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"ANTICANCEROUS AND ANTIOXIDANT INVESTIGATIONS ON THE HERBAL EXTRACT OF CATHARANTHUS PUSILLUS (MURRAY) G. DON".

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ABSTRACT

Catharanthus pusillus (Murray) G. Don of the family Apocynaceae, is a common weed growing widely in various parts of Kerala. It is used as one of the important drug in traditional system of medicine to treat various ailments. Phytochemical analysis of whole plant extract showed the presence of alkaloids, tannins, phenol, flavonoids, steroids, carbohydrate and proteins. A few studies showed the extract possess anti-cancerous and pain relieving properties. Hence the present study is concentrated on the anti-cancerous and antioxidant activity of this. The antioxidant activity was determined by three methods DPPH (1, 1-diphenyl -2- picryl hydroxyl) assay, super oxide radical scavenging assay and reducing power assay. Anticancer activity was done by using cell line MCF-7.

KEYWORDS: Catharanthus pusillus, Apocynaceae, Phytoconstituents, Antioxidant.

INTRODUCTION

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as a raw material for various scientific investigation. Many secondary metabolites of plants are commercially find and use in a number of pharmaceutical compounds. Plant extracts contain many chemical compound which are biologically active in the human body. For centuries human had used plants and plant extracts to treat various disease conditions and more recently to produce new drugs. Most of the plants can provide biologically active molecules that are inevitable for the sustenance of life. World Health Organization estimated that 70-80% of people worldwide

rely chiefly on traditional knowledge of herbal medicine to meet their primary healthcare needs.

Catharanthus pusillus is a perennial decumbent herb grows up to 1m tall, with white latex. The genus Catharanthus consists of eight species of which seven are endemic to Madagascar and one, C. pusillus to India .It is known with various names in India and all over the world. The synonyms are Lochnera pusilla and Vinca pusilla. C.pusillus is commonly known as Tiny Periwinkle in English and Chupa vela in Malayalam. Catharanthus pusillus is widely used as various treatments of diseases and traditionally used as herbal medicine. The roots, leaves and latex of these plants are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumor ear aches, meningitis in children, rheumatic, heart diseases, hernia, infantile malnutrition, dyspepsia and asthma. Further its action is antimicrobial, anticancerous antioxidant and anthelmintic properties also have been reported.

MATERIALS AND METHODS

Collection of Plant material

Catharanthus pusillus (Murray) G. Don was collected from Vellimon, Kollam District, Kerala during early morning. The collected plant was identified at the Herbarium, Department of Botany, University College, Palayam, Trivandrum.

Extraction of plant material

The phytochemicals present in the plant material was extracted by the distillation method using soxhlet apparatus. Different solvent systems were used for the separation of chemical (methanol). About 500 gm of plant tissue were weighed and shade dried for 10 days. The dried materials were powdered and 100g of powder sample was packed in a thimble and kept in soxhlet apparatus. The solvent was taken separately for the extraction and the powdered material was siphoned by 3 times. The whole apparatus was kept over a heating mantle and was heated continuously for 8 hours at boiling point of each solvent. The extract was concentrated to dryness and the residues were transferred to a pre weighed sample bottle and were stored in a desiccators for further studies.

3.7 Antioxidant Activity

DPPH Assay (1, 1-diphenyl -2-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was

measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol.

Procedure

Different volumes (1.25-20µg/µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm.3 ml of DPPH was taken as control.

Super oxide free radical scavenging activity

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Over production of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan product. 0.02 ml of extracts, 0.05 ml of Riboflavin solution (0.12mM) 0.2 ml of EDTA solution [0.1M], and 0.1 ml NBT (Nitro-blue tetrazolium) solution [1.5mM] were mixed in test tube and reaction mixture was diluted up to 2.64 ml with phosphate buffer [0.067M]. The absorbance of solution was measured at 560nm using DMSO as blank after illumination for 5 min and difference in OD was determined after 30 minutes incubation in fluorescent light. Absorbance was measured after illumination for 30 min. at 560 nm on UV visible spectrophotometer.

