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Full Length Research Paper

Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species

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This study evaluated the antifungal activity of essential oil of *Cymbopogon citratus* (Poaceae) against five mycotoxigenic species of the genus *Aspergillus* (*Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Aspergillus niger* and *Aspergillus fumigatus*) isolated from maize samples. The oil of *C. citratus* was obtained by hydro-distillation and analysed by Gas Chromatography – Mass Spectrometry (GC-MS). The oil was dominated by monoterpene hydrocarbons which accounted for 94.25% of the total oil and characterised by a high percentage of geranial (39.53%), neral (33.31%), and myrecene (11.41%). The antifungal activity tests showed that the oil was active against all the five *Aspergillus* species. The extent of inhibition of fungal growth was dependent on the concentration of the oil. The activity of the oil against the mycotoxigenic fungi had Minimun Inhibitory Concentration (MIC) values ranging from 15 to 118 mg/ml. These results show that the essential oil of *C. citratus* has antifungal activities against fungi that are the producers of poisonous mycotoxins found in foods. This oil can be used in food preservation systems to inhibit the growth of moulds and retard subsequent mycotoxin production.

Key words: *A. flavus, A. fumigatus, A. niger, A. ochraceus, A. parasiticus,* antifungal.

INTRODUCTION

Moulds cause extensive damage on foods, feeds and other agricultural commodities in the field, during transportation, storage and processing, leading to postharvest losses. They are known to destroy 10 to 30% of the total yield of crops and more than 30% for perishable crops in developing countries by reducing their quality and/or quantity (Agrios, 1997). They also lower the nutritional and sale value of the produce. In addition, moulds produce mycotoxins that can cause illness or even death to the consumers. Mycotoxins contaminate 25% of agricultural crops worldwide and are a source of morbidity and mortality throughout Africa, Asia and Latin America (Smith et al., 1994). Mycotoxicosis causes acute liver damage, liver cirrhosis, induction of tumours and attack on the central nervous system, skin disorders and hormonal effects (Ibrahim et al., 2000; Oguz et al., 2003).

Most of the deterioration of grains and legumes after harvest is caused by several species of *Aspergillus* which are responsible for many cases of food and feed contamination (Abarc et al., 1994; Katta et al., 1995; Agrios, 1997). Several mycotoxins are produced by *Aspergillus* species. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and are among the economically most important mycotoxins. Acute aflatoxicosis epidemics occur in several parts of Africa and Asia leading to the death of several hundred people (Varga et al., 2009). Aflatoxins are known to be potent hepatocarcinogens in animals and humans (Dvorackova, 1990). Ochratoxin A, which has been experimentally shown to be teratogenic, a potent renal carcinogenic and immunosuppressive is largely produced by *Aspergillus ochraceus* and less frequently by *Aspergillus niger* (Nielsen et al., 2009). As an enzyme

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inhibitor, ochratoxin A affects lipid peroxidation and has been implicated in Balkan Nephropathy (BEN) in humans (Hohler, 1998). Mycotoxins produced by other *Aspergillus* species include malformins by *A. niger*; fumitoxins and gliotoxin by *Aspergillus fumigatus* (Hof and Kupfahl, 2009).

The wide and indiscriminate use of chemical preservatives has been cause of the appearance of resistant microorganisms, leading to occurrence of emerging food borne diseases (Gibbons, 1992; Akinpelu, 2001). Due to this, there is an increasing interest to obtain alternative antimicrobial agents from natural sources for use in food preservation systems. It is well established that some plants contain compounds able to inhibit the microbial growth (Matasyoh et al., 2007, 2009). One of the main procedures used in the search for biologically active substances is the systematic screening of interaction between micro-organisms and plant products. This procedure has been useful in identifying agents to control microbial survival in different microbiology applications (Salvat et al., 2001). Plant products of recognized antimicrobial spectrum could appear in food preservation systems as the main antimicrobial agents (Kaur and Arora, 1999). Considerable interest has developed on the preservation of grains by the use of essential oils to effectively retard growth and mycotoxin production (Bullerman et al., 1977; Chatterjee 1989).

The aim of this study was, therefore, to evaluate the bioactivity of the essential oil of *Cymbopogon citratus* (lemon grass) against five species of the genus *Aspergillus* (*A. flavus, A. parasiticus, A. ochraceus, A. niger* and *A. fumigatus*) isolated from maize, which is the staple food for most African and Latin American communities. The genus Cymbopogon (Poaceae) has about 55 species of grasses and is largely distributed in tropical and sub-tropical regions of the world.

MATERIALS AND METHODS

Plant material

Fresh plant materials of *C. citratus* were collected from the equatorial rainforest in Kakamega, Kenya. A voucher specimen was deposited at the Department of Biological Sciences, Egerton University.

Isolation of essential oils

Fresh whole plant materials of *C. citratus* were subjected to hydro-distillation in a modified Clevenger-type apparatus for a minimum of 4 h. The essential oil was obtained in a yield of 0.8% w/w after drying over anhydrous sodium sulphate (Na_2SO_4).

