

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/ml, 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.

32
33 **KEY WORDS:** Uropathogens, Castor bean oil, Palm kernel oil, Phytochemicals, Antimicrobial activities and
34 inhibition zone diameter.

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37 1.0 INTRODUCTION

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

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Commented [H6]: add: wide use....

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Commented [H8]: Replace with: increasing incidence of antimicrobial resistance

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

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Commented [H10]: the adverse side effects associated with several chemically synthesized drug.

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

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

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Commented [H17]: Provide a clear definition of UTIs at its initial usage

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56 Urinary tract infections (UTIs) are broadly divided into two groups; uncomplicated (uUTIs) and complicated
57 (cUTIs). uUTIs usually affect individuals who are in good health and with having intact immune system, but
58 do not have with no any underlying structural or neurological issues with their urinary tract. This usually
59 occurs when the intestinal microbiomes accidentally enter the urethra, thereby colonizing the bladder.
60 Those UTI infections which in persons with deficiencies in the urinary tract that placesing them to-be at a
61 higher risk of infections, like those on catheter or those with functional or anatomical abnormalities are
62 known as cUTI [4] (Mancuso et al., 2023).

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

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

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73
74 This high prevalence and rapid spread of drug and multi-drug-resistant pathogens including the
75 uropathogens have also increased the threat of untreatable microbial infections [8] (Sani *et al.*, 2017). This
76 has added urgency to the hunt for novel antimicrobial strategies, such as extracting plant materials, owing
77 to its chemical properties, ~~and the~~ bioactive compounds, minerals and the vitamin content which gives
78 necessary protection from many diseases [9] (Micha *et al.*, 2017). This involves the extraction of the
79 phytochemicals from the plant materials and the use of the bioactive compounds as an antitumor, anti-
80 inflammatory, antioxidant, and antimicrobial. Plant extracts have been proven by several studies to be more
81 effective against pathogens that show progressive failure to synthetic drugs and also have less side effects
82 unlike chemically synthetic drugs that may come with side effects like allergic reactions (skin reactions),
83 immune suppression, heart problem, depletion of beneficial gut and mucosal microorganisms [10] (Namita
84 and Mukesh 2017).

85
86 Essential oils produced by plants are equally used as alternative antimicrobials. They are oleaginous plants,
87 which have mostly been traditionally used for cooking as they impact colour and good flavour to foods. In
88 addition to maintaining food quality, essential oils are important raw materials in cosmetics, pharmaceutical,
89 and oil industry fields [11] (Yapi *et al.*, 2020). These oils contain several kinds of phenolic compounds which
90 helps to minimize oxidative stress thereby maintaining oxidative stability on human health. Several
91 researches done in the past have shown the health benefits of flavonoids and phenolic acids [12] (Bouras
92 *et al.*, 2015).

93 Palm tree (*Elaeis guineensis*) produces two major types of vegetable oil - palm oil from the mesocarp of
94 the fruit while PKO is obtained from the inner seed of the palm fruit. Two sub-species of this plant (dura and
95 tenera) have successfully produced both oils, but the sub-specie, pisifera does not, due to its shell-less
96 nature [13] (Onyebuchi-Ogwuegbu *et al.*, 2023). The palm oil is manually extracted from the fiber of the ripe
97 palm fruit of the oil palm. The PKO is popularly known for its use in folk medicine for treating hypersensitivity
98 reactions and fungal infections. Locally, it is equally used in treatment of convulsions especially in children,
99 ulcers, wounds, intestinal disorder, and skin diseases in Nigeria or West African countries [11] (Yapi *et al.*,
100 2020).

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101 The bioactive compounds in castor bean oil (CBO) include phospholipids, phenolics, monounsaturated fatty
102 acid and vitamin E, and therefore are known to possess high nutritional value [14] (Sbihi *et al.*, 2018). The
103 presence of natural antioxidants and tocopherols, confers the anti-proliferative and anti-inflammatory

104 properties to CBO. The CBO is a brand of triglycerides, with ricinolein acid (70-90 mg/ml of the fatty acids),
105 and also contains oleic acid, stearic acid, as well as minute quantity of linolenic acid, and linoleic acid [15]
106 (Wikipedia 2024).

107 According to recent research, unsaturated fatty acids in plant-based vegetable oils can help reduce
108 individual risks associated with various diseases like asthma, cancer, diabetes, cardiovascular diseases,
109 cancer, HIV/AIDS and other life-threatening diseases [16] (Ganesan *et al.*, 2018). Several studies have
110 reported that CBO have effective antimicrobial activities against pathogenic bacteria such as *Pseudomonas*
111 *aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* as well as antifungal
112 activities against some fungal agents like *Candida albicans* and *Aspergillus niger* [17] (Rashimi and Kumar
113 2019).

