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Physicochemical, Volatile Organic Composition, Phenolic, Flavonoid and Ascorbic Acid Contents, Antioxidant, Anti-Arthritic and Anti-Inflammatory Properties of *Cocos nucifera* Juice

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Abstract- Various parts of *Cocos nucifera* are locally used for treatment of diseases and production of some foods and beverages for man and animals. This study examined the physicochemical properties, phytochemical and multi therapeutic potentials of juice of *C. nucifera* from Nigeria. These were measured using GC-MS, pH meter, specific gravity, UV-Vis spectrometry, Folin-Ciocalteu's, aluminium chloride, DPPH, PTAC and egg albumin methods respectively. GC-MS analysis revealed the presence of nitroisobutylglycerol as the most abundant volatile organic compounds in the juice. The pH, clarity, turbidity, TPC, TFC and TAA were 5.09, 1.34, 1.07, 2,261.5±0.00 μgmg^{-1} GAE, 20.00±0.0 μgmg^{-1} QE and 66.75±0.00 μgmg^{-1} AAE, respectively. The antioxidant IC_{50} and AAI values of the juice were 0.25 mgml^{-1} and 160 and it was capable of scavenging free radicals at a range between 33.09- 76.26%. The TAC was 645.38±0.00 μgmg^{-1} AAE. The protein denaturation inhibition capacity was at the range between 42.0-73.4%. Therefore, the juice of *C. nucifera* could be used as multi therapeutic agent.

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I. Introduction

Natural products are good drug leads and phytochemical probes to explore mechanisms associated with infection of diseases (Kingston, 2011; Ibekwe and Ameh, 2014). Numerous plant polyphenols reportedly afford multiple health benefits. They are regarded as healthy food and nutrient sources because of their many beneficial components (Voravuthikunchai and Howe, 2014; Zhang *et al*., 2016). Secondary metabolites in the natural products are considered to be responsible for positive health outcomes (Gechev *et al*., 2014). Particularly, it is widely noted that plants produce a great deal of antioxidants to combat the oxidative stress induced by oxygen and light in the natural environment (Cartea *et al*., 2011; Li *et al*., 2016). Natural products possess antimicrobial and antioxidant activities responsible for the prevalence of dermatophytosis (Thebo *et al*., 2016). Many approved therapeutics and drugs are derived from natural sources (Cragg and Newman, 2013; Lahlou, 2013).

Cocos nucifera Linn commonly known as coconut is an important fruit tree in the tropical regions and the fruit can be made into a variety of foods and beverages (Yong *et al*., 2009). *C. nucifera* is an important member of the family *Arecaceae*. The juice of *C. nucifera* has the ability to prevent diseases and sickness. This is due to the free radical scavenging abilities of the antioxidant phytochemicals in it. *C. nucifera* has long been used in traditional medicine for different kind of illness and almost all parts have their uses. The juice inside the fruit is sterile but when it is extracted and exposed to air, it becomes subjected to quick oxidation and microbial contamination leading to depletion of nutrients and spoilage (Matsui *et al*., 2008; Queiroz *et al*., 2008; Jean *et al*., 2009; Nakono *et al*., 2012; Adubofuor *et al*., 2016). Juice of *C. nucifera* is one of the natural food products to quench thirst and easily available in most of the countries. Both water and meat of coconut refresh the body by providing nutritious content. Traditionally, it has been used to protect the body against infection by dangerous diseases. It has been found to improve digestion and hasten the absorption of nutrients including vitamins, minerals, and amino acids. Recently, the health and medicinal uses of *C. nucifera* products get research interest because it contains several metabolites such as sugars, proteins, free amino acids, vitamins, minerals and growth promoting factors (Reddy and Lakshmi, 2014). The juice of *C. nucifera* contains many enzymes including acid phosphatase, catalase, dehydrogenase, diastase, peroxidase and RNA polymerase. Juice of *C. nucifera* is locally consumed fresh, directly from the fruit. (Adubofuor *et al*., 2016). This study aimed at evaluations of physiochemical, phytochemical and therapeutic efficacies of *C. nucifera* juice.

II. Materials and Methods

The fruit of *C. nucifera* was gotten from Ota, Ogun State, Nigeria and the juice was collected and then stored in vial at 5 °C temperature to prevent contamination.

