https://doi.org/10.48047/AFJBS.6.14.2024.1888-1902

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

PHYTOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF ROOT EXTRACTS OF ASPARAGUS RACEMOSUS

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Volume 6, Issue 14, Aug 2024

Received: 01 June 2024

Accepted: 11 July 2024

Published: 29 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.1888-1902

ABSTRACT

Plants have the potential to produce new, helpful medications for humans. The World Health Organization (WHO) estimates that traditional medicines are used by 80% of the world's population. The tribal and rural populations of several parts of India rely heavily on medicinal plant therapy to address their health-care demands. The therapeutic characteristics of plants have piqued the interest of countless biotechnologists, botanists, and plant scientists, who are conducting research to find or rediscover several medicinal plants, as well as their medicinal cures for various disorders. In Ayurveda, *A. racemosus* is known as the queen of herbs because it has a strong rejuvenating, nurturing and stabilizing effect on excessive air, gas, dryness, gastric ulcers, boost immune system and agitation in body and mind. The Satavari root produces three antioxidants, racemofuran, asparagamine A, and racemosol. Women use shatavari to treat conditions related to hormone imbalance, increases lactation. Bacterial infections cause millions of infection-based morbidities and mortalities annually and became the worldwide open health matter. In this scenario, herbal medicines are prepared from plant materials as a remedial point of view. The present study was an effort to investigate and proof out the phytochemical constituents and antibacterial activities of plant roots extract of *Asparagus racemosus*. Preliminary qualitative Phytochemical screening of four different extracts of *Asparagus racemosus* confirms the presence of proteins, flavonoids, steroids, glycosides, phenol & tannin and saponins in all the extracts. The Evaluation of the antibacterial potential of plant extracts by all the total of three bacterial strains i.e. one gram positive and two gram negative bacteria viz. *Staphylococcus aureus*, *Escherichia coli* & *Pseudomonas aeruginosa*. In the antibacterial assay, better activity was found in acetone extracts for dry sample and in ethyl acetate for boil sample against all the three bacterial strains. Therefore from the present studies, it can be concluded that the root extract of *Asparagus racemosus* would be recommended in future for the treatment of various ailments.

Keywords: *Escherichia coli*, Lead Acetate, *Pseudomonas aeruginosa*, Saponins, *Staphylococcus aureus*

INTRODUCTION

Since, ancient time plants have been admonitory sources of medicine. Ayurveda and other Indian literature mention the uses of plant in treatment of various human diseases. Plants have the major sources of drugs in Indian system of medicine and other ancient systems in world. Earliest description of curative properties of medicinal plants is found in Rig veda, Charaka samhita, and Sushruta samhita give extensive description on various mediational herbs. Information about medicinal plants in India provides a great deal of information on folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is basedon various systems including Ayurveda, Siddha and homeopathy.

Plants have the potential to produce new, helpful medications for humans. The World Health Organization (WHO) estimates that traditional medicines are used by 80% of the world's population (Anonymous, 1998). The tribal and rural populations of several parts of India rely heavily on medicinal plant therapy to address their health-care demands. The therapeutic characteristics of plants have piqued the interest of countless biotechnologists, botanists, and plant scientists, who are conducting research to find or rediscover several medicinal plants, as well as their medicinal cures for various disorders. Natural product research to cure diseases is a hot topic, with plants serving as the primary source (Borate & Disale, 2013).

Many plants are reported to have pharmacological properties because they contain various secondary metabolites such as glycosides, saponins, flavonoids, steroids, alkaloids, terpenes, phenols, and tannins, which should be used to combat disease-causing pathogens (Kamali et al., 2010, Hussain et al., 2011). The search for natural substances with antibacterial capabilities is on the rise due to their therapeutic use in the treatment of a variety of ailments. The fast rise of different drug-resistant pathogen strains to present antimicrobial drugs has prompted an active quest for novel antibiotics derived from medicinal plants (Chopra et al., 1997). Antibiotics and chemically manufactured treatments treat microbial illnesses quickly, but they can disrupt the body's natural immunity and induce a variety of negative effects. This has aroused a strong interest in plants and plant products that may supplement or replace pharmaceutical medications. Plant

extracts with target sites other than those employed by antibiotics are likely to be active against drug-resistant bacteria (Ahmad et al., 2001). Researchers have increasingly focused on safer phytochemicals and physiologically active substances extracted from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs.

In Asparagales, the (asparagus or orchid) order of flowering plants, containing 14 families, 1,122 genera, and more than 36,200 species. Asparagales contains many garden plants and several types of bulbs and cut flowers that are commercially important. Asparagus is a genus in the plant family Asparagaceae, sub family Asparagoideae. It comprises up to 300 species. Most of the species are evergreen long – lived perennial plants growing from the understoryas lianas, bushes or climbing plants. The best known species is the edible *Asparagus officinalis*, commonly referred to as just asparagus. Other members of the genus are grown as ornamental plants. Species in this genus vary in their appearance, from unarmed herbs to wiry, woody climbers with formidable hooked spines that earn them vernacular names such as "cat thorn" and "wag 'n bietjie" (literally "wait a bit"). Root tubers are storage organs developed by *Asparagus* species and are a valuable source of moisture and nutrition for species growing under drought conditions. It is widely cultivated as a vegetable crop. The genus is common at low altitudes in shade and in tropical climates throughout India, Asia, Australia and Africa.

Asparagus species may be erect or climbing, and most of the species are more or less woody. The rhizome like or sometimes tuberous, roots give rise to conspicuous fern like branchlets. True leaves are reduced to small scales. Many species are dioecious; the small greenish yellow flowers in the spring are followed by red berries in the fall. Thesered berries are toxic to humans. Members of the genus are characterized by the presence of cladodes, which are leaf like organs in the axils of the true leaves i.e. the 'leaves' are in fact needle-like cladodes (modified stems) in the axils of scale leaves. The root system, often referred to as a 'crown', is adventitious and the root type is fasciculate. Flowers are showy and "lily-like," with identical sepals and petals (tepals), as well as stamens in multiples of three. Ovaries are superior and generally have three compartments that mature into dry capsules. The leaves are the reduced to small bracts, leaving the green stems as the primary structure responsible for photosynthesis. The flowers may be bisexual and unisexual, and they grow on stalks from the junction of leaf and stem.

