	Diamine derivative	Antimicrobial, anticancer, anti-inflammatory properties
34	3-Deoxy-d-mannoiclactone	$C_6H_{10}O_5$	162.14	18.058	1.56	Cyclic ester	Antibacterial, antimetabolite, anticancer property
35	1H-Pyrrole-2-carboxylicacid,4-(benzylaminomethyl)-3,5-dimethyl-,ethylester	$C_{17}H_{22}N_2O_2$	286.37	18.157	0.38	Pyrrole derivative	Antimicrobial, anti-inflammatory, anticancer and neuroprotective effects
36	3-Deoxy-d-mannoiclactone	$C_6H_{10}O_5$	162.14	18.433	3.02	Cyclic ester	Antibacterial, antimetabolite, anticancer property
37	6-O-Methyl-2,4-methylene-.beta.-sedoheptitol	$C_9H_{18}O_7$	238.23	18.715	0.27	Sugar alcohol	Antibacterial, antiviral, antioxidant, enzyme modulating properties
38	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	19.713	0.30	Phenols	Antioxidant, anti-inflammatory, antimicrobial and anticancer properties
39	2-Amino-3-hydroxypyridine	$C_5H_6N_2O$	110.11	19.890	0.37	Pyridine derivative	Neuroprotective, antioxidant, antimicrobial, anticancer effects
40	Undecanoicacid	$C_{11}H_{22}O_2$	186.29	21.896	0.75	Saturated fatty acid	Antimicrobial, anti-inflammatory, neuroprotective properties
41	3,5-Dimethoxy-4-hydroxyphenethylamine	$C_{10}H_{15}NO_3$	197.23	22.101	0.24	Phenethylamines	Neurotransmitter-modulating, antioxidant property
42	5.beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol	$C_{15}H_{26}O$	222.37	23.626	0.29	Sesquiterpene alcohol	Anti-inflammatory, antioxidant, antimicrobial, anticancer, neuroprotective effects
43	Dotriacontane	$C_{32}H_{66}$	450.9	26.008	0.44	Hydrocarbon	Antimicrobial, antioxidant, antispasmodic, antibacterial and antiviral
44	Pent-3-ene-2-one,3-phenyl-,oxime	$C_{11}H_{13}NO$	175.23	26.945	0.58	Oxime	-
45	Campesterol	$C_{28}H_{48}O$	400.7	27.118	0.40	Sterol	Cholesterol-lowering, antioxidant, anti-inflammatory, anticancer properties
46	1-Ethyl-2-pyrrolidinone	$C_6H_{11}NO$	113.16	27.503	0.27	Cyclic amide	Anti-inflammatory, antimicrobial, anticancer, neuroprotective effects

47	Stigmasterol	$C_{29}H_{48}O$	412.7	28.011	2.71	Steroid	Anti-inflammatory, antioxidant, antimicrobial, anticancer, diuretic properties
48	Eicosane	$C_{20}H_{42}$	282.5	28.311	0.39	Saturated hydrocarbon	Antioxidant property
49	Dotriacontane	$C_{32}H_{66}$	450.9	28.966	4.65	Hydrocarbon	Antimicrobial, antioxidant, antispasmodic, antibacterial and antiviral
50	Hexatriacontane	$C_{36}H_{74}$	507.0	29.318	0.22	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
51	Cholest-5-en-3-ol(3.beta.)-, carbonochloridate	$C_{28}H_{45}ClO_2$	449.1	29.713	2.24	Cholesterol ester	Anti-inflammatory, antimicrobial, cytotoxic activities
52	Hexatriacontane	$C_{36}H_{74}$	507.0	30.516	0.22	Saturated hydrocarbon	Antimicrobial, anti-inflammatory property
53	Trimethylsilyl-di (timethylsiloxy)-silane	$C_9H_{27}O_2Si_4$	279.65	33.232	0.24	Organ silicon compound	-
54	4-(5-Bromo-3-tert-butylsalicyl)-2,6-di-tert-butylphenol	$C_{25}H_{35}BrO_2$	447.4	33.827	0.36	Phenol	Antioxidant, anti-inflammatory, antimicrobial properties

FTIR Analysis: FTIR analysis provide information about the functional groups of the compounds [27] and the *A. racemosus* plant extracts revealed several functional groups based on the peak value ratio. The functional groups present in the chloroform, methanol, and aqueous extracts of *A. racemosus* plant were identified based on the peak values in the IR region. The results showed the presence of C=C, C-H, C=O, O-H, C-N, C-O, C-F, S=O, S-C≡N, and N-H groups. The peak values, their respective functional groups and their bond nature are represented in **Table 6-8** and **Fig. 6-8**.

