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STUDY OF ANTIMICROBIAL ACTIVITY OF *Rosa indica* AGAINST GRAM POSITIVE AND GRAM NEGATIVE MICROORGANISMS

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Abstract- Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary.
The present study reveals the antibacterial potential of crude extracts of different parts of *Rosa indica*. Extracts from almost all parts of the plant showed antibacterial potential and produced zone(s) of inhibition thus; it should be further studied to determine the active component (s). Furthermore, Gram-negative bacteria were found to have more susceptibility as compared to Gram-positive bacterial species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms. Data also showed that some antimicrobial substances could be extracted by organic solvents, suggesting that organic solvents are clearly better solvents of antimicrobial agents.
Key words- Herbal medicines, Antimicrobial, Rosa indica, Zone of inhibition, active components.

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Introduction

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. Antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.
Emerging and reemerging infectious diseases and spread of deadly drug-resistant strains pose a challenge to public health care services. Several micro organisms' derived antibiotics are currently in use to treat a variety of infectious human disease. Most of them have a limited antimicrobial species of the pathogen, some even lead to serious side effects. Broad spectrum antibiotics kill useful bacteria in the colon, allowing yeast and other unfriendly microbes to multiply out of control. Some are synthetic compounds. Therefore action must be taken to control the use of antibiotic, develop new drugs either synthetic or natural. Efforts are thus being directed to identify antibiotic sources other than traditional microorganisms.
For a long period of time, plants have been valuable sources of natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs. According to World Health Organization (WHO) any plant which contain substances that can be used for therapeutic purposes or which are precursor of chemo-pharmaceutical semi synthetic new drugs is referred as medicinal plants. Medicinal plants would be the best source to obtain a variety of drugs as the phyto-chemicals are more specific, biodegradable and are suppose to have fewer side effects. Phyto-chemicals offer unique platform for structural diversity and biological functionality which is indispensable for drug

discovery [1,2]B**e**cause of the appearance of bacterial resistance to antimicrobial agents more effort is being made to find alternative antimicrobial components, so the natural products are more preferred than synthetic ones [3]. Also on the pharmacological side, if a chemical compound can be isolated showing a wide range antifungal and antibacterial property, it could be put into good use to alleviate. On a long term basis these chemical components showing activity could be synthesized in the laboratory, formulated and screened taking this project on a more commercial / development stage [4].

The Present investigation deals with the study of antimicrobial activity of *Rosa indica,* a perennial flower shrub or vine of the genus Rosa, within the family Rosaceae, that contains over 100 species and comes in a variety of colours. The species form a group of erect shrubs, and climbing or trailing plants, with stems that are often armed with sharp prickles.

Materials and Methods

Collection of Plant Material

The fresh and healthy leaves stem and root from three different varieties of *Rosa indica* "red", "Pink" and "yellow" were collected. The plant materials were washed thoroughly with tap water followed by sterilized distilled water. The plant materials were dried in hot air oven at 50 °C and used as the raw materials for the extraction of antimicrobial compounds from the plant.

Extract Preparation

5 g of each dried plant part was ground in mortar and pestle in 50 ml of 70% ethanol.They were then kept for 2-3 days. This was then filtered through Whatman's filter No. 1. The filtrate was then concentrated by complete evaporation of solvent at room temperature to yield the pure extract.Stock solutions of crude extracts were prepared by mixing well the 50 mg of the dried extracts with 50 ml of Tris-HCl buffer. The solution was stored at 4°C after collecting in eppendrof until use.

Antibacterial Susceptibility Assay

Extracts obtained were evaluated for their potential antibacterial activities by the standard agar well diffusion assay. Sterilized distilled water was used as negative control. Tetracycline was used as a standard antibiotic (*i. e.* positive control) in the present study for a comparative analysis with the effectiveness of various plant extracts against selected microflora. The antibiotic was procured from local chemist. On the basis of claim *i.e.* .500 mg/capsule; an appropriate amount of tetracycline powder was dissolved in sterilized distilled water to obtain a final concentration of 1mg/ml; this solution was used as a standard antibiotic throughout the study.

