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Preservative potentials of essential oils of three Nigerian spices in mixed fruit juice and their antioxidant capacity

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The study was conducted to evaluate the preservative potentials of essential oils (EOs) of *Piper guineense*, *Xylopicia aethiopica* and *Tetrapleura tetraptera* in mixed fruit juice and determine their antioxidant capacity. The preservative effect at varying concentrations was evaluated using *S. cerevisiae*, *S. aureus* and *B. cereus* as test isolates. The antioxidant properties were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric ion reducing power assay (FRPA) methods. Concentrations (10 and 5%) of *P. guineense* and *X. aethiopica* EOs reduced the number of *S. cerevisiae* by one log cycle on the 10th day of storage, while 3.3% reduced it by one log cycle after 14 days. The 10% concentration produced a log cycle reduction in the growth of *S. aureus* and *B. cereus* on the 12th day. However, 5% concentration reduced the growth of *B. cereus* by one log cycle on the 14th day. *T. tetraptera* had the largest amounts of vitamin A (544.41 iu/100 mL), phenol (1.81%) and flavonoids (0.45%). *P. guineense* had the highest value for vitamin E (37.03 iu/100 mL). *X. aethiopica* EO produced the highest scavenging activity (46.04%), while *P. guineense* EO produced the strongest reducing power activity (38.64%). The EOs can act as natural preservative in mixed fruit juice. Their inclusion in such products can improve the products thereby serving as functional foods/beverages, and thus can reduce the risks of cancer formation and chronic diseases in humans.

Key words: Mixed fruit juice, preservation, microbial isolates, essential oils, antioxidant ability.

INTRODUCTION

There has been an increase in the consumption of unpasteurized fruit juices and minimally processed fruit products in recent times (Estrada et al., 2013). This increase is due to the high nutrient content of the juice and their freshness (Rathnayaka, 2013). Several fresh

fruit products are not normally heat processed and do not contain preservatives, thus they can easily be contaminated and spoiled by various microorganisms especially fungi and bacteria. These fruits are very good substrates that support their growth where they cause

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spoilage, produce flavour changes, odour defects as well as product discoloration, and can cause illness in humans if they are pathogens (Tournas et al., 2006). These could originate from soil particles, manure, animals, fruit processing equipment, human handlers, faeces or during transportation (Johnnessen et al., 2002) which include various pathogenic bacteria such as *Staphylococcus aureus*, *Shigella* species, *Bacillus cereus*, *Salmonella* species, enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Campylobacter* species, *Yersinia enterocolitica*, *Listeria monocytogenes* as well as parasites: *Cyclospora cayetanensis*, *Giardia lamblia* and *Cryptosporidium parvum* (De Roever, 1998; Kumar et al., 2006).

This increase in demand for non-thermally processed fruit juices with high quality therefore requires the application of different treatments for elimination of contaminating microbes. More so the increasing occurrence of food-borne disease outbreaks and growing interest in consumer demand for safe, fresh and ready to eat high quality foods/beverages raise considerable challenges (Tajkarinmi et al., 2010; Chana-Thaworm et al., 2011). Some reported cases of food borne disease outbreaks in some countries have been linked to unpasteurized fruit juices (Raybaudi-Massilia et al., 2009; Ghenghesh et al., 2005). The possible health hazards associated with the consumption of fruit juices preserved by synthetic preservatives has brought a significant change in the attitude of people towards such beverages. Thus, there is need to search for more antimicrobial compounds from plant sources.

Recently, there is an increase in the interest to use plant antioxidants in scientific research and possibly food preservation (Satish et al., 2014; Pandey et al., 2017). This is possibly due to their strong biological activity which has been found to exceed those of many synthetic antioxidants which are possible carcinogens (Suhaj, 2006). Thus, there is need to replace them with potent, more powerful and natural antioxidants from plant sources. Spices, medicinal plants and herbs are therefore continuously investigated for their antioxidant activities.

Essential oils (EOs) are usually complex but volatile compounds which have strong/pungent odour. They are produced by aromatic plants as secondary metabolites in many parts of the plants (Burt, 2004; Bakkali et al., 2008). Several EOs elicit antibacterial and antifungal effects (Ayafor et al., 1994; Barre et al., 1997; Cui et al., 2015; Sonker et al., 2015; Beatovic et al., 2015). They have also been found to possess antioxidant properties (Beatovic et al., 2015), and have cancer suppressive activity when applied on some human cancer cell lines (Adaramoye et al., 2011; Kuete et al., 2015; Sado et al., 2015). Such spices include *Piper guineense* (Uziza), *Xylopiya aethiopica* (Uda) and *Tetrapleura tetraptera* (Oshorisho).

P. guineense (Uziza) commonly called black pepper belongs to the Piperaceae family. The EOs of *P.*

guineense from Nigeria (Ekundayo et al., 1988; Oyedeji et al., 2005) and Cameroon (Amvam et al., 1998; Jirovets et al., 2002) have been previously studied. They were found to be composed largely of monoterpenoid alcohol linalool which represents 52.2% of the EO content (Owolabi et al., 2013).

