International Journal of Basic Science and Technology
April, Volume 10, Number 2, Pages 119 – 124 http://www.ijbst.fuotuoke.edu.ng/ 119
https://doi.org/10.5555/WDBP4912 **ISSN 2488-8648**

Antibacterial Efficacy of Coconut Oil Against Specific Isolates

Anyiam, I.V. and Opara, C.N.
Department of Microbiology, Federal University Otuoke, Bayelsa State Nigeria

Article Information

Article # 10014
Received: 15th Jan., 2024
1st Revision:14h Feb. 2024
2nd Revision:24th Feb. 2024
Acceptance:9th April 2024
Available online:
2nd May 2024.

Key Words
Phytochemical analysis,
Antibacterial activity,
Zone of inhibition,
Coconut oil.

Abstract

Phytochemical analysis and antibacterial activities of the coconut oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp were determined using microbiological standard and disc diffusion methods. Qualitative phytochemical analysis showed the presence of flavonoids, tannins, phenols, anthocyanins, terpenoids, and alkaloids. Quantitative phytochemical analysis revealed total phenolic and total flavonoid constituents as 0.822 ± 0.011 GAE/mg and 19.83 ± 1.69 GAE/mg, respectively. Significant dose-dependent DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging ability was demonstrated by the coconut oil. At 125 µg/ml, it inhibited 91.01 ± 1.53 percent of DPPH, while ascorbic acid inhibited 94.18 ± 3.22 percent at the same dose. Against every test organism, the coconut oil exhibited strong antibacterial activity ($p<0.05$). In contrast, the coconut oil's zone of inhibition against the test microorganisms revealed the biggest zone of inhibition—22 mm and 13 mm, respectively when tested against *Staphylococcus aureus* at concentrations of 40 mg/ml and 20 mg/ml. In contrast, the lowest was 2 mm at a 20 mg/ml concentration when it came to *Klebsiella* species. The results of this study showed that coconut oil possesses antibacterial properties that could be used to create novel antimicrobials to treat infections and other illnesses, as well as to fight antibiotic resistance

***Corresponding Author:** Opara, C.N.; oparacn@fuotuoke.edu.ng

Introduction

Coconut (*Cocos nucifera*) is a common fruit in the tropics cultivated in nearly 90 different countries (Pires *et al.*, 2004). Coconut is cultivated for its multipurpose values (nutritional and medicinal). It is a unique source of various natural products for the development of drugs and industrial products (Floriana *et al.*, 2015). According to reports, traditional medicinal herbs and plant parts—leaves, stems, roots, and bark—can effectively treat wounds and fend off major diseases worldwide in addition to offering healthcare services to rural residents (Ezeigbo *et al.*, 2016). Alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds are among the important phytochemicals found in plants that have important antibacterial characteristics (Odinakachukwu, *et al.*, 2019). Research on medicinal plants used as traditional therapies has thus attracted significant attention in the scientific community in an attempt to unearth new solutions to the problems of diverse resistance to the present synthetic and conventional antimicrobials (Taiwo *et al.*, 2011). The diseases that had previously ravaged humanity had been eradicated by antibiotics, but their indiscriminate use has led to the rise of multidrug-resistant microbes (Shanmugan *et al.*, 2008).

The only recognized species in the genus Cocos, which is often referred to as coconut—a phrase used to refer to the complete coconut palm, the seed, or the fruit—is Cocos nucifera, a member of the Arecaceae (palm) family. Local names for it in Nigeria are Kyewe (Tiv), Agbon (Yoruba), Aki beke (Igbo), and Kawakawa (Hausa). It is a huge palm that may reach a height of 30 meters (98 feet) and has pinnate leaves that are 4 to 6 meters (13 to 20 feet) long and 60 to 90 cm long. The old leaves break off cleanly, leaving a smooth husk on the stem (Nevin and Rajamohan, 2004). One of the many amazing qualities of coconut oil is its antibacterial activity. Monolaurin, found in coconut oil, has a long history of being used as an insect repellent. It may be included in breast milk to help protect the developing infant from illness (Clarke and May, 2000). Studies by Clarke and May (2000) and Carpo *et al.* (2007) suggest that coconut milk may also help protect against a range of clinically important bacteria and fungi. The three medium-chain fatty acids in this virgin coconut oil—lauric acid (50–53%), caprylic acid, and capric acid—are said by Abbas *et al.* (2017) to be powerful antibacterial and antifungal agents against lipid-coated bacteria like *Staphylococcus* species and fungi like *Candida spp.*
Locals in several states in the north-central region of Nigeria employ extracts from the shell of Cocos

https://doi.org/10.5555/WDBP4912 ISSN 2488-8648

nucifera for a variety of purposes, including antibacterial, anti-ulcer, and anti-inflammatory effects. The purpose of this research was to identify the phytochemical characteristics of coconut oil and assess its antibacterial efficacy against clinical isolates.

