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Extraction, phytonutrients, the nutraceutical, and mineral analysis of coconut (*Cocos nucifera*) oil

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Abstract

Coconut oil is edible oil which is derived from the kernels, meat, and milk of the coconut palm fruit. This study aimed at evaluating the physicochemical properties, mineral elements, phytochemical content of coconut oil and determines the susceptibility pattern of coconut oil against bacteria. The results revealed the average values of the coconut oil contains for alkaloid, tannin, saponins, flavonoid, phenol, steroid, terpenoid, and glycoside were 38.230 mg/100g, 28.124 mg/100g, 16.185 mg/100g, 29.745 mg/100g, 79.167 mg/100g, 0.915 mg/100g, 1.251 mg/100g, and 0.506 mg/100g respectively; which are considered to be responsible for the many benefits attributed to coconut consumption. The mineral elements such sodium (75.350 ppm), calcium (105.400 ppm), potassium (502.55 ppm), magnesium (24.502 ppm), phosphorus (20.372 ppm), and copper, zinc, iron and manganese were present in moderate amounts of 0.442 ppm, 0.393 ppm, 0.772 ppm, 0.629 ppm respectively. The physicochemical analysis reveal the pH level to be neutral, while other parameters such as nitrate, phosphate and ammonia were not detected. 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. 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.

Keywords: Antibacterial; Coconut oil; Phytochemicals; Physicochemical

1. Introduction

The coconut palm has been recognized as one of the world's most useful plants (Chan and Elevitch, 2006). Its products have received attention of the scientific community because water, milk, and oil all have nutritional and medicinal properties (Enig, 2010). The benefits reported for coconut oil are mainly related to antibacterial properties associated to the presence of fatty acids (Dufour *et al*., 2007). Additionally, coconut oil fermentation has been considered in food and pharmaceutical industries (Khoramnia *et al*., 2013). However, health and manufacturing benefits are attributed to coconut oil.

Coconuts are known for their versatility of uses, ranging from food to cosmetics. The inner flesh of the mature seed forms a regular part of the diets of many people in the tropics and subtropics. Coconuts are distinct from other fruits because their endosperm contains a large quantity of clear liquid, called "coconut milk" in the literature, and when immature, may be harvested for their potable "coconut water", also called "coconut juice" (Patil, 2016).

Mature, ripe coconuts can be used as edible seeds, or processed for oil and plant milk from the flesh, charcoal from the hard shell, and coir from the fibrous husk. Dried coconut flesh is called copra, and the oil and milk derived from it are

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commonly used in cooking – frying in particular – as well as in soaps and cosmetics. The hard shells, fibrous husks and long pinnate leaves can be used as material to make a variety of products for furnishing and decorating (Patil, 2016).

Currently, there is a growing interest based on the development of techniques and the search for products for the treatment and prevention of infectious diseases, which is highly prevalent worldwide (Lemos *et al*., 2019; Romero-González., 2020). Although the use of drugs helps to treat various medical conditions, some of these have adverse effects (Tartaglia *et al*., 2019). For this reason, there is a constant search for and use of natural resources to help prevent disease and maintain good health, especially in developing countries such as Nigeria (Rupani *et al*., 2018).

One of the most commonly used oils in this technique is coconut (*Cocus nucifera*) oil (Woolley *et al*., 2020), which contains predominantly medium-chain saturated fatty acids (Chatterjee *et al*., 2020) that are metabolized more easily and quickly than long-chain fatty acids because they are smaller, more soluble and more stable against oxidation. Coconut oil is 50% lauric acid, to which antibacterial, antiviral and antiprotozoal properties are attributed, especially to its monoglyceride, monolaurin (Joshi *et al*., 2020).

In Nigeria, little is known about the antibacterial action of coconut oil on pathogenic organisms and, since this is related to the high prevalence of diseases, it is necessary to include easily extractable, low-risk natural substances in daily hygiene products that help to slow the development and progression of infections. Hence, the aim of this study is to evaluate the phytochemical, mineral composition, physicochemical properties and the antibacterial susceptibility pattern of coconut oil extract against some clinical isolates.

