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ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT CLINICAL ISOLATE OF *STREPTOCOCCUS PNEUMONIAE* IN INDIA

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ABSTRACT

Streptococcus pneumoniae is the leading cause of invasive diseases such as pneumoniae, meningitis and sepsis. Furthermore the emergency of multidrug-resistant streptococcus pneumoniae has been focused worldwide. One of the most important bacterial pathogens that affect respiratory system. The occurrence of multidrug resistant strains of *S. pneumoniae* necessitates the discovery of new classes of anti-pneumonia drugs. As some of the medicinal plants and their extracts have antibacterial activity, we aimed to investigate the antibacterial potential of four Indian medicinal plants including D.elata, S.Campanulata, Phoenix dactylifera L., and Pteruspermum Canescens against multidrug-resistant strain of *S. pneumoniae*, which was screened for its antibiotic resistance profile showing 50% resistance of the tested antibiotics. In this research micro-broth dilution method was used to study the inhibitory activity of ethanol, distilled water and Zamzam water extracts of all plants under investigation. The obtained results showed that all Zamzam extracts of the four studied plant species inhibited the growth of *S.pneumoniae*. It was clearly noticed that there was a difference in the antibacterial activity between distilled water and Zamzam water extracts recording higher activity in the Zamzam water extract of some of the studied plant species. *Pteruspermum Canescens* distilled water extract was the most effective one with MIC equal to 0.391 mg/ml. In general, both water extracts were more bioactive than the ethanol extracts for all the examined plants. These results confirmed the possibility of using these plant species in medicine and pharmaceutical industry of new drugs for the treatment of multidrug-resistant clinical isolates of *S. pneumoniae.*

KEYWORDS: *Streptococcus pneumoniae,* Antimicrobial Activity, Medicinal Plant Extracts, India.

INTRODUCTION

In 1881, the organism, known later in 1886 as the pneumococcus.[1] for its role as an [etiologic agent] of pneumonia, was first isolated simultaneously and independently by the U.S. Army physician George Sternberg,[2] and the French chemist Louis Pasteur.[3] The bioactive materials isolation concept from natural wild plants was based on the consumption of many medicinal plants in traditional medicine. Therefore, the herbal medicine market has spread and became prosperous in pharmacies against many types of bacteria,[4] The development of new antimicrobial agents for the treatment of many infections is an urgent demand in recent years due to the cumulative bacterial resistance against common antibiotics.[5] This challenge posed by the resistant strains of microorganisms can be countered by the increase of the chemotherapy efficacy depending on the continuous search for new drugs. Recently, the range of available antibacterial agents against Gram-positive bacteria is significantly lower than the range effective against Gram-negative pathogens.[6] Thus researchers are faced with major obstacles to develop drugs effective against problematic Gram-positive organisms. Hence the antimicrobial assay investigation of indigenous plants may yield the discovery of new potential bioactive plant components, which aid significantly in the therapeutic applications against human pathogens bacteria, fungi or viruses.[7] One of the most common Gram-positive pathogens that cause pulmonary inflammations is *Streptococcus pneumoniae*, which is known as pneumococcal in medical microbiology. It was recognized as a major cause of pneumonia diseases in the late 19th century causing many types of pneumococcal infections in children and the elderly other than pneumonia in many communities acquired respiratory infections,[8-9] Streptococcus pneumonia is a major source of morbidity and mortality worldwide. It is estimated that about 1 million children die of pneumococcal disease every year in WHO report.[10] Pneumococcal infections are the leading cause of death from a caccine-preventable illness in children aged less than 5 years[11] Invasive diseases caused by pneumococcai include meningitis, sepsis and pneumonia,[12] Risk factors for invasive pneumococcal disease (IPD) include age (with incidence being highest in young children aged less than 2 years and the elderly aged over 65 years), ethnicity, geographic location, concomitant chronic illnesses, and attendance in day care centers.[13]

India is premeditated as one of the most countries containing a wide variety of vegetation in the world. The utilization of complementary and alternative medicine in India is very common.[14-15] Several studies have been published concerning many plant extracts biological active properties such as antibacterial, antitumor, antifungal and antioxidant of wild plants in India.[16] Nevertheless, Documentation of scientific information about the efficacy and safety of wild plants in ethno botanical bank. India is one of our research goals based on their accurate taxonomical identification. Systematic screening of folk medicine plants may result in the discovery of novel effective compounds exploring unknown medicinal wild plants. The objective of this study was to determine the Antimicrobial Activity of some medicinal plants against multi-drug resistant clinical isolate of *Streptococcus pneumoniae* in India.

