PHYTOCHEMICAL SCREENING, MACRONUTRIENTS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF WATER FROM MATURE COCONUT FRUIT GROWN IN SANDY AND LOAM SOILS AT COAST OF KENYA

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ABSTRACT
Mature coconuts fruits were collected from two blocks in Kilifi and Kwale Counties at the Coast in Kenya. They were broke open and the water inside extracted using n-hexane, ethanol and ethyl acetate for 18 hours. For macronutrients analysis, plain (untreated) coconut water was used, n-hexane and ethanolic extracts for phytochemical screening and n-hexane and ethyl acetate extracts for antimicrobial activity against bacteria (*E. coli*, *S. aureus*, *P. aureginosa*) and fungi (*C. albicans*) with tetracycline, gentamycin, streptomycin and fluconazole as the standards, respectively. The results of different extracts and soils were compared using student's t-test at 95% confidence level. Coconut water had macro-nutrients: carbohydrates, proteins, fats and oils. Phytochemicals present included: flavonoids, tannins, phenols, alkaloids, glycosides and acidic compounds. The macro-nutrients and proximate composition of coconuts from palm growing in sand and loam soils were significantly different (p<0.05) with higher concentration found in coconuts from palm growing in sand soils. However, the macro-nutrients proximate composition of the different extracts from the same soil had no significant difference (p>0.05). The antimicrobial of different extracts from different soils had no significant difference (p>0.05). Thus, growth of coconut palms in sandy soils may be encouraged for better quality products from the coconut palms.
Key words: *Coconut water, Phytochemicals, Proximate composition*

INTRODUCTION
Cocos nucifera belongs to the Aracaceae (Palm family) and one of the most extensively grown and used nuts in the world. It is rated as one of the most important of all palms and the only accepted specie of the genus *Cocos*. (Onifade and Jeff-Agboola, 2003; Popenoe, 1969). Coconut is grown throughout the tropics for decorative, as well as culinary and non-culinary uses. Virtually all parts of coconut palm can be utilized by humans. It provides almost all the necessities of life-food, drink, oil, medicine, fiber, timber, thatch, mats, fuel, and domestic utensils. For good reason, it has been called the 'tree of heaven' and 'tree of life' (Chan *et al*., 2006). It produces products that are directly or indirectly important in world trade. These are whole coconut, copra, coconut oil, coconut oil cake, coir, desiccated shredded coconut, coconut skim milk and coconut protein (Onifade and Jeff-Agboola, 2003). Desired texture in cookies, candies, cakes, pies, salads and desserts has been produced using coconut. Coconut is commercially viable because of its rich nutritive values (Akubugwo *et al*., 2008; Kyari, 2008; Child, 1964).

The medicinal use of *C. nucifera* aqueous extract has been reported in a large extent (Esquenazi *et al*., 2002; Mendonca- Filho *et al*., 2004). The extracts of coconut oil obtained from the kernel consist of monolaurin and lauric acid, which helps the immune system in a beneficiary manner. The monolauric acids are used by the body to produce high levels of antimicrobial (Mid-American Marketing Corp., 2004). Lauric acid is the basic of monolaurin and a part of the chemical constituent of sodium lauryl sulfate that promote health and used in adjunct treatment of viral diseases. It is also found to be one of the active chemicals in controlling Human Immunodeficiency Virus (HIV) disease (Davrit, 2004). The extracts also possess antibacterial properties (Alaris *et al*., 2005) and antiproliferative effect on animal lymphocytes (Kirszberg *et al*., 2003). Coconut oil possesses antimicrobial, antiviral and antiprotozoal activities (Isaacs and Thormar, 1991; Thormar, 1996; Enig, 2003). However, comprehensive studies on coconut water nutritional and pharmacological properties are still inadequate.

Coconut water is a clear, sterile, colourless, slightly acidic and naturally flavoured drink. The flavor of the water varies with the stage of maturity. Each coconut may contain about 200-1000 ml of water depending on the cultivar type and size. However, any nuts young than 5 months of age tend to be bitter in taste and devoid of nutrients. The water has adequate natural minerals and high quality proteins, which are valuable for growth and repair of the body (Pehowich *et al*., 1992), certain carbohydrates, fats and oils, amino acids and active biological compounds making useful both as food and medicine (Fife, 2008). It has been demonstrated that *C. nucifera* natural water present antioxidant properties (Evans and Hallowell, 2001; Mantena and Alvies, 2003).

The water is known to be a heart tonic, anti-hypertensive agent (Alleyne *et al*., 2005), a rehydrating agent (Saat *et al*., 2001; Ismael *et al*., 2007), promote dehydration when other pyrogen-free fluids are not available (Campbell-Falck et al., 2000; Effiong *et al*., 2010) and skin smoothener (Enig, 2004). Studies also demonstrate that its consumption reduces the risk of heart failure in heart disease patients (Hemanth *et al*., 2011). The kernel of tender coconut exhibit antibacterial, antifungal, antiviral, antiparastic, antidermatophytic, antioxidant, hyperglycemic, lepatoprotective and immunostimulant (DebMandal and Mandal, 2011). Phytohormones; cytokinins, auxins, tans-zeatins and gibberilins present in coconut water are essential in the callus formation (Overbeek *et al*., 2007*)*. This extends the application of the coconut water in plant tissue culture as a growth-promoting component in tissue culture medium.

