A short review and study on antimicrobial activity of Unani drug - Ushba (Smilax ornata)

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ABSTRACT
The study was planned to investigate the antimicrobial activity of Alcoholic and Aqueous extract of Smilax ornata, and brief information about this medicinal herb was also reviewed. Result was compared statistically and it showed significant p<0.001 antimicrobial as compared to control (Plain water) and standard Ciprofloxacin (for gram positive strains) and gentamicin (for gram negative strains).

Keywords: Antimicrobial activity, Smilax ornata, Extract, Strain

INTRODUCTION
Plants are used for treating various diseases through ancient time in most part of the world especially in developing countries and dependence on traditional medicine for various diseases¹. The drug Sarsaparilla consists of the dried roots and sometimes contains the rhizomes. It is difficult to explain about exact geographical and Botanical sources of varieties of the drug but according to National Formulary description is as follows².

The National Formulary (1960) are as follows:

| Variety and geographical source | Synonyms       | Botanical source      |
|---------------------------------|----------------|-----------------------|
| Mexican                         | Vera Cruz or Grey | Smilax aristolochiaeefolia |
| Honduran                        | Brown          | Smilax regelii        |
| Ecuadorian                      | Guayaquil      | Smilax febrifuga      |
| Central American                | Costa Rica or Jamaica | Smilax ornata         |

There are number of species of sarsaparilla, the most common are smilax ornata and smilax regelii, which contain saponins, sarasaponin and parallin and yield isometric sapogenins, sarsapogenin and smilogenin³.

Several varieties of sarsaparilla are important but the one known as Jamaica sarsaparilla is the most esteemed in this country. This variety is obtained from smilax ornata Hooker-filus, Family Liliaceae, a climbing plant with woody stems ascending lofty trees and springing from a stout, knotty rhizome³,4.

Sarsaparilla is the common name for Smilax ornata (formally smilax regelii), which has been used medicinally to treat everything from chronic pain to toe fungus. Other common names include sarsaparilla, Honduran sarsaparilla and Jamaican sarsaparilla⁵. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines⁶. Over 50% of all modern clinical drugs are of natural product origin and natural products play a vital role in modern drug development in the pharmaceutical industry⁷. Interest in a large number of traditional natural products has increased⁸.

There was a great interest in plants with antimicrobial properties, had revived as a result of current problems such as resistance associated with the use of antibiotics⁹. These extracts show various medicinal properties, among which one has the antimicrobial property¹⁰. Over the past several years, intensive efforts have been made to discover clinically
useful antimicrobial drugs, which have been reviewed by many researchers\(^1\).

**MATERIAL AND METHODS**

**Collection of Sample**

The crude drug material was procured from the local market of Baraduari in Aligarh city. The drugs were properly identified by the Botanical literature available.

**Preparation of Sample**

10 gram of crude drug powder and 150 ml of 95\% ethanol were put into a Soxhlet apparatus. The solvent was boiled at 40-50°C on heating mantle with thermostat and refluxed for a period of 6 hours. The extract was filtered and evaporated to dryness on the water bath.

Then one gram of extract was dissolved in 1 ml Dimethyl Sulphoxide (DMSO) by vigorous shaking and stirring which resulted 1gm/ml of stock solution. A total volume of 50\µl/well from test drug extract, taken from stock solution.

RESULT AND OBSERVATION

The highest antimicrobial activity was observed in the alcoholic extract of Ushba than in the aqueous extract. One concentration (5 mg/ml) of test drug taken in alcoholic and aqueous solvent; it was observed that as the test drug in alcoholic solvent show more ZI0 than aqueous solvent. This indicates that test drug has significant effect in alcoholic solvent then aqueous solvent.

