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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *Catharanthus roseus* PLANT EXTRACTS

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ABSTRACT: ***Catharanthus roseus* is one plant recognized well in ayurveda. The therapeutic role of plant materials in multi-disease conditions has necessitated its continued exploration and consumption. This is led us to evaluate the antimicrobial property and phytochemical components of *Catharanthus roseus* plant extracts. Using the protocols, the phytochemical screening of different extracts namely methanol and ethyl acetate extracts of *C.roseus* plant was done. This study was conducted to determine the effect of ethyl acetate and methanol extract on *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi and Candida albicans*. The ethyl acetate extract of cantharanthus roseus showed no activity against all 4 pathogens. The 10ul methanolic extract showed good activity against *Staphylococcus aureus* comparing to other oraganisms.**

Index terms – Antimicrobial activity, *B. subtilis, C. albicans, C. roseus, S. aureus, S. typhi.*

I. INTRODUCTION

Catharanthus roseus (Madagascar periwinkle) is a species of Catharanthus native and endemic to Madagascar synonyms include vinca rosea. Ammocallis rosea, and lochnera rosea; other English names occasionally used include cape periwinkle, rose periwinkle, Rosy periwinkle, and “old–maid”. In india it is known as “nithyakalyani” Studies in the 1950s revealed that *C.roseus* contained 70 alkaloids, many of which are biologically active.(Parameswari. P* *et al* 2015). While initial studies for its use in diabetes mellitus were disappointing, the discovery that it caused myelosuppression (decreased activity of the bone marrow) led to its study in mice with leukemia, whose lifespan was prolonged by the use of a vinca preparation. The other scientific name for “Vinca rosea” is *C.roseus*. Vincristine is vinca alkaloids from *C.roseus*. It is a mitotic inhibitor and used in cancer chemotheraphy.(parameswari.P* *et al* 2015).

C.roseus is an important medicinal plant of the apocynaceae family which contains more than 70 different type of alkaloids and chemotherapeutic agents that are effective in treating various type of cancer – breast cancer, lung cancer, ulterine cancer, melanomas, hodgkin's and non – hodgkin's lymphoma.(Parameswari. P* *et al 2015*) Generally, it is known as Vinca rosea, Ammocallis rosea and Lochnera rosea. *C.roseus* is an Indian originated herb which grows wild in the Indian subcontinent in southern asia.

C.roseus are cultivated two common names, which is named on the basis of their flower colours, pink : Rosea, White : Alba. Traditionally, leaves of *C.roseus* are used as medicine for the treatment of following diseases, they menorrhagia, rheumatism, Dyspepsia, indigestion, dysmenorrheal, diabetes, hypertension, cancer, menstrual disorder, skin diseases, bleeding diarrhea and has sedative and antiviral properties.

The plant has historically been used to treat a wide assortment of diseases. It was used as folk remedy for diabetes in Europe for centuries. In India, juice from the leaves was used to treat wasp stings. In Hawaii, the plant was boiled to make a poultice to stop bleeding. In china, it was used as an astringent, diuretic and cough remedy. In central and south America, it was used as a homemade cold remedy to ease lung congestion and inflammation. Throughout the That plant leaves contains more than 70 types of chemical constituents such as indole type of alkaloids, ajmalicine, serpentine and reserpine. Due to presence of those alkaloids in *C.roseus* , it has antihypertensive and antispasmodic properties.(Kabesh, K. *et al* 2015) One of the important types of alkaloids is the vinblastine produced from *C.roseus* to produce modern chemotherapeutic agent for their pain – relieving properties.

Apocyanaceae is native to the Caribbean historically used to treat assortment of diseases. European herbalists used the plant for conditions as varied as headache to a folk remedy for diabetes.(Kabesh, K. *et al* 2015) Wound is a disrupt an anatomical structure of normal living tissues and its functions due to physical, chemical, microbiological or immunological injury. It acts as a wound healer.(Kabesh, K. *et al 2015).*

II. MATERIALS AND METHODS

2.1 Collection of plant materials

Fresh leaves, flowers and stem of *C.roseus* were collected from the garden. The plant materials were washed thoroughly with tap water and then with sterilized distilled water. The plant materials were dried in shade at room temperature for about 1 hour and used as the raw materials for the extraction of antimicrobial compounds from the plant.