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

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

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

124 2.0 MATERIALS AND METHODS

125 2.1 Plant Collection and Preparation

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

131 2.1.1 *R. communis* and *E. guineensis* seeds oil extraction

132 *E. guineensis* seed (palm kernel) oil was extracted using the thermal extraction process. The kernel was
133 washed, dehulled and dried. It was then roasted directly till a black-coloured oil came out and the oil was
134 scooped with a clean spoon into clean sample bottle, ~~and stored in an airtight container and~~ properly
135 labelled and stored at (what temperature?) until needed for further analysis.

136 *R. communis* seed (Castor bean) oil was also extracted using the thermal extraction process. The castor
137 bean was washed, dried and dehulled. The dehulled bean was then ground to a smaller size and was

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Commented [H37]: Specify the part used e.g. leaves, fruits, stem etc

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Commented [H44]: Since the method of oil extraction for both plant materials is identical, the procedure can be described collectively

138 heated by adding it to water boiled at 100°C and was further boiled till the water separated from the oil. The
139 oil appeared on the top while the water and residue stays at the bottom. The oil was then scooped with a
140 clean spoon and properly stored for further analysis.

141 2.2 Qualitative Phytochemical Analysis

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

147 2.3 Urine Sample Collection and Processing

148
149 Fifty (50) midstream urine samples were collected using sterile universal containers patients suspected of
150 having UTIs at the University of Nigeria, Nsukka, Medical Centre. The samples of urine collected from UTI
151 patients of varying ages were immediately taken to the Microbiology Laboratory for analysis.

152 2.3.1 Urine macroscopy and microscopy of the urine deposit

153
154
155 Macroscopically, the urine samples were examined to determine the cloudiness, colour and presence of
156 blood which will provide necessary information about potential urinary tract infections (UTIs) as well as
157 other health conditions. The urine deposits were examined microscopically for the presence of red blood
158 cells and the presence of pus cells (pyuria) where more than 10 pus cells per high power field of the
159 microscope is a sign of UTI [20] (Fang *et al.*, 2023).

160 2.2.2 Isolation of uropathogens

161
162 The urine samples were inoculated on eosin methylene blue agar (EMB), mannitol salt agar (MSA) and
163 potato dextrose agar (PDA) using a sterile wire loop. The cultured plates were incubated at a temperature
164 of 35°C 24-hrs. Mixed colonies were observed. Discrete colonies observed were sub-cultured onto the 3
165 different media to obtain a pure culture and kept at 35°C in an incubator for 24 hours. The pure culture was
166 preserved in a bijou bottle slant agar and kept in a refrigerator at 4°C until required for analysis.

167 2.3 Identification of the Isolated Uropathogens

168 The Preliminary identification of the isolates was determined was done based on by morphological growth
169 characteristics on the media plates ~~by viewing the discrete colonies macroscopically based on~~
170 ~~characteristics~~ such as colony colour, size, form of the surface and texture. The bacteria isolates were
171 further identified using biochemical tests while the fungal isolate was identified by germ tube test. The

Commented [H45]: 1.What was the criteria for collection of 50 urine samples?
2.Duration of sample collection

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Commented [H56]: Gram staining was not done. How did you selected the required biochemical test needed for further identification.

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2.What is the significance of Germ tubes in the identification process

172 biochemical tests which include catalase test and citrate utilization test as described by Cheesebrough,
173 2006 [21].

174 2.4 Antimicrobial Activities

175 2.4.1 Standard inoculum preparation

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

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183

184 2.4.2 Plant extract concentration preparation

185 A two-fold serial dilution was carried out using 2ml of DMSO and the oil extract as the stock culture. Four
186 test tubes were prepared, one containing the stock culture which is 100 mg/ml oil extract and three
187 containing 2ml of DMSO. Then, 1ml of the stock culture is collected with a micropipette transferred to the
188 second test tube and shaken-vortexed. The solution was serially diluted to the fourth test tube. This is
189 carried out for varied concentrations of the inhibitory effect by diluting them subsequently, to get 100 mg/ml,
190 50 mg/ml, 25 mg/ml and 12.5 mg/ml oil extract.

