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EVALUATION OF METABOLIC COMPOUNDS OF *CATHARANTHUS ROSEUS* AND ITS ANTICANCER ACTIVITY

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ABSTRACT

The aqueous and methanol extract of *Catharanthus roseus* (leaves) was accessed for their antimicrobial activity. The Antimicrobial activity was performed against pathogens such as *Salmonella*, *Klebsiella*, *Proteus*, *E.coli and Shigella*. The phytochemical analysis showed the presence of flavonoids, Saponins, Alkaloids, Amino acids, Tanins and Terpenoids in both aqueous and ethanol extracts. The active components present in the extract are found to be amino acids, Alkaloids, Lipids and terpanoids. The antimicrobial activity of active components present in the leaf extract shows inhibition of *Salmonella* and *Klebsiella*. *Catharanthus roseus* exhibit anticancer activity in *in-vitro* in Breast cancer cell line (MCF-7) and it was found effective. The inhibitory concentration found at 18.67µg/ml. The research work which is going on this direction is a proof of benefits by reducing toxicity of chemo and radio therapies and providing better and healthier life style by *Catharanthus roseus*.

KEYWORDS: *Catharanthus roseus*, Terpenoids and MCF-7.

1. INTRODUCTION

Cancer is a complex genetic disease that is caused primarily by environmental factors. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers occur in epithelia. In 1996 there were 10 million new cancer cases worldwide and six million deaths attributed to cancer. In 2020 there are predicted to be 20 million new cases and 12 million deaths. Part of the reason for this is that life expectancy is steadily rising and most cancers are more common in an ageing population. More significantly, a globalization of unhealthy lifestyles, particularly cigarette smoking and the adoption of many features of the modern western diet (high fat, low fibre content) will increase cancer incidence.

So there has been an intense search on various biological sources to develop a novel anti-cancer drug to combat this disease. Plants have proved to be an important natural source of anti-cancer therapy for several years. About 30 plant derived compounds have been isolated so far and are currently under clinical trials. These anti-cancer compounds have been found to be clinically active against various types of cancer cells (Joyce Nirmala *et al.*, 2011[1]).

There are around 460 species of plants that can be used as herb for remedy, including plant healer various types of cancer. Various types of anticancer plant are Zedoary (*Curcuma zedoaria*), Rodent Tuber (*Typhonium flagelliforme*), God's Crown (*Phaleria macrocarpa*), Madagaskar Periwinkle (*Catharanthus rosens*), Artocarpus Integer (*Selaginella corymbosa*), Bamboo Grass (*Loathatreum Gràcies*) , handsome (*Taraxacum mongolicum*), fruit makasar (*Brucca javanica*), Garlic (*Allium sativum*), Echo China (*Smilax china*), Sunflower (*Helianthus annus*), Leunca (*Solanum nigrum*), Job's Tears (*Coix Lachryma-Jobi*), Bamboo Rope (*Asparagus cochinchinensis*), and others.

Catharanthus roseus (L) G. Don known as "The Madagascar periwinkle". It is a popular ornamental plant found in gardens and homes across the warmer parts of the world. *Catharanthus roseus* (L.) G. Don, which also known as "an anti cancerous drug yielding plant" is a tropical and subtropical plant belonging to the family *Apocynaceae.* This review highlights the marvelous properties of this plant. The alkaloids like Vinblastine and vincristine are mainly present in aerial parts of *C. roseus*, which are used in treatment of various human cancers, so it is considered as mile stone in cancer chemotherapy. Apart from this it shows antidiabetic, antibacterial, antioxidant, antiulcer and antidiarrheal properties. Stems and leaves of *C.roseus* have enormous

amount of phytochemical constituents. It is found to be endangered so, it is the topic of conservation also (Monika Sain and Vandana Sharma 2013[2]).

The discovery of the anticancer properties of vinblastine and vincristine from *Cataranthus roseus* soon followed (Noble *et al.*, 1958[3]) and gave the impulse for wide range of investigations of plant extracts and plant derived compounds for possible anticancer activity. Similar useful drugs like diterpenetaxol obtained from plant *Taxus brevifolia* (Suffness 1987[4]), pyridocarbazole alkaloid ellipticine from *Ochrosia elliptica* and pyrrolo (3,4-β)-quinoline alkaloid camptothecin were obtained from *Camptotheca acuminata* (Hamburger *et al.*, 1991[5]). *Catharanthus roseus* was investigated from the ancient time for their phytochemical components and their therapeutic effect. Hence most work could be done on the above plant to reveal the unknown mysteries which would help the need of the present pharmaceutical world.

