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ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF FRACTIONATED LEAF EXTRACTS OF *CATHARANTHUS ROSEUS*

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ABSTRACT: Antioxidant effectiveness of indigenous medicinal plant *C. roseus* leaves extracts and fractions with solvents of different polarity (ethanol, methanol, acetone, hexane, butanol, and water) were assessed for DPPH radical scavenging activity. The *C. roseus* extracts and fractions contained appreciable levels of the antioxidant activities of leaves of *C. roseus* at various concentrations. The leaves of *C. roseus* showed good antioxidant activity (81.70%). The phytochemical analysis of the *C. roseus* shows the presence of alkaloids, terpenoids, steroids, flavonoids, and other plant secondary metabolites. GC-MS result shows that the important secondary metabolites and their derivatives present in *C. roseus* leaves extract. The results of the present comprehensive analysis demonstrated that *C. roseus* extracts and fractions are a viable source of natural antioxidants and might be exploited for functional foods and nutraceutical applications.

INTRODUCTION: An important element of drug development using plants is the accumulation and analysis of pertinent research data, and purported ethnomedical (folkloric) uses for plants. There is considerable scientific and commercial interest in the continuing discovery of new antioxidant agents from natural product sources. The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute and has since made major contributions to the discovery of new naturally occurring antioxidant agents. The semi-synthetic and synthetic derivatives of active constituents derived from plants are important sources of antitumor drugs.

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Over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources or are related to the natural sources, for example, *Vinca* alkaloids, vinblastine, and vincristine, were isolated from *Catharanthus roseus* (Apocynaceae). Plants are an essential part of human society since the civilization started. Medicinal plants are the boon of nature to cure a number of ailments of human beings.

In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Evaluation of plants bearing efficiency in healing various diseases is growing in recent years [1-4]. Innumerable biologically active compounds of plants are found to possess antibacterial properties. Practitioners of Ayurveda and Unani system of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents and over the last 40 years, intensive efforts have been made to discover clinically used herbal antibacterial, antifungal drugs and antioxidant drug. An antioxidant is a molecule capable of inhibiting

the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent.

Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves; antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Although, oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase, and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells [5].

C. roseus (*Vinca rosea*) is known as the common or Madagascar periwinkle. It is a perennial herb of the Apocynaceae family originally native to Madagascar. It measures about two feet in height and has dark green glossy leaves and pale pink or white flowers. The organic extracts of *C. roseus* are used in the folklore treatment of diabetes, malaria, leukemia, wasp stings, sore throat, eye irritation, infections. It is also used as an astringent, diuretic and expectorant. Antioxidants are radical scavengers which give protection to the human body against free radicals by inhibiting the oxidizing chain reactions. When these substances are present at low concentration in the body, they markedly delay or prevent the oxidation of an oxidizable substrate [7].

These antioxidants always play important roles in delaying the development of chronic diseases such as cardiovascular diseases (CVD), cancer, atherosclerosis, inflammatory bowel syndrome and Alzheimer's diseases Bozin *et al*., 2006. [1] A variety of different alkaloids is present in C. roseus and more than 130 different compounds have been reported including about 100 monoterpenoid indole alkaloids Pereira *et al*., 2010. [2] As an important medicinal plant, it has a good antioxidant potential throughout its parts under drought stress. Hence, if the compound having antioxidant potentials and phytochemical activity additionally; it can be a good therapeutic agent for accelerating then wound healing process. Little information is available about the antioxidant potential of *C. roseus*. The objective of this study was to determine the antioxidant potential and phytochemical activity of different fractions of *C. roseus* leaf extract [6].

MATERIALS AND METHODS:

Plant Material: The selected plant *C. roseus* leaves were collected from the Herbal Garden, PRIST University, Thanjavur, Tamilnadu, India and it was taxonomically identified and authenticated by Rev Fr. Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph College (Autonomous), Tiruchirapalli, Tamilnadu, India. The voucher specimen was deposited at the Rapinat Herbarium, and the voucher number is RHPSB.001.

Preparation of Plant Extracts: The fresh leaves of *C. roseus* plant were air-dried, and the dried plant material was weighed and ground with Blender. The leaves were cleaned and shade dried for 7 days, then ground well to get a fine powder. A mass of 500 g of dried powder was exhaustively extracted with 1 L of methanol by continuous hot percolation using Soxhlet apparatus. After filtration, the solvent was evaporated under reduced pressure in a rotary evaporator at 45 °C to afford them methanol extract (100 g). The residue was stored separately in airtight containers and stored in a deep freezer.

