Original Research Article

EVALUATION OF THE ANTIMICROBIAL ACTIVITIES OF PALM KERNEL OIL (*Elaeis guineensis*) AND CASTOR OIL (*Ricinus communis*) ON UROPATHOGENS

ABSTRACT

Aim: This study investigated the antimicrobial properties of castor bean (CBO) and palm kernel oils (PKO) against common uropathogens.

Study Design: This study is a cross sectional research.

Place and Duration of Study: Midstream urine samples were collected from UTI suspected patients at University of Nigeria Nsukka, Medical Centre. Fresh Castor bean (*Ricinus communis)* and palm kernel (*Elaeis guineensis)* were bought from the market. The analyses were done in Microbiology laboratory, University of Nigeria, Nsukka from January to May, 2024.

Materials and Methods: The urine samples were cultured on Eosin methylene blue agar, Mannitol agar and potatoes dextrose agar. The isolates were identified by morphological growth characteristics, biochemical tests and by Gram staining reaction. Castor bean and palm kernel oil extraction was done by semi-thermal process at a low temperature.

Results: The presence of terpenoid, flavonoids and glycosides were observed in both PKO and CBO among others. The isolated uropathogens were identified as *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. The antimicrobial testing indicated that PKO had 100% against *S. aureus* at all concentrations, 50% sensitivity against *E. coli* at 100mg/ml and 50mg/ml; whereas *C. albicans* had 50% sensitivity at 100mg/ml and 50mg/ml, but 100% resistant at 25mg/ml and 12.5mg/ml. PKO showed inhibition zone diameter (IZD) against *S. aureus* (16.5 mm) at 100 mg/m, 11mm against *C. albicans* at 100 mg/ml and 50 mg/ml whereas 0mm was observed at 25mg/ml and 12.5mg/ml. CBO had lower antimicrobial activities than PKO. The maximum IZD for CBO was against *S. aureus* (14 mm) and *E. coli* (11mm) at 100 mg/ml concentration, while 0mm IZD was observed against *C. albicans*. However, the combination of the 2 oils had no synergistic effect.

Conclusion: The sensitivity of mostly *S. aureus,* then, *E. coli* to these oil extract particularly PKO highlights a viable alternative in treating UTIs.

KEY WORDS: Uropathogens, Castor bean oil, Palm kernel oil, Phytochemicals, Antimicrobial activities and Inhibition zone diameter.

1.0 **INTRODUCTION**

Recently, the need for natural products as potential sources of therapeutic agents with multifaceted benefits for human health is on the increase. Utilization of natural resources like therapeutic plants has been present since ancient times, with medicinal plants being of crucial importance from the beginning. Even our ancestors used these plants to treat infections, heal wounds, and inhibit cancer cells and microorganisms, among other things, without a proper knowledge of their chemical constituents. According to the United States Department of Agriculture [1] (USDA. 2023), most people living in rural areas of Nigeria rely heavily on natural herbs/plants for the treatment of most common diseases.

The use of these natural plants is due to the rising global population, increase in antimicrobial resistant, coupled with inadequate supplies of conventional drugs, their prohibitive costs, and the side effects of several chemical synthetic drugs [2] (Siddiqui, 2018).

The urinary tract harbors several microorganisms which can be beneficial, pathogenic and opportunistic pathogen. Any form of imbalance in these urinary tract microbiomes can lead to diseases or infection of the urinary system. Such conditions include urgency urinary incontinency, infection of the urinary tract, use of antimicrobials, prostrate disorders and others [3] (Hawra et al., 2024).

For instance, UTIs usually occur when microorganisms from the gastrointestinal (GIT) or reproductive system (their normal flora or pathogens) leave those sites to inhabit the urethra, ureter or periurethral area and finally move to the bladder. Microorganisms in the bloodstream can equally result to UTIs when they migrate to the kidneys or bladder. However, this is a rare occurrence [3] (Hawra et al., 2024).

