Original Research

Volume 3, Issue 2, pp 95-103, December 2021

ACHIEVERS JOURNAL OF SCIENTIFIC RESEARCH

Open Access Publications of Achievers University, Owo

Available Online at *www.achieversjournalofscience.org*

Phytochemical Properties and Antibacterial Analysis of Aqueous and Alcoholic Extracts of Coconut Husk Against Selected Bacteria

T. Temikotan[1], A.O. Daniels, [1] and A.O. Adeoye[1]

1 Department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo state

E-mail: toyosidanny@yahoo.com

Submitted: October 20, 2021 Revised: December 1, 2021 Accepted: December 4, 2021 Published: December 13, 2021

ABSTRACT

Cocos nucifera (L.) (Arecaceae) is commonly called the ‘‘coconut tree’’ and is the most naturally widespread fruit plant on Earth. Throughout history, humans have used medicinal plants therapeutically, and minerals, plants, and animals have traditionally been the main sources of drugs. The constituents of C. nucifera have some biological effects, such as antihelminthic, antiinflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor activities. The objective in the present study was to review the phytochemical profile, and antibacterial analysis of *C. nucifera* husk to guide future preclinical and clinical studies using this plant. This study is aimed at evaluating the antibacterial activity of coconut husk fiber extracted from aqueous ethanol, and ethyl acetate on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Klebsiella pneumoniae. The antimicrobial activity of the coconut husk and the antibiotic susceptibility of the organisms were done on Muiller Hinton agar using agar diffusion method. 10 g of crude extract was reconstituted in 100ml of Dimethyl sulfoxide (DMSO). Comparative antibiotic susceptibility test was carried out on test organisms using commonly used antibiotics discs. The coconut husk extract had the antimicrobial activity against *E. coli* (10mm for ethyl acetate extract and 8mm for ethanol extract) and *K. pneumoniae* (11.2mm for ethanol extract and 5mm for distilled water extract). Antibiotics resistance profile of the tested organisms subjected to standard antibiotics showed that *E. coli* had 80%, *P. aeruginosa* was resistant to 60%, *K. pneumoniae* was resistant to 90% and *S. aureus* was resistant to 70% to the antibiotics used. The phytochemical analyses carried out on the coconut husk extracts showed the presence of Flavonoids, alkaloids, resins, glycosides, saponins and tannins. Terpenoids and steroids were not detected. This study shows that *Cocos nucifera* (coconut) husk has the ability to inhibit the growth of specific bacteria which are *E. coli* and *K. pneumoniae.*

Keywords: Phytochemical, Antibacterial, Coconut husk, Aqueous extract, Alcoholic extract, Agar diffusion method, Antibiotic sensitivity disc.

1.0 Introduction

Coconut (*Cocos nucifera* linn) belongs to the family Arecaceae (Palmae) and it has a wide family which consists of 217 genera and 2,500 species. Cocos nucifera belongs to the order Arecales and it is the sole species of the genus cocos which belongs to the subfamily cocoideae, which includes 27 genera and 600 species (Evans, 2012). Coconut is planted for different purpose (nutritional and medicinal) and that is the reason why it's called the fruit of life (Khan *et al.,* 2013; Foale, 2013). *C. nucifera* produced different products which include coconut water, coconut husk, copra, coconut oil, raw kernel, coconut cake and coconut milk. It is a unique source of different natural products in the development of drugs and industrial products that is effective against fungi, bacteria, viruses, parasites and dermatophytes (Floriana *et al*., 2015). It is also an antioxidant.

Phytochemicals act in numerous ways to assist the human body in combating disease and health problems. In recent times quite a number of some plant parts such as palms, leaves, stems and roots have been used due to the presence of phytonutrients in them (Hasler, 2018). Plants have been reported to be excellent sources of secondary metabolites such as tannins, flavonoids, alkaloids which can be used in the production of modern medicines to fight against microbial attacks (Chandra *et al.,* 2017).

