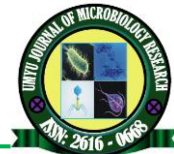
UJMR, Volume 5 Number 1, June, 2020, pp 54 - 59 **ISSN: 2616 – 0668**
https://doi.org/10.47430/ujmr.2051.009



Received: 24/03/2020 **Accepted:** 12/06/2020

Physicochemical and Antibacterial Screening of Coconut Oil on some Clinical Bacteria Isolates

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Abstract
Coconut (Cocos nucifera) is a palm tree found worldwide mainly used as a staple food crop, source of wood and in traditional medicine. This study investigates physicochemical properties and antibacterial screening of coconut oil on some clinically isolated bacteria; Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes and Escherichia coli from sputum, faeces and urine. The isolates were characterized based on biochemical tests according to standard methods. Coconut oil was extracted using the wet processing method and physicochemical properties involving Colour, pH, Moisture content, free fatty acid and Peroxide values were also determined accordingly. These properties were found to fall with standard range values of the Association of Official Agricultural Chemists (AOAC). Agar-well diffusion assay was used for sensitivity assessment and zones of inhibition diameter were measured in millimetres. Antibacterial activity of coconut oil was minimal at low volumes, but demonstrated significantly appreciable antimicrobial effect on the test organisms with increase in oil volume. The highest antimicrobial activity of 12.10 mm mean zone of inhibition diameter observed for Staphylococcus aureus, followed by Streptococcus pyogenes having 10.13mm, Escherichia coli 6.96mm, whereas, Salmonella typhi had the lowest inhibition zone of 5.05 mm. Salmonella typhi showed relatively low zone of inhibition even at the higher volume. Coconut oil has demonstrated antibacterial activity on bacterial isolates used for this study and a potential source of antimicrobial agent.
***Keywords**: Antibacterial, Coconut oil, physicochemical properties.*

INTRODUCTION

The importance of natural products in health and treatment of diseases has been recognised throughout the human evolution (Ramana, *et al.,* 2014). Several natural products derived from plants for thousands of years have been traditionally used to treat various types of human illnesses including general injuries, wound healing and several infections. Natural products have been shown to possess significant pharmacological and anti-bacterial activities that regulate various vital cell signalling pathways that cause mitogenic, cytotoxic and genotoxic reactions leading to various disease pathologies (Ramana, *et al.,* 2014). These plants and plant products that possess anti-bacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, steroids and polyphenolic acids (Jean *et al.,* 2009). The anti-bacterial activity may also be due to various chemical components and hydrophobic activities of essential oils (Samy and Gopalakrishnakone, 2010).

Plant oils and extracts have been used for a wide variety of purposes for many years, and have recently generated widespread interest as a source of natural antimicrobials (Rakholiya *et al.,* 2013). Essential oils and plant extracts are of particular interest because; they are relatively safe, increase the shelf-life of foods, widely accepted by consumers and have the potential to be exploited for multiple uses (Rakholiya *et al.,* 2013).Coconut, (*Cocos nucifera*) is a palm tree growing in the tropics and mainly used as a staple food crop, source of wood and handicrafts. It is thought by many to be the world's most useful medicinal plant in tropical and subtropical countries (Chen and Elevitch, 2006).

Over the years, coconut and its products are believed to possess anti-bacterial, antifungal, anti-viral, anti-inflammatory properties (Zakaria *et al.,* 2006; Jean *et al.,* 2009) and antioxidant activity (Rajeev *et al.,* 2011), and have been employed in traditional medicine (Mandal and Mandal, 2011). Coconut oil is very stable in high heat, low oxidation point and the oxidation begins after 2 years of storage. This stability is due to the higher content of saturated fat (Oladeji *et al.,* 2010). Saturated fats are considered essential to health and comprise 50% of the human cell membranes.

Some proportions of saturated fats are found in all fats and oils, whether plant based or animal based (Che Man and Marina, 2006). Coconut oil is semi-solid at room temperature as a soft, almost waxy substance. It is composed of 92% saturated fatty acids, 6% monounsaturated and 2% polyunsaturated fatty acids. It has the largest concentration of Medium Chain Fatty Acids (MCFAs) compared to other oils which contains Long Chain Fatty Acids (LCFAs). The three valuable Medium Chain Fatty Acids in coconut fat are (C12:0) lauric acid, (C10:0) capric acid and (C8:0) caprylic acid (Oladeji, *et al.,* 2010). Lauric acid has higher antimicrobial activity than caprylic acid (C8:0) and capric acid (C10:0) (Loung *et al.,* 2014). It is generally reported that fatty acids and monoglycerides inactivate bacteria by damaging the plasma membrane (lipid bilayer) (Kabara *et al.,* 1972; Widiyarti *et al.,* 2009).

