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Assessment of the Nutrient Quality and Fungi Pathogen Associated with Lime (*Citrus aurantiifolia*) fruit in Port Harcourt Metropolis

Chuku, E.C[1]., Emiri U.N[2]. & Wekhe, O. E[3].
[1&3]Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. [2]Department of Crop Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
***Corresponding Author:** *ucheemiri@gmail.com*

ABSTRACT

Lime (*Citrus aurantiifolia*) is known for its various distinctive features and importance in every aspect of life. It is notable for its great medicinal and nutritional importance. Study on the nutrient quality, phytochemical constituents and associated fungi of lime fruit was carried out in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. Nutrient and Phytochemical screening were conducted using the methods of the Association of Official Analytical Chemist (AOAC) method, while standard mycological technique was used for fungi isolation. Proximate composition revealed the presence of moisture, ash fibre, fat, carbohydrate and protein in lime fruits. However, highest (2.25 ± 0.05%) and lowest (1.18± 0.03%) values were observed for ash and fibre respectively. Minerals and vitamin assessment revealed the availability of thiamin, calcium, iron, phosphorus, magnesium, potassium and sodium. However, magnesium recorded highest value (32.5± 10.5 mg/l00g) while vitamin C had the lowest value 1.2 ± 0.00mg/100g). Phytochemical screening indicated the presence of glycoside, oxalate, saponin, tannin, carotenoid, phenol, flavonoid and lignan. Flavanoid had the highest value (13.3 ± 0.1 %) and the lowest (0.01 ± 0.00%) values were observed for tannin and glycoside. Two fungal organisms (*Candida* sp. and *Mucor* sp.) were isolated from spoilt lime fruits. *Candida* sp had the highest incidence (75.55%) while the lowest incidence (24.44%) was recorded for *Mucor* sp. Generally, despite the susceptible nature of lime fruit to fungal spoilage, it still contains valuable nutrients, minerals and phytochemicals. It is therefore recommended for consumption for its culinary, therapeutic and health benefits.

Keywords: *Citrus aurantiifolia*, Lime, Medicinal, Nutrient, Phytochemical, Minerals, Fungi, Pathogen.

Introduction

Lime (*Citrus aurantiifolia*) belongs to the family of evergreen tree species Rutacea that is commonly found in South Asia and primarily in North East India. Lime is known for its various distinctive features and importance in every aspect of life. Its medicinal and nutritional value is of utmost importance (Siebert, 2011).

All parts of the plant is utilizable; its juice is helpful in many ways including reducing fever, blood pressure that is considered as main step toward chronic disorders (Azantsa *et al.,* 2017). Its leaf that was treated as waste is also utilizable for extracting oil to serve as medicinal and nutritional property, also have great importance as natural preserving agent.

Not only lime is used individually but it is proved to be more beneficial when added with honey for sore throat and water for reducing weight (Adokoh *et al.,* 2019).To prove it's beneficial values, scientists and researchers have done many experiments on lime, not only on mice' and rats but also practically on humans (Vivien *et al,* 2020). Many disorders are treated using it at home, due to its special properties and also it is inexpensive, easy and quick way to cure many aches and even diseases (Adokoh *et al.,* 2019).

The genus Citrus belongs to the subtribe Citrinae tribe Citreae, subfamily limoniodeae of the family Rutaceae. This genus may be further divided into two subgenera (Citrus and Papeda) based on leaf, flower and fruit properties. Sweet orange and its relatives are members of the subgenus Citrus (Nicolosi *et al.,* 2000).

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Within this taxon there is no sterility barrier, although there are various impediments to sexual hybridization including cross-compatibility, nuclear embryonic and isolation by time of flowering (Abugroun *et al.,* 2019).

True limes form a diverse group with two natural clusters; acidic or sour limes, and non-acidic limes. Acid limes can be either small fruited or large fruited types. The sour and sweet limes were considered by certain taxonomists to be distinct enough to merit creation of a different species (Adriano, 2018). These valuable resources need to be preserved and broadened for future crop improvement programmes by the breeders and geneticists (Hamed *et al.*,2017). Although, the production of lime has faced numerous setbacks in the past, there is massive potential as the demand from both the domestic and industrial consumer grows. It has been reported that the consumption and trading of limes between countries has been on an upward trend in recent years (Plattner, 2014**)**. Keeping in view the customer and industry demands, new lime cultivars or hybrids are expected to emerge to meet the demands of different countries across the world. Recently, seedless lime hybrids, including C4-5-27 (a cross of 'Key lime' and tetraploid lemon) with superior fruit and dried lime being sold in Middle East countries.

