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COMPARATIVE STUDIES ON PHARMACOLOGICAL ACTIVITIES OF *C. roseus* LEAF & FLOWER EXTRACT

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ABSTRACT

Catharanthus roseus (periwinkle) is an important medicinal plant for novel pharmaceuticals sincemost of the bacterial pathogens are developing resistance against many of the currently available anti microbial drugs. This study aims to investigate some of the anti microbial properties, phytochemical analysis, antidiabetic activity, antiviral activity in this plant. The findings show that the extracts from the leaves and flower of this plant can be used as prophylactic agent in many of the diseases, which sometime are of the magnitude of an epidemic.

KEYWORDS

vinblastine, vincristine, C. roseus, antioxidant, antidiabetic, antiviral.

INTRODUCTION

Medicinal plants are the source of various pharmaceutical products that shows pharmacological effect on the human beings. Instead of using chemical drugs which cause side effect, we can use

ancient medicine to identify the novel drug formulations that are more effective with lesser side effects and also cheaper cost. Though, many of the traditional drugs were used without understanding the basic mechanism, their effect could be proved further with the help of the present technology and tools. The active compound that is responsible for the pharmacological effect could be found very easily and also commercialized as a drug product itself with proper approval from the respective organizations. *Catharanthus roseus* is one of the important medicinal plants found. It is used for the treatment of a number of diseases such as diabetes, sore mouth, mouth ulcers, and leukemia. It produces about 130 alkaloids such as reserpine, vinceine, raubasin and ajmalcine. Anti-leukemic activity is shown by vinblastine and vincristine. There are a number of reports supporting its anti-microbial activity against *Staphylococcus albusi, Bacillus megatarium, Shigella, Pseudomonas*, etc. Its anti-oxidant and antimutagenic effects have also been reported. Further studies need to be done to explore its anti-tumour effects (Jai Narayan Mishra *et al.*, 2017).

MATERIALS AND METHODS

Collection of Plant sample

The samples for research *Catharanthus roseus* (Madagascar periwinkle) were collected from Singanallur, Coimbatore Tamil Nadu, India and then subjected for morphological, microscopical and physicochemical analysis.

Fig 1 - Sample plant *C. roseus*

Drying and powedering of *Catharanthus roseus*

The Plant material was collected and the dust particles of plant material were removed by washing the *Catharanthus* with water and shade dried for one week. The leaves and flowers of *Catharanthus roseus* were cut into small pieces. Thus, the chopped plant material was pulverized by a mechanical grinder, and then it is sieved through 40 mesh and stored in an air tight and light resistant container for further use.

Phytochemical Screening

The crude fractions of the selected herbs were checked for the presence of Phenol test, Reducing sugar test, Saponin test, Flavonoid test, Phyto steroid test, Ninhydrin test, Steroid test, Tannin test, Glycoside test. The results are expressed as (+) for the presence and (-) for the absence of phytochemicals. The qualitative phytochemical study was performed on the extracts by using below standard tests.

Phytochemical constituents extraction

The finely powdered *Catharanthus roseus* (Madagascar periwinkle) is subjected to a soxhlet extraction. For the soxhlet extraction water is used as solvent due to its non-toxicity nature.

Phytochemical Analysis

Various biochemical compounds that play a vital role in maintenance of human health and diet which also present in the fruits of the trees or plants. The biochemical compounds, especially secondary metabolites that found and isolated from several plants have shown that these compounds have anticancer, antihaemolytic, antimicrobial, analgesic, antitumor, antinuclear, and anti- inflammatory. These phytochemical compounds including Phenol test, Reducing sugar test, Saponin test, Flavonoid test, Phyto steroid test, Ninhydrin test, Steroid test, Tannin test, Glycoside test. Phytochemical compounds shall be effectively obtained on the solvent extraction from plants. The phytochemical study was performed on the *Catharanthus* extract standard and the results were observed.