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Therefore, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years, especially due to the constant emergence of microorganism's resistance to conventional antimicrobials. Apparently, bacterial species possess the genetic ability to acquire and transmit resistance against currently available antibacterial drugs and thus there are frequent reports on the isolation of bacteria that are known to be sensitive to routinely used drugs and became multi-resistant to other medications available in the market (Nascimento *et al*., 2020; Sakagami and Kajimura, 2017). Consequently, common strategies adopted by pharmaceutical companies to supply the market with new antimicrobial drugs include changing the molecular structure of the existing medicines in order to make them more efficient or recover the activity lost due to bacterial resistance mechanisms (Chartone-Souza, 2018).

The aim of the research is to analyze the phytochemical properties and antibacterial activities of aqueous and alcoholic extracts of coconut husk fiber against specific organisms

Figure 1**:** Coconut husk (*Cocos nucifera* linn)

2.0 Materials and Methods

2.1. Collection of coconut husk:

Coconut husk were collected in a sterile container from coconut seller in Owo market, Ondo State, Nigeria and transported to the Microbiology laboratory, Achievers University Owo, Ondo State for further analysis.

2.2 Collection of test organisms:

The clinical strains of *Escherichia coli, Staphylococcus aureus Pseudomonas aeruginosa,* and *Klebsiella pneumoniae* were collected on slants from Federal University Technology, Akure (FUTA) and then transported to Microbiology laboratory for isolation by using selective media.

2.3 Preparation of coconut husk sample:

The coconut husk was oven-dried at 50^{o}C. The dried husk fiber was then pulverized to powder using electric blender (Master Chef Blender) and put inside a sterile container.

2.4. Extraction procedure:

An 80g portion of the plant powder (dried coconut husk fiber) was soaked in 600 ml of sterile distilled water (For the preparation of aqueous extract (7.5ml/g) in a conical flask for 48 hours. Same procedure was employed for the ethanol and ethyl acetate extracts. The solution was filtered using Whatman no 1 filter paper. The filtrate was transferred into the evaporating dish. The gel-like extract of each extracts was reconstituted with 5% DMSO and kept in the refrigerator for further analysis. The 10g of the crude extracts were reconstituted in 10 mls of 5% DMSO.

2.5 Phytochemical screening of extracted coconut husk:

These phytochemical tests were done to detect the presence of secondary metabolites, such as alkaloids, tannins, saponins, resins, flavonoids, steroid, glycosides and terpenoids using standard known methods as described by Obidoa *et al*., (2017).

2.6. Antibacterial analysis of the extracts:

The agar diffusion method of Perez, (2006) was adopted in this test using Mueller Hinton agar. 1 ml of nutrient broth containing test organisms is inoculated into a Petri dish and Mueller Hinton agar (when cooled) was poured into the Petri dish by using pour-plate method. Holes were bored on the gelled agar by using 5 mm cork-borer and the different extracts were introduced into holes. The Petri dishes were incubated for 24 hours at 280C after which the zone of inhibition were observed, measured and recorded.

2.7. Comparative antibiotic susceptibility test:

Comparative antibiotic susceptibility test was carried out on the test organisms using the disc diffusion procedure of Kirby-Bauer (1996). Commonly used antibiotics discs used include; Gentamycin (30µg), Pefloxacin (30µg), Tarivid (10µg), Streptomycin (30µg), Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (30µg), Amoxacillin (30µg), Augmentin (10µg), Erythromycin (10µg), Ampiclox (30µg), Zinnacef (20µg), Rocephin (25µg). The values obtained were standardized using the guidelines of the CSCL (NCCLS modified disc diffusion technique 2008)

3.0 Results and Discussion

3.1 Antibacterial Screening of the extract:

Results obtained for the antibacterial tests performed on aqueous, ethanol and ethyl acetate extracts of *C. nucifera* (Coconut) husk are presented in the Table 1. The results showed that the ethanol extract of the plant showed a narrow spectrum of activity, being active only to Gram-negative organisms *(E. coli* and *K. pneumoniae*) with zones of inhibition of 8 mm and 11.2.mm respectively. *E.coli* was sensitive to ethanol and ethyl acetate extract but resistant to the aqueous extract. Only *K. pneumoniea* was sensitive to the aqueous extract. *Pseudomonas* was resistant to all the extracts used.

