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Phytochemical Analysis of *Catharanthus roseus* Plant Extract and its Antimicrobial Activity

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ABSTRACT

More than 3000 plant species that have reportedly been used in the treatment of cancer and other diseases. Plant derived compound have played an important role in the development of several clinically useful anticancer agents. Catharanthus roseus is an important medicinal plant of the Apocynacae family. Catharanthus roseus plant leaves are used for cancer treatment. Aim of the present study is to investigate the phytochemical analysis and anti microbial activity of aqueous and methanol extracts of Catharanthus roseus. The enzymatic and non enzymatic (DPPH) method was employed to analyze the antioxidant property. Qualitative analysis of phytochemical screening reveals the presence of Alkaloids, Phenol, Saponins and Protein. Further presents of phytochemicals were detected by Thin Layer Chromatography (TLC), which is the standard technique for separating organic compounds. The extracts were purified using Column chromatography (silica gel). The fraction 3 was subjected to GC-MS analysis to the compounds presents in the extract.

Keywords*: Catharanthus roseus, Phytochemical analysis, Antioxidant activity, Antibacterial activity.*

INTRODUCTION

Plants, mainly used for variety of disease related to cancer treatment. Plants produce several secondary metabolites including alkaloids, flavonoids, saponins, steroids cyanogenic glycosides and terpenoids to protect themselves from the attack of naturally occurring pathogen, insects' pests and environmental stresses. Above activity of those compounds should depend on the methods and solvent used for extraction[4,10].

Most probably herbal plants used in traditional medicine consist of wide range of bioactive compounds that can be used as alternative therapeutic tools for the prevention or treatment of many contagious diseases. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotic[2,9].

Catharanthus roseus is an evergreen sub herb plant growing to 1 m tall. The leaves are oval to oblong, 2.5- 9.5 cm. long and 1-3.5 cm. broad glossy green hairless with a pale midrib and a short petiole about 1-1.8 cm. long and they are arranged in the opposite pairs. The flowers are white to dark pink with a dark red center, with a basal tube about 2.5-3 cm. long and a corolla about 2-5 cm. diameter with 5 petal like lobes. The fruit is a pair of follicles about 2-4 cm. long and 3 mm broad[6].

Catharanthus 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 cancers-breast cancer, lung cancer, ulterine cancer, melanomas, Hodgkin's and non-hodgkin's lymphoma[12]. Generally, it is known as Vinca rosea, Ammocallis rosea and Lochnera rosea. *Catharanthus roseus* is an Indian originated herb which grows wild in the Indian subcontinent in southern Asia[1].

Catharanthus roseus are cultivated two common names, which is named on the basis of their flower colours, Pink: Rosea, White: Alba[6]. Traditionally, leaves of *Catharanthus roseus* are used as medicine for the treatment of following diseases, they Menorrhagia, Rheumatism, Dyspepsia, Indigestion, Dysmenorrheal, Diabetes, Hypertension, Cancer, Menstrual disorders, Skin diseases, Bleeding diarrhea and has sedative and antiviral properties.

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 *Catharanthus roseus*, it have antihypertensive and antispasmodic properties. One of the important types of alkaloid is the vinblastine produced from *Catharanthus roseus* due to its antitumour activity and wide pharmaceutical use[7]. *Catharanthus roseus* to produce modern chemotherapeutic agent for their pain-relieving properties[5].

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. 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. Experimentally, tried to prove the antibacterial activity of *Catharanthus roseus* against clinical wound isolates. From this, produce many antibiotics against for treat wound pathogens but they also cause undesirable adverse effects[11]. Probably, most medicinal plants contains wide variety of natural antioxidants namely phenolics, flavonoids and tannins than dietary plants.

Catharanthus roseus contains significant amounts of volatile and phenolic compounds including caffeoylquinic acids and flavonal glycosides which are known to antioxidant activity. It has a important role in the body defence system that is acts as a antioxidants against reactive oxygen species (ROS), which are harmful by forming such products through normal cell aerobic respiration[8]. Accumulation of free radicals can cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degeneration, parkinson's diseases, mongolism, aging process and perhaps dementia[9].

The flower petals, seeds and other parts of *Catharanthus roseus* exhibit antioxidant properties. Thus phenolic compounds have redox properties that act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. It has multiple applications in foods, cosmetics and pharmaceutical industries. Besides antioxidant activity, these compounds exhibits antiallergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio protective and vasodilatory effects[1]. This is influenced by number of geographical and environmental factors.

