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Effects of Selected Leaf Extracts on the Microbial Quality of Stored Orange and Pineapple Juices

Oladapo Sinmiat Abiodun*[1]
Akinyosoye Felix Akinsola [2]
Adebolu Tinuola Tokunbo[2]
Ojokoh, Anthony Okhonlaye[2]

[1]*Department of Food Science and Technology, Osun State Polytechnic, Osun State*
[2]*Department of Microbiology, Federal University of Technology, Ondo State*

Abstract

Several preservation methods have been developed to assure microbial fruit juices safety; however, the demand for natural antimicrobial agents for food preservation is increasing due to consumers' concern on health issues. This study therefore aims at evaluating the effects of aqueous extracts of the leaves of some plants (*Ocimum gratissimum* Linn*, Cymbopogan citratus* Linn and *Anthoteista djalonensis* Linn) on the microbial qualities of two fruit juices: orange and pineapple juices stored at 30±2ºC using standard techniques. Each of the extracted fruit juices was divided into four parts, one part was treated with aqueous leaf extract of *O. gratissimum*, the second part was treated with the aqueous leaf extract of *C. citratus,* the third part was treated with the aqueous leaf extract of *A. djalonensis* while the fourth part was left untreated. Different concentrations of the extracts which ranged from 5 % to 40 % were used for sub-portions of each of the juices respectively. The juices were then stored at ambient temperature (30±2ºC) for 10 days. Seven different bacterial species and six fungal species were isolated namely*;* *Bacillus subtilis* MH769494, *Bacillus thuringiensis* KJ9343380, *Bacillus cereus* KX057595, *Acinetobacter indicus* MF497336 *Corneybacterium* sp MF285789, *Micrococcus luteus* MG733544 and *Staphylococcus aureus* MH 938044 as bacteria and *Pichia kudriavezeii* MH593830, *Saccharomyces cerevisiae* CAA39055, *Candida krusei* HE861376, *Aspergillus aculeatus* HE861781, *Curvularia lunata* KU681406, and *Curvularia aeria* PSU 06 as fungi. The total viable bacterial count of untreated orange and pineapple juices ranged from 2.27±0.01 $\log_{10}$ CFU/ml to 5.50±0.20 $\log_{10}$ CFU/ml and 5.34±0.02 $\log_{10}$ CFU/ml to 5.63±0.03 $\log_{10}$ CFU/ml respectively while fungal count ranged from 2.65±0.02 $\log_{10}$ SFU/ml to 4.49±0.30 $\log_{10}$ SFU/ml and 2.68±0.02 $\log_{10}$ SFU/ml to 3.03±0.01 $\log_{10}$ SFU/ml respectively. Out of the three aqueous extracts used, *O. gratissimum* extract (40% concentration) reduced the bacterial count to 1.83±0.02 $\log_{10}$ CFU/ml with total fungal counts of 1.04±0.01 $\log_{10}$ CFU/ml for the stored pineapple juice but no significant reduction was observed in orange juice. It is concluded that *O. gratissimum* extract has the potential to improve the microbial quality, preserve and extend the shelf life of pineapple juice for 10 days at ambient temperature.

Keywords: Fruit juices, Selected plants extracts, Microbial quality, Shelf life.

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***Corresponding author:** Oladapo Sinmiat Abiodun, Department of Food Science and Technology, Osun State Polytechnic, P.M.B. 301, Osun State. Tel: +2348035743082; Email: oladapo.ospoly@gmail.com

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Introduction

Consumers are clamoring for food preserved with natural preservatives owing to the awareness of the fact that chemical preservatives have deleterious effects on health [1].The nutrition and safety of fruit juices pose a major concern to the consumer nowadays due to hazardous implications by the chemical preservatives. Among the food consumed, fruits and vegetables have been categorized as one of the vital sources of chief dietary nutrients for humans [2].There is an increasing demand for nutritious foods such as fresh-cut fruits and juices owing to their contents which play vital roles in the prevention of heart diseases, cancer, and diabetes [3]. However, an investigation has revealed that consumption of unpreserved fruit juices causes illnesses [4]. Although, pasteurization techniques have been employed in reducing the infection pathogens in fruit juices to the FDA recommended standard of 5log10. Pasteurization is effective against *E. coli* and *Salmonella* but is not effective against ascospores of heat resistant fungi, yeasts, and bacteria [5]. The high occurrences of bacteria and fungi have been reported in NAFDAC approved fruits juices [6,7]. Therefore thermal pasteurization damages the nutritional, physicochemical properties and sensory qualities of fruit juices [8]. Non-thermal preservative methods are receiving good attention because of their potential for quality and safety improvement of food. The conventional non-thermal technique of preservation includes the use of weak organic acids: citric, lactic, and acetic and benzoic acids. The use of some plant leaves extract in the preservation of fruit juices is emerging [3,12].

