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Phytochemistry, Antioxidant and Antimicrobial Studies of Endosperm Tissues of *Cocos nucifera* L.

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Abstract

The phytochemical composition, antioxidant and antimicrobial potency of endosperm tissues of *C. nucifera* were investigated. The phytochemical constituents were revealed to include phenols (0.19 %), flavonoids (0.53 %), alkaloids (0.29 %), tannins (0.18 %) and saponins (0.35 %). Vitamins contents were ascorbic acid (0.08 %), riboflavin (0.27 %), niacin (0.66 %), β-carotenoid (0.03 %) and thiamine (0.18 %). Proximate compositions were moisture (22.33 %), ash (7.76 %), crude protein (11.59 %), crude fibre (12.79 %) and lipids (41.16 %). Mineral constituents detected were calcium (2.87 %), magnesium (0.27 %), potassium (0.53 %), sodium (0.18 %), phosphorus (0.23 %), iron (0.39 %), copper (0.005 %) and zinc (0.007 %). The endosperm tissue extract of *C. nucifera* showcased significant free radical scavenging activity (using 1,1-diphenyl-2-picrylhydrazyl method) at minimum and maximum concentrations of 4.0 and 20.0 mg/mL (38.2 – 64.4 %) using ascorbic acid as a standard free radical scavenger (40.4 – 89.6 %). The extract also exhibited potent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* and *Penicillium notatum*. Ciprofloxacin was used as an antimicrobial standard. The *in vitro* bioprotective properties demonstrated by the extract of *C. nucifera* endosperm tissues could be as a result of the presence of certain vitamins and phytochemicals.

Keywords: *Cocos nucifera*, phytochemicals, antimicrobial activity, endosperm tissue, free radical scavenging

1. Introduction

Before the advent of what is now called 'orthodox medicine', different cultures of the world possessed (and most still possess) methods of handling, treating and preventing diseases which emanated from long time beliefs and practices that have been handed down from generation to generation. One of the man's many desires is to live without disease and infection which since many years ago he relies on plants to achieve this and of course plants have always played an important role to preserve a healthy living. However, the therapeutic potentials of many plants are yet to be verified while verified ones come with inconsistent documentation. Coconut botanically known as '*Cocos nucifera* L.' (*C. nucifera*) is one of such plants. *C. nucifera* belongs to the family 'Palmae' [1]. The plant is widely distributed around the tropical regions of the world. When the fruit is fully ripe, the husk turns brown, dry and very fibrous with a high content of pentosans, cellulose and lignin [1, 2]. *C. nucifera* fruit is called the fruit of life due to its numerous nutritional, health and economic benefits [3]. Virtually all parts of the coconut trees are utilised; from the nuts, husks, inflorescences, stems and even the roots [3]. In Nigeria and most other parts of Africa, *C. nucifera* products include nuts, leaves for roofing, brooms, coco wood, and copra which are processed into oil mainly for the soap industry, cosmetics, and candle wax. *C. nucifera* trunks are used for house and bridge construction [3]. In addition to the production of vegetable oil for industrial uses from cosmetics and explosives to bio-fuels, and health and

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wellness products, it is also a fibre crop, a food and beverage crop, and a visual amenity palm for tourist hotels, golf courses and city parks and village gardens throughout the tropics [4].

