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Phytochemical Screening, Antimicrobial and Elemental Analyses of Crude Extracts from *Cocos nucifera* (Coconut) Shell

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Abstract

Crude extracts from the shell powder of Cocos nucifera (coconut) - a perennial large palm, were obtained by successive extraction using the solvents n-hexane, ethyl acetate, dichloromethane and ethanol and water. These were subjected to antibacterial activities, phytochemical screening and elemental analysis. It was found that the crude extracts contained alkaloids, flavonoids, glycosides, saponins, steroids, tannins and phenols; these were more present in the solvents with higher polarity, terpenoids were completely absent in all the extracts. In addition, the antimicrobial activities of the crude extracts of Cocos nucifera shell were investigated using eight test including Staphylococcus aureus, Escherichia coli, Pseudomonas aeriginosa, Klebsiella pneumonia, Candida albicans, Aspergilus funigatus, Cryptococcus neoformans and Aspergillus subtilis. The highest zones of inhibition were observed for ethanol and water crude extracts. The crude extracts of dichloromethane, ethyl acetate and n-hexane showed no susceptibility for all the test organisms. Fluconazole was used as control for the fungi while Levofloxacin and dimethylsulfoxide (DMSO) were the positive and negative control for the bacteria respectively. The elemental analysis showed high concentration of lead (Pb) 0.237 mg/L and copper (Cu) 0.331 mg/L; nickel (Ni) and chromium (Cr) were not detected. The results suggested that the crude extracts of Cocos nucifera might contain some biologically active chemical compounds which could be further explored.

Keywords: Antimicrobial activity, Cocos *nucifera,* Crude extracts, Phytochemical screening, Shell powder

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INTRODUCTION

Cocos nucifera is a member of the family Arecaceae (palm family), the only accepted species in the genus *Cocos* and commonly called coconut a term used to refer to the entire coconut palm, the seed, or the fruit, which, botanically, is a drupe. In Nigeria, it is locally called kwakwa (Hausa), Agbon (yoruba), Aki beke (Igbo) and Kyewe (Tiv). It is a large palm, growing up to 30 m (98 ft) tall, with pinnate leaves 4 – 6 m (13 – 20 ft) long, and pinnate 60 – 90 cm long; old leaves break away cleanly, leaving the trunk smooth. It is found throughout tropic and sub tropic area and known for its great versatility as seen in the use many parts. Coconuts are daily diets of many people, it is different from any other fruits because it contains a large quantity of "water" and when immature it is known as tender-nut or jelly-nut and may be harvested for drinking. When matured, it still contains some water and can be used as seed nuts or processed to give oil from the kernel, charcoal from the hard shell and coir from the fibrous husk (Nevin & Rajamohan 2004). The coconut has three layers: the exocarp, mesocarp, and endocarp; the exocarp and mesocarp make up the "husk" of the coconut. Coconuts sold in the shops of non-tropical countries often have had the exocarp (outermost layer) removed. The mesocarp is composed of a fiber, called coir, which has many traditional and commercial uses. The endocarp (shell) has three germination pores (stoma) or "eyes" that are clearly visible on its outside surface once the husk is removed (Bourke and Tracy 2009).

It has been reported that coconuts and their husks can be converted into useful energy in three ways. First, the oil in the copra of the coconut can be expelled and used to make coconut bio-diesel to fuel a diesel generator to make electricity. Alternatively, the coconut oil can be burned directly in a modified diesel generator. Second, the coconut husk and shell might be used in a biomass converter to produce combustible gases that can then be used in a gas turbine to produce both electricity and heat. Third, the coconut shell can be converted to charcoal and combustible gases to be used in cooking or heating (Walter 2006).

Fixed-bed slow pyrolysis experiments of coconut shell have been conducted to determine the effect of pyrolysis temperature, heating rate and particle size on the pyrolysis product yields. The results indicate that the effects of pyrolysis temperature and particle size on the pyrolysis yield are more significant than that of heating rate and residence time. The various characteristics of pyrolysis oil obtained under the optimum conditions for maximum liquid yield were identified on the basis of standard test methods (Ganapathy *et al*., 2009). A new compound identified as propyl(2-nitromethylphenyl)ethanoate was isolated from the mesocarpic fiber (coir) of dried coconut (*Cocos nucifera*) husk using soxhlet extraction method (Sofowora 1993).

