International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 2 Issue 1, January 2015

ISSN: 2394-4404

Antibacterial And Toxicological Properties Of Essential Oils Of Cymbopogon Citratus Stapf And Khaya Ivorensis Chev

Fadipe, D. O.

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

Onifade, A. K.

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

Abstract: ***Aim: There is need to scientifically proof the traditional claim of the medicinal use of these plants materials and to add that the use of the essential oils may be better than crude extracts of these plants materials.***

Study Design: 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.

Methodology: The essential oils of the leaves of Cymbopogon citratus and seeds of Khaya ivorensis were extracted using Petroleum ether as solvent in Soxhlet apparatus. The antibacterial activities of both oils were determined in vitro, against six bacterial species. Laboratory animals were fed orally with these oils for some days to determine their level of toxicity when ingested.

Result: Minimum Inhibitory Concentration (MIC) of C. citratus were found to range from 0.38mg/ml to 1.46mg/ml, while that of K. ivorensis varied from 0.36mg/ml to 1.32mg/ml. When different concentrations of these two essential oils were administered orally to albino rats for 14days to test their toxicity and biosafety, there were no significant changes (P≥0.05) in the haematological parameters and liver enzymes estimation done using blood specimens from the used rats.

Conclusion: Both oils possess antibacterial activities against the used organisms. There appears to be no toxic effect in the use of the essential oil of Cymbopogon citratus, while the essential oil of the seeds of Khaya ivorensis must be used with caution when to be used orally.

Keywords: Essential oil, in vitro, antibacterial properties, Minimum inhibitory concentration, Toxicity.

I. INTRODUCTION

Over the years, there have been alarming reports of multiple drug resistance in medically important strains of bacteria and fungi (Ozumba, 2003; Aibinu *et al.*, 2004). The persistent increase in antibiotic-resistant strains of organisms has led to the development of more potent synthetic antibiotics such as the third and fourth generations of cephalosporins and the quinolones by pharmaceutical companies. These new antibiotics are costly and are not affordable particularly in developing countries and therefore make compliance to the required dosage difficult. There is therefore the strong need for continuous search for new, effective and affordable antimicrobial drugs. Local medicinal plants provide a source of new possible antimicrobial drugs.

Essential oils are volatile compounds which are extracted or hydrodistilled from odorous (aromatic) plant materials. The oil is basically essential because of its unique odour or scent which is regarded as the essence of the plant (Onifade, 2010). Aromatic plants and their products, particularly essential oils, are becoming more important and numerous essential oils have been found to display a whole spectrum of biological activities.

Cymbopogon citratus, otherwise known as lemon grass ("kooko-oba" or "ewe tea" in Yoruba) and other *Cymbopogon* spp belong to the family Poaceae. It is a tall, coarse grass with a strong lemon taste used for cooking, medicinal tea and

ISSN: 2394-4404

potpourri. Lemon grass stalks are commonly used in the cuisines of Africa, the Middle East and South East Asia. *Cymbopogon citratus* is a native of Sri Lanka and South India and is now widely cultivated in the tropical areas of America and Asia. The oil is used as a culinary flavouring, a scent and medicine. A tea made from the leaves of West Indian lemon grass has been used to treat fevers, colds and upset stomachs (Hatch, 1995).

Khaya ivorensis, also known as African mahogany ("Oganwo" in Yoruba), belongs to the family Meliaceae of the class Magnoliopsidae and kingdom Plantae (Bell and Hemsley, 2000). Lagos mahogany (as fondly called), is a tall forest tree with buttressed trunk in the Meliaceae family. It is found in Angola, Cameroon, Cote-d'ivoire, Gabon, Liberia, and Nigeria where it grows primarily in lowland tropical rainforests. It is now being threatened by habitat loss. There are over 1, 400 species in the family Meliaceae, which are mostly trees and rarely shrubs. Members of this plant family are generally used as timbers, which are moderately hard and are suitable for furniture. Notably among the family is *Azadirachta indica* Linn, also called neem ("Dogonyaro" in many parts of Nigeria), which is of tremendous medicinal importance. Essential oil extracted from neem is efficacious in the treatment of sores and ulcers.

