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Extension of shelf-life of *Brassica oleracea* using ethanol extracts of *Citrus aurantifolia*

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Abstract

Many studies have been conducted on the use of plant extracts to control microbial surface contamination of foods, enhance product microbial safety and extend its shelf life. The study was designed to extend the shelf- life of *Brassica oleracea* using ethanol extract of *Citrus aurantifolia.* The phytochemical analysis of the *Citrus aurantifolia* leaf extract was carried out using gas chromatography–mass spectrophotometry. The chromatogram result revealed the presence of seventy-four (74) bioactive compounds in *Citrus aurantafolia*. The bioactive compounds present includes alkaloid, phenols, alkenes, alkanes, sesquisterpernoid, alcohol, benzamides, terpenes and phthalic acid. Agar –well diffusion method was used to determine the antimicrobial activities of the plant extract against gram positive bacteria (*Staphylococcus aureus, Micrococcus roseus* and *Bacillus subtillis)* and gram-negative bacteria (*Pseudomonas aeruginosa, Citrobacter multilinea, Salmonella enterica, Bacillus lichenformis)*. The extract exhibited both antibacterial and antifungal activities against tested microorganisms. Ethanolic extract of *Citrus aurantafolia* showed significant antibacterial effect while no inhibitory effect on the antifungal. Staphylococcus appears to be a sensitive strain to the extract. Further studies of the antimicrobial studies were carried using inhibition of total dehydrogenase activity as measure of toxicity *C. aurantifolia.* The toxicity of the *C.aurantifolia* leaf extract and Ciprofloxacin on the microbial isolate reveals that *Bacillus subtilis* was more susceptible to the plant extract than the other microbial isolates. Total microbial counts of the cabbage after 12 days incubation period was used to assess the shelf-life of *Brassica oleracea* using two samples of the cabbage, identified as Sample A and sample B respectively. The citrus aurantifolia extract has significant effect on the cabbage. The results suggest that the ethanoic extracts of *Citrus aurantifolia* exhibit a considerable number of phytochemicals and antimicrobials activities. Hence, plant extracts have demonstrated potential as natural antimicrobials and can be used safely as food preservatives.

Keywords: *Citrus aurantafolia; Brassica oleracea;* Phytochemicals; Antimicrobial activity; Inhibition of total dehydrogenase; Activity; Shelf-life

1. Introduction

Currently plants- derived food preservatives is the new trend used in food industry for preservation of foods. In the olden days, some of the natural preservatives such as citrus fruits, salts and vinegar were traditionally used to preserve food but with the current changes, the food industry uses plants and their extracts as a potential means to preserve food. Plants constitute source of bioactive compounds which are the essential raw materials in production of foods, cosmetics and drugs. Different plant parts have been used for this purpose. Plant extract as biostimulant is essential in modern and sustainable agriculture. Plants possess antifungal, antimicrobial, medicinal and anti-inflammatory properties. Utilization of plant extract in preserving food has different impacts on the quality of the food. Some methods used in food preservation are known to be carcinogenic. Traditional methods used in preserving foods have lower energy input in contrast to modern method. The food and agriculture organization FAO) anticipates that the deterioration in the world ecosystem and world's population will lead to inability of humans to feed themselves (FAO, 2010)

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Bioactive compounds occurring in plants have antioxidant and antimicrobial properties and hence, play important role in food preservation. The bioactive compounds present in plants are grouped as phenolic compound which are present in plants, fruits etc (Ahmad et al, 2015). The bioactive compounds of plant parts like the seed, leaf, root, bark and stem may differ in terms of quality because of the presence of chemical compounds present. The bioactive compounds of plants are attributed to phenolic compounds like flavonoids, terpenoid, and other phenolic compounds. They have the ability to inhibit the growth of microorganisms and slow down the enzymatic activities in food (Brewer, 2011).

Shelf-life of food products refers to the length of time a food can be kept under storage condition while maintaining its safety and quality. The shelf-life of food is the time the food remains acceptable to eat. However the recent trend in the use of plant extracts in extending the shelf-life of foods has improved the physiological properties of foods when added under appropriate conditions (Baptista et al, 2019 *Citrus aurantafolia* (Lime leaf) is a traditional plant, originated from tropical Southeast. Asia and South Asia. *Citrus aurantifolia* has spread throughout the world through migration and trade. *Citrus aurantifolia* belongs to *Rutaceae* family, its common name is lime or swing, the lgbo name is oroma nkirisi. *Citrus aurantifolia* is widely used as antibacterial (Roka et al, 2016).

Citrus aurantifolia leaf is gotten from citrus fruit called Lime. Lime fruit is typically round, greenish in colour and contains acidic juicy vesicles. Lime leaf contains lower amount of vitamin C but higher content of vitamin A. Lime leaf contains flavonoids and other phytochemicals that are effective antimicrobials and chemotherapeutic medicinal agent (Dongmo et al*,* 2009). Phytochemical components of lime leaf include alkaloids, tannins, steroids and flavonoids such as rutin, quercetin and essential oils which function as antioxidants. (Dongmo et al 2009).

Brassica oleracea (cabbage) is a cruciferous Brassica vegetable and a member of *Brassicaceae* and mustard family (Delahaut. 1997). Raw Cabbage and other cruciferous vegetables contain small amounts of thiocyanate (a compound associated with goitre formation when iodine intake is deficient (Vanderpas, 2006).