2. MATERIALS AND METHODS

2.1 Collection of samples and extraction

Leaves and stem were collected from the *Catharanthus roseus* in the college Herbal Garden. It was ensure that the plant was healthy and uninfected. The leaves and stem were washed under running tap water to eliminate dust and other foreign particles and to clean the leaves thoroughly. The fresh leaves and stem were trodden into small pieces, powdered, and mixed in 1:10 ratio with distilled water and methanol separately. The extractions were obtained through continuous grind using mortar and pestle followed by filtration using Whattman No.1 filter paper. The residues were re-dissolved with the appropriate solvents for the antibacterial assay.

2.2 Antibacterial activity

Salmonella typhi, Klebsiella, Proteus, Escherichia coli and Shigella was used for the study. Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard [0.5%] as recommended by WHO(2009). Antibacterial activity of the different extracts was determined by cup diffusion method on Muller Hinton agar medium by Anon (1996).[6] Wells are made in Muller Hinton agar plate using cork borer (5 mm diameter) and inoculums containing bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. 20 and 40 µl of the working suspension/solution of plant extract and same volume of distilled water for control were filled in the wells with the help of micropipette. Plates were kept for some time till the extract diffuse in the medium and incubated at 37°C for 24 h. After incubation, the plates were observed for the zone of inhibition [ZI], the diameter of the inhibition zone were measured and recorded.

2.3 Minimum Inhibitory Concentration (MIC) – Dilution Method

To determine Minimum Inhibitory Concentration (MIC) 1 ml of nutrient broth was taken in 10 test tubes. Different sets were prepared for each bacterium. Different concentrations of plant extracts ranging from 1mg to 10mg/ml were added into test tubes. To this 50µl of an overnight broth culture of each bacterium were inoculated in the respective tubes and the tubes were incubated for 24 hours at 37°C. One tube was inoculated with 1ml sterile nutrient broth with the 50µl of an overnight broth culture of each bacterium and placed at +4°C in a refrigerator overnight to be used as standard for the determination of complete inhibition. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

2.4 Analysis of Phytochemical Components

Phytochemical analyses were carried out according to the methods described by Trease and Evans (1989)[7] of the crude extract of leaves and stem for the identification of phytochemicals like, alkaloids, saponin, steroids, flavonoids, cardiac glycosides, cynogenic glycosides and phlobatannins.

2.5 Protein Determination

Protein content in the plant extracts were estimated by the method described by Lowry *et al*., (1951).[8] 0.02ml and 0.04ml of extracts were used to determine the protein content.

2.6 Estimation of total Antioxidant (Phosphomolybdenum method- Prieto *et al.*, 1999[9])

Aliquot of 0.1ml sample was obtained with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling waterbath at 95ºC for 90 mints. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each tube was measured at 695 nm against blank, a typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same as rest of the sample. For sample of unknown composition, water soluble antioxidant capacity was expressed as equilates of ascorbic acid (1 ml of extracts).

2.7 Phenolic content as tannic acids (Jayaprakasha *et al.*, 2001[10])

The extracts were dissolved in a mixture of methanol and water (6:4 v/v).sample (0.2 ml) were mixed with 1 ml of tenfold diluted folin-ciocalteus reagents and 0.8 ml of 7.5% sodium carbonate solution, after standing for 30 mints at room temperature, the absorbance was measured at 765 nm. The estimation of phenolic compound in the

fractions was carried out in triplicate and the results were averaged.

2.8 Separation of Active Compound from *Catharanthus roseus* extracts by Thin Layer Chromatography (TLC)

The glass slides were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel with double the volume of distilled water in a clean beaker. One drop of slurry was placed on the slide by using another slide edge, the drop of slurry was scattered all over to make thin film. The slides werekept as such for few minutes. Then the chromo plates were activated by heating in hot air oven at 120°C for 30 min. The slides were allowed to cool at room temperature and marked about 2 cm from the bottom as the origin. The working suspensions were loaded at the center of the each slide above from the edge. The development tank was saturated with suitable solvent according to Eskil Hultin (1966).[11]

Alkaloids : Benzene/ Methanol-80:20.
Flavanoids : Chloroform/Methanol-70:30.
Lipid : Chloroform/Methanol/water-10:10:3.
Terpeniods : Acetic acid/water-1:3.