Column Chromatography: The crude extract (50 gm) was fractionated with different solvents *viz*., ethanol, methanol, acetone, hexane, butanol and water using column chromatography under reduced pressure over silica gel Moorthy *et al*., 2011. [3] These fractions were then stored in a refrigerator until further use [8].

Phytochemical Analysis (Qualitative Method): Phytochemical analysis of the plant extracts was undertaken using standard qualitative methods as described by various authors Kapoor *et al*., 1969. [4] The plant extracts were screened for the presence of biologically active compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, proteins, phenolics, tannins, and saponins.

Alkaloids: [5]

Wagner's Reagent (Iodine-Potassium Iodide Solution) Iodine: (1.2 g) and of potassium iodide (2.0 g)were dissolved in 5 ml of H_2SO_4, and the solution was diluted to 100 ml. 10 ml of plant extract was acidified by adding 1.5% v/v HCl and a few drops of Wagner's reagent. The formation of a yellowish brown precipitate confirmed the presence of alkaloids.

Flavonoids: [6] In a test tube 0.5 ml of plant extract, 5-10 drops of diluted HCl and a small piece of zinc or magnesium were added, and the solution was boiled for a few minutes. In the presence of flavonoids, a reddish pink or dirty brown color was produced.

Carbohydrates:

Fehling's Test Solution A: [7] 34.65 g of copper sulphate was dissolved in water and made up to 500 ml Solution B: 125 g of potassium hydroxide and 173 g of Rochelle's salt (sodium potassium tartrate) were dissolved in water and made up to 500 ml. The solutions 'A' and 'B' were added. The contents were boiled for a few minutes. The formation of a red or brick red precipitate indicated the presence of carbohydrates.

Benedict's Test: 173 g of sodium citrate and 100 g of sodium carbonate were dissolved in 500 ml of distilled water. 17.3 g of copper sulphate dissolved in 100 ml of distilled water was added to the above solution. To 0.5 ml of plant extract, 5 ml of Benedict's reagent was added and boiled for 5 min. The formation of a bluish green color showed the presence of carbohydrates.

Phenols: [8] To 1 ml of plant extract, 2 ml of distilled water followed by a few drops of 10 percent aqueous $FeCl_3$ solution were added. Formation of a blue or green precipitate indicated the presence of phenols.

Saponins: [9] In a test tube containing about 5 ml of plant extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. Formation of a honeycomb like froth showed the presence of saponins.

Tannins: [10]

Lead Acetate Test: In a test tube containing about 500 ml of plant extract, a few drops of 1 percent solution of lead acetate was added. Formation of yellow or red precipitate indicated the presence of tannins.

Phytosterols: [11] About 0.5 ml of the test solution was mixed with a minimum quantity of chloroform, and the 3-4 drops of acetic acid and one drop of concentrated H_2SO_4 were added. Formation of a deep blue or green color showed the presence of steroids.

Determination of Antioxidant Activity (Radical Scavenging Activity): Experiments were carried out in triplicate, according to the method of Babushok 1953 [12] with a slight modification Sabir *et al*., 2008 [13] by Cakir. Briefly, 25 mg/l solution of DPPH radical in methanol was prepared, and then 2 ml of this solution was mixed with different concentration (200, 400, 600, 800 and 1000 µg) of *C. roseus* extract was added to the solution to achieve the final volume of 3 ml. After 30 min the absorbance was measured at 517 nm.

The antioxidant activity was calculated using the equation:

$$AOA = (Ao-As)/Ao \times 100$$

Ao = DPPH solution without the sample, As = DPPH solution with the sample.

GC-MS Analysis: The methanol fraction of C. roseus extract was analyzed by GC-MS [14] (Kapoor *et al*., 2004) according to Babushok.

