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IN VITRO SCREENING OF C. *ROSEUS* ALKALOIDS FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT
The present work is to study the antibacterial activity of *Catharanthus roseus* on common human pathogens like *Salmonella, Proteus*, *Klebsiella, Pseudomonas* and *Staphylococcus*. This study involves extraction of active ingredients from roots of *C. roseus* and its antibacterial activity were tested by disc diffusion method. It is inferred that ethanol extract of the roots of *C. roseus* containing alkaloids has satisfactory antibacterial activity against the entire organism taken in the study, except *Pseudomonas* which is resistant to the alkaloids of *C. roseus*. Further, HPLC alkaloid profile of the *C. roseus* root extract has been studied. The present finding suggests that *C. roseus* root alkaloids can be used as antibacterial agent in new drugs for therapy against these pathogens.

INTRODUCTION

Medicinal plants are nature's gift to humans to make disease free healthy life. Infectious diseases continue to represent a significant challenge to human medicine. Clearly there is an urgent need for new and efficient drugs to treat the life threatening diseases. Large preparations of drugs currently used to treat infectious diseases are mostly natural products. Despite the progress in the field of Microbiology, incidence of epidemics due to drug resistant microbes and the emergence of unknown disease causing agents still occur. Herbal products remain highly effective instruments in the fight against microbial infections. Plant based antimicrobials represent a vast untapped source for medicine and they have enormous therapeutic potential. They are effective in treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

The Indian subcontinent abounds with wide variety and diversity of health traditions. We have with us the largest unbroken tradition, which has not only a stream of practices but also a textual and theoretical backing in terms of the Ayurvedic, Homeopathic and Siddha systems of medicines. Indian people have an incredible knowledge of phytomedicine driven apparently by a tremendous passion for the study of medicinal plants. (Cowan, 1999)

Medicinal herb is a biosynthetic laboratory as it is containing a number of chemical compounds like alkaloids, glycosides, resins, carbon, hydrogen, oxygen, nitrogen, essential oil, fatty oil, gums, mucilage and tannins etc. These active principles are present in plants viz roots, seeds, leaves, bark and wood. Various compounds used as antimicrobials are secondary metabolites such as coumarins, flavonoids, anthocyanin, tannins, alkaloids etc. The alkaloids are a large group of secondary plant products that possesses a wide range of pharmacological activities (Kutchan, 1995)

The tropical plant Madagasker Periwrinkle (*C. roseus*) (L.) G. Don is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities. (El-Sayed and Cordell, 1981). A large number of alkaloids are present in *C. roseus*. Out of them, about 20 dimeric indole alkaloids possess oncolytic activity and among them, vincristine and vinblastine are most significant. (Chtatopadhyay *et al.,* 1991) The other alkaloids like catharantine, vindoline, vindolinine, perivine, vindolinic acid are also present (Jaleel *et al.,* 2006). Microbiological analysis of Vindolinine and some of its structural changes have been analyzed (Rojas Hernandez and Cuellar, 1976). *C. roseus* flower extract has wound-healing activity in Sprague Dawley rats (Nayak *et al.,* 2006) Kulkarni *et al.,* 1999. Inheritance of morphological traits of periwinkle mutants leads to modified contents and yields of leaf and root alkaloids in *C. roseus* (Kulkarni *et al.,* 1999). Antimicrobial activites of *Emblica officinalis* and *Coriandum sativum* against gram positive bacteria and *Candida albicans* have been reported (Saed and Tariq, 2007). The antimicrobial activity of various plant extracts

like Cinnamomum (Chaudhry and Tariq, 2006) and Ocimum extract against diverse microbial flora has also been reported (Ekmea and Elizabeth, 2009).