Urinary tract infections (UTIs) are broadly divided into two groups; uncomplicated (uUTIs) and complicated (cUTIs). uUTIs usually affect individuals who are in good health and with intact immune system, but do not have any structural or neurological issues with their urinary tract. This usually occurs when the intestinal microbiomes accidentally enter the urethra, thereby colonizing the bladder. Those UTI infections which in persons with deficiencies in the urinary tract that places them to be at a higher risk of infections, like those on catheter or those with functional or anatomical abnormalities are known as cUTI [4] (Mancuso et al., 2023).

A major factor promoting the increased focus on new plant-based medicines is the growing prevalence of life-threatening infections caused by resistant and multi-resistant pathogenic microorganisms. This is currently a major health challenge worldwide as infections caused by this category of microorganisms are increasing rapidly, which subsequently leads to higher morbidity and mortality [5] (WHO, 2022).

Infections like, urinary tract infections (UTI) have become increasingly resistant to most first-class antibiotics. This has created the need for alternative antimicrobials. According to research conducted by Ahmed et al., [6] (Ahmed et al., 2019), the majority of bacteria causing UTIs have developed resistance to at least one widely-used antibiotic or at least two, have shown resistance at 92mg/ml and 80 mg/ml concentrations respectively. Due to the high prevalence of UTIs, antibiotic resistance poses a significant challenge to the treatment of UTIs compared to other types of infections [7] (Seaton, 2023).

This high prevalence and rapid spread of drug and multi-drug-resistant pathogens including the uropathogens have also increased the threat of untreatable microbial infections [8] (Sani et al., 2017). This has added urgency to the hunt for novel antimicrobial strategies, such as extracting plant materials, owing to its chemical properties, and the bioactive compounds, minerals and the vitamin content which gives necessary protection from many diseases [9] (Micha et al., 2017). This involves the extraction of the phytochemicals from the plant materials and the use of the bioactive compounds as an antitumor, anti-inflammatory, antioxidant, and antimicrobial. Plant extracts have been proven by several studies to be more effective against pathogens that show progressive failure to synthetic drugs and also have less side effects unlike chemically synthetic drugs that may come with side effects like allergic reactions (skin reactions), immune suppression, heart problem, depletion of beneficial gut and mucosal microorganisms [10] (Namita and Mukesh 2017).

Essential oils produced by plants are equally used as alternative antimicrobials. They are oleaginous plants, which have mostly been traditionally used for cooking as they impact colour and good flavour to foods. In addition to maintaining food quality, essential oils are important raw materials in cosmetics, pharmaceutical, and oil industry fields [11] (Yapi et al., 2020). These oils contain several kinds of phenolic compounds which helps to minimize oxidative stress thereby maintaining oxidative stability on human health. Several researches done in the past have shown the health benefits of flavonoids and phenolic acids [12] (Bouras et al., 2015).

Palm tree (*Elaeis guineensis*) produces two major types of vegetable oil - palm oil from the mesocarp of the fruit while PKO is obtained from the inner seed of the palm fruit. Two sub-species of this plant (dura and tenera) have successfully produced both oils, but the sub-specie, pisifera does not, due to its shell-less nature [13] (Onyebuchi-Ogwuegbu et al., 2023). The palm oil is manually extracted from the fiber of the ripe palm fruit of the oil palm. The PKO is popularly known for its use in folk medicine for treating hypersensitivity reactions and fungal infections. Locally, it is equally used in treatment of convulsions especially in children,

ulcers, wounds, intestinal disorder, and skin diseases in Nigeria or West African countries [11] (Yapi et al., 2020).

The bioactive compounds in castor bean oil (CBO) include phospholipids, phenolics, monounsaturated fatty acid and vitamin E, and therefore are known to possess high nutritional value [14] (Sbihi et al., 2018). The presence of natural antioxidants and tocopherols, confers the anti-proliferative and anti-inflammatory properties to CBO. The CBO is a brand of triglycerides, with ricinolein acid (70-90 mg/ml of the fatty acids), and also contains oleic acid, stearic acid, as well as minute quantity of linolenic acid, and linoleic acid [15] (Wikipedia 2024).

