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Research Article
Effects of Leaf Extracts of *Ocimum gratissimum* L. on Quality of Fresh Cut *Cucumis sativus* L.

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Abstract

Background and Objective: The consumption of fruits and vegetables is recommended for humans to increase immunity and reduce the risk of many chronic diseases. High moisture content and minimal processing of fruits and vegetables with high demand by consumers bring to fore the need for preservation. This study was carried out to ascertain the preservation potential of the leaf of *Ocimum gratissimum* (*O. gratissimum*) on fresh cut *Cucumis sativus* (*C. sativus*). **Materials and Methods:** *Ocimum gratissimum* leaves were collected and extracted with different solvents: Ethanol and hexane. The extracts were used to determine the quality of fresh cut *C. sativus* stored for 9 days using dipping method. The following quality parameters were assessed: Carotenoids, ascorbic acid, total phenolic acid, pH, total soluble solids, turbidity, microbial loads and moisture contents. Furthermore, qualitative and quantitative phytochemical analyses of the extracts were determined and essential oil constituents were established with the aid of GC-MS. Data obtained were subjected to descriptive analysis using SPSS analysis (Version 20.0). **Results:** *Cucumis sativus* treated with *O. gratissimum* extracts had lower moisture content, pH and brix values of the total soluble solids than untreated *C. sativus*. The ascorbic acid, phenolic and carotenoids contents of *C. sativus* treated with *O. gratissimum* extracts had lower values than untreated *C. sativus*. The total load of microbes in *C. sativus* treated with *O. gratissimum* extracts was lower than untreated *C. sativus*. Flavonoid, alkaloid, cardiac glycoside, phenol, tannin, steroid, anthraquinone and saponin were present in *O. gratissimum* leaves. The essential oils isolated from *O. gratissimum* included γ-terpinene, caryophyllene, oleic acid and (E)-9-octadecenoic acid. **Conclusion:** This study established that the leaf extracts of *O. gratissimum* have bio-preservation potential that can be used to enhance the shelf of *C. sativus*.

Key words: Bio-preservation, *Cucumis sativus*, GC-MS, *Ocimum gratissimum*, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ocimum gratissimum L. commonly known as clove basil is an aromatic perennial herb native to Africa, Southern Asia, the Bismarck Archilepago, is cultivated and naturalized in several regions. Its ethnobotanical importance ranges from antioxidants, antiinflammatory to antimicrobial activities[1-3]. The plant has been established to have preservative activities against food spoilage organisms and its activity has been noted on several fruits and vegetables[2]. It is within this context that the preservative potential of *O. gratissimum* on a commonly used vegetable, *Cucumis sativus* L. was evaluated. *Cucumis sativus* is a member of the *Cucurbitaceae* family, it is commonly known as cucumber and widely grown for its sweet and juicy fruits in warm climates all over the world.

The need for safe and quality fresh fruits and vegetables has led to dynamic growth in acceptance and sales of fruit and vegetables products. The acceptance has raised greater opportunities for production and marketing[4-6]. The demand boils down from the fact that substantial portion of vitamins and minerals in the diet comes from the consumption of fruits and vegetables[6] and these are needed for human's health. Research by Mahajan *et al.*[7] revealed that 32% of all food produced in the world was lost or wasted in 2009 when converted into calories. The global loss represents about 24% (weight basis) in estimate of food produced. There is need for continuous production of food to meet the teeming population of consumers in the world. The reduction of food arises through loss of postharvest fruits and vegetables due to activities of microorganisms, these encourage scarcity of food and add to menace of food shortage. The high moisture content of most fruits and vegetables makes them more accessible to microbial attack which hastens spoilage of the fresh produce.

The utilization of the nutrients of fresh fruits and vegetables occurs during processing operations such as cutting, shredding and slicing. The process affords the microbes the opportunity to grossly contaminate fruits and vegetables[8-10]. The needs to avert the activities of microbes start with proper monitoring of the chains of activities of distribution from the farms through the growers to the consumers[7,11]. The incursion of microbes in fruits and vegetables reduced their availability to consumers and encourage the incursion of microbes that cause foodborne diseases[12-14]. Foodborne illnesses are major concern for consumers, food industries and food safety authorities. This raised unease tension considering the significant implication of pathogenic microorganisms to fruits and vegetables. This happens in addition to the apprehension with the use of chemical preservatives and artificial antimicrobials to inhibit the growth of spoilage and pathogenic microorganisms[15-18]. The trepidation raised the need for alternative process of enhancing characteristics such as colour, firmness, juiceness, absence of decay, total soluble sugars, pH, titratable acidity, flavor and aroma quality of fruits and vegetables[6,16]. The use of natural antimicrobials have not been reported to have any side effects such as cancerous diseases and this brought to fore the search for preservative agents derived from a variety of natural sources.

