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ANTIOXIDANT PROFILES OF CYMBOPOGAN CITRATUS (DC) STAPF. LEAVES

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Abstract

The study aimed to investigate the antioxidant activcities from *Cymbopogan citratus*. The acetone extract were analyzed by the methods of DPPH, phosphomolybdenum reduction assay, FRAP, metal ion chelating activity, lipid peroxidation inhibiting assay, reducing power assay and superoxide radical scavenging assay. The acetone extract of *Cymbopogan citratus* recorded IC $_{50}$ value for DPPH assay to be 38.49 µg/ml, FRAP content was observed at 709.73 ± 6.21 mmol Fe(II)/g, phosphomolybdenum value was 535.16 ± 50.26 mg AAE/g extract, chelating ability 37.32± 1.07 mg/EDTA/g, LPO activity of the extract with IC_{50} value as 31.67 ± 0.93 µg/ml, The IC $_{50}$ value of superoxide radical scavenging assay was observed as 238.84 ±3.57 µg/ml. Therefore, the results suggest that the acetone extract of *Cymbopogan citratus* have promising therapeutic value for natural antioxidant.

Key words: *Cymbopogan citratus*, antioxidant, DPPH, free radicals, FRAP

I.INTRODUCTION

Oxygen is one of the most essential factors required to bolster life. Reactive Oxygen Species (ROS) or free radicals are alarming substances produced in the body; it may cause disruption of membrane fluidity, lipid peroxidation, protein denaturation, etc by creating oxygen stress (Anderson *et.al*., 1996). Generating free radical beyond the antioxidant present in biological system which gives rise to oxidative stress (Zima *et al*., 2001). Free radicals produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as carcinogenesis, neuro-degenerative diseases, cardiovascular diseases, aging, etc (Gyamfi *et al*.,2002).

In a normal cell there is balance between formation and removal of free radicals. However this balance can be shifted towards more formation of free radicals or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive and prolonged. Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Lian, 2008).Antioxidants are chemicals

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that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers". Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet (Osowas *et al*., 1994)

The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life. These antioxidants may help to relieve oxidative stress, i.e. Preventing free radicals from damaging biomolecules such as proteins, DNA, and lipids (Middleton *et al*., 2007).

Endogenous compounds in cells can be classified as enzymatic antioxidants and non-enzymatic antioxidants. The major antioxidant enzymes directly involved in the neutralization of ROS and RNS are: superoxide dismutases (SOD), catalase (CAT), glutathione peroxidise (GPx) and glutathione reductase (GRx) (6-12). SOD, the first line of defense against free radicals, catalyzes the dismutation of superoxide anion radical (O_2 $\bullet^-$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into selenoprotein GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH) (Bahorun, 2006). The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants are produced by metabolism in the body, such as lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc (Droge 2002). While nutrient antioxidants belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc (Willcox, 2004).

The antioxidant activity of extracts of several plants, including their leaves, bark, roots, fruits, and seeds has been extensively studied. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as food additive because of consumer preference. Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several medicinal plants extracts. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins .

The genus *Cymbopogan* comprises 140 species that are widely distributed in the world. Approximately 45 species have been reported to occur in India. *Cymbopogan citratus* (DC) Stapf. Known as West Indian lemon grass is an important species of Poaceae family commonly found in Southeast Asia, which its origin can be

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tracked from India. It is a tall, clumped perennial grass growing to a height of 1 m and it is commonly growing grass in Kodaikanal hills, Tamil Nadu. The leaf blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm. The plant is commonly used in folk medicine in many countries since it exhibit antioxidant properties which in turn inhibits the propagation of free radical reactions and protects the human body from disease. It has been used in traditional medicine for treatment of several ailments. Infusions of the leaves are used in traditional medicine as antimicrobial, anti-inflammatory and sedative (Figueirinha *et al*., 2010. *Cymbopogon citratus* has been generally supported for its relieving effect against fever, flu, headaches, pain and oral thrush (Wright *et al*., 2009).

