

Qualitative phytochemicals screening and antimicrobial susceptibility patterns of coconut oil extract on some selected bacteria and fungi

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Abstract

Focuses on its high values, coconut product; nutritional and health benefits have currently been attributed to its intake, antioxidant and anticancer properties. This research study is aimed at evaluating the phytochemical content of coconut (*Cocos nucifera*) oil and determines the susceptibility pattern against some selected microbes. The findings revealed that it contained phytochemicals; alkaloid, glycosides, resins, saponins, tannins and terpenoid. Subjected to susceptibility testing on some bacterial and fungal; agar diffusion method was being applied and inhibition zones which indicated its antimicrobial properties. Assay of antibacterial activity of standard bacteria organisms showed that *Staphylococcus aureus* had the highest susceptibility to coconut oil while *Pseudomonas aeruginosa* had the least, *Candida albicans* had a higher susceptibility to coconut oil more than *Aspergillus fumigatus* in antifungal testing. This was concluded from their average zones of inhibition; 14.55mm (32%) for *Staphylococcus aureus*, 12.1 mm (27%) for *Streptococcus pneumoniae*, 10.95 mm (24%) for *Escherichia coli* and 7.7 mm (17%) for *Pseudomonas aeruginosa*. And for the fungal are 18.5mm (55%) for *Candida albicans* and 15.1mm (45%) for *Aspergillus fumigatus*. The utilization of coconut oil should be promoted as a functional food in Nigeria and the use of coconut seed flesh in our diets should be encouraged for health supporting functions and considered to be responsible for the many benefits attributed to its consumption.

Keywords: Antimicrobial; Coconut oil; Inhibition; Phytochemical; Susceptibility; Test organisms

1. Introduction

Some plants such as medicinal plants play an important role in the management of many diseases, especially in the developing countries where a small number of several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases. These medicinal plants comprise of the plant parts such as fruits, leaves, stem barks, roots, flowers, seeds, plant juice, oil, etc. where some of the proper knowledge of their functions are yet to be known, although, the phyto-therapy is continuing to be in use in several countries despite its many side effects. Plant base drugs have been in use against various disease causing organisms and diseases since the time memorial. The primitive human being use herbs as therapeutic agents and medicaments, which they were able to procure easily. In nature, there are abundant plants made for the welfare of man and animals (phyto-therapy and ethno-therapy). The some essential (phytochemicals or phyto-nutrients) values of some of these plants have long been established and published, but a large number of them remain unexposed.

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According to Ivora *et al.*, [1]; British Pharmacopoeia [2] stated that few plants have received scientific or medicinal scrutiny, a large number of medicinal plants possess some degree of toxicity and about one third of the medicinal plants used in the treatment of various diseases are considered to be toxic. The natural products have been interesting and important sources of biologically active (antimicrobial) substances and the major sources of which are still left undiscovered [3-6]. The existence of plants with therapeutic uses (medicinal plants) could be traced to as far as the time of creation [7-10]. The plant constituents which function to cure various diseases are termed secondary metabolites [11- 14]. Plants have been major source of medicine and presence of plant secondary metabolites (phytochemicals) has been implicated for most plants therapeutic activities [15-18].

There are more than 35,000 plant species with various phytochemicals in them being used in various human cultures around the world for medicinal purpose [19, 20, 21, 22]. Phytochemicals are biologically active compound found in plants such vegetables, fruits, grains or plants parts in small amount (quantity), these compounds are not established nutrients, but significantly protect the development of lots of degenerative diseases [6, 23- 25]. However, the presence of phytochemicals constituents such as alkaloids, flavonoids, tannin, and phenolic, etc, compounds in edible plants parts have been reported to be important compounds in many other medicinal plants [14, 26-27].

Coconut (*Cocos nucifera*) is a monocotyledon belonging to the Arecaceae Family and the order Arecales [16]. It is referred to as the “Tree of Life” because of its many uses. *Cocos nucifera* is a fruit tree found in warm, humid climates with well drained soils. Coconut oil is made from fresh coconuts. The oil retains its naturally occurring phytochemical which produce a distinctive coconut taste and smell since chemical solvents are not used [16]. Coconut oil contains potential medium chain fatty acids (lauric acid, capric acid, caprylic and myristic acid) which have an inhibitory effect against bacterial, edible coconut oil exhibit antimicrobial activity against bacteria and fungi; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas spp*, *Streptococcus spp*, *Candida albicans*, *Aspergillus spp*, etc [19].

