Journal of Applied Research on Medicinal and Aromatic Plants 3 (2016) 105–111

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Effects of storage time on the antimicrobial activities and composition of lemon grass oil

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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 3 February 2016
Accepted 28 February 2016
Available online 21 March 2016

Keywords:
Lemon grass oil
Essential oil constituents
Storage
Antimicrobial activities
Cympobogon citratus
Citral

ABSTRACT

The composition and antimicrobial activities of fresh and stored essential oil of *Cympobogon citratus* (lemon grass oil) were studied. Three samples of steam distilled oil from the aerial parts of well grown plants collected before 12 noon in Ile-Ife, Nigeria between December and April of year 2004, 2011 and 2013 were screened. The first two oils were stored after extraction half-full in well closed amber colored bottles at 4 °C and were screened along with the freshly extracted 2013 oil. Antimicrobial studies included Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) determinations against nine strains of type and clinical bacteria isolates and two fungi. Time-kill analysis was done at MIC concentrations. A total of eight, twenty and thirty-six constituents were identified by GC–MS, accounting for 95.25%, 85.92%, and 93.07% in the fresh, two-year and nine-year old oil respectively. Geranial (51.70%), neral (32.62%) and nerol (4.40%) were the major constituents in the fresh oil while geranial (18.19%), neral (10.58%), 3-heptanol-4-methyl (10.50%), epoxy-linalooloxide (5.57%) and *trans*-7-oxabicyclo [4.3.0] nonane (6.75%) were those of the two-year old oil. Major constituents in the nine-year old oil include 1,6-octadien-3-ol, 3,7-dimethyl—(32.36%), geraniol (16.03%) and eucalyptol (10.81%). The MIC and MBC of the two stored oil were similar. The minimum antibacterial MIC:MBC of the fresh and the stored oils was 0.625%v/v:1.25%v/v and 1.25%v/v:10.0%v/v respectively. *Pseudomonas aeruginosa* was not inhibited by any of the three oil samples. Antifungal activities were higher in the stored oils. The nine year old oil killed the *Staphylococcus aureus* and *Klebseilla pneumoniae* strains faster than the fresh oil. In conclusion, there were extensive differences in the chemical constituents of fresh and stored lemon grass oil which affects its antimicrobial activities. Storage of the oil made available many components that were not found in the fresh oil.

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1. Introduction

Cymbopogon citratus Stapf belong to the family Graminae and it is commonly cultivated in home gardens across Southern Nigeria. Decoction of the leaves is widely consumed as antimalarial locally in tropical West Africa (Oliver, 1960). Lemon grass oil is the volatile principle extracted from the leaves of *C. citratus*. The antimicrobial properties of freshly extracted lemon grass oil (LGO) against a wide range of microorganisms have been reported by many authors (Onawunmi et al., 1984; Bassolé et al., 2011; Ekpenyong et al., 2014).

Reports indicated that antimicrobial activities reflect the chemical compositions of the oil. Investigations have also shown that the chemical composition of the oil is affected by various factors including the geographical origin, genetic differences, part of the plant used, method of extraction, age/stage of maturity, and season of harvest of the plant (Figueiredo et al., 2008; Hanaaa et al., 2012; Tajidin et al., 2012). Irrespective of these factors however, most reports of chemical compositions indicated that the oil consists mainly of citral, a mixture of two stereoisomeric monoterpene aldehydes. This is particularly the case in most reports of oil composition from Nigeria (Onawunmi et al., 1984; Adeleke et al., 2001; Owolabi et al., 2008). In this wise, citral has remained the key compound in evaluating the quality of LGO (Onawunmi, 1989; Bassolé et al., 2011). In most

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http://dx.doi.org/10.1016/j.jarmap.2016.02.005
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reported studies, the *trans* isomer of citral, geranial dominates over the *cis* isomer, neral.

Other reported constituents of the oil include geranyl acetate, limonene, luteolin and its 6-C and 7-*O*-glycosides, isoorientin 2′-*O*-rhamnoside, flavonoids: quercetin, kaempferol and apiginin (Chisowa et al., 1998; Ekpenyong et al., 2014). The phenolic compounds elimicin, catecol, chlorogenic acid, caffeic acid and hydroquinone have also been reported isolated from the plant (Chisowa et al., 1998; Ekpenyong et al., 2014).

There have been concerns expressed on the biological activities of certain essential oils following storage (Wabner et al., 2006). This is more important as it has been documented that exposure to light, oxygen, heat and cold leads to a wide variety of chemical reactions and interactions in the constituents of essential oils resulting in their modifications and breakdowns on storage (Figueiredo et al., 2008; Burfield, 2004; Bråred-Christensson et al., 2009). Orafidiya (1993) have reported that LGO autoxidizes on storage and by the use of oxidizing agents. It was further indicated that the oxidized oil demonstrated reduced activities against bacteria and that this reduction in activities can be reversed by the addition of antioxidants to the oil (Orafidiya, 1993). Other workers have also shown that terpenic compounds such as limonene and linalool form stable hydroperoxides when oxidized and that the hydroperoxide formation is caused by autoxidation of chemicals during handling and storage (Börje et al., 2004).

