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THE ANTIBACTERIAL ACTIVITY OF SOME SELECTED MEDICINAL PLANTS AGAINST UPPER RESPIRATORY TRACT INFECTIONS

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Abstract:

Upper respiratory tract infections (URTIs) pose significant health challenges globally, often caused by bacterial pathogens. This study investigated the antibacterial activity of three medicinal plants—*Ocimum sanctum*, *Adhatoda vasica*, and *Solanum trilobalum*—traditionally used to treat URTIs in village communities. The plant materials were processed into aqueous extracts using a Soxhlet apparatus. Antibacterial activity was assessed against clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, common URTI pathogens, putting to use the agar well diffusion method. The least inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for each extract. The combined effect of the extracts was evaluated using the checkerboard method. Furthermore, the efficacy of the extracts against patient sputum samples was assessed. The results demonstrated significant antibacterial activity for all plant extracts against the tested pathogens. Importantly, the combined action of the extracts exhibited a synergistic effect, enhancing their antibacterial efficacy compared to individual extracts. These findings bring to notice that these medicinal plants can be promising candidates for the making of alternative or adjunct therapies for URTIs. However, further research is warranted to explain their mechanisms of action and to optimize their therapeutic potential.

Keywords: antibacterial, medicinal plants, sputum, diffusion method, check board method, Mechanisms...

INTRODUCTION

Nature always resembles the golden treasure in which the wealth of the world is hidden. And since ages, man finds all possible remedies for all his ailments from this treasure. Hence plants are necessary to man in life. The information on drugs has been piling up for thousands of years. Herbal medicine in the past is as aged as mankind. Most of the paperwork from classical times discloses that plants were medicinally used in China, India, Egypt, and Greece even before the start of the Christian era. India is home to 45,000 plant varieties and 550 tribal communities associated with 160 linguistic groups and inhabited by various geographic and climatic zones with different plant breeds and diverse cultures, a rich conservative knowledge system, wisdom, and an ethnobotanical store.

Various medicinal practices in India include, Ayurveda, Siddha, Unani, and Amchi, and regional health practices, use a huge amount of plants for the cure of human disease, throughout the history of mankind and even today. Many of these health-giving vegetation have been recognized and their uses are well preserved in the documentation and explained by various authors (Nadkarni, 1976; Dastur, 1985; Saradamma, 1990; Jain, 1991; Kirtekar and Baru, 1991; Ambasala, 1992;). Plants as always are still the most absolute origin of drugs for the maximum number of people living in the world (Hamburger and Hostellmann, 1991). But efficacy of many of this vegetation by scientific investigation, a versatile program has begun with the intention of discovering plants for their antimicrobial activity. The plants from written works are picked based on frequent utilization in the cure of diseases that are infectious like fever, bronchitis, ulcer, diarrhea, dysentery, and skin disease.

Among several infectious diseases, upper respiratory tract infections are found to be more common and quite significant in the world's population. Hence the present-day analysis is done to verify the antimicrobial activity of four plants (*Oimum sanctum*, *Adhatoda varica*, and *Solamum trilobalum*) traditionally used by the villages as remedy for upper respiratory tract infections and their combined action against the upper respiratory tract bacteria and also against sputum of a patient directly.

The main purpose and objectives of the present study are due to 3 main factors. Firstly the present-day medical amenities are still not appropriately distributed and hence are inaccessible to most of the people in the rural regions despite their rapid progression.

Secondly, the plant beneficial systems are keenly woven together with the beliefs and lifestyle of people's rural living. Thirdly, even where modern medical facilities are available to the people they are at times too costly and therefore are replaced with alternations from herbal medicines.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The plants put to use under study *Solanum trilobatum* L., *Adhatoda vasica* Nees., and *Ocimum sanctum* L., were collected in different parts of Palayamkottai in Tirunelveli district in Tamilnadu. The plant specimens were identified taxonomically at St.Xaviers's College Herbarium. From the collected plant materials the leaves were separated and subsequently shade-dried at ambient temperature of 29-30°C. The dried materials were powdered with the help of a mixer grinder.

PREPARATION OF THE EXTRACT

The shade-dehydrated and finely powdered plant materials (10 gm / 150 ml) were aqueous extracted using Soxhlet apparatus at 90 – 100°C for 5-7 hours until the solvent came back colorless. An aqueous suspension of the leaves represents the formation usually taken in conventional medicine (Satyavathi, 1987). The drawn-out was stored at 4°C until used just before use the extract was lyophilized to remove the traces of water by a hot sand bath and then used for the test.

