Journal of Applied and Natural Science
12(1): 25 - 29 (2020)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Chemical composition and antioxidant activity of essential oil of *Cymbopogon flexuosus*

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Abstract
Cymbopogon species from the Poaceae family are widely distributed in the Himalayan region of India and commonly used as flavors, fragrances, cosmetics, and pharmaceuticals. It is known to contain compound citral, which give the lemon scent to many of the plants of the cymbopogon genera. The essential oil of *Cymbopogon flexuosus* has high polyphenolic content which is responsible for antioxidant properties. Beside citral is also used for the synthesis of vitamin B and Ionones. The bioactive potential of Lemongrass and constituent are rapidly increasing which is reflected from growing number of reports being published. The present study was to know the chemical composition and in vitro antioxidant activity of essential oil of *C. flexuosus* from Uttarakhand. The essential oils of Cymbopogon collected in the region of Uttarakhand were obtained by hydrodistillation of the leaves and analyzed for chemical composition by GC/MS. The antioxidant activity of essential oils at different concentrations was determined against DPPH radical activity and vitamin C as the standard antioxidant compound. The IC_{50} value and percentage of DPPH inhibition were recorded. Twenty-five compounds were identified in essential oil extracted from leaves representing 93.15% of the oil composition. The yield of essential oil of Cymbopogon was 0.6 $\pm$ 0.1 %and the major compound in the essential oil was citral (a racemic mixture of two isoforms geranial and nearl) followed by heptenone(1.98%) , linalool(1.65%), geraniol (1.47%), β-caryophyllene (1.14%) , limonene (0.92%), nearl acetate (0.82%), citronellal(0.44 %) and citronellol (0.22%). Radical scavenging capacity (Inhibition, %) of the *C. flexuosus* essential oil was high (78.19$\pm$1.11) at the concentration level of 150 µg/ml and IC_{50} value of the essential oil was 43.67µg/ml. The data of this study encourages to consider the essential oil of *C. flexuosus* as a source of bioactive compounds which may add great industrial value to this crop.

Keywords: *Cymbopogon flexuosus*, Bioactivities, Antioxidant activity, Citral, Monoterpene

Article Info
https://doi.org/10.31018/jans.v12i1.2207
Received: December 25, 2019
Revised: February 23, 2020
Accepted: March 3, 2020

How to Cite
Bhatnagar, A. *et al.* (2020). Chemical composition and antioxidant activity of essential oil of *Cymbopogon flexuosus*. *Journal of Applied and Natural Science*, 12(1): 25 - 29 https://doi.org/10.31018/jans.v12i1.2207

INTRODUCTION

The genus Cymbopogon family Poaceae is known for their essential oils of immense commercial significance in flavors, fragrances, perfumery and pharmaceuticals (Ganjewala,2009). Cymbopogon contain about 140 species worldwide, out of which 45 species are occur in India. The members of the genus Cymbopogon occur abundantly from mountains and grassland to arid zones in tropics and sub tropic regions of Asia, Africa, and America. (Bor, 1960; Soenarko, 1997). *Cymbopogon* species show large variation in morphological features and composition of essential oil at inter and intra specific level over the years. The prevalent economic species viz., *C. flexuosus, C. citrates, C. winterianus, C. martini var. motia and sofia, C. nardus var. nardus, , C. pendulus, C. warancusa, C. khasianus.* The essential oil, such as palmarosa oil, lemongrass oil, citronella oil, ginger grass or rusa oil extracts from different species of Cymbopogon, are of commercial interest (Gupta and Jain, 1978). These species differ from each other in oil content, oil composition *and* quality, however morphological difference at the intra and interspecies level are often blurred (Sangwan et. al.,2001). Lemongrass (*C.flexuosus*) is cultivated in large scale in Brazil, Mexico, Dominica, Haiti, Indonesia, and China. In India, it is mainly cultivated in Kerala, Assam, Maharashtra, and Uttar Pradesh.

The three *Cymbopogon* species such as Java citronella (*Cymbopogon winterianus),* lemongrass (*Cymbopogon flexuosus* and *Cymbopogon pendulus*) and palmarosa (*Cymbopogon martinii var. motia*) are mainly cultivated for their essential oils of commercial importance (Gupta and Daniel,1982). The essential oils from Cymbopogon species contain a large variety of terpenoids, some of which are important perfume materials like geraniol and its ester, citronellol and citron-

ellal. Another terpenoid such as citral is used in ionone synthesis and vitamin A . (Khanuja *et al*., 2005).