Although several studies have investigated the various effects of storage on factors such as allergic reactions (Börje et al., 2004), reports of effects of storage on the antimicrobial activities of the oil are rare in literature. Therefore, it was the objective of this study to determine the composition as well as the antimicrobial efficacy of freshly distilled and stored essential oil of *C. citratus*. For the purpose of the study, LGO samples stored in amber colored well closed glass bottles in the refrigerator over a period of two years and nine years were compared with that of freshly extracted oil.

2. Materials and methods

2.1. Collection of plant and extraction of oil

For the first oil sample, the aerial parts of lemon grass were collected at Ile-Ife in the morning in December, 2004. It was identified and authenticated at the herbarium of Botany Department, Obafemi Awolowo University, Ile-Ife with the voucher number 10232, where a sample specimen was deposited. For the second, the plant was collected in March, 2011 while in the case of the third, plant parts were collected in April, 2013 and authenticated with the earlier specimen. All the samples were collected between 8.00am and 11.00am. The oils were extracted by steam distillation of the fresh aerial parts of the plant in an all-glass still following the procedure described in the British Pharmacopoeia (1980). In all cases, a mobile, light yellow oil with the characteristic "lemon" odor was obtained. The stored oils used for the experiment were kept in the refrigerator at 4 °C in tightly half full closed amber colored bottles and were used together with the freshly extracted 2013 oil.

2.2. Test organisms

The organisms used were *Bacillus subtilis* (NCIB 3610), *Clostridium sporogenes* (NCIB 532), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Klebseilla pneumoniae* (clinical strain), *Proteus mirabilis* (clinical strain), *P. aeruginosa* (NCIB 950), *Bacillus cereus* (NCIB 6349), *Candida albicans* (ATCC 24433), *Candida pseudotropicalis* (NCYC 6). These were from the stock collection in our laboratory stored in Nutrient and Sabouraud Dextrose Agar Slope and subcultured in the appropriate broth when needed.

Table 1
Antimicrobial activity of the *C. citratus* essential oils.

Organisms	Mean zone of inhibition ± SD (mm)				MIC %v/v				MBC %v/v			
	Fresh oil	2 year old oil	9-year old oil	Ofloxacin (10 µg/disc)	Fresh oil	2 year old oil	9-year old oil	Streptomycin (µg/L)	Fresh oil	2 year old oil	9-year old oil	Streptomycin (µg/L)
E. coli (ATCC 25922)	12.72 ± 0.08	8.00 ± 0.00	0.00	15.0	5.0	5.0	2.5	0.0625	5.0	10.0	10.0	0.5
Pseudomonas fluorescence (NCIB 3756)	8.70 ± 0.04	6.70 ± 0.10	0.00	23.0	5.0	5.0	>10.0	0.25	5.0	10.0	10.0	0.5
P. aeruginosa (NCIB 950)	0.00	0.00	0.00	21.0	>10.0	>10.0	>10.0	0.50	>10.0	>10.0	>10.0	0.5
Proteus mirabilis (clinical strain)	12.00 ± 0.05	8.00 ± 0.00	0.00	22.0	2.5	2.5	2.5	0.125	5.0	10.0	10.0	0.5
B. cereus (NCIB 6349)	18.30 ± 0.10	10.00 ± 0.10	0.00	16.0	5.0	5.0	5.0	0.125	5.0	10.0	10.0	0.5
Staphylococcus aureus (ATCC 29213)	17.00 ± 0.00	12.50 ± 0.7	8.00 ± 0.50	26.0	0.625	2.5	5.0	0.0625	2.5	10.0	10.0	0.5
Klebseilla pneumoniae clinical strain	12.30 ± 0.80	8.00 ± 0.50	0.00	18.0	2.5	2.5	5.0	0.125	10.0	10.0	10.0	0.5
Clostridium sporogens (NCIB 532)	18.00 ± 0.05	10.00 ± 0.50	10.00 ± 0.50	18.0	2.5	5.0	2.5	0.125	5.0	10.0	10.0	0.5
Bacillus subtilis (NCIB 3610)	17.00 ± 0.00	4.00 ± 0.10	0.0	16.0	0.625	1.25	1.25	0.125	1.25	10.0	10.0	0.5
Candida albicans (ATCC 24433)	10.0 ± 0.50	12.0 ± 0.0	11.0 ± 0.0	24.0 ± 0.50*	0.625	<0.3125	<0.3125	16.0**	0.625	<0.3125	<0.3125	16.0**
Candida pseudotropicalis (NCYC 8)	12.0 ± 0.10	14.0 ± 0.0	14.0 ± 0.0	30.0 ± 0.50*	0.625	<0.3125	<0.3125	16.0**	0.625	<0.3125	<0.3125	16.0**

* Acriflavin 6.3 mg/ml/disc.
** Concentration of acriflavin (mg/L).

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