

Mineral Composition, Phytochemicals and Anti-microbial Activity of Coconut Water (Cocos nucieferal) on Candida albicans and Lactobacillus acidophilus

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Accepted June 10 2021

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ABSTRACT

The phytochemicals and anti-microbial activity of coconut water on Candida albicans and Lactobacillus acidophilus and also its mineral composition were investigated for its medicinal potential. The study revealed the presence of Pb, Cu, Zn, Cd, Fe, Ca, Na, K, Mg and Mn. The concentration of the elements in ppm were as follows Pb (0.058), Cu (0.012), Zn (0.202), Cd (0.028), Fe (0.109), Ca (0.368), Na (1.300), K (1.740), Mg (0.947), Mn (0.229). Saponins, alkaloids, steroids, cardiac glycosides and carbohydrates were the phytochemicals detected. The coconut water was tested against Candida albicans and Lactobacillus acidophilus using Agar well diffusion method. Inhibitory zone diameter ranges from 13-25 mm. The results showed a concentration-dependent antimicrobial activity against the tested organisms. Inhibitory activity exhibited by coconut water against these micro-organisms is an indication of the presence of bioactive compounds which can be identified, isolated and incorporated into modern oral care systems for controlling dental caries.

Keywords: Coconut water, phytochemicals, Candida albican, Lactobacillus acidophilus.

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INTRODUCTION

Coconut (*Cocos nucifera*) is a common fruit in the tropics cultivated in nearly 90 different countries (Pires et al., 2004). The endosperm contains water referred to as coconut water, which contains carbohydrates (glucose, fructose and sucrose), vitamin C, minerals, amino acids, enzymes, hormones, and phytochemicals (Bandalam and Galvez, 2016).

Coconut is cultivated for its multipurpose values (nutritional and medicinal). It is a unique source of various natural products for the development of drugs and industrial products (Floriana et al., 2015). It was reported to have antioxidant properties (Manisha and Shyamapada, 2011).

Coconut water has been used to treat various ailments, it is used as antibacterial, antifungal, antiviral and antidermatophytes agents (Fawole and Oso, 2004). The antimicrobial property of coconut water is because of its high lauric acid content which has been used as a medication for certain oral infections. Several studies reported that sucrose monolaurate and glycolipid compound present in coconut water has anti-caries properties (DebMandal and Mandal, 2011). This effect is probably due to condensed glycolysis and sucrose oxidation in a non-competitive method affected by streptococcus mutants and hence inhibits in-vitro dental plaque.

Due to the antimicrobial resistance of microorganism to antibiotics as a result of broad-spectrum antibiotics and immunosuppressive agents and other factors, the use of plants as an alternative is being studied globally especially in developing countries like Nigeria, since plants are considered nutritionally safe, biodegradable and possess antimicrobial phytochemicals (Berahou et al., 2007; Chika et al., 2007). Plants have also been reported to be an excellent source of secondary metabolites which can be used in the production of modern medicines (Chandra et al., 2017). Many of the secondary metabolites such as tannins, flavonoids, alkaloids have been demonstrated in many studies to have antimicrobial activities (Akinpelu et al., 2015; Akinyemi et al., 2006).

Phytochemicals are bioactive compounds produced by plants as a result of primary or secondary metabolism (Molyneux et al., 2007). Some phytochemicals have

been used as poisons and others as traditional medicines. Phytochemicals present in plant such as alkaloids, phenols, steroids, saponins, cardiac glycosides, flavonoids, etc, have played several roles in disease prevention (Babosaa et al., 2013). Chemical compounds derived from plants play important role in preventing activities such as anti-inflammatory, antianti-aging. antimicrobial. diabetic. anti-parasitic. antidepressant, anticancer, antioxidant and wound healing (Bahramsoltant et al., 2018).

Priyanka et al. (2017) reported that candidiasis is a widespread oral and perioral opportunistic infection that usually results from overgrowth of endogenous Candida fungal microorganisms. Though many species of Candida exists, *Candida albicans* is the most frequently (>80%) encountered organism in the ambulatory general practice in dental patient. It is clinically presented in various forms such as angular chelitis, denture stomatitis, hyperplasic (*candidial leukoplakia*), chronic atrophic (erythematous), median rhomboid glossitis, inflammatory papillary hyperplasia, muco cutaneous and pseudo membranous (Greenberg et al., 2008; Dangi et al., 2010).

