



Phytochemical Screening and *In Vitro* Antioxidant Activity of Methanol Extract of *Catharanthus roseus* leaves

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ABSTRACT

This study was undertaken to evaluate the phytochemical analysis and *In vitro* antioxidant activity of methanol extract of *Catharanthus roseus* leaves. The preliminary phytochemical screening of methanol extract of leaves of *Catharanthus roseus* (L.) was carried out using Harbone method. Various concentrations (20, 50, 100 and 200 µg/ml) of methanol extract of *Catharanthus roseus* were taken for *In vitro* antioxidant activity. The methanol extract showed the presence of alkaloids, flavonoids, glycosides, steroids, terpenoids, phenol, saponins, phenols and tannins. The methanol extract of leaves exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, 2, 2'-azino-bis(diammonium salt) (ABTS) radical cation, hydrogen peroxide radical scavenging activity and hydroxyl radical. The results of the present study revealed strong antioxidant potential of methanol extract of *Catharanthus roseus* in a dose dependent manner.

Keywords: *Catharanthus roseus*, methanol, phytochemical analysis, antioxidant.

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INTRODUCTION

Plant-derived medicines continue to occupy an important niche in the treatment of diseases worldwide. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [3]. In all systems of traditional medicine, the fundamentals are to use the medicinal plants in the treatment and prevention of disease, as well as in the maintenance of health [1].

Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases [15]. Production of reactive oxygen species (ROS) and defense system against them are balanced well in the living body. This balance is very much important for the maintenance of physiological condition. There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, several non-transmissible degenerative diseases in humans [19,8]. Thus, there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals [9].

The present study was undertaken to evaluate the phytochemical analysis and *In vitro* antioxidant activity of methanol extract of *Catharanthus roseus* leaves.

MATERIAL AND METHODS

Plant material and Preparation of extract:

The whole plant of *Catharanthus roseus* (L.) was collected from Tiruvannamalai district. The leaves were shade dried, coarsely powdered and was extracted with methanol. The solvents in leaves extract were evaporated under reduced pressure and extracts were stored. The methanol extract was taken for phytochemical analysis and *in vitro* antioxidant activity.

Preliminary phytochemical analysis:

The qualitative chemical analysis of various extracts was carried out for the presence of alkaloids, saponins, tannins, phenols, proteins using the method adopted in similar surveys. [5].

In-Vitro Antioxidant

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of [9]. To 1ml of 100 μ M DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1 ml of methanol instead of test sample was added to the control tube. Different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation $[(\text{Absorbance of control} - \text{Absorbance of test}) / \text{Absorbance of control}] \times 100$. IC₅₀ value was calculated using Graph pad prism 5.0.

ABTS Radical Scavenging Activity

ABTS radical scavenging activity was performed as described by [12] with a slight modification. 7mM ABTS in 14.7mM ammonium peroxy-disulphate was prepared in 5 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.02 at 734nm. Various concentration of the sample solution dissolved in ethanol (20 μ l)

Was added to 980 μ l of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20 μ l of ethanol and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of [13]. A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 200 μ l of sample containing different concentration was mixed with 0.6 ml of H₂O₂ solution. Absorbance of H₂O₂ was determined 10 minutes later against a blank solution containing phosphate buffer without H₂O₂. A test tube containing 200 μ l of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the test sample was estimated according to the method of [4]. The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2ml of sample in varied concentration to which, 0.1ml EDTA (1mM)-FeCl₃ (10mM) mixture, 0.1ml H₂O₂ (10mM), 0.36ml deoxyribose (10mM), 0.33 ml phosphate buffer (50mM, pH 7.4) and 0.1ml of ascorbic acid (1mM) was added in sequence. The mixture was incubated at 37°C for 1h. To this mixture was added 1.0ml each of TCA (10%) and TBA (0.67%) and kept in boiling water bath for 20 minutes. The color developed was read at 532nm. The control tube contains phosphate buffer, instead of sample. Different concentration of ascorbic acid was used as reference compound.

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical screening of methanol extract of *Catharanthus roseus* (L.) was carried out. The methanol extract showed the presence of alkaloids, Flavonoids, glycosides, steroids, phytosterols, terpenoids, saponins, phenols and tannins.

