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PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF COCONUT WATER (Cocos nucifera L.) AGAINST BACTERIA ISOLATES FROM STUDENTS URINE SAMPLES IN A UNIVERSITY COMMUITY

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Abstract

Coconut is a special source of diverse natural compounds that are used in the creation of different pharmaceuticals and commercial goods that are often effective against dermatophytes, bacteria, viruses and fungi. Coconut water is high in vitamins, minerals, amino acids, carbohydrate, enzymes and phytochemicals. Research has suggested the use of coconut water in the creation of innovative antimicrobial medicines by highlighting its potential as a natural antibacterial agent against bacteria that are resistant to drugs. The aim of this study was to determine the antimicrobial and phytochemical properties of coconut water against bacterial isolates from students urine samples at Chukwuemeka Odumegwu Ojukwu University, Anambra state. A total of 56 urine samples were collected from students while matured Cocos nucifera (L.) samples were purchased from a local market in Anambra state. The test organisms were isolated and identified following standard laboratory procedure. Biochemical test such as indole, citrate, catalase and oxidase were performed. The phytochemical property of the coconut water was determined by testing for tannins, saponins, flavonoids, amino acid, glycosides, terpenes and steroids using the required reagents. The antimicrobial property of the coconut water was determined using agar well diffusion technique and result compared with other antibiotics disc.

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Twenty-six (26) test isolates were obtained and they were 17 (65%) Escherichia coli and 9 (35%) Pseudomonas aeruginosa. The phytochemical result showed that saponins, flavonoids, steroids and glycosides were detected in the coconut water sample. The coconut water exerted no antibacterial effect on all of the test isolates. The phytochemical analysis of the coconut water revealed the presence of saponins, flavonoids, steroids and glycosides. The coconut water sample showed no antimicrobial activity against Pseudomonas aeruginosa and Escherichia coli. Therefore coconut water cannot serve as a potential antimicrobial agent.

Keywords: *bacteria, coconut water, urine, hostel, phytochemicals*

INTRODUCTION

Medicinal plants have been used for thousands of years as traditional treatments for numerous human diseases in different parts of the world. Natural products gotten from medicinal plants have shown to be a rich source of biologically active compounds, which makes them effective source for alternative medicines (Jose *et al.*, 2014). Products derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phyto-medicine (Hassan *et al.*, 2018). Phytochemicals are natural bioactive compounds found in plants and they include vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines and betalains (Hassan *et al.*, 2018).

The coconut tree, *Cocos nucifera* (*L.*) is a member of the family Arecaceae of which they belong to the cocoideae subfamily (Lopez, 2023). It is native to the coastal areas of Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. From these regions, the coconut palm is believed to have been brought to India, then to East Africa, West Africa and from here, dispersed to the American continent (Lima *et al.*, 2015). It is a unique source of different natural products that can be used in the development of drugs and industrial products that is very effective against fungi, bacteria, viruses, parasites and dermatophytes (Kiran *et al.*, 2024). The main target food products derived from coconut trees are coconut milk, coconut oil and coconut water (Atiq *et al.*, 2022).

Coconut water is sometimes called the "fluid of life" due to its medicinal benefits such as oral rehydration, treatment of childhood diarrhea, gastroenteritis and cholera (Lopez, 2023). In its purest form, coconut water is a delightful and nutrient-dense drink. It has long been utilized due to its positive benefits on human health, which include anti-hyperlipidemic, antiulcerogenic, antibacterial and cardio protective properties (Anurag and Raamohan, 2014). According to Bandalam and Galvez (2016), coconut water is high in vitamins, minerals, amino acids, enzymes, hormones and phytochemicals. Study has showed that coconut water has antimicrobial properties against a variety of harmful bacteria, such as *Escherichia coli*, *Staphylococcus aureus* (Silva *et al.*, 2016) and *Candida albicans* (Gopal *et al.*, 2017).