X. aethiopica (Uda) called Guinea pepper belongs to the Annonaceae family. The oil is dominated by the monoterpene fraction (EL-Kamali and Adam, 2009). Almost all parts of *X. aethiopica* possess great medicinal values in traditional medicine. In most parts of Africa, it is used in the treatment of cough, rheumatism, dysentery, malaria, uterine fibroid, boils, wounds, etc. The various parts of *X. aethiopica* possess a wide diversity of phytochemicals which include essential oils, alkaloids and diterpenes. Extracts and isolates from parts of the plant possess one bioactivity or another which include antimicrobial activity (Fleischer et al., 2008), thus confirming its traditional uses, and have largely shown to be of low toxicity (Fetse et al., 2016).

The nutritional and health potential of the seed oils of the two spices (*P. guineense* and *X. aethiopica*) have been evaluated (Ogbonna et al., 2015). Water extracts of the spices possessed better antioxidant properties while ethanolic extracts of the spices exhibited best antibacterial activities (Dada et al., 2013). The EOs from these spices have been demonstrated to inhibit the growth of several microorganisms (Boakye-Yiadom et al., 1977; Tatsadjieu et al., 2003; Asekun and Adeniyi, 2004; Okigbo et al., 2005; Okigbo and Igwe, 2007).

T. tetraptera (Schumach. and Thonn) belongs to the Mimosaceae family and is commonly known as Aridan (fruit). The plant is widely distributed over several parts of tropical Africa, especially in the rain forest belt of West, Central, and East Africa. The fruit extracts have been shown to possess molluscicidal activity. Two saponin glycosides, oleanolic acid, umbelliferone and ferulic acid have been isolated from the extract (Adesina et al., 1980). The nutritional value of fresh mature fruits harvested in Southern Nigeria has been found to be rich in some macro-elements such as potassium, iron, magnesium and phosphorus while the physical and chemical properties of the oil indicated that it could be used as drying, and it has few unsaturated bonds (Dosunmu, 1997). The nutritional quality of the dry fruit has also been studied by Essien et al. (1994) and Okwu (2003) where it has been found to contain crude protein, crude lipid, crude fiber, carbohydrate, and food energy. They also contain tannins, phenolic compounds, saponins, alkaloids, steroids, and flavonoids.

Due to the increasing importance of spice EOs in the world market, there is the need to further study their biological potentials and their application in fruit juice preservation. This work therefore aims at determining the preservative potentials of the EOs of the spices *P. guineense*, *T. tetraptera* and *X. aethiopica* in mixed fruit juice as well as their antioxidant capacity.

MATERIALS AND METHODS

Collection of spices

Fresh fruits of *X. aethiopicum* (Uda), *P. guineense* (Uziza) and *T. tetraptera* (oshriho) were obtained from a farm in Obinze and Ihiagwa, Owerri West Local Government Area (latitude 5° 15'N - 5° 34'N and longitude 6° 52'E - 7° 05'E), Imo State, Nigeria in December, 2016. They were cleaned and sorted to remove sand, spoilt spices and dirt.

Test microorganisms

Pure cultures of *S. aureus* and *B. cereus* were obtained from the laboratories of Federal Medical Centre, Owerri, Imo State, Nigeria, while *Saccharomyces cerevisiae* was isolated from spoilt fresh fruit juice. These were stored at 4°C on the appropriate agar slants until required for use.

Preparation and extraction of EOs

Fruits of *X. aethiopicum*, *P. guineense* and *T. tetraptera* were destalked and sorted. These were milled with attrition mill (Landers YCLA, S.A Colombia), while the EOs were extracted using the Clevenger hydro distillation method (Selim, 2011). The spices (300 g of the milled spices) were put in a flask (4 L) and 1.5 L distilled water was added. The mixture was later boiled for 3 h and after the EOs were collected, dried with anhydrous sodium sulphate and stored at -10°C until required for use.

Preparation of stock solution of the EOs

Stock solutions of the essential oils were prepared by diluting with 5% dimethyl sulphoxide (DMSO) and different concentrations in percentage series were obtained, which corresponded to 10.0, 5.0, and 3.3% of the EOs, respectively. The various concentrations obtained were stored in sample bottles and stored in the refrigerator until required for use. Sterility of the prepared concentrations of EOs was ensured by plating out on Nutrient agar and Potato Dextrose agar plates, and incubated at 37 and 30°C, respectively for 24 and 72 h.

Standardization of the inoculum

A sterilized wire loop was used to scrap the surface of the agar containing the microorganism in Bijou bottles. This was introduced into a nutrient broth, one for each organism and incubated at 37°C for 24 h in a shaker incubator. After incubation, the broth was centrifuged for 30 min and the top decanted. The broth supernatant (nutrients) were washed with sterilized distilled water and centrifuged again. This procedure was repeated three times. At the end of the centrifugation, the microorganisms were harvested and re-suspended in sterilized distilled water in sample bottles, and the cultures adjusted to 0.5 Mac Farland's standard (Approximately 10⁸ cfu/ml). This was stored at 4°C and used for the analysis.

Dilution susceptibility testing (Determination of minimum inhibitory concentration [MIC] and minimum bactericidal concentration MBC)

The method of Salah-Fatnassi et al. (2017) was adopted for the evaluation of the MIC and MBC of the EOs. 0.01 mL of the bacterial

suspensions that have been adjusted to 0.5 McFarland standard (1.5×10⁸ cfu/mL) were inoculated into test tubes of Mueller-Hinton broth containing different concentrations of the EOs. The tubes containing the broth cultures were incubated at 37°C for 24 h. Bacterial growth was determined visually by the turbidity of the culture. The least concentration which showed no visible growth was taken to be the MIC.

