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ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT LEAVES OF *CYMBOPOGON CITRATUS* (LEMON GRASS)

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ABSTRACT

Cymbopogon citartus family Myrtaceae also called Tea Tree oil. In traditional system of medicine the plant have various uses for aromatherapy, skin problems, inflammation hair care, stress reduce, and anxiety. Hence the present studies were designed to evaluate the antioxidant activity of methanol extract of *Cymbopogon citartus* leaves. Antioxidant activity carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl, ABTS(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), NO (Nitric oxide) and SO (Super oxide) assay and phytochemical screening of the methanol leaves extract were carried out. The results shows that *Cymbopogon citartus* methanol extracts of leaves shows significant antioxidant activity which is higher when compared with ascorbic acid. In DPPH of IC_{50} value of methanol extract of leaves is 67.69 µg/ml. In superoxide assay IC_{50} value of methanol extract of leaves is 89.73 µg/ml ABTS assay shows the IC_{50} of methanolic extract of *Cymbopogon citartus* of leaves 51.70 µg/ml and, Nitric oxide assay shows IC_{50} of methanolic extract of *Cymbopogon citartus* of leaves is 56.11.The methanol extract of leaves shows the existence of various phytoconstituent such as terpenoids, phenolic compounds, flavonoids and steroids**.** Thus the study suggest that methanol extract of *Cymbopogon citartus* leaves shows strong antioxidant activity might a possible source.

KEYWORDS: *Cymbopogon citartus,* Myrtaceae, inflammation.

1. INTRODUCTION

Human use natural products from history times such as animals, plants, marine and microoranisms in order to treat several diseases.[1] The use of traditional drugs increase day by day because of its availability and lower side affects. As indicated by World health organization (WHO)more than half population depend upon herbal drugs.[2] Many traditional drugs are antioxidant as they contain large amount of phenolic compunds i.e flavonoids, carotenoids, vitamin C.[3-4] These traditional drugs are effective to prevent oxidative stress.[5]

The term oxidative stress use to describe the steady state level of oxidative damage in tissue, organ and cell and free radical production is increase and low antioxidant activity.[6-8] The low and moderate concentration of Reactive nitrogen species (RNS) and Reactive oxygen species (ROS) play an important role in cell life and death, pathogen protection and cellular pathways. But when the concentration of ROS and RNS is high the antioxidant mechanism in the body is low and cause oxidative stress.[9-11] Oxidative stress cause many disease such as cancer, Parkinson, diabetes, atherosclerosis and aging.[12-15] Therefore, antioxidant prevent oxidative stress and control the various diseases.

Tea tree oil also known as *Cymbopogon citartus* (Maiden and Betche cheel) belonging to the family myrtaceae. This plant is grown in Australia, found in new southwales grown above 300 m sea level. In ayurveda system of medicine the Tea tree oil from leaves have been used for, wounds treatment, fungal disease,[16] sore throats, skin diseases[17] and acne.[18] *Cymbopogon citartus* contain large number of terpenes hydrocarbons[19] arjunic acid, betulinic acid and melaleulic acid and fatty acid mixtures.[20]

The Tea tree oil (TTO), has been found to have several medicinal effects as an anti-inflammatory effect, antibacterial, onychomycosis, candidiasis, clearance of bronchial congestion; effective in asthma, coughs, sinusitis, whooping cough, antioxidant, antifungal, antianxiety but there is no work done on extract of leaves. Therefore keeping the application of the oil the present study evaluate antioxidant, activity of methanol extract of leaves.

2. MATERIAL AND METHODS

2.1 Plant material

The *Cymbopogon citartus* (Myrtaceae) leaves were collected from Nilgiris hills in Tamil Nadu in April

2016.The healthy plant material was prepared and Prof Madhava Chetty, Botany Department S.V university, which is situated in Tirupati, A.P, India identify the plant species. The Voucher specimen no is 1241, 28 May,2016. A plant material of Voucher specimen stored in Khalsa college of pharmacy,Amritsar,Punjab,India

2.2 *Extraction*

1600 gm leaf powder was taken in round bottom flask and subjected to soxhlet extraction with methanol solvent for 12hour. The methnol extract was concentrated under reduced pressure at 50-60˚C until the extract is complete drying. The extract yield is 347 gm and stored in vessel 4^{o}C.