Statistical Analysis: The experiments were carried out in triplicate, and the statistical software package (SPSS 12.0) was used for the statistical analysis and the results were given as a mean ± standard deviation (SD). Regression analysis was carried out for the comparison of concentration dependency, and one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$. [15]

RESULTS:

Phytochemical Analysis of the Methanolic Fraction of *C. roseus* Leaf Extracts (Qualitative Method): Phytochemical screening was performed to check the presence of different secondary metabolites. The aqueous extracts revealed that the presence of alkaloids, carbohydrates, flavonoids,

phenols, phytosterols, saponins, and tannins. Phytosterols and terpenoids were absent in the test **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF METHANOLIC FRACTION OF *C. ROSEUS* LEAF EXTRACT

S. no.	Phyto - Compounds	*C. roseus* leaf extract
1	Alkaloids	+
2	Phenolic compounds	+
3	Phytosterols	-
4	Terpenoids	-
5	Flavonoids	+
6	Carbohydrates	+
7	Quinone	+
8	Tannins	+
9	Saponins	+

+ Present; - absent

Antioxidant Activity: DPPH assays determined the antioxidant activity of *C. roseus* at different concentrations (200, 400, 600, 800 and 1000 µg). Among the five concentrations tested, 800 µg shows the maximum antioxidant activity (81.70%) **Table 2**. One way analysis of variance (ANOVA) was used to test the level of significance between absorbance and concentration. The experiments were conducted in triplicate and the probability factor (P) was <0.05 between different concentration and absorbance for the leaves of *C. roseus* and it was considered as significant [15].

TABLE 2: ANTIOXIDANT ACTIVITY OF METHANOLIC FRACTION OF *C. ROSEUS* LEAF EXTRACT

Concentration(µg)	Antioxidant Activity (%
200	78.00
400	80.34
600	80.54
800	81.70
1000	80.24

Values are expressed as Mean ± Standard deviation

GC-MS Analysis of the Extract of *C. roseus*: GC-MS analysis of methanolic fractions of *C. roseus* showed that confirmation of the presence of active compounds like alkaloids, flavonoids and other plant secondary metabolites **Fig. 1**.

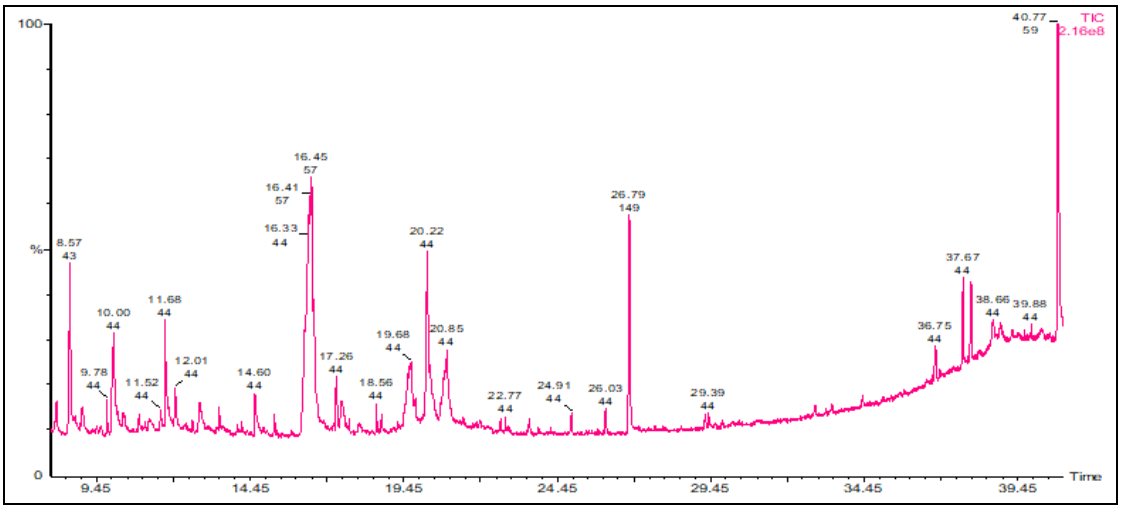

FIG. 1: GCMS ANALYSIS OF METHANOLIC FRACTIONS OF *C. ROSEUS* SHOWED THAT CONFIRMATION OF THE PRESENCE OF ACTIVE COMPOUNDS