Literature has shown that the nutrient composition and fungal spoilage of lime is influenced by the environmental and the genetic make-up (Abugroun *et al,* 2019). However, there is dearth of information on the phytochemical contents of lime fruits sold in Port Harcourt metropolis. This study seeks to add to the work of earlier researchers; to assess the nutrient and phytochemical contents of lime fruits and to ascertain the fungi pathogen associated with the spoilage of lime fruits.

Materials and Methods

Study Area

The study was carried out at the Department of Plant Science and Biotechnology, Rivers State University Port Harcourt. Rivers State has a mean annual rainfall of 2000-3000 mm and mean annual temperature of 25 -28ºC area, while relative humidity varies between 70-85%.

Collection of Lime Fruit Samples

Fresh and partially spoilt lime fruits were procured from the Fruit Garden Market in Kaduna Street, D-Line, Port Harcourt, and brought to the Department of Plant Science and Biotechnology, Rivers State University for processing and analysis. Upon arrival, the fruits were thoroughly washed under running tap water to eliminate dirt and reduce potential contaminants. The fruits were then peeled, and the seeds carefully removed. The pulp was homogenized using an electric blender to obtain a uniform extract. This crude extract was stored in air-tight, stoppered glass containers to preserve its integrity prior to analysis. For solvent extraction, aliquots of the extract were placed in test tubes and treated with ethanol. The mixture was thoroughly agitated and then filtered using Whatman filter paper The resulting filtrates were collected and used for subsequent quantitative phytochemical screening.

Determination of Proximate Composition of Lime Fruit

Standard Analytical Method AOAC, (2005) was the method used to determine the proximate composition comprising; Moisture, Ash, Crude Fiber, Protein, Lipid, and Carbohydrate.

Determination of Moisture (%)

For moisture content, a gravimetric (measurement by weight) was used. In this method, 40 grams of the pulp sample was dried to a constant weight of 120ºC in a crucible and left to stay overnight. Then crucible was transferred to oven again and weighed after 2 hours, this was repeated until constant weight was obtained.
Calculation:
Moisture Content % = $(W_2\text{-}W_1)$- $(W_3\text{-}W_1)$ x 100 $(W_2\text{-}W_1)$
Where:
W_1= weight of empty crucible,
W_2= weight of crucible + sample,
W_3= weight of crucible + dry sample.

Determination of Ash (%)

The gravimetric method was also used in the determination of ash content. 20 grams of the sample was added in a clean pre-weighed crucible for 24 hours at 550ºC.

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An empty crucible was accurately weighed, and then 10ml of sample was weighed in it using sensitive balance. The sample in crucible was placed in muffle furnace at 550°C for more than 3 hours until white to grey ash was obtained, then crucible was removed from furnace to a desiccator to cool, then weighed.

Determination of Crude Fiber (%)

For determination of crude fiber, Acid Alkaline Hydrolysis was used in boiling 20 grams of sample with 0.1m of H_2SO_4 in a beaker and then filtered through a Büchner funnel. The residue was washed with hot water till it was free from acid. The material was transferred to the same beaker and added 200ml of 1.25% NaOH solution and refluxed for 30 minutes. Again filtered and the residue was washed with hot water till it was free from alkaline.

The total residue was transferred to a crucible and placed in hot air oven, allowed to dry to a constant weight at 80-110 °C and weighed. The residue was ignited in muffle furnace at 550- 600°C for 2-3hrs, cooled and weighed again. The loss of weight due to ignition was the weight of crude fiber.

Determination of Protein (%)

Kjeldal Method of Nitrogen Analysis was used to determine the protein content of both samples. K_2SO_4 was used as a catalyst for concentrated H_2SO_4 to digest approximately 20 grams of sample. Ammonia produced in the digested sample was afterward distilled into boric acid and titrated with 0.1m of HCl. To ascertain the amount of protein in each sample, the crude protein formula is used.

$$\text{Crude Protein} = \frac{\text{Titre value x 1.4 x 50 x 100 x 65}}{(1000 \text{ x } 10 \text{ x } 1)N_2}$$

Determination of Lipid (%)

Lipid (total fat) content is determined through the Sochlet Solvent Extraction Technique. Gravimetrically, the residue was dried to a constant weight and calculated as:

$$\text{Percentage (\%) of Ether Extract} = \left[\frac{\text{Weight of extract}}{\text{Weight of Sample}}\right] \text{ x } 100$$

Determination of Carbohydrate (%)

Carbohydrate content was calculated as the difference between the totalities of all other proximate content and 100.
i.e. 100 - (moisture + ash + fibre + protein + lipid).

