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Bio-preservation Potential of Leaf Extracts of *Ocimum gratissimum* L. on Fresh-Cut Fruits of *Citrullus lanatus* (Thunb)

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ABSTRACT: This study was conducted to assess the retail market edibility quality of *Citrullus lanatus* fresh-cut fruits enhanced with the extracts from the leaves of *Ocimum gratissimum*. Analytical graded ethanol and hexane were used to extract *O. gratissimum* leaves differently. The fresh-cut fruits were dipped in the extract obtained from the leaves and reconstituted with Tween 80, fruits treated with Tween 80 and untreated fruits were used as controls. The treated fruits with the extracts, fruits treated with Tween 80 and fruits without treatment were analysed for quality test using the following parametres: carotenoids, ascorbic acids, total phenolic acid, pH, total soluble solids, microbial loads and moisture contents at day 0 and at an interval of 3, 5 and 9 days. The quantitative estimations of the phytochemicals in the extracts were determined and the constituents in the essential oil of the hexane extracts were established with the use of GC-MS system, and the data generated from the study were analysed with SPSS 20.0. This study showed that there was lower reduction in moisture content, pH, ascorbic acid and potential browning values of the treated fresh-cut fruits of *C. lanatus*, compared to higher reduction in untreated and tween 80 treated fresh-cut fruits during the storage intervals for nine days. The treated fresh-cut fruits had higher Brix values of TSS, total phenolic acid and carotenoids contents, compared with the untreated fresh-cut fruit, which was low during the period of storage for nine days. The microbial loads in the untreated fresh-cut fruit of *C. lanatus* were higher than what was obtained in the treated fresh-cut fruits of *C. lanatus* after nine days of storage. The yield of Alkaloids from ethanol and hexane extracts were 54.25±0.09 $^{mg}/_{100g}$ and 51.86±0.06$^{mg}/_{100g}$ respectively. γ-Terpinene (17.21 %) and (E)-9-Octadecenoic acid (11.848 %) had the highest percentage composition of phytoconstituents present in the essential oil from *Citrullus lanatus*. This study was able to establish the preservation potential of *Ocimum gratissiumum* on fresh-cut fruits of *Citrullus lanatus*.

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The growing enlightenment among consumers in developing nations due to the nutritional and safety profiles of fruits and vegetables enhanced with synthetic preservatives has raised a need for healthy and nutritive fruits and vegetables devoid of side effects in the form of headaches, cancer and palpitations. Fruits and vegetables have been classified as one of the important sources of major dietary of nutrients for humans (Qadri *et al*., 2015). One of the examples is watermelon; watermelon with a botanical name: *Citrullus lanatus*, is of the Cucurbitaceae family. It is related to the cantaloupe, squash and pumpkin, and it is a vine-like flowering plant which originated from sub-Saharan Africa. It is typically found in grasslands and bushlands in close proximity to water (Alam *et al*., 2012). Watermelon is rich in Vitamins A, B, C, amino acid and carotene lycopene. The presence of these nutritional elements accords the fruits the ability to assist with the boosting of the energy levels, aids in the synthesis of collagen, which assist in protecting against oxidative damage in humans. It has been established that the consumption of watermelon protects humans against stomach, oral and lung cancers, improves cholesterol and prevents scurvy (Alam *et al*., 2012). Minimal processing of the watermelon is in the form of fresh-cut and this will deteriorate rapidly, compared with the respective intact fruits as a consequence of the cutting of the fruits. The deterioration might be due to post-processing increases in hydrolytic enzymes (Mao *et al*., 2006), and this might reduce the shelf life of the fruits; the reduction in shelf life might be ascribed to fewer compartments of the fruits which are obvious with the juice leakage and water soaking and thus contribute to the reduction of soluble solids and lycopene (Perkins-Veazie and Collins, 2004).

We carried out this study to determine the preservation potential of leaf extracts of *Ocimum gratissimum* on fresh-cut fruits of *Citrullus lanatus*. This test plant is *Ocimum gratissimum* L.; it is commonly known as clove basil and is an aromatic perennial herb native to

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Africa, Southern Asia and the Bismarck Archipelago; its cultivation is now widely acknowledged in most parts of the world. Its ethnobotanical importance ranges from antioxidants, anti-inflammatory to antimicrobial activities (Nakamura *et al*., 1999, Nguefack *et al*., 2009 and Pandey *et al*., 2014).

