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CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF (*CYMBOPOGON CITRATUS)* ESSENTIAL OIL CULTIVATED IN MADINAH MONAWARA, SAUDI ARABIA AND ITS COMPARISON TO THE EGYPTIAN CHEMOTYPE

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ABSTRACT

Volatile compounds of Lemongrass (*Cymbopogon citratus)* cultivated in both Egypt and Madinah, Saudi Arabia, were hydrodistilled and identified using Gas Chromatography-Mass Spectrometry (GC-MS). Fourty-nine components were detected in the Egyptian Lemongrass essential oil, whereas geranial (20.9%), neral (16.2%) and geraniol (8.3%) constitute the major aroma compounds. On the other hand, geranial (37.8%) and neral (33.6%) were the major constituents among 18 compounds identified in Madinah oil. *Cymbopogon citrates* can be used as an easily source of natural antioxidant and antimicrobial, as well as a possible food supplement and as phytochemical. Antioxidant activity and radical scavenging were investigated using DPPH and β-Carotene linoleic bleaching assays. The obtained results revealed a higher antioxidant activity in Egyptian Lemongrass essential oil with inhibition constant 50, IC_{50} 1.0 mg ml^{-1}, in comparison to the Madinah volatile oil IC_{50} 6.9 mg ml^{-1}. This is in agreement with total phenolic content which measured for both oils, and in accordance with differences in chemical constituents between them. *Fusarium melanoform* showed a higher sensitivity against oils in comparison to bacteria types investigated during testing the antimicrobial activity. Egyptian Lemongrass volatile oil has a superior antimicrobial activity than Madinah cultivar essential oil due to its higher phenolic content.

Keywords : *Cymbopogon citrates;* essential oil composition; GC-MS; geranial; neral; DPPH ; Total phenolic content

INTRODUCTION

Lemongrass is an aromatic plant belonging to the *Gramineae* family, with a commercial importance for the industries of flavors, fragrances, cosmetics, perfumes, soaps, detergent and pharmaceuticals (Akhila, 2010). There is a great interest on the essential oils of *Cymbopogan Citratus* (commonly named: Lemongrass), due to using in food technology as well as in traditional medicine (Mirghani et al., 2012).

Bioactivity of Lemongrass have been extensively studied, especially as antioxidant, antimicrobial, antibacterial, antifungal, insecticidal and insect repellent activities (Mirghani et al., 2012 , Helal *et al.,* 2006 and Ganjewala, 2009). Consumers interests in natural food additives especially antioxidant, have been increased due to the toxicological studies which showed the promoting of synthetic antioxidants in the development of cancerous cells in rats (El-Massry *et al;* 2002). Several reports published earlier have revealed the presence of many chemical constituents in lemongrass essential oil responsible for such bioactivities for example citral (mixture of neral and geranial), geraniol, citronellol, citronellal, 1,8-cineol…..etc (Kumar et al., 2008). Such chemical constituents influenced by factors for example temperature, light intensity, soil moisture, fertilizer, and maturity stage (Miyazaki, 1965). Many studies have been carried out concerning the chemical composition as well as antimicrobial and antifungal activates of Egypt lemongrass but not the antioxidant activity (Helal *et al.,* 2006, Hanaa *et al.,* 2012 and Al Yousef *et al.,* 2013).

Saudi Arabia which separated from Egypt by the red sea and located in Arab peninsula has a lot of commodities and crops with different geography and environmental conditions. However, the chemical constituents and bioactivity of lemongrass cultivated in Saudi Arabia have not been investigated yet. So, the aim of this study was to evaluate the antimicrobial, antioxidant properties and the chemical composition of Saudi

lemongrass essential oil cultivated in Madinah (the west region) and its comparison to the Egyptian type.

MATERIAL AND METHODS

MATERIALS AND PLANTS

The fresh green parts of Lemongrass (*Cymbopogon citratus*) which shadow dried, were collected from Abiar Ali, Madinah, Saudi Arabia, while the Egyptian type purchased from a local market in Cairo. Diethyl ether and Methanol purchased from (Fisher chemicals). Mixture of n-Alkanes C_6-C_{26}, authentic compounds, Sodium bicarbonates, linoleic acid ≥ 99% , Tween 40, β-Carotene ≥ 97%, Folin-Denis reagent for total phenolic, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and gallic acid (97.5-102.5%) were obtained from Sigma Aldrich Chemical Co . (St Louis, MO, USA). Petriplates 9cm, PDA media and Micro-organisms isolated from Plant Pathology Disease from Agriculture research center, Egypt.