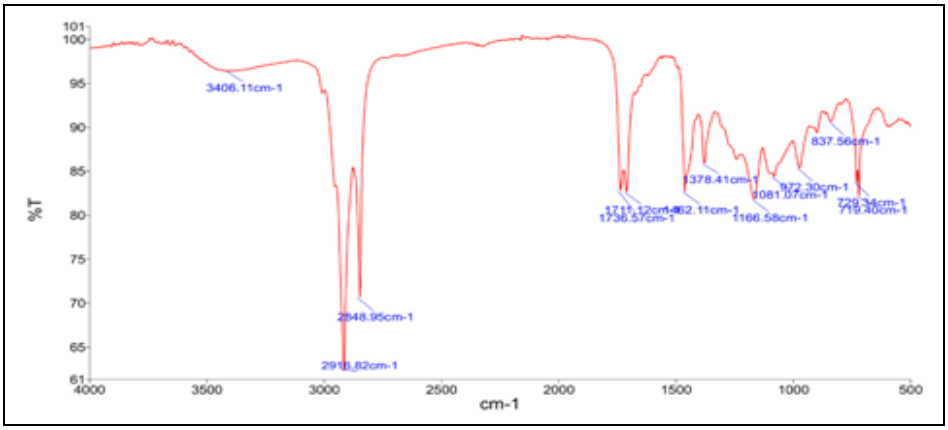

FIG. 6: FTIR SPECTRA OF THE WHOLE PLANT CHLOROFORM EXTRACT OF *A. RACEMOSUS*

TABLE 6: FTIR INTERPRETATION OF COMPOUNDS OF WHOLE PLANT CHLOROFORM EXTRACT OF *A. RACEMOSUS*

Frequency (cm^{-1})	Functional group	Bond strength
3406.11	Alcohol	O-H stretching
2916.82	Alkane	C-H stretching
2848.95	Alkane	C-H stretching
1736.57	Esters	C=O stretching

1711.12	Aliphatic ketone	C=O stretching
1462.11	alkane	C-H bending
1378.41	Alcohol	O-H bending
1166.58	Esters	C-O stretching
1081.07	Amine	C-N stretching
972.30	Alkene	C=C bending
837.56	Alkene	C=C bending
729.34	Alkene	C=C bending
719.40	Alkene	C=C bending

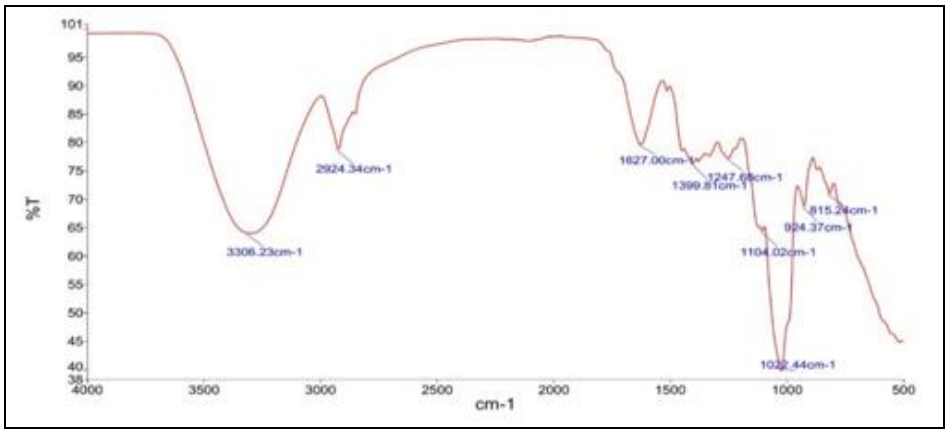

FIG. 7: FTIR SPECTRA OF THE WHOLE PLANT METHANOLIC EXTRACT OF *A. RACEMOSUS*

TABLE 7: FTIR INTERPRETATION OF THE WHOLE PLANT METHANOLIC EXTRACT OF *A. RACEMOSUS*

Frequency (cm^{-1})	Functional group	Bond strength
3306.23	Primary amine	N-H stretching
2924.34	Amine salt	N-H stretching
1627.00	Alkene	C=C stretching
1399.81	Carboxylic acid	O-H bending
1247.68	Amine	C-N stretching
1104.02	Secondary alcohol	C-O stretching
1022.44	Amine	C-N stretching
924.37	Alkene	C=C bending
815.24	Alkene	C=C bending