Ayurveda, *A. racemosus* is known as the queen of herbs because it has a strong rejuvenating, nurturing and stabilizing effect on excessive air, gas, dryness, gastric ulcers, boost immune system and agitation in body and mind. The Satavari root produces three antioxidants, racemofuran, asparagamine A, and racemosol. Antioxidants can prevent damage and disease in your body. Satavari has antiviral properties and could be used to treat or prevent viral infections. Breastfeeding mothers use shatavari powder to increase their milk production. Shatavari increases the production of prolactin, a hormone that is important for breastfeeding. Women use shatavari to treat conditions related to hormone imbalance such as polycystic ovarian syndrome and infertility (Acharya *et al.*, 2012; Anupam *et al*., 2012, Bhatnagar and Sisodia, 2006).
The present study on phytochemical characterization and Antibacterial Activity of Root Extracts of *Asparagus racemosus* comprised of collection of samples of Asparagus from agricultural field of Shri Guru Ram Rai University, Dehradun, Uttarakhand for phytochemical estimation and antibacterial analysis against human pathogens.

MATERIALS AND METHODS

Location of the experiment and climatic conditions:

The present investigation was carried out during February-April 2023 at Department of Botany, School of Basic and Applied Science of Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand. Dehradun is a picturesque city with mild climate. It is the capital of Uttarakhand, and is located between the latitude 29055' and 38031'N and longitude77035' and 78020'E, covering an areaof 2002.4sq.km with an elevation of 2000 m above the sea level. The climate of Dehradun is generally temperate, although it varies from tropical, to severely cold, depending upon the season, and the altitude of the area. The nearby hilly regions often get snowfall during winter but the temperature in Dehradun does not go under 0^0C. During summer the temperature here is usually between 27-40^0C whereas during winter it is between 2 -24^0C. During monsoon, there is often constant and heavy rain falls. The main synclinal trough receives an average of 210 cm rainfall annually. The weather is considered to be good during winter in the hilly regions but it is often hot in the Doon valley. The agriculture is good here due to the fertile alluvial soil and the adequate water drainage and rainfall.

Materials:

Fresh and disease free plant roots of *Asparagus racemosus* were collected from agricultural field of

S.G.R.R. University and collected samples were cleaned. The half of plant root sample was subjected for morphological study and from the rest half were dried in shade at 25°C to 30° C for 10-15 days and half were boiled ,peeled off and dried in shade at 25°C to 30°C for 10 to 15 days . After that both dry and boil sample, then crushed to coarse powder using grinder. The dried and boiled root material of plant was stored in paper bags for Phytochemical and antibacterial analysis.

Figure no. 1. Study area- Dehradun (Uttarakhand)

a. b.

Figure 2. (a) Whole plant (b) Twigs of Asparagus racemosus

Experimental methodology:

a) Collection of plants: The fresh plant roots were collected from agricultural field of S.G.R.R. University, Dehradun on February, 2023. The half roots were dried in shade at 25^0C to 35^0C for 15-20 days in the laboratoryand half roots were washed, boiled, peeled off and dried in shade at 25°C to 35°C then crushed to coarse powder using grinder. The both dry and boil plant root material were stored in paper bags.

b) Extraction Procedure for successive solvent extraction: The dried plant root material powder was subjected to successive infusion method with different solvents in increasing order of polarity (i.e. Ethyl acetate < Acetone < Ethanol < Distilled water). In the method of infusion extraction, a small volume of solvent is taken in beaker and plant material was dipped in it for 48 hours. After that, the sample was filtered and filtrate heated to dryness, and Followed by other solvent in the order of their increasing polarity with the same menstrum. Thus the same quantityof menstrum was recycled every time and complete extraction is achieved with a very small volume of menstrum.

c) Steps involve in extraction:

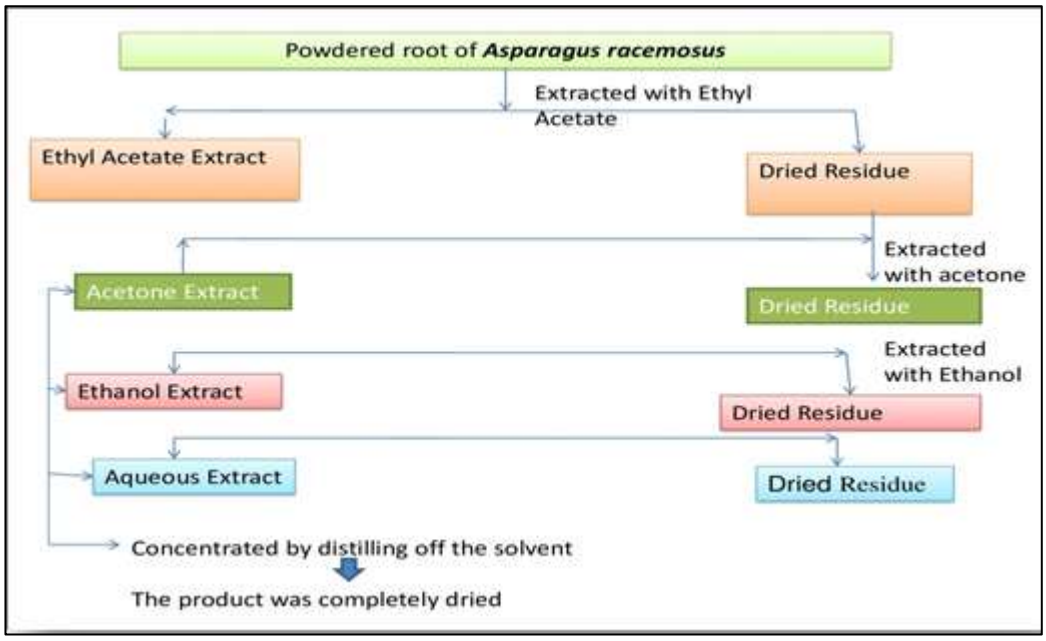

Figure no. 3. Flow chart for extraction of crude drug from *A. racemosus*

Calculation of percentage yield: Percentage yield of the crude extracts were calculated with the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Powder Drug}} \times 100$$

