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STUDIES ON PHARMACOLOGICAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Plant based drugs are in great demand due to their major contributions in pharmaceutical industries. *Catharanthus roseus* popularly known as Madagescar periwinkle is an evergreen shrub or herbaceous plant which exhibits the anti cancer activity due to the presence of vincristine and vinblastine. More than 3000 plants species were reported for the treatment of diabetics, blood pressure, asthma, constipation, cancer and menstrual problem. The main focus of the present study is to investigate the phytochemical, antimicrobial, and anticancer activity of *C. roseus.* Phytochemical screening reveals the presence of alkaloids, phenols, saponins and proteins. The alkaloids like vinblastine and vincristine are mainly present in the aerial part of *C. roseus*. The methanol extracts showed high antimicrobial activity against human pathogenic organisms. The extract also showed significant anticancer activity against L929 mouse Fibroblast cell lines type L, so it is considered as milestone in cancer chemotherapy. The studies implicates that bio-active compounds of *C. roseus* could potentially be exploited as anti-bacterial agents and anti-cancer agents.

KEYWORDS: *Cathranthus roseus*, Phytochemical analysis, Antibacterial activity, Vinblastin, Anti-cancer, L929.

INTRODUCTION

Catharanthus roseus is an important medicinal plant of the family Aposynaceae. Emerging and reemerging infectious diseases and spread of deadly drug- resistance strains possess a

challenge to public health care services. The emerging resistance of the antibiotics has hampered the place by which newer antibiotics are being introduced into the public domain. This drives the discovery of novel antimicrobial therapeutic agents from the medical herbs (Gootz, 1990).[1] A large population of plant based compounds are used as lead molecules in drug discovery, this implicates that phytochemicals play a critical role in diversity oriented synthesis (DOS) of natural product like pharma- compounds (Marcaurelle and Johannes, 2008).[2] Traditionally, *Cathranthus roseus* has been used in medicine to treat diabetics and high blood pressure. The anti cancer drugs vincristine and vinblestine are obtained from alkaloids of *C. roseus.* Besides anti cancer activity, alkaloids from this plant are known for their anti hypertensive and anti spasmodic properties (Verpoorte -2002).[3] Ironically, amidist such anormous data only handful of publications details with the antimicrobial potential of this plant (Govindaraji, 2007).[4] In view of the medical importance of *C. roseus*, the extracts (Benzene, chloroform, ethanol, ethyl acetate and methanol) of *C. roseus* were aimed assess the phytochemical analysis, anti bacterial activity against human pathogenic bacteria and anti cancer activity.

MATERIALS AND METHOD

Fresh leaves of *Catharanthus roseus* was collected and washed under running tap water, air-dried at room temperature and then reduced to coarse powder using an electric blender. The powders obtained were stored in airtight containers prior to extraction. The dried powder of leaves was successively extracted using Soxhlet apparatus involving different solvents. The soxhlet extractor was placed on to a flask containing 250ml of solvent. Solvents such as Benzene, chloroform, ethanol, ethyl acetate and methanol are used for extraction. They were run in a soxhlet apparatus for 12-24 hours after 5 cycles the extracts were collected from the distillation flask. The extract obtained was evaporated completely for dryness. 2g of dried sample were mixed with 10ml of DMSO and stored for further studies (Sukhdev Swami Handa *et al.*, 2008*).*[5]

Screening of phytochemicals

Qualitative chemical tests were carried out using the extracts obtained from plant in solvents namely Benzene, chloroform, ethanol, ethyl acetate, methanol and aqueous using standard procedures to identify the constituents.[6-10]

Antimicrobial screening

Antibacterial screening of plant extracts

The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method. Pathogenic bacteria (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology and Chandigarh. The bacterial strains *Enterobacter aerogenes* (MTCC 2823), *Bacillus subtilis* (MTCC 121), *Pseudomonas aeruginosa* (MTCC 4676), *Escherichia coli* (MTCC 40) and *Salmonella typhi* (MTCC 3224) were spreaded over the medium. Filter paper disc of uniform size (4mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Bacterial colonies were allowed to grow overnight at 37^0c, then the inhibition zone around the disc was measured (Bauer AN, 1966).[11]

Thin layer chromatography

TLC was conducted on silica gel G coated plates with a view to ascertain the number of phytochemical constituents present in the ethanol extract. Chromatographic studies were performed in the following manner.[12-14]

Thin layer preparation was made by using silica gel slurry (prepared by mixing 25g of silica gel in methanol and chloroform in the ratio 2:1 in 50ml). The plate was air dried and kept in hot air oven at 80^0C for 1 to 2 minutes. Sample was loaded in plate using capillary tube. The spots should be generally placed above 1.5cm from the bottom edge of the plate. Plate development was done by using solvent, which is the mobile phase (93ml Toluene + 7ml ethyl acetate) was taken in a glass tank. It was closed with a glass plate. The TLC plate was placed vertically on the tank. It should be noted that the sample spot should not touch the solvent. The tank was again covered and the set up was kept for another 4 to 5 hours until the mobile phase travels more than ¾ of the plate. Then the plate was taken out and allowed to dry. The solvent front was immediately marked.