Natural antioxidants are the source of finding the potentially safe, cheap and effective antioxidants. From this, these are collectively called as free radical scavengers. This type of plant antioxidants mainly applied to prevent lipid peroxidation in the food industries[9].

MATERIALS AND METHODS

COLLECTION AND PROCESSING OF PLANT:

Matured leaves of ***Catharanthus roseus*** were collected from Coimbatore city during the flowering and fruiting period. That plant leaves were washed with tap water to remove soil and unwanted dust particles. Then the leaves were shaded, dried, and then powdered by using mechanical blender and stored in air tight bottles.

EXTRACT PREPARATION:

The powdered plant leaves were soaked with (10g/100ml) in different solvent (aqueous and methanol), for overnight in rotator shaker.

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Test for Alkaloids:

To 1 mL of extract added 1 mL of Mayers reagent and few drop of Iodine solution. Formation of yellow colour precipitate indicates the presence of Alkaliods.

Test for Terpenoids:

To 1 mL of crude extract add 1 mL of concentrated H_2SO_4 and heated for 2 minutes. A grayish colour indicates the presence of terpenoids.

Test for Phenol and Tannins:

To 1 mL of crude extract added 1 mL of $FeCl_3$. A blue green or black colour indicates presence of tannins.

Test for reducing Sugar:

To 1 mL of extract added 1 mL of Fehling's A solution and 1 mL of Fehling's B solution. Formation of red colour indicates the presence of sugar.

Test for Saponins:

To 1 mL of extract added 2 mL of distilled water, shaken well and formation of 1 cm layer of foam indicates presence of saponins.

Test for Flavonoids:

To 1 mL of extract added few fragments of magnesium ribbon and added few drops of concentrated HCl drop wise. Appearance of pink scarlet colour confirmed the presence of flavonoids.

Test for Quinines:

To 1 mL of extract added 1 mL of 1% NaOH and mixed well. Appearance of blue green or red indicates presence of Quinines.

Test for Protein:

To 1 mL of extract added few drop of mercuric chloride. Formation of yellow colour indicates the presence of protein.

Test for Steroids:

1 mL of extract mixed with 1 mL of chloroform and concentrated H_2SO_4 sidewise. A red colour presence at the lower chloroform layer indicates presence of steroids.

Antibacterial assay:

The agar well diffusion method was used for antibacterial assay. Petri plates were prepared by pouring 20 ml of Nutrient Agar medium and allowed to solidify. Plates were solidified and 20 µl of bacterial culture *E. coli, S. aureus, P. aeruginosa* and *B. subtilis* was poured and uniformly spread. The excess inoculum was drained away and the inoculum was allowed to dry for 5 minutes. Agar well of 5 mm in diameter were prepared with the help of a sterilized stainless cork borer. The wells were labelled appropriately and to each well were loaded with 10 µl, 20 µl and 30 µl plant extract, along with disc and plant extract using a micro-pipette. Standard reference antibiotic Amikacin(methanol) and Neomycin(water) (25 mcg/disc) was used as controls for the tested bacteria. The plates were incubated at 37° C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria.

ANTI-OXIDANT ACTIVITY:

DPPH radical scavenging activity:

This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. 0.1 ml, 0.2 ml and 0.3 ml of methanol leaf extracts were mixed with 1 ml of 0.1 mM DPPH. To all the tubes added 0.4 ml of 50 mM Tris-HCl. Incubate the reaction mixture at room temperature for 30 minutes. The absorbance of the reaction mixture was read at 517 nm. The percentage of free radical scavenging was calculated as formula mentioned below.

Estimation of total flavonoids:

1 ml of methanol and aqueous extract was mixed with 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled H_2O. The mixture was incubated in room temperature for 30 minutes. Then the absorbance was measured at 415 nm in spectrophotometer.

Estimation of total phenol:

Total phenol content was determined by the Folin- ciocalteau reagent method. 1 ml of methanol and aqueous extract was mixed with 1 ml of folin's phenol reagent and 1 ml of 20% sodium carbonate. The mixture was allowed it for incubation at 45° C for 45 minutes and the absorbance was measured at 765 nm in spectrophotometer.

Thin layer Chromatography:

The aqueous and methanol extracts were added as spot using capillary tubes on the one end of the thin layer plate at above 1 cm. Plate was allowed it for air dry, then it was placed in a beaker containing solvent Ethyl acetate: Methanol in the ratio of 6: 4. The samples 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 minutes. The plate was then visualized under the UV light and violet colour spot was absorbed on the plate.

