Plant Archives Volume 20 No. 2, 2020 pp. 4187-4192 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ESTIMATION OF QUALITATIVE AND QUANTITATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF *CATHARANTHUS ROSEUS* (L).

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Abstract

Catharanthus roseus is an alkaloid plant that contains alkaloid constituents such as Vincristine and Vinblastine. In the present study we report the Total phenol content (DPC), total flavonoid content (TFC), free radical scavenging activity (DPPH) of the entire plant (shoot, flower, Root (SFR) extract obtained from *C. roseus* and evaluated for cancer treatment. *C. roseus* plants were identified, collected the plants, separated the shoot, flower and roots and dried under laboratory conditions and powdered. The temperature specific suitable solvents were used to and extraction was done using conical flasks for one week and filtered through whatman No. 1 paper. The filtrate was stored and used for further experimentation. Analysis of solvent extracts was subjected to phytochemical and free radical scavenging activity of DPPH assay. Various concentrations of extract containing 25❍g to125 ❍g were taken for all experimental analysis, and were carried out in triplicate and the values are entered on the mean ± SD. The IC50 values in the DPPH estimate were calculated using ANNOVA. Methanolic extraction from respectively solvent has a high value of antioxidant properties.

Key words : Total Phenol, Total Flavonoid, DPPH, C. roseus.

Introduction

Ayurveda is an important system of alternative and complementary medicine. Various medicinal formulas are utilized in the treatment of Ayurvedic systems. Also, for other herbal medicines, majority of its medicines belong to domestic herbs. It is essential for everyone working in Ayurveda to have a complete and recent knowledge of herbal plants to find out specific plant suitable for a specific disease (Ballabh and Chaurasia, 2007). In current years, interest in medicinal plants has increased tremendously, and the West has taken the problem vigorously (Perumal *et al.,* 1998). The World Health Organization (WHO) is presently promoting folk medicine in national health programs that are cheaply obtainable and culturally accepted. Furthermore, WHO consider that one third of the world's population utilize herbs and other folk medicines to treat diseases (Leena and Sreelakshmi, 2017). Plant-based treatments are safe because they have very little or no side effects (Sreesha *et al.,* 2017). However, the lack of quality control knowledge for accepting Ayurvedic medicines is less acceptable to receive Ayurvedic medicines. Hence the end product analysis form has an impact on its functionality and safety (Shaikh and Jain, 2018).

Plant formulas are chiefly utilized for a diverse of diseases related with cancer treatment. Plants fabricate numerous secondary metabolites comprise alkaloids, steroids, flavonoids, cyanogenic glycosides, saponins, terpenoids to guard for themselves from an attack of obviously occurring disease, pests, environmental stresses. Compounds are separated by techniques based on the

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ingredients of the solvents utilized for mixing and extraction (Cragg and Newman, 2005; Shalini and Prema, 2012). Herbal plants, often used in folk medicine, contain many bioactive compounds that can prevent many infections or used as alternative treatments. Medicinal plants are considered an effective and safe alternative to artificial antibiotics (Chinnavenkataraman and Rajendran, 2012; Sarabjot and Poonam 2014; Angelin Jebamalar *et al.,* 2019).

Polyphenolic compounds are generally found in eatable and inedible plants, which have numerous biological effects and also antioxidant activity (Sivakumar and Panneerselvam, 2011; Sivakumar and Gajalakshmi, 2013; Sivakumar and Gajalakshmi, 2014; Jothi *et al.,* 2019). Plant phytochemicals of flavonoids have been classified into six classes such as (flavones, flavonones, isoflavonoids, flavonols, anthocyanins and flavans) based on their structural originality around the heterocyclic oxygen ring. Structurally, flavonoids are commonly classified with C&S carbon skeletons. Flavonoids can cause aglycones (without sugar moieties) and glycosides (with sugar moieties).

Oxidative stress is an inequity among the reactive oxygen species (ROS) formation and permeate ability to eliminate ROS. DNA, RNA, fatty tissues, vitamins, carotenoids and proteins are extremely harmful to all living cells, including microorganisms (Dastmalchi *et al.,* 2007). Antioxidant-induced free radicals can cause cell membrane and membrane protein degradation and mutation, which can persist to development of numerous diseases, like as lipofuscinosis, oxygen toxicity, aging, atherosclerosis, and liver injury (Iyer and Devi, 2009; Smerq and Sharma 2011). Free radicals are not only a cause to human disease but also lipid oxidation in the food system. Lipids oxide is the main cause of a quality decline in numerous dietary practices, the formation of objectionable reproduction, few toxic substances, and diminishes food quality and nutritional values.