The slides were kept in the tank without touching baseline by solvent. The final solvent front was marked and the slides were dried. For visualization of Flavanoids 1% ethanolic solution of Aluminium chloride was used and viewed under 560nm UV light. Alkaloids and Terpenoids were visualized under UV light and they were visible as yellow and orange fluorescent spots. Few pieces of iodine crystals were kept in the tank and covered with glass plate to saturate the tank with iodine vapor for detecting lipids. The plate was then kept in iodine vapor saturated tank and left for few hours and brown colored spots were visualized.

2.9 Retrieval of the active compound

Spots on the preparative silica gel slides were scratched with the help of clean and dry spatula and collected in beaker containing appropriate solvents (Bishnu Joshi, 2011[12]) and left overnight. The content in the beaker was stirred and filtrated through Whattsman no.1 filter paper. The filtrate was collected in clean and dry beaker. The filtrate containing active compound was used for the determination of antimicrobial effect against *Salmonella* and *Klebsiella* by cup diffusion method.

2.10 MCF -7 cell line study

The human breast cancer cell line MCF -7 was carried out in KMCH College of Pharmacy, Coimbatore. The cell line was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air, and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.10.1. Cell line treatment procedure (Monks, A. *et al.*, 1991[13])

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a haemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the methanol extract of *Catharanthus roseus* (leaves and stem separately). They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the methanol extract of *Catharanthus roseus* (leaves and stem) was diluted to twice the desired final maximum test concentration with serum free medium. Additional four 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.10.2. MTT assay (Mosmann, T., 1983[14])

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

3. RESULTS

3.1 Phytochemical analysis

Qualitative phytochemical analyses were performed for the detection of alkaloids, saponin, steroids, flavonoids, cardiac glycosides, cynogenic glycosides and phlobatannins. The observed results were tabulated (Table 1).

3.2. Protein Determination

Standard graph was plotted for bovine serum albumin using spectrophotometric reading at 660 nm. Table 2

represented the total protein content in the *Catharanthus roseus.*

3.3. Total anti oxidant and Phenolic content

The absorbance of the aqueous solution of each tube was measured for total anti oxidant content at 695 nm against blank it was observed that leaves contain 13.71 mg/g and 10.70 mg/g of anti oxidant in methanol and aqueous extract. Stem contained 5.34mg/g and 5.16 mg/g of anti oxidant content in both methanol and aqueous extract. The absorbance was measured at 765 nm for Phenolic content. The estimation of phenolic compound in the fractions was carried out in triplicate and the results were averaged. Leaves contain 11.56 mg/g and 12.55 mg/g of phenolic content in methanol and aqueous extract. Stem contained 6.39 mg/g and 5.16 mg/g of phenolic content in both methanol and aqueous extract. The results were tabulated in table 3.

3.4. Antibacterial Property

Minimal inhibitory concentration assay was carried out for methanolic extraction *Catharanthus roseus Shigella, Proteus, Klebsiella, Escherichia coli* and *Salmonella*. Table 4 illustrated the minimal concentration level which required to kill the pathogen. The pathogens *Shigella, Proteus, Klebsiella, Escherichia coli* and *Salmonella* were tested for antibacterial assay. The methanol extract of *Catharanthus roseus* leaves showed 2 cm the maximum zone of inhibition in *proteus* followed by *Salmonella typhi* and minimum zone of inhibition found in methanol extract in *Eschrichia coli* and methanol extracts of stem showed maximum zone of inhibition found in *Eschrichia coli* followed by *Proteus* which is tabulated in table 5.

3.5. Separation of Active Compound from Thin Layer Chromatography (TLC)

Table 6 represented the methanol extract of leaf and stem contain alkaloids, flavonoids, lipids and terpenoids. The separated active compounds alkaloid, flavonoids, lipids, and terpenoids were found that effective against *Salmonella* and *Klebsiella* the zone of inhibition tabulated in table 7 which represented the maximum zone of inhibition of leaves extract found in *Klebsiella* in the active compound of flavanoids and minimum zone of inhibition found in active compound of lipid. maximum zone of inhibition of stem extract found in *Klebsiella* in the active compound of flavanoids and minimum zone of inhibition found in active compound of lipid Figure 1 represented the zone of inhibition of active compounds of alkaloids and lipids.