Determination of Mineral Composition

A digestion mixture containing concentrated nitric acid and concentrated tetraoxosulphate (VI) acid (H_2SO_4) in a ratio of 3:1 was used as wet digestion to be able to carry out mineral analysis. 0.2 gram of sample was weighed and put into a conical flask and 5cm^3 of digestion mixture was added and placed in a fume cupboard for digestion at a temperature of 150°C-200°C for 2 hours, the mixture became digested and was allowed to cool. Distilled water (30cm^3) was added to the digest, shaken vigorously, and filtered. In a volumetric flask, the filtrate is marked up into 100 cm^3. In the digest, the Flame photometer was used to determine sodium (Na), Potassium (K), Calcium (Ca), Iron (Fe), Magnesium (mg), and Phosphorous (P), but when EDTA complexometric fixation (ASTM, 2004) is used in the digest, it determines calcium.

Determination of Phytochemicals

Some phytochemicals determined in the sample of lime include glycoside, oxalates, saponin, tannins, carotenoids, polyphenols, flavonoids, and lignan. Standard Analytical Method, AOAC, (2005) was used in the determination of the phytochemicals.

Determination of Saponin

For Saponin determination, 100cm^3 of 20% aqueous ethanol was added to 5grams of each sample in a 250cm^3 conical flask; the mixture was heated over a hot water bath for 4 hours and stirred continuously at a temperature of 55°C. Extraction of the residue from the mixture was repeated with another 100cm^3 of 20% aqueous ethanol after filtration and heated for another 4 hours at a constant temperature of 55°C with continuous stirring. The combined extract was evaporated to 40cm^3 over a water bath at 90°C. 20cm3 of diethyl ether was added to the concentrate in a 250cm^3 separation funnel and agitated vigorously from which the aqueous layer was recovered while the ether layer was discarded and the purification process was repeated twice.

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60cm^3 of n-butanol was added and extracted twice with 10cm^3 of 5% sodium chloride after discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes after which, the solution was transferred into a crucible and was dried in an oven to a constant weight.

Determination of Polyphenol

Determination of polyphenols took a different means as 0.5 grams of the samples were boiled for 15 minutes with 50cm^3 of ether to extract the polyphenolic components. 10cm^3 of distilled water, 2cm^3 of 0.2m of ammonium hydroxide solution, and 5cm^3 of concentrated amyl alcohol were also added to the 5cm^3 of the extract and left to react for 30 minutes for color development. Optical density was measured at 505nm. In preparation for the phenol standard curve, 0.2 grams of tannic acid dissolved in distilled water and diluted to 200 marks (1mg/cm^3).

Determination of Flavonoid

For Flavonoid determination, 0.5ml of appropriately diluted was mixed with 0.5ml methanol, 50μl of 10% Alcl, 50μl of 1m of potassium acetate and 1.4 ml of water and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently calculated while non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

Determination of Tannin

1gram of the sample was put into a conical flask of 100cm^3 of distilled water and set to boil gently for 1 hour on an electric hot plate and filtered using 125nm Whatman filter paper into a 100cm^3 volumetric flask. For color development, 5.0cm^3 of Folin-Denis reagent and 10cm^{3}of saturated Na_2CO_3 solution were added to distilled water of 50cm^3 and 10cm^{3}of diluted extract of aliquot volume was pipetted into the 100cm^3 conical flask. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25 after thorough agitation. With the aid of a Spectrum Lab 23A Spectrophotometer, optical density was measured at 700nm and compared on a standard tannic acid curve. To obtain the tannic standard curve, dissolution of 0.20g of tannic acid in distilled water and dilution to 2 00cm^{3}marks (1mg/cm^3) were used.

Varying concentrations (0.2-1.0mg/cm^3) of the standard tannic acid solution were pipette into 5 different test tubes of 5cm^3 of Folin-Denis reagent and 10cm^3 saturated Na_2CO_3 solutions were added and made up to 100cm^3 marks with distilled water. Optical density (absorbance) against tannic acid concentration was plotted.

Determination of Glycoside

For glycoside determination, 5g of both samples were weighed into a clean distilled flask, to which 20 ml of distilled water was added and the samples were left to stand overnight for proper hydrolysis to occur. The samples were distilled into 20ml sodium hydroxide containing 0.5g crystal. The distillate was filtered with 0.02N silver nitrate in the presence of 0.2ml 5% potassium iodide and 1ml 6N ammonium hydroxide solution to a permanent turbidity. The formula below is used in calculating glycoside.

