FUDMA Journal of Sciences (FJS)
ISSN: 2616-1370
Vol. 2 No. 2, June, 2018, pp 192 - 202

PRESERVATIVE ACTIVITIES OF *EUCALYPTUS CITRIODORA* ESSENTIAL OIL ON SOME PROCESSED FOODS

***[1]Bukar, A. and [2]Sani, H. M.**
*[1]Department of Microbiology, Bayero University, PMB 3011, Kano
[2]National Board for Technology Incubation, Kano Office
*Correspondence author: abukar.bio@buk.edu.ng; +2348023567064

ABSTRACT
The evaluation of plant constituents for antimicrobial and preservative activities on food products is part of the ongoing search for natural food preservatives. In line with that, detection of phytochemical constituents and evaluation of antimicrobial, GC – MS analysis and preservative properties of *Eucalyptus citriodora* essential oil on boiled white rice and bread was carried out using standard procedures. The results of the phytochemical screening revealed presence of saponins, tannins, flavonoids and phenolic compounds. Antibacterial activity of the *E. citriodora* oil showed highest zone of inhibition diameter against *Shigella* spp (21.66mm) while antifungal activity was more pronounced against *Aspergillus fumigatus* (31mm) using 100% oil. GC – MS analysis of the 100% petroleum ether fraction of the oil revealed compounds, which include cyclohexene and 1 – isopropyl – 2 – methylbenzene. Results of sanitizing activity of the 100% oil revealed 3.12 log count (cfu/g) reduction (bacteria), 3.22 log count (cfu/g) reduction (fungi) for boiled white rice and 1.50 log count (cfu/g) reduction (bacteria), 0.77 log count (cfu/g) reduction (fungi) for bread after 72 hours of storage. Results of organoleptic assessment and general acceptability showed that treatment with 50% w/w of oil revealed 74.4% likeness by judges as compared to 11.1% for control (untreated) after 96 hours (4 days) for boiled white rice and 61.1% likeness by judges as compared to 45.5% for control (untreated) after 96 hours (4 days) for bread. Findings from this study showed that *E. citriodora* essential oil possess antimicrobial and preservative activities on boiled white rice and bread, which makes the oil a candidate for food preservation.

Keywords: *Antimicrobial, Judges, Organoleptic, Preference, Preservative*

INTRODUCTION

The consumer quest for natural means of preserving foods has concentrated attention on the wide range of extremely effective naturally-occurring antimicrobial systems that are employed by animals, plants and microorganisms, with the aim of exploiting some of them in food preparation and preservation (Davidson and Brannen, 1991; Dillon and Board, 1994). Preservation involves the control of growth and rate of metabolic activities of spoilage organisms in food as well as the ability to extend shelf life of preserved foods (Jay, 2003). One of the plant extracts that are extensively used in the cosmetic, perfumery, food and pharmaceutical industry is *Eucalyptus* (Babu and Singh, 2009). *Eucalyptus* is a large genus of the Myrtaceae family which includes over 700 species (Batish *et al.,* 2008). *Eucalyptus* oil is a complex mixture of a variety of monoterpenes, sesquiterpenes, and aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. They are extracted from the foliage of *Eucalyptus* trees and the quantity and strength of the oil vary across *Eucalyptus* species (Batish *et al.,* 2008).*Eucalyptus* oil has been shown to contain very high amounts of 1,8-cineole, which has chemical and physical properties that make it suitable for a range of applications. The most-known compound, terpenoid, gives *Eucalyptus* foliage its characteristic smell. *Eucalyptus* oil is used widely as an ingredient in many general pharmaceutical products (e.g. liniments, inhalants, expectorants) due to its broad biological properties including anti-inflammatory, anti-allergenic, antiasthmatic, anticonvulsant, antiseptic, aquaculture antiviral, anti-bacterial and anti-malarial. It

has also been used as a flavor and aroma enhancer in food and cleaning products and in cosmetic formulations (Gilles *et al.*, 2010). A number of studies have demonstrated the antimicrobial properties of *Eucalyptus* essential oils against a wide range of microorganisms. The essential oils produced by *E. citriodora* are used for medicinal and pharmaceutical purposes (Gilles *et al.*, 2010). Also, *E. citriodora* oil has been shown to have a wide spectrum antifungal activity. Despite the well-reported antimicrobial activity *in vitro*, the food industry has applied *Eucalyptus* essential oils mainly as flavoring agents. Therefore, the aim of this study is to determine the preservative properties of *Eucalyptus citriodora* essential oil on boiled white rice and bread samples.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

The leaves of *Eucalyptus citriodora* were collected from various trees located at Technology Incubation Center, Tarauni Local Government Area, Kano State. Sample was first identified in the field using standard keys and descriptions (Dalziel, 1916*).* Further confirmation and authentication of *E. citriodora* (BUKHAN/0028) was carried out at the Herbarium of the Department of Biological Sciences, Bayero University, Kano.