2. Materials and methods

2.1. Sample Collection and Preparation

Samples of fresh coconut fruits (*Cocos nucifera*) were bought from Oja Bisi market in Ado-Ekiti metropolis, and brought to the laboratory for extraction of oil. Before the extraction of oil was done, the working area was thoroughly cleaned to avoid any contamination. The coconut fruits were broken and dehusked, and the seed flesh was removed from the shell with a sterile kitchen knife which were then cut into small pieces and thereafter grated. The grated flesh was grinded with warm water in a blender which resulted to coconut milk; this was separated from the chaff by sieving. The bowl of coconut milk was covered and kept in a refrigerator overnight at 20 ^{o}C.

After the overnight storage of the coconut milk, there was formation of white caked coconut oil. The caked white coconut oil was separated from the water and placed in a clean dry stainless pot, this was places on steam bath and stirred 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 chiffon cloth to remove the charred bits and this was collected using a sterile bottle for analysis, and thereafter stored at 4 ^{o}C for further analysis.

2.2. Phytochemical analysis

Phytochemical tests were carried out to detect the presence of secondary metabolites such as alkaloid, tannin, saponins, flavonoid, phenol, steroid, terpenoid and glycoside. This was performed according to the procedures and method outlined by Trease and Evans 1983.

- **Alkaloid:** Two milliliter (2 mL) of the coconut oil sample was added and stirred with 5 mL of aqueous HCl. 1 mL each of the extract was tested with Dragendorff's reagents, Mayer's reagent, Wagner's reagent, Picric acid (1%). The presence of precipitate and various color changes as seen in the result indicated the presence and level of intensity of alkaloids.
- **Tannin:** Two milliliter (2 mL) of coconut oil extract was boiled with 5 mL of 45% ethanol for 5 min. The mixture was cooled and filtered. Ferric chloride reagent added to the filtrate. A blue-black green precipitate indicated the presence of tannins.
- **Saponins:** Five milliliter (5 mL) of the coconut oil extract was shaken and boiled with equivalent amount of water for 5 minutes. Frothing that persists on warming was taken as evidence of the presence of saponins.
- **Flavonoid:** Five milliliter (5 mL) of dilute NH_3 solution was added to 3 mL of coconut oil extract followed by the addition of concentrated tetraoxosulphate (VI) (H_2SO_4). The various color changes as seen in the result indicated the presence and level of intensity of flavonoids.
- **Phenol:** Five milliliter (5 mL) of coconut oil was added to 5 mL of distilled water and 2 mL of 1% solution of Gelatin containing 10% NaCl was added to it. White precipitate indicates the presence of phenol compounds.

- **Steroid:** Analytical method was used to determined 2.0 mL of coconut oil and was added to 2 mL of Chloroform and few drops of Sulphuric acid was added to form a lower layer. A reddish brown color at the interface indicates the presence of steroid.
- **Terpenoids:** Five milliliter (5 mL) of coconut oil was mixed with chloroform (2 ml); thereafter, 3 mL of concentrated H_2SO_4 was carefully added to the solution to form a thin layer. A reddish brown coloration at the interface indicates the presence of terpenoids
- **Glycosides:** Two milliliter (2 mL) of coconut oil was added to 1 mL of glacial acetic acid containing one drop of ferric chloride solution, it was then under layered with 1mL of concentrated sulphuric acid, a brown ring obtained at the interface indicate the presence of de-oxysugar characteristic of cardenolides.

2.3. Mineral analysis

The mineral elements of the extracted coconut oil were determined according to the method described by AOAC (2015). 2 mL of the sample was separately measured into conical flasks, followed by addition of aqua regia (HNO_3,HCl 1:3). The conical flasks were placed in the fume cupboard and then heated on a hot plate, at the temperature of 60 ^oC until the materials dissolved. The samples were filtered using filter paper, the filtrate were diluted into 100 mL volumetric flask and was made to mark with distilled water. The diluted sample was used for metals determination using Atomic Absorption Spectrophotometer (Buck 210VGP).