Test for Alkaloids (Wagner's test)

About few ml of plant extract was treated with 4-5 drops of Wagner's reagent. The formation of reddish brown precipitate confirms the presence of Alkaloids

Test for Phenol (Ferric chloride test)

About 2ml of the extract was treated with 10% ferric chloride solution and observed for the formation of deep blue / black colour.

Test for reducing sugars (Fehling's Test)

To 1 ml of the extract added few drops of Fehling's reagent and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of blue color.

Test for Saponins (Foam test)

To 2 ml of the plant extract added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins.

Test for Flavonoids

To about 2ml of plant extract, few drops of 10% ferric chloride solution was added. The formation of green or blue color indicates the presence of flavonoids.

Test for Phytosterols (Salkowski's Test)

One ml of the plant extract was treated with 2 ml of chloroform and few drops of acetic anhydride were added. To that mixture added equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols

Test for Aminoacids and Proteins (Ninhydrin test)

To a few ml of plant extract added small amount of Ninhydrin reagent. A purple or violet colour formed indicates the presence of amino acids and proteins

Test for Steroids

About 2 ml of chloroform and 0.2 ml of concentrated sulphuric acid was added to 1ml of flower extract. The formation of red colour precipitate indicates the presence of steroids.

Test for Tannin

To about 1ml of plant extract added few drops of dilute ferric chloride solution. The presence of tannin is confirmed by the formation of dark green or blue color.

Test for glycosides

To 1ml of plant extract added few ml of concentrated sulphuric acid. Formation of red colour indicates the presence of glycoside.

Antioxidant Activity

DPPH radical scavenging activity

The free radical scavenging activity of methanolic extract of plant extract was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca *et al.,* 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.5 to 2.5 µl of plant extract/ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)} = 045}{\text{A518 (control)}} \times 100$$

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of plant extracts samples were estimated according to the procedure described by Benzie and Strain (1996) and as modified by Pulido *et al*. (2000). FRAP reagent (900 µl), prepared freshly and incubated at 37 ºC, was mixed with 90 µl of distilled water and 30 µl of test sample, or acetone (for the reagent blank). The test samples and reagent blank were incubated at 37 ºC for 30 minutes in a water bath. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl3.6H2O and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At the end of incubation period the absorbance readings were recorded immediately at 593 nm using a spectrophotometer. The known Fe (II) concentration ranging between 100 and 2000 µmol/l (FeSO4.7H2O) was used for the preparation of the calibration curve. The parameter Equivalent Concentration (EC1) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Nitric oxide (NO) scavenging activity

NO scavenging activity (Garrat DC., 1964) of sample was determined by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH - 7.4) and 100 µL of different concentration of plant extract. This reaction mixture was kept for incubation at 25°C for 150 minutes. To 0.5 mL of above solution, 0.5 mL of Griess reagent was added (0.1 mL of sulfanilic acid and 200 µL naphthylethylenediamine dichloride (0.1%) w/v)). This was kept on incubation at room temperature for 30 minutes, and finally absorbance is observed at 540 nm. All the reactions were performed in triplicates, and their percentage inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/A_{control}) \times 100$$

Hydrogen peroxide scavenging activity (H_2O_2)

This activity of the plant was evaluated by the method of Ruch *et al*. 1989. 850 µL of the aqueous plant extract was added to 150 µL of 4 mM hydrogen peroxide solution prepared in phosphate buffer (0.1 M, pH-7.4). This was incubated for 10 minutes, and absorbance was read at 230 nm. BHT was taken as a positive control and the reaction was carried out in triplicates. Percent inhibition of the assay was calculated.

% Inhibition = (A control-A sample) /A control) X 100

Antibacterial Activity

To perform antimicrobial activity using various bacterial and fungal species were selected viz., *Escherichia coli, staphylococcus aureus, pseudomonas species, bacillus species, klebsiella species; proteus species* are bacterial cultures.