The study additionally assesses the antimicrobial, phytochemical constituents of the leaves through the employment of the GC-MS technique, with the aim of augmenting the potential applications of these plants, particularly in extension of shelf life of *Brassica oleracea.*

2. Materials and method

2.1. Materials

2.1.1. Chemicals and Reagents

All chemicals are at the best analytical crude commercially available. , Ethanol, cotton blue stain, distilled water, sterile tryptone water, kovac's reagent, Methyl red–Voges Proskauer, naphthol, Sabouraud dextrose agar (SDA), pipeton , lactophenol. Bench centrifuge, gas chromatography column (Agilent 6890N) and mass spectrophotometer (5975B MSD), spectrophotometer, water bathe, distillation apparatus, Unicam Spectronic 20-DR spectrophotometer, curvette, grinder, UV/VIS Spectrophotometer

2.1.2. Collection and Identification of Plant Materials

The present study involved the harvest of fresh plant leaves from *Citrus aurantafolia* from Umuezegwu in Ihitte-Uboma Local Government of Imo State. It was identified and assigned voucher numbers by a plant taxonomist, Mr. Francis Iwueze, who is affiliated to the Department of Forestry and Wildlife at the Federal University of Technology Owerri (FUTO), located in Imo State , the plant was authenticated with voucher no FUTO/FWT/ERB//2023/100

2.1.3. Plant sample preparation

The leaves were washed using running water in order to remove dirts accumulated on the surface of the leaf. The leaves were dried at room temperature for two weeks, They were later grounded into fine powder using an industrial-grade grinding machine.

2.1.4. Ethanol extraction

Powdered *Citrus aurantafolia* powder weighing 200 gm was extracted using ethanol in which the *Citrus aurantafolia* powder was soaked in 1500ml of ethanol for 48 hours. After 48 hours the solution was filtered using filter paper and subjected to water bath for evaporation to 48 hours. The solution was later evaporated for 24 hours in order to get the extract.

2.2. Methods

2.2.1. Phytochemistry

Gas Chromatography- Mass Spectrometry Analysis (GC-MS)

Ten grams (10g) of the grounded sample was soaked in 30ml aqueous solution overnight and filtered through filter paper. Two microliter of the sample extract was injected into the GC column for analysis. The GC (Agilent 6890N) and MS (5975B MSD) were equipped with DB-5ms capillary column (30m×0.25mm; film thickness 0.25µm). The initial temperature was set at 40 ^{0}C but increased to 150^{0}C at the rate of 100^{0}C/min. The temperature was further increased to 23^{0}C at the rate of 5^{0}C/min

The process continued till the temperature reached 28^{0}C at the rate of 20^{0}C /min and was then held for 8 minutes. The injector port temperature remained constant at 280^{0}C and detector temperature was 250^{0}C. Helium was used as the carrier gas with a flow rate of 1ml/min. Split ratio and ionization voltages were 110.1 and 70eV respectively.

Identification of Unknown Compounds Present in the Extract

To identify the unknown components in the extract, their individual mass spectral peak values were compared with the database of National Institute of Science and Technology 2014 (NIST, 2014). Then the phytochemicals were identified after comparing the unknown peak value and chromatogram from the GC-MS against the known chromatogram, peak value from the NIST library database. Subsequently, the details about the molecular formula, molecular weight, retention time and percentage content were also obtained.

Antimicrobial Susceptibility Test

The antibacterial activities of the extracts against the test bacteria were evaluated by modified disc diffusion methods (Agu *et al*., 2013; Adindu *et al.*2016) Exactly 25 µl of 0.5 McFarland standardized suspension of test bacteria (1.5x10^8 cfu ml^{-1}) were cultured onto the Mueller-Hinton plates by pour plate method. Exactly 50 µl of the extracts were used to impregnate the 6mm filter paper discs and placed on two portions of the agar plate. The Inhibition zone diameters of the various plates were measured and recorded in millimeters. All experiments were done in triplicates. Negative controls were set up with sterile physiological saline and positive controls were set up using 50 µg/ml Ciprofloxacin

Antimicrobial assay using Inhibition of total dehydrogenase Activity

Antimicrobial studies was carried using inhibition of total dehydrogenase activity as a measure of the toxicity as previously described (Alisi et al, 2008; Nweke *et al,* 2014) . The toxicity of *C. aurantifolia* leaf extract and ciprofloxacin on the microbial isolate was determined in 1.0ml volume containing varying concentrations of respective test material in requisite volumes of distilled water, nutrient broth, 0.02% MTT and isolate. The toxicity of the compounds was determined for *C. aurantifolia* leaf extract (0-5000 µg/ml), and Ciprofloxacin (0 –200 µg/ml).

Briefly, total dehydrogenase activity was assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Germany) as the artificial electron acceptor, which was reduced to the purple-coloured diphenyl-formazan (DPF). The set-up consisted of varying concentrations of the toxicants in corresponding volumes of sterile distilled water, 0.25ml nutrient broth (x 4 strength) and 0.1 ml of inoculum. Thereafter, 0.1 ml of 0.02 % (w/v) MTT in sterile distilled water was added to each tube to obtain final volume of 1.0ml and corresponding final concentration in different each test tubes. The controls consisted of the respective isolate, sterile distilled water and the media void of toxicants. The set-up was incubated in the dark for 24 hrs at 25 ± 2^{o}C. The formazan produced, was extracted by shaking vigorously in 4ml of butanol. The extracted formazan was measure in a UV-visible spectrophotometer at 575nm. The absorbance readings were converted into percentage inhibition of dehydrogenase activity relative to control.