Extraction of essential oil

The extraction of *E. citriodora* essential oil was carried out using the steam distillation method, where 0.1Kg of fresh leaves and young stems of *E. citriodora* were placed on a perforated plate (Srikrishna and Satyanarayana, 2005), which was located some centimeters away from the extractor bottom. The extractor was then filled with water up to the plate and heated by a hot-plate at 100^{O}C producing water vapor (Singh *et al.*, 2002). After passing over the plant material, the vapor was condensed in a cooling system (Deguerry *et al.,* 2006), collected in a funnel together with the oil and then separated (Betts, 1994). The separated oil was collected in an Erlenmeyer flask (Food and Drug Administration (FDA), 2002) and weighed immediately after collection. The extraction experiment was carried out for a period of 120minutes.

Phytochemical Screening

Phytochemical analysis for the detection of alkaloids, saponins, tannins, flavonoids, phenolic compounds and reducing sugars were carried out according to methods described by Ciulci (1994), Sofowara (1993) and Trease and Evans (2008).

Sources of Microorganisms

Bacteria isolates; *Staphylococcus aureus, Escherichia coli, Salmonella* spp., *Shigella* spp., *Pseudomonas aeruginosa* and *Bacillus cereus* and fungal isolates; *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger*, *Penicillium* spp and *Mucor* spp were isolated from stale foods and drinks such as 'zobo drink', raw beef, boiled rice, bread, and fresh tomatoes. Homogenized food samples were streaked on appropriate media for isolation of the microorganisms. Further microscopic and biochemical characterization of the organisms were carried out according to methods described by Cheesebrough (2002).

Antimicrobial Disc Preparation

Antimicrobial susceptibility was determined by the disc method. Disc of about 6mm diameter were punched from Whatman No.1 filter paper and batches of 100 discs were transferred into Bijou bottles and sterilized in an autoclave at 121^{0}C for 15minutes. Different concentrations of the *E. citriodora* essential oils were prepared using Dimethyl Sulphoxide (DMSO) to obtain the working concentration of 25%(0.25ml of oil + 0.75ml of DMSO), 50% (0.5ml of oil + 0.5ml of DMSO), 75% (0.75ml of oil + 0.5ml of DMSO) and 100% concentration of oil with no addition of DMSO (Lima *et al*., 1993; Shamsuddeen *et al.,* 2014).

Standardization of Innoculum

Inoculum where prepared from the cultures maintained on a slant of nutrient agar (Bacteria) and potato dextrose agar (PDA) for fungal isolates. Density of bacterial suspension was adjusted to 0.5 McFarland standard (Barium sulphate solution) (1.5×10^{8} cfu/ml) as described by Cheesebrough (2002). Aloopful of fungal spores from an overgrown plate was taken and shaken thoroughly in 10ml of 20% Tween 80 solution to arrive at 6.0×10^{5}cfu/ml of the spore suspension (Murugan *et al.*, 2007).

Susceptibility Testing

Agar diffusion technique described by Bauer and Kirby (1996) as demonstrated by Cakir *et al.,* (2004) was employed for antibacterial and antifungal bioassay. The assessment of antibacterial and antifungal activity was based on measurement of the diameter of the inhibition zone formed around the disc.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the active concentrations were determined using the tube

dilution technique described by Akinyemi *et al.* (2005) and Shamsuddeen *et al.* (2014). Appropriate test concentrations were prepared and added to sterile capped test tubes of Mueller Hinton Broth (Bacteria) and Potato Dextrose Agar (fungal isolates) to cover the range of dilutions chosen in duplicate After overnight incubation at 37ºC, the lowest concentration of the extract at which no turbidity was observed and recorded as the Minimum Inhibitory Concentration.

Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC)

Sterile Mueller-Hinton agar plates were inoculated with samples from the MIC tubes that show no visible bacterial growth .The lowest concentration in which no growth occurred on the medium was considered as the MBC (Lima *et al*., 1993)

Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis

GC-MS analysis was conducted on the most active fraction of *E. citriodora* from the bioactivity guided fractionation to determine the molecular weight, IUPAC name and chemical structures of some of the compounds that were identified during the analysis.

Evaluation of sanitizing activity of *E. citriodora* on Boiled white rice

Commercially prepared boiled white rice was purchased from a local vendor at Kabuga, Gwale LGA, and Kano. Five (5) different concentrations of the essential oils were applied on 5g of the rice and bread, each in different sterile plastic container, arriving at a concentration of 0%,(w/v)(control), 25%(w/v), 50%(w/v) and 75%(w/v). Enumeration of Aerobic mesophilic bacteria and fungi was carried out in triplicate determinations. The mean value was taken, and the room temperature was monitored throughout the period of experiment as described by Bukar (2011).

Sensory Evaluation

The setup for the sanitizing activity of *E. citriodora* was replicated and three (3) different concentrations of the essential oil was applied on 5g of rice and bread, each in different sterile plastic container, arriving at a concentration of 0%, (w/v) 25%, (w/v), 50% (w/v). Number of days taken for each treatment to spoil was recorded. Organoleptic parameters namely, taste, texture, odor, and color were assessed by a panel of ten (10) judges. The score were graded on a Hedonic scale and after 120hrs of storage, the treatment most preferred by the judges was determined (Bukar, 2012).

Statistical Analysis

Data generated from the sanitizing activity tests and the scores generated based on the assessment of judges (from the preservative experiments) using Hedonic scale, were statistically analyzed using Analysis of Variance (ANOVA) at 0.05 probability level using the software package developed by Microsoft corporation. Where significant difference was observed, the means separation was carried out using least significant difference (LSD).