Media and culture condition

Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani (LB) were used throughout the study for determining the antibacterial assay. The media was adjusted to the pH and autoclaved at 121°c for 15 minutes.

Preparation of the Bacterial Inoculum

Stock cultures were maintained at 4°c on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37°c on shaking incubator and fungal cultures were incubated at 27°c for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37°c for 24 hours and fungal incubated at 27°c for 3-5 days. A single colony was transferred to nutrient agar media slantswere incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27°c for 3-5 days. These stock cultures were kept at 4°c. For use in experiments, a loop of each test

organism was transferred into 50ml nutrient broth and incubated separately at 37°c for 18-20 hours for bacterial culture.

Well Diffusion method

The antibacterial activity and antifungal activity of crude extract extracts was determined by Well Diffusion method (Bauer *et al*., 1996). MHA plates were prepared by pouring 20ml of molten media into sterile petriplates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. Then 10-50 µl of plant extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on mullerhintonagar plate and potato dextrose agar. Well were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°c for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Antidiabetic Activity

Plants have always been a source of drugs for humans since time immemorial. The Indian traditional system of medicine is replete with the use of plants for the management of diabetic conditions. According to the World Health Organization, up to 90% of population in developing countries use plants and its products as traditional medicine for primary health care. The present review is aimed at providing in-depth information about the antidiabetic potential and bioactive compounds present in *Ficus religiosa, Pterocarpus marsupium, Gymnema sylvestre, Allium sativum, Eugenia jambolana, Momordica charantia*, and *Trigonella foenum-graecum*. The review provides a starting point for future studies aimed at isolation, purification, and characterization of bioactive antidiabetic compounds present in these plants.

Antiviral Activity

Despite successful vaccination programs and effective treatments for some viral infections, humans are still losing the battle with viruses. Persisting human pandemics, emerging and re-emerging viruses, and evolution of drug-resistant strains impose continuous search for new antiviral drugs. A combination of detailed information about the molecular organization of viruses and progress in molecular biology and computer technologies has enabled rational antivirals design. Initial step in establishing efficacy of new antivirals is based on simple methods assessing inhibition of the intended target. We provide here an overview of biochemical and cell-based assays evaluating the activity of inhibitors of clinically important viruses.

RESULTS AND DISCUSSION

Phytochemical Analysis

The qualitative phytochemical study was performed on the *Catharanthus roseus* extracts by using the standard tests. The result reviews that medically active compounds were present in the *c. roseus* extract.

The formation of reddish-brown precipitate confirms the presence of Alkaloids. The formation of deep blue / black colour indicates the presence of phenol. Presence of reducing sugars observed by the appearance of blue colour. Formation of persistent foam for few seconds confirms the presence of saponins.The formation of green or blue colour indicates the presence of flavonoids. Formation of red colour precipitate indicates the presence of steroids. The presence of tannin is confirmed by the formation of dark green or blue colour. The results are expressed as (+) for the presence and (-) for the absence of phytochemicals in the table given below (Table-4) (k Kabesh *et al*., 2015).

Fig 2 – Phytochemical Analysis- *C. roseus*

Test	Observation	Leaf extract Result	Flower extract result
Alkaloids	Reddish brown precipitate	+ + +	+ + +
Phenol	Deep blue colour	+ + +	+ + +
Reducing sugar	Blue colour	+ +	+ +
Saponin	Foam formation	Ab	+ +
Flavonoids	Dark blue	+ + +	+ + +
Phyto sterols	Blueish green colour	+ +	+ + +
Protein	Purple or violet colour	+ + +	+ +
Steroids	Red colour precipitate	+	+ + +
Tannins	Dark green or blue colour	+ + +	+ + +
Glycoside	Red colour	+ +	+ + +

Table 4 – Results of phytochemical screening done for *Catharanthus roseus* extracts