For the determination of the MBC, 0.1 ml inoculum was taken from the tubes in which there was no growth and sub cultured on Mueller-Hinton agar plates. The plates were examined for bacterial growth after incubation at 37°C for 24 h. The least concentration that showed no bacterial growth was taken as the MBC.

Antioxidant properties of EOs

1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay

Total free radical scavenging capacity of the EOs against DPPH free radical was evaluated as described by Gyanfi et al. (1999). A solution of the radical was prepared by dissolving 2.4 mg DPPH in 100 ml methanol. A 1:10 dilution of the EOs was prepared and 1 ml of it was mixed with 1 ml 0.4 mM methanolic solution containing DPPH radicals. The mixture was shaken vigorously and kept at ambient temperature for 30 min in the dark. Absorbance of the reaction mixture was measured at 516 nm wavelength using UV-Visible spectrophotometer. Absorbance of the DPPH radical without antioxidant (blank) was also measured. All determinations were performed in duplicate.

The capability to scavenge the DPPH radical was calculated using the following equation (Yen and Duh, 1994).

$$\text{DPPH Scavenged (\%)} = ((A_B - A_A)/A_B) \times 100$$

where A_B = absorbance of blank at t = 0 min and A_A = absorbance of the antioxidant at t = 30 min.

Ferric ion reducing antioxidant power assay (FRPA)

The antioxidant capacity of the EOs was also estimated spectrophotometrically using the method of Benzie and Strain (1996). The method was based on the reduction of Fe³⁺ tripyridyltriazine complex (colourless) to Fe²⁺ tripyridyltriazine (blue) formed by the action of electron donating antioxidants at low pH. The reaction was monitored by measuring the change in absorbance at 700 nm wavelength. A 2.5 ml aliquot of each EO was mixed with 2.5 ml 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. The supernatant was collected and 5 ml aliquot of each EO was mixed with an equal volume of water and 1 ml 0.1% Ferric chloride. The absorbance was measured at 700 nm wavelength. All the determinations were performed in duplicates. The ferric ion reducing antioxidant property was calculated and expressed as percentage mg equivalent per millilitre of sample.

Determination of antioxidant components in EOs

Determination of Vitamin A

This was determined spectrophotometrically using the modified standard method of AOAC (2000). Each EO (0.5 g) was homogenized and saponified with 2.5 ml 12% alcoholic KOH in a water bath at 60°C for 30 min. The saponified EO was transferred

to a separating funnel containing 10 to 15 ml of petroleum ether. This was properly mixed. The lower aqueous layer was transferred to another separating funnel while the upper petroleum ether layer containing the carotenoids was collected. The extraction process was repeated until the aqueous layer became colourless. Excess moisture was removed from the petroleum ether fraction by adding small amount of anhydrous sodium sulphate and the final volume of the petroleum ether fraction noted. The absorbance of the yellow colour was then read on a visible spectrophotometer at 450 nm. Petroleum ether was used as blank.

Determination of Vitamin E (Tocopherol)

The determination was done spectrophotometrically using the modified standard method of AOAC (2000). 1.5 ml of EO, 1.5 ml of standard tocopherol, and 1.5 ml of water were pipette into three stoppered centrifuge tubes, respectively. Into all the centrifuge tubes, 1.5 ml of ethanol and 1.5 ml of xylene were added, properly mixed and centrifuged at 300 rpm. The xylene (1.0 ml) layer was then transferred into another stoppered tube. To each tube, 1.0 ml of dipyrindyl reagent was added and mixed properly. The mixture (1.5 ml) was pipetted into a cuvette. The extinction was read at 460 nm. Ferric chloride solution (0.33 ml) was added to all the tubes and mixed properly. The red colour that developed was read after 15 min at 520 nm using a visible spectrophotometer.

Determination of flavonoids

This was done according to the method described by Boham and Kocipai (1994). Five grams of the ground spice sample was weighed in a 250 ml titration flask and 100 ml of 80% aqueous methanol added at ambient temperature. This was shaken for 4 h in an electric shaker. The solution was filtered twice through Whatman filter paper No. 1 (125 mm). The filtrate was then transferred into a crucible and evaporated to dryness over a water bath and weighed.

Determination of phenols

The method of Harborne (1998) was used for the assay. Two grams of the ground samples were defatted with 100 ml of diethyl ether using Soxhlet apparatus for 2 h. The fat free samples were boiled with 50 ml of ether for 14 min. Five millilitres of the extract was pipetted into a 50 ml flask after which 10 ml of distilled water was added. Two millilitres of $\text{NH}_4(\text{OH})_2$ solution and 5 ml of concentrated ethyl alcohol were then added. The samples were then made up to mark and left to react for 30 min for colour development. The absorbance of the solutions was read using a visible spectrophotometer at 505 nm wavelength.

