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An Assessment of Medicinal *Cocus Nucifera* Plant Extracts as Natural Antibiotic Phytotherapies.

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Abstract
The natural antibiotic phyototherapeutic value of *Cocus nucifera* were screened against five bacteria isolates (*Staphylococcus. aureus, Escherichia. coli, Streptococcus. pneumoniae, Proteus. mirabilis* and *Pseudomonas. aeruginosa*) using the agar-well diffusion method. The root extracts were screened for antibacterial activities at concentrations of 100µl/ml. The antibiotic value of the extract against the bacterial strains was indicated by the appearance of clear zone of inhibition around the wells. The percentage yield for the various solvent was found to range between 17.32 – 32.60 with ethanol having the highest value (35.60). The susceptibility of the organism to the antibiotic effect of the extract vary among the different organism tested. The highest zone of inhibition was recorded in *Escherichia coli* (13.08±0.14). While the least was observed in *Pseudomonas aeruginosa* (0.08±0.00). Comparison of the antibiotic effect of the extract with some commercial antibiotic. Gentamycin, penicillin and Trimethoprin showed that the commercial antibiotic was found to be more effective in inhibiting the growth of the tested bacterial isolates than the *Cocus nucifera* extract. Phytochemical screening of the extract revealed the presence of saponin, tannis, Glycosides, Alkaloids and flavonoids which has been associated with the antimicrobial activities of several herbs. Thus, these preliminary results support the folkloric claims of *cocus nucifera* preparations are effective against some disease.
Keywords: Natural antibiotic the Phytoetherapies, *Cocus nucifera,* Phytochemical. Corresponding authors: email: immaugo@yahoo.com.

1. Introduction

In many parts of the world, there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases (Williamson, 2001). The use of complementary and alternative medicine has been on the increase extensively over the past fifteen years (Romers *et al,* 2005). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities (Barbour, *et al,* 2004, Yasunaka *et al.,* 2005). In Nigeria, many populations still utilize traditional medicine for their psychological and physical health requirement (Rabe and Van Staden, 1997). However, medicinal plant remedies have not been comprehensively investigated and should be studied for its safety and efficacy (Eloff, 1998). Numerous assays are available to evaluate the safety and efficacy of promising natural products firstly *In-vitro* and later *In-vivo* (Fernell *et al.,* 2004).

As reported by Barbour *et al.,* (2004). Many bacterial pathogens are rapidly becoming resistant to a number of the originally discovered Antimicrobial drugs as a result of indiscriminate the use of these drugs. The Antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1998). There is thus, a continuous search for new antibiotics and medicinal plants may offer a new source of antibacterial agents.

Combinations of two or more antibiotics with different mechanisms of action are sometimes tested in an attempt to improve efficacy through synergy and prevent the development of antibiotic resistance (Beringer, 1999). The increase cost of new and more effective antimicrobial remedies together with their side effects and lack of health care facilities in some rural areas. Makes the search for safer, more effective and affordable alternative remedies imperative (Griggs *et al.,* 2001). The plant investigated in this study (*Cocus nucifera*) is an economic tree planted within living compounds for decorative purpose. Every part of the plant is medicinal. It contains glycerol of caprylic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic acids (Adode, 2002). The bark has been reported to be effective against skin ailments like rashes, black spots, scabies and measles. The root are also effective against fibroid, bronchits and hepatitis (Gill, 1992). The nutshell contains phenols and is antifungal against 3 *Microsporium* and 4 *Trichophyton* species, and 1 *Epidermophyton* specie (Bever, 1986).

2. Materials and Methods

2.1 Plant Extraction

Fresh roots of *C. nucifera* were collected and washed with distilled water and then cut into smaller sizes. 50g of

the plant material was soaked into 200ml 96% ethanol and then left for about 36 hours at room temperature wit occasional shaking. The same amount of the plant material was boiled with 100ml of water and then allowed to cool. The preparations were filter with No. 1 Whatmann filter paper, evaporated to dryness in a steady air current and the residue were exposed to U.V rays for 18 hrs after which it was checked for sterility by streaking on nutrient agar plate. The residue was stored in clean sterile labeled container until there were required.