Some Phytochemicals with significant health potentials include flavonoids, carotenoids, phenolic acids, saponins, glycosides, tannins and alkaloids. Coconut has been found a typical example of plants possessing such quality [19]. The antimicrobial susceptibility testing of coconut oil in a rapid means of determining the response of microorganisms to coconut oil, it utilizes the diffusion method which is simple and widely used for sensitivity testing. This test resists actively identifies organisms that were susceptible to coconut or certain antimicrobial agents such as the nut oil by visual means [13].

Chemical are few techniques for oil extraction such as physical, includes, fermentation or enzymatic process using microbial as remains enzymatic starter. The benefits of coconut oil reported by Enig, [28], include quick energy, falling hair, stress management, treatment of tropical disease, premature aging, weight loss, digestion related problem, wound healing, cardiac disorder. Immunity helps fighting harmful bacterial, infection control due to antimicrobial properties. It reduces incidence of liver diseases, kidney and gall bladder diseases are control, diabetes, and coconut oil helps in controlling blood sugar and bones improvement [29]. The aim of this study is to evaluate the phytochemical components of coconut oil and determine the susceptibility of its extract against some selected bacteria and fungi. Hence, there it is necessary to explore their uses and to conduct pharmacokinetic and pharmacological studies in order to ascertain their therapeutic properties, nutritional and health benefits, and their side effects as well if any.

2. Material and methods

2.1. Area of study

All research was carried out at the general microbiology laboratory at NnamdiAzikiwe University, Awka, Anambra State of Nigeria.

2.2. Materials

All Materials, reagents and media used for this analysis were of analytical grade and were all obtained from National Agency for Food and Drug Administration and Control (NAFDAC), Zonal Laboratory, Agulu, Anambra State of Nigeria.

2.3. Sampling and sample collection

The fresh coconut fruits (*Cocos nucifera*) were carefully examined, selected for ripened ones and obtained commercially from Eke-Awka market, Anambra State (Nigeria), and brought to the laboratory for the processing and extraction of the oil (Coconut oil) for the study.

2.3.1. Authentication of the sample collected

Before processing, some of the samples collected were taken to the Department of Forestry Technology, Mohamet Lawan College of Agric., Maiduguri, Nigeria for authentication. The coconut fruit was authenticated by Shitima Umar Kyari as *Cocos nucifera* fruit. Some of the sample were numbered and stored under shelve in herbarium laboratory for feature reference.

2.3.2. Sample preparation

The working area was thoroughly hygienically cleaned and cutting utensils were aseptically cleaned. The coconut fruits were broken and dehusked. The seed flesh was removed from the shell with a kitchen knife, cut and graded into small pieces. The graded flesh was grinded with warm water in a blender. After the grinding, the coconut milk was separated from the chaff by pouring in to a cleaned, sterile plastic bowl through a sterile plastic sieve. The bowl of coconut milk was covered and kept in a fridge at temperature of 20 °C overnight.

2.3.3. Coconut oil extraction

Caked white coconut oil was placed on a hot plate and separated from the water and placed in a sterile clean - dried stainless steel pot and stirred with sterile plastic spatula for a period of time to reduce the moisture content giving an aqueous extract of coconut oil. The stirring was stopped when charred bits come were seen in the oil and the pot was set aside to cool to a comfortable temperature. Coconut oil was sieved with a sterile chiffon cloth to remove the charred bits, was collected by using sterile plastic funnel and this was dispensed into a sterile bottle for subsequent analysis. It was stored at 4 °C, ready for further analysis.

2.4. Test for phytochemical analysis

For the phytochemical analysis is the extraction method involved non-polar solvent. The tests were carried out to determine the active constituents according to the procedures and methods outlined by Harborne [24]; Trease and Evans [25]. These phytochemical tests were done to detect the presence of secondary metabolites such as Alkaloids, tannins, saponins, flavonoids, steroid, glycosides, terpenoids, etc, in the coconut oil. These phytochemicals are chemical substances that occur naturally in plants which have antimicrobial properties [(Harborne, [24]; Trease and Evans, [25].

2.4.1. Alkaloids test

2ml of the coconut oil sample was stirred with 5ml of aqueous HCl. 1ml each of the extract was tested with Dragendorff's reagents, Mayers reagent, Wagners reagent, Picric acid (1%). The presence of precipitate and various colour changes as seen in the result indicated the presence and level of intensity of alkaloids.