Natural antimicrobials can be obtained from different sources including plants, animals, bacteria, algae and fungi[13,19]. The knock-on effects of synthetic preservatives bring a good attention on the explorative need for inhibitory activities of natural compounds against a number of microorganisms responsible for spoilage of fruits and vegetables. This enhances the shelf-life, reduce the development of antibiotic resistance by pathogenic microorganisms or strengthening of immune cells in humans[18,20-21]. Several studies have established great efficacy of plant derived compounds in food application as well as general acceptance of its constituents such as essential oils and plant extracts as being safe[18,19-21]. Plant extracts and essential oils are naturally occurring antimicrobials found in many plants that have shown to inhibit the activities of microbes and they have been acknowledged as a substitute to synthetic preservative in food preservation[22-25]. This study investigated the preservative potential of *O. gratissimum* on fresh cut vegetable, *C. sativus*.

MATERIALS AND METHODS

Glassware, consumables and equipment: The glassware were products of Labchem (Pty) Ltd, Johannesburg and the consumables were also obtained from Labchem (Pty) Ltd, Johannesburg. The rotary evaporator (Heidolph Laborota 4000-efficient, United States of American) used was from laboratory and analytical supplies (PTY), Durban, South Africa, platform shaker (Stuart SSM 1, scientific orbital Shaker) from Labcon laboratory consumables (PTY), Durban, South Africa and the Hewlett Packard 6890 GC-MS system used was used for the analysis of the oil.

Plant materials: Fresh *C. sativus* was obtained from a local market in Lagos in August, 2016. Fresh *O. gratissimum* was obtained from the Botanical Garden of the University of Lagos in August, 2016. They were authenticated and deposited at the Lagos University Herbarium.

Preparation of plant extracts: The leaves of *O. gratissimum* were shade-dried for 10 days and ground with the aid a specialized grinding machine at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos. Two hundred grams each of the ground leaves were soaked in analytical graded ethanol and hexane with different conical flasks plugged with cotton plugs, respectively and observed on a shaker for 72 h. The extracts were filtered through a Whatman No. 1 filter paper and Muslin cloth for several times and concentrated to dryness with the aid of a rotary evaporator. The extracts were kept at 4°C in a refrigerator until further use[8,26].

Preparation of fresh cuts of *Cucumis sativus*: The preparation of fresh cuts of *C. sativus* was based on adopted method of Da Silva *et al.*[26] with slight modifications. They were washed with water separately, later dipped in 20% hypochlorite solution for 5 min and rinsed with water, later allowed to dry at room temperature. Cleaned *C. sativus* were then peeled and cut into 1 cm thick slices.

Extraction of the essential oils: The oil extraction was obtained with the adoption of methods described by Adeogun *et al.*[8]. The oil was obtained via hydrodistillation of 0.5 kg of dried leaves for 3 h using Clevenger-type apparatus[8].

Gas chromatography-mass spectroscopy analysis of the leaves of *Ocimum gratissimum*: The analysis of the constituents of the essential oils followed the method outlined by Adeogun *et al.*[8]. The oils were analyzed using Hewlett Packard 6890 Gas Chromatography linked with Hewlett Packard 5973 mass spectrometer system which was equipped with a HP5-MS capillary column (30 m×0.25 mm, film thickness 0.25 m, Agilent Technologies Wilmington, DE, USA). The oven temperature was programmed from 70-240°C at the rate of 5°C min^{-1}. The ion source was set at 240°C with ionization voltage of 70 eV. 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 *Cucumis sativus*: The ethanol and hexane extracts were reconstituted in 0.05% of Tween 80 differently and this was with slight modifications to method employed by Hyun *et al.*[27]. The sliced cuts of *C. sativus* were dipped into the solution for 10 min and later transferred and packaged in Nylon films. The samples were stored for 9 days at 4°C and analyzed at day 0, 3, 5 and 9 using the following quality parameters.