Reducing power activity

Reducing power of extract was determined by the method of different concentrations of extract (1.25-20µg/µl) were mixed with 2.5 ml of phosphate buffer (200mM), (pH 6.6) and

2.5ml of 1% potassium ferric cyanide was added and boiled for 20 minutes at 50°C. After incubation, 2.5 ml of 10% TCA were added to the mixtures followed by centrifugation at 650 xg it for 10 minutes. The upper layer (5ml) was mixed with 5 ml of distilled water and 1ml of 0.1% ferric chloride was added and the absorbance was read at 700nm.

Anticancer Activity

Determination of In vitro and proliferative effect of-extracts on cultured MCF-7 cell line

MCF-7 breast cancer cell lines were purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 in a humidified atmosphere in a CO2 incubator (NBS, EPPENDORF, GERMANY). The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia)) for 2 minutes and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50 µg/ml and 100µg/ml from a stock of 1mg/ml and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

MTT Assay

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilised formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37^0C for 3 hours. MTT was removed by washing with 1x PBS and 200µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISASCAN, ERBA).

RESULT

Antioxidant Activity

Table-1 DPPH Assay of Methanol extract of Catharanthus pusillus (Murray) G. Don

Sample concentration(µg/ml)*	% inhibition (extract)	% inhibition (standard)
Control		
1.25	11.151	17.84
2.50	11.981	25.12
5	14.353	32.36
10	17.675	46.85
20	29.775	61.35

***µg/ml:microgram per millilitre

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of plant extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased. In the present study, the whole plant extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance.

In the present study, the whole plant extract of Catharanthus pusillus was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the plant extract was given in (Table1).The methanolic extract of Catharanthus pusillus showed the highest DPPH scavenging activity (29.775%) at 20µg/ml and the lowest percentage of inhibition (11.151%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (61.35%) at 20µg/ml and the lowest percentage of inhibition (17.84%) at 1.25µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and plant extract. The plant extract has lower DPPH scavenging activity than that of standard. The methanolic extract of plant has a good antioxidant activity at higher concentrations.

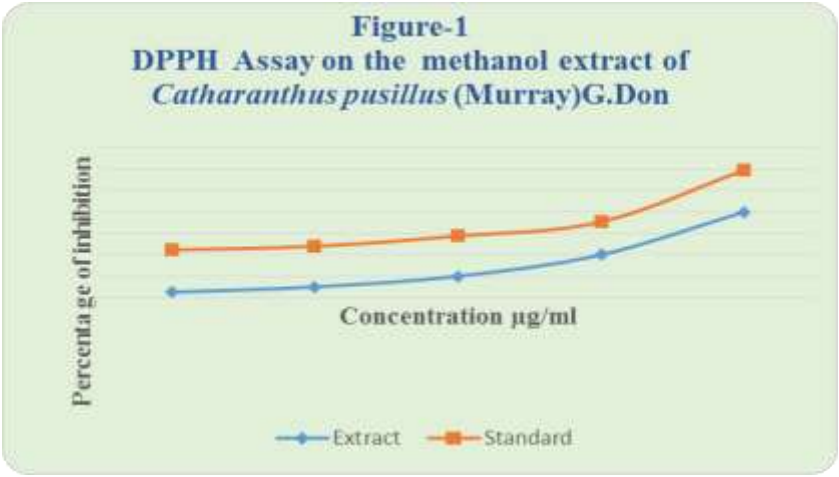


Table-2 Super oxide Free radical Scavenging Activity of Methanol extract of Catharanthus pusillus (Murray) G .Don

Sample concentration(µg/ml)	% of Scavenging (extract)	% of Scavenging (Ascorbic acid)
1.25	47.646	41.41
2.5	47.928	54.54
5	60.028	63.63
10	70.527	72.72
20	75.895	81.81

µg/ml:microgram per millilitre

The methanolic extract of Catharanthus pusillus showed the highest percentage (75.895%) of super oxide free radical scavenging activity at 20µg/ml and the lowest percentage of inhibition (47.646) at 1.25µg/ml but this lowest percentage of inhibition higher than that of standard at same concentration (1.25µg/ml). Ascorbic acid (Standard) showed highest percentage of inhibition (81.81%) at 20µg/ml and the lowest percentage of inhibition (41.41%) at 1.25µg/ml (Table 2). Super oxide free radical scavenging activity of both plant and standard increased with increase in concentration of extract.