Column chromatography

1.0 gm of Silica gel 100- 200 Mesh was added to 50 ml sterile distilled water and kept for overnight soaking. To this 10 ml of methanol leaf extract was added carefully to the top of the gel and allowed to pass into the gel by running the column. After 30 minutes the samples were eluted and the fractions were collected at 15 minutes interval each in 5 eppendorf tubes and stored at -20º C.

GC-MS analysis

GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and GasChromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silicacapillary column (30 m 0.25 mm ID ´ 1 m df, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volumeof 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with anincrease of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle massspectra and chromatograms was a Turbo mass.

RESULTS

Preliminary phytochemical analysis of *Cathanthus roseus*:

Phytochemical analysis of *C. roseus* was carried out in Aqueous, Methanol extracts and results are shown below.

Fig.1: Phytochemical tests of methanol extract

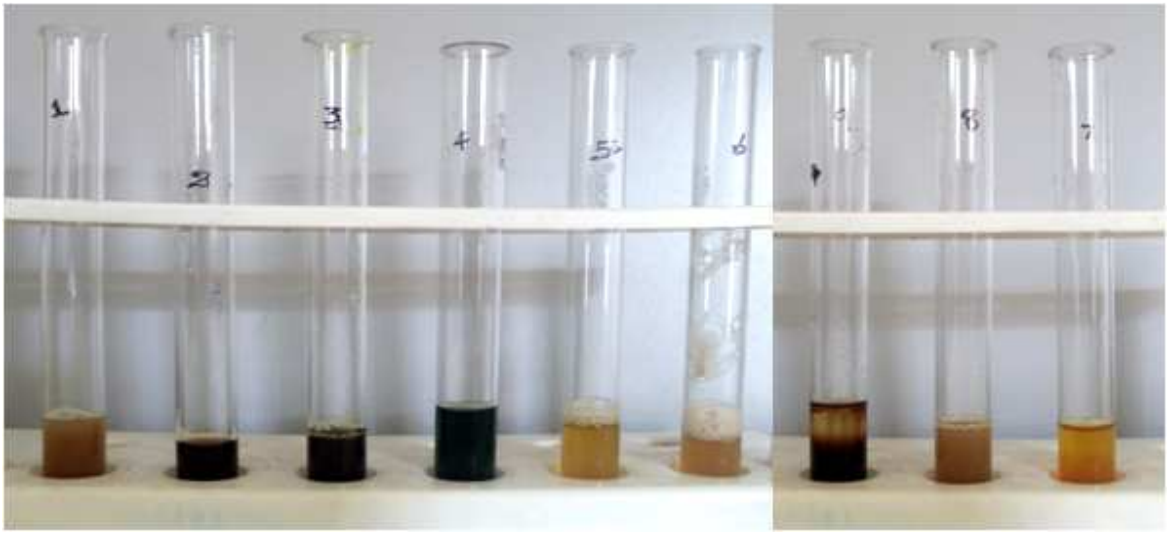