Kodaikanal is situated on top of palani hills and always cool (18^{o}-22^{o}C) throughout the year due to high elevation of the city (2,200m). It comprises unique jungle, grassland and shola. Number of environmental factors such as climate, altitude, rainfall and other conditions may affect growth of plants which in turn affect the quality of herbal ingredients present in a particular species (Kokate *et al*., 2004). In the present investigation an attempt has been taken to determine the antioxidants in Cymbopogan *citratus* leaves.

II. MATERIALS AND METHOD

2.1 Collection of plant material

The aerial parts of the plant *Cymbopogan citratus* were collected from Department of Biotechnology Garden at Mother Teresa Women's University, Kodaikanal. The plant was authenticated by Dr. N.Jayaraman, Director, National Institute of Herbal Science, Plant Anatomy Research Center, Tambaram, Chennai and the voucher Specimen (PARC/2014/2049) has been deposited in our department herbarium for future reference.

2.2 Preparation of the plant extracts

The leaves were washed under running tap water to remove the surface pollutants and the leaves were air dried under shade. The powdered leaf samples were subjected to successive extraction with chloroform, methanol and acetone using soxhlet apparatus. Fresh leaf material was ground using distilled water and filtered and used as an aqueous extract. The extracts obtained using solvents were concentrated using rotary vacuum evaporator and then dried. The extract thus obtained was used for various analyses.

2.3 DPPH Radical Scavenging assay

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, 1958). 1ml of the sample was diluted to 50ml with distilled water and five different concentrations (40-200µl) were taken from it. 2.5 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 517nm against the blank (methanol). Percentage radical scavenging activity of the sample was calculated as follows.

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

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

2.4 *Phosphomolybdenum reduction assay*

The total antioxidant capacity of the extract was evaluated by the green phosphomolybdenum method according to the method of Prito *et al*., 1999. An aliquot of 0.1 ml sample solution was combined in a 4 ml vial with 1ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95 ^{o}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank (methanol). The results reported are mean values expressed as mg of ascorbic acid equivalents (AAE) /g extract.

2.5 *Ferric reducing antioxidant power (FRAP) assay*

The FRAP assay was used to estimate the reducing capacity of the sample extract, according to the method of Benzie and Strain, 1996. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37°C. 900µl FRAP reagent was mixed with 90µl water and 10µl of the extract. The reaction mixture was incubated at 37°C for 30 minutes and the absorbance was measured at 593 nm against the reagent blank (methanol). The results reported are mean values expressed as millimoles of Fe (II) equivalents per gram sample.

2.6. *Metal ion chelating activity*

The chelating of ferrous ions of extract was estimated by the method of Dinnis *et al*.,1994. 400 µl of extract were added to 50 µl of solution of 2 Mm $FeCl_2$. The reaction was initiated by the addition of 200 µl of 5Mm ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). The results reported are mean values expressed as mg EDTA/g extract.

2.7. *Lipid peroxidation inhibiting assay*

The lipid peroxidation inhibition ability of the sample extracts was carried out using a modified procedure of Ohkawa *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 extracts (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 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

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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.

2.8. *Reducing Power*

The reducing power of the sample extract was determined by the method reported by Siddhuraju *et al*. (2002). 40 - 200 µg 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.

2.9. *Superoxide Radical Scavenging Assay*

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (1971).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. 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 (50 - 250µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract 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:

% Superoxide radical scavenging activity = (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.

Statistical Analysis

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

III. RESULT

In the present investigation, we have evaluated the free radical scavenger activity of acetone extract of *Cymbopogan citratus* leaves .The DPPH radical is a stable radical with a maximum absorption at 517 mm that can readily undergo reduction by an antioxidant. IC $_{50}$ value of the extract was found to be 38.49 µg/ml. The IC_{50} value of extract and standard was calculated by dose response-curve of DPPH radical scavenging activity in Fig 1. In the present study the FRAP content was observed at 709.73 ± 6.21 mmol Fe (II)/g (Table 1).

