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Phytochemical, screening, characterization using HPLC and FTIR Techniques and analysis of Antioxidant activity of Lemon Grass (Cymbopogon schoenanthus)

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Abstract

Cymbopogon schoenanthus commonly called lemon grass is claimed to possess diverse medicinal value among different cultures. The present study determined the phytochemicals and evaluated the antioxidant potential of *Cymbopogon schoenanthus* leaves. The phytochemical and proximate analysis of the powdered leaves were carried out using standard methods. The total phenolic, flavonoid contents and antioxidant activity were assessed using the Folin-Ciocalteu, aluminum chloride colorimetric methods and ascorbic acid. The phytochemical analysis revealed the presence of carbohydrates, reducing sugars, saponins, flavonoids and other phenolics compounds. The procedure involves a stationary phase using a 10:10 methanol: water pH 5 mobile phase containing 0.1%H3PO4 water solution (36:64) UV detector (set at 365 nm). Antioxidant activity of ethanolic extract of *Cymbopogon schoenanthus* is found to be very high. This can be due to the high flavonoid and high phenolic content present in ethanolic extract of *Cymbopogon* schoenanthus. These results were also analyzed by HPLC and FTIR which are very sensitive method and the same results were confirmed.

Keywords: - *Cymbopogon schoenanthus*, Flavonoids, Phenol, HPLC, FTIR

1. INTRODUCTION

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc.

The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Tonthubthimthong *et al*., 2001).

Bioactive compounds of plants are produced as secondary metabolites (Bernhoft *et al*., 2010). Every living body, from one cell bacterium to million cell plants, processes diverse chemical compounds for their survival and subsistence .The presence of secondary metabolites in plants is characterized by their ability to provide defense against biotic and abiotic stress (Ballhorn *et al*., 2009).The mechanism of defense varies from plant to plant, their environmental conditions and climatic variations.

The genus *Cymbopogon* is widely distributed in the tropical and subtropical regions of Africa, Asia and America. Comprised of 144 species, this genus is famous for its high content of essential oils which have been used for cosmetics, pharmaceuticals, and perfumery applications (Khanuja *et al*., 2005). Two main species, *C. Flexuosus* and *C. citratus* (lemongrass) are commercially cultivated in the Democratic Republic of Congo (DRC), Madagascar, and the Comoros Island. The commercial value of some *Cymbopogon* species is further enhanced by their ability to grow in moderate and extremely harsh climatic conditions (Padalia *et al*., 2011).

1.1 Species information

Scientific name: *Cymbopogon schoenanthus*

Common name: Lemon grass, citronella

Conservation status: Least Concern (LC) according to IUCN Red List criteria.

Habitat: Tropical grassland.

Key Uses: Food and drink, medicine, perfumery, ornamental, insect-repellent

Known hazards: Lemon grass oil can cause contact dermatitis.

Figure 1.1 ***Cymbopogon schoenanthus (lemon grass)***

Species	Region	Common Name	Parts	Medicinal Uses	Refrences
C. nardus (L.) **Rendle**	India	Citronella oil	Leaves	Insect repellent and as perfumes	(Noor *et al*., **2012**)
C. parkeri **(Stapf)**	Pakistan	Lemon grass	Aerial	Antiseptic and stomachic treatment	(Bagheri *et al*., **2007)**
C. Excavatus **(Hoscht)**	South Africa	Bread-leavened Turpentine grass	Sheaths	Used as insecticides	(Govere *et al.*, **2000**)
C.oliveri **(Boss)**	Pakistan	Pputar	Aerial	Pyretic, vomit, diuretic, rheumatism, and as anti-malaria condiment.	(Mahboubi *et al*., **2012**) (Abbas *et al*., **2003**)
C. validus **(Stapf)**	Eastern and Southern Africa	African bluegrass	Essential oils	skin toner, anti-ageing in men, fumigant and for rodent control	(Kepe *et al*., **2004**)
C. Winterianus **(Jowitt)**	Brazil	Java grass	Fleshy leaves	Treatment of epilepsy and anxiety	(Leite *et al*., **2011**)
C. Marginatus **(Steud.)**	South Africa	Lemon-Scented grass	Root	They are used as moth repellent	(Secoy *et al*.,**1983)** .
C. Giganteus **(Hochst.) Chio-v.**	Cameroon	Tsauri gras-s	Decoctions of leaves and flowers	Cough and arterial hypertension	(Jirovetz *et al*., **2007**)
C. Ambiguous **(Hack.) A. Camus.**	Australi-a	Native lemon grass	Leaves and stems	Headache remedychest infections, muscle cramp and scabies	(Grice *et al*.,**2011**) (Dayalan et al.,**2000**)