MATERIAL AND METHODS

Plant Materials: Fresh *Citrullus lanatus* was obtained from a local market in Lagos, and the vendor acknowledged the uniformity in the variety of the fruit because they were from the same source, and the fruits were later authenticated at the Lagos University Herbarium (LUH) to confirm the uniformity; it was observed that the fruits were of uniform quality, maturity and size and devoid of a symptom of infection. Fresh *Ocimum gratissimum* was obtained from the Botanical Garden of the University of Lagos. They were authenticated and deposited at the Lagos University Herbarium.

Preparation of Plant Extracts: The leaves of *Ocimum gratissimum* were shade-dried for ten days and ground with the aid of an industrial miller. Ethanol and Hexane were used to extract the weighed leaves separately; 200g of the plant samples was soaked in 500mL of the test solvent in a conical flask and plugged with cotton wool and kept on a shaker for 72 hours. The extracts were filtered using a Whatman, No.1 filter paper and a muslin cloth several times, and the extracts were concentrated to dryness with the aid of a rotary evaporator. The extracts were kept at 4°C in a refrigerator prior to use (Silva *et al*., 2014 and Adeogun *et al*., 2016).

Preparation of Fresh Cuts of Citrullus lanatus: The fresh-cut fruits of *Citrullus lanatus* were rinsed under an aseptic condition with distilled water, later dipped in 20% Sodium hypochlorite solution for five minutes before rinsing again with distilled water. The fruits were then processed minimally into one centimetre thick slices (Bitencourt *et al*., 2014).

Gas Chromatography-Mass Spectroscopy Analysis of the Essential oil from the Leaves of Ocimum gratissimum

The hexane extract was analyzed for the essential oil phytoconstituents using the method adopted by Adeogun *et al*. (2016). The hexane extract was selected because of the recovery rate, non-polar nature, low latent heat of vaporization and high selectivity of the solvent. The oils were analyzed using Hewlett Packard 6890 Gas Chromatograph linked with the Hewlett Packard 5973 mass spectrometer system which was equipped with an HP5-MS capillary column (30m×0.25 mm, film thickness 0.25 m, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 70ºC to 240ºC at the rate of 5°C min^{-1}. The ion source was set at 240ºC with an ionization voltage of 70eV. Helium was used as a carrier gas. Spectra were analyzed using the Hewlett Packard Enhanced Chem Station G1701 program for windows.

Application of the Extracts to Fresh Cuts of Citrullus lanatus: The ethanol and hexane extracts were reconstituted in 0.05% of Tween 80 separately, and this was with slight modifications to the method employed by Hyun *et al*. (2015). The sliced cuts of *Citrullus lanatus* were dipped into the solution for 10 minutes and later transferred and packaged in nylon films. The samples were stored for nine days at 4ºC and analyzed at day 0, 3, 5 and 9 using the following quality parametres:

Carotenoids: The total carotenoid content of the samples was determined using Rodriguez–Amaya (2001) methods with slight modifications. Ten millilitres of cold pure acetone was added to four grams of each test sample and homogenized until the residue became colourless. The homogenate was filtered under suction using Whatman's filter paper. Ten millilitres of petroleum ether was added to the extract, and the residue was rinsed with distilled water several times to remove the acetone. The petroleum ether extract was passed through a test tube containing anhydrous sodium sulfate. Afterwards, the test tube was shaken for homogeneity of the mixture, then it was left for 10mins to settle. Each tube had two layers and five millilitres of the upper layer of each tube was poured in a separate tube. The determination of the total carotenoid content was carried out using a spectrophotometer at 435nm with petroleum ether as a blank sample. The total carotenoid content was expressed as mg/100g.

Ascorbic Acid Content: The ascorbic acid content was determined with 20g of the test fresh-cut fruits using the 2, 6-dichlorophenolindophenol titrimetric method (AOAC 2006). The results were expressed as $^{mg}/_{100g}$ Fresh Weight (FW).

