

IN VITRO ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF *CATHARANTHUS ROSEUS* LEAVES EXTRACT AGAINST IMPORTANT PATHOGENIC ORGANISMS

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ABSTRACT

Catharanthus roseus (periwinkle) is an important medicinal plant for novel pharmaceuticals since most of the bacterial pathogens are developing resistance against many of the currently available anti microbial drugs. Plants have proved to be significant natural resources for effective chemotherapeutic agents and offering a broad spectrum of activity with greater emphasis on preventive action. This study aims to investigate some of the anti microbial, antifungal property of this leaves extract. The antimicrobial activity has been checked against microorganisms like *Escherichia coli* (MTCC - 443), *Klebsiella oxytoca* (MTCC - 4676), *Klebsiella pneumoniae* (MTCC- 7162), *Proteus mirabilis* (ATCC -49565), *Pseudomonas aeruginosa* (MTCC -5210), *Salmonella typhimurium* (ATCC -13311), *Salmonella paratyphi* (MTCC-735), *Staphylococcus aureus* (MTCC -740) and antifungal activity against *Aspergillus niger* (MTCC- 2425), *Aspergillus fumigatus* (MTCC -9657), *Candida albicans* (MTCC- 183), *Penicillium chrysogenum* (MTCC- 160), *Aspergillus flavus* (AFG3) by disc diffusion method. The finding shows that this leaf extracts having the potential to inhibit the growth of bacteria and fungi. From the result, the leaves of this plant can be used as prophylactic agent in many of the diseases, which sometime are of the magnitude of an epidemic.

Keywords: *Catharanthus*, Leaf extracts, Periwinkle, Bacterial and Fungal strains.

INTRODUCTION

Natural products including plants, animals and minerals have been the basis of treatment of human diseases. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be acquired to the patients and the community (Patwardhan and Hopper, 1992). The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized in the laboratory data back to the early 2000. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents (Krishnaraju *et al.*, 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. The Greek physician, Galen (AD129-200) devised the first pharmacopoeia describing the appearance, properties and use of many plants. Approximately 20% of the plants found in the world have been submitted pharmacological or biological activities.

The plants are having capacity to produce a large number of organic chemicals so called secondary metabolites ; which are divided into different categories based on their mechanism of function like Chemotherapeutic, bacteriostatic, bactericidal and antimicrobial agent. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanism of activities for new and re-emerging infectious disease (Parekh and Chanda, 2007). Therefore researchers are increasingly looking for new leads to develop better drug against microbial infections. Approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery. Investigations of plants used in traditional and modern medicine in china serve as a source of inspiration and as models for the synthesis of new drugs with better therapeutic, chemical or physical properties than the original compounds. The world health organization also has recognized the importance of traditional medicine and has been active in crating strategies, guidelines and standards for botanical medicines (WHO, 2002).

Medicinal plant products could prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health

(Kaushik *et al.*, 2002). Medicinal plants are widely used in management of diseases all over the world (Aliyu *et al.*, 2007). Historically, the use of medicinal plants is as old as mankind and medicine. *Catharanthus roseus* is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al.*, 2009). (Muhammad *et al.*, (2009) reported that the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of *Catharanthus roseus* against clinically significant bacterial strains. Emerging and reemerging infections and spread of deadly, drug-resistant strains of organisms pose a challenge to the global public health for their treatment. Bacterial resistance to antibiotics is a major therapeutic problem and the rate at which new antibiotics are being produced is slowing, (Russell *et al.*, 2002). Thus, the search for novel antimicrobial agents is of the utmost importance (Gootz *et al.*, 1990). Global attention has been shifted towards finding new chemicals, specific the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery (Nisbet and Moore, 1997). The present investigation is focused on the screening of leaf extracts of *Catharathus roseus* for antibacterial, antifungal activity.

MATERIALS AND METHODS

Plant collection and extract preparation

Catharanthus roseus was collected in the month of July, 2010 collected from the herbal garden at the PRIST University, Thanjavur, Tamilnadu, India. It was taxonomically identified and authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph College (Autonomous), Tiruchirappalli, Tamilnadu, India. The voucher specimen was deposited at the Rapinat herbarium and the voucher number is RHPSB.001. The leaves were air-dried and the dried plant material was weighed using Gallenkamp (FA2104A, England) electronic weighing balance and grinded with Blender/Miller III, (model MS - 223, China). The leaves were cleaned and dried in shade for 7 days, then ground well to fine powder. About 500 g of dry powder was extracted with 1 litre of methanol solvents by continuous hot percolation using soxhlet apparatus. The extraction was continued for 24 h. The different extracts were filtered and kept in hot air oven at 40°C for 24 h to evaporate the methanol from it. A dark brown residue was obtained. The residue was kept separately in air tight containers and stored in a deep freezer.