According to recent research, unsaturated fatty acids in plant-based vegetable oils can help reduce individual risks associated with various diseases like asthma, cancer, diabetes, cardiovascular diseases, cancer, HIV/AIDS and other life-threatening diseases [16] (Ganesan et al., 2018). Several studies have reported that CBO have effective antimicrobial activities against pathogenic bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* as well as antifungal activities against some fungal agents like *Candida albicans* and *Aspergillus niger* [17] (Rashimi and Kumar 2019).

Palm kernel oil from oil palm (*Elaeis guineensis*) and castor oil from castor bean (*Ricinus communis*) (used in the production of the popular *ogiri*, a food condiment majorly produced in Anambra state in the eastern part of Nigeria) have been used for years for treating skin diseases, infections, convulsion and joint pain, especially when the chemical synthetic alternatives failed [11] (Yapi et al., 2020).

This study was designed to explore castor oil and palm kernel oil efficacy against various uropathogenic bacteria and fungi, suggesting their potentials as natural disinfectant or therapeutic agent for infectious diseases [12, 18] (Bouras et al., 2015; Ekwenye and Ijeoma 2015).

The specific objectives include- isolation and identification of uropathogens from urine samples of suspected urinary tract patient, qualitative phytochemical analysis of CBO and PKO and evaluation of the antimicrobial activities of CBO and PKO against the uropathogens.

2.0 MATERIALS AND METHODS

2.1 Plant Collection and Preparation

Castor bean (*R. communis)* and palm kernel (*E. guineensis)* were bought from Orba main market in Orba, Udenu Local Government of Enugu state. The plant materials were transported to the laboratory for extraction and analysis. The plant products were identified by a plant taxonomist from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu state.

2.1.1 *R. communis and E. guineensis* seeds oil extraction

E. guineensis seed (palm kernel) oil was extracted using the thermal extraction process. The kernel was washed, dehulled and dried. It was then roasted directly till a black-coloured oil came out and the oil was scooped with a clean spoon and stored in an airtight container and properly labelled for further analysis.
R. communis seed (Castor bean) oil was also extracted using the thermal extraction process. The castor bean was washed, dried and dehulled. The dehulled bean was then ground to a smaller size and was heated by adding it to water boiled at 100℃ and was further boiled till the water separated from the oil. The oil appeared on the top while the water and residue stays at the bottom. The oil was then scooped with a clean spoon and properly stored for further analysis.

2.2 Qualitative Phytochemical Analysis

Phytochemical analysis was done on the plant oils to detect the presence or absence of specific classes of bioactive compounds according to the method described by Jigna *et al.,* 2007 [19]. Various chemical tests were carried out to detect compounds like alkaloids, flavonoids, tannins, saponins, phenols and glycosides. The phytochemical analysis provides necessary information about the chemical composition of the oils and their potential medicinal or nutritional properties**.**

2.3 Urine Sample Collection and Processing

Fifty (50) midstream urine samples were collected using sterile universal containers patients suspected of having UTIs at the University of Nigeria, Nsukka, Medical Centre. The samples of urine collected from UTI patients of varying ages were immediately taken to the Microbiology Laboratory for analysis.

2.3.1 Urine macroscopy and microscopy of the urine deposit

Macroscopically, the urine samples were examined to determine the cloudiness, colour and presence of blood which will provide necessary information about potential urinary tract infections (UTIs) as well as other health conditions. The urine deposits were examined microscopically for the presence of red blood cells and the presence of pus cells (pyuria) where more than 10 pus cells per high power field of the microscope is a sign of UTI [20] (Fang et al., 2023).

2.2.2 Isolation of uropathogens

The urine samples were inoculated on eosin methylene blue agar (EMB), mannitol salt agar (MSA) and potato dextrose agar (PDA) using a sterile wire loop. The cultured plates were incubated at a temperature of 35°C 24-hrs. Mixed colonies were observed. Discrete colonies observed were sub-cultured onto the 3 different media to obtain a pure culture and kept at 35°C in an incubator for 24 hours. The pure culture was preserved in a bijou bottle slant agar and kept in a refrigerator at 4°C.