ESSENTIAL OIL EXTRACTION

Essential oils from Dried Lemongrass for both herbs under study were obtained by hydrodistillation for 3h using a Clevenger-type apparatus according to the method of (Giray et al., 2008). The obtained essential oil was closed under nitrogen gas and stored in airtight glass vials covered with aluminum foil at – 20°C.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS

The obtained essential oils analyzed using GC-MS apparatus. Separation was performed on Trace GC Ultra Chromatography (Thermo Scientific, USA), equipped with ISQ-Mass (Thermo Scientific, USA) and 60 m x 0.25 mm x 0.25 µm film thickness TG-5MS capillary column (Thermo Scientific, USA). The column separation programmed from 50°C with hold time 3 minutes and temperatures increase at rate 4°C/minute to 140°C with hold time 5 minutes, then at rate 6°C /minute to 260°C with 5 minutes. Isothermal hold. The injector temperature was 180°C, Ion source temperature 200°C and the transition line temperature was 250°C. The carrier gas was helium with constant flow rate 1.0 ml $minutes^{-1}$. The mass spectrometer had a scan range from m/z 40 to m/z 450. Ionization energy was set at 70 eV. The identification of compound based on the comparison the MS computer library (NIST library version 2005), compared with those of authentic compounds and published data [20], and the relative percentage of the oil constituents was calculated from GC peak areas. A linear retention was calculated for each compound using the retention times of a homologous series of $C_6 – C_{26}$ n-alkanes (Adams, 1995).

ANTIOXIDANT ACTIVITY MEASURMENTS

SCAVENGING EFFECT ON 2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) RADICAL

The potential antioxidant activity of *Cymbopogon citratus* oils were assessed according to (Hatano et al., 1988) in comparison to synthetic antioxidant used in food industry, tert-butylhydroquinone (TBHQ). The absorbance was measured at 517nm using spectrophotometer (Evolution 300 Thermo UV-VIS); all tests were run in three replicates and averaged.

β- CAROTENE BLEACHING ASSAY

Antioxidant activity of the aqueous solution was determined by a β -carotene / linoleic acid system as described by (Taga et al., 1984) in comparison to TBHQ. The absorbance was measured at 470nm over a 60 minutes period.

DETERMINATION TOTAL PHENOILC CONTENT

Total phenolic content of the essential oils obtained from *Cymbopogon citratus* was determined using Folin–Ciocalteu reagent according to a modified method of (Singleton et al., 1999), using gallic acid as the standard. The reaction mixtures were incubated in a thermostat at 45°C for 45 minutes before the absorbance at 765 nm was measured.

ANTIMICROBIAL ACTIVITY

Micro-organisms isolated from Plant Pathology Disease Research from (ARC) were tested for antagonism with essential oil of lemongrass on PDA plates for fungi and bacteria with added into PDA media at the rates of (1, 3, 6 and 10 mg ml^{-1}) before plating. Plates were inoculated with 0.5 cm diameter discs of *Fusarium moniliforme* grown on PDA media for 12 days. Control of the experiment was non-amended exudate PDA plates. Growth diameter was recorded when fungus growth filled up a plate. The testing of the bacterial cultures for the inhibitory essential oil of lemongrass for different concentration the rates of (1, 3, 6 and 10 mg ml^{-1}) effect of for each treatment was replicated. Isolates exhibited convenient and antagonisms in accordance to (Haenseler and Allen, 1934).

RESULTS AND DISCUSSION

GC-MS analysis of Egyptian Lemongrass (*Cymbopogon citratus*) essential oil showed the identification of 49 compounds accounting for (92.6 %) of the total amount (Figure 1, Table 1). The major compounds which identified were geranial (20.9 %), neral (16.2), geraniol (8.3%) and linalool (5.6%), in accordance to (Helal *et al.*, 2006, Hanaa *et al.*, 2012 and Al Yousef *et al.*, 2013). In comparison to the present study, the above references identified only between (12 –18) components, so, the newly identified compounds may affect the bioactivity of the essential oil for example antioxidant, antimicrobial or antifungal. There are variations in the percentage of essential oil composition; due to variety of factors for example locations, season, plant age and parts. With respect to the above findings, neral and geranial found as a total of 68.3% by (Helal et al., 2006), collected from Sharkia (north east of Egypt), (34.5%) and (39.9%) by (Hanaa et al., 2012, Nazni and Dharmaligam, 2013), collected from Banha (Nile Delta of Egypt), and (23.9%) and (46.3%) by Al-Yousef collected from Assuit (south of

Egypt) (Al Yousef et al., 2013). It is therefore concluded that, such variations reflect environmental differences among the populations (El-Massry et al; 2002). The volatile oil obtained from the leaves of *Cymbopogon citratus* cultivated in Madinah Monawara, Saudi Arabia was pale yellow with pleasant and distinct odour. The percentage of constituents is shown in (Table 1, Figure 2). Eighteen compounds could be identified in the oil accounting for about (95.0%) of it. The major compounds which identified by GC-MS were granial (37.8%), neral (33.6%), α- myrcene (8.4%) and geranial acetate (3.5%).