De Vos et al. (2009), described *Lactobacillus acidophilus* as a normal flora commonly found in the human mouth, vagina and gastrointestinal tract. They are gram-positive, obligate homo-fermentative and produce lactic acid. Lu, (2020), ascertained that it is a well-documented probiotic species with numerous health benefits. *Lactobacillus acidophilus* is commonly used with a starter culture to make probiotic yogurt. The end products from *Lactobacillus acidophilus* are lactic acid, acetic acid and H_2O_2 , which make the intestinal environment become less favorable for pathogens. Therefore, this study was carried out to determine the mineral composition, phytochemical and antimicrobial activity of coconut water on *Candida albican* and *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Sample collection

The coconut fruits were obtained from Chaha household garden in Jos South Local Government Area of Plateau State, Nigeria.

Collection of coconut water

Coconut water was collected by cracking the coconut shell and the water drained directly into Buchner funnel filled with a Whatman filter paper 42 mm. The filtrate was transferred into 250 ml screw-capped plastic bottles and stored in the refrigerator at 40°C.

Collection of isolates

The microbial isolates were collected from National Veterinary Research Institute (NVRI) Vom stock culture. They were cultured on nutrient agar and incubated at 37°c for 24 hours before usage.

Confirmation of isolates

The identification of Lactobacillus acidophilus isolate

was confirmed after incubation using morphological cultural and biochemical characteristics which include gram staining and catalase test while *Candida albican* was identified and confirmed after incubation using morphological cultural and biochemical characteristics which include gem tube test and sugar fermentation test.

Preparation of standard inoculums

Suspension of each test organism was made by inoculating a loopful of a colony into peptone water and incubated at 37° C for 24 hours. The overnight broth culture of the organism was diluted with sterile distilled water to inoculum loads of 1×10^{6} cfu/ml. It was standardized by comparing the turbidity with 0.5 mc far land turbidity standards (Murray et al., 2016).

Antimicrobial susceptibility test

Antimicrobial susceptibility test was done using agar disc diffusion method (Abbas et al., 2017). Different concentrations (12.5, 25, 50, 100%) of the coconut water sample were made in a sterile universal container. A sterile Whatman no. 1 filter paper disc was impregnated with an appropriate concentration of coconut water sample. The disc (equally spaced) were placed on a freshly and uniformly inoculated nutrient agar plate. The plate was incubated at 37°C for 24 hours. After incubation plates were examined for zones.

Sample digestion for mineral analysis

Digestion of coconut water was performed by optimizing the procedure of Hseu (2004). About 5 ml of coconut water was measured into a digestion flask which was followed by the addition of 5 ml HNO₃ and 2.5 ml of per chloric acid. This was heated on a digester in a fume cupboard at the temperature of 125°C until a transparent color was obtained. This was made up to 100 ml with deionized water.

Determination of mineral in the coconut water

The digested sample was analyzed for minerals using atomic absorption spectrophotometer (buck scientific model 210 VGP) and flame photometer (Jenway model).

Preparation of extracts for phytochemicals screening

This was done according to the method reported by Osuagwu and Eme (2013).

Acidic extract preparation

10 ml of 2 M HCl was added to 10 ml of coconut water in a beaker, covered and allowed to stand for 20 minutes. Filtration was done and the filtrate was set aside for analysis.

Alcohol extract preparation

10 ml of coconut water was measured into a flask containing 10 ml of methanol, covered and left for 30

 Table 1: Mineral element concentration (ppm) of coconut water.

Mineral element	Concentration (ppm) in Coconut Water
Pb	0.058
Cu	0.012
Zn	0.202
Cd	0.028
Fe	0.109
Ca	0.368
Na	1.300
К	1.740
Mg	0.947
Mn	0.229

PPM: Part Per Million.

minutes. It was filtered, poured into porcelain dish and evaporated on a water bath. It was removed and allowed to cool followed by the addition of 10 ml of chloroform, scratched and stirred to obtain a homogenized mixture.

Phytochemical screening

The phytochemical screening was done according to the method reported by Osuagwu and Eme (2013) as follows:

Saponins

About 1 ml of coconut water was shaken with water in a test tube. A layer of foam indicated the presence of saponins.

Alkaloid

About 1 ml of acid extract was measured into a test tube followed by the addition of few drops of picric acid. The appearance of precipitate indicated the presence of alkaloids.

Steroids

About 2 ml of the methanolic extract was measured into a test tube followed by 2 drops of H_2SO_4 on the wall of the test tube (to prevent boiling of the acid). A reddishbrown ring indicated the presence of steroids.

Cardiac glycosides

3 drops of ferric chloride were added to 1 ml of the acid extract in a test tube. The formation of a brown ring indicated the presence of cardiac glycosides.

Carbohydrate

2 ml of the coconut water was measured into a beaker and heated with 1 ml of H_2SO_4 blackness and effervescence indicated the presence of carbohydrate.

