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ORIGINAL RESEARCH ARTICLE

Screening for Antibacterial Activity of *Turnera subulata* Extracts against Human Pathogens

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ABSTRACT

The herbal plants are considered as hidden treasures and explored for novel drugs for more than a decade. *Turnera subulata* was one of the least explored medicinal plants which are assumed to have antimicrobial potential because they are used in Tamil medicinal (Siddha) preparations. Leaves extracts were obtained by hexane, petroleum ether, chloroform and acetone. Discs were prepared by impregnating 5%, 10% and 15% concentration of extract. The positive controls were Chloromphenical, Kanamycin and Erythromycin. The extracts were tested against *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *Proteus vulgaris* showed sensitive to acetone extract, *Staphylococcus aureus* and *Klebsiella pneumoniae* to chloroform extract. Other two species showed resistant to all the extract tested.

Key words: *Turnera subulata*, leave extract, human pathogens and positive control.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [1]. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry [2].

Medicinal plants are a source of great economic value all over the world. Nature has been showed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the main stay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [3]. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines.

Communicable diseases are one of the major threats to human kind which belongs to nearly 50% death of the tropical countries. Sudden outbreaks of new diseases and resistance developed by microorganisms to drug add to the severity. The main reason for the increase in human diseases particularly caused by bacteria and fungi in the last decade is due to increase in respiratory tract infections and an increase in antibiotic resistance nosocomial and community

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acquired infections. Though several commercial antifungal and antibacterial drugs have been used it has lead to induced toxicity rather than cure. Hence a scientific search for viable alternatives to chemical drugs (antibiotics) is highly essential to create a sustainable livelihood. One such alternative is using less explored higher plants as medicine. The process of identification of suitable candidates and processing it for its hidden value is called "Bio-prospecting" [4].

Secondary metabolites of medicinal plants serve as the source of novel drugs and have contributed to human health in a large scale [5]. Since past few decades antibiotics of microbial origin and other synthetic chemotherapeutic agents have been effectively used for control of bacterial diseases. However due to indiscriminate use of these drugs various pathogenic bacteria have developed resistance to many antibiotics currently available in the market. Emergence of multidrug resistant bacteria has created clinical problems in the treatment of infectious diseases. Other drawbacks with these drugs are their high cost and undesirable side effects of many of these drugs. Considering these problems efforts have been intensified for searching new bacterial drugs.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The present study is focussed on the pharmacological screening of *Turnera subulata* for its antibacterial activity against bacterial pathogens.

MATERIALS AND METHODS

Collection and Extraction of leaf extract:

The leaves of *Turnera subulata* were collected from sub-urban of Chennai (Tamilnadu), shade dried and powdered. 20g of powder was extracted successively with Hexane (Extract I, 66-62ºC), Petroleum ether (Extract II, 40-60ºC), Chloroform (Extract III, 60-62ºC) and Acetone (Extract IV, 60-62ºc) in Soxhelet apparatus for 48 hours. All the filtrate were pooled and evaporated under vacuum in a rotary evaporator at 190 rpm/min [6].

Disc preparation:

Concentrates of extracts obtained by 4 different extractions were weighed and dissolved in their respective solvents (5%, 10%, and 15%). Sterile discs of 6mm diameter were soaked in these extract for 18 hours and were completely dried from the solvents under laminar airflow. Controls also maintained throughout the study.

Antibacterial assay:

The following clinical isolates were used in this study for the determination of antibacterial activity, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa***.** Colonies were picked up from pure cultures, made an emulsion to give a standard density of inoculum of 10^4 Colony-forming units (CFU) were inoculated on the agar medium. A 0.5 McFarland standard was used for visual comparison of the suspension to a density equivalent to approximately 10^8 CFU/ml.

The plant extract was tested against the pathogens by Disc diffusion method [7]. Sterile discs of 6mm diameter was obtained from Hi Media, impregnated with extracts for 18 hours and were completely dried from the solvents under laminar airflow at room temperature. The bacterial cultures were adjusted to 0.5 McFarland standards and swab was made on Muller Hinton Agar plates. The inoculated plates were kept at room temperature for 30 minutes. The extract impregnated disks were placed on Muller Hinton Agar plates along with positive control, and incubated at 37^{o}C for 18 hours. Disc with Dimethyl sulfoxide (DMSO) act as negative control. The incubated plates were observed for anti-bacterial activity by measuring the zone of inhibition (in mm).

RESULTS AND DISCUSSION

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [8].

In this present research, the antibacterial activity of plant extract was observed using the Disc diffusion method by measuring the diameter of the growth inhibition zone. The results were depicted in the (**Table 1, Fig 1 & 2**). The acetone extract of the plant showed high degree of inhibition against *Proteus vulgaris* and moderate to *Klebsiella*

pneumoniae. No inhibition was noted in the same extract against *Escherichia coli* and *Pseudomonas aeruginosa*. Although the leaf extract with chloroform showed moderate inhibition against *Staphylococcus aureus* and less activity against *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* and *Escherichia coli* was resistant to all the crude extracts of *Turnera subulata*. It was evident from the above results the greater activity resides in acetone and chloroform extract of *Turnera subulata*. It exhibited greater activity against *Proteus vulgaris*, *Staphylococcus aureus* and satisfactory results against other tested bacteria.

