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The Evaluation of the In-Vitro Antibacterial and Anti-Inflammatory Potentials of the Tender Water of *Cocosnucifera* (*L.*)

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ABSTRACT

The aim of this study was to evaluate the antibacterial and anti-inflammatory potentials of the tender water of Cocosnucifera (L.). Antibacterial potentials of tender coconut water were assessed utilizing the agar well diffusion method, and the anti-inflammatory test was carried out by the Human Red Blood Cell (HRBC) membrane stabilization method, and the supernatant solution estimated using a UV-Visible spectrophotometer at a wavelength of 560 nm.The highest antimicrobial activity was observed against Pseudomonas aeruginosa with the minimum inhibitory concentration (MIC) being 50% concentrate of tender coconut water. The anti-inflammatory results revealed a notable anti-inflammatory activity of 30% as measured by hypotonicity induced hemolysis of the HRBC membrane using the UV-visible spectrophotometer at a wavelength of 560 nm. Qualitative phytochemical analysis indicated that tender coconut water contained major bioactive compounds such as alkaloids, polyphenols (flavonoids), and terpenoids which indicate high potentials of Cocosnucifera (L.) as a resplendent material for future ethnopharmacological research.

Keywords: *Antibacterial, Anti-inflammatory, Potentials, Tender water, Cocosnucifera*

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INTRODUCTION

Mankind has used herbal plants therapeutically, as plant, animal, and mineral resources have been key sources of drugs. As the world turns once more to natural products as safer remedy for ailments, ongoing research is increasing into use of plants (and their derivatives) which have been claimed to possess preventive and therapeutic effects. The study of medicinal plants and plant derivatives used in folklore remedies have therefore attracted great attention in the field of drug making in an attempt to find possible solutions to the diseases that plague mankind.

Pinner *et al*. in 1996 reported that infectious diseases are on the rise in developed countries, thus the search for antibacterial properties in higher plants is being pursued. DebMandal *et al*. in 2011 reported that there exists an increasing problem of resistance to existing synthetic and conventional antibacterial agents as well as emergence of multi-drug resistant pathogens.Inflammation is a frequent clinical observation, and the drugs currently used to treat it have a high profile list of adverse effects and toxicity profiles. Hence, there is need for development and screening of new or complementary anti-inflammatory agents and thus the look to plant sources is necessitated.

Cocosnucifera is credited with numerous positive health benefits, such as antibacterial activity, ability to reduce blood pressure, ability to fight free radicals, prevention of tumors, and diuretic activities according to Lima and Block (2019). Coconut water is a tasty, clear, naturally sweet beverage with a slight acidity that is refreshing. Research spanning decades has demonstrated the nutritional value of coconut water, which includes dietary minerals, palmitic and oleic acids, and essential amino acids (tryptophan, phenylalanine, tyrosine, leucine, cysteine, and histidine). Others minerals such as iron, zinc, and manganese are also present at substantial levels. The primary sugars in coconut water are glucose, fructose, and sucrose, and tartaric, citric and malic acids are its abundant organic acids (Adolf *et al*., 2012; Jose, 2014). Around the world, Coconut water is a taken as a beverage. It contains B vitamins, including nicotinic acid (vitamin B3), pantothenic acid (vitamin B5), biotin (vitamin B7), riboflavin (vitamin B2), folic acid (vitamin B9), trace amounts of thiamine (vitamin B1), and pyridoxine (vitamin B6). Coconut water also contains sugar alcohol, vitamin C, free amino acids, phyto-hormones, enzymes, and growth promoting factors (Subramaniam *et al*., 2012). Coconut water in its envelope is sterile and comprises both organic and inorganic compounds (almost all minerals found in food) (Jose, 2014).

In Nigeria, Coconut water is commonly employed as an antidote for poisons, as a hydration booster for treating cholera, stomach aches, diarrhea, and dysentery. Cocosnuciferapossesses multiple benefits including astringent,

diuretic, and anthelmintic properties. It has found application in addressing issues like sore throat, uterine disorders, excessive vaginal discharge, bronchitis, liver problems, difficulty in passing urine, and worm infections (Loperito Loki & Rajamohan, 2003; Naskar *et al*., 2011; Verma *et al*., 2012).