3.6. MCF -7 Cell line study

After treatment with various concentrations of *Catharanthus roseus* (6.25 µg/ml, 12.5µg/ml, 25 µg/ml, 50µg/ml, 100µg/ml) parameters like cell viability, growth of growth and morphological changes of the cell line were noted and compared with control (untreated) cell sample. From the results, it is observed that the cell viability was decreased and increased in inhibition of growth by the *Catharanthus roseus*

With respect to different concentrations of the extracts, significant decreases in cell viability were observed in the concentrations 6.25 µg/ml, 12.5µg/ml and 25 µg/ml (Fig.2 and Fig 3). The growth inhibitory activity was more significant than the percentage viability. IC_{50} was found at 18.67 µg/ml for leaves and 39.47 µg/ml for stem. The percentage of growth inhibition of the treated cells with different doses of *Catharanthus roseus* was seen in Fig 4a, Fig 4b, Fig 5a fig 5b and Table 8 and Table 9.

Table 1: Phytochemical analysis of *Catharanthus roseus.*

Phytochemical constituents	Aqueous		Methanol	
	Leaves	Stem	Leaves	Stem
Alkaloids	+	+	+	+
Steriods	+	+	+	+
Saponin	+	+	+	+
Cynogenic glycosides	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	-	-	-	-
Phenols	+	+	+	+

'+' – Present '-'–Absent

Table: 2 Total protein content of *Catharanthus roseus.*

Concentration of proteins (µg/ml) OD at 660nm (mg/g)			
Aqueous		Methanol	
Leaves	Stem	Leaves	Stem
2.8	1.9	5.0	3.8

Table 3: Total anti oxidant and phenol content of *Catharanthus roseus.*

Sample extraction	Anti oxidant content OD at 695 nm (mg/g)		Phenolic content OD at 765 nm (mg/g)	
	Leaves	Stem	Leaves	Stem
Methanol	13.71	5.34	11.56	6.39
Water	10.70	5.16	12.55	5.16

Table 4: Minimal Inhibitory Concentration (Methanol extraction) of *Catharanthus roseus.*

Test organisms	Leaves OD at 540 nm			Stem OD at 540 nm		
	60µl	80µl	100µl	60µl	80µl	100µl
Salmonella typhi	0.57	0.35	0.13	-	-	-
Proteus sp.	0.42	0.32	0.10	0.41	0.13	0.09
Escherichia coli	0.60	0.50	0.42	0.18	0.10	0.08
*Klebsiella*sp.	0.79	0.21	0.19	-	-	-
Shigella sp.	0.53	0.21	0.09	-	-	-

Table 5: Antimicrobial activity of Methanolic extracts of *Catharanthus roseus.*

Test organisms	Zone of inhibition(in diameter)								Standard drug (ampicillin 1mg/ml)
	Leaves (in cm)				Stem (in cm)				
	20µl	40µl	60µl	80µl	20µl	40µl	60µl	80µl	20 µl
Salmonella typhi	0.8	1.3	1.4	1.7	-	-	-	-	1.2
Shigella	-	1.2	1.3	1.5	-	-	-		2
Escherichia coli	-	1.2	1.4	1.6	-	-	-	1.2	2
Proteus	-	1.2	1.6	2.0	-	-	-	1	1
Klebsiella	-	0.8	1	1.5	-	-	-		-

Table 6: Rf values of metabolic compounds of *Catharanthus roseus* by TLC.

Methanol extraction of *Catharanthus roseus*	Flavonoids	Alkaloids	Lipids	Terpenoids
Leaves	0.5	0.45	1.2	0.66
Stem	0.5	0.45	1.2	0.66

Table 7: Antimicrobial activity of active compounds from TLC.

