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IN VITRO ANTIOXIDANT ACTIVITY OF *CHROMOLAENA ODORATA* (L.) KING AND ROBINSON LEAF EXTRACTS

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ABSTRACT

The aim of the present study was to investigate the antioxidant activity of different extracts (Petroleum ether, chloroform, methanol and aqueous) of *Chromolaena odorata* leaves (Family*: Asteraceae*). Antioxidant activity was evaluated by using *in-vitro* antioxidant assays like DPPH radical-scavenging activity, Superoxide radical-scavenging activity, Nitric oxide radical scavenging activity and Lipid peroxidation inhibiting assay. All the antioxidant activities were compared with standard antioxidant such as gallic acid. Methanolic extracts of the plant showed effective free radical scavenging activity than other extracts. The IC_{50} values of the methanolic extract of *Chromolaena odorata* leaves were found to be 18.34µg/ml in DPPH radical-scavenging activity, 50.14µg/ml superoxide radical-scavenging activity model, 29.34µg/ml in Nitric oxide radical scavenging activity and 49.14µg/ml Lipid peroxidation inhibiting assay. The results obtained in this study showed that the leaves of *Chromolaena odorata* have antioxidant properties which provide a basis for the traditional use of the plant.

KEYWORDS: *Chromolaena odorata*, *In vitro* antioxidant activity, DPPH, IC_{50} and gallic acid.

1. INTRODUCTION

Free radicals are highly reactive due to the presence of unpaired electrons.Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Reactive oxygen

species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors.[1] This ROS acts on lipids, proteins and nucleic acids may trigger various diseases including cancer, cardiovascular diseases, cataracts, diabetes immune deficiency diseases and ageing.[2] Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.[3] Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources.[4] Synthetic Antioxidant cause toxicity therefore recently there has been an upsurge of interest in therapeutic potential of medicinal plants as antioxidants in reducing such free radicals.

Chromolaena odorata (L) King and Robinson Asteraceae commonly known as Siam weed, is a fast-growing perennial and invasive weed native to South and Central America. It has been reported to have antispasmodic, aniprotozoal, antitrypanosomal, antibacterial and antihypertensive activities. It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatotropic activities.[5] Thus, the aim of this study was to evaluate the potential antioxidant activity of different extracts. The study provides scientific evidence on the use of these plants which are being utilized traditionally as herbal medicines.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample

Chromolaena odorata leaves were collected from the Mother Teresa Women's University campus, Kodaikanal and washed thoroughly with distilled water to remove the dust particles. The thoroughly washed leaves were air dried for a week at room temperature. The dried leaves were ground into fine powder and stored in a dry air tight container to avoid any other contaminations. The powder thus prepared was used for further analyses.

2.2 Solvent extraction

A 50 g of air dried, coarsely powdered sample was successively extracted with different solvents (petroleum ether, chloroform, methanol and water) in the increasing order of polarity using soxhlet apparatus. Each time, before extracting with the next solvent, the powdered

material was dried in hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 h and the water extract filtered. The different solvent extracts were concentrated to dryness under reduced pressure using rotary vacuum evaporator and weighed.

2.3 Free radical scavenging assays

2.3.1 DPPH radical scavenging activity

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH.[6] The extracts at various concentrations was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH• was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as follows.

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.2 Superoxide radical scavenging activity

The assay was based on the capacity of the sample to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system.[7] Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations of extracts. Reaction was started by illuminating the reaction mixture with extracts for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in dark served as blank. The percentage inhibition of superoxide anion generation was calculated as.

% Inhibition = (control OD-sample OD / control OD) x 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.3 Nitric oxide radical scavenging activity

The nitric oxide scavenging activity of the sample was measured.[8] 3ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations of the samples and incubated at roomtemperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H3PO4) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage radical scavenging activity of the sample was calculated as follows.

