



RESEARCH ARTICLE

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**INVESTIGATION OF ANTIBACTERIAL ACTIVITY AND
PHYTOCHEMICAL ANALYSIS OF CATHARANTHUS ROSEUS
AGAINST ISOLATED WOUND PATHOGENS.**

***S.THENMOZHI¹, S.ARUNOTHAYAM², M.SANGEETHA², D.AMUTHA²**

1. Lecturer, Department of Microbiology, Srimad Andavan Arts and Science College,
Trichy-620 005.Tamilnadu.India.
2. Department of Microbiology, Srimad Andavan Arts and Science College,
Trichy-620 005.Tamilnadu.India.

Corresponding Author mail: honey.sam@rediffmail.com

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Abstract: The aim of the present study is to investigate the antimicrobial activity and phytochemical analysis of different extracts of *Catharanthus roseus* leaves against the wound isolates. Two different solvents such as ethanol and methanol were used to extract the bioactive compounds from the leaves of *Catharanthus roseus* and screened for their antimicrobial activity against the isolated wound pathogens under well diffusion method. The maximum antibacterial activity was observed in crude Ethanolic extract of *Catharanthus roseus* against *Pseudomonas aeruginosa* (29mm). Qualitative analysis of phytochemical screening reveals the presence of Tannin, Flavonoids, alkaloids and Terpenoids.

Key words: *Catharanthus roseus*, Antibacterial activity and Phytochemical analysis

INTRODUCTION

A wound is a disruption in the normal anatomical structure and function of living tissue that can be caused by physical, chemical, Microbiological or immunological injury. Wounds represent a significant burden on the patients and health care professionals worldwide. Current estimates indicate that worldwide nearly 6 million people suffer from chronic wounds (Kumar *et al.*, 2007). Chronic wounds may even lead to multiple organ failure or death of the patient. Wound infections often contain multiple organisms, including both aerobic and anaerobic gram-positive cocci and gram-negative bacilli and yeast. Organisms like *Streptococcus sp*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus sp*, *Klebsiella*, *Enterobacter*, *Clostridium*, *Peptostreptococcus*, *Fusobacterium* and *Aeromonas* are highly predominant. (Henry, John, 2001). Many antibiotics used to treat wound pathogens but they also cause undesirable side effects. Bacterial resistance to antibiotic is a major therapeutic problem (Russell, 2002). Search towards safety medicines is must, scientists turn towards the herbal medicines to overcome side effects.

According to World Health Organization medicinal plants with various life sustaining constituents would be the best source to obtain a variety of potential, safe and novel drugs. (Natarajan *et al.*, 2003). Bioactive compounds are exploited on a large scale because of their more systemic and no toxic effects. *Catharanthus roseus L* (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 .it also act as a wound healer (Fischhof *et al.*, 1996 and Hindmarch *et al.*, 1991). In our investigation an attempt was made to analyze the antibacterial activity of *Catharanthus roseus* against the clinical wound isolates.

MATERIAL AND METHODS

Plant Materials

Matured leaves of *Catharanthus roseus* were collected from in and around Trichy District, Tamilnadu .The plant was authenticated by Prof. Dr. S. Ahmed John, and it has been deposited in the Herbarium, Department of Botany, Jamal Mohamed College, Tiruchirapalli, Tamil Nadu, India, for reference.

Leaves were surface sterilized with 70% ethyl alcohol followed by 0.1% mercuric chloride. *Catharanthus roseus* leaves were shade dried and powdered with mechanical blender. The air dried plants powders (100 g) were successively extracted with water and alcohol. The extracts were dried in vaccum desicator and were stored in a sterile container for further use (Kelmanson *et al.*, 2000).

Determination of antibacterial activity

Collection of Samples

Fifty Pus samples were collected from the Wound of patients admitted in Maruthi Hospital, Trichy, and Tamilnadu. Pus samples were collected from wall of an abscess with the help of sterile swab. Samples were transported to the laboratory for microbiological analysis.

Isolation and identification of wound samples

For the isolation of causative agents, the wound samples were inoculated in Blood agar and MacConkey agar. Plates were incubated at 37°C for 24-48 hrs. Colonies were analyzed by physiological and biochemical test for conformation (Koneman *et al.*, 1998).

Agar Well Diffusion Assay

Agar well diffusion method was followed by using Muller-Hinton Agar (MHA). The plates were seeded with 18 hours old culture of the isolates. The organic fractions were dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using sortorious syringe filter of pore size 0.22µm. various concentrations of the extracts (250µl, 500µl, 750µl and 1000µl) were added into the sterile 8mm diameter well. Incubation was made at 37°C for 24hrs. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The experiment was repeated thrice and the average values were calculated for antibacterial activity (Perez *et al.*, 1990)

Phytochemical screening

Ethanollic extracts were analyzed for the presence of alkaloids, saponins,

triterpenes, and/or steroids, flavonoids, and tannins according to standard methods (Harborne, 1973)

Thin layer chromatography

Preparation of TLC Plates

25x10 cm glass plates were washed with distilled water followed by smearing with acetone. After drying the plates were placed on the template in row. The slurry of silica gel G prepared with glass distilled water in the ratio 1:2 (w/v) was poured in the applicator. The glass plates were immediately coated with a layer of silica gel in 500 μ m thickness. The coated plates were activated at 80°C for 3 hours. Then the plates were stored in a plate chamber for further study. In that study chloroform and methanol (solvent) was used in 96:4 ratio.