Sample Analysis on Cabbage Spoilage Bacteria after 12 days incubation period

One gram (1g) of the spoilt Cabbage samples were weighed out aseptically and introduced into 10ml of sterile peptone water for bacteria, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10^{-2}) of the sample were pour plated in sterile plates of Ceramide Agar, Mannitol Egg Yolk Polymyxin (MYP) agar plates for the culture of bacteria. The culture plates were incubated at 37°C aerobically for 24-48hours for bacteria. Discrete colonies for the bacteria were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods. The colonies were counted and recorded as colony forming units per millilitre (cfu/ml¬^{1}) after 48 hour for 12 days.

3. Result

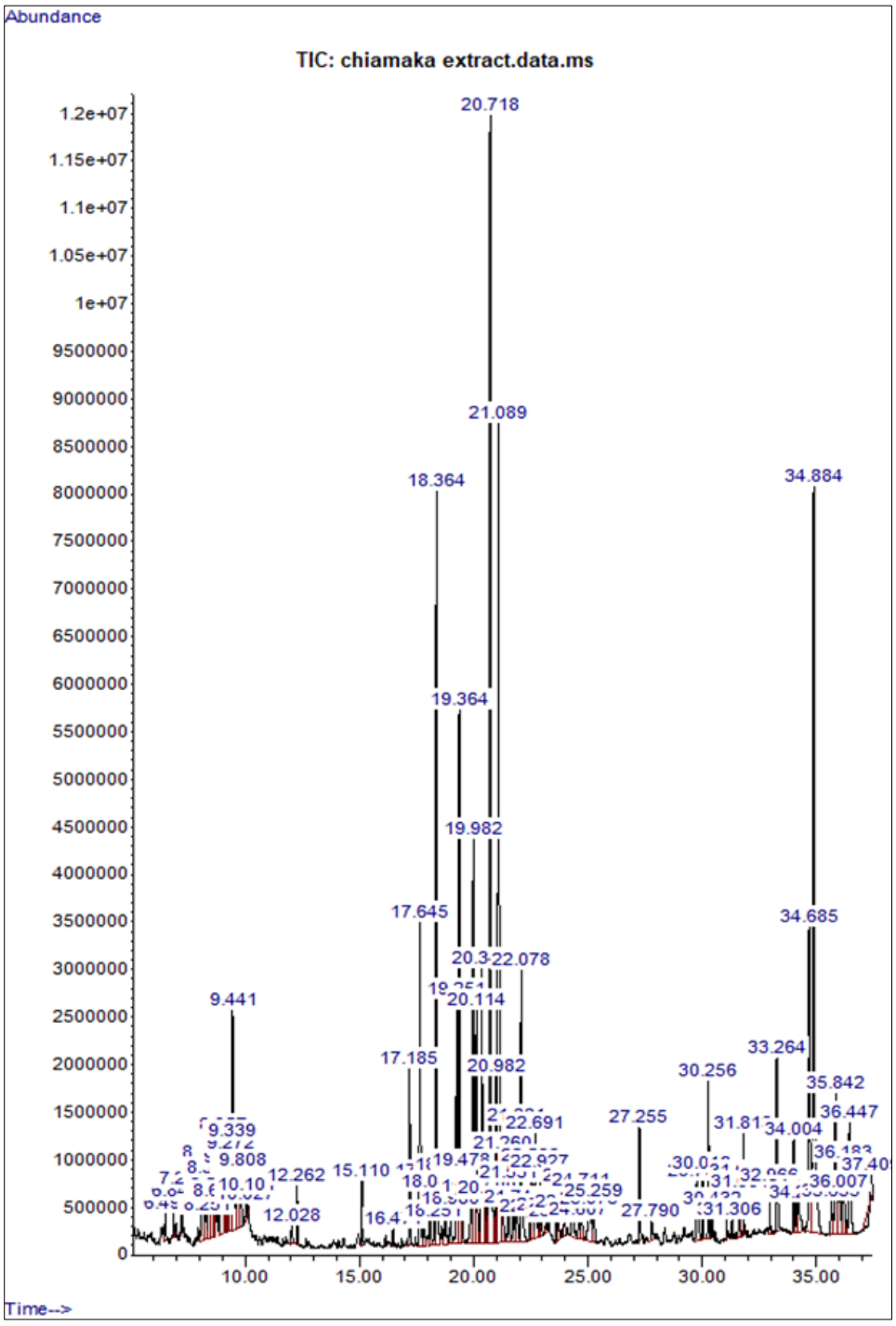

Figure 1 Chromatogram of GC-MS of *Citrus aurantafolia* leaves

Table 1 Result showing bioactive compounds present in *Citrus aurantafolia* using GC-MS

