Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2025, Volume 16, Issue 7, Page 1311-1323

http://www.jmaterenvironsci.com

Determination of the Proximate Content, Phytochemical Composition and the Antimicrobial Properties of the Aqueous Extract of *Citrus sinensis* on some Microorganisms

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Received *15 May 2025,*
Revised *20 June 2025,*
Accepted *21 June 2025*

Keywords:
- ✓ *Nanocomposite;*
- ✓ *Citrus sinensis,*
- ✓ *Orange,*
- ✓ *Proximate,*
- ✓ *Phytochemical,*
- ✓ *Bacterial,*
- ✓ *Fungi*

Citation*: Udinyiwe, C.O., Salami, D.A. (2025) Determination of the proximate content, phytochemical composition and the antimicrobial properties of the aqueous extract of Citrus sinensis on some microorganisms, J. Mater. Environ. Sci., 16(7), 1311-1323*

Abstract: *Citrus sinensis*, commonly known as sweet orange, has many medicinal properties and is widely used against various ailments, such as colic, upset stomach, diuretic, immune enhancing, stomachic, tonic to digestive system, immune system, and skin. This study was aimed at determining the proximate composition, phytochemical composition and antimicrobial potential of the aqueous extract of *Citrus senenis* (pomace) against a range of bacterial and fungal isolates. The pomace extract was also subjected to qualitative phytochemical screening to detect the presence of various phytochemicals. such as phenols, terpenoids, alkaloids, eugenols, flavonoids, tannins, and reducing sugars. Antimicrobial activity was evaluated using an agar well dilution technique against five bacterial strains and three fungal strains using the Mueller Hinton Agar (MHA) method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were determined using the MIC and MBC. The antibacterial activity of the crude extract against selected bacterial isolates was also evaluated using the MHA method. The phytochemical screening detected the presence of phenols, terpenoids, alkaloids, eugenols, flavonoids, tannins and reducing sugars It was demonstrated that the microbial strains of public health importance, *Escherichia coli* and *Staphylococcus aureus*, were significantly inhibited by the extract, suggesting its potential applications in combating bacterial infections.

1. Introduction

Long before man ever recognised the potential that microbes may exist, there was a belief that certain plants were therapeutic or even possessed what we now refer to as antimicrobial properties (Rios and Receo, 2005). The increasing prevalence of antibiotic resistance has become a significant challenge in the treatment of infectious diseases globally. As a result, the exploration of alternative sources of antimicrobial agents, particularly from plants has garnered much attention. Plants are rich in bioactive compounds that have long been used in traditional medicine to treat various ailments. These natural substances, including alkaloids, flavonoids, saponins and tannins have demonstrated significant antimicrobial, anti-inflammatory, and antioxidant properties (Santiago *et al*., 2021; Aourabi *et al*., 2021). *Citrus sinensis*, commonly known as the sweet orange, is one of the most widely cultivated fruit

species worldwide. *Citrus sinensis* is mostly enjoyed for its sweet flavour and abundant vitamin C, yet the peel, leaves and seeds of the plant has also been researched for its medicinal properties. Studies have indicated that the peel of the plant contains multiple bioactive compounds, which contribute to its antimicrobial properties, antioxidant effects and potential health benefits (Aboaba and Efuwape, 2001; Alams *et al*., 2005; El Ouadi *et al*., 2015; Diass *et al*., 2023; Haddou *et al*., 2023; Nouioura *et al*., 2024).

For instance, the methanolic extracts of citrus peels have exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (Nair *et al*., 2005; Lawal *et al*., 2013). The potential of *Citrus sinensis* as a natural treatment for illnesses brought on by both Gram-positive and Gram-negative bacteria is highlighted by these findings. *Citrus sinensis* plant is known to be rich in bioactive compounds. Proximate analysis, which evaluates the nutritional content of plant materials, is essential for understanding the potential health benefits of *Citrus sinensis*. The nutritional components typically assessed in proximate analysis include moisture content, crude protein, fat, fiber and ash content (Dubey *et al*., 2011). These components are important not only for understanding the nutritional value of the plant but also for determining its suitability for various therapeutic applications. The demand for alternative antimicrobial agents has increased due to the growing worry over antibiotic resistance. Although synthetic antibiotics remain the mainstay in treating bacterial infections, their overuse has led to resistance, making infections harder to treat (Chohan *et al*., 2010; Vahabi *et al*., 2011; Mahajan *et al*., 2012; Titi *et al*., 2021). The search for natural antimicrobial agents from plant sources offers a promising solution to this problem. *Citrus sinensis*, with its wide availability and reported medicinal properties, remains an understudied source of potential antimicrobial agents, especially when compared to other more commonly studied plants. Comprehensive research on the proximate content, phytochemical composition, and antibacterial activities of *Citrus sinensis* in connection to infections is limited, knowing that numerous studies have concentrated on other well-known identified medicinal plants with antimicrobial qualities.