Fig.2: Phytochemical tests of water extract

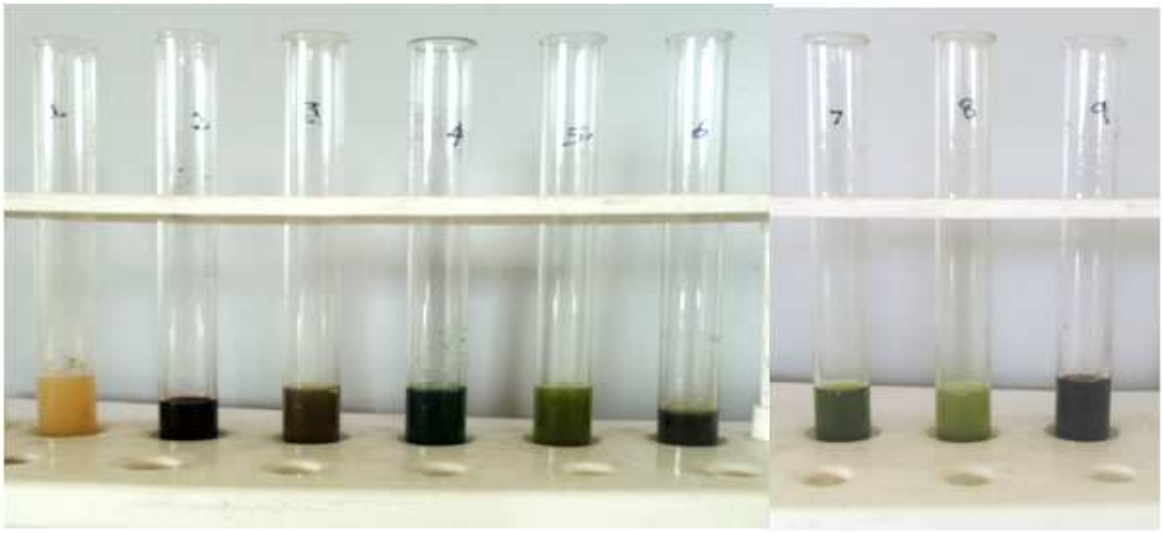


Table 1. Phytochemical analysis of methanol and water extracts

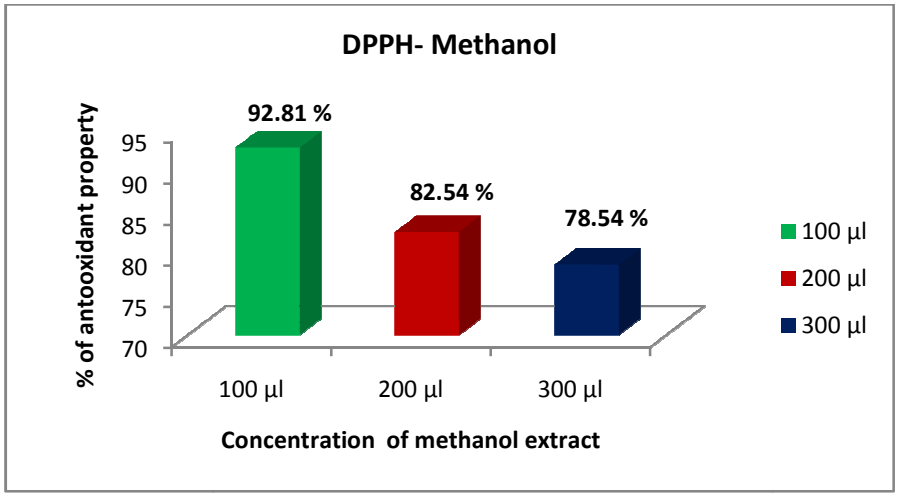
Extracts and tests	Leaf	
	Aqueous	Methanol
Alkaloids	+	+
Terpenoids	+	-
Phenols & Tannins	+	+
Sugar	-	-
Saponin	+	+
Flavonoids	-	-
Quinines	-	+
Proteins	+	+
Sterols	-	-

+ symbol indicates presence and – indicates absence with respect to extractive solvents.

DPPH assay:

DPPH radical scavenging activity was done for Methanol extract with required concentrations. After 30 minutes of incubation in room temperature, absorbance was measured at 517 nm and percentage of free radical scavenging was calculated as formula mentioned below.

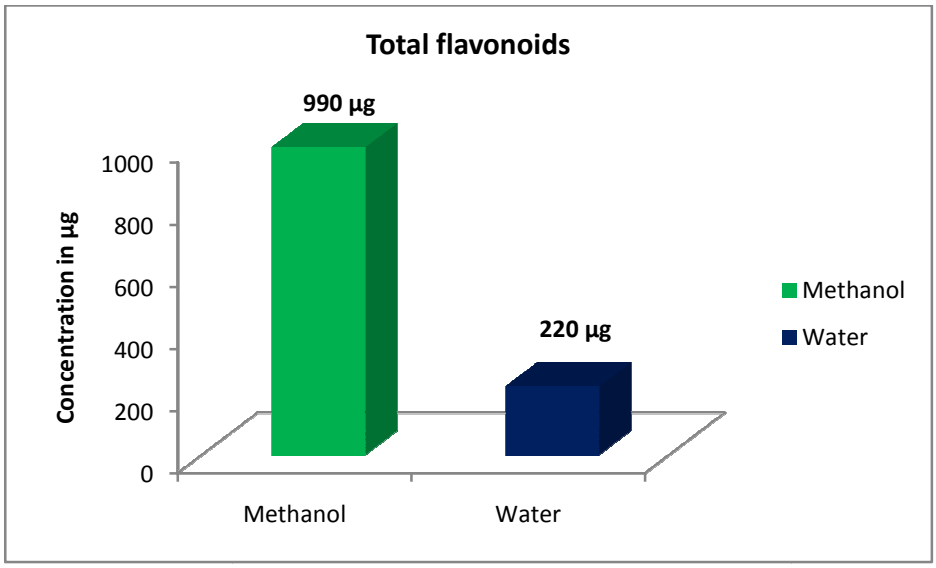
Chart 1. Estimation of antioxidant- DPPH



Estimation of total flavonoids:

The aluminum chloride method was used for the determination of the total flavonoid content of the methanol and aqueous extract. After incubation period the sample and control was measured at 415 nm and readings were calculated using standard graph.

