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Evaluation of antimicrobial activity of *Rosmarinus officinalis* leaf extract on selected bacterial strains

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ABSTRACT: Background: Herbal medicine is the oldest known healthcare system known to mankind. India has rich medicinal plants of nearly 7500 species. Many medicinal plants were with a long history of use in folk medicine against a variety of diseases. Plants and plant extracts have an important role in modern medicine as their chemical and medical constituents are found in natural form. Plants and plant-based products are bases of many modern pharmaceuticals that are currently in use for various diseases. *Rosmarinus officinalis* is a medicinal plant that belongs to the *Lamiaceae* family and is commonly known as rosemary. **Aim:** The aim of this study was to examine the antimicrobial properties of methanolic extract of *R. officinalis* leaves against some bacterial isolates. **Method:** The antimicrobial activity of *R. officinalis* was evaluated against bacterial strains in methanolic extract by using agar well diffusion method. **Results:** The present study showed the effectiveness of the crude plant extract against the tested bacterial strains and indicates the potential use of the extract as antimicrobial agent for the control of infectious diseases. **Conclusion:** This study has revealed that the leaves extract of *R. officinalis* contains antimicrobial and phytochemical substances which can be harnessed in satiation of human quest for better and healthier living.

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INTRODUCTION:

The continuous evolution of multidrug resistant pathogens is a global clinical concern. In recent years there has been an increasing incidence of multiple drug resistance in human pathogenic microorganisms due to the indiscriminate uses of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has led to the increase in search and research for new antimicrobial substances from various sources like medicinal plants. The search for new antibacterial agents by the screening of many plant families is encouraged. Additionally, using antibiotics is sometimes associated with adverse effects. Therefore,

phytomedicine could be an alternative treatment method for bacterial infections which may decrease such problems [1,2].

Among ancient civilizations, India has been known to be a rich repository of medicinal plants. The forest in India is the principal repository of a large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayus systems in India. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India [3,4].

Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these compounds. This field is maturing and the health effects of these compounds are now getting the careful study they warrant at both a chemical and a molecular biological level. Identifying bioactive compounds and establishing their health effects are active areas of scientific inquiry [5-7].

Recent trends, however, show that the discovery rate of active novel chemical entities is declining. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethnomedicinal plants in India [8,9].

Rosmarinus officinalis (*R. officinalis*) is a medicinal plant that belongs to the *Lamiaceae* family and is commonly known as rosemary (Fig 1 and 2). Besides the culinary uses due to the characteristic aroma, this plant is also widely employed by indigenous populations, where it grows wild. *R. officinalis* is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple or blue flowers. This is a powerful herb that originates from the Mediterranean region [10-13].

Rosemary has been named the herb of the year in 2001 by the international herb association. It was introduced to Britain by the Romans and is still particularly loved today by the Italians and the British, who use it frequently in their cooking. In ancient Greece and Rome rosemary was believed to strengthen the memory, which accounts for it's been known as the herb of remembrance and fidelity. Rosemary was an essential part of the apothecary's repertoire during the Renaissance. Hippocrates, Galen, Dioscorides prescribed rosemary for liver problems. Rosemary is not a popular plant in India. It was introduced by the Europeans as a garden plant due to its present fragrant scented leaves [14,15].

Fig 1. *Rosmarinus officinalis* plant.

Fig 2. *Rosmarinus officinalis* leaves.

The aim of the present project work is to assess the *in-vitro* antimicrobial activity of *R. officinalis* leaves using methanol as a solvent.

MATERIALS AND METHODS:

Methanol of analytical grade was procured from SD Fine Chem., Mumbai. All other chemicals and reagents used in this research were of analytical grade and procured from an authorized dealer.

Collection of plant:

The plant material was collected from surrounding areas in Ghatkesar, Hyderabad, Telangana, India. The fresh leaves were collected and washed with tap water and later with deionized water and dried under shade. The plant material was regularly checked for fungal growth

or rotting. After the plant material was dried it was powdered with the help of an electric blender and sieved through size 80 sieves to obtain a uniform fine particle size. This plant material was stored in airtight containers at 4 ºC for future usage [15]

Preparation of extract:

About 200 g of the sieved powder was accurately weighed and extracted with solvent methanol in a Soxhlet extractor (Fig 3) for 72 h. The extract thus obtained was concentrated under reduced pressure to yield crude plant extract. The extract was stored at 4 ºC in amber coloured glass stoppered vials for further use [16-19].

Fig 3. Extraction of leaves by Soxhlation.

Bacterial strains used:

The following strains were used for the present research that are *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa),* and *Staphylococcus aureus (S. aureus)*.

***In vitro* antimicrobial activity:**

The antimicrobial activity of *R. officinalis* was evaluated against bacterial strains in methanolic extract by using agar well diffusion method. Nutrient agar plates were prepared for all extracts, 50 µL inoculum of each selected bacterium was uniformly spread on agar plates with the help of glass spreader, after 5 min three wells approximately 5 mm diameter were bored with the help of borer. The equal volume (50 µL) of antibiotic (tetracycline), distilled water and plant extract were poured into the wells. The plates were incubated at 37 ºC for 24 h [20-23].

Determination of minimum inhibitory concentration (MIC) of methanol extract:

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation at 37 ºC in the shaker incubator. MIC of all samples was determined by the broth dilution method. A two-fold serial dilution of methanol extract was prepared and optical density was measured at 600 nm [22-25].

