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RESEARCH ARTICLE

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PRESERVATION OF *CITRUS SINENSIS* L (SWEET ORANGE) JUICE USING ESSENTIAL OIL FROM TWO MEDICINAL PLANTS

ABSTRACT:

The preservation of freshly squeezed orange juice with essential oils from the leaves of *Cymbopogon citratus* and *Eucalyptus globulus* was investigated. Extraction was carried out using volatile oil distillator at 80°C. The quality assessments of juice were analyzed after 28 days of storage at 4°C. The volatile constituents of test plants were analyzed via GC-MS. *Aspergillus flavus, Aspergillus niger, Candida* sp, *Saccharomyces cerevisiae, Trichoderma* sp. were isolated from the freshly squeezed orange juice and branded orthodox juices. Antifungal assay of the oil showed *A. flavus* exhibiting highest mean zone of inhibition of 10.58 ± 1.10 mm in *C. citratus*. Quality assessment as days of storage increased indicated an increase in pH values in the control (3.70 to 4.02) while acidity reduced from 3.50 to 3.70 and 3.60 to 3.80 in *C. citratus* and *E. globulus*, respectively. Potential browning increased in *C. citratus* from 1.324 to 1.798 and from 1.215 to 1.764 in *E. globulus* within the same time of storage. Citronelle (9.5%) and Apha-phellande (8.40%), respectively had highest percentage composition of volatile oils in *C. citratus* and *E. globulus*. This study indicated that the volatile oils in the plants have preservative potentials in extending the shelf-life of orange juice.

KEY WORDS:

Natural preservatives, Volatile oil, *Cymbopogon citratus*, *Eucalyptus globulus*, shelf-life

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INTRODUCTION:

Preservation of food for long shelf-life is giving people concern the world over. The use of synthetic preservatives such as Sodium Benzoate, Aspartame, Saccharine, Sodium cyclamate and Ascorbic acid which have lately been queried in the markets because of established information of repercussive effects on human's health tends to provide the urge for an alternative (Abdulmumeen *et al.*, 2012; Al-Shammari *et al.* 2014). Nowadays, many people of the world eat an orange or drink juice every day; because it is one of the best and cheapest sources of vitamin C which is a powerful antioxidant (Barry-Ryan *et al.*, 2009). Oranges are very good source of dietary fibre (pectin), but do contain high amount of minerals (Clemens *et al.*, 2015). Oranges are marketed in many forms including orange juice concentrate, fresh-squeezed juice mixed, mixed fruit juice, smoothie (orange and yoghurt mixtures), and marmalades. According to Sánchez-Moreno *et al.* (2003) the nutritional values of orange

include vitamin C, fibre, potassium, calcium, potassium, and foliate. A reliable orange preservation requires the application of effective preservation methods that can extend the shelf life along with maintaining the nutritional qualities. Plants which are readily available sources of bioactive compounds have been established to possess bioactive compounds against microbes that are responsible for deterioration in foods (Adekunle *et al.* 2005; Aliero and Shehu, 2010; Mohanka and Priyanka, 2014). Essential oil which are highly concentrated, non-water based phytochemicals is one of the bioactive compounds of plants (Gutierrez *et al.*, 2009). Kabara (1991) had stated that essential oil of many plants are widely used in food, health and personal care industries and are classified as generally regarded as safe substances or permitted as food additives. According to the mode of extraction, mostly distillation, essential oils contains a variety of volatile molecules such as Terpenes, phenolic-derived aromatic and aliphatic components. The leaves of *Cymbopogon citratus* and *Eucalyptus globulus* comprises of medicinal plants with high essential oil (Sellar, 2001). Activities of these plants' essential oil against microorganisms have been reported (Adekunle, 2000; Ghalem and Mohamed, 2008; Matasyoh *et al.*, 2011; Martins *et al.*, 2013). Phytochemicals constituent of the leaves of *Cymbopogon citratus* are alkaloids, saponins, tannins, phenols, and flavonoid. (Sofowora, 1982). The aim of this study is to preserve orange juice using essential oil from *Cymbopogon citratus* and *Eucalyptus globulus*.