The antibacterial properties of coconut water have been studied almost extensively. The presence of Lauric acid and the ability to extract antimicrobial peptides Cn-AMP (1, 2, and 3) from tender coconut water have drawn attention to its effectiveness as an antibacterial agent (Rukmini *et al*., 2017). Hence the study of the antibacterial potentials of concentrated Coconut water on the selected microorganisms will add useful data to the information available.

Coconut water also contains cytokinins, which have been associated with anti-inflammatory effects (Lazim *et al*., 2015). However, more studies are needed to fully understand how these compounds interact with the human body and contribute to any potential anti-inflammatory properties. The study by Rao *et al*. published in the Journal of Intercultural Ethnopharmacology in 2016, explored anti-inflammatory potentials of coconut water of different maturity stages and found that coconut water exhibited anti-inflammatory effects in rats using the paw edema model. The researchers observed potently reduced inflammation in the rats that were given tender coconut water and mildly reduced inflammation the rats given mature coconut water (Rao & Najam, 2016). Another study published by Bhagya *et al*. in the Asian Pacific Journal of Tropical Medicine in 2012 also studied the anti-inflammatory activity of tender coconut water in rats. The researchers found that coconut water reduced inflammatory markers significantly by inhibiting certain enzymes involved in the inflammatory process as well as increased activity of antioxidant enzymes (Bhagya *et al*., 2012). In-vitro anti-inflammatory test methods have been performed using the Human Red Blood Cell (HRBC) Membrane Stabilization Method (Anosike *et al*., 2012; Karthik *et al*., 2016; Parameswari *et al*., 2019; Yesmin *et al*., 2020). The method evaluates the activity of the test inflammatory agent on preventing hemolysis of human red blood cell which triggers an inflammatory response via release of cytokines and other chemicals. In an in-vivo experiment carried out at the University of Karachi, Pakistan, it was documented that young and mature coconut water showed remarkable anti-inflammatory effect. However, the intensity of the effect varied with several different coconut fruit evaluated (Rao & Najam, 2016). This study aroused interest to studying the anti-inflammatory potentials of tender Coconut water especially as an anti-poison agent using in-vitro models.In local settings in Nigeria, Coconut water is commonly used as an antidote to poisons. The mechanism of its anti-poison activity is not yet elucidated and this research seeks to assess if the resolution of poisoning symptoms is possibly via an anti-inflammatory mechanism.

MATERIALS AND METHODS

Plant sample collection and preparation

The mature fruits of Cocosnucifera(L.) were collected from a single 13 year old Coconut tree in Udu, Delta State, Nigeria in May 2023. The fruits were identified by the curator of the herbarium at the Department of Pharmacognosy, Faculty of Pharmacy, Delta State University, Abraka. The coconut shell was drilled into mechanically and the fresh Tender Coconut Water (liquid endosperm) was obtained from the coconuts, filtered using a filter paper and collected into sterile vials which were stored at 4ºC until use

Phytochemical analysis

Phytochemical screening of tender coconut water for its active constituents was done according to the methods of Trease and Evans (2002) to detect secondary metabolites present in it.

In-vitro evaluation of antibacterial activity

Test bacteria used

The bacterial isolates used in this study were Escherichiacoli, Bacillussubtilis, Pseudomonasaeruginosa and Staphylococcus aureus. The isolates were part of the culture collection of the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Microbiology, Delta State University, Abraka. The bacterial isolates used were environmental strains (2) and clinical isolates (2).

A suspension of each of the test organisms was made by collecting a loop-full colony from each isolate and inoculating them into Peptone broth which was then incubated overnight at 37ºC. These served as the test organism suspensions.

Agar well diffusion assay

According to Boyanova *et al*. (2005) and Akinpelu *et al*. (2008), the agar-well diffusion method was used to screen the test bacteria's susceptibility to tender coconut water. A control of 2 mg/ml of ciprofloxacin was employed. Using sterile swab sticks, the inocula produced in Peptone broth were seeded onto Mueller-Hinton agar and left to stand at room temperature. Afterwards, a 6 mm stainless steel cork borer was used to drill wells with a 6 mm diameter into the agar plates. The wells were then filled with 20ụl of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% tender coconut water respectively and 20ụl of Ciprofloxacin (2 mg/ml) as the control in the center well, taking care to prevent spillage of the solutions onto the agar surface. The plates were allowed to stand on the work bench for an hour to allow proper diffusion of the coconut water and antibiotic into the media and then the plates were incubated at 35ºC for 24 hours, after which they were observed for zones of inhibition. This procedure was carried out in duplicate and the diameters of the zones of inhibition were measured in millimeters.