C. Procerus **(R.Br.)Domin**	Australi-a	Scent grass	Leaves and stems	Leaves and stem are pounded and used as medicinal body wash; used for headache	(smith *et al.*,**1991**)
C .flexeosus **(Nees ex Steud.)Wats.**	India	Lemon grass	Leaves	Cosmetics,antiseptic and for treatment of fever	(Desai *et al.*,**2012**)
C. pendulus **(Nees ex Steud.)Wats**	India	Jammu Lemongrass	Leaves	Antiseptic and for perfumery	Jayasinha *et al.*,**1999**)
C. Schoenanthus **(L.) Sprens.**	Saudi Arabia	Ethkher	Leaves	Antidiarrheal, to treat fever, treatment of jaundice and tonic	Al – ghamdi *et al.*,**2007**)
C. Obtectus **(S.T.Blake)**	Central Australia	Silky-heads	Mixture	Cold and flu,headaches,fever and sore throat	(Dayalan *et al.*,**2000**)
C. Refractus **(R.Brown) A.Camus.**	Australia	Barbed wire grass	Leaves	Feed for animals	(Beefalkt.*et al.*,**2015**
C. Densiflorus **(Steud.) Stapf**	Congo	Lemongrass	Leaves and rhizomes	Employed against asthama, epilepsy, abdominal cramps and pains and also for interpreting dreams by which doctors	(Takaisi-kikuni *et al.*,**1996**) (De-Smet *et al.*,**1996**)
C. Jwarancusa **(Jones) schult**	Egypt	The Limon	The whole plant	Condiment and for medicinal purpose	(El-bakry *et al.*,**2012***)*

Table 1.1: Several Cymbopogon species, common name, regions, plant part used and the uses

Traditional uses of *Cymbopogon* genus in various countries shows huge applicability as a common tea, medicinal supplement, insect repellant, insecticide, in flu control, and as anti-inflammatory and analgesic. Table 1.1 shows the common names of some species and their significance. *C. Citratus* is one of the most widely distributed of the genus which is used in every part of the world. Its uses in Nigeria include cures for upset stomach, malaria therapy, insect repellent and as an antioxidant (tea) (Aibinu *et al*., 2007). *C. citratus*

and *C. flexuosus* are the usual species in Eastern and Western India have been used locally in cosmetics, insecticides, and for the treatment of digestive disorders and fevers (Jeong *et al*., 2009; Desai *et al*., 2012).

2. MATERIALS AND METHODS

2.1 Collection of plant material

Fresh plants of *Cymbopogon schoenanthus* were gathered from Botanical garden of Mata gujri college, Fatehgarh sahib. Then, plants were washed with distilled water and then dried. After this period, leaves and stems of the plant have been grinded and transformed to powder by a grinder. The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

2.2 Preparation of extract

1 g of powdered stems and leaves of *Cymbopogon sps.* Was added into a 100 ml and 50 ml solvent (methanol and water). Ultrasound Assisted Extraction (UAE) was performed at 400 W, at 60°C for 10, 20, 25, 40 and 60 min. Then this was centrifuged to separate the liquid extract from the pellet.