Survey of literature on *C. roseus* extracts indicted that information on antibiogram studies is scarce. Experiments were conducted on the antibiogram of *C. roseus* on *B.cereus* and *B.megaterium* and it was found that among various extraction procedures with different solvents, crude ethyl acetate extract was very effective antimicrobials (Sathiya *et al.,* 2008). The present study has been designed to isolate alcoholic extract from roots of *C. roseus* and separation of active ingredients through HPLC and to screen the antibacterial activities of *C. roseus* against some human pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant material

C. roseus plants were collected at Vellandi valasu near by Idappadi, in Nammakal district, Tamilnadu, India. The roots of *C. roseus* were washed thoroughly and kept for shade drying. The air dried material was crushed in mortar and pestle to obtain coarse particles. The air dried roots weigh upto 1kg.

Preperation of *C. roseus* extract

The coarse material of dried roots was taken and weighed upto 13g. The root powder was moistened with ammonia for efficient extraction of alkaloids. The soxhlet apparatus was rinsed with acetone and was further rinsed with the solvent (ethanol) used for extraction.

The coarse powder of *C. roseus* was packed in soxhlet apparatus. Porcelin beads were added in the round bottom flask to prevent bumping. The solvent ethanol was added in the round bottom flask and was heated to extract the plant extract over a heating mantle using the adjustable rheostat. Hot vapor of ethanol passes through the percolator tube and then vapor drops in the soxhlet tube and it degrade the cell wall of the roots and constituents of plants will be extracted out. This method is called hot percolation method and the extraction was done upto 72hr. The extract was collected and concentrated by evaporating ethanol.

Extraction of alkaloids

The *C. roseus* extract was shaken with successive quantities of dilute sulphuric acid and the acid portion was collected, which contain the alkaloid in sulphate form. To it ammonia was added and shaken to get free alkaloids in the alkaline fraction. Confirmatory test for the presence of alkaloids was done using Wagner's reagent. The reddish brown precipitate shows the presence of alkaloids.

Purification

The *C. roseus extract* was purified and to check the active ingredients present in it. HPLC (High Pressure Liquid Chromatography), Shimadzu model, Japan, was performed in CECRI (Central Electrochemical Research Institute), Karakudi. 0.2g of *C. roseus* root extract was mixed with 10 mL of methanol and then 20μL of *C. roseus* extract was injected for HPLC analysis.

Culture used

Microorganisms for the study were collected from the laboratory of Department of Biotechnology, Vivekananda College of Arts and Science, Thriuchengode. They were sub cultured, identified by biochemical tests and their sensitivity of Antibiotics was analyzed. The strains of bacteria used are *Proteus* vulgaris, *Salmonella typhi*, *Shigella* sonnei, *Klebsiella* pneumoniae, *Pseudomonas* aeruginosa and *Staphylococcus aureus*. The stock cultures of microbial strains were maintained in nutrient agar slants at 4ºC in refrigerator. All the chemicals and reagents used in the present investigation were of analytical grade.

Screening of antimicrobial activity

Disc diffusion method was used to determine the growth inhibition of bacteria by using antibiotic discs and the discs was prepared from alkaloids of *C. roseus* root extract. Muller Hinton Agar pH 7.2 (Himedia) was prepared and sterilized at 121ºC for 15 min and poured in sterile Petri dishes. The clinical isolate of the microbes were inoculated by streak plate method.

Alkaloid disc preparation

The disc were prepared form the Whatmann filter paper no.1 was sterilized and the disc was 1.28 mm in diameter. 0.1 mg of extract was mixed in 100μL of ethyl alcohol. The disc was prepared in 10μg and 20μg of alkaloid concentration. Control paper discs were prepared by using 1% ethanol. The alkaloids disc were taken with sterile forceps and placed carefully in the microbes inoculated Muller Hinton agar plate, at least 25 mm away from the edge. Equal distance of placement is must, to avoid the overlapping of the zone of inhibition. Then Petri dishes were incubated at 37ºC for 16–18 hr. After incubation the results were noted for the zone of inhibition which was measured in mm.