Among them organic extracts especially acetone and chloroform leaf extracts showed greater activity than other solvents. This is because most of the bacterial principles which are either polar or non-polar and can be extracted only through the organic solvent medium. On the basis of the results obtained it is suggested that the antibacterial principles present in *Turnera subulata* may either polar or non-polar or this plant have both polar and non-polar compounds. These observations suggest that the organic solvent extraction method is better for the isolation of antibacterial compounds. In addition the effectiveness of plant extract was not due to one main active constituent but to the combined action of other chemical compounds involved in it. Some examples include alkaloids, flavinoids, triterpinoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds. The present study showed the efficacy of antibacterial activity exclusively for bacterial pathogens which really showed the presence of biological principles [9].

Table 1: Antibacterial activity of *Turnera subulata* extracts against test organism

S.No	Test organism	Extract conc./mcg												Std antibiotics		
		Extract I			Extract II			Extract III			Extract IV			Chl	Kan	Ery
		5	10	15	5	10	15	5	10	15	5	10	15	30	30	15
1.	*Proteus vulgaris*	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R
2.	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R
3.	*Staphylococcus aureus*	R	R	R	R	R	R	S	S	S	R	R	R	S	R	R
4.	*Escherichia coli*	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
5.	*Pseudomonas aeruginosa*	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R

R – Resistant; S - Sensitive

Fig 1: Antibiogram of test organism against standard antibiotics

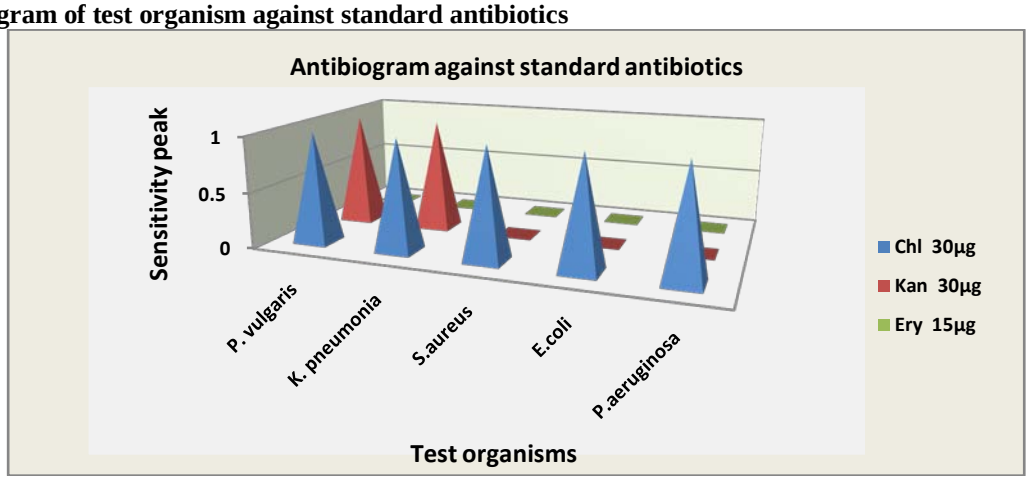
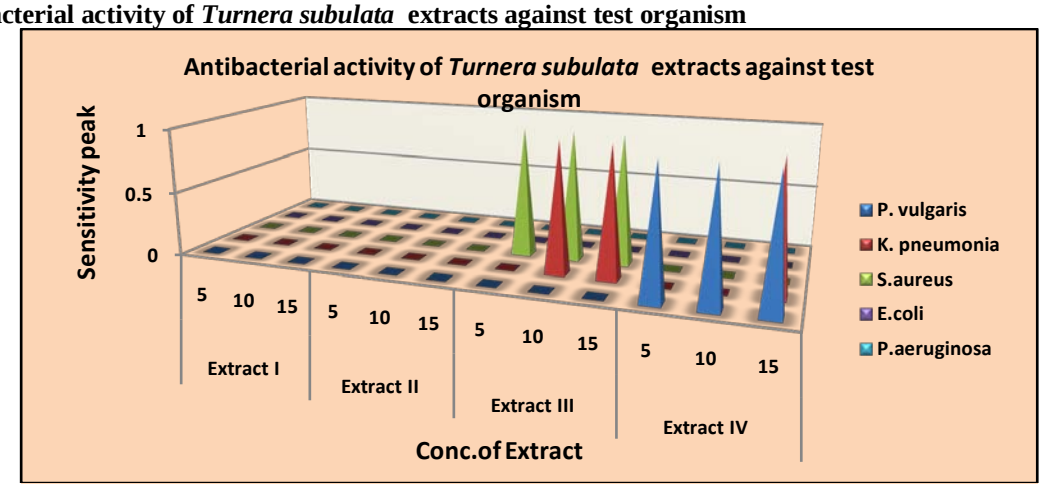


Fig 2: Antibacterial activity of *Turnera subulata* extracts against test organism



CONCLUSION

From this preliminary investigation it was concluded that the plant *Turnera subulata* possess antibacterial activity and further study is required to establish exact principles and minimum inhibitory concentrations of the same. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents. The present study justifies the claimed uses of leaves in the traditional system of medicine to treat various infectious diseases caused by the microbes.

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