Materials and Methods

Sample Collection: Mature coconuts were purchased at the Otuoke community market in Bayelsa state.

Sample Preparation: Every inch of the workspace was cleaned. The coconuts were cracked open and their shells removed. Using a kitchen knife, the seed meat was extracted from the shell and either sliced into tiny pieces or grated. In a blender, the grated flesh was mashed with warm water. Following the grinding, the coconut milk was poured into a sieve to remove the chaff. The coconut milk bowl was covered and refrigerated at 200C for the entire night. To produce an aqueous extract of coconut oil, caked white coconut oil was extracted from the water and put in a clean, dry stainless-steel saucepan. The oil was then agitated for a while to lower the moisture content. When burnt particles appeared in the oil, the stirring was stopped, and the pot was allowed to cool until it reached a comfortable temperature. After the burned particles were removed from the coconut oil using a chiffon cloth, the oil was collected in a sterile vial for examination. This was kept for later examination at 40C.

Test bacterial isolates: The study employed bacterial strains (*Klebsiella* spp., *Pseudomonas aeruginosa*, *S. aureus* and *E. coli*) that were sub-cultured overnight at 37^0C in selective media, such as Eosine Methylene Blue (EMB) agar for *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, and Mannitol salt agar for *Staphylococcus aureus*. Using the Kigler Iron Agar, Motility, Indole, and Urea, as well as the catalase, citrate, oxidase, and coagulase tests, the organisms were further identified. The results were interpreted following Cheesebrough (2006) and WHO (2003) before determining if the cultures were susceptible to extracts, they were purified on nutrient agar.

Qualitative phytochemical analysis

The overall contents of anthocyanins, tannins, flavonoids, and phenols were ascertained using quantitative analysis. With a few minor adjustments, the total phenolics were calculated using the Folin-Ciocalteau reagent (FCR), following Velioglu *et al*. (1998)'s instructions. Using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins according to Makkar *et al.'s* (1993) description, the amount of tannin in each sample was ascertained. With a few minor adjustments, the approach outlined by Kumaran and Karunakaran (2006) was used to determine the flavonoid content. The creation of a flavonoid-aluminum combination, which absorbs a maximum at 415 nm, served as the foundation for this technique. With a few minor adjustments, the spectrophotometric pH differential procedure published by Giusti and Wrolstad (2001) and Wolfe *et al*. (2003) was used to determine the total anthocyanin contents of the plant extracts.

Antibacterial Susceptibility Test: Pure cultures of isolated bacteria were tested for ethanol extract sensitivity using the well-in-agar diffusion technique. The turbidity criterion of McFarland was modified to 0.5 using sterile normal saline to create a suspension of bacteria (NCCLS, 2010). The agar surface was uniformly sliced into wells using a conventional sterile cork borer with a 6 mm diameter. One milliliter of coconut oil extract at various strengths was added to each well individually. The dish plates were left to stand at room temperature for 45 minutes to give the extract time to properly diffuse. Ciprofloxacin was used to set up the control experiment. After a 24-hour incubation period at 37°C, all of the plates were measured and recorded in millimeters to check for zones of inhibition.

Statistical Analysis: Data was expressed as mean standard deviation. The data obtained were subjected to an Analysis of Variance (ANOVA) test to determine the significant difference at a 95% confidence limit.

Result and Discussion

Table 1 displays the zones of inhibition (mm) for the positive control (ciprofloxacin) and negative control (2.5 % dimethylsulfoxide; DMSO) against test bacteria. Compared to the zones created by the plant extracts, the positive control produced bigger zones of inhibition.

Table 1: shows the zones of inhibition (mm) for the positive control (ciprofloxacin) and negative control (2.5 % dimethyl sulfoxide (DMSO) against test bacteria.

