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EFFECT OF SOME MEDICINAL HERBAL EXTRACTS ON CLINICALLY IMPORTANT BACTERIAL PATHOGENS

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ABSTRACT

Medicinal plant products when compared with synthetic antibiotics minimize the infections at a major part. Phytochemical screening of medicinal plants showed positive results for most of the phytochemical constituents namely tannins, saponins, flavonoids, carbohydrates, alkaloids, anthraquinones, protein and aminoacids, mixed oils and fats etc., This study provided a good medicinal plant based treatment strategy and will create social awareness among the clinically infected patients. Five important bacterial spp., identified from them namely *P. aeruginosa, K. pneumoniae, E. coli, S. aureus* and *B. subtilis.* Antibacterial activity of the plant extracts showed different inhibition spectrum against the isolated bacterial pathogens. Among them leaf methanolic extract of *Azadirachta indica* showed good antibacterial activity.

INTRODUCTION: In developing countries, microorganisms are frequently a cause of prevailing diseases, presenting a serious public health issue in a significant segment of the population as uncovered by either private or official health care systems. Infectious diseases or transmissible diseases comprise clinically evident illnesses which are of characteristic medical signs and or symptoms of disease resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism.

Infectious diseases that are especially infective are sometimes called contagious and can be easily transmitted by contact with an ill person or their reactions. Infectious disease results from the interplay between those few pathogens and the defences of the host they infect [1]. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments [2]. Indigenous herbal remedies are widely used against many infectious diseases, but only few of them have been scientifically investigated with their active components isolated and characterized [3]. In modern medicinal practice, the alarming world wide evidences of antibiotic resistance causes and increasing need for new compounds that can act either by inhibiting resistance mechanism of microorganisms of medical importance [4]. Bacterial resistance is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics [5].

The most common side effects from antibiotics are diarrhoea, nausea, vomiting. Fungal infections of the mouth, digestive tract and vagina can also occur with antibiotics because they destroy the protective good bacteria in the body as well as the bad ones responsible for the infection being treated. Medicinal plant products when compared with their synthetic counterparts minimize the adverse side effects. The global interest in therapeutic potential of phytochemicals during the last few decades is therefore quite obvious. Plants have a great potential for producing new drugs for human benefit. There is a continuous development of resistant strains which pose the need for the search and development of new drug to cure diseases [6]. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds [7]. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development [8]. Secondary metabolites are frequently accumulated by plants in smaller quantities than primary metabolites.

The present study states that the use of locally and commercially available medicinal plants such as *Acalypha indica, Adhatoda vasica, Azadirachta indica, Cynodon dactylon* and *Withania somnifera* to analyse their effect on pathogenic bacterial organisms. These plants are considerably useful and economically essential. They contain active components that are used in the treatment of many human diseases[9]. There is a high level of positive correlation between the traditional medicinal use off the plants and the current therapeutic use of the chemical extracted from the plant. Numerous studies have been conducted with the extracts of various plants, screening of antimicrobial activity as well as for the discovery of new antimicrobial compounds [10].

MATERIALS AND METHODS: Fresh herbal plants namely *Acalypha indica, Adhatoda vasica, Azadirachta indica*, *Cynodon dactylon* and *Withania somnifera* were collected from the Herbal Garden of PRIST University, Thanjavur, Tamil Nadu, India, were identified by a botanist. The plant materials were cleaned, shade dried and powdered. Fresh plant material were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles.

Extraction: The medicinal herbal plant leaves and root extracts were obtained by Soxhlet's apparatus. For this extraction ethanol solvent was used. After extraction, the solvent was removed by condensation of the extracts.

Collection of Biosamples: 25 biosamples were collected from clinically suspected patients of various Hospitals, Medical Colleges and Social Welfare Centres of Thanjavur. All the patients were thoroughly evaluated by detailed history, clinical examination and biochemical parameters. All the strains were confirmed by cultural characteristics and maintained in slants for further use.

Phytochemical screening: The herbal extracts were subjected to various phytochemical tests separately. Different qualitative chemical tests were performed for determining the phytoconstituents present in the plant extracts. Phytochemical analyses were done according to the procedure of phytochemical methods by Harborne, 1984 [11] and Sofowara, 1993 [12]. Ethanolic extract of plants were used for qualitative phytochemical analysis.

Antibacterial susceptibility test: Agar well diffusion assay is used widely to determine the antibacterial activity of crude extract containing unknown components. Nutrient agar was prepared and poured in petridishes. 24 hours growing culture such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* were swabbed on it. The wells about 8mm in diameter were made by using cork borer. The different concentrations (10µl, 25µl and 50µl) of the crude extract were loaded in the wells. The plates were then incubated at 37^{o} for 24 hours. The diameter of inhibition zones was measured.