Test Microorganisms

The strains of bacteria which was collected from private labs in Salem, Tamil Nadu , Staphylococcus *aureus, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus* licheniformis. were selected to assess susceptibility patterns against the extracts prepared in the present study. Each of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37° C.

The zone of inhibition was measured and expressed in millimeter. Antibacterial activity was recorded if the zone of inhibition was greater than 6mm [5,6].

Results

The result showed that the extract screened against two gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and two grampositive bacteria *Bacillus licheniformis* and *Staphylococcus aureus* for three different varieties and parts of *Rosa indica* using agar well diffusion assay method shows that *Rosa indica* possessed bactericidal properties. The percentage inhibition varied with the type of rose varieties and plant part as well as the type of bacteria. The results showed that inhibition of microbial growth was greater in the stem extract of the plant irrespective of the variety(Fig 1,2&3).

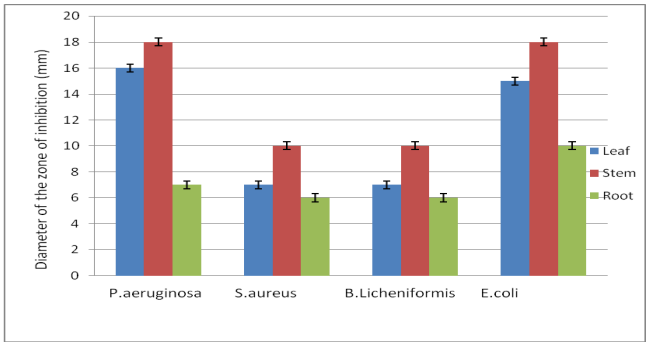

Fig.1- Graph showing effect of leaf, stem and root extract of red rose plant on four different microorganisms

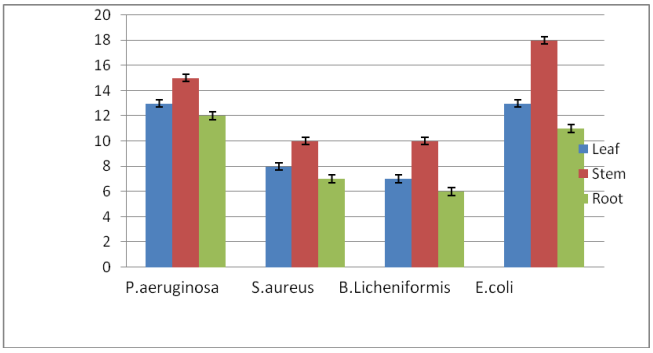

Fig. 2- Graph showing effect of leaf, stem and root extract of Pink rose plant on four different microorganisms

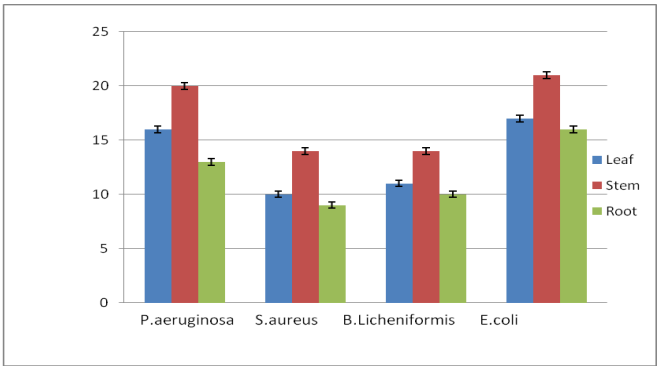

Fig. 3- Graph showing effect of leaf, stem and root extract of Yellow rose plant on four different microorganisms

The leaf extract also has considerable inhibition in comparison to root extract. The yellow variety of *Rosa indica* shows more inhibition than red and pink variety(Fig 4,5 &6).