2.3 Qualitative phytochemical analysis

Preliminary qualitative phytochemical screening was carried out with the following methods:

2.3.1 Detection of carbohydrates

- **Molisch's Test-** For the detection of carbohydrates filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of Carbohydrates.

2.3.2. Test for Saponins

Added 0.5 ml of filtrate to 5 ml of distilled water and shake well. If foam produced persists for ten minutes it indicates the presence of saponins.

2.3.3. Test for Phenols

To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color indicated the presence of phenols.

2.3.4 Test for flavonoids

3 ml of the filtrate was mixed with 4 ml of 1% aluminum chloride in methanol in a test tube and the color was observed. Formation of yellow color indicated the presence of flavanols, flavones and chalcones.

2.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF STANDARD QUERCETIN AND PLANT EXTRACT (Song *et al.*, 2009).

Materials

Following materials were needed to carry out the analysis. Apparatus used: HPLC apparatus, Syringe filters of pore size 0.45µm.

Reagents used:

Standard solution of quercetin: A stock solution of quercetin (100ppm) was prepared by dissolving 10mg quercetin in 10 ml of methanol and then from the stock solution 100ppm of standard solution was loaded in the HPLC column. The wavelength for maximum absorption of quercetin is 365 nm (Chen et al., 2012) and the flow rate was maintained at 0.5ml/min.

HPLC Conditions

The following conditions were used

HPLC Column : 5µm, C_{18} column with dimensions 250*2.0 mm

Column temperature : 25°C

Mobile phase : Acetonitrile: 0.1%H3PO4 water solution (36:64),(v/v)

Flow rate : 0.5ml /min

Injection volume : 20 µl

Detection wavelength : 365 nm

Running time : 20 min

Calculation:

Amount of quercetin was calculated

Quercetin (mg/g) = concentration of standard * Peak area of sample/Peak area of standard

2.5 FTIR Spectroscopic analysis:

The extracts were examined under visible and UV light for proximate analysis. For FTIR spectrophotometer analysis, the sample is diluted to 1:10 with the same solvent. Then the FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

2.6 Determination of total phenolic content

The amount of total phenolic content in extract was determined using the folin - Coicalteu assay. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE) (Lim *et*

al., 2006). Concentration of 0.01, 0.02, 0.03, 0.05 ppm of gallic acid were prepared in methanol. The blue produced has a maximum absorption in the region of 760nm and proportional to the total quantity of phenolic compounds originally present. All determination was performed in triplicate.

2.7 Determination of total flavonoid content

Aluminum chloride method was used for flavonoid determination (Quideau *et al*., 2011). In this method quercetin was used as standard and flavonoid contents were measured as quercetin equivalence. For this purpose, the standard curve of quercetin was prepared. 1 ml of standard or extract solution (20, 40, 60, 80, 100 ppm) was taken into 10 ml volumetric flask, containing 4 ml of distilled water. 0.3 ml of 5% $NaNO_2$ added to the flask. After 5 min, 0.3ml 10% $AlCl_3$ was added to the mixture. At the 6^{th} min, 2ml of 1M NaOH was added and volume was made up to 10 ml with distilled water. The absorbance was noted at 510nm using UV-Visible spectrophotometer.

2.8 Antioxidant Activity

The antioxidant assay was estimated by ferric reducing antioxidant power method. To the 2.5 ml of extract, 1ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1 % potassium ferricyanide was added. The reaction mixture was incubated in water bath at 50°C for 20 minutes. Afterward, reaction mixture was rapidly cooled and 2.5 ml of 10 % trichloroacetic acid was added to stop the reaction and was centrifuged for 10 minutes. 2.5 ml of aliquots was pipetted out and 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride solution was added. The color changes to green. The mixture was allowed to stand for 10 minutes and absorbance was measured at 593 nm spectrophotometrically UV-Visible spectrophotometer. The blank was performed using reagent blank and solvent. Ascorbic acid was used as standard. The extracts were performed in triplicate (Pourmorad F *et al*., 2006).