RESULTS AND DISCUSSION: Eight types of bacterial pathogens were identified. The isolated bacterial pathogens with percentages showed in **Table 1.** The predominant isolates were *E. coli, P. aeruginosa, S. aureus, K. pneumoniae* and *B. subtilis* shown in **Table 1**. The mean value observed was 51.2.

TABLE 1: ISOLATED BACTERIAL PATHOGENS

Bacterial pathogens	Isolated colonies (cfu)	Percentage (%)
Bacillus subtilis	32	13
Escherichia coli	75	29
Klebsiella pneumoniae	43	17
Pseudomonas aeruginosa	57	22
Staphylococcus aureus	49	13
Mean	51.2	

The results of the phytochemical analysis shown in **Table 2** represented that the medicinal plants showed positive results for saponins, flavonoids, glycosides, phenolic compounds, fixed oils and fats, alkaloids and protein and amino acids.

Figure 1 represented the antibacterial susceptibility test results for *Bacillus subtilis*. For *Acalypha indica,* the highest zone of inhibition 11mm was noted in the concentration of 50µl. For *Adhatoda vasica*, 12mm was noted in the concentration of 50µl. For *Azadirachta indica*, 18 mm was noted in the concentration of 25 µl. For *C. dactylon*, 9 mm was noted in the concentration of 50µl. For *Withania somnifera*, the highest zone of inhibition 5 mm was noted in the concentration of 50µl.

TABLE 2: QUALITATIVE PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS

Phytochemicals	*Acalypha indica*	*Adhatoda vasica*	*Azadirachta indica*	*Cynodon dactylon*	*Withania somnifera*
Tannins	-	+	+	+	+
Saponin	+	+	-	-	+
Flavonoids	+	+	+	+	+
Terpenoids	-	-	-	-	-
Glycosides	+	-	+	+	-
Anthraquinones	-	+	+	+	+
Phenolic compounds					
i) Gelatin	+	+	+	+	+
ii) Ferric Chloride Test	-	+	-	-	-
iii) Lead acetate test	-	-	-	-	+
Fixed oils and fats	+	+	+	+	+
Alkaloids					
i) Mayer's test	-	-	-	-	+
ii) Wagner's test	+	+	+	+	+
iii) Hagner's test	-	-	-	-	-
Carbohydrates					
i) Molisch's test	+	+	+	+	+
ii)Fehling test	-	-	-	-	+
iii)Benedict's test	-		-	-	+
Protein and Amino acids					
i) Millons test	-	-	-	-	+
ii) Biuret test	+	+	+	+	+
Phytosterols	-	+	+	+	+

(+) = indicates presence ; (-) = indicates absence

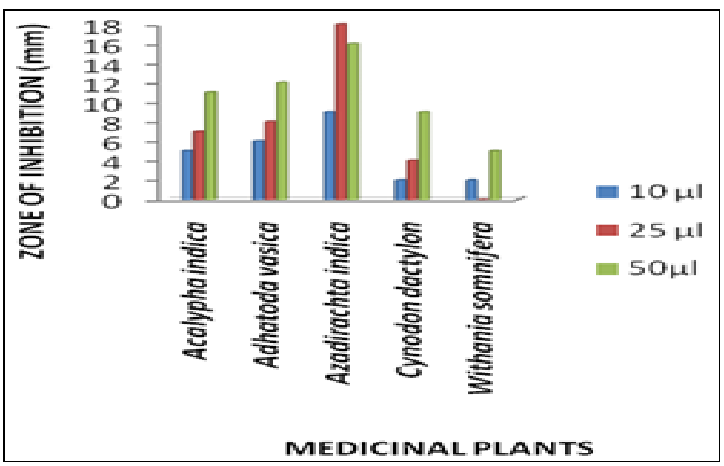

FIG. 1: ***BACILLUS SUBTILIS***

Figure 2 represented the antibacterial susceptibility test results for *Escherichia coli.* For *Acalypha indica,* the highest zone of inhibition 15mm was noted in the concentration of 50µl. For *Adhatoda vasica* 18mm was noted in the concentration of 25µl. For *Azadirachta indica* 19 mm was noted in the concentration of 25µl followed by 16 mm in the concentration of 50µl. For *C. dactylon* 9 mm was noted in 50µl. For *Withania somnifera*, the isolated *E. coli* produced no inhibition zones in 10 and 25µl concentrations, 2mm only in 50µl concentrations.