Class of compound	AREA (%)	RT	Bioactive compound	Molecular formula	molecular weight
Alkenes	8.251	0.27	Octane, 3,4,5, 6-tetramethyl-Heptadecane	C_8H_{18}	14.22g/mol
	8.380	0.82	Decane, 2, 6,7-trimethyl-undecane	$C_{10}H_{12}$	142.28g/mol
	8.534	0.55	Nonane	C_9H_{20}	170.33g/mol
	8.591	0.19	Tetradecane	$C_{14}H_{30}$	198.39g/mol
	8.644	0.23	Undecane-2-methyl- hexadecane	$C_{12}H_{26}$	282.5g/mol
	8.957	2.14	Heptadecane	$C_{10}H_2$	142.28g/mol
	9.118	0.46	Octane, 4-ethyl- Carbonic acid	$C_{10}H_{22}$	212.41g/mol
	9.176	0.72	Tetradecane Undecane, 4- Tridecane	$C_{15}H_{32}$	212.41g/mol
	9.272	0.78	Heptadecane, 2, 6, 10, 14- 2, 8-dimethyl	$C_{29}H_{44}$	296.57g/mol
	9.339	0.89	Tridecane	$C_{44}H_{90}$	619.18g/mol
	9.735	0.42	Oxalic acid	$C_2H_20_4$	90.03g/mol
	9.808	0.50	Nonane, 3, 6-dimethyl- Nonane	$C_{12}H_{26}$	142.28g/mol
	0.027	0.11	Undecane,5-methyl- Octane $C_{11}H_{24}$	$C_{11}H_{24}$	170.33glmol
	9.691	0.67	2, 3,7-trimethyl- Carbonic acid	$C_2H_{22}O_3$	244,30g/mol
	15.110	0.49	Tridecane	$C_{13}H_{78}$	184.37g/mol
	10.10	0.09	Octane, 2-methyl- Dimethyldecane	$C_{10}H_{16}$	142.28/mol
	17.83	0.50	Tetradecane1H Cycloprop[e]azulene	$C_{13}H_{28}$	184.36g/mol
	31.62	0.26	9-Oxabicyclo [6.1.0] nonane $C_8H_{12}O$	$C_8H_{12}O$	126.20g/mol
Alkene	12.028	0.15	1-Dodecene	$C_{12}H_{26}$	170.33g/mol
	12.26	0.44	Dodecene	$C_{12}H_{24}$	168.32g/mol
	31.813	0.36	1-Octadecene		
	20.98	1.45	Cyclohexene	C_6H_{12}	84/16g/mol
	31.664	0.26	1-Docosene	C_2H_{44}	308.58g/mol
	21.089	7.59	1- cyclohex-1-ene ethyl	$C_7H_{12}0$	136.23g/mol
Phthalic acid	29.764	0.83	L 1,2-B carboxylic Benzamide acid	C_2H_3NaO	114.09g/mol
	33.264	1.59	Diisooctyl phthalate	$C_{26}H_{42}O_4$	390.56g/mol
Alcohols	27.790	0.22	Ethanone	C_9H_4O	134.17g/mol
Linoleic acid	31.306	0.11	Linoleic acid	$C_{20}H_{36}$	308.41g/mol
Ether	6.496	0.23	Oxirane	C_2H_4O	44.05g/mol
Benzamide	36.18	0.94	Benzamide	C_7H_7NO	121.139g/mol
Alkaloids	35.84	1.79	piperine	$C_{17}H_{19}NO_3$	285.34g/mol
Pyrrolidine	36.47	1.45	L Pyrrolidine	C_4H_9N	71.123g/mol
Terpene	21.88	1.00	1, 6,10-Dodecatrien-3-ol	$C_{15}H_{24}$	204.35g/mol
	22.078	2.59	1H-Cycloprop[e]azulen-7-ol	$C_{11}H_8$	140.18g/mol
	8.162	1.0	gamma.-Terpinene	$C_{10}H_{14}$	134.21g/mol

	23.181	0.11	Apiol	$C_{12}H_{14}0_4$	222.37g/mol
	24.29	0.80	Beta.-Bisabolene	$C_{15}H_{24}$	204.35g/mol
Monoterpiene	21.748	0.37	Alloaromadendrene	$C_{15}H_{24}$	204.35g/mol
	8.162	1.07	gamma.-Terpinene	$C_{10}H_{14}$	134.21g/mol
	23.59	0.16	Aromandendrene Naphthalene	$C_{10}H_8$	128.17g/mol
	21.26	0.90	1, 3,6-Octatriene	C_8H_{12}	180.18g/mol
Aromatic compound	20.114	2.07	Naphthalene	$C_{10}H_8$	128.17g/mol
	25.073	0.79	L- Naphthalene	$C_{10}H_8$	128.17g/mol
	20.481	0.37	alpha-Muurolene	$C_{15}H_{24}$	204.18g/mol
	20.588	0.37	Benzene, 1, 4- dichloro-benezene	$C_6H_4Cl_2$	47.00g/mol
	7.202	0.26	p-Cymene	$C_{10}H_{14}$	134.21g/mol
	22.078	2.59	1H-Cycloprop[e]azulen-7-ol	$C_{11}H_8$	140.18g/mol
	22.450	0.19	1, 3-Bis-(2-cyclopropyl-2-methyl	$C_{18}H_2CO$	250.33g/mol
	19.364	4.34	beta-Famesene	$C_{15}H_{24}$	204.35g/mol
Sesquisterpenoid	19.251	1.90	Humulene	$C_{15}H_{24}$	204.35g/mol
	18.98	0.28	7-Isopropyl-1,4-dimethyl-	$C_{20}H_{32}O$	204.36g/mol
	18.737	0.63	Santolina triene 1,3-Cyclo- pentadiene hexahydroazulene	$C_{10}H_{16}$	130.23g/mol
	18.364	6.27	Aromandendrene	$C_{15}H_{24}$	204.35g/mol
	18.251	0.17	alpha-Farnesene	$C_{15}H_{24}$	204.35g/mol
	18.083	0.46	1H-Cycloprop[e]azulene	$C_{11}H_{18}$	140.18g/mol
	17.645	2.76	gamma-Elemene	$C_{13}H_{28}$	184.36g/mol
	17.185	1.34	alfa-Copaene	$C_{15}H_{24}$	204.35g/mol
	9.441	2.52	Linalool	$C_{10}H_{180}$	154.25g/mol
	16.471	0.13	alpha-Cubebene	$C_{15}H_{24}$	204.35g/mol