Cymbopogon flexuosus is a perennial, tall, fast growing grass of 1.5m long having lemon like smell. It has distinct, dark green foliage and also produces seed. Lemongrass mainly prefers tropical and subtropical climate, it grows well at a temperature between of 10 ^{O}C to 33^{O}C and needs enough sunshine for the development of oil in the plant. The grass is sensitive to cold weather and cannot withstand frost. The oil is extracted from fresh plant material mainly stalks and leaves of plant, by hydrodistillation method (Lawrence, 1988).

In recent years medicinal and pharmacological significance of lemongrass EO and its major constituent citral has been rapidly increased. A number of studies have revealed many useful bioactivities such as antimicrobial, allelopathic, anthelmintic, anti-inflammatory, anticancer, antioxidant, insect and mosquito repellent of lemongrass extract, oil, citral, and citral derived compounds. The goal of this study was to investigate the chemical composition and antioxidant activity of essential oil of *C. flexuosus* collected in the northern plain of Uttarakhand.

MATERIALS AND METHODS

Plant material: The aerial parts of *C. flexuosus* were collected from cultivated crops at experimental field of CAP Research Center, Dehradun in September 2016 and duly identified by Botany Department, Forest Research Institute, Dehradun. The location of experimental site is at an altitude of 30 N, longitude of 78.03 E and it experiences climate with hot and dry summer and chilled winter. After identification, the all sample were kept in the herbarium of D.B.S. (P.G.) College Dehradun, Uttarakhand (India).

Extraction of essential oil: The fresh leaves of 100 gm was used to extract the oil by hydrodistillation method in 3 replicates by using Clevenger apparatus. The evaporated essential oil is collected as oil drop after condensation into a closed tube attached to Clevenger apparatus. Aqueous layer was separated from the essential oil with the help of separating funnel. The essential oil was dried over anhydrous Na_2SO_4 and stored in sealed vials under refrigeration prior to analysis. Oil content was calculated in terms of oil percentage as the mean of 3 samples. The fresh weight of the material was used to calculated the oil yield on the basis of (v/w)(Kulkarni, et.al., 2003)

GC-MS analysis: It is performed using an Perkin Elmer Clarus 500 Gas Chromatograph interfaced with Perkin Elmer Clarus 500 mass spectrometer fitted with an RTX-5 capillary columns (60 m × 0.32 mm i.d., film thickness 0.25 µm). The range of oven column temperature is 60°C–210°C, programmed at flow rate is 3°C/min, with 2 min is hold time , Helium using as carrier gas at 10 psi constant pressure, 1: 50 is a split ratio, an injection volume was 0.02 µL neat, injector, transfer line, source temperatures were 210°C; ionization energy 70 eV; mass scan range 40-450 m/z. The Identification of constituents was done on the basis of retention time. The Retention Index [RI, calculated with reference to n-alkanes and its homologous series (C_9-C_{26}, Polyscience Corp., Niles IL) under identical experimental condition], co-injection with standards (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature database (Adams,1995; Davies, 1990). The relative quantities of individual components were calculated based on peak areas without using correction factors.

Antioxidant activity

Radical scavenging activity by DPPH: DPPH (2,2 –diphenyl -1-picryl hydrazyl) radical scavenging activity was measured with the Akter (2010) method. For each determination, stock solution (1mg/ml) was diluted to a dilution series (50 µg - 1000µg /ml)with 60% (V/V) ethanol. A methanolic solution of DPPH (5ml, 0.06 mM) was mixed in an aliquot of each dilution (0.5 ml). The mixture was vigorously shaken and kept in the incubator for 30 Min at 37^{0}C. A control was run, containing 60% (v/v) ethanol (0.5 ml) and methanolic solution of DPPH (5 ml, 0.06 mM). The absorbance of methanol as blank was measured at 517 nm. The percentage of DPPH scavenging was calculated as follows:

DPPH radical scavenging activity (%) = {(Abs control-Abs sample)/Abs control} x 100 ……Eq.1

A graph was plotted between scavenging percentage of DPPH and different concentration of samples. The concentration of the sample necessary to decrease the DPPH concentration by 50% was obtained by interpolation from linear regression analysis and denoted IC_{50} value (µg/ml). All determinations were taken in triplicate. Data were expressed as means $\pm$SD. Ascorbic acid was used as reference compound.