3.2 Comparative antibiotic screening of organisms

Antibiotic sensitive disc was also observed against the test organisms; E. coli was resistant to 80%, P. aeruginosa was resistant to 60%, K. pneumoniae was resistant to 90% and S. aureus was resistant to 70%. The result of this test as presented in table 2. Following the recommendation of the CSCI (2008), *S. aureus* had intermediate susceptibility to gentamycin, K. pneumoniae, *E. coli* and *S. aureus* had intermediates susceptibility to Pefloxacin, *P. aeruginosa* had intermediates susceptibility to Septrin and Sparfloxacin, while *K. pneumoniae* also had intermediate susceptibility to Ciprofloxacin and *S. aureus* had intermediate susceptibility to Erythromycin.

3.3 Phytochemical Screening of coconut husk:

The results of the phytochemical analyses carried out on *Cocos nucifera* husk revealed the presence and the absence of some secondary plant metabolites. From the result it was detected that 75% of the phytochemical test were present in husk fiber, Phytochemical analysis showed the plant to contain Alkaloids, flavonoids, Glycosides, Saponins, Tannins and Resins whereas Steroids, Terpenoids were absent (Table 3).

Table 1: Antibacterial screening of coconut husk extract on tested organisms

Test Organisms	Ethanol extract	Ethyl-acetate extract	Distilled water extract	(5% DMSO)
S. aureus	-	-	-	-
K. pneumoniae	11.2mm	-	5mm	-
E. coli	8mm	10mm	-	-
P. aeruginosa	-	-	-	-

Table 2: Antibiotic Sensitivity test of certain organisms to standard antibiotic.

A/B Zones of inhibition in mm	*K. pneumonia*		*E. coli*		*P. aeruginosa*		*S. aureus*	
Gentamycin	R	6	R	2	R	2	I	10
Pefloxacin	I	10	I	10	R	2	I	12
Tarivid	R	6	R	8	R	-	R	-
Streptomycin	R	4	R	-	R	-	R	8
Septrin	R	1	R	2	I	12	R	6
Chloranphenicol	R	4	R	4	R	10	R	-
Sparfloxacin	R	4	R	8	I	12	R	-
Ciprofloxacin	I	10	R	8	R	2	R	8
Amoxacillin	R	4	R	4	R	-	R	-
Augmentin	R	-	R	-	R	-	R	-
Erythromycin	R	-	R	-	R	-	I	10
Ampiclox	R	-	R	-	R	-	R	-
Zinnacef	R	-	R	-	R	-	R	-
Rocephin	R	-	R	-	R	-	R	6

Legend;

R – Resistant

I—Intermediate

Table 3: Qualitative phytochemical screening of the husk fiber

Phytochemical test	Observation	Inference	Intensity
1. Alkaloid			
(a) Dragendorff's Reagent	Red precipitate	Alkaloids present	High
(b) Mayer's Reagent	Creamy-white colored precipitate	Alkaloids present	High
2. Flavonoid			
(a) Ammonium test	Yellow coloration	Flavonoids present	Moderate
3. Glycosides	Dense red precipitate	Glycosides present	Low
4. Steroids			
(a) Conc. H_2SO_4 test	No reddish-brown interface	Steroids absent	---
5. Terpenoids			
(a) Conc. H_2SO_4 test	No grey colour	Terpenoids absent	---
6. Saponins			
(a) Emulsion test	Emulsion formed	Saponins present	Low
7. Tannins			
(a) Lead Sub-acetate test	Cream gelatinous precipitate	Tannins present	High
8. Resins			
(a) Precipitate Test	White precipitate	Resins present	Moderate
(b) Color Test	Pink color	Resins present	Moderate

3.4 Discussion

Essential oils and extracts have been used for thousands of years by mankind as natural therapies. Plant extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens (Hammer *et al*., 1999).