In a study, coconut shell powder was used as a biosorbent for the removal of Cu (II) from aqueous solutions with the adsorption capacity investigated by batch experiments. The influence of pH and particle size was studied and adsorption isotherm models were fitted. An appropriate kinetic model was also attempted leading to the achievement of high adsorption (>90 %) (Ramesh and Ramesh 2012). Verma *et al.,* (2012) obtained and identified active bio-components as tocopherol, palmitoleyl alcohol, cycloartanol and β-sitosterol from *Cocos nucifera* mesocarp powder and their anti-bacterial activity was determined using disc diffusion method. *Cocus nucifera* shell extracts is been used by locals of some states in the north central zone of Nigeria for antimicrobial, anti-ulcer and anti-inflammatory purposes among others uses. This research work investigated the possible phytochemicals present in different extracts from the shell of *Cocos nucifera* and evaluated their biological activities as a possible lead to the discovery of new drugs.

MATERIALS AND METHODS

Cocos nucifera fruits were obtained from Gombe local market, North Eastern Nigeria. They were dehusked, cleaned and opened up to remove the endocarp. The shells obtained were further cleaned, dried and crushed into powder and stored in sterile containers for use.

Accurately measured quantities of *Cocos nucifera* shell powder were exhaustively extracted with analytical grade of *n*-hexane, ethyl acetate (ETOAc), dichloromethane (CH_2Cl_2) and ethanol (EtOH) in increasing polarity. Water was also used to extract separately mimicking the process used by the locals. The resulting extracts were concentrated to recover solvents and to obtain solvent-free extracts which were used for various screenings.

Phytochemical Screening

Standard methods were adopted for qualitative detection of some important phyto-constituents as described by Sofowora, (1993) and Trease and Evans, (2002).

Test for Alkaloids

The aqueous (3ml) was stirred with (3ml) of 1% HCl on a steam bath. Meyer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids

Test for Terpenoids

The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids on both extract.

Test for Flavonoids

About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids on both extract.

Glycosides

10 cm_3 of 50% H_2S0_4 was heated in boiling water for 5 min. 10 cm_3 of Fehlings solution (5 cm_3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

Test for Steroids

Acetic anhydride (2 ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2 ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids on both extract.

Test for Phenols

To 1 ml of solvent extracts, 2 ml of distilled H_2O was added. To this, a few drops of neutral 10% $FeCl_3$ solution was added. Formation of a dark green colour indicated the presence of phenols

Test for Saponin

About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of Saponins in Methanol while red in Distilled water.

Test for Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue solution indicated the absence of tannins in distilled water and dark green colour indicating presence in methanol.

Antimicrobial screening

Standard methods described by (Sanchez *et al*., 2005 and Ochei, & Kolhatkar, 2007) was adopted to test for the susceptibilities of some microorganisms obtained from Abubakar Tafawa Balewa Teaching Hospitals (ATBU) with standard codes: *Staphylococcus aureus* (ATCC 25921), *Escherichia coli* (ATCC 25922), *Pseudomonas aeriginosa* (ATCC 26733), *Klebsiella pneumonia* (ATCC 33521), *Candida albicans* (ATCC 67821), *Aspergilus funigatus* (ATCC 67822), *Cryptococcus neoformans* (ATCC 68721) *and Aspergillus subtilis* (ATCC 68722).

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ensitivity test of crude extact of Agar Well diffusion method

The organisms used were standardized using Mc-Farland turbidity standard scale 1,to obtain a bacterial cell density of 106 colony forming unit milliliter(cfu/mL).The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextro agar plates respectively for the bacterial and fungal growth. For wells were punched on the inculated plate with a cork borer (8mm in diameter). The wells were properly labeled according to different number of concentration prepared. The well were then fill up with the extracts about 0.2Ml per well. The plates were allowed to stay on the bench for 1hr for extract to diffused on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37^{o}C for three days were the potato dextro agar plates for fungi were incubated at room temperature (drawer) for three days. At the end of incubation period, all the plates were observed for any evidence of inhibition, which will appear as a clear zone that were completely devoid of growth around the wells (zone of inhibition). The diameters of zones were measured with transparent ruler calibrated in millimeter (mm).

RESULTS

This study has revealed the presence of phytochemicals (secondary metabolites) considered as active medicinal chemical constituents (Table 1).

