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CHARACTERIZATION OF ANTIBACTERIAL, ANTICANCER PROPERTIES AND BIOACTIVE COMPOUNDS OF METHANOLIC LEAF EXTRACT OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Phytomedicines have been always the main principle form of medicine since traditions in India. Plants are the source of enormous drugs which are used directly/indirectly. *Catharanthus roseus* is one of the medicinal plants is a member of Apocynaceae family and the whole part of plant is an evergreen shrub and herbal in nature. It has anti-inflammatory, anti-bacterial, anti-fungal, anti-diabetic, anti-cancer, anti-oxidant, anti-hypertensive and anti-mitotic activities due to the presence of indole alkaloids and other biologically active compounds. The methanolic leaf extract was extracted by soxhlet extraction process. Thin Layer Chromatography was performed for methanolic leaf extract and the presence of alkaloid was detected by using Cerium Ammonium Sulphate as spraying reagent. The extract was subjected to GC-MS analysis and the identification of compounds was done by comparing the chromatogram, peak value of the unknown compounds with entries in NIST database. Five compounds were identified such as Vitamin d_3, 14-Hydroxy-14-methyl-hexadec-15-enoic acid, methyl ester, Ethanoperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester, 10-Octadecenoic acid, methyl ester, Dasycarpidan-1-methanol, acetate (ester). In which 10-Octadecenoic acid, methyl ester being the major compound in *C. roseus*. The anti-bacterial activity of the extract was performed for various pathogenic bacteria such as *Staphylococcus aureus, Eschericia coli, Bacillus megaterium, Bacillus subtilis, Bacillus flexus, Bacillus* sp. (KF781350), *Bacillus* sp. (KF772943), *Bacillus* sp. (KF746386), *Pseudomonas* sp. (KF762388), *Staphylococcus* sp. (KF782792), *Klebseilla pneumoniae, Pseudomonas stutzeri, Pseudomonas fluorescens, Proteus vulgaris, Enterobacter aeruginosa* by agar well diffusion method and the obtained results were compared with streptomycin as a positive control. Anticancer activity of methanolic leaf extract of *Catharanthus roseus* was determined through Colorimetric MTT assay against human breast adenocarcinoma (MCF-7) cell line. Study confirms the antibacterial activity and anticancer activity of methanolic leaf extract of *Catharanthus roseus* and identification of bioactive compounds which have the applications in therapeutic interventions

KEYWORDS: *Catharanthus roseus*, Methanolic Leaf

INTRODUCTION

Herbal drugs have the antibiotic properties and have no side effects. The medicinal plants are the source of many potent and powerful drugs for relief from illness. Particular secondary metabolites present in the medicinal plants are used for various disease treatments. Plant derived substances are used as pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction method used pharmaceutically for active compounds separation from inactive compounds by using selective solvents. Phytotheraphy is the study of the use of extracts from natural origin as medicines or health promoting agents. The term “phytochemicals” refers to a wide variety of compounds made by plants. Phytochemicals are

non-nutritive plant chemicals that works with nutrients dietary fiber to protect one against disease**.** Alkaloids that are isolated from *C. roseus* are found to be hypotensive, sedative and possess tranquilising and anti cancerous properties. *Catharanthus roseus* is a medicinal plant belonging to the family Apocynaceae. It is commonly known as Madagascar periwinkle, vinca rosea.. It exhibits the anti-cancer, anti-bacterial, anti-mutagenic, anti-fungal, anti-viral, anti-hypertensive activities due to the presence of indole alkaloids like vinblastine, vindoline, vincristine, catharanthine, Ajmalicine, etc.

Mechanism of Action of Indole Alkaloids from *Catharanthus roseus*

Vinca alkaloids are indole alkaloids which produce a wide range of biochemical effects in cells and tissues, the principal mechanisms of cytotoxicity relate to their interactions with tubulin and disruption of microtubule function. The vinca alkaloid's mechanism in a nutshell: by occupying tubulin's building block structure, vinca alkaloids prevent cancer cells from successfully dividing. Many of effects that do not involve microtubule disruption occur only after treatment of cells with clinically irrelevant doses of the Vinca alkaloids, whereas nanomolar concentrations induce typical anti microtubule effects. Additional support of anti microtubule actions, or more specifically, anti mitotic actions, as being the principal cytotoxic effect of the Vinca alkaloids is that the dissolution of the mitotic spindle apparatus, appearance of mitotic figures, and cytotoxicity strongly correlate with both the duration and concentration of drug treatment.

MATERIALS AND METHODS

Catharanthus roseus leaves were collected. The leaves were washed with distilled water and were air dried in shade.

Extraction of *Catharanthus roseus* Leaves by Soxhlet Extraction Method

50g of sample was taken in a thimble of the soxhlet apparatus for extraction using 80% methanol as solvent. The chlorophyll was removed by centrifuge the crude extract with equal volume of dichloromethane at 4000 rpm for 10 minutes. The final concentrated leaf extract was stored in the sterile tubes for further studies.