In Madinah herb, neral, geranial and myrcene detected in higher concentrations in comparison to the Egyptian lemongrass, while other compounds found in lower concentrations e.g. linalool (0.7%) and carvone (0.4%). However, other constituents are found only in the essential oil Egyptian of *Cymbopogon citratus* e.g., terpin-4-ol (2.1%), cineole (0.6) % and carveol (0.5) %.

The antioxidant activity of Lemongrass (*Cymbopogon citratus)* essential oil cultivated in both Egypt and Madina Monawara tested by DPPH radical scavenging and β-Carotene-linoleate bleaching assays. Recorded activities are presented in (Table 2), where lower IC_{50} values indicate higher activity. Both essential oils of lemongrass under the present study, exhibited antioxidant activity in agreements with (Mirghani *et al.*, 2012 , Salem *et al.*, 2010 , Jumepaeng *et al.*, 2013 and Guerrero-Beltran et al., 2015). However, Egyptian herb essential oil exhibited a higher scavenging ability for DPPH (IC_{50} 1.0 mg ml^{-1}) in comparison to Madinah Lemongrass volatile oil, (IC_{50} 6.9 mg ml^{-1}). Again, the inhibiting effect for linoleic acid oxidation and the subsequent bleaching of β-carotene was higher for Egyptian Lemongrass essential oil (55.1%) than Madinah one (36.3%), which assured the DPPH radical scavenging assay results. The remarkable antioxidative activity might be due to the considerable citral isomers (neral and geranial) which found as major components in both essential oils under investigation (Xiaolin et al., 2013 and Godwin et al., 2014). However, antioxidant effectiveness in natural sources was reported to be mostly due to phenolic compounds (Moller et al., 1999). Pure phenolic compound for example linalool and eugenol exhibited a higher antioxidant activity (IC_{50} 0.6 and 2.8 µg ml^{-1}) in comparison to essential oils e.g. sweet basil (IC_{50} 26.5 µg ml^{-1}), lemongrass presented in this study (IC_{50} 1.0 and 6.9 mg ml^{-1}), or citral (IC_{50} 0.7 mg ml^{-1}) (Pripdeevech et al., 2010 and Wanga et al., 2012, Dharmalingam and Nazni, 2013). Therefore, Egyptian Lemongrass essential oil showed a stronger scavenging ability for DPPH radicals than Madinah Monawara type; due to the presence of phenolic compound for example Linalool (5.6%), geraniol (8.3%), terpin-4-ol (2.1%) and eugenol (0.4%).

Total phenolic constituents of the lemongrass essential oils under investigation were determinutesed by experimental methods involving Folin-Ciocalteau test. The total phenoilc concentration was calculated as Gallic acid equivalents. Results indicated that Egyptian lemongrass essential oil had a concentration of 15.8 mg/g GAE followed by Madinah oil with 6.1 mg GAg^{-1} (Table 2). A great number of reports have established that plant phenolic compounds including flavonoids are potent antioxidants with reported anti mutagenic and anti carcinogenic effects (Middleton and Kandaswami, 1994) and (Rice-Evans *et al.*, 1997).

Data presented in (Table 3) indicated that, essential oils under test process showed antimicrobial activities against fungus and bacteria. Both oils delayed condition of fungus and bacteria, while the feature of antimicrobial activity varied between them as well as among Microorganisms. The antimicrobial activity of tested essential oils was generally higher against fungi than bacteria. *Fusarium melanoform* revealed the highest sensitivity toward essential oil, while fecal coliforms revealed the lowest sensitivity. The above findings are in agreement with (Tabassum and Vidyasagar, 2013). (Table 3) showed that, Egyptian lemongrass essential oil have a higher antimicrobial activity than Madinah herb essential oil. It is well known that terpenoids possess antimicrobial activity (Singh et al., 2002 and Vagi *et al.*, 2002) for example citrals which constitute major components in both essential oils. However, presence of more phenolic compounds in the Egyptian essential oil for example linalool and linalyl acetate, exhibited antifungal activity against many other potent microorganisms for example fusarium oxysporum, which may revealed the higher activity during study (Pitarokili *et al.*, 2002).