2.4.2. Flavonoids test

About 5ml of dilute NH₃ solution was added to 3ml of coconut oil extract followed by the addition of concentrated tetraoxosulphate (VI) i.e. H₂SO₄. The various colour changes as seen in the result indicated the presence and level of intensity of flavonoids.

2.4.3. Glycosides test

5ml of dilute sulphuric was added to 1ml of coconut oil extract and shake to mix, and boiled for 15minutes in a water bath. It was cooled and neutralized with 20% potassium hydroxide solution (KOH). 10 ml of equal parts of Fehling's solution A and B was added and boiled for 5 minutes. The colour change as seen in the result indicated the presence, absence or intensity.

2.4.4. Saponins test (Frothing test)

About 5ml of the coconut oil extract were shaken ant boiled with equivalent amount of water for 5 minutes. Frothing that persists on warming was taken as evidence of the presence of saponins.

2.4.5. Resins test

5ml of the coconut oil extract was added to 20ml of distilled water and the mixture was shaken vigorously. The presence of precipitate and various colour changes as seen in the result indicated the presence and level of intensity of resins.

2.4.6. Tannins test

2ml of coconut oil extract were boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and filtered. The filtrates were subjected to the following tests: (i) lead acetate test and (ii) Ferric chloride test. The result indicated presence and the intensity of tannins or the absence of tannins.

2.4.7. Acidic compounds test

Blue litmus paper was used to test the extracts. Colour changes indicated the presence or absence of the acidic compounds.

2.4.8. Steroids and terpenoid test

9 ml of ethanol was added to 1 g of the coconut oil extract and filtered. The filtrate was concentrated to 2.5 ml in a boiling water bath. 5 ml of distilled water was added and the mixture was allowed to stand for 1 hour and the waxy matter was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of the extract, 1ml conc. H_2SO_4 was carefully added. To another 0.5 ml of extract, it was evaporated to dryness on a water bath and heated with 3 ml of conc. H_2SO_4 for 10 minutes in a water bath. To colour changes in both tests indicated the presence, absence and intensity of steroids and terpenoids.

2.5. Bacterial isolates

Standard bacterial organisms were obtained from the Zonal Laboratory, National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Anambra State of Nigeria. The bacterial strains used for the study include; Gram positive bacteria [G+] (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram negative bacteria [G-] (*Escherichia coli* and *Pseudomonas aeruginosa*).

2.6. Fungal isolates

Also Standard fungal organisms were obtained from Zonal Laboratory, National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Anambra State. The fungal strains used for the research include *Candida albicans* and *Aspergillus fumigatus*.

2.7. Media used for study

The following media were used for the analysis which includes: Nutrient broth (NB), Nutrient agar (NA), MacConkey broth (MB), MacConkey agar (MA), Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA)

2.7.1. Preparation and dispensing media

Preparation of media was done according to the manufacturer's instruction which is used for culturing of microorganisms in the microbiology laboratory. Required amounts of media were accurately weighed in a beaker and distilled water was poured to make up to mark.

The growth medium was dispensed into appropriate containers i.e. Petri-dishes for agar and McCartney bottles for broth. For the broth, 9 ml of it was dispensed into the McCartney bottles. It was tightly covered and autoclaved at 121 °C for 15 minutes according to manufacturer's instruction. For the agar, the process was a little different. The agar was covered with foil and autoclaved before dispensing or pouring into the Petridish.

2.7.2. Purity test of the extracted coconut oil

Purity test is a quantitative test carried out on samples to evaluate their microbial quantity and to ensure that the sample complies with the specifications for the test. Extracted coconut oil was cultured on Sabouraud dextrose agar (SDA), MacConkey agar (MA) and Nutrient agar (NA) plates and incubated for seven days to ensure that the extract was completely pure, not contaminated.

2.8. Inoculation of the test organisms on the media used

2.8.1. Procedure

Using a sterile syringe, 1 ml each of the coconut oil sample were inoculated into the McCartney bottles containing 9ml each of Sabouraud dextrose broth (SDB), MacConkey broth (MB), Nutrient broth (NB). It was capped and shaken to mix. 1 ml each of the broth containing the sample were inoculated into the corresponding agar plates i.e. NB to NA, SDB

to SDA and MB to MA by pour plate method. The agar plates were incubated for 7 days to check for growth (NA at 37 °C for aerobic mesophilic bacteria, MA at 37 °C for pathogenic bacteria and SDA at 25 °C for fungi).

