WebLog Journal of Nutrition and Food Sciences

Research Article
Published: 26 Nov, 2026

Assessment of Antibacterial and Nutritional Value of Lime

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Abstract

Citrus juice is widely consumed because of its many health benefits and antimicrobial properties. This study investigated the antibacterial potentials of lime (*Citrus aurantifolia* Swingle) against some selected bacterial organisms as well as its nutritional value. Some lime fruits were purchased from Eke Awka Market and taken to Docchy Analytical Laboratories for analysis. The proximate analysis of the lime was determined using standard techniques. Data were analyzed using descriptive statistics. The results revealed the presence of moisture (58.30%), ash (4.40%), fat (7.5%), crude fibre (2.60%), protein (5 60%) and carbohydrate (22.15%). The lime juice was subjected to antibacterial screening by disc diffusion methods against the test bacterial isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, *Citrobacter murliniae*, *Bacillus licheniformis*, *Micrococcus roseus* and *Bacillus subtilis*) at varying concentration of the extract. Lime juice demonstrated highest inhibition on both *Micrococcus roseus* and *Bacillus subtilis* with zones of inhibition (22.33 mm) respectively while the lowest inhibitory activity was recorded against *Pseudomonas aeruginosa* with zone of inhibition of (21.00 mm). The antibacterial activity of lime juice revealed their potentials nutritional benefits.

Keywords: Assessment; Antibacterial; Nutritional; Value; Lime

WebLog Open Access Publications
Article ID : wjnfs.2026.k2606
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OPEN ACCESS

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Received Date: *28 Oct 2026*
Accepted Date: *24 Nov 2026*
Published Date: *26 Nov 2026*

Citation:
Remigius Obinna Ezebo. Assessment of Antibacterial and Nutritional Value of Lime. WebLog J Nutr Food Sci. wjnfs.2026.k2606. https://doi.org/10.5281/zenodo.24532532

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Introduction

Lime (*Citrus aurantifolia*) belongs to the Rutaceae family and the Citrus genus [1]. It measures between 4-6 cm in diameter, green in colour and having a sour and fresh taste. Citrus is the world's principal fruit crop, with about 60 million megatons grown annually [2]. There are five groups of cultivated Citrus: sweet oranges, madarins, grapefruit, pommelo, lemons and limes [3].

Limes account for 5% of global Citrus production [2] and countries in the Middle East as well as India, Pakistan, Brazil, Argentina and Mexico grow lime as a key part of their agricultural economies [4, 5].

In 100 g, lime contains nutritional components including 30 kcal of energy, 0.019 g of fatty acids, 2.8 g of dietary fiber, 11 g of carbohydrates, 29.1 mg of total sugar [6]. Besides its nutritional content, lime also contains phytochemicals such as flavonoids and alkaloids, as well as essential oils [7].

The use of lime fruit is increasingly widespread worldwide, both fresh fruit for consumption and for making juice and other drinks. It is also used in making jams and candies. Essential oils obtained from the peel are widely used in the pharmaceutical and cosmetic industries for medicines, perfumes, soaps, body lotions, detergents and for food and drinks. The peel is also widely used in cooking [8]. Lime juice has been reported to contain flavonoids and ascorbic acid, which have several bioactivities, including antioxidant properties [9]. Hesperidin and eriocitrin have been reported as major flavonoids in lime varieties [10, 11]. The bioactive components of cold-stored lime fruit juice-maintained hesperidin and eriocitrin content for up to 21 days [12]. The aim of this research was to investigate the antibacterial and nutritional value of lime.

Materials and Methods

Sources of sample materials

The fresh lime fruits were purchased from Eke Awka Market, Anambra State, Nigeria. The test bacterial strains; Salmonella enteric, Escherichia coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the Reference Laboratory Section of Conig-Simonne Laboratories, Awka, Anambra State, Nigeria. The organisms were maintained on nutrient broth for 24 hours.

Preparation of samples

Lime fruits were washed several times with distilled water to remove dirt on the surface and

cleaned with 70% ethanol. It was cut open with sterile knife and the juice was extracted using manual juice extractor. The extracted juice was kept in a sterile container and filtered using Sigmur membrane filter paper (0.45 milipore). The crude juice was freshly used without refrigeration using the method of [13].

Standardization of test bacteria

The test bacteria were standardized by using a sterile wire loop to pick 3-5 pure cultures of the test microorganism and emulsified in 3-4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland standard was recorded as Absorbance in a Spectrophotometer at 540 nm, while the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standard at the same wave length, using physiological saline. NB: 0.5 McFarland contains 1.5x108 cfu/ml.

Antimicrobial susceptibility test

The antibacterial activities of the extracts against the test bacteria were evaluated by modified disc diffusion methods [14-17]. Exactly 25 μl of 0.5 McFarland standardized suspension of test bacteria (1.5x108 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.

Proximate analysis

Moisture Content [18].

Procedure: A Petri-dish was washed and dried in the oven. Approximately 2 g of the sample was weighed into Petri dish. The weight of the Petri dish and sample was noted before drying. The Petri dish and sample were put in the oven and heated at 105^{o}C for 2 hrs. The result was noted and heated another 1 hr until a steady result is obtained and the weight was noted.

The drying procedure was continued until a constant weight was obtained.

% moisture content = (W1-W2/ Weight of sample) x 100

Where W1 = weight of Petri dish and sample before drying; W2 = weight of Petri dish and sample after drying.