The antimicrobial test result showed the efficacy of the solvent extract of the coconut husk. The aqueous extract did not show any appreciable activity against the test organisms. Several authors have established the fact that solvent extracts are usually more effective that the aqueous extract, However, Cyriac *et al.* (2013), recorded activity at 100-200mg/ml of the extract. Jose *et al*. (2014) observed appreciable antimicrobial effect of the

ethanol extract which correlates with the result obtained in this work. Suggestion have been made that coconut husk extract or tea extracted from coconut husk can be used to prevent the growth of these aetiological agents (*E. coli* and *K. pneumoniae*) that might cause diseases in human.

Studies have also shown that the antimicrobial activity of husk extract increased with increasing concentration and was found to be more effective against Gram-negative than Gram-positive organism, Rusdi *et al*. (2019) reported a broad spectrum activity against selected pathogen. S. aureus was resistant to all the solvent extracts. S. aureus is notorious for quick response to antimicrobials with the development of resistance mechanisms. Resistance to penicillins, methicillin, linezolid and daptomycin have been recorded (Pantosi *et al.*, 2007).

The high level of resistance against standard antibiotic is an indication of the danger of resistance of bacteria to antimicrobials. Who (2020) has declared that AMR is one of the top 10 global public health threats facing humanity. It was also reported that the rate of resistance to ciprofloxacin, an antibiotic commonly used to treat urinary tract infections, varied from 8.4% to 92.9% for *Escherichia coli* and from 4.1% to 79.4% for *Klebsiella pneumoniae* in countries reporting to the Global Antimicrobial Resistance and Use Surveillance System (GLASS) (WHO, 2020).

Phytochemical analysis showed the plant to contain Alkaloids, flavonoids, Glycosides, Saponins, Tannins and Resins whereas Steroids, Terpenoids were absent. The presence of these phytochemicals are partly responsible for the biological action recorded for this plant. Phytochemicals are non-nutrient chemical compounds produced by cells from plants to protect the plant from environmental attack. These phytochemicals have been reported to have biological activity against pathogens (Mazaya *et al.,* 2020). Some authors have reported the presence of presence of phenols, tannins, leucoanthocyanidins, flavonoids, triterpenes, steroids, and alkaloids in coconut husk (Costa *et al.*, 2009) which agrees with some of the phytochemicals identified in this work. Alkaloids have been reported to have broad spectrum biological activity such as antiviral, antibacterial, anti-inflammatory, and anticancer properties (Adamski *et al*., 2020). Flavonoids act as free radical acceptors and potent antioxidants (Rice-Evans *et al.,* 1995) and also possess wound healing properties (Cowan, 1999). Generally, the presence of phytochemicals in the food chains has significant role which is to work with nutrients and dietary fibre to protect against disease (Gordon *et al.*, 1995).

Diverse diseases have been caused by these aetiological agents (*E. coli* and *K. pneumoniae*) such as diarrhea, pneumonia, urinary tract infection and food borne illness, which has increased the morbidity rate of human's population. Most of this pathogen have exhibited multi-drug resistance.

This study revealed the used of coconut husk in traditional folk medicine and to compare their antimicrobial potency with the commercial antibiotics.

4 Conclusion

Cocos nucifera has a significant inhibitory action against common pathogens, indicating the presence of highly effective antimicrobial compounds. The observed antimicrobial profile of *Cocos nucifera* husk fiber extracts against the test bacteria supports the use of the plant in folk medicine.

This study recommends that coconut husk extracts should be used in the treatment of human diseases.

References

Akinyemi K. O., Oluwa O. K., Omomigbehin E. O. (2016). Antimicrobial activity of crude extracts of three medicinal plant used in South west Nigeria folk medicine on some food bacterial pathogens. AfricanJournal of Traditional Compounds and Alternative Medicine. 3(4): 13 – 22.

Annalisa P., Andrea S., Monica M. (2007). Mechanisms of antibiotic resistance in

Staphylococcus aureus. Future Microbiology. 2(3): 323 – 334.

Ashraf A. M., Abdulaziz A. A., Khalid S. A., Turki M. D., Essain N. S., Marwah M. B. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences. 25(2): 361 – 368.

Chandra H., Bishnol P., Yodav A., Mishra A. P., Nautiyal A. K. (2017). Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials a review Plant. Critical Reviews in Plant Sciences. 6(2): 16.