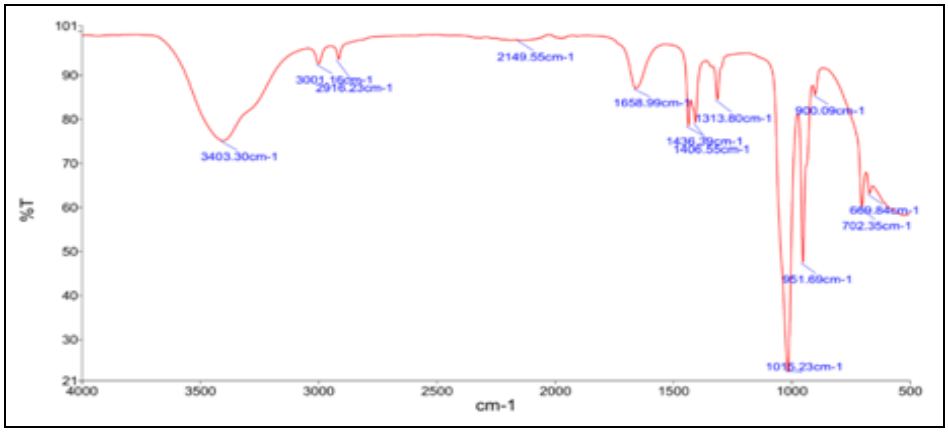

FIG. 8: FTIR SPECTRA OF WHOLE PLANT AQUEOUS EXTRACT OF *A. RACEMOSUS*

TABLE 8: FTIR INTERPRETATION OF WHOLE PLANT AQUEOUS EXTRACT OF *A. RACEMOSUS*

Frequency (cm^{-1})	Functional group	Bond strength
3403.30	Primary amine	N-H stretching
3001.16	Alkene	C-Hstretching
2916.23	Alkane	C-Hstretching
2149.55	Thiocyanate	S-C≡N stretching
1658.99	Imine/oxime	C-N stretching

1436.39	Carboxylic acid	O-H bending
1406.55	Sulfonyl chloride	S=O stretching
1313.80	Phenol	O-H bending
1015.23	Fluoro compound	C-F stretching
951.69	Alkene	C=C bending
900.09	Alkene	C=C bending
702.35	Alkene	C=C bending
669.84	Alkene	C=C bending

Antioxidant Activity: The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation. Natural antioxidants are more popular these days because of their potential to improve health and fend off diseases [28]. The antioxidant activity of *A. racemosus* whole plant extract was measured and compared with ascorbic acid in different concentrations. DPPH radical scavenging ability of the *A. racemosus* whole plant extracts exhibited potent antioxidant activity. The result revealed that the aqueous extract exhibited highest percentage of inhibition (73.42 ± 0.531%) at 250 µg and lesser IC_{50} value of 146.84and in comparison, the standard ascorbic acid had 89.01 ± 0.173% inhibition with IC_{50} value of 84.61. The percentage inhibition of DPPH and IC_{50} values are represented in **Fig. 9** and **Table 9** respectively.

TABLE 9: ANTIOXIDANT ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT BY DPPH

Concentration (µg/mL)	Standard Ascorbic acid	Chloroform	Methanol	Aqueous
50	41.82 ± 0.725	8.85 ± 0.816	21.47 ± 0.660	24.59 ± 0.387
100	52.92 ± 1.568	17.60 ± 0.645	33.74 ± 0.669	39.47 ± 0.646
150	65.94 ± 0.850	28.20 ± 0.495	49.74 ± 0.562	54.28 ± 0.598
200	79.20 ± 0.682	41.62 ± 0.766	59.68 ± 0.715	61.99 ± 0.399
250	89.01 ± 0.173	53.11 ± 0.705	70.20 ± 0.442	73.42 ± 0.531
IC_{50} (µg/mL)	84.61	239.36	162.27	146.84