2.3 *Antioxidant activity*

2.3.1 *DPPH radical scavenging activity*[21]

1. Prepare 0.1mM DPPH solution (4mg/100ml) in methanol.
2. Prepare different concentration of extract with methanol.
3. Add 2ml of extract and 1ml of DPPH solution.
4. Incubated at room temperature for 10 min.
5. Take absorbance at 515 nm against blank (methanol).
6. Calculate % Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100] (AC=Absorbance control, AS=Absorbance sample).
7. Plot a curve for % Inhibition and concentration and using line of regression estimate IC_{50}

2.3.2 *ABTS Radical Cation Scavenging Method*

1 mL of distilled DMSO was added to 0.2 mL of various concentrations of the extract or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. Absorbance was measured spectrophotometrically, after 20 min at 734 nm using UV spectrophotometer. Control was maintained without sample. IC_{50} value obtained is the concentration of the extract required to inhibit 50 % ABTS radical mono cation[22]

2.3.3 Scavenging of Superoxide radical by Alkaline DMSO Method

To the reaction mixture containing 1 mL of alkaline DMSO, 0.3 mL of the drug samples and standard was added in DMSO at various concentrations followed by 0.1 mL of NBT (0.1 mg) to give a final volume of 1.4 mL, and reaction mixture without extract (water in place of extract) used as control. The absorbance was measured at 560 nm[23]

2.3.4 Nitric oxide radical inhibition

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), PBS (pH 7.4, 1 ml) and different concentration of extract or standard solution (1 ml) were incubated at room temperature for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to for min for completion of diazotization. Then, 1ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm. [24]

2.3.5 Phytochemical screening

Methanol extract of *Cymbopogon citartus* leaves was subjected to phytochemical screening.[25]

3. RESULTS AND DISCUSSION

3.1 *DPPH radical scavenging activity*

The values of % inhibition of extract were compared with that of ascorbic acid. However, the % inhibition values in ascorbic acid started at 51.01% at 20 µg/ml and 75.76% at 100 µg/ml while the methanol extract it was 39.56 % at 20 µg/ml and 60.44 % at 100 µg/ml The methanol extract have high IC_{50} value 67.59 ug/ml when compared to ascorbic acid which is 16.53 ug/ml. These results show that methanol extract of plant have significant DPPH radical scavenging activity (Table 1 and Table 2 and Figure 1 and Figure 2).

Table 1: % Inhibition of DPPH by Ascorbic acid.

Conc. (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
20	0.291	0.594	51.01	16.53
40	0.253		57.41	
60	0.217		63.47	
80	0.172		71.04	
100	0.144		75.76	