Following table contains the summary of complete extraction procedure:

Table.1. Extraction of crude by drug different Solvents

S.No	Solvent Used	Quantity of powder taken	Method Applied	Extraction Time
1	Ethyl acetate	50gm	Simple filtration	48 hrs
2	Acetone	50gm	Simple filtration	48 hrs
3	Alcohol	50gm	Simple filtration	48 hrs
4	Water	50gm	Simple filtration	48 hrs

5. Phytochemical analysis: - The various solvent extracts of *Asparagus racemosus* plant extract were subjected to preliminaryqualitative phytochemical investigation. The various tests performed were given below:

a. **Test for proteins by Lowry method:** 2ml of extracted sol and 5ml of reagent 1 taken in a test tube. Then add 0.5ml of Follin's reagent in it, put it in dark for half an hour and check the appearance of violet color which indicates presence of proteins.
b. **Test for flavonoids by Lead Acetate:** 1ml of extract sol taken in a test tube and addition of 1ml of lead acetate in it. Appearance of yellowish green color indicates the presence of flavonoids.
c. **Test for Steroids:** 1ml of plant extract sol was dissolved in 2ml of chloroform in test tube and equal volume of concentrated sulphuric acid and 1ml of acetic acid. Appearance of a greenish color indicates the presence of steroids.
d. **Test for Glycosides:** 1ml of plant extract was dissolved in 2ml of chloroform in a test tube and addition of 1ml of conc. Sulphuric acid. Reddish brown coloration of the upper layer indicates the presence of glycosides.
e. **Test for Phenolic Compounds and Tannins by Ferric Chloride Test:** Ferric chloride sol was added drop by drop to 1ml of plant extract in a test tube. Appearance of bluish black color indicates the

presence of tannins and phenolic compounds.

f. Test for Saponins by Foam Test: To a small quantity of water in a test tube the plant extract was added and shaken vigorously. The Appearance of foam for 10 min indicates the presence of saponin.

6. Antibacterial assay: Substance that kills or inhibits the growth of microbes such as bacteria, viruses and fungi called antimicrobials. Antimicrobial drugs that kill microbes called microbicidal or prevent the growth of microbes called as microbistatic. The term chemotherapy is used for the treatment of microbial infection. Chemotherapeutic Agents are the drugs that inhibit or kill invading parasite or malignant cell and have no or minimal pharmaco dynamic effect in the recipient. All the crude extracts were first screened for preliminary test to know whether they were active against the particular bacteria or not. Only active extracts then assayed further at different concentrations and finally for MIC test. The plant extracts of *Asparagus racemosus* were dissolved in DMSO; for maintain their concentration and it is a highly polar solvent and is nontoxic to the microbes.

i. Source of bacterial strains:-The antibacterial assay of different extracts was performed. All the bacterial strains were procured fromthe Department of Microbiology, S.G.R.R. University, Dehradun. The study was performed at Botany laboratory in School of Basic & Applied Sciences, S.G.R.R. University, Dehradun.

ii. Evaluation of the antibacterial potential of plant extracts: All total of three bacterial strains i.e. one gram positive and two gram negative bacteria viz. *Staphylococcus aureus*, *Escherichia coli* & *Pseudomonas aeruginosa* were taken to evaluate antibacterial potential of the different 4 extracts in both dry and boil extract. All the three bacterial strains are pathogenic to human being they cause diseases like food poisoning, diarrhea, fever, pneumonia, meningitis, etc.

All the crude extracts were first screened for preliminary test with the concentration 1000mg/ml to know whether they were active against the particular bacteria or not. The sensitivities against standard drugs such as Streptomycin were also observed. Only extracts with good activity were then assayed further at different concentrations for MIC test. All the extracts were made to dissolve in DMSO, a highly polar, organic solvent miscible with water and majority of organic solvents and are nontoxic to the microbes.

Table.2. Detail characteristics of bacterial strains used for the studies

S.No.	Bacterial Strains	Family	Characteristics	Disease caused
P1	*Pseudomonas aeruginosa*	Pseudomonadaceae	Aerobic, saprophyte, gram positive, rod shaped, motile, non sporing	Wound, burn and eye infection, UTI, intestine diarrhea.
P2	*Escherichia coli*	Enterobacteriaceae	Anaerobic, gram negative, rod shaped, non motile, non sporing	Abdominal & pelvic infection, urinary tract infection
P3	*Staphylococcus aureus*	Staphylococcaeae	Aerobic, facultative and aerobic, cocci, non motile, Gram positive, parasite.	Boils, abscess, wound infection, food poisoning.

iii. Requirements for antibacterial assay

1. Broth media: Broth is a liquid containing nutrients for culturing microorganisms. The mentioned bacteria were cultured in broth media from pure cultures maintained in the Botany laboratory of Department of Life sciences, S.G.R.R.University Dehradun.

a. For the preparation of broth take the weight amount of peptone, beef extract and NaCl in aconical flask and make the volume 500ml with distilled water.

b. The mixture was then heated with agitation to dissolve all the constituents.

c. Make the volume 1000ml by adding distilled water.

d. pH of the medium was adjusted to 7.0 using a pH meter; by adding alkali (acid may be added if pH comes out to be less).

e. The prepared media was covered with cotton plug and then autoclaved at 121^0C, 15lbs pressure

for 15min.

f. The autoclave was allowed to cool. The broth was removed and brought to room temperature.

2. Agar media: Agar is a dried hydrophilic, colloidal substance obtained from various species of red algae. When suspended in a liquid medium and heated to 212^0F (100^0C), it dissolves. When allowed to cool to 110^0F (43^0C), the medium becomes a solid gel. It is used as base in culture media for growth of bacteria and other microorganism.

a. For the preparation of agar media, the weight amount of peptone, beef extract NaCl and agar, put into a conical flask and mix the 500ml of distilled water.

b. The mixture was then heated with agitation to dissolve all the constituents.

c. Distilled water was added to make up the volume 1lit.

d. pH of the medium was adjusted to7.0 using a pH meter; by adding alkali (acid may be added if pH comes out to be less).

e. Prepared media was covered with cotton plug and autoclaved it at 121^0C, 15lbs pressure for 15min.

f. The autoclave was allowed to cool. Nutrient agar was removed and brought to room temperature.

iv. Sub-culturing of bacterial strains by slant preparation- The food material on which microorganisms are grown is called culture medium and the growth is called culture. The Nutrient Agar Media (NAM) was used for the preparation of slants for routine sub-culturing of the studied bacterial strains.