RESULTS AND DISCUSSION

Table 1 show that *E. citriodora* essential oil had a yield of 2.3ml per 100g (2.3% v/w) of the leaves. This is in accordance with the studies conducted by Manika *et al*. (2012) who reported relative low yield of 2.5 % of the extracting leaves. The oil was equally observed to be pale yellow in color, with a strong, lemon scent and camphor like taste. Phytochemical analysis of *E. citriodora* oil revealed saponins, flavonoids, reducing sugars, tannins and Phenolic compounds, while alkaloids were not detected (Table 2). This corroborated the work of Batish *et al.* (2008) who reported the presence of these same phytochemicals and attributed the antimicrobial activity of the *E. citriodora* oil to the presence of these compounds. The presence of these phytochemicals gives an indication of their medicinal value. Flavonoids, tannins and phenolic compounds have been found to have antioxidant and antimicrobial properties (Qian and Nihorimbere, 2004).

Table 1: Physical characteristics of Essential oil of *Eucalyptus citriodora*

Variable	
Initial weight of leaf (g)	100
yield of oil (ml)	2.3
Colour	Pale yellow

Table 2: Phytochemical Constituents of Essential oil of *Eucalyptus citriodora*

Parameters	**Inference**

Saponnins	+
Reducing sugars	+
Alkaloids	-
Phenolic Compounds	+
Tannins	+
Flavonoids	+

Key: + = Presence, - = Absence.

Table 3 presents the results of the antimicrobial activity of *E. citriodora oil* against bacterial and fungal isolates. The oil exhibited highest antibacterial activity against *Shigella* spp and *Bacillus cereus* (21mm) and antifungal activity against *Penicillium* spp and *Aspergillus niger* (28mm). The fungal isolates showed the least MIC and MFC (10%) compared to bacterial isolates with 35%. This finding is similar to report by Su *et al.* (2006) where *E. citriodora* was reported to have wide spectrum of antifungal activity. Findings from studies by Fiori *et al*. (2000) also described *Eucalyptus* essential oils and their major constituents to have toxicity against a wide range of microbes including bacteria and fungi, both soil-borne and post-harvest pathogens. They have been found to reduce mycelial growth, and inhibit spore production and germination (Fiori *et al*., 2000; Oluma and Garba, 2004).

Compounds and aroma of *E. citriodora* essential oil identified from the results of the Gas chromatography-mass spectrometry (GC-MS) analysis were found to corroborate the findings of Tayyab (1990). Compounds were found to be hydrocarbons, which were either acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Aromatic compounds like the cyclohexene characterized by sharp smell, and unstable compound which is a precursor of Maleic acid and Adipic acids (Reed *et al*, 2000). Another constituent identified during the GC-MS analysis of *E.citriodora* oil is 1-Isopropyl-2-methylbenzene also called p-Cymene. It is a naturally occurring, volatile, aromatic organic compound, classified as an alkylbenzene related to a monoterpene (Bakkali *et al.,* 2008). It is used as food flavor, pharmaceutical ingredients as it has expectorant efficacy, fungicide, pesticide, analgesic and anti-inflammatory properties according to reports by Selvaraj *et al.* (2002), Bakkali *et al.* (2008), Santana *et al.* (2011) and Xie *et al.* (2012).

Table 3: Zone Diameter of Inhibition (mm) of *E.citriodora* Essential Oil against Bacterial and Fungal Isolates

Organisms	100%	75%	50%	25%	MIC(%)	MBC/ MFC(%)
S. aureus	19.00±2.00	15.66±2.08	10.66±1.15	0.00± 0.00	35	40
Salmonella spp.	19.00±1.50	0.00± 0.00	0.00± 0.00	0.00± 0.00	85	90
Shigella spp.	21.66±0.57	16.00±1.14	12.25±2.06	0.00± 0.00	45	45
P. aeruginosa	19.33±1.15	16.00±1.73	0.00± 0.00	0.00± 0.00	60	60
B. cereus	21.00±4.32	15.00±3.55	9.00±2.16	0.00± 0.00	45	45
E. coli	9.75±1.25	0.00± 0.00	0.00± 0.00	0.00± 0.00	60	60
Penicillium spp	28.25±6.65	26.00±5.88	22.00±3.26	18.50±1.00	15	15
Mucor spp.	26.00±1.63	20.00±1.63	17.75±1.25	14.00±1.63	10	15
A. flavus	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	-	-
A. niger	28.25±1.25	23.75±2.63	19.75±1.70	15.75±0.50	10	10
A. fumigatus	31.00±0.81	29.25±0.95	25.25±2.06	16.25±1.25	10	10

KEY- MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, MFC=Minimum Fungicidal Concentration. Values are mean ± Standard error and each value is mean of three determinations

Figures 1 and 2 showed the result of the sanitizing activity of *E. citriodora* on boiled rice. At 0hr (before treatment), the rice had mean Aerobic Bacterial Count (ABC) of 4.65*log* and Fungal Count (FC) of 4.85*log*. Untreated rice (A) had ABC of 4.76*log* and FC of 4.94*log* after 72hrs of storage indicating an increase in the counts. Treatments with 100% *E. citriodora* (E) reduced the ABC and FC to 1.63*log* (3.22 *log* count reduction) and 1.53*log* (3.12 *log* count reduction) after 3 days of storage.