2.4. Physicochemical analysis of the coconut oil

The extracted coconut oil quality parameters which include pH was determined using the pH meter. The spectrophotometric method was used in the determination of the nitrate (NO^-_3), phosphate (PO^{3-}_4) and ammonia (NH_3) as described by Oko *et al.* (2017).

2.5. Bacteriological analysis

2.5.1. Collection of bacterial isolates

Standard bacterial organisms were obtained from Microbiology Laboratory of Ekiti state university teaching hospital, Ado-Ekiti, Ekiti State (EKSUTH). The bacteria strains used for the study include: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The bacteria were identified using conventional methods and were maintained on Nutrient agar slants at 4 ^oC in the refrigerator until required.

2.5.2. Preparation of standard inoculum

Different cultures of the test bacteria were taken and sub-cultured on nutrient agar for pure culture. Standard inoculum of 0.1% was made by comparing with standard turbidity.

2.5.3. Determination of antibacterial activity

The extract coconut oil was screen for its antibacterial activity, that is, to determine zones of inhibition against tested bacteria by agar well diffusion method as described by Jayana *et al*. (2010). Sterile Mueller-Hinton Agar plates were inoculated with prepared inoculum with sterile cotton swab. Then with the help of sterile cork borer, wells were made in the inoculated media plate. 1.0 mL of the coconut oil was transferred into the well with the aid of micropipette. The control was also placed in the separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well.

2.5.4. Antibacterial susceptibility test

The antibiotic discs were aseptically, carefully and firmly placed on the inoculated plates using sterile forceps. The plates were then inverted and incubated for 24 hours at temperature of 37 ^oC. After incubation, the plates were examined for growth and the diameters of zone of inhibition were measured. The bacterial isolates were screened for resistance to ten (10) gram-positive antibiotic discs which comprise; Chloranphenicol (CH 30µg), Ciprofloxacin (CPX 10µg), Amoxil (AMX 20µg), Gentamycin (CN 10µg), Streptomycin (S 30µg), Rifampicin (RD 20 µg), Erythromycin (E 30µg), Levofloxacin (LEV 20µg), Norfloxacin (NB 10µg) and Ampiclox (APX 20µg). Gram-negative discs contains additional constituent such as Tarivid (OFL 10µg), Reflacine (PEF 10µg), Ciproflox (CPX 10µg), Augmentin (AU 30µg), Gentamycin (CN 10µg), Streptomycin (S 30µg), Ceprorex (CEP 10µg), Nalidixic acid (NA 30µg), Septrin (SXT 30µg) and Ampicilin (PN 30µg).

3. Results

Table 1 shows the result of quantitative phytochemical constituents of the extracted coconut oil sample. The average values for Alkaloid, Tannin, Saponins, Flavonoid, Phenol, Steroid, Terpenoid, and Glycoside were 38.230 mg/100g, 28.124 mg/100g, 16.185 mg/100g, 29.745 mg/100g, 79.167 mg/100g, 0.915 mg/100g, 1.251 mg/100g, and 0.506 mg/100g respectively.

The mineral analysis of the extracted coconut oil is depicted in Table 2. The mineral elements analyzed were Sodium (Na), Calcium (Ca), Potassium (K), Magnesium (Mg), Copper (Cu), Zinc (Zn), Iron (Fe), Manganese (Mn), and Phosphorus (P). The average values of the amount of these elements varies from 75.350 ppm, 105.400 ppm, 502.55 ppm, 24.502 ppm, 0.442 ppm, 0.393 ppm, 0.772 ppm, 0.629 ppm, and 20.372 ppm respectively. The physicochemical analysis of the extracted coconut oil reveals the average value of pH to be 7.535 and Ammonia (NH_3) 0.010 mg/kg (Table 3). Nitrate (NO^{-}_{3}) and phosphate (PO^{3-}_{4}) were not detected.