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a) Determination of Clarity and Turbidity

Clarity and turbidity of the juice was determined by measuring the absorbance at 525 and 660 nm respectively using a UV-Vis spectrophotometer (Surajbhan *et al*., 2012).

b) Colour Determination

Colour of the juice was determined by physical observation in day light (Barkatullah *et al*., 2012).

c) Odour Determination

Odour of the juice was determined by organoleptic evaluation (Aloko *et al*., 2017).

d) Determination of pH

The pH of the juice of *C. nucifera* was determined immediately after extraction at room temperature using digital pH meter (Paz *et al*., 2016).

e) Determination of Specific Gravity (SG)

A clean specific gravity bottle was weighted (W_0). Then the bottle was filled to the brim with water and stopper was inserted. The water on the stopper and bottle were carefully wiped off and reweighed (W_1). Same process was repeated, but using juice samples instead of water and weighted again (W_2). The specific gravity of the juice was calculated using the formula below.

Specific gravity = $(W_2-W_0)/(W_1-W_0)$

Where:

W_0 = Weight of empty specific gravity bottle
W_1 = Weight of water + specific gravity bottle
W_2 = Weight of test sample + specific gravity bottle.

f) GC-MS Analysis

The juice of *C. nucifera* dissolved in methanol was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280 °C (temperature at 60 °C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280 °C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature (Ololade *et al*., 2017).

g) Determination of Total Phenolic Content (TPC)

The TPC of the juice of *C. nucifera* was determined using Folin-Ciocalteau method. 1 ml of juice was mixed with 1 ml of 10% Folin-Ciocalteu reagent in distilled water and then neutralized with 4 ml of 7.5% sodium carbonate solution. The sample was maintained at room temperature for 3 hrs with periodical mixing, the absorbance at 760 nm was measured using UV-vis spectrophotometer. Gallic acid was used as a reference and for the calibration curve; result was expressed in micrograms per gram of gallic acid equivalent (Vasudevarao and Sravanthi, 2017).

h) Total Flavonoid Concentration (TFC)

The total flavonoid content of the juice of *C. nucifera* was determined by spectrophotometry, using aluminium chloride method. Briefly, 1.0 ml of the juice, 0.10 ml of 10% aluminium chloride, 0.10 ml of sodium acetate (1 M) and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using a UV-Vis-spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The index of TFC concentration is expressed as quercetin equivalents (QE) in μg per mg of juice. All assays were carried out in triplicate (Formagio *et al*., 2015).

i) Determination of Total Ascorbic acid content (TAA)

1 ml of the juice was added to 1.0 ml 2,4-dinitrophenylhydrazine (2,4-DNPH). It was allowed to stand for 30 min. and the absorbance was read in triplicate at 515 nm, using distilled water as blank. Ascorbic acid was used as a reference and for the calibration curve; result was expressed in milligram per gram of ascorbic acid equivalent (Benites *et al*., 2015).

j) Determination of Free Radical Scavenging and Antioxidant Activities

i. *In vitro 2,2'- Diphenyl-1-picryl-hydrazyl Assay*

The antioxidant and free radical scavenging of the juice of *C. nucifera* was measured by using DPPH. Briefly, the reaction mixture of 2.0 ml; consist of 1.0 ml of DPPH in methanol (0.004%) and 1.0 ml of various concentrations of juice. Then incubated for 30 min. in dark, and the absorbance was measured at 517 nm. The control was prepared by DPPH and methanol in place of sample. In this assay, the positive control is ascorbic acid. The percentage of inhibition can be calculated using the formula:

$$I\% = [(A_{blank} - A_{juc})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of blank solution and A_{juc} is the absorbance of the juice. The dose response curve was plotted and IC_{50} value for the juice and the standard were calculated (Ololade *et al*., 2016).

Antioxidant Activity Index: The antioxidant activity index (AAI) was calculated as:

$$AAI = [DPPH \text{ initial concentration}]/[IC_{50}]$$

AAI was classified as weak, when AAI < 0.5, moderate, when AAI ranged between 0.5-1.0, strong, when AAI ranged between 1.0-2.0, and very strong, when AAI > 2.0 (Arulpriya and Lalitha, 2014).

ii. *Phosphomolybdate Total Antioxidant Capacity (PTAC) Assay*

The PTAC of the juice of *C. nucifera* was determined with phosphomolybdenum using ascorbic acid as the standard. An aliquot of 1.0 ml of juice solution is combined with 1.0 ml of reagent (0.6 M sulphuric acid, 28 μM sodium phosphate and 4 μM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ^{0}C for 90 min. after the samples had cooled to room temperature, the absorbance of the aqueous solution of each were measured at 695 nm in UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as the rest of the samples. The total antioxidant capacity was expressed as equivalents of ascorbic acid (Bulus *et al*., 2017).