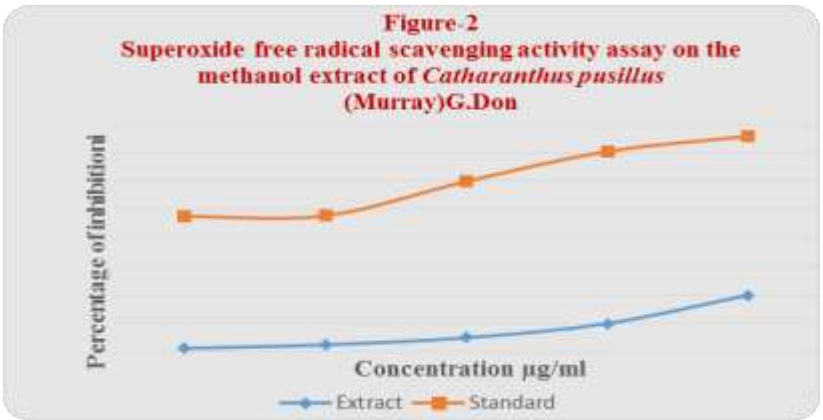
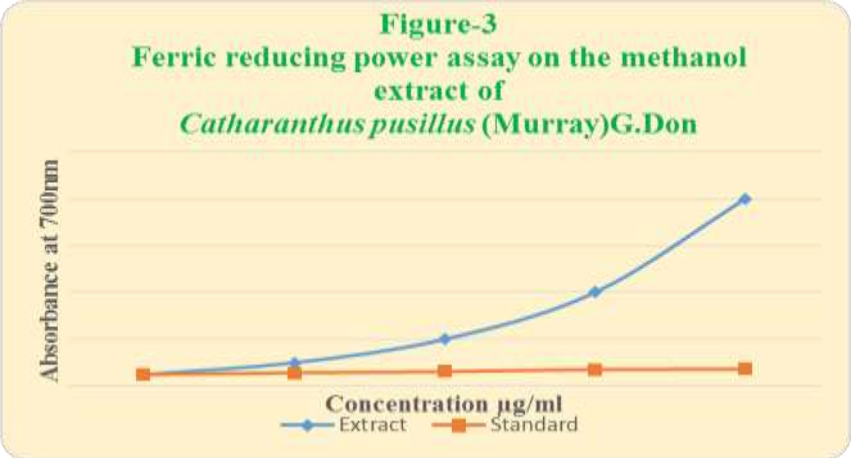


Table-3 Reducing Power Aactivity of Methanol extract of Catharanthus pusillus (Murray) G .Don

Sample concentration (µg/ml)	% of inhibition(extract)	% of inhibition (standard)
1.25	0.25	0.26
2.5	0.421	0.52
5.0	0.56	0.58
10	0.78	0.66
20	0.801	0.89

µg/ml:microgram per millilitre

Methanolic extract of plant showed higher reducing activity (0.8%) at higher concentration (20µg/ml). The lower reducing activity (0.25%) at 1.25 µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (0.89%) at 20µg/ml and the lowest percentage of inhibition (0.26%) at 1.25µg/ml (Table3). It has agreement with the fact that increased concentration also increases the reducing activity.



Anticancer Assay

Table-4: Anticancerous Assay of Methanol extract of Catharanthus pusillus (Murray)G. Don

Sample Concentration (µg/ml)	Percentage cell Viability (Extract)	Percentage Cell Inhibition (Extract)	Percentage cell viability (Doxorubicin)	Percentage cell Inhibition (Doxorubicin)
6.25	98.94	1.06	88.00	12
12.5	93.69	6.31	55.36	44.64
25	84.41	15.59	41.86	58.14
50	71.76	28.24	33.52	66.48
100	53.76	46.24	26.01	73.99