C. nucifera plant has also found wide application in traditional herbal medicine. The husk fibre decoction has been used in north-eastern Brazil traditional medicine for treatment of diarrhoea and arthritis [1, 2]. Heating *C. nucifera* shells gives an oil that is used against ringworm infections in the popular medicine of India [1, 2]. The alcoholic extract of ripe dried *C. nucifera* shell has been reported to have antifungal activity against *Microsporum canis*, *Microsporum gypseum*, *Microsporum audouinii*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans* and *Trichophyton violaceum* [1, 5]. The activity was mainly attributed to the high content of phenolic compounds [1, 5]. The *C. nucifera* water is reported to be used in the Indian ayurvedic medicine to increase semen, promote digestion and clear the urinary path [6, 7]. It is also traditionally prescribed for burning pain during urination, dysuria, gastritis, burning pain of the eyes, indigestion, and hiccups or even expelling of retained placenta [6, 8, 9]. In the case of emergency in the remote part of the world, *C. nucifera* water is used as a short-term intravenous hydration and resuscitation fluid [6, 8, 9]. The young *C. nucifera* endosperm is semisolid and jelly-like but as the fruit matures, it becomes solid and fibrous, developing into the firmer coconut meat from which coconut oil is extracted [10]. The meat may also be grated and mixed with water to make coconut milk, fried to make coconut snack or used in cooking and as a substitute for cow's milk [10]. Zakaria *et al.* [11] showed that the aqueous extract of coconut meat exhibited anti-inflammatory and wound healing properties when tested on mice.

Compared to coconut water, there are only limited studies on the aqueous extract of coconut solid endosperm [12]. Waziri *et al.* [10] reported the mineral elements composition of different cultivars of *C. nucifera* from different states in Nigeria; none was reported from the south-east. Solangi and Iqbal [13] also reported the mineral composition and physicochemical parameters of coconut meat and water of major cultivars at the coastal area of Pakistan. In the same vein, the bio-nutritional constituents of coconut fruit and its possible medicinal applications were reported by Ishiaq and Odeyemi [14], but they focused mainly on the coconut milk and oil, not endosperm tissues. In this research, therefore, the West Africa Tall coconut from south-east Nigeria was used. The phytochemicals, vitamins, proximate and mineral elements compositions of *C. nucifera* endosperm tissues as well as the free radical scavenging activity and antimicrobial potency of its oil extract are hereby reported.

2. Materials and Methods

2.1. Sample Collection and Preparation

C. nucifera fruits (the West Africa Tall coconut) were sourced and collected at Obollo-Afor, Enugu State, Nigeria. The fruits were identified and authenticated at the Taxonomy Unit of Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were dehusked and the nuts were removed and broken with the aid of a hammer. The solidified endosperm tissues were cut out with a clean and sharp kitchen knife. The endosperm was washed, dried and pulverized using a laboratory mill.

2.2. Extraction of Oil

In a typical extraction of oil from *C. nucifera* endosperm tissues, the method explained by Igwe and Onuoha [15] was employed. 200 g of the powdered sample was introduced into a conical flask and 500 mL of methanol was added followed by continuous stirring which lasted for about 5 h. The flask was covered with filter paper and cotton fastened with a paper tape to make it airtight. Stirring continued the next day for another 5 h. The mixture was separated using a Whatman No. 1 filter paper. The process was repeated one more time on the residue to ensure exhaustive extraction. The filtrate was pooled together and the solvent was evaporated to get the oil.

2.3. Determination of Plant Chemicals

Alkaloids and phenols were determined according to the method of Harborne [16] while tannin was determined using the method of Van-Burden and Robinson [17]. Saponin was determined using the method of Obadoni and Ochuko [18]. Flavonoids were determined according to the method of Boham and Kocipia [19]. Ascorbic acid was determined using the method of the Association of Vitamin Chemists described by Kirk and Sawyer [20]. The B-complex vitamins (thiamin, riboflavin and niacin) were determined according to SKALAR Analyzers method of Baraket *et al.* [21] while carotenoid was determined according to the method described by James [22]. The macro and micro elements comprising potassium, sodium, magnesium, calcium, phosphorus, iron, copper and zinc were determined according to the method of Shahidi *et al* [23]. Protein, crude fibre, lipids, ash, and moisture were determined by the method described by James [22].