3. RESULTS AND DISCUSSIONS

Biologically active compounds sometimes occur in low concentration in plants. Associate in nursing extraction technique is that which ready to get extracts with high yield and with minimal changes to the functional properties of the extract required. Many studies have reported variation within the biological activities of extracts prepared by different extraction techniques and solvents. Therefore, it is necessary to select the suitable extraction methodology as well as solvent supported on sample matrix properties, chemical properties of the analytes, matrix-analyte interaction, potency and desired products.

3.1 Yield of extract

Extract yield of *C. schoenanthus* prepared by ultrasonication technique using water and ethanol as solvent is 30.97% w/v and 41.11% w/v respectively. The percentage yield of extract was calculated by using the formula

$$\text{Percentage yield of extract} = \frac{W_1 - W_2}{W_0}$$

Where, W_1 =weight of the container

W_2 =weight of the extract and the container

W_0 =weight of initial dried sample

From the present study we can say that as percentage yield is maximum when we have used plant product as solvent, therefore it is the higher solvent than the water. Moreover, it is a polar solvent having polarity index of 5.2 and its solubility is more as compared to water. Also, ethanol has low boiling point of about 78.37°C. So, extraction and concentration of bioactive compound is easy by using ethanol as solvent.

3.2 Qualitative phytochemical analysis

Importance of phytochemical as candidate for drug development is known (Gurumurthy *et al*., 2008). Phytochemicals can as well act as a source of precursor for synthetic drug. *Cymbopogon schoenanthus* gained special attention of Phyto chemists due to their traditionals medicinal uses.

Figure 3.1: Showing result of Phytochemical analysis

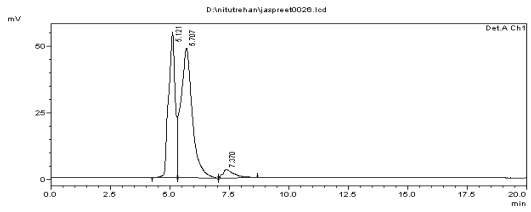
Table 3.1: Presence and absence of bioactive components

S. No.	Bioactive Components	Presence /Absence
1.	Carbohydrates	+ve
2.	Saponins	+ve
3.	Phenolics	+ve
4.	Flavonoids	+ve

Screening study discovered presence of phenolics, flavonoids, carbohydrates, saponins in the extract of *Cymbopogon schoenanthus* as shown in Table 3.1.

3.3 Extraction of bioactive compounds by Ultrasound Assisted method followed by HPLC

After ultrasound assisted extraction the extract was analyzed using HPLC with respect to standard quercetin. The HPLC chromatogram of standard quercetin of 100 ppm showed a peak area of 1638047 and retention time at 5.707 respectively as shown in figure 3.2.



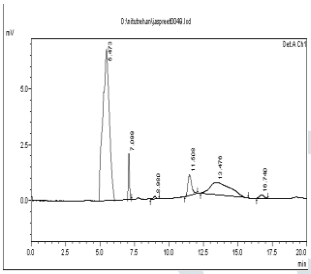
Detector A Ch1 365nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.121	1045150	54804	37.527	51.281
2	5.707	1638047	48660	58.815	45.699
3	7.370	101883	3215	3.658	3.020
Total		2785080	106479	100.000	100.000

Figure 3.2: HPLC Chromatogram for quercetin 100ppm

3.3.1 High Performance Liquid Chromatography of plant extract

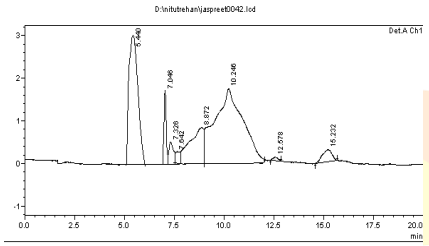
Chromatograms of Lemon grass with methanol and water extracts are shown in figure 3.3 and 3.4. When chromatogram of extracts was compared with standard chromatogram both showed the presence Quercetin.



