BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

Phytochemical Screening and Combined Invitro Anti-Microbial and Anti-Oxidant Studies of Various Extracts of Vinca Rosea Leaves

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Abstract:

Medicinal plants are important species of plants that according to the traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases, make human health more invigorating. These plants are contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs.Phytochemical screening is the scientific process of analysing, examining, extracting, experimenting, and thus identifying different classes of phytoconstituents present in various parts of the base for the discovery of drugs, the active components could be further taken for investigation and research. The process was qualitative which is termed phytochemical screening. The outcome of the research could be fruitful in developing potent drugs against various diseases.There are many naturally grown plants around us which may be used for medicinal purpose. Among those Catharanthus roseus is the plant which globally found in tropical areas.

Key Words: Medicinal plants, Phytochemical screening, Discovery of drugs, Various diseases, Catharanthus roseus.

Introduction:

Catharanthus roseus Linn is a perennial plant which are mostly found in southern Asia, tropical countries and are native to Madagascar catharanthus roseus L. has many common names like vinca rosea, Madagascar periwinkle, bright eyes, Cape periwinkle, graveyard plant, old maid, pink periwinkle, rose periwinkle myrtle .It is used for ornamental purpose which has different colours of pink, purple and white and it is also used as medical plants.The oldest group of the plant alkaloids groups that used to treat cancer are the vinca alkaloids.. Among them there are two anti –neoplastic compounds derived from plants of vinblastine and vincristine.It also have antimicrobial activity, antioxidant activity, anti-diarrheal activity, hypolipidemic activity and also wound healing activity.

Materials and Methods:

The leaves of the plant Catharanthus roseus us were collected from our college Sree Vidyanikethan College of pharmacy Medicinal garden, rangampet.A, Chittoor district, Andhra

Pradesh, India. The fresh leaves were recognized and authentified in the Sree Venkateshwara University. The fresh leaves were dried for 10 days in shade. The powdered sample was stored in a bottle at room temperature before analysis.

Plant Profile

Botanical Description:

Catharanthus roseus is commonly known as bright eyes (English name)

- Kingdom: Plantae
- Angiosperms Class: Dicotyledenae
- Class: Dicotyledenae
- Division: Angiosperms
- Sub class: Gamopetalae
- Series: Bi-carpellatae
- Order: Gentiales
- Family: Apocynacea
- Genus: Catharanthus
- Species: roseus
- Other Common Names: Vinca rosea, cape periwinkle, graveyard plant, Madagascar periwinkle, old maid, pink periwinkle, rose periwinkle.

Methodology

1. Identification of the Plant.

- Selection of the plant was based on through literature survey.
- The plant was identified to haveinvitro antioxidant and invitro antimicrobial activity .
- The plant was identified and cultivated near Tirumala hills.

2. Authentication of the Plant :

Authentification is process or action that is mainly used to verify the identification of specific medicinal plants .

3. Extraction of the Plant :

Materials and Methods

The leaves of the plant Catharanthus roseus us were collected from our college Sree Vidyanikethan College of pharmacy Medicinal garden, rangampet.A, Chittoor district, Andhra Pradesh, India. The fresh leaves were recognized and authentified in the Sree Venkateshwara University. The fresh leaves were dried for 10 days in shade. The powdered sample was stored in a bottle at room temperature before analysis.

Maceration:

Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time. Moreover, this technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction. The whole or coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. This process is done in a closed vessel where an appropriate solvent (menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes:

(1) Promotes diffusion,

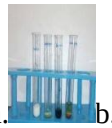
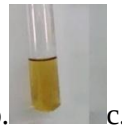
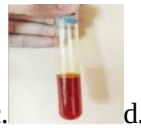
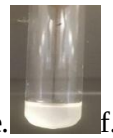
(2) Separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

Preparation of Extract:

- The fresh leaves are collected from a healthy plant of *Catharanthus roseus.*
- Thereafter the leaves were air-dried in shade at room temperature for 10 days.
- Then the dried leaves were grinded with an electrical grinder to obtain fine powder.
- The obtain powder was stored in a sealed bottle at room temperature.
- 15gms of powdered extract was weighed and mixed with the solvent 150ml of butanol.
- The mixture was allowed to macerate for a whole day. Whatsmann filter paper is used to filter the mixture after 24 hours.
- Finally, pressure was applied to the residue in order to extract the last bit of solvent.