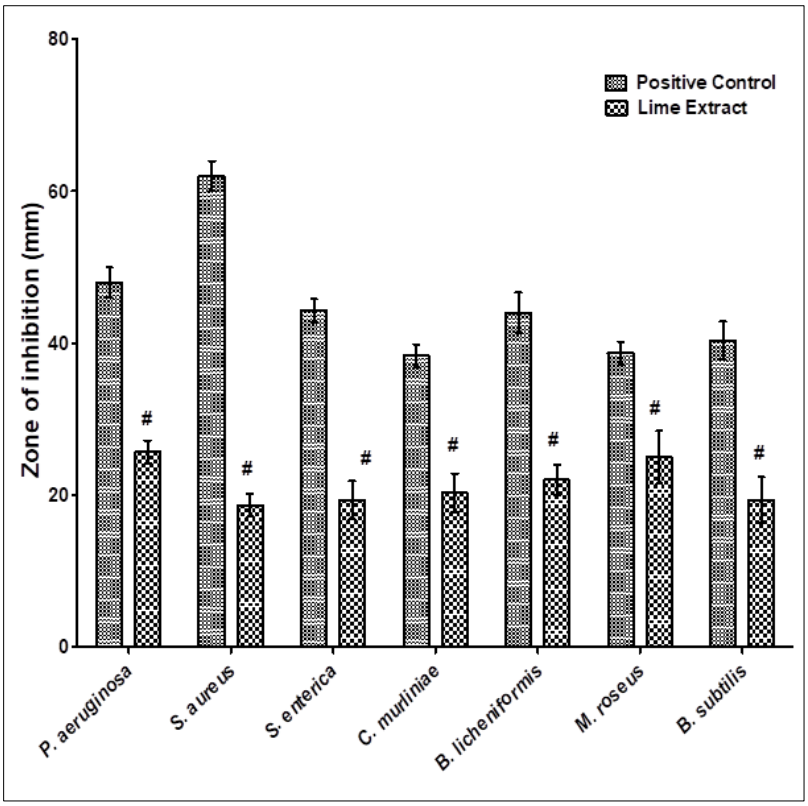

Figure 2 Zones of inhibition (cm) of microbial isolates exposed to *Citrus aurantafolia* extract

Bars are mean ± standard deviation of triplicate determinations. Bars are significantly ($p<0.05$) lower than their respective control values

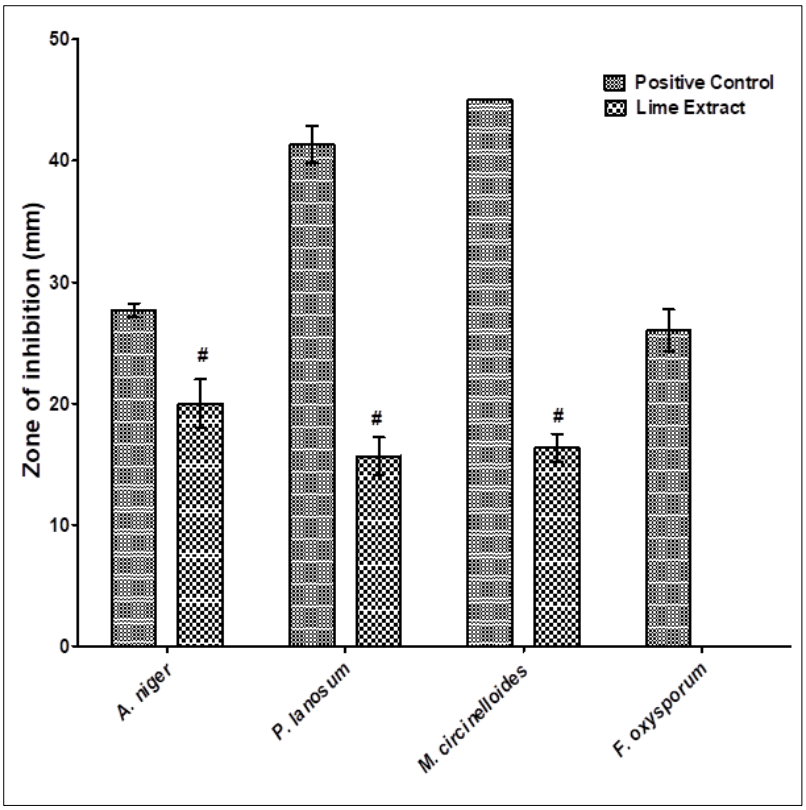

Figure 3 Antifungal susceptibility pattern of various Fungi Isolates exposed to Lime extract.

