



COMPARATIVE ANALYSIS OF ANTIFUNGAL AND HISTOPATHOLOGICAL PROPERTIES OF ESSENTIAL OILS OF *CYMOPOGON CITRATUS* STAPF AND *KHAYA IVORENSIS* CHEV.

Fadipe DO*¹ and Onifade AK²

¹Health Centre, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

²Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

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Abstract: This study was done to determine or otherwise the possible use of the essential oils of these plants against clinically isolated fungi, especially the dermatophytes noted for their resistance to commonly used antifungal agents. The essential oils of Mahogany (*Khaya ivorensis*) seeds and the leaves of Lemon grass (*Cymbopogon citratus*) were extracted using Soxhlet apparatus in Petroleum ether. The *in vitro* antifungal activity of the oils was compared with Fulcin (150µg) against four fungal species including *Candida albicans*, *Epidermophyton floccosum*, *Microsporium canis* and *Trichophyton tonsurans*. Generally, the growth inhibitory potential of the oils increased with a corresponding increase in concentration. The Minimum inhibitory concentration of these oils against the fungal biotest ranged between 0.12mg/ml and 0.21mg/ml for *C. citratus* oil and 0.18mg/ml - 0.33mg/ml for *K. ivorensis* oil. When different concentrations of the oils were administered orally to albino rats for 14days to test their toxicity and biosafety, there were no observable changes in the histological sections of internal organs of the groups fed with *C. citratus*, while the groups fed with *K. ivorensis* oil showed some pathological changes in their internal organs.

Key Words: Essential oil; *in vitro* assay; antifungal activity; Histopathological effects; inhibitory concentration.

INTRODUCTION

Volatile compounds from angiospermic plants, especially essential oils, have been demonstrated to possess outstanding fungi toxicity against mycelial growth or spore germination *in vitro* (Alvares-Castellanos *et al.*, 2001; Letessier *et al.*, 2001; Cho *et al.*, 2002; Bouchra *et al.*, 2003; Nguefack *et al.*, 2004; Al-Burtanami *et al.*, 2005; Fatope *et al.*, 2006; Onifade, 2007). Chemically, essential oils are a mixture of monoterpenes and sesquiterpenes, although a variety of low molecular weight hydrocarbons, acids, alcohols, aldehydes, acyclic esters of lactones and exceptionally nitrogen and sulphur containing compounds could be present (Al-Burtanami *et al.*, 2005 and Fatope *et al.*, 2006). Both alcohol and water extracts of *C. citratus* have been found to exhibit remarkable antifungal properties on three genera of dermatophytes, namely *Microsporium canis*, *Trichophyton rubrum* and *Epidermophyton floccosum* (Nwadiaro and Nwachukwu, 2007). The essential oil of *C. citratus* has also been found to be an effective alternative substance for rice seed treatment to control seed-borne fungi of rice (Nguefack *et al.*, 2005). Moreover, essential oil from the seed of *K. ivorensis* has been found to significantly reduce aflatoxin production by the fungi *Aspergillus flavus* and *A. parasiticus* (Onifade, 2007). Medicinal uses of *K. ivorensis* (African mahogany) have only traditional claims. These include treatment of malaria, jaundice, skin infections, anaemia and arthritis, also being used as antihelmintic, emetic, and emmenagogue (Odugbemi, 2006).

When proposing the use of plant extracts for medicinal purpose, there is the strong need to consider the effect(s) of their use on vital organs such as the liver, heart, kidney and intestine. The diseases in these organs are often terminal, leading to the eventual death of the person or animal (Robinson *et al.*, 2006). Sequel to this, the present study was designed to focus on the *in vitro* antimycotic activities and histopathological effects of essential oils from *Khaya ivorensis* and *Cymbopogon citratus* on vital organs of albino rats.

*Corresponding Author:

Mr. Fadipe DO,
Health Centre,
Adekunle Ajasin University, Akungba-Akoko,
Ondo State, Nigeria.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves of lemon grass (*Cymbopogon citratus* Stapf) and the seeds of mahogany tree (*Khaya ivorensis* A. Chev) were harvested from the Garden of Federal Department of Forestry, Ado-Ekiti. The plant samples were authenticated at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife Nigeria.

