PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COCONUT OIL ON SOME SELECTED CLINICAL ISOLATES.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY, (MICROBIOLOGY TECHNIQUES), FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

FEBRUARY, 2025

CERTIFICATION

We certify that this thesis titled PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COCONUT OIL ON SOME SELECTED CLINICAL ISOLATES was carried out by Halima Ummi SHUAIBU with matriculation number LSC1807334 in the Department of Science Laboratory Technology, University of Benin, Benin City, in partial fulfillment of the requirements to be awarded a Bachelor in Science Laboratory Technology.

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DEDICATION

This project is dedicated to God Almighty for his guidance, protection, mercy and favor upon my life throughout my years of study.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all these individuals for mentoring and supporting me in completing this project.

My supervisor Mr. Haruna. O. for providing me with invaluable insights and direction. His patience and dedication towards providing guidance has made this project such a huge success.

Our esteemed Head of Department Prof. Oshomoh for providing the assistance I needed. My lecturer and friend, Mr. Ekhator for his constant support and counsel and other lecturers, who pushed and supported me towards the smooth completion.

To my parent and my uncle and the entire family, their constant encouragement, patience, and understanding have been the pillars, thank you for your immense support, I am eternally grateful.

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ABSTRACT

Coconut, as food is usually called perfect diet since it contains almost all of the vitamins the body requires. The phytochemical characteristics of coconut oil was examined to determine the presence of Alkaloid, Flavnoid and Saponin quantitatively. Antimicrobial activities were also determined against several bacterial isolates. The tested organisms were sourced from the University of Benin Teaching hospital (UBTH) and they include *Klebsiella species, Streptococcus species, Escherichia coli,* and *Staphylococcus aureus.* and *Pseudomonas aeroginosa*. The phytochemical examination provided confirmation of the existence of alkaloids, flavonoid and saponin. The Kirby- Bauer disk diffusion method was employed to evaluate sensitivity, with zones of inhibition measured in millimeter diameter. Coconut oil showed activities as well as resistant on the isolates at the various dilution concentrations with the zone of inhibition ranged from 5 mm for *Pseudomonas aeruginosa,* 3 mm for *Escherichia coli and* 2.5 mm for *Streptococcus spp* while *Staphylococcus aureus* and *Klebsiella spp,* showed no activities in all the concentrations tested against them. The result of Minimum Inhibitory Concentration (MIC) revealed activities at 400 mg/ml for *Pseudomonas aeruginosa* and *Escherichia coli,* while *Streptococcus spp* was seen to have activity at 600 mg/ml. The oil was seen to be bacteriostatic up to the highest concentration (1000 mg/ml) used. Since this study identified some bioactive components that are known to be bacteriostatic, it suggests using coconut oil as a medicinal agent and in the battle against antibiotic resistance. To understand the mechanisms of action of the oil and its derivative, more in vitro and in vivo research should be conducted.

CHAPTER ONE

1.0 **INTRODUCTION**

Plants have historically been significant in the management of diseases affecting both humans and animals. Medicinal plants can be utilized in various ways: (i) as primary sources for the extraction of active compounds, or (ii) for the extraction of plentiful yet inactive components that can be partially synthesized into active compounds, such as traditional preparations or extracts (Mukherjee, 2008).

The coconut palm (Cocos nucifera), a member of the monocotyledonous family Palmae, produces coconut, a drupe. Often called a "wonder food," it is regarded as the perfect nutritional component since it contains a wide range of vital elements that the human body needs. Over 80 nations throughout the world are home to coconut trees, which produce 61 million tons a year (FAO, 2010). India is the world's third-largest coconut grower with approximately 1.78 million hectares dedicated to this crop. Tamil Nadu, Karnataka, Andhra Pradesh, and Kerala are the four southern states that produce around 90% of India's coconuts. (FAO, 2010). This fruit is highly versatile and essential for many individuals living in tropical regions. It is a complete diet that is high in calories, vitamins, and minerals and is well-known for its ability to fortify, nourish, and increase body fat. In tallying to the coconut great oil content, coconuts are known for their high-quality protein that contains all of the key amino acids needed for body growth and maintenance. The elements potassium, sodium, magnesium, and sulfur are especially abundant in it. About 662 calories are included in 100 grams of dried coconut (Bakhru, 2000). Although each species has a different nutritional makeup, nuts typically include significant amounts of vegetable protein, dietary fiber, monounsaturated and polyunsaturated fatty acids, vitamins E and K, folate, magnesium, copper, selenium, and potassium.