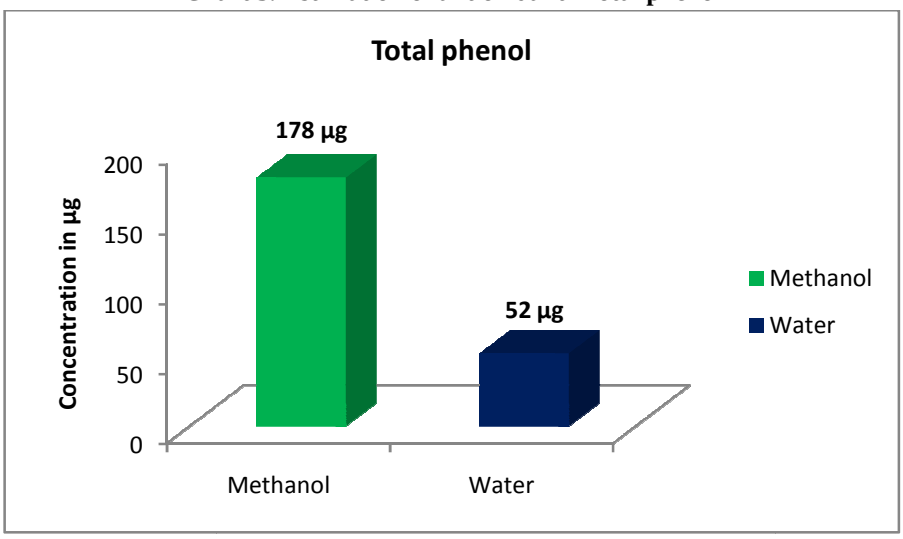
Chart 2. Estimation of antioxidant- Total flavonoids



Estimation of total phenol content:

Total phenol content was estimated by Folins ciocalteau method for Methanol and aqueous extract. After 45 minutes of incubation at 45°C, it was measured at 765 nm in spectrophotometer.

Chart 3. Estimation of antioxidant- Total phenol



Antibacterial studies:

Antibacterial assay was carried out for water and methanol extracts of *Catharanthus roseus* leaves with test organism *P.aeruginosa*, *B.substilis*, *S.aureus*, and *E.coli*, were swabbed on Muller hinton plate . Plant extracts 10 µl, 20 µl and 30 µl and commercial antbacterial disc Amikacin(methanol), Neomycin(water) (25 mcg) with plant extract and Amikacin and Neomycin (25 mcg) disc was placed in nutrient agar plate. After 24 hours incubation, it was found to be that plant I and Plant III has more antibacterial activity when compared to the plant II and IV and disc.