% NO radical scavenging activity = (control OD-sample OD / control OD) x 100

The analysis was performed in triplicate. The extract concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.4 Lipid peroxidation inhibiting assay

The lipid peroxidation inhibition ability of the extracts was carried out using a modified procedure .[9] Goat liver was washed thoroughly in cold phosphate buffersaline (pH 7.4) and homogenized to give a 10% homogenate. The homogenate was filtered and centrifuged at 10000 rpm for 10 min and the supernatant used to carry out the assay. To 0.5 ml of 10% homogenate, 0.5 mL of the sample at various concentrations was added. To this, 0.05 mL of 0.07M ferrous sulphate was added and incubated at room temperature for 30 min. To the incubated solution, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TCA (in 1% SDS) were added. The tubes were incubated at 100°C for 1 hr and cooled to room temperature. About 5 ml of butanol was added and centrifuged at 3000 rpm for 10 min. The upper layer was used to read the absorbance at 532 nm. The percentage inhibition was calculated as follows.

% inhibition = [(Control OD – Sample OD)/ Control OD] × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.5 Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhibition (I%) was calculated using the formula.

I % = (Ac-As) x 100/Ac

where Ac is the absorbance of the control and As is the absorbance of the sample.

2.3.6 Statistical Analysis

All experiments were performed in triplicate (n=3) and results were expressed as mean ± SEM. Statistical analysis was carried out with (SPSS package version 10.0) using ANOVA followed by Turkey's test ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1. Dpph Radical Scavenging Activity

The free radical scavenging activity of different extracts of *C.odarata* was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maximal of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2- diphenyl-1-picryl hydrazine resulting in decrease in absorbance. The aqueous and methanol extracts were showed maximum activity of 93.48% and 97.13% respectively at 500 µg/ml, whereas gallic acid at the same concentration exhibited 88% inhibition respectively. Four extracts exhibited considerable DPPH free radical scavenging activity as indicated by their IC_{50} values are shown in Table 1 and Figure 1. IC_{50} indicates the potency of scavenging activity. Standard gallic acid was found to have an IC_{50} of 4.42µg/ml. In comparison to standard gallic acid, methanol, aqueous, chloroform and petroleum ether extract of *C.odarata* leaves were showed IC_{50} of 18.34, 44.16, 128.63 and 134.89 µg/ml respectively. The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants.[10] DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants.[11] The stable free radical DPPH has been widely used to test the free radical-scavenging ability of various dietary antioxidants.[12] Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolourization is stoichiometric with respect to the number of electrons taken up. The highest scavenging effect was showed by methanol extract with a value of 18.34µg/ml. This result indicated that extract has a noticeable effect on scavenging the free radicals.

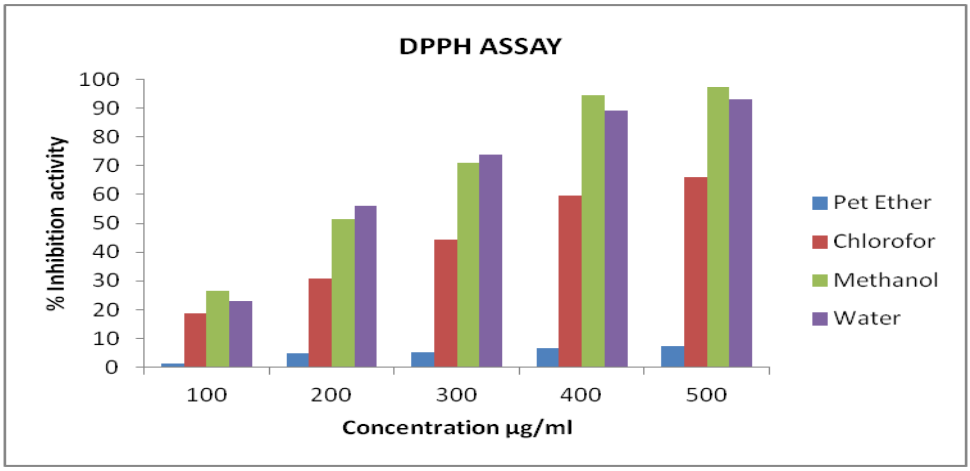

Figure 1. Comparative DPPH radical scavenging activity of *C.odarata* leaves extracts.