Antioxidant Activity

DPPH Assay Cathranthus roseus

Table: 1 DPPH Assay *C. roseus*

Extract Concentration	OD at 518nm
5 µl	0.015
10 µl	0.180
15 µl	0.255
20 µl	0.341
25 µl	0.482

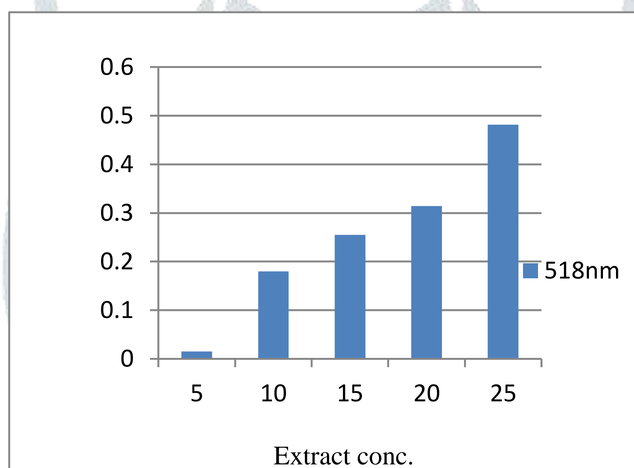


Figure:-3 DPPH Assay *Canthranthus roseus*

FRAP Assay:

The concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

Table: 2 FRAP Assay *Catharanthus roseus*

Extract Concentration	OD at 590nm
0	0
5 µl	0.270
10 µl	0.311
15 µl	0.385
20 µl	0.437
25 µl	0.438

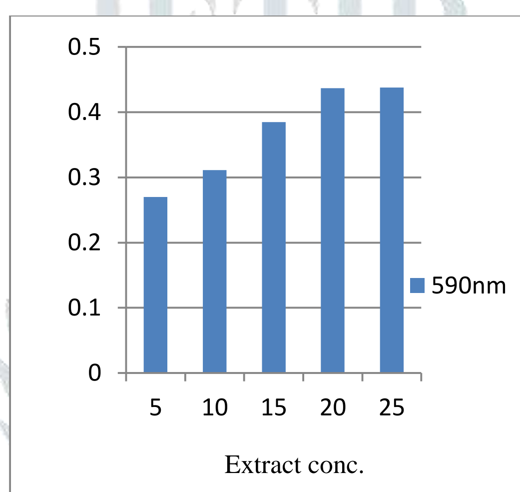


Figure: 4- FRAP Assay *Catharanthus roseus*

Hydrogen peroxide scavenging activity (H_2O_2)

The hydrogen peroxide scavenging activity was measured absorbance was read at 610 nm. BHT was taken as a positive control and the reaction was carried out in triplicates. Percent inhibition of the assay was calculated.