Results are expressed as mean ± standard error

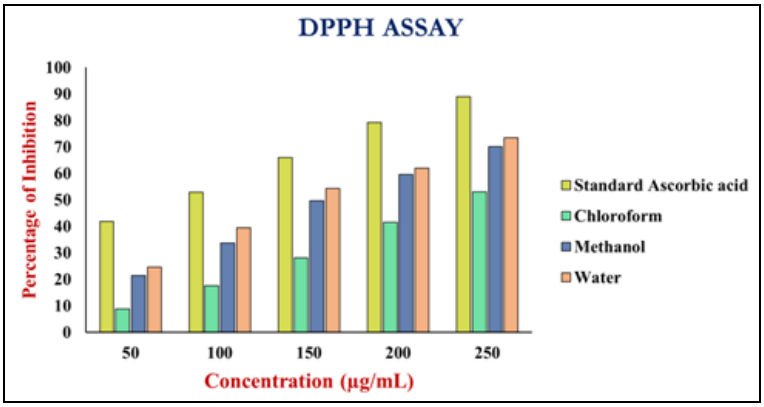

FIG. 9: ANTIOXIDANT ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT BY DPPH

The FRAP assay was performed to determine the reducing power of *A. racemosus* plant extracts. Greater the absorbance of the extracts corresponds to their greater antioxidant activity. Among the extracts, aqueous extract exhibited the highest activity with varying absorbance between 0.331 ± 0.010 to 0.786 ± 0.004. The results are represented in **Fig. 10** and **Table 10** respectively.

TABLE 10: ANTIOXIDANT ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT BY FRAP ASSAY

Concentration (µg/mL)	Standard Ascorbic acid	Chloroform	Methanol	Aqueous
50	0.388 ± 0.004	0.198 ± 0.008	0.271 ± 0.006	0.331 ± 0.010
100	0.620 ± 0.011	0.284 ± 0.005	0.398 ± 0.007	0.435 ± 0.007
150	0.744 ± 0.007	0.368 ± 0.008	0.486 ± 0.004	0.547 ± 0.007
200	0.892 ± 0.003	0.477 ± 0.004	0.583 ± 0.006	0.688 ± 0.002
250	1.037 ± 0.042	0.541 ± 0.009	0.649 ± 0.010	0.786 ± 0.004

Results are expressed as mean ± standard error

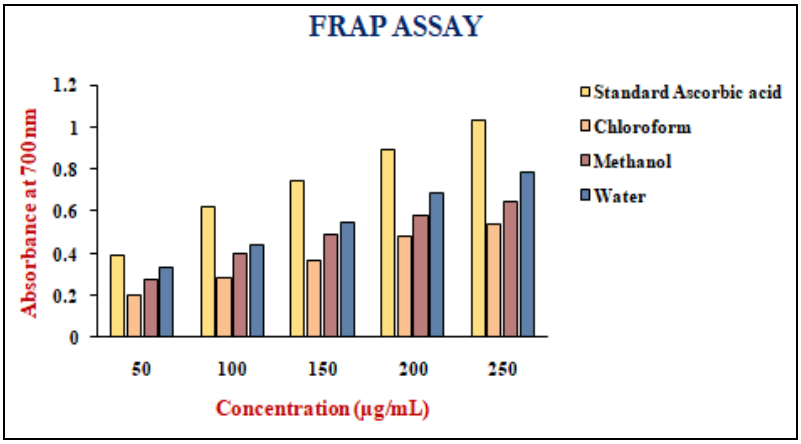

FIG. 10: ANTIOXIDANT ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT BY FRAP ASSAY

Anti-inflammatory Activity: The non-steroidal anti-inflammatory drugs function by protecting albumin protein denaturation in response to heat treatment [29]. Hence, the chloroform, methanol and aqueous extracts of *A. racemosus* plant was evaluated for their ability to inhibit the denaturation of albumin protein which was attributed to the presence of flavonoid and sterols present in the plant extract. In our study, the chloroform extract showed maximum anti-inflammatory activity (66.71 ± 0.384%at 250 µg) with an IC_{50} value of 152.88whereas the standard Aspirin revealed an IC_{50}value of 51.63 with the protein inhibition percentage of 87.41 ± 0.262%. The methanolic, chloroform and aqueous extract of *A. racemosus* plant significantly inhibits the production of nitric oxide which shows a key role in inflammation. The results are represented in the **Table 11** and **Fig. 11**.