MATERIAL AND METHODS:

Source of plant materials:

Healthy oranges for this study were plucked from a local farm in Ado-Odo Otta LGA of Ogun state, Nigeria. Branded orange juice such as Chivita orange juice, Fumman orange juice and Dansa orange juice of not more one month from production date were purchased from Yem-yem supermarket on University of Lagos, Akoka campus.

For extraction of essential oil, fresh matured healthy leaves of *Eucalyptus globulus* and *Cymbopogon citratus* (lemon grass) were collected from University of Lagos Botanical garden and samples were authenticated at the Lagos University herbarium, University of Lagos, Lagos.

Oil extractions and assay:

Using a modified method described by Martins *et al.* (2013), essential oil was extracted by hydro-distillation using a Clevenger-type apparatus. Distilled water was mixed with healthy leaves cut into 1 x 2 cm at the ratio of 1: 10 (w/v). Sterile cotton wool wrapped in Aluminium foil was put at the top of the extraction flask to prevent the evaporation of the oil which is volatile. The oil was extracted for 2-3 hrs to ensure complete extraction from each sample. Substantial oil was extracted with a Pasteur pipette from an outlet.

The oil was analysed using Hewlett Packard 6890 Gas Chromatograph linked with Hewlett Packard 5973 mass spectrometer system which was equipped with a HP5-MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 70-240^{o}C at the rate of 5^{o}C min^{-1}. The ion source was set at 240^{o}C with ionization voltage of 70 eV. Helium was used as a carrier gas. Spectra were analysed using the Hewlett Packard Enhanced Chem Station G1701 programme for windows. The components of the oils were identified by matching their spectra and retention indices with those of the Wiley 275 (Wiley, New York) in the computer library and literature. Percentage composition was calculated using the summation of the peak areas of the total oil composition.

Preparation of agar and isolation of microorganisms from orange juice:

Commercially produced Potato dextrose agar (PDA) was prepared according to manufacturer's specification. Chloramphenicol was added to the medium before pouring the melted medium into sterile Petri plates under aseptic conditions and left to solidify. Serial dilution of freshly squeezed orange juice and the branded juice were carried out.

From the stock of 10^{-3} for each sample, 0.1 ml was pipette and spread on PDA plate in replicates. These were incubated for 48 hours at 28-30 °C. Developing fungal colonies were sub-cultured aseptically by streaking into fresh Potato dextrose agar plates until pure cultures of the isolates were obtained.

Identification of fungi from the orange juice and branded juice:

The identity of the fungi were observed via morphological studies which is the examination of the size, shape, colour, spore formation and the number of days taken for the fungus to reach maximum diameter (9 cm) of the Petri dish. The texture of fungal growth was also observed. After 2 – 4 days of growth, the spore–bearing mycelia were then carefully sectioned, teased out and stained on a slide, then observed with a light microscope. The fungi identified were confirmed by comparing their morphology and cultural characteristics with descriptions given by Deacon (1980) and Bryce (1992). The photomicrographs of the fungi were obtained via the Motic Camera.

Antifungal assay:

A modified method of Adekunle and Ikumapayi (2006) was adopted by soaking

sterile Whatman No 1001/25 filter paper disc of 5 mm in diameter in the oil extract for 3 hrs. Cork borer was used to bore fungi layer and dropped into 5ml of saline water inoculated with the fungi *Aspergillus niger*, *A. flavus*, *Trichoderma* sp. and *Saccharomyces cerevisiae* in different test tubes. A hockey stick was used to ensure an even spread on the Petri dishes in a sterilized condition. Four replicates of the soaked discs were placed on the fungi plates at different portion using sterile forceps. They were then incubated for 48 hrs at 28°C. The zones of inhibition were measured and the results were analyzed statistically as described by Adekunle and Ikumapayi (2006).