MATERIALS AND METHODS

Bacterial Cultures

Specimens for the recovery of Streptococcus pneumoniae from nasopharyngeal, throat and wound swabs, ear and eye discharges, cerebrospinal fluid, pleural, peritoneal and ascetic fluids, pus, urine, sputum, and blood were collected aseptically with sterile cotton plagued applicator sticks and sterile test tubes as per the routine clinical management of the patients at Indian Private and government Hospitals. All streptococcal isolates were identified by standard conventional biochemical methods or the VITEK2 system (biomerieus, Durham NC, USA).

Collection of Plants

Four Plants Species (D.elata, S.Campanulata, Phoenix dactylifera L., Pteruspermum Canescens) were collected from different area of India. The plants were identified by Botanical Department, Bharathidasan University, and Trichy, India. For the antimicrobial activity, plant materials were washed, air tried, ground into powder and stored at room temperature until use.

Antibiotic Screening assay

The in-vitro antimicrobial activities of the plant extracts were evaluated against a multidrug resistant clinical isolate of S.pneumoniae, which was obtained from biotechnology Laboratory and identified by catalase, a-hemolytic, optochin and Gram stain tests.[17] The susceptibility of this clinical isolate was tested on blood agar plates by using ten antibiotics which are: AMPC. Amoxicillin, CP. Chloraphenicol, CTX. Cefotaxime, EM. Erythromycin,

GM. Gentamycin, OFLX. Ofloxacin, PCG. Pencillin G, Streptomysin, TC Tetracycline, VCM Vancomycin.[18]

Extraction of Aqueous

Five grams of each plant powder were soaked in 100 ml distilled water that previously warmed to 70°C and then incubated at 37°C with interval shaking for one week. The mixtures were centrifuged for 5minutes at 5000 rpm and the supernatants were frozen at -20°C and evaporated by freeze-drying. The extracted powder of each plant species was dissolved in sterile distilled water to obtain 200 mg/ml final concentration and the extracts were stored at -20°C. The same procedure was carried out using Zamzam water as extract solvent instead of distilled water.[19]

Extraction of Ethanol

Five grams of each plant powder were soaked in 100 ml of 70% Ethanol for one week with interval shaking at 37°C. The mixtures were centrifuged for 5 minutes at 5000 rpm and the supernatants were evaporated by rotary evaporator then by freeze-drying. The extracted powder of each plant species was dissolved in 10% dimethyl sulfoxide (DMSO) to obtain 200 mg/ml final concentration.

Preparation of Media

Thirty percent of LSB (lysed sheep blood) media was prepared. Five freeze-thaw cycles were performed using sheep blood (to obtain lysed blood) by complete freezing the blood at -20°C and then full thawing the blood at room temperature. LSB was prepared using sheep lysate diluted in distilled water to obtain final concentration 50% and centrifuged at 20,000 rpm for clarification. Six ml of supernatant was added to 94 ml nutrient broth supplemented with Ca++ and Mg++ ions at 1 µg/ml final concentration of each ion.[20]

Antimicrobial activity against *Streptococcus pneumoniae*

The in-vitro antibacterial activities of the plant extracts were evaluated using clinical isolate of Gram-positive *S. pneumoniae*. Minimum inhibitory concentrations (MIC) were determined using micro-broth dilution method. Extracts were serially diluted two folds in LSB broth medium in microtiter plates. Then duplicates of each dilution (100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391 and 0.195mg/ml) were inoculated with 1µl of 5×10^7 (CFU/ml) of *S. pneumoniae*. The last two wells were used as positive and negative controls. Also, Zamzam water control was used to study its effect on bacterial growth. The inoculated microtiter

plates were incubated at 35°C for 48 h using CO_2 incubator. The lowest extract concentration (highest dilution) that inhibited the growth of tested microorganisms was considered as MIC.

RESULTS AND DISCUSSION

The tested clinical isolate *S. pneumoniae* had showed susceptibility to five antibiotics, which are Ampicillin, Chloramphenicol, Cefotaxine, Gentamicin, and Streptomycin**.** However, it was resistant to the other screened antibiotics in this study, which are Amoxicillin, Erythromycin, Tetracycline, Pencillin G, and Vancomycin (Table 1), which represents 50 % resistance of the examined antibiotics. The antimicrobial activity for the four plant species in this study was recorded by measuring their minimum inhibitory concentrations (MIC) (Table 2). The MIC results of the conducted experiment showed that both aqueous extracts (distilled water and Zamzam water) were more effective than the ethanol extracts for all plants *(*Figure 1). The lowest MIC was for the distilled water extract of *Phonenix dactylifera L.* indicating its highest antibacterial activity among the studied plant species. Of great concern, there was a remarkable difference in the bioactivity of both aqueous extracts of *D.elata and Pteruspermum Canescens* as Zamzam water extract have had lower MIC values than distilled water extracts. However, an opposite phenomenon was recorded in the distilled water extract of *S. campulata*, *Phonenix dactylifera L.* as they have had lower MIC range (1.563-6.25 mg/ml) than Zamzam Water extracts (6.25-25mg/ml). While the other species have antimicrobial effect with MIC values ranging from 12.5-100mg/ml. The Significant finding in this experiment was that all Zamzam extracts of the four plants showed antimicrobial activity against *S.Pneumoniae*.