Preparation of inoculums- Take the 1 loop full culture and transfer it into 4-5ml of broth in a test tube and incubate it for 2 hours at 37^0C. After 2 hours inoculums was prepared.

Preparation of disc:-Whattman filter paper No.41 was punched out to prepare disc of 6mm diameter. All the disc were collected in a glass bottle and were autoclaved at 121°C, 15lbs pressure for 15 minutes. These pre sterilized disc were used to impregnate plant extract onto the agar media against studied the bacterial strains.

v. Antibacterial assay procedure: The nutrient agar media for antimicrobial assay was prepared by dissolving the 5 gm peptone, 3 gm beef, 5 gm NaCl and 15 gm of agar in 1000 ml of distilled water. The media was dissolve on hotplate by continuous stirring. Then media was then autoclaved at 15 lbs (121^0C) for 15 minutes. It was pouredquickly into sterile petri dishes while hot to give a depth of 3-4 mm, under aseptic condition and allowed to cool and solidify. The activated bacterial culture (100µl) was introduced to the solid surface of agar media with the help of micropipette. It was then spread across the surface of solid agar mediaby means of a sterile spreader and kept at room temperature for 15 min for absorption to occur. The pre sterilized discs dipped in different extracts were then placed on the surface of the agar media. The petri dish was then incubated in BOD incubator for 24 hrs at temperature 37^0C. After incubation the degree of sensitivity was determined by measuring the zone of inhibition around the disc in mm using aruler. To follow the above procedure, all the glasswares were sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. All the above procedure was carried out in laminar air flow in well aseptic condition with great care.

Minimum Inhibitory Concentration (MIC) analysis: Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent (drug) that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Inhibitory Concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge et al., 2003).

Here the MIC values of extracts were determined only against those bacterial strains which showed high sensitivity during the preliminary antibacterial testing. MIC analysis was performed by serial dilution of the active concentrated extract in pure DMSO to achieve a decreasing concentration range of1000 mg/ml to 15.62 mg/ml. By using different concentration of the active extract i.e. the growth around the disc with lowest concentration to which the organism is susceptible would be determined as MIC of the extract against the particular organism. The degree of activity of extracts was determined by measuring the zone of inhibition of growth around the disc. The MIC was determined for extract as the concentration, which showed the maximum inhibition at minimum concentration against maximum number of bacteria, when compared with the slandered drug. Highest zone of inhibition at minimum concentration gave the measure of MIC value.

RESULT

Asparagus racemosus revealing its phytochemical uses, the experimental methodology that has been adopted for the present study includes infusion extraction using different solvents in increasing order of polarity, concentration of the extracts followed by their phytochemical evaluation, antimicrobial screening and the determination of the MIC value of the active extracts against various pathogenic microbes. The findings of the present study were described under the following headings:

Appearance and Percentage yield of root extract for dry and boil sample: Plant extracts isolated from the root powered of *Asparagus racemosus* by using different solvents showing variation in its color. It is light yellowish brown. 50gm of powdered *Asparagus racemosus* plant was subjected to infusion extraction. The extract was concentrated on water bath and it was finally reduced to dryness to get dry extract. The yield of various crude extract with different solvents was as follows-

Table.3. Appearance and Percentage yield of root extract for dry sample

S.No.	Solvents	Appearance	Weight of powdered roots (gm)	Weight of extract (gm)	%Yield w/w
1.	Ethyl acetate	Yellowish brown	50	1.3	2.6
2.	Acetone	Yellowish brown	50	3.2	6.4
3.	Ethanol	Yellowish brown	50	1.2	2.4
4.	Water	Yellowish brown	50	2.4	4.8

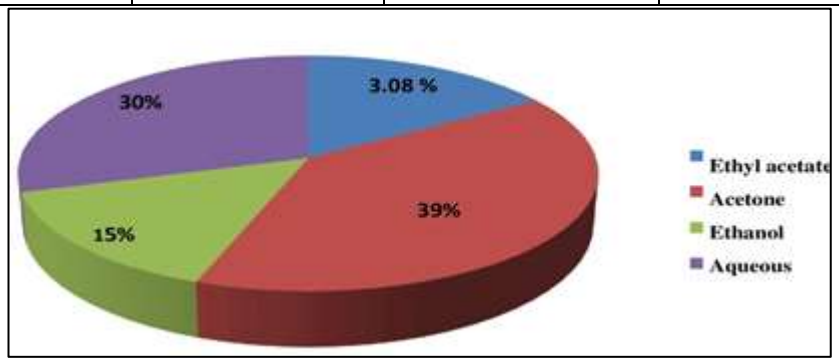

Figure no. 4. Graphical representation of percentage yield of crude extract of dry sample

Table.4. Percentage yield of root extract for boil sample

S. No.	Solvents	Weight of powdered leaf (gm)	Weight of extract (gm)	%Yield
1.	Ethyl acetate	50	1.9	3.8
2.	Ethanol	50	0.9	1.8
3.	Alcohol	50	1.9	3.8
4.	Water	50	3.2	6.4

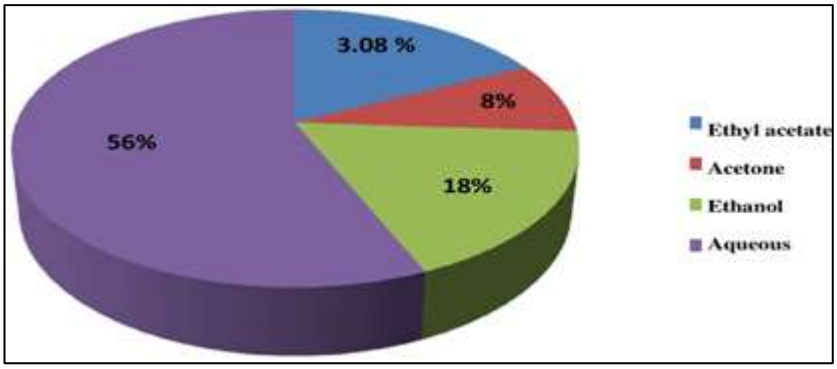

Figure no. 5. Graphical representation of percentage yield of crude extract of boiled sample

Results of phytochemical analysis: Phytochemical are the chemicals which are derived from the plant sources, generally affect health, but are not yet established as essential nutrients. Alkaloids, terpenoids, coumarin, tannins, quinines, flavonoids, glycosides, steroids, saponins are some classes ofphytochemical.

