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Phytochemical Screening and Antimicrobial Studies of Unripe Coconut Fluid (*Cocus Nucifera* L)

[1]Ikyenge B.A., [1]Weor T.T., [1]Aho N. T., [1]Adoga S.O and [2]Shiriki D

[1]Department of Chemistry, Benue State University, Makurdi, Benue State
[2]Department of Biological Sciences, Benue State University, Makurdi, Nigeria
Email: bkyenge@bsum.edu.ng; donbkyenge@gmail.com
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A B S T R A C T

In this research, phytochemical screening and antimicrobial studies of unripe coconut fluid (Cocus nucifera L) was carried out. Coconut fruits were collected and the mesocarp was opened to expose the endocarp which harbours the clear fluid. The unripe coconut fluid was used for the preliminary phytochemical screening and antimicrobial studies. Findings reveals that the fluid contains Alkaloids, Terpenoids, Steroids, Phenols, Flavonoids, Tannins, Anthraquinone and Saponins. The GCMS analysis revealed 16 bioactive components with 5-Eiicosene, an alkene, with highest composition with the percentage area of 31.95 %. FTIR results of the unripe coconut fluids showed absorption bands corresponding to N-H, O-H, C-H and C-C double bonds.Antimicrobial studies was carried out on the unripe coconut fluid (UCF) and was screened against Aspergillus fumigatus, Candidas Spp, Staphylococcus Aureus (Gram positive Bacteria) and Escherichia coli (Gram negative bacteria). The result of the Zone of Inhibition (ZI) adopting agar diffusion method indicates that, Unripe Coconut Fluid (UCF) was effective against both gram positive and negative bacteria and to a small extent on fungi. The research therefore infers that unripe coconut fluid has bioactive compounds that elicited antibacterial and antifungal activities and hence, can be employed in pharmaceutical and cosmetic industries as well as herbal medication.

Keywords: Keywords: *Anti-bacterial, anti-fungal, phytochemical screening, unripe coconut fluid, zone of inhibition*

1. Introduction

As a result of bacterial resistance to antibiotics, which can be attributed to genetic flexibility of resistant isolates, indiscriminate use of antimicrobial drugs, adulterated drugs and other factors has now become a global challenge [1]. There is therefore a continuous search for new antimicrobial agents, to which medicinal plants provides a promising alternative with a different mechanism of action from antibiotics and can have an important role for treating diseases caused by resistant microbial strains [2].

Novel antibiotics and lead compounds in drug design and development have been reported to be present in plant. These compounds are mainly synthesized during secondary metabolism and have potential health benefits because of their antiaging, anti-atherosclerotic, anti-inflammatory, and antioxidant properties [3]. These bioactive compounds constitute the defense mechanism against attack by microorganisms and allelopathy [4]. They provide protection against biotic and abiotic stresses, which includes adaptation to changing environments and the maintenance of structural integrity [5]. The most common classes of these chemicals are alkaloids, coumarins, essential oils, flavonoids, glycosides, gums, phenols, polysaccharides, saponins, tannins, terpenes and terpenoids, which are widely distributed among various plant families in abundant quantities [6].

Coconut is a member of the family arecaceae (family of palm). It is a common species in the genus “cocos” commonly called coconut [7]. After germination, the fruiting of the coconut palm begins between 6 to 10 years and attains full production at 15 to 20 years of age. The fruit require about a year to develop and mature and are generally produced regularly throughout the year [8].

Unripe coconut is the same variety as the ripe one, both from coconut palm (Cocos nucifera L.). The difference lies in the age of the coconut. Unripe coconuts are young mostly green and contains fluids, whereas the ripe coconuts are fully matured with meat development [9].

Due to the fact that various parts of coconut palm have been significantly used as supplement in herbal medicine, reports on antimicrobial capacity and phytochemical screening of unripe coconut fluid is still very scanty. This research therefore, seek to carry out phytochemical screening and antimicrobial studies of the unripe coconut fluid.