Antioxidants are compounds that reduce the oxidative or antioxidant ruin of free radicals, thus they are possible transporter of free radicals or reactive oxygen species. Therefore, antioxidants respond as one or more of the following: inhibition of free radical activity, cleaning free radicals, mixing of pro-oxidant metals and slacking single oxygen (Tachakittirungrod *et al.,*2009). Synthetic antioxidants like as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl galactate (PG) have been used for decades around the world (Almey *et al.,* 2010), but their use in food products is prohibited because they are suspected of being cancer causing (Chanda and Dave, 2009).

Catharanthus roseus (Apocynaceae) is a conventionally vital medicinal plant, commonly known as *Vinka rosea*, Ammocellis rosa, and Lochnera rosea. Indian originated herbal plants like *C. roseus* raise naturally in the Indian subcontinent in southern Asia (Asheesh Kumar *et al.,* 2012). *C. roseus* leaves contains more than 70 types of chemicals compounds such as indole types of alkaloids, ajmalicine, serpentine and reserpine. The vital types of alkaloid, vinblastin produced by *C. roseus* is considered to have antitumour function and is broadly used in pharmaceutical industry (Rischer *et al.,* 2006). *Catharanthus roseus* were developed to produce a modern chemotherapeutic agent for their pain-relieving activities (Kratika Kumari and Sharmita Gupta, 2013).

Therefore, new medicines from herbal resources are expected to provide better and cheaper alternatives to natural products. Therapeutic ability of these plants depends on the diverse phytochemicals creating a consistent physiological action in the human body (Edeoga *et al.,* 2005). Hence, there is instant and emerging need for identification of antimicrobial compounds, diverse chemical structures and the therapeutic effects on infectious diseases. Thus, the present work estimates the total Flavonoid content, total phenol content, and the antioxidant activities of whole plant extract (shoot, flower, root) of *Catharanthus roseus.*

Materials and Methods

Plant Materials

Catharanthus roseus (shoot, flower, root) were collected from Bharathiar University, Coimbatore 2013-2014 Tamil Nadu, India, and also identified by the Department of Botany, Annamalai University, Tamil Nadu, India. Uprooted whole plants were separated and washed out in tap water, dried in the shade and finely powdered, stored in zip lock covers.

Chemicals

All chemicals were purchase in the SD chemicals Company Mumbai and each chemical was Scientific grade.

Total Phenolic Content

Total phenol content in C. roseus was determined by the method described by (Dewanto *et al*., 2002). Three different parts of the plants; *viz.,* shoot, Flower, and root were taken in equal quantities and 25µg, 50µg, 75µg, 100µg, and 125 µg were used to make an aliquot of extract using methanol 0.25ml and Folin-Ciocalteu reagent was added to it. The resulted solution was adjusted with distilled water to a final volume of 3mL and shaken

thoroughly. The solution was incubated and kept in the dark and the absorbance was measured using spectrophotometer at a wavelength of 760nm and was read against prepared blank. The total phenol content of plant parts was expressed as milligrams of gallic acid equivalents per gram of dry weight. All the samples were analyzed in replicate.

Total Flavonoid Content

Total Flavonoid content in *C. roseus* whole plant extract (From three different parts of the plants; *viz.,* shoot, flower, root) was analyzed using aluminum chloride colorimetric system (Mervat *et al.,* 2009). 0.5ml of entire plant extract of different concentrations like 25µg, 50µg, 75µg, 100µg, and 125 µg were taken and the final volume was made up to 3mL with methanol. 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8ml of distilled water were added simultaneously and test solution was shaken vigorously for getting a homogenous solution. After 30 minutes of incubation period, the absorbance was recorded at 415 nm. The concentration of flavonoids in test samples were calculated and expressed as the equivalent of quercetin (QE) / g of sample. The experiments were repeated thrice and analyzed and the average is taken.