Fig. 3: Antibacterial effect in water extract of *C. roseus*

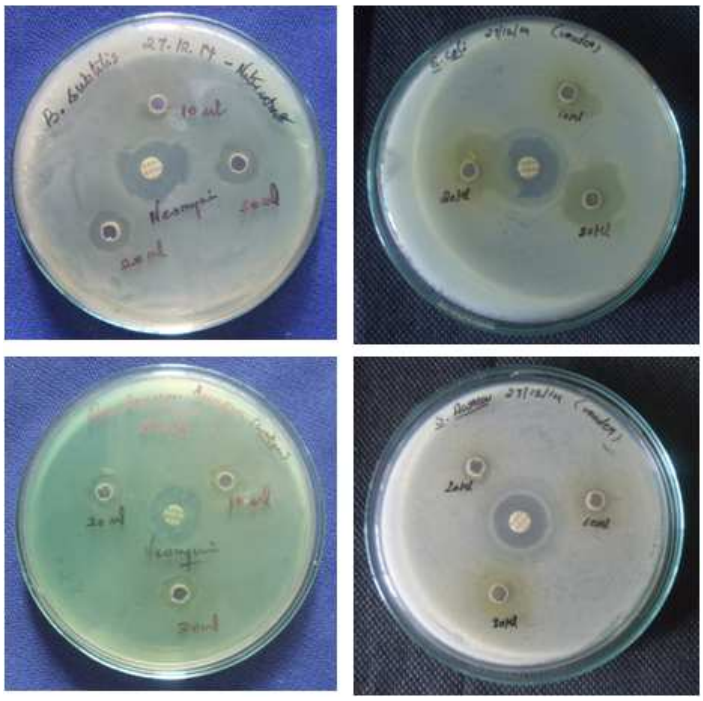


Table 2: Antibacterial activity of aqueous extract

Aqueous Extract (µL)	*Zone of inhibition in mm*			
	P. aeruginosa	***B. subtiIis***	***S. aureus***	***E. coli***
10	2	2	3	2
20	4	4	2	3
30	6	6	4	8
Antibiotic Disc	6	10	9	9

Fig. 4: Antibacterial effect in methanol extract of *C. roseus*

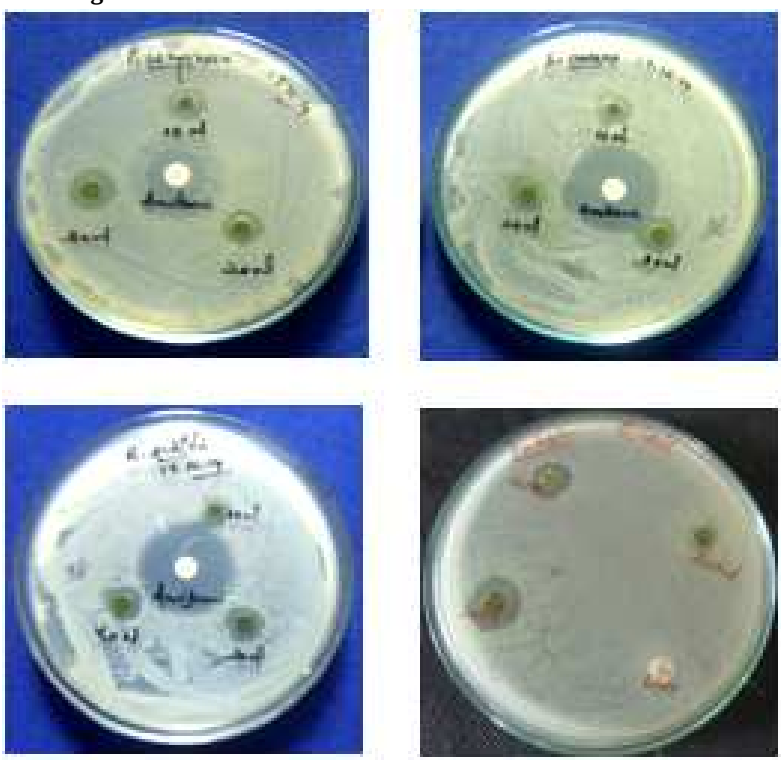


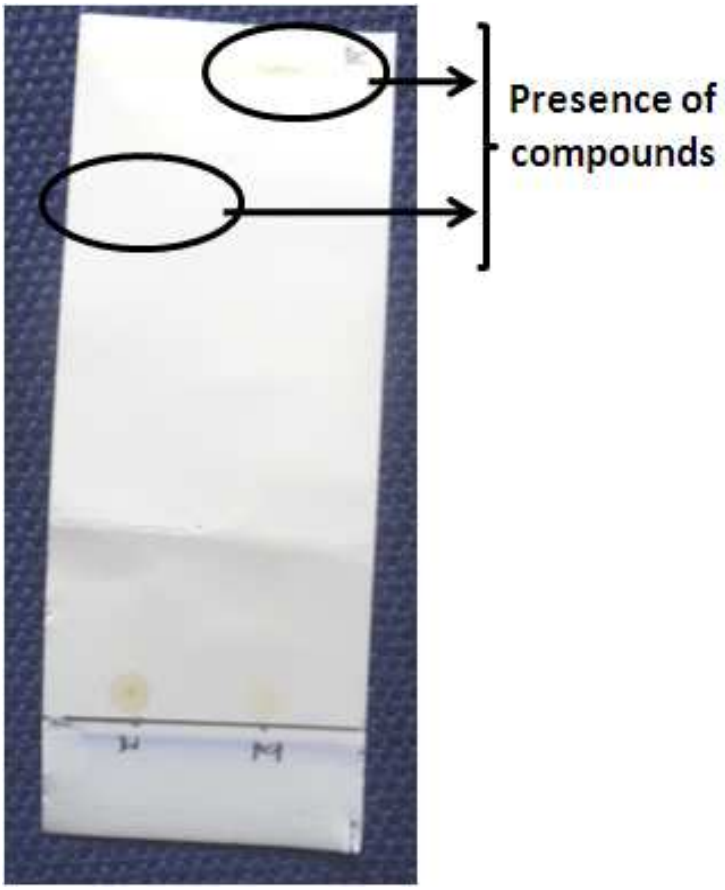
Table 3: Antibacterial activity of methanol extract