Organism	Concentration (µl)	DMSO	Ciprofloxacin
Klebsiella spp.	30.0	-	14.0
Pseudomonas aeruginosa	30.0	-	19.0
Escherichia coli	30.0	-	17.0
Staphylococcus aureus	30.0	-	25.5

Key: µl = microliter, mm = millimeter

https://doi.org/10.5555/WDBP4912 ISSN 2488-8648

Klebsiella species, *E. coli*, *S. aureus*, and *P. aeruginosa* served as the test organisms in this investigation. When the organisms were tested against 2.5% dimethylsulfoxide, which was utilized as the negative control, they showed no zones of inhibition. At a concentration of 30µl, Ciprofloxacin, which was utilized as the positive control, inhibited *S. aureus* with the greatest zone of inhibition at 25.5mm, while *Klebsiella* showed the least amount of inhibition at 14mm. When tested on bacterial isolates, the coconut oil's antibacterial properties demonstrated efficacy. At 22 mm, *S.aureus* showed the largest zone of inhibition, while *Klebsiella sp.* showed the smallest zone. Lauric acid, which has been shown to have antibacterial, antifungal, and antiviral properties, is found in coconut oil and palm kernel oil (Manisha and Shyamapada, 2011). The zone of inhibition that the coconut oil displayed against the test bacteria is displayed in Table 2. By contrast, when it came to *Staphylococcus aureus*, the biggest zone of inhibition was 22 mm at a dose of 40 mg/ml, while the smallest zone of inhibition measured 2 mm at a concentration of 20 mg/ml for *Klebsiella spp*. All organisms showed resistance to coconut oil at various dilution concentrations as opposed to the study of Ogbolu *et al*. (2007), who reported the antimicrobial potential of coconut oil on fungal organisms. Nasimuddin, *et al* (2016) investigated the antimicrobial activity of coconut water and oil on Gram-positive and Gram-negative bacteria including *S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa*. The results showed that coconut oil had antibacterial properties which is in line with our study

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Table 2: zones of inhibition of coconut oil against test bacteria at 20mg/ml and 40mg/ml

Organism	Concentration (20mg/ml)	Concentration (40mg/ml)
Klebsiella spp.	2.0	5.0
P. aeruginosa	9.0	15.0
E. coli	6.0	13.0
S. aureus	13.0	22.0

The qualitative analysis of the coconut oil extract is displayed in Table 3. Important phytochemical elements were found in the coconut oil extract, according to a qualitative analysis that was conducted. The main phytochemical elements that were present in reasonably high concentrations were flavonoids, tannins, phenols, anthocyanins, terpenoids, and alkaloids; resin and protein were not present. One possible explanation for the phytochemical discovery could be that plants naturally create secondary metabolites to fend off microbial invasions. One significant class of bioactive substances found in coconut oil are phenolic compounds (Fowoyo and Alamu, 2018).

Table 3: The phytochemical constituents in coconut oil

S/N	Phytochemicals	Result
	Flavonoids	++
	Tannins	+
	Phenols	+
	Anthocyanins	+
	Alkaloids	++
	Terpenoids	+
	Carbohydrates	+
	Glycosides	+
	Saponins	+
	Steroids	+
	Resin	-
	Protein	-

+ = Presence of phytochemicals; ++ = Strong presence of Phytochemicals; - = Absence of phytochemical

https://doi.org/10.5555/WDBP4912 ISSN 2488-8648

Table 4 presents the results of a quantitative investigation of the phytochemical elements of coconut oil. One significant class of bioactive substances found in coconut oil was phenolic compounds. The quantitative analysis of phytochemical constituents revealed the phenolic compounds found to be the major class of bioactive components in the coconut water. The amount of total phenolics was 0.822 ± 0.011 mg GAE/mg of aliquot of coconut oil extract and total flavonoid of 9.91 ± 0.72 rutin equivalents /g dry weight extract of coconut oil. Over the years, coconut has been extensively explored for its use in different fields. Results from this study also revealed high phenol and flavonoid contents contribute to the inhibitory effect of the coconut oil, consistent with a study on the phytochemical analysis of *Cocos nucifera* endosperm by Anyiam, and Mounmbegna, (2020), conferring its antioxidant ability to significantly lower cellular oxidative stress. Phenolic compounds derived mostly from plants possess an antioxidant potential to manage oxidative stress-associated diseases like Alzheimer's and other neurodegenerative diseases (Barreira, *at el.*, 2008).

Table 4: Quantitative analysis on phytochemical constituents

Extract	Phenolic contents *			Total anthocyanin †	Total flavanols ‡	Total flavonoids ‡
	Total Phenols	Non-tannins	Tannins			
Coconut oil	0.822 ± 0.011	0.387 ± 0.002	0.423 ± 0.004	4.67 ± 0.14	9.91 ± 0.72	19.83 ± 1.69

The process of scavenging free radicals is depicted in Figure 1. Significant dose-dependent DPPH radical scavenging activity was demonstrated by aliquots of coconut oil extract (Fig. 4.1). At a concentration of 125µg/ml, it inhibited 91.01 ± 1.53% of DPPH, while ascorbic acid inhibited 94.18 ± 3.22% at the same concentration.