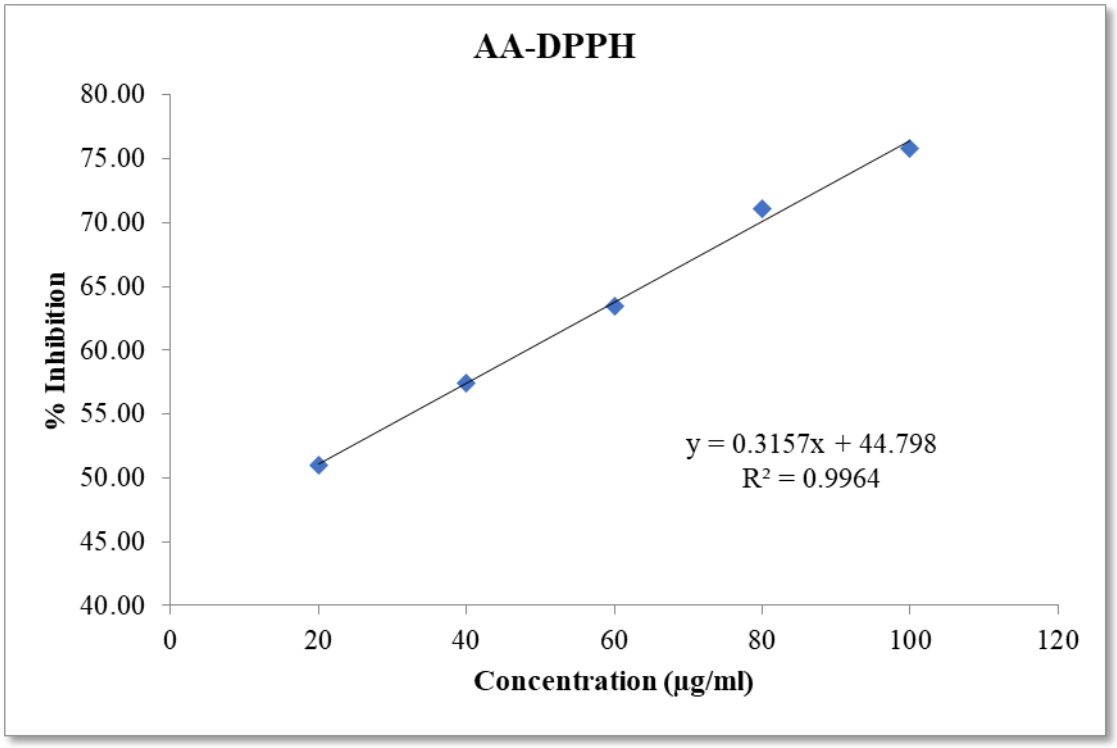

Figure 1: Graph represent regression curve of Ascorbic acid by DPPH assay method.

Table 2: % Inhibition of DPPH by *Cymbopogon citartus* methanol leaves Extracts.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
20	0.359	0.594	39.56	67.69
40	0.336		43.43	
60	0.315		46.97	
80	0.295		50.34	
100	0.235		60.44	