GC, GC-MS analysis

Samples of essential oils were diluted in methyltert-butylether (MTBE) (1:100) and analyzed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m × 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. Helium (at 0.8 ml/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 – 1: 100. The injector was kept at 250°C and the transfer line at 280°C. The column was maintained at 50°C for 2 min and then programmed to 260°C at 5°C/min and held for 10 min at 260°C. The MS was operated in the EI mode at 70 eV, in m/z range 42-350. Identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature (Adams, 1995) and supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil constituents are expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Test fungi

Maize samples were collected from various households and market centres in Western Kenya. Moulds were isolated from these samples using the direct plating technique. The grains were surface-sterilized in 2.5% sodium hypochlorite for 30 s and rinsed in three changes of sterile distilled water. The grains were blotted with sterile filter paper and plated on Czapek Dox and potato dextrose agar containing 7.5% sodium chloride and 1gm streptomycin sulphate (for 1 L of media). The plates were incubated at 25°C and monitored daily for fungal growth for seven days. Identification of the moulds was done based on morphological and cultural characteristics using taxonomic keys (Kozakiewiez, 1989; Klich, 2002). Target moulds were sub-cultured to obtain pure single-spore cultures.

Antifungal assays

Paper disc diffusion inhibition test was used to screen for antimicrobial activity of the essential oil as described by Souza et al. (2005). One hundred microliters of mould suspension (approximately 10^6 spores/ml) was uniformly spread on sterile potato dextrose agar media in Petri dishes. Sterile sensitivity discs were soaked with 10 µl of the essential oil and placed at the center of the inoculated culture plates. The plates were incubated at 25°C for 7 – 10 days. At the end of the incubation period, diameters of the inhibition zones were measured to the nearest millimeter (mm). Nystatin discs (100 µg) were used as the reference standard. The Minimum Inhibitory Concentration (MIC) was determined using the paper disc diffusion method as described above. Serial dilutions of the essential oil were done using dimethyl sulfoxide (DMSO) which was also used as the negative control.

Statistical analysis

Data on inhibition zones was analysed using Microsoft Office Excel 2003 to derive means and standard deviations.

RESULTS

The fresh whole plant material of *C. citratus* yielded an essential oil on hydro-distillation which was analyzed by gas chromatography-mass spectrometry (GC-MS) and also evaluated for antifungal activity against five species of the genus *Aspergillus*, namely *A. flavus, A. parasiticus, A. ochraceus, A. niger* and *A. fumigatus*. Twenty four compounds which constituted 95.41% of the total oil were

Table 1. Chemical composition of *C. citratus* oil.

S/N	Monoterpene hydrocarbons			
	Compound	**KI**	**Concentration %**	**Method of identification**
1.	α-Pinene	925	0.01	RI, GC-MS
2.	6-methyl-5-Hepten-2-one	981	1.25	RI, GC-MS
3.	Myrcene	988	11.41	RI, GC-MS
4.	(Z)- β-Ocimene	1036	0.69	RI, GC-MS
5.	(E)- β-Ocimene	1047	0.34	RI, GC-MS
6.	6,7-Epoxyocimene	1094	0.27	RI, GC-MS
7.	Linalool	1104	1.29	RI, GC-MS
8.	Citronellal	1157	0.12	RI, GC-MS
9.	Silo from lemone grass	1166	0.58	RI, GC-MS
10.	Rosefuran epoxide	1175	0.07	RI, GC-MS
11.	Silo from lemongrass 2	1186	0.88	RI, GC-MS
12.	Nerol	1232	0.34	RI, GC-MS
13	β-Citronellol	1235	0.34	RI, GC-MS
14.	Neral	1247	33.31	RI, GC-MS
15.	Geraniol	1258	3.05	RI, GC-MS
16.	Geranial	1277	39.53	RI, GC-MS
17.	2-Undecanone	1299	0.53	RI, GC-MS
18.	Geranyl acetate	1385	0.24	RI, GC-MS
19.	(E)-Caryophyllene	1429	0.15	RI, GC-MS
20.	α-(E)-Bergamotene	1442	0.13	RI, GC-MS
21.	2-Tridecanone	1503	0.37	RI, GC-MS
22.	δ-Cadinene	1527	0.10	RI, GC-MS
23.	5-epi-7-epi-a-Eudesmol	1617	0.12	RI, GC-MS
24.	M.w 222	1634	2.41	RI, GC-MS
25.	α-Cadinol	1667	0.28	RI, GC-MS
	Total % in oil		97.82	

identified. The constituents identified by GC-MS analysis, their retention times and area percentages are summarized in Table 1. The oil was dominated by monoterpene hydrocarbons which accounted for 94.25% of the oil. This monoterpene fraction was characterized by a high percentage of geranial (39.53%), neral (33.31%), myrecene (11.41%) and geraniol (3.05%). Only 0.78% of the components identified were sesquiterpenes.