2.3 Identification of the Isolated Uropathogens

The identification of the isolates was determined by morphological growth characteristics on the media by viewing the discrete colonies macroscopically based on characteristics such as colour of the surface and texture. The bacteria isolates were further identified using biochemical tests while the fungal isolate was identified by germ tube test. The biochemical tests which include catalase test and citrate utilization test as described by Cheesebrough, 2006 [21].

2.4 Antimicrobial Activities

2.4.1 Standard inoculum preparation

An 18-h culture was prepared using nutrient agar and potato dextrose agar. This was carried out by using a wire loop to collect a loopful of the stored isolate and inoculate onto the nutrient and potato dextrose agar. Viable discrete colonies were observed. Then, the inoculum was prepared by taking a loopful from the 18-h plates and dispensing in to test tubes containing 2ml normal saline for each test organism. The resulting turbidity was adjusted to already prepared 0.5 McFarland turbidity standard which typically corresponds to a bacterial concentration of approximately 1.5×10^8 Cfu/ml for *E. coli* and 1.0×10^8Cfu/ml for *S. aureus* 1and 1.5×10^6Cfu/ml for *C. albicans* [22] (Tankukar and Maharjan 2017).

2.4.2 Plant extract concentration preparation

A two-fold serial dilution was carried out using 2ml of DMSO and the oil extract as the stock culture. Four test tubes were prepared, one containing the stock culture which is 100 mg/ml oil extract and three containing 2ml of DMSO. Then, 1ml of the stock culture is collected with a micropipette transferred to the second test tube and shaken. The solution was serially diluted to the fourth test tube. This is carried out for varied concentrations of the inhibitory effect by diluting them subsequently, to get 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml oil extract.

2.4.3 Antimicrobial activity test

The antimicrobial properties of the plants were carried out using the agar well diffusion method according to Mounyr *et al.,* (2015) (23). Mueller-Hinton agar was prepared according to the manufacturer's instruction. After allowing the media to solidify, a sterile swab stick was used to seed the freshly prepared standardized viable organisms into the solidified onto sterile Mueller–Hinton agar plates, which were labelled appropriately for each organism. A sterile cork borer was used to make a well in the inoculated plates creating four holes per plate. The wells were labelled A, B, C and D for different dilutions and were impregnated with 0.1ml of the CBO and PKO on different plates respectively were allowed to diffuse into the agar. After 30 minutes of introducing the CBO and PKO. The inoculated plates were incubated for 24h at 37°C and the different inhibition zones diameter (IZD) were measured using a transparent meter rule and recorded.

3.0 RESULTS

3.1 Morphological and Biochemical Identification of Uropathogens

Twelve isolates were identified morphologically, of which 5 had smooth and metallic sheen green colour, 5 had yellow colonies with yellow zones and 2 had smooth and creamy colour. The biochemical tests done showed that out of the 10 samples analysed, 5 were both catalase and citrate negative, and Gram negative while 5 were catalase, citrate and Gram stain positive. The results indicate the presence of three distinct isolates, which have been presumptively identified *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* as shown in table 1.

Table 1: Biochemical identification of the uropathogens

No of isolates	Gram's reaction	Biochemical test		Agar	Morphological features	Microscopic features	Germ tube test	Probable organism
		Catalase	Citrate					
5	-	-	-	EMB	Smooth and metallic sheen green colour	Pink rod in single	NA	*E. coli*
5	+	+	+	Mannitol salt	Yellow colonies with yellow zones	Purple cocci in clusters	NA	*S. aureus*
2	NA	NA	NA	PDA	Smooth and creamy colour	NA	+	*C. albicans*

Keys:

+ = Positive

- = Negative

NA = Not Applicable

3.3 Phytochemical Analysis

The result in table 2 shows the phytochemical constituent of the CBO and the PKO. From the table, flavonoids were abundantly present in CBO and moderately present in PKO. Then, Terpenoid was highly present in CBO and abundantly present in PKO. Tannin was absent in CBO and moderately present in PKO. Glycoside was moderately present in CBO and highly present in PKO. Alkaloid was moderately present in CBO and highly present in PKO. Then, saponins were moderately present in CBO but absent in PKO whereas phenol is absent in both CBO and PKO.