Total Phenolic Content: The total phenolic content of the fruit extract was determined using the Folin–Ciocalteu assay as described by Zlotek *et al*. (2016) with slight modifications. A 40µL aliquot from the extract of diluted test fresh-cut fruit was mixed with 1.8ml of Folin–Ciocalteu reagent. After five minutes of equilibrium at 25ºC, 1.2ml of 7.5g/100ml Na_2CO_3 solution was added to the extract. The solutions were mixed and allowed to stand for one hour at 25ºC, and thereafter; the absorbance was measured at 765nm

using a microplate reader. Total phenolic content was calculated using a standard curve of Gallic acid and expressed as mg of Gallic acid equivalents.

pH Measurement: The acidity of the test fruit samples was determined with constant agitation of 20mL of the samples on a pH metre at room temperature. It was expressed as the negative logarithm of the hydrogen ion concentration in a solution (Martin-Diana *et al*., 2009).

Total soluble solids (Brix): The total soluble solids of the samples were determined by measurement of the refraction index with a refractometer at 20^{o}C. Refractive index was recorded and expressed as Brix (Martin-Diana *et al*., 2009).

Moisture Content: The moisture content was determined using the method described by the Association of Official Analytical Chemists (AOAC, 2006).

Microbial Load: The treated sample was diluted in 225ml of 0.1% ($^{w}/_{v}$) sterile water and homogenized for 60 secs. One millilitre of diluted samples was 10-fold serially diluted with nine millilitres of sterile peptone water. A drop of the suspension was placed on the ruled area of a clean Neubauer counting chamber. A coverslip was placed first, and the cells were permitted to run underneath by capillary action from Pasteur pipette tip. The counting cell was allowed to stand about 10 mins to permit the viable cells to settle into the same focal plane as much as possible. Using a light microscope, the spores were counted with 10x ocular and 5mm objective and this was done in the total grid in triplicate (Adeogun *et al*., 2016). The cell count was calculated thus:
Viable cells/ml= Number of cells in the total grid x dilution factor x 10^{-4}.

Browning Potential: An approximate volume of 10mL of the test fresh-cut fruit was treated with ethanol for 60 minutes and then centrifuged at 4830rpm at 10^{o}C for 10 minutes, retaining the supernatants. After, a further amount of ethanol was added to bring the final measure to 25mL of the aliquot. The aliquot was read with a spectrophotometre at 320nm, and the results were expressed as absorbance units (AU) mL^{-1} (Martin-Diana *et al*, 2009).

Quantitative Phytochemical Screening: The quantitative phytochemical determination of the ethanol and hexane extracts of *O. gratissimum* was carried out using the standard methods described by Trease and Evans (2009); Mbaebie *et al*. (2012); Adeogun *et al*. (2016) and Bankole *et al*. (2016).

Statistical analysis: The treatments for the test fresh-cut fruit samples were prepared in triplicate for each of the analyses and the data obtained were subjected to ANOVA and differentiated with Duncan Multiple Range Test. The statistical application used was Statistical Package for the Social Sciences tool 20.0.