Table 1- Chemical composition of Egyptian and Madinah Lemongrass volatile oils

S/N	Compound	Kovat's index	% Area Egyptian oil	% Area Madinah oil	Identification method
1.	6-methyl-5-heptene-2-one	985	3.0	0.6	MS[b] & KI[a]
2.	α-Myrecen	991	0.8	8.4	MS & KI & ST[c]
3.	Cineol (Eucalyptol)	1016	0.5	--	MS & KI
4.	D-Limonene	1031	--	0.2	MS & KI
5.	Z-β- Ocimene	1040	0.4	0.3	MS & KI
6.	E-β- Ocimene	1050	0.6	0.2	MS & KI
7.	Dihydro Tagetone	1054	--	0.1	MS & KI & ST
8.	Myrcenol	1064	0.2	0.2	MS & KI
9.	Cis-linalool oxide	1074	2.8	--	MS & KI
10.	Trans-linaloloxide	1088	2.5	--	MS & KI
11.	Linalool	1089	5.6	0.7	MS & KI& ST
12.	Cis - Thujone	1102	0.8	--	MS & KI

13.	Rose Oxide	1110	0.1	--	MS & KI
14.	Phenyl ethyl alcohol	1112	0.2	--	MS & KI & ST
15.	Menth-3-en-8-ol	1149	0.4	--	MS & KI
16.	Cintronellal	1153	---	0.3	MS & KI
17.	Cis - verbenol	1155	---	1.3	MS & KI
18.	β – pinene oxid	1156	---	1.2	MS & KI
19.	Cis-Chrysanthenol	1162	0.1	--	MS & KI & ST
20.	Trans- β -Terpineol	1163	0.6	--	MS & KI & ST
21.	1,3- Dimethoxy benzene	1164	0.7	--	MS & KI
22.	Borneol	1165	0.8	--	MS & KI
23.	terpin-4-ol	1177	2.0	--	MS & KI & ST
24.	pinocarveol	1183	0.2	--	MS & KI
25.	α-terpineol	1189	1.9	--	MS & KI
26.	Estragole	1195	0.5	--	MS & KI & ST
27.	Trans - carveol	1217	0.8	--	MS & KI
28.	Citronellol	1228	0.58	0.3	MS & KI
29.	Nerol	1235	0.36	--	MS & KI& ST
30.	Neral	1240	16.2	33.6	MS & KI & ST
31.	Carvon	1242	2.5	0.4	MS & KI
32.	Geraniol	1255	8.3	5.76	MS & KI& ST
33.	Methyl Citronellate	1261	1.8	--	MS & KI
34.	Geranial	1270	20.9	37.8	MS & KI
35.	Linalool acetate (dihydro)	1275	0.6	--	MS & KI
36.	Methyl Nerolate	1279	2.0	--	MS & KI
37.	Carvecrol	1281	0.3	--	MS & KI
38.	α – Terpinene-7-Al	1281	0.4	--	MS & KI & ST
39.	E- Anethole	1283	0.7	--	MS & KI
40.	Undecan-2-one	1291	0.2	--	MS & KI & ST
41.	Methyl Geranate	1323	2.4	--	MS & KI
42.	Eugenol	1356	0.4	--	MS & KI
43.	Geranyl acetate	1383	4.1	3.5	MS & KI
44.	Z-Caryophyllene	1404	0.6	--	MS & KI & ST
45.	E-Caryophyllene	1418	0.5	--	MS & KI
46.	Trans-α -bergamotene	1436	0.7	--	MS & KI
47.	Cinnamic acid methyl ester	1437	0.7	--	MS & KI & ST
48.	α-guaiene	1439	0.6	--	MS & KI
49.	α-Bulensene	1505	0.6	--	MS & KI
50.	Gamma- Cadinene	1513	0.5	--	MS & KI
51.	Delta- Cadine	1524	0.6	--	MS & KI
52.	Caryophellene oxide	1582	0.2	--	MS & KI
53.	Globulol	1583	0.2	--	MS & KI & ST
54.	α- Cadinol	1653	----	0.1	MS & KI & ST
55.	Phytol	1949	0.2	--	MS & KI
	Total	---	92.6 %	95.0%	MS & KI

a Confirmed by comparison with Kovat's index on DB5 column (Adams, 1995). b Identification by comparison with data obtained from NIST mass spectra library. C -Confirmed by comparison with mass spectrum of authentic compound.

