STUDY OF ANTIBACTERIAL ACTIVITY AND BIOCHEMICAL ANALYSIS OF SOME MEDICINAL PLANTS AGAINST SELECTED PATHOGENS CAUSING HUMAN INTESTINAL DISTURBANCES.

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ABSTRACT
The present study is based on the investigation of antibacterial activity of aqueous and ethanolic extracts of Cymbopogon citratus and Asparagus racemosus. The microorganisms used in the study are human intestinal disturbance causing pathogens viz. Staphylococcus aureus, Proteus vulgaris and Escherichia coli. The susceptibility of bacterial strains against the two extracts was determined using disk diffusion method. The most susceptible microorganism was S. aureus while the least susceptible organisms was E.coli. Highest antibacterial activity was observed with ethanolic extract of Cymbopogon citratus against S.aureus (12mm) while minimum activity was observed with aqueous extract of Asparagus racemosus against P.vulgaris (1mm).

Ethanolic extracts of both the samples were more effective in comparison to the aqueous extracts in inhibiting the growth of pathogens under study. They were less potent when compared with ciprofloxacin used as positive control. Biochemical analysis shows the presence of alkaloid, flavanoids and tannins in both the samples where as steroids were detected only in aqueous extract of Cymbopogon citratus & Asparagus racemosus.

KEY WORDS: C.citratus, A. racemosus, Antibacterial activity, Biochemical analysis, Pathogens.

INTRODUCTION
For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems. Herbs are safe, less toxic,
economical and a reliable key natural resource of drugs all over the world. Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medicine facilities. The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compound. The medicinal value of plants lies in some chemical substances that produce a definite physiological action in human body. The most important of these bioactive constituents are alkaloids, tannins, flavonoids, phlobotannins, saponins and cardiac glycoside.\[1\]

*Cymbopogon citratus* (lemon grass) is an aromatic perennial tall grass with rhizomes and tufted fibrous root. It is a native herb from India and also cultivated in other tropical and subtropical countries. It is used as a traditional folk medicine in the treatment of malaria, typhoid fever, nervous and gastrointestinal disturbances, fever, hypertension, stomach ache. It has applications in soap, perfumery industries and cosmetics.

*Asparagus racemosus* commonly called as shatavari in hindi. It belongs to the family of asparagaceae and genus asparagus. This plant is widely cultivated in tropical and sub-tropical India. Its medicinal use has been reported in Indian traditional medicine as Ayurveda, Unani and Siddha. *A.racemosus* is widely used as antioxidant\[2,3\], antitussive\[4\], gastro protective\[5\], neuroprotective\[6\] and to cure depression.\[7\]

The aim of our study is to examine the antibacterial activity and biochemical analysis of ethanol and aqueous extracts from leaves of *Cymbopogon citratus* and *Asparagus racemosus*. *A.racemosus* is spinus under shrub with numerous succulent roots and grows at an altitude of 1500 m.

**MATERIAL and METHODS**

1. **Collection of plant material**

Plants (*Cymbopogon citratus* and *Asparagus racemosus*) was collected from a local nursery in the month of October. Further whole plant material containing leaves and stems were planted, cultivated and harvested.

2. **Pretreatment of the samples**
The leaves of the respective plants were collected and washed with clean water for 3-4 times and further with distilled water for 2 times. It was chopped and then spread on dried clean muslin cloth under the room temperature for 15 days.

3. Extraction
The dried samples were grinded and sieved. The powdery sample were then partitioned in two parts

a) ETHANOLIC EXTRACT
(12 & 16 gm) powdered samples were extracted with 100ml of ethanol. The powdered sample was soaked in the solvent for 24 hours and plant extract was prepared using reflux and steam distillation method. In this method, plant material is immersed in a solvent in the round- bottomed flask, which is connected to a condenser. The solvent is heated up to its boiling point. As the vapors are condensed, the solvent is recycled into the flask.

b) AQUEOUS EXTRACT
(12 & 16 gm) powdered sample were extracted with distilled water. The powdered sample was soaked in 100 ml water for 24 hours and plant extract was prepared using the same above mentioned method.

4. Bacterial Strains
The test organisms used for the study were E.coli, P.vulgaries, S.aureus. These organisms were collected from Choithram Hospital and research center, Indore (M.P.), India. The organisms were sub-cultured and maintained at 4°C.