Orange is one of the popular fruit widely accepted for its high vitamin C content, carotenoids and desirable flavour. Consumption of these vitamins correlated with a reduction in the incidence of certain cancers [9]. The recent trends show that the consumer preference towards unpasteurized orange juice has increased due to its superior taste, aroma and nutritive values. However, the juice is less stable during storage and may become unsafe for consumption due to the growth of micro-organisms. Microorganisms associated with the spoilage of orange juices have long been studied [10]. Orange juice has become one of the most widely accepted natural beverages due to its invigorating flavours and as an energy source to consumers [11,12]. Consumption of orange juice significantly improves blood lipid profiles in people living with hypercholesterolemia [13,14]. Pineapple is important in the human diet as a source of micronutrients.

In this study, the potentials of aqueous extracts of some plants as a preservative of freshly prepared orange and pineapple juices were explored. The selected plants for the study are *Ocimum. gratissimum, Cymbopogan. citratus* and *Anthotecista. djalonensis*

Ocimum gratissimum extract has been reported to have inhibited *Staphylococcus aureus* [15]. *O gratissimum* L [clove basil] belongs to the family Labiatae and is an aromatic perennial herb native to Africa and Southern Asia it's widely cultivated in most parts of the world. In the southern part of Nigeria, it is called "Efirinnla" by the Yoruba speaking tribe. "Nichonwu" in Igbo while in the northern part of Nigeria, is called "Daidoga" [16]. The extract from the leaves of *O. gratissimum* possesses good anti-inflammatory, antimicrobial and antioxidant potentials which may be attributed to its phytochemical compositions [17,18].

Cymbopogon citrates Linn. belongs to the family *Gramineae.* It is an herb worldwide known as lemongrass. Studies indicate that *C. citratus* possesses various pharmacological activities such as anti-amoebic, anti-diarrheal, anti-filarial, anti-inflammatory and antimicrobial properties [19]. The flavonoids and tannins found in the *Cymbopogon citratus* extract are responsible for the antimicrobial activity [20].

Anthocleista djalonensis Linn. belongs to the family of *Gentianaceae* consists of 14 species of trees and shrubs plant-like distributed in tropical Africa [21]. *Anthocleista* spp. is called the cabbage tree in English, *SapoSapo* in the Western part of Nigeria while it is called *Kwari* by Hausa and *Mpoto* by the Igbos. It was also established that *A. djalonensis* possess phytochemicals that have strong inhibitory effects on microbes which justify the use of it as an antimicrobial [22,23].

Consumers are clamoring for food products devoid of contamination and toxicological problems hence, the preference for natural preservatives. Natural substances have been employed as preservatives [24-26]. Many leaves extracts have been screened for their antimicrobial activity, but only a few have been exploited as food preservatives on a commercial basis [27-29]. The natural antimicrobial agents in leaves extract are polyphenolic compounds with potent inhibitory effects on both food spoilage microorganisms and food-borne pathogens [30]. Therefore, their potential as food preservatives is of great interest. The potency of natural preservatives is influenced by factors such as concentration, temperature, pH, and product shelf life. The present study aimed at investigating the potency of selected leaves extracts on the microbial quality of stored orange and pineapple juices.

Materials and Methods

Mature and healthy fruits (orange and pineapple) were bought at the *Oba Adesida* market in Akure, Ondo State, Nigeria. The leaves of *O.gratissimum* Linn. and *C. citrates* Linn. were obtained from a garden in *Ede* in Osun State, Nigeria, while *A. djalonensis* Linn. were collected in July 2018 in the forest along *Obanla* road, Federal University of Technology campus, Akure (FUTA). The leaves of the plants were authenticated and identified by a Botanist at the Forestry and Wood Technology Department, FUTA. Organic acids of *BDH Analar* specification (citric, lactic, acetic, and benzoic acids) were collected at Microbiology Department, FUTA.

Samples preparation

Mature and healthy leaves of *O. gratissimum, C. citrates* and *A. djalonensis* were manually separated, cleaned and air-dried at 30±2ºC for 7 days and pulverized. The powder obtained was extracted at 70±2ºC with sterile distilled water for 48 hours in a shaker water bath. The resulting mixture was filtered with *Whatman* filter paper and the filtrate was

evaporated to dryness at 30±2ºC and the resulted cake was ground into a fine powder using mortar and pestle. The dried crude extracts were thereafter kept in an air-tight plastic container and labeled. Different concentrations of the crude leaves extracts (5 % to 40 % w/v) were prepared using sterile distilled water [31,32]. The fruit juice for the study was extracted from cleaned and peeled fruits (orange and pineapple) using a juice extractor (Russell Hobbs model number 13889-220-24N-5Hz-350-400N) under an aseptic condition. The extracted fruit juices were treated with different concentrations (5 % - 40 % w/v) of the extracts.

Microbial analysis

Bacterial isolate's identities were determined using standard methods [33]. The bacterial and fungal isolates were identified at room temperature based on their cultural, morphological and biochemical characteristics [33,34].The microbial analysis was performed at room temperature to ensure the growth of micro-organisms in the fruit juice as storing the juices at colder temperatures may hinder the growth of micro-organisms. The molecular identification of microorganisms was also determined [35].