Table 2: Phytochemical analysis of castor bean oil and pam kernel oil

Phytochemical	**CBO**	**PKO**
Terpenoid	++	+++
Flavonoid	+++	+
Tannin	-	+
Glycoside	+	++
Phenol	-	-
Alkaloid	+	++
Saponins	+	-

Keys:

- = Absent

+ = Moderately present

++ = Highly present

+++ = Abundantly present

3.4 Antimicrobial Activity of Castor and Palm Kernel Oils on Uropathogens

The sensitivity result showed that *E. coli, S. aureus* and *C. albicans* were all sensitive to PKO; 50%, 100% and 50% sensitivity across the different oil extract concentrations, whereas for CBO, *E. coli, S. aureus,* was 50% sensitive to CBO, but *C. albicans* was resistant to CBO across the different oil extract concentrations. The result also showed that the Gram-positive bacteria, *S. aureus* was more sensitive to PKO than the Gram-negative, *E. coli*. Also, with the CBO, *E. coli,* and *S. aureus* have equal sensitivity but have higher sensitivity than *C. albicans* as shown in table 3.

Result of the antimicrobial activities of CBO and PKO, prepared in 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml and their activities against the uropathogens are shown in figs. 1 and 2. The inhibition zone diameter (IZD) was measured in millimeter, and the result showed that PKO has an IZD ranging from 10 mm to 16.5 mm, while CBO has an IZD ranging from 10mm to 14mm as shown in figures 1 and 2 respectively. The combination of the 2 oils (CBO: PKO; 1:1) did not show any synergistic effect on the uropathogens as shown in table 4.

Table 3: Sensitivity of the uropathogens to castor oil and palm kernel oil

Test organisms	**CBO**	**Sensitivity (%)**	**Resistance (%)**	**PKO**	**Sensitivity (%)**	**Resistance (%)**
E. coli	+	50	50	+	50	50
S. aureus	+	50	50	+	100	0
C. albicans	-	0	100	+	50	50

Key:

+ = Sensitive

- = Resistant

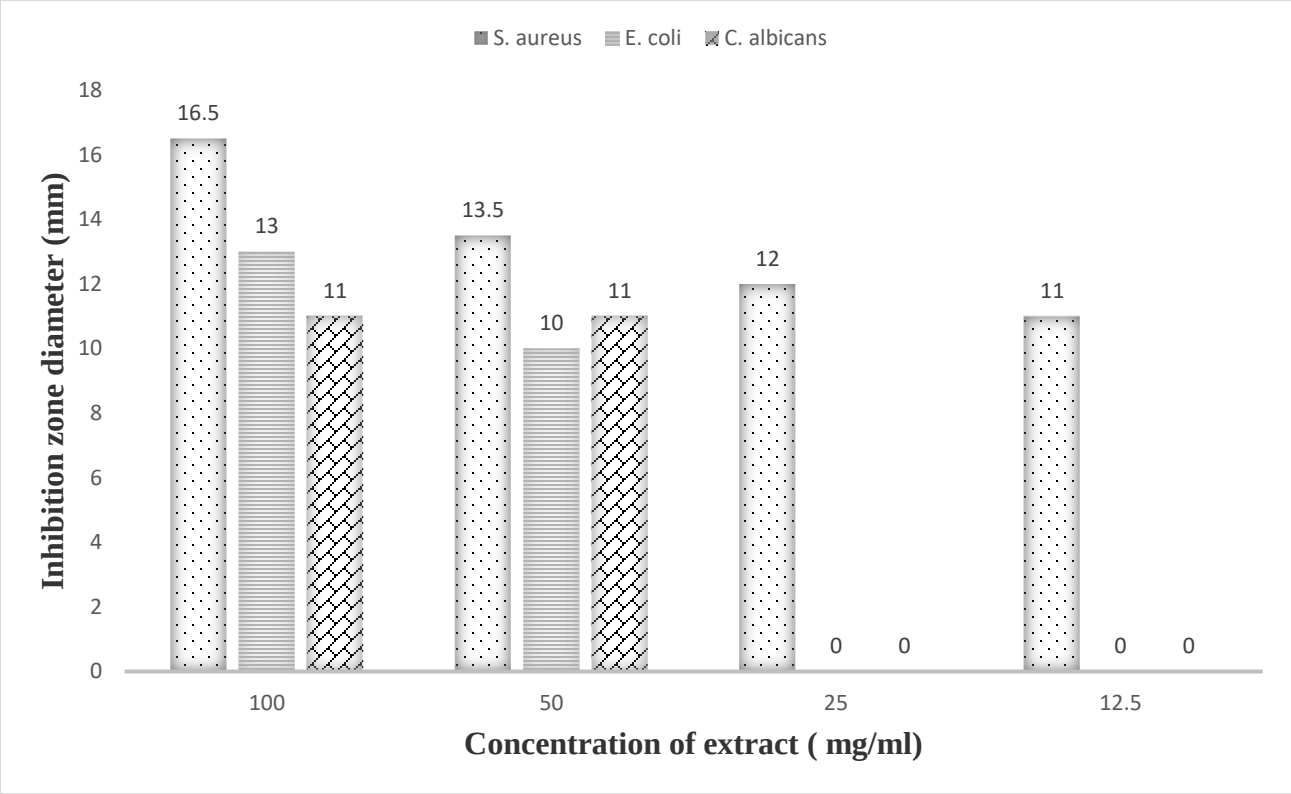

Figure 1: Antimicrobial effect (IZD) of palm kernel oil against uropathogens in mm