Preparation of mixed fruit juice

Three different moderately ripe fruits (oranges, paw paw and pineapple) were used in the juice preparation. They were obtained from a plantation in Ohaji/Egbema Local Government Area, Imo State, Nigeria. The fruits were thoroughly washed with distilled water and aseptically peeled with a kitchen knife. All the materials used were pre sterilized to keep off possible contaminants from utensils. The edible portions of the three fruits were mixed in equal ration and blended in an electric blender (sterilized) without the addition of extra water. The resulting juice was filtered through a sterile muslin cloth, pasteurized at 60°C for 30 min while another set was not pasteurized which served as control. They were stored at ambient temperature (30 to 32°C) and used for the preservative potential assay.

Preservative potential of the EOs in mixed fruit juice

The EOs used for the determination of preservative potentials were those that sufficiently inhibited the test isolates (*X. aethiopica* and *P. guineense* EOs). The procedure described by Careaga et al. (2005) was used with slight modification. The mixed fruit juice from orange, paw paw and pineapple was prepared as described earlier. The juice was dispersed in 90 ml portions in heat stable sterile sample bottles. A total of 63 bottles of 90 ml portions of the juice was used for each of the EOs. The experiment was set in such that each EO was used at three concentrations of 10.0, 5.0, and 3.3% against the three isolates. The juice samples were replicated into seven places for each isolate used against each EO at different concentrations. This was done to ensure that different replicate of the same sample was taken at two days interval for microbiological assay without having to open and close the sample over the time. This made it possible to avoid contamination from the environment. The fruit juice samples in their respective labeled bottles were inoculated with the different EOs at the appropriate concentrations. 1 ml of standardized broth cultures of the isolates were added, mixed thoroughly and capped. Mixed fruit juice without the EOs served as controls. The microbial load at onset was recorded as the standard concentration added (1 Macfarland unit = 1.5×10^8 cfu/ml). Thereafter, the inoculated samples were allowed to be stored aseptically at ambient temperature (30 to 32°C) for 14 days and monitored at two days interval to determine the microbial population by plating out samples on MHA agar plates using spread plate method as described by Ogueke and Aririatu (2004). The colony forming unit (cfu) per ml of sample was calculated at the end of the incubation period.

Statistical analysis

The data were means of triplicate determinations and were analyzed using Analysis of Variance (ANOVA). Statistical Package for Social Scientists (SPSS) version 20 was used for statistical analysis of data obtained. The means were separated using Fisher's Least Significant Difference (LSD) at 95% confidence level ($p < 0.05$).

RESULTS

Dilution susceptibility testing of EOs

Results obtained from dilution susceptibility testing (Table 1) show that the least MIC value of 8.0 mg/mL was recorded for *P. guineense* and *X. aethiopica* on *B. cereus* and *S. cerevisiae*, while *X. aethiopica* had MIC value of 8.0 mg/mL on *S. aureus*. The highest MIC value of 46 mg/mL was produced by *T. tetraptera* on *B. cereus*. The least MBC value (16 mg/mL) was produced on *B. cereus* and *S. cerevisiae* by *P. guineense* and *X. aethiopica*, respectively. The highest MBC value (60 mg/mL) was observed on *B. cereus* by *T. tetraptera*.

Antioxidant properties of the EOs

The EOs contained varying amounts of vitamin A, vitamin E, phenols and flavonoids (Table 2). *T. tetraptera* had the largest amount of vitamin A (544.41 iu/100 mL), phenol (1.81%) and flavonoids (0.45%) contents, while *P. guineense* had the largest value for vitamin E (37.03

Table 1. MIC and MBC of *X. aethiopica*, *P. guineense* and *T. tetraptera* EOs on isolates (mg/ml).

Essential oils	Microorganism	MIC	MBC
<i>P. guineense</i>	<i>B. cereus</i>	8	16
<i>P. guineense</i>	<i>S. aureus</i>	16	32
<i>P. guineense</i>	<i>S.cerevisiae</i>	8	16
<i>X. aethiopica</i>	<i>S. cerevisiae</i>	8	16
<i>X. aethiopica</i>	<i>S. aureus</i>	8	32
<i>X. aethiopica</i>	<i>B. cereus</i>	8	32
<i>T. tetraptera</i>	<i>S. cerevisiae</i>	36	48
<i>T. tetraptera</i>	<i>S. aureus</i>	42	56
<i>T. tetraptera</i>	<i>B. cereus</i>	46	60

Table 2. Mean values of antioxidant components of EOs

EO type	Vitamin A (iu/100 ml)	Vitamin E (iu/100 ml)	Phenol (%)	Flavonoids (%)
<i>X. aethiopica</i>	283.59±0.47 ^a	13.37±0.50 ^a	1.29±0.0 ^a	0.29±0.02 ^a
<i>P. guineense</i>	464.31±0.81 ^b	37.03±0.40 ^b	0.48±0.0 ^b	0.26±0.02 ^b
<i>T. tetraptera</i>	544.41±0.36 ^c	8.49±0.40 ^c	1.81±0.0 ^c	0.45±0.01 ^c
LSD	1.19	1.33	0.02	0.02

^{a,b,c}Values with different superscript on the same column are significantly different ($p < 0.05$).

Table 3. Mean percentage values of antioxidant activity of EOs.