RESULTS AND DISCUSSION

This study examined the potential of the ethanol and hexane extracts of *Ocimum gratissimum* leaf as an effective shelf-life enhancement agent for *Citrullus lanatus* (watermelon). The effect of the leaf extracts on the quality of fresh-cut fruits of *Citrullus lanatus* is illustrated in Table 1. Table 1 shows that there was a decrease as the days of storage increased, and it also indicates there is a lower reduction in the pH of the fruits treated with extracts. The addition of the extracts to *C. lanatus* caused a reduction in the pH value. As noted in this study, this addition slowed down the rate of depreciation and increased the acidic values compared to *C. lanatus* stored without the extracts. The moderation in acidity with the addition of the extracts showed that the extracts can contribute to the delay in physiological changes of the fresh cut of *C. lanatus* (Marpudi *et al*., 2011). Browning potential is known to depend on the total amount of phenolic compounds and the level of polyphenol oxidase enzymes, which triggers browning in fresh fruits. The non-browning effect noted in the treated watermelon might be due to the effect of the extracts in hindering the interaction of the polyphenol oxidase with the polyphenol present in the fruit. The antioxidant activity of *O. gratissimum* could explain the browning potential condition through inhibition of oxidative processes as reported by Akinmoladun *et al*. (2007). Table 1 also indicates that there was a reduction in the ascorbic acid content in the test fruits, with a lower reduction with treated fruits. There was a slow decrease in the ascorbic acid content of the watermelon stored with the extracts. Klimczak *et al*. (2007) noted a loss of about 22 % ascorbic acid in fresh-cut orange juice stored over a long period. This loss was attributed to the use of ascorbic acid to avoid enzymatic browning by reducing quinones to phenolic compounds before they undergo reactions to produce pigment. The reduction observed in this study might also be due to the influence of *O. gratissimum* leaf extracts in reducing respiration as well as oxidation in the stored watermelon. This corresponds to the study of Gupta and Jain (2014) who also noted a reduction in ascorbic acid content in the stored mango fruit pulp, which they ascribed to the rapid conversion of L-ascorbic to dehydro-ascorbic acid in the presence of the enzyme ascorbinase. Oxidation of antioxidants is a phenomenon that always occurs during processing of fresh-cut fruits and vegetables due to wound stress.

Table 1: Quality Parametres of Stored Fresh Cut of *Citrullus lanatus* for 9 days

Parameter	Test Sample	*Citrullus lanatus*			
		Day 0	**Day 3**	**Day 5**	**Day 9**
pH (Log_{10} H^+)	Ethanol Extract	5.00±0.02	5.05±0.01	4.80±0.01	4.60±0.01
	Hexane Extract	4.40±0.01	4.44±0.06	4.22±0.03	4.09±0.04
	Citrullus lanatus	4.40±0.01	4.27±0.01	4.22±0.00	4.05±0.07
	Tween 80	5.40±0.01	5.24±0.04	5.18±0.06	5.26±0.04
Moisture (%)	Ethanol Extract	72.00±0.03	69.84±0.01	58.28±0.01	54.36±0.03
	Hexane Extract	62.01±0.02	60.14±0.01	58.84±0.08	54.56±0.01
	Citrullus lanatus	97.00±0.03	93.12±0.01	91.20±0.04	85.36±0.06
	Tween 80	85.00±0.02	82.40±0.01	81.10±0.06	77.20±0.01
TSS (Brix)	Ethanol Extract	12.33±0.03	15.12±0.04	16.39±0.03	18.49±0.01
	Hexane Extract	16.50±0.01	16.85±0.01	18.67±0.01	19.14±0.01
	Citrullus lanatus	8.50±0.06	13.61±0.08	17.23±0.01	21.34±0.00
	Tween 80	7.00±0.01	12.48±0.05	16.08±0.06	19.56±0.05
Ascorbic Acid (AA)	Ethanol Extract	15.99±0.01	15.83±0.01	15.25±0.01	14.74±0.01
	Hexane Extract	21.99±0.01	20.04±0.01	19.57±0.01	15.66±0.01
	Citrullus lanatus	18.18±0.03	22.37±0.08	27.21±0.02	32.85±0.05
	Tween 80	19.41±0.04	25.83±0.03	29.10±0.07	33.87±0.09
Phenolic Content (mg of Gallic Acid)	Ethanol Extract	39.81±0.04	42.16±0.01	43.78±0.01	44.49±0.01
	Hexane Extract	41.71±0.32	42.94±0.01	44.37±0.01	45.89±0.01
	Citrullus lanatus	18.37±0.01	12.85±0.05	7.93±0.04	7.37±0.02
	Tween 80	15.41±0.01	12.74±0.01	9.36±0.04	5.10±0.02
Carotenoid ($^{mg}/_{100g}$)	Ethanol Extract	11.62±0.03	13.90±0.01	14.03±0.05	14.08±0.07
	Hexane Extract	12.68±0.03	13.65±0.09	13.85±0.01	14.01±0.00
	Citrullus lanatus	4.34±0.01	2.54±0.04	2.54±0.01	1.97±0.07
	Tween 80	2.97±0.01	3.53±0.01	13.54±0.01	13.53±0.01
Potential Browning (AU) mL^{-1}	Ethanol Extract	0.17±0.04	0.16±0.01	0.16±0.00	0.14±0.00
	Hexane Extract	0.14±0.02	0.14±0.01	0.14±0.01	0.13±0.00
	Citrullus lanatus	0.30±0.01	0.25±0.04	0.20±0.05	0.16±0.04
	Tween 80	0.22±0.01	0.18±0.04	0.12±0.03	0.06±0.01
Microbial Load (CFU mL^{-1})	Ethanol Extract	$2.62x10^4$	$3.12 x10^4$	$3.26 x10^4$	$3.42 x10^4$
	Hexane Extract	$2.43 x10^4$	$2.97 x10^4$	$3.02 x10^4$	$3.05 x10^4$
	Citrullus lanatus	$2.34 x10^4$	$3.78 x10^4$	$5.02 x10^4$	$5.15x10^4$
	Tween 80	$2.27 x10^4$	$3.88 x10^4$	$5.06 x10^4$	$5.28x10^4$