The Minimum Inhibitory Concentration (MIC) is determined.

Tender coconut water's Minimum Inhibitory Concentration was determined using Akinpelu and Kolawole's (2004) procedures. The tender coconut water was serially diluted twice, and two millilitre aliquots of each of the various concentrations were introduced to thirteen millilitres of sterilised, melted Mueller-Hinton agar. After that, the medium was transferred into clean Petri plates and left to harden. The surfaces of the agar were streaked with the bacterial cultures and the plates were incubated at 37ºC for 24 hours after which they were examined for the presence or absence of growth. The MIC was taken as the least concentration of tender coconut water that prevented visible growth of the test bacteria.

In-vitro evaluation ofanti-inflammatory activity

The HRBC membrane stabilization method was carried out using the method of Karthik *et al*. (2016) with modification.

5 ml of blood was collected from a healthy human volunteer who had not taken any anti-inflammatory medication for 2 weeks prior to the testing and collected into EDTA tubes. The blood was mixed with equal volume of Alsever's medium (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride). Alsever's medium is a balanced salt solution for suspending and stabilizing red blood cells. The blood sample was then refrigerated at 4ºc for 24 hours.

The blood sample was retrieved and centrifuged at 2500 rpm for 5 minutes and the supernatant was decanted. The cell sediment was washed with sterile saline solution (0.9% sodium chloride) and centrifuged again at 2500 rpm for 5 minutes. The process of removing the supernatant and washing with sterile saline, followed by centrifugation at 2500 rpm for 5 minutes was repeated until a clear, colorless supernatant was observed.

The Packed Cell Volume (PCV) was measured with the formula:

$$\text{PCV} = (\text{height of red blood cell column})/(\text{total height of blood column}) \times 100 \quad (1)$$

The cell sediment was then reconstituted to 10% v/v suspension with sterile saline. This formed the HRBC suspension used in the assay.

6 sterile test tubes were prepared and 1 ml of tender coconut water (test sample) of concentrations 100%, 50%, 25%, 12.5%, 6.25% and 3.125% was put into respectively labeled test tubes. 1 ml of 0.2 M phosphate buffer, 0.5 ml 10% HRBC suspension, and 0.5 ml hyposaline (0.45% sodium chloride) were added into each test tube.

The test tubes were incubated for 30 minutes using a water bath maintained at 37ºC. The test tubes were then centrifuged at 3000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated using a UV-Visible spectrophotometer at a wavelength of 560 nm.

For the standard, Diclofenac was used by replacing the test sample with Diclofenac in the procedure above.

A control group was also prepared by using sterile water to produce 100% hemolysis.

The percentages of HRBC hemolysis and membrane stabilization or protection were calculated using the equations:

$$\%\ \text{hemolysis} = (\text{Absorbance of test sample})/(\text{Absorbance of control}) \times 100 \quad (2)$$

$$\%\ \text{protection} = 100 - (\%\ \text{hemolysis}) \quad (3)$$

RESULTS AND DISCUSSION

In the quantitative phytochemical analysis of the fresh tender coconut water, the fimdingsdemonstrated the availabilityof alkaloids, flavonoids, glycosides, saponnins, steroids, tannins and terpenoids. These phytochemicals are reported to have a diversity of biological activity such as anti-inflammatory, analgesic, anti-oxidant, astringent and antibacterial properties (Akinyemi *et al*., 2006). The antibacterial activity of tender coconut water was investigated on four bacteria strains, Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), and Pseudomonas aeruginosa (P. aeruginosa), using the agar well diffusion method. Fresh tender coconut water demonstrated antibacterial activity by preventing growth of Pseudomonas aeruginosa, with a zone of inhibition ranging between 5 and 10 mm at all concentrations of the tender coconut water **(Table 2)**. The findings of the evaluation of the minimum inhibitory concentration (MIC) of fresh tender coconut water are displayed in **Table 3**. The fresh tender coconut water showed activity against Pseudomonas aeruginosa with a Minimum Inhibitory Concentration of 50% concentrate. This suggests that at this concentration (equal parts coconut water and sterile water) tender coconut water is bacteriostatic. It is hypothesized that certain components of the coconut water (lauric acid) cause it to exhibit antibacterial property against certain pathogens (DebMandal & Mandal, 2011).