β -Carotene bleaching assay: To know lipid peroxidation activity of the samples, β-carotene bleaching assay was performed by Geckil et al**.** method. Crystalline β –carotene (0.02 mg) was dissolved in chloroform (10 ml) and then added linoleic acid (20mg) and 200 mg of Tween 80 reagent. After removal of Chloroform by rotary evaporator under vacuum at 40^{o}C for 5 min, 50 ml of distilled water was added with vigorous stirring to form an emulsion. Add 0.1 ml of essential oil extract (1ug/ml) in 5 ml of this emulsion. BHT was taken as standard. The test tube containing standard and sample were kept in water bath in Incuba-

tor at 50°C and record the absorbance at an interval of 20 min till 2 hrs.

RESULTS AND DISCUSSION

The essential oil of aerial parts of *C. flexuosus* viz. , Krishna cultivated Dehradun, a Tarai area of Uttarakhand, isolated by hydrodistillation indicated that the essential oil yield was0.6 ± 0.1 %on dry weight. Table -1 showed that twenty-five volatile constituents, representing 93.58 % of the total composition in the essential oil. The most abundant components found in the essential oil were citral { a isomeric mixture of geranial (40.29%) and neral (34.29%)}, followed by Geraniol (1.47%), Linalool (1.65%), Limonene (0.92%),β-Caryophyllene (1.14%), Neryl acetate (0.82%) ,camphene (2.01%), borneol (1.01%), and Caryophyllene oxide (1.10%) as a major constituents. The minor constituents were citronellal (0.44%), α-terpineol (0.35%), β-myrcene (0.42%), Citronellol (0.22%) and γ-cadinene (0.62%) These results agree with those reported by Negrelle and Gomes (2007). Adukwuallen and Phillipa (2012) informed that citral was the main component in *C.flexuosus* and *C.nardus*. The Essential Oil of lemongrass has been investigated for chemical compositions (Sharma *et al*., 1999; Khanuja *et al*., 2005; Ganjewala *et al*., 2009Padalia, *et al*., 2011, Bhatnagar, 2018).

Table 1. Chemical composition of essential oil of *C. flexuosus* harvested in September from Uttarakhand.

S.N.	Retention Index	Name of constituents	Area %
1	942	α –Pinene	0.74
2	1160	β-myrcene	0.42
3	956	Camphene	2.01
4	931	Hept-5-en-2 one	1.98
5	1034	Limonene	0.92
6	1036	β-ocimene	0.05
7	942	α –Pinene	0.63
8	1225.8	Carven	0.14
9	668	Pentyl propyl ketone	1.29
10	1154	Citronellal	0.44
11	1098	Linalool	1.65
12	1109	Borneol	1.01
13	1148	Verbenol	1.07
14	1191	α-terpineol	0.35
15	1210	Mentha	0.44
16	1241	Neral	34.29
17	1257	Geraniol	1.47
18	1270	Geranial	40.29
19	1359	Neryl acetate	0.82
20	1418	β-Caryophyllene	1.14
21	1429	Isoeugenol	0.01
22	1226	Citronellol	0.22
23	1514	γ-Cadinene	0.62
24	1229	Nerol	0.05
25	1584	Caryophyllene oxide	1.10
		Oil content (%)*	93.15

The major component of the essential oil of the herbal plant are mainly comprised with monoterpenes, sesquiterpenes, and their oxygenated derivatives. These compounds are also known to exhibit diverse biological activities. It is described in an article of Mata *et al*., 2007, that geraniol and eugenol are known for their antioxidant activity. Most of the bioactivities of lemongrass oil has been attributed to its one or more major chemical constituents namely citral and geraniol (Gangwala, 2009).

Retention time was determined by GC-MS on a RTX - 5 capillary columns.

Retention Index determined with homologous series of n-alkanes(C_9-C_{26}).

