# Extraction of *Asparagus Racemosus* and its Performance along with Antibiotics for Antimicrobial activities

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Abstract:
*Various diseases are caused by different pathogenic microorganisms. Antibiotics are used for treatment of infectious diseases, but indiscriminate use of it leads development of resistant microbes. It is required to find new ways to fight against diseases causing microbes. One of them is therapy of plant extract with antibiotics and this may be effective for treatment of various diseases. This study has been carried out to evaluate interaction of methanol extract of* ***ASPARAGUS RACEMOSUS*** *with different antibiotics against bacteria and fungi.*

**Key words:** ***ASPARAGUS RACEMOSUS****, antibiotics, antibacterial, antifungal.*

## 1. INTRODUCTION

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 4,000 BC.[1,2]Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system.[3] Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The *Sushruta Samhita* attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources.[4]

**Ayurveda** (Sanskrit: आयुर्वेद, *Āyurveda*, "life-knowledge";[5] **Ayurveda medicine**, is a system of medicine with historical roots in the Indian subcontinent.[6] Globalized and modernized practices derived from Ayurveda traditions are a type of complementary or alternative medicine.[7,8] In the Western world, Ayurveda therapies and practices (which are manifold) have been integrated in general wellness applications and as well in some cases in medical use.[9]

***Asparagus racemosus*** (**satavar**, **shatavari**, or **shatamull**) is a species of asparagus common throughout Nepal, Sri Lanka, India and the Himalayas. It grows one to two metres tall and prefers to

take root in gravelly, rocky soils high up in piedmont plains, at 1,300–1,400 metres elevation.[10] It was botanically described in 1799. Because of its multiple uses, the demand for *Asparagus racemosus* is constantly on the rise. Because of destructive harvesting, combined with habitat destruction, and deforestation, the plant is now considered "endangered" in its natural habitat.

*Asparagus racemosus* (Shatavari) is recommended in Ayurvedic texts for the prevention and treatment of gastric ulcers and dyspepsia, and as a galactogogue. *A. racemosus* has also been used by some Ayurvedic practitioners for nervous disorders.[11]A few recent reports demonstrated some additional beneficial effects of this herb including antihepatotoxic, immunomodulatory, immunoadjuvant and antilithiatic effects.[12]

Shatawari has different names in the different Indian languages, such as shatuli, vrishya and other terms. In Nepal it is called kurilo. The name "shatawari" means "curer of a hundred diseases" (shatum: "hundred"; vari: "curer").

**Plant taxonomy**[13]

Kingdom: Plantae

*Clade*: Angiosperms

*Clade*: Monocots

Order: Asparagales

Family: Asparagaceae

Subfamily: Asparagoideae

Genus: *Asparagus*

Species: ***A. racemosus***

**Chemical properties**

Asparagamine A, a polycyclic alkaloid was isolated from the dried roots[14,15] and subsequently synthesized to allow for the construction of analogs.[16]

Two new steroidal saponins, shatavaroside A and shatavaroside B together with a known saponin, filiasparoside C, were isolated from the roots of *Asparagus racemosus*.[17]

Five steroidal saponins, shatavarins VI-X, together with five known saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparanin A), have been isolated from the roots of *Asparagus racemosus*.[18]

## 2. METHODOLOGY

*Asparagus racemosus* is extracted by using soxhlet extraction. Here different solvents like acetone, methanol and ethanol used. These extracts are combined with different Antibiotics and their combinations were applied for zone of inhibition test against different microorganism.

**Antimicrobial Activity :**

Antimicrobial activity and antifungal activity of plant extract alone, antibiotics alone and combination of both was carried out by a well diffusion method. In vitro antibacterial activity of the crude extracts was studied by the agar well diffusion method. After getting the turbidity equal to 0.5 McFarland standards, inoculums were aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 10.0 mm diameter. The plant extract and antibiotic drug were dissolved in DMSO to get desired concentration. The wells were filled with plant extract 25µl, 50µl and antibiotic drug (100µl). The plates are incubated at 37°Cfor 48 hours and then zone of inhibition was measured. In case of combination of plant extract and antibiotic, equal volume of each was added in the well and zone of inhibition was measured. The experiment was replicated two times. (Zone of Inhibition = ±1mm)

**Qualitative Phytochemical Analysis**

Preliminary qualitative analysis of all plant extract was carried out as per standard methods as follows.

**1. Test for alkaloids**

Extracts were dissolved in dil.HCl and filtered.

A. **Dragendroff's Test:** Filtrate was treated with Dragendroff's reagent (Potassium Bismuth Iodide solution). Formation of red precipitate indicates the presence of alkaloids.
B. **Wagner's Test:** Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**2. Test for flavonoids**

**A. Lead acetate Test:**

Extract was treated with 10% lead acetate solution. Yellow colour precipitate formation indicates the presence of flavonoids.