EO type	Scavenging activity (%)	Reducing power activity (%)
<i>X. aethiopica</i>	46.04±0.01 ^a	28.72±0.02 ^a
<i>P. guineense</i>	22.74±0.01 ^b	38.64±0.04 ^b
<i>T. tetraptera</i>	41.44±0.04 ^c	29.55±0.05 ^a
LSD	0.16	2.97

^{a,b,c}Values with different superscript on the same column are significantly different ($p < 0.05$).

iu/100 mL). *X. aethiopica*, *T. tetraptera* and *P. guineense* had the least values for vitamin A, vitamin E, phenols and flavonoids, respectively. Statistical analysis revealed that the values obtained for the components differed significantly ($p < 0.05$) among the different EOs obtained from the spices.

The EOs possessed varying degrees of antioxidant activity (Table 3). *X. aethiopica* EO produced the highest scavenging activity (46.04%), while *P. guineense* EO produced the strongest reducing power activity (38.64%). However, *P. guineense* and *X. aethiopica* had the least values for scavenging activity (22.74%) and reducing power activity (28.72%), respectively. Value obtained for scavenging activity differed significantly ($p < 0.05$). However, the values for reducing power obtained from *X. aethiopica* and *T. tetraptera* did not differ while they differed significantly ($p < 0.05$) with the value obtained from *P. guineense* EO.

Preservative potentials of EOs in mixed fruit juice

Inhibitory effect of the EOs was concentration dependent

(Figures 1 to 6). The different concentrations of the EOs reduced the number of the test isolates during storage; however, the 10% had the highest inhibitory effect. On *S. cerevisiae* the 10 and 5% concentrations of the EOs produced a log cycle reduction in the growth of the yeast on the 10th day of storage, while 3.3% reduced their growth by one log cycle on the 14th day (Figures 5 and 6). Thus, *S. cerevisiae* was most susceptible to the EOs. For the other bacterial isolates, the 10% concentration produced a log cycle reduction on the 12th day of storage (Figures 1 to 4). However, on the 14th day of storage the 5% concentration reduced the growth of *B. cereus* by one log cycle.

DISCUSSION

Fresh fruit juices contain a diverse group of microflora which is normally present on the surface of fruits during harvest and postharvest processing (Tournas et al., 2006). Many microorganisms especially the acid tolerant bacteria and fungi (moulds, yeasts) use them as

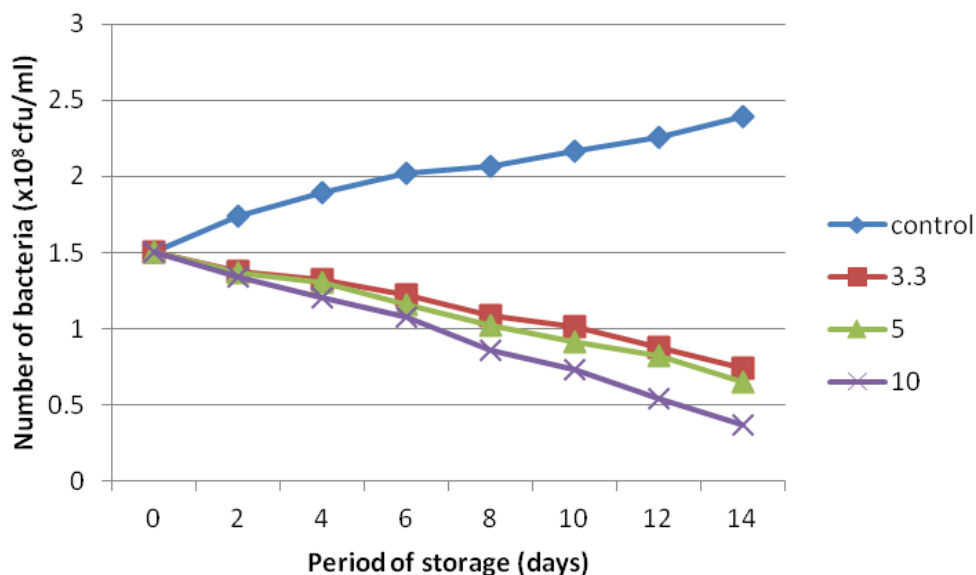


Figure 1. Effect of *X. aethiopicum* EO on *S. aureus* in mixed fruit juice.

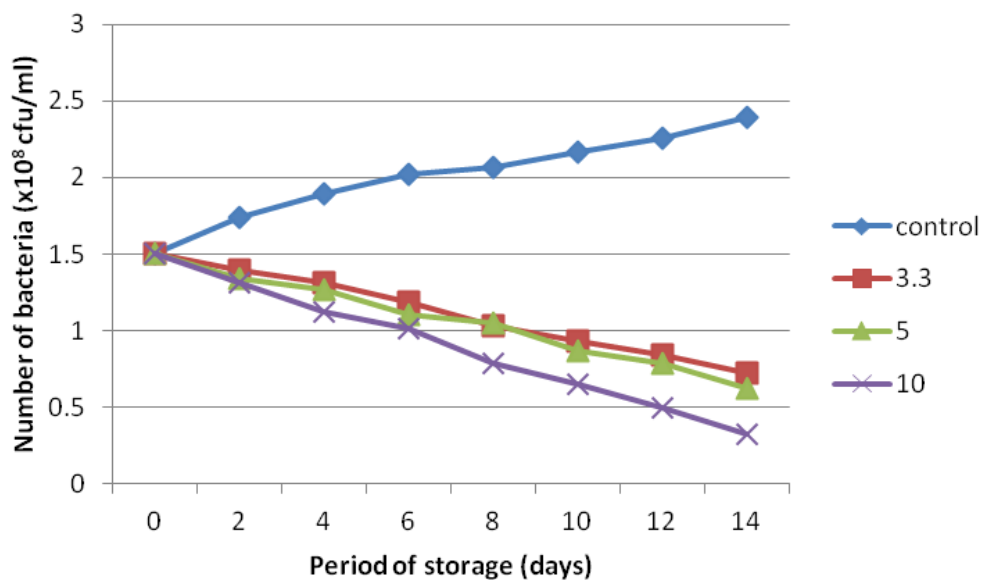


Figure 2. Effect of *P. guineense* EO on *S. aureus* in mixed fruit juice.

