PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIOXIDANT ACTIVITY OFMETHANOL EXTRACT OF CATHARANTHUS ROSEUSLEAVES

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ABSTRACT

This study was undertaken to evaluate the phytochemical analysis and *in vitro* antioxidant activitymethanol extract of*Catharanthusroseus*leaves.The preliminary phytochemical screening of methanol extract of leaves of*Catharanthus roseus (L.)*was carried out using Harbonemethod.Various concentrations (20, 50, 100 and 200µg/ml) of methanol extract of *Catharanthusroseus* was taken for *in vitro* antioxidant activity. The methanolextract 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-bisdiammonium salt (ABTS) radical cation, hydrogen peroxide radical scavenging activity and hydroxyl radical. The results of the present study revealed strong antioxidant potentials of methanol extract of *Catharanthusroseus* in dose dependent manner.

Key words:*Catharanthusroseus,* methanol*,* phytochemical analysis, antioxidant.

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(Davis J.,1994).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 (Acharya D *et al*., 2008).

Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases (Savithramma*et al.,* 2011).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 (Yuan *et al.,* 2008; Lai *et al.,* 2010). Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals (Mishra *et al.,* 2009).

The present study was undertaken to evaluate the phytochemical analysis and *in vitro* antioxidant activityofmethanol extract of *Catharanthusroseus*leaves.

MATERIALS AND METHODS

Plant material and Preparation of extract:

The whole plant of *Catharanthus roseus (L.)*was collected from Thiruvannamalai 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 methanolextract were taken for phytochemical analysis and *invitro* antioxidant activity.

Preliminary phytochemical analysis:

The qualitative chemical analysis of various extracts were carriedout for the presence of alkaloids, saponins, tannins, phenols, proteins using the method adopted insimilar surveys. (Harbone 1973).

IN-VITRO ANTIOXIDANT

DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1 ml of 100 µM 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 [(Absorbance of control - Absorbance of test)/ Absorbance of control)] X 100. IC_{50} value was calculated using Graph pad prism 5.0.

ABTS RADICAL SCAVENGING ACTIVITY

ABTS radical scavenging activity was performed as described by Re*etal*., (1999) with a slight modification. 7mM ABTS in 14.7mM ammonium peroxo-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 734 nm. Various concentration of the sample solution dissolved in ethanol (20µl) wasadded to 980µl 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 20µl 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 Ruch*et al*., (1989). A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 200µl of sample containing different concentration was mixed with 0.6 ml of H_2O_2 solution. Absorbance of H_2O_2 was determined 10 minutes later against a blank solution containing phosphate buffer without H_2O_2. A test tube containing 200µl 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 Halliwell*et al*.,

(1992). The hydroxyl radical was generated by a fenton-type reaction. The reaction mixture contained 0.2 ml of sample in varied concentration to which, 0.1ml EDTA (1mM)- $FeCl_3$ (10mM) mixture, 0.1 ml H_2O_2 (10mM), 0.36 ml deoxyribose (10mM), 0.33 ml phosphate buffer (50mM, pH 7.4) and 0.1 ml of ascorbic acid (1mM) was added in sequence. The mixture was incubated at 37°C for 1 h. To this mixture was added 1.0 ml each of TCA (10 %) and TBA (0.67 %) and kept in boiling water bath for 20 minutes. The colour developed was read at 532 nm. 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 methanolextract showed the presence of alkaloids, Flavonoids, glycosides, steroids, phytosterols, terpenoids, saponins, phenols and tannins.

Table 1: Phytochemical screenings of methonal extract of *catharanthus roseus (l.)*

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

INVITRO ANTIOXIDANT ASSAY:

Radical Scavenging Activity:

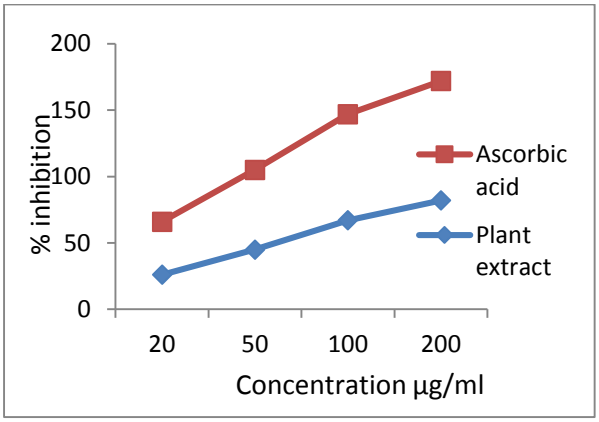
Different concentrations ranging from 75-500µg/ml of the methanol extract of leaves of *Catharanthusroseus*were tested for their antioxidant activity in different *in vitro* models. The percentage of inhibition was observed and found that the free radicals were scavenged by the test compounds in a concentration 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 fig 1. The percentage of inhibition in DPPH in different concentration like 25, 50, 100, 200 µg/ml were observed in

26,45,67,82,respectivelywhere as the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 µg/ml were found to be 40,60,80,90 respectively. The IC_{50} values for DPPH scavenging activity for methanol extract of leaves of *Catharanthusroseus*and ascorbic acid were 57µg/ml and 40µg/ml respectively.