Loading of substances:

The concentrated plant extract of 2.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. After, the solvent front reached approximately 18cm height. The plates were removed and allowed at room temperature for 30 min. Then the plates were also observed under UV light (240 and 300 nm) and recorded the Rf value of fluorescence substances (Anushia *et al.*, 2009).

RESULTS & DISCUSSION

Antibacterial activity:

Different concentrations (250 μ g to 1000 μ g) of all the four extracts were recorded. Crude ethanolic fractions of *Catharanthus roseus* were tested against all the isolates. *Pseudomonas aeruginosa* (29mm), were highly sensitive to the ethanol fraction followed by *Staphylococcus aureus* (25mm), *Escherichia coli* (24mm), *Klebsiella pneumoniae* (18mm) and *Streptococcus pyogens* (15mm). In case of Crude methanolic extract *Staphylococcus aureus* (25mm), *Klebsiella pneumoniae* (24mm), *Escherichia coli* (21mm), *Pseudomonas aeruginosa* (20mm), *Streptococcus pyogens* (16mm) also shows their sensitivity. Soxhlet methanolic extract shows highest sensitivity against *Staphylococcus aureus* (16mm) followed by *Escherichia coli*(13mm), *Klebsiella pneumoniae* and *Streptococcus pyogens* (12mm) *Pseudomonas aeruginosa* (9mm). Under Soxhlet ethanolic extraction sensitivity was recorded in *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Streptococcus pyogens* as 22mm,21mm,18mm,15mm and 12mm respectively (Table 1).

Phytochemical analysis:

Phytochemical such as Tannin, Flavonoids, Alkaloids, saponin and Terpenoids were reported from *Catharanthus roseus*. Alkaloids and flavonoids found to be present in all the four extracts whereas Terpenoids present in crude ethanol. Tannin shows their presence in Crude methanol and ethanol. Saponin present in soxhlet ethanolic and methanol. Piovan and Fillipini also reported the presence of various alkaloids, viz. Vincristine, Vinblastine, Yohimbine is an alkaloid and another flavonoids hirsutidin in *Catharanthus roseus* (Table 2).

Thin layer chromatography:

Crude ethanol (Rf 0.9) and soxhlet ethanol (0.77) shows brown colour spots whereas yellow spots were identified in crude methanol extracts .Olive green (Rf 0.15) spot was observed in soxhlet methanol extract (Table 3).

Table 1

Antibacterial activity of *Catharanthus roseus* against isolated wound Pathogens.

Sr. No	Organisms	Concentration of Extracts /Zone of inhibition in mm															
		Crude methanolic extract (µl)				Crude ethanolic extract (µl)				Soxhlet methanolic extract (µl)				Soxhlet ethanolic extract (µl)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
1.	<i>Escherichia coli</i>	12	14	18	21	15	18	21	24	5	7	10	13	9	10	12	15
2.	<i>Klebsiella pneumoniae</i>	14	17	20	24	12	15	16	18	-	4	8	12	-	5	10	18
3.	<i>Pseudomonas aeruginosa</i>	12	15	18	20	16	18	23	29	-	5	7	9	9	15	18	22
4.	<i>Staphylococcus aureus</i>	12	17	21	25	19	20	23	25	5	7	9	16	8	14	17	21
5.	<i>Streptococcus pyogens</i>	8	12	14	16	9	10	12	15	5	7	10	12	-	-	6	12

Table 2
Phytochemical screening of *Catharanthus roseus* extracts

Sr. No	Phytochemicals	Crude methanolic extract	Crude ethanolic extract	Soxhlet methanolic extract	Soxhlet ethanolic extract
1	Alkaloids	+	+	+	+
2	Flavanoids	+	+	+	+
3	Steroids	-	-	-	-
4	Glycosides	-	-	-	-
5	Terpanoids	-	+	-	-
6	Tannins	+	+	-	-
7	Quinine	-	-	-	-
8	Coumarin	-	-	-	-
9	Starch	-	-	-	-
10	Saponins	-	+	-	+
11	Phenols	-	-	-	-

Table 3
Rf value of various extracts of *Catharanthus roseus* Leaves.

Sr. No	Extracts	Observation of spots	Rf value
1	Crude Methanol	Yellow	0.4
2	Crude Ethanol	Brown	0.9
3	Soxhlet Methanol	Olive green	0.15
4	Soxhlet Ethanol	Brown	0.77

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