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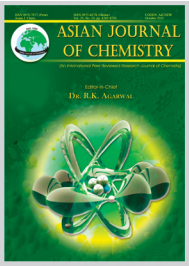

Antimicrobial Activity of Chloroform Extract of Leaves of *Catharanthus roseus* (L) G.DON

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Catharanthus roseus popularly known as Madagascar periwinkle is a potential source for antileukemic alkaloids. The present study aims to evaluate the possibility for the presence of novel bio-active compounds against pathogenic bacteria, as most of the pathogens develop drug resistance against commonly used antibiotics. To determine antimicrobial activity, crude extract from leaves of *Catharanthus roseus* were tested against bacterial and fungal strains of clinical significance. Extraction of bio-active components in appropriate solvent was followed by evaluation of antimicrobial activity by cup plate method against selected bacterial and fungal strains. Extract prepared from leaves showed significantly higher efficacy. Among the extract that were significantly active. The extract obtained using chloroform exhibited maximum activity against bacterial and fungal strains tested. Gram (+) stains and fungal strains were more sensitive when compared to Gram (-) bacteria. The study implicates that bio-active compound(s) of *Catharanthus roseus* could potentially be exploited as antimicrobial agents.

Key Words: ***Catharanthus roseus, Escherichia coli*.**

INTRODUCTION

The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners[1]. Natural products are a source of synthetic and traditional herbal medicine. They are still the primary health care system in some parts of the world[2]. The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications.

Catharanthus roseus (apocyanaceae) also known as *Vinca rosea*, is native to the Caribbean basin and has historically been used to treat a wide assortment of diseases. European herbalists used the plant for conditions as varied as headache to a folk remedy for diabetes. It has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms tumor and other cancers. Its vasodilating and memory-enhancing properties have been shown to alleviate vascular dementia and Alzheimer's disease[3,4]. The two classes of active compounds in *Vinca* are alkaloids and tannins. The major alkaloid is vincamine and its closely related semi-synthetic derivative widely used as a medicinal agent, known as ethyl-apovincaminate or vinpocetine, has vasodilating, blood thinning, hypoglycemic and memory-enhancing actions[5,6]. The extracts of *Vinca* have demonstrated significant anticancer activity against numerous cell types[7]. *Catharanthus roseus* possesses known antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. The extracts have demonstrated significant anticancer activity against numerous cell types[8].

The anticancer drugs vincristine and vinblastine are synthesized from alkaloids of *Catharanthus roseus*. The plant shows the presence of various alkaloids, *viz.* 1) Vincristine, which binds to tubulin dimers, inhibiting assembly of microtubule structures. Disruption of the microtubules arrests mitosis in metaphase. The *Vinca* alkaloids therefore affect all rapidly dividing cell types including cancer cells, but also intestinal epithelium and bone marrow[9]. 2) Vinblastine is an antimicrotubule drug used to treat certain kinds of cancer[10]. 3) Yohimbine (Procomil) is an alkaloid with stimulant and aphrodisiac effects found naturally in *Pausinystalia yohimbine*[11]. *C. roseus* also shows the presence of this compound along with another flavonoid hirsutidin[12]. The plant is also known for its antihypertensive and antispasmodic properties. Considering the medicinal value that this plant has already been shown to have, we evaluated the antimicrobial potential in crude extract of leaves of this plant against clinically significant bacterial and fungal strains.

EXPERIMENTAL

Mature and healthy leaves of. *Catharanthus roseus* were collected from the medicinal garden of Mohamed Sathak A.J. College of Pharmacy, Sholinganallur, Chennai and were

identified by Prof. P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Chennai, India.

Preparation of plant extract: The leaves were washed in tap water, shade dried for 10 days and made into a fine powder of 40 mesh size using the laboratory mill. The coarse powder of shade dried leaves of. *Catharanthus roseus* (200 g) was extracted with 500 mL of chloroform by cold maceration in a narrow mouthed bottle with occasional shaking for three days. It was filtered and the solvent was removed by distillation under reduced pressure. The dried extract was redissolved in ethanol to yield solutions containing 100 µg/disc concentration.