A urinary tract infection (UTI) is an inflammatory disorder of the urinary tract that is caused by the existence and growth of microorganisms throughout the urinary tract. This is due to bacteria moving from the gastrointestinal region to the urethra, multiplying (Al-Hilu & Al-Shujairi, 2024). Several factors affect the prevalence of UTIs such as gender, age, urological instruments and immunosuppression (Rahman *et al.*, 2019). Sexual activity, certain types of birth control, menopause, urinary tract abnormalities, blockages in the urinary tract and a recent urinary procedure are other risk factors for UTIs. It is one of the most prevalent bacterial infections in women and elderly individuals (Fazly Bazzaz *et al.*, 2021). Poor personal hygiene and urinary tract abnormalities have been attributed as some factors that predispose one to urinary tract infection (Ekwealor *et al.*, 2016). Gram-negative bacteria, particularly *Escherichia coli*, are primarily responsible for spreading UTIs (Al-Hilu & Al-Shujairi, 2024). Worldwide, the prevalence of antimicrobial resistance in urinary pathogens is on the rise (Ekwealor *et al.*, 2016). The development of antibiotics resistance results from the indiscriminate and widespread use of antibiotics (Said *et al.*, 2021). Due to this resistance, the use of plant as an alternative is being studied globally, especially in developing countries like Nigeria, since plants are considered nutritionally safe, biodegradable and possess phytochemicals with antimicrobial property (Anurag and Raamohan, 2014). The aim of this study is to provide information about the antimicrobial and phytochemical properties of coconut water (*Cocos nucifera*) against bacterial isolated from urine samples obtained from students in Chukwuemeka Odumegwu Ojukwu University, Anambra state.

MATERIALS AND METHODS

METHODS

Ethical clearance

Ethical approval for the study was obtained from the ethics committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka, Anambra state. Verbal consent was obtained from the students prior to specimen collection. Confidentiality of the participants was maintained throughout the study.

Study design

This study was conducted in the microbiology laboratory of the department of Pharmaceutical Microbiology and Biotechnology, Chukwuemeka Odumegwu Ojukwu University Igbariam, Anambra State.

Area of study

The study was carried out in Igbariam, Anambra East L.G.A., Anambra state in southeast Nigeria. The coordinates of Igbariam town are 6°24\ 0"N and 6°56\ 0"E.

Collection of coconut samples

Matured coconuts (*Cocos nucifera*) samples were purchased from a local market in a rural community in Igbariam, Anambra East Local Government Area of Anambra State, Nigeria. They were identified in the Department of Pharmacognosy and Traditional Medicine, Chukwuemeka Odumegwu Ojukwu University Igbariam.

Preparation of the coconut water

The coconut husk was fractured, which allowed for the extraction of fresh coconut water samples. Afterwards the samples were filtered to remove any solid particles and sterilized by filtering through sterile filter. It was later then transferred into a container that had been sterilized.

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Collection of urine specimen

A total of 56 urine samples were randomly obtained from students in Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus between 11th June and 30th June 2024. The urine samples were collected using a specimen bottle and were cultured immediately after collection.

Culture media preparation

The different media used in the study are: nutrient agar, nutrient broth, MacConkey agar, Cetrimide agar and Mueller Hinton Agar. Each medium was prepared following the manufacturer's specification.

Isolation and identification of bacteria

The urine samples were inoculated using a sterilized loop by streak plating method onto the surface of labeled Petri dishes that contained nutrient agar that had already been sterilized and then incubating the petri dishes for 24 h at 37°C.

Purification of bacterial isolates

At the end of the incubation period, those nutrient agar Petri dishes showing growth were picked and discrete bacterial colonies were further purified. The streak plating method was used to further purify discrete bacterial colonies. This was done by aseptically using a sterile inoculating wire loop to sub-culture on properly labeled Cetrimide agar and MacConkey agar in Petri dish. The Petri dishes were incubated for 24 h at 37°C.

Biochemical identification of test organisms

The biochemical test was performed following the method described by Poudel *et al.,* (2019) and is shown below.

Indole Test

This experiment was performed in order to confirm the presence of probable *Escherichia coli* on MacConkey agar. It assesses the bacterial isolate's capacity to hydrolyze tryptophan to indole that has accumulated in the medium. In 10 ml test tubes, 5 ml of sterile peptone water was added to each bacterial isolate, and the tubes were then incubated for 24 hrs at 35°C. Using a sterile Pasteur pipette, 0.5ml of Kovac's reagent was then added to the peptone broth. A vivid pink to red color at the top of the reagent layer indicates a positive result, whereas no color change indicates a negative result. This reaction occurs between aldehyde and indole in the test reagent.

Citrate Test

This test is carried out in the confirmation of *Escherichia coli*. Simmons citrate agar is a specialized medium that contains citrate as the sole carbon source. Using a sterile inoculating loop, a small amount of the bacterial isolate was transferred into the citrate medium, the inoculated medium was placed in an incubator at 37°C for 24-48 hrs. After incubation, the medium was observed for color change, in which blue color signifies negative.