Active compounds	Solvents	Zone of Inhibition of active compounds (diameter in cm)	
		Test organism	
		Salmonella sp.	*Klebsiella sp.*
Lipid	Leaves	1.9	2
	Stem	1.8	1.9
Flavonoids	Leaves	2	2.5
	Stem	2.2	2.1
Alkaloids	Leaves	2.3	2.1
	Stem	1.8	1.9
Terpenoids	Leaves	2.2	2.4
	Stem	2.2	2.1

Table 8: MTT effect on MCF- 7 cell line- % of viability *Catharanthus roseus* (leaves)

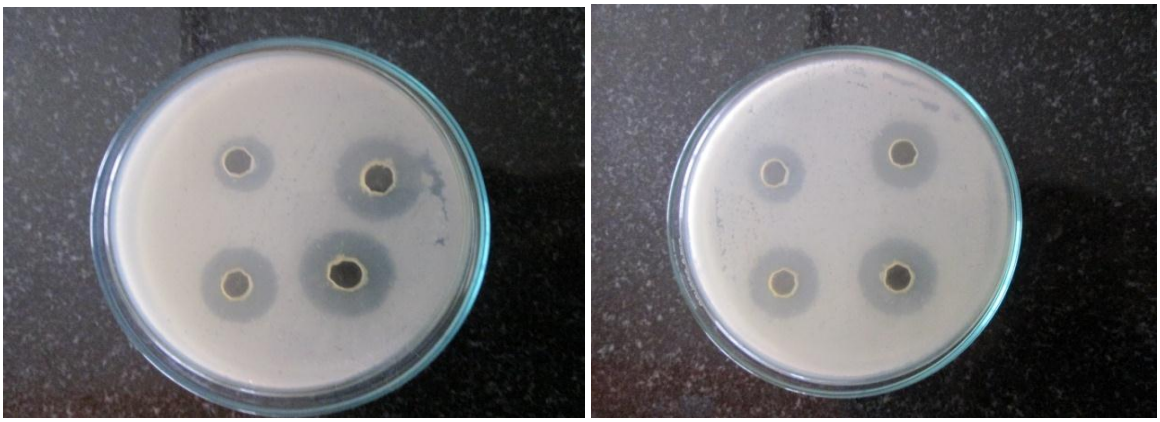
Conc (µg/ml)	% Cell viability
6.25	35.20457
12.5	43.19696
25	52.902
50	66.1275
100	72.78782

IC 50 18.67 µg/ml

Table 9: MTT effect on MCF- 7 cell line- % of viability *Catharanthus roseus* (stem).

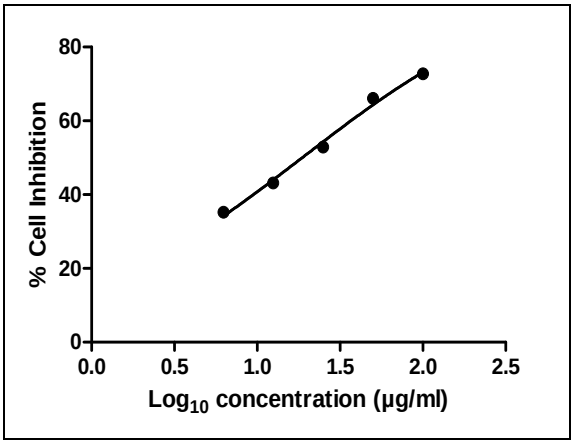
Conc (µg/ml)	% Cell viability
6.25	14.46242
12.5	23.59657
25	42.24548
50	55.75642
100	69.07707

IC 50 39.47 µg/ml



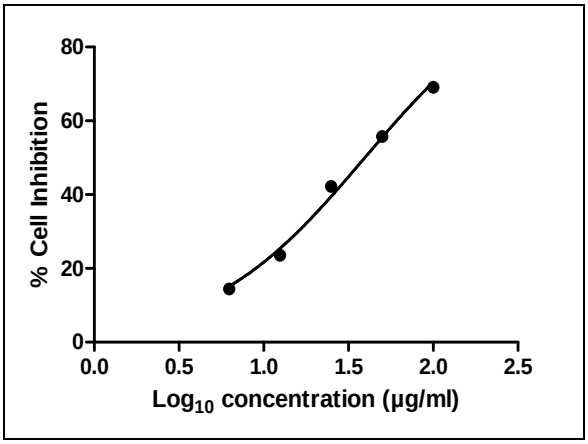
Salmonella ***Klebsiella***

Fig. 1 Antimicrobial activity of active compounds from *Catharanthus roseus* (leaves and stem).