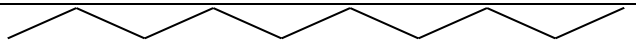
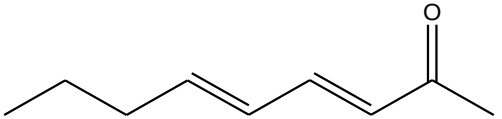
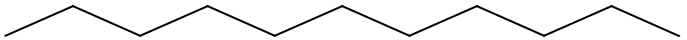
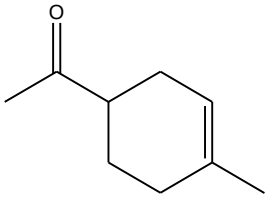
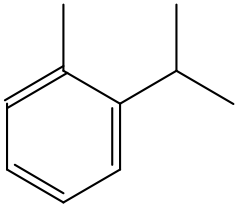
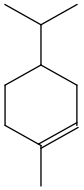
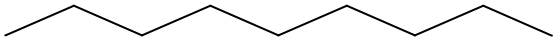
Figures 3 and 4 showed the results of the sanitizing activity of *E. citriodora* on bread. At 0hr (before treatment), the bread had mean Aerobic Bacterial Count (ABC) of 1.59 *log* and Fungal Count (FC) of 1.43 *log*. Untreated bread (A) had significant ($p<0.05$) increase of ABC and FC to 3.47 *log* and 3.50 *log* respectively after 3 days of storage, storage indicating an increase in the counts. Treatments with 100% *E. citriodora* (E) reduced the ABC and FC to 0.00 *log* (1.50 *log* count reduction) and 0.66 *log* (0.77 *log* count reduction) after 3 days of storage. *E. citriodora* prevented the growth of molds on the bread, thus sanitizing it for a period of 3 days. This finding is not surprising owning to the fact that the MIC and MFC of most of the fungal isolates were between 10% and 15% respectively. The results showed that treatments of bread and boiled rice with 100% oil proved the most effective in reducing the counts of both bacteria and fungi followed by 75% and the least was 25% treatments. This shows that the activity of the oil against the microorganisms is dose – dependent, implying increase in activity with corresponding increase in quantity of the oil. Average environmental temperature of the storage area ranged between 28^0C and 30^0C respectively.

Results from the sanitizing activity of boiled rice and bread showed high bacterial and fungal counts at 0hr. This could be due to poor processing as well as pre and post production contamination (Shamsudeen and Ameh, 2008; Bukar *et al.*, 2009; Kawo and Abdulmunin, 2009). Research has shown that the nutritional value of rice is provided by its content in carbohydrate, sugar, energy, fat, protein and a host of nutrients and vitamins (Kaneko *et al.*, 1999). The cooked rice is highly perishable due to its high water activity. It is easily affected by microorganisms which ferment the rice starch under room temperature, which in turn result in food poisoning and infection (Frazier, 1995).

From the results in Tables 5 and 6, the organoleptic assessment showed that the judges rejected the untreated boiled rice after 48hrs (2days) of storage with a percentage likeness of 34.4% compared to 92.2% at 0hr. It also showed that the bread was rejected by judges after 48hrs (2days) of storage with a percentage likeness of 55.5% compared to 97.7% at 0hr.

Treatment C (50% oil) recorded 74.4% percent acceptance (boiled rice) and 61.1% (bread) before the foods were rejected with percentage likeness of 47.7% (boiled rice) and 45.5% (bread) at 102hours. Based on comparison of the treatments, treatment C was found to be most effective, as the judges gave excellent ratings, reporting that treatment C has maintained its moisture, texture as well as lack of objectionable odour at the end of the study.

Table 4: GC-MS analysis of 100% Pet-ether fraction of *E. citriodora* essential oil showing some of the fractions

S/N	Molecular weight	IUPAC Name	Molecular formula	Chemical structure
1.	142	n-Decane	$C_{10}H_{22}$	
2.	138	Nona-3,5-dien-2-one	$C_9H_{14}O$	
3.	156	n-Undecane	$C_{11}H_{24}$	
4.	138	4-Acetyl-1-methylcyclohexene	$C_9H_{14}O$	
5.	134	1-Isopropyl-2-methylbenzene	$C_{10}H_{14}$	
6.	138	1-Methyl-4-1-methylethyl (Cyclohexene)	$C_{10}H_{18}$	
8.	128	n-Nonane	C_9H_{20}	