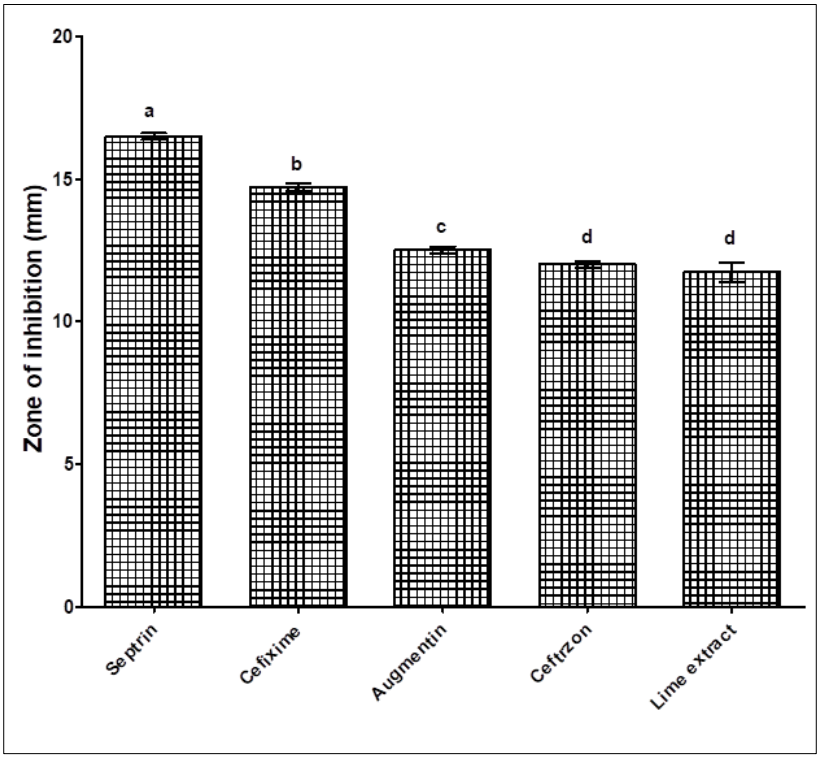

Figure 4 Antimicrobial sensitivity pattern of *pseudomonas aeruginosa* against lime extract and standard antibiotics

3.1. Dehydrogenase Activity Assay

Table 2 Percent inhibitory effects of aqueous extract of lime against microbial isolates.

Extract Conc. (µg/ml)	***Bacillus subtilis***	***Salmonella sp.***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Aspergillus niger***
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
100	1.89 ± 0.55a	11.37 ± 1.42b	15.10 ± 0.29c	3.70 ± 0.28d	2.00 ± 0.08a
150	6.36 ± 0.69a	14.37 ± 2.36b	18.39 ± 1.62c	7.99 ± 3.17a	4.56 ± 0.62a
200	14.95 ± 2.02a	17.32 ± 0.96b	25.11 ± 1.58c	15.90 ± 0.25a	10.33 ± 0.55d
500	22.26 ± 1.73a	22.70 ± 0.52a	28.96 ± 0.58b	23.15 ± 3.08a	17.62 ± 0.40c
800	27.02 ± 0.24 a	30.74 ± 4.66ab	32.12 ± 0.41b	29.28 ± 0.80a	33.66 ± 1.66b
1000	33.18 ± 0.22a	40.11 ± 0.16b	40.43 ± 0.18b	36.42 ± 1.89c	40.82 ± 1.75b
2000	64.79 ± 0.25a	56.16 ± 0.14b	55.38 ± 1.86b	46.97 ± 1.45c	48.60 ± 2.24c
3000	87.61 ± 0.13a	61.27 ± 1.72b	61.02 ± 0.51b	50.80 ± 0.68c	53.83 ± 1.19d
5000	91.26 ± 1.35a	81.58 ± 0.79b	74.26 ± 0.31c	61.22 ± 0.24d	55.95 ± 2.30e

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$)

Table 3 The Threshold inhibitory concentration (IC_{50}) of extract and standard

Isolates	***C. aurantifolia* extract (*µg/ml*)**	**Non-linear model**	**R^2**	**Ciprofloxacin (*µg/ml*)**	**Non-linear model**	**R^2**
Bacillus subtilis	1364.90 ± 129.44	Sigmoid, abc	0.9813	1.92 ± 0.29	Sigmoid, abc	0.9922
S. aureus	1195.66 ± 114.63	Logistic, abc	0.9900	1.91 ± 0.20	Sigmoid, abc	0.9967
P. aeruginosa	1727.11 ± 169.19	Logistic, abc	0.9953	1.9608 ± 0.17	Sigmoid, abc	0.9984
Salmonella spp	1356.93 ± 178.35	Logistic, abc	0.9904	2.1455 ± 0.05	Logistic, abc	0.9998
A. niger	679.39 ± 62.59	Logistic, abc	0.9955	2.0236 ± 0.040	Sigmoid, abc	0.9978