2.2 Preparation of catharanthus plant extracts

The dried leaf, stem, and flower (15mg) were powdered manually. Three hundred ml of methanol and ethyl acetate separately were used for the extraction of 15mg in the soxhlet apparatus followed by the standard procedure. The plant material was loaded in the inner tube of the soxhlet apparatus and the fitted into a round bottomed flask containing methanol and ethyl acetate separately. The solvents were boiled gently (40°c) over a heating mantle using the adjustable rheostat. The extraction continued for 8 hour and the solvent was removed at the reduced pressure with the help of rotary vaccum evaporator to yield a viscous dark green residue of each of methanol and ethyl acetate of stem, flower, and leaf extracts.

2.3 Antibacterial assay

The following bacterial strains were used in this study.., *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella*. Sterile Muller – Hinton Agar (MHA) was prepared. diluted bacterial cultures was spread evenly over the entire surface of the agar plates using sterile cotton swab. The plates were incubated for 15min at room temperature. Using a well puncher 1-5 wells of 5mm diameter were made for drug loading. The plant extracts (methanol and ethyl acetate) were prepared and dissolving in dimethyl sulfoxide (DMSO). The wells were labeled approximately and each well were loaded with 10µl, 20µl plant extracts using a micro-pipette. The plates were incubated at 37°C for 24 hours. Antibacteial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria.

2.4 Antifungal assay

The following fungal strains were used in the study. *Aspergillus niger, Candida albicans*. Well diffusion method was carried out by using standard protocol. Plant extract (2 mg) was dissolved in a sterile tube with 1 ml of 2% dimethyl sulfoxide (DMSO) to obtain a stock solution. Sabouraud dextrose agar was prepared. The fungal spores was spread over the entire surface of the agar plates using sterile cotton swab. The plates were incubated for 15min at room temperature. Using a well puncher 1-5 wells of 5mm diameter were made for drug loading. The plant extracts (methanol and ethyl acetate) were prepared and dissolving in dimethyl silfoxide (DMSO). The wells were labeled approximately and each well were loaded with 10µl, 20µl plant extracts using a micro-pipette. The plates were incubated at 27±2°C for 7 days and fungal growth (mm) in each plates was measured and the diameter of zones of inhibition against the fungi will be measured.

2.5 qualitative analysis of the plant extracts for phytoconstituents

The extracts of leaves and flowers of *Catharanthus roseus* were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant. Alkaloids, protein, tannin, saponins, terpenoids, steroids, carbohydrate tests are should be done.

2.6 Thin layer chromatography

The methanol and ethyl acetate extracts were added as spot using capillary tubes on the one end of the thin layer plate at above 1 cm. Plate was allowed to air dry, then it was placed in a beaker containing solvent ethyl acetate : methanol in the ratio of 6 : 4. The sample were allowed to run towards the other end of the plate. The sheet was removed and allowed it to air dry and 2 % of ninhydrin was sprayed and again allowed to air dry for 10 mins. The plate was then visualized under the uv light and violet colour spot was absorb on the plate.

III. RESULT AND DISCUSSION

Antibacterial activity, the plant extracts were prepared in dimethyl sulfoxide (DMSO). The methanol extract of *C. roseus* showed antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa, s. typhi*. It showed maximum inhibition zones. Among different concentration, 20µl.,1:1 & 1:2 of plant extracts of C. roseus tested. The highest inhibition zones occurs in *S. aureus, B. subtilis, S. typhi* . The maximum inhibitory effect in terms of the area of the zone of inhibition was *S. aureus* 1:1 (20mm), *B.subtilis* 1:1 (19mm), *S.typhi* 1:2 (19mm) in the extract of *C. roseus*. The growth inhibition exhibited by plant extract and DMSO. The ethyl acetate extract of *C. roseus* does not showed antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa, S. typhi.*

Antifungal activity, the plant extracts prepared in dimethyl sulfoxide (DMSO). The methanol extract of *C. roseus* showed antifungal activity against *C. albicans*. It showed maximum inhibition zones. Among different concentration, 20µl.,1:1 & 1:2 of plant exracts of *C. roseus* tested. The growth inhibition exhibited by plant extract and DMSO. The ethyl acetate extract of *C. roseus* does not showed antifungal activity against *C. albicans*. The maximum area of the inhibition was 1:1 (18mm) in the extract of *C. roseus* against *C. albicans.*

Phytochemical screening, the preliminary phytochemical screening test of *C. roseus* plant extracts revealed the presence of tannin, saponins, alkaloids, protein, terpenoids, carbohydrates. Besides alkaloids were present high amount. But steroid was completely absent in both solvent. Tannin is present in both extracts. In saponins the methanol is positive. In protein the ethyl acetate is positive. In carbohydrates both are positive.