In-vitro Anti-Arthritic and Anti-Inflammatory Activities of the Juice on Inhibition of Protein Denaturation (Egg Albumin Assay): in vitro anti-arthritic/anti-inflammatory activity of the juice was evaluated against protein denaturation method using fresh hen's egg albumin. About 5 ml reaction mixtures (0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) add 2 ml of test sample at 1000, 500, 250 and 125 μgml^{-1}). Distilled water with same volume (0.2 ml) was used as control. The mixtures were incubated at 37 ^{o}C in BOD incubator for about 15 min. followed by heating at 70 ^{o}C for 5 min. After cooling to the room temperature, absorbance was measured spectrophotometrically at 660 nm using vehicle as blank. Aspirin (3000 μgml^{-1}) was used as reference drug. The inhibition percentage of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt/Vc) - 1$$

Where:

V_t = absorbance of test sample,
V_c = absorbance of control.

The drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration. (Smitha *et al*., 2017).

III. Results and Discussion

a) Physicochemical Properties of the Juice of C. nucifera

Determination of different physicochemical properties showed the practical importance and provides bases for suitability, consumption, utility, nutritional and physical qualities of the natural juice of *C. nucifera* in daily life (Angaye and Maduelosi, 2015). Physicochemical properties of the juice such as colour, odour, pH, clarity, turbidity, specific gravity (Table 1) showed the quality of the juice of *C. nucifera* from Nigeria.

b) Colour and Odour of the Juice of C. nucifera

The colour of the fresh juice of *C. nucifera* was milky in nature with sweet aromatic odour.

c) Clarity and Turbidity of the Juice of C. nucifera

The clarity and turbidity of the juice of *C. nucifera* were determined as 0.74 and 0.66 respectively using UV-Vis spectrophotometer.

d) pH of the Juice of C. nucifera

The pH value of the *C. nucifera* juice was 5.09; which was within the standard limit (pH 3.40–6.10) that insures freshness of the juice (El-Sohaimy *et al*., 2015). The acidity of juice might be due of present organic acid in the juice, which is responsible for important characteristics of juice: flavour and stability against microbial spoilage and this may confer longer keeping quality of the juice (Nadzirah *et al*., 2012; Offia-Olua and Ekwunife, 2015). Furthermore, it might also indicate that the juice of *C. nucifera* have high content of minerals. pH is a very important parameter in the conduct of fermentation. A pH of 4 is the optimum for the growth of fermentative yeast. This also inhibits the development of undesirable microbial flora (Ahoussi *et al*., 2015; Walker and Stewart, 2016).

e) Specific Gravity of the Juice of C. nucifera

The specific gravity of the juice of *C. nucifera* was 1.01 (Table 1). This was in line with the amount stated for beverages (including soft drinks and juices) and fruit drinks (low calories and undiluted) as 1.01-1.03. The more sugar present in a juice, the denser the juice becomes. Juice is low dense foods because of its high water content, which provides high volume and weight. To stay within low density guidelines, it is important to either consume natural fruit juice that has not been dehydrated than to eat processed fruit juice that contains added sugar (Swinburn *et al*., 2004; Ledikwe *et al*., 2006; Slavin and Lloyd, 2012; Babajide *et al*., 2013).

Table 1: Colour, Odour, Turbidity, pH, and Specific Gravity of the Juice of *C. nucifera*

Colour	Odour	Clarity	Turbidity	pH	SG
milky	Sweet aromatic smell	0.74	0.66	5.09	1.01

f) Organic Composition

GC-MS analysis revealed the presence of nitroisobutylglycerol ($C_4H_9NO_5$) as the most abundant volatile organic composition of the juice. The mass spectrum of the compound with retention time 14.942 and retention index 1444 gave 8 major peaks (m/z) at 27, 29, 31, 55, 57, 73, 85 and 86. Nitroisobutylglycerol is a low molecular weight (151) medicinal compound with oxytocin-induced, antioxidant, anti-staphylococcal activities (Rane and Anusha, 2012).

g) Total Phenolic Content, Total Flavonoid Contents and Total Ascorbic Acid

The TPC, TFC and TAA analyses of the investigated juice of *C. nucifera* showed the presence of high amount phenolic, flavonoid compounds and ascorbic acid (Table 2). Natural phenolic compounds and ascorbic acid play many significant roles in human health as evident from their therapeutic properties (Dimitros, 2006; Ansari *et al*., 2013; Dzialo *et al*., 2016). Plants consumed by humans may contain thousands of different amounts of ascorbic acid, phenolic and flavonoid components (Saxena *et al*., 2013; Kasote *et al*., 2015; Zhang *et al*., 2015). The effect of dietary ascorbic acid and phenolics is currently of great interest due to their antioxidative and possible anticarcinogenic activities (Nahak *et al*., 2014; Pereira *et al*., 2009). Ascorbic acid, Phenolic and flavonoid compounds are chain breaking antioxidant, free radicals scavenger and quenchers of singlet oxygen formation in the process of formation of intracellular substances throughout the body (Mitra and Uddin, 2014; Ozcan *et al*., 2014). Moreover, ascorbic acid, phenolic and flavonoid components play important roles in the control of cancer and other human diseases (Ghasemzadeh and Ghasemzadeh, 2011).