Thin Layer Chromatography (TLC)

A drop of methanolic leaf extract of *Catharanthus roseus* was applied onto the TLC plate and allowed for migration. After migration of sample, the plate was air dried and sprayed with Cerium Ammonium Sulphate - chromogenic reagent (1% CAS in 85% phosphoric acid).

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of methanolic leaf extract was quantitatively performed by Gas Chromatograph-Mass Spectrometer. Hp5-Ms column was used. High pure helium was the carrier gas and the flow rate was 1ml/min. Temperature at the front inlet was 220°C. The oven temperature used was maintained at 50°C to 250°C, gradually raised at 10°C/min. Ion chamber and GC interface temperature was maintained at 250°C. The identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST database) library and by direct comparison with published data.

Agar Well Diffusion Method

After sterilization 20ml of nutrient agar medium was poured into the plates under aseptic conditions and allowed to solidify. After solidification, all the plates were marked and were inoculated with 100µl of respective bacterial culture.

Then the plates were incubated for 5minutes in inverted position and the wells were made using gel puncture on the agar plates. 20µl of methanolic leaf extract was poured into the wells. Similarly 20µl of streptomycin sample (Conc: 500µg/ml) was also poured into the wells of plates containing different bacterial strains. All the plates were incubated at 37ºC for 24 hours.

Anticancer Activity

All the cells were grown in standard cell medium (RPMI 1640) supplemented with 5% fetal bovine serum in a 5% CO2 atmosphere. The cells were then transferred into microplate at the concentration of 10^2 cells per well for cytotoxicity test of the plant extract. At 48 h, proliferation was measured by the colorimetric MTT assay. The half maximal inhibitory concentration (IC50) value was calculated from the following formula:

$$\log_{10}(IC_{50}) = \frac{\log_{10} C_L (I_H - 50) + \log_{10} C_H (50 - I_L)}{I_H - I_L}$$

IC50 = 10 log10 (IC50) Where: IH : I% above 50% IL : I% below 50% CH : High drug concentration CL : Low drug concentration.

Colorimetric MTT Assay

Colorimetric MTT (3-(4, 5-dimethythiazol-2-yl)-2,5- diphenyl tetrazolium bromide) assay was carried out as described by Mosmann (1983). Ten micro liters of MTT solution (5 mg/ml) was added to all wells of 96 wells micro plate followed by 4 h incubation at 37°C. Acid isopropanol was added to all wells to dissolve the dark blue crystals. The micro plate was then read with an ELISA reader at wavelength 570 nm within 1 h after addition of isopropanol.

RESULTS

Extraction of *Catharanthus roseus* Leaves by Soxhlet Extraction Method

The crude extract of *Catharanthus roseus* leaves was extracted by soxhlet extraction and the chlorophyll was removed by using dichloromethane are shown in

a) Soxhlet Extraction Process

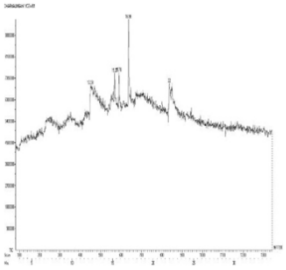
b) Stages of Chlorophyll Removal

Thin Layer Chromatography (TLC)

TLC was performed for methanolic leaf extract and the result was obtained. CAS reagent has shown the pink around yellow colour band on TLC plate shown in figure 3.2. R_f value was calculated for the identified band as 0.84. R_f value = Distance travelled by the compound / Distance travelled by the solvent. R_f values for alkaloid standards were compared with that calculated R_f value for the methanolic leaf extract and was found that value related with vindoline alkaloid.

Gas Chromatography- Mass Spectrometry (GM-MS)

The GC-MS analysis of methanolic leaf extract was quantitatively performed by GC-MS (The GEOL GCMATEII System). variety of bioactive compounds and compositions were also screened. The GC/MS spectral results and the comparison of results with library search successfully enabled the identification of five compounds: Vitamin $d_{3,}$ 14-Hydroxy-14-methyl-hexadec-15-enoic acid, methyl ester, Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1, 3-dioxolan-2-yl)ethyl]pentyl ester, 10-Octadecenoic acid, methyl ester, Dasycarpidan-1-methanol, acetate (ester). In which 10-Octadecenoic acid, methyl ester being the major compound in *C. roseus*. The structures of all compounds were given in figure and the composition % of identified compounds was given in table.



The GC-MS Chromatogram of Bioactive Compounds in the Methanolic Leaf Extract

Antibacterial Activity

Antibacterial activity of isolated methanolic leaf extract of *C. roseus* was performed against various pathogenic bacteria such as Staphylococcus aureus, Eschericia coli, *Bacillus megaterium, Bacillus subtilis, Bacillus flexus, Klebseilla pneumoniae, Pseudomonas stutzeri etc.,* by agar well diffusion method. The zone of inhibition of every plate was measured. The results were shown in the figure and the zone of inhibition of the methanolic leaf extract against various bacterial pathogens was shown in the table.