Source and maintenance of test organisms

Clinical isolate of *Candida albicans* was obtained from Microbiology Laboratory of Adekunle Ajasin University Health Centre, Akungba-Akoko Ondo State. Cultures of *Epidermophyton floccosum*, *Microsporium canis* and *Trichophyton tonsurans* were obtained from the Mycology Unit of Lagos University Teaching Hospital, Idi-Araba, Lagos. All the isolates were maintained by regular transfers unto freshly prepared agar media and kept in slants at 4°C until used.

Extraction of essential oil from plant materials

Five hundred grams (500g) of the seeds of *K. ivorensis* were air-dried for two months, the pericarps were maximally removed and the seeds pulverised to a fine powder using an electric grinding machine (Marlex CM/L7371373). Fresh leaves of *C. citratus* were thoroughly washed in clean water, air-dried for two weeks and pulverised into a fine powder using the Marlex electric grinding machine. The powdered plant materials were subjected to distillation in Soxhlet apparatus equipped with Clevenger type distillation arm for 3h using Petroleum ether as solvent to recover the oils (Nguefack *et al.*, 2005; Jirovetz *et al.*, 2007 and Onifade *et al.*, 2008). The oils were dried over anhydrous Sodium sulphate and stored in darkness at 4°C until needed for use.

Antimicrobial sensitivity testing of the oils

The agar diffusion technique of Juliani *et al.* (2002) was employed; sterile Sabouraud dextrose agar (SDA) contained in Petri dishes were inoculated with the standardized fungal

inocula using sterile cotton swabs. Wells of 6mm diameter were cut and filled with 0.3ml of each extract at concentrations ranging from 25mg/ml to 100mg/ml. Tween-20 (50%) was used as control. The extracts were allowed to diffuse into the medium for 1h, after which the plates were incubated at 37°C for 24h (for *Candida albicans*) and 25°C for 7days (for moulds). The diameter zones of inhibition were measured (Minimum Fungicidal concentration) (MFC). The minimum inhibitory concentrations of the oils were determined by using the tube dilution method (Atlas, 1995). The least concentration (highest dilution) of the oils that showed inhibitory effect on any of the test dermatophytes was recorded as the minimum inhibitory concentration (MIC). The experiment was conducted in five replicates.

Histopathological effect of the Oils

A total number of 40 albino rats were used to determine whether any of these two oils will be toxic to humans. Five animals in each group of three different groups and another five as a control group were used for each of the extracts as five replicates. Prior to the experiment, the animals were weighed and stabilized for a period of 7days by giving them water and grower's mash obtained from Guinea feed Nig. Ltd. This was done to ascertain that the animals were apparently healthy. Different concentrations of the oils were administered orally to each of the three groups of rats for a period of 14 days, according to Laurence *et al.* (2002) and Oladunmoye (2007). Clean water and grower's mash were administered to the control group.

During the days of extracts' administration, the animals were observed for clinical presentations like salivation, nervousness, vomiting and diarrhoea and none was observed. After the expiration of fourteen days, the animals were sacrificed and some vital organs; heart, liver, kidney and small intestine were examined for any pathological changes. These organs were fixed in 10% Formal-saline to preserve them before processing. They were later dehydrated in different percentage (50%, 70%, 90% and 100%) of ethanol (Analar) for 1½ hours each. After, they were cleared with 100% xylene and left for 2hours to remove any remnant alcohol and then impregnated in liquid wax for 2hours for embedding. These embedded tissues were sectioned using microtome machine and sections were mounted on clean microscopic slides and stained with haematoxylin-eosin (Baker *et al.*, 2001). Excess stains were removed under running tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The slides were dry in oven at 40°C and then placed under the microscope with a digital camera connected to a computer system which was examined by a Histopathologist. The photomicrographs taken were interpreted according to the methods of Baker *et al.*, 2006.

Statistical Analysis of Data

The experimental design used is complete randomized block and data obtained from this study were subjected to analysis of variance. Each treatment was replicated five times and treatment means were compared

using Duncan New Multiple Range Test (DNMRT) at 5% level of Significance on computer aided SPSS version 17.