Table 1 Phytochemical screenings of methanol extract of *catharanthus roseus*(L.)

S.No	Phytochemicals	Methanol solvent
1	Alkaloides	+
2	Flavonoids	+
3	Glycosides	+
4	Tannins	+
5	Steroids	+
6	Phytosterols	+
7	Terpenoids	+
8	Phenols	+
9	Saponins	+

***In vitro* Antioxidant Assay:**

Radical Scavenging Activity:

Different concentrations ranging from 75-500 $\mu\text{g/ml}$ of the methanol extract of leaves of *Catharanthus roseus* were their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration dependent up to the given concentration in all the models.

DPPH Radical Scavenging Activity

The activity of DPPH radical scavenging of the leaves extract was presented in fig1. The percentage of inhibition in DPPH in different concentration like 25, 50, 100, 200 $\mu\text{g/ml}$ were observed in 26, 45, 67, 82, respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 $\mu\text{g/ml}$ were found to be 40, 60, 80, 90 respectively. The IC₅₀ values for DPPH scavenging activity for methanol extract of leaves of *Catharanthus roseus* and ascorbic acid were 57 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$ respectively.

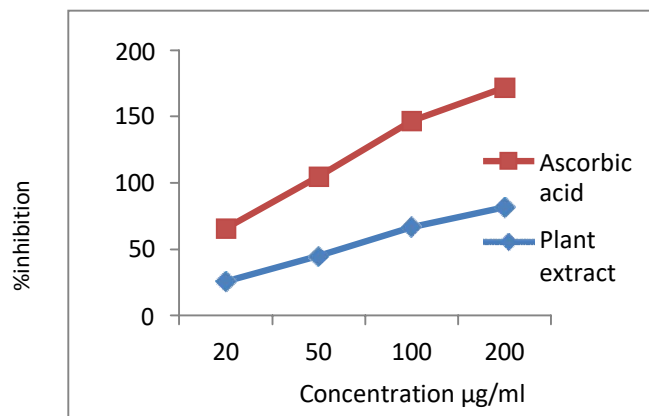


Fig.1 DPPH radical scavenging activity of methanol extract of leaves of *Catharanthus roseus*
ABTS Radical Scavenging Activity

Fig2 shows the percentage of inhibition in ABTS in different concentration like 25, 50, 100, 200 $\mu\text{g/ml}$ were observed in 37, 48, 74, 90, respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 $\mu\text{g/ml}$ were found to be 47, 67, 94, 98 respectively.

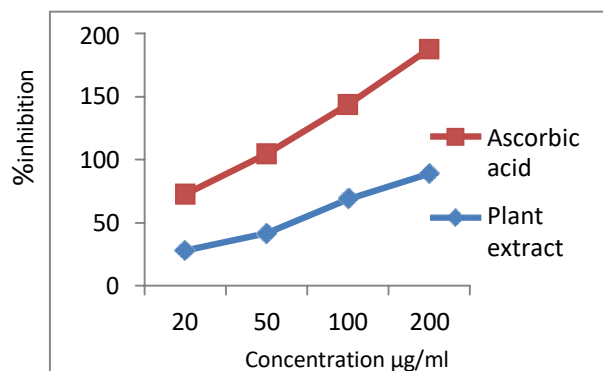


Fig. 2 ABTS radical scavenging activity of methanol extract of leaves of *Catharanthus roseus*

The IC₅₀ values for ABTS scavenging activity for methanol extracts of leaves of *Catharanthus roseus* and ascorbic acid were 60 $\mu\text{g/ml}$ and 32 $\mu\text{g/ml}$ respectively.

Hydrogen Peroxide Radical Scavenging Activity

Figure 3 shows the percentage of inhibition of H₂O₂ in different concentration like $\mu\text{g/ml}$ were observed in 28, 42, 69, 89 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 $\mu\text{g/ml}$ were found to be 45, 63, 75, 99 respectively. The IC₅₀ values for hydrogen peroxide scavenging activity for methanol extract of leaves of *Catharanthus roseus* and ascorbic acid were 62 $\mu\text{g/ml}$ and 32 $\mu\text{g/ml}$ respectively.