Table 4 revealed the antibacterial activity of the extracted coconut oil on the test bacteria isolates (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Staph. aureus*). *E. coli* had the average diameter of zone of inhibition of 10.50 mm and 8.50 mm; *K. pneumoniae* had average diameters of zones of inhibition of 10.00 mm and 9.50 mm; *P. aeruginosa* had the same average diameters of zone of inhibition of 9.50 mm on both agar plates respectively; and *Staph. aureus* had average diameters of zones of inhibition of 8.00 mm and 10.50 mm at concentration of 1.00 mL.

Table 1 Phytochemical analysis of the extracted coconut oil sample

Parameters	x(mg/100g)	y(mg/100g)	Average (mg/100g)
Alkaloid	38.240	38.220	38.230
Tannin	28.118	28.130	28.124
Saponins	16.190	16.180	16.185
Flavonoid	29.770	29.720	29.745
Phenol	79.180	79.154	79.167
Steroid	0.910	0.920	0.915
Terpenoid	1.260	1.242	1.251
Glycoside	0.503	0.509	0.506

Table 2 Mineral analysis of the extracted coconut oil sample

Parameters	x(ppm)	y(ppm)	Average(ppm)
Sodium (Na)	75.500	75.200	75.350
Calcium (Ca)	105.800	105.000	105.400
Potassium (K)	502.800	502.300	502.55
Magnesium (Mg)	24.504	24.500	24.502
Copper (Cu)	0.439	0.444	0.442
Zinc (Zn)	0.390	0.396	0.393
Iron (Fe)	0.769	0.775	0.772
Manganese (Mn)	0.632	0.626	0.629
Phosphorus (P)	20.373	20.370	20.372

The susceptibility pattern of conventional antibiotics to the test bacteria is presented in Table 5. The gram negative test bacteria (*E. coli* and *Pseudomonas*) were resistant to Nalidixic acid (NA) and Ampicilin (PN). However, *Klebsiella* was

resistant to the entire antibiotics, except Streptomycin (S). The gram positive test bacterium (*Staph aureus*) was susceptible to the gram positive antibiotic disc, but only resistant to Streptomycin (S).

Table 3 Physicochemical analysis of the extracted coconut oil sample

Parameters	**x**	**y**	**average**
pH	7.520	7.550	7.535
NO^{-}_{3} (mg/kg)	ND	ND	ND
PO^{3-}_{4} (mg/kg)	ND	ND	ND
NH_3 (mg/kg)	0.010	0.010	0.010

ND = not detected

Table 4 Antibacterial activity of the extracted coconut oil on test bacteria

Test bacteria	**Zones of inhibition (mm)**		
	x	y	average
Escherichia coli	10.00 8.00	11.00 9.00	10.50 8.50
Klebsiella pneumonia	9.00 9.00	11.00 10.00	10.00 9.50
Pseudomonas aeruginosa	10.00 10.00	9.00 9.00	9.50 9.50
Staphylococcus aureus	8.00 11.00	8.00 10.00	8.00 10.50

Table 5a Susceptibility pattern of conventional antibiotics to the test bacteria (Gram negative)

Test bacteria	**Diameter of zones of inhibition (mm)**									
	Conventional antibiotics disc									
	OFL	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
E. coli	*Suc*	*Suc*	*Suc*	*Suc*	*Suc*	*Suc*	*Res*	*Res*	*Suc*	*Res*
Pseudomonas spp.	*Res*	*Suc*	*Suc*	*Res*	*Res*	*Suc*	*Suc*	*Res*	*Suc*	*Res*
Klebsiella	*Res*	*Res*	*Res*	*Res*	*Res*	*Suc*	*Res*	*Res*	*Res*	*Res*