2.8.2. Controls

Controls are sanitary and sterility measures taken to make sure there are no pre-existing contamination in the environment and growth media, but it is not always like that. The introduction of control in analysis involves dispensing a well prepared media into the respective containers without the inoculation of the sample. There are two types of control. These two controls were used as measuring yardsticks for judging the results of the analysis.

2.8.3. Open control

In the open control, the Petri dishes containing the well prepared media is kept open throughout the analysis. The reason for leaving them open is to checkmate the environment because during analysis, environmental contaminants enter together with the air.

2.8.4. Closed control

In closed control, the Petri dish containing the well prepared media will never be opened all through the analysis. This type of control evaluates the integrity of the media used for analysis.

2.9. Antimicrobial susceptibility testing (AST)

This is a rapid means of determining the response of microorganisms to different antimicrobial agents. Antimicrobial susceptibility testing (AST) mostly utilizes the Kirby-Bauer agar diffusion method [30]. This test simply, rapidly and effectively identifies organisms that are susceptible or resistant to certain antimicrobial agent through visual means. This method is used when the test substance can diffuse through the agar gel. Generally, this is preferred to serial dilution methods because of ease with which quantitative results can be obtained. This leads to the successful treatment of bacterial and fungal infection [31].

2.10. Antimicrobial susceptibility testing of the test organisms

The antibacterial susceptibility testing was carried out on bacterial strains both gram positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) to check the effectiveness of coconut oil on bacterial infections. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in terms of millimeter. The zone of inhibition of the coconut oil was compared to that of commercially produced antibiotic (Ciprofloxacin 500 mg).

Ciprofloxacin is a broad spectrum antibiotic that has antibacterial activity against *Staphylococci*, *Streptococci*, *Escherichia coli*, *Pseudomonas*, *Salmonella* and used for treatment of bacterial infections such as respiratory infections urinary tract infections, genital infections, gastrointestinal infections and wound infections.

The reconstitution and dilution of the commercial antibiotic (Ciprofloxacin 500 mg) needed for the analysis was carried out according to National Agency for Food and Drug Admission and Control (Nigeria) method book from (USP 28). The dilution of commercial antibiotic was performed in order to get a final concentration of 50 µg/ml. This 50 µg/ml are taken to be the lowest concentration of the antibiotic at which the organism cannot produce visible growth after 24 hrs. This is the lowest concentration that is effective against bacterial infections.

2.10.1. Procedure

The Nutrient agar (NA) was prepared according to the manufacturer's instruction, poured into the Petri-dishes and allowed to gel. The Petri-dishes were labeled accordingly for the analysis for the coconut oil sample and commercially produced antibiotic (Ciprofloxacin). A perpendicular line crossing the middle of the Petri dish was made using a marker and a mark was made in the center of each of the two sectors (X and Y). Ten - fold serial dilution of the already subcultured test organisms. Different swab sticks were dipped into the bottles of the 10⁻² dilutions of the test organisms and smeared on the surface of the gelled nutrient agar. A well/hole was created in the center of each sector using a sterile Cork borer (10 mm). 1 ml each of the coconut oil sample was dispensed into the two holes using the sterile syringes. This is repeated for ciprofloxacin. The plates were kept for 30 minutes for the inoculum to diffuse into a considerable area of the medium. Plates were incubated for 24 hours at 37°C. After incubation, the susceptibility of the sample or organism is indicated by the diameter of the zone of inhibition in mm, subtracting the diameter of the hole and finding average of the two zones.

2.11. Antifungal sensitivity testing

The antifungal sensitivity testing was carried out on fungal strains (*Candida albicans*, *Asperigillus spp*) to check the effectiveness of coconut oil and fungal infections. The antifungal activity of the test agent was determined by measuring the diameter of the zone of inhibition in terms of millimeter. The higher the zone of inhibition is the more sensitive the test organism and the better the antimicrobial agent. The zone of inhibition of the coconut oil was compared to that of commercially produced antifungal (Ketoconazole). Ketoconazole is a broad spectrum Imidazole antifungal that has potent antimycotic action against *Candida albicans* and dermatophytes.