2. Experimental

This study was carried out in the Department of Chemistry and Biological Sciences Laboratories of the Benue State University and the materials and method used for this study are as described hereafter.

2.1 Sample Collection

The unripe coconut fruit was collected at Daudu, Guma local government area of Benue State. The sample was authenticated by a Botanist at the Department of Biological Sciences, Benue State University, Makurdi. Four microorganisms, two fungi (*Candida Spp* and *Aspergillus fumigatus*) and two bacteria (*Staphylococcus aureus* and *Escherichia coli*) already isolated were obtained from the department of Biological Sciences, Benue State University, Makurdi.

2.2 Sample preparation

The coconut fruit was washed, first with 3 % Sodium hypochlorite solution and rinsed thoroughly with sterile (autoclaved) water. The mesocarp of the unripe coconut was then removed aseptically using a sterilized cutlass to expose the endocarp which contains the clear liquid. The fluid was aseptically turned into a sterile bottle and corked. All the equipment used were highly sterilized to avoid microbial contamination.

2.3 Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the unripe coconut fluid were carried out on the fluid extracts using analytical grade reagents and the following phytochemicals were analyzed using standard procedures; Terpenoids (10). Flavonoid (11). Alkaloids (12). Tannins (13). Saponins (14). Steroids (15). Phenols (16) and Anthraquinones (17) respectively.

Instrumental analysis of the extracts was carried out using GC-MS (gas chromatography hyphenated to a mass Spectrophotometer, 5975C) and Fourier Infrared Spectroscopy (FTIR)

2.4 Antimicrobial Studies

2.4.1 Preparation of Culture Media

Two different media were prepared; The Nutrient agar for the bacteria growth was prepared by weighing 23.5 g of the nutrient agar into a beaker and dissolving it with 1000 mL of distilled water to obtained 1000 mL of the agar. The Sabourand-dextrose agar media was prepared by weighing 65 g of Sabourand-dextrose into a beaker and dissolving it with 1000 mL of distilled water to obtained 1000 mL of the agar. Both media were boiled to dissolve the medium completely and autoclave at 121^{o}C for 15 minutes. The media were brought down from the autoclave and allowed to cool to touch. Antibiotic chloramphenicol capsule was added to the Sabourand –dextrose agar to prevent bacteria growth. It was then poured into petri dishes and allowed to solidify and were marked in accordance with the serial dilution prepared.

2.4.2 Inoculation of the Organism

The S. Auereus (Gram positive bacteria), E. Coli (Gram negative bacteria), Candida's (Fungus) and A. Fumigatus (Fungus) were inoculated evenly on each of the agar loop labeled plates respectively. The inoculation wire loop was heated to red hot sing spirit lamp after each inoculation to avoid contamination of the other plates

2.4.3 Zone of Inhibitory

Disc diffusion method was applied to determine the zone of inhibition of the extracts and Ciprofloxacin and Ketoconazole as positive control for bacteria and fungi respectively, while sterilized water was used as a negative control as described by Bazerque et al, (18).

3. Results and Discussion

The results of the phytochemical screening conducted on the Coconut fluid extracts by conventional methods is presented in Table 1

Table 1: Phytochemical Screening of Unripe Coconut Fluid

Parameter	Observation
Terpenoid	++
Flavonoids	+

Alkaloids	+
Tannins	+
Saponins	-
Steroids	++
Phenols	+
Anthraquinones	-

Keys: + (low Concentration), ++ (moderate Concentration), – (absent)

Table 1 Shows the presence of secondary metabolites in the coconut fluid extracts. The presence of terpenoids and phenols is an indication of antioxidant potentials of the fluid extracts, which helps in reducing free radicals or reacting with these free radicals and neutralizing them thus protecting the cell from oxidative damage (19). The other secondary metabolites; alkaloids, flavonoids, tannins are known for their anti-bacterial properties (21).