Table 1: Result for Phytochemical Screening of the Crude Extracts

Phytochemicals	Crude extracts					
	Hexane	EtOAc	CH_2Cl_2	Ethanol	Water	
Alkaloids	+	+ + +	-	+ +	+ +	
Terpenoids	-	-	-	-	-	
Flavonoids	+	+ + +	+	+ + +	+ +	
Glycosides	+	+ +	-	+ +	+	
Phenols	-	+ +	+	+ +	+	
Saponins	+	+ + +	-	+ + +	+ +	
Steroids	+	+ +	+	+ + +	+ +	
Tannin	-	+ + +	+	+ + +	+ +	

Key: + + + Highly present, + + moderately present, + slightly present, - Completely absent

The antimicrobial activities of the ethanol and water crude extracts as shown in Table 2 and 3 revealed significant activities against the test organisms used.

Table 2: Result for Antibacterial Activities of the Crude Extracts

Test organism	Zone of inhibition (mm)						
	Hexane	EtOAc	CH_2Cl_2	Ethanol	Water	DMSO (-) control	Levofloxacin (+) control
S. aureus	0.00	0.00	0.00	14.00	10.00	0.00	20.00
P. aeruginosa	0.00	0.00	0.00	18.00	13.00	0.00	20.00
K. pneumonia	0.00	0.00	0.00	0.00	5.00	0.00	20.00
E. coli	0.00	0.00	0.00	0.00	7.00	0.00	20.00

Table 3: Result for Antifungal Activities of the Crude Extracts

Test organism	Zone of inhibition (mm)					
	Hexane	EtOAc	CH_2Cl_2	Ethanol	Water	Fluconazole (+) control
C. albicans	0.00	0.00	0.00	6.00	5.00	20.00
A. fumigatus	0.00	0.00	0.00	0.00	8.00	20.00
C. neoformans	0.00	0.00	0.00	4.00	4.00	20.00
A. subtilis	0.00	0.00	0.00	0.00	6.00	20.00

Table 4: Result for Elemental Analysis *Cocos nucifera* Shell

Elements Determined	Concentration (mg/L)
Copper (Cu)	0.311
Lead (Pb)	0.237
Chromium (Cr)	0.00
Nickel (Ni)	0.00

DISCUSSION

Different phytochemicals have been found to possess a wide range of activities which may help in the protection of chronic diseases (Mir *et al.*, 2013). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponin, steroids etc. Hence presence of these medicinal chemical constituents in the extracts justifies its antimicrobial, anti-ulcer and anti-inflammatory properties, which confirms the claim of the local users that the plant can be used for the treatment of ulcer.

The *Cocos nucifera* are known to carry out important medicinal roles in human body as a result of the presence of flavonoids in the extracts. Flavonoids have inherent ability to modify the body's reaction to allergens viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial, antioxidants, Salah *et al*.; 1995 Okwu 2004 (Cushnie and Lamb 2005, Salah *et al*.; 1995 Okwu 2004). *Cocos nucifera* is a very useful medicinal plant because saponins were detected. There is tremendous, commercially driven promotion of saponin as dietary supplements and nutriceuticals. It can be emphasized that mistletoe leaf has a medicinal value since it contain saponin which is useful in medicine and pharmaceutical due to its foaming ability that produce frothy effects in the food industry (George 1965). The presence of terpernoids was detected in the mistletoe leaf and hence can be used in herbal medicines (Edeoga *et al.;* 2005).

The antimicrobial activities of the ethanol and water crude extracts as shown in Table 2 and 3 revealed significant activities against the test organisms used. The non activities of the ethyl acetate and dichloromethane could probably be as a result of low concentration (100 µL of 4 mg/mL) of the extracts used. The ability of the crude extracts to inhibit the growth of several bacterial and fungal species is an indication of the wide spectrum antimicrobial potential of *Cocos nucifera* which makes the plant an important source for bioprospecting antibiotic and

antifungal drugs. Earlier studies reported that the presence of tannins, alkaloids, flavonoids, saponins and steroids in plants inhibited the growth of these micro organisms (Kendeson *et al*., 2014).

The presence of copper (Cu) and lead (Pb) as indicated in Table 4 shows that the shell of *Cocos nucifera* could be due to pollution and geographical environment of the plant, hence detrimental to health as the action level for Pb and Cd as recommended by WHO has been exceeded (Abdulkadir *et al*., 2017).