Therefore, this study aims at determining the proximate composition, identifying the phytochemical constituents and evaluating the antimicrobial activity of the aqueous extract of *Citrus sinensis* against a range of bacterial and fungal isolates.

2. Methodology

2.1 Collection and Identification of Plant Materials

The sweet orange fruits were purchased from Ekiosa market in Benin City, Edo State, Nigeria. The fruits were identified by a taxonomist, Prof. H.A. Akinnibosun in the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria as Linnaeus Osbeck variety of *Citrus sinensis*.

2.2 Preparation of Sample (*Citrus sinensis* Pomace)

The fruit *Citrus sinensis* was peeled and the juiced remove. Afterwards, the peels, fibers and seeds were dried in a dehydrator at a controlled temperature (37 ^{o} C). The dried samples were then blended with electronic milling machine grinder, Lab. Mill, Serial No. 4745, Christy and Norris Ltd, England into powder. The extraction was carried out according to the methods of Igbe *et al.* (2009). Four hundred grammes (400 g) of the pulverised samples were macerated in a 1.5 litre of aqueous (sterile distilled water) contained in big bottle. The duration of the maceration was 48 hours. After extraction, the liquid was filtered using Whatman paper. The filtrate was then concentrated using crude method to

get a semi solid form. It was dispensed in small sample container and labelled, and then stored in a refrigerator.

2.3 Proximate Determinations

2.3.1 Moisture content

Two (2) grams of the sample was weighed and dried in an oven continuously. The dried sample was constantly re-weighed at 10 minutes intervals until a constant weight was obtained. The ratio of the change in weight to the original weight expressed in percentage gives the moisture content given by:

moisture content = $\frac{W0 - Wdry\ (\%)}{W0}$ (A.O.A.C. 1990)

2.3.2 Fat content

Three (3) grams of the sample (moisture-free) was placed into fat-free thimble, which was then weighed and sealed with glass wool. This thimble was placed into Soxhlet extractors containing 160 mL of petroleum ether (b.p. 60-80 °C). Clean, dry receiver flask was also weighed and attached to the extractor. After assembling the system, the water bath was heated to 60 °C and cold water was cycled. It took eight hours for the extraction. The thimble containing the sample was then taken out and dried for three hours at 70 °C in an oven until its weight remained consistent. Finally, the weight of the thimble and its contents was measured using an analytical balance.

Calculation: The crude fat was obtained as the difference in weight before and after the exhaustive extraction. Hence the percentage fat was therefore calculated as:

% Fat = $\frac{X-Y}{Z}$, where, X = Weight of sample and thimble and oil, Y = Weight of empty thimble

Z = Weight of sample

2.3.3 Ash content

Two (2) grams of the dried sample was placed in a porcelain crucible, which was first weighed. The crucible was then placed into a preheated muffle furnace set to a temperature of 900 °C. The furnace was left running for one hour, after which the crucible and its contents were transferred to a desiccator to cool. Once cooled, the crucible and its contents were re-weighed, and the weight was recorded. The percentage of ash content was then determined using the appropriate calculation (A.O.A.C. 1990).

$$\text{Ash} = 100\frac{W_{ash}(\%)}{W_0}$$

W_{ash} = content weight after final drying, W_o = the dried weight of the sample