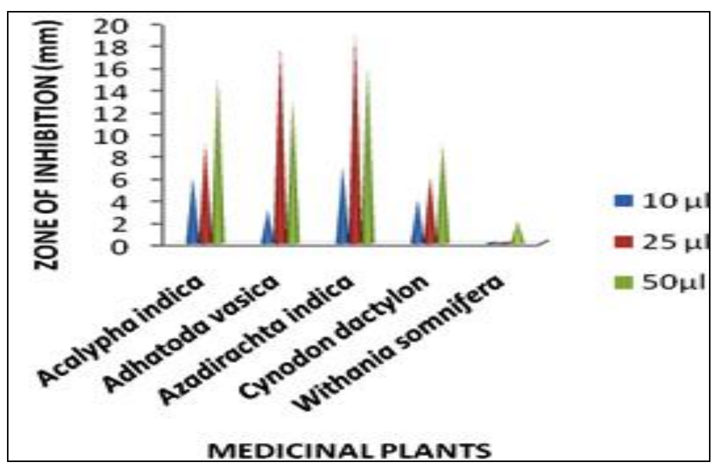

FIG. 2: ***ESCHERICHIA COLI***

Figure 3 represented the antibacterial susceptibility test results for *Klebsiella pneumoniae* For *Acalypha indica,* the highest zone of inhibition 18mm was noted in the concentration of 50µl. For *Adhatoda vasica*, 19mm was noted in the concentration of 50µl. For *Azadirachta indica*, 23 mm was noted in 50µl. For *C. dactylon*, the highest zone of inhibition 5 mm was noted in the concentration of 10µl. For *Withania somnifera*, 4 mm was noted in the concentration of 25µl.

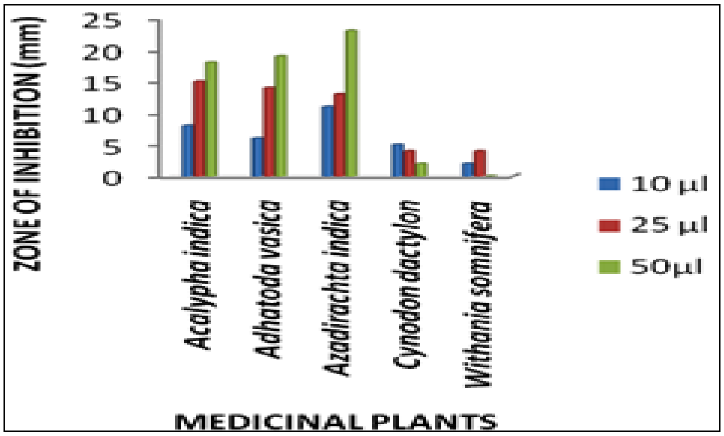

FIG. 3: *KLEBSIELLA PNEUMONIAE*

Figure 4 represented the antibacterial susceptibility test results for *Pseudomonas aeruginosa.* For *Acalypha indica,* the highest zone of inhibition 9 mm was noted in the concentration of 50µl. For *Adhatoda vasica*, 11 mm was noted in the concentration of 50µl. For *Azadirachta indica*, 16 mm was noted in the concentration of 25µl. For *C. dactylon*, the highest zone of inhibition (8 mm) was noted in the concentration of 25µl. For *Withania somnifera*, no zones of inhibition were noted for 25 and 50µl concentrations and least zone about 2 mm was noted for 10µl.

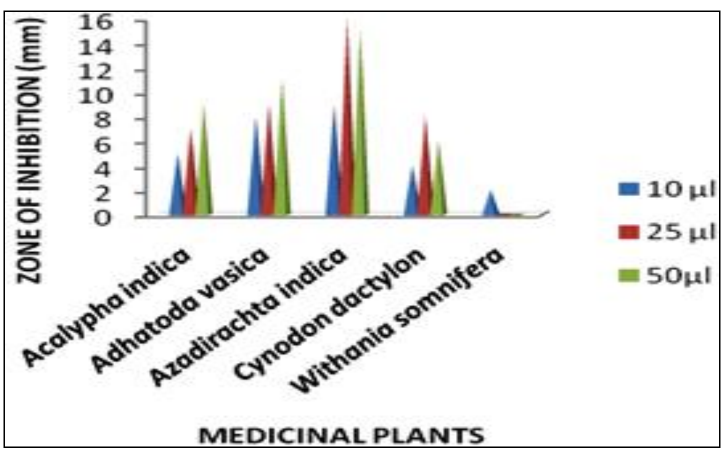

FIG. 4: *PSEUDOMONAS AERUGINOSA*

Antibacterial activity of ethanol extract of 5 medicinal plants against the isolated *S. aureus* represented in **Figure 5**. For *Acalypha indica,* the highest zone of inhibition 18 mm was noted in the concentration of 50µl. For *Adhatoda vasica*, 18 mm was noted in the concentration of 25µl. For *Azadirachta indica*, 19 mm was noted in the concentration of 25µl. For *C. dactylon*, 9 mm was noted 50µl. For *Withania somnifera*, the moderate zone of inhibition 5 mm was noted in the concentration of 2µl.