Fractionation (Moorthy et., al 2011)

500g of powdered plant material was soaked and then extracts with ethanol in a soxhlet apparatus. After, collected extracts were evaporated to dryness in desiccators. From that 250 gm of crude extract was fractionated with water, ethanol, methanol, acetone, hexane and butanol using column chromatography under reduced pressure over silica gel. These fractions was collected and stored in a refrigerator until used for the phytochemical and antimicrobial screening.

Cultures used for antimicrobial activity

The microorganisms used were as follows. The antimicrobial activity has been checked against microorganisms like, *Escherichia coli* (MTCC- 443), *Klebsiella oxytoca* (MTCC - 4676), *Klebsiella pneumoniae* (MTCC- 7162), *Proteus mirabilis* (ATCC - 49565), *Pseudomonas aeruginosa* (MTCC - 5210), *Salmonella typhimurium* (ATCC -13311), *Salmonella paratyphi* (MTCC-735), *Staphylococcus aureus* (MTCC -740). *Catharanthus roseus* leaves extract were screened for their antifungal activity against *Aspergillus niger* (MTCC- 2425), *Aspergillus fumigatus* (MTCC -9657), *Candida albicans* (MTCC- 183), *Penicillium chrysogenum* (MTCC- 160), *Aspergillus flavus* (AFG3) by disc diffusion method.

Antimicrobial testing (Bauer et al., 1966)

Culture supernatants with fractions and crude extract of the plants were used in the disc diffusion method separately. Pathogenic organisms were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of *C. roseus* leaves extract plant sample was placed on the surface individually. To compare the antibacterial activities, streptomycin (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent followed by drying was used. The plates (triplicates) were incubated 28°C for 72 h. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

Antimycotic assay by Disc Diffusion Technique

Different concentration of each leaves extract i.e., 100 µg, 50 µg, and 25 µg µg/disc were used for assay. The fungal cultures were grown on czapexdox broth (diffco). The cultures 7-day old culture was washed, suspended in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of suspension of the test fungus was determined and test inoculum was adjusted 1-5 X 10 ml. These conidia were used for antifungal assay 5 tests. Inocula (0.1ml) were applied on the surface of the Czapek's dox agar (Diffco) plate and spread by using sterile glass spreader. The sterile discs (5mm diameter, Whatman filter paper No.42) were soaked in added concentrations (100µg / disc, 50µg /disc, 25µg /disc) of *Catharanthus roseus* leaves extract. The test was performed in triplicate. These dishes were incubated for 48 h at 28°C. Zone of inhibition in mm were determined after 48 h. Standard antibiotics Nystin (20 µg/disc) used as standard antibiotic for fungus and negative control, a blank disc impregnated with solvent followed by drying was used in order to control the sensitivity of the tested fungus.

RESULTS AND DISCUSSION

Antimicrobial activity

Antimicrobial activity of *Catharanthus roseus* extract against pathogenic organism tested, ethanolic fractions gave better results

when compared to other organism tested to other fractions tested. Among eight organism tested *E. coli* (MTCC- 443), *Klebsiella oxytoca* (MTCC- 4676) two organisms showed better result (Table-1) and (Figure-1). Plants are a repository of various biomolecules responsible for different biological activities. India is endowed with rich plant biodiversity. Many plants have been evaluated for the different biological activities all over the world. Considering the biodiversity of higher plants, the number of plants screened for antimicrobial and antifungal activity. As can be seen from the literature survey that this plant has been mostly studied with respect to its anti cancer properties and its anti diabetic properties. Till date, very little studies have been done on the anti microbial properties of the plant extracts. Therefore, this study focuses on the anti microbial properties of the leaf extracts. These extracts may not find a therapeutic use in immediate future but definitely it can be used as a prophylactic agent in regions where certain diseases can occur as endemic if not in pandemic scale. It can be seen from the results above that the leaf extract contained many indole alkaloids, and some phenolic compounds.) The phenolic compounds are known for their antimicrobial properties.