RESULTS

Isolation of Ethanol extracts of *C. roseus* roots

Alkaloids from the total ethanol extract were isolate using Soxhlet apparatus. The presence of alkaloids was confirmed with Wagner's reagent. Reddish brown precipitate developed when Wagner's reagent was added, which confirmed the

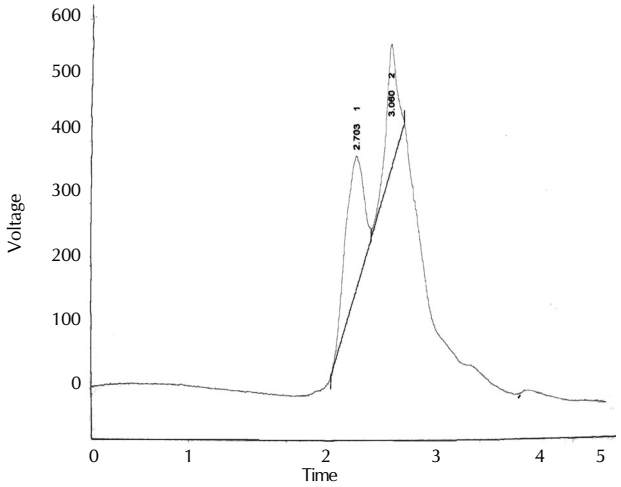
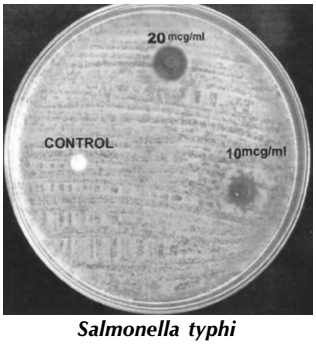
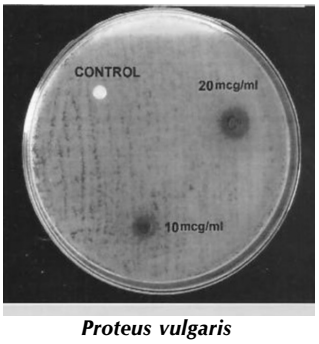


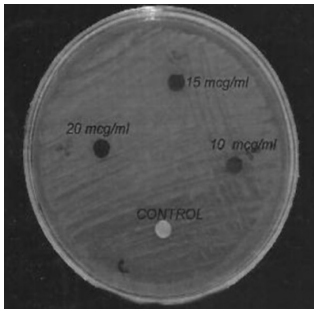
Figure 1: Two peaks by HPLC at 270 nm absorbance



Salmonella typhi

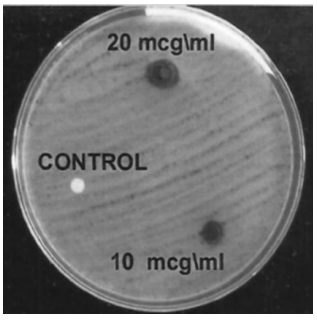


Proteus vulgaris

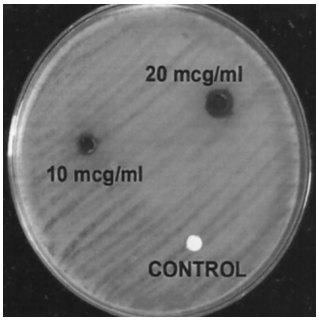


Pseudomonas Aeruginosa

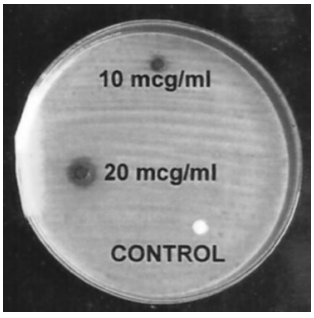
Figure 2: Antibacterial Activity of *Catharanthus Roseus*



Klebsiella pneumoniae



Shigella sonnei



Staphylocooccus aureus

Figure 3: Antibacterial Activity of *Catharanthus Roseus*

Table 1: Sensitivity/ resistance pattern of *C. roseus* root alkaloids on pathogenic bacteria

