



## **RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITY OF METHANOLIC LEAF EXTRACT OF *CHROMOLAENA ODORATA* (L) KING AND ROBINSON BY *INVITRO* ASSAYS**

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### **ABSTRACT**

Objective: To evaluate the antioxidant activity of methanolic extract of *chromolaena odorata* leaves using in vitro models. Methods: The *invitro* antioxidant was evaluated for DPPH radical scavenging activity, Hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, reducing power, lipid peroxidation inhibiting activity and total antioxidant assay were studied. Ascorbic acid and Quercetin were kept as standards. Results: IC<sub>50</sub> values were observed for DPPH radical scavenging activity, Hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, reducing power, lipid peroxidation inhibiting activity and total antioxidant assays were  $40.99 \pm 0.05$ ,  $75.37 \pm 0.41$ ,  $100.71 \pm 3.98$ ,  $62.23 \pm 1.48$  ( $\mu\text{g/ml}$ ) and  $194.34 \pm 13.07$  (mg ascorbic acid eq/g extract) respectively. Conclusion: The results clearly indicate that the methanolic extract of the study species is effective in scavenging free radicals and has the potential to be powerful antioxidant.

### **KEY WORDS**

*chromolaena odorata* leaves, Antioxidant activity- DPPH, Reducing power, Hydroxyl radical scavenging activity, Total antioxidant capacity and Lipid peroxidation inhibition assay.



## INTRODUCTION

Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, no side effects and economic viability. Several compounds widely distributed in plants which have been reported to exert multiple biological activity. According to the World Health Organization, 80% of Asian and African population still depends on traditional medicine for primary health care. Globally, India has been acknowledged as a major resourceful area in traditional medicine. The primary benefits of using plant derived medicine are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment. Many commercially proven drugs used in modern medicine are from traditional medical plants, with ethnobotanical and ethnomedical knowledge.(Tilahunand Mirutse.,2007).

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. In recent years, natural antioxidants, particularly those present in fruits and vegetables have gained increasing interests among consumers and the scientific community. Epidemiological studies have demonstrated that frequent intake of fruits and vegetables are associated with a lower risk of age-related disease such as coronary heart diseases and cancer (Temple NJ., 2000 and La Vecchia *et al.*, 2001).Natural food usually contains dietary antioxidants that can scavenge free radicals.

Antioxidant supplements are vital to combat oxidative damage by free radicals in many oxidative stress-mediated disease conditions such as cancer, atherosclerosis, diabetes, inflammation and aging. Recently, natural antioxidants are in high demand for application as nutraceuticals and as food additives (Tawaha *et al.*, 2007; Jayasri *et al.*, 2009; Kalimet *et al.*, 2010). Exertion of oxidative stress on human cells by free radicals which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells cause protein and DNA damage along with lipid peroxidation resulting in pathological processes ( Braca *et al.*, 2002; Hazra *et al.*, 2008). While plants serve as rich, natural, and safer sources of



antimicrobials, the rapid incidences of increased resistance to available antibiotics worldwide have turned the attention of researchers and the pharmaceutical industries to plants in search of viable alternatives.

*Chromolaena odorata*(L)King & Robinson belongs to the family Asteraceae. It is widely spread over many parts of the world including Florida,Texas,USA,West Indies, Argentina, India, Ceylon, China, Malaysia,Indonesia,Philippines,Asia,Ghana, Nigeria,SriLanka,Thailand and Ivory coast (Muniappan R and Jesse B, 1999).The leaves are used in traditional medicine decoction as a cough remedy. It is also used with other leaves like guava and lemon grass for the treatment of malaria .The plant is used as an anti-diarrheal, antispasmodic, diuretic, anti-inflammatory and antihypertensive. The plant is a rapidly growing perennial herb which has some common names such as Siam weed, Christmas Bush, Devil weed, Common floss flower and Camphur grass (Pierangeli GV and Windell LR, 2009). It has been reported that an aqueous extract of *Chromolaena odoratum* leaves affected the fertility of male rats. Antimicrobial activity and cytotoxicity of ethanol extracts of leaves of *chromolaena odorata* and ethyl acetate extracts of stem bark has been reported. This revealed that the extracts can be used to produce alternative forms of antimicrobials (Yakubu MT, 2007). Thus, the aim of this study was to carry out the radical scavenging antioxidant activity of methanolic extract of the leaves of *chromolaena odorata* (Linn.)King Robinson.