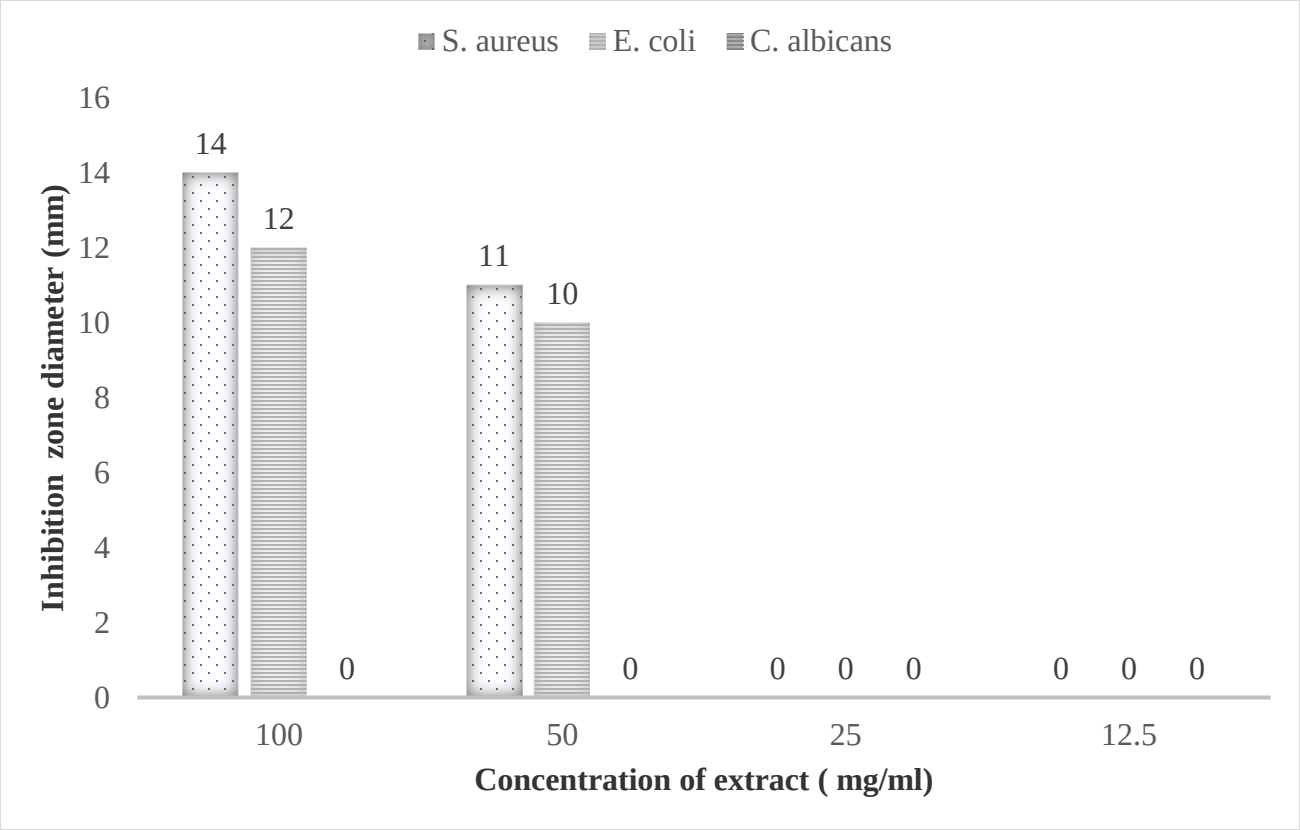

Figure 2: Antimicrobial effect (IZD) of castor bean oil against uropathogens

Table 4: Sensitivity of the uropathogens to the combination of castor bean oil and palm kernel oil in the ratio 1:1

Test organisms	Pos/Neg	Sensitivity (%)	Resistance (%)
E. coli	+	50	50
S. aureus	+	100	0
C. albicans	-	0	100

Key:

+ = Sensitive

- = Resistant

4.0 DISCUSSION

Several antimicrobials have been developed in the past to deal with antimicrobial-resistant infectious microorganisms such as uropathogens that cause UTI. In this study, oil extracts from castor bean and palm kernel were prepared and their antimicrobial activities on uropathogens isolated from the urine samples evaluated.

Most of the urine samples that had up to 10 pus cells per high power field of the microscope during microscopy, yielded microbial growth after 24 h incubation at 37^0C. The study by Fang *et al*., 2023 [20], supports these findings, that the presence of pus and blood in urine, along with its colour, are reliable indicators of UTIs.

The growth morphological features of the colonies on EMB, MSA and PDA were used to identify the isolated uropathogens. In all, twelve isolates were identified morphologically from the urine samples. The isolates were further identified using biochemical tests and Gram staining reaction. The yeast isolate was confirmed using germ tube test. Five of the isolates have smooth and metallic green colour, Gram-negative rods, negative to both catalase and citrate tests and were identified as *Escherichia coli*. Another 5 have yellow colonies with yellow zones, Gram-positive cocci in clusters, positive to both catalase test and citrate test, and were identified as *Staphylococcus aureus.* The remaining 2 isolates were different, were tiny smooth and creamy colour, budded yeast under the microscope, and positive to the germ tube test and were identified as *Candida albicans.* The morphological identification agrees with the reports of Sugumaran *et al*., 2020 [24] and Hassan *et al*., 2020 [25], where similar colony characteristics were observed for these uropathogens.

The CBO and PKO used in this study were prepared at a very low heat to avoid denaturation or destruction of the bioactive compounds by high temperature. In other words, this preparation method preserved the bioactive compounds in the oils, essential for their antimicrobial properties. The phytochemical analysis result of this study showed that flavonoid was the most abundant bioactive compound in CBO which contradicts the result of Onyebuchi-Ogwuegbu *et al*., [13], which showed tannin as the most abundant bioactive compound. However, terpenoids were the most abundant in PKO which corresponds with the result given by Momoh *et al.,* 2012 [26].

The abundance of flavonoid and terpenoid in CBO and PKO respectively, is attributed to their antimicrobial activities against uropathogens. Flavonoids can inhibit bacterial growth by disruption of the microbial cell walls and its synthesis. Terpenoids has the ability to penetrate microbial membranes and disrupt their integrity (Donadio et al., 2021) [27].