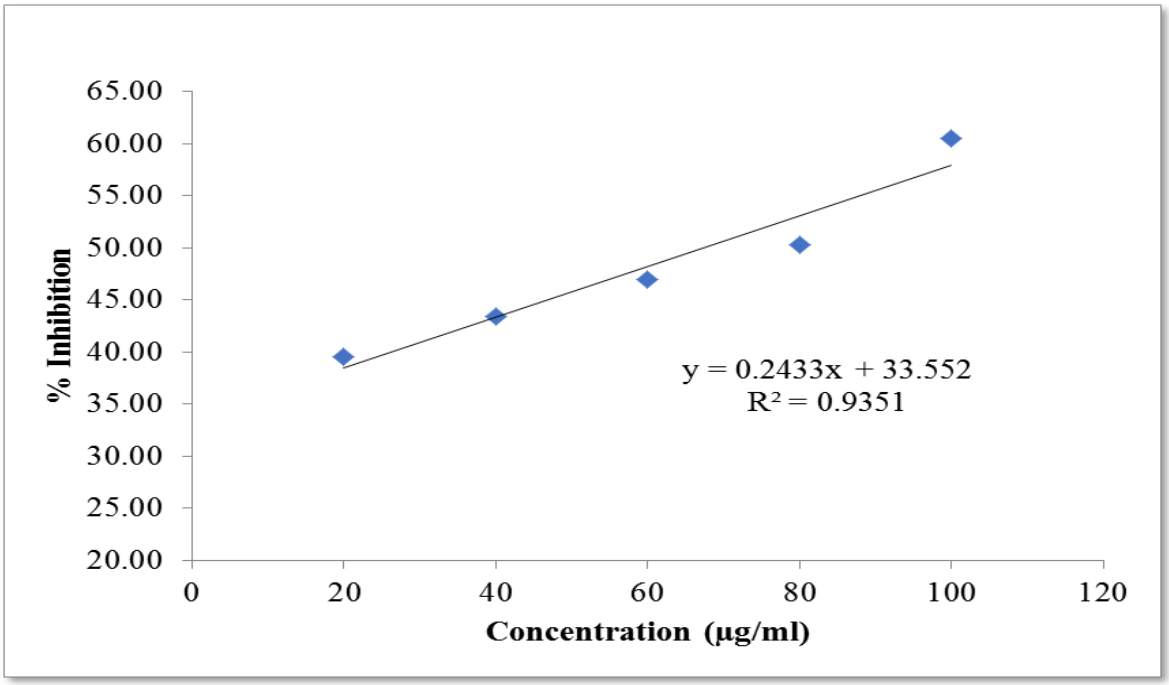

Figure 2: Graph represent regression curve of DPPH by *Cymbopogon citartus* methanol leaves Extracts

3.2 ABTS Assay.

The methanol extract of shows significant ABTS radical scavenging method. The % inhibition of ascorbic acid started at 10ug/ml is 50.89 and for 50 ug/ml is 73.94 whereas in methanol extract the % inhibition started at 10ug/ml is 25.50 and for 50 ug/ml is 50.11.The IC_{50} value of methanol extract is 51.70 which is higher as compared to ascorbic acid. (Table 3 and Table 4; Figure 3 and Figure 4)

Table 3: ABTS assay % inhibition by Ascorbic acid.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
10	0.441	0.898	50.89	8.64
20	0.396		55.90	
30	0.328		63.47	
40	0.285		68.26	
50	0.234		73.94	

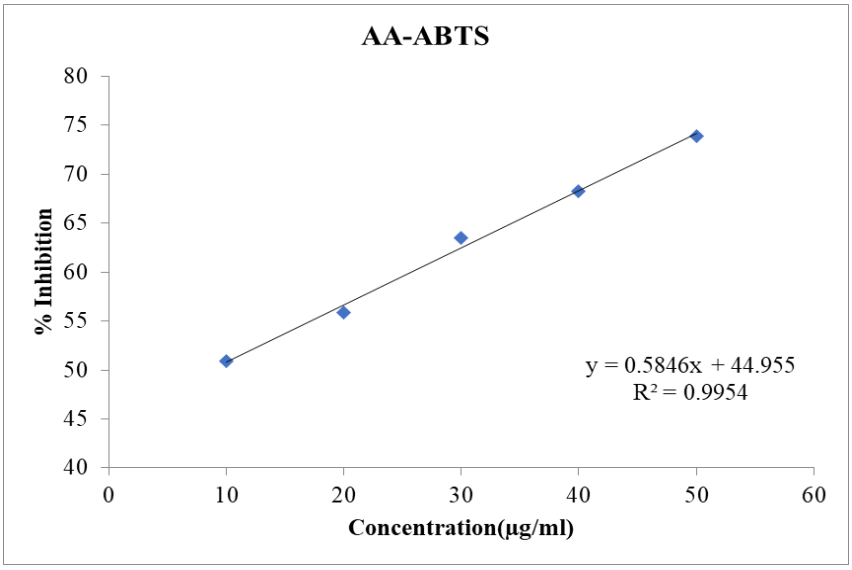

Figure 3: Graph represent regression curve of Ascorbic acid ABTS assay method.

Table 4: % ABTS assay % inhibition by *Cymbopogon citartus* methanol leaves Extracts.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
10	0.669	0.898	25.50	51.70
20	0.619		31.07	
30	0.564		37.19	
40	0.526		41.43	
50	0.448		50.11	