3.2 Superoxide Radical Scavenging Activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system. Due to oxidative enzymes superoxide are formed in our body as well as auto oxidation of non enzymatic reactions such as catecholamines.[13] The Superoxide anion scavenging activity of the extracts from *C.odarata* was increased markedly with the concentrations. The chloroform and methanol extracts were showed maximum activity of 28.48% and 31.68%, respectively at 500µg/ml, whereas gallic acid at the same concentration exhibited 57% inhibition respectively.The percentange inhibition activity and IC_{50} values are shown in Table 1 and Figure 2. The IC_{50} values of methanol, chloroform, aqueous and petroleum ether extracts of *C.odarata* leaves were showed IC50 of 50.14, 145.16, 183.63 and 304.89 µg/ml respectively. The IC_{50} value of standard gallic acid found to be 17.25 µg/ml. The methanol extracts showed considerable super oxide radical scavenging activity when compared to other extracts of *Chromolaena odarata*.

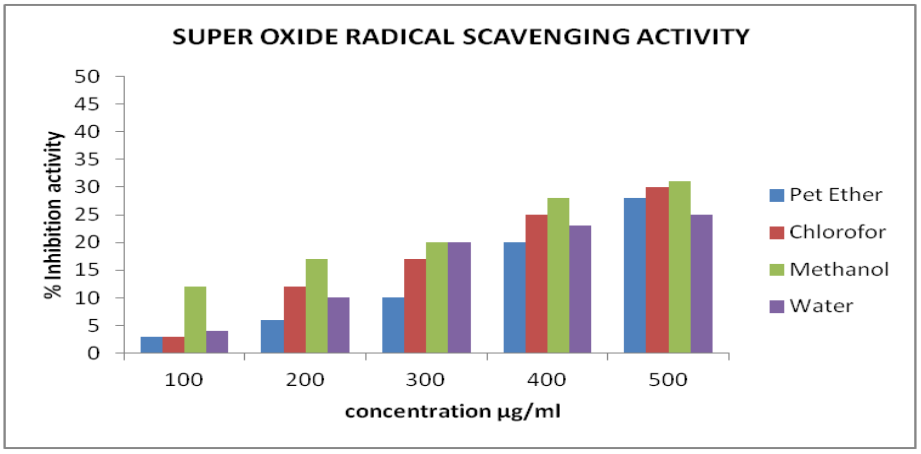

Figure 2. Comparative Superoxide radical scavenging activity of *C.odarata* leaves Extracts.

3.3 NITRIC OXIDE SCAVENGING ACTIVITY

Nitric oxide (NO) is a potent inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities.[14] The methanol and aqueous extracts were showed maximum activity of 72.13 and 71.48%, respectively at 500 µg/ml, where as gallic acid at the same concentration exhibited 57% inhibition respectively. Four extracts exhibited considerable nitricoxide radical scavenging activity as indicated by their IC_{50} values are shown in Table 1 and Figure 4. IC_{50} indicates the potency of scavenging activity. Standard gallic acid was found to have an IC_{50} of 17.25µg/ml. In comparison to standard gallic acid, methanol, aqueous, chloroform and petroleum ether extracts of *C.odarata* leaves were showed IC_{50} of 21.34, 54.16, 154.63 and 334.89µg/ml respectively.