Methanol Extract (µL)	***Zone of inhibition in mm***			
	P. aeruginosa	***B. subtiIis***	***S. aureus***	***E. coli***
10	3	3	3	4
20	6	5	4	3
30	8	8	4	7
Antibiotic Disc	7	14	11	2

Thin layer chromatography:

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

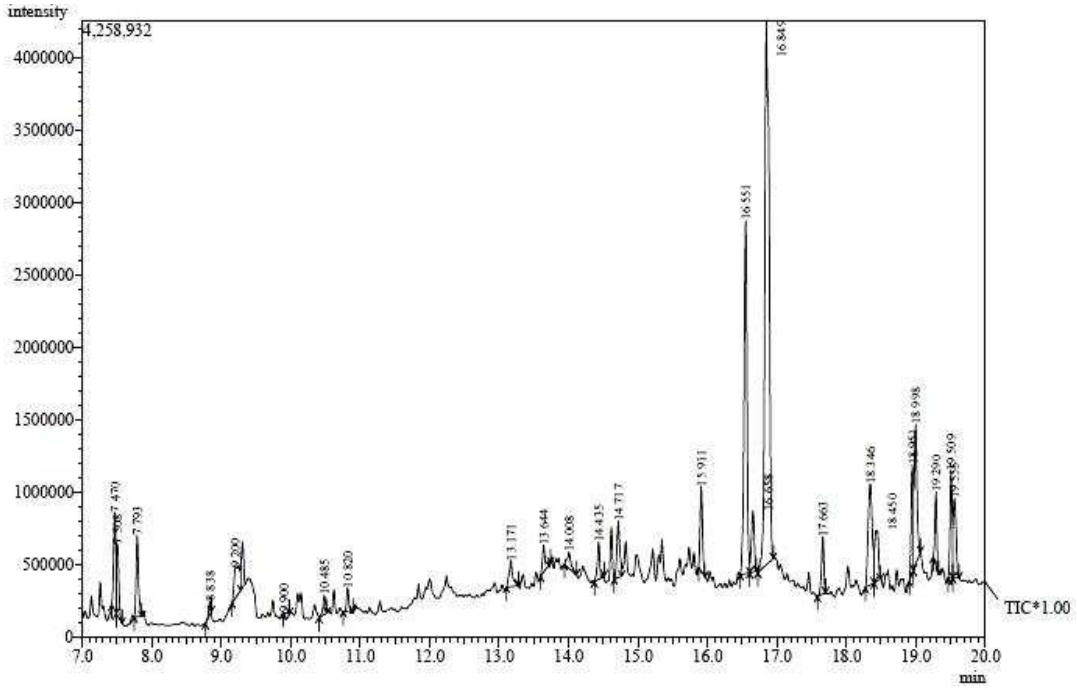
Fig.4: Thin Layer Chromatography



Column chromatography:

Plant compounds were purified by silica gel-mesh 100 - 200 column chromatography. Different fragments of purified plant compounds were collected in 5 eppendorf tubes at the interval of 15 minutes each and it was stored at -20º C.