Table 2 displays the results of the antifungal tests. The oil was found to be active against all the fungal species tested. The MIC of the oil ranged from 15 to 118 mg/ml. The concentrations of the oil were generally in the range of ten times more than the standard antifungal (Nystatin) and they showed marked antifungal activities as evidenced by their zones of inhibition (Table 2). In general, the oil was more active against the five species of *Aspergillus* than the reference standard. The highest activity of the oil was observed against *A. niger* with the largest inhibition zone of 46.33 mm and an MIC of 15 mg/ml as compared to 20.5 mm inhibition by Nystatin. This was followed by activity against *A. ochraceus* with an MIC of 59 mg/ml. Although, the oil had lower inhibition zones with *A. parasiticus* and *A. fumigatus* as compared to *A. ochraceus*, similar MIC values were observed for the three species. The least antifungal activity of the oil was observed against *A. flavus* with an MIC of 118 mg/ml.

DISCUSSION

The essential oil of *C. citratus* analyzed in this study was dominated by monoterpene hydrocarbons which accounted for 94.25% of the total oil and characterised by a high percentage of geranial (39.53%), neral (33.31%), and myrecene (11.41%). The oil showed antifungal activities against mycotoxigenic species *A. flavus, A. parasiticus, A. ochraceus, A. niger* and *A. fumigatus*. The antifungal activity of this oil could be due to the presence of several components known to have biological activities. These major components of the oil, geranial, neral and geraniol, have been reported to have high antifungal activity (Lee et al., 2008). A natural mixture of geranial and neral, which are two isomeric acyclic

Table 2. Antifungal activity of *C. citratus* oil on five *Aspergillus* species seven days after inoculation.

Fungus	Inhibition zone (mm)									MIC mg/ml
	Essential oil µg × 10^{2*}							STD[a]	Control (-)	
	94.0	47.0	23.5	11.8	5.9	3.0	1.5			
A. flavus	19.6 ± 1.50	11.67 ± 0.58	9.67 ± 0.58	0	0	0	0	12 ± 2.83	0	118
A. parasiticus	20.67 ± 0.58	17 ± 2.08	13 ± 0.58	6.67 ± 0.00	0	0	0	14.5 ± 0.71	0	59
A. ochraceus	35 ± 3.00	15 ± 0.00	10.67 ± 1.15	8.33 ± 0.58	0	0	0	17 ± 1.41	0	59
A. niger	46.33 ± 2.08	39.67 ± 1.53	24 ± 1.00	10.67 ± 0.58	10.33 ± 0.58	8.67 ± 0.58	0	20.5 ± 0.71	0	15
A. fumigatus	25.33 ± 2.08	12.67 ± 0.58	10.33 ± 0.58	8 ± 0.00	0	0	0	20.5 ± 0.71	0	59

a-Nystatin (100 µg),*-The oil concentration values are multiplied by 10^2.

monoterpene aldehydes, is referred to as citral. Geranial and neral is actually *trans* and *cis* citral respectively. Citral has been reported to show various antifungal activities (Onawunmi, 1989). The high antifungal activity of this oil could be mainly due to the presence of these two isomers, although, other minor constituents like α-pinene have been reported to be the main cause of the antifungal activity of the oil from *Pistacia lentiscus* (Anacardiaceae) (Magiatis et al., 1999).

The other major component myrecene is not known to show any antifungal activity. Aldehydes and alcohols have been known to be active but with differing specificity and levels of activity, which is related not only to the functional group present but also to hydrogen bonding parameters (Skaltsa et al., 2003). Another minor monoterpene alcohol, linalool, is reported to have a wide range of antibacterial and antifungal activity (Pattnaik et al., 1997). Linalool is known to inhibit spore germination and fungal growth. The inhibition of sporulation appears to arise from respiratory suppression of aerial mycelia (Lahlou and Berrada, 2001). The fourth major constituent of this oil, geraniol, has been reported to show antifungal activity and its mode of action involves enhancing the rate of potassium leakage out of the cells (Bard et al., 1988). In general, the inhibitory action of natural products on mould cells involves cytoplasm granulation, cytoplasmic membrane rupturing and inactivation and/or synthesis inhibition of intercellular and extracellular enzymes (Souza et al., 2005). These actions can occur alone or in combination and culminate in inhibition of mycelium germination (Cowan, 1999).

The antifungal activity of the oil varied with its concentration and the kind of fungal species, indicating that its activity is proportional to its concentration. The highest activity of the oil was observed against *A. niger* with an MIC of 15 mg/ml and the highest resistance was observed from *A. flavus* with an MIC of 118 mg/ml. *A. flavus* has also been known to show resistance to basil and spearmint oils (Solimana and Badeaa, 2002). However, the inhibition level of the oil against this species in the current study was higher than that of the reference Nystatin indicating that, despite the resistance, the oil is relatively effective. The findings of this study clearly show that the essential oil of *C. citratus* has antifungal activity and can be used in practical application in the inhibition of mould growth and mycotoxin production in stored grain. It would be important to establish the actual bioactive component/s and the mode of action of the essential oil against the *Aspergillus* species. Of interest also would be to determine the effect of the oil on mycotoxin production ability of the moulds.

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