2.4. Antioxidant Activity Determination

The free radical scavenging activity of the oil fraction of the sample extract was determined using the

1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) method described by Man-zocco *et al*. [24]. 1.0 g of DPPH, a stable radical was dissolved in 100 mL of methanol. 3.0 mL of different concentrations of the test sample were added to 3.0 mL of a 0.004 % methanol solution of DPPH and incubated for 30 min at room temperature. The decrease in absorbance of the solution brought about by the test samples was measured at 517 nm using a spectrophotometer. Ascorbic acid, which is a known antioxidant, was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using Equation 1.

$$\% \text{ Inhibition of DPPH radical} = \left[\frac{\left(A_{blank} - A_{sample}\right)}{A_{blank}}\right] \times 100 \qquad 1$$

where A_{blank} is the absorbance of the control reaction solution (containing all reagents except the test sample) and A_{sample} is the absorbance of the test sample.

2.5. Antimicrobial Screening

The *in vitro* antimicrobial activity of the sample extract was carried out on three selected bacteria and fungi. The bacteria organisms used were *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The fungi organisms used were *Candida albicans*, *Aspergillus niger*, and *Penicillium notatum*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Central Laboratory services Unit of National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No. 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilised in an autoclave at 121^{o}C for 15 min. They were however used within 48 h of production. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique [25, 26]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs were carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h in an incubator at 37^{o}C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimetre rule.

2.6. Statistical Analysis

Data were replicated three times and statistically analysed and expressed as mean ± SD.

3. Results and Discussions

The phytochemical constituents of *C. nucifera* endosperm tissues are shown in Table 1. The tissue showed the presence of phenols, flavonoids, alkaloids, tannins and saponins. The highest among the five phytochemicals were the flavonoids followed by saponins. The least being tannins. These classes of compounds are known to have therapeutic and pharmacological activities *in vivo*. Phenolic compounds are usually found in plants and they have been reported to exhibit biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [27, 28].

Table 1. Phytochemical Constituents of *C. nucifera* Endosperm Tissue

Phytochemicals	Composition (%)
Phenols	0.19 ± 0.04
Flavonoids	0.53± 0.08
Alkaloids	0.29 ± 0.01
Tannins	0.18 ± 0.04
Saponins	0.35 ± 0.06

Table 2. Vitamins Composition of *C. nucifera* Endosperm Tissues

Vitamins	Composition (%)
Ascorbic acid	0.08 ±1.27
Riboflavin	0.27± 0.03
Niacin	0.66 ± 0.09
β-carotenoid	0.03 ± 2.11
Thiamine	0.18 ± 0.01

Flavonoids which are polyphenolic compounds are antioxidant and antimicrobial agents [29]. Alkaloids have been reported to possess analgesic, cytotoxic, antispasmodic and antibacterial activities [28]. They are also used in medicine as anaesthetic agents [30]. Saponins are potentially useful for the treatment of hyperglycaemia [31] and possess anti-inflammatory and anti-diabetic properties as well as reduction of cardiovascular diseases [31]. Saponins have the property of precipitating and coagulating red blood cells [32]. Some of the characteristics of saponins include the formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [32]. Tannins, on the other hand, aid in wound healing [33] and prevention of urinary tract infection and intestinal disorders such as dysentery and diarrhoea [34]. Tannins have also been found to possess anti-inflammatory and antibacterial properties [35]. The presence of these phytochemicals in the endosperm tissue of *C. nucifera* indicates the phytochemical richness of the sample.

The sample showed the presence of vitamins such as ascorbic acid, riboflavin, niacin, β-carotenoid and thiamine (Table 2). These vitamins are necessary for the maintenance of sound health. This investigation reveals the endosperm tissue to be rich sources of water-soluble vitamins. The presence of ascorbic acid could be the reason why the aqueous extract of the endosperm tissue was reported to possess anti-inflammatory and wound healing properties [11]. Proximate analysis conducted on the endosperm tissue of *C. nucifera* showed the presence of crude protein, ash, lipids, crude fibre and moisture (Table 3). The endosperm tissue of *C. nucifera* contained high protein, lipids, and fibre. Protein is essential to the human system because it functions in the growth, support and movement [36]. Lipids especially fats, give palatability to food and provide energy, and are essential emulsifiers for a number of drug preparations [36]. Dietary fibre helps to form softer bulky stools and has also been associated with protection against colon and rectal cancer [36]. The endosperm tissues of *C. nucifera* proved to be a good source of minerals such as Ca, Mg, K, Na, P, Fe, Cu and Zn (Table 4). These mineral elements are necessary for healthy living. This investigation reveals that Ca was the highest element followed by K while the least detected element was Cu.