Nuts are also naturally low in sodium and saturated fats (O'Neil *et al.*,, 2012). Benefitful substances like phenols, phytosterols, flavonoids, proanthocyanidins, resveratrol, and arginine are also present; these bioactive substances, in addition to micronutrients like vitamin E and selenium, (Bolling *et al.*,, 2010). According to Pushpan *et al.,* (2013), the coconut tree is frequently referred to as Kalpavriksha because of its many uses. As a unique source of many natural ingredients, it aids in the creation of medications for a range of illnesses and has industrial uses. The fruit's constituents, especially the coconut kernel and its soft water, have a variety of therapeutic uses, such as antiviral, antifungal, antibacterial, Antipyretic, antidermatophytic, antioxidant, hypoglycemic, hepattective and immunostimulant properties. A staple meal for people all throughout the world, especially in tropical areas, coconuts are also high in microminerals and nutrients that are essential for human health in their water and kernel (Deb Mandal and Mandal 2011).

1.1 Coconut Oil

Coconut oil is obtained through the processing of copra, the dried kernel, which contains approximately 60–65% oil. This oil exhibits the natural sweetness typical of coconuts and is composed of 92% saturated fatty acids, predominantly in the form of triglycerides, which are relatively rare in other vegetable oils. Lauric acid, which makes up 45–56% of the MCFAs present in coconut oil, holds particular importance. Various fractions of coconut oil include medium-chain triglycerides, recognized for their efficacy as solvents for flavors, essences, and emulsifiers. These fatty acids are utilized in the development of emulsifiers, pharmaceuticals, and cosmetics (Krishna *et al.,* 2010). Several methods for extracting coconut oil have been developed, including both dry and is subjected to pressure through wedge presses, screw presses, or hydraulic presses to extract the oil, which is then refined, bleached, and deodorized (O’Brien and Timms 2004). This chapter

will explore the various techniques for oil extraction from coconuts, the chemical characteristics of coconut oils, the blending of coconut oil with other vegetable oils, and the biological activities associated with coconut oils.

1.2 Different Coconut Oils Preparation

Various types of coconut oils are derived from distinct parts of the coconut using different techniques. Copra oil, along with refined, bleached, and deodorized (RBD) oils, is obtained from the dried coconut kernel; however, RBD oil is subjected to chemical refinement and bleaching processes. The brown testa of the coconut is utilized in the production of coconut testa oil (CTO), which is essentially a byproduct of coconut oil extraction. In contrast to copra oil (CO) and RBD oils, virgin coconut oil (VCO) is produced through a "wet method" that employs fresh coconut milk. Since there is no universally accepted method for the preparation of VCO, any process that does not involve refinement or modification of the oil is classified as virgin (Narayanankutty *et al.,* 2018).

1.2.1 Copra Oil

Coconut kernels are dried and used as copra. After being dried in the sun or an oven, the fresh coconut kernel's oil is extracted using a machine. The moisture content of the oil is eliminated by collecting it and letting it dry in the sun (Narayanankutty *et al.*, 2018).

1.2.2 Testa Oil

The new form is coconut testa oil (CTO), which may be extracted from the coconut testa using isopropyl alcohol (Zhang *et al.,* 2016). With a yield of up to 63–76% and a substrate to solvent ratio of 1:4, CTO is best produced at 60 °C for three hours. CTO hasn't been utilized much for food uses yet because the extraction process requires chemical solvents.

1.2.3 Virgin Coconut Oil

Without the use of chemicals or high temperatures, virgin oil of coconut (VOC) is naturally produced from the kernel of fresh coconut. There are several kinds of VCO depending on how it is prepared (Narayanankutty *et al.*, 2018).

1.3 Extraction of oil

1.3.1 Cold Extraction

The extraction of VCO without the use of heat is known as cold processing. This process involves freezing the coconut milk at 2 to 8 degrees Celsius for the whole night, after which the separated oil is recovered by centrifugation, filtered, and stored. According to Narayanankutty *et al.*, (2018), this is the most straightforward and affordable approach accessible.

1.3.2 Hot Extraction

Regarding the preparation of VCO using the heat extraction technique, Southern India has a lengthy history. The coconut milk is heated by this process to a comfortable temperature of up to 100 degrees Celsius. When the oil has been processed for 60 minutes, or until it is completely separated from the milk, it is recovered by filtering. This heating technique helps increase the evacuation of trapped phenolic acids onto the oil while also yielding a significantly higher production. The oil produced in this way is used to treat skin disorders, especially in babies, in the Ayurvedic medical system (Narayanankutty *et al.*, 2018).