The various solvent extracts of *Asparagus racemosus* were subjected to preliminary qualitative phytochemical investigation (table 5).

Table.5. Results of phytochemical analysis analysis

S.NO.	Constituents	Extracts			
		Ethyl acetate	**Acetone**	**Ethanol**	**Aqueous**
1.	Phenol &Tannin compounds	+	+	+	+
2.	Saponin	+	+	+	+
3.	Proteins	+	+	+	+
4.	Glycosides	+	+	+	+
5.	Steroids	+	+	+	+
6.	Flavanoids	+	+	+	+

*(+) and (-) signs indicate presence or absence of the compound, respectively.

Result of antibacterial test: Initially all solvent extracts like petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol, aqueous extract of *Asparagus racemosus* plant were tested at 1000mg/ml concentration against various gram positive and gram negative pathogenic bacteria. The different extracts of *Asparagus racemosus* showed antibacterial activity against the bacterialstrains. The following table 6 and 7concludes the results:-

Table.6. Results of antibacterial test for dry sample:

S.NO.	Bacterial strains	Zone of inhibition (in mm)					
		Ethyl acetate	Acetone	Ethanol	Aqueous	Positive Control	Negative control (DMSO)
1.	*Staphylococcus aureus*	-	12	5	2	15	-
2.	*Pseudomonas aeruginosa*	-	-	-	-	18	-
3.	*Escherichia coli*	-	5	-	8	18	-

Table.7. Results of antibacterial test for boiled sample:

S.NO.	Bacterial strains	Zone of inhibition (in mm)					
		Ethyl acetate	Acetone	Ethanol	Aqueous	Control	Negative control(DMSO)
1.	*Staphylococcusaureus*	10	2	6	5	15	-
2.	*Pseudomonas aeruginosa*	-		-	2	16	-
3.	*Escherichia coli*	10	2	-	8	18	-

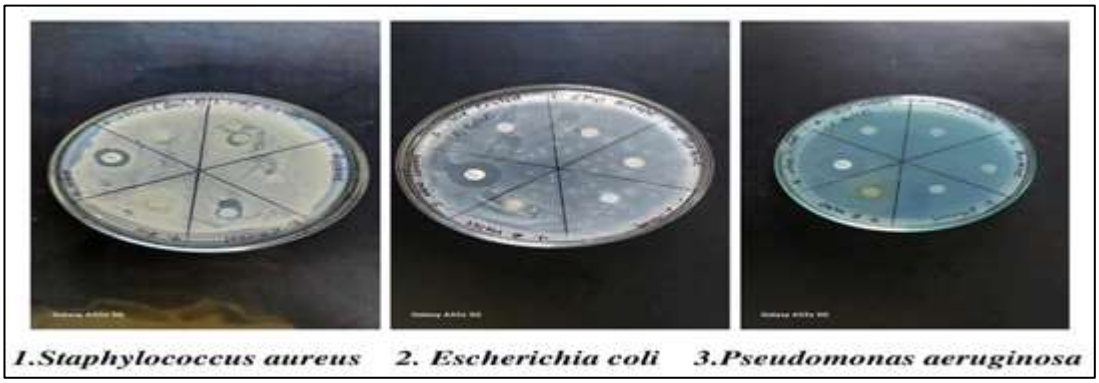

Figure no.6. (a) Plates of antibacterial assay for acetone extract (dry)

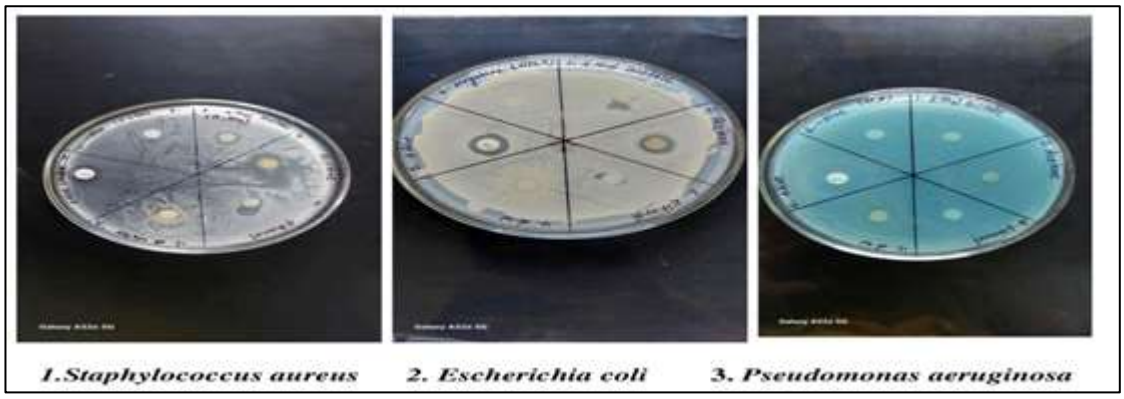

Figure no.7. (a) Plates of antibacterial assay for ethyl acetate (boil)