Detector A Ch1 365nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.473	267066	7242	71.969	61.395
2	7.099	23182	2811	6.247	23.830
3	8.980	1818	121	0.490	1.029
4	11.508	17536	927	4.726	7.863
5	13.476	58258	546	15.699	4.628
6	16.740	3227	148	0.870	1.255
Total		371085	11795	100.000	100.000

Figure 3.3: HPLC Chromatogram for methanol extract



Detector A Ch1 365nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.440	129024	3367	24.474	27.068
2	7.046	30892	2675	5.860	21.504
3	7.326	27565	1406	5.229	11.304
4	7.642	17590	1115	3.336	8.964
5	8.872	89668	1438	17.009	11.563
6	10.246	221530	2077	42.021	16.696
7	12.578	1409	76	0.267	0.614
8	15.232	9515	284	1.805	2.287
Total		527192	12439	100.000	100.000

Figure 3.4: HPLC Chromatogram for water extract

Table 3.2: Comparison of water and Methanol extract and the standards using HPLC

SAMPLE/STANDARD	PEAK AREA	QUERCETIN EQUIVALENT (Mg/g)
Quercetin 100ppm	1045150	104515000
Methanol extract	267066	26706600
Water extract	129024	12902400

Also, the comparison of chromatogram demonstrate that flavonoid is present in more concentration in ethanol extract than in water extract as methanol extract i.e. 12902400 mg/g and 26706600 respectively as shown in table 3.2.

3.4 FTIR Analysis

FTIR spectroscopic analysis reveals the presence of different functional groups of the bioactive compounds present in the ethanolic extracts, once the extract passed within the FTIR region, the functional groups were

separated based on its bonding positions, the resulting peaks confirms the presence of wide range of functional groups of bioactive compounds.

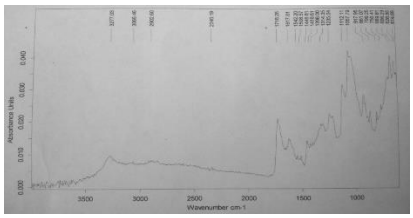


Figure 3.5: FTIR spectrum of Cymbopogon schoenanthus methanolic extract ssband positions.

Table 3.3: FTIR – Band position and their functional groups of *Cymbopogon schoenanthus* methanolic leaf extract

S.No	Peak area	Functional group
1.	3315.40	Hydrogen bonded alcohols
2.	3132.25	Phenols
3.	2797.34	Hydrogen bonded acids
4.	1668.37	Alkanes
5.	1609.84	Alkenes
6.	1560.56	Aromatic ring
7.	1521.19	Aromatic ring
8.	1456.46	Nitro compound
9.	1382.63	Nitro compound
10.	1318.75	Amines
11.	1263.50	Carboxylic acid
12.	1207.13	Alcohols
13.	1169.83	Ethers
14.	1135.11	Esters
15.	1011.93	Alcohols
16.	820.66	Alkanes
17.	795.09	Alkanes

18.	725.15	Alkanes
19.	679.18	Alkanes
20.	643.79	Alkanes

The results of FTIR spectrum of leaf extract of *Cymbopogon schoenanthus* confirmed the presence of phenols and alcohols with a peak at 3132.25 cm^{-1} and 1011.93 cm^{-1} corresponded to hydroxyl and O-H bonding frequency respectively. The peak at 1609.84 cm^{-1} assigned to the C-H stretching which means that some alkene compounds are present. The peak value at 1318.75 cm^{-1} confirms primary amines. The peak value at 1560.56 cm^{-1} and 1521cm^{-1} confirms aromatic rings. The peak value at 3315.40 and 2797.34 confirms hydrogen bonded alcohols, the peak value at 1263.50 cm^{-1} confirms carboxylic acids, and the peak value at 1668.37 cm^{-1}, 820.66 cm^{-1}, 795.09 $cm^{-1,}$ 725.15 $cm^{-1,}$ 679.18 $cm^{-1,}$ 643.79 cm^{-1} confirms alkanes. The peak value at 1169.83 cm^{-1} and 1135.11 cm^{-1} confirms esters and ethers respectively. Also peak at 1456.46 cm^{-1} and 1382.63 cm^{-1} confirms the nitro Compound are given in Figure 3.5 and Table 3.3.