5. Antibacterial Activity
The antibacterial activity of aqueous and ethanolic extracts of both the plants (C.citratus and A.racemosus) was performed by agar disc diffusion method.[8,9] The plates were prepared by spread plate method by using Muller Hinton agar medium. Plates were inoculated with 0.1 ml of the inoculums of test organisms used in the study. The Hi Media sterile susceptibility test discs were used. These discs were saturated with 100 µl of test compounds, allowed to dry and were seeded on the inoculated agar plates. The plate were incubated overnight at 37 0C in incubator. The zone of inhibition of plant extracts was observed and measured. The results were recorded by measuring the diameter (mm) of the zone of growth inhibition surrounding
the disc. Sterile water disc was used as negative control and antibiotic ciprofloxacin disc was used as positive control.\textsuperscript{[8,9]}

6. **Biochemical analysis of extracts of (C. citratus)**

Biochemical analysis for the major phyto-constituents of the plant extracts was done using standard qualitative methods.\textsuperscript{[10]}

**A) Steroids and Terpenoids** - 10mg of extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of con Sulphuric acid. Blue color in chloroform layer which changes to green shows the presence of steroids, whereas the appearance of pink color in chloroform layer shows the presence of terpenoids.

**B) Alkaloids** - 10mg of extract is dissolved in con HCL and filtered. A few drops of solution is poured into the centre of watch glass. Mayer reagent is added in the sides of the watch glass with the help of a glass rod. Formation of a gelatinous white precipitate at the junction of the two liquid shows the presence of alkaloids.

**C) Flavonoids** - 10mg of extract was dissolved in methanol. Magnesium turnings were added into this followed by con HCL. A magenta color shows the presence of Flavonoids.

**D) Tannins** - 10 mg of extract was boiled with 1 ml water for 30 min. The extract is filtered clear and to this 0.5 ml 2% gelatin was added. A curdy white precipitate indicates the presence of tannin.

2. **Biochemical analysis of extracts (A. racemosus)**

Biochemical analysis for the major phyto-constituents of the plant extract was done using standard qualitative methods described as-

**A) Steroids** - 1 ml of extract was dissolved in 10 ml of methanol, to this volume of concentrated sulphuric acid was added. Red coloration of the upper layer and yellow with green fluorescence of the sulphuric acid layer indicates presence of steroids.

**B) Alkaloids (Dragendroff’s test)** - The extract was dissolved in methanol and this was acidified by adding few drops of Dragendroff's reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicates presence of alkaloids.\textsuperscript{[11]}
C) Flavonoids (Ferric chloride test)- Appearance of green coloration on addition of a few drops of neutral ferric chloride solution to the alcoholic solution of the plant extract, indicates presence of flavanoids.

D) Tannins (Ferric chloride test)- Ferric chloride solution was added drop by drop to 2 ml of extract in a test tube. Appearance of bluish black precipitate indicates presence of tannins and phenolic compounds.

RESULTS
1) Ethanolic and aqueous extracts of *C.citratus* and *A.racemosus* were isolated and phytochemical analysis was performed (Table 1). It has been observed that most of the secondary metabolites were identified in the polar extracts. Biochemical analysis showed the presence of alkaloids, flavonoids and tannins in both the extracts of *Cymbopogon citratus* and *Asparagus recemosus*, while the presence of steroids was found only in the aqueous extract.

<table>
<thead>
<tr>
<th>Biochemical constituents</th>
<th><em>Cymbopogon citratus</em></th>
<th><em>Asparagus recemosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

2) The antibacterial activity of *Cymbopogon citratus* and *Asparagus racemosus* showed varied results with solvents used for extraction (Table 2). The test organisms showed inhibition activity with the zone range of 5-15mm for *C. citratus* and 1-10mm for *A. recemosus*. The zone of inhibition was found to be more in the ethanolic extracts in comparison with the aqueous extracts. The zones of inhibition for aqueous extract of *C. citratus* are 5mm, 9mm, 6mm and for aqueous extract of *A. recemosus* are 2mm, 3mm, 1mm for *E.coli*, *S.aureus*, *P.vulgaries* respectively. The zones of inhibition for ethanolic extract of *C. citratus* are 8mm, 12mm, 10mm and ethanolic extract of *A. recemosus* are 10mm, 7mm, 5mm for *E.coli*, *S.aureus*, *P.vulgaris* respectively. Hence in the study of *C. citratus*, *S. aureus* was found to be the most susceptible microorganism, while the least susceptible organism was *E.coli*. Whereas, in the study of *A. recemosus*, *E.coli* was found the most susceptible while, *P.vulgaris* was the least susceptible. Ciprofloxacin was used as a positive
control. Test organisms were more susceptible to ciprofloxacin as compared to both the extracts of *C. citratus* and *A. racemosus*.