Khaya ivorensis is greatly used as timber and little is known about its extracts especially the essential oil of the seed. The present study was carried out to determine the antibacterial properties of the essential oils of these plants against the selected bacterial isolates and to establish or otherwise their toxicity if used by humans for medicinal purpose.

II. MATERIALS AND METHODS

A. 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.

B. SOURCE AND MAINTENANCE OF TEST ORGANISMS

The clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Enterococcus faecalis,* and *Salmonella typhimurium* were obtained from different clinical samples of wound swab, high vaginal swab, urine and stool in Adekunle Ajasin University Health Centre, Akungba-Akoko. The viability of the bacterial isolates was maintained by regular transfers unto Nutrient agar slants. The agar slants were kept in the refrigerator until when needed for use.

C. EXTRACTION OF ESSENTIAL OILS FROM PLANT MATERIALS

Five hundred grams (500g) of the seeds of *K. ivorensis* were air-dried for two months, the pericarp was 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 extraction 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.

D. ANTIBACTERIAL SENSITIVITY TESTING OF ESSENTIAL OILS

The agar diffusion technique of Juliani *et al*. (2002) was employed; sterile Nutrient agar (NA) contained in Petri dishes were inoculated with the standardized bacterial 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. In addition 60% Tween-20 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. Thereafter, the diameter of zone of inhibition was measured in millimetre. The experiment was conducted in five replicates.

E. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Minimum inhibitory concentration is defined as the minimum concentration of the extract that will not allow any viable growth or turbidity of the test organism (Cheesbrough, 2004).The Agar dilution Technique was used to determine the minimum inhibitory concentration of the oils. The extracts were incorporated into Molten Nutrient Agar to make final concentration ranging from 3.1mg/ml to 6.25, 12.5g and 25mg/ml. The untreated but inoculated agar plates (without oil extracts) served as controls. Each test organism was radially streaked on the agar plate and incubated at 37°C for 24 h and clearance zones around any of the wells were noted and measured in millimetres. The lowest concentration of the oil extracts that inhibited the growth of the test organisms was recorded as MIC.

F. TOXICOLOGICAL TESTING 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

ISSN: 2394-4404

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 blood samples were collected to test for blood parameters; Packed Cell Volume (PCV), white blood cell (Total WBC) Erythrocyte sedimentation rate (ESR) and haemoglobin estimation (Hb) according to Cheesbrough (2004). Three liver enzymes, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were quantified in the Blood samples of the animals to detect the level of toxicity to the livers of the used animals.

G. 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.

III. RESULTS

The yields of the essential oils as percentage of plant material weight were 0.80% and 20% for *C. citratus* leaves and seeds of *K.ivorensis* respectively after the extraction. Essential oil of *C. citratus* inhibited the growth of *S.aureus* with a diameter of zone of inhibition of 7.40mm, *E.coli* with the diameter of zone of inhibition of 10.60mm, *P.aeruginosa* with a diameter of zone of inhibition of 8.20mm, *K.oxytoca* with diameter of zone of inhibition of 5.80mm, *E.faecalis* with a diameter of zone of inhibition of 5.80mm and *S.typhimurium* with a diameter zone of inhibition of 7.60mm. These results are shown on Table 1.

Test Organisms	*C. citratus*	*K. ivorensis*	Ofloxacin (5µg)
Staphylococcus aureus	7.40 ± 0.547^{a}	10.04 ± 0.261^{c}	8.50 ± 0.500^{b}
Escherichia coli	10.60 ± 1.917^{d}	12.60 ± 0.158^{e}	10.40 ± 0.570^{c}
Pseudomonas aeruginosa	8.20 ± 0.570^{b}	5.36 ± 0.114^{a}	7.10 ± 0.547^{b}
Klebsiella oxytoca	5.80 ± 0.837^{a}	6.60 ± 0.100^{b}	10.50 ± 0.500^{c}
Enterococcus faecalis	5.80 ± 0.837^{a}	10.50 ± 0.084^{b}	12.40 ± 1.140^{c}
Salmonella typhimurium	7.60 ± 1.140^{b}	15.04 ± 0.167^{d}	14.90 ± 0.894^{c}