Table 1: The Zone of Inhibition of Methanolic Leaf Extract of *C. roseus* against Various Bacterial Pathogens

Bacterial Isolates	Zone of Inhibition	
	Streptomycin (500µg/Ml) (Dia Inmm)	Methanolic Leaf Extract (Dia Inmm)
S. aureus	32	29
E. coli	26	-
B. megaterium	35	27
B. subtilis	29	18
B. flexus	26	24
Klebseilla pneumonia	25	-
Pseudomonas stutzeri	32	28
Proteus vulgaris	26	-
Pseudomonas fluorescens	28	19
Enterobacter aeroginosa	12	-

Table 1: Contd.,

Bacillus sp. (KF781350)	39	32
Staphylococcussp. (KF782792)	38	34
Pseudomonas sp. (KF762388)	18	-
Bacillus sp. (KF746386)	37	30
Bacillus sp. (KF772943)	31	29

A) Antibacterial Activity Against S. Aureus (Control)

b) Antibacterial Activity Against S. Aureus (Sample)

a)Anti bacterial Activity Against *B. megatarium* (Control)

b) Antibacterial Activity Against *B. megatarium* (Sample)

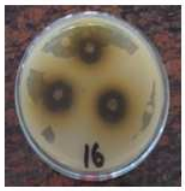
a) Antibacterial Activity Against P. *fluorescens* (Control)

b) Antibacterial Activity Against *P. fluorescens* (Sample)

a) Antibacterial Activity Against *B. sp*(KF781350) (Control)

b) Antibacterial Activity Against *B.* sp. (KF781350) (Sample)

a) Antibacterial Activity Against B. sp (KF746386)



b) Antibacterial Activity Against B. sp (KF746386)

a) Antibacterial Activity Against *S. sp* (KF78279)

b) Antibacterial Activity Against S. sp (KF782792)

a) Antibacterial Activity Against *P. stutzeri* (Control)

b) Antibacterial Activity Aganist *P. stutzeri* (Sample)

a) Antibacterial Activity Against *K. pneumoniae* (Control)

b) Antibacterial activity against *K. pneumoniae* (Sample)

a).Antibacterial Activity Against *P. sp.(*KF762388*)* (Control)

b) Antibacterial Activity Aganist *P. sp*(KF762388) (Sample)

a)Antibacterial Activity Against *E. aeruginosa* (Contol)

b) Antibacterial Activity Aganist *E. aeruginosa* (Sample)

a)Antibacterial Activity Against *P. vulgaris* (Control)

b) Antibacterial Activity Against *P. vulgaris* (Sample)

a)Antibacterial Activity Against *E. coli* (Control)

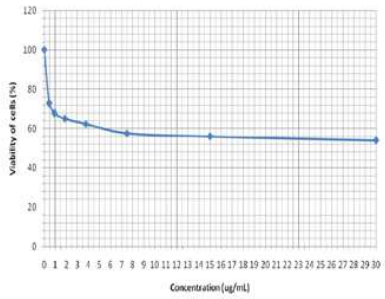
b) Antibacterial Activity Against *E. Coli* (Sample)

Figure 1: Antibacterial Activity of Methanolic Leaf Extract of *C. roseus* with Streptomycin as Positive Control

Anticancer Activity

Anticancer activity of *C. roseus* leaf extract was anticipated following the result of cytotoxicity test against human breast adenocarcinoma (MCF-7) cell line. Viability of cells decreased sharply to 72% when concentration of the extraction

was applied at 0.5 µg/ml. Patterns of decrease was rather constant and reach up to 54% viability at the concentration 30 µg/ml.



Graph 1: Anticancer Activity of Methanolic Leaf Extract of *C. roseus*

CONCLUSIONS

India is one of the most promising regions for discovering novel biologically-active substances from its flora. Herbal medicine in treatment of various severe diseases as complementary and alternative therapy is one of the most extensively studied areas of recent research. Task of modulating the adverse affect is feasible only through requisite perspective regarding the specificity of these natural molecules with combination therapy. The increased economic burden along with unintentional side effects limits the research and development of pure, novel phytoceuticals as therapeutic drug against the cancer and other severe diseases. Hence bioprofiling of least explored plants can aid in the development of novel therapeutic agents. As an alternative, purified crude extracts of significant plants as dietary supplements and botanical drug products are generating increased acceptance. In the current study the antibacterial and anticancer potential of *Catharanthus roseus* was studied in detail and the chemical compositions of methanolic leaf extract of *C. roseus* were identified. The methanolic leaf extract of *C. roseus* shows significant antibacterial and anticancer activities. The extract is antibiotic in nature and it has the cytotoxic effect on MCF-7 cell line. More efforts are needed to explore potent anticancer plants from the nature and save humans around the world from various injurious diseases

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