RESULTSAND DISCUSSION:

1. Phytochemical screening:

a.  b.  c.  d.  e.  f.  g. 

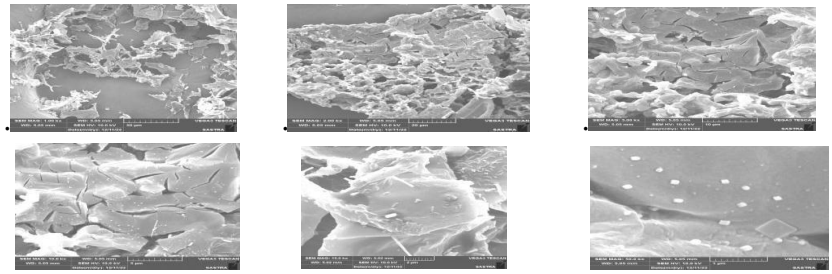
a.protein b.phytosteroids c.phenols d.carbohydrates e.tannis f.gums&mucilage g.alkaloids

S.NO	Phytoconstituents	Butanolic extract of catharanthus roseus
01	Alkaloids	+
02	Glycosides	+
03	Phenols	+
04	Flavonoids	+
05	Saponins	+
06	Gums/Mucilage	+
07	Carbohydrates	-
08	Amino acids	-
09	Proteins	-
10	Steroids	+
11	Tannins	+

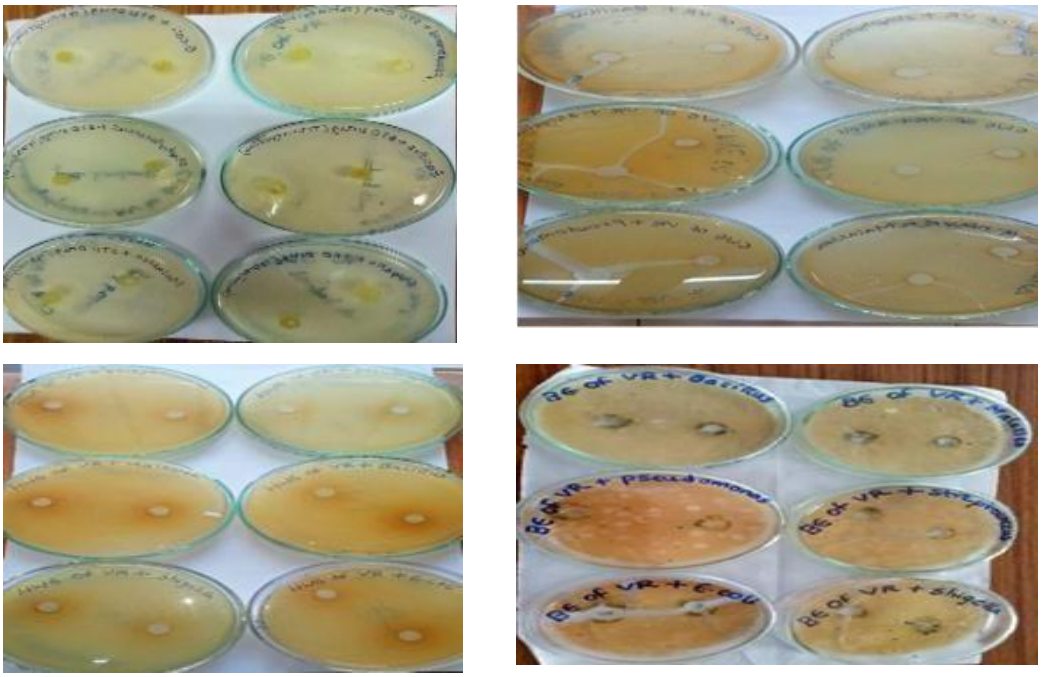
2. Scanning Electron Microscopy (SEM):