**B. Test with Sodium hydroxide:**

Extract was treated with few drops of NaOH solution. The yellow colour indicates the presence of flavonoids.

**3. Test for Glycosides**

**A. Keller-Kiiani Test:**

Add glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4 to the extract. Formation of reddish brown color at junction of the two liquid layers and bluish green at upper layer indicates the presence of glycosides.

**B. Legal Test:**

On treatment of Extracts with sodium nitropruside in pyridine and sodium hydroxide, observed pink to blood red colour that indicates the presence of cardiac glycosides.

**4. Test for Phenolics**

**Test with Ferric Chloride:**

Extract was treated with few drops of ferric chloride solution and formed bluish black colour indicates the presence of phenols.

**5 Test for Saponins**

**Froth Test:**

The extract was diluted with 20 ml of distilled water and then shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

**6. Test for Tannins**

**Test with Ferric Chloride:**

Extract was boiled with water and filtered it. Added FeCl3 to the filtrate and formation of brownish green or a blue-black coloration indicates the presence of tannins.

**7. Test for Terpenoids**

**Salkowski Test:** To the extract, added 2ml chloroform and 3ml Conc.$H_2SO_4$ by the wall of test tube carefully. Formation reddish brown colour at interface indicates the presence of terpenoids.

## 3 Results and Discussion of *Asparagus Racemosus*

### A Qualitative phytochemical analysis of *Asparagus Racemosus*

| Name of Phytochemical | Test | Acetone Extract | Ethanol Extract | Methanol Extract |
|---|---|---|---|---|
| Alkaloids | Dragondrorff's test | --- | --- | --- |
| | Wagner's test | --- | --- | --- |
| Tannins | With $FeCl_3$ | + | ++ | ++ |
| Saponins | Froth Test | + | + | + |
| Glycosides | Killer- kiiani Test | --- | --- | --- |
| | Legal Test | --- | --- | --- |
| Flavonoids | Lead acetate | + | + | + |
| | With NaOH | + | + | ++ |
| Terpinoids | Salkowski Test | --- | --- | + |
| Phenolics | With $FeCl_3$ | + | + | + |

**--- = No Presence**

**+ = Less Presence**

**++ = Medium Presence**

**+++ = Complete Presence**

**Antimicrobial activity of *Asparagus Racemosus* and its combination with antibiotics for 25µl.**

**Table 1 shows antibacterial activity of *Asparagus Racemosus* extract for 25 µl.**

| Bacteria | Acetone extract | | | | Ethanol extract | | | | Methanol extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration ( µg/ml ) | | | | | | | | | | | |
| | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| *S. aureus* | 12 mm | 11 mm | 10 mm | 9 mm | 14 mm | 13 mm | 11 mm | 9 mm | 17 mm | 15 mm | 12 mm | 9 mm |
| *B. subtilis* | 11 mm | 10 mm | 9 mm | 8 mm | 12 mm | 11 mm | 10 mm | 9 mm | 15 mm | 13 mm | 11 mm | 8 mm |
| *Pseudo Aeruginosa* | 15 mm | 14 mm | 13 mm | 11 mm | 18 mm | 16 mm | 15 mm | 14 mm | 19 mm | 17 mm | 16 mm | 15 mm |
| *E. coli* | 17 mm | 16 mm | 15 mm | 14 mm | 18 mm | 17 mm | 16 mm | 15 mm | 19 mm | 18 mm | 17 mm | 16 mm |

**Table 2 shows antibacterial activity of combination of of *Asparagus Racemosus* extract and amoxicilline for 25 µl .**

| Bacteria | Amox | Acetone extract + Amoxicilline | | | | Ethanol extract + Amoxicilline | | | | Methanol extract + Amoxicilline | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration ( µg/ml ) | | | | | | | | | | | |
| | | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 100 µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| *S. aureus* | 31 mm | 37 mm | 36 mm | 35 mm | 34 mm | 39 mm | 38 mm | 36 mm | 35 mm | 38 mm | 37 mm | 36 mm | 35 mm |
| *B. subtilis* | 35 mm | 43 mm | 42 mm | 40 mm | 38 mm | 43 mm | 42 mm | 40 mm | 39 mm | 43 mm | 42 mm | 40 mm | 39 mm |
| *Pseudo Aeruginosa* | 2 mm | 33 mm | 32 mm | 30 mm | 28 mm | 35 mm | 33 mm | 32 mm | 29 mm | 35 mm | 33 mm | 31 mm | 29 mm |
| *E. coli* | 2 mm | 29 mm | 28 mm | 26 mm | 24 mm | 29 mm | 27 mm | 26 mm | 24 mm | 32 mm | 30 mm | 28 mm | 25 mm |