OFL – Tarivid; PEF - Reflacine; CPX – Ciproflox; AU – Augmentin; CN – Gentamycin; S – Streptomycin; CEP – Ceprorex; NA – Nalidixic acid; SXT – Septrin; PN – Ampicilin ; *Suc* – Susceptible; *Res* – Resistant

Table 5b Susceptibility pattern of conventional antibiotics to the test bacteria (Gram positive)

Test bacteria	**Diameter of zones of inhibition (mm)**									
	Conventional antibiotics disc									
	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
Staph aureus	*Suc*	*Suc*	*Suc*	*Suc*	*Res*	*Suc*	*Suc*	*Suc*	*Suc*	*Suc*

CPX – Ciproflox; NB – Norfloxacin; CN – Gentamycin; AMX – Amoxil; S – Streptomycin; RD – Rifampicin; E – Erythromycin; CH – Chloranphenicol; APX – Ampiclox; LEV – Levofloxacin; *Suc* – Susceptible; *Res* – Resistant

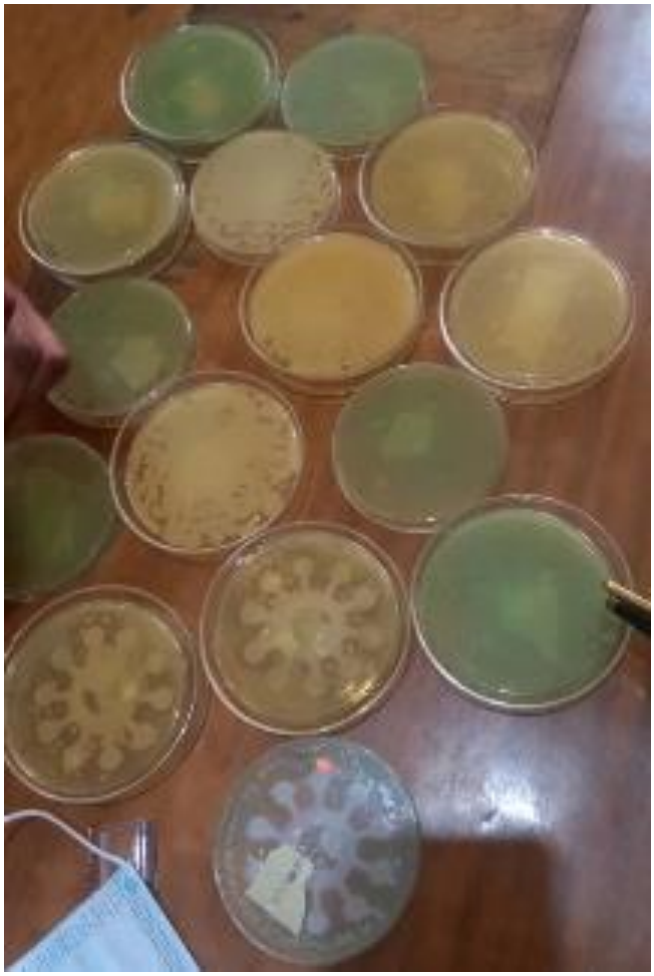

Figure 1 Susceptibility patter of the test bacteria

4. Discussion

The result of this study revealed the presence of various phytochemical constituents in the coconut oil sample. This signifies the potentials of coconut and coconut oil as a functional food. The alkaloids, tannins and other phytochemicals found in this study as shown in Table 1 correlates with the findings of Effiong *et al*. (2018). The very low values of steroids, glycoside and terpenoid in the studied coconut oil sample implies that Nigerian coconut seed flesh is not a good dietary source of steroids which agrees with the study of Effiong *et al*. (2018).