RESULTS

There is a clear distinction in the antifungal activities of these oils as shown from the values of the zones of inhibition and MIC in Tables 1&2. For all the fungal species tested, the essential oil of *C. citratus* has lower MIC than that of *K. ivorensis* and higher diameter zones of inhibition, conferring a higher antifungal activity on the essential oil of *C. citratus*.

Histological examinations of the stained sections of the small intestines, liver, kidney and the heart of the experimental animals did not show any level of abnormality in the groups fed with different concentration of essential oil of *C. citratus* indicating that the toxicological effect of this extract is near zero. Also there were no appreciable abnormal features seen in the group fed with the diluted oil of *K. ivorensis*. However, there were observable abnormalities resulting from the ingestion of the undiluted and high concentration of the oil of *K. ivorensis*. The intestinal abnormalities seen in the group fed with the full strength included; extreme washing of the intestinal villi of one end, slight necrosis at the other end with the finger like projections still in place. Those fed with half strength of the extract showed reduced necrosis of the extreme end, without washing of the intestinal villi and finger like projections also being in place. These abnormalities were absent in the control group.

In addition, the full and half concentrations of this oil used, showed various degrees of toxicity in the liver, heart and kidney of the animals. The kidney sections showed the presence of hyaline cast, varying degree of cellular infiltration in the glomeruli and slight tubular necrosis, while the control group showed presence of hyaline cast with normal kidney nephron. The histological sections of the liver showed depletion of sinusoids with hepatic washing of cells leading to the red colouration of the liver cells. There is slight necrosis of centre sinusoids in the full strength unlike in the half strength. There were inflammation of cardiac myocytes, infiltration of cells, high dilation of heart muscular strata and extreme necrosis seen in the histological sections of the heart (Plates 1-4).

CONCLUSION

Both essential oils have remarkable antifungal activities against the tested fungi. However, essential oil of *C. citratus* showed a better antifungal activity over that of *K. ivorensis*. This study agrees with earlier work done by Nguetack *et al.*, 2005 who found out that the essential oil of *C. citratus* exhibited antifungal activity against fungal disease of Rice. And also, agrees with the work of others such as Nwadiaro *et al.*, 2007 and Sonker *et al.*, 2014 who had work on the antifungal activities of the essential oil of *C. citratus*. Also, Onifade, 2007 found out that the essential oil of *K. ivorensis* possess antiaflatoxin properties against the production of aflatoxin in *Aspergillus flavus* and *A. parasiticus*.

From this work the diameter zones of inhibition as shown in Table 1, doubles in the oil of *C. citratus* as against the oil of *K. ivorensis* and far outweigh that of Fulcin used as positive control. This shows that the antifungal property of *C. citratus* is superlative in this work. In addition, the histological studies, also showed that the essential oil of *C. citratus* appears non-toxic to internal organs of Laboratory animals, while that of *K. ivorensis* showed defects on some organs of albino rats especially at higher concentrations. This then makes the oral use of the oil of *C. citratus* safe while the oral use of the essential oil of *K. ivorensis* calls for caution.

Table 1: Diameter of Zone of Inhibition (mm) induced by the oils *Cymbopogon citratus*, *Khaya ivorensis* and Fulcin on Fungi

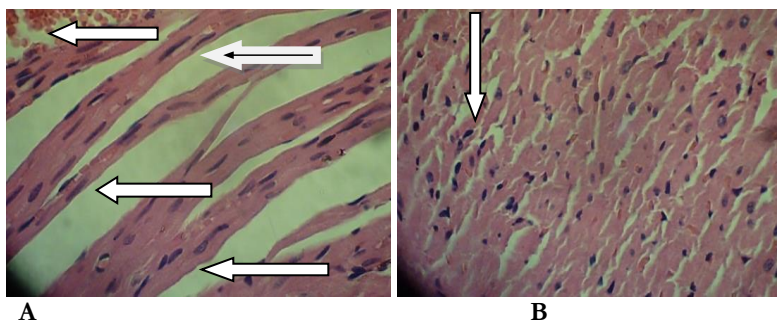
Test Organisms	<i>C. citratus</i>	<i>K. ivorensis</i>	Fulcin (150µg)
<i>Candida albicans</i>	6.40±0.114 ^c	2.36 +0.055 ^b	1.60 ± 0.418 ^a
<i>Trichophyton tonsurans</i>	7.30±0.447 ^d	3.52 + 0.084 ^c	2.40±0.652 ^b
<i>Microsporum canis</i>	5.60 ± 0.652 ^c	2.08 + 0.084 ^b	1.30±0.477 ^a
<i>Epidermophyton floccosum</i>	5.20 ± 0.447 ^c	2.16 + 0.114 ^b	1.24 ± 0.167 ^a