**Analysis of Antibacterial activity of Ethanolic and Aqueous Extracts of C. citratus and A. recemosus**

**Table 2**

<table>
<thead>
<tr>
<th>Pathogens</th>
<th><em>Cymbopogon citratus</em></th>
<th><em>Asparagus recemosus</em></th>
<th>Ciprofloxacin (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extract</td>
<td>Ethanol Extract</td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>E.coli</td>
<td>5mm</td>
<td>8mm</td>
<td>2mm</td>
</tr>
<tr>
<td>S.aureus</td>
<td>9mm</td>
<td>12mm</td>
<td>3mm</td>
</tr>
<tr>
<td>P.vulgaries</td>
<td>6mm</td>
<td>10mm</td>
<td>1mm</td>
</tr>
</tbody>
</table>

**Fig:** Inhibition zone of *A. recemosus* against selected pathogens.

**Fig:** Inhibition zone of *C. citratus* against selected pathogens.
DISCUSSION

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting this into herbal remedy depends upon the solubility of these compounds in various solvents.\(^{[12]}\) Laboratories of the world have found literally thousands of phytochemicals having inhibitory effects on microorganisms.\(^{[13]}\) Different types of plants extracts like crude, methanolic, ethanolic, phenolic etc have been used for many thousands of years in food preservation\(^{[14]}\) alternative medicines and natural medicines\(^{[15,16]}\), Gopinath S.M et al. studies observed the absence of steroids in both the aqueous as well as ethanolic extracts and presence of alkaloids, flavonoids and tannis in both the polar solvents.\(^{[17]}\) Phytochemical analysis by Jayshree G.V. et al revealed the presence of various active phytochemical constituents viz. alkaloids, flavanoids, tannins in methanolic extract while steroids were absent\(^{[18]}\), whereas Nagamani et al in their study showed the absence of alkaloids, flavanoids, tannins and presence of steroids in ethanolic extract while all the phytochemical constituents were present in aqueous extract\(^{[19]}\) which is in contrast with our study showing the presence of alkaloids, flavanoids, tannins and absence of steroids in the ethanolic extract whereas all the active phytoconstituents were present in aqueous extract. These active components usually interfere with growth and metabolism of microorganisms in a negative manner.\(^{[20]}\) Phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens.

The use of plant extract with known antibacterial properties can be of great significance in therapeutic treatment. Several researchers have reported that many plants possess antimicrobial properties including the parts i.e. flower, bark, stem, leaf\(^{[21]}\), etc. Various antibacterial investigations were carried out on the crude extract obtained from leaves of A. recemosus and C. citrtatus for the screening of antimicrobial potential. Antibacterial activity obtained in Sooad Al-Daihan et al study varied with solvent used for extraction.\(^{[12]}\) Sankarnarayan et al reported that ethnolic extract of A. recemosus showed the zone of inhibition against E.coli and S.aureus ranging from 16-24 mm and zone of inhibition with chloroform extract against same organism ranging from 16-20 mm.\(^{[22]}\) Rajendra R et al examined methanolic extract of A. recemosus for the antibacterial activity against isolated human pathogens and reported the zone of inhibition ranging from 3-15 mm, hence he concluded that the methanolic extract of A. recemosus showed the maximum activity against
three pathogens viz. *E. coli*, *P. vulgaris*, *S. aureus*. In correlation to this researches our study for the extract of *A. recemosus* showed moderate inhibition activity with the zone range from 1-10 mm. Maximum zone of inhibition was observed with ethanolic extract against *E. coli* (10 mm) and minimum zone of inhibition with aqueous extract against *P. vulgaris* (1mm). Against all the tested bacterial strains we observed ethanolic extracts of *A. recemosus* showing much better antibacterial activities in contrast to aqueous extract which may be because of organic nature of ethanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds.

In the study of Adegbegi Ademuyiwa Joshua et. al. showed that, the ethanolic extract of *C. citratus* stem inhibited the entire tested organisms more than the leaves with varied concentrations. Whereas, In vitro studies of C.K. Hindumathy, showed that the methanolic and aqueous extracts of *C. citratus* inhibited bacterial growth of *E. coli*, *P. vulgaris* and *S. aureus*, but their effectiveness varied with the concentration. In parallel to this, our study showing the antibacterial activity of *C. citratus* against three pathogens viz. *E. coli*, *P. vulgaris*, *S. aureus*. The zone of inhibition ranging from 5-12 mm. Maximum zone of inhibition was observed with ethanolic extract against *S. aureus* (12 mm) and minimum zone of inhibition with aqueous extract against *E. coli* (5mm). Against all the tested bacterial strains we observed ethanolic extracts of *C. citratus* showing much better antibacterial activities in contrast to aqueous extract which may be because of organic nature of ethanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds.

Several studies have reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals. As a result sterilization is needed especially for aqueous extracts before use to get rid of this contaminations. Keeping this thought alive in our study we have given pre-treatment to the plant material by continuous washing with distilled water under sterile conditions.

**CONCLUSION**

In conclusions of this study it is possible to state that *C. citratus* and *A. recemosus* possess antibacterial activity. On comparing with the literature and present work indicates that, most diverse results can be obtained. Plants extracts have shown inhibitory effects on the growth of the test organisms studied. Further research is needed toward isolation and identification of
active principles present in the extracts which could possibly be exploited for pharmaceutical use.

REFERENCES


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