The surface characteristics of Cold water extract of Catharanthus roseus which was studied by SEM (Vegan 3 tescan). The specimens were scanned with an electron beam of acceleration potential of 10 kV and the images were collected as secondary electron mode.

1) SEM:



ANTI-MICROBIAL STUDIES OF VARIOUS EXTRACTS OF VINCA ROSEA LEAVES



Evaluation of Antioxidant activity by in vitro Techniques

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao (1990). The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA –H_2O_2system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat

(1964).The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization.

Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Total phenol

The measurement of total phenol is based on Mallick and Singh (1980) [14]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2°C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

Results And Discussion

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases. They are also involved in autoimmune disorders like rheumatoid arthritis etc.

Hydroxyl radical scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The percentage of Hydroxyl radical scavenging activity of Cold Water extract of *Catharanthus roseus* presented in Table 1. The Cold Water extract of *Catharanthus roseus* exhibited a maximum Hydroxyl radical scavenging activity of 48.27 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the Cold Water extract *Catharanthus roseus* and ascorbate were found to be 1100µg/ml and 410µg/ml respectively.

Table 1: Hydroxyl radical scavenging activity of Cold Water extract *Catharanthusroseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Cold Water extract)	Standard (Ascorbate)
1	125	24.11 ± 0.040	28.87 ± 0.074
2	250	35.21 ± 0.030	32.30 ± 0.054
3	500	41.36 ± 0.075	56.64 ± 0.020
4	1000	48.27 ± 0.021	75.23 ± 0.020
		IC_{50} = 1000 µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The percentage of hydroxyl radical scavenging activity of Cold water extract of *Catharanthus roseus* in Table 2. The Hot water extract of *Catharanthus roseus* was exhibited a maximum hydroxyl radical scavenging activity of 56.21 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the Hot water extract *Catharanthus roseus* and ascorbate were found to be 1000µg/ml and 410µg/ml respectively.

Table 2: Hydroxyl radical scavenging activity of Hot Water extract of *Catharanthus roseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Hot Water extract)	Standard (Ascorbate)
1	125	23.18 ± 0.081	26.87 ± 0.076
2	250	38.59 ± 0.067	30.30 ± 0.054
3	500	48.38 ± 0.042	60.64 ± 0.022
4	1000	56.21 ± 0.039	75.23 ± 0.014
		IC_{50} = 1000 µg/ml	**IC_{50} = 400 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The percentage of hydroxyl radical scavenging activity of alcoholic extract of *Catharanthus roseus* presented in Table 3. The alcoholic extract of *Catharanthus roseus* was exhibited a maximum hydroxyl radical scavenging activity of 89.64 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} of the alcoholic extract of *Catharanthus roseus* and ascorbate were found to be 1000µg/ml and 400µg/ml respectively.

Table 3: Hydroxyl radical scavenging activity of alcoholic extract of *Catharanthus roseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Alcoholic extract)	Standard (Ascorbate)
1	125	46.72 ± 0.012	26.87 ± 0.076
2	250	54..87 ± 0.049	30.30 ± 0.054
3	500	62.42 ± 0.036	60.64 ± 0.022
4	1000	69.64 ± 0.024	75.23 ± 0.014
		IC_{50} = 1000 µg/ml	**IC_{50} =600 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the above result alcoholic extract of *Catharanthus roseus* (IC_{50} = 1000 µg/ml) was found more effective than that of standard (IC_{50} = 600 µg/ml). The alcoholic extract of *Catharanthus roseus* was found to more effective than that of Cold water and Hot water extracts.