Table 2- Antioxidant activity of Egyptian and Madinah Lemongrass volatile oils

Material	**IC_{50} / mg ml $^{-1}$ (DPPH)[a].**	**β-Carotene / bleaching % [a], for 1mg ml $^{-1}$**	**Total phenolic content (mg GA g^{-1})[a] , for 1mg ml $^{-1}$**	**(DPPH)[a] %, for 1mg ml $^{-1}$**
Madinah volatile Oil	6.9	36.3	15.8	31.4
Egyptian volatile Oil	1.0	55.1	6.1	49.8

[a]Values represent averages ± standard deviations for triplicate experiments

Table 3- Effect of Madianh and Egyptian Cymbopogon citrates essential oils on the Inhibition of Microorganisms growth diameter

Microorganisms	Control Growth Diameter	*C. citrates oil* 1mg /ml		*C. citrates oil* 3mg /ml		*C. citrates oil* 6mg /ml		*C. citrates oil* 10mg /ml	
		Medinah	Egypt.	Medinah	Egypt	Medinah	Egypt	Medinah	Egypt
E.coli	**2.8 cm**	3.7 cm	0.1cm	0.0cm	0.0cm	0.0	0.0cm	0.0cm	0.0cm
Fecal Coliform	**3.6cm**	1.1 cm	0.8 cm	0.9cm	0.6	0.4	0.0cm	0.3 cm	0.0cm
Staphylococcus	**3.1cm**	0.7 cm	0.1 cm	0.0cm	0.0	0.0 cm	0.0cm	0.0cm	0.0cm
Fusarium melanoform	**9. 0 cm**	0.5 cm	0.2 cm	0.0cm	0.0	0.0 cm	0.0cm	0.0cm	0.0cm

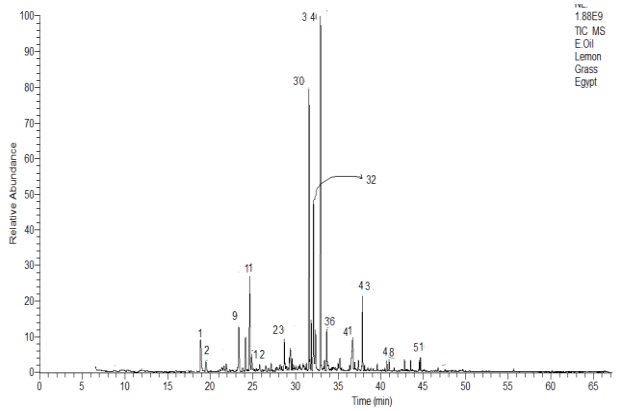

Figure 1. GC-MS Chromatogram for Egyptian Lemongrass volatile oil

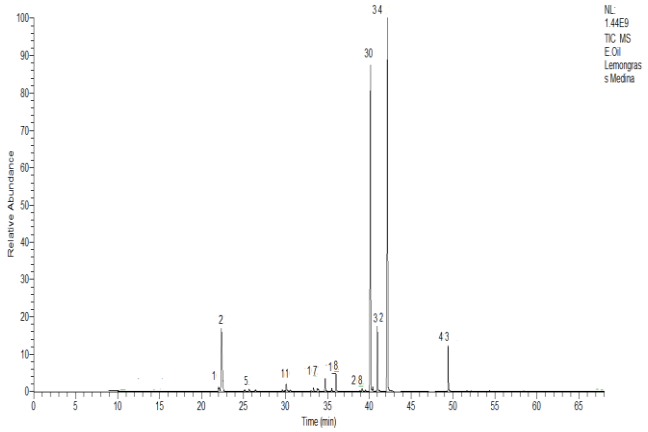

Figure 2- GC-MS Chromatogram for Madinah, Saudi Arabia Lemongrass volatile oil

CONCLUSIONS

Due to differences in chemical compositions, geranial, neral and geraniol were the predominant in the Egyptian essential oil of Cymbopogon citratus, while Madinah limongrass oil has geranial and neral as the major constituents. The higher concentrations of phenolic content, presence of linalool and eugenol in addition to citral isomers are responsible for the higher antioxidant activity of the Egyptian herb oil in comparison to Madinah one. DPPH and β-Carotene linoleic bleaching assays were used and confirmed the difference in the antioxidant activity.

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