Carotenoids: The total carotenoid content of the samples was determined following Rodriguez-Amaya[28] methods with slight modifications. Ten millilitres of cold pure acetone was added to 4 g of each 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 10 min to settle. Each tube had two layers and 5 mL 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 435 nm with petroleum ether as blank sample. The total carotenoid content was expressed as mg/100 g.

Ascorbic acid content: Ascorbic acid content of the fruit samples was determined from 20 g flesh using the 2, 6-Dichlorophenolindophenol titrimetric method[29]. The results were expressed as mg/100 g fresh weight (FW).

Extraction of polyphenols: Five grams of fruit samples was homogenized with 4 mL of 80% ethanol. The homogenate was left for 15 min and centrifuged at 10,000 rpm at 5°C for 15 min. Afterwards, the mixture was filtered through Whatman No. 1 filter paper in order to extract maximum polyphenols, the method was repeated 3 times. Thereafter, 80% methanol was added to the collected extracts to make up the volume of 50 mL and further dilutions were made up with 80% methanol. The final concentration of the extract was 0.4 g mL^{-1} of the original fruit sample and was used for total phenols content[30].

Total phenolic content: The total phenolic content of the fruit extract was determined using the Folin-Ciocalteu assay as described by Zlotek *et al.*[30] with modifications. A 40 µL aliquot of diluted fruit extract was mixed with 1.8 mL of Folin-Ciocalteu reagent. After 5 min of equilibrium at 25°C, 1.2 mL of 7.5 g/100 mL Na_2CO_3 solution was added to the extract. The solutions were mixed and allowed to stand for 1 h at 25°C and thereafter, the absorbance was measured at 765 nm using a microplate reader. Total phenolic compounds were calculated using a standard curve of gallic acid and expressed as mg of gallic acid equivalents.

pH measurement: The pH of 20 mL samples were determined at room temperature and constant agitation using a pH meter. It was expressed as the negative logarithm of the hydrogen ion concentration in a solution[31].

Total soluble solids (Brix): The total soluble solids of the samples were determined by measurement of the refraction index with a refractometer at 20°C. Refractive index was recorded and expressed as Brix. Measurements were performed at 20°C[31].

Turbidity: The turbidity of each sample was measured using a direct reading spectrophotometer. The wavelength of the instrument was brought to 810 nm and deionised water was used as a blank. The measurements of the samples were done in triplicate with a solution of 1:25 (homogenized sample/water), to work within the detectable range. The results were given in milligrams of suspended solids per liter of solution[31].

Browning potential: Ten millilitres of sample was treated with ethanol for 60 min and then centrifuged at 4,830 rpm at 10°C for 10 min, retaining the supernatants. After, a further amount of ethanol was added to bring the final volume to 25 mL. Absorbance at 320 nm of aliquots of these extracts was measured. The results were expressed as absorbance units[31] (AU) mL^{-1}.

Moisture content: The moisture content were determined using the method described by Association of Official Analytical Chemists (AOAC)[32].

Microbial load: The treated sample were diluted in 225 mL of 0.1% (w/v) sterile water and homogenized for 60 sec. One millilitres of diluted samples was 10-fold serially diluted with 9 mL of sterile peptone water. A drop of the suspension was placed on the ruled area of a clean Neubauer counting chamber. A cover slip 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 for 10 min 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 10× ocular and 5 mm objective and this was done for the total grid in triplicate. The cell count was calculated[31] thus:

$$\text{Viable cells } mL^{-1} = \text{Number of cells in total grid} \times \text{dilution factor} \times 10^{-4}$$

Qualitative and quantitative phytochemical screening: The ethanol and hexane extracts of *O. gratissimum* were subjected to qualitative preliminary phytochemical screening and later subjected to quantitative analysis of the phytochemical constituents by adopting the standard methods of Trease and Evans[33] as described by Adeogun *et al.*[8], Bankole *et al.*[34] and Mbaebie *et al.*[35].

Qualitative phytochemical screening

Test for alkaloids: Briefly, 0.5 g of the extract was stirred in 5 mL of 1% aqueous hydrochloric acid, heated on a water bath and filtered. Then, 1 mL of the filtrate was treated with a few drops of Mayer's reagent and a second portion was treated the same way only with Dragendroff's reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in the extract.