The rate at which TSS content increase in the treated fresh-cut was slow compared with the rate of an increase in the untreated fresh cut of the *C. lanatus*. The addition of the extracts increases the total soluble solids of the *C. lanatus* compared to *C. lanatus* without the extract and the stored sample with Tween 80. The increase in TSS indicates hydrolysis of starch or complex carbohydrate into soluble sugars such as glucose, fructose and sucrose. There is usually an increase in sugar content during ripening. The hydrolysis process was noticed in the works of Tehrani *et al*. (2011) and Hossain *et al*. (2014) who noticed an increase of TSS in stored *Syzygium agueum* and *Mangifera indica* respectively. This, however, runs contrary to the study of Kasim and Kasim (2014) who noted decreased in TSS level during the storage of *Galia melon* cultivar previously treated with UV light. They went further to state that cutting induces degradative changes associated with plant tissue senescence, and consequently, decrease in the shelf life of fresh-cut product compared to the unprocessed product. There was a reduction in the phenolic and carotenoid contents of fresh-cut fruits of *C. lanatus*, treated with *O. gratissimum* extracts while there was a significant reduction in the untreated fresh cut fruit, this is illustrated in Table 1. It was noted that the total phenolic content increased as the storage days increased in the sample treated with the plant extracts while there was a decrease in the untreated sample, likewise, in the sample stored with Tween 80. The breakdown of tannin in the extracts and the watermelon could have triggered the increase in phenolic content. The result of this present study confirms the report of Rivera *et al*. (2006). The breakdown could also be ascribed to the slowdown of the metabolic process in the *C. lanatus*. There is a reduction in the total phenolic content of stored *C. sativus* without extracts and *C. sativus* with Tween 80. The decrease can be associated with a series of physical and chemical changes, including slow inactivation of oxidative enzymes such as polyphenol oxidases and peroxidases (Lutz *et al*., 2015).

The addition of the plant extracts increased the carotenoid contents of *C. lanatus* which could be the reason for the reduction in oxidation. It is a well-known fact that lycopene, a carotenoid found in watermelon also enhances the antioxidant properties of watermelon. With the extracts, there is prevention of the formation of free radicals and reduce their reactions thus minimizing oxidative damage (Hayashi *et al*., 2005). The increase in the carotenoid content of treated watermelon as noted in this study might as well be responsible for the reduction in speedy oxidation.

The reduction in carotenoid content observed in the untreated watermelon lay credence to the work of Dea *et al*. (2012) who observed that the degradation of carotenoids of fresh-cut fruits might be the inducement of ethylene production by occurrence of the wound, and this hastens tissue senescence, including fatty acid oxidation by lipoxygenase which in turn contributes to carotenoid co-oxidation.