In addition, aqueous extracts of each plant sample were prepared by boiling the ground tissue in water for 2-3 hours and then filtering to separate the residue filtrate. The filtrate was subsequently lyophilized and all removed or taken out were stored at 5°C till put to use in antibacterial activity tests.

TEST ORGANISMS

The test micro-organisms for determination of antibacterial activity were *Staphylococcus*, *Klebsiella pneumonia*, *Pseudomonas* and *Salmonella typhi*. The pure bacterial cultures were obtained from the Microbiology Department of Paramakalyani College, Alwarkurichi, and were maintained in the nutrient agar medium. Samples from each

test bacterial culture were sub-cultured in individual tube slants of nutrient agar medium and incubated for 48 hours at room temperature.

In addition to the above bacterial culture the sputum from the upper respiratory tract of the patient was collected aseptically for the test.

MEDIUM PREPARATION

The antibacterial tests were conducted using 10cm diameter Petri dishes containing suitable sterile nutrient media. The nutrient agar medium was prepared by taking the following materials (Gunasekaran, 1995).

Peptone	- 2.5 gm
Beef-extract	- 1.5 gm
Agar agar	- 10 gm
Glucose	- 2.5 gm
Distilled water	- 500 ml

pH was adjusted to 7.2.

The above chemicals were taken in a conical flask and 500 ml of water that was distilled was added. The contents were heated till they were completely dissolved and then plugged with cotton and autoclaved.

EXPERIMENTAL

ANTIBACTERIAL ASSAY

The antibacterial functions of the plant extracts were evaluated using an agar-diffusion way. A suspension of the bacterium was prepared in a sterilized saline solution to get a turbid bacterial culture. The prepared suspension of each bacterium was flood inoculated onto the surface of nutrient agar medium contained in plates (10 cm in diameter) and dried for 2-3 minutes. After which, solubilized drawn outs were permitted to soak up into sterile 6 – 9 mm diameter Whatman No. 1 filter paper discs for 2 – 3 minutes, then air dried at 35°C and put onto the new inoculated nutrient agar plates having bacterial, midway between the plate center and edge. The same procedure was repeated with the rest of the discs. The disc impregnated with aqueous solvent, dried and kept on the agar surface, worked as a negative control and the disc impregnated with antibiotics – streptomycin and chloramphenicol were put on the same agar surface, resulted as a positive control. All discs

were placed equidistantly from each other. The plates containing discs were placed in an incubator for 24-28 hours at 35°C - 37°C and the inhibition of bacterial increase was determined by evaluating the size of the transparent area around every disc.

In the case of sputum inoculation, the streak plate method was used and the rest of the procedure was the same as that of the antibacterial assay mentioned above.

BACTERIAL STAINING

Method of Identification of Bacteria into Gram-Positive and Gram Negative

The identification of bacteria as gram-positive and gram-negative was done by the following method (Gunasekaran, 1995).

- A loop full of bacterial inoculums was taken and smeared on a glass slide.
- The smear was heat-fixed and subjected to four different reagents.
- The smear was stained with primary stain, crystal violet, for 20 seconds.
- After this period it was washed with distilled water and then subjected to a mordant stain iodine solution for 1 minute.
- The bacterial stain was then washed with decolourizing agent alcohol which was followed by the staining with counter stain safranin.

The color changes that occur in bacterial cells at each stage were observed. The bacteria that hold on to the primary stain and appear dark blue are called gram-positive, although those that lost the crystal violet and counter-stained by safranin appear red, are called gram-negative bacteria.

RESULTS AND DISCUSSION

RESULTS

The result achieved from the antibacterial screening of extracts from various plant species at different concentrations (60 mg/ml, 120 mg/ml) reported in Tables – 1 and 2.

The aqueous extracts of all the tested plant species showed greater antibacterial activity against gram-negative bacteria *Klebsiella pneumonia* (Fig. 1), *Pseudomonas aeruginosa* (Fig. 3), *Salmonella typhi* (Fig. 4), and the gram-positive bacteria *Staphylococcus aureus* (Fig. 2).

ANTIBACTERIAL ACTIVITY OF ADHATODA VASICA NEES.