Test for tannins: In the test for tannins, 0.5 g of extract was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration as indication of tannins.

Test for phlobatanins: In brief, the extract was reconstituted in water and boiled with 1% aqueous hydrochloric acid and observed for deposition of red precipitate as indication of phlobatanins.

Test for saponins: Approximately 1 g of extract was boiled in 20 mL of distilled water in a water bath and filtered. Next, 5 mL of the filtrate was mixed with 2.5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as indication of saponin.

Test for flavonoids: A portion of the extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of diluted ammonia solution. Development of yellow colouration is an indication of the presence of flavonoids.

Test for steroids: In this test, 2 mL of acetic anhydride was added to 0.5 g of the extract with 2 mL concentrated H_2SO_4. The colour change from violet to blue or green is indication of steroids.

Test for terpenoids (Salkowski's test): In brief, 5 mL of extract was mixed with 2 mL chloroform and 3 mL H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

Test for anthraquinone (Borntrager's test): About 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red, or violet coloration in the ammonical phase indicated the presence of anthraquinone.

Test for cardiac glycosides (Keller-Kiliani test): In this test, 5 mL of the extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Phenolic compounds: The extract (500 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

Quantitative determination

Determination of alkaloids content: Five grams of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4 h. It was then filtered and the extract was concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.

Determination of tannin content: Five hundred milligrams of the sample was weighed into a 50 mL plastic bottle. Fifty millilitres of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of saponin content: Ten grams of the sample was put into a conical flask followed by the addition of 50 mL of 20% aqueous ethanol. They were then heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 100 mL of 20% ethanol. The combined extracts were reduced to 20 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel and 10 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty milliliters of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. Saponin content was calculated as percentage.

Determination of total phenols content by spectrophotometric method: The fat-free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 min. Five milliliters of the extract was pipetted into a 50 mL flask, then 10 mL of distilled water was added. Two milliliters of ammonium hydroxide solution and 5 mL of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for color development. This was measured at 505 nm.

Determination of cardiac glycosides content: One gram of fine powder was added to 10 mL of 70% alcohol for 2 h and filtered. Four millilitres of 12.5% lead acetate was added to the solution with 8 mL of 4.77% Na_2HPO_4 (disodium hydrogen phosphate) to make the volume to 32 mL with distilled H_20 and 5 mL of Buljet's reagent[35] was added to it. The absorbance was read at wavelength 495 nm.

Determination of steroid content: A portion of 2 mL was taken from a reconstituted extracts in 50 mL of distilled water after vigorous shaking for 1 h. The solution was washed with 3 mL of 0.1 M NaOH (pH: 9) and later mixed with 2 mL of chloroform and 3 mL of ice cold acetic anhydride followed by adding two drops of concentrated H_2SO_4 cautiously. The absorbance of both sample and blank were measured spectrophotometrically at 420 nm.

Statistical analysis: The data obtained were subjected to descriptive analysis using Statistical Package for the Social Sciences tool 20.0 analysis[36,37]. This was done at 95% confidence interval.

RESULTS

Quality parametres: The effect of the parametres analyzed on fresh cut *C. sativus* L. prepared with leaf extracts of *O. gratissimum* at 4°C is depicted in Table 1. The results showed that there was variation in values as the day of storage increased from day 0-9.

The pH values decreased as the days of storage increased. The moisture content decreased as the days of storage increased. There was increase in the total soluble solid as the days of storage increased.

There was reduction in the ascorbic acid content as the days of storage increased. The carotenoid content in the *C. sativus* treated with extracts increased as the days of storage increased. There was no significant browning in the stored fruits treated with extracts and there was stable turbidity range with the stored *C. sativus* treated with the extracts as the days of storage increased. The microbial load increased as the days of storage increased.

Phytochemical screening: The results of the screened extracts for qualitative and quantitative phytochemical constituents are shown in Table 2 and 3.

Qualitative phytochemical screening: The qualitative phytochemical constituents as shown in Table 2 indicates the presence of alkaloids, cardiac glycosides, phenol, tannin, steroids, anthraquinone and saponin in ethanol and hexane extracts while flavonoids, terpenoid and phlobatanins are absent in the two extracts.