Component detection

After drying, the plate was kept in chamber containing iodine till a brown colour develops (for the detection of saponins) or was sprayed with Folin- Ciocalteau reagent till an orange colour develops (for the detection of alkaloids). Then the distance moved by sample from the bottom edge of the plate was measured. From this the R_f value was measured by the formula,

$$R_f = \frac{\text{Distance moved by the sample (cm)}}{\text{Distance moved by the solvent (cm)}}$$

Anti-Cancer Activity

Determination of invitro antiproliferative effect of extracts on cultured L929 Cells. L929 cells were maintained in eagle media supplemented with 10% FBF and grown in 5% carbon dioxide. The % difference in viability was determined by standard MTT assay after 24 hours of incubation (Daniel sypniewsket *al.*, 2013).[15]

The cell culture suspension was washed with 1x PBS and 300 µl cell culture with plant extract (SAB6 and SAB43) were taken in a sterile micro titter plates and incubated for 4 hrs in optimum conditions. Then 200µl MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) solution was added to the culture flask (MTT 5 mg/volume dissolved in PBS, Filtered through 0.2µm filter before use). Then incubated at 37^{o}c for 3 hrs, removed all MTT solution, washed with 1x PBS and added 300µl DMSO to each culture flask, incubated at room temperature for 30 mins until all cells get lysed and homogenous colour was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 mins to precipitate cell debris. OD was measured at 540 nm using DMSO blank.

RESULTS

Phytochemical Analysis

In the preliminary phytochemical screening eight compounds (alkaloids, cardiac glycosides, flavanoid, phenol, protein, saponins, tannin and terpenoids) were tested for their presence or absence in five different extracts of *Catharanthus rosueus*. The results were summarized in table 1.

Table: 1. Phytochemical Screening of *Catharanthus rosueus.*

Test	Benzene Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract	Methanol Extract	Aqueous Extract
Alkaloids	+	+	+	_	_	_
Cardiac Glycosides	_	+	+	+	_	_
Flavanoid	_	_	+	+	+	_
Phenol	_	_	_	+	+	_
Protein	+	_	_	-	_	_
Saponin	+	+	_	+	+	_
Tannin	+	+	+	_	_	_
Terpenoids	_	+	_	_	_	_

+' indicates positive and '-'indicates negative.

Thin Layer Chromatography

The bioactive compounds from crude chloroform extract were separated by TLC. The Rf values of the spots (0.77 for vinblastine and 0.74 for vincristine) derived upon TLC of the crude extract using chloroform:methanol (8:2) solvent system when compared with the standard Rf value of vinblastine and vincristine were found to be identical when sprayed with ceric ammonium sulphate reagent. The identified compounds are shown in table: 2

Table 2: Thin layer chromatography.

Colour of compound	Rf value(cm)
Dark purple	0.77
Dark violet	0.74

Antibacterial Activity

The antibacterial activity of *C. roseus*were studied against five human pathogenic microorganism. Methanol extract of *Catheranthus roseus* showed highest antibacterial activity against *Salmonella typhi* (2 cm). The least activity was showed against *P. aerogenosa* (1 cm). Benzene extract showed highest antibacterial activity against *E. aeroginosa* (1.2 cm) and did not show any activity against *B. subtilis*. Chloroform extract showed maximum antibacterial activity against *B. subtilis* (1.3cm) and ethanol extract showed highest activity against *E. coli* (1.8 cm) In ethyl acetate extract of *Catheranthus roseus* showed highest antibacterial activity against *S. typhi* (1.6 cm). The results of antibacterial screening are summarized in table 3.

Table 3: Antibacterial Activity of *Catheranthus roseus* against human pathogens.

S. No.	Bacteria	(Plant extract) Zone of inhibition (cm)				
		Benzene	Chloroform	Ethanol	Ethyl acetate	Methanol
1	*E.coli*	0.5	1.2	1.8	1.3	1.5
2	*Enterobacter aerogenosa*	1.2	1.2	1.5	0.5	1.1
3	*Pseudomonus aerogenosa*	1.1	1	1.2	1.3	1
4	*Salmonella typhi*	1	1.1	Nil	1.6	2
5	*Bacillus subtilis*	Nil	1.3	0.7	1.3	1.1

Anticancer Activity

The cytotoxic effect of chloroform extract on L929 cell were tested by MTT cell viability assay. The viability decreases with increasing concentration of chloroform extract of

Catharanthus roseus. The viability of the carcinoma cells with an increase in concentration of the extract is shown in the following graph.