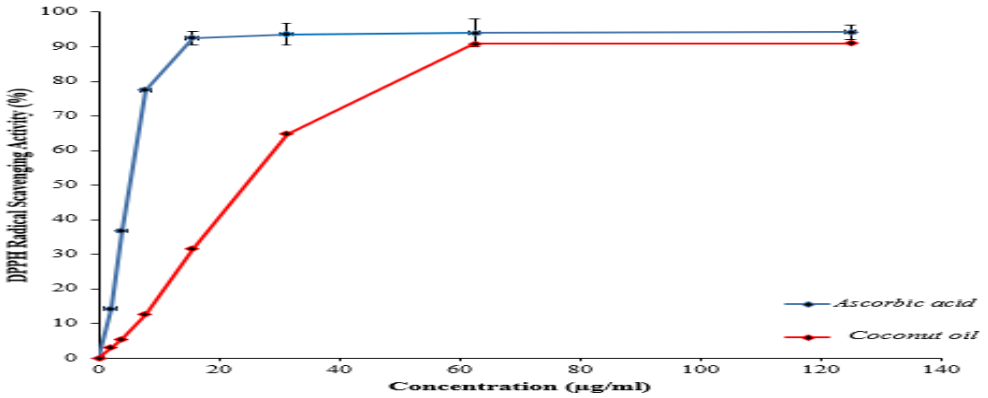

Figure 1: DPPH radical scavenging activity of coconut oil extract. Data represented as mean ± SEM (n = 3)

The effect of coconut oil on DPPH radicals showed significant dose-dependent DPPH radical scavenging capacity. The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-colored ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent (Bandeira, *at el.*, 2006). The scavenging ability and the reducing power of coconut oil could be attributed to the total flavonoid and total phenol content. The increase in the scavenging ability and the reducing power of coconut oil in a dose-dependent manner could related to the total phenolic concentration (Anyiam, and Mounmbegna, 2020).

https://doi.org/10.5555/WDBP4912 ISSN 2488-8648

Table 5: Free radical and lipid peroxidation inhibitory potency (IC50)

	IC50 value for inhibitory potential (µg/ml)			
Sample	DPPH radical	Hydroxyl radical (.OH)	Superoxide anion (O2.-)	Lipid peroxidation
Coconut oil	19.6 ± 1.9	825.7 ± 9.9	285.3 ± 3.4	432.1 ± 7.9
Standard anti-oxidant	4.1 ± 0.3 *	38.9 ± 2.8 #	3.3 ± 0.2 β	24.3 ± 1.4 £

Data represented as mean ± SEM (n = 3).

* Compared to ascorbic acid; # compared to α-Tocopherol; β compared to rutin; £ compared to butylated hydroxytoluene

The IC50 values for lipid peroxidation and free radical inhibition are displayed in Table 5 above. The efficacy of the extract was ascertained by measuring the concentration of coconut oil aliquots that inhibited 50% of the free radicals and lipid peroxidation (IC50). The more potent the extract, the lower the IC50 value. When it came to inhibiting various free radicals, coconut oil outperformed conventional antioxidants. 19.6 ± 1.9 µg/ml was the IC50 value for DPPH radical inhibition. The inhibition of OH radicals was measured at 825.7 ± 9.9µg/ml, that of O2.-anion at 285.3 ± 3.4µg/ml, and that of lipid peroxidation at 432.1 ± 7.9µg/ml.

Conclusion

A plant that is extensively distributed and has significant pharmacological effects while being lowly poisonous is *cocos nucifera*. Additionally, C. nucifera is frequently utilized in the food business for therapeutic purposes. Because it is used to treat waste body parts, this application will also assist in reduce environmental pollution. This study supports the use of Cocos nucifera in the treatment of numerous crippling conditions, including diabetes, cancer, ulcers, obesity, heart disease, and microbial infections. However, the oil found in the plant and its non-nutrient (phytochemical) content, which function as antioxidants against harmful free radicals in the human system, are what provide *Cocos nucifera* its medical and pharmacological uses.

While proteins, carbohydrates, reducing sugar, fats, and oil were found in the endosperm of Cocos nucifera L., the phytochemical analyses of the same material revealed the presence of alkaloids, resins, steroids, terpenoids, and the absence of flavonoids and acidic compounds.

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