Table 2: TPC, TFC and TAA of the Juice of *C. nucifera*

TPC	TFC	TAA
2,261.5±0.00 μgmg^{-1} GAE	20.0±0.00 μgmg^{-1} QE	66.75±0.00 μgmg^{-1} AAE

Data are presented as the mean value ± S.D. of triplicate

h) Free Radical Scavenging and Antioxidant Potentials

The percentage inhibitions of the juice at various concentrations (1000, 750, 500 and 250 μgml^{-1}) were 76.26, 71.22, 71.00 and 33.09% respectively; while the IC_{50} value was found to be 0.25 $mgml^{-1}$ in comparison to ascorbic acid with IC_{50} value of 9.0 μgml^{-1}.

i) Phosphomolybdate Total Antioxidant Capacity (PTAC)

The PTAC of the juice of *C. nucifera* was 645.38±0.00 μgmg^{-1} AAE (Table 3). The measure of the ability of natural products to delay oxidative stress in a controlled system is defined as total antioxidant capacity (Apak *et al*., 2016; Zhang *et al*., 2016; Pieme *et al*., 2017; Tyagi and Agarwal, 2017). The juice showed high antioxidant potential and this can be related to the high amounts of ascorbic acid, flavonoids and phenolic compounds in juice. Antioxidant play definite roles in many pathological conditions and they are known to fight against these free radicals and protects body from various diseases (Aprioku *et al*., 2013; Lone *et al*., 2013). Their mechanism of action is either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms (Birben *et al*., 2012). The total antioxidant potential is a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress (Pisoschi and Negulescu, 2011).

Table 3: Antioxidant Properties of the Juice of *C. nucifera*

Juice and Reference Drug	DPPH IC_{50} $mgml^{-1}$	AAI	PTAC μgmg^{-1} AAE
Juice	0.25	160	645.38±0.00

j) In-vitro Anti-Arthritic and Anti-Inflammatory Potential

The juice of *C. nucifera* possesses potentially useful anti-arthritic and anti-inflammatory activities at the doses tested. The juice exhibited significant inhibition of egg albumins denaturation of protein between 42.0-73.4% at concentrations between 125-1000 μgml^{-1} and with the IC_{50} value of 6.0 μgml^{-1}. The result was comparable to that of standard drug aspirin (89.4%) (Table 4). The juice showed inhibition of heat-induced protein (albumin) denaturation and prominent effects on protein denaturation was produced. Natural products that can prevent protein denaturation would be very useful for the development of anti-arthritic and anti-inflammatory drugs (Sowjanya *et al*., 2013; Janakiraman and Parameswari, 2014; Obaseki *et al*., 2016). Therefore, the juice is a promising anti-arthritic agent of natural origin in the treatment of inflammatory disorders. It shows that the juice is capable of reducing the production of auto-antigen which indirectly reduces the protein denaturation and hence alleviate arthritis (Alamgeer *et al*., 2017; Boddupally *et al*., 2017). Protein denaturation is one of the leading causes of inflammatory as well as arthritic diseases, which led to production of auto antigens, progressing to certain rheumatic diseases (Jayaprakasam and Ravi, 2012; Pashikanti *et al*. 2014; Elisha *et al*., 2016; Mahabaland

Kaliwal, 2017). The main mechanism involved in protein denaturation is characterized by changes or alterations in hydrophobic, electrostatic, hydrogen and disulphide bonding among the protein molecules (Zavodszky *et al*., 2001; Sangeetha and Vidhya, 2016).

Table 4: Egg Albumin Anti-Arthritic/Anti-Inflammatory Activity of the Juice of *C. nucifera* and Reference Drug

Conc. μgml^{-1}	% Inhibition	IC_{50} μgml^{-1}	% Inhibition of Aspirin 3000 μgml^{-1}
1000	73.4		
500	71.4	6.0	89.4
250	48.0		
125	42.0		

IV. Conclusion

The results of this study showed that the juice of *C. nucifera* can be an accessible source of promising therapeutic agents that can be used in Combating some infectious diseases caused. The study showed the presence of significant antioxidant, anti-arthritic anti-inflammatory activities of the juice. The activities were due to the presence of pharmacologically active phytochemicals in the juice. Fresh juice of *C. nucifera* is a fluid that could be consumed for health, refreshment and nutritional purposes. Therefore the juice can include the production and commercialization of foods and drugs.

Conflict of Interest Statement: The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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