1ml 0.02N $AgNO_3$= 20N or 1.08HCN.

Determination of Oxalate

In the determination of oxalate constituents, 5g of the sample was weighed and collected. In a conical flask, 100ml of distilled water was added and left to heat for an hour and allowed to cool. The samples were filtered and 25ml of the filtered samples was measured into a container.

The 20ml of $H_2SO_4$was added to the 25ml of filtered samples. Using a heating mantle, the sample was left to heat till it got to 70^{o}C, temperature (using a thermometer to check the temperature in between heating), after the flask was brought down, the sample was titrated with $KMnO_4$ till its color changed to pink.

Determination of Vitamins

Standard Analytical Method AOAC, (2005) was used to determine the vitamin composition of the sample.

Determination of Vitamin a

Determination of Vitamin A was done in a test tube in the test tube, 0.5rnl of the sample, 0.5mI of chloroform and 2m1 of Trilluoroacetate reagent (TFA) are put into it. The absorbance as recorded at 600nm.

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Determination of Vitamin C

For Ascorbic acid also known as vitamin C, its determination is done in a test tube. In a test tube, O.5m1 of the samples, 0.2ml of distilled water and 0.5m1 of DTCS reagent were added and mixed, the test tubes were incubated at 37°C for 3 hours. Afterwards, I.5rnl of 65% ice cold sulphuric acid was added and mixed very well and then incubated for another 30 minutes at 37°C.

Preparation of Mycological Medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120°C for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Chuku, 2009). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Potato Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with nonabsorbent cotton wool and wrapped with aluminum foil. The conical flask containing the mycological medium was autoclaved at 121° C and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40^0C and dispensed into Petri dishes at l5mls per plate and allowed to further cool and solidify.

Isolation of Fungi from Lime Fruit

A threefold serial dilution was used in accordance to the method of Mehrotra & Aggrawal, (2003) where Ig of the spoilt lime fruit samples were transferred into the first test tube containing 9mls of normal saline. Iml of the solution was transferred to the second test tube and finally from the second to the third. 0.1 ml aliquots from the second and third dilutions were plated onto Sabouraud Dextrose Agar in Petri dishes containing ampicillin to hinder the growth of bacteria and this was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25°C ± 30 °C (Chuku, 2009). The entire set tip was observed for 7 days to ensure full grown organisms.

Pure cultures of isolates were obtained after a series of isolations.

Identification of Fungal Organisms from Lime Fruit

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology mycelia structure and other associated structures using the keys of (Barnett & Hunter, 1998).

Determination of Percentage Incidence

The percentage incidence of fungal occurrence was determined by the formula stated below (Chuku *et al* 2019):

$\frac{x}{y} \; x \frac{100}{1}$ = % incidence. Where:

X = total number of each organism in a variety

Y = total number of all identi1ed organism in a variety.

Statistical Analysis

Data obtained were subjected to mean and standard deviation analysis with the aid of SPSS software version 22.

Results

The result of proximate composition of lime presented in Table 4.1, revealed the presence of moisture, ash, lipid, fibre, carbohydrate and protein in varying concentrations. Highest (75.68 ±0.17%) and lowest (1.18±0.0 3%) values wore observed for fibre.

Table 1: Proximate Composition of Lime Fruits

Parameter	Composition %
Moisture	75.68 ±0.17
Ash	2.25 ±0.05
Lipid	4.45 ± 0.05
Fibre	1.18 ±0.03
Carbohydrate	4.73 ±0.23
Protein	12.05±0.05

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The result of mineral and vitamin composition lime fruit shown in Table 2 revealed that the availability of thiamin, calcium, iron, phosphorus, magnesium, potassium and sodium. However, Magnesium recorded the highest value (32.5± 0.5-mg/I00g) while vitamin C has had lowest value (1.2 ± 0.00mg/I00g).

Table 2: Mineral and Vitamin Composition Lime Fruit

Parameter	Composition (mg/100g)
Calcium	15.25 ±0.25
Iron	4.1 ±0.1
Magnesium	32.5 ± 0.5
Phosphorus	18.55 ± 0.45
Potassium	11.15 ± 0.05
Sodium	1.5 ±0.00
Vitamin A	2.5 ± 0.00
Vitamin C	1.2± 0.00

Phytochemical composition result (Table 3) revealed Glycoside, Oxalate, Saponin, Tannin, Carotenoid, Polyphenol, Flavonoid and lignan to be available. Flavonoid had the highest value (13.3 + 0. 1%) while the lowest value (0.01±0.00) was recorded for Glycoside and tannin.