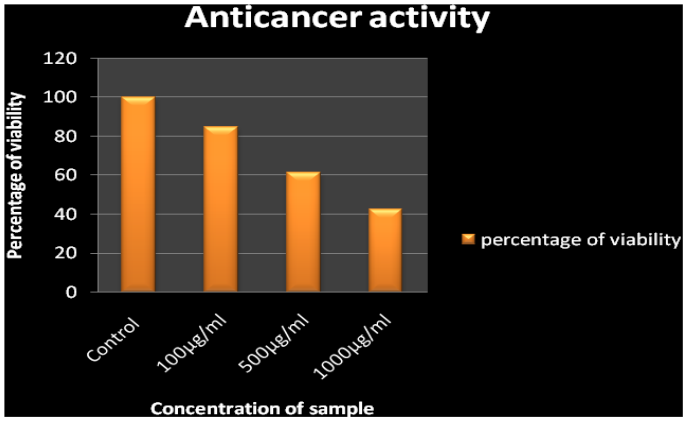

Fig. 1: Cytotoxic activity of chloroform leaves extract of *C. roseus* by MTT assay.

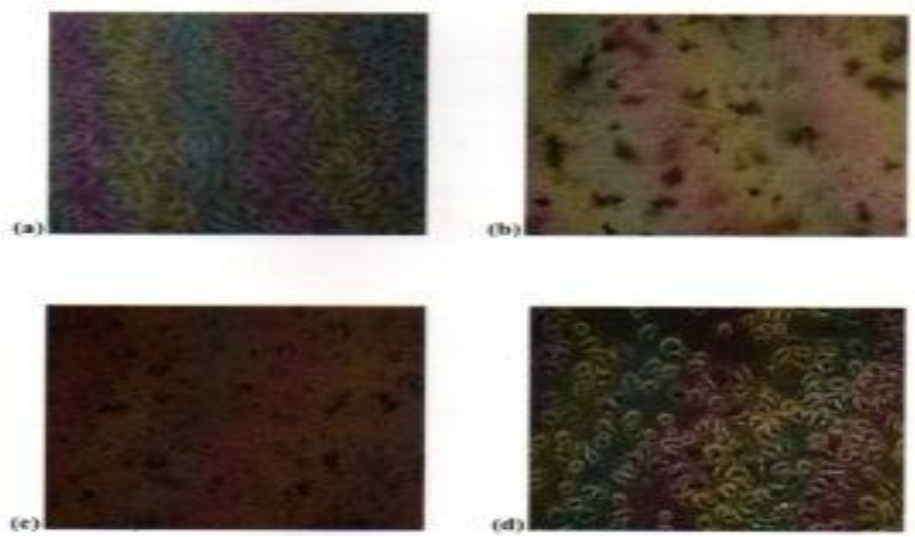

Figure 1: a) Control (DMSO), b) *C. roseus* in 1000 µg/ml, c) *C. roseus* in 500 µg/ml, d) *C. roseus* in 100 µg/ml.

DISCUSSION

Herbal medicines are valuable and readily available resource for primary health care and complementary health care system. Nair *et al.*, 2005[16] reported the presence of phytochemical from plants promise to be used in allopathic medicine as they are potential sources of antiviral, anti tumour, antimicrobial agent. In this study phytochemical analysis of *Catheranthus roseus* shows the presence of alkaloids, cardiac glycosides, flavanoid, phenol, protein, saponins, tannin and terpenoids.

In the present study methanolic extract of *Catheranthus roseus* showed the highest antibacterial activity against *Salmonella typhi*. Similarly Chinnavenkataraman *et al.*, 2012[17] reported the antibacterial with ethanol and methanol extract of *Catheranthus roseus* against *B. subtilis, E. coli, S. aureus, S. typhi* and *P. aeuroginosa*. Dash *et al.*, 2011[18] also reported the antibacterial with methanol and acetone extract of *Trigonella foenum* and *Coridrum sativum* against *P. seudomonas species, Shigella dysentiriae, Salmonella typhi* and *E. coli.*

In the present cytotoxicity test, the chloroform extract of *C. roseus* showed viability of L929 cell line decreased with increased concentration of the extract of *C. roseus*. Similarly, EL-Sayed and Cordel *et al.*, 1981[19] reported the extracts of *C. roseus* have demonstrated significant anticancer activity against numerous cell types. Hence this study holds the importance in using medicinal plants as an alternative source for treating various diseases.

CONCLUSION

Medicinal plant is the most exclusive source of life saving drugs for majority of the world's population. In the present study phytochemical analysis of *Catheranthus roseus* shows the presence of secondary metabolites which is responsible for treatment of various antimicrobial diseases. Among the extract methanolic extract shows highest antibacterial activity against *Salmonella typhi*. The cytotoxicity study of *Catharanthus roseus* by MTT assay showed decrease in viability of L929 cell line. It shows the plant extract act as excellent drug for the treatment of L929 mouse fibroblast cancer. Today, there is a renewal interest in traditional medicine and an increasing demand for more drugs from plant sources because green medicine is safe and herbal medicines are valuable resource.

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