Table 2: Quantitative Phytochemical Analysis of Leaf Extracts of *Ocimum gratissimum*

Phytochemical Constituents	Hexane Extract ($^{mg}/_{g}$)	Ethanol Extract ($^{mg}/_{g}$)
Alkaloid	51.86±0.06	54.25±0.09
Cardiac Glycoside	11.30±0.10	6.39±0.64
Phenol	11.32±0.70	4.61±0.64
Tannin	3.52±0.12	16.58±0.09
Steroid	2.45±0.49	11.60±0.73
Saponin	53.81±0.08	86.07±0.34

Values are expressed as mean ± SEM for three replicates

It was established that the rate of microbial growth in the watermelon stored with *O. gratissimum* was insignificant compared to the watermelon stored alone or that stored with Tween 80. This could have been due to the synergistic effects of the phytoconstituents of the extracts. Several works have laid credence to the effective antimicrobial activity of *O. gratissimum* as the leaf's extracts had been successfully used in the inhibition of some food pathogens like *Salmonella enteritica*, *Vibrio paraha emolyticus*, *Escherichia coli*, *Listeria monocytogenes*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii*, *Penicillium citrinum*, *Penicillium oxalicum*, *Rhizopus nigricans* and *Rhizopus oryzae* (Bankole and Somorin, 2010; Rathnayaka *et al*., 2013). It was noted that the saponin and alkaloid contents had the highest percentage compositions respectively at 86.07±0.34 mg/g and 54.25±0.09 mg/g in the ethanol extracts while tannin and steroid had the lowest percentage compositions in the hexane extract of *O. gratissimum* leaves. The result of the quantitative phytochemical determination of leaf extracts is depicted in Table 1.

Table 3: Essential Oil analysis of Hexane Extract of the Leaf of *Ocimum gratissimum*

Peak No	Plant Constituents (Essential Oils)	RT	RI	Composition (%)	Mode of Identification
1	γ-Terpinene	3.30	0	17.210	MS
2	Caryophyllene	10.70	0	6.084	MS
3	Humulene	11.26	0	0.810	MS
4	n-Hexadecanoic acid	19.33	0	0.329	MS
5	Oleic Acid	21.23	0	10.021	MS
6	(E)- 9-Octadecenoic acid	21.56	0	11.848	MS
7	cis-Vaccenic acid	21.78	814	1.149	MS
8	Squalene	23.47	0	2.215	MS
9.	Thymol methyl ester	25.67	918	0.216	MS
10.	β-Elemene	27.24	0	0.421	MS
11.	3,4- Xylenol	30.72	0	0.126	MS
12	ρ-Cymenene	34.14	0	0.246	MS

Table 3 depicts the presence of 12 phytochemicals in the essential oil of the hexane extract of *O. gratissimum* leaf analysed. It was observed from the analysed oil that γ-Terpinene had the highest percentage composition of 17.210% while 3,4-Xylenol had the lowest percentage composition of 0.126%. The leaves of *O. gratissimum* have been documented in literature to have antimicrobial property (Nakamura *et al*., 1999; Nguefack *et al*., 2009; Mbata and Soukia, 2007; Dambolena *et al*., 2010; Dimic *et al*., 2014). Several reports have established that the essential oil with the high amount of γ-Terpinene exhibit anti-inflammatory, antimicrobial antioxidant, anti-proliferative and biopreservation properties (Giweli *et al.* 2012; Silva *et al.* 2014). The potential of the leaf of *Ocimum gratissiumum* as preservation agents of fresh-cut of watermelon might be due to the synergistic effects of the phytoconstituents in the extracts. This is in correlation with the work of Adeogun *et al*. (2016) who confirmed that the activity of natural antimicrobials presents in *Thaumatococcus daniellii* might be due to the synergy among the avalanche of phytoconstituents present in the plant extracts.

*Conclusion***:** This study shows that the leaf extracts of *O. gratissimum* have the potential to preserve the fruits of *Citrullus lanatus*. The bio-preservation activity on fruits of *Citrullus lanatus* might be due to the presence of phytochemicals in the leaf extracts of *O. gratissimum*. This work has been able to provide a guide for the purification of the extracts to ascertain the responsible compounds for the biopreservation activities. This study will contribute to conscientious efforts and advocacy of various food agencies for the use of natural antimicrobials for the preservation of fruits and vegetables.

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