Nitric oxide scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems. The animal studies suggested the role for NO in pathogenesis of inflammation and pain. So it is worthful to investigate the NO scavenging potential of the plant extract.

The reduction of nitric oxide radical by the Cold water extract of alcoholic extract of *Catharanthus roseus* and ascorbate were illustrated in Table 4. The maximum nitric oxide scavenging activity of Cold water extract and ascorbate at 1000 µg/ml were found to be 46.37 % and 75.23%

respectively. The IC_{50} value of Cold water extract and ascorbate were recorded as 1000µg/ml and 450µg/ml respectively.

Table 4: Nitric oxide scavenging activity of Cold water extract of *Catharanthus roseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Cold water extract)	Standard (Ascorbate)
1	125	11.58 ±0 .015	26.87 ± 0.076
2	250	20.06 ± 0.049	30.30 ± 0.054
3	500	31.48 ± 0.030	60.64 ± 0.022
4	1000	46.37 ± 0.027	75.23 ± 0.014
		IC_{50} = 1000 µg/ml	**IC_{50} = 450 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The reduction of nitric oxide radical by the Hot water extract of *alcoholic* extract of *Catharanthus roseus* and ascorbate were illustrated in Table 5. The maximum scavenging activity of Hot water extract and ascorbate at 1000 µg/ml were found to be 64.37% and 75.23% respectively. The IC_{50} value of Hot water extract and ascorbate were recorded as 1000µg/ml and 500µg/ml respectively.

Table 5: Nitric oxide scavenging activity of Hot water extract of *Catharanthus roseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Hot water extract)	Standard (Ascorbate)
1	125	32.25 ± 0.051	26.87 ± 0.076
2	250	48.61 ± 0.029	30.30 ± 0.054
3	500	52.03 ± 0.031	60.64 ± 0.022
4	1000	64.37 ± 0.019	75.23 ± 0.014
		IC_{50} = 1000 µg/ml	**IC_{50} = 500 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The reduction of nitric oxide radical by the Alcoholic extract of *Catharanthus roseus*and ascorbate were illustrated in Table 6. The maximum scavenging activity of Alcoholic extract of *Catharanthus roseus*and ascorbate at 1000 µg/ml were found to be 79.31% and 75.23% respectively. The IC_{50} value of Alcoholic extract of *Catharanthus roseus*and ascorbate were recorded as 1000µg/ml and 600µg/ml respectively.

Table 6: Nitric oxide scavenging activity of Alcoholic extract of *Catharanthus roseus*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	39.42 ± 0.015	26.87 ± 0.076
2	250	47.58 ± 0.029	30.30 ± 0.054
3	500	56.60 ± 0.032	60.64 ± 0.022
4	1000	69.31 ± 0.028	75.23 ± 0.014
		IC_{50} = 1000 µg/ml	**IC_{50} = 600 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the above results the Alcoholic extract of *Catharanthus roseus* was found more effective in scavenging nitric oxide radical than that of Hot water and Cold water extracts. But when compared to the all the three extracts with Ascorbate (standard), the Alcoholic extract of *Catharanthus roseus* showed the better result.

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to anti-oxidative action. The total phenolic content of various extract of whole plant *Catharanthus roseus* was presented in Table

Table 7: The total Phenolic content of various extracts of *Catharanthus roseus*

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Cold water extract of *Catharanthus roseus*	1.50 ± 0.022
2	Hot water extract of *Catharanthus roseus*	2.60 ± 0.072
3	Alcoholic extract of *Catharanthus roseus*	4.80 ± 0.039

*All values are expressed as mean ± SEM for three determinations

Based on the result the Alcoholic extract of *Catharanthus roseus* found higher content of phenolic components than that of Cold water and Hot water extracts of *Catharanthus roseus.*

Summary And Conclusion

The present study was clearly indicated the Alcoholic extract of *Catharanthus roseus* showed strong antioxidant activity by inhibiting Hydroxyl radical scavenging, Nitric oxide radical scavenging activities when compared with standard Ascorbate.