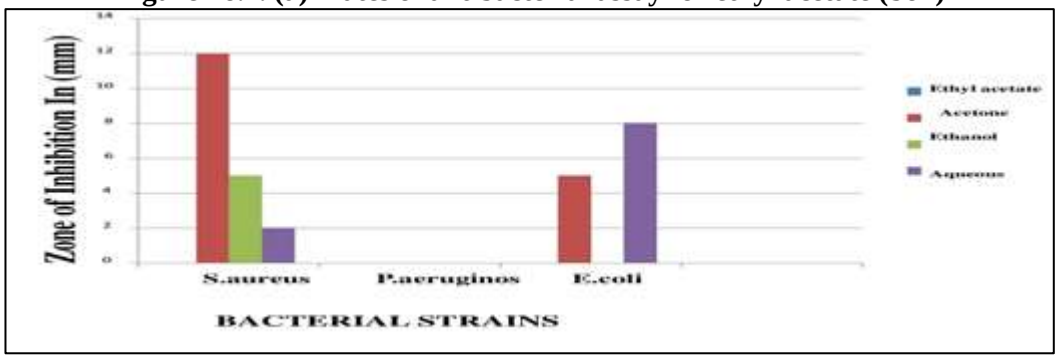

Figure no. 6. Graphical representation of Antibacterial test for dry sample

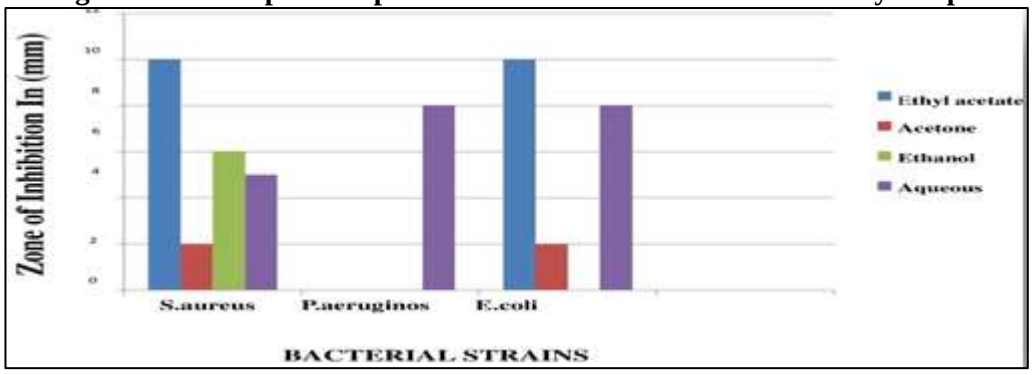

Figure no.8. Graphical representation of Antibacterial test for boiled sample

Results of Minimum Inhibitory Concentration (MIC) analysis: - Out of the four extracts that were tested against bacterial strains i.e. *Escherichia coli, Pseudomonas aeruginosa & Staphylococcus aureus* maximum zone of inhibition was obtained in aqueous extract. Thus, MIC was carried out only for aqueous extracts against the bacterial strains which showed maximum zone of inhibition. MIC was analyzed against *Staphylococcus aureus* for the acetone extract (dry) and ethyl acetate in boil sample. Results of MIC analysis for acetone extract shown in the table 8 and 9 for dry and boil sample respectively as follows:

Table.8. Minimum Inhibitory Concentration (MIC) Analysis for acetone extract [Dry]

S. No.	Bacterial strains	Zone of inhibition in mm					
		1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
1.	*Staphylococcus aureus*	12	8	6	-	-	-

Table.9. Minimum Inhibitory Concentration (MIC) Analysis for ethyl acetate extracts [Boil]

S. No.	Bacterial strains	Zone of inhibition in mm					
		1000mg/ml	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
1.	*Staphylococcus aureus*	10	8	4	-	-	-

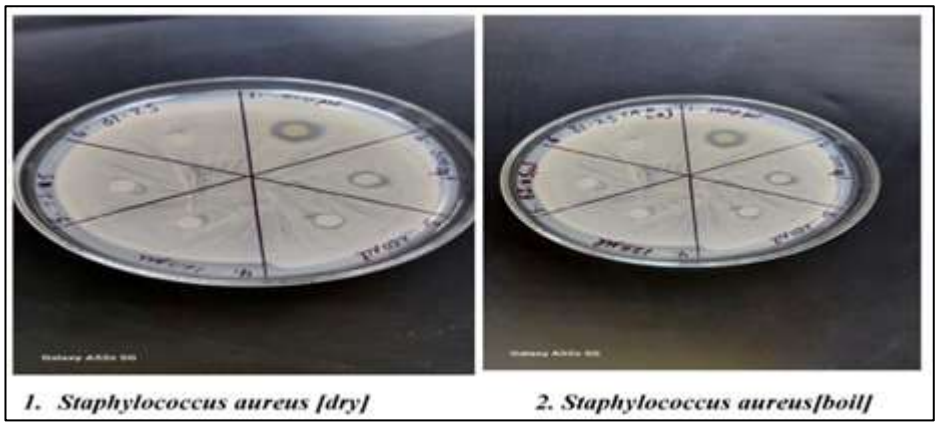

Figure no.9. (1) Plate of MIC Analysis for acetone extract (dry)
(2) Plate of MIC Analysis for ethyl acetate extract (boil)

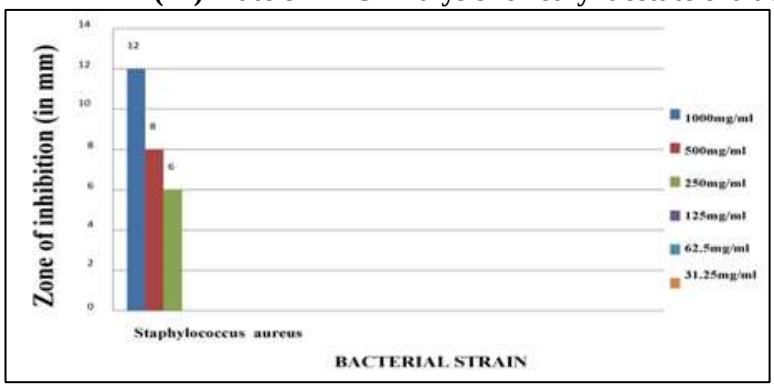

Figure no.10. Graph of MIC Analysis for acetone extract [dry]