NB: Values are means of five replicates, ± Standard deviation
Values with the same superscript on the same row are not significantly (p≤0.05) different

Table 2: Minimum Inhibitory Concentration (mg/ml) of the Oils on Test Organisms

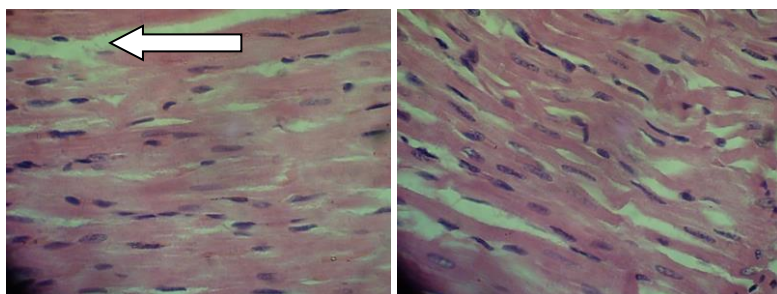
Test organisms	<i>C. citratus</i> Oil	<i>K. ivorensis</i> seed oil
<i>Candida albicans</i>	0.21 ± 0.022 ^b	0.33 ± 0.027 ^c
<i>Trichophyton tonsurans</i>	0.14 ± 0.055 ^a	0.25 ± 0.055 ^b
<i>Microsporum canis</i>	0.15 ± 0.027 ^c	0.18 ± 0.005 ^d
<i>Epidermophyton floccosum</i>	0.12 ± 0.017 ^a	0.30 ± 0.017 ^b

Notes: Values are means of five replicates, ± Standard deviation
Values with the same superscript on the same row are not significantly (p≤0.05) different



A

B

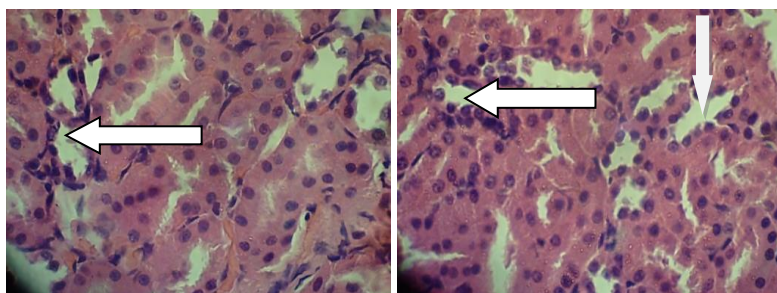


C

D

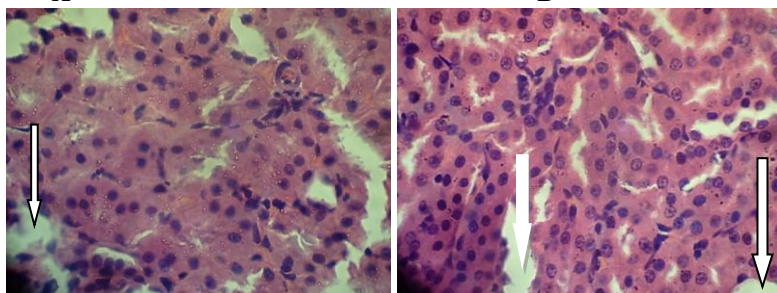
Plate 2: Photomicrograph of the histological sections of the heart of rats fed with *K. ivorensis* oil