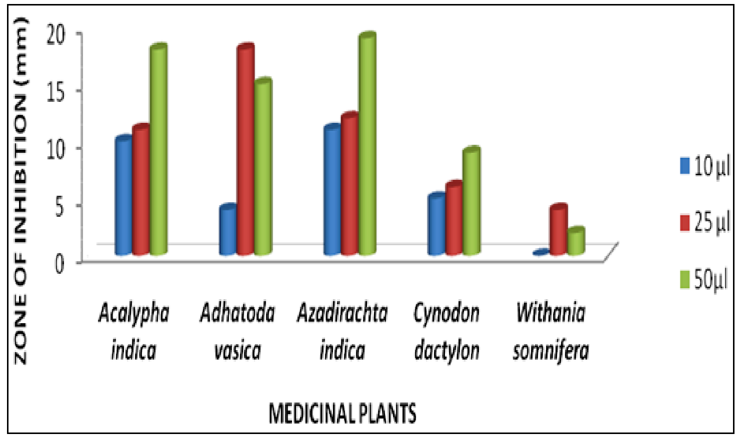

FIG. 5: *STAPHYLOCOCCUS AUREUS*

Many antibiotics are used nowadays to control the diseases. The increased awareness of the environmental problems associated with these antibiotics has led the search for nonconventional chemicals of biological origin, for the management of disease. Bactericides of plant origin can be one approach to disease management because of the eco-friendly nature [13]. Screening is important not only for therapeutic efficacy of the medicinal plants, but also for the validation of their historical utilization by traditional healers and herbalists.

The present study is an attempt to evaluate plans as source of potential chemotherapeutic agents and antimicrobial activity [14]. The search of novel bioactive compounds including antimicrobial ones continues. This is largely so because some pathogens have developed resistance to certain currently used drugs and some disease have yet to be treated chemotherapeutically [15]. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development. Medicinal plants are necessity for the scientific point of view, to establish a rational relationship between chemical biological and therapeutical activities of folklore medicine [16].

Our study stated that the antimicrobial activities of *A. calamus* due to the presence of the following phytochemicals namely, Tannins, Saponins, flavonoids, anthraquinones, fixed oils and fats, Alkaloids, carbohydrates, protein and amino acids and phytosterols. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism [17]. *A. vasica* showed that the presence of tannins, saponins, flavonoids, phenolic compounds, fixed oils and fats, alkaloids and carbohydrates, protein and amino acids in the leaves of *A. vasica*.

It has also been shown that tannins are biologically active, against *E. coli*, *S. aureus*, *S. paratyphi* and *C. albicans* [18]. These classes of compounds in the bark extracts are known to show curative effects against several pathogens. The principal active medicinals are asclepin and mudarin. The antimicrobial activities of methanolic extracts of *C. procera* showed highest antimicrobial activity against various phytopathogens [19].

C. dactylon leaf extract showed positive results for tannins, saponins, flavonoids, terpenoids, anthraquinones, phenolic compounds, fixed oils and fats, alkaloids, carbohydrate, proteins and amino acids, phytosterols. *W. somnifera* root extract showed the presence of tannins, saponins, flavonoids, phenolic compounds, phytosterols, alkaloids, Carbohydrate, proteins and amino acids and phytosterols. According to Bhattacharya *et al*., two new Glycowithanolides, Sitoindoside IX and Sitoindoside X isolated from *W. somnifera* were evaluated for their immunomodulatory and CNS effects [20].

The main constituents of Ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somnifera, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudotropine, cuscohygrine, anferine and anhydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. Studies also reveal ashwagandha to be a potential antimicrobial agent, with antifungal activity and moderate antibacterial activity against *S. aureus* and *P. aeruginosa* [21].

CONCLUSION: To conclude that, this study would definitely create awareness among the infectious patients for taking control or preventive measures based on herbal plants against the common infectious bacterial pathogens. However, due to changing pattern of infections depending upon the degree of immunosuppression, constant monitoring of infections in HIV positive population is important for better management and to improve the quality of life of such patients. Alternative to antibiotics, the patients should be recommended to take the herbal based medicines surely minimize the bacterial related infections and increase the life span of the patients. Thus, this study is a scientific validation for the therapeutic use of these plants and it suggests that the synergistic studies can be further extended to other plant sources to enhance their curative properties.

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