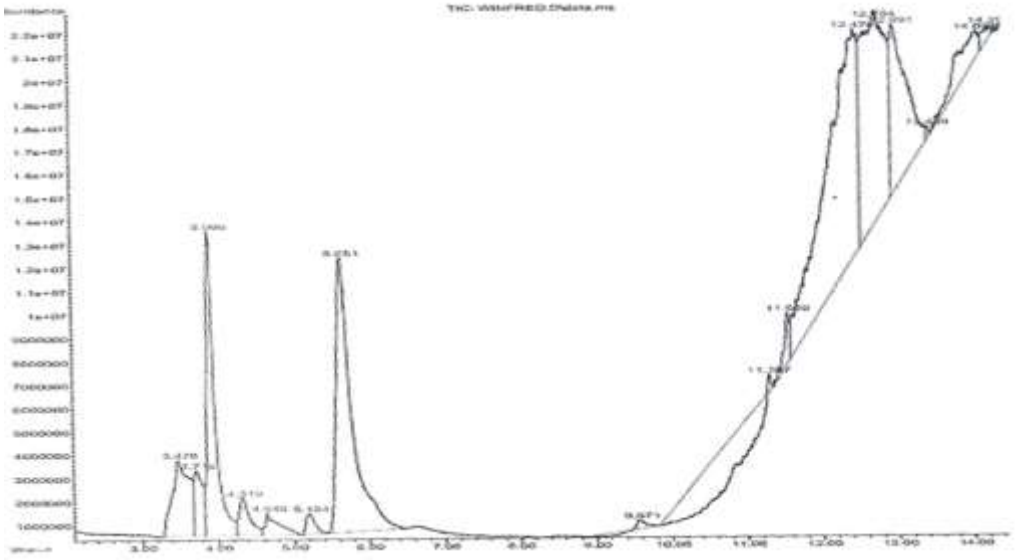

Figure 1: *GC-MS Spectrum of Unripe Coconut Fluid.*

The GC-MS revealed the presence of 16 bioactive compounds corroborating the preliminary phytochemical screening. Notable among these compounds is 5-Eicosene reported for its antibacterial activity (22). Also, the presence of carbamic was evidence, which is a major constituent of the class of pesticide known as carbamate which functions as inhibiting the synthesis of acetycholinesterease, a neurotransmitter (23).