Phytochemical screening of extracts

The crude extracts of *O. gratissimum, C. citrates* and *A. djalonensis* were subjected to qualitative phytochemical analysis. The phytochemical tests for phenols, terpenoids, flavonoids, alkaloids, steroids, saponin, tannins and terpenes, flavonoids, steroids and lycopene were determined based on colour reactions with chemical reagents [36-38].

Quantitative estimation of phytochemicals

Total phytates content: The phytin content was quantified by the titrimetric method under acidic condition using ferrous chloride solution (0.195 mg/ml) and ammonium thiocyanate indicator (0.3 % w/v). The end-point of titration is a brownish yellow colouration that persisted for 5 minutes. Phytin phosphorous was determined by relating each milligram of iron to 1.19 mg of phytin-phosphorous equivalent. The phytin content of the sample was calculated by multiplying with a factor of 3.55 [39].

Total oxalate contents: Oxalate content was determined by the gravimetric method and calcium chloride as precipitating agent (5 %) under acidic conditions. The precipitate was dissolved by warming in 1.5 moldm^{-3} H_2SO_4 solutions in a water bath at about 80ºC [40]. The dissolved precipitate was titrated with freshly prepared 0.005N potassium permanganate solution at 29ºC until the first pink colour appeared throughout the solution. The solution was allowed to stand until it was colourless. It was warmed to 70ºC – 80ºC and titration continued until a pink colour persisted for at least 30 seconds.

$$\%Oxalate = \frac{Wx100}{5}$$

W = Mass of oxalate in 100 ml of extract

Total Tannins content: Tannin was determined as total phenols after de-fatting with 0.5 ml *Folin-Ciocalteu's* reagent (Sigma) and 2.5 ml sodium carbonate using a visible spectroscopic method at 725 nm. The tannin equivalent in the form of phenol was calculated from a standard curve.

Total flavonoids content: The total flavonoids content was estimated by using spectroscopic method at 510 nm with sodium nitrite solution (5 %). The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve

Statistical analysis

All the determinations were done in triplicate and the data generated were subjected to One Way Analysis of Variance (ANOVA), while the means were separated by *Duncan's* New Multiple Range Test using SPSS version 23 at 95% confidence interval.

Results

Bacterial types isolated from orange and pineapple juices stored at ambient temperature [30±2ºC] for 72 hours:

Prior the addition of the selected leaf extracts concentrations; six bacteria were isolated from orange and pineapple juices namely; *Bacillus subtilis, Bacillus thuringiensis, Bacillus cereus, Acinetobacter indicus, Corney bacterium* sp MF285789and *Staphylococcus aureus.* Out of all the bacteria isolated, *Acinetobacter indicus* MF497336 is the only Gram-negative (-ve) bacteria, the remaining five are Gram-positive (+ve) bacteria. The results of morphology, biochemical and molecular characteristics of bacteria isolated from orange and pineapple juice stored at ambient temperature (30±2ºC) for 72 hours are shown in table 1.

Fungal types isolated from orange and pineapple juices stored at ambient temperature (30 ± 2ºC) for 72 hours

Before the addition of the selected leaf extracts concentrations, six fungi were isolated from orange and pineapple juices namely: *Pichiakudriavezeii, Saccharomyces cerevisiae, Candida krusei, Aspergillus aculeatus, Curvularialunata* and *Curvulariaaeria,* The results of cultural and microbiological characterization of fungi isolated from the different fruit juices stored at ambient temperature (30 ± 2ºC) for 72 hours are shown in table 2.

Qualitative and quantitative phytochemical constituents of plant leaf extracts used to preserve selected fresh fruit juices at 30±2ºC for 10 days

Qualitative analysis of the phytochemical constituents of the hot aqueous extracts of the selected plants revealed the presence of terpenoids, phenols, flavonoids, alkaloids, steroids, cardiac glycoside, saponin, tannins and anthraquinone (Table 3). *O. gratissimum* contained all the tested phytochemicals except cardiac glycoside and anthraquinone. Anthraquinone was also absent in *C. citrates* while steroid was absent in *A. djalonensis.* The highest concentration of phytate (5.76±0.06 %) was found in *O. gratissimum*. The plant's extract contained oxalate

Juice Samples	Gram stain	Cell shape	VP	MR	Citrate	Motility	Indo	Catalase	Sugar fermentation					Types of bacteria Present
									Lactose	Sucrose	Glucose	Manito	Maltose	
Orange	+	Rod [chains]	-	-	+	+	-	+	-	-	+	-	-	*Bacillus cereus* KX057595
	+	Rod [chains]	+	-	+	+	-	+	-	+	+	+	+	*Bacillus subtilis,* MH769494
	+	Rod [chains]						+	-	+	+	-	+	*Corneybacterium* sp MF285789
	+	Cocci[Tetra	-	+	-		+	+	+		+	-	-	*Micrococcus luteus* MG733544
	+	Cocci[grapes bunches]	+	+	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus* MH938044
Pineapple	+	Rod [chains]	+	-	+	+	-	+	-	+	+	+	+	*Curvularialunata* KU681406
	+	Rod [chains]		+	-	-	-	+	-	+	+	+	+	*Bacillus thuringiensis* KJ9343380
	+	Rod [chains]	-	-	+	+	-	+	-	-	+	-	-	*Bacillus cereus* KX057595
	-	Rod [chains]	-	-		+	-	+	-	+	+	+	-	*Acinetobacter indicus* MF497336
	+	Rod [chains]						+	-	+	+	-	+	*Corneybacterium* sp MF285789
	+	Cocci	+	+	+	-	-	+	+	+	+	+	+	*Staphylococcus aureus* MH938044
Key: MR = methyl red, VP = Voges-Proskaeur, + = positive to the test, - = negative to the test														