1.3.3 Technique of Fermentation

Fermentation approach using microbes especially bacteria and its activity to make VCO was additionally proposed. It is generally divided into two types-natural ferment in addition to forced fermentation.

To obtain the coconut milk, the freshly shredded coconut kernel and its water are extracted using the natural fermentation technique. After that, it is allowed to ferment and separate the oil layer for 24 to 48 hours at room temperature (or as high as 45 °C). The oil layer is then removed, filtered,

and stored (Nevin and Rajamohan 2010). Using the induced fermentation approach, Masyithah (2017) extracted VCO from coconut milk using Lactobacillus plantarum (strain 1041 IAM) and Saccharomyces cerevisiae. The fermentation method also uses L. plantarum and L. delbrueckii. VCO generated by the natural fermentation process is sometimes referred to as F-VCO, and research employing induced fermentation are somewhat uncommon.

1.3.4 Enzymatic Extraction Technique

Because (Shah *et al.,* 2005), it is possible to extract oil using the enzymes in the water-based extraction process. About 10% of the carbohydrate in coconut meat is cellulose, of which 50% is cellulose, and 75% of the cellulose is made up of α-cellulose (Rosenthal *et al.,* 1996; Shah *et al.,* 2005). Oil is found inside plant cells, linked with proteins and a variety of carbohydrates, including cellulose, starchy carbohydrates, hemicellulose, and pectins. Cell-wall breaking down enzymes are substances that can be used to extract oil by solubilizing structure cell wall components of the oilseed. Man *et al.,* (1996) used a 1% enzyme combination consisting of cellulase, α-amylase, polygalacturonase, and the protease to successfully extract coconut oil with a 74% oil yield. The study found that different enzymes were needed to break down components of the structural cell wall, such as mannan, galactomannan, arabinoxylogactan, and cellulose. The polygalacturonase hydrolyzes α-linkages of polygalacturonic acid of the polymers randomly from the ends, while an α-amylase hydrolyzes α-linkages to liquefy starch and produce maltose.

1.3.5 Wet Extraction

Aqueous processing, another name for wet processing, is the process of separating coconut oil from coconut milk. This method can drastically save energy consumption and investment costs because it does not require the use of solvents. The enhanced, discolored, or deodorized (RBD) process is also avoided (Villarino *et al.,* 2007). Although this technology is attractive, its economic

feasibility has been hampered due to its relatively low oil output (Rosenthal *et al.*, 1996). The wet processing procedure depends on the emulsion in coconut milk breaking down, which is made more difficult by the emulsion's natural resilience. A destabilization can happen in three main ways. The first process is creaming, in which two separate phases are created by gravitational forces. The phase with a higher specific gravity rises to the top, while the phase with a lower specific gravity descends. The second process, known as flocculation or clustering, maintains the structural integrity of the initial globules by allowing the oil phase to collect without rupturing the interfacial membrane that surrounds each globule. Coalescence, the last and most important process, causes the interfacial area to be disturbed, allowing globules to combine and reduce the contact area (Onsaard *et al.,* 2005). When opposed to solvent extraction, the wet processing approach is more noticeable in producing natural oil.

1.4. Chemical Composition of Coconut Oil

1.4.1 Composition of Fatty Acid and Triacylglycerol

Coconut oil is characterized by a significant presence of glycerides derived from short-chain fatty acids. This oil exhibits remarkable stability against oxidation in the atmosphere. It is noted for its low iodine value, high saponification value, and elevated content of saturated fatty acids, remaining in a liquid state at 27 °C. Medium chain triglycerides (MCT) represent a category of lipids where three saturated fatty acids are linked to a glycerol backbone. The defining feature of MCT, in contrast to other triglycerides, is that each fatty acid molecule consists of 6 to 12 carbon atoms in length (Babayan 1988). MCT are prevalent in various food sources, with coconut and palm oils being the richest dietary sources of these compounds. Additionally, MCT are available as dietary supplements (Heydinger and Nakhasi 1996). The absorption and utilization of MCT differ from that of long-chain triglycerides (LCT), which constitute 97% of dietary fats. For LCT absorption, the fatty acid chains must be cleaved from the glycerol backbone by the enzyme lipase.