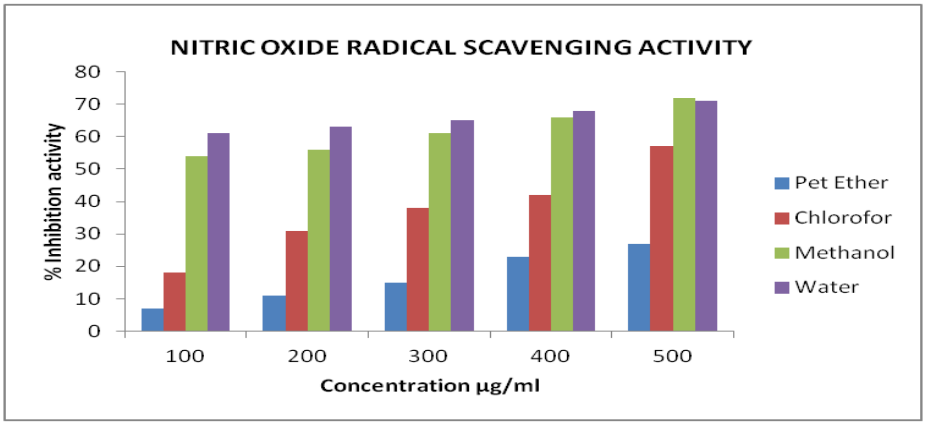

Figure: 3 Comparative nitric oxide scavenging activity of of *C.odarata* leaf extracts.

3.4 Lipid Peroxidation Inhibition Activity

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membrane that generates no of degradation products. SLipid peroxidation consists of free radical mediated chain reaction which causes several type of cellular damage.[15] The Lipid Peroxidation inhibition activity of the extracts from *C.odarata* was increased markedly with dose dependent manner. The chloroform and methanol extracts showed maximum activity of 36.48% and 42%, respectively at 500µg/ml, whereas gallic acid at the same concentration exhibited 94% inhibition respectively. The percentage inhibition activity and IC_{50} values are shown in Table 1 and Figure 4. The IC_{50} values of methanol, chloroform, aqueous and

petroleum ether extract of *C.odarata* leaves were showed IC_{50} of 49.14, 55.16, 59.63 and 75.89 µg/ml respectively. The IC_{50} value of standard gallic acid found to be 13.37µg/ml. The methanol extract showed maximum lipid peroxidation inhibition activity when compared with other extracts of *Chromolaena odarata.*

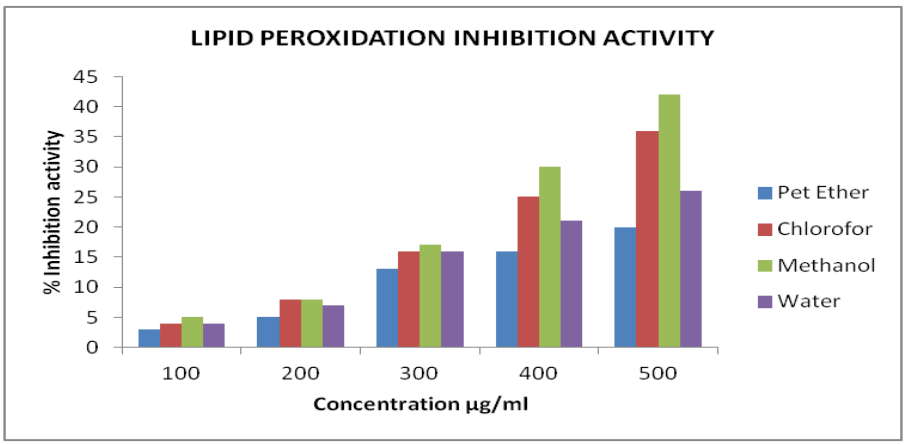

Figure 4. Comparative lipid peroxidation inhibition activity of *C.odarata* leaves Extracts

Table 1. 50% Inhibition concentrations (IC_{50}) of different extracts of *Chromolaena odarata* leaves against DPPH,Superoxide, Nitric oxide and Lipid peroxidation radicals.

S.No	Antioxidant Assay	50 % Inhibition concentration IC_{50}(µg/ml)				
		Pet ether	Chloroform	Methanol	Aqueous	Gallic acid
1	DPPH Assay	134.89	128.63	18.34	44.16	4.42
2	Superoxide Radical Scavenging Assay	304.89	145.16	50.14	183.63	17.25
3	Nitric Oxide Scavenging Assay	334.89	154.63	21.34	54.16	17.25
4	Lipid Peroxidation Inhibition Assay	75.89	55.16	49.14	59.63	13.37

4. CONCLUSION

The findings of this study supports the view that the methanolic leaf extract of *Chromolaena odarata* is a promising sources of potential antioxidant and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry. Further detailed studies on isolation of phytoconstituents of this methanolic extract are essential to characterize them as biological antioxidants.

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