Test organisms: The extract was tested on the following two gram positive bacteria: *Staphylococcus aureus*, *Bacillus cereus* and two gram negative bacteria including *Salmonella typhi, Escherichia coli*. A fungal strain, *Candida albicans* was also tested.

Phytochemical investigation: The presence of various chemical constituents in plant extracts was determined by preliminary phytochemical screening as described by Trease and Evans[13]. By this analysis, the presence of several phytochemicals like flavonoids and alkaloids were confirmed.

Antimicrobial screening: The leaf extract (1000 µg/mL) was tested for antimicrobial activity using agar cup-plate. The strains of microorganisms obtained were inoculated in conical flask containing 100 mL of nutrient broth. These conical flasks were incubated at 37 ºC for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. The cups each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish, which was streaked with the organisms. The solutions of test compound (0.1 mL) were added separately in the cups and petri dishes were subsequently incubated. Ciprofloxacin and ketoconazole were used as standard reference drugs respectively and dimethyl sulphoxide as a control, which did not reveal any inhibition. Zone of inhibition produced by plant extract was measured in mm.

The preliminary phytochemical analysis of the leaf extract revealed the presence of alkaloids as presented in Table-1. The results obtained from the cup-plate method showed that there has been an increasing effect on bacterial and fungal growth. The extract showed good inhibitory activity on almost all the bacteria and fungi used. It has been found that among all the tested organisms, the gram positive bacterial strain, *Staphylococcus aureus* and *Bacillus cereus* were found to be more susceptible to the plant extract by showing inhibition zone of 19 mm and 20 mm, respectively. The fungal strain of *Candida albicans* was more susceptible with the inhibition zone of 19 mm. The antimicrobial activity in terms of zone of inhibition was presented in Table-2. The observed activity may be due to the presence of potent phytoconstituents in the leaf extracts.

Antibiotics provide the main basis for the therapy of microbial infections. However, the high genetic variability of microbes enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics[14]. According to world health report of infectious diseases 2000[15], overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management. *Catharanthus roseus* showed notable antimicrobial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

TABLE-1
PHYTOCHEMICAL PROFILE

S. No	Phytochemicals	Presence/absence
1	Phenol	-
2	Flavonoids	+
3	Alkaloids	+
4	Glycosides	-
5	Tannins	-
6	Steroids	-

- Absence; + Presence

TABLE-2
ANTIMICROBIAL ACTIVITY OF CHLOROFORM LEAF EXTRACT OF *Catharanthus roseus*

Strains	Organisms	Drugs	STD	1000 (mcg/mL)	Control
Bacterial strains	*S. aureus*	Ciprofloxacin	30	19	Nil
	B. cereus		30	20	Nil
	E. coli		25	10	Nil
	S. typhi		23	10	Nil
Fungal strain	*C. albicans*	Ketaconazole	20	19	Nil

RESULTS AND DISCUSSION

As can be seen from the literature survey that this plant has been mostly studied with respect to its anticancer 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 antimicrobial properties of the leaf extracts. These extracts may not find a therapeutic use in immediate future but definitely it can be used as prophylactic agent in regions where certain diseases can occur as endemic if not in pandemic scale. It would go a long way to remove the stress on specific vaccine production to protect such population (which is mostly in developing nations) from the pathogen, which changes its invasive strategies by varying it antigenic nature. 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. The significance of these compounds are that these can substitute long term antibiotic therapy like in case of chronic kidney infection, bacterial endocarditis, carrier conditions of typhoid (where the organisms resides in gall bladder). These compounds having minimum side effect can be easily substituted for antibiotics. Actually the indole alkaloids do not show direct antimicrobial actions but strengthens the immune system and it is this system that takes care of the pathogens. Similarly, the strong immune

system also will take care of many initiation steps in certain oncogenesis. However, if the immune system is too weak or the organism is too virulent then certain other medication will have to be given along with these compounds to show significant antimicrobial activity.

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