Table 1: Quality parameters of stored fresh cut of *Cucumis sativus* for 9 days

		Cucumis sativus			
Parameters	Test sample	Day 0	Day 3	Day 5	Day 9
pH [-log10 (H+)]	Ethanol extract	5.58±0.01	5.36±0.01	5.36±0.01	5.13±0.02
	Hexane extract	5.37±0.02	5.27±0.01	4.91±0.32	5.28±0.02
	Cucumis sativus	5.20±0.01	4.39±0.01	4.24±0.02	4.12±0.00
	Tween 80	5.20±0.02	5.20±0.01	5.01±0.02	4.68±0.01
Moisture (%)	Ethanol extract	60.92±0.04	59.16±0.01	57.31±0.02	53.65±0.01
	Hexane extract	53.85±0.01	52.36±0.01	50.76±0.01	47.50±0.01
	Cucumis sativus	93.85±0.01	84.16±0.01	72.19±0.08	62.70±0.01
	Tween 80	85.95±0.03	80.40±0.01	74.84±0.01	66.66±0.01
Total soluble solid (Brix)	Ethanol extract	14.48±0.01	15.23±0.01	16.36±0.02	16.80±0.01
	Hexane extract	17.47±0.02	18.98±0.02	18.74±0.34	19.56±0.01
	Cucumis sativus	10.98±0.01	14.14±0.01	18.29±0.02	20.30±0.01
	Tween 80	8.48±0.01	12.88±0.01	14.15±0.01	18.76±0.01
Ascorbic acid (mg/100 g)	Ethanol extract	14.96±0.02	14.70±0.01	14.06±0.07	13.45±0.02
	Hexane extract	25.33±0.02	24.62±0.01	22.92±0.02	20.81±0.68
	Cucumis sativus	19.35±0.01	22.71±0.01	26.07±0.02	33.77±0.01
	Tween 80	13.68±0.01	16.68±0.02	19.07±0.01	24.67±0.36
Phenolic content (mg of gallic acid)	Ethanol extract	44.08±0.01	47.21±0.02	47.62±0.01	50.27±0.01
	Hexane extract	52.64±0.02	56.28±0.02	56.87±0.02	60.04±0.01
	Cucumis sativus	11.25±0.04	10.08±0.02	8.21±0.01	6.90±0.01
	Tween 80	26.76±0.11	22.33±0.02	18.10±0.01	14.75±0.01
Carotenoid (mg/100 g)	Ethanol extract	13.99±0.02	14.20±0.02	14.22±0.01	14.24±0.11
	Hexane extract	11.56±0.14	13.12±0.01	13.09±0.01	13.10±0.01
	Cucumis sativus	2.35±0.02	0.51±0.01	0.53±0.01	0.51±0.01
	Tween 80	2.17±0.11	3.18±0.01	2.84±0.01	2.23±0.01
Potential browning (AU) mL^{-1}	Ethanol extract	0.13±0.00	0.13±0.00	0.13±0.00	0.03±0.00
	Hexane extract	0.27±0.01	0.24±0.01	0.23±0.01	0.24±0.01
	Cucumis sativus	0.45±0.01	0.38±0.01	0.34±0.01	0.29±0.01
	Tween 80	0.10±0.00	0.10±0.00	0.06±0.00	0.04±0.00
Turbidity (mg L^{-1})	Ethanol extract	0.32±0.01	0.32±0.01	0.29±0.01	0.27±0.01
	Hexane extract	0.36±0.01	0.36±0.01	0.39±0.01	0.39±0.01
	Cucumis sativus	0.35±0.01	0.31±0.01	0.41±0.01	0.49±0.01
	Tween 80	0.52±0.01	0.44±0.01	0.38±0.01	0.31±0.00
Microbial load (CFU mL^{-1})	Ethanol extract	2.85×10^4	3.02×10^4	3.46×10^4	3.69×10^4
	Hexane extract	2.69×10^4	3.03×10^4	3.32×10^4	3.63×10^4
	Cucumis sativus	2.77×10^4	3.68×10^4	1.02×10^5	2.23×10^5
	Tween 80	2.53×10^4	2.99×10^4	1.06×10^5	2.26×10^5

Average Mean±Standard errors indicate that the experiment was conducted in triplicate

Table 2: Qualitative phytochemical screening of leaf extracts of *Ocimum gratissimum*