## **MATERIALS METHODS**

### **Free radical scavenging activity on DPPH**

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (Blois MS.1958).The sample extracts at various concentrations (100 - 500 $\mu$ g) was taken and the volume was adjusted to 100  $\mu$ l 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:

$$\% \text{ DPPH radical scavenging activity} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$$

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

### **Nitric oxide radical scavenging activity**

The nitric oxide scavenging activity of the sample was measured according to the method of Sreejayan and Rao (1997). 3ml of 10mM sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (50 - 250 $\mu$ g) of solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediaminedihydrochloride in 2%  $H_3PO_4$ ) was added. The absorbance of the chromophore formed was read at 546 nm. Percentage radical scavenging activity of the sample was calculated as follows:

$$\% \text{ NO radical scavenging activity} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$$

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

### **Reducing power**

The reducing power of the sample extract was determined by the method reported by (Prieto P *et al.*,1999). 100 - 500  $\mu$ g of extract was taken in 1 ml of phosphate buffer and 5 ml of 0.2M phosphate buffer (pH 6.6) was added. To this, 5 ml of 1% potassium ferricyanide solution was added and the mixture was incubated at 50°C for 20 min. After the incubation, 5ml of 10% TCA was added. The content was then centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5ml) was mixed with 5ml of distilled water and 0.5ml of 0.1% ferric chloride. The absorbance of the reaction mixture was read spectroscopically at 700 nm.



### **Hydroxyl radical scavenging activity**

The scavenging activity of the sample on hydroxyl radical was measured according to the method of (Siddhuraju P *et al.*,2002). Different concentrations of the extract (100 - 500µg) were added with 1ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1ml of dimethyl sulfoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated as follows:

$$\%HRSA = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100$$

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

### **Lipid peroxidation inhibiting assay**

The lipid peroxidation inhibition ability of the sample was carried out using a modified procedure of (Ohkawa, H *et al.*,1979). Goat liver was washed thoroughly in cold phosphate buffer saline (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 (50 -250µg) 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.



### Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

## RESULTS AND DISCUSSION

The successive antioxidant activity were done for DPPH radical scavenging activity, Hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, reducing power, lipid peroxidation inhibiting activity and total antioxidant assay using standard procedure. The results are given below.

### DPPH free radical scavenging activity

DPPH is a purple colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour stoichimetrically with the number of electrons taken up. (Chidambara Murthy *et al.*, 2003).

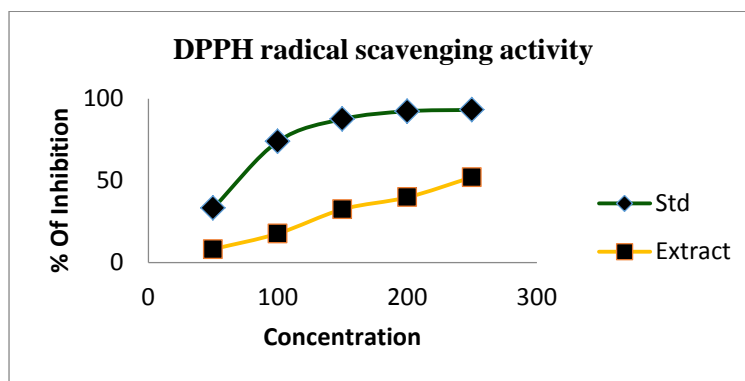


Figure: 1 DPPH free radical scavenging activity

The DPPH radical scavenging activity of methanolic leaf extract of *Chromolaena odorata* (MECO) were detected and compared with Ascorbic acid. The percentage inhibition (%)



inhibition) at various concentration (50- 250 µg/ml) of as well as standard Ascorbic acid (2-10 µg/ml) were calculated and plotted in Fig .1 The IC<sub>50</sub> values are calculated from graph and were found Ascorbic acid (3.77 ± 0.00 µg/ml) and the The IC<sub>50</sub> values of MECO is 40.99 ± 0.05 µg/ml). The IC<sub>50</sub> values of the extract showed good DPPH scavenging activity and it was evident that the extract could serve as free radical inhibitors or scavengers when compared to ascorbic acid.