substrate for their growth and cause spoilage in the juices which includes cloudiness, development of off-flavours, gas formation, colour change and texture defects (Lawlor et al., 2009; Sospedra et al., 2012). Fresh fruit juices usually have pH in the acidic range (less than 4.5) which serve as important barrier to growth of various microbes, however, food borne pathogens and spoilage microorganisms survive in the environment due to acid stress response. The acid survival mechanisms of these microorganisms have been found to be through induction

of enzymes that are involved in increasing the internal pH and activation of enzymes responsible for the protection and repair of proteins and DNA (Bearson et al., 1997).

Plant phytochemicals such as phenolics exhibit strong radical scavenging activity. These compounds in some cases have been established to express synergistic actions where they inhibit the formation of hydroxyl radicals, reactive O₂ species, superoxide anions and hydrogen peroxide which usually result in oxidative damage to biomolecules (Satish et al., 2014). It has also

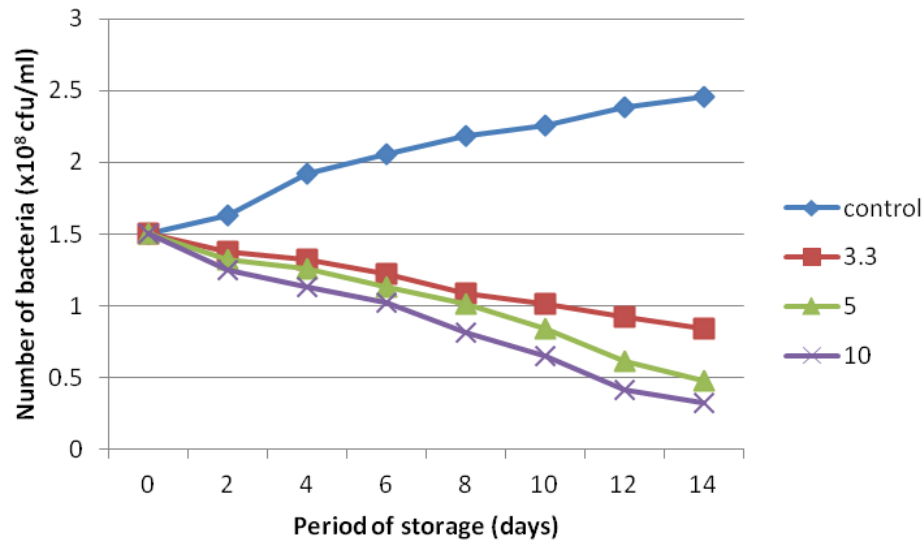


Figure 3. Effect of *X. aethiopica* EO on *B. cereus* in mixed fruit juice.

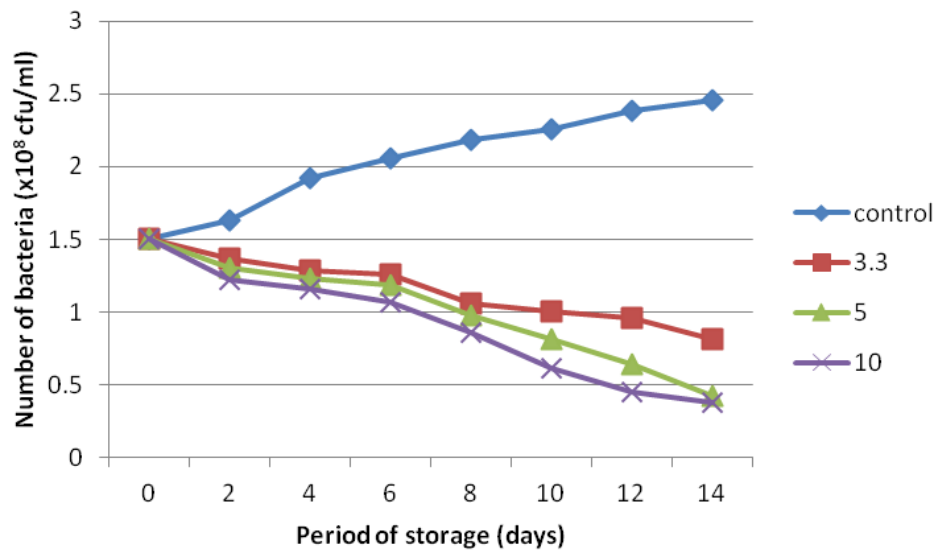


Figure 4. Effect of *P. guineense* EO on *B. cereus* in mixed fruit juice.

been established that these phytochemicals possess antimicrobial activities against some groups of microbes (Cowan, 1999; Swamy et al., 2016). This present research work studied *P. guineense*, *X. aethiopica* and *T. tetraptera* EOs for their antioxidant components and their preservative potentials in mixed fruit juice. These spices contain phenolics which contribute to their medicinal value and the mechanisms for bring about their antioxidant actions have been studied (Upadhyay et al., 2014).