Table 1: Morphology, biochemical and molecular characteristics of bacteria isolated from fruits (orange and pineapple) juice stored at ambient temperature (30± 2ºC) for 72 hours.

Colour on PDA	Colour on MEA	Configuration	Margin	Microscopic features	Fungi
Off white	Off white	Hemispherical	Irregular	Ellipsoidal to cylindrical, reproducing by irregular budding	*Pichia kudriavezeii*MH593830
Dark-brown to black	Black	Spherical	Irregular	Conidiophores, spherical vesicles and lightly pigmented hyphae	*Aspergillus aculeatus* KM45873
White	Off white	Circular	Irregular	Ellipsoidal to long cylindrical, reproducing by irregular budding	*Candida krusei* HE861376
Off white	White	Circular	Irregular	Spherical to sub spheroidal, reproducing by irregular budding	*Saccharomyces cerevisiae* CAA39055
Green to black	Off white to grey	Circular	Irregular	Simple conidiophores bearing spores apically, Conidia dark end cells 3-5 cells more or less fusiform and typically bent	*Curvularia lunata* KU681406
Green to black	Grey	Circular	Irregular	Curved cell from the base, sometimes straight, branch, pyriformis, elongated towards rounded ends	*Curvularia aeria* PSU06
Keywords PDA = Potato Dextrose Agar, MEA = Malt Extract Agar					

Table 2: Cultural, morphological and molecular characteristics of fungi isolated from pineapple juice stored at ambient temperature (30±2ºC).

Phytochemical constituent	*O. gratissimum*	*C. citratus*	*A. djalonensis*
Terpenoids	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Cardiac glycoside	-	-	-
Saponin	+	+	+
Tannins	+	+	+
Anthraquinones	-	-	-
Lycopene	-	-	-
[+] = Present [-] = Absent			

Table 3: Qualitative phytochemical constituents of selected plant leaves extracts used to preserve fruit juices (orange, pineapple) at 30±2ºC for 10 days.

(0.74±0.04 %), tannins (2.40±0.10 %) and flavonoids (1.72±0.050 %). The highest concentration of phenols (1.0±0.20 %) was found in *C. citratus* crude (Table 4).

Effect of different concentrations of selected plant leaf extracts on the total bacterial counts [log_{10}cfu/ml] of orange juice stored at 30±2 ºC for 10 days

The addition of crude aqueous leaves extract of *O. gratissimum* at (5 and 30 %w/v) concentration to freshly prepared orange juice did not significantly (p≤0.05) reduce the bacterial counts during the storage. However, the addition of 40 % w/v concentration of the extract to the juice significantly (p≤0.05) reduce the bacterial load from 2.27±0.01 log_{10} cfu/ml to 2.23±0.03log_{10} cfu/ml on day 1 and from 5.50±0.20log_{10} cfu/ml to 2.09±0.02 log_{10} cfu/ml of the juice stored for 10 days at 30±2ºC. The extracts of *C. citratus* and *A. djalonensis* follow similar patterns. Out of three extracts used, 40 % w/v of *O. gratissimum* was found to be the most effective in reducing the total bacterial counts in the stored juice samples for 10 days. The results of different concentrations of selected plant leaves extracts on total bacterial counts [log_{10}cfu/ml] of orange juice stored at 30±2ºC for 10 days is shown in table 5.

Effect of different concentrations of selected plant leaves extracts on the total fungal counts (log_{10} sfu/ml) of orange juice stored at 30±2ºC for 10 days

The addition of *O. gratissimum* aqueous leaves extract at concentrations (5-30 % w/v) to freshly prepared orange juice did not significantly (p≤0.05) reduce the fungi counts during the storage. However, the addition a 40 % w/v concentration of aqueous crude extract (*O. gratissimum*) significantly (p≤0.05) reduce the fungal load from 2.65±0.02 log_{10}sfu/ml to zero counts on day 1 and from 4.49 ± 0.30 log_{10}sfu/ml to 1.45±0.04 log_{10}sfu/ml of the juice stored for 10 days at 30±2ºC. The addition of *C. citratus* and *A. djalonensis* extracts to the orange juice follows similar patterns on the fungal counts of orange juice. However, out of the three extracts used, 40 % w/v *O. gratissimum* was found to be the most effective in reducing the total fungal counts in the stored juice samples for 10 days. The results of different concentrations of selected plant leaves extracts on the total fungal counts (log_{10}sfu/ml) of orange juice stored at 30±2ºC for 10 days are shown in table 6.