Key: Arrowed = Pathological changes seen in the sections.
A= Rats fed with undiluted oil.
B= Rats fed with 50mg/ml of oil.
C = Rats fed with 25mg/ml of oil.
D = Rats used as control (not given oil)



A

B

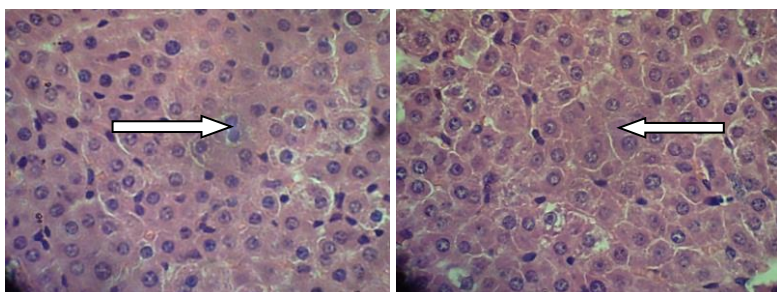


C

D

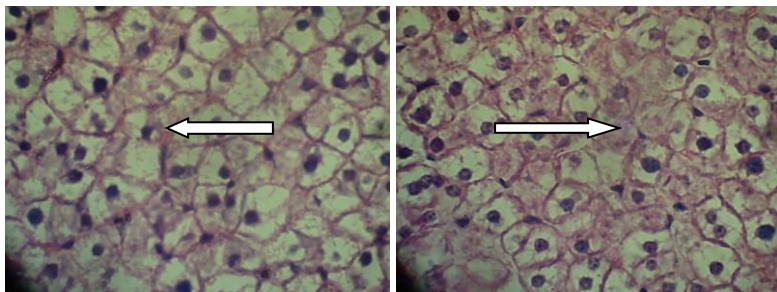
Plate 3: Photomicrograph of the histological sections of kidney of rats fed with *K. ivorensis* oil

Key: Arrowed = Pathological changes seen in the sections.
A= Rats fed with undiluted oil.
B= Rats fed with 50mg/ml of oil.
C = Rats fed with 25mg/ml of oil.
D = Rats used as control (not given oil)



A

B



C

D

Plate 1: Photomicrograph of the histological sections of the liver of rats fed with *K. ivorensis* oil

Key: Arrowed = Pathological changes seen in the sections.
A= Rats fed with undiluted oil of *K. ivorensis*
B= Rats fed with 50mg/ml of oil.
C = Rats fed with 25mg/ml of oil.
D = Rats used as control (not given oil)

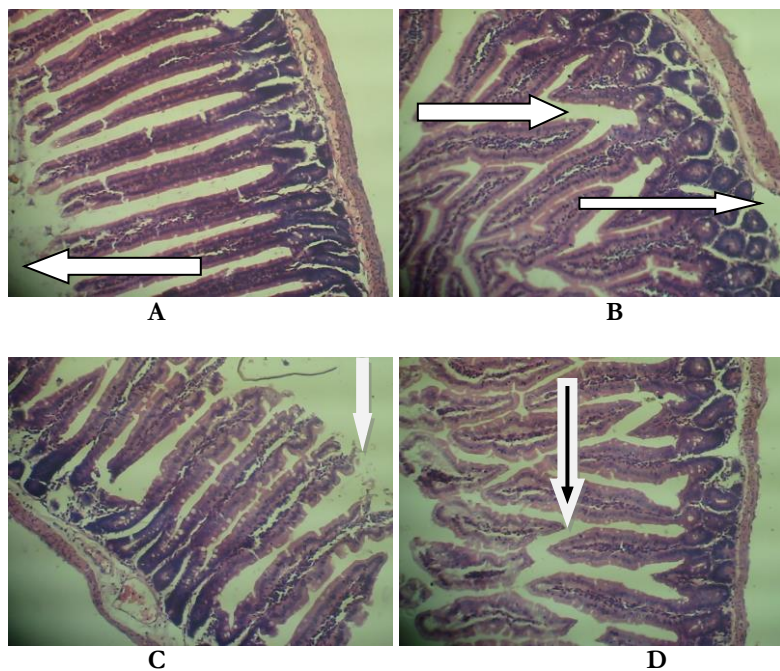


Plate 4: Photomicrograph of the histological sections of small intestine of rats fed with *K. ivorensis* oil

Key: Arrowed = Pathological changes seen in the sections.