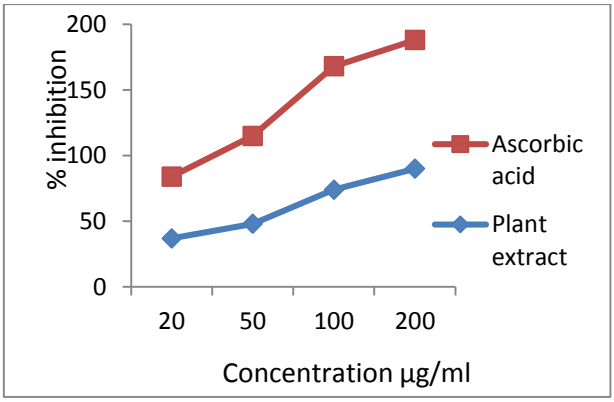
Fig: 1 DPPH radical scavenging activity of methanol extract of leaves of *Catharanthusroseus*



ABTS Radical Scavenging Activity

Fig 2shows the percentage of inhibition in ABTS in different concentration like 25, 50, 100, 200µg/ml were observed in 37,48,74,90, respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200 µg/ml were found to be 47,67,94,98 respectively.

Fig 2: ABTS radical scavenging activity of methanol extract of leaves of *Catharanthusroseus*

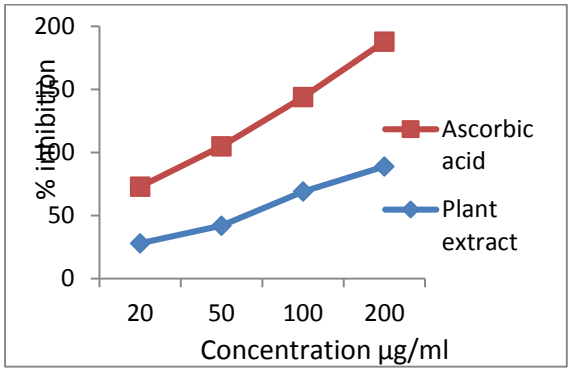


The IC_{50} values for ABTS scavenging activity for methanol extract of leaves of *Catharanthusroseus*and ascorbic acid were 60µg/ml and 32µg/ml respectively.

Hydrogen PeroxideRadical Scavenging Activity

Table 4shows the percentage of inhibition of H_2O_2 in different concentration like µg/ml were observed in 28,42,69,89 respectively where as the percentage inhibition of ascorbic acid in concentration like 25, 50, 100, 200µg/ml were found to be 45,63,75,99 respectively. The IC_{50} values for hydrogen peroxide scavenging activity for methanol extract of leaves of ***Catharanthusroseus***and ascorbic acid were 62µg/ml and 32µg/ml respectively.

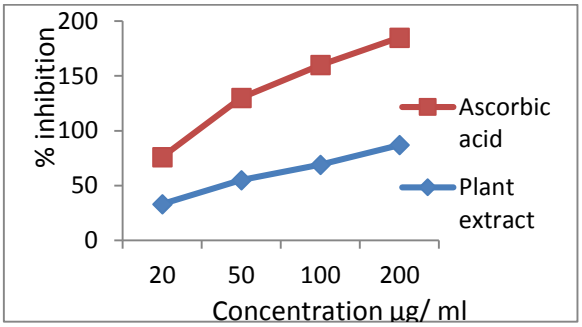
Fig: 3 H_2O_2 radical scavenging activity of methanol extract of leaves **of *Catharanthusroseus***



Hydroxyl Radical Scavenging Activity

The 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 where as 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 IC_{50} values for hydroxyl radical scavenging activity methanol extract of leaves of ***Catharanthusroseus***and ascorbic acid were 45µg/ml and 26µg/ml respectively.

Fig: 4 Hydroxyl radical scavenging activity of methanol extract of leaves **of *Catharanthusroseus***



DISCUSSION

The present work discusses the phytochemical investigation and the evaluation of antioxidant activity of *Catharanthusroseus*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 byproducts. They lead to oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA (Steenkamp*et al*., 2005). 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(Osawa T 1994, Noda Y*et al* 1997)

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 *Catharanthusroseus*leaves have highest radical scavenging activity (Srinivasan M *etal*2007). Phenolic compounds have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Jorgensen LV*et al* 1999).

*Invitro*assays were used to characterize the radical scavenging the antioxidant activity of the methanol extract of *Catharanthusroseus*leaves.*Invitro* systems are easier, faster and more cost effective compared to traditional bio assay *invivo*. The testing of the antioxidant activity of compounds *invitro* is useful, because if a substrate is poorly effective in vitro will not be better under *invivo* conditions (Aruoma, 1999).

SUMMARY AND CONCLUSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The present work is undertaken to produce some phytochemical standards and *in vitro* antioxidant activity of *Catharanthusroseus*.

The results obtained in the present study indicate that methanol extract of *Catharanthusroseus*leavesexhibit significant free radical scavenging and antioxidant activity. Theoverall antioxidant activity might be attributed to its polyphenolic content andother phytochemical constituents. The findings of the present study suggestthat*Catharanthusroseusleaves* could be a potential source of natural antioxidant thatcould have great importance as therapeutic agents in preventing or slowing theprogress 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*Catharanthusroseusleaves* 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*Catharanthusroseus.*

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