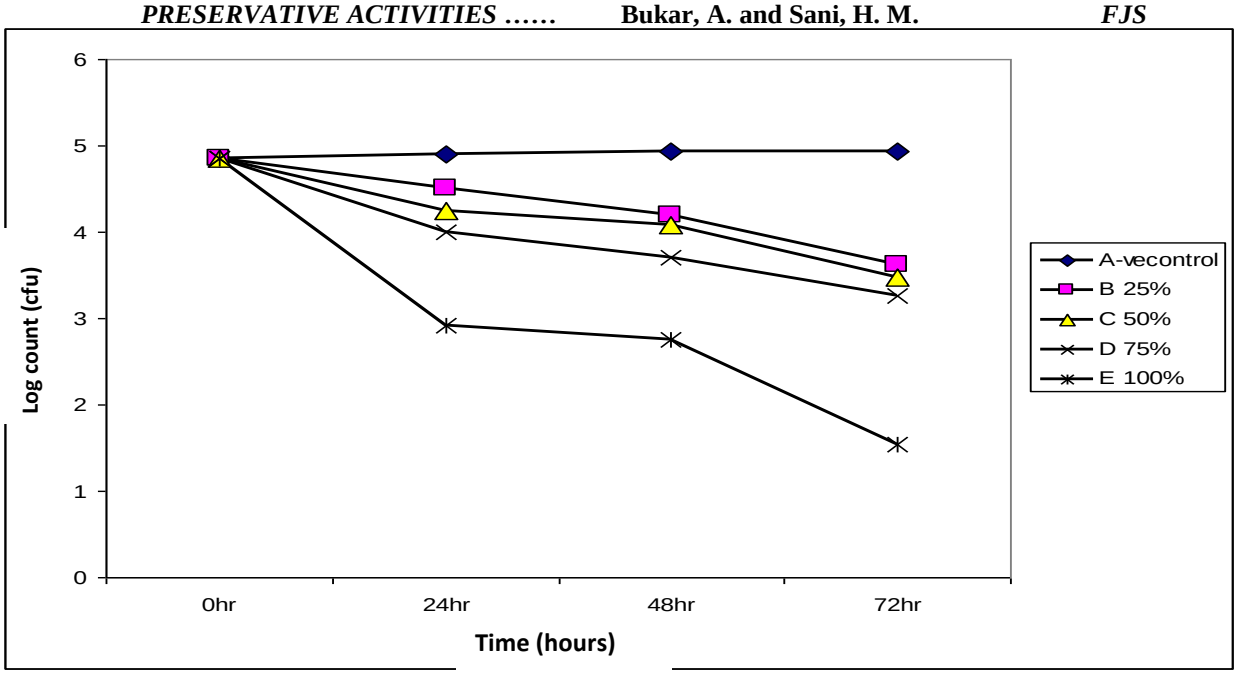


Fig. 2: Sanitizing activity (bacterial count reduction) by E. citriodora on boiled rice stored for 72hrs

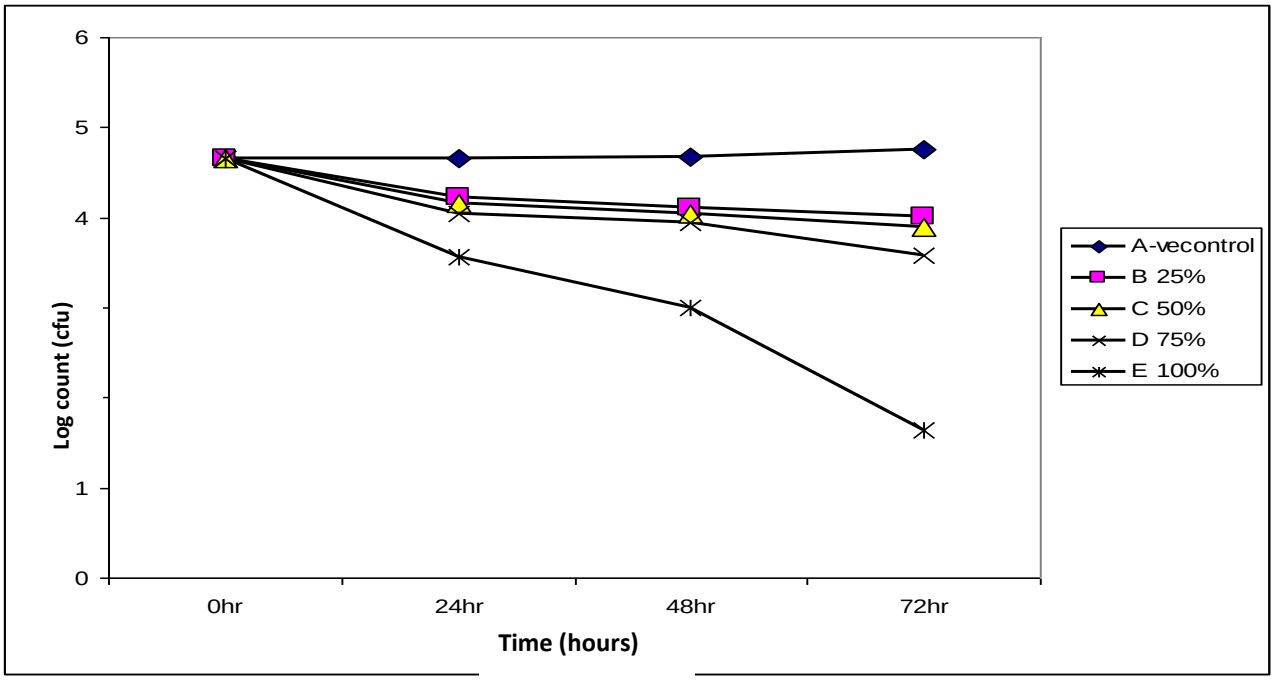
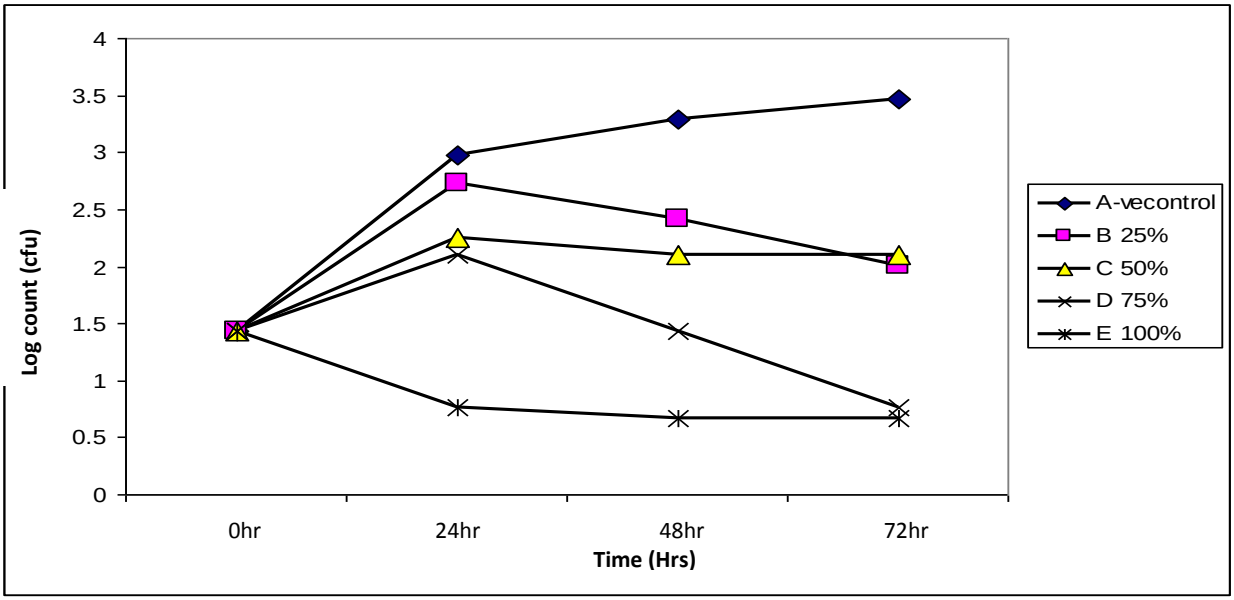