Phytochemical constituents	Hexane extract of *Ocimum gratissimum*	Ethanol extract of *Ocimum gratissimum*
Flavonoid	-	-
Alkaloid	+	+
Cardiac glycoside	+	+
Terpenoid	-	-
Phenol	+	+
Tannin	+	+
Steroid	+	+
Anthraquinone	+	+
Saponin	+	+
Phlobatanins	-	-

+: Present, -: Absent

Table 3: Qualitative phytochemical determination of leaf extracts of *Ocimum gratissimum*

Phytochemical constituents (mg/100 g)	Hexane extract	Ethanol extract
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 represent the Mean±Standard errors

Table 4: Essential oils constituents in hexane extracts of 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

*RT: Retention time, RI: Retention index, MS: Mass spectroscopy

Quantitative phytochemical determination: The result of quantitative phytochemical determination of leaf extracts is depicted in Table 3.

Essential analysis of hexane extract of leaf of *Ocimum gratissimum*: The list of the constituents of essential oils in hexane extract of leaf of *O. gratissimum* as shown in Table 4.

DISCUSSION

This study was able to establish the preservation potential of *O. gratissimum* on fresh cut fruits of *C. sativus*. The need to preserve fresh cut fruits occurred due to high consumption of fresh cut fruits because of its low calorie and fresh-like attributes[29].

The pH of *C. sativus* was affected by the addition of extracts and this raised the pH and reduced the acidity of *C. sativus*. The addition of the extract as observed in the study indicated slow depreciation and higher acidic values compared to the stored *C. sativus* 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. sativus*. Several studies have reported the delay in spoilage of fruits due to the modification of respiration rate by plant extracts and edible coating. Marpudi *et al.*[38] reported the delay in the pH of papaya (*Carica papaya* L.) fruits using *Aloe vera* based antimicrobial coating. The delay in pH as observed in Marpudi *et al.*[38] supports the finding in this study.

The reduction in moisture content can be attributed to transpiration, starch hydrolysis and evaporation loss. The rapid reduction in moisture content might be the incursion of microorganisms into the fruits. This study showed that the moisture content of the extracts with *C. sativus* was low which

signaled the activity of the extracts on the *C. sativus*. The high moisture contents in stored *C. sativus* without the extracts indicate tendency for short shelf life and such findings corroborate observations made by Agbeniyi and Ayodele[39]. They revealed that reduction in moisture contents of *Cola nitida* and *Cola acuminata* was due to fungal attack and high water contents which influenced the hyphal growth of fungi.

Total soluble solid is an important parameter that is used to assess the quality of many fresh fruits and vegetables because solids include soluble sugars (sucrose, fructose and glucose). The addition of the extracts increase the total soluble solids of the *C. sativus* compared to *C. savitus* without the extract and the stored sample with Tween 80. The increase in TSS indicates hydrolysis of starch to soluble sugars such as glucose, fructose and sucrose. The hydrolysis process was noticed in the works of Hossain *et al*.[40] and Tehrani *et al*.[41], who noticed an increase of TSS in stored *Syzygium agueum* and *Magnifera indica*, respectively.

There is general reduction of ascorbic acid content of the test *C. sativus*. It was observed during storage that there is gradual reduction in test *C. sativus* treated with the extracts compared to the samples without the extracts. The rate of reduction was higher in stored untreated *C. sativus*. The reduction might be due to the rapid loss through oxidation because of greater availability of oxygen. Mgaya-Kilima *et al*.[42] mentioned that vitamin C content of Roselle-Mango juice blend decreased with increased storage period because vitamin C can easily be oxidized in the presence of oxygen by both enzymatic and non-enzymatic catalyst. The slight retention of ascorbic acid by *C. sativus* stored with extracts might be due to the influence of *O. gratissimum* leaf extract on reducing respiration as well as oxidation of stored fresh cut of *C. sativus*.

The total phenolic content in the stored extracts with *C. sativus* increased and this agrees with observations made by Rivera *et al*.[43] and this increase could be attributed to possible breakdown of the tannins present in *O. gratissimum* and *C. sativus*. The breakdown can also be ascribed to slowing down of the metabolic process in the *C. sativus*. However, there was a reduction in total phenolic content of stored *C. sativus* without extracts and *C. sativus* with Tween 80. The decrease can be associated with series of physical and chemical changes including slow inactivation of oxidative enzymes such as polyphenol oxidases and peroxidases[44].