2.3.4 Protein content

The crude protein content was determined using a modified micro-Kjeldahl method, as described by the AOAC (1990). For digestion, three grams of defatted sample was weighed into pre-weighed micro-Kjeldahl digestion flasks along with a few anti-bumping granules. Each flask received 2 grams of a catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5.1:0.2 w/w), followed by 10 mL of nitrogen-free concentrated H_2SO_4. The flasks were placed in an inclined position on a heating mantle inside a fume hood. The digestion started at 30 °C, continuing until frothing stopped, then the temperature was raised

to 50 °C for an additional 30 minutes. The temperature was finally increased to 100 °C, and the heating continued until a clear solution formed. Digestion was carried on below boiling for another 30 minutes to ensure complete breakdown and conversion of nitrogen to ammonium sulfate. After digestion, the samples were allowed to cool, then transferred quantitatively to 100 mL volumetric flasks, washed, and cooled to room temperature. The volumes were then adjusted to the mark with distilled water. Then, 5 mL of the filtrate was transferred into a 25 mL standard flask using a 10 mL pipette. To this, 2.5 mL of Alkaline Phenate was added, and the solution was mixed thoroughly. Then, 1 mL of Sodium Potassium Tartrate was added, followed by shaking, and then 2.5 mL of sodium hypochlorite was added. The solution was made up to the 25 mL mark with distilled water, and the absorbance was measured at 630 nm using a UV/visible spectrophotometer. Nitrogen standards were processed in the same way as the sample.

Calculation

$$\% \text{ N} = \frac{\text{Instrument.Reading X Slope Reciprocal X Color Vol.X Digest Vol.}}{\text{Weight of Sample X Aliquot Taken X 10000}}$$

% Crude Protein = % Nitrogen × 6.25

2.3.5 Crude Fiber

The procedure followed was based on the AOAC (1990) method. Four (4) grams the powdered moisture-free sample was weighed into a 250 mL beaker, and 50 mL of 4 % H_2SO_4 was added, followed by distilled water to reach a total volume of 200 mL. The mixture was then heated to boiling and kept boiling for exactly 30 minutes on a Bunsen flame, with constant stirring using a rubber-tipped glass rod to ensure all particles were removed from the sides of the beaker. The volume was maintained by adding hot distilled water. After 30 minutes of boiling, the contents were poured into a Buchner funnel fitted with ashless Whatman No. 40 filter paper and connected to a vacuum pump. The beaker was rinsed several times with hot distilled water and the contents were transferred quantitatively using a jet of hot water. Washing continued on the funnel until the filtrate was acid-free, as indicated by litmus paper. The acid-free residue was then quantitatively transferred from the filter paper into the same beaker, with any remaining traces removed using 5 % NaOH solution and hot water to a volume of 200 mL. The mixture was boiled for another 30 minutes with constant stirring, as previously described, and the volume was kept constant with hot water. The mixture was then filtered and washed as before until it was alkaline-free. Finally, the residue was washed twice with 2 mL of 95 % alcohol. The filter paper remains were moved to a porcelain crucible that had been previously weighed. After being dried to a constant weight in an oven set at 110 °C, the contents of the crucible were let to cool in a desiccator. The crucible and its contents were chilled, then lit for eight hours at 550 °C in a muffle furnace, cooled, and weighed. A triplicate analysis was performed for the sample. The percentage of crude fiber was then calculated as:

$\% \text{ Crude Fibre } = \frac{100\,(y - a)}{x}$, x = Weight of sample (g), y = Weight of insoluble matter (g)

a = Weight of Ash (g)

2.3.6 Carbohydrate content

2.3.6.1 Estimation of total carbohydrate: The total carbohydrate content of the diet samples was determined by subtracting the combined percentages of crude protein, crude fat, moisture, fiber, and ash from 100.

2.4 Qualitative Phytochemical Screening

The method used for the qualitative phytochemical screening was according to A.O.A.C (1990).

2.4.1 Detection of Alkaloids Content

Two (2) millilitres of the plant extract were first evaporated to dryness. Then the resultant residues were dissolved in 5 ml of HCl (2 mol/dm^3) and filtered. Two test tubes were filled with the filtrate. A few drops of Mayer's reagent were added to the first test tube, the presence of alkaloids is indicated by the production of a yellow precipitate. The second test tube was treated with few drops of Wagner's reagent and the brownish-red precipitate formation indicates alkaloids.

2.4.2 Detection of Glycoside

This was carried out by dissolving 0.5 mg of the extract in approximately 1 mL of water, followed by the addition of an aqueous NaOH solution. The appearance of a yellow colour indicates the presence of glycosides.

2.4.3 Detection of Tannins

1.0 ml of 1 % gelatin solution containing Sodium Chloride was added to 1.0 ml of the extract. The formation of a white precipitate indicates the presence of tannins.