2.11.1. Procedure

The Sabourauds dextrose agar (SDA) was prepared according to the manufacturer's instruction, poured into the Petri dishes and allowed to gel. The Petri-dishes were labeled accordingly for the analysis for the coconut oil sample and commercially produced antifungal (Ketoconazole). A perpendicular line crossing the middle of the Petri dish was made using a marker and a mark was made in the center of each of the two sectors (X and Y). Ten-fold serial dilution of the already subcultured test organisms. Different swab sticks were dipped into the bottles of the 10^{-2} dilutions of the test organisms and smeared on the surface of the gelled agar. A well/hole was created in the centre of each sector using a sterile cork borer (10 mm). 1 ml each of the coconut oil sample was dispensed into the two holes using the sterile syringes. For ketoconazole, the topical cream is pressed directly into the hole. The plates were kept for 30 minutes for the inoculum to diffuse into a considerable area of the medium. Plates were incubated for 24 hours at 25 °C. After incubation, the susceptibility of the sample or organism is indicated by the diameter of the zone of inhibition in mm, subtracting the diameter of the hole and finding average of the two zones.

3. Results

Plants and plants materials produce a diverse range of biological active elements or molecules (bioactive) which are very rich sources of different types of foods and medicinal used in nutrition and medicines. Focuses on its high values, coconut product provides nutritional and health benefits have currently been attributed to its intake (antioxidant and anticancer properties) which was evaluated the phytochemical content of coconut (*Cocos nucifera*) oil and determined the susceptibility pattern against bacteria and fungi. These analyses involved various experiments; the phytochemical content of coconut (*Cocos nucifera*) oil were evaluated, determined the susceptibility pattern against bacteria and fungi conducted by agar diffusion method and inhibition zones which indicated its antimicrobial properties as shown in tables 1. Table 1 shows the phytochemicals content which were detected in the coconut oil sampled analyzed, polar solvent was used, the following phytochemicals were present such as alkaloids, glycosides, saponins, resins, tannins, terpenoids, while acidic compounds, flavonoids, steroids and were absent.

Table 1 Qualitative phytochemical test of coconut oil sample using polar solvent (Water)

Phytochemicals	Test used	Observations	Inferences
Alkaloids	Wagners reagent	Reddish brown Precipitate	Alkaloids present
	Mayers reagent	Milky precipitate	Alkaloids present
	Dragendorff reagent	Brick red precipitate	Alkaloids present
	Picric acid test	Light yellow precipitate	Alkaloids present
Acidic compounds	Blue litmus paper	No colour change to red	Acidic compounds absent
Flavanoids	Alkaline – Acid test	No colour change	Flavanoids absent
Glycosides	Fehling's solutions	Dense red precipitate	Glycosides present
Resin	Precipitate test	Precipitate present	Resin present
	Colour test	Light pink colour	Resin present
Saponins	Frothing test	A stable froth	Saponins present
Tannins	Lead sub-acetate test	White gelatinous precipitate	Tannins present
	Ferric chloride	Light green colouration	Tannins present
Terpenoids	Conc. H ₂ SO ₄ test	Grey colouration	Terpenoids present
Steroids	Conc. H ₂ SO ₄ test	Brownish red colouration	Steroids absent

Table 2 showed the result of the purity test after 7 days' incubation. Extracted coconut oil cultured on Nutrient agar, MacConkey agar and Sabourauds dextrose agar incubated at 25 °C and 37 °C complied with the specifications for purity testing. The coconut oil showed satisfactory results of purity.

Table 2 Purity test of coconut oil sample

Media used	Temperature of Incubation (°C)	Number of Colonies (cfu/ml)	Inference
Nutrient Agar	37	11	Satisfactory
MacConkey Agar	37	0	Satisfactory
Sabourauds Dextrose	25	3	Satisfactory

Table 3 showed the maximum contamination limit as shown in indicates whether the test sample complies with specifications for the analysis. The result is termed 'unsatisfactory' if the colony forming unit exceeds the maximum limit while the result is satisfactory' if the test complies with the specifications.

Table 3 Maximum concentration limit for purity test

Media used	Temperature of Incubation (°C)	Maximum contamination Limit (cfu/ml)
Nutrient agar	37	1000
MacConkey agar	37	0
Sabouraud Dextrose agar	25	100

Table 4 showed the result of susceptibility test of Coconut oil on bacteria, in which X and Y represent the diameter zones of inhibition in mm of the two agar well which contains the coconut oil on standard bacteria organisms. 'Average' shows the sum of the two zones of inhibition divided by two, i.e., $[X + Y] / 2$. The percentage (%) and percentage average zone of inhibition gotten from the average showed that *Staphylococcus aureus* has the highest sensitivity to coconut oil at of 14.55 mm (32%), followed by *Streptococcus pneumoniae* with 12.1 mm (27 %), *Escherichia coli* had 10.95 mm (24 %), while *Pseudomonas aeruginosa* was the least sensitive to coconut oil at 07.7 mm (17%) respectively.