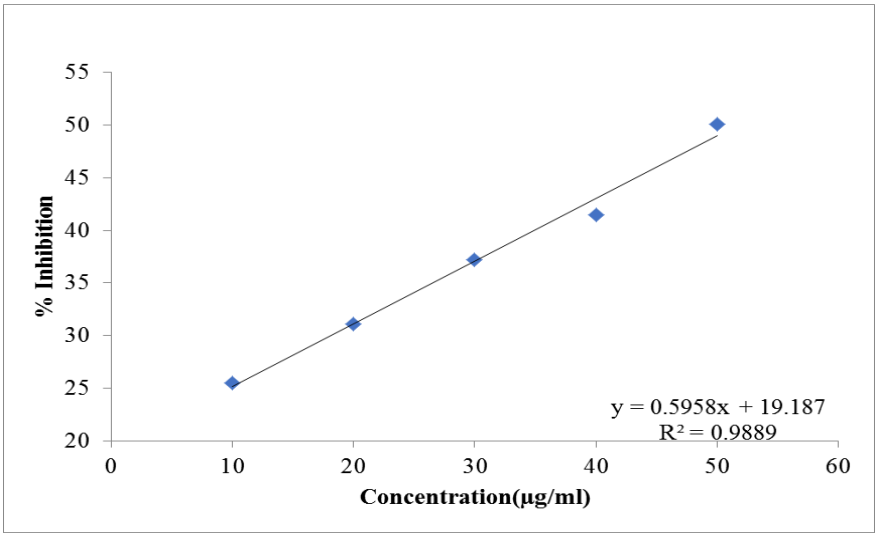

Figure 4: Graph represent regression curve of ABTS assay by *Cymbopogon citartus* methanol leaves Extracts.

3.3 Scavenging of Superoxide radical by Alkaline DMSO Method

Super oxide assay was performed where ascorbic acid was taken as standard. Values of test and sample were compared by measuring % inhibition from concentration 20 µg/ml to 100 µg/ml and then the values were compared to evaluate antioxidant activity of the sample. The IC_{50} value of methanol extract of plant is 89.73 which is higher as compare to ascorbic acid. (Table 5 and Table 6; Figure 5 and Figure 6).

Table 5: % Inhibition of Superoxide assay by Ascorbic acid.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
20	0.688	1.307	27.52	27.50
40	0.599		33.93	
60	0.572		39.98	
80	0.526		44.88	
100	0.473		48.71	

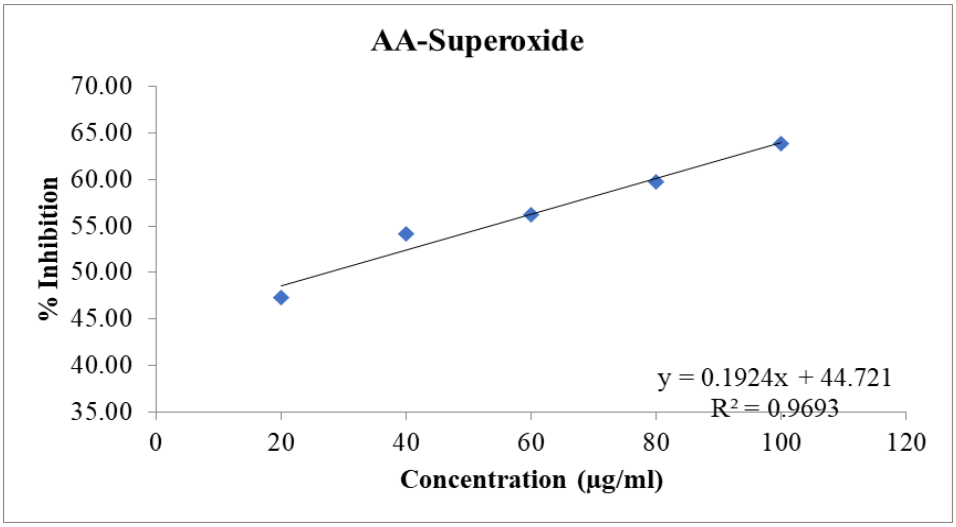

Figure 6: Graph represent regression curve of Ascorbic acid by superoxide assay method.