2.4.4 Identifications of Phenolic Compounds

This was done by treating 1.0 ml of the plant extract with 4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of Phenols.

2.4.5 Detection of Saponins

The presence of saponins was detected using foam test method. In the foam test, 0.5 g of the plant extract was shaken with 2.0 mL of distilled water. The formation of foam that lasts for 10 minutes indicates the presence of saponins.

2.4.6 Screening for Flavonoids

Flavonoids were detected using the alkaline reagent test and the lead acetate test. In the alkaline reagent test, a few drops of a 2 mol/dm^3 sodium hydroxide solution were added to the extract. The development of an intense yellow colour, which turns colourless upon the addition of dilute hydrochloric acid (2 mol/dm^3), indicates the presence of flavonoids.

2.4.7 Detection of Eugenols

5 mL of a 5 % KOH solution was mixed with around 2 mL of the extract. Next, the aqueous layer was filtered and separated. A light-yellow precipitate formed when a few drops of HCl were added to the filter, indicating a positive result.

2.4.8 Detection of Steroids

To 0.5 g of the extract, 2 mL of acetic anhydride and 2 mL of H_2SO_4 were added. A colour change from violet to blue or green in some samples indicated the presence of steroids.

2.4.9 Detection of Terpenoid

A 0.2 g portion of the plant extract was combined with 2 mL of chloroform ($CHCl_3$), and 3 mL of concentrated H_2SO_4 was carefully added to create a separate layer. The appearance of a reddish-brown colour at the interface indicates a positive result for the presence of terpenoids.

2.4.10 Detection of Reducing Sugars (Carbohydrates)

To ascertain this parameter, Fehling's test was employed. Fehling's solutions A and B were heated in equal volumes for one minute each, followed by the addition of a volume of the plant extract and another boiling for five minutes. A bricked- red precipitate is required.

2.5 Quantitative Phytochemical Analysis

2.5.1 Determination of Total Phenolic Contents

The method used was according to A.O.A.C (1990) with slight modifications, using tannic acid as the standard. Briefly, 1.0 mL of the extract solution (250 µg/mL) was placed in a test tube. Then, 1.0 mL of Folin-Ciocalteu reagent was added, and the mixture was stirred thoroughly. After 5 minutes, 15.0 mL of 20% Na_2CO_3 was added, and the mixture was allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K.). The total phenolic content was expressed in µg of tannic acid equivalents (TAE), based on a calibration curve prepared with standard tannic acid.

2.5.2 Determination of Total Alkaloids Content

The total alkaloid content was determined using the method outlined by A.O.A.C (1990). 100 mL of 20 % acetic acid in ethanol was added to five grams (5 g) of the extract in a 250 mL beaker, which was then covered and allowed to stand for two hours. Following the filtration process, the extract was concentrated to a quarter of its initial volume in a water bath. Prior to precipitation, concentrated ammonium hydroxide was progressively added. The mixture was allowed to settle, and the precipitate was collected by filtration, washed with 1 % ammonia solution, dried and weighed. The sample was analyzed in triplicate:

Alkaloid (%) = Weight of residues x 100 weight of sample

2.5.3 Flavonoid Content Determination

The flavonoid content was measured using triplicate samples of the homogenized cabbage extract (1.5 g) as described by Ilahy *et al*. (2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. The samples were diluted with 90 µL of methanol, followed by the addition of 6 µL of 10% Aluminium Chloride ($AlCl_3$), 6 µL of 1 mol/L Sodium acetate (CH_3CO_2Na), and 170 µL of methanol. After 30 minutes, the absorbance was measured at 415 nm. Quercetin was used as the standard to calculate the flavonoid content (µg Qe/g). For the lead test, the plant extract was treated with a few drops of lead acetate solution, and the formation of a yellow precipitate indicated the presence of flavonoids.

2.5.4 Estimation of Total Saponins Content

Estimation of total saponins content was determined by the method described by Makkar *et al*. (2007) based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant

extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 ml of 99.5 % ethanol) was added. Then 2.5 ml of 72 % sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 570 nm. 0-25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples.