These fatty acids then form micelles, are absorbed, and subsequently reattached to glycerol, resulting in triglycerides that travel through the lymphatic system before entering the bloodstream. All fats and oils consist of triglyceride molecules, which are triesters formed from glycerol and fatty acids. Upon hydrolysis, these fats yield fatty acids and glycerol. Fatty acids can be classified into two categories: monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The second classification method is predicated on the molecular size or the length of the carbon chain present in fatty acids. The majority of fats and oils, regardless of whether they are saturated or unsaturated, or derived from animal or plant sources, predominantly consist of long-chain triglycerides (LCT). All dietary fats are primarily composed of LCT, with the exception of coconut oil, which is notably characterized by its high content of medium-chain triglycerides (MCT). The size of the fatty acid is crucial, as the physiological effects of medium-chain fatty acids found in coconut oil differ significantly from those of the long-chain fatty acids that are more prevalent in typical diets (Furman *et al.,* 1965). In addition to coconut oil, MCT are also natural and crucial components of human breast milk. MCT are regarded as essential nutrients for infants and individuals with significant digestive disorders, such as cystic fibrosis (St-Onge *et al.,* 2003; Illam *et al.,* 2017).

1.4 AIM AND OBJECTIVES

This study aimed at evaluating the phytochemical constituents and antimicrobial properties of coconut oil on some selected clinical isolates.

The specific objectives of this study were to;

i. test for sterility of the coconut oil sample.
ii. quantitatively analyze the phytochemical constituents of coconut oil.
iii. determine the antibacterial properties of coconut oil on some clinical isolates using well

CHAPTER TWO
LITERATURE REVIEW

2.1 Biological Effects of Coconut Oil

Anti-inflammatory

According to research, the hepatorenal damage linked to methotrexate (MTX) may be influenced by inflammatory mediators including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa B (NF-κB) expression (El-Sheikh *et al.,* 2015). Since virgin coconut oil's (VCO) anti-inflammatory qualities have been known for a number of years, several investigations have been conducted to examine its mechanisms of action in various animal models. In chemically induced ear and paw edema models, Intahphuak *et al.,* (2010) showed that fermented VCO (F-VCO) prevented the development of granulomas. Furthermore, F-VCO was demonstrated to reduce the production of TNF-α, cyclooxygenase, and inducible nitric oxide synthase in rats with adjuvant-induced arthritis (Vysakh *et al.,* 2014).

F-VCO successfully decreased acute inflammation, but its effectiveness was lessened in chronic models, according to a research by Zakaria *et al.,* (2011). F-VCO has been shown to have analgesic and anti-nociceptive effects in addition to its anti-inflammatory qualities (Intahphuak *et al.,* 2010; Zakaria *et al.,* 2011). The ability of other VCO formulations to reduce inflammation has not yet been evaluated. The in vitro anti-inflammatory and skin-protective qualities of VCO were examined by Varma *et al.,* (2019). Historically, people in tropical areas have been using VCO as a moisturizer for generations. According to clinical research, VCO reduces the symptoms of skin conditions by having calming and moisturizing properties. The advantages of VCO for the skin and its mechanisms of action have not, however, been fully clarified in vitro. VCO's cytotoxicity (CTC50) in THP-1 (human monocytes) and HaCaT (human keratinocytes) cells was found to be 706.53 mg/mL and 787.15 mg/mL, respectively. TNF-a (62.34%), IFN-g (42.66%), IL-6

(52.07%), IL-8 (53.98%), and IL-5 (51.57%) were all shown to be inhibited by VCO in THP-1 cells. Furthermore, HaCaT cells showed increases in involucrin (INV) and filaggrin (FLG) of 47.53% and 40.45%, respectively. Additionally, VCO increased the expression of filaggrin (FLG), involucrin (INV), and aquaporin-3 (AQP3) while offering HaCaT cells a modest level of UV protection. VCO is neither irritating to the skin (IC50 >1000 mg/mL) or phototoxic (PIF <2), according to in vitro tests of skin irritation performed on Reconstructed Human Epidermis (RHE) and NIH3T3 cells. According to these results, VCO's anti-inflammatory qualities could result from its capacity to reduce inflammatory indicators and strengthen the skin's protective layer. All of the findings point to the benefits of using VCO in skin care products.

2.2 Beneficial uses of coconut in dentistry

Coconut oil is employed in the practice known as Oil Pulling or Oil Mulling. This traditional method involves the action of "swishing or swirling" oil in the mouth, effectively cleansing the oral cavity with natural oils. Originating from Ayurvedic practices in India, it has been used to enhance both oral hygiene and overall health. The process requires the individual to swish a tablespoon of coconut oil in the mouth for approximately 20 minutes. Following this duration, the oil, which now contains bacteria, should be expelled, and it is advisable for the person to brush their teeth as normal. The swirling action creates negative pressure in the mouth, which, along with the oil's thickness, aids in trapping food particles, bacteria, and other microorganisms that may be present on the teeth and gums. The debris becomes encapsulated within the oil and is subsequently removed from the surfaces of the teeth into the swirling oil mass (Athlone Institute of Technology, 2012).