GC-MS



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	7.470	7.425	7.492	1593075	1.60	680206	2.24	2.34		1,3-CYCLOHEXADIENE, 2-
2	7.508	7.492	7.575	948575	0.95	490180	1.62	1.94	V	Sulfuric acid, diethyl ester
3	7.793	7.750	7.858	1294531	1.30	560866	1.85	2.31		BENZENE, METHYL(1-ME
4	8.838	8.775	8.858	201819	0.20	94615	0.31	2.01	MI	BENZENE, 1-METHYL-4-(1
5	9.200	9.158	9.283	1234933	1.24	209287	0.69	5.90		Levoglucosenone
6	9.900	9.892	9.983	-133412	-0.13	-2107	-0.01	0.00	MI	Goitrin
7	10.485	10.408	10.525	325795	0.33	132209	0.44	2.44	MI	ETHYL 2-ACETYLOCTANO
8	10.820	10.758	10.900	354698	0.36	167468	0.55	2.12	MI	Benzaldehyde, 2,4-dimethyl-
9	13.171	13.108	13.283	662666	0.66	182953	0.60	3.62	MI	1-DODECANOL
10	13.644	13.600	13.742	507581	0.51	185354	0.61	2.68	MI	TRICYCLO[4.4.0.0(2,7)]DE
11	14.008	13.942	14.117	425850	0.43	112301	0.37	3.85	MI	1H-CYCLOPROP[E]AZULE
12	14.435	14.375	14.517	618064	0.62	260677	0.86	2.36	MI	2-Isopropenyl-4a,8-dimethyl-
13	14.717	14.658	14.767	1139966	1.14	391600	1.29	2.91	V	.ALPHA.-SELINENE
14	15.911	15.875	15.967	1367992	1.37	585603	1.93	2.34		1,1,4,7-TETRAMETHYLDE
15	16.551	16.475	16.608	6897366	6.92	2437487	8.04	2.83		2-Naphthalenemethanol, 1,2,3
16	16.658	16.608	16.708	1198127	1.20	425277	1.40	2.82	V	NAPHTHALENE, 1,2,3,4,4A,
17	16.849	16.725	16.950	16012785	16.06	3756432	12.39	4.26		2-Naphthalenemethanol, decal
18	17.663	17.583	17.700	951304	0.95	392281	1.29	2.42	MI	Benzenepropanamide, N-(2-b
19	18.346	18.275	18.400	2870087	2.88	697953	2.30	4.11		Benzene, 1-(1,1-dimethylethy
20	18.450	18.400	18.492	1270150	1.27	348323	1.15	3.65	V	2-Naphthalenemethanol, 2,3,4
21	18.951	18.908	18.967	1656635	1.66	750677	2.48	2.21		ROSIFOLIOL

DISCUSSION

Medicinal plant is the most exclusive source of life saving drugs for majority of the world's population. They continue to be an important therapeutic aid for alleviating the ailments of human kinds. India has a rich and diverse flora of flowering medicinal plants. Plants have been used as medicines by all cultures from arced times to the resent days. Medicinal plants play a vital role in human health care, about 80% of the world population role on the use of traditional medicine, concomitantly based on plant materials. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes is now 150 billion, which will double by the year 2025. The results of the maximum antibacterial activity was identified with ethanolic and methanolic leaf extract of *C. roseus* against *S. typhi* and the antimicrobial activity of the ethanolic extract might be due do the presence of the unique phytochemical constituents[3].

The plant of *C. roseus* has a very great medicinal value. The vast collection of literature and publication and about 295 patents dealing with the plant and its products, very well illustrated this fact. This plant has been mostly studied with respect to its anticancer, anti hypertension and anti diabetic properties. Therefore, there is need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as the medicinal plants.

The antimicrobial activity found in this present study may be attributed to the presence of secondary metabolites of varies chemical types present in the plant material either individually. The discovery of a potent remedy from plant origin will be great advancement in microbial infection therapies.

In order to realize the health benefits from potential plant sources, it is important to measure the antioxidant activity using various radical and oxidation systems. The four bacterial strains (*B. subtilis, P. aeruginosa, S. aureus* and *E. coli*) used in this experiment are responsible for human diseases such as cholecystitis, urinary infection, skin infections etc. However, these human pathogenic strains were significantly inhibited by the methanolic leaf extracts of the medicinal plants.

There are, the study provides support to the plant's traditional and alternative use against various diseases and infections. Further, the biomolecules present in the extract which are active against these microbes needs to be characterized. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance to conventional synthetic antibiotics. Hence, this study holds importance in using medicinal plants as an alternative source for treating various diseases.

CONCLUSION

In the present study phytochemical, antioxidant, antimicrobial and anticancer cell line study have been done using *Catharanthus roseus* plant water and methanol extracts. Among the two extract tried methanol extract was found to be the best extract for the all studies. The methanol extract was further purified using silica gel column followed by GC-MS studies. TLC study revealed the presence of compound in the plant extract. The anti bacterial studies showed the anti microbial activity against *Pseudomonas aeruginosa* and *Bacillus subtiIis* among the pathogen used. The He La cell line cytotoxins study was also conducted and the cell death was 23%. GC-MS study revealed the presence of compounds in the extracts towards the anti microbial, anti cancer and anti inflammatory effect.

Acknowledgement

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