3.5 Total Phenolic content

The amount of total phenol as determined with the folin- ciocalteu reagent. Polyphenols in plant extract with specific redox reagents (folin ciocalteu reagent) to form a blue complex that can be quantified by visible - light spectrophotometry at 760 nm.

Table 3.4 Absorbance of Standard Compound (Gallic Acid)

Concentration (ppm)	Absorbance at 760 nm
20	0.218
40	0.490
60	0.617
80	0.796
100	0.967

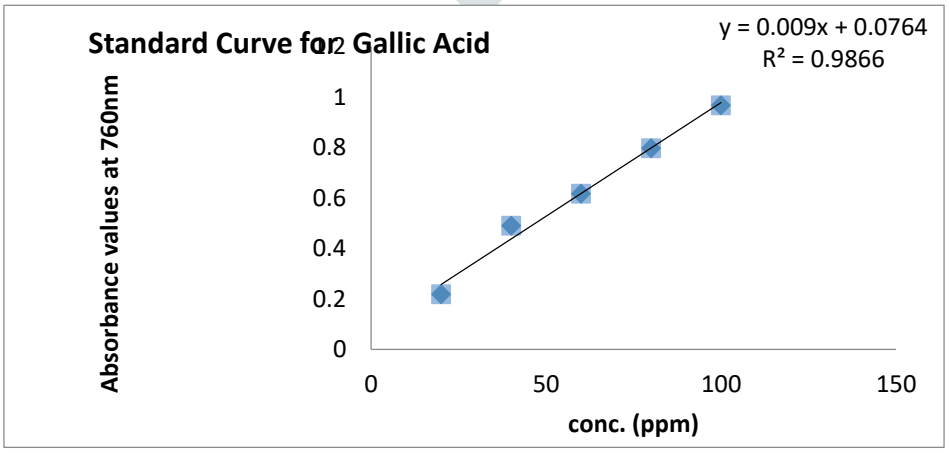


Figure 3.6: Gallic acid standard graph for total phenolic content.

As in Table 3.4, Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using standard curve equation: y = 0.010x, R^2 = 0.970(fig. 3.6). The total phenolic of leaves of *Cymbopogon schoenanthus* found in the ethanol extract and water extract *was* 10.4 mg/g and 8.9 mg/g. This result shows that the phenolic content of methanolic leaf and flower extracts is correlated with the activity of gallic acid and showed that the moderate amount of phenolics are present.

3.6 Total flavonoid content

The total flavone and flavanol contents of the samples were determined using aluminum chloride. The method uses aluminum chloride is based on the formation of a complex between the aluminum ion, Al (III), and the carbonyl a hydroxyl groups of flavones and flavanols that produce a yellow color.

Table 3.5: Absorbance of standard compound (Quercetin)

S. No.	Concentration (ppm)	Absorbance (nm)
1.	10	0.114
2.	20	0.181
3.	30	0.242
4.	40	0.292
5.	50	0.425
6.	60	0.487
7.	70	0.611
8.	80	0.683
9.	90	0.789
10.	100	0.804