2.5.5 Estimation of Tannins Content

Exactly 0.20 mL of sample was added to 20 mL of 50 % methanol and placed in a water bath at 77 ˚C – 80 ˚C for 1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin Denis reagent and 10 ml 17 % Na_2CO_3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

2.6 Antimicrobial Activity Assay

The antimicrobial properties of *Citrus sinensis* were evaluated using the agar diffusion method in Mueller Hinton Agar (MHA), with microbial isolates obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The microbial isolates used in this study were *Pseudomonas aeruginosa*, *Klebsiella* spp., *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Penicillium notatum*, *Aspergillus niger* and *Candida albicans*.

2.7 Standardization of Test Organisms

The MacFarland 0.5 turbidity standard was prepared by mixing 0.5 mL of a 1 % w/v solution of Barium Chloride dihydrate ($BaCl_2.2H_2O$) with 99.5 mL of 1 % sulfuric acid (H_2SO_4). The mixture was thoroughly mixed and then divided into several test tubes. A sterile wire loop was used to pick a loopful of inoculum from a pure culture of the test organism, which was transferred into sterile normal saline in separate test tubes and standardized. The turbidity of the solution was then compared with the MacFarland turbidity standard and adjustments were made by adding either more organism or sterile saline to match the standard (Zapata and Ramirez-Arcos, 2015).

2.8 Agar Well Diffusion Method

This method was carried out on Muller Hinton agar for selected bacterial and fungal organisms. After sterilizing the agar, it was allowed to cool, and then 30 mL of the agar was poured into sterile Petri dishes and left to solidify. After solidifying, the agar plates were placed in an oven for ten minutes at 50 °C to eliminate any remaining moisture from the surface. A standardized inoculum suspension was then evenly swabbed onto the agar plates, including both bacterial and fungal inocula on the Muller Hinton agar. An 8-10 mm sterile cork borer was used to create wells in the agar and the base of the Muller Hinton agar plate was sealed. Using a calibrated micropipette with a rubber teat, 0.2 mL of the plant extract was added to the well. The plates were allowed to stand for 30 minutes to allow for proper diffusion, then incubated at 37 °C for 24 hours. The control for bacterial organisms was Ciprofloxacin (0.5 µg/mL), and the control for fungal species was Ketoconazole. The inhibition zone was measured using a meter rule after a 24-hour period.

2.9 Determination of Minimum Inhibitory Concentration

The agar dilution method (Afolayan and Meyer, 1997) was used for the determination of minimum inhibitory concentration (MIC) of the extracts and Ciprofloxacin and Ketoconazole as the standard antibacterial and antifungal on susceptible test microorganisms. The MIC was regarded as the lowest concentrations of extracts of ciprofloxacin that inhibited the growth of the test microorganisms.

2.10 Determination of Minimum Bactericidal Concentration

The plates showing no visible growth after determining the MIC were swabbed and streaked onto fresh Muller Hinton agar plates containing the same predetermined concentrations of extracts. All the plates were then incubated at 37°C for 18-24 hours. The MBC was identified as the lowest concentration of extracts and ciprofloxacin that inhibited the growth of the test organisms.

2.11 Statistics

The research data was analysed with Graph pad prism version 6.0 Data were presented as Means=S.E.M. and statistical significance was calculated using one-way ANOVA.

3. Results and Discussion

The findings of an analysis of the proximate composition of orange pomace aqueous extracts are displayed in Table 1. The aqueous extract contains varying levels of proximate components, including moisture content (51.0837 ± 0.1310%), ash content (4.8953 ± 0.0707 mg/kg), crude fat (8.1967 ± 0.1073 mg/kg), crude fiber (9.2112 ± 0.0112 mg/kg), protein (7.1567 ± 0.1027 mg/kg), and carbohydrate (70.5401 ± 0.0669 mg/kg). The aqueous pomace extract was also subjected to qualitative phytochemical screening to detect the presence of various phytochemicals such as phenols, terpenoids, alkaloids, eugenols, flavonoids, tannins, and reducing sugars. As shown in Table 2, all these components, except for glycosides, were detected in the extract. The levels of specific phytochemicals, including total tannins, total phenolics, total flavonoids, and alkaloids, were determined through quantitative analysis, with the results presented in Table 3.