Table 6: % Superoxide assay % inhi by *Cymbopogon citartus* methanol leaves Extracts.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
20	0.911	1.307	30.30	89.73
40	0.853		34.74	
60	0.783		40.09	
80	0.694		46.90	
100	0.602		53.94	

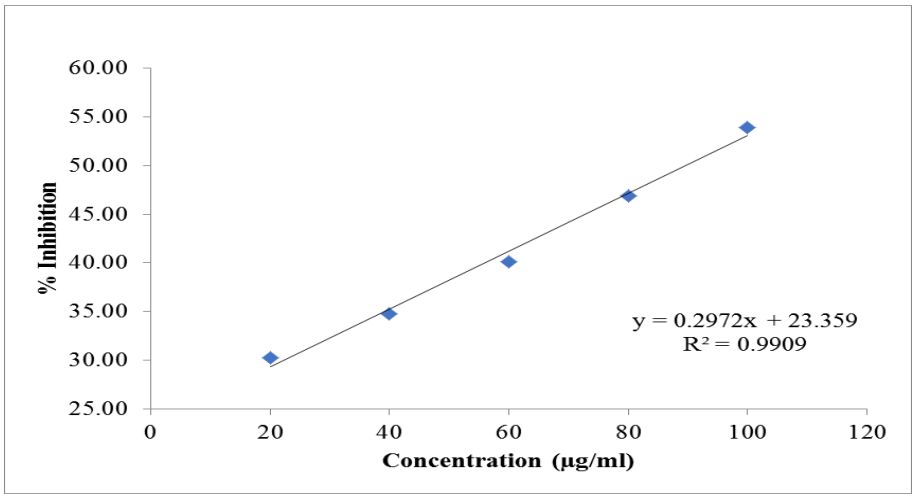

Figure 7: Graph represent regression curve of *Cymbopogon citartus* methanol leaves extracts by Superoxide assay method.

3.4 Nitric oxide assay

In this assay the % inhibition of ascorbic acid started at 10ug/ml is 48.99 and for 50 ug/ml is 68.32 whereas in methanol extract the % inhibition started at 10ug/ml is 25.60 and for 50 ug/ml is 56.04.The IC_{50} value of methanol extract is 56.11 which is higher as compared to ascorbic acid which is 10.60 which shows significant nitric oxide antioxidant activity (Table7 and Table 8; Figure 7 and Figure 8).

Table 7: NO assay % Inhibition by Ascorbic acid.

Conc. (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
10	0.277	0.543	48.99	10.60
20	0.242		55.43	
30	0.223		58.93	
40	0.193		64.46	
50	0.172		68.32	

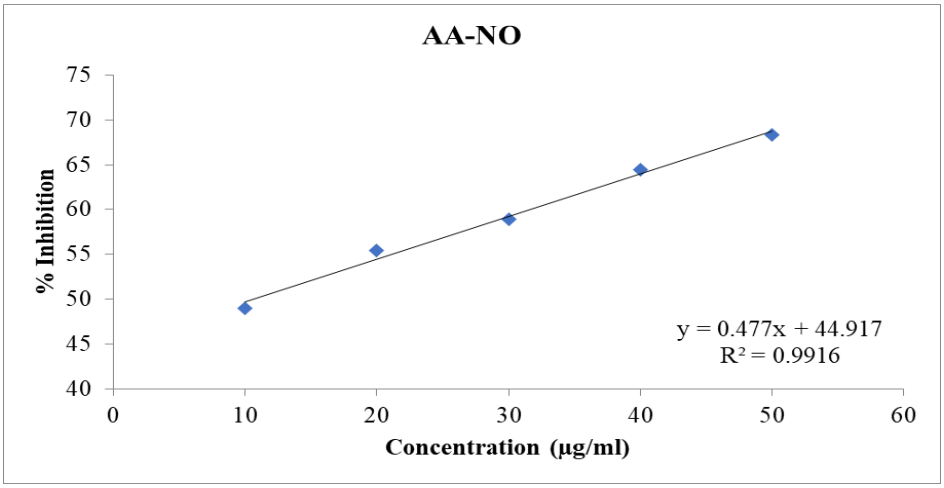

Figure 7: Graph represent regression curve of Ascorbic acid NO assay method.