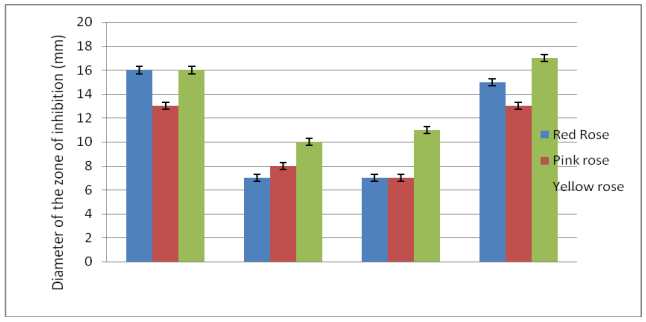

Fig. 4- Graph showing effect of leaf extract of Red, Pink and Yellow rose plant on four different microorganisms

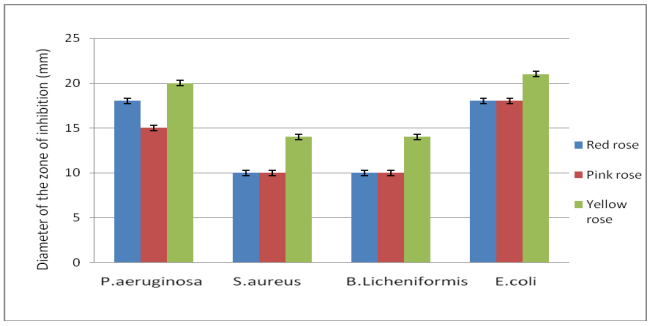

Fig. 5- Graph showing effect of stem extract of Red, Pink and Yellow rose plant on four different microorganisms

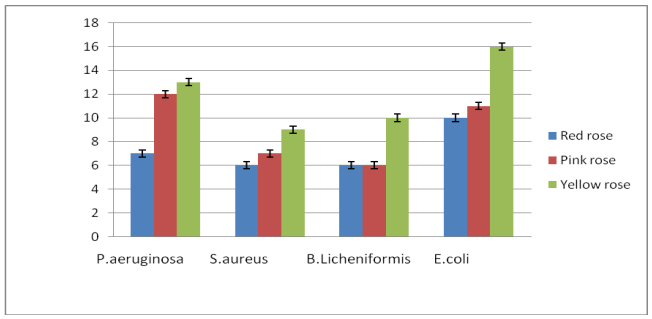

Fig. 6- Graph showing effect of root extract of Red, Pink and Yellow rose plant on four different microorganisms

The diameter of the zone of inhibition in petriplates inoculated with E.coli and Pseudomonas aeruginosa is comparatively more than that of other petriplates inoculated with *Bacillus licheniformis* and *Staphylococcus aureus* respectively (Fig7,8&9).

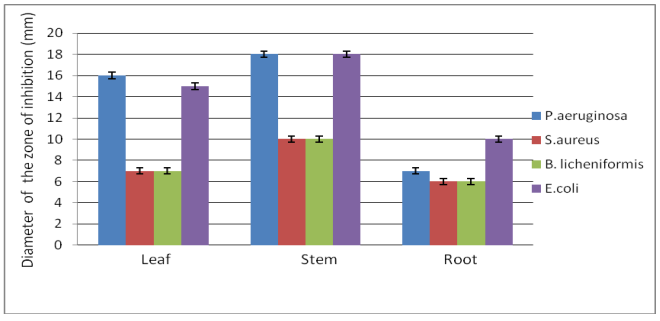

Fig. 7- Graph showing effect of Red rose extract of leaf, stem and root on four different microorganisms

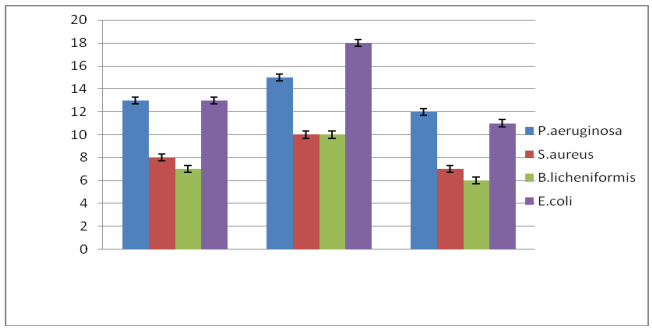

Fig. 8- Graph showing effect of Pink rose extract of leaf, stem and root on four different microorganisms

