PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON 
ASPARAGUS RACEMOSUS

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ABSTRACT

Asparagus racemosus is the one of the important medicinal plant and commonly known as Shatavari in Hindi and Sanskrit. Asparagus racemosus is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers, dyspepsia and as a galactotogogue. It is also used successfully for nervous disorders, inflammation, liver diseases and certain infectious diseases. In present study, in vitro antimicrobial activity of crude ethanol and acetone extract of the roots of Asparagus racemosus were investigated. The Phytochemical screening of the extracts revealed the presence of Alkaloids, Glycosides, Phenolic compounds, Tannins, Saponins, Steroids, Flavonoids and Carbohydrates. The result obtained from agar well diffusion method indicate that, ethanolic and acetonie root extract of Asparagus racemosus showed the spectrum of inhibition on Staphylococcus aureus, Bacillus subtilis, E. coli, Klebsiella pneumonia, Salmonella typhi. Thus results of phytochemical and antimicrobial study have shown that, the Asparagus racemosus acts as promising plant for developing new medicine.

KEYWORDS: Asparagus racemosus, Phytochemical, antimicrobial activity.
INTRODUCTION
In many developing countries, there has been an increasing interest in use of herbal medicine for primary health care. About 80 % worldwide peoples mainly use herbal medicine (WHO).[1] The herbal extracts have been a chief source of medicinal agents from long time ago. In worldwide, about thousands of plant species are known to have medicinal value and used to cure specific ailments.[2] According to Lakshmann[3] and Ravishankar[4], plants extracts from 157 families have been reported to be active against microorganisms. Asparagus racemosus belongs to family Asparagaceae. It is an under shrub climbing herbs with a tuberous root. In the Traditional System of Medicine the herb is used mainly to promote milk secretion.[5] It also possesses anthelmintic and antiseptic properties.[6] Goyal et al.,[7] reported that A. racemosus possess much more important biological properties such as immunostimulants, anti-inflammatory, anti-hepatotoxic, antimicrobial and reproductive agent. Hayes et al.,[8] investigated that, A. racemosus is also used in the treatments of various disorders including stomach ulcers, lung abscess, menopause, herpes and chronic fevers. In addition to this, roots of this plant can be used in nervous disorders, dyspepsia, diarrhoea, dysentery, tumors, inflammations, neuropathy, hyperdipsia, cough, bronchitis, hyperacidity and infectious diseases.[9] Generally, root of this plant is bitter in taste, emollients, cooling, nervine tonic, constiputing, diuretic and rejuvenating.

MATERIAL AND METHOD
Plant Material
The whole plant of Asparagus racemosus were collected from botanical garden of Y. C. Institute of Science, Satara. The collected plant was identified and authenticated by Department of Botany, Y. C. Institute of Science, Satara.

Preparation of plant root extract
The collected roots were thoroughly washed; shade dried and powdered using a mechanical grinder. The powder kept in air tight bottle for further study. The solvent extraction was done in accordance with Dhairyasheel et al.,[10] with some modifications.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies
The five Bacterial cultures of Staphylococcus aureus, Bacillus subtilis, E. coli, Klebsiella pneumoniae, Salmonella typhi were procured from Department of Biotechnology of Y. C. I. S. Satara. They were identified on the basis of cultural and morphological characteristics. The
collected bacteria were maintained on nutrient agar slant at 37°C and sub-cultured at regular interval for further studies.

**Antibacterial activity**
The antibacterial activities of the leaf extracts were determined by using the agar well diffusion method of Dhairyasheel et al.,[10] to determine the zone of inhibition.

**Antimicrobial Agent**
The reference standard Ciprofloxacin was procured from Hi media Pvt. Ltd, Mumbai.

**Phytochemical screening test**
The different extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, flavonoid, terpenoids (Salkowski Test), alkaloids, cardiac glycosides and carbohydrates in accordance with Njoku and Obi,[11] Abba et al.,[12] and Edeoga et al.,[13] with some modification.