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191 2.4.3 Antimicrobial activity test

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

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202 2.5 Data Analysis

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204 **3.0 RESULTS**

205 **3.1 Morphological and Biochemical Identification of Uropathogens**

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

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212

213 **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	<i>E. coli</i>
5	+	+	+	Mannitol salt	Yellow colonies with yellow zones	Purple cocci in clusters	NA	<i>S. aureus</i>
2	NA	NA	NA	PDA	Smooth and creamy colour	NA	+	<i>C. albicans</i>

214

215 Keys:

216 + = Positive

217 - = Negative

218 NA = Not Applicable

219

220 3.3 Phytochemical Analysis

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

227

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

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

229 Keys:

230 - = Absent

231 + = Moderately present

232 ++ = Highly present

233 +++ = Abundantly present

234

235 3.4 Antimicrobial Activity of Castor and Palm Kernel Oils on Uropathogens

236 The sensitivity result showed that *E. coli*, *S. aureus* and *C. albicans* were all sensitive to PKO; 50%, 100%
 237 and 50% sensitivity across the different oil extract concentrations, whereas for CBO, *E. coli*, *S. aureus*, was
 238 50% sensitive to CBO, but *C. albicans* was resistant to CBO across the different oil extract concentrations.
 239 The result also showed that the Gram-positive bacteria, *S. aureus* was more sensitive to PKO than the
 240 Gram-negative, *E. coli*. Also, with the CBO, *E. coli*, and *S. aureus* have equal sensitivity but have higher
 241 sensitivity than *C. albicans* as shown in table 3.
 242 Result of the antimicrobial activities of CBO and PKO, prepared in 100 mg/ml, 50 mg/ml, 25 mg/ml, and
 243 12.5 mg/ml and their activities against the uropathogens are shown in figs. 1 and 2. The inhibition zone
 244 diameter (IZD) was measured in millimeter, and the result showed that PKO has an IZD ranging from 10
 245 mm to 16.5 mm, while CBO has an IZD ranging from 10mm to 14 mm as shown in figures 1 and 2
 246 respectively. The combination of the 2 oils (CBO: PKO; 1:1) did not show any synergistic effect on the
 247 uropathogens as shown in table 4.

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248

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

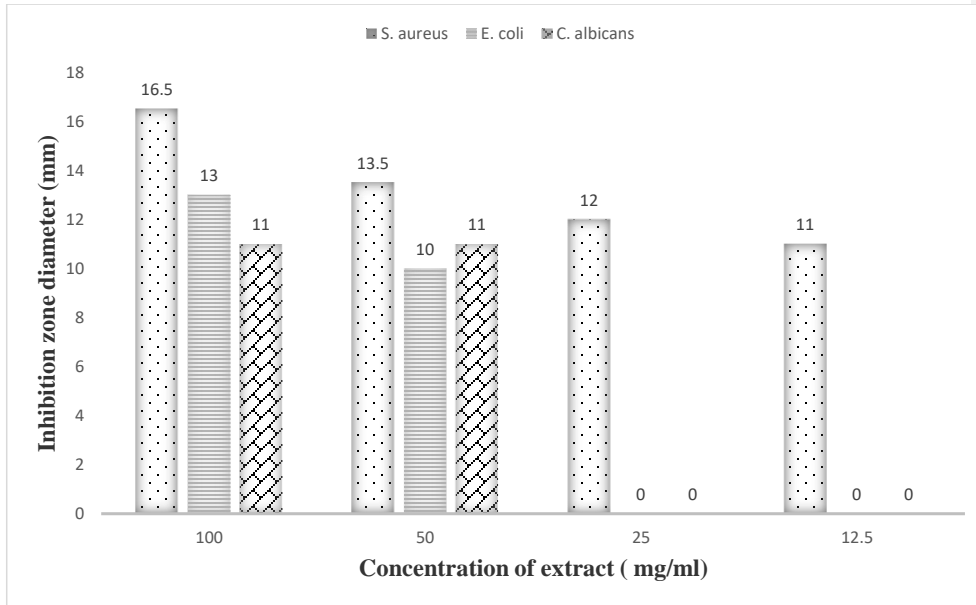
Test organisms	CBO		PKO	
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)
<i>E. coli</i>	+	50	+	50
<i>S. aureus</i>	+	50	+	100
<i>C. albicans</i>	-	0	+	50