The addition of plant extracts increased the carotenoids contents and might also be responsible for the reduction in speedy oxidation. It was observed in this study that the rate of degradation was faster with the *C. sativus* without the extracts and the one stored with Tween 80. Dea *et al*.[45] 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. The reduction in carotenoids in this study corroborates the reduction in carotenoids content of orange juice as seen in the work of Martin-Diana *et al*.[31]. They observed that storage time determines the degradation of carotenoids content of juice and it was succinctly stated that the antioxidant effect of chitosan used did not protect the carotenoids from oxidation over storage time.

The browning potential of the fresh cut of *C. sativus* with the extracts did not show any sign of browning as depicted in Table 1. The indistinct state of the browning potential might be as a result of the activity of the extracts on the control of interaction of polyphenol oxidase with polyphenols and oxygen. Martin-Diana *et al*.[31] posited that the control of browning could be associated with the capacity to coagulate solids to which browning related enzymes are bound. The antioxidant activity of *O. gratissimum* could explain the browning potential condition through inhibition of oxidative process[46]. The extracts and storage time also affected the turbidity of *C. sativus*. Table 1 shows that *C. sativus* without the extracts and *C. sativus* with Tween 80 become more turbid along the storage time from their initial turbidity levels compared to *C. sativus* with the extracts that had slowly increase in turbidity. Akkarachaneeyakorn and Tinrat[47] and Martin-Diana *et al*.[31] noted that polysaccharides are responsible for turbidity as observed in orange juice and the loss of turbidity can be ascribed to high acidity and/or the activity of any remaining pectin esterase.

The activity of the extracts on *C. sativus* could be well justified based on previous established activities of *O. gratissimum* on microbes associated with food spoilage[48-49]. This study shows that there was less considerable growth of microbes associated with *C. sativus* stored with extracts compared with the one without the extracts. Bankole and Somorin[50] and Rathnayaka[51] in their studies confirmed the activity of leaf extracts of *O. gratissimum* on some food pathogens: *Salmonella enteritica*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Listeria monocytogenes*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii*, *Penicillium citrinum*, *Penicillium oxalicum*, *Rhizopus nigricans* and *Rhizopus oryzae*.

The presence of phytochemical compounds in the extracts indicates that some of the phytochemical constituents might be responsible for the potential

bio-preservative property of the leaf extract of *O. gratissimum*. The presence of alkaloids, cardiac glycoside, terpenoids, phenol, tannin, steroids, anthraquinone and saponin in the leaf extracts of *O. gratissimum* have been confirmed by several authors[46,52-53]. The bioactivity of this plant laid credence to several reports that have been done on the inhibition of microbes responsible for spoilage which gives the plant the ability as preservation agents. This study was able to establish that the two extracts from leaves of *O. gratissimum* showed good inhibitory activities.

The antimicrobial properties of the essential oils in the plants have been documented in several literature to have antimicrobial property[1,2,52,54-57]. The preservation adeptness of the plant might be as a result of synergy among the phytoconstituents in the extracts and this was confirmed by Adeogun *et al*.[8], they cited in their report that the activity of natural antimicrobials might be due to the synergy among the avalanche of phytoconstituents present in the plant.

CONCLUSION

This study accounted for the bio-preservation potential of leaf extracts of *O. gratissimum*. The quality assessment of the parameters of the *C. sativus* was assessed for 9 days using hexane and ethanol leaf extracts of *O. gratissimum*. The presence of phytochemical constituents portends the extracts as antimicrobial agent and was qualified them as preservation agents. There is need for further purification of the extracts via biomonitoring to ascertain if the activities of all the constituents or certain constituents are responsible for the inhibition. This study contributes to drive towards the use of natural antimicrobials for preservation of foods against the established synthetic preservatives which have been acknowledged with concern regarding safety to human health.

SIGNIFICANCE STATEMENTS

This study discovered the potential of leaf extract of *Ocimum gratissimum* as a preservative agent for the enhancement of fresh cut *Cucumis sativus*. This study was able to put forward the use of leaf of *O. gratissimum* for antimicrobial activity due to the presence of phytochemicals. This will contribute to the existing research on the need for exploration of natural antimicrobials for the preservation of fresh fruits and vegetables. Results of this study will contribute to the bio-preservation and management of postharvest crops.

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