Values are means of five replicates, ± Standard deviation
Values with the same superscript along the same column are not significantly different ($p \leq 0.05$)

Table 1: Diameter of Zone of Inhibition (mm) induced by oils of Cymbopogon citratus, Khaya ivorensis and Ofloxacin on bacteria

The essential oil of *K. ivorensis* also demonstrated good antibacterial activities against both Gram-positive and Gram-negative bacteria used for this study, with diameter of zone of inhibition of 10.04mm against *S. aureus*, *E. coli* with mean diameter of zone of inhibition of 12.60mm, *K. oxytoca* with diameter of zone of inhibition of 6.60mm. *E. faecalis*, *P. aeruginosa* and *S. typhimurium* have diameter zones of inhibition of 10.50mm, 5.36mm and 15.04mm respectively (Table 1).

The Minimum Inhibitory Concentration is the least concentration of the essential oil needed to completely inhibit the growth of the test organism. It was observed that the essential oil of *K. ivorensis* had minimum inhibitory concentration (MIC) of 0.37mg/ml for *S. aureus* and 1.32mg/ml for *P. aeruginosa* while the MIC of the oil of *C. citratus* for the same organisms is 1.46mg/ml and 0.97mg/ml respectively. While the essential oil of *C. citratus* had lower MIC of 0.46mg/ml for *E. coli*, 0.38mg/ml for *E. faecalis* as against 0.56mg/ml and 0.63mg/ml respectively for the same organisms by the oil of *K. ivorensis.* This makes the antibacterial activity of both essential oils to compare favourably with one another as shown in Table 2. But there is noticeable disparity in the MICs of both oils on *Staphylococcus aureus* and *Salmonella typhimurium* as presented.

Test organisms	*C. citratus* Oil	*K. ivorensis* oil
Staphylococcus aureus	1.46 ± 0.055^{d}	0.37 ± 0.01^{c}
Escherichia coli	0.46 ± 0.022^{d}	0.56 ± 0.084^{e}
Pseudomonas	0.97 ± 0.027^{c}	1.32 ± 0.027^{b}
aeruginosa	0.70 ± 0.071^{e}	0.76 ± 0.005^{f}
Klebsiella oxytoca	0.38 ± 0.027^{c}	0.63 ± 0.027^{d}
Enterococcus faecalis	1.46 ± 0.055^{d}	0.36 ± 0.055^{e}
Salmonella typhimurium		

Values are means of five replicates, ± Standard deviation
Values with the same superscript along the same column are not significantly different ($p \leq 0.05$)

Table 2: Minimum Inhibitory Concentration (mg/ml) of essential oils on test organisms

A. HAEMATOLOGICAL PROPERTIES OF BLOOD SAMPLES OF EXPERIMENTAL RATS

The haematological parameters, after the administration of the essential oil of *C.citratus* did not show marked difference from that of the control group as seen in Table 3, while the administration of the oil of *K.ivorensis* showed varied difference in the value of Packed Cell Volume (PCV) Red blood cell (RBC) count and the Haemoglobin (Hb) estimation, whereas the white blood cell (WBC) count and the Erythrocyte sedimentation rate (ESR) appeared as shown in Table 4.

ISSN: 2394-4404

TREATMENT	ESR(mm/1h)	PCV (%)	RBC(10^{12}/L)	WBC mm^{-3})	Hb (g/dl)
A (CONTROL)	1.00 ± 0.000[b]	40.67 ± 0.578[c]	6.79 ± 0.012[a]	6200 ± 0.000[d]	13.63 ± 0.057[d]
B (UNDILUTED)	1.17 ± 0.298[c]	39.00 ± 0.000[a]	7.12 ± 0.029[b]	4833 ± 57.74[a]	13.25 ± 0.050[a]
C (50mg/ml)	0.80 ± 0.288[a]	40.00 ± 0.000[b]	7.05 ± 0.042[b]	5683 ± 28.87[c]	13.43 ± 0.057[b]
D (25mg/ml)	1.17 ± 0.298[c]	40.33 ± 0.577[b]	6.84 ± 0.053[a]	5670 ± 57.74[b]	13.47 ± 0.057[b]