Quality assessment of orange juice:

Total soluble solid:

The Brix was determined by measurement of the refraction index with a refractometer (Bellingham and Stanley, England) at room temperature. Refractive index was recorded and expressed as °Brix. Measurements were performed at room temperature as described by Barry-Ryan *et al*. (2009)

pH measurement:

The pH of 10 ml of the orange juices was determined at room temperature by constant agitation on a pH meter (model 420A, Orion, USA). This process was carried out thrice in the month to determine the shelf life of the orange juices.

Potential browning:

Potential browning was measured according to the methodology of Viña and Chaves (2006) by treating 5 ml of fresh orange juice with ethanol for 60min and then centrifuged at 4800×g at 10°C for 10 min, retaining the supernatants. After a further amount of ethanol was added to bring the final volume to 10ml. Absorbance at 320 nm of aliquots of these extracts was measured. The results were expressed as absorbance units (AU) mL^{-1} fresh orange juice.

Turbidity:

The turbidity of each sample of juice was measured using a direct reading spectrophotometer (model DR/2000, Hach, USA). The wavelength of the instrument was brought to 810 nm and deionised water used as a blank. The measurements of the samples of orange juice were carried out in triplicate with a solution of 1:25 (juice/water), to work within the detectable range. The results were given in milligrams of suspended solids per litre of solution (Barry-Ryan *et al.*, 2009).

Statistical analysis:

The data generated from the antifungal assay and quality assessments were analysed using analysis of variance and compared for significance using Duncan multiple range test.

RESULTS:

The result of the isolation of fungi from the squeeze orange juice and brandedprocessed orange juice (Fumman, Dansa, and Chivita) showed the presence of *Aspergillus niger* in all tested samples while *Trichoderma* sp. was only isolated from the Chivita juice. *Aspergillus flavus* and *Saccharomyces* cerevisiae were isolated from the freshly squeezed juice and the Dansa juice sample (Table 1).

Table 1. Fungal pathogens isolated from fresh orange and branded orange juice

Types of juice	Fungal Isolates
Fresh orange juice	*Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae*
Dansa	*Aspergillus flavus, Aspergillus niger, Saccharomyces cerevisiae*
Chivita	*Aspergillus niger, Trichoderma* sp.
Fumman	*Aspergillus niger, Candida* sp.

From the quality assessment analysis results revealed that the pH values in untreated fresh orange juice increased from 3.70 to 4.02 while that of *C. citratus* increased from 3.50 to 3.70 and *E. globulus* from 3.60 to 3.80 which infers that the acidity decreased. The branded juice also showed slight increase in pH, all readings taken at day 0, 14 and 28 (Fig. 1). The turbidity of the orange juice was reduced in fresh orange juice from 1.723 to 1.066 compared to that of *C. citratus* which reduced from 1.920 to 0.854 and *E. globulus* reduced from 1.898 to 0.815 which showed that essential oil inhibited spoilage within 28 days shelf life (Fig. 2). Potential browning of orange juice assay at 4 °C also increased as the days of storage increased, in fresh orange juice from 1.236 to 1.762 while that of *C. citratus* increased from 1.324 to 1.798 and *E. globulus* increased from 1.215 to 1.764. However, this was significantly higher in *C. citrates* extract. Antifungal analysis of the oil extracts of the leaves of *C. citratus* and *E. globulus* showed high variation against the tested fungi. From table 2, the oil of *C. Citratus* had highest zones of inhibition against the *Trichodema sp*. (10.33 ± 0.13 mm) and *A. flavus* (10.58 ± 1.10 mm). The oil of *E. globulus* was however more potent on *S. cerevisiae* than *C. citratus.* The highest zone of inhibition was recorded in *A. flavus*. All zones of inhibition for all the extracts on the fungi tested were obviously over 5 mm. Phytochemical screening revealed that the fresh leaves of the plants samples have several important constituents in varying

concentration. Alpha-phellande (8.40%), Epiglobulol (7.3%) showed high percentage composition in *E. globulus* while Citronelle and Lycopene, respectively showed highest percentage of 9.50 % and 7.0% in *C. citratus* (Table 3 a&b and Fig. 4)).