Antifungal activity

The antifungal activities of *C. roseus* plant leaves extract obtained by the disc diffusion method are shown in the table. The leaves extract were tested exhibited the antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Candida albicans* and *Penicillium* species. Among the species tested *Aspergillus flavus* gave better results. Ethanolic extract of *Catharanthus roseus* extract showed better result on *Aspergillus flavus* pathogen. (Table 2) and (Figure 2.). Antibiotic (Nystatin), C- Control, S- Sample. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal part (Ramani, 2008). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated a number estimated to be less than 10% of the total. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. This plant is the Madagascar periwinkle, *Catharanthus roseus*. It is an herb or sub shrub found throughout the Tropics and cultivated as a garden plant worldwide. Ruiz, (2009) The development of the periwinkle anticancer drugs from isolation and purification of several *Catharanthus* alkaloids to laboratory and clinical testing and subsequent marketing of two of them represents a tremendous success story in the field of plant-derived pharmaceuticals. In the short time since first clinical use, these alkaloids have become two of the most valuable cancer chemotherapy treatments available. The Madagascar periwinkle was one of 440 plants selected for study in an Eli Lilly plant screening program for plant-derived drugs. The plants were chosen on the basis of carefully assessed reports of folklore use and reported alkaloid contents. Alkaloids, complex nitrogen containing plant compounds, are often physiologically active. They are the most common active ingredients in many plant-derived medicinal.

Table 1: Antimicrobial activity of *Catharanthus roseus* extract

Antibacterial activity Name of the bacteria	Zone of Inhibition (mm)					
	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
<i>Escherichia coli</i>	-	6.3	-	1	-	-
<i>Kelbsiella oxytoca</i>	-	1.6	8	-	T	-
<i>K. pneumoniae</i>	-	-	T	1	-	T
<i>Proteus mirabilis</i>	-	8	-	1	-	-
<i>Pseudomonas aeruginosa</i>	T	T	1	-	-	-
<i>Salmonella typhi</i>	-	1	6.2	T	1	T
<i>S. paratyphi</i>	T	3	1	-	-	-
<i>Staphylococcus aureus</i>	-	-	7.3	1	1	-

Table 2: Antifungal activity *Catharanthus roseus* extracts (mm)

Test organism	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
<i>Aspergillus niger</i>	-	4.2	T	-	-	T
<i>Aspergillus flavus</i>	-	3	-	T	-	-
<i>Aspergillus fumigatus</i>	-	1.8	-	0.8	-	-
<i>Candida albicans</i>	T	1.6	0.5	-	-	-
<i>Penicillium</i> species	-	3.3	-	-	T	T