Values are means of five replicates, ± Standard deviation
Values with the same superscript along the same column are not significantly different ($p \leq 0.05$)
Key:
A: Given growers mash and water only
B: Growers mash mixed with undiluted oil
C: Growers mash mixed with 50mg/ml of oil
D: Growers mash with 25mg/ml of oil.

Table 3: Effects of Administration of C. citratus Oil on Haematological Parameters of Albino Rats

TREATMENT	ESR(mm/1h)	PCV (%)	RBC(10^{12}/L)	WBC mm^{-3})	Hb (g/dl)
A (CONTROL)	0.80 ± 0.45[b]	37.6 ± 2.59[a]	6.460 ± 0.85[a]	6480 ± 0.50[a]	12.1 ± 1.01[a]
B (UNDILUTED)	1.00 ± 0.71[c]	37.0 ± 2.55[a]	6.92 ± 0.28[c]	9060 ± 1.89[c]	12.3 ± 0.62[b]
C (50mg/ml)	0.60 ± 0.55[a]	37.6 ± 2.51[a]	6.82 ± 0.45[b]	8880 ± 2.01[b]	12.6 ± 0.054[c]
D (25mg/ml)	0.80± 0.45[b]	37.2 ± 1.92[a]	6.38 ± 0.36[a]	8880 ± 1.31[b]	12.2 ± 0.60[a]

Notes:
Values with the same superscript along the same column are not significantly different ($p \leq 0.05$)
Values are means of five replicates ± Standard deviation
Key:
A: Given growers mash and water only
B: Growers mash mixed with undiluted oil
C: Growers mash mixed with 50mg/ml of oil
D: Growers mash with 25mg/ml of oil.

Table 4: Effects of Administration of K. ivorensis oil on haematological parameters of Albino rats

B. LIVER ENZYME OF THE BLOOD SAMPLES

The albino rats fed with the essential oil of *K. ivorensis* were investigated for possible changes in their liver enzymes; to determine the level of the hepatic toxicity of this oil after oral administration. The enzymes estimated are Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). The results obtained for these three parameters both for the control group and the groups fed with the oil did not show any significant difference as shown in the Table 5.

TREATMENT	Aspartate Aminotransferase (AST) U/L	Alanine Aminotransferase (ALT) U/L	Alkaline Phosphatase (ALP) U/L
A (CONTROL)	84.2±10.13[c]	62.6 ± 3.50[c]	2.00 ± 0.71[a]
B (UNDILUTED)	70.6 ± 8.96[a]	47.6 ± 24.3[a]	2.00 ± 1.00[a]
C (50mg/ml)	78.0 ± 15.6[b]	55.0 ± 19.3[b]	2.00 ± 0.84[a]
D (25mg/ml)	79.0 ± 17.1[b]	55.0 ± 16.1[b]	2.00 ± 1.00[a]

Values are means of five replicates ± Standard deviation
Values with the same superscript along the same column are not significantly different ($p \leq 0.05$)

Table 5: Effects of Administration of K. ivorensis Seed Oil on Liver Enzymes of Albino Rats

IV. DISCUSSION

The family *Poaceae* to which *C. citratus* belongs is a widely distributed plants-family among which many contain aromatic oils which are gaining use in Aromatherapy. The information presented in this study on the antibacterial and toxicological properties of its essential oil provides incentive for its continuous and cautions safe use in humans. This is in tandem with the work of Mahdi *et al.* (2012) where essential oil of lemon grass was used as preservatives for cream-filled cakes and pastries. Contaminating organisms, such as, S. *aureus, E. coli, Candida albicans, Bacillus cereus* and *Sal. typhimurium* were all inhibited in the baked cake, making this oil a safe natural preservative and food spoilage inhibitor. There is an additional claim of its antihypertensive and anti-inflammatory properties making it broad-spectrum in activity. The result of the haematological parameters of the laboratory animals used for the toxicological test revealed that there were little or no significant changes in the body physiology after oral ingestion of the essential oil of *C. citratus*. These largely confirm that the use of the crude extract from the ancient times and up on till now, is safe. In the case of *K. ivorensis*, the result of this study proved that essential oil of the seeds can be an effective antimicrobial agent against some bacteria but its toxicity and bio safety raises questions. In the family *Meliaceae*, only the essential oil of neem has been adequately studied and there will be need to explore the over 1000 species of plants in this family for possible medicinal value.