### Hydroxyl radical scavenging activity

Hydroxyl radical are the major active oxygen causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein(Srikanth G *et al.*,2010.)

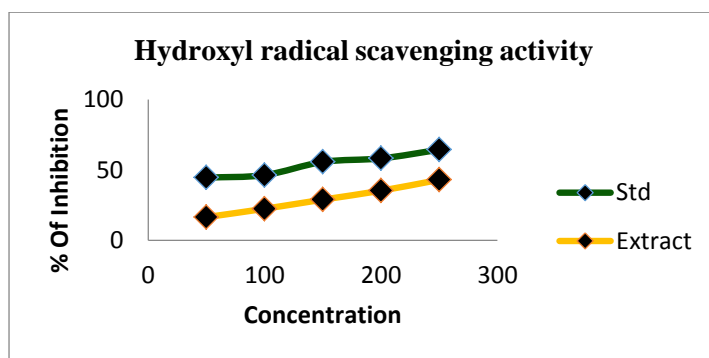


Figure: 2 Hydroxyl radical scavenging activity

The Hydroxyl radical scavenging activity of methanolic leaf extract of *Chromolaena odorata* (MECO) were detected and compared with Quercetin. The percentage inhibition (% inhibition) at various concentration (50- 250 µg/ml) of as well as standard Quercetin (20-100 µg/ml) were calculated and plotted in Fig. 2 The IC<sub>50</sub> values are calculated from graph and were found Quercetin (17.81 ± 0.12µg/ml) and the The IC<sub>50</sub> values of MECO is 75.37 ± 0.41µg/ml). In this study *Chromolaena odorata* was found to scavenge significantly and in dose dependent manner and may protect the DNA, protein and lipid from damage.



### Nitric oxide radical scavenging activity

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is associated with several diseases. It would be interesting to develop potent and selective inhibitors of NO for potential therapeutic use (Hepsibha BT *et al.*,2010).

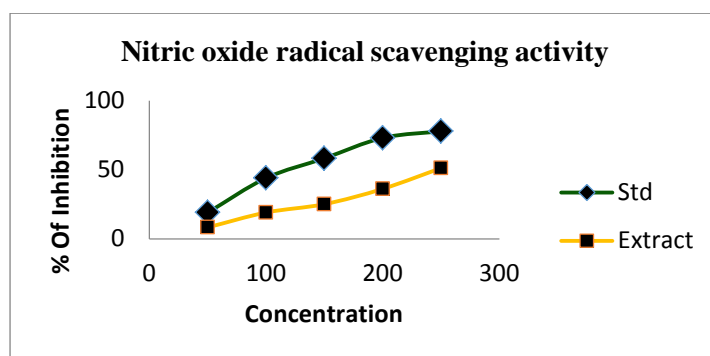


Figure: 3 Nitric oxide radical scavenging activity

The Hydroxyl radical scavenging ac Nitric oxide radical scavenging activity of methanolic leaf extract of *Chromolaena odorata* (MECO) were detected and compared with Ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 250 µg/ml) of as well as standard Ascorbic acid (10-50 µg/ml) were calculated and plotted in Fig. 3 The IC<sub>50</sub> values are calculated from graph and were found Ascorbic acid ( $11.32 \pm 0.03$  µg/ml) and the IC<sub>50</sub> values of MECO is  $100.71 \pm 3.98$  µg/ml). In this study, MECO exhibited potent nitric oxide radical scavenging activity, which competes with oxygen to react with nitric oxide and thus inhibits the generation of nitrite.

### Reducing power

A reducing power is an indicative of reducing agent having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction (Ganu GP *et al.*,2010).