RESULTS AND DISCUSSION:

The result obtained from the antimicrobial activity of the methanolic extract of *R. officinalis* showed varying degrees of inhibition. A Zone of Inhibition test (ZOI), also called a Kirby-Bauer Test, is a qualitative method used clinically to measure antibiotic resistance and industrially to test the ability of solids and textiles to inhibit microbial growth. Researchers who develop antibacterial textiles, surfaces, and liquids use this test as a quick and easy way to measure and compare levels of inhibitory activity.

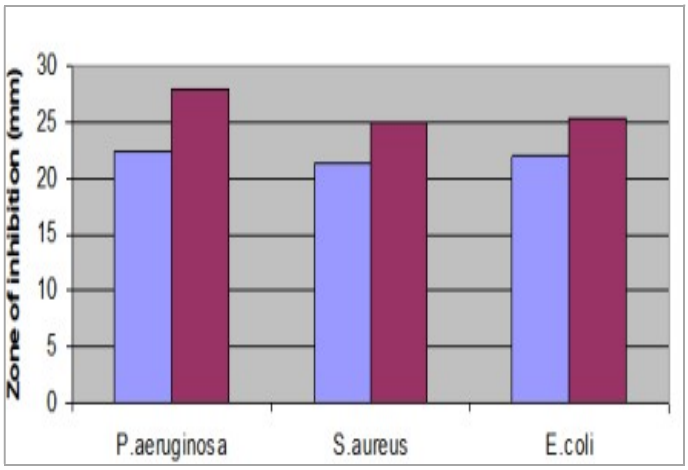

Fig 4. Zone of inhibition observed in *P. aeruginosa, S. aureus,* and *E. coli.*

Zone of inhibition testing is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms. In the world of antimicrobial substances/surfaces, the degree to which these materials are inhibitory can be of vital importance to the health of the consumer. This test is an outstanding qualitative way for manufacturers of antimicrobial

surfaces/substances to be able to compare the inhibition levels of their products. The methanolic leaf extract of *R. officinalis* was selected to screen for its antimicrobial activity against different bacterial strains. Table 1 and Fig 4 and 5 shows the susceptibility pattern of the organisms to the crude extract of *R. officinalis*.

This study supports previous findings in the literature that the antimicrobial activities have a direct relation to increasing the extract concentration (%). As put forward, the difference in MIC of plant extract is due to variation in their chemical constituents and volatile nature of their components. Moreover, it has been reported that large number of different chemical compounds such as (phenolic compounds and its derivative compounds, the esters of weak acid, fatty acid, terpenes, and others) are presented in methanolic extracts of spice, and thus these chemical components can affect multiple target sites against the bacterial cells.

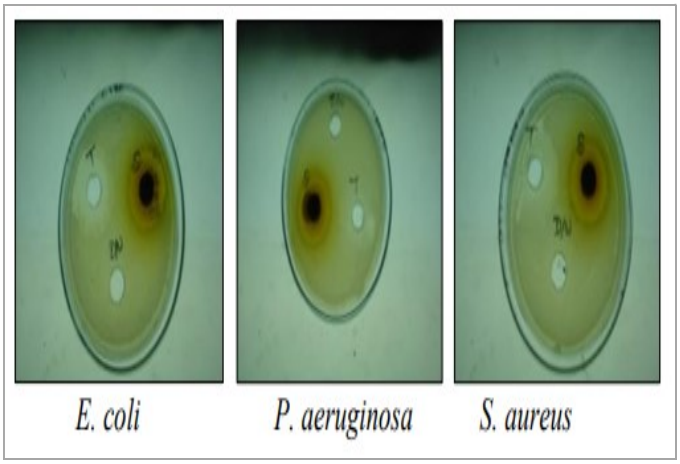

Fig 5. *P. aeruginosa* was having higher zone of inhibition compare to *E. coli* and *S. aureus.*

Table 1. Antimicrobial activity of methanol leaves extract of *Rosmarinus officinalis.*

Sl. No.	Bacterial strain	Zone of inhibition (mm)	Tetracycline (mm)
1	*P. aeruginosa*	22.5	27.5
2	*S. aureus*	21.5	25.0
3	*E. coli*	22.0	25.5

The antimicrobial assay showed that a zone of inhibition was observed 27.5 mm against *P. aeruginosa* for methanolic extract. The methanol extract of *R. officinalis* leaves has demonstrated promising antimicrobial properties. Increasing awareness, promotion and utilization of these leaves for public benefits are highly encouraged and identification of active phyto-constituents in the extract will serve as a natural cytotoxic agent against various cancers. This study confirms that *R. officinalis* leaf possesses antimicrobial activity.

CONCLUSION:

The phytochemicals and antimicrobial studies of *R. officinalis* leaf extract provided scientific evidence for the rational use of *R. officinalis* leaves in prevention of disorders due to presence of some useful phytochemicals, and in treatment of diseases caused by some bacterial pathogens such *as P. aeruginosa, E. coli,* and *S. aureus*. Further research is necessary to reveal its detailed molecular mechanism behind these phytochemical and antimicrobial activities. While the initial results have been encouraging, additional research is needed to confirm the safety and efficacy of *R. officinalis* as a preservative and therapeutic agent.

The findings of this study support the view that the methanolic extracts of plants are promising sources of potential antimicrobial and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in the pharmaceutical industry. Further detailed studies on isolation of phyto-constituents of the plant extracts are essential to characterize them as biological antimicrobial agents. This knowledge about the medicinal plant's usage can also be extended to other fields like the field of pharmacology. In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phyto-pharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind.

Further research is necessary to reveal its detailed molecular mechanism behind these phytochemical and antimicrobial activities. Our aim is to find plants which have antimicrobial activity without many side effects. A detailed study needs to be carried out to isolate bioactive compounds that show antimicrobial activity.

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