Previously pneumococcal infections are treated with penicillin as the first choice drug, and erythromycin is also frequently used.[21] Since then, resistance of pneumococcal to a variety of antimicrobial agents has evolved from an ominous medical curiosity to a worldwide health problem. The infection incidence by multidrug-resistant Gram-positive bacteria is increasingly causing the treatment of the diseases caused by them by the currently available antibacterial agents is extremely difficult.[22] Although many researchers reported only antimicrobial susceptible patterns in multidrug-resistant Streptococcus Pneumoniae.

Table 1: Susceptibility of *Streptococcus pneumoniae* clinical strain to 10 conventional antibiotics

SL.NO	ANTIBIOTICS	INDUCIBILITY	
		Resistant	Sensitive
1.	Amoxicillin	R	-
2.	Ampicillin	-	S
3.	Chloramphenicol	-	S
4.	Cefotaxine	-	S
5.	Erythromycin	R	-
6.	Gentamicin	-	S
7.	Penicillin G	R	-
8.	Streptomycin	-	S
9.	Tetracycline	R	-
10.	Vancomycin	R	-

Streptococcus pneumoniae tested on LSB medium, R: Resistant; S: Sensitive

Table 2: Antimicrobial activity of the tested plant extracts against *S. pneumoniae*.

Extract Method	Plant Species			
	D.elata	***Phonenix Dactylifera***	***S.Campanulata***	***Pteruspermum Canescens***
Zamzam Water	3.125	25	6.25	0.391
Distilled Water	12.5	6.25	1.563	25
Ethanol	12.5	25	50	100

MIC: Minimum Inhibitory Concentration in mg/ml. (-): no inhibition of growth

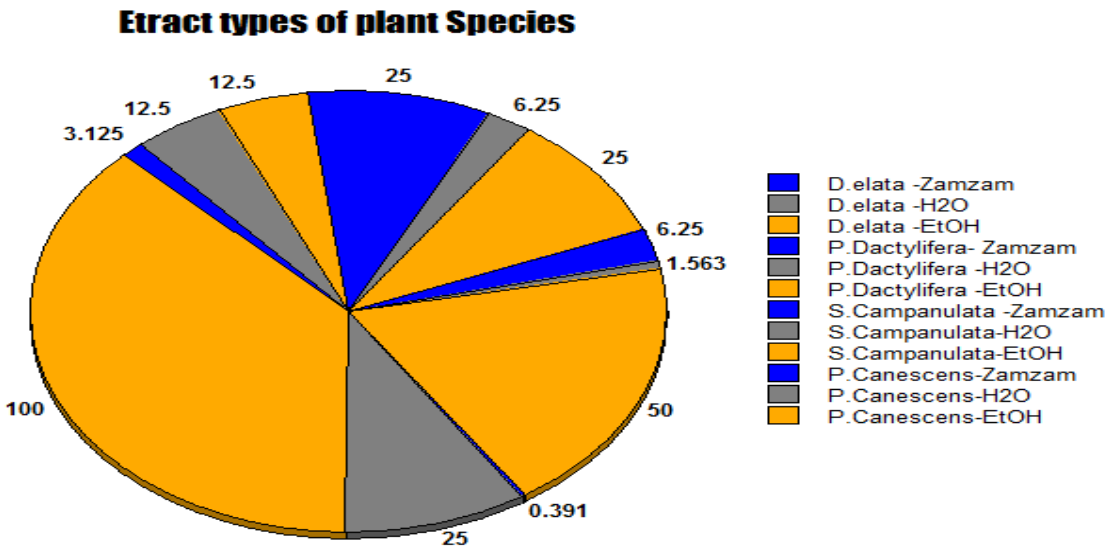

Figure 1. Minimum Inhibitory Concentration (mg/ml) of selected plant extracts against *Streptococcus Pneumoniae.*

Similar case was recorded in *S. pneumoniae* antibacterial resistance, which became widespread in many parts of the world during 1980s. However, its resistance has increased to a point that it is clinically relevant in the following classes of antibiotics: Beta-lactams (penicillins, cephalosporins and carbapenerns), Macrolides (erythromycin, azithromycin, clarithromycin and clindamycin), Tetracyclines and folate inhibitors (trimethoprim-sulfamethoxazole) and Fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin).[23] Owing to the continued clinical pressure for novel approaches to combat antibiotic-resistant bacterial infections, there has been a resurgence of interest in the search for new antibacterial agents that are able to overcome multidrug-resistant mechanisms emanating the approach of screening natural products from plants. Plants have already successfully yielded compounds with activities suggesting that they inhibit efflux pumps of Gram-positive bacteria, which may be referred to the presence of alkaloids, flavonoids, tannins, polyphenols and many other bioactive chemicals.[24] Therefore, in this study we sought to identify medicinal plants that could provide compounds for further antimicrobial drug development.