Effect of different concentrations of selected plants leaves extracts on total bacterial counts [log_{10}cfu/ml] of pineapple juice stored at 30 ± 2ºC for 10 days

The addition of aqueous leaves extract of *O. gratissimum* at concentrations (5-30 % w/v) to the freshly extracted pineapple juice did not significantly (p≤0.05) reduce the bacterial counts during the storage. The addition of the 40 % w/v concentration of the extract significantly (p≤0.05) reduced the bacterial load from 5.34±0.02log_{10} CFU/ml to 2.24±0.0log10 CFU/ml on day 1 and from 5.63±0.02log_{10} CFU/ml to 1.83±0.00log_{10} CFU/ml of the juice stored for 10 days. The extract of *C. citratus* and *A. djalonensis* follow similar patterns on the bacterial counts of pineapple juice. Out of the three extracts used, 40 % (w/v) concentration of *O. gratissimum* aqueous extract was found to be the most effective in reducing the total bacterial counts in the stored juice samples for 10 days. The result of different concentrations of selected plants leaves extracts on the total bacterial counts [log_{10}cfu/ml] of pineapple juice stored at 30±2ºC for 10 days is shown in table 7.

Effect of different concentrations of plant leaf extracts on the total fungal counts [log_{10} sfu/ml] of pineapple juice stored at 30 ± 2ºC for 10 days

The addition of aqueous leaves extract of *O. gratissimum* at concentrations (5 -30 % w/v) to freshly prepared pineapple juice did not significantly (p≤0.05) reduce the fungi counts during the storage. There was a significantly [p≤0.05] reduction in the fungal load from 2.68±0.02log_{10}sfu/ml to zero counts on day 1 and from 3.03±0.01 log_{10}sfu/ml to 1.04±0.01log_{10}sfu/ml upon the addition of 40 % w/v concentration of *O. gratissimum leaves extract to* the pineapple juice stored for 10 days at 30±2ºC. A similar pattern of fungal load reduction was observed upon the addition of *C. citratus* and *A. djalonensis* to the pineapple juice stored under the same conditions for 10 days. However, out of the three plant extracts used, 40 %w/v *O. gratissimum* was found to be the most effective in reducing the total fungal counts in the stored juice samples for 10 days. The results of different concentrations of selected leaves extracts on the total fungal counts [log_{10}sfu/ml] of pineapple juice stored at 30 ± 2ºC for 10 days are shown in table 8.

Discussion

Microorganisms are associated with raw food such as fruits [40]. The high microbial load found in juices during the storage can be a result of the surface flora added during harvest and post-harvest processing, transport and storage [41,42]. The bacteria isolated in this investigation are *Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Acinetobacterindicus, Corneybacteium* sp *Staphylococcus aureus* and *Micrococcus luteus.* The presence of these bacteria in fruit juices is of great concern because some are

Phytochemical constituent	*O. gratissimum*	*C. citratus*	*A. djalonensis*
Phytates	5.76±0.06^{c}	0.48±0.01^{b}	0.24±0.04^{a}
Oxalates	0.74±0.04^{c}	0.46±0.02^{b}	0.14±0.03^{a}
Tannins	2.40±0.10^{c}	0.52±0.21^{b}	0.18±0.01^{a}
Flavonoids	1.71±0.01^{c}	1.14±0.04^{b}	1.10±0.10^{a}
Phenols	0.71±0.01^{b}	1.00±0.20^{c}	0.34±0.01^{a}
Values are mean ± SD of triplicates (n = 3), Means with the same superscript letters along the row are significantly different (p ≤ 0.05).			

Table 4: Quantitative phytochemical constituents of selected plant leaves extracts [mg/100ml] used to preserve fresh fruit juice (orange and pineapple) at 30±2ºC for 10 days.