The phosphomolybdenum value was 535.16 ± 50.26 mg AAE/g extract. The acetone extracts of lemon grass leaves were found to have the maximum chelating ability (37.32± 1.07 mg/EDTA/g extract (Table 1). Antioxidant activity of extracts is measured to inhibit lipid peroxidation (LPO) by TBA methods and the results

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are expressed in IC_{50} value as 31.67 ± 0.93 µg/ml in Fig. 2. The results of reducing power assay are given in Table 2 .The IC_{50} value of superoxide radical scavenging assay was observed as 238.84 ±3.57 µg/ml.

IV.DISCUSSION

4.1 DPPH radical scavenging activity

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. Relative stable DPPH radical has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorption at 517 mm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment Eyob *et al*., 2008. The DPPH scavenging capacity of the plant extracts may be related to the phenolic compounds present. The result of DPPH assay was expressed in IC_{50} value. IC_{50} values are negatively related to the antioxidant activity, as it express the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC_{50} value represents the higher antioxidant activity of the tested sample. In the present investigation, we have evaluated the free radical scavenger activity of acetone extract of Cymbopogan *citratus* leaves. IC_{50} value of the extract was found to be 38.49 µg/ml (Fig- 1). The results were in agreement with reports of Rao *et al*., 2009 , Chandan Prasad and Akande *et al*., 2012

4.2 *Phosphomolybdenum assay*

The Phosphomolybdenum method is based on the reduction of Mo (VI) and to Mo (V) by the antioxidant compound with the formation of green phosphate / Mo (V) complex with the maximal absorption at 695 nm. The phosphomolybdenum value was 535.16 ± 50.26 mg AAE/g extract (Table-IV). Phosphomolybdenum assay has been routinely used to evaluate the antioxidant capacity of extracts [Prieto *et al*.1999]. Higher Phosphomolybdenum value indicates higher antioxidant activity.

4.3 *FRAP assay*

The assay measures the reducing potential of an antioxidant reacting with a ferric (Fe (III)-TPTZ) complex and producing a colored ferrous (Fe) III-TPTZ) complex by a reductant at low pH. This complex reaction was monitored at 593 nm. Halvorsen *et al*., 2006 suggested most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay. In the present study the FRAP content was observed at 709.73 ± 6.21 mmol Fe(II)/g (Table-IV). From our results, it is apparent that the reducing ability of lemon grass, as determined by FRAP assay, seems to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds. The data obtained from this assay demonstrated that the extracts of revealed an effective capacity for metal-binding, suggesting that they may play a protective role against oxidative damage by sequestering Fe^{2+} ions [Naidu, 2008].

4.4 *Metal ion chelating activity*

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Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress. An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. The methanolic extracts of lemon grass leaves were found to have the maximum chelating ability (37.32± 1.07 mg/EDTA/g extract). From the iron chelating data, it is evident that the extracts may be able to play a protective role against oxidative damage by sequestering Fe (II) ions that may otherwise catalyze Fenton type reactions or participate in metal catalyzed hydroperoxide decomposition reactions. The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups Pazos *et al*., 2005.

4.5 *Lipid peroxidation assay*

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver. Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals.

Antioxidant activity of extracts is measured to inhibit lipid peroxidation (LPO) by TBA methods and the results are expressed in IC_{50} value as 31.67 ± 0.93 µg/ml (Fig- 2). The tested plant extracts showed strong antioxidant activity to inhibit LPO in biological system. Formation of lipid peroxide has been shown to result from the cumulative effect of reactive oxygen species, disrupting the assembly of the membrane causing changes in fluidity and permeability, alterations in ion transport and inhibition of metabolic process (Nigam, 2000) .