TABLE 11: ANTI-INFLAMMATORY ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT

Concentration (µg/mL)	Standard Aspirin	Chloroform	Methanol	Aqueous
50	47.38 ± 1.556	28.33 ± 1.314	6.39 ± 0.595	17.60 ± 0.548
100	61.39 ± 1.019	42.08 ± 0.746	20.83 ± 1.320	31.70 ± 0.882
150	70.89 ± 0.869	50.72 ± 1.188	32.22 ± 0.651	50.13 ± 1.392
200	79.19 ± 0.475	59.39 ± 0.513	49.44 ± 0.785	58.68 ± 1.124
250	87.41 ± 0.262	66.71 ± 0.384	60.46 ± 0.869	66.75 ± 0.543
IC_{50} (µg/mL)	51.63	152.88	208.95	170.07

Results are expressed as mean ± standard error

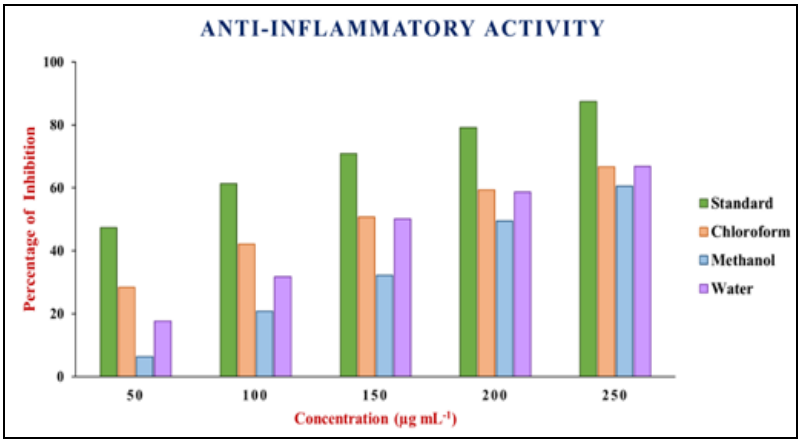

FIG. 11: ANTI-INFLAMMATORY ACTIVITY OF *A. RACEMOSUS* WHOLE PLANT EXTRACT

Antibacterial Activity: Plant extracts are a fantastic source of pathogen-fighting antibacterial compounds. They can therefore be utilised to treat a variety of infectious disorders brought on by virulent microorganisms [30]. The antibacterial activity of *A. racemosus* whole plant extract was studied against *E. coli*, *P. aeruginosa* and *Xanthomonas* sp. The methanolic extract exhibited the highest zone of inhibition against *Xanthomonus* sp. with maximum inhibitory zone of 14 mm

followed by *E. coli* with 11 mm and *P. aeruginosa* with 9 mm zone of inhibition at 1200 µg. The aqueous extract was sensitive against *P. aeruginosa* (11 mm). Whereas, the chloroform extract was insensitive against the tested pathogens. **Table 12** and **Fig. 12-14** display the measured zone of inhibition for *A. racemosus* plant extracts.

TABLE 12: ZONE OF INHIBITION (IN MM) OF *A. RACEMOSUS* WHOLE PLANT EXTRACTS

Extracts	Zone of inhibition (mm)											
	E. coli				*P. aeruginosa*				*Xanthomonas sp.*			
	30 µg	60 µg	90 µg	120 µg	30 µg	60 µg	90 µg	120 µg	30 µg	60 µg	90 µg	120 µg
Methanol	4	6	9	11	2	4	6	9	3	7	10	14
Aqueous	-	-	-	-	3	5	7	11	-	-	-	-

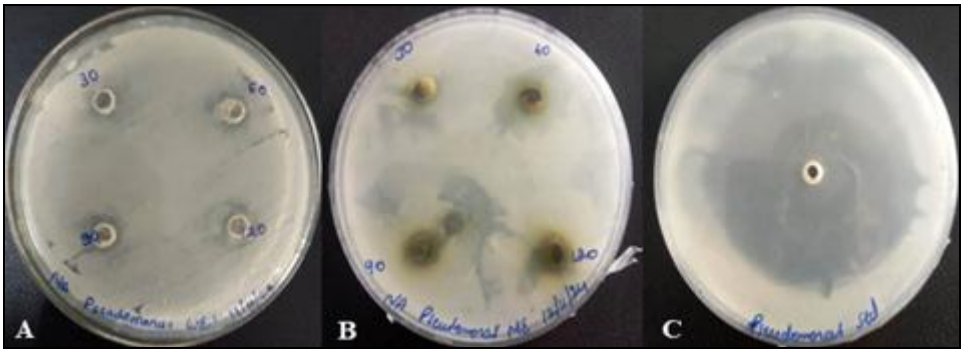

FIG. 12: ANTIBACTERIAL ACTIVITY OF THE A. RACEMOSUSPLANT EXTRACTS AGAINST *P. AERUGINOSA*; A. AQUEOUS EXTRACT, B. METHANOL EXTRACT, C. STANDARD CIPROFLOXACIN