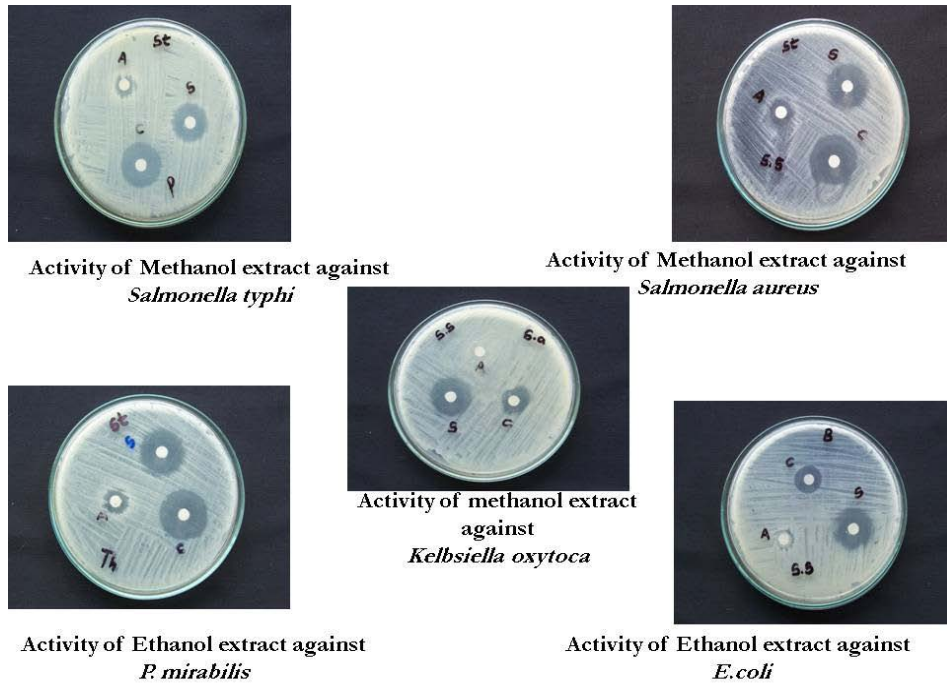


Fig. 1: Antimicrobial activity of selected pathogens

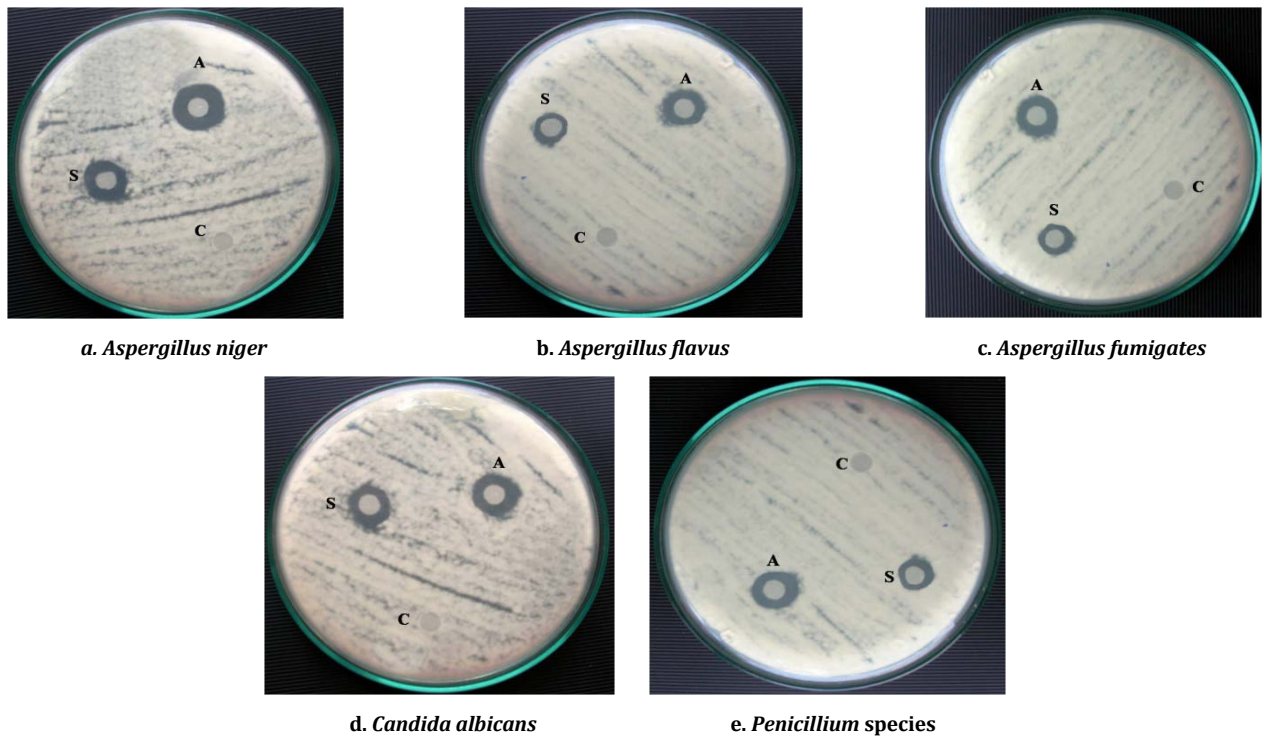


Fig. 2: Antifungal activity of *Catharanthus roseus* (Ethanollic extract)

Antibiotic (Nystatin), C- Control, S- Sample, T-Trace

CONCLUSION

This study has revealed the presence of secondary metabolites like alkaloids in the leaves of *Catharanthus roseus*. It has further confirmed that the leaf extract could be used for the treatment of infections caused by the fungi *Aspergillus* spp. and other fungal diseases. The result shows that to the folkloric use of this plant in treating microbial infections *Catharanthus roseus* could be exploited for new potential antibiotics.

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