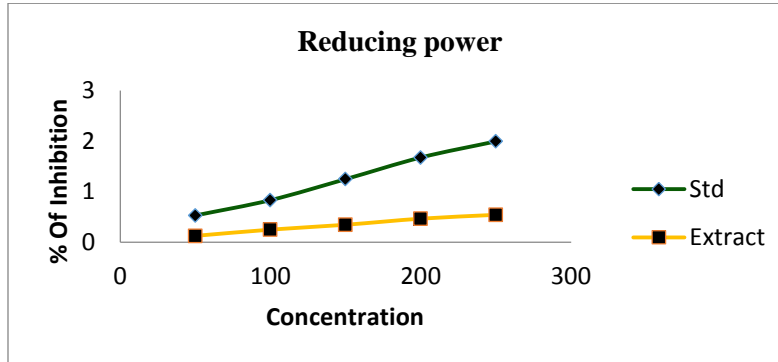


Figure: 4 Reducing power of *Chromoleana odorata*

Accordingly, *Chromoleana odorata* might contain a sizable amount of reductants which may react with the free radicals to stabilize and terminate from free radical chain reaction.

### Lipid peroxidation inhibiting activity

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) which in turn generate hydroxyl radicals (OH) resulting in initiation and propagation of lipid peroxidation.

(Mustafa AG *et al.*,2010)

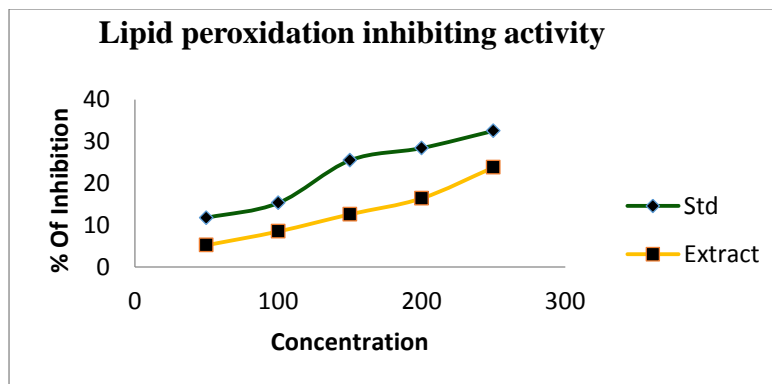


Figure: 5 Lipid peroxidation inhibiting activity



The Lipid peroxidation inhibiting activity of methanolic leaf extract of *Chromolaena odorata* (MECO) were detected and compared with Ascorbic acid. The percentage inhibition (% inhibition) at various concentration (50- 250  $\mu\text{g/ml}$ ) of as well as standard Ascorbic acid (50-250  $\mu\text{g/ml}$ ) were calculated and plotted in Fig 3 The IC<sub>50</sub> values are calculated from graph and were found Ascorbic acid ( $38.42 \pm 2.45 \mu\text{g/ml}$ ) and the The IC<sub>50</sub> values of MECO is  $62.23 \pm 1.48 \mu\text{g/ml}$ ). The ability of the extracts to quench OH seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.

#### **Total antioxidant activity (Phosphomolybdenum assay)**

Phosphobdenum assay is mainly based on the reduction of MO (VI) in the presence of antioxidant compounds and the subsequent formation of a green phosphate MO (V) complex at acidic pH (Prieto.,1999). The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid. Total antioxidant capacity of MECRP was found to be  $194.34 \pm 13.07 \text{ mg ascorbic acid equivalence /gram extract}$ . This good antioxidant activity might be due to the presence of phytochemicals in the extract.

#### **CONCLUSION**

Based on the results obtained, it may be concluded that all the fractions of the methanolic extract of the leaves of *C. odorata* showed strong antioxidant activity, DPPH radical scavenging activity, Hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, reducing power, lipid peroxidation inhibiting activity and total antioxidant assay when compared to standards such as ascorbic acid and quercetin .Further studies to evaluate the *in vivo* potential of the fractions in various animal models and the isolation and identification of the antioxidant principles in the leaves of *Chromolaena odorata* are being carried out.



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