To assess the antimicrobial efficacy of the oils, they were diluted to various concentrations: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The results of this study, showed that at higher concentrations, the oils gave better antimicrobial activities than at lower concentrations. This implies that the efficacy of the oils is

dependent on the concentration, with higher concentrations providing more substantial antimicrobial effects (Balouiri et al., 2016) [28]. During the antimicrobial activity testing using agar well diffusion method, it was observed that the oil did not totally diffuse into the medium, which may limit the accuracy of the antimicrobial activities of the oils. Therefore, the need arises for a solvent with high dissolution strength than the DMSO used in this study, to ensure complete diffusion of the oil into the medium for better antimicrobial properties.

The 3 uropathogens isolated in this study (*S. aureus, E. coli* and *C. albicans*) were all sensitive to PKO, while only *S. aureus* and *E. coli* exhibited sensitivity to CBO at a lower rate. *C. albicans* was completely (100%) resistant to CBO at all concentrations. PKO showed a more significant antimicrobial activity against the Gram-positive bacterium *S. aureus*, with IZD of 16.5 mm, than against the Gram-negative bacterium *E. coli* and the fungus *C. albicans*, with IZDs of 13 mm and 11 mm, respectively. These reports agree with the result given by Yapi *et al*. [11] (Yapi et al., 2020), which also reported higher antimicrobial effects of PKO against Gram-positive bacteria than Gram-negative bacteria.

From the result of this study, *E. coli* and *S. aureus* showed moderate sensitivity to CBO, with IZDs of 14 mm and 12 mm, respectively. However, *C. albicans* displayed complete resistance, showing no inhibitory zone (0 mm). This resistance contradicts the findings of Momoh *et al*., 2012 [26] which reported that *C. albicans* was sensitive to CBO. On the other hand, Hassan *et al*., 2020 [25] and Dulal *et al*., 2021 [29] reported 0mm as IZD of CBO against both *E. coli* and *S. aureus* in their own studies.

The result of the antimicrobial activity of PKO in this study showed IZDs of 16.5, 13. 5, 12.0mm and 13, 10.0, 0mm against S. aureus and E. coli respectively at 100mg/ml, 50mg/ml and 25mg/ml dilutions respectively. This result tallies with the report of Akpan *et al*., 2020 [30] that PKO inhibited *S. aureus* in concentrations of 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml which they rated as little, intermediate and immense inhibition respectively.

The combination of CBO and PKO in this study did not give better antimicrobial activities on the uropathogens which corresponds with the report of Padalia et al., 2017 (31) but does not tally with the findings of Hassan et al., 2020 (25) that reported synergistic effects of the oils on the uropathogens.

Several factors could explain these discrepancies, geographical variations in pathogen strains, differences in the dissolving agents used, variations in the oil extraction processes, as well as geographical variation of oil trees and season of collection. In this study, the solvent used in the dissolution of CBO and PKO might limit their antimicrobial activities and using a more suitable solvent with higher dissolution strength might produce better antimicrobial effects on the uropathogens, just as stated by Dulal et al., 2021 [28] that proper dissolution of essential oils is crucial for accurately assessing the oils' efficacy, as incomplete diffusion can lead to underestimation of their true antimicrobial effect (Dulal et al., 2021) [28].

CONCLUSION

The treatment of UTIs especially UTIs caused by drug resistant or multi-drug resistant strains of microorganisms is becoming a global health challenge. The search for novel drugs or alternative therapy

prompted the search for plants and its' materials that can be used in the treatment of those diseases caused by drug resistant pathogens. Some of the alternative include using medicinal plants such as CBO and PKO against uropathogens as done in this study. The PKO used in this study showed antimicrobial effects on *E. coli, S. aureus*, and *C. albicans*, which indicates its potential as natural antimicrobial agents. For better understanding of the antimicrobial properties of CBO and PKO, more intensive researches are needed to further fractionate the extracts and know the antimicrobial potency of each of the fractions. There is also need the check the dissolution power of other solvents rather than DMSO used in this study.

CONSENT

Before the sampling, informed consent forms were filled by the participants.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies like Large Language Models (ChatGPT, COPILOT etc) and text-to-image generations have been used during writing or editing of the manuscript.

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