Oxidase Test

To confirm that *Pseudomonas aeruginosa* were present, this test was conducted. It determines whether an organism is capable of producing cytochrome oxidase. Using a Pasteur pipette, 2-3 drops of oxidase reagent (1% tetra methyl-p-phenylenediamine dihydrochloride) were applied to a tiny region of filter paper. The test isolate colony was then selected using a flamed inoculating loop, and it was rubbed over the moistened area to create a smear. The colony was fresh, having grown for 18 to 24 hrs. Within 10 to 30 seconds of the inoculation, a deep blue or purple hue shift indicates a positive result.

Catalase Test

The catalase test is a biochemical procedure that tests for the catalase enzyme in bacteria. It's a test used to confirm the presence of *Pseudomonas aeruginosa*. Hydrogen peroxide (H_2O_2) is converted to water (H_2O) and oxygen (O_2) in the presence of catalase. In order to conduct this test, a colony of the test isolate was selected using a sterile inoculating loop and deposited on one side of the clean, grease-free slide. Using a Pasteur pipette, a drop of 3% hydrogen peroxide was placed to the slide. When air bubbles form without mixing, the catalase test results are positive.

Phytochemical Screening

Phytochemical tests for the screening and identification of bioactive chemical constituents' in the medicinal plant under study were carried out on the coconut water using the standard procedures described by (Olasehinde *et al.,* 2018).

Tannins

The coconut water sample was boiled in a test tube, 0.1% $FeCl_3$, was added to the coconut water sample and observed for brownish green or a bluish black coloration which shows the presence of tannins.

Saponins

In a test tube, 1-2 ml of lead acetate solution (10%) was combined with 1-2 ml of coconut water. A white precipitate or cloudiness was seen, signifying the presence of saponins. Absence of precipitation or cloudiness, which suggests saponins are not present.

Flavonoid

A few drop of 1% ammonia solution was added to the sample in a test tube. A yellow coloration is observed if flavonoids compound is present.

Amino acid

A 1 ml of coconut water sample was mixed with 1 ml of ninhydrin reagent (0.2% in ethanol), the mixture was heated in a boiling water bath for 5-10 min. The color change was observed, purple or blue color indicates the presence of amino acids and no color change indicates the absence of amino acids.

Glycosides

A test was done to detect the presence of glycosides. The extract was combined with 2 ml of glacial acetic acid solution containing 2 drops of 2% $FeCl_3$. The solution was transferred into a separate tube containing 2ml of pure sulfuric acid. The presence of glycosides may be indicated by the observation of a brown ring during the interface.

Terpenes (Liebermann-Burchard reaction)

To 2 ml of the sample, about 2ml of acetic anhydride and few drops of concentrated sulphuric acid were added in the test tube. Pink- purple ring indicates the presence of terpenes.

Steroids

About 2ml of acetic anhydride were added to 3ml of the sample with the addition of 2ml H_2SO_4. A color change from violet to blue or green indicated the presence of steroids.

Antimicrobial activity of the coconut water samples

The purpose of this test was to ascertain the isolated bacteria's resistance and susceptibility profile to both coconut water extract and commercial antibiotics. The antimicrobial screening was carried out using the agar diffusion method described Jennifer & Perpetua, (2023) with slight modifications. Each of the previously

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isolated and identified organisms was inoculated into freshly prepared sterile nutrient broth in test tubes. The test tubes were then incubated till the growth in the broth was equivalent to the Mac Farland standard (0.5). Using a sterile cotton swab, the inoculums were then swabbed on the surface of sterile Muller Hinton agar in Petri dish labeled properly. Five wells were bored in each of the medium with the help of a sterile cork-borer and labeled properly. The first well was then filled with 0.3ml of the coconut water sample while standard drugs like gentamicin, levofloxacin and ofloxacin were placed in another three different wells and used as positive control. Sterile water was used added in the fifth well and used as negative control. The Petri dish lid was closed to allow for extract diffusion throughout the medium. The Petri dishes were then incubated for 24 h at 37°C. After incubation, the Petri dishes were brought out and carefully examined before using a millimeter rule to measure the diameter of the zone of inhibition (clearance). The result was recorded accordingly.

RESULTS

Morphological characteristics of the cultured organisms

A total of fifty-six (56) samples were collected from students living at Dvora lodge located in Chukwuemeka Odumegwu Ojukwu University Igbariam Campus. A total of twenty-six (26) isolates were obtained, seventeen (17) were *Escherichia coli* and nine (9) were *Pseudomonas aeruginosa*. The detailed results are presented in table 1 and 2.