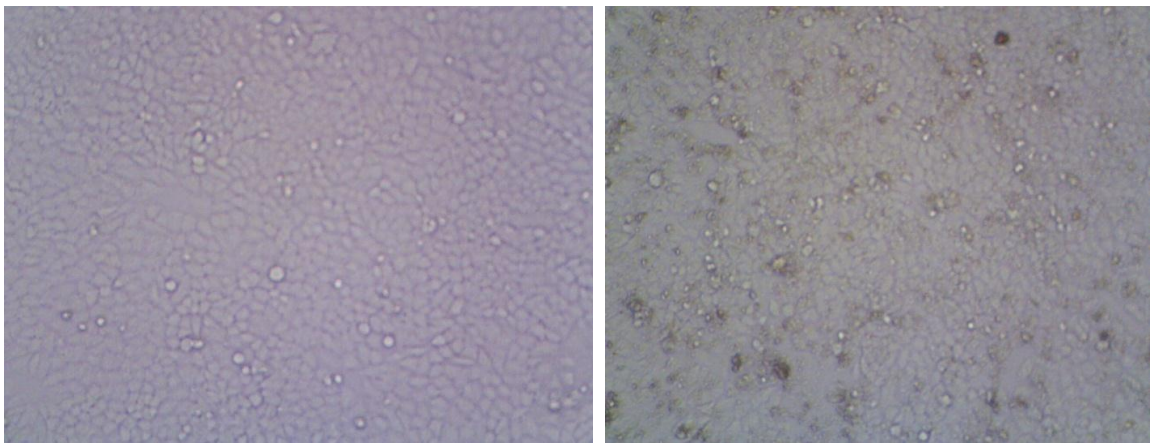
IC 50 18.67 µg/ml

Fig. 2: Inhibitory level of *C. roseus* (Leaves) at different concentration in MCF-7 cell line.



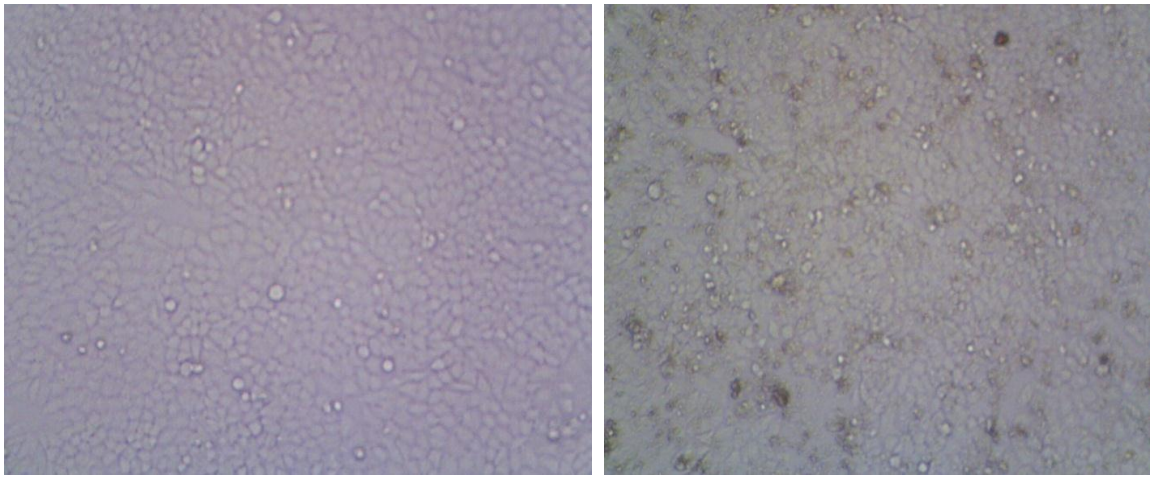
IC 50 39.47 µg/ml

Fig. 3 Inhibitory level of *C. roseus* (stem) at different concentration in MCF-7 cell line.



Control ***C. roseus* (leaves) treated cell line**

Fig. 4a and 4b MCF-7 Cell line- Cell viability



Control ***C. roseus* (stem) treated cell line**

Fig. 5a and 5b MCF-7 Cell line- Cell viability

4. DISCUSSION

The aqueous and methanolic extract of both leaves and stem of *Catharanthus roseus* contain alkaloids, saponin, steroids, flavonoids, cardiac glycosides and cynogenic glycoside. Phlobatannins was absent in both methanol and aqueous extract of *Catharanthus roseus.* Thenmozhi *et al.,* (2012)[15] reported the phytochemical screening with the *Catharanthus roseus* was showed that presence of tannin, flavonoids, alkaloids, saponins and terpenoids. In the Present investigation the methanol and chloroform extract showed more number of phytoconstituents of *Catharanthus pusillus*.