Results are mean ± standard deviation of 3 determinations

3.2. Total microbial counts of the Cabbage (shelf life)

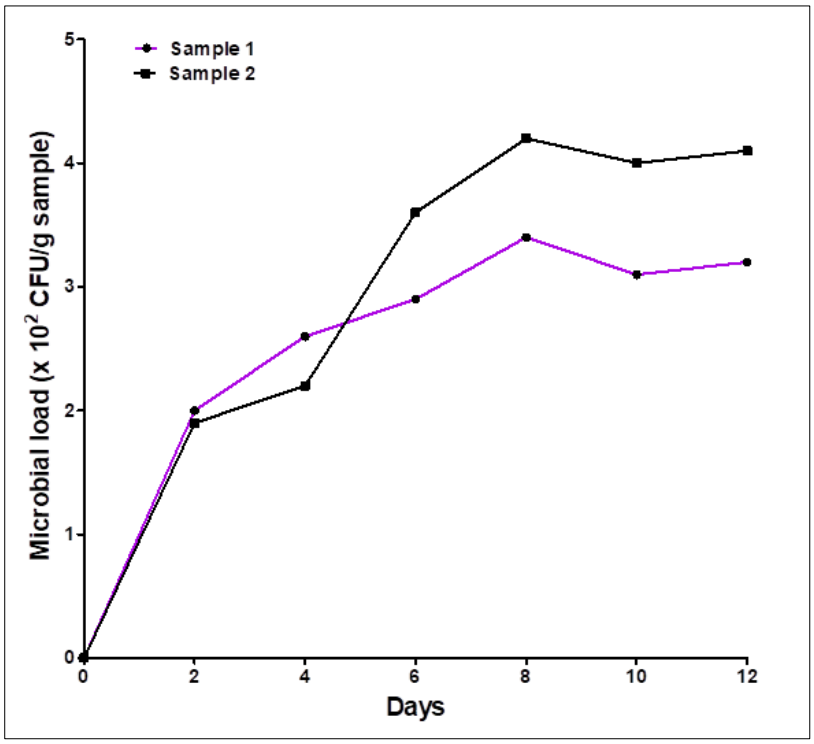

Figure 5 Microbial load (CFU/g) in plate succession (Shelf life) of samples stored at Room Temperature

4. Discussion

Plant extracts are the most pressing sources of biomolecules, which can be screened from plant parts. Due to the high chemical diversity of natural compounds, plant extracts could control the growth of microbes in food products. The use of extracts obtained from different sources and parts of plant perform various functions in food industry as antioxidants, antimicrobial agents, flavouring agents, enzymes, extension of shelf life of foods and packaging additives. The part of plant extracts to perform these function is linked to the array of phytochemicals produced and accumulated in them. GC-MS profile of citrus aurantifolia revealed presence of a number of secondary metabolites. The result revealed that *Citrus aurantafolia* produced different bioactive compounds such as alkaloids, monoterpenes, sesquistererpernoid and phenols. Alkaloids was present in the Citrus aurantifolia leaves. The presence of alkaloids supports the findings of Oyeleke et al, 2008, that the microbial activity of this plant may be attributed to the presence of alkaloids. Alkaloids have been reported to possess various pharmacological activities such as anaesthetics, cardio protective, antimalarial and anticancer activity (Saxena et al. 2013). The presence of monoterpenes in *Citrus aurantafolia* acts as antioxidant, anticancer and antidiabetic. Monoterpenes are effective in treating early and advanced cancers (Crowell et al, 1994). Linalool an example of sesquisterpernoid. Was found in the Citrus autrantifolia leaf extract., Linalool is a class of terpenes that is use in treatment of ailments such as diarrhoea, burns, influenza and neurodegradation , analgesic, antimigraine activity, sedative actions and antitumor potential (Guzman et al., 2012). Benzamide was also present, it was observed that Benzamide possess antimicrobial, anti-inflammatory, anti-cancer, cardiovascular and other biological activity. Bnzamides are used in treatment of arthritis (Raffa et al, 2002). Alkenes such as tetradecane, hexadecane, heptadecane, and octadecane identified in the studied plants' extracts had earlier been reported to have antibacterial, antiepileptic, and therapeutic properties (Mbongue et al, 2005). These alkanes are biologically important raw materials for the chemical industry and are the principal constituents of gasoline and lubricating oils (Isaac, 2012). Similarly, 2, 4-Di-tert-butylphenol, Cyclohexene. 1-Methyl-4-(6-methylhept) ene, Cyclohexanemethanol Benzene, 1, 4-dichloro-benzene which are examples of phenols, were also detected. They have a wide range of pharmacological properties which include antioxidant and radical scavenging activity, hepatoprotective effect. 4-Di-tert-butylphenol an example of phenols is used in the production of phenolic resins (Fiege et al 2000). Terpenes was also present in C.aurantifolia. Previous studies have shown that terpenes exhibits anti –inflammatory effects by inhibiting various proinflammatory pathways in ear edema and skin inflammation. Gamma terpinene, an example of terpenes is widely used in the pharmaceutical industry (Maj et al, 2015). Linoleic acid present in C.aurantifolia have the tendency of lowering the risk of cardiovascular disease. Linoleic acid serves as anti-inflammatory, acne reduction and skin lightening (Diezel et al 1993). Ethanone is used in the production of medical wipes and in antibacterial hand sanitizers

gel as an antiseptic for antibacterial and antifungal effects (McDonnell et al 1999). Aromatic compounds present in the plant include p-cymene, Naphthalene, alpha muurolene, Benzene, 1, 4- dichloro-benzene. These aromatic compounds possess the natural scent found in plants and are pervasive in nature (Balaban et al 2004).