**Antibacterial Activity of Ushba against Gram Positive Bacterial Strains**

| S.No | Test Strains (Clinical Isolate) | Zone of inhibition (in mm) expressed as Mean±S.E.M | Probability of error |
|------|--------------------------------|---------------------------------------------------|---------------------|
|      | Alcoholic extract (50µl) | Aqueous extract (50µl) | Gentamicin (SDO16) 5µg/disc | Plain control DMSO (50µl) |
| 1.   | *Streptococcus mutans* | 8.33±0.33*** | 11.33±0.33*** | 35.33±0.33*** | 6.33 ±0.33*** |
| 2.   | *Staphylococcus aureus* | 8.33±0.33*** | 12.33±0.33*** | 32.33±0.33*** | 6.33±0.33*** |
| 3.   | *Streptococcus viridians* | 8.33±0.33*** | 8.33±0.33*** | 34.33±0.33*** | 6.33±0.33*** |
| 4.   | *Streptococcus pyogenes* | 16.33±0.33*** | 8.33±0.33*** | 32.33±0.33*** | 6.33±0.33*** |
| 5.   | *Corynebacterium diphtheriae* | 7.33±0.33*** | 10.33±0.33*** | 32.33±0.33*** | 6.33±0.33*** |
| 6.   | *Corynebacterium xerosis* | 7.66±0.33*** | 10.33±0.33*** | 27.33±0.33*** | 6.33±0.33*** |

**Antibacterial Activity of Ushba against Gram Negative Bacterial Strains**

| S.N | Test Strains (Clinical Isolate) | Zone of inhibition (in mm) expressed as Mean±S.E.M | Probability of error |
|-----|--------------------------------|---------------------------------------------------|---------------------|
| 1.  | *Escherichia coli* | 6.33±0.33*** | 7.66±0.33*** | 26.66±0.33*** | 6.33±0.33*** |
| 2.  | *Klebsiella pneumoniae* | 10.33±0.33*** | 10.33±0.33*** | 27.33±0.33*** | 6.33±0.33*** |
| 3.  | *Pseudomonas aeruginosa* | 10.33±0.33*** | 10.33±0.33*** | 27.66±0.33*** | 6.33±0.33*** |

Significance:*** =p<0.001 ** =p<0.01 * =p<0.05 NS=Not significant

Media preparation

Mueller-Hinton agar (Himedia Lab Pvt. Ltd., Mumbai, India) was autoclaved and 25-30 ml was poured into sterile disposable (90mm in diameter) petridishes on a level surface to give a uniform depth of 4mm. The prepared plates were stored in inverted position at 4°C.

Statistical Analysis: All the statistical analysis was done using gpaid soft ware one way ANOVA & post test named Bonferroni, p-value ≤ 0.05 was taken to be statistically.
Antibacterial activity of Ushba against clinical isolates respective bacterial strains with alcoholic (1) and aqueous (2) extract at 5 mg/ml concentration compared with plain control (PC) and standard drug (S) which includes antibacterial disc ciprofloxacin for gram positive and gentamicin for gram negative bacterial strains.
DISCUSSION

There are in discrimination use of antimicrobial drugs for the treatment of infectious disease. As a result most infective microbes have developed resistance to many antibiotics. The need of hour to search newer antibiotics that can help in fighting the dreadful microorganisms causing such infectious diseases.

For the purpose of antibacterial screening, drug extracts viz alcoholic and aqueous were used. Unani drugs have diversity of phytoconstituents with soluble in different solvents based on their polarity. Aqueous extract has some limitations i.e. it cannot extract oils, resins, alkaloids with are water insoluble. Therefore, in the present study two solvents (Aqueous and 95% alcoholic) were used for extraction of test drugs. Although sensitivity was found higher in alcoholic extract, which indicate that maximum phytoactive constituents are present in this extract, and it is suggested that this extract can be used therapeutically to achieve the maximum benefits.

The antimicrobial screening was conducted by Agar well method using different gram positive and gram negative bacterial strains. Zone of Inhibition (ZoI) produced by them was measured as criteria to evaluate their antimicrobial activities. It was compared with the standard antibiotic ciprofloxacin (SD060) 5µg/disc for gram positive and Gentamicin (SD016) 10µg/disc for gram negative bacterial strains and Plain control i.e. DMSO (Dimethyl sulphoxide) was also as the solvent used to exclude out any activity to dissolve the plant extract.

CONCLUSION

Alcoholic extract showed good antibacterial activity against the gram positive bacterial strains and these results higher against the Streptococcus pyogenes while the aqueous extract showed moderate antibacterial activity against the gram positive bacterial strains and it has better result against the staphylococcus aureus.

Alcoholic and aqueous extract of Ushba, both show moderate results against the gram negative bacterial strains of Klebsiella pneumoniae and Pseudomonas aeruginosa while aqueous extract have mild activity against E.coli.

The study confirms that the drug Ushba has mild and moderate antibacterial activity and inhibit the growth of such micro-organisms. On the basis of result the drug can be used against various micro-organisms as revealed by this study.

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