2.3 Antibacterial Properties of Coconut

Coconut endocarp extracts, derived from both methanolic and aqueous solutions, demonstrated significant antibacterial activity against Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Micrococcus luteus (Singla *et al.,*, 2011). Additionally, the antimicrobial properties of coconut shell were shown to exert considerable antibacterial effects on Escherichia coli and Salmonella typhi (Verma *et al.,*, 2012). Research into the antibacterial efficacy of crude aqueous and n-Hexane extracts from the husk of Cocos nucifera revealed their effectiveness against forty-five strains of Vibrio pathogens and twenty-five other bacterial isolates commonly associated with food and wound infections. The aqueous extract was effective against 17 of the tested bacterial strains and 37 of the Vibrio isolates, while the n-Hexane extract exhibited antibacterial activity against 21 of the tested bacteria and 38 of the Vibrio species (Akinyele *et al.,*, 2011).

Tender coconut water is administered to individuals suffering from cholera due to its content of saline and albumin (Effiong *et al.,*, 2010). It is commonly utilized in the treatment of urinary infections and diarrhea. Through reversed phase-high performance liquid chromatography (RP-HPLC), three peptides with molecular weights under 3 kDa were isolated and characterized from green coconut water, specifically with molecular weights of 858 Da and 1249 Da, and were assessed for their effectiveness against B. cereus, which has a molecular weight of 950 Da. These peptides hold significant potential for the development of new antibiotics derived from natural sources (Mandal *et al.,*, 2009). Additionally, bacterial isolates obtained from fermented Listeria monocytogenes and E. coli, both prevalent foodborne pathogens affecting the gastrointestinal tract,

were examined. The findings indicated that two out of ten isolates were capable of inhibiting the indicator organisms, although the degree of inhibition varied (Krishnamoorthy and Arjun, 2012). The antimicrobial properties of coconut leaf extracts were evaluated against a variety of pathogens, including Acinetobacter spp., B. cereus, E. coli, S. typhi, Shigella dysenteriae, S. aureus, Aspergillus flavus, and A. niger. The results indicated that the leaf extracts demonstrated activity against all tested pathogens, with the exception of S. aureus and A. flavus (Ifesan *et al.*,, 2013). Phytochemical analysis of coconut flowers revealed the presence of alkaloids, flavonoids, phenols, phytosterols, tannins, amino acids, and carbohydrates (Dyana and Kanchana, 2012). These phytochemicals are recognized for their therapeutic effects against various human pathogens. Furthermore, a study examining the antibacterial properties of aqueous and methanolic extracts from 26 medicinal plants utilized in Mexico for gastrointestinal disorders found that coconut exhibited significant bactericidal activity against the tested species (Alanis *et al.*,, 2005).

The research further assessed the effects of oil pulling on oral microorganisms within biofilm models using different edible oils. The results revealed that oil pulling with coconut oil demonstrated antimicrobial effects against S. mutans and C. albicans, which are the primary microorganisms associated with dental caries (Thaweboon *et al.*,, 2011). Additionally, toddy has been acknowledged for its potential therapeutic advantages in the treatment of oral diseases (Alviano *et al.*,, 2008). Lauric acid, a naturally occurring compound and the predominant fatty acid in coconut oil, is also found in human breast milk. Studies have validated its efficacy against a variety of harmful pathogens. In 2009, Batovska *et al.,* investigated the antibacterial properties of medium-chain fatty acids and their corresponding monoglycerides. Due to its high lauric acid content, coconut flour has been utilized as a treatment for oral sores, as noted by Taheri *et al.*, in 2010. Furthermore, extracts from coconut husk fibers, particularly monolaurin, exhibited

significant antibacterial activity against various Gram-positive bacteria. The antimicrobial effects of lauric acid on Propionibacterium acnes were examined both in vitro and in vivo. In vitro findings indicated that lauric acid effectively inhibited the growth of P. acnes while having minimal impact on the skin's resident flora. Moreover, cytotoxicity studies demonstrated that higher concentrations of lauric acid did not compromise cell viability; rather, they successfully eliminated the bacteria responsible for acne, as reported by Nakatsuji *et al.,* in 2009.