Similarly, Govindasamy (2012)[16] reported the phytochemical analyses of *Catharanthus roesus* were showed the presence of soluble sugar, reducing sugar, protein, amino acids, lipids, total chlorophyll, phenol and ortho-dihydroxyphenols in the ethanolic extract.

The methanol extract of *Catharanthus roseus* leaves showed 2 cm the maximum zone of inhibition in *Proteus* followed by *Salmonella typhi* and minimum zone of inhibition found in methanol extract in *Eschrichia coli* and methanol extracts of stem showed maximum zone of inhibition found in *Eschrichia coli* followed by *Proteus*.

Gram negative stains were more sensitive when compared to Gram positive bacteria. The study implicates that bio-active compound(s) of *C. Roseus* could potentially be exploited as antibacterial agents (Ramya *et al.,* 2008[17]).

The methanol extract of leaf and stem contain alkaloids, flavonoids, lipids and terpenoids in the Rf value of 0.45, 0.5, 1.2 and 0.66. The separated active compounds alkaloid, flavonoids, lipids, and terpenoids were found that effective against *Salmonella* and *Klebsiella* the zone of inhibition. Chromatographical analysis of *Catharanthus roseus* revealed that the presence of terpenoids, lipids, alkaloids and flavanoids.

The methanolic extract was further purified for specific alkaloids present using modified Shams *et al.,* (2009)[18] method extraction procedure. Vincristine, Vinblastine, Vindoline and Ajmalicine could be chromatographically identified in extracts of *C. roseus* intact plant after individual alkaloid purification procedure. This has confirmed the anti-neoplastic and anti-hypertensive properties these alkaloids have in making the plant effective in healing cancer and high blood pressure as it was originally used by traditional people (Magagula *et al.,* 2012[19]). Aqueous extracts of *Azadirachta indica* (Neem) was subjected to Thin layer chromatography (TLC) by using different solvent system for the analysis of lipid, alkaloids, flavonoids present in plant extract. The active components separated through TLC were subjected to antimicrobial activity against the pathogens. The present study will be successful in identifying candidate plant with different antimicrobial activity which could be further exploited for isolation and characterization of the novel phytochemicals in the treatment of infectious diseases especially in light of the emergence to produce more effective antimicrobial agents (Susmitha. S. *et al.,* 2013).

Different concentrations of the extracts, significant decreases in cell viability were observed in the concentrations 6.25µg/ml, 12.5µg/ml and 25µg/ml. The growth inhibitory activity was more significant than the percentage viability.

Tapakdara or Madagascar Periwinkle (*Catharanthus roseus*[L] G.Don), a natural plant, is empirically reported to have promising anticancer activity. To elucidate its mechanism, a research was conducted to investigate the possible ethanol extract of *C. roseus* in inducing apoptosis on breast cancer cell line (T47D). Antioxidant activity of *C. roseus* was investigated as well. Sub-G1 flowcytometric apoptotic analysis result showed that extract of *C. roseus* at 6.25 µg/ml induced apoptosis for 26.365%. Increasing extract concentration resulted an increasing apoptotic level as well, extract at concentration of 12.5 µg/ml induced apoptosis for

22.235%. Meanwhile doxorubicin at concentration of 10 µg/ml induced apoptosis for 36.055%. *C. roseus* extract had a potential anticancer activity by inducing apoptosis (WahyuWidowati *et al.*, 2010[20]).

The discovery of the anticancer properties of vinblastine and vincristine from *Cataranthus roseus* soon followed (Noble *et al.*, 1958[3]) and gave the impulse for wide range of investigations of plant extracts and plant derived compounds for possible anticancer activity.

5. CONCLUSION

Traditional medicine has a long history of serving peoples all over the world. Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed awe-inspiring. The ethno botany and ubiquitous plants provide a rich resource for Natural drug research and development. In recent years, the use of traditional medicine information on plant research received considerable interest. The medicinal plants contain several phytochemicals such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals etc. These phytochemicals possess antioxidant activities, which, prevent or can be used in the treatment of many diseases, including cancer. There are the several medicinal plants all over the world, including India, which are being used traditionally for the prevention and treatment of cancer.

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