MATERIALS AND METHODS

Collection of sample and Preparation

Ten (10) fresh mature coconuts fruits (12-13 months) were purchased from Jimeta Modern Market, Adamawa State. The fruits were washed using clean water and de-husked to obtain the fleshy part.

Coconut Oil Extraction (Wet Processing Method)

The method of Davina and Keith (2006), was employed for oil extraction. De-husked fleshy fruits were shredded using a manual grater in a clean bowl and mixed with hot water (at about 70ºC), allowed to cool to tolerable temperature, and using the hands, the mixture was kneaded and decanted to obtain coconut milk. The coconut milk was collected, transferred into a 1000 ml volumetric flask and heated. After one hour of heating, the mixture was allowed to cool to 80ºC to allow milk protein to coagulate. When the oil began to form, the heat was lowered to about 60ºC. The oil was collected by decantation.

Drying of Oil

The oil was dried by heating at 40º C for about15 min. This is characterized by a change from turbid to water-clear colour (Davina and Keith, 2006).Acid, peroxide value was carried out as described by the (AOCS, 2009).

Physicochemical Analyses of Coconut Oil

Physicochemical properties such as; pH, Moisture and volatile content, free fatty acids were determined according to methods of (AOCS, 2009).

Determination of pH

The pH of extracted oil was determined using a pH strip. Universal pH strip was taken from its container and one end of the strip was dipped into the oil and was set on a dry surface for 60 seconds, this was compared to the colour chart that accompanied the universal indicator paper packaging (AOCS, 2009).

Moisture and Volatile Content

Samples (20 g) were heated at a temperature of 110$\pm$5˚C in a pre-dried beaker until cessation of rising bubbles of steam and incipient smoking. Heated samples were cooled to room temperature in a desiccator and reweighed. Moisture and volatile content was calculated by difference.

$$\text{Moisture and Volatile (\%)} = \text{Initial weight} - \text{Final weight} \times 100$$

(AOCS, 2009).

Free Fatty Acid (FFA)

For estimation of Free Fatty Acid (FFA) content, 30 g oil was weighed into 250 ml flask. 50 ml of ethanol was added, which was previously neutralized by adding 0.1N NaOH and 1 ml of 1% phenolphthalein solution. The samples were titrated against 0.1N NaOH until faint pink colour appeared. The free fat acids are expressed as % of lauric acid as indicated in the following equation:

$$FFA\ (as\ \%\ lauric\ acid) = V\ x\ N\ x\ 20/W$$

Where: V = the volume of NaOH solution, N = the normality of NaOH, W = the weight of the oil and20 = the equivalence factor of lauric acid (AOCS, 2009).

Peroxide Value

Peroxide Value (PV) was determined by first weighing 2 g oil in an Erlenmeyer flask. 25 ml of chloroform-acetic acid mixture (2:3) was added and mixed thoroughly. Saturated potassium iodide solution (1 ml) was added and left in the dark for exactly 5 min. 30 ml of distilled water and 1 ml (0.5%) starch indicator was added. The mixture was titrated against sodium thiosulfate (0.01 N) until blue colour disappears. Blank was determined by titration following the above procedure without oil. The peroxide value is expressed as miliequivalents available oxygen/kg (meqO_2/kg) of sample and calculated from the following formula:$P.V = (Vt - Vo)\ x\ N\ x\ 1000/W$

Where: Vt and Vo = the volumes in (ml) of sodium thiosulphate solution used to titrate the test sample and blank, respectively. N = the normality of sodium thiosulphate solution and W = the weight of oil sample (g)(AOCS, 2009).

Microbial Culture, Isolation and Identification

Clinical isolates used for this study were isolated from sputum, stool and urine specimen. A loop-full of specimens were aseptically inoculated onto already prepared i.e. Eosin methylene blue agar (EMB), Manitol Salt Agar (MSA) blood agar and Salmonella-Shigella agar. The plates were incubated at 35 -

37°C for 18 - 24 hrs.

Gram Stain

A drop of normal saline was dispensed on a clean grease-free glass slide and using a sterile wire loop, a loop full of the isolate was picked and a smear was made on the slide. This was allowed to air-dry and then heat-fixed. Smear was stained with crystal violet for 1min, and then washed off with water. Iodine solution was added for 1min and washed off. The smear was decolorized with 95% acetone for 10-30seconds and then rinsed. Finally, the smear was stained with safranin for 1 min, rinsed and allowed to air dry (Cowan and Lunde, 2002).