2.4 Anti-fungal activity

Heating coconut shells produces an oil that is utilized in traditional Indian medicine for addressing ringworm infections. The alcoholic extract derived from mature, dried coconut shells exhibits antifungal properties against various fungi, including Microsporum canis, M. gypseum, M. audouinii, Trichophyton mentagrophytes, T. rubrum, T. tonsurans, and T. violaceum. This extract has demonstrated antifungal efficacy against all tested dermatophytes, requiring only half the concentration needed to inhibit Epidermophyton floccosum (200 µg/ml). The antifungal effects are primarily attributed to the high levels of phenolic compounds present (Venkataraman *et al.*,, 1980). A comprehensive study by Ogbolu *et al.,* (2007) indicated that coconut oil exhibits antifungal activity against six Candida species isolated from clinical environments. Furthermore, they discovered that coconut oil was effective against these Candida species at a 100% concentration, exceeding the effectiveness of fluconazole.

2.5 Antiviral activity

Coconut oil has demonstrated significant efficacy against various lipid-coated viruses. The medium-chain fatty acids present in coconut oil disrupt viral membranes, thereby hindering their assembly and maturation (Arora *et al.*,, 2011). In a clinical trial involving 15 HIV-positive patients, coconut oil was administered in different concentrations over a period of six months. After three months, 50% of the participants showed a reduction in viral load, and by the end of the six-month period, eight individuals had both a decreased viral load and an improved CD4/CD8 ratio (Conrado, 2000). Additionally, polyphenols derived from coconut husk fiber were evaluated for their antimicrobial and antiviral effects. The specific antibacterial properties of C. nucifera against S. aureus and Herpes Simplex Virus 1 (HSV-1) indicate that this plant may hold potential for topical applications in wound healing (Esquenazi *et al.*,, 2002).

2.6 Antiprotozoan activity

The antihelmintic study carried out by Mariselvam *et al.*, in 2013 utilized chironomus larvae as part of its methodology. The crude extract obtained from coconut inflorescence not only demonstrated the capacity to inactivate helminths but also exhibited a faster mortality rate in comparison to the standard medication, albendazole. Furthermore, the in vitro leishmanicidal effects of coconut husk extracts against Leishmania amazonensis were investigated. The results showed that at a concentration of 10 µg/ml, the coconut husk extract displayed significant leishmanicidal activity, effectively inhibiting the growth of both the promastigote and amastigote forms of L. amazonensis within 60 minutes, without causing any allergic reactions in vivo or cytotoxic effects in vitro in mammalian systems. In addition, a combination of specially formulated extracts from onion (Allium cepa) and coconut was assessed for its effectiveness against pathogens

that cause gastrointestinal infections in animals. Sheep afflicted with gastrointestinal helminthic infections received the extract over an 8-day period, which included 60 g of both coconut and onion extracts. The findings indicated that the larval stages of the worms were absent from the feces, with no traces found 9 and 20 days after the treatment was discontinued (Mehlhorn *et al.*,, 2011).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Sample Collection

Freshly picked, completely ripe coconut fruits were purchased from Oba market in Benin City, Edo State, is where. After that, they were sent to the microbiological lab for examination.

3.2 The process of extracting coconut oil

The workspace was thoroughly sanitized, and the cutting instruments were sterilized before initiating the procedure. The coconuts were then opened and dehusked, with the seed flesh being removed from the shell using a kitchen knife. The flesh was subsequently chopped into smaller pieces and blended with warm water. Following this, the coconut milk was separated from the remaining chaff by pouring it into a sterile plastic bowl through a sterile stainless sieve. The coconut milk was then transferred into a pot and heated to obtain the coconut oil. The separated coconut oil was then transferred into a separate sterile plastic bottle after cooling and stored for further analysis.

3.3 Phytochemical screening of the coconut oil

3.3.1 Determination of Alkaloids Content

The approach developed by Harborne (1973) was used to evaluate the total alkaloid content. Five grams of the extract and one hundred milliliters of 20% acetic acid in ethanol were added to a 250 milliliter beaker. After that, the mixture was allowed to stand for two hrs. Afterward, filtration was done, and the extract was concentrated to a fourth of its original volume in a water bath. After that, drops of concentrated ammonium hydroxide were added to the extract until full precipitation had taken place and the precipitate was filtered out, cleaned with 1% solution of

ammonia, dried, and then weighed. Every sample was examined three times.

Alkaloid (%) = residual weight x 100

sample weight.

3.3.2 Flavonoid content determination

Three identical aliquots of a homogeneous cabbage extract (1.5 g) were used to measure the flavonoid concentration, in accordance with Ilahy *et al.*, (2011). Aliquots of the methanolic extract containing thirty microliters were used to determine the flavonoids. Following a 90 µl methanol dilution, 6 µl of 10% aluminum chloride (AlCl3) and 6 µl of 1 mol/l sodium acetate (CH_3CO_2Na) were added to the samples. A 30-minute incubation time was followed by the addition of 170 µL of methanol and the measurement of the absorbance at 415 nm. The flavonoid content was calculated using quercetin as the standard (Ug Qe/g).