Some of the important bioactivities of citral are antimicrobial, antiviral, anti-inflammatory, allelopathic, antiparasitic and cognitive activities. Other oil constituents, such as limonene and borneol has immune-stimulatory, analgesic and anesthetic properties whereas geraniol, geranyl acetate and bisabolol possess different types of bioactivities such as antimicrobial, antifungal, anti-inflammatory, anticancer and antioxidant (Padalia, *et al.,* 2011; Ganjewala, 2009).

Antioxidant activity of *C. flexuosus*: The free radical scavenging activity of *C. flexuosus* oil was measured by DPPH method is given in Table 2. To evaluate the anti oxidative activities in a relatively short time, the model of scavenging stable DPPH free radicals can be used. The absorbance value decreases as radical is scavenged through a donation of hydrogen by antioxidants to form the stable DPPH-H molecule, due to this color change from purple to yellow. The effect of antioxidants on DPPH radical scavenging activity was thought to be due to their hydrogen donating ability. In aromatic plants, antioxidant activity of essential oil is mainly attributed to the active compounds present in them. This activity can be due to the high amount of main constituents, but also to the presence of other constituents in small quantities or synergy among them. In this study, the antioxidant activity of essential oil was compared with Vitamin C as a reference antioxidant compound, determined by the method of DPPH radical scavenging assay and the results are summarized in Table 2.

It was found that the essential oil *C. flexuous* showed similar antioxidant capacities as compared to vitamin C (Standard antioxidant compound). The results indicated that the radical scavenging activity (%inhibition) of the essential oil from *Cymbopogon flexuous* was highest (78.19±1.11) at the concentration of 150 µg/ml, Table 2. It was noticed that the scavenging activity of the essential oil was increased with increase the oils concentrations. The results clearly indicate that essential oil of lemongrass is effective in scavenging free radical and has the potential to be a powerful antioxidant.

Table 2. Scavenging effect (%) of essential oil of *C. flexuosus* as well as vitamin C on DPPH at different concentrations.

S.N.		Concentration (ug/ml)	% Inhibition of DPPH	IC_{50} (ug/ml)
1.	Essential oil	10	30.01±1.00	43.67
2		20	40.27±0.11	
3		40	56.01±0.89	
4		60	60.11±0.21	
5		100	71.02±1.02	
6.		150	78.19±1.11	
7		10	31.01±0.35	36.32
8	Vitamin C	20	51.11±0.11	
9		40	70.02±0.15	
10		60	80.57±0.79	
11		100	85.21±0.15	
12		150	90.03±1.88	

β-Carotene bleaching activity of C. flexuous: The β-Carotene anti-bleaching activity of sample was measured by monitoring the color intensity of emulsion at 470 nm for every 15 min for 2 hrs. The concentration was taken to be 1µg/ml. The initial concentration was considered to be 100 %. In the first 15 minutes the sample showed 94.56 % effect and 84% of the effect shown by standard. In one hour of incubation, percentage decrease was found to be 72.18%. The decrease of standard percentage is 54.7 % in one hour time. By the end of second hour the percentage decrease was 60.2% and 28.2 % for oil and standard respectively. Reena et. al., 2015; reported that In vitro antioxidant activity of lemongrass essential oil by using DPPH assay is 0.5mg/ml and β -Carotene bleaching is 84.1% bleaching in I^{st} hour and went to 46.8 % by the completion of 2^{nd} hour which is relatively similar in comparison of *C. flexuosus*.

Conclusion

The GC-MS analysis identified twenty-five volatile compounds in the essential oil of *C. flexuosus*. The essential oil composition was mainly constituted of monoterpene fractions with a large proportion of citral and geraniol. Citronellal, β-Caryophyllene, camphene, limonene, neryl acetate, borneol and caryophyllene oxide as minor constituents. The free radial scavenging activity of essential oil of *C.flexuosus* through its ability to quench the synthetic DPPH radical and beta-carotene bleaching method showed that essential oil of C. flexuosus had a good antioxidant capacity compared with Standard antioxidant compound. Thus, *C. flexuosus* can be used as an easily accessible source of natural antioxidants.

ACKNOWLEDGEMENTS

This work was supported by UGC, New Delhi, India under the Minor Research Project Grant. Also, I duly acknowledge the CIMAP-CSIR research center, Pantnagar, Uttarakhand, India for providing necessary facilities and support.

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