The analysis of the mineral element composition of the extracted coconut oil revealed the presence of sodium, calcium, potassium, magnesium, and phosphorus, while copper, zinc, iron and manganese were not well pronounced. The most abundant element found in the coconut oil is potassium with concentration of 502.55 ppm. This oil is a poor source of potassium compared to the daily requirement of 2000 – 3500 mg/day (Sani *et al*., 2014). The oil cannot serve as nutritional supplement for this element but can be recommended for hypertensive patient. The other most abundant elements in coconut oil are calcium and sodium with the concentration of 105.4 ppm and 75.35 ppm; sodium keeps fluids and electrolytes balanced in the body and it is essential for muscular contraction and nervous cell communication Thus, this oil is a poor source of calcium and sodium cannot serve as a nutritional supplement since a reference range of 2300 mg/day is recommended by (Sani *et al*., 2014). Hence the oil can be recommended for hypertensive patient.

Magnesium serve as a co-factor of many enzyme, involved in energy metabolism, protein synthesis, RNA and DNA synthesis, maintenance of electrical potential of nerve cells and cell membrane. 24.502 ppm was obtained and the daily requirement of 150-500mg is needed, indicating that this oil is a poor source of magnesium. The copper content of the oil is 0.442 ppm. Copper is an essential constituent of several enzymes, such as cytochrome oxidase, catalase, tyrosinase, superoxide dismutase etc. Deficiency of copper causes demineralization of bones, demyelination of neural tissue, fragility of arteries, myocardial fibrosis etc., the daily requirement of copper is 900 mg/day, as such this oil is not a good

source of copper (Sani *et al*., 2014). The value of iron obtained was 0.772 ppm which indicates that the coconut seed oil is a fair source of iron since the recommended daily allowance for iron is 6 – 8 mg/day (Sani *et al*., 2014).

Zinc concentration is 0.393 ppm, therefore, this coconut oil can serve as a nutritional supplement for zinc because it has the moderate concentration that is required daily as reported by Sani *et al*. (2014). Zinc is an essential trace element which plays many biological roles. The manganese content of the coconut oil is 0.629 ppm. Manganese functions as a cofactor for several enzymes, such as arginase, pyruvate carboxylase, isocitrate dehydrogenase, superoxide dismutase and peptidase. Manganese is also required for the formation of bone, proper reproduction and normal functioning of the nervous system. Hence, coconut oil can be a good source of manganese.

The quality of coconut oil was analyzed by evaluating the physicochemical properties such as pH, nitrate, phosphate and ammonia. The pH value of the coconut oil is somewhat neutral however slightly basic, which is preferred to being acidic. pH is a measure of the relative amount of free hydrogen and hydroxyl ions in the coconut oil. Nitrate and phosphate were not detected in the coconut oil sample, and ammonia was found in a minute amount (0.010 mg/kg); their presence in the coconut oil would have been deleterious in the human digestive system if consumed.

In this study, the assay of antibacterial activity of standard bacteria organisms showed that *Staphylococcus aureus* had the highest susceptibility to coconut oil while *Klebsiella pneumoniae* had the least (Table 4). The antibacterial activity of the coconut oil against gram positive and gram negative organisms was consistent with previous research by Effiong *et al*. (2018). The antibacterial activity of gram positive and negative conventional antibiotics as a control gave higher zone of inhibition than coconut oil (Table 5). The action of coconut oil is attributed to the emulsification, saponification, and antimicrobial action of contents action of coconut oil. A recent study by Shino *et al*. (2016) has also shown that coconut oil has antimicrobial activity against various gram positive and gram negative organisms such as *Escherichia vulneris*, *Enterococcer* spp, *Helicobater pylori*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candia albicans*.

5. Conclusion

This study has shown that coconut oil is effective against bacterial pathogens. The phytochemical constituents and mineral elements present in the oil are generally moderate in concentration. Therefore, this has the advantage of inferring pharmacological attributes on the oil.

The coconut (*Cocos nucifera*) oil can be a good source of oil because it has moderate oil content. The oil is a good source of iron, sodium, potassium and calcium because the concentrations of these elements in the oil meet up with the adequate quantity needed by the body daily. Thus, coconut oil has both nutritional and pharmacological benefits and being free from lead, it is safe for human consumption. 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.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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