While medicinal plants provide possible alternatives to commercial antimicrobials that are being resisted by medically important bacteria and fungi, Nigerian Government should be committed to the preservation of our natural forest and re-afforestation of plants lost due to urbanisation and timber use otherwise, medicinal plants will gradually go into extinction.

REFERENCES

[1] Aibinu, I., Adenipekun, E. and Odugbemi, T. (2004). Emergence of quinolones resistance amongst Escherichia coli strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. Nigerian Journal of Health and Biomedical Sciences, 3(2):73-78.

ISSN: 2394-4404

[2] Bell, P. R. and Hemsley R. A. (2000). The kingdom Embryophyta (cont.): division Tracheophyta, part 4. In Green Plants: Their origin and diversity. 2nd Ed Cambridge University Press, UK. Pp349.
[3] Cheesbrough, M. (2004). District Laboratory Practice in Tropical Countries. Part 2 Low price edition, Cambridge University Press, U.K. Pp434.
[4] Hatch, E. (1995): Asthma, inhaled oxidants, and dietary antioxidants. American Journal of Clinical Nutrition, 61 (3): 625S-630S.
[5] Jirovetsz, L., Buchbauer, G., Eller, G., Ngassoum, M. B and Maponmetsem, P. M. (2007). Composition and Antimicrobial activity of Cymbopogon giganteus (Hochst.) Chiov. Essential flower, Leaf and Stem Oils from Cameroon. Journal of Essential Oil Research, 12: 207-212.
[6] Juliani Jr, H. R., Biuruun, F., Koroch, A. R., Olivia, M. M., Demo, M. S., Trippi, V. S. and Zygadlo, J.A. (2002). Chemical constituents and antimicrobial activity of the essential oil of Lantana xenica. Planta Medica,68:762-764.
[7] Laurence, D. R., Bennett, P. N., Brown M. J. (2002). Clinical Pharmacology. 9thEdition. Churchill Livingstone, Edinburgh, London. Pp. 719.
[8] Mahdi, V., Somayeh, T. K., Mohammad, R., Shams, A., Mahnaz, K., Hossein, J., Mohammad, R. F. and Abolfazl, N.T. (2012). Antimicrobial activity of Lemon grass (Cymbopogon citratus DC Stapf.) essential oil against food-borne pathogens added to cream-filled cakes and pastries. Journal of Essential Oil Research, 24 (6): 579-582.
[9] Nguefack, J., Somda, I., Mortensen, C. N., and Amvam, Z. P. H. (2005). Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (Oryzae sativa L.). Seed science and Technology, 33:397-407.
[10] Oladunmoye, M. K. (2007). 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): 200-204.
[11] Onifade, A. K. (2010). Phytotheraphy: A random walk through a random forest? Inaugural Lecture
[12] Series 57, Federal University of Technology, Akure, Nigeria, Delivered on Tuesday 16th
[13] February 2010, p32.
[14] Onifade, A. K., Fatope, M. O., Deadman, M. L., and Al-Kindy, S. M. L. (2008). Nematicidal activity of Haplophyllum tuberculatum and Plectranthus cylindraceus oils against Meloidogyne javanica. Biochemical Systematics and Ecology, 36:697-683.
[15] Ozumba, U. C. (2003). Antibiotic sensitivity of isolates of Pseudomonas aeruginosa in Enugu. Nigeria. African Journal of Clinical and Experimental Microbiology, 4:48-51.