The findings from this study revealed that the EOs especially those from *P. guineense* and *X. aethiopica*

exerted broad spectrum antimicrobial activity which is in consonance with previously published work (Boakye-Yiadom et al., 1977; Tatsadjieu et al., 2003; Konning et al., 2004; Asekun and Adeniyi, 2004; Dada et al., 2013; Okigbo et al., 2005). However, EOs of *X. aethiopica* and *P. guineense* expressed stronger antimicrobial activity than *T. tetraptera* EO on the test isolates. Microbicidal and microbistatic activities of EOs have been studied severally and these activities are due to the phytochemicals they contain. These activities however can be affected by the composition of critical chemical compounds contained therein which differ significantly

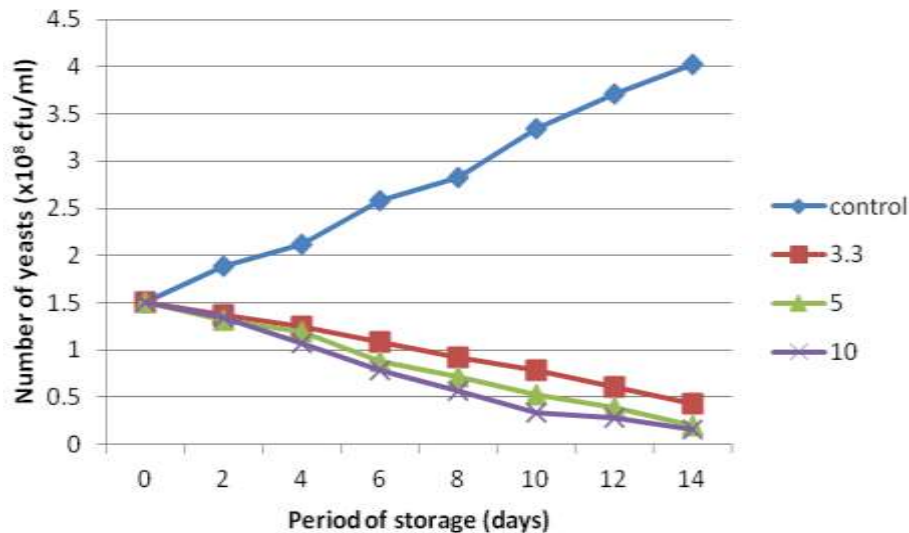


Figure 5. Effect of *X. aethiopica* EO on *S. cerevisiae* in mixed fruit juice.

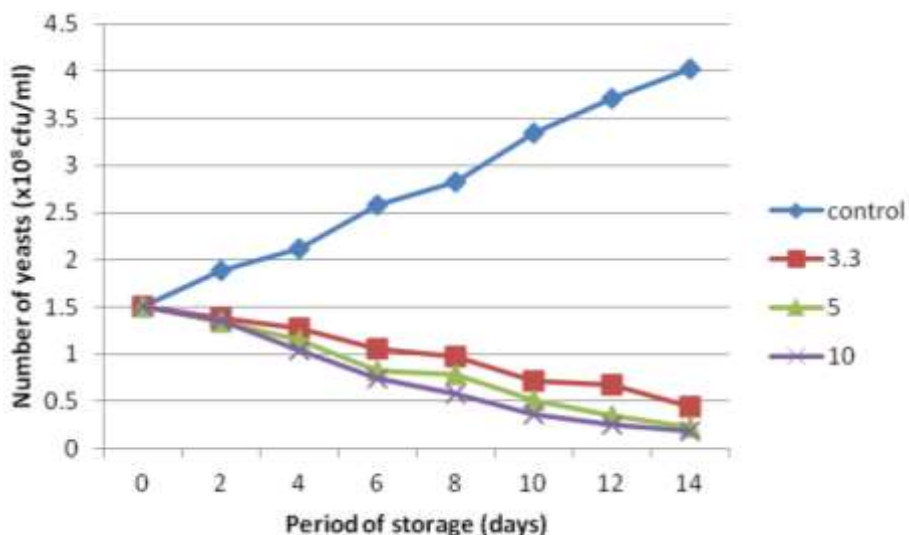


Figure 6. Effect of *P. guineense* EO on *S. cerevisiae* in mixed fruit juice.

due to variations and differences in genetic blueprint, agro-practices, type of extracting solvent, extraction method and environmental factors, thus changing their antimicrobial efficacy against food borne pathogens (Hao et al., 1998; Cowan, 1999; Chao et al., 1999; Abu-Shanab et al., 2004; Okigbo and Ajalie, 2005; Okigbo et al., 2005). Their configuration, composition and amount could possibly affect their interaction which could be additive, synergetic or antagonist (Lis-Balchin et al., 1998; Wang et al., 2016). Weaker antimicrobial activity could also be attributed to the volatile characteristics of the components of the EOs which are simultaneously lost with the solvent during evaporation/drying. These may have been responsible for the weaker antimicrobial action

recorded for *T. tetraptera* EO. EOs obtained from other plants have also been established to inhibit the growth of diverse group of microorganisms (Ilori et al., 1996; Lopez et al., 2005; Bisht et al., 2014; Lawal et al., 2014).