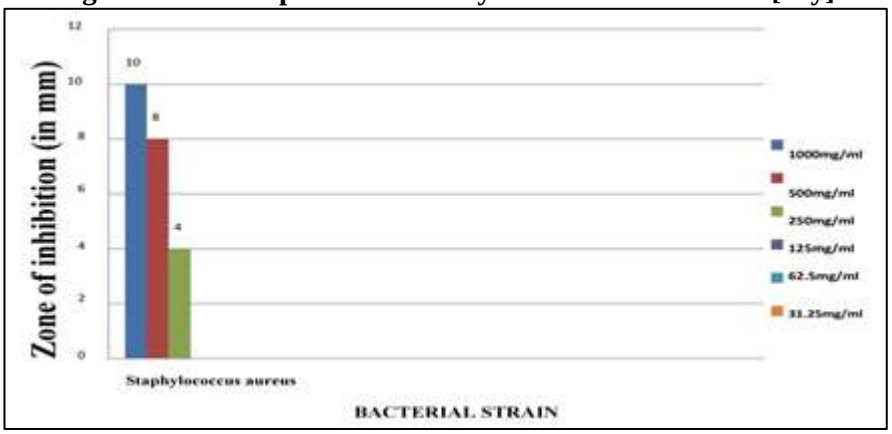

Figure no.11. Graph of MIC Analysis for ethyl acetate extract [boil]

Results of positive and negative control against bacterial strains: Three ready to use antibiotics impregnated disc i.e. Streptomycin were used as a positive control in order to check the sensitivity of the bacterial cultures. All of them showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotics. In contrast to this, DMSO (99% pure) was used as a negative control. Results obtained that wereshowing sensitivity of the bacteria against positive and negative control is tabulated as follows:

Table.10. Positive and negative control against bacterial strains for acetone extract[dry]

S.No.	Bacterial Strains	Positive control	Negative control
		Streptomycin	DMSO
1.	*Staphylococcus aureus*	15	-
2.	*Pseudomonas aeruginosa*	18	-
3.	*Escherichia coli*	18	-

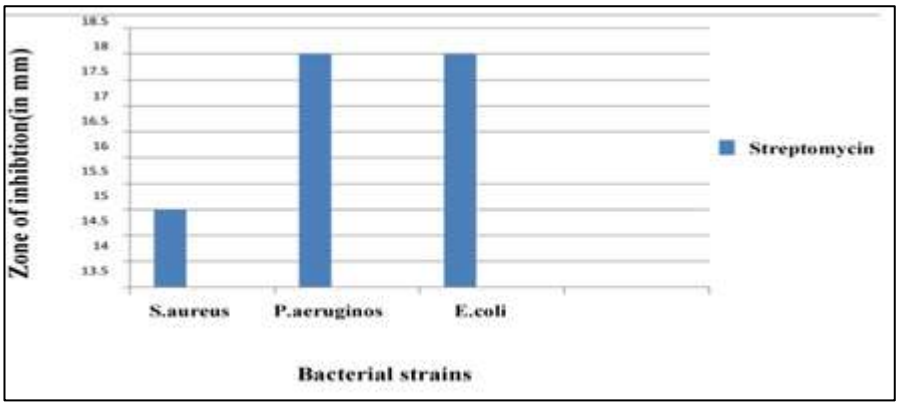

Figure no.12. Graph of positive and negative control against bacterial strains foracetone extract [dry]

Table.11. Positive and negative control against bacterial strains for ethyl acetateextract (Boiled)

S.N0.	Bacterial Strains	Positive control	Negative control
		Streptomycin	DMSO
1.	*Staphylococcus aureus*	15	-
2.	*Pseudomonas aeruginosa*	18	-
3.	*Escherichia coli*	16	-

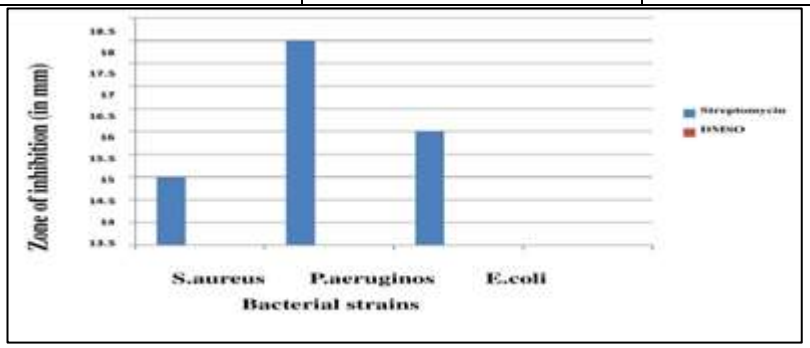

Figure no.13. Graph of positive and negative control against bacterial strains forethyl acetate extract [boiled]

DISCUSSION

In India most of the traditional knowledge on medical plants is in the oral form carried over generations to generations without any standard inventory. Necessary steps are needed for proper documentation, systematic regulation and widespread application. Since herbal medicines are prepared from materials of plant origin, they are prone to contamination, deterioration and variation in composition. Hence, before proceeding to clinical studies, scientists need to authenticate plants and also to detect their potency. A lot of analytical techniques have been developed for quality control of drug from plant origin. The Asparagaceae combines seven previously recognized families, 114 diverse genera, and approximately 2900 individual species worldwide. Similar to the Amaryllidaceae, until 2003 species now classified as Asparagaceae were found in Liliaceae. The present study was an effort to investigate and proof out the phytochemical constituents and antibacterial activities of *Asparagus racemosus* plant roots (Deepika and Dimple, 2014). The findings of the present study were discussed under the following headings:

Phytochemical analysis: Plant roots have an almost limitless ability to synthesize various chemical substances as primary and secondary metabolites. These phytochemicals are proteins, flavonoids, steroids, glycosides, phenol & tannin and saponins etc. The presence of these phytochemicals can be used as an indicator for various biological activities like antioxidants, antibacterial, antifungal etc. In the present study, plant roots were collected, dried and grinded out into a powdered form which was then subjected to infusion method using four different solvents in the increasing order of their polarity ethyl acetate, acetone, ethanol and water. Various extracts obtained showed yellowish-brown colour appearances. Total percentage yield of crude extract for dry sample varies from 2.4 %to 6.4% & for boiled sample percentage yield of crude extract varies from 1.8% to 6.4%. Out of the four extracts, the acetone crude extract showed the highest yield (6.4 %) while ethanol showed the lowest (2.4 %) for dry sample. Similarly, Out of the four

extracts, the aqueous crude extract showed the highest yield (6.4 %) while acetone showed the lowest (1.8 %) for boil sample. Preliminary qualitative phytochemical screening of four different extracts of *Asparagus racemosus* confirms the presence of proteins, flavonoids, steroids, glycosides, phenol & tannin and saponins in all the extracts. Devendra *et al.*(2013) showed the presence of favonoids, steroids, glycosides and saponins in roots of *Asparagus racemosus.* Similar, study was conducted by Behera *et al.,* (2018) on theroots of *Asparagus racemosus*, which shows the presence of steroids, flavonoids, tannins etc.