Table 8: NO assay % Inhibition by *Cymbopogon citartus* methanol leaves extracts.

Conc (µg/ml)	Absorbance of Sample	Absorbance of Control	% Inhibition	IC_{50} (µg/ml)
10	0.404	0.543	25.60	56.11
20	0.371		31.68	
30	0.335		38.31	
40	0.316		41.80	
50	0.293		46.04	

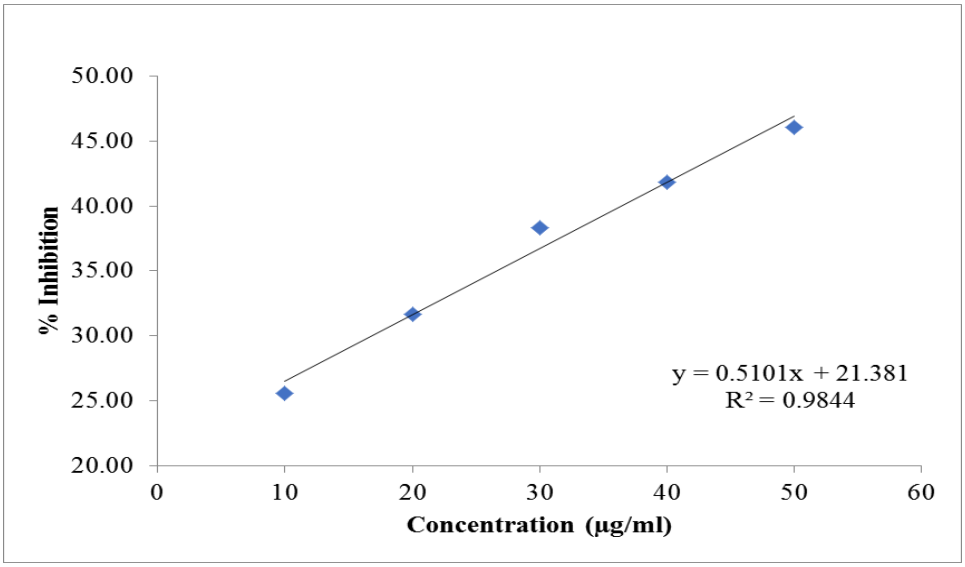

Figure 8: Graph represent regression curve of *Cymbopogon citartus* methanol leaves extracts by NO assay method.

3.5 Phytochemical evaluation

Methanol extract containing leaves shows existence of various phytoconstituent such as terpenoids, phenolic compounds, flavonoids and steroids (Table 9).

Table 9: Preliminary phytochemical analysis for methanolic extract of *Cymbopogon citartus* leaves extract.

S. No.	Test	Crude Extract
1	**Carbohydrates** Molisch's test	 -
2	**Glycosides** Keller-Killiani test	 -
3	**Saponins** Foam test	 +
4	**Alkaloids** A.Mayer's test B.Dragendrodroff's test	 - -
5	**Flavonoids** A. Alkaline reagent test	 +
6	**Phenolics and Tannins** A. Ferric chloride test B. Test for Tannins	 + +
7	**Phytosterols and Triterpenoids** A. Leiberman-Bucharat test B. Salkowaski test	 + +
8	**Test for fixed oils and fats** Oily spot test	 -

CONCLUSION

The various results shows that *Cymbopogon citartus* leaves have antioxidant activity due to presence of terpenoids, phenolic compounds, flavonoids and steroids. In DPPH,ABTS, NO, and SO assay the methanol extract of leaves shows various antioxidant activity because the IC_{50} value of methanol extract is high when compared to ascorbic acid. We can say that that the plant might useful for free radical disorder like, aging, carcinoma etc. Hence further studies are required for plant extracts for identification of phytoconstituents which is responsible for pharmacological activity.

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