Table 1. Results of the phytochemical screening of tender coconut water

TEST	OBSERVATION	INFERENCE
Alkaloids - Dragendorff's reagent	Brick red precipitate	**Alkaloids present**
Flavonoids - Ammonium test	No color change	**Flavonoids absent**
Glycosides - Keller-kilani test	Brick red precipitate	**Glycosides present**
Saponins - Emulsion test	Emulsion formed	**Saponins present**
Steroids - Concentrated Sulphuric Acid test	Reddish brown ring at interface	**Steroids present**
Tannins - Ferric Chloride test	Light green color observed which then changed to black	**Tannins present**
Terpenoids - conc. sulphuric acid test	Grey color	**Terpenoids present**

Table 2. Antibacterial activity of fresh tender coconut water on test bacteria.

	Mean Zones of Inhibition (mm)						
Organism	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**Control (Ciprofloxacin)**
S. aureus	_	_	_	_	_	_	16
B. subtilis	_	_	_	_	_	_	26
E. coli	_	_	_	_	_	_	16.5
P. aeruginosa	8.5	9.5	8.5	6	5	5.5	20.5

Table 3. Minimum Inhibitory Concentration of fresh tender coconut water on test bacteria.

Organisms	Concentration (%)					
	100	**50**	**25**	**12.5**	**6.25**	**3.125**
S. aureus	+	+	+	+	+	+
B. subtilis	+	+	+	+	+	+
E. coli	+	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+
Key:						
+	-					
growth	no growth				MIC	

In the in-vitro anti-inflammatory test, tender coconut water was assayed for its ability to prevent hypotonicity induced hemolysis and protect the erythrocyte membrane. Blood samples collected from a healthy human volunteer was mixed with Alsever's medium and this mixture was centrifuged and washed to remove all potential contaminants and unwanted substances from the cell surfaces. Normal saline was used to wash the cell sediment because it has similar osmolarity to body fluids and does not disrupt the erythrocyte membrane. For the assay, 1 ml of the test sample (tender coconut water) was added at different concentrations to 1 ml of phosphate buffer (dipotassium hydrogen phosphate and potassium dihydrogen phosphate pH 7.4) in a test tube. Phosphate buffer was used to prevent osmotic lysis of the cells. Then 0.5 ml of the red blood cell suspension was added into the test tube and finally, 0.5 ml of hyposaline was added into the test tube to induce hemolysis of the erythrocyte membrane. For the control, instead of hyposaline, distilled water was added into the test tube to cause 100% hemolysis due to the hypotonic environment. These mixtures were incubated, centrifuged and assayed using a UV spectrophotometer and the Absorbance values were used to calculate the percentages of hemolysis and membrane protection as specified by Karthik *et al*. (2016).

According to the logic, the test sample has strong anti-inflammatory properties since it stabilises the erythrocyte membrane, preventing hemolysis. By preventing the red blood cell membrane from being lysed by hypotonicity, tender coconut water demonstrated a minimal membrane stabilisation effect.

Since the lysosomal membrane and erythrocyte membrane are comparable, stabilising the erythrocyte membrane suggests that the sample may also stabilise the lysosomal membranes, albeit figuratively. Lysosomes release pro-inflammatory cytokines during immune reactions. By stopping the release of lysosomal components that trigger inflammation, stabilising the lysosome membrane is crucial for reducing the inflammatory response (Parvin *et al*., 2015) although the exact mechanism of the membrane stabilization by the sample is not yet known. At the minimum concentration of 3.125%, the tender coconut water showed a hemolysis of 78% which is quite high whereas the conventional drug used as positive control (Diclofenac) showed a hemolysis of only 31% **(Table 4)** at a concentration of pf 3.125 mg/ml. With increase in concentration, hemolysis activity of both coconut water and Diclofenac decreased. Tender coconut water at a concentration of 100% had a 70% hemolysis activity which is still quite high whereas Diclofenac at 25 mg/ml had a hemolysis of only 10%.