Leaves extract Concentrations (% w/v)				
O. gratissimum	Day 1	Day 2	Day 5	Day10
0	2.27±0.01^{Bb}	2.29±0.02^{Bb}	5.43±0.20^{Aa}	5.50±0.20^{Aa}
5	2.26±0.01 ^{Bb}	2.29±0.02 ^{Bb}	5.40±0.40 ^{Aa}	5.44±0.30^{Aa}
10	2.26±0.01 ^{Bb}	2.27±0.01 ^{Bb}	5.38±0.02 ^{Aa}	5.33±0.11 ^{Aa}
20	2.25±0.02 ^{Bb}	2.26±0.03 ^{Bb}	2.24±0.10 ^{Bb}	3.18±0.41 ^{Aa}
30	2.25±0.02^{Bb}	2.29±0.02^{Bb}	2.15±0.10^{Bb}	3.14±0.22^{Aa}
40	2.23±0.03^{Aa}	2.19±0.03^{Aa}	2.12±0.01^{Bb}	2.09±0.02^{Bb}
C. citrates				
0	2.27±0.01^{Bb}	2.29±0.02^{Bb}	5.43±0.20^{Aa}	5.50±0.20^{Aa}
5	2.27±0.01^{Bb}	2.28±0.02^{Bb}	5.42±0.50^{Aa}	5.45±0.40^{Aa}
10	2.26±0.03^{Bb}	2.28±0.01^{Bb}	5.41±0.40^{Aa}	5.34±0.21^{Aa}
20	2.26±0.01^{Bb}	2.24±0.03^{Bb}	3.27±0.40^{Aa}	3.21±0.31^{Aa}
30	2.25±0.21^{Bb}	2.27±0.03^{Bb}	3.16±0.11^{Aa}	3.15±0.20^{Aa}
40	2.25±0.20^{Aa}	2.21±0.02^{Aa}	2.13±0.01^{Bb}	2.23±0.05^{Aa}
A. djalonensis				
0	2.27±0.01^{Bb}	2.29±0.02^{Bb}	5.43±0.20^{Aa}	5.50±0.20^{Aa}
5	2.27±0.01^{Bb}	2.29±0.00^{Bb}	5.44±0.14^{Aa}	5.45±0.30^{Aa}
10	2.27±0.02^{Bb}	2.27±0.02^{Bb}	5.42±0.11^{Aa}	5.44±0.22^{Aa}
20	2.26±0.01^{Bb}	2.22±0.00^{Bb}	3.27±0.05^{Aa}	3.23±0.04^{Aa}
30	2.26±0.01^{Bb}	2.29±0.02^{Bb}	3.18±0.03^{Aa}	3.16±0.20^{Aa}
40	2.26±0.02^{Bb}	2.26±0.00^{Bb}	3.13±0.05^{Aa}	3.11±0.04^{Aa}
Values are mean ± SD of triplicates (n = 3). Means with the superscript letters (a, b, c, d) along the row are significantly different ($p \leq 0.05$). Means with the different superscript letters (A, B) along the column are significantly different ($p \leq 0.05$).				

Table 5: Effect of different concentrations of selected plant leaves extracts on total bacterial counts [$\log_{10}$cfu/ml] of orange juice stored at 30±2ºC for 10 days.

Plants extract Concentrations (% w/v)				
O. gratissimum	Day 1	Day 2	Day 5	Day10
0	2.65±0.02^{Bb}	3.42±0.01^{Aa}	3.66±0.05^{Aa}	4.41±0.30^{Aa}
5	NG	2.36±0.06^{Ac}	3.53±0.06^{Ab}	3.68±0.05^{Aa}
10	NG	1.53±0.02^{Ab}	1.78±0.05^{Aa}	1.71±0.03^{Aa}
20	NG	1.72±0.03^{Ac}	1.98±0.01^{Aa}	1.61±0.02^{Ab}
30	NG	1.60±0.02^{Ac}	1.32±0.01^{Bb}	1.48±0.04^{Ba}
40	NG	NG	NG	NG
C. citratus				
0	2.65±0.02^{Bb}	3.42±0.00^{Aa}	3.66±0.05^{Aa}	4.49±0.30^{Aa}
5	NG	2.34±0.02^{Ac}	2.48±0.31^{Ab}	2.60±0.21^{Aa}
10	NG	1.48±0.02^{Ab}	1.85±0.01^{Aa}	1.78±0.03^{Aa}
20	NG	1.72±0.01^{Ac}	2.07±0.02^{Aa}	1.87±0.01^{Ab}
30	NG	1.61±0.02^{Aa}	1.56±0.03^{Bb}	1.54±0.01^{Bb}
40	NG	NG	NG	NG
A. djalonensis				
0	2.65±0.02^{Bb}	3.42±0.01^{Aa}	3.66±0.05^{Aa}	4.49±0.03^{Aa}
5	NG	3.30±0.05^{Ac}	3.49±0.31^{Ab}	3.63±0.10^{Aa}
10	NG	1.56±0.01^{Ab}	1.88±0.02^{Aa}	1.79±0.03^{Aa}
20	NG	1.69±0.05^{Ab}	1.43±0.02^{Ac}	1.93±0.01^{Aa}
30	NG	1.63±0.02^{Aa}	1.58±0.02^{Ab}	1.61±0.03^{Aa}
40	NG	1.62±0.02^{Aa}	1.57±0.03^{Bb}	1.57±0.01^{Bb}
Values are mean ± SD of triplicates (n = 3). Means with the superscript letters (a, b, c, d) along the row are significantly different ($p \leq 0.05$). Means with the different superscript letters (A, B) along the column are significantly different ($p \leq 0.05$) NG = No Growth.				

Table 6: Effect of different concentrations of selected plants leaves extracts on the total fungal counts [$\log_{10}$sfu/ml] of orange juice stored at 30±2ºC for 10 days.