Table 1: Morphology and biochemical test result of organism on MacConkey agar

No of isolates	Colony features	IND	CIT	OXI	Probable organism
17	Pink, smooth, convex, opaque, shiny	+	-	-	*Esherichia coli*

KEY: + Positive; - Negative, IND- Indole, CIT- Citrate, OXI- Oxidase

Table 2: Morphology and biochemical test result of organism on Cetrimide agar

No of isolates	Colony features	IND	CIT	OXI	Probable organism
9	Green, smooth, opaque, mucoid, convex	+	+	+	*Pseudomonas aeruginosa*

KEY: + =Positive, - = Negative, CAT= Catalase, OXI = Oxidase, IND = Indole

The presence and absence of various secondary plant metabolites were identified by the phytochemical analysis performed on coconut (*Cocos nucifera*) water. Phytochemical analysis of the coconut water sample showed that saponins, flavonoids and steroids were highly present. Glycosides were detected in minute quantity while secondary metabolites like terpenes, tannins and amino acid was not present. The results obtained are presented in table 3.

Table 3: Phytochemical screening of coconut water

S/N	Phytochemicals	Result
1	Saponins	+ +
2	Flavonoids	+ +
3	Terpenes	-
4	Steroids	+ +
5	Tannins	-
6	Glycosides	+
7	Amino acid	-

Keys: ++: Highly present, +: Slightly present, -: Absent.

Antimicrobial susceptibility testing

The coconut water was tested against the identified isolated bacterial strains using sterile water as the negative control and gentamicin, ofloxacin, and levofloxacin as positive controls. The chosen bacterial isolates were evaluated using freshly made coconut water. The following results were obtained:

Table 4: Antimicrobial activity of the coconut water on *Pseudomonas aeruginosa* isolates

			Positive Control(mm)			Negative Control (mm)
S \ N	**Organism**	**CWS**	**GN**	**LBC**	**OFX**	**STW**
1	*Pseudomonas aeruginosa*	0	0	21	18	0
2	*Pseudomonas aeruginosa*	0	15	23	24	0
3	*Pseudomonas aeruginosa*	0	0	23	35	0
4	*Pseudomonas aeruginosa*	0	17	28	27	0
5	*Pseudomonas aeruginosa*	0	0	23	35	0
6	*Pseudomonas aeruginosa*	0	9	20	21	0
7	*Pseudomonas aeruginosa*	0	17	38	35	0
8	*Pseudomonas aeruginosa*	0	15	28	23	0
9	*Pseudomonas aeruginosa*	0	10	17	15	0

KEY: CWS = Coconut water sample, + CTL = Positive Control, - CTL = Negative Control, MM= Millimeter, STW= Sterile water, GN= Gentamicin, LBC= Levofloxacin, OFX= Ofloxacin.

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Table 5: Antimicrobial activity of the coconut water on *Escherichia coli* isolates

			Positive Control(mm)			Negative Control (mm)
S\N	**Organism**	**CWS**	**GN**	**LBC**	**OFX**	**STW**
1	*Escherichia coli*	0	20	26	25	0
2	*Escherichia coli*	0	0	20	17	0
3	*Escherichia coli*	0	13	20	18	0
4	*Escherichia coli*	0	0	21	20	0
5	*Escherichia coli*	0	18	27	25	0
6	*Escherichia coli*	0	0	21	20	0
7	*Escherichia coli*	0	0	22	24	0
8	*Escherichia coli*	0	19	30	28	0
9	*Escherichia coli*	0	0	13	15	0
10	*Escherichia coli*	0	0	15	10	0
11	*Escherichia coli*	0	23	29	23	0
12	*Escherichia coli*	0	0	17	0	0
13	*Escherichia coli*	0	0	25	23	0
14	*Escherichia coli*	0	0	0	18	0
15	*Escherichia coli*	0	13	28	31	0
16	*Escherichia coli*	0	0	22	19	0
17	*Escherichia coli*	0	0	20	17	0

KEY: CWS = Coconut water sample, + CTL = Positive Control, - CTL = Negative Control, MM= Millimeter, STW= Sterile water, GN= Gentamicin, LBC= Levofloxacin, OFX= Ofloxacin.

DISCUSSION

A total of twenty-six (26) isolates were obtained out of fifty-six (56) samples which were collected from students in the campus. The results of the urine culture showed that 26 (46.4%) of urine samples were found to be positive. In a study by Mahdi *et al.*, (2020), 318 (31.8%) of urine samples were found positive for microbial growth. In a similar research by Akter *et al.*, (2016), 48.67% of samples used were found to be positive for bacterial infection, which agrees with this study. This contradicts a study done by Priyadharsini *et al.*, (2014) where 48 (77.4%) samples were showed to be urine culture positive.