Antibacterial assay: The curative effect of plant extract in the study has been variables referred to as resistance modifying/modulating activities. This ability of plant extracts to potentiate alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics (Jeung *et al*., 2004). Plant have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plant are frequent. Plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Bag *et al*., 2008). It has good antimicrobial activity against both gram positive (+) and gram negative (-) organismswhereas maximum inhibition was shown in case of gram negative (-) organisms. The results of antimicrobial activity of ethanol fraction was compared with the positive control (Standard drugs: Chloramphenical) for evaluating their relative percentage inhibition. It was showed that the fraction have maximum relative percentage inhibition against *Klebsiella pneumonia* (91.93), followed by *E. coli* (89.73), *Streptococcus mutans* (84.28) and *Staphylococcus aureus* (78.4)(Roy *et al* ,2014).

Similarly, study done by Uddin *et al*., 2012on root extract of *A. racemosus* at the concentration of 10000 µg/ml showed highest activity against all bacterial strains ranging from 26±1.41 (Proteus species) to 14±1.41 (*Salmonella typhi*). Similarly, extract at the concentrationof 1000 µg/µl showed activity against four strains including *Proteus* species, *Vibreo cholera, Staphylococcus aureus* and *Pseudomonas alkaligenes*. The maximum antibacterial activity at 100 µg/ml was assessed for *Staphylococcus aureus* (14.5±0.707) while it was found lowest 2.5±3.53 for both *Proteus* species and *Vibreo cholera*. At other extract concentrations including 10 µg/ml and 1 µg/ml, no activity was observed against all the strains. All the four extracts i.e. ethyl acetate, acetone, ethanol and aqueous extract of *Asparagus racemosus* were subjected for their preliminary antibacterial screening at 1000mg/ml concentration against one gram positive and two gram negative bacterial strains. Different extracts of *Asparagus racemosus* showed antibacterial activity against all the studied bacterial strains that are pathogenic to human being causing several diseases like food poisoning, diarrhea,Urinary Tract Infection, meningitis, fever etc.

For dry sample, out of the four extracts assayed, the acetone extracts of *Asparagus racemosus* were found to be most active against all studied bacterial strains. The maximum activity was observed in *Staphylococcus aueus* with zone of inhibition for aqueous extract was 15mm. Therefore, Minimum Inhibitory Concentration (MIC) was carried out with acetone extract against *Staphylococcus aureus*.

For boiled sample, out of the four extracts assayed, ethyl acetate extracts of *Asparagus racemosus* were found to be most active against all studied bacterial strains. The maximum activity was observed in *Staphylococcus aueus* with zone of inhibition for aqueous extract was 15mm. Therefore, Minimum Inhibitory Concentration (MIC) was carried out with ethyl acetate extract against *Staphylococcus aureus*.

Minimum inhibitory concentration is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge *et al*., 2003). MIC analysis was performed by serial dilution of the concentrated aqueous extract in pure DMSO to achieve a decreasing concentration range of 1000mg/ml to 31.62mg/ml. For dry sample, on performing MIC for the acetone extract of *Asparagus racemosus* the results revealed that all selected bacterial strains i.e., *Staphylococcus aureus* were sensitive against the 250mg/ml concentration of the extract, there by exhibiting 250mg/ml as their MIC value. No zone of the inhibition was obtained around the disc impregnated with 125mg/ml, 62.5 mg/ml and 31.25 mg/ml concentration interpreting that all bacterial strains could resist this concentration of the extract. For boil sample, on performing MIC for the ethyl acetate extract of *Asparagus racemosus* the results revealed that all selected bacterial strains i.e., *Staphylococcus aureus* were sensitive against the 250mg/ml concentration of the extract, there by exhibiting 250mg/ml as their MIC value. No zone of the inhibition was obtained around the disc impregnated with 125mg/ml, 62.5 mg/ml and 31.25 mg/ml concentration interpreting that all bacterial strains could resist this concentration of the extract. In order to check the susceptibility of bacterial strains ready to use antibiotics impregnated disc i.e. streptomycin were used as a positive control in order to check

the sensitivity of the bacterial cultures. All of them showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotics. In contrast to this, DMSO (99% pure) was used as a negative control.

CONCLUSION

Asparagus racemosus is being used from Pre-Vedic times and mentioned in ayurvedic literature. Root of *A. racemosus* has been referred as bitter- sweet, emollient, cooling, nervine tonic, constipating, galactogogue, and aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic and as tonic. Beneficial effects of the root of *A.racemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentery, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases. According to present study, *Asparagus racemosus* contain various phytochemicals such as proteins, flavonoids, glycosides, tannins and phenolic compound, steroids and saponins in all four extract of both sample. *Asparagus racemosus* is an important medicinal plant studied fromancient period. At present the plant is widely used for making allopathic, ayurvedic and homeopathic medicines. Numerous specific chemical constituents of this plant are used in different pharmaceutical formulations have raised its demand. A systematic cataloguing and identification of different phytochemicals provides a meaningful way to explore the indigenousknowledge of this medicinal herb. In this review a brief evaluation of Satavari properties are discussed in order to explain the practical clinical applications. In the antibacterial assay, better activity was found in acetone extracts for dry sample and in ethyl acetate for boil sample against all the three bacterial strains. So, Minimum Inhibitory Concentration was tested for *Staphylococcus aureus* against and measured zones were ≥ 6mm. In positive control the antibiotic streptomycin was active against all the three bacterial strains and the measured zones were ≥14 and in negative control DMSO was used as a negative controland there was not any activity. So, the acetone extract for dry sample and ethyl acetate for boil sample were the best solventsfor analyse the Minimum Inhibitory Concentration of extract against *Staphylococcus aureus* bacterial strain. Thus from the above studies, it can be concluded that the root extract of *Asparagus racemosus* would be recommended in future for the treatment of various ailments.

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