The leaf extracts of *A. vasica* at a concentration of 60 mg/ml showed different degrees of antibacterial activity. The maximum activity of inhibition was shown against *Klebsiella pneumonia* (12 mm) and the other bacterial species showed decreased activity in the descending order viz., *Pseudomonas* (7.5 mm), *Staphylococcus aureus* (7 mm), *Salmonella typhi* (5 mm).

At high concentrations (120 mg/ml) the plant extract showed greater inhibition against *Staphylococcus* (11 mm) and reduced inhibition against *Klebsiella pneumonia* (8 mm).

ANTIBACTERIAL ACTIVITY OF *SOLANUM TRILOBATUM* L.

The different degrees of antibacterial activity at a concentration of 60 mg/ml shown by the *S. trilobatum* L. were in descending order, *Pseudomonas* (24 mm). The plant extracts at low concentration (60 mg/ml) showed their maximum inhibition between 10 – 12 mm which is more or less equal to antibiotics activity.

DISCUSSION

Although all the plants used in this study are known and used as remedies for upper respiratory tract infections, the testing for antibacterial activity of these plants individually and collectively on various bacteria and sputum samples is able to provide a clear cut idea on their effects in comparison with the commercially available antibiotics.

The *Adhatoda vasica* Nees., a small evergreen shrub flourishing all over the plains of India. It is a famous drug in Ayurvedic and Unani of medicine. According to Nagaraju and Rao (1990), the decoction of these leaves and roots with pepper is an excellent remedy for cough, bronchitis, and asthma. The study here provides a scientific basis for the utilization and substitution for antibiotics and informs that vaccine and vasicinone extracted from *Adhatoda vasica* are good for asthmatic bronchitis due to their bronchodilatory activity and hence are used widely in preparing cough syrup like glycogen, zecuf, and crux, etc. This plant inhibited the growth of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

The *Solanum trilobatum* L. is a much-branded climbing shrub with sharp recurved, short, compressed spines used in chronic bronchitis and as a treatment for cough. Govindhan et al., (1999) suggest that *S.trilobatum* L. could be used to treat respiratory diseases. The leaf extracts in this study showed their inhibition of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, and this paved the way for its role in upper respiratory tract diseases.

Ocimum sanctum Linn., an upright, herbaceous much branched, softly hairy, annual, 30-75 cm high is seen throughout India ascending to 1,800 m in the Himalayas. Kokate et al., suggest that the juice could be used as anticatarrhal, spasmolytic, and diaphoretic. Parida et al., (1997) informed about its polivirus type replication. Tulasi is very effective in the cure of tropical pulmonary eosinophilia in children (Sharma et al., 1987b). Its antispasmodic and antiasthmatic activity have been studied their antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. The maximum inhibition was found against *Klebsiella pneumonia*.

The individual and collective antibacterial activities of plants are compared with each other for their effects. This in turn compared with the commercially used antibiotics to check the possibilities of substitutions.

The above-mentioned 4 plants showed their antibacterial activity against the sputum sample which has gram-positive bacteria, *Neisseria*, *Staphylococcus*, *Streptococcus*, and gram-negative *Bacilli*. Aneja (1996) reported that the potential pathogens, such as *Staphylococcus aureus*, *S.epidermidis*, *Haemophilus influenza*, *Streptococcus pneumonia*, and *Neisseria meningitis* in the upper respiratory tract of an infected person as well as in healthy persons.

The most frequent microorganisms found in acute bacterial infections of the respiratory tract are *Pneumococcus pneumonia*, *Klebsiella* species, *Haemophilus influenza*, *Staphylococcus aureus*, and *Streptococcus* species. The antibacterial venture of the extract from the plant used here indicated that these are good substitutes for commercially available antibiotics namely chloramphenicol and streptomycin. Michael et al., (1993) reported that streptomycin's antibacterial spectrum includes many gram-negative bacteria including, *Franciella tularensis* and some organisms in the *Salmonella* group. It is inhibitory for several species of *Mycobacteria*. Chloramphenicol is a broad spectrum that acts as opposed to many gram-

positive and gram-negative bacteria. The possibility of serious side effects such as blood dyscrasias has limited the use of general antibiotics.

According to Kumar and Swatikumar (1998), streptococcus causes tonsilitis, scarlet fever, and cellulitis, staphylococcus is responsible for carbuncles, and boils, Salmonella typhi causes typhoid fever and Klebsiella and is responsible for pneumonia. The present study in the plant extracts is a remedy for all the above-mentioned ailments and the combined action of these plant extracts is more effective than the individual extracts.

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