Plants extract Concentrations (% w/v)				
O. gratissimum	Day 1	Day 2	Day 5	Day10
0	5.34 ± 0.02^{Aa}	5.39 ± 0.03^{Aa}	5.60 ± 0.02^{Aa}	5.60 ± 0.03^{Aa}
5	2.28 ± 0.11^{Ac}	3.36 ± 0.03^{Ac}	5.40 ± 0.04^{Ab}	5.50 ± 0.02^{Aa}
10	2.28 ± 0.02^{Ac}	2.30 ± 0.03^{Ac}	5.36 ± 0.01^{Ab}	5.43 ± 0.01^{Aa}
20	2.28 ± 0.01^{Ac}	2.30 ± 0.01^{Ac}	5.36 ± 0.01^{Ab}	5.43 ± 0.01^{Aa}
30	2.25 ± 0.01^{Ac}	2.39 ± 0.02^{Aa}	2.33 ± 0.02^{Ab}	2.04 ± 0.01^{Ad}
40	2.24 ± 0.01^{Bb}	2.30 ± 0.04^{Aa}	2.24 ± 0.01^{Aa}	1.83 ± 0.02^{Bc}
C. citratus				
0	5.34 ± 0.02^{Aa}	5.39 ± 0.03^{Aa}	5.63 ± 0.02^{Aa}	5.60 ± 0.03^{Aa}
5	2.30 ± 0.03^{Ad}	3.34 ± 0.05^{Ac}	5.38 ± 0.02^{Ab}	5.40 ± 0.30^{Aa}
10	2.28 ± 0.02^{Ad}	2.28 ± 0.05^{Ac}	5.32 ± 0.10^{Ab}	5.42 ± 0.02^{Aa}
20	2.28 ± 0.05^{Ac}	2.23 ± 0.03^{Ad}	5.34 ± 0.04^{Aa}	5.17 ± 0.02^{Ab}
30	2.27 ± 0.02^{Ac}	2.34 ± 0.06^{Ab}	3.30 ± 0.02^{Aab}	3.00 ± 0.02^{Aa}
40	2.24 ± 0.05^{Bb}	2.30 ± 0.02^{Aa}	2.25 ± 0.03^{Bb}	2.23 ± 0.02^{Bb}
A. djalonensis				
0	5.34 ± 0.02^{Aa}	5.39 ± 0.03^{Aa}	5.63 ± 0.02^{Aa}	5.60 ± 0.03^{Aa}
5	2.29 ± 0.02^{Ac}	5.45 ± 0.05^{Ab}	5.46 ± 0.02^{Ab}	5.54 ± 0.05^{Aa}
10	2.29 ± 0.05^{Ac}	5.27 ± 0.02^{Ab}	5.36 ± 0.01^{Ab}	5.51 ± 0.02^{Aa}
20	2.29 ± 0.30^{Ac}	5.27 ± 0.05^{Aa}	5.24 ± 0.20^{Aa}	5.21 ± 0.03^{Ab}
30	2.29 ± 0.04^{Ad}	5.44 ± 0.01^{Aa}	5.38 ± 0.03^{Ab}	5.09 ± 0.01^{Ac}
40	2.29 ± 0.01^{Bc}	5.35 ± 0.01^{Aa}	5.24 ± 0.01^{Bb}	5.21 ± 0.03^{Bb}

Values are mean ± SD of triplicates (n = 3). Means with the superscript letters (a, b, c, d) along the row are significantly different ($p \leq 0.05$). Means with the different superscript letters (A, B) along the column are significantly different ($p \leq 0.05$).

Table 7: Effect of different concentrations of selected plants leaves extracts on total bacterial counts [$\log_{10}$cfu/ml] of pineapple juice stored at 30±2ºC for 10 days.

Plants extract Concentrations (% w/v)				
O. gratissimum	**Day 1**	**Day 2**	**Day 5**	**Day10**
0	2.68 ± 0.02^{Ad}	2.72 ± 0.02^{Ac}	2.84 ± 0.03^{Ab}	3.03 ± 0.01^{Aa}
5	NG	1.51 ± 0.01^{Ac}	1.56 ± 0.03^{Ab}	1.72 ± 0.01^{Aa}
10	NG	NG	1.38 ± 0.02^{Ab}	1.51 ± 0.05^{Aa}
20	NG	NG	1.30 ± 0.01^{Ab}	1.36 ± 0.03^{Aa}
30	NG	NG	NG	1.30 ± 0.04^{Aa}
40	NG	NG	NG	1.04 ± 0.01^{Aa}
C. citratus				
0	2.68 ± 0.02^{Ac}	2.72 ± 0.02^{Ac}	2.84 ± 0.03^{Ab}	3.03 ± 0.01^{Aa}
5	NG	1.49 ± 0.01^{Ab}	1.67 ± 0.02^{Aa}	1.18 ± 0.04^{Ac}
10	NG	NG	1.81 ± 0.05^{Ab}	1.89 ± 0.02^{Aa}
20	NG	NG	1.79 ± 0.02^{Aa}	1.73 ± 0.01^{Aa}
30	NG	NG	1.60 ± 0.03^{Aa}	1.58 ± 0.02^{Aa}
40	NG	NG	NG	1.30 ± 0.01^{Aa}
A. djalonensis				
0	2.68 ± 0.02^{Ad}	2.72 ± 0.02^{Ac}	2.84 ± 0.03^{Ab}	3.03 ± 0.01^{Aa}
5	NG	1.56 ± 0.01^{Ac}	1.71 ± 0.02^{Ab}	1.80 ± 0.01^{Aa}
10	NG	NG	1.80 ± 0.01^{Ab}	1.88 ± 0.02^{Aa}
20	NG	NG	1.78 ± 0.03^{Aa}	1.26 ± 0.02^{Ab}
30	NG	NG	1.60 ± 0.01^{Aa}	1.60 ± 0.03^{Aa}
40	NG	NG	NG	1.48 ± 0.01^{Aa}