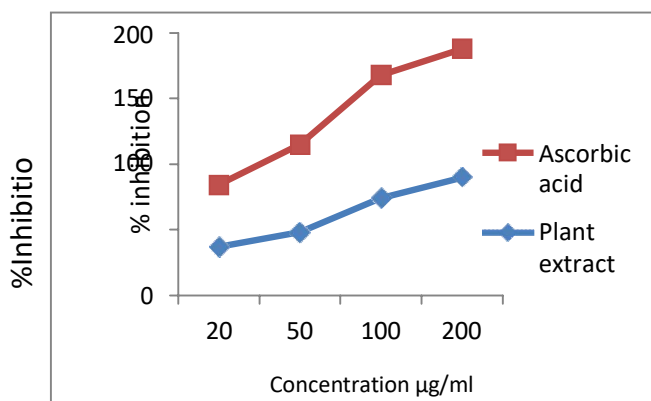


Fig. 3: H₂O₂ radical scavenging activity of methanol extract of leaves of *Catharanthus roseus* Hydroxyl Radical Scavenging Activity

Figure 4 shows the percentage of inhibition of hydroxyl radical scavenging activity of plant extract was presented in Fig. 4. Hydroxyl radicals were scavenged in different concentration like 25, 50, 100, 200 µg/ml were observed in 33, 55, 69, 87 respectively whereas the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 µg/ml were found to be 43, 75, 91, 98 respectively. The IC₅₀ values for hydroxyl radical scavenging activity methanol extract of leaves of *Catharanthus roseus* and ascorbic acid were 45 µg/ml and 26 µg/ml respectively.

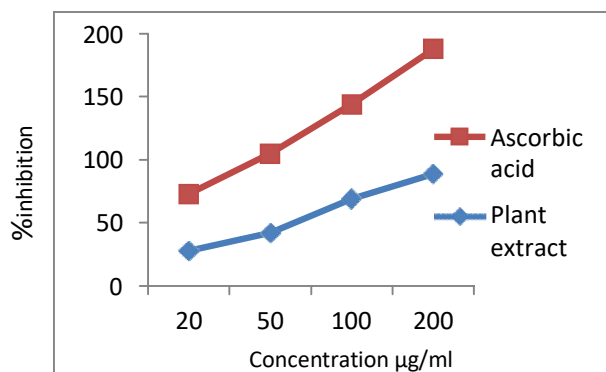


Fig. 4: Hydroxyl radical scavenging activity of methanol extract of leaves of *Catharanthus roseus*

DISCUSSION

The present work discusses the phytochemical investigation and the evaluation of antioxidant activity of *Catharanthus roseus* leaves in the light of recent research on medicinal properties.

Antioxidants are important in the prevention of human diseases. The reactive oxygen species (ROS) generated during normal cellular metabolism is harmful by products. They lead to oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA [18]. This has inspired much interest in antioxidant activity of phytochemicals.

There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants [12,13].

Many of the therapeutic potential of the phenolic compounds can be attributed to its antioxidant activity. The result of this study on in vitro antioxidant activity of *Catharanthus roseus* leaves have highest radical scavenging activity [17]. Phenolic compounds have been reported to be associated with anti oxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals [12].

In vitro assays were used to characterize the radical scavenging the antioxidant activity of the methanol extract of *Catharanthus roseus* leaves. *In vitro* systems are easier, faster and more cost effective compared to traditional bio assay *in vivo*. The testing of the antioxidant activity of compounds *in vitro* is useful, because if a substrate is poorly effective *in vitro* will not be better under *in vivo* conditions [1].

CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be including in the pharmacopoeia, the standards must be established. The present work is under taken to produce some phytochemical standards and *In vitro* antioxidant activity of *Catharanthus roseus*. The results obtained in the present study indicate that methanol extract of

Catharanthus roseus leaves exhibit significant free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that *Catharanthus roseus leaves* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. The above studies provide information in respect of their chemical constituents which may be useful for standardization of herbal drugs and having an essential role in medicine

To conclude that methanol extract of *Catharanthus roseus leaves* showed a significant *In vitro* antioxidant and also showed the presence of various phytoconstituents in the leaves. This shows that the present research carried out paves a pathway for further pharmacological studies and isolation and novel components from the leaves of *Catharanthus roseus*.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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