Name of the Antibiotic	Strength	Diameter of Zone of Inhibition	Sensitivity/ Resistance
Staphylococcus aureus	10μg	9mm	
	20μg	14mm	Sensitive
Pseudomonas aeruginosa	10μg	-	
	20μg	-	
Klebsiella pneumoniae	10μg	13mm	
	20μg	14mm	Sensitive
Proteus vulgaris	10μg	8mm	Sensitive
	20μg	16mm	
Shigella sonnei	10μg	10mm	Sensitive
	20μg	15mm	
Salmonella typhi	10μg	11mm	Sensitive
	20μg	14mm	

extract also contains alkaloids along with other constituents of *C. roseus* root.

High pressure liquid chromatography analysis of *C. roseus* root extract

Two peaks were observed in *C. roseus* root extract. This HPLC system detected the separated compounds based on their absorbance at 270 nm. They were significant peaks after two minutes. The retention time observed to be 2.703 and 3.060. These two peaks signify that they are the only active substances in *C. roseus* root extract that is involved in antibacterial activity. The result is shown in Fig. 1

Antibacterial effect of *C. roseus* root extract

The antibacterial effect of *C. roseus* root extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella sonei*, *Salmonella typhi*, *Proteus vulgaris*. The Result showed some antibacterial activity against common human pathogens. The antibacterial effect of *C. roseus* root extract against these organisms is shown in Table 1.

Proteus vulgaris formed a zone of inhibition of 8 mm in diameter in minimal concentration and at the maximum concentration formed a zone of 16mm in diameter. *Klebsiella pneumoniae* produced good zone of inhibition than other microbes. It produces zone of 13 mm in diameter and 20mm zone at maximal concentration. *Salmonella typhi* at 10 μg/mL concentration formed a zone of 11 mm in diameter and at a 20 μg/mL formed a zone of 14 mm in diameter. *Staphylococcus aureus* at a 10 μg/mL concentration formed a zone of 9 mm in diameter and at a 20 μg/mL concentration formed a zone of 16 mm in diameter. *Shigella sonnei* at a 10 μg/mL concentration formed a zone of 10 mm and 20 μg/mL concentrations formed a zone of 15 mm. The results are shown in Fig. 2 and 3.

DISCUSSION

Alkaloids of *C. roseus* are well known for their hypoglycemic, sedative and antihyperglycemic properties and used in anti-cancer therapy (Chtatopadhyay *et al.,* 1991). Even at a minimal concentration of 10 μg and 20 μg the extracts of *C. roseus* have shown a good zone of inhibition. *Shigella sonei* showed sensitivity by forming a zone of 15 mm in diameter at a concentration of 20μg/mL and formed a zone of 10mm at a concentration of 10 μg/mL. *Proteus vulgaris* at a concentration of 10 μg/mL formed a zone of 8mm in diameter and at a concentration of 20μg/mL formed a zone of 16mm in diameter. *Klebsiella pneumoniae* at 10μg/mL formed a zone of 13mm in diameter and at 20μg/mL formed a zone of 14mm in diameter. *Staphylococcus aureus* at 10μg/mL formed a zone of 9mm in diameter and 20μg/mL formed a zone of 16mm in diameter. The trace elements in the *C. roseus* root extract may be directly or indirectly helpful in the management of many diseases. These compounds may play a major role in health care and disease control of human body, there is ample scope for their exploitations in further investigations.

Analytical methods allow the quantification of the active substances in the plant extract with good confidence. HPLC analysis showed the presence of the two active ingredients in the root. These active ingredients may be responsible for the antibacterial activity of *C. roseus* root extract. Further isolation of the specific alkaloid responsible for the activity can be preceded. This preliminary work on the *C. roseus* alkaloids can be extended by testing for safety, toxicity and used as a commercial herbal medicine.

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