Catalase Test

A loopful of colony was transferred from solid culture media onto a clean slide and a drop of 3% hydrogen peroxide was placed on the colony. Immediate bubbling indicates a positive test whereas, no bubbles signifies a negative test (Abiola and Oyetayo, 2016).

Oxidase Test

A filter paper soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride was moistened with sterile distilled water. A colony of test organism was picked with platinum loop and smeared on the filter paper. The inoculated area on the filter paper was observed for colour change to deep blue or purple within 10-30seconds which indicates a positive result (Cowan and Lunde, 2002).

Indole Test

The test organism (growth from 18 – 24hrs) was inoculated into a test tube containing 4ml of sterile tryptone water. Incubation was done at 37°C for 24hrs,0.5ml of Kovac's reagent was added and shaken gently. Examination of a red ring-like colour on the surface of the layer within 1minutes indicates a positive result, a negative result will appear yellow (Sagar, 2015).

Voges Proskauer Test

A colony of the test organism was inoculated into glucose phosphate peptone water in a bijou bottle and incubated at 37°C for 24 hrs. 0.6ml of alpha-naphthol solution and 0.2ml of potassium hydroxide was added. The tubes were allowed to stand for 10-15min. A red colour formation indicates a positive result (Abiola and Oyetayo, 2016).

Sugar fermentation test

Ten grams (10 g) of trypticase peptone, 5 g of sodium chloride, 1 g of beef extract, 7.2 ml of 0.25% of phenol red and 10 g each of carbohydrates (glucose, sucrose, mannose and lactose) were mixed in 1000 ml of distilled water and gently heated to dissolve. 4-5 ml of the mixture was transferred into test tubes. Inverted Durham tubes were inserted and autoclaved at 121°C for 15 min to sterilize. A colony of test organisms was aseptically inoculated to the sterile broth and incubated at 35-37°C for 18-24 hrs. A positive test is indicated by a change in colour from red to yellow, with bubble for gas production while negative test retains the red colour of phenol without bubbles (Acharya, 2016).

***In vitro* antibacterial Activity**

In vitro antibacterial activity of Coconut oil against bacterial growth was performed by agar well diffusion method. Tests organisms were inoculated in Nutrient broth, incubated for 18 hrs at 37° C and were adjusted to 0.5 McFarland standards, giving a final inoculum of 1.5 x 10^5 CFU/ml. Standardized inoculum were spread over Mueller Hinton agar (MHA) plates. Holes were bored in the centre of each plate using a sterile cock borer (2mm) and 0.1ml, 0.15 ml and 0.2ml of the coconut oil were dispensed into the wells using sterile syringe. The plates were then incubated for 24 hrs at 37°C. Susceptibility of organisms was determined by measuring the diameter of zone of inhibition in mm with a meter rule. Control plates for Organism Viability Control (OVC), Medium Sterility Control (MSC) were provided (CLSI, 2015).

RESULTS

Coconut Oil Extraction

In this study, ten (10) coconuts fruits were processed (using heat) to yield 650 ml of coconut oil.

Table 1 shows physicochemical properties of coconut oil. Values obtained falls within normal standard range values of coconut oil as described by the Association of Official Agricultural Chemists (AOAC, 2009).

Table 1: Physicochemical Analyses of Coconut Oil

Properties	Standard	Sample
pH	(5.5 - 6.0)	5.8
MC	0.20% max	0.1%
FFA (as lauric acid)	0.20% max	0.12%
PV	3.0meq/kg oil max	1.5meq/kg oil
Food additives	None permitted	Absent
Colour	Colourless	Colourless

Key: MC = Moisture Content, FFA = Free Fatty Acid, PV = Peroxide Value

Table 2.0 Shows Antimicrobial susceptibility of coconut oil against test isolates. Highest mean zone of inhibition was 12.10 mm at 0.2 ml (oil) on *Staphylococcus aureus* while lowest zone of mean inhibition diameter was 5.00 mm at 0.2 ml (oil) on *Salmonella typhi*.

Table 2: Antimicrobial Susceptibility Testing of Coconut oil

Organisms	Zones of Inhibition (mm)		
	0.1ml (oil)	0.15 ml (oil)	0.2 ml (oil)
E. coli	NIZ	6.06 ± 05	6.96 ± 05
S. typhi	NIZ	NIZ	5.00 ± 05
S. aureus	6.10 ± 01	10.50 ± 05	12.10 ± 01
S. pyogenes	5.13 ± 15	8.10 ± 01	10.13 ± 15

Key: NIZ = No Zone of Inhibition

DISCUSSION

The oil yield from the wet processing method of coconut oil extraction using 10 coconut fruits (each weighing an average of 1.2 kg) was 650ml. It is expected50 coconuts to yield approximately 3-5litres of oil. Thus, on the minimum, 10 coconut fruits are expected to yield 0.6 litre (600ml) or 1 litre (1000ml) on the maximum. Therefore, this method of extraction gave a fairly good yield of oil (Davina and Keith, 2006).

