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EVALUATION OF ANTIOXIDANTS FROM *CYMBOPOGON CITRATUS.*

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ABSTRACT

Lemon grass (Cymbopogon citratus) is a traditionally used in food ingredient because of its refreshing aroma. The objectives of study were to determined antioxidant contents of this plant. The plant was collected from Botanical garden Institute of Science, Nagpur. Identification of Plant was done with the help of the standard floras. Estimation of antioxidants like flavanols, ortho-dihydric phenol, Bond phenol, Leuco-anthocyanin, anthocyanin ands lycopene were carried out by using spectrophotometer.

KEY WORDS: *Cymbopogon citratus, Antioxidants, Spectrophotometer.*

INTRODUCTION

The most essential and safest drugs since several are medicinal plants and they play remarkable role in public and primary health care. Medicinal herbs are therapeutic agents crucial in the primary health care system in maintaining exceptional well-being and health condition. Plant synthesizes number of chemical compounds such as secondary metabolites which have enormous medicinal properties. In recent times, approximately 10,000 medicinal herbs have been recorded and about 4500 medicinal herbs have been examined for the bioactive components and pharmacological assays (JAYASINHA., 1999). One of the medicinal plants with innumerable pharmacological activities is *Cymbopogon citratus. Cymbopogon citratus* (DC) Stapf. It is an aromatic plant belongs to the Poaceae (Graminaea) family, it is a native herb of India and also cultivated in other tropical and subtropical countries. It has been used to extract essential oils. *C. citratus* have tremendous record in the folk and Ayurvedic medicine. Scientific investigations have reported the antifungal, antibacterial, anti-protozoal, anti-inflammatory, anti-carcinogenic, antioxidant, anti-rheumatic and cardio-protective activities of *C. citrates* (Ajayiet al., 2016; Ntonga et al., 2014).Phenolics rich plant are a good alternative to synthetic antioxidants. The most of the natural antioxidants are phenolic compounds and the most important are the tocopherols, flavonoids and phenolic acids antioxidant (Kumar et al., 2015). *C. citrates* has a citrus flavor and widely used as an herb in Asian cuisine, can be used fresh, dried or powdered. It is commonly used in soups, curries and teas, also is suitable for fish, beef, poultry and seafood. In many countries it has been used to treat feverish conditions and act as a relaxant and sleeping

aid (Figueirinha et al., 2008). it acts as an antidepressant agent and also helps with emotional states (Gupta,1995).

MATERIALS AND METHODS

Collection and Preparation of Plant Materials: Fresh *Cymbopogon citratus* leaves were collected from the garden of institute of science, Maharajabagh Nagpur. The plant identification was carried out with the help of standard floras.

Estimation of anthocyanin:

Plant extract was prepared by using 1 gm of washed leaves was grind in mortal and pestle with alcohol, centrifuged it and collected the supernatant and prepared the volume up to 10ml with alcohol. 1ml of alcoholic extract was pipette out into the test tube and added 3ml of HCL methanol, 1ml of anthocyanin reagent was added into sample. Blank was prepared in the same manner by adding 1ml of methanol- HCL instead of anthocyanin reagent. Incubate the sample for 15 min in the dark, measure the absorbance at 525 nm against the blank. The standard formula of cyanin hydrochloride was used for calculation.

10µg of cyanin hydrochloride/ml in methanol-HCl = Absorbance of 0.405 in a 1.0 cell at A_{525}

Estimation of Leuco- anthocyanin:

Plant extract was prepared by using 1 gm of washed leaves was grind in mortal and pestle with ethanol, filtered with the help of muslin cloth and collected the filtrate and centrifuged it and collected the supernatant and prepared the volume up to 10ml with ethanol. 1ml of extract was pipette out in test tube. Reduced the volume to 0.5 ml on a hot water bath than 0.5 ml of water and 10 ml of leuco- anthocyanin reagent, mixed thoroughly, heated the tubes in water bath at 97±1^{0}C for 3 min. then covered the tubes with glass stoppers and continue heating for 40 min. blank was prepared by same method but without heating. Absorbance was measured at 550nm.Leuco- anthocyanin was calculated as follow.

10µg of cyanin hydrochloride/ml in ethanol-HCl = Absorbance of 0.405 in a 1.0 cell at A_{525}

Estimation of Lycopene:

1 gm of washed leaves was grind in mortal and pestle with acetone until the residue became colorless. Transfer to the separating funnel containing about 20ml petroleum ether and mix gently. Add 20ml of 5% sodium sulphate solution. Add 20ml more petroleum ether to the separating funnel for clear separation of two layers. Most of the color will be noticed in the upper petroleum ether layer separate the two phases and re extract the lower aqueous phase with additional 20ml petroleum ether until the aqueous phase is colorless. Pooled the petroleum ether extract and washed with little distilled water. Poured the washed petroleum ether extract containing carotenoids into a brown bottle containing about 10 gm anhydrous sodium sulphates. Kept it aside for 30min or longer. Decant the petroleum ether extract into a 100 ml volumetric flask. Wash sodium sulphate slurry with petroleum ether until it became colourless and transferred into volumetric flask. Make up the volume and absorbance was measured at 503nm . lycopene was calculated by using following formula:

Absorbance(1 unit)= 3.1206µg lycopene/ml

$$mg \text{ lycopene in 100 g sample} = \frac{3.1206 \times \text{Absorbance}}{Weigt\ of\ sample\ (g)}$$

Estimation of Flavanols:

1 gm of washed leaves was grind in mortal and pestle with ethanol, and centrifugation at 10,000rpm for 20 min and supernatant collected, evaporated to dryness. dissolved the residue and made up the volume up to 5 ml.1ml extract pipette out into 25 ml conical flask .added 1 ml of water and 4 ml of vanillin reagent

from burette within (10- 15 sec) to flask A and 4ml of H_2SO_4 to flask B. Blank prepared in flask C containing 4ml of vanillin reagent and added 2ml of water. Shake the flask A and B in water bath at temperature below 35^0C. kept the flask at room temperature for 15 min. measured the absorbance of flask A,B and C at 500nm against 47% H_2SO_4 (Flask D). Subtract the absorbance of the Flask B and C from that of A(alternatively read the absorbance of flask A+D against B+C). The total phenolic content was calculated from the calibration curve.

Estimation of Bound Phenol:

1 gm of washed leaves was grind in mortal and pestle with 5ml SD S solution., and centrifugation at 2,000rpm for 5 min. wash the residue successively once with 5ml SDS solution, twice with 5ml of water, twice with 5ml of ethanol and twice with 10 ml of diethyl ether (after each washing centrifuged and discarded the supernatant). Allow the residue to dry and suspended in 3ml of 0.5M NaOH. Kept overnight at room temperature, centrifuge and save the supernatant. Diluted 1:10 with 0.5 M NaOH and absorbance measured at 290nm. Standard catechol prepared by dissolving10mg catechol in10ml Distilled water. The total phenolic content was calculated from the calibration curve.

Estimation of Ortho-dihydric phenol:

1 gm of washed leaves was grind in mortal and pestle in 10 time volume of 80%ethanol. Centrifuge at 10,000 rpm for 20 min, saved the supernatant. Evaporate the supernatant to dryness. Dissolve the residue in 5ml .pipette out different aliquots (0.2-1.0 ml) into test tubes. Make up the volume in each tube to 1 ml with water. Add 1ml of 0.05N HCl, 1 ml of Arnows reagent, 10ml of water and 2ml of 1N NaOH. Mix thoroughly. Absorbance was read at 515nm. Standard catechol prepared by dissolving10mg catechol in10ml Distilled water. The total phenolic content was calculated from the calibration curve.

RESULT AND DISCUSSION

Sr.no	Secondary Metabolites	Wt of sample	Volume of extract	Wavelength	Absorbance	Amount of secondary metabolites in 1 g
1	Anthocyanin	1g	10ml	A_{550}	0.388	9.580 µg
2	Leuco-anthocyanin	1g	10ml	A_{525}	0.165	4.074 µg
3	Lycopene	1g	5ml	A_{500}	0.203	63.34 µg
4	Flavonols	1g	60ml	A_{503}	0.188	2500 µg
5	Bound phenol	1g	10ml	A_{290}	0.019	140 µg
6	Ortho-dihydric phenol	1g	5ml	A_{515}	0.057	2100 µg

In the present investigation of secondary metabolites were quantitatively analyzed using *Cymbopogon citratus* leaves. Results are shown in Table. The different phytochemicals have been found to possess a wide range of medicinal properties. The table shows that the flavanols and ortho-dihydric phenol was present in highest amount that is 2500µg and 2100 µg.

Flavonoids is one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Manisha *et al., 2014)*. Phenolic com-pounds have redox properties, which allow them to act as antioxidants (Soobrattee *et al.,* 2005). Phenolic com-pounds with a C3side chain at a lower level of oxidation and containing no oxygen have often been reported to be antimicrobials (Cowan., 1999).

The leuco-anthocyanin was present in lowest amount i.e., 4.074 µg. bound phenol, Anthocyanin and lycopene content was 140 µg, 9.580 µg. and 63.34 µg respectively. Lycopene is among the carotenoids

with the highest power of quenching singlet oxygen (Di Mascio et al., 1989). Anthocyanins have a higher antioxidant activity than other flavonoids, due to their positively charged oxygen atom (Kong et al., 2003).

CONCLUSION

The study of secondary metabolites of *Cymbopogon citratus* revealed that the presence of medicinally important constituents in the plant. On the basis of the results obtained in the present study, it was concluded that the leaf extract of *Cymbopogon citratus* which contains enough amount of phenolic, flavonols, anthocyanin and lycopene compounds exhibit antioxidant activity

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