Fig. 4: Sanitizing activity (bacterial count reduction) by E. citriodora on bread stored for 72hrs



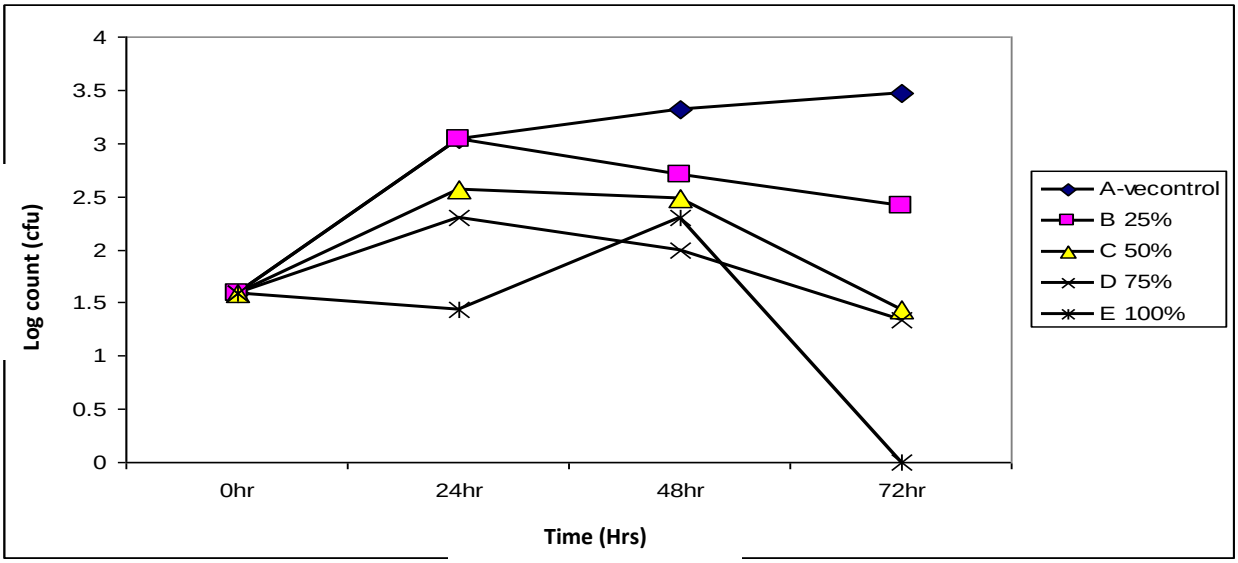


Table 5: General acceptability (percentage likeness) of preserved Boiled rice by Judges (scale 0-9)