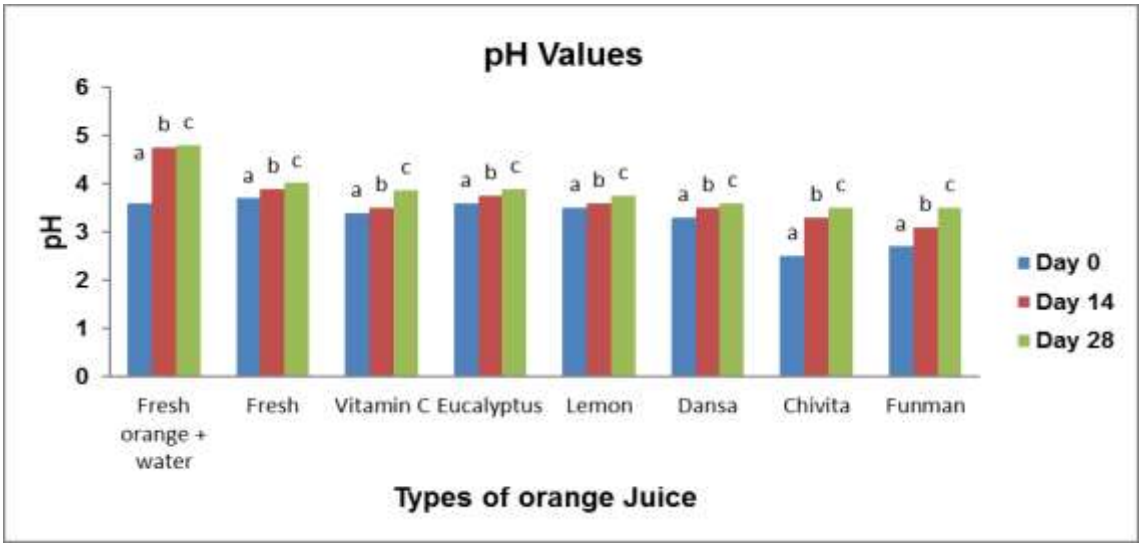

Fig. 1. pH values of orange juice in 28 days shelf life.