3.3.3 Estimation of total saponins content

With certain adjustments, the Makkar *et al.,* methodology—which includes a vanillin-sulphuric acid colorimetric reaction—was used to determine the total saponin concentration. The plant extract was first combined with 250 µL of distilled water in 50 µL. Once 800 mg of vanillin had been dissolved in 10 mL of 99.5% ethanol, 250 µL of vanillin reagent was added to the mixture. The solution was then well stirred after 2.5 mL of 72% sulfuric acid was added.

After that, the mixture was incubated for ten minutes at 60°C in a water bath. The solution was chilled in ice-cold water following this incubation, and the absorbance at 570 nm was measured. From a saponin stock solution, standard saponin solutions with concentrations ranging from 0 to 25 ppm were made and handled similarly to the test samples. Parts per million (PPM) were used to report the findings.

3.4 Sterility Test

A laboratory technique called the sterility test is performed to determine if a material contains any live bacteria. Nutrient agar and MacConkey agar plates were used to cultivate the extracted coconut oil, and it was incubated at 37 °C for 24 hr. The purpose of this procedure was to determine the oil's purity and find any possible contaminants.

3.5 Evaluation of Antibacterial Activity

In order to create bacterial inocula, bacterial colonies were transferred using a sterile inoculating loop and then suspended in a sterilized test tube containing sterile water. The inocula was then adjusted to a 0.5 MacFarland standard prior to sensitivity test.

Each plate underwent an antimicrobial susceptibility test using the Bauer-Kirby (2008) agar disc diffusion technique. The test organisms were inoculated onto an agar plate in this procedure. A susceptibility examination was conducted using agar that had been uniformly inoculated with the test organism and appropriately spaced on the inoculation plate. A disc of Whatman filter paper was saturated with a predetermined concentration of coconut oil. The antimicrobial agent diffused from the disc into the medium, resulting in the inhibition of the test organism's growth at a specified distance from the disc. Following the incubation period, the agar plate was examined for zones of inhibition, which are areas devoid of growth surrounding the discs. The presence of a zone of inhibition signifies the effectiveness of the antimicrobial against the organisms, while the lack of such a zone suggests that the acid was ineffective against the test organisms or that the organisms exhibit resistance to the acid. Strains that are susceptible to the antimicrobial agent displayed inhibition at a distance from the disc, whereas resistant strains exhibited smaller zones of inhibition or continued to grow up to the edge of the disc (Cheesbrough, 2006).

CHAPTER FOUR

4.0 RESULTS

The phytochemical content identified in the examined samples of coconut oil is represented in Table 1. It was observed that the quantitative analysis of the three parameters analyzed varies in content. Table 2 displays the result of sterility test carried out on the oil used for the study. The oil that was obtained was cultivated on Nutrient agar, and the absence of growth seen indicates good sterility. The antimicrobial activities of the coconut oil screened against some clinical pathogenic isolates using disc diffusion and antibiotics as positive control is represented in Table 3. Among the tested organism susceptibility was recorded higher in the case of *Pseudomonas aeruginosa* while the least was recorded in *Streptococcus spp*. Antibiotics pefloxacin and tarivid did not inhibit *Escherichia coli* but was seen to be highest in *Pseudomonas aeruginosa* and least in *Streptococcus spp*; while ciprofloxacin was seen to be highest in *Staphylococcus* spp. with gentamycine showing the least activity. This is depicted in Table 3.

Table 1: Quantitative analysis of phytochemical constituents of coconut oil (%)

Phytochemicals	Replicate 1	Replicate 2	Replicate 3	Mean±SD.
Total Alkaloids	0.193	0.230	0.221	0.215±0.015
Total Flavonoids	0.04	0.030	0.04	0.037±0.004
Total Saponins	0.272	0.280	0.308	0.29±0.015

Table 2: Sterility Test of Coconut Oil Sample

Media	Temperature of incubation	Number of colonies	Inference
Nutrient agar	37^0C	0	Satisfactory
MacConkey agar	37^0C	0	Satisfactory

Table 3: zone of inhibition of various concentration of coconut oil in (mm) diameter using agar disc diffusion against selected species of bacteria isolates and test control using sensitivity disc method.