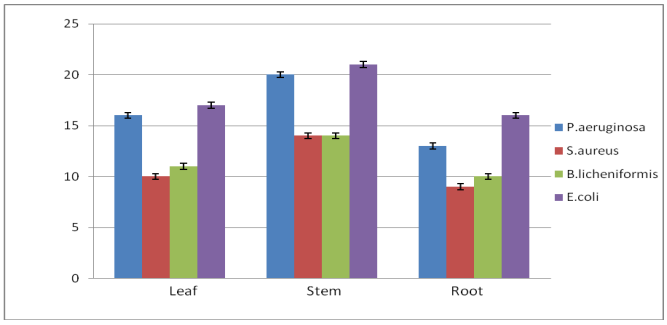

Fig. 9-Graph showing effect of yellow rose extract of leaf, stem and root on four different microorganisms

This implies that the inhibitory compounds of the plant extracts are more efficacious for Gram negative bacteria (Table1,2&3). The control did not inhibit growth of all the bacteria.

Table 1- The diameter of the zone of inhibition (mm) of leaf extract of different rose varieties tested on four different microorganisms

Rose variety	Microorganisms			
	P. aeruginosa	*S. aureus*	*B. licheniformis*	*E. coli*
Red	16	7	7	15
Pink	13	8	7	13
yellow	15	10	11	17

Table 2- The diameter of the zone of inhibition (mm) of stem extract of different rose varieties tested on four different microorganisms

Rose variety	Microorganisms			
	P. aeruginosa	*S. aureus*	*B. licheniformis*	*E. coli*
Red	18	10	10	18
Pink	15	10	10	18
yellow	20	14	14	21

Table 3- The diameter of the zone of inhibition (mm) of root extract of different rose varieties tested on four different microorganisms

Rose variety	Microorganisms			
	P. aeruginosa	*S. aureus*	*B. licheniformis*	*E. coli*
Red	7	6	6	10
Pink	12	7	6	11
yellow	13	9	10	16

Discussion

Plant extracts have been used for many thousands of years [7], in food preservation, pharmaceuticals, alternative medicine and natural therapies [8,9]. It is necessary to investigate those plants

scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds [10] especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extract s inhibited bacterial growth but their effectiveness varied with the concentration . The antimicrobial activity of many plant extract has been previously reviewed and classified as strong, medium or weak [11]. This study indicated that plant extract may possess antibacterial activity and can be exploited as an ideal treatment for future human disease management programs eliminating bacterial spread. The present study reveals the antibacterial potential of crude extracts of different parts of *Rosa indica*. Almost all parts of the plant showed antibacterial potential and produced zone(s) of inhibition. Extracts from leaves of *R. indica* demonstrated antibacterial activity thus; it should be further studied to determine the active component(s). Furthermore, Gram-negative bacteria were found to have more susceptibility as compared to Gram-positive bacteria species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms. In conclusions of this study it is possible to state to confirm the validity of the use of this plant as medicine in ancient medicinal traditions. Because of the diversity and complexity of the natural mixtures of bioactive compounds in the crude plant extract and fungal cultures, it is rather difficult to characterize every compound present and elucidate its structure in a single study. According to the information presented, the compounds considered characteristic of *C. fusiforme* are produced by the microorganisms, at least in the selected culture medium. If cultured in other conditions, such as different cultivation time and water content, very different compounds could be produced. However, further investigation is still needed to discover the unidentified/ unknown bioactive constituents in the endophytic fungal isolates and its host.The bacteriostatic activities of extracts from *Nerium indicum* leaves and provided references for its reasonable development. With *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis* and *Sarcina lutea* as tested bacteria, the bacteriostatic effects of ethanol extracts of *N. indicum* leaves with different concentrations were studied. The ethanol extracts from *N. indicum* leaves with 3 kinds of concentrations had bacteriostatic effects on 6 kinds of tested bacteria to different degrees. The bacteriostatic effect of 70 % ethanol extracts was most obvious and strongest on *S. aureus* [12,13]. Antibacterial resistance especially among gram-negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drugs or natural anti-bacterials [14]. The results of the present study supports the traditional usage of the studied plants and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Further study on the analysis of the most active extracts using NMR, Mass Spectrometry, UC-MS, LC-MS, HPLC, etc. will help in isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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