Table 4 Zone of inhibition (mm) of susceptibility testing of coconut oil on some bacteria strains

Bacteria strains	X	Y	Average	Percentage average
<i>Staphylococcus aureus</i>	14.5	14.6	14.55	32
<i>Streptococcus pneumoniae</i>	12.2	12.0	12.1	27
<i>Escherichia coli</i>	11.0	11.9	10.95	24
<i>Pseudomonas aeruginosa</i>	07.6	07.8	07.7	17

Table 5 showed the result of susceptibility testing on Ciprofloxacin on bacteria, X and Y represent the diameter zone of inhibition in millimeter of the two agar well which contains the coconut oil on standard bacterial organisms. 'Average' is the sum of the zones of inhibition divided by two, i.e. $[X + Y] / 2$ and the percentage (%) zone of inhibition gotten from the average in table 5 showed that *Staphylococcus aureus* had the highest sensitivity of coconut oil at 28.95 mm (28 %), then followed by *Pseudomonas aeruginosa* has 26.0 mm (26 %) , followed by *Streptococcus pneumoniae* with 25.1 mm (24 %) while *Escherichia coli* was the least sensitive to coconut oil had 24.4 mm (23 %).

Table 5 Zone of inhibition (mm) of susceptibility testing of Ciprofloxacin on some bacteria strains

Bacteria strains	X	Y	Average	Percentage average
<i>Staphylococcus aureus</i>	29.0	28.9	28.95	28 %
<i>Streptococcus pneumoniae</i>	25.2	25.0	25.1	24 %
<i>Escherichia coli</i>	24.3	24.5	24.4	23 %
<i>Pseudomonas aeruginosa</i>	26.0	26.0	26.0	26 %

Table 6 showed the result of susceptibility test of Coconut oil on fungi (microbe), in which X and Y represent the diameter zones of inhibition in mm of the two agar well which contains the coconut oil on standard bacteria organisms. 'Average' shows the sum of the two zones of inhibition divided by two, i.e., $[X + Y] / 2$. The percentage (%) and percentage average zone of inhibition gotten from the average showed that *Candida albicans* is highly sensitive to coconut oil at 18.5 mm (55%), while *Aspergillus* was moderately sensitive to coconut oil at 15.1 mm (45 %).

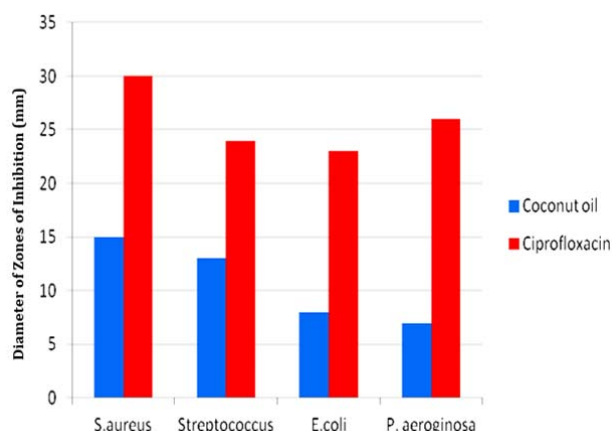
Table 6 Zone of inhibition (mm) testing of coconut oil on some fungi strains

Fungal strains	X	Y	Average	Percentage average
<i>Candida albicans</i>	18.7	18.3	18.5	55 %
<i>Aspergillus fumigatus</i>	15.0	15.2	15.1	45 %

Table 7 showed the result of susceptibility test of Ciprofloxacin on fungi (microbe), in which X and Y represent the diameter zones of inhibition in mm of the two agar well which contains the coconut oil on standard bacteria organisms. 'Average' shows the sum of the two zones of inhibition divided by two, i.e., $[X + Y] / 2$. The percentage (%) and percentage average zone of inhibition gotten from the average showed that *Candida albicans* is highly sensitive to coconut oil at 20.85 mm (57%), while *Aspergillus* was moderately sensitive to ketoconazole at 16.0 mm (43 %).