**Test for Sterols**
In different extracts, few drops of concentrated sulphuric acid (H₂SO₄) was added, shaken and allowed to stand, Instead of aqueous extract, in all extract appearance of red color indicates the presence of sterols.

**Test for Saponins**
The saponin content in root extract was determined by adding small amount of 2N HCl, Shake extract with little quantity of water and finally adds few drops of Mayer’s reagent. If foam produced persists for 10 minutes; it indicates the presence of saponins.

**Test for Alkaloids**
For alkaloid test, heat 2 ml of root extracts by adding 10% NaOH solution. The white precipitate was taken as positive test for alkaloids.

**Test for Tannins**
For tannins, heat 2 ml of root extract by adding concentrated HNO₃ along with excess ammonia. The formation of white precipitation indicates the presence of tannins.
Test for Carbohydrate
The root extract was treated with Molisch reagent and concentrated sulphuric acid was added from the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

Test for Flavonoids
To alcoholic solution added few drops of sodium hydroxide solution. Intense yellow color which disappeared after adding dilute HCl indicates the presence of flavonoids.

Test for Amino acid
About 3 ml of extract and 3 drops of ninhydrin solution in boiling water bath for 10 minutes. If purple color appear that shows the presence of amino acids.

RESULT AND DISCUSSION
The results obtained in the present investigation are summarized in table 1, and 2. The Phytochemical analysis of root extract of Asparagus racemosus reveals the presence of important bioactive secondary metabolites such as steroid, saponin, alkaloid, tannins, flavonoids and amino acids. In present investigation, the steroid, saponin, tannins, carbohydrate, flavonoids and amino acids were observed in ethanol extract.

Our results show close conformity with findings of Sanker et al.[14] According to them, ethanolic extracts of A. racemosus is rich in bioactive secondary metabolites. The flavonoids content was fairly present in acetone extract.

The antimicrobial activity of Asparagus racemosus was studied by Agar Well Diffusion Method. The activity of different extract of root was observed on different Gram positive and Gram negative bacteria. The result revealed that, ethanol extract of the Asparagus racemosus showed moderate to strong antimicrobial activity against E. coli, Staphylococcus aureus, Klebsiella pneumoniae and Salmonella typhi over the standard antibiotic ciprofloxacin and acetone extract. However, Acetonic extract of A. racemosus showed maximum inhibitory zone against Bacillus subtilis, than ethanolic extract. The result presented from present investigation indicates that root extracts from Asparagus racemosus exhibited antimicrobial properties. Thus justifying scientifically their traditional used as medicinal plant.
Table-1. Phytochemical analysis of roots Extract of *Asparagus racemosus*

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Phytochemical analysis</th>
<th>Ethanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Sterols/ Triterpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2)</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3)</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4)</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5)</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6)</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7)</td>
<td>Amino acid/ Protein</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; - = Absent

Table-2. Antimicrobial Activity of *Asparagus racemosus* on different organism on Nutrient Agar

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Test Microorganism</th>
<th>Zone diameter in mm</th>
<th>Zone diameter in mm</th>
<th>Zone diameter in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
<td>Ethanol</td>
<td>Standard Antibiotic-Ciprofloxacin</td>
</tr>
<tr>
<td>1)</td>
<td><em>E. coli</em></td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2)</td>
<td><em>Bacillus subtilis</em></td>
<td>20</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>3)</td>
<td><em>Staphylococcus aureus</em></td>
<td>21</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>4)</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>14</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>5)</td>
<td><em>Salmonella typhi</em></td>
<td>16</td>
<td>18</td>
<td>15</td>
</tr>
</tbody>
</table>

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco friendly in nature effective as antimicrobial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the microorganism. From the antibacterial activities of *Asparagus racemosus*; higher effect was showed on *E. coli* and *S. aureus* than on *Klebsiella pneumoniae*. This gives an insight, into the phytochemistry of the test plant. The antibacterial activities of leaf and root are found due to the presence of the phytochemicals. Hence it can be used as an antibacterial agent.

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