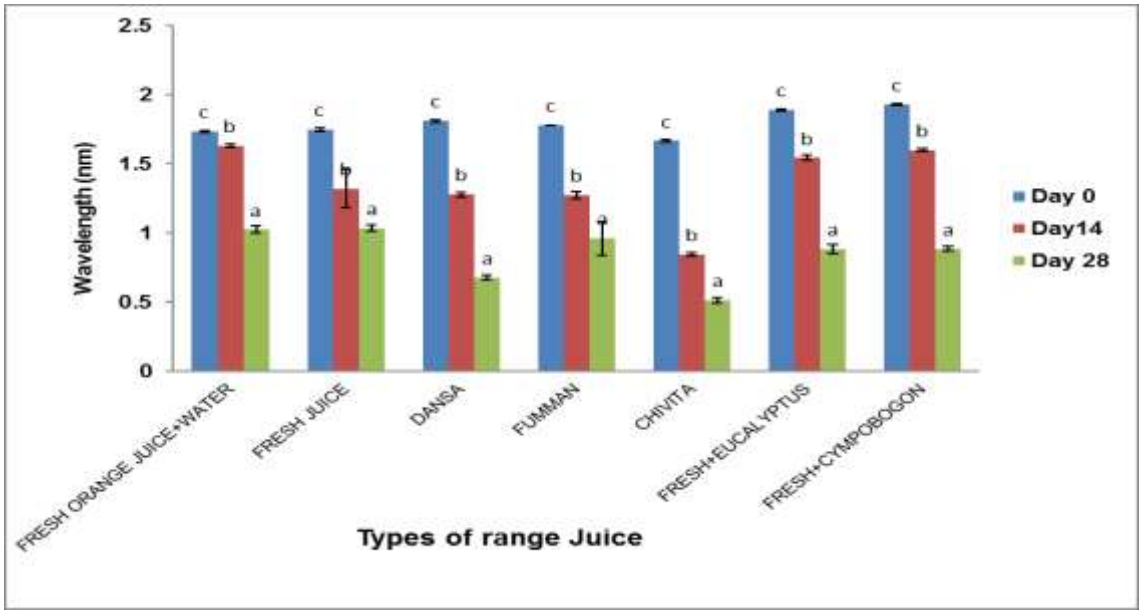

Fig. 2. The turbidity of the orange juice from 0 – 28 days shelf life

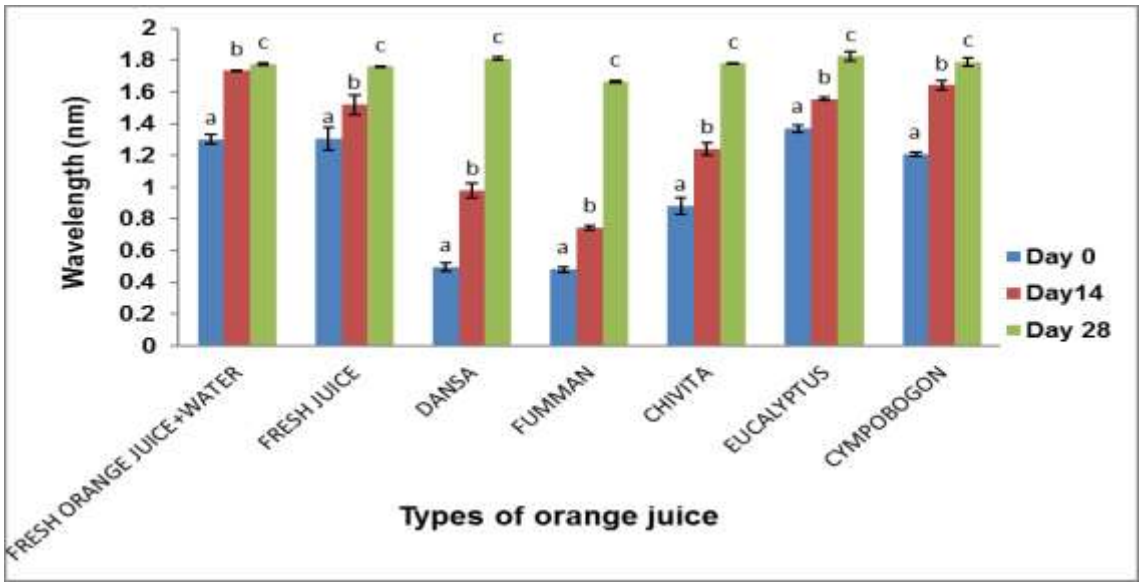

Fig. 3. The potential browning of the orange juice between 0- 28 days shelf life

Table 2. Zone of inhibition (mean ± S.E mm) produced by extracts

Fungal isolates	*C. citratus*	*E. globulus*	Control
Aspergillus niger	9.93 ± 0.63	9.78 ± 0.52	0.00 ± 0.00
Trichoderma sp.	10.33 ± 0.13		0.00 ± 0.00
Aspergillus flavus	10.58 ±1.10	10.25 ± 0.84	0.00 ± 0.00
Saccharomyces cerevisiae	9.63 ± 0.72	10.28 ± 0.95	0.00 ± 0.00
Candida sp.	9.98 ± 0.66		0.00 ± 0.00

Table 3a. Chemical constituent of *C. citrates* essential oil obtained through GC-MS