No.of hour	A	B	C
0	8.33±0.16(92.2)	7.22±0.22(80.0)	6.50±0.17(72.2)
24	5.50±0.31(61.1)	7.30±021(81.0)	6.8±0.13(75.5)
48	3.10±0.17(34.4)	6.60±0.16(73.0)	7.7±0.15(85.5)
72	1.00±0.00(11.1)	6.40±0.16(71.1)	7.20±0.29(80.0)
96	1.00±0.00(11.1)	1.70±0.15(18.8)	6.70±0.21(74.4)
120	1.00±0.00(11.1)	1.00±0.00(11.1)	4.30±0.21(47.7)

Key: A= untreated food/drink (control), B= 25% *E. citriodora oil*, C= 50% *E. citriodora oil*
SD= Significant difference at 5% probability level. Scale (0-9)

Table 6: General acceptability of preserved Bread by Judges (scale 0- 9)

No. of hours	A	B	C
0	8.80±0.13(97.7)	7.00±0.20(84.4)	7.00±0.25(77.8)
24	6.70±0.26(74.4)	7.00±0.01(77.7)	7.80±0.13(86.7)
48	5.00±0.21(55.5)	6.00±0.21(66.7)	6.40±0.22(71.1)
72	4.70±0.39(52.2)	5.40±0.16(60.0)	5.90±0.10(65.5)
96	4.10±0.23(45.5)	4.80±0.20(53.3)	5.50±0.22(61.1)
120	3.00±0.21(33.3)	3.70±0.26(41.1)	4.10±0.23(45.5)

Key: A= untreated food/drink (control), B = 25% *E. citriodora oil*, C= 50% *E. citriodora oil* Values in parenthesis ( ) are percentage likeness by judges

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study has provided evidence on the antimicrobial, sanitizing and preservative properties of *E. citriodora* essential oil due to the presence of phytochemicals in different combinations. GC-MS analysis revealed the identity of the active bio compounds (terpenes and other aromatic compounds) contained in the fractions. Furthermore, results from the preservative activities of *E. citriodora* oil indicate their ability to reduce microbial count from foods, as well as increase its shelf life with preferred organoleptic parameters. The findings of this study clearly indicates the prospects of *E. citriodora* oil as plant based substitute for chemically synthesized preservatives with higher level of acceptability. However, there is need for further studies on the toxicity and mode of actions of the *E. citriodora* essential oil, with further preservative studies on other food models and systems.

REFERENCES

Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. (2005): Screening of crude extracts of six medicinal plants used in South West Nigerian unorthodox medicine for anti-methicillin resistant activity. *BMC Complimentary Alternative Medicine*, 5:6

Babu, G.D.K. and Singh, B. (2009): Simulation of *Eucalyptus cinerea* oil distillation: a study on optimization of *1,8-cineole* production, *Biochem. Eng. J*. 44 (2009) 226–231

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008): Biological effects of essential oils e a review. *Food and Chemical Toxicology*. 46,446-475.

Batish, D.R., Singh, H.P., Kohli, R.K., Kaur, S. (2008): *Eucalyptus* essential oil as a natural pesticide, *For. Ecol. Manage*. 256: 2166–2174.

Bauer, A.W., Kirby, W.M., Serris, J.C. and Turek, M. (1996); Antibiotic susceptibility testing by a standard single disc method. *AmericanJournal of c;inical Pathology*; 42:493-496

Betts, T.J. (1994) : Evaluation of a "Chirasil-Val" capillary for the gas chromatography of volatile oil constituents, including sesquiterpenes in patchouli oil, *Journal of Chromatography* :664 295–300.

Bukar, A., Uba, A. and Oyeyi, T. I. (2009): Occurrence of some enteropathogenic bacteria in some minimally and fully processed ready - to - eat foods in Kano metropolis, Nigeria. *African Journal of Food Science* 4(2): 32 – 36. www.academicjournals.com/ajfs

Bukar, A. (2012). *Preservative properties of Plant Extracts and oils on some foods*. Lambert Academic Publishing, Germany. ISSBN: 978-3-659-26829-8. www.get-morebooks.com

Cakir, A., Kordali, S., Zengin, H., Izumi, S. and Hirata, T. (2004): Composition and antifungal activity of essential oils isolated from *Hypericum hussopifolium* and *H. heterophyllum*. *Journal of Flavor Fragrance*. 19:62-68

Cheesebrough, M. (2002): *District Laboratory Practice for Tropical Countries*, Part 2. Cambridge University Press, UK. Pp 180-197

Ciulci, I. (1994): Methodology for the analysis of Vegetable Drugs. Chemical Industries Branch, Division of Industrial Operations. *UNIDO*, Romania: 24, 26 and 67

Dalziel, J.M. (1916): *A Hausa Botanical Vocabulary*. T, Fisher Unwin Ltd, London. Pp 5-167

Davidson, P.M. and Brannen, A.L. (1993): *Antimicrobials in Foods*. Marcel Dekker, New York.

Deguerry, F., Pastore, L., Wu, S., Clark, A., Chappell, J. and Schalk, M (2006): The diverse sesquiterpene profile of patchouli, Pogostemoncablin, is correlated with limited number of sesquiterpene synthases, *Archives of Biochemistry and Biophysics* 454: 123–126.

Dillon, V.M. and Board, R.G. (1994): *Natural Antimicrobial Systems and Food Preservation*. CAB International,Wallingford. Oxon.