Free radical scavenging activity (DPPH)

The antioxidant activity of methanolic whole plant extract (shoot, flower, root) of C. roseus was analyzed spectrophotometrically using stable [1, 1-diphenyl -2-Picryl hydrazyl radical (DPPH)] as explained in literature (Blois, 1958). Stock solution of each plant having different concentrations like 25µg, 50µg, 75µg, 100µg, and 125 µg were taken and was extracted using methanol solution and brought to a concentration of 1 mg / ml. This solution was mixed with equal volume of Methanolic solution of DPPH (0.1 mM). 0.5ml of methanol was added to each sample solution and mixed with 2.5 ml of 0.5 mM of Methanolic DPPH solution. The mixture was vortexed vigorously and kept in dark under room temperature for 30 minutes. The absorbance was analyzed at 517nm against a blank using a UV spectrophotometer. Ascorbic acid was used as control and the experiments were conducted in triplicate.

Statistical analysis

One-way ANNOVA was used to statistically analyze SPSS 17.0. Variance was considered significant when $P<0.005$. Three assays were performed for each set of the experimental conditions. All values were expressed as mean ± SD (standard deviation). The IC50 value is calculated for all test conditions.

Results

Determination of Total phenol contents

Total phenol content of entire plant extract (shoot, flower, root) of *Catharanthus roseus* was estimated with various amounts of entire plant *viz.,*25 µg, 50 µg, 75 µg, 100 µg and 125 µg concentrations were found to be (shoot, flower, root) 2.135 µg/ml, 1.854 µg/ml and 1.985 µg/ml, respectively. Gallic acid was taken as control, which has a total phenol content of 1.652 µg / ml is shown in fig. 1.

Determination of Total flavonoids

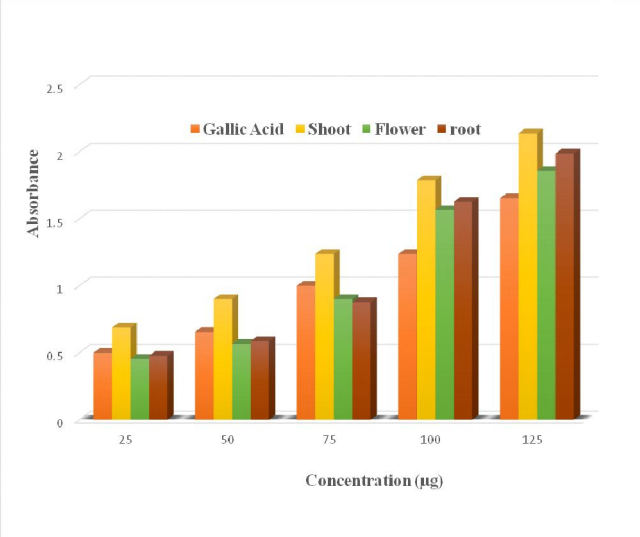

Fig. 1: Total phenol content analyzed with whole plant extract of C. roseus.

Total flavonoid content in *C. roseus* entire plant extract was evaluated as various amounts of different parts of entire plant *viz.,* 25 µg, 50 µg, 75 µg, 100µg and 125 µg) was shoot (2.325 µg/ml), flower (1.965 µg/ml) and root (1.768 µg/ml) while standard Gallic acid was taken as positive control which has (1.056 µg/ml) of total flavonoid contents are shown in fig. 2.

Determination of Antioxidant activity of DPPH assay

DPPH radical scavenging activity of different concentrations of methanolic extracts from different amounts of plants parts such as shoot, flower, root *viz.,* 25µg, 50 µg 75 µg100 µg and 125 µg of *C. roseus* was evaluated as 80.55µg/ml, 72.15 µg/ml, 64.71 µg/ml, respectively. Ascorbic acid (AA) was taken as positive control which showed activity of 50.21 µg/ml (Fig. 3).

Discussion

Plants are potential sources of natural antioxidants. Epidemiological studies have shown that natural antioxidants in fruit and vegetables in foods safeguard from numerous chronic diseases associated with aging, such as cancer, heart disease, cataracts, cardiovascular

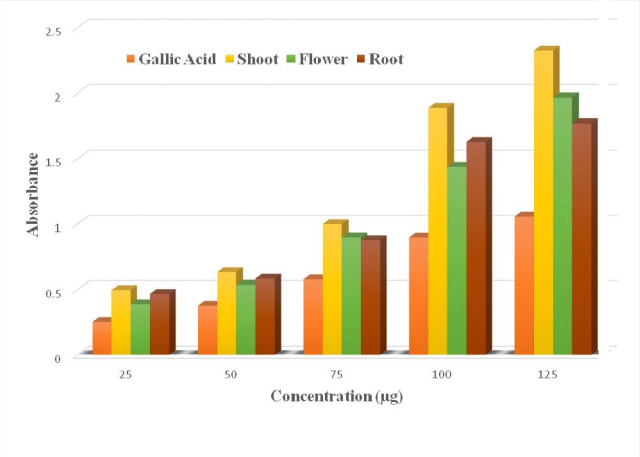

Fig. 2: Total flavonoid content analyzed with whole plant extract of C. roseus.