Compound	Percentage
Geranoil	1.92
Citronelle	9.50
Trans-b-ocimene	1.50
Myrcene	3.50
Chrysanthetin	5.10
Tetrahydroxy	3.60
Citral	1.20
Neral	5.50
Myrtillin	3.14
Sabdaretine	4.20
Daphoniphylline	3.30
Delphinidine	1.40
B-ionone	4.80
Linalool	1.10
Lycopene	7.0
Phytoene	1.60

Table 3b. Chemical constituent of *E. globulus* essential oil obtained through GC-MS

Compound	Percentage
Piperitone	1.1
Epiglobulol	7.3
Alpha-pinene	2.0
Beta-pinene	4.30
Trans-b-ocimene	3.50
Alpha-phellande	8.40
Aromadendene	2.20
Globulol	1.20
Limonene	3.80
B-caryophyllene	1.50
Germacrene	6.50
Bicyclogermacrene	2.50

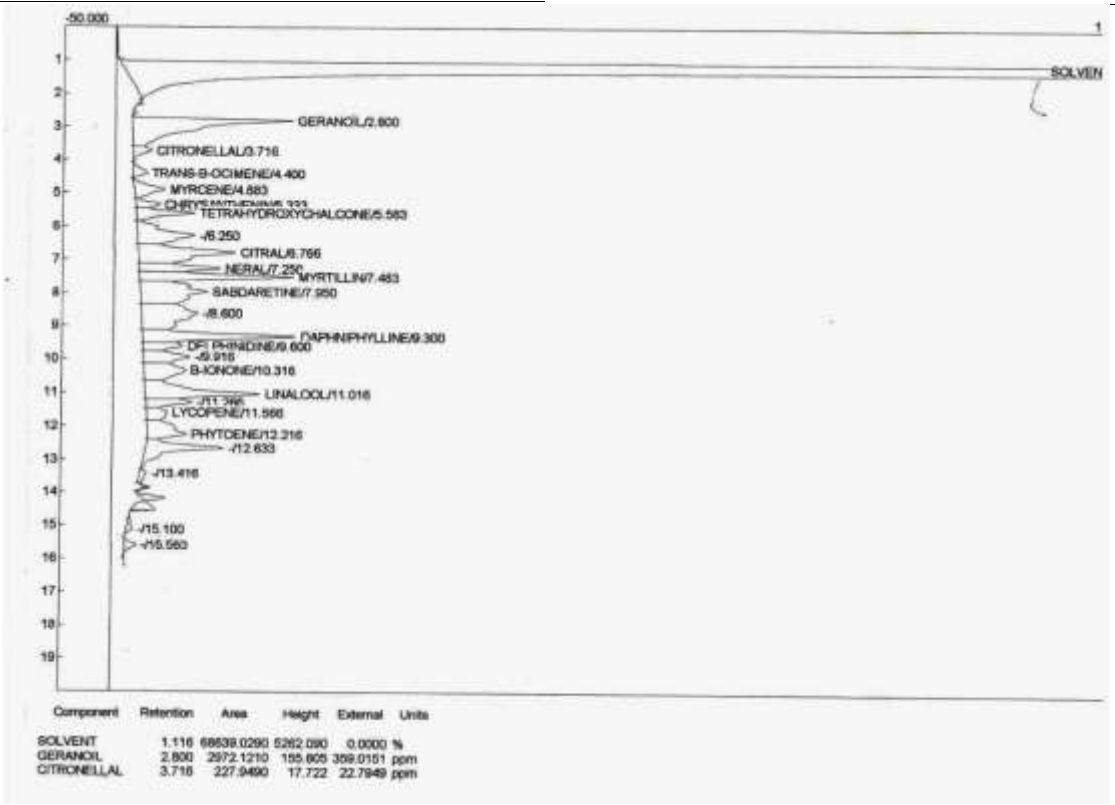

Fig. 4. Chromatography of *C. citratus*

DISCUSSION:

Several reports have established the efficacy and usefulness of plants for several antimicrobial activities and preservative purposes (Adekunle and Ikumapayi, 2006; Ghalem and Mohamed, 2008; Matasyoh *et al.*, 2011; Martins *et al.*, 2013). This study established the preservative potential of *E. globulus* and *C. citratus* volatile oil on the shelf life of

orange juice. *Aspergillus flavus*, *Aspergillus niger*, *Candida* sp. *Saccharomyces* cerevisiae and *Trichoderma* sp were isolated from the juices. The presence of *A. flavus* and *A. niger* in orange juice had earlier been reported by Tafinta *et al.* (2013) who isolated these organisms among others from orange fruit sold in the open market. The inhibitory activity of the plants' oil showed that it was able to inhibit the fungal growth and reduce the viability of the fungi. This could be attributed to the presence of volatile compounds present in the plant. The presence of 1, 8-cineole and α-pinene in the test plants agrees with the works of Canhoto and Graça, (1999) and Martins *et al.* (2013) that the inhibitory effect of *E. globulus* on the tested fungi is due to the activity of the volatile oil even at a low concentration. Mohanka and Priyanka (2014) had also reported the inhibitory activity of volatile oil of *Citrus* fruit against spoilage fungi like *Mucor* and *Rhizopus* species. High antifungal activity was recorded on *A. flavus* in both essential oils. *A. flavus* is known to produce mycotoxin which causes deterioration of food. The effects of the oil against this organism also gives credence to the report of Sinha *et al.* (1993) which showed that oil from clove and cinnamon hinders the growth and aflatoxin productions by *A. flavus.* Also, this correlates the report of Matasyoh *et al.* (2011) who showed that the oil of *C. citratus* is effective against the growth and activities of five *Aspergillus* spp which included *A. flavus* and *A. niger*. The zones of inhibition showed that the essentials oils were very effective in inhibiting the growth and activities of the fungi thereby preserving orange juice.

The preservative potential of *E. globulus* and *C. citratus* on orange juice were assayed based on their inhibitory activities on the tested fungus. The available phytochemical constituents in the volatile oils seem to be responsible for the inhibition. The increase in pH values of the juice with oil extract as the days of storage increases was slow in comparison with increase in orange juice without treatment which had an increase that was higher. This can be attributed to increase in microbial spoilage of the juice where the growth of microbes was delayed in the juice with the extracts. Barry-Ryan *et al.* (2009) reported that the increase in pH values of orange juice treated with chitosan compared with untreated orange juice might be due to the capacity of chitosan to reduce fruit juice acidity based on its acid-binding properties. That the turbidity in juice is mainly caused by the polysaccharides present in the juice. Increase in turbidity values of the juice with the extract during storage disagree with earlier works Barry-Ryan *et al.* (2009) that reported the reduction in turbidity values during storage using chitosan. The high increase of the browning potential values of the ordinary juice compared with the treated can be attributed to enzymatic properties of the extracts and the control of browning could be associated with the capacity to coagulate solids to which browning-related enzymes are bound (Barry-Ryan *et al.*, 2009). Barry-Ryan *et al.* (2009) explained that browning reduction in oranges clarified with chitosan which had antioxidant capacity similar to the capacity associated with phenolic compounds could explain this browning reduction by inhibiting the oxidative process.

The GC-MS analysis revealed the presence of some volatile oils that have been identified to be associated with antifungal activity. Citronelle had the highest percentage composition in *C. citratus* while in *E. globulus*, Apha-phellande had highest percentage composition. The presence of these oils in these plants respectively agrees with the works of Matasyoh *et al*. (2011) and Martins *et al.* (2013) who showed the extraction of different groups of active compounds from these plants. The antimicrobial effects of the extract could also be attributed to the synergistic effects of the compounds.

The use of alternative approaches for orange juice stabilization appears as a promising trend, due to increase in awareness of consumers towards natural, fresh and nutrient enriched foods free of additives. The use of naturally driven preservatives for preservation of orange juice will reduce the use of synthetic preservatives which have been established to be carcinogenic. The use of plants to preserve orange juice will scale up the cultivation of the plants and provide employment opportunity.

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