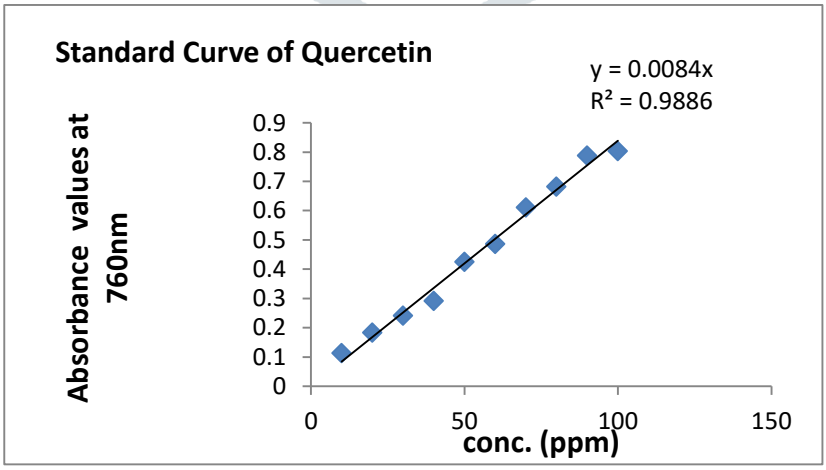


Figure 3.7: Standard curve for the determination of total flavonoid content

Quercetin was used as a standard compound as in Table 3.5 and the total flavonoid were expressed as mg/g quercetin equivalent using the standard curve equation y = 0.008x, R^2 = 0.988(fig.3.7). The total flavonoid

content of *Cymbopogon schoenanthus* found in water and ethanol extract was 1.9 mg/g and 2.0 mg/g equivalents of quercetin respectively. Thus, more flavonoid content was extracted in ethanol than in water.

3.7 Antioxidant activity

The antioxidant assay for aqueous, and methanolic extracts were estimated by ferric reducing antioxidant power method using ascorbic acid as standard (Table 3.6). Reducing power is a measure of ability of the extract to reduce Fe^{+3} to Fe^{+2}.Substance which have reduction potential react with potassium ferricyanide (Fe^{+3}) to form potassium ferrocyanide (Fe^{+2}) and then react with ferric chloride to form ferric-ferrous complex.

Table 3.6: Absorbance of standard compound (Ascorbic Acid)

Concentration (ppm)	Absorbance
10	0.164
20	0.272
30	0.457
40	0.674
50	0.892

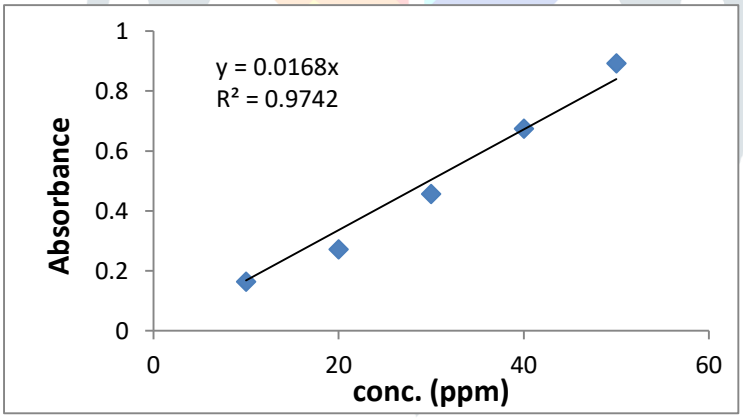


Figure 3.8: Standard curve for Ascorbic acid

The ascorbic acid solution of concentration (10-60 ppm) conformed to Beer's Law at 593 nm with a regression co-efficient (R^2) = 0.974. The plot has a slope (m) = 0.0016 and intercept = 0 (Fig. 3.8). The total antioxidant content found in water extract was 17.625 mg/g and ethanolic extract was 17.812 mg/g. Clearly more antioxidant content extracted by ethanol than water.

4. *CONCLUSION*

Antioxidant activity of ethanolic extract of *Cymbopogon schoenanthus* is found to be very high as compared to the other medicinal plants. This can be due to the high flavonoid and high phenolic content present in ethanolic extract of *Cymbopogon schoenanthus.*

These results were also analyzed by HPLC and FTIR which are very sensitive method and the same results were confirmed.

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