Table 1: Result for Proximate Content in Aqueous Extract of *Citrus sinensis*

S/N	PARAMETERS	AQUEOUS
1	Moisture Content	51.0837 ± 0.1310
2	Ash Content	4.8953 ± 0.0707
3	Crude Fat	8.1967 ± 0.1073
4	Crude Fibre	9.2112 ± 0.0112
5	Protein	7.1567 ± 0.1027
6	Carbohydrate	70.5401 ± 0.0669

The antibacterial activity of the aqueous pomace extract was evaluated using the agar well diffusion method, testing against five bacterial strains and three fungal strains. The results, displayed in Table 4, show that the aqueous extract inhibited all the bacterial strains, but not the fungal strains. The largest inhibition zone observed for the aqueous pomace extract was 20 mm against *Staphylococcus aureus*, compared to 30 mm for the standard antibiotic, Ciprofloxacin, against the same organism. The fungal strains showed no inhibition zones. The minimum inhibitory concentration (MIC) of the aqueous

extract against selected bacterial isolates is presented in Table 5, while the minimum bactericidal concentration (MBC) results for the aqueous extract of orange pomace are provided in Table 6.

Table 2: Result for Qualitative Phytochemical Screening for *Citrus sinensis* Pomace Extracts

S/N	PARAMETERS	TEST METHOD	AQUEOUS
1	Glycosides	General Test	-
2	Saponins	Frothing Test	-
3	Phenols	Ethanol/Ferric Chloride	+
4	Eugenols	Ethanol/Ferric Chloride	+
5	Terpenoids	Salkowski Test	+
6	Steroids	KOH Test	-
7	Alkaloids	Pieric Test	+
8	Flavonoids	Lead Acetate	+
9	Tannins	Ferric Chloride	+
10	Reducing Sugars	Fehlings A&B	+

Key: = - NEGATIVE = +PRESENT =++ LARGELY PRESENT

Table 3 Result for Quantitative Phytochemical Analysis of *Citrus sinensis* Pomace Extracts

S/N	PARAMETERS	UNIT	AQUEOUS
1	Alkaloids	%	5.4063 ± 0.2985
2	Total Tannins	TAE mg/kg	5.9620 ± 3.6112
3	Total Saponin	mg/kg	0.1077 ± 0.0012
4	Total Phenolics	TAE mg/kg	0.1533 ± 0.0023
5	Total Flavonoids	QE mg/kg	6.9993 ± 0.0127

Key: Tannic Acid Equivalent (TAE)

Table 4: Result for Zone of Inhibition (mm) of Aqueous Extracts of *Citrus sinensis*

ORGANISMS	CONTROL (mg/ml)	AQUEOUS (mg/ml)
Escherichia coli	15	NZ
Pseudomonas aeruginosa	35	18
Bacillus subtilis	38	18
Staphylococcus aureus	30	20
Klebsiella spp.	28	11
Candida albican	31	NZ
Penicillum notatum	28	NZ
Aspergillus niger	27	NZ

Key: NZ= No Zone, CONTROL= Ciproflxacin for Bacteria, Ketoconazole for Fungi

Table 5: Result for Minimum Inhibition Concentration

ORGANISMS	CONCENTRATION			
mg/ml	200	100	50	25
Pseudomonas aeruginosa	NG	NG	G	G
Bacillus subtilis	NG	NG	G	G
Staphylococcus aureus	NG	NG	G	G
Klebsiella spp.	NG	NG	G	G

Key: NG = No Growth; G = Growth

Table 6: Result for Minimum Bactericidal Concentration

ORGANISMS	CONCENTRATION	
mg/ml	**200**	**100**
Pseudomonas aeruginosa	NG	G
Bacillus subtilis	NG	G
Staphylococcus aureus	NG	G
Klebsiella spp.	G	G