Fiori, A.C.G., Schwan-Estrada, K.R.F., Stangarlin, J.R., Vida, J.B., Scapim, C.A., Cruz, M.E.S. and Pascholati, S.F. (2000). Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. *J. Phytopathol.* 148, 483–487.

Food and Drug Administration (2002): *Summary of Bioterrorism Act of 2002 and its Effects on U.S. Imports*. Section 305.

Frazier, and Westhoff, (1995): *Food Microbiology*.Tata and McGraw hill edition, Cambridge University Press. Pp 521-540

Gilles, M., Zhao, J., An, M., and Agboola, S. (2010): Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. *Food Chemistry*, 119, 731–737

Jay, M.J. (2003): *Modern Food Microbiology*. 4th ed. Chapman and Hall inc. New York. Pp 187-195

Kaneko, K.I., Hayashidani, H., Ohtomo, Y., Kosuge, J., Kato, M., Takahashi, K., Yasuo, S. and Ogawa, M. (1999): Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot.*, 62(6): 644-649.

Kawo, A.H. and Abdulmumin, F.N. (2009): Microbiological quality of pre-packed sweet sold in metropolitan Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences* 2(1):154-159

Lima, E.O., Gompertz, O.F., Geisbrecht, A.M. and Paulo, M.Q. (1993): *Invitro* antifungal activity of essential oils obtained from Official plants against dermatophytes. *Mycoses.* 36:333-336.

Manika , N., Mishra, P., Kumar, N., Chanotiya, C.S. and Bagchi, G.D., (2012) : Effect of season on yield and composition of the essential oil of *Eucalyptus* citriodora Hook. Leaf grown in sub-tropical conditions in north India. Journal of Medicinal Plants. 6(14) 2875-2879

Murugan, S., Anand, R. Uma Devi, P., Vidhya, N. and Rajesh, K.A. (2007): Efficacy of *Euphobiamilli and E.pulcherrima* on aflatoxin producing fungi (*Aspergillus flavus* and *A.parasiticus*). *African Journal of Biotechnology*. 6(6):718-719.

Oluma, H.O.A., and Garba, I.U. (2004): Screening of *Eucalyptus globulus* and *Ocimumgratissimum* against *Pythium aphanidermatum*. *Nigerian J. Plant Prot*. 21, 109–114

Qian, H. and Nihorimbere, U. (2004): Antioxidant power of phytochemicals from *Psidium guajava* leaf. *Zhejiang Univ. Sci* : 5: 676-683

Reed, S.M. and Hutchinson, J.E.(2000): Green Chemistry in the Organic Technical Laboratory: An environmentally benign synthesis of the adipic acid. *J.Chem.Educ*. 77(12): 1627-1629

Santana, L.J., Quintans-Junior, S.C.H., Cavalcanti, M.G.B., Oliveira, A.G. and Guimaraes, E.S. (2011): p-Cymene reduces orofacial nociceptive response in mice. *Revista Brasiteria Farmacogen*. 21: Pp 1138-1143

Selvaraj, M., Pandurangan, A., Seshadri, K.S., Sinha, P.K., Krishnasamy, V. and Lal, K.B. (2002): Comparison of mesorporous A1-MCM-41 molecular sieves in the production of p-Cymene for Isopropylation of toluene. *Journal of Mocular Catalysis A Chemistry*. 186:173-186

Shamsudeen, U. and Ameh, J.B. (2008): Survey of the possible critical control points during the production of' 'Balangu' in Kano. *Bayero Journal of Pure and Applied Sciences* 1(1): 76-79

Shamsudeen, U., Bassey, E.E. and Sheshe, K.I (2014) :Inhibitory Activity of Clove and Neem Essential oil on the growth of some Molds Isolated from Foods. *International Journal of Applied Sciences and Engineering*. 2(1): 10-12.

Singh, G., Kapoor, I. P. S., Pandey, S. K., Singh, U. K., and Singh, R. K. (2002): Studies on essential oils: Part 10; antibacterial activity of volatile oils of some spices. *Phytotherapy Research*, 16, 680–682

Sofowara, E.A. (1993): *Medicinal Plants and Traditional Medicines in Africa.* Spectrum Books Ltd., Ibadan, Nigeria: 2. 81-85.

Srikrishna, A., Satyanarayana, G. (2005) An enantiospecific total synthesis of patchouli alcohol, A*symmetry* 16: 3992–3997

Su, Y. C., Ho, C. L., Wang, E. I., and Chang, S. T. (2006): Antifungal activities and chemical compositions of essential oils from leaves of four eucalyptus. *Taiwan Journal of Forest and Science,* 21, 49–61

Tayyab, S. (1990): Biotechnology: *A Textbook of Industrial Microbiology*. Biochemical Education, *18*(3), 151–152.

Trease, G.E. and Evans, W.C. (1989): *Pharmacognosy.* 15th edition. Lea and Fabiger, Philadepia.

Xie, G., Chen, N., Soromou, F., Liu, Y., Xiong, Q., Wu, H., Li, H., Feng, H. and Liu, G (2012): p-Cymene protects mice against lipopolysaccharide induced acute lung injury by inhibiting inflammatory cell activation. *Molecules.* 17 Pp 8159-8173