A= Rats fed with undiluted oil.

B= Rats fed with 50mg/ml of oil.

C = Rats fed with 25mg/ml of oil.

D = Rats used as control (not given oil)

REFERENCES

- Al-Burtamani SK.S, Fatope MO, Marwah RG, Onifade AK. and Al-Saidu SH. Chemical composition, antibacterial, and antifungal activities of the essential oils of *Haplophyllum tuberculatum* from Oman. *Journal of Ethnopharmacology* 96 (2005): 107-112.
- Al-varez-Castellanos PP, Bishop CD and Pascual-Villaloboa MJ. Antifungal activity of essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* 57 (2001): 99-102.
- Baker FJ, Breach MR and Chris P. Medical Laboratory Science Chris Publisher (2006) United Kingdom. Pp487.
- Baker FJ, Silvertown RE and Pallister CJ. Introduction to Medical Laboratory Technology 17th edition (2001) Arnold Pp 448.
- Bouchra C, Achouri M, Idrissi HLM, Hmamouchi M. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. Fr. *Journal of Ethnopharmacology* 89. 1 (2003):165-169.
- Cho J, Kim M and Lee H. Fungicidal activities of 67 herb-derived oils against six phytopathogenic fungi. *Agricultural Chemistry and Biotechnology* 45.4 (2002): 202-207.
- Fatope MO, Marwah RG, Onifade AK., Ochai JE and Al-Mahrogi YK. NMR Analysis, Antifungal and Insecticidal activities of Oman Dill herb oil. *Pharmaceutical Biology*, 44.1 (2006): 44-49.
- Jirovetsz L, Buchbauer G, Eller G, Ngassoum MB and Maponmetsem PM. Composition and Antimicrobial activity of *Cymbopogon giganteus* (Hochst.) Chiov. Essential flower, Leaf and Stem Oils from Cameroon (dagger). *Journal of Essential Oil Research* 12 (2007): 207-212.
- Laurence DR, Bennett PN, Brown MJ. Clinical Pharmacology. 9th Edition. (2002): Churchill Livingstone Edinburgh, London. Pp. 719.
- Letessier MP, Svoboda KP and Walters DR. Antifungal activity of the essential oil of Hyssop (*Hyssopus officinalis*). *Phytopathology* 149 (2001): 673-678.
- Nguefack J, Somda I, Mortensen CN and Amvam ZPH. Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (*Oryzae sativa* L.). *Seed science and Technology* 33 (2005): 397-407.
- Nwadiaro PO and Nwachukwu I. Inhibition of Pathogenic fungi by ethnobotanical extracts of *Cymbopogon citratus*, *Ceiba pentandra* and *Loranthus bengwelenis*. *Nigerian Journal of Biotechnology* 18 1-2 (2007): 61-65.
- Odugbemi T. Outlines and Pictures of Medicinal Plants from Nigeria. University of Lagos Press (2006) Lagos Pp283.
- Oladunmoye MK. The Immunostimulatory Effect of Ethanolic Extract of *Cassia alata* on immune system of Albino Rats Dosed with *Staphylococcus aureus* (NCIB8588). *Journal of Pharmacology and Toxicology* 2.2 (2007): 200-204.
- Onifade AK. Effect of essential oils of some Meliaceae plants on aflatoxin production and growth of *Aspergillus parasiticus*. *Journal of Food Technology* 4.4 (2007): 322-324.
- Onifade AK, Fatope MO, Deadman ML and Al-Kindy SML. Nematicidal activity of *Haplophyllum tuberculatum* and *Plectranthus cylindraceus* oils against *Meloidogyne javanica*. *Biochemical systematic and Ecology* 36 (2008): 697-683.
- Robinson CM, Sinclair JF, Smith MJ and O'Brien AD. "Shiga toxin of enterohemorrhagic *E. coli* type O157:H7 promotes intestinal colonization" *Proc. Natl. Acad. Sci, USA*, 20103 25 (2006): 9667-9672.

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