This study was conducted on a clinical multidrug-resistant isolate of *S. pneumoniae*. The studied strain of *S. pneumoniae* performed an antibiotics resistance, which coincides with or differs from the recorded antimicrobial agent's resistance profile of *S. pneumoniae* in the literature.[22-29] Out of the ten-screened antibiotics, only three have had effect against the studied *S. pneumoniae* representing 70% resistance to the tested antibiotics, most of which are commonly used to treat *S. pneumoniae* infections. Hence, this spring the examination of four plants extracts in India. In addition to that, due to unique properties of the Zamzam water, which have been discussed previously, it spouts the idea of the examination of the Zamzam water extract antibacterial efficacy of the studied plant species. The recorded results confirmed the difference in using Zamzam water from distilled water as extract solvent, which has been illustrated via the higher antibacterial effect of Zamzam water extract of *pteruspermum canescens* than its water extract.

Moreover, Zamzam water extract of *D.elata has* showed antibacterial effect in contrast to the water extract, which has had no antibacterial effect at all. Therefore, we can recommend that using Zamzam water as extract solvent may reveal different antibacterial agents with higher activity potency taking into account the observed growth of *S. pneumoniae* in Zamzam water alone. All studied plant species extract types showed antibacterial effect against *S.pneumoniae* except for *D.elata* water extract and *pteruspermum canescens* ethanol extract.

Nonetheless, distilled water extract of *S.Campulata* and Zamzam water extract of *D.elata* have had very low MIC values, 1.563 and 3.125 mg/ml, respectively.

The results of antimicrobial activity of petroleum ether, chloroform and methanolic leaf and stem extracts of pterospermum canescens Roxb, against the streptococcus pneumoniae. It was found that petroleum ether leaf and stem extracts exhibited significant activity against the various pathogens like (25, 50,100mg/ml).[30] However, this is the first study to provide data that *pteruspermum canescens* possess potential antibacterial activity against *S. pneumoniae*. Methanol and aqueous extracts of two different plant species were examined for their antimicrobial activity against the isolated human pathogens. Both crude methanol and aqueous extracts of *D. elata* exhibited varying degrees of antimicrobial activities against the test organisms. The 200 µg/ml crude methanol extract showed higher inhibition zone than crude aqueous extract against S. aureus, S. pneumoniae, B. cereus and S. flexneri. Similarly, 200 µg/ml methanol extract of S. campanulata exhibited inhibition zone of 18 mm (AI = 0.666) for S. pneumoniae and 14 mm (AI = 0.615) for S. aureus.

However, in this research both aqueous extracts of *Pteruspermum Cancscens* showed inhibitory effect against *S. pneumoniae*, which is a Gram-positive bacterium. This out finding add a scientific base for the traditional use of *Pteruspermum Cancscens* in the treatment of pulmonary diseases caused by *S. pneumoniae* in India as it has been consumed locally for other diseases treatment. *Phonenix dactylifera* was not previously detected for its antibacterial effect in spite of its herbal value in the treatment of many diseases. Therefore, it was studied in this research. Although we cannot simply compare our results and previous reports because of different types of antibiotics and different definitions of multi drug – resistance, further investigation will be necessary to elucidate the antimicrobial activity of some medicinal plants against multi-drug resistant clinical isolate of *Streptococcus pneumoniae* in India.

CONCLUSION

Resistance to commonly used antibiotics and multidrug resistance of S. pneumoniae in the investigated area are remarkably high. Plants have not been completely investigated, nevertheless, data from previous literatures as well as our results revealed the great potential of plants for therapeutic treatment. Therefore, more studies need to be conducted to search new compounds. Moreover, once they are extracted and before being used in new therapeutic treatments, they should have their toxicity tested in vivo. However, these plant extracts were

active against the multidrug-resistant *S. pneumoniae* under very low concentration Values, thus minimizing the possible toxic effects. Adding to that, some of these plant extracts were more effective than the traditional antibiotics to combat the pathogenic tested microorganism. The overall evaluation of the present study results in which all extracts types of the same plant species showed varying degrees of antibacterial activity against *S. pneumoniae* in combination to the variations among the studied plant species. Therefore, the results suggest the presence of either good antibacterial potency or high concentration of an active principle in the extracts. In conclusion, this study supports to a certain degree the traditional medicinal uses of the plants in diseases therapy and reinforces the concept that ethno botanical approach to screen plants as potential sources of bioactive substances is successful. However, an extensive study would be needed to extrapolate laboratory results into hospital settings for the benefit of mankind.

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