CONCLUSION

This present work reveals the phytochemicals present in *Cocos nucifera* shell which are alkaloids, flavonoids, glycosides, saponins, steroids, tannins and phenols; these phytoconstituents provide proof for the biological activities - antimicrobial and antiulcer exhibited by the plant shell and validates the pharmacological properties. But with this research it can be concluded that the plant shells not only have antibacterial activity but also antifungal activity which can be useful for the preparation of antibacterial and antifungal cream. It is very helpful for researcher to explore more about this valuable plant especially the shell which has not fully been explored in this aspect.

REFERENCES

Abdulkadir, A. G., Ibrahim H., Kendeson, A. C, and Abdullahi, Y (2017). Comparative Analysis of Physicochemical Parameters of Borehole, Pond and Well Water in Kashere Metropolis. *European Journal of Engineering Research and Science*. 2(6), 42-27.

Bourke, R. M. and Tracy H (2009). Food and Agriculture in Papua New Guinea. Australian National University. Pp; 327.

Cushnie, T. P. T, Lamb, A. J (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents,* **26** (5): 343–356.

Edeoga, H.O.; Okwu, D. E. and Mbaebie, B.O. (2005). Phytochemical Constituent of Some Nigerian plants, *African Journal of Biotehnology*, 4 (7), 685-688.

Ganapathy, S. E. and Natarajan, E (2009). Pyrolysis of Coconut Shell: An Experimental Investigation. *The Journal of Engineering Research*. 6(2) 33-39.

George, A.G. (1965). Legal status and toxicity of saponins in food and cosmetics. *Toxicol*., **3**: 85-92

Ibidun, J.; Olatunji, G. and Atolani, O (2009). Propyl ethanoate derivative from coconut coir, *Research Journal of Applied Sciences.* 4(6), 217-230.

Kendeson, A. C., Isyaka, M. S. and Dashak, D. A (2014). Antimicrobial Activity of the Crude Ethylacetate and Methanol Extacts from the Root Bark of *Jatropha Curcas* (LINN). *International Journal of Scientific and Research Publications*. 4(10), 1-3.

Mir, M. A., Sawhney, S. S. and Jassal, S. M. M (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacun officinale.Wudpecker Journal of Pharmacy and Pharmacology* 2(1), 001-005.

Nevin, K. G and Rajamohan T (2004). Beneficial effects of virgin coconut oil on lipid parameters and *in vitro* LDL oxidation. *Clinical Biochemistry.* 37(9), 830-835.

Ochei, J and Kolhatkar, A. (2007). Medical Laboratory Science. Theory and Practice. 6th Ed. Tata McGraw-Hill publishing company ltd. pp 801-807.

Okwu, D.E, 2004, Phytochemical, and vitamin contents of two indigenous species of South Eastern Nigeria J. Sustain Agric. Environ, 6: 30-34

Ramesh, P. and Ramesh, A (2012). Study of Biosorption of Cu (II) from Aqueous Solutions by Coconut Shell Powder. *Chemical Sciences Journal.* 17, 1-9.

Salah, W, Miller, N.J, Pagauga,G., Tijibung, Bolwel, A.P., Rice, E and Evans, C. (1995) Prlyphenolic flavonis as scavenger of aqueous phase radicals as chain breaking oxidant. Arch Biocem. Biorh, 2:339-346

Sanchez, N. R.; Garcia,D. A.; Shiavini, M. S.; Nakamura, C. V and Filho, B. P. D (2005). An evaluation of antibacterial activities of *Psidium guajava*. *Brazilian Journal of Biotechnology*. 48, 429-436.

Sofowora, A. E (1993). Medicinal Plants and Traditional Medicines in Africa. 2nd edition. Spectrum Books, Ibadan, Nigeria. Pp. 2-5, 97-145, 289.

Trease, G. E. and Evans, W. C (2002). Pharmacognosy. Harcourt Publishers Ltd., London. Pp. 26, 127, 147.

Ushie O.A., Egwaikhide P.A., and Longbab B. D. (2016) Phytochemical Screeing and Antimicrobial Activity of Tamarindus indica. *International Journal of Traditional and Complementary Medicine (*2): 10010-0017

Verma, V., Bhardwaj, A., Rathi, S. and Raja, R.B (2012). A Potential Antimicrobial Agent from *Cocos nucifera* mesocarp extract; Development of a New Generation Antibiotic, *ISCA Journal of Biological Sciences*. 1(2), 48-54.