**Table 3 shows antibacterial activity of combination of *Asparagus Racemosus* extract with Ciprofloxacin for 25 µl.**

| Bacteria | Cipro µg/ml | Acetone extract + Cipro. | | | | Ethanol extract + Cipro. | | | | Methanol extract + Cipro. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration ( µg/ml ) | | | | | | | | | | | |
| | | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 25 µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| S. aureus | 24 mm | 33 mm | 33 mm | 32 mm | 31 mm | 36 mm | 35 mm | 33 mm | 32 mm | 36 mm | 35 mm | 34 mm | 32 mm |
| B. subtilis | 29 mm | 36 mm | 35 mm | 34 mm | 33 mm | 38 mm | 37 mm | 36 mm | 35 mm | 38 mm | 37 mm | 36 mm | 35 mm |
| Pseudo Aeruginosa | 27 mm | 33 mm | 32 mm | 31 mm | 30 mm | 34 mm | 33 mm | 32 mm | 31 mm | 33 mm | 32 mm | 31 mm | 30 mm |
| E. coli | 27 mm | 35 mm | 34 mm | 32 mm | 30 mm | 35 mm | 33 mm | 31 mm | 30 mm | 35 mm | 34 mm | 33 mm | 31 mm |

**Table 4 shows antibacterial activity of combination of *Asparagus Racemosus* extract with Ceftadizime for 25 µl .**

| **Bacteria** | **Cefta µg/ml** | **Acetone extract + Cefta** | | | | **Ethanol extract + Cefta** | | | | **Methanol extract + Cefta** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Concentration ( µg/ml )** | | | | | | | | | | | |
| | | **1000** | **500** | **250** | **125** | **1000** | **500** | **250** | **125** | **1000** | **500** | **250** | **125** |
| | **25 µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** | **25µl** |
| ***S. aureus*** | **0 mm** | **12 mm** | **10 mm** | **9 mm** | **8 mm** | **12 mm** | **10 mm** | **9 mm** | **8 mm** | **12 mm** | **11 mm** | **9 mm** | **8 mm** |
| ***B. subtilis*** | **0 mm** | **12 mm** | **11 mm** | **9 mm** | **8 mm** | **12 mm** | **11 mm** | **10 mm** | **9 mm** | **12 mm** | **11 mm** | **10 mm** | **9 mm** |
| ***Pseudo aeruginosa*** | **5 mm** | **11 mm** | **10 mm** | **9 mm** | **8 mm** | **12 mm** | **10 mm** | **9 mm** | **8 mm** | **12 mm** | **10 mm** | **9 mm** | **8 mm** |
| ***E. coli*** | **16 mm** | **22 mm** | **21 mm** | **20 mm** | **18 mm** | **22 mm** | **21 mm** | **20 mm** | **19 mm** | **23 mm** | **21 mm** | **20 mm** | **19 mm** |

Table 5 shows antibacterial activity of combination of *Asparagus Racemosus* extract with Erythromycin for 25 µl .

| Bacteria | Erythro µg/ml | Acetone extract + Erythro | | | | Ethanol extract + Erythro | | | | Methanol extract + Erythro | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration ( µg/ml ) | | | | | | | | | | | |
| | | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 25 µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| S. aureus | 16 mm | 31 mm | 30 mm | 29 mm | 27 mm | 31 mm | 30 mm | 29 mm | 28 mm | 31 mm | 30 mm | 29 mm | 28 mm |
| B. subtilis | 22 mm | 32 mm | 32 mm | 31 mm | 29 mm | 33 mm | 31 mm | 30 mm | 29 mm | 31 mm | 29 mm | 28 mm | 27 mm |
| Pseudo aeruginosa | 1 mm | 27 mm | 26 mm | 24 mm | 22 mm | 28 mm | 27 mm | 26 mm | 25 mm | 28 mm | 27 mm | 26 mm | 25 mm |
| E. coli | 5 mm | 21 mm | 20 mm | 19 mm | 18 mm | 24 mm | 23 mm | 21 mm | 19 mm | 24 mm | 23 mm | 21 mm | 20 mm |

Table 6 shows antifungal activity of *Asparagus Racemosus* extract for 25 µl.

| Bacteria | Acetone extract | | | | Ethanol extract | | | | Methanol extract | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration ( µg/ml ) | | | | | | | | | | | |
| | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| Aspergillus Niger | 14 mm | 13 mm | 12 mm | 10 mm | 13 mm | 12 mm | 11 mm | 10 mm | 14 mm | 13 mm | 12 mm | 11 mm |
| Candida Albicum | 13 mm | 12 mm | 11 mm | 10 mm | 14 mm | 13 mm | 12 mm | 11 mm | 15 mm | 14 mm | 13 mm | 12 mm |