Table 3: Phytochemical Composition of Lime Fruits

Parameter	Composition (%)
Glycoside	0.01 ± 0.00
Oxalate	0.02 ± 0.00
Saponin	0.05 ± 0.00
Tannin	0.01 ± 0.00
Carotenoid	7.3 ± 0. I
Polyphenol	6.75 ± 0.5
Flavonoid	13.3 ± 0.1
Lignan	9.25 ± 0.05

The Incidence and characteristics of the fungi isolated are as shown in Table 4. The result revealed *Candida* sp and *Mucor* sp as the two fungal organisms associated with spoilt lime fruit, although at different percentage incidence. *Candida* had 75.55% incidence while 24.44% incidence was recorded for *mucor* sp.

Table 4: Fungi Characterization and Incidence of Spoilt Lime Fruit

Fungal Isolate	Microscopic Examination	Probable Organism	(%) Incidence
Isolate A	Colonies with a small budding spherical circular, convex, undulate, elongate cells, smooth opaque and pseudo-mycelium.	*Candida* sp	75.55
Isolate F	Growth rate is rapid and hyphae are septate and bearing cottony colonies and produced fluffy white.	*Mucor* sp	24.44

Discussion

The present study has revealed the numerous valuable proximate nutrients in Lime fruits including moisture, fibre, lipid, carbohydrate and protein. Although, moisture recorded the highest value as opposed to fibre that had the lowest value. Vital minerals and vitamin, such as calcium, magnesium, iron, phosphorus, potassium, sodium and thiamin were all present in the assessed lime fruits at varying proportions. The vitamins recorded were vitamins A and C. However, highest minerals value was observed for magnesium whereas sodium had the lowest mineral values.

Phytochemical screening also revealed the availability of several phytochemicals at different concentrations. Highest and lowest concentration, were clearly seen in flavonoid and tannin respectively. This is in tandem with the findings of Ezinne & Uzoamaka (2023), who observed similar trend of phytochemical in lime fruit.

Notwithstanding, two fungal organisms associated with the spoilage of lime fruits in the present study. *Candida* sp showed the highest incidence, compared to *Mucor*.

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The phytochemical results from this study negates the assertion of Okonkwo *et al.*, (2025), who reported higher amount of Tannin, 4.4mg/l, Saponin 0.88% and absence of Flavonoid and Phenol in lime fruits. The difference could be due to the plant part analyzed (leaves versus juice), variations in environmental growing conditions, or the superior sensitivity of chromatographic methods over basic colorimetric assays. However, Ezinne & Uzoamaka (2023) identified a broader array of phytochemicals, including flavonoids, saponins and cardiac glycosides in citrus juices and peels, which is tandem with the current study. Both studies agree, however, on the presence of saponins and cardiac glycosides, affirming their role in the therapeutic potential of lime as an antioxidant and cardioprotective agent. Further comparison with Oikeh *et al.,* (2020) who focused on *Citrus sinensis* (orange) peel extracts, indicates consistent detection of tannins, saponins, and phenolic compounds, alongside flavonoids. This partial agreement supports the universality of certain phytochemicals like tannins and saponins in citrus species.

More researches have been documented for the presence of essential oil in lime fruits (Lemes *et al.*, 2018; Almeida *et al.*, 2018). Literatures have also shown the use of lime leaves, peel, and fruits as a source of antibacterial compounds, each with different compositions of phytochemical compounds. Lemes *et al*. (2018) and Al-Amari *et al.* (2018), reported a dose-dependent effect of lime leaf essential oil with a more pronounced effect against *Staphylococcus*. The essential oil in lime fruits was revealed to possess antifungal property against *Aspergillus flavus* and *Penicillium* sp. It is therefore considered a good preservative against spoilage of food crops. Almeida *et al.*, (2018) reported lime fruits, and oil serves as antibacterial activities as it was used against gram negative Bacilli. Similarly, Lemes *et al.*, (2018) reported that lime oil exhibit antioxidant property.

Conclusion

Lime fruit contains nutrients (moisture, ash, crush, fibre, lipid, protein and carbohydrate), Vitamins and Minerals (thiamin, calcium, iron, phosphorus, magnesium, potassium and sodium). Several phytochemicals; Glycoside, oxalates, saponin, tannins, carotenoid, polyphenol, flavonoids and lignan, are also embedded in lime fruits. However, the fruit is still susceptible to fungi attack. It is recommended that, Lime should be utilized as a good source of nutrient. It should be exploited for pharmaceutical purposes.

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