In addition, the Alcoholic extract of *Catharanthus roseus* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

Reference

[1] Geeta watal et al, Preliminary phytochemical screening of six medicinal plants used in traditional medicine. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6,(5) 539-542
[2] M Vishwa Rohini et al, Preliminary phytochemical screening of selected medicinal plants of polyherbal formulation. Journal of pharmacognosy and phytochemistry. 2016; 5(5): 277-282.
[3] Sathiyadevi M et al, Preliminary Phytochemical Screening of Methanol Extract of Indigofera trita Linn. Journal of Molecular Histology & Medical Physiology, 2017, 2(1)1000112: 1-5.
[4] Aye tree Rai Basumatary, Preliminary Phytochemical Screening of some compounds from plant stem bark extracts of Tabernaemontana divaricate Linn. Used by Bodo Community at Kokrajhar District, Assam, India. Archives of Applied Science Research, 2016, 8 (8):47-52.
[5] Joseph BS et al, Preliminary phytochemical screening of selected Medicinal Plants. Int. Res. J. of Science & Engineering, 2013, Vol. 1(2): 55-62.
[6] S. R. Kane, Preliminary phytochemical screening of Glochidion ellipticum. European Journal of Experimental Biology, 2016, 6(4):41-45.
[7] Nidal Jaradat et al, Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of Ephedra alata Decne.journal Mater. Environ. Sci. 6 (6) (2015) 1771-1778.
[8] Ruchi R. Sharma, preliminary phytochemical screening of some indigenous medicinal plant leaves extract in regulation of ant diabetic activity. Science Research Reporter. 2012- 2(3):307-310.
[9] Benson Oloya et al, Phytochemical screening, ant mycobacterial activity and acute toxicity of crude extracts of selected medicinal plant species used locally in the treatment of tuberculosis in Uganda. Research of Tropical Medicine and Health. (2022) 50:16, 3-13.
[10] Mohammad Qadi et al, Phytochemical screening, ant obesity, ant diabetic and antimicrobial assessments of Orobanche aegyptiaca from Palestine, BMC Complement Med There (2021) 21:256.
[11] Nidal Jaradat et al, Ephedra alata fruit extracts: phytochemical screening, anti-proliferative activity and inhibition of DPPH, α-amylase, α-glycosidase, and lipase enzymes. BMC Chemistry (2021) 15:41.
[12] Okoh et al, Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of Helichrysum long folium DC. Research of BMC Complementary and Alternative Medicine 2010, 10:21.
[13] Rahman Gull et al, Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermediate Indigenous to Baluchistan, 2017, Article ID 5873648, 7 pages.
[14] Thite SV et al, preliminary phytochemical screening of some medicinal plants, IJPCBS 2013, 3(1), 87-90.
[15] Pervaz Anwar et al, Phytochemical Screening and Analysis of Selected Medicinal Plants in Gujarat. Journal of J Photochemistry & Biochemistry, 2018, 2:1 1000108.
[16] Jun aid R Shaikh et al, Qualitative tests for preliminary phytochemical screening, International journal of chemical studies, 2020; 8(2): 603-608.
[17] Nabin Kark et al, phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination, 2020; 35(2)1-11.
[18] Ammar Altemimi et al; Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts, Journal of plants, 2017, 6, 42; 10-3390.
[19] Priya Panchal et al; Phytochemical analysis of medicinal herb (ocimum sanctum).International journal of nanomaterial, nanotechnology and nanomedicine.2019- 5(2): 008-011.
[20] Mohamed Amine Gacem et al, Phytochemical screening, antifungal and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies. SN Applied Sciences (2019) 1:1721