Data analysis

Data obtained were analyzed using SPSS version 23. One way Anova was used to test the significant difference among the concentrations. The results were considered significant at 95% and P<0.05.

RESULTS AND DISCUSSION

The result of mineral analysis of the coconut water in Table 1 revealed the presence of microelements such as Mn, Fe, Zn, Cu, and macro elements such as Mg, Na, Ca and K. These elements played important roles in the body metabolism. Though two heavy metals were detected during the analysis. This may be as a result of the geographical location. The high concentration of Na and K made it a good electrolyte and can be used in protection against dehydration and maintenance of osmotic pressure in the body. The use of coconut water in dehydration as a sport drink and in the treatment of a number of diseases such as congestive cardiac diseases, diabetes and infectious diseases has been reported (Bruce, 2005; Adam and Bratt, 1992). Na also plays a role in the normal irritability of muscles and cell permeability (Grim et al., 1987) while K is a major cation of intracellular fluid involved in protein synthesis, calcium on the other hand is an indispensable component of the bone and teeth. These hold their hardness and strength to the presence of calcium (Arnaud, 1988). Other minerals such as Zn, Fe, and Mg were also detected in appreciable quantities. Mg helps to move calcium and potassium into muscles to aid in contraction and relaxation. It also helps in energy production and supports organ function (Mandy, 2017). Fe is a constituent of hemoglobin, myoglobin and several enzymes, therefore is an essential nutrient to humans (Bothwell et al., 1979). The presence of iron in the coconut water analyzed might be connected to the ironrich minerals in the soil.

Lead (Pb) is said to be the commonest metal found in coconut water. It is known to be harmful to the body especially in children at high concentrations; it causes convulsions, brain attacks, central nervous system coma and even death. According to the U.S Center for Diseases Control and Prevention and WHO, a blood 10 ug/l or above, is a cause of concern. However, the value of lead obtained in the result as shown in Table 1 is low. Thus, the consumption of coconut water is considered safe. Cadmium (Cd) is also poisonous and toxic to the human body. Zinc is necessary for the activity of over 300 enzymes that aid in metabolism, digestion, nerves function, development and also function in immune cells, skin health, DNA synthesis and protein production (Jillian, 2018). Since it is essential element coconut water can be a source of zinc based on the amount found in the course of this research.

Table2:Qualitativephytochemical screening.

Parameters	Results	
Saponins	+	
Tannins	+	
Alkaloids	+	
Steroids	+	
Cardiac glycosides	+	
Carbohydrates	+	
Kev: +: Detected		

Table 3: Anti-microbial activity of coconut water on Candida alibican and lactobacillus acidophilus.

	Concentration	n of the sample	Control Gentamycin			
Organism	12.5	25	50	100	(20mg/ml)	P- value
Candida albicans	0.00 ±0.00 D	0.00 ±0.00 D	15.00±0.141C	25.00±0.707B	36.00±141A	0.000
lactobacillus acidophilus	0.00 ±0.00 D	0.00 ±0.00 D	13.00±0.57C	25.00±0.707B	35.00±0.70A	0.000

Mean \pm SD along each row with different letters are significantly different.

The anti-microbial activity of coconut water in Table 3 shows that concentrations of 100 % and 50 % were able to inhibit the activities of Candida albicans and Lactobacillus acidophilus. The inhibition efficacy of coconut water can be attributed to the presence of phytochemicals, such as saponins, steroids, cardiac glycosides, alkaloids and carbohydrates as shown in Table 2. Saponins have been reported in several studies to inhibit the growth of candida albican and lactobacillus acidophilus. Ojha and Gupta (2003) reported that saponins extract obtained from Sapindus fruits were active against lactobacillus acidophilus and depend on the dosage. In a study by Arora and Sood (2017) cardiac glycoside exhibited a broad range of antimicrobial potential with inhibition zone ranging from 13-35 mm on candida albican. Noguti et al. (2019) reported that alkaloids have positive activity against Candida albican. These isolates were reported to be common causes of dental caries among others. The results in Table 3 also show that the antimicrobial efficacy of coconut water was significantly lower when compared to the positive control that is gentamycin which is used as a gold standard against other antimicrobial agents.

Conclusion

Cocos nucifera (coconut) is a widely dispersed plant that has important pharmacological effects with low toxicity. Medicinal use of *cocos nucifera* has an environmental appeal since this plant is widely used in the food industry. The pharmacological effects of the plant differ according to the plant or fruit used. Within the limitations of the study, coconut water was proved to be a valuable antifungal and antibacterial agent against *Candida albicans* and *Lactobacillus acidophilus*.

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