Test isolates	Concentration of extract in mg/ml				Control antibiotics in µg (+ve)			Control antibiotics in µg (-ve)		
	400	600	800	1000	CH 30µg	PEF 30µg	OFX 10µg	SXT 30µg	CN 10µg	CPX 10µg
Escherichia coli	2mm	2.5mm	2.95±0.071mm		12mm	—	—	NA	NA	NA
Klebsiella spp,	—	—	—	—	11mm	15mm	13mm	NA	NA	NA
Pseudomonas aeruginosa	2mm	2.7mm	3.75±0.53mm	5mm	—	3mm	4mm	NA	NA	NA
Staphylococcus aureus	—	—	—		NA	NA	NA	5mm	9mm	15mm
Streptococcus spp	1.7±0.353mm	2mm	2.15±0212mm	2.5mm	NA	NA	NA	3mm	4mm	5mm

Values are mean ± SEM; n=2.
µg: microgram, **NA**: not applicable, —: no inhibition, **CH**: chloramphenicol, **PEF**: pefloxacin, **OFX**: Tarivid, **SXT**: septrin, **CN**: Gentamycin, **CXP**: ciprofloxacin.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Research has shown that phytochemicals possess a wide range of characteristics that may help avoid chronic diseases. Alkaloids, flavonoids, and saponins were found in coconut oil's phytochemical components, according to a quantitative examination. Table 1's results, which demonstrate a moderate presence of alkaloids, flavonoids, and saponins in coconut oil, are in line with those published by Obidoa *et al.,* (2010).

This study looked at the antibacterial qualities of coconut oil against a number of bacterial isolates, such as *Pseudomonas aeruginosa, Klebsiella spp., Streptococcus spp., Escherichia coli, and Staphylococcus aureu*s. The mentioned isolates have been documented as prevalent pathogens that are responsible for some notable infections including urinary tract infections (Odoki *et al.,*, 2019) as well as nosocomial infections (Khan *et al.,*, 2019), among other conditions. It is worth noting that *Escherichia coli* is recognized as an opportunistic pathogen in individuals receiving hospital care. The efficacy of coconut oil in inhibiting bacterial growth was shown in all test organisms, with the exception of two. This research demonstrates that *Pseudomonas aeruginosa* has the greatest vulnerability to coconut oil, whilst *Escherichia coli* and *Streptococcus spp* displays the lowest susceptibility; *Klebsiella spp*. and *Staphylococcus aureus,* exhibits resistance. The findings shown in Table 3 align with the prior study conducted by Effiong *et al.,* (2018), which reported notable antibacterial effects of coconut oil against *Pseudomonas aeruginosa* and *Escherichia coli*. The findings of Florianna *et al.,* (2015) about the efficacy of coconut oil against *Escherichia coli* and *Pseudomonas aeruginosa* are consistent with the results obtained in this research. Table 4 presents the inhibition zone in millimeter diameters of the antibacterial activity of the coconut oil

for several bacterial species with pefloxacin, tarivid, spafloxacin, ciprofloxacin, and chloramphenicol as (control) for gram negative bacteria while septrin, erythromyci, pefloxacin and gentamycin as (control) for gram positive bacteria. Antibiotics pefloxacin and tarivid were not effective on *Escherichia coli* but were seen to be effective on *Pseudomonas aeruginosa* and *Streptococcus spp* for the Gram negative bacteria; while ciprofloxacin was seen to be highest in *Staphylococcus* spp. with the zone of inhibition of 15mm diameter while septrin was seen to have the the least activity on same organism.

CONCLUSION

In conclusion, it can be inferred that the aforementioned points collectively support the notion that the motivation for the initiation of this investigation stemmed from the escalating phenomenon of antibiotic resistance. Plant extracts have recently gained attention as potential antibacterial agents due to their rich assortment of phytochemicals with significant therapeutic capabilities that might be used in the management of both developing and re-emerging illnesses. This research presents an analysis of the phytochemical composition of coconut oil, highlighting their biological activities and confirming their pharmacological qualities. This study also demonstrates the antibacterial capabilities of coconut oil and, highlighting their potential use in the formulation of antibacterial soaps and lotions. It is possible to promote the frequent use of coconut. From the research carried out, it is possible to commercialize and promote food compositions including coconut and its compounds as functional foods. Coconut products can be used to manufacture medications and supplements for both preventative and therapeutic uses. Since this study identified some bioactive components that are known to be bacteriostatic, it suggests using coconut oil as a medicinal agent and in the battle against antibiotic resistance. To determine its mechanisms of action, more research on the oil and its derivatives should be conducted both in vitro and in vivo.

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