4.6 *Reducing Power*

Reducing power is closely blended with antioxidant activity and the compounds with reducing power indicate that they are electron donors so they can act as primary and secondary antioxidant (Prieto, 1999). The reductive capabilities were found to increase with increasing of concentration in extract as well as standard ascorbic acid. The remarkable reducing power may be associated with the presence of a large amount of reductone, it play an important role in reducing power (Ohawaka, 1996). The yellow colour of the test solution changes to green and blue colour which depends upon the reducing power of each compound. The presence of radicals (i.e. antioxidant) causes the conversion of Fe^{3+}/ ferricyanide complex into ferrous, by measuring the formation of pearls prussian blue at 700nm, reducing power can be monitored.

4.7 *Superoxide Radical Scavenging Assay*

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan. The IC_{50} of the extract was found to be 238.84±3.57. Results are graphically represented in figure 3. From the graph, it was observed that as concentration increases, the % scavenging is increasing linearly for the extract and ascorbic acid (standard

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preparation), revealed by the regression analysis. *In-vitro* super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formazan that can be measured at 560 nm. From results, it was found that the extract showed potent free radical scavenging activity compared to the ascorbic acid (standard) at low IC50.

Antioxidant results showed the capability of the extract to scavenge different free radicals. Therefore leaf extract of lemon grass may be useful as therapeutic agents for treating free radical related diseases. The same type of analyses was reported earlier using this plant (Asaolu, 2009).The lemon grass samples collected from different locations of world showed significant variations in proximate composition, mineral contents and total antioxidants capacity. The present investigation also showed significant variation in the contents like fibre, protein and some of the total antioxidants capacity when compared to above mentioned reports. These variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned by Kokate *et al*., 2004.

V. CONCLUSION

Antioxidants may be actively reducing the free radicals by chain breaking reaction, reducing the concentration, scavenging the initiation, or chelating the metal catalyst. The plant *Cymbopogan citratus* extracts having any one of the above potency separately or combines with other potency and ultimately it shows the good antioxidant activity. This study solidly concludes the plant having significant antioxidant property which is coincidentally comparable with quantity of phenolics, hence it should be with some pharmacological importance. From the observed results it could be concluded that the acetone extract of *Cymbopogan citratus* may be a probable source of natural antioxidant revealed from various *in vitro* assays.

Fig: 1 ***DPPH Radical scavenging activity of acetone extracts***

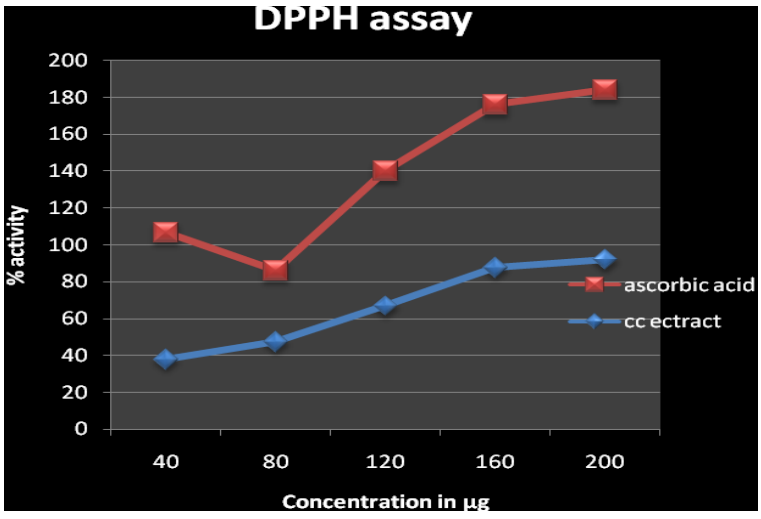

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Fig: 2 ***Inhibition of Fe^{2+}- ascorbate induced lipid peroxidation***