Table 7 Zone of inhibition (mm) testing of ciprofloxacin on some fungi strains

Fungal strains	X	Y	Average	Percentage average
<i>Candida albicans</i>	20.8	20.9	20.85	57 %
<i>Aspergillus Fumigatus</i>	16.0	16.0	16.0	43 %


Figure 1 Graphical presentation showing the diameter of zones of inhibition of Coconut oil to ciprofloxacin as a positive control tested on standard bacteria organisms

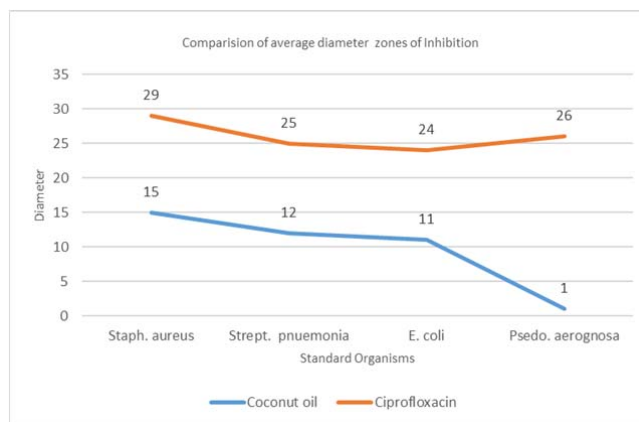


Figure 2 Graphical presentation showing the diameter of zones of inhibition of Coconut oil to that of ciprofloxacin as positive control tested on standard bacteria organisms

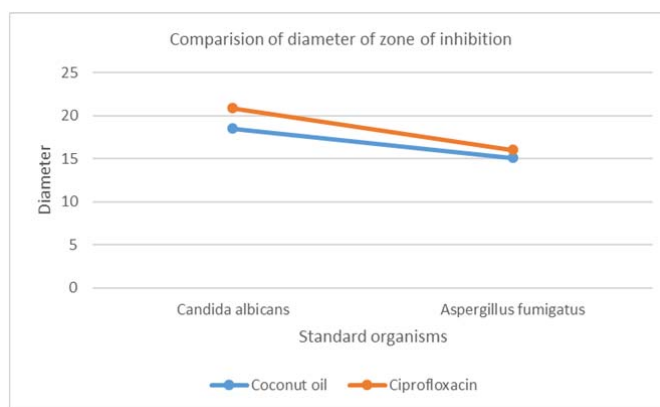


Figure 3 Graphical presentation showing the diameter of zones of inhibition of coconut oil to that of ciprofloxacin as positive control tested on standard fungal organisms

4. Discussion

Plant base drugs have been in use against various disease causing organisms and diseases since the time memorial. The primitive human being use herbs as therapeutic agents and medicaments, which they were able to procure easily. In nature, there are abundant plants made for the welfare of man and animals in phyto-therapy and ethno-vet-therapy. Kolo *et al.*, [33] stated that the essential values of some of these plants have long been published, but a large number of them remain unexposed. In search for drugs with less or no toxicity and side effects, such plants need to be and become necessary to be exposed.

Focuses on its high values, nutritional and health benefits of coconut products have currently been attributed to its intake, antioxidant and anticancer properties. Research study, aimed at evaluating the phytochemical content of coconut (*Cocos nucifera*) oil and was determined the susceptibility pattern against some selected microbes. The findings revealed that it contained phytochemicals such as alkaloid, glycosides, resins, saponins, tannins and terpenoid.

The coconut oil was subjected to susceptibility testing on some bacterial and fungal by agar diffusion method being applied and inhibition zones which indicated its antimicrobial properties. Assay of antibacterial activity of standard bacteria organisms revealed that *Staphylococcus aureus* and *Candida albicans* had the highest susceptibility to coconut oil while *Pseudomonas aeruginosa* and *Aspergillus fumigatus* had the least susceptibility to coconut oil testing. This was concluded from their average zones of inhibition; 14.55 mm (32%) for *Staphylococcus aureus*, 12.1 mm (27%) for *Streptococcus pneumonia*, 10.95 mm (24%) for *Escherichia coli* and 7.7 mm (17%) for *Pseudomonas aeruginosa*. And for the fungal are 18.5 mm (55%) for *Candida albicans* and 15.1 mm (45%) for *Aspergillus fumigatus*. After 24hrs incubation, the antibacterial activity of the coconut oil extract on the test organisms was determined by measuring the

diameter of zone of inhibition in millimeter with a meter rule. Ciprofloxacin was used as positive control to compare the sensitivity of the strains to coconut oil.