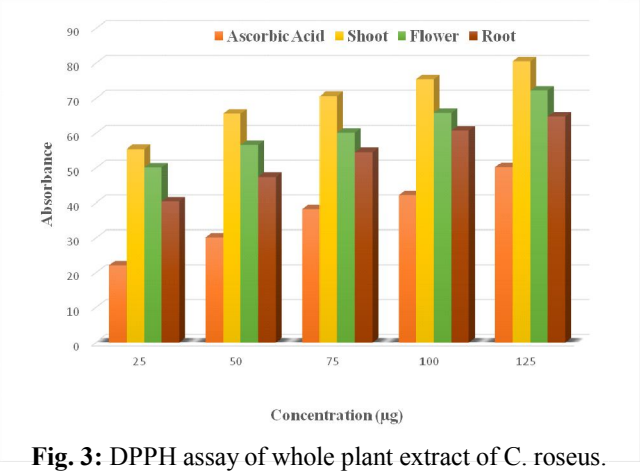

Fig. 3: DPPH assay of whole plant extract of C. roseus.

disease, brain and immune dysfunction (Ames*et al.,* 1993; Block*et al.,* 1992; Vinson *et al.,* 1995). These natural protection properties are associated with varying components like as carotenoids, vitamins C and E, and phenolic and thiol (SH) compounds (Paganga *et al.,* 1999). Polyphenols and phenols found in plants are two secondary metabolites that are thought to be natural antioxidants. They are often measured with folin's regeneration. They are seen as an effective hydrogen donor that is commonly distributed in the plant kingdom. There is a wide difference between phenolic compounds as a result of antioxidants (Robards *et al.,* 1999). There are phytochemicals that can have serious scavenger activity, especially polyphenols that are hydrogen atom donors (Sagbo *et al.,* 2005).

Lee, (2007) explained flavonoids as plant pigments authentic for the color of flower parts. Flavonoids are ketonic compounds that can excite anti-inflammatory activity and inhibit oxygen compounds, enzyme cyclooxygenase-dependent inflammatory activity. Moreover, flavonoids have potential anti-inflammation activity by blocking prostaglandin synthesis. Flavonoids in higher plants are indistinguishable with antioxidants that can treat cardiovascular disease and cancer (Noroozi and Angerson, 1998; Humaid *et al.,* 2010). Flavonoids and antioxidants origin in vitamins A, C, E and plant raw foods (Pietta, 2000; Senthilkumar *et al.,* 2017; Sivakumar, 2019; Sivakumar and Panneerselvam, 2011; Senthilkumar *et al.,* 2015).

According to (Huang *et al.,* 2005), DPBH is one of the antioxidant assays that is responsible for the formation of violet color solutions in ethanol treatment due to the conversion of electrons to Superoxide. Anions are involved in the formation of reactive species and other reactive oxygen species, such as H_2O_2, hydroxyl radical or singlet oxygen, with the exchange of a single electron (Stief, 2003). This is because the antioxidants effectively reduce diseases such as stomach problems, ulcers, cancer and AIDS. Antioxidants react with nitric oxide to form peroxynitrite, which can produce radicals such as hydroxyl radicals (Halliwell, 1997; Sivakumar *et al.,* 2015).

Conclusion

The current study on the analysis of the estimation of total phenol content, flavonoid, and antioxidant assays of whole plant parts of *C. roseus* revealed the concentration of various phytochemicals in different parts individually and in combination under various amounts with different extraction solvents. The inhibitory concentration of this extract for these phytochemicals like as phenols and flavonoids may be determined. Based on the dosage prescribed for different diseases the extract concentration can be quantified and can be used as an effective medicine for control of various diseases that can be contained by these chemicals.

Acknowledgement

The authors are appreciative to all the faculty members of the Department of Botany, Annamalai University, to provide all facilities to carry out this research.

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