Table 3 – H_2O_2 Assay

Flower extract	OD at 610 nm
5	0.090
10	0.129
15	0.211
20	0.382
25	0.477

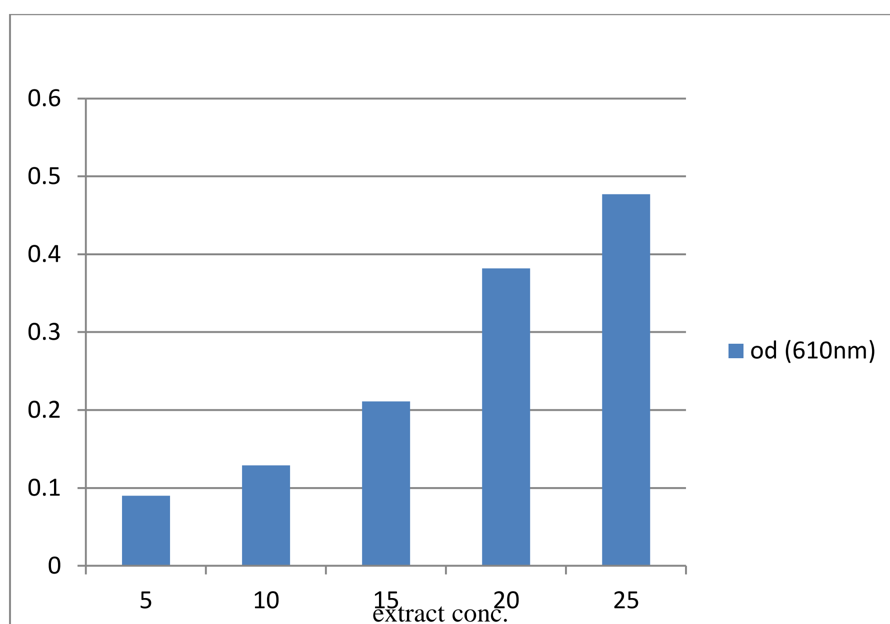


Figure 5 – H_2O_2 Assay

Antidiabetic Activity

The hydrogen peroxide scavenging activity was measured absorbance was read at 610 nm. The antidiabetic activity is increase with adding increase concentration.

Extract Concentration	OD at 610nm
0	0
10µl	0.060
20µl	0.122
30µl	0.231
40µl	0.303
50µl	0.416

Table: 3 Antidiabetic activity of *C. roseus*

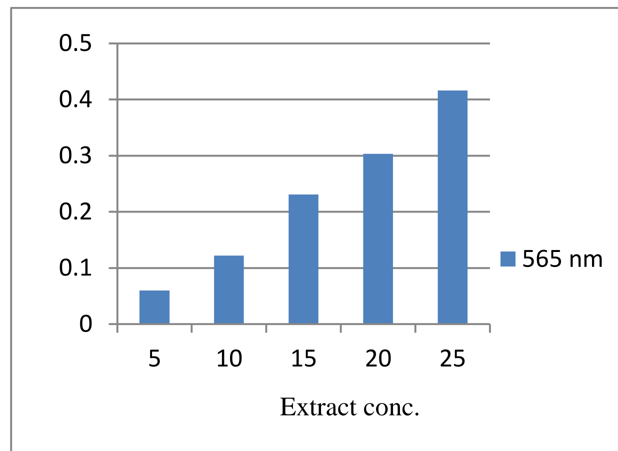
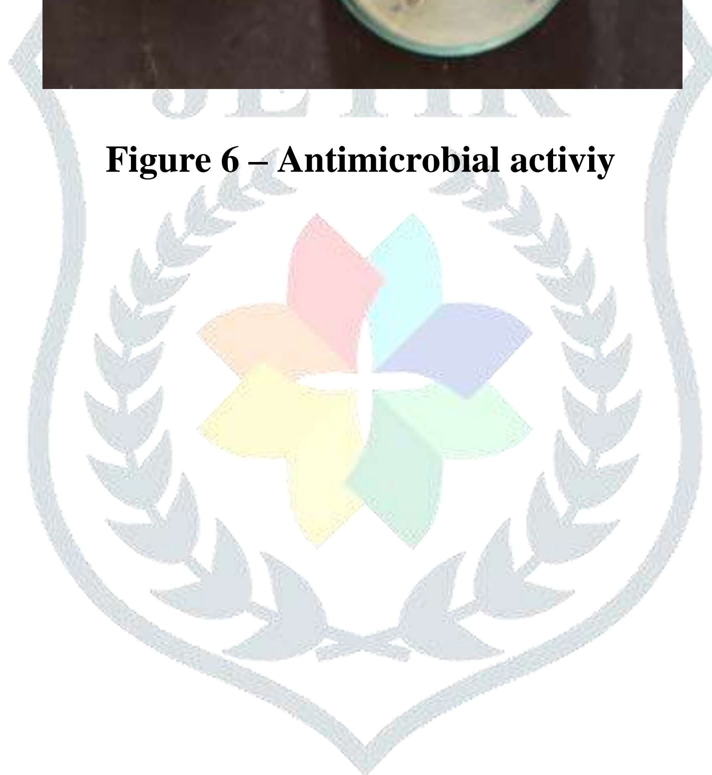