The study revealed that application of the EOs of *X. aethiopica* and *P. guineense* in mixed fruit juice inhibited the isolates used in the study. This can be attributed to the various compounds contained in the EOs which have been known to have inhibitory effects on microbial growth (Cowan, 1999; Swamy et al., 2016). From other research works reported on the preservation of food using EOs from oregano and thyme, results have shown that their EOs inhibited the growth of *Salmonella* spp. up to a significant level (Hayouni et al., 2008; Bajpai et al., 2012;

Seow et al., 2014). Selim (2011) also studied the preservative properties of EOs of some plants on vancomycin resistant Enterococci and *E. coli*. The EOs produced one log cycle reduction in the number of bacteria used in the study after three days of storage. In this study, a log cycle reduction was obtained from the 10th day of storage. Ogueke et al. (2015) in their study with *Alstonia boonei* and *Euphorbia hirta* extracts obtained a log cycle reduction in the growth of *E. coli* and *Bacillus subtilis* in minced meat at 0.1, 0.2 and 0.3% inclusion of extract. Ogbonna et al. (2013), however, obtained a microbial concentration of 4 cfu/mL in orange juice containing 100 ppm of ethanolic extract of *X. aethiopica*, after storage for 28 days at ambient temperature. Sonker et al. (2015) improved the shelf life of table grapes by up to 9 days through application of EO obtained from *Artemisia nilagirica*. Other researchers have shown that EOs from plants can extend the shelf life of certain foods (Pandey et al., 2017).

The insolubility of active compounds in media and water as well as presence of inhibitors to the antimicrobial components may play major roles in the antimicrobial activity of EOs (Janssen et al., 1987; Kalemba and Kunicka, 2003; Burt, 2004). The thickness of the oils and rate of diffusion may also significantly affect their ability to act as antimicrobial agents in juices.

This present study has revealed that the EOs possess potent antioxidant property which can be harnessed in the food/beverage industry. Such EOs with strong antioxidant properties are usually good natural antioxidants. These properties could be attributed to the bioactive compounds, phytochemicals and pigments which are contained in the EOs. Osuala and Anyadoh (2006) have shown that *P. guineense* contains elemicine, safrole, 5 to 8% piperine, 10% myristicine and dillapiol. Previous work on antioxidant mechanisms of spices in model systems has been studied and elucidated. It has been identified that binding of transition metal ion is one of the mechanisms by which antioxidants inhibit lipid peroxidation in biological and food systems. Thus, the transition metal ion's oxidation state is reduced from high to low oxidation number, thereby reducing their carcinogenic effects in humans (Mittal et al., 2012).

The presence of flavonoids in the EOs still buttresses the fact that they possess potent antioxidant activity. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals could be their most important function (Iniaghe et al., 2009) and such property could be harnessed in the food/beverage industry for production of foods/beverages especially now that functional foods are being emphasized and popularized. Flavonoids have been reported to exert multiple biological properties which include antimicrobial activity (Peres et al., 1997), however, the best-described property of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals which are clearly associated with etiology of

cancers and other chronic diseases, and reactive O₂ species, thereby inhibiting oxidative cell damage, provide strong anticancer activity and protect cells against carcinogenesis (Adaramoye et al., 2011, Kuete et al., 2015). Flavonoids in the intestinal tract reduce the risk of heart disease (Okwu, 2005). Some studies have also shown that flavonoids contain hypolipidemic potential (Narender et al., 2006; Harnafi and Amrani, 2007). Therefore, dietary antioxidants with the ability to scavenge free radicals may likely reduce the risks of cancer and chronic diseases (Yen and Duh, 1994).

The EOs were found to contain appreciable levels of vitamins A and E. Since there has been an increase in the consumption of fresh fruit juices, because of their high vitamin C content, freshness and low caloric value (Rathnayaka, 2013); the inclusion of the EOs will further improve the juice nutritionally and increase the health benefits derivable from consumption of such juice through provision of the much needed antioxidant capabilities and increased levels of the vitamins. Therefore, consumption of such fruit juice product containing these EOs could probably inhibit the formation of free radicals that are usually associated with the initiation of certain cancer cells in human beings. With the high vitamins A and E, content consumption of the fruit juice may contribute significantly to the intake of the vitamins and consequently may help consumers achieve the required dietary intake (RDI) for these vitamins. Thus, in addition to providing nutrients they could also provide certain health benefits that include anti-tumour, anti-asthmatic, anti-inflammatory, antimicrobial (Okigbo et al., 2005; White, 2006; Okigbo and Igwe, 2007), hypotensive and coronary vasodilatory effects (Fleischer, 2003) which could qualify such juice product as a functional food/beverage. Use of these spices with very long history of usage in many parts of the world are usually preferred to chemical preservatives which often have undesirable side effects on consumers (Ogbonna et al., 2013). Although the antimicrobial activity of *T. tetraptera* EO was low, the high content of vitamin A as well as the appreciable content of vitamin E, phenols and flavonoids make the EO a useful source of antioxidants and can be added to fruit juice for improvement of functionality and enhancement of human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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