% viability = (OD of Test/ OD of Control) X 100 Control OD: 0.945

µg/ml:microgram per millilitre

Anticancer activity of methanol extract against MCF-7 was performed using MTT assay. In this, the concentration of formazan formed by reduction of MTT by succinate dehydrogenase in living cells was measured spectrophotometrically. Results of anticancer activity on MCF-7 Breast cancer cell line is shown in (Table4 and fig4,5) The extract showed a potent cytotoxic activity against MCF-7 cancer cell line. Methanol extract of Catharanthus pusillus at 6.25, 12.5, 2.5, 50 and 100mg/ml showed % of cell inhibition 1.06, 6.31, 15.59, 28.24 and 46.24% and % of cell viability 98.94, 93.69, 84.41, 71.76 and 53.76 respectively. The highest % of cell inhibition (46.24%) was observed at 100mg/ml and lowest % of cell inhibition (1.06) at 6.25mg/ml. In the present study increase in the concentration of sample, percentage of cell inhibition also increases and decreases cell viability. The results showed that percentage of cell inhibition was lower than compared to standard Doxorubicin.

Figure-4
Effect of methanol extract of *Catharanthus pusillus* (Murray) G. Don on MCF-7 cell Inhibition

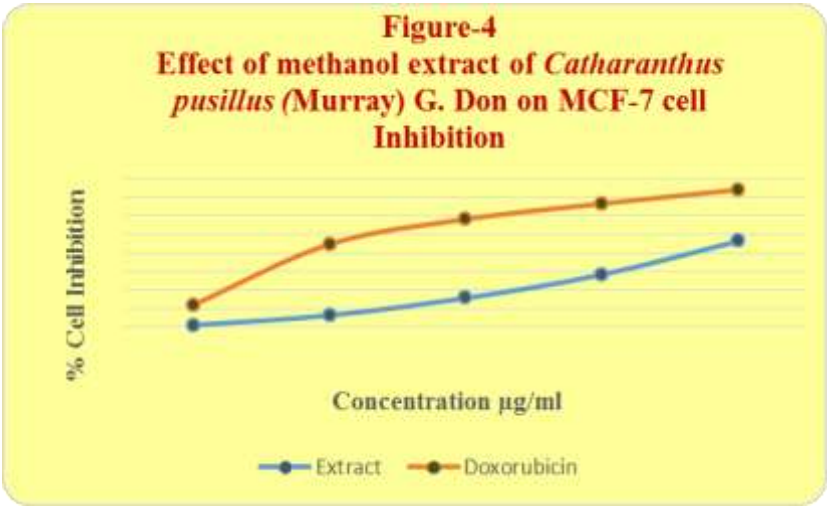
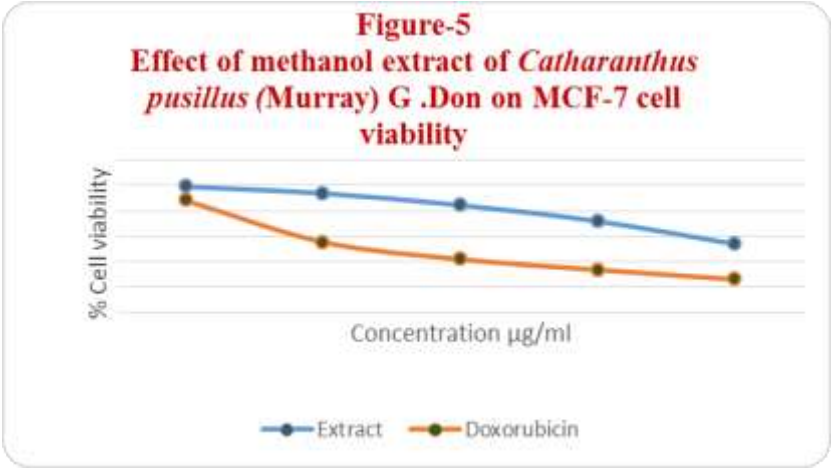


Figure-5
Effect of methanol extract of *Catharanthus pusillus* (Murray) G .Don on MCF-7 cell viability



CONCLUSION

Nowadays the demand for natural products and plant- based medicines is growing throughout the world. Catharanthus pusillus is a remarkable herb owing to its broad spectrum

of applications. The current study describes the antioxidant and anticancer activities of crude extracts of C. pusillus. The data obtained shows that C. pusillus possesses very high free radical scavenging and anti proliferative properties. In future the compounds present in the plants are proposed to be purified from the crude extract to identify the potential anticancer compounds that will be tested against various cancer cell lines. Hence, more work could be done on the above plant to reveal the unknown mysteries which would help the need of the present pharmaceutical world.

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