2.2 Screening Of Extracts for Antibacterial Activity

The extracts were checked for antibacterial activity using the agar well diffusion techniques (Okoli *et al.,* 1989). A 0.5 McFarland equivalent standard of the test organisms were aseptically streaked on the surface of a sterile Muller-Hinton agar plate (Oxoid UK) with a sterile wire loop. A 100µl volume of 200mg/ml concentration of the extracts was used to fill agar wells made in Muller Hinton agar plates. The plates were allowed to stand for 1 hr to allow the "drug" to pre-diffuse into the agar and were incubated at 37^0c for 18-24 hrs. After incubation, the zones of inhibition were recorded to the nearest millimeter (mm).

2.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The MIC was determined by Macro broth dilution techniques. A twofold serial dilution of the reconstituted extract was prepared in a Muller-Hinton broth. 100 µl of 0.5 MacFarland equivalent standards of test organisms were introduce into each of the test tube containing the broth and were incubated at 37^0c for 18-24hrs. After incubation the test tubes were observed for growth by checking for turbidity. The highest dilution of the extract where there was no growth in tube was taken as the MIC. The MBC was determined by transferring nine 0.1ml volume of broth from each macro. broth MIC testing sharing no bacterial growth into plates containing sterile Muller Hinton agar and incubated at 37^0c for 18-24hrs. The MBC was taken as the least concentration showing no growth on subculture.

2.4 Statistical Analysis

Paired sample T-test was conducted to analyze the diameter of the zone of inhibition values are reported as means of duplicate determination ± standard deviation.

3. Results

C. nucifera root contained Flavonoids, Tannins, Saponin and Glycosides. Ethanol extract produces more yield (35.60%) than the hot water extract (17.32%). The pH of the ethanol and hot water extracts are 3.7 and 4.3 respectively.

As observed from table 3, ethanol extracts showed greater activity against the test isolates than the hot water extract with diameter of inhibition zone range of 5.55±0.03 to 13.08±0.14 against that of hot water (0.18±0.08 – 5.71±0.07). *Escherichia. coli* and *Streptococcus. pneumoniae* were mostly inhibited with inhibition zone diameter of 13.08±0.14 and 11.50 ±0.07 respectively by the ethanol extract. *Pseudomonas. aeruginosa* showed resistance to both the extracts.

4. Discussion

The result of this study revealed the presence of bioactive components such as saponin, tannis, flavonoids etc in the plant sample. The presence of these bioactive compounds in plant have been reported to make them relevant as potential therapeutic agents in folkloric medicine (Adobolu and Olalimeyi, 2005). The bioactive compounds of any medicinal plant differ in their solubility depending on the extractive solvents used (Oloke and Kolawole, 1988). Hence, the need to employ broad ranges of extractive solvents in the extraction of possible phytochemical from medicinal plants. As reported by Kordeli *et al.,* (2003), the percentage recovery from plants are dependent on the type of solvent used for extraction. This study reveals that ethanol was the best extracting solvent than hot water. This finding agrees with the result of Jonathan and Fasidi (2003) who reported that ethanol was the best solvent for extracting antimicrobial substances from *Lycoperdon pusidium* and *L. giganteum*. These variations were probably due to the type and nature of bonding present in the solvent in relation to the chemical constituent of the plant. Polar solvents have been show to be more promising in extracting organic and inorganic materials from plant (Campos *et al.,* 2002). Hot water though is the most polar solvent than ethanol was not frontier in the extraction process. This is in accordance with Cowan (1999), who reported that the most active components are generally water insoluble. Hence it is expected that the solvent, ethanol will yield more active extracts.