Figure 4.1 shows the antimicrobial activities of some bacterial isolates in which Staphylococcus aureus exhibited significantly high resistant compared to other isolates. The antibacterial potential of *Citrus aurantafolia* extracts has been elucidated by the result of this study. The ethanolic extracts of citrus aurantifolia were also bactericidal towards *pseudomonas aeruginosa*, *salmonella enterica, Citrobacter mutilinea, Bacillus Lichenformis. Micrococcus roseus, Bacillus Subtillis.* The findings with that of Obasi et al, 1993 who reported that the roots and leaves of Citrus aurantifolia were medicinal

Similarly, the antifungal activity of the plant extract was isolated and identified. The fungal strains involved in this study *include Aspergillus niger, Penicillum lanosum, mucor circinelloides and Fusarium oxysporum*. The fungal strains used in this study were identified by the morphologic characteristics of the spores and structures. Figure 3 illustrates the different fungi involved in this study in which aspergillus niger shows resistance against the antifungal drug. The result showed that *Citrus aurantafolia* had more inhibition o*n Aspergillus niger* and *Penicillum lanosum* than other fungal isolates. *Citrus aurantafolia* possess antifungal activities. A scenario was also observed for septrin, cefuroxime, ceftrazion, Augmentin and lime extract which demonstrated significantly higher antibacterial activity against *pseudomonas aeruginosa*. The organisms show remarkable resistant against septrin and cefixime. Combination of Augumentin with Citrus autrantifolia leaf extract shows significant decrease in resistance to pseudomonas aeruginosa. Ceftriaxone is also resistant against strains of Pseudomonas aeruginosa. Pseudomonas aeruginosa shows inherent resistant to many antimicrobial agents due to the synergy between multi drug efflux system. Pseudomonas aeruginosa on the lime extract shows that the lime extract inhibits pseudomonas aeruginosa and the extract has excellent antibacterial activity against pseudomonas aeruginosa. Pseudomonas aeruginosa causes a wide range of infections which lead to mortality in immune-comprised patients (Obritsch et al, 2004).

Researchers can evaluate the antimicrobial properties of plant extracts by utilising the capacity of the dehydrogenase enzyme in organisms to convert triphenyltetrazolium chloride into triphenylformazan. The toxicity of *C. aurantifolia* leaf extract and Ciprofloxacin on the microbial isolate were observed that the plant extract shows varying degrees of effectiveness against the microbial isolates. Bacillus subtilis had more inhibitory effect than the other microbial isolates. Table 2 depicts the impact of the extracts and standard on the dehydrogenase activity of different microbial isolates. The findings indicate that these plants exhibited efficacy as antimicrobial agents. However at 5000 ug/ml Bacillus subtilis showed the highest susceptibility than the other microbial isolates. The findings indicate that these plants exhibited efficacy as antimicrobial agents. This further means that these extracts were observed to have a negative impact on the respiration and carbon metabolism of the isolated microbes, which are known to interact with dehydrogenase activity.

Table 4.4 shows the Threshold inhibitory concentration (IC_{50}) of extract and standard. The IC_{50} had a significant impact on the inhibition rates of the extract compared to the standard owing to their low $IC50_{5\ 0}$ values. The plant extracts were observed to have varying levels of effectiveness against different microbial isolates. Pseudomonas aeruginosa displayed the highest degree of resistance, as indicated by its elevated IC_{50} concentrations.

Figure 5 shows the total microbial count method which was used to analyse the shelf-life of the cabbage using two different samples of the cabbage. The plate with sample A was identified as the cabbage coated with lime leaf extract while sample B was identified as cabbage not coated with lime leaf extract. Application of the lime leaf extract inhibits the growth of different fungi specie. Treatment of the cabbage with the lime leaf extract affects the microbial parameters of the cabbage. Figure 5 shows the total microbial counts of the cabbage after treatment with the lime extracts every two days for 12 days. As the total microbial count increases, the cabbage deteriorates and the deterioration was caused by physiological breakdown of plant tissue rather by microorganisms. The result obtained from treatment of the cabbage with lime leaf showed that the microbial load of the treated or coated cabbage is smaller compared with the microbial load of the untreated cabbage. Figure 5 showed that the microbial load of sample B (untreated cabbage) is significantly higher than sample A (treated cabbage). The result revealed that lime leaf can prolong the shelf life of cabbage by reducing microbial load. A findings by Hugo and Rusell showed that the bioactive component of lime know as P-hydroxylbenzoic acid esteri exhibited a wide range of activities against microorganisms and that lime serves as preservatives in food industry, toiletries and in pharmaceutical industry. Cabbage are mainly exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing and they harbor a diverse range of microorganisms including plant and human pathogens. (Sagagi et al, 2022)

5. Conclusion

The Presence of various bioactive compounds in appreciable concentrations in *C. aurantafolia* revealed their potential biological, pharmacological and industrial applicability. The plant extracts exhibited antibactericidal and antifungicidal properties. The plant extract exhibited a higher degree of susceptibility towards *Staphylococcus aureus* bacterial strains and *Aspergillus niger* fungal strain. The plants extract exhibited potential as natural drug agents against diabetics, hypertensive and also in prevention of urinary diseases

The research validates the traditional usage of the plant leaf extract in extending the shelf –life of brassica oleracea in which the plant extract have significant effect on Brassica oleracea after 12 days incubation period. Hence *C. aurantifolia* has the tendency to preserve or extend the shelf –life of cabbage. The high microbial load of the cabbage does not show any sign of spoilage and thus, physical appearance may be poor criterion for analysing spoilage in cabbage. The high microbial load present in the cabbage is attributed to improper handling, unhygienic storage and transportation conditions. Microbiological control is important in the food industry to prevent food poisoning and other health hazards.

Consequently, the research shows that the plant extract can be considered in the development of new antimicrobial drugs and applied in different research areas.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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