DISCUSSION: The phytochemical activity of *C. roseus* leaf extract In our investigations, phytochemical constituents of leaf extracts of *C. roseus* aqueous extracts revealed that the presence of alkaloids, carbohydrates, flavonoids, phenols, phytosterols, saponins, and tannins. Phytosterols and terpenoids were absent in the test. These compounds are described as potent biologically active compounds found in medicinal plant parts which are precursors for clinically useful drugs Steiling *et al*., 1993. [16] The potency of medicinal plants is attributed to the action of the phytochemical constituents. Plants produce these as secondary metabolites in response to environmental pressure or as a defense mechanism to animal or plant diseases. For instance, many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infection actions are assigned to tannins Velazquez *et al*., 2003. [17] Medicinal plants having tannins as the main components are astringent and are used for the treatment of intestinal disorders

such as diarrhea and dysentery Rhee, 2003. [18] Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for development of drugs Aruoma, 2003. [19]

The present study showed that the presence of biologically active secondary metabolites in *C. roseus*. Plants have long been used for medicine, and many plants have been screened if they contained active compounds with therapeutic activity. Therefore, it is vital to evaluate the antioxidant and phytochemical activity of *C. roseus*. The phytochemical activity of *C. roseus* was evaluated by various authors Bauer, 1996. [20] This study revealed that the presence of active compounds like alkaloids, carbohydrates, flavonoids, phenols, phytosterols, saponins, tannins. The methanolic fraction of *C. roseus* leaf extracts was further studied for antioxidant properties.

Antioxidant Activity of *C. roseus* Leaf Extracts: The present study indicating that antioxidant activity of *C. roseus* determined by DPPH assays at different concentrations (200, 400, 600, 800 and 1000 µg). Among the five different concentrations tested 800 µg shows the maximum antioxidant activity (81.70%). Medicinal plants are an important target of patent claims since they have proved to be great interest to the International drug and cosmetic industry. Several plants have been identified for their antioxidant properties.

In general, there are two major strategies which convey partial resistance against oxidative stress to most cell types: small antioxidant molecules like ascorbate, polyunsaturated fatty acids or sugars mainly mannitol and ROS- scavenging enzymes, such as superoxide dismutase (SOD), catalase and various peroxidase Okwu, 2005. [21] The role of free radicals reactions in biology has become an area of intense interest. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in the living organism Okwu *et al*., 2004. [22] An antioxidant is defined as any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate Dharmananda *et al*., 2003. [23]

DPPH radical scavenging assay, we investigated the free radical scavenging activity of extracts and fractions of *C. roseus*. Fractions of *C. roseus* leaves showed excellent radical scavenging activity, the extract concentration providing 82% of inhibition when compared to other concentrations. All the concentration showed that the lower level of antioxidant activity. [17] To best of our knowledge, no earlier reports are available regarding the DPPH radical scavenging activity of *C. roseus* leaves of which to compare with the present values. There are many pieces of evidence that natural products and their derivatives have efficient anti-oxidative characteristics, consequently linked to anti-cancer, hypolipidemic, anti-aging and anti-inflammatory activities Kapoor *et al*., 1969. [14] Results obtained in this study confirmed that the antioxidant activity of *C. roseus*. Antioxidant activity was assessed by DPPH scavenging method where methanolic extract was found to be the most potent antioxidant. Moreover, the higher alkaloid content was observed in the methanolic extract of *C. roseus* [24].

GC-MS Analysis: The result of GC-MS analysis shows that the presence of biologically active compounds. In *C. roseus* leaves extract alkaloids compounds is highly present in the derivatives of secondary metabolites. The methanol extracts of *C. roseus* leaves contains rich phytochemical constituents which in turn resulted in the identification of twenty-eight different compounds by GC-MS analysis. The individual names of compounds identified by GC-MS concerning their individual peak value were also listed with their peak number, area, retention time in column chromatography and percentage of the area.

CONCLUSION: Different pharmacological studies and the traditional uses proved the high medicinal properties of the *C. roseus*; which continuously being used in the treatments for numbers of diseases. Various important alkaloid mostly the monomers were successfully identified. It would also be interesting to considering *C. roseus* for medically important and for development of new drugs for preventive treatment against various diseases. *C. roseus* was taken to isolate the novel antioxidant compounds. The result indicated that leaf extract possesses the highest antioxidant as well as phytochemical activity.

However, further pharmacological and toxicity studies are necessary to understand the metabolic compounds present in this plant. This study demonstrated that *C. roseus* leaf extract may be considered as a useful source of material for human health, as an antioxidant and phytochemical agent.

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CONFLICT OF INTEREST: Nil

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