Figure: 5 - Antidiabetic activity *Catharanthus roseus*

Antimicrobial Activity

Antibacterial activity was performed by agar diffusion method. The stock culture of bacteria (Pseudomonas, Bacillus,) were received by inoculating in nutrient broth media and grown at 37% for 18 hours. The agar plates of the above media were prepared. Each plates was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. Cut the 5 wells Pour the plant extract in ratio. All the plates were incubated at 37°C for 24 hrs. Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory conc. or plant extracts against Gram positive, Gram negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

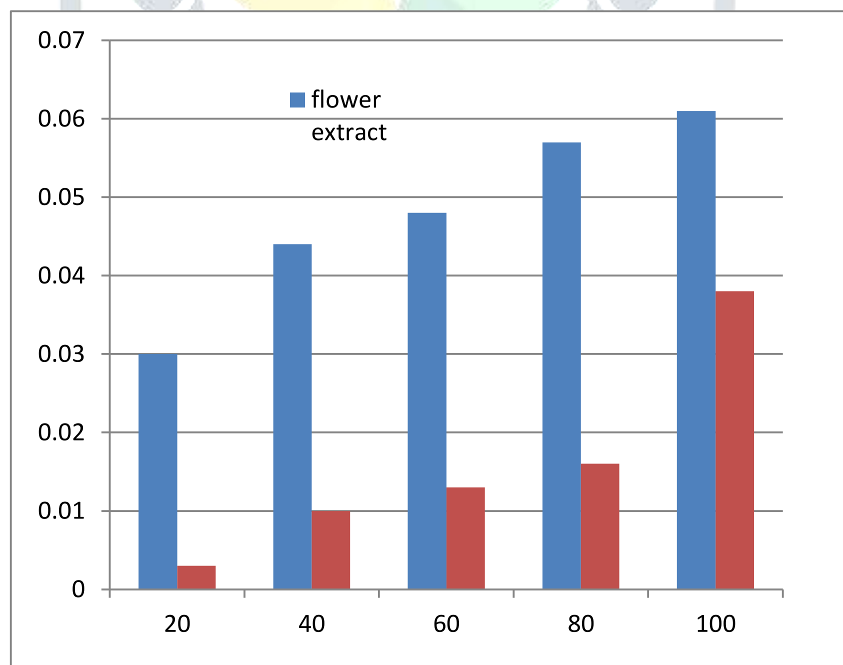
Figure 6 – Antimicrobial activiy



Antiviral Activity

Extract concentration	Flower extract (od at 545nm)	Leaf extract (od at 545nm)
20	0.030	0.003
40	0.044	0.010
60	0.048	0.013
80	0.057	0.016
100	0.061	0.038

Table 5 – Antiviral Activity



CONCLUSION

In the present study phytochemical, antioxidant, antimicrobial, antidiabetic and antiviral have been done using *Cathranthus roseus* plant water extracts. The antibacterial studies showed the antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* among the pathogen used. Comparing to the leaf, the pharmacological activites are highly present in flower extract. We make syrup from *Cathranthus roseus* flower extract.

REFERENCES

1)Akhtar, M.S., Panwar, J. and Yun, Y.S., 2013. Biogenic synthesis of metallic nanoparticles by plant extracts. ACS Sustainable Chemistry & Engineering, 1(6), pp.591-602.

2) Ameh, T. and Sayes, C.M., 2019. The potential exposure and hazards of copper nanoparticles: a review. Environmental toxicology and pharmacology, 71, p.103220.

3) Awwad, A.M., Albiss, B.A. and Salem, N.M., 2015. Antibacterial activity of synthesized copper oxide nanoparticles using Malva sylvestris leaf extract. SMU Med J, 2(1), pp.91-101

4) Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., 1985. Natural plant chemicals: sources of industrial and medicinal materials. Science, 228(4704), pp.1154-1160.

5) Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., 1985. Natural plant chemicals: sources of industrial and medicinal materials. Science, 228(4704), pp.1154-1160.

6) Bhutkar, M.A. and Bhise, S.B., 2011. Studies on Antioxidant Properties of Catharanthus rosea and Catharanthus alba. Journal of Current Pharma Research, 1(4), p.337.

7) Baskar, G., Sakthivel, D. and George, G.B., Synthesis, characterization and anticancer activity of copper nanobiocomposite synthesized by leaf extract of Catharanthus roseus.

8) Čermák, V., Dostál, V., Jelínek, M., Libusová, L., Kovář, J., Rösel,

D. and Brábek, J., 2020. Microtubule-targeting agents and their impact on cancer treatment. European journal of cell biology, p.151075.

9) Chattopadhyay, R.R., Sarkar, S.K., Ganguly, S., Banerjee, R.N. and Basu, T.K., 1991. Hypoglycemic and antihyperglycemic effect of leaves of Vinca rosea linn. Indian Journal of Physiology and Pharmacology, 35(3), pp.145-151.

10) Cheirmadurai, K., Biswas, S., Murali, R. and Thanikaivelan, P., 2014. Green synthesis of copper nanoparticles and conducting nanobiocomposites using plant and animal sources. RSC Advances, 4(37), pp.19507-19511.

11) Chung, I.M., Abdul Rahuman, A., Marimuthu, S., Vishnu Kirthi, A., Anbarasan, K., Padmini, P. and Rajakumar, G., 2017. Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidant and cytotoxic activities. Experimental and therapeutic medicine, 14(1), pp.18-24.

12) Farquar, J.N., 1996. Plant Sterols, their biological effects in human.

Handbook of lipids in human nutrition.

13) Gajalakshmi , S. Vijayalakshmi, S. And Devi Rajeswari, V (2013) 'Pharmacological activites of catharanthus roseus', International Journal of pharma and Biosciences.

14) Hu, N., Li, R., Wu, Z.L., Huang, D. and Li, H.Z., 2015. Intensification of the separation of CuO nanoparticles from their highly diluted suspension using a foam flotation column with S type internal. Journal of Nanoparticle Research, 17(10), pp.1-11.

15) Issaabadi, Z., Nasrollahzadeh, M. and Sajadi, S.M., 2017. Green synthesis of the copper nanoparticles supported on bentonite and investigation of its catalytic activity. Journal of cleaner production, 142, pp.3584-3591.

16) Kabesh, K., Senthilkumar, P., Ragunathan, R. and Kumar, R.R., 2015. Phytochemical analysis of Catharanthus roseus plant extract and its antimicrobial activity. Int. J. Pure App. Biosci, 3(2), pp.162-172.

17) Khatami, M., Heli, H., Jahani, P.M., Azizi, H. and Nobre, M.A.L., 2017. Copper/copper oxide nanoparticles synthesis using Stachys lavandulifolia and its antibacterial activity. Iet Nanobiotechnology, 11(6), pp.709-713.

18) Krishnaraju, A.V., Rao, T.V., Sundararaju, D., Vanisree, M., Tsay, H.S. and Subbaraju, G.V., 2005. Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. Int J Appl Sci Eng, 3(2), pp.125-34.

19) Maki, H., Ando, S., Kodama, H. and Komamine, A., 1991. Polyamines and the cell cycle of Catharanthus roseus cells in culture. Plant physiology, 96(4), pp.1008-1013.

20) Mari, A., Vincent, M.V., Mookkaiah, R., Subramani, R. and Nadesan, K., 2020. Catharanthus Roseus Leaf Extract Mediated Facile Green Synthesis of Copper Oxide Nanoparticles and Its Photocatalytic Activity. Chemical Methodologies, 4(4), pp.424-436.