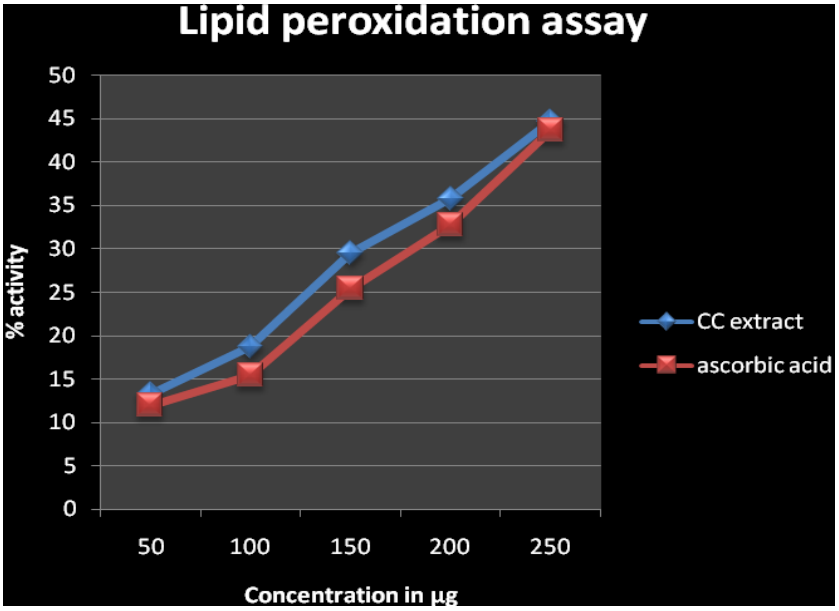

Fig : 3 ***Superoxide Radical scavenging activity of acetone extracts***

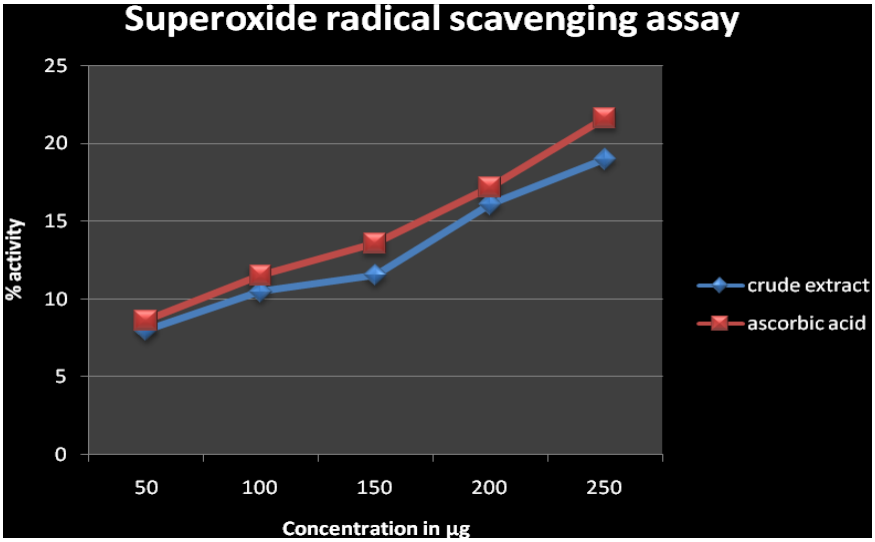

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Table1 : Phosphomolybdenum, FRAP and metal ion chelating activity

S.N0	Phosphomolybdenum (mg AAE/g extract)	FRAP (mmol (Fe(II)/g extract)	Metal ion chelating (mg/EDTA/g extract)
	535.16 ± 50.26	709.73 ±6.21	37.32± 1.07

Values are means of three independent analyses of the extract ± SD (n = 3)

Table 2: Reducing power activity of Lemon grass extract

Concentration (µg/ml)	Absorbance at 700nm
50	0.071
100	0.141
150	0.200
200	0.291
250	0.317

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