The antibacterial activity variously exhibited particularly by the ethanol extracts of the root parts of *Cocus nucifera* is significant for two reasons with respect to their traditional medicinal use in south-eastern Nigeria. First, the more active preparations are the ethanolic extracts, the form in which medicinal roots preparations are popularly administered in ethno-medical practice. Secondly, the bacterial strains used in this study which sowed susceptibility to the extracts have been implicated in some of the diseases such as diarrohea, wound infections, bronchitis and food poisoning against which *C. nucifera* is the preferred herbal remedy. Because of the resistant therapeutic problems posed by *Proteus. mirabilis* and *Pseudomonas. aeruginosa*, this study try to check the

antibacterial activity of *C. nucifera* against these bacterial strains and the findings showed that *Proteus. mirabilis* and *Pseudomonas. aeruginosa* are still resistant to hot water extract and ethanol extract of *C. nucifera* despite its recorded antimicrobial activity against other bacteria.

The limited spectrum of activity of the hot water extracts compared with the ethanolic extracts is difficult to explain since both extracts contained relatively the same type of metabolites though not in the same proportions. Perhaps, the paradox may be resolved when the active constituents have been isolated and the molar activity of the purified form determined. At that stage, a study of the interactions between the active and non-active components may throw even more light onto the differential activity of the various extracts.

Agar-well diffusion method used in this study was based on diffusion of soluble constituents of the extract in the agar medium. Consequently, a low zone of inhibition may not actually be a measure of actual activity but may have arisen on account of low solubility of the constituent of the extracts and low rate of diffusion (Jen-chyl *et al.,* 1997).

The antibacterial activities of extracts on the test organisms used compare favourably with that of commercial antibiotics as recorded in table 3. This is because the active compounds in the commercial antibiotics are well refined and concentrated while the active components are not refined thus the presence of impurities might have been responsible for the narrow zone of inhibition exhibited against the Microorganisms (Sayed *et al.,* 1987).

Pharmacological basis for plants must be thoroughly screened before they can be fully exploited as sources of therapeutic agents. The tests are necessary so as to identify or ascertain the potentials of the plants *C. nucifera* root extract.

5. Conclusion

The present study has verified the usefulness of *C. nucifera* root extracts for medicinal purposes. This partly explained the use of these plant materials in herbal medicine. As rich source of Phytochemical, *C. nucifera* can be seen as a potential source of useful drugs.

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Table 1: Yield (mg) and pH of the extracts of *C. nucifera*

Solvent of extraction	Yield (mg)	Percentage yield	pH
Ethanol	17.80	35.60	5.7
Hot water	8.66	17.32	4.3

Table 2: Preliminary photochemical screening of the root extract of *C. nucifera*

	Solvent of extraction	
Component	**Ethanol**	**Hot water**
Alkaloids	-	-
Flavonoids	+	-
Tannins	+	-
Saponin	+	+
Glycosides	+	+
Reducing sugar	-	+
Anthraquinone	-	-

Key: - = not detected, + = present.

Table 3: Mean zone inhibition (mm) of root extracts of *Cocus nucifera*

Test isolates	Extracts		Control antibiotics		
	Ethanol extract	**Hot water extract**	**Genta.**	**Pen.**	**Tri.**
E. coli	13.08± 0.14	0.18± 0.08	31.31±0.01	24.78±0.08	6.79±0.12
S. aureus	5.55±0.03	3.04±0.01	26.50±0.06	13.17±0.09	7.29±0.09
S. pneumoniae	11.50±0.07	5.71±0.07	29.17±0.08	13.73±0.06	6.94±0.04
P. mirabilis	9.45±0.21	1.30±0.06	25.85±0.01	9.07±0.02	5.07±0.01
P. aeruginosa	0.08±0.00	0.23±0.01	14.75±0.21	4.49±0.01	2.61±0.06

Key: Genta = Gentamycin, Pen = penicillin, Tri = Trimethoprin

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract of *C. nucifera* (mg/ml).

Test isolates	Ethanol extracts			Hot water extracts		
	MIC	**MBC**	**MIC/MBC**	**MIC**	**MBC**	**MIC/MBC**
E. coli	100	>100	<1.00	>200	>200	<0.50
S. aureus	100	>200	<0.50	>100	>200	<0.50
S. pneumoniae	50	>100	<0.50	>100	>200	<0.50
P. mirabilis	100	>200	<0.50	>200	>200	<0.50
P. aeruginosa	200	>200	<0.50	>200	>200	<0.50