Carbohydrate determination (Differential method)

100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

Ash content [18].

Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.

Procedure: Empty platinum crucible was washed, dried and the weight was noted. Approximately 2 g of sample was weighed into the platinum crucible and placed in a muffle furnace at 5500c for 3 hours. The sample was cooled in a dessicator after burning and weighed.

Calculations:

% Ash content = (W3 -W1/W2 -W1) x 100

Where:

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning; W3 = weight of platinum and ash.

Crude fibre [18].

Procedure: Defat about 2 g of material with petroleum ether (if the fat content if more than 10%). It was boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution. The solution was filtered through linen, washed with boiling water until the washings were no longer acid. The residue was transferred to a beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25g of carbonate free NaOH per 100 ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven, weighed, incinerated and cooled. The loss in weight after incineration x 100 is the percentage of crude fibre.

% Crude Fiber = (Weight of Fibre/Weight of Sample Crude fat) x 100

Soxhlet fat extraction method

This method was carried out by continuously extracting a food with non- polar organic solvent such as petroleum ether for about 1 hour or more.

Procedure: 250 ml clean boiling flasks were dried in oven at 105-110^{o}C for about 30 minutes. They were transferred into a dessicator and allowed to cool. Correspondingly labeled, cooled boiling flasks were weighed. The boiling flasks were filled with about 300 ml of petroleum ether (boiling point 40-60^{o}c). The extraction thimble was lightly plugged with cotton wool. The Soxhlet apparatus were assembled and allowed to reflux for about 6 hours. The thimble was removed with care and the petroleum ether was collected in the top container of the set-up and drained into a container for re- use. When flask was almost free of petroleum ether, it was removed and dried at 105^{o}C-110^{o}C for 1 hour. It was transferred from the oven into a dessicator and allowed to cool then weighed.

% Fat = (Weight of Flask + Oil - Weight of Flask/Weight of Sample) x 100

Crude proteins [18].

Principle: The method is the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline, and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

Procedures: Exactly 0.5 g of sample was weighed into a 30ml Kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5 g of the Kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, it was made up to 100 ml with distilled water to avoid caking. 5ml was transferred to the Kjedahl distillation apparatus, followed by 5 ml of 40% sodium hydroxide. A 100 ml receiver flask containing 5 ml of 2% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methlene blue was added under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution and

Table 1: Mean zone of inhibition of bacterial isolates with lime juice (mm).

Bacterial Isolates	Lime Juice	Control
Pseudomonas aeruginosa	21.00	48.00
Staphylococcus aureus	21.33	53.67
Salmonella enterica	22.00	44.33
Citrobacter murliniae	21.67	37.33
Bacillus licheniformis	22.00	41.33
Micrococcus roseus	22.33	38.67
Bacillus subtilis	22.33	41.67
Mean	21.81±0.50	43.57±5.68

Table 2: Proximate composition of the lime fruit.

Proximate Composition (%)	Lime Juice
Moisture	58.30
Ash	4.40
Fat	7.5
Crude Fibre	2.60
Protein	5.60
Carbohydrate	22.15

distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

%Nitrogen = Titre value x 0.01 x 14 x 4

%Protein = %Nitrogen x 6.25

Data analysis

The data were analyzed using descriptive statistics. The results were compared numerically to describe the observed differences in antibacterial activity.

Results

Mean zone of inhibition of bacterial isolates with lime juice

The results of the in vitro assessment of bacterial isolates with lime juice revealed that *Micrococcus roseus* and *Bacillus subtilis* showed highest zone of inhibition (22.33 mm) respectively while the lowest zone of inhibition (21.00 mm) was observed on *Pseudomonas aeruginosa*. The results of the control in the study recorded highest zone of inhibition (53.67 mm) against *Staphylococcus aureus* whereas the lowest zone of inhibition (37.33 mm) was noticed on *Citrobacter murliniae* (Table 1).

Proximate analysis of lime fruit

Table 2 shows the results of the proximate composition of the lime obtained from Eke Awka Market, Anambra State, Nigeria. The study indicated that moisture content of lime was 58.30%. The ash content of lime investigated in the study was 4.4%. Ash refers to the inorganic residue in any food material and it directly signifies the total amount of minerals present within the food. The lipid content of lime 7.5%. The crude fibre content of lime was 2.60%. Fibre in foods are plant non-digestible carbohydrate and lignin and have been shown to have health benefits. The protein content of lime was 5.60%. The carbohydrate content of lime in this study was 22.15%.

Discussion

The antimicrobial susceptibility of the lime juice against gram positive (*Staphylococcus aureus*, *Micrococcus roseus*, *Bacillus subtilis*) and gram-negative bacteria (*Salmonella enterica*, *Pseudomonas aeruginosa*, *Citrobacter murliniae*, *Bacillus licheniformis*) by disc diffusion methods is shown in Table 1. Lime juice showed highest antibacterial activities on both *Micrococcus roseus* and *Bacillus subtilis* with zones of inhibition of (22.33 mm) respectively whereas it had lowest activity on *Pseudomonas aeruginosa* with zone of inhibition of (21.00 mm). It was observed that the susceptibility of the test organisms increased with increase in concentration which is similar to the finding of [19]. The differences in the susceptibility of gram positive and gram-negative bacteria to lime juice is as a result of the differences in their cell wall composition [20].

Conclusion

In the light of the foregoing, the results of the study revealed that lime possesses substantial antimicrobial properties and are effective against some food borne pathogens.

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