Values are mean ± SD of triplicates (n = 3). Means with the superscript letters (a, b, c, d) along the row are significantly different ($p \leq 0.05$). Means with the different superscript letters (A, B) along the column are significantly different ($p \leq 0.05$) NG = No Growth.

Table 8: Effect of different concentrations of leaves extracts on the total fungal counts [$\log_{10}$sfu/ml] of pineapple juice stored at 30±2ºC for 10 days.

pathogens that may cause disease outbreaks associated with fruit juices [43]. The survival of pathogens in an acidic environment of juices is attributed to their ability to control their internal pH and kept at neutral pH by the combination of passive and active homeostasis mechanisms [44,45]. The presence of *Bacillus* sp. in fruit juices may aid the fermentation of carbohydrates [46]. Also, *Bacillus subtilis* is known to produce a diversity of enzymes. Their metabolic activities can contribute to flavour and aroma generating reactions [47]. The presence of *Staphylococcus aureus* could be a result of contamination during processing *Staphylococcus aureus* was reported to be present only at the early stages of the storage [48,49]. *Bacillus cereus* occurrence in some juices might be due to contamination from human and animal wastes that may have been used for fertilizing the soil on which such fruits were planted or contamination from the irrigation water [50]. The presence of *Acinetobacter indicus* could originate from the soil and they can withstand the acidic environment. The presence of *Corynebacterium* sp could be a result of acid produced during the fermentation [51].

The observed yeasts in the juice samples were *Pichia kudriavezeii, Saccharomyces cerevisiae* and *Candida tropicalis.* These organisms have the ability to grow at low pH, high sugar concentration, and low water activity (Table 2). Fruit juices are generally rich in simple carbohydrates and nitrogenous compounds which are ideal substrates for yeast growth [52]. The following moulds were observed in the fruit juices studied; *Aspergillusaculeatus, CurvularialunataandCurvulariaaeria.* These moulds were aerobic in nature; grow at low pH values, high sugar concentration and at high pasteurization temperature [53,54]. Pasteurized packaged fruit juices have been reported to contain moulds that adhered to package interior, cart or seals and produce pectinolytic enzymes that influence juice stability causing musty and stale off-flavours [3,54]. The presence of *Aspergillus aculeatus* originated from the soil and these fungi contain cellulose and hemicelluloses, can degrade food substance [55].

The bacteria and fungi isolated from juice samples and stored at ambient temperature after being subjected to both cultural and molecular characterization showed that molecular characterization supported the results obtained from the cultural method. Some bacteria and fungi identified culturally aligned with the molecular characterization results and this was in agreement with the previous study [56].

The mechanism underlying the inhibition of bacterial growth is thought to be charged amino-group present in the extracts which may suppress fungal growth by impairing the exchanges with the medium, chelating transition metals ions and inhibiting enzymes due to the positive charge, resulting in increased permeability of the membranes and leakage of cell material from tissue or due to water binding capacity and inhibition of various constituents within the extracts [57,58].

Previous studies have established the effectiveness of *O. gratissimum* leaves extract as an antimicrobial agent used in the inhibition of some food pathogens: *Escherichia coli, Listeria monocytogenes, Salmonella enteritica, Vibrio parahaemolyticus, Aspergillus niger, Aspergillus flavus, Aspergillus tamarii, Rhizopus nigricans, Rhizopus oryzae, Penicillium citrinum* and *Penicillium oxalicum* [59,60].

The mode of action of the extracts on the growth of fungi might be due to the interaction of extracts with membranes or cell wall components. The juice preservative tendency of the selected plants leaves extracts may be microbicidal or microbiostatic each of which simply prevents the organism from growing, thus improving the self-life of the product [61]. The presence of the extract might inhibit the formation of the cell wall resulting in the death of the fungi [62]. The activity of the plant leaf extracts used in this study has laid credence to several works that have identified the role of plant leaves constituents as agents of preservation in the inhibition of food spoilage fungi [63].

Conclusion

The selected plant leaves extracts used (*Ocimum gratissimum, Cymbopogan citratus* and *Anthocleista* sp.) exert different degrees of potency at different concentrations in reducing the microbial load in orange and pineapple juices. *Ocimum gratissimum* at 40 % w/v concentration however has the highest preservative potency in pineapple juices during storage at 30±2ºC for 10d.

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Declaration of Conflicts

None

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