KEY: NG = No Growth; G = Growth

The use of medicinal plants has provided quite a number of natural remedies to treat and manage diseases in both human and livestock (Rios and Receo, 2005). Through studies, the potency of this plant has been established and has been identified to contain certain bioactive compounds which are responsible for their therapeutic effects on the human body. The analysis of the proximate composition of *Citrus sinensis* aqueous pomace extract reveals valuable insights into its nutritional value, bioactive compounds, and antimicrobial potential. The aqueous extract is composed mainly of moisture (51.08 %), carbohydrates (70.5401 %), crude fat (8.20 %), crude fibre (9.21 %) and protein (7.16 %). These results suggest that the pomace extract is an excellent source of energy, especially due to its carbohydrate content. The high moisture content in the aqueous extract (51.08 %) is consistent with common plant-based extracts, where moisture aids in enhancing nutrient solubility and bioavailability. The carbohydrate content is notably high (70.54 %), positioning the extract as an efficient energy source. Although the protein content is moderate (7.16 %), it suggests that the pomace may provide supplementary protein but is unlikely to be a primary protein source compared to other more protein-dense plant extracts. With an ash content of 4.89 %, the extract suggests a moderate mineral presence, which is typical for plant extracts. The crude fat content (8.20 %) further indicates the presence of beneficial lipids, contributing essential fatty acids (Udinyiwe and Aghedo, 2022). The rich phytochemical results of *Citrus sinensis* in Table 2 of this study aligns with the phytochemical results of other medicinal plants (Udinyiwe and Osemwegie, 2025; Udinyiwe and Omoregie, 2025; Udinyiwe and Aghedo, 2022). The proximate content results of *Citrus sinesis* in this study aligns with the proximate results of previous studies, such as Uraku (2015) and Oikeh *et al*. (2013), which reported that citrus pomace contains significant macronutrients essential for human health. The research of Uraku (2015) found that orange peel has a high carbohydrate content (61.07%), supporting the findings

of this study that citrus pomace can serve as an energy source. Additionally, Oikeh *et al*. (2013) demonstrated that citrus seeds have notable protein content (6.13 %), which is comparable to the 7.16 % found in this study. The quantitative phytochemical results in Table 3 in this study confirmed the presence of phenols, terpenoids, alkaloids, eugenols, flavonoids, tannins and reducing sugars, whereas glycosides, saponins, and steroids were absent. These results are consistent with previous studies (Moyo *et al.*, 2023), which highlighted that *Citrus sinensis* contains flavonoids, alkaloids, essential oils and phenolic acids known for their medicinal benefits. The presence of flavonoids (6.99 mg/kg) and alkaloids (5.41%) in the quantitative analysis supports findings by Dubey *et al*. (2011), who demonstrated that citrus peels contain bioactive compounds with potential antimicrobial properties. Flavonoids have been extensively studied for their antioxidant and antimicrobial effects (Pandey and Rizvi, (2009). The detection of phenols and terpenoids further supports the findings of Rao *et al*. (2013), who reported that these compounds contribute to anti-inflammatory and antimicrobial properties (Bouslamti *et al*., 2023). The absence of saponins in this study contrasts with findings from other studies that suggested their presence in citrus extracts (Loizzo *et al*., 2012). This difference could be attributed to variations in solvent types and extraction techniques used in different studies. The antimicrobial assay of the aqueous pomace extract evaluated through the agar well diffusion method (Table 4), showed promising results against various bacterial strains. The extract demonstrated inhibition against all bacterial strains tested, with the strongest inhibition observed against *Staphylococcus aureus* (20 mm). These results align with previous research with Ahmed and Beg (2001), who found that citrus extracts required moderate concentrations to exhibit bactericidal effects. The concentration-dependent nature of the antimicrobial effect further supports past research on the necessity of higher extract concentrations for stronger antimicrobial action. However, the extract showed no activity against fungal strains, including *Candida albicans*, *Penicillium notatum*, and *Aspergillus niger*, which indicates that the aqueous pomace extract may not be an effective antifungal agent. These findings are consistent with other studies where citrus extracts exhibited limited antifungal properties (Prabuseenivasan *et al*., 2006). Bacterial growth could be inhibited by the aqueous extract at concentrations as low as 50 mg/mL, according to the Minimum Inhibitory Concentration (MIC) values (Table 5). However, the Minimum Bactericidal Concentration (MBC) data (Table 6) suggest that higher concentrations (100-200 mg/mL) are necessary to completely eradicate bacterial growth. This implies that while the extract demonstrates antimicrobial activity, its bactericidal effects are concentration-dependent.

Conclusion

The aqueous extract of *Citrus sinensis* pomace appears to be a promising source of bioactive compounds, including flavonoids, alkaloids, and phenolics, which contribute to its potential therapeutic properties. Its nutritional composition, particularly its high carbohydrate content, indicates that it could serve as an energy source. The extract's antibacterial activity, particularly against *Staphylococcus aureus*, suggests that it may have applications in combating bacterial infections. However, further research is needed to optimize its antimicrobial efficacy and to explore its potential against fungal pathogens.

Acknowledgement: I want to appreciate the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin for their assistance with the laboratory space, consumables and equipment during the study.

Disclosure statement: The authors declare that there is no conflict of interest.

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