From the result presented in table 1 and table 2, about 17 (65%) of the identified bacterial isolates were *Escherichia coli* and 9 (35%) were *Pseudomonas aeruginosa*. The uro-pathogens identified in this study are similar to those of many other studies conducted in different countries around the world. The microbes isolated from a study included *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus sp.*, *Staphylococcus epidermidis* and *Candida albicans* (Priyadharsini *et al.*, 2014). Similar uro-pathogen were isolated in a study by Akter *et al.*, (2016) as the following organisms were isolated *E. coli* (50.68%), *Pseudomonas sp.* (17.81%), *Streptococcus sp.* (13.70%), *Staphylococcus aureus* (10.96%), *Klebsiella sp.* (4.11%) and *Proteus sp.* (2.74%). The study done Mahdi *et al.*, (2020) demonstrated that E. coli (40.5%) was the most frequent uro-pathogen isolated. The results show that *E.coli* were predominant isolates isolated from the urine specimen followed by *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *P. mirabilis* and *E. faecalis* (Madeeha & Malik, 2021). These studies are in agreement with this current study as *Escherichia coli* was the most prevalent organism isolated and *Pseudomonas aeruginosa* was also isolated. Urinary tract infection (UTI) is a collective term used to describe the microbial invasion of any part of the urinary tract (Mahdi *et al.*, 2020). It involves bacterial infections on one or more parts of the urinary system. Majority of UTI is caused by *E. coli*, while other causative agents are *Pseudomonas sp.*, *Proteus sp.*, *Klebsiella sp.*, *Citrobacter sp.*, group B Streptococci, *Staphylococcus aureus*, *Staphylococcus saprophyticus* (Akter *et al.*, 2016), *Serratia sp.* and *Salmonella sp.* (Madeeha & Malik, 2021). Most of the antibiotics used in treating UTI have become resistant. It is therefore very necessary to develop new antimicrobials that are effective with no side effects, easily available and less expensive (Priyadharsini *et al.*, 2014).

Saponins, flavonoids, steroids and glycosides were detected while terpenes, tannins and amino acid were not detected in this study. This is in line with the report of Anyiam & Opara, (2023) where flavonoids, tannins, phenols, anthocyanins, alkaloids, carbohydrates, glycosides, saponins where the phytochemical constituents detected in the coconut water used in their study. Terpenoids, steroids, resin and protein were not detected. The finding of this study is partially consistent with another finding reported by Fatema *et al*, (2017) were the phytochemical analysis of coconut water revealed the presence of alkaloids, saponins, tannins and glycosides while the absence of flavonoids was observed. According to Akpomie *et al.*, (2020), the absence of phytochemicals in the coconut water used in their study may be attributed to the very high content of the water which may have diluted the phytochemicals to undetected levels. Coconut water with different coconut variations is made up of diverse concentration of compounds and that the phytochemical constituents also vary during the various stages of maturity (Rukmini *et al.*, 2017).

Results presented in table 4 shows the antibacterial activity of the coconut water on *Escherchia coli* and *Pseudomonas aeruginosa* isolates. In this study, the coconut water exerted no antibacterial effect on any of the test isolates. This contradicts previous work done by Anyiam & Opara, (2023) where coconut water used showed growth inhibitory effect on *Klebsiella sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* used. It also in contrast with the report of Nasimuddin *et al.*, (2016) where coconut water had antimicrobial activity on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Based on study by Jennifer & Perpetua, (2023), *Staphylococcus aureus*, *Escherichia coli* and *Bacillus sp.*, showed no resistance to coconut water which contradicts this current study. The research conducted by Rukmini *et al.*, (2017) supports this current study as no observed antibacterial effects of coconut water against the organism used. In another study by Akpomie *et al.*, (2020), the bacterial isolates used exhibited resistance to all the tested concentrations of coconut water used. Though the antimicrobial property of coconut is due to its high lauric acid content, the lack of antibacterial effect observed in this current study might be as a result of the phytochemical constituents of coconut water being affected by numerous factors. The soil and

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environmental condition disturbs the phytochemical constituents of coconut water (Rukmini *et al.*, 2017).

CONCLUSION

Phytochemical analysis of the coconut water used in this study showed the presence of saponins, flavonoids, steroids and glycosides. It also showed no antimicrobial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. That means that coconut water cannot serve as a potential antibacterial agent.

RECOMMENDATION

From the findings above, further research should be done on the antifungal activity of coconut water. Studies should be done to isolate and characterize bioactive compounds present in coconut water.

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