Thin layer chromatography, compound identification was done using silica gel coated thin layer chromatography in methanol and ethyl acetate extracts. Light violet colour at visible light mode was present in the tracks of paper identified as compound in the sample.

Table.1: Phytochemical screening:

PLANT EXTRACTS	METHANOL	ETHYL ACETATE
Test for alkaloids	Positive	Positive
Test for protein	Negative	Positive
Test for tannin	Positive	Positive
Test for saponins	Positive	Negative
Test for terpenoids	Positive	Negative
Test for steroids	Negative	Negative
Test for carbohydrates	Positive	Positive

Table.2: Antibacterial activity of methanol extracts

ORGANISM	EXTRACTS	ZONE OF INHIBITION	
		1:1	1:2
S. aureus	Methanol	20mm	19mm
P. aeruginosa	Methanol	13mm	15mm
B. subtilis	Methanol	19mm	15mm
S. typhi	Methanol	15mm	19mm

Table.3: Antifungal activity of Methanol extracts

ORGANISM	EXTRACT	ZONE OF INHIBITION	
		1:1	1:2
C. albicans	Methanol	18mm	14mm

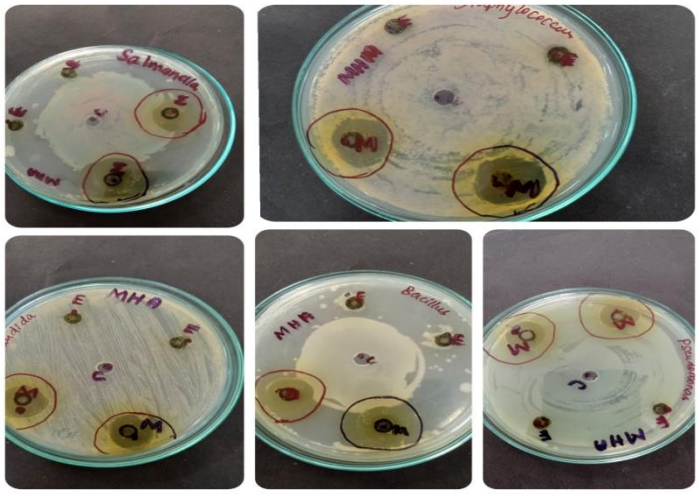

Figure.1

Antimicrobial activity of C. roseus plant extract against *B.subtilis, S.aureus, S.typhi, C.albicans*

Thin layer chromatography

Compound identification was done using silica gel coated thin layer chromatography in methanol and ethyl acetate extracts. Light violet colour at visible light mode was present in the tracks of paper identified as compound in the sample.

Retention factor (thin layer)

Ethyl acetate

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}} = \frac{2.9}{5.8} = \textbf{0.5 - Aspargine}$$

Methanol

3 / 5.3 = 0.56 = Tryptophan

IV. SUMMARY AND CONCLUSION

In the present investigation medicinal plant catharanthus roseus was collected from the garden. The plant leaf, flower, stem were subjected for organic solvent extract preparation to accomplish in vitro bioassays. The phytochemical analysis of all organic solvent extracts revealed the presence varying degree of major phytoconstituents such as alkaloids and tannin.

The antibacterial and antifungal activity of the plant extracts in the in vitro level. All the four extracts from selected plant species, all the extracts were active at varying degree against bacterias with an exception of ethyl acetate extract, which showed no bacterial inhibition.

The methanolic plant extract of C. roseus was found to actively impeding with bacteria at varying inhibitory levels. The methanolic plant extract of C. roseus were observed to be more active against the fungal species used in the assay.

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VI. CONFLICTS OF INTEREST

The author has no conflicts of interest to publish this research article in this journal.

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