250 Key:

251 + = Sensitive

252 - = Resistant

253

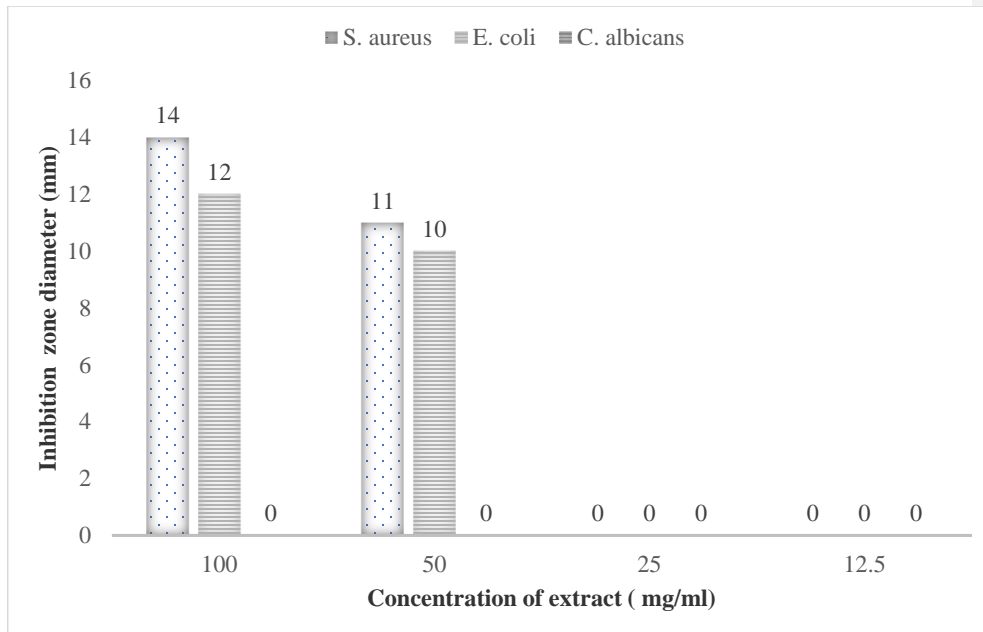


254

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

256

257



258

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

260

261

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

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

264 Key:

265 + = Sensitive

Commented [H76]: What is the criteria for grouping as Sensitive or Resistant

266 - = Resistant

267 **4.0 DISCUSSION**

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

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

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276 ~~The growth morphological features of the colonies on EMB, MSA and PDA were used to identify the isolated
277 uropathogens. In all, twelve isolates were identified morphologically from the urine samples. The isolates
278 were further identified using biochemical tests and Gram staining reaction. The yeast isolate was confirmed
279 using germ tube test. Five of the isolates have smooth and metallic green colour, Gram-negative rods,
280 negative to both catalase and citrate tests and were identified as *Escherichia coli*. Another 5 have yellow
281 colonies with yellow zones, Gram-positive cocci in clusters, positive to both catalase test and citrate test,
282 and were identified as *Staphylococcus aureus*. The remaining 2 isolates were different, were tiny smooth
283 and creamy colour, budded yeast under the microscope, and positive to the germ tube test and were
284 identified as *Candida albicans*. The morphological identification agrees with the reports of Sugumaran *et al.*,
285 2020 [24] and Hassan *et al.*, 2020 [25], where similar colony characteristics were observed for these
286 uropathogens.~~

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

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

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

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

306 The 3 uropathogens isolated in this study (*S. aureus*, *E. coli* and *C. albicans*) were all sensitive to PKO,
307 while only *S. aureus* and *E. coli* exhibited sensitivity to CBO at a lower rate. *C. albicans* was completely
308 (100%) resistant to CBO at all concentrations. PKO showed a more significant antimicrobial activity against
309 the Gram-positive bacterium *S. aureus*, with IZD of 16.5 mm, than against the Gram-negative bacterium *E.*
310 *coli* and the fungus *C. albicans*, with IZDs of 13 mm and 11 mm, respectively. These reports agree with the
311 result given by Yapi et al. [11] (Yapi et al., 2020), which also reported higher antimicrobial effects of PKO
312 against Gram-positive bacteria than Gram-negative bacteria.

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

318 The result of the antimicrobial activity of PKO in this study showed IZDs of 16.5, 13.5, 12.0mm and 13,
319 10.0, 0mm against *S. aureus* and *E. coli* respectively at 100mg/ml, 50mg/ml and 25mg/ml dilutions
320 respectively. This result tallies with the report of Akpan et al., 2020 [30] that PKO inhibited *S. aureus* in
321 concentrations of 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml which they rated as little, intermediate and immense
322 inhibition respectively.

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

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

333 CONCLUSION

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

Commented [H80]: 1.A solubility testing ought to have been done before proper analysis
2.Try other Solvents such as Tween – 20, Lower concentration of DMSO

Commented [H81]: Not in the method

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

343 **CONSENT**

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

345 **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

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

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UNDER PEER REVIEW