Antimicrobial activity was observed by the existence of clear zone formed on the media grown with colonies of tested microbial strains. It was found that the clear zone on the media fully grown with strains of *Escherichia coli*, *Staphylococcus aureus* indicated that this oil had activity against the growth of tested strains. It is now clear and scientifically validated that the inclusion of coconut oil in the diet could and should be utilized for its preventive and healing properties. The antimicrobial properties from coconut oil have shown promise in this study. Calbom and Calbom, [34] stated that the present of high concentration of antimicrobial properties of coconut oil in the animal body can inhibit the growth of pathogenic microorganisms.

According to Bassey *et al.*, [4] stated that, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines and foods. Plant that produces such product, antimicrobial activities should be tested against appropriate microbes to confirm the activity and to ascertain the parameters associate with, i.e. phytochemicals.

The antifungal susceptibility was determined by using the agar-well diffusion technique. The research focused on *Candida albicans* and *Aspergillus*. The solutions were allowed to diffuse for 30 minutes in the agar medium, after which the plates were then incubated at 25 °C for 24 hrs. The zones of inhibition were measured in millimeters. The susceptibility pattern measures as zones of inhibition of the organisms to the coconut oil extract was compared with those of ketoconazole, a topical cream that is also effective against *Candida albicans* and *Aspergillus spp.*

The presence of various phytochemicals in these coconut oil samples revealed the potentials of coconut and coconut oil as a functional food. The alkaloids, glycosides and other phytochemicals found in this study is similar with the findings of Obidoa *et al.*, [19]; Ogbolu [32]. In this research study, the absence of steroids, flavonoids and acidic compounds in the samples implies that Nigerian coconut seed flesh is not a good dietary source of steroids which agrees with the work of Obidoa *et al.*, [19].

In this study, it revealed the assay of antibacterial activity of standard bacteria organisms showed that *Staphylococcus aureus* had the highest susceptibility to coconut oil while *Pseudomonas aeruginosa* had the least susceptibility. The results obtained in this research work were in consistent with the work of Carpo *et al.*, [35]. It was also found from the results obtained that the antibacterial activity of ciprofloxacin as a control gave higher zone of inhibition than coconut oil.

The antifungal testing studied showed that *Candida albicans* had a higher susceptibility to coconut oil more than *Aspergillus fumigatus*. The effect of coconut oil on fungal organism is as effective as that of ketoconazole as revealed by this study. This correlates with the work of Ogbolu *et al.*, [32] in which *Candida* species were sensitive to 100% coconut oil. Other studies e.g. Abeyundara *et al.*, [36] have shown that there might be better activity when these naturals are made into creams. The utilization of coconut oil should be promoted as a functional food in Nigeria and the use of coconut seed flesh in our diets should be encouraged for health supporting functions and considered to be responsible for the many benefits attributed to its consumption.

5. Conclusion

Secondary metabolites from plant constituents are said to cure various diseases. As a result of this statement, research study was conducted on the qualitative phytochemicals screening and antimicrobial susceptibility patterns of coconut oil extract on bacteria and fungi, it revealed very excellent results (health benefits) and very informative. The phytochemical content of coconut (*Cocos nucifera*) oil was evaluated and the susceptibility pattern against some selected microbes was determined. The findings revealed that it contained phytochemicals, susceptibility testing was performed on the microbes which indicated its antimicrobial properties. Assay of antibacterial activity of standard bacteria organisms and fungi showed that *Staphylococcus aureus* had the highest susceptibility to coconut oil while *Pseudomonas aeruginosa* had the least. *Candida albicans* had a higher susceptibility to coconut oil more than *Aspergillus fumigatus*. It was concluded from their average zones of inhibition; *Staphylococcus aureus* > *Streptococcus pneumonia* > *Escherichia coli* > *Pseudomonas aeruginosa* respectively. *Candida albicans* > *Aspergillus fumigatus* sequentially. The use of coconut seed flesh in our diets should be encouraged for health supporting functions and for many benefits attributed to its consumption.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no any interest of conflict between the authors of this piece of research work. The authors agreed and assigned in hand to all matter arise to this piece of research work.

Statement of ethical approval

'The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

This research studies do not involve any information about any individual person or group of persons.

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