In essence, both tender coconut water and the positive control used showed anti-inflammatory activity, with the positive control being far more potent **(Figures 1 and 2)** because as established, the greater the percentage of hemolytic activity, the lesser the anti-inflammatory activity. The ability of the test sample to protect or stabilize the erythrocyte membrane was also nominal. At the minimum concentration of coconut water tested - 3.125% coconut water showed a protection value of 22% **(Table 5)** while the positive control showed a protection activity of 69%. When the concentrations were increased to the maximum for the test, both the test sample and the standard drug showed increased protective ability on the erythrocyte membrane. Coconut water showed a protection of 30% while Diclofenac showed a protection activity of 89%. The findings of this assay have significant implications for future research and potential applications. Coconut water has the potential to be used as a natural agent to parlay conventional anti-inflammatory drugs. However, it must be noted that further studies are needed to fully evaluate its effectiveness.

Table 4. Shows the percentage hemolysis observed with the test sample - Tender Coconut Water and the Positive Control – Diclofenac.

Sample Extract - Tender Coconut Water		Positive Control - Diclofenac	
Concentration (%)	**% hemolysis**	**Concentration (mg/ml)**	**% hemolysis**
3.125	77.71	**3.125**	31.43
6.25	74.07	**6.25**	26.98
12.5	76.84	**12.5**	20.00
25	75.89	**25**	16.92
50	71.58	**50**	13.84
100	69.95	**100**	10.88

Table 5. Shows the percentage protection of the erythrocyte membrane by the test sample - TENDER COCONUT WATER and the POSITIVE CONTROL – Diclofenac

Sample Extract - Tender Coconut Water		Positive Control - Diclofenac	
Concentration (%)	**% protection**	**Concentration (mg/ml)**	**% protection**
3.125	22.29	**3.125**	68.56
6.25	25.92	**6.25**	73.01
12.5	23.16	**12.5**	80.00
25	24.12	**25**	83.07

50	28.41	**50**	86.15
100	30.05	**100**	89.11

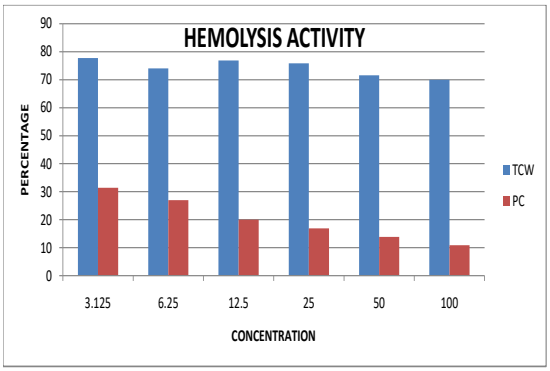

Figure 1. Hemolysis Activity of Test Sample - Tender Coconut Water (TCW) and Positive Control - Diclofenac (PC)

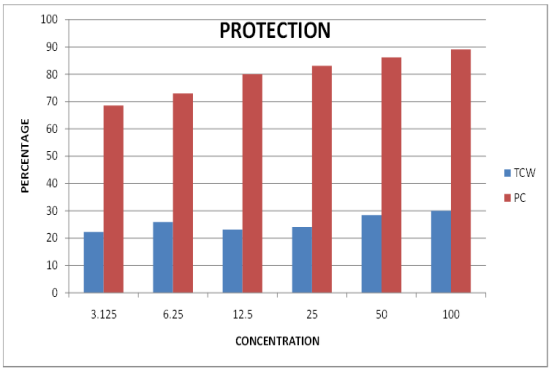

Figure 2. Protection Activity Of Test Sample - Tender Coconut Water (TCW) And Positive Control - Diclofenac (PC)

CONCLUSION

The results of this research revealed some significant findings on the therapeutic potentials of the fresh liquid endosperm of Cocosnucifera L. thus:

- Coconut water has significant activity against Pseudomonas aeruginosa infections and this supports its use in the treatment of human infections.
- The antibacterial potentials of Coconut water opens up a scope for future utilization of other parts of this nut for therapeutic purposes.
- Coconut water has marked anti-inflammatory activity and is a potential candidate for complementary treatment of mild inflammation or in preventing inflammation.

In conclusion, the readily available coconut water not only quenches thirst but also showcases promises as a natural antibacterial resource. While its anti-inflammatory activity seen in this study was only modest, the discovery still holds value and contributes further to a deeper role plants in our locales play in the existence and survival of humankind.

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