From Table 1, the pH of coconut oil was 5.8, Moisture and volatile Content (MVC) was 0.1%, Free Fatty Acid (FFA) was 0.12 (% lauric acid) and Peroxide Value was 1.5meq/kg. These values are within the normal standard limits of (MVC: 0.20% (w/w,), FFA: 0.20 % lauric acid and PV: 3.0meq/kg max (AOAC, 2009).The pH of an environment is important for bacterial growth and moderate changes in the pH modify the ionization of amino-acid functional group and disrupts hydrogen bonding which in-turn promotes changes in the folding of molecules, promoting denaturation and destroying activity (Thompson *et al.*, 2017). High moisture content in foods provides an excellent environment for many bacteria to grow. Removal of water from foods permits longer storage without bacterial growth. As such, foods with lower moisture content would have a slower bacterial growth and have longer storage time compared to those with high moisture content. In addition, high moisture content promotes hydrolytic rancidity of fats and oils (Oseni *et al.*, 2017).

Free fatty acid found in coconut oil (expressed as lauric acid) has been shown to demonstrate antibacterial activity, as free fatty acids have the ability to disrupt the electron transport chain and oxidative phosphorylation (Loung *et al.*, 2014). Free fatty acid may also result in inhibition of enzyme activity, impairment of nutrient uptake, generation of peroxides and auto-oxidation degradation products as well as direct lysis of bacterial cell wall such are considered as the main inhibitory substance in coconut oil (Abbas *et al.*, 2017).

Antimicrobial screening of coconut oil at lower volumes of 0.1 ml on *Escherishia coli* and *Salmonella typhi* showed no zone of inhibition whereas; *Staphylococcus aureus* and *Streptococcus pyogenes* gave 6.10 mm and 5.13 mm respectively. However, increasing oil volume to 0.15 ml and 0.20 ml, *Escherichia coli* gave 6.06mm and 6.96 mm mean inhibition diameter and *Salmonella typhi* showed clearance of 5.05 mm at 0.2 ml. *Staphylococcus aureus* and *Streptococcus pyogenes* showed an increase in inhibition diameter of 10.05 mm and 8.10 mm at 0.15 ml and 12.10 mm and 10.13 mm at 0.20 ml accordingly. This shows that coconut oil has more antibacterial effect against gram positive organism compared to gram negative organisms used in this study. This can be attributed to the basic difference between gram positive and gram negative bacterial cell walls.Gram positive bacteria have a thick peptidoglycan layer; they lack an outer membrane and virtually have no lipopolysaccharide content. Whereas, Gram negative bacteria have a thin layer of

peptidoglycan, an outer membrane and high It has also been shown that, gram positive bacteria are more susceptible to antibiotics whereas, gram negatives are more resistant to antibiotics This can be due to the high lipopolysaccharide content in gram negatives as opposed to the low (or even no) lipopolysaccharide content in gram positive bacteria (Sader *et al.*, 2016).

Lipopolysaccharide is the major component of the outer membrane of Gram negative bacteria, which serves as a physical barrier, providing the bacteria protection from its surroundings (Ronsenfeld and Shai, 2006). In addition to this, gram negative bacteria have a high lipid and lipoprotein content due to the presence of the outer membrane (Kerlly *et al.*, 2017). Lipopolysaccharide, lipid and lipoprotein content contribute to the structural integrity of the bacteria. This explains why coconut oil has more antibacterial effect against gram positive bacteria compared to Gram negative bacteria. The higher susceptibility of *Staphylococcus* lipopolysaccharide content. *aureus Streptococcus pyogenes* even at low volumes of oil compared to *Escherichia coli* and *Salmonella typhi*(Sodha *et al.*, 2015).

CONCLUSION

Coconut oil was extracted from coconut fruits and its' physicochemical properties were determined. Antimicrobial activity of coconut oil was also evaluated against some pathogenic bacteria; an increase in inhibitory activity with increasing oil volume was observed. However, antibacterial activity of the oil was lower in Gram negative organisms (*Escherichia coli* and *Salmonella typhi*) than Gram positive bacteria (*Staphylococcus aureus and Streptococcus pyogenes*) used for this study.

Acknowledgement

We wish to acknowledge Dr. J. Ewansiya and Dr. O. Ajunwa for their contribution towards the completion of this study.

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