Chartone-Souza E. (2018). Bactérias ultra-resistentes: uma guerra quase perdida. Ciencia Hoje. 23(138): 127 – 135.

Costa C. T., Bevilaqua C. M., Morais S. M., Camurca-Vasconcelos A. L., Maciel M. V., Braga R. R. (2010). Anthelmintic activity of Cocos nucifera L. on intestinal nematodes of mice. Research in Veterinary Science. 88: 101 – 103.

Cowan M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews. 12(4): 564–582.

Cyriac M. B., Pai V., Shantaram M., Jose M. (2013). Antimicrobial properties of coconut husk aqueous extract on cariogenic bacteria. Archives of Medicine and Health Sciences. 1: 126 – 30.

Evans W. C. (2012). Elsevier Science limited, New York. Trease and Evans Pharmacognosy. 15: 156 – 200.

Floriana S. L., Jansen S., Duri S. (2015). Antibacterial activity of enzymatic hydrolysed virgin coconut oil and palm kernel oil against Staphylococcus aureus, Salmonella typhi and Escherichia coli. International Journal of Pharmaceutical Technology and Research. 6(2): 628-633.

Foale M. (2013). The Coconut Odyssey: The Bounteous possibilities of the tree of life. Australian Centre for International Agricultural Research (ACIAR). 101: 132.

Gebila M., Karseno F., and Tri Y. (2020). Antimicrobial and Phytochemical Activity of Coconut Shell Extracts. Turkish Journal of Agriculture - Food Science and Technology. 8(5): 1090 – 1097.

Gordon D. T., Stoops D., and Ratliff V. (1995). Dietary fiber and mineral nutrition, in Dietary Fiber in Health & Disease. Kritchevsky D. and Bonfield C. (Eds.) Eagan Press, Eagan, Minn, USA.

Hammer K. A., Carson C. F., Riley T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 86: 985 – 990.

Hasler C. M. (2018). Functional Foods: their role in disease prevention and health promotion. Food Technology. 52: 63 – 70.

Khan M., Nasiruddin M. U. and Khurram K. W. (2013). A study of chemical composition of Cocos nucifera L. (Coconut) water and its usefulness as rehyderation fluid. Pakistan Journal of Botany. 35(5): 925 – 930.

Maji J., Maria B. C., Vidya P., Ipe V., Manjula S. (2014). Antimicrobial properties of Cocos nucifera (coconut) husk: An extrapolation to oral health. Journal of Natural Science, Biology and Medicine. 5(2): 359 – 364 doi: 10.4103/0976-9668.136184

Mazaya G., Karseno M., and Yanto T. (2020). Antimicrobial and phytochemical activity of coconut shell extracts. Turkish Journal of Agriculture - Food Science and Technology. 8(5): 1090 – 1097.

Mohammad A. (2018). Determination of MIC by Broth Dilution Method. Head of Microbiology & Immunology Department, Faculty of Pharmacy, Mansoura University Press. Pp 739 – 785.

Nascimento G., Locatelli P., Freitas C., and Silva G. (2020). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic resistant Bacteria. Brazilian Journal of Microbiology. 31: 247 – 256.

Obidoa O., Joshua P. E., and Eze N. J. (2017). Phytochemical Analyses of Cocos Nucifera L. Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. Research Article Archives of Pharmaceutical Sciences & Research.1: 87 – 96.

Rice-evans C. A., Miller N. J., Bolwell P. G., Bramley P. M., Pridham J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research. 22: 375 – 383.

Rusdi R., Asriani H., Rosmiaty A. (2019). In vitro Evaluation of Coconut Husk Potential as Phytobiotics for Poultry. International Journal of Poultry Science. 18: 109 – 115.

Sakagami Y., and Kajimura K. (2017). Bactericidal activities of disinfectants against vancomycin-resistant enterococci. Journal of Hospital Infection. 50(2):140 – 204.

WHO (2020). Antimicrobial resistance. Fact sheet. On line article. Assessed July, 2021.

Zbigniew A., Linda L. B., Luigi M., and Sabino A. B. (2020). Biological Activities of Alkaloids: From Toxicology to Pharmacology. Toxins 12: 210