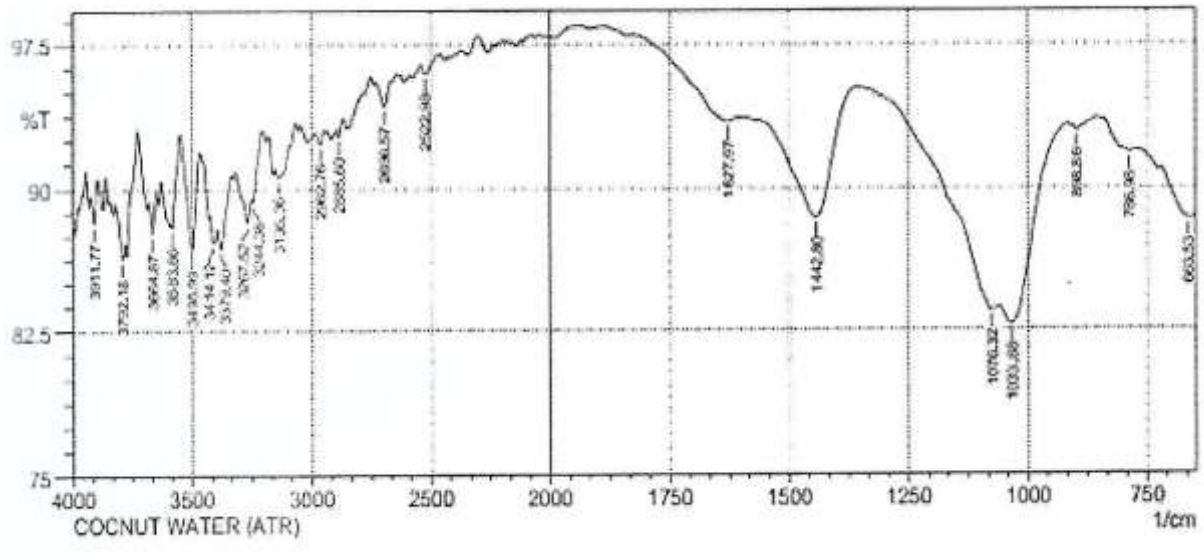

Figure 2: *FTIR Spectrum of Unripe Coconut Fluid*

The Fourier infrared Spectroscopy shows the presence of four N-H functional groups within the range of 3500cm^{-1} to 4000 cm^{-1} at 3583.88 cm-1 3664.87 cm$^{-1,}$ 3792.18 cm^{-1} and 3911.73cm^{-1} respectively. This indicates that the bioactive compounds within this range have nitrogen heteroatoms. Other functional groups of interest identified include; O-H at 3267.52 cm^{-1}, 3372.18 cm^{-1} 3414.99 cm^{-1} and 3499.99 cm^{-1} which could be due to alcoholic, carboxylic compounds. These functional groups corroborated the GC-MS spectrum.

The results of the antimicrobial sensitivity test and that of the minimum inhibitory concentration is shown in table 2 and 3 respectively.

Table 2: *Antimicrobial Sensitivity Test*

Test Sample/ Serial Dilution	**Test Organisms**			
(+ve) and (-ve) Control (%)	E.coli	S. Aures	C. Spp	A. Fumigatus
100	+	+	+	-
50	+	+	-	-
25	+	+	-	-
12.5	+	+	-	-
6.25	+	+	-	-
+ve Control	+	+	+	+
-ve Control	-	-	-	-

Keys: + Sensitive, - Not sensitive, 100% Full strength of Unripe Coconut Fluid, 50, 25, 12.5, 6.25 are serial dilution of UCF per mL of sterilized water, +ve control; Ciprofloxacin and Ketoconazole tablet for bacteria and Fungi respectively, -ve control; sterilized water.

Table 3: *Zone of Inhibition (ZI) Results (mm)*

Test Sample/Serial Dilution	**Test Organisms**			
(+ve) and (-ve) Control (%)	E.coli	S. Aures	C. Spp	A. Fumigatus
100	23.70 ± 0.5	8.00 ± 0.5	6.67 ± 0.2	-
50	11.90 ± 2.0	7.10 ± 0.7	-	-
25	11.00 ± 0.7	6.40 ± 0.1	-	-
12.5	5.90 ± 0.5	6.00 ± 0.1	-	-
6.25	5.50 ± 0.6	5.80 ± 0.1	-	-
+ve Control	29.70 ± 0.3	20.30 ± 0.1	43.80 ± 0.4	17.02 ± 2.70
-ve Control	-	-	-	-

Keys: - Not sensitive, 100 % Full strength of Unripe Coconut Fluid, 50, 25, 12.5, 6.25 are serial dilution of UCF per mL of sterilized water, +ve control; Ciprofloxacin and Ketoconazole tablet for bacteria and fungi respectively, -ve control sterilized water.

From table 2, it is shown that the Unripe Coconut Fluid exhibited sensitivity against both Gram positive and Gram-negative bacteria even at the lowest concentration. It also shows sensitivity to Candida Spp, a fungus at its full strength. However, on serial dilution, sensitivity was not observed.

Table 3 shows the zone of inhibition of unripe coconut fluid. The result indicate sensitivity for E. coli, S. Aureus and C. Spp respectively but not sensitive to A. fumigatus corroborating the antimicrobial sensitivity.

4. Conclusion

The research established that Unripe Coconut Fluid (UCF) contains phytochemicals that provide proof for biological activities i.e., exhibited antimicrobial activities that validates its pharmacological properties against both gram +ve and -ve bacteria and even fungus. Hence, can be employed in pharmaceutical and cosmetic industries as well as herbal medication.

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