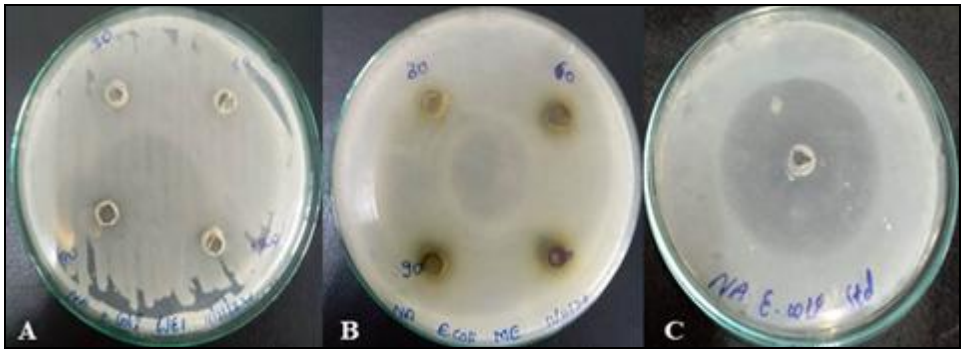

FIG. 13: ANTIBACTERIAL ACTIVITY OF THE *A. RACEMOSUS*PLANT EXTRACTS *AGAINST E. COLI*; A. AQUEOUS EXTRACT, B. METHANOL EXTRACT, C. STANDARD CIPROFLOXACIN

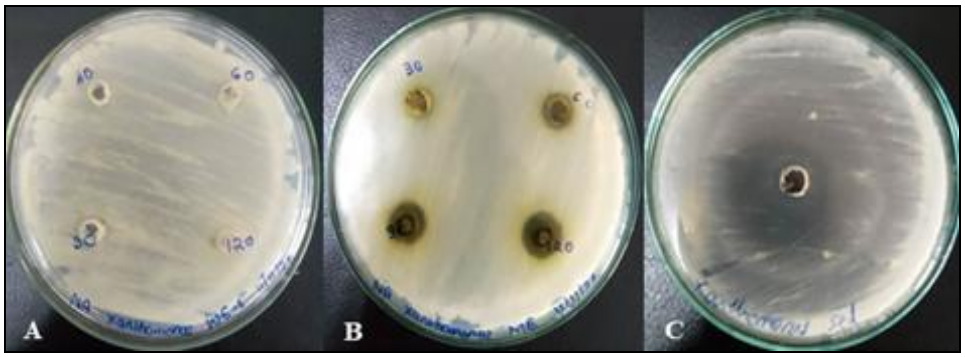

FIG. 14: ANTIBACTERIAL ACTIVITY OF THE *A. RACEMOSUS*PLANT EXTRACTS *AGAINST XANTHOMONAS* SP.; A. AQUEOUS EXTRACT, B. METHANOL EXTRACT, C. STANDARD CIPROFLOXACIN.

CONCLUSION: Several bioactive compounds found in the plants were thought to have medicinal qualities. These were considered as crucial components by modern pharmaceutical companies for manufacturing one-fourth of all medications. Screening medicinal plants for their therapeutic uses is therefore gaining importance in recent years. In the current study, the aqueous extract was

found to be high in phenols and flavonoids and was also more effective in free radical scavenging and anti-inflammatory actions which is supported by the presence of various bioactive compounds identified through GC-MS analysis. Thus, the study suggests that the extracts could be utilized as an excellent source of natural antioxidants and a new molecule in the creation of anti-inflammatory drugs. This study adds a vital component to the pharmacological uses of *A. racemosus* plant and the identification of the novel plant metabolites from A. *racemosus* paved the way to discovery of new drugs. However, further research could be encouraged to isolate the bioactive compounds to determine the mechanism(s) behind their pharmacological effects.

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CONFLICT OF INTEREST: The authors hereby declare no conflict of interest.

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