Table 3. Proximate Composition of *C. nucifera* Endosperm Tissue

Constituents	Composition (%)
Crude protein	11.59 ± 1.45
Ash	7.76 ± 1.60
Lipids	41.16 ± 1.42
Crude fibre	12.79 ± 0.19
Moisture	22.33 ± 1.60

Table 4. Mineral Elements Composition of *C.nucifera* Endosperm Tissues

Mineral elements	Composition (%)
Calcium	2.87 ± 1.8
Magnesium	0.27 ± 0.03
Potassium	± 0.07
Sodium	0.18 ± 0.07
Phosphorus	0.23± 0.02
Iron	0.39 ± 0.51
Copper	0.005± 0.04
Zinc	0.007 ± 0.06

Table 5. Antimicrobial Activity of Extract of *C. nucifera* Endosperm Tissues

Microorganisms	Extract (mm)	Ciprofloxacin (mm)
S. aureus	11.00±1.45	20.00±1.41
E. coli	10.00 ±0.15	19.00±1.41
P. aeruginosa	16.00 ±0.70	17.00±0.50
C. albicans	22.00 ±0.69	28.00±1.41
A. niger	12.00 ±0.14	20.00±0.71
P. notatum	16.00 ±0.70	30.00±0.50

Table 6. Free Radical Scavenging Activity of Extract of *C. nucifera* Endosperm Tissues

Concentration (mg/mL)	Radical scavenging (%)	
	Ascorbic acid	Extract
4.0	40.4 ± 1.20	38.2± 6.2
8.0	47.5 ± 1.25	41.7 ± 3.6
12.0	58.4 ±1.0	51.6 ± 1.52
16.0	88.8 ± 2.1	62.7± 1.1
20.0	89.6 ± 1.0	64.4 ± 2.1

The antimicrobial activity of the extract of *C. nucifera* endosperm tissue is shown in Table 5. The extract showed potent antimicrobial activity against the tested organisms and its efficacy is comparable to that obtained with ciprofloxacin as a standard antimicrobial agent. The activity of the extract against these organisms could be as a result of the presence of alkaloids, phenols, flavonoids and tannins in the sample. This is because these phytochemicals have been reported as antimicrobial agents [27, 28, 29, 35]. The extract also showed significant free radical scavenging activity (Table 6). This again is attributed to the presence of phenolic and flavonoid compounds which are often reported to possess free radical scavenging activity [27, 29]. Ascorbic acid detected in the sample might have contributed to the free radical scavenging activity shown by the extract since ascorbic acid possesses antioxidant activity [36]. It is worthy of note that the antimicrobial and free radical scavenging activities of flavonoids are probably due to their ability to complex with bacterial cell wall and radical species [37]. Flavonoids as antioxidants neutralise free radicals, which attack the cells of human body every day [37]. Free radical damage is believed to contribute to a variety of health problems, including cancer, heart disease and ageing [37]. The presence of flavonoids and ascorbic acid in appreciable amounts in the sample was suspected to be largely responsible for its free radical scavenging activity.

4. Conclusions

The results of this investigation reveal that the endosperm tissue of *C. nucifera* contains medicinally important constituents. And these constituents are responsible for the antimicrobial and free radical scavenging activities shown by the extract. Moreover, *C. nucifera* endosperm tissue is a rich source of vitamins and minerals and is hereby recommended as a dessert food to ensure a balanced diet. The tissues could be processed and used in industrial foods as a source of nutraceuticals and as an antimicrobial and antioxidant agent.

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