**Table 7 shows anti fungal activity of combination of *Asparagus Racemosus* extract and Amphoteracin B for 25 µl.**

| Bacteria | Ampho (µg/ml) | Acetone extract + Ampho | | | | Ethanol extract + Ampho | | | | Methanol extract + Ampho | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration ( µg/ml ) | | | | | | | | | | | |
| | | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 100 µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| *Aspergillus Niger* | 16 mm | 28 mm | 26 mm | 25 mm | 23 mm | 29 mm | 27 mm | 26 mm | 24 mm | 29 mm | 27 mm | 26 mm | 25 mm |
| *Candida Albicum* | 15 mm | 30 mm | 28 mm | 26 mm | 24 mm | 30 mm | 28 mm | 26 mm | 25 mm | 31 mm | 29 mm | 27 mm | 26 mm |

**Table 8 shows antifungal activity of combination of *Asparagus Racemosus* extract and Fluconazole for 25 µl.**

| Bacteria | Fluco (µg/ml) | Acetone extract + Fluco | | | | Ethanol extract + Fluco | | | | Methanol extract + Fluco | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration ( µg/ml ) | | | | | | | | | | | |
| | | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 | 1000 | 500 | 250 | 125 |
| | 100 µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl | 25µl |
| *Aspergillus Niger* | 2 mm | 12 mm | 11 mm | 10 mm | 9 mm | 15 mm | 13 mm | 12 mm | 10 mm | 15 mm | 14 mm | 13 mm | 11 mm |
| *Candida Albicum* | 0 mm | 12 mm | 11 mm | 10 mm | 8 mm | 14 mm | 12 mm | 10 mm | 9 mm | 14 mm | 12 mm | 10 mm | 9 mm |

**Discussion**

Table A showed qualitative phytochemical analysis of *Asparagus Racemosus.* Results showed that Tannins, Saponins, Phenolics, Steroids are present while Alkaloids and glycosides are absent in plant extracts. Here methanol, ethanol and acetone extracts used.

Table 1 showed antimicrobial activity of *Asparagus Racemosus* extract for 25µl. Methanol and ethanol extract has little more value of zone of inhibition observed compare to ethanol extract. At higher concentration value of zone of inhibition found little more but as concentration decreased value of zone of inhibition decreased.

Table 2 showed antimicrobial activity of combination Asparagus Racemosus extract with amoxicillin for 25µl. Value of zone of inhibitions are indicated more compare to pure amoxicillin against all mentioned bacterial culture. Combination showed synergic effect and it is more powerful compare to pure antibiotic.

Table 3 showed antimicrobial activity of combination of Asparagus Racemosus extract with Ciprofloxacin for 25 µl. Pure Ciprofloxacin have zone of inhibition 24mm against S.aureus while combination showed 36mm, 35mm, 34mm and 32mm for 1000µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml respectively for methanol extract. Further against E.coli good synergic effect observed.

Table 4 showed antimicrobial activity of combination of Asparagus Racemosus extract with Ceftadizime for 25µl. Methanol, ethanol and acetone extract showed good antagonistic effect against different bacterial.

Table 5 showed antimicrobial activity of combination of Asparagus Racemosus extract with Erythromicin for 25 µl. Results showd that all three extracts showed synergic effect at all conctrations. All have strong value of zone of inhibition against all for bacterial cultures.

Table 6 showed antifungal activity of Asperagus Racemosus extract for 25µl. All extract showed good resistance against A.niger and C. albicum. Methanol extract and ethanol extract showed good resistive properties.

Table 7 showed antifungal activity of combination of Asperagus Racemosus extract with Amphoteracin B for 25 µl. Methanol, ethanol and acetone extract showed good synergic effect against A.niger and C.Albicum.

Table 8 showed antifungal activity of combination of Asperagus Racemosus extract with Fluconazole for 25 µl. Methanol extract has good resistance potential compare to acetone extract. Value of zone of inhibition for methanol extract are found15mm, 14mm, 13mm and 11mm against A.niger.

## 4. CONCLUSION

Plant extracts have showed effective antimicrobial activity against bacterial various culture. To decrease side effect of antibiotics and to increase sensitivity of plant extract, combination of Herbal extract and antibiotics used. Day by day bacteria regaining antibody against traditional antibiotics and gaining strength. To stop their regenerating power, combination of plant extracts and antibiotics provide one of the best results in such direction. If nontoxic plant extracts have been taken in suitable doses, it may prove best supplementary remedies patient.

This is in vitro study and such combination must be followed by toxicity test and in vivo tests to determine its therapeutic application

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