Coconut water is highly versatile and its chemical profile is affected by soil, altitude and environmental conditions. Different varieties of *C. nucifera* contain water with varying concentration of compounds that varies at different stages of maturity (Yong *et al*., 2009). The physical properties of coconut water were affected by varying nitrogen and potassium application. Although coconut water is already well studied in terms of its chemical content, there may still be unknown solutes which contribute to its special biological effects. This study was therefore designed to identify phytochemicals, comparing macronutrients composition and antimicrobial activity of coconut water from sandy and loam soils. This may contribute knowledge that can have a bearing on promotion on the use of the coconut water for primary health care, solving malnutrition problem and food security through the production of various products for food and animal feeds and development of new drugs.

MATERIALS AND METHODS

Healthy mature coconuts were collected from specific farms in Kilifi and Kwale counties. The nuts were taken to national museum of Kenya, where they were authenticated by a taxonomist. The nuts were then transported to TUM laboratory in the department of pure and applied sciences where they were broken and water collected for analysis.

Qualitative analysis macronutrients was done as described by Obidao *et al*., 2010 with modification to test the presence of carbohydrates (s**ulfuric acid test** and Molisch test**)**, reducing sugars (Fehlings' test and Benedicts' test), non-reducing sugars, starch (iodine solution test), fats and oils (translucency test) and proteins (Millon's test and Biuret's test).

Macro-nutrients analysis was carried out on the coconut water to determine concentration of different sugars (glucose, sucrose, maltose and fructose) using **c**olorimetric method-phenol sulfuric acid method as described in Current Protocols in Food Chemistry, (2001) with some modifications.

Phytochemicals in the coconut water were subjected to solvent extraction as described by Obidoa et al., 2010, with modification using n-hexane, ethanol, ethyl acetate and water for 18 hours in a separation funnel and the aqueous phase collected for phytochemical screening.

The phytochemical tests below were carried out on the liquid extract of coconut water to determine the active constituents according to the procedures and methods described by Trease and Evans, 1983 and Harborne, 1973. Flavonoids (Pew's test, Shinoda test, ammonium test and NaOH test), Tannin (gelatin test), Phenols (phenol test), resins, saponins (foam test and emulsion test), Alkaloids (Iodine test and picric acid test), glycosides (Keller-Kiliani test, concentrate H_2SO_4 test and Molisch's test) and acidic compounds (litmus test).

Four test microorganisms were used in antimicrobial sensitivity tests. They were standard strains and clinical isolates; gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and the yeast *Candida albicans* obtained from the Department of Pure and Applied Sciences, Mombasa Polytechnic University College.

The microbes were inoculated on specific media and incubated for 24-36 hrs. Muller and Sabouraud Dextrose Agar were used for antibacterial and anti-fungal analysis. Paper discs of diameter of 6mm prepared from punching Whatman No. 1 filter paper were used. These discs were sterilized at 15 lbs pressure at 121^{o}C for 15 minutes in a well sealed universal bottle. The sterile paper discs were soaked in the coconut water extracts (hexane, ethyl acetate and water) for five minutes then using a sterile wire loop, transferred to culture plates inoculated with test microorganisms. The impregnated paper discs were transferred to Petri dishes with test organisms and the plates incubated at 35^0 C for 24-36 hours and the microbial susceptibility assayed by observing and measuring the zones of inhibitions. Standard antimicrobial drugs-streptomycin for *S. aureus,* fluconazole for *C. albicans*, gentamycin for *P. aureus* and tetracycline for *E. coli*, were used as positive controls while sterilized distilled water was used as a negative control.

All extractions and analysis were performed in triplicates. Results were expressed as Mean±Standard error (SE) Tables were used to present the experimental results. Positive (+) and negative (-) signs were used to indicate the presence and absence of phytochemicals respectively. Comparisons were made using student's t-test with a $p < 0.05$ being considered significantly difference between two parameters.

RESULTS

The results are presented in two parts reflecting analysis using both polar solvents (ethanol, ethyl acetate, water) and non-polar solvent (n-hexane) extracts of coconut water from palms growing in sandy and loam soils. Both polar and non-polar extracts revealed the presence of macronutrients; carbohydrates, proteins, fats and oils [Tables 1].

Table 1: Macronutrient analysis of coconut water extracts using polar and non-polar solvents

Macronutrient	Test	Coconut water extract			
		Water		n-hexane	
		Loam soils	Sandy soils	Loam soils	Sandy soils
Carbohydrates	Sulfuric acid test	++	+++	++	+++
Starch	Iodine test	++	+++	++	+++
Fats and oils.	Translucency test	+	++	+	++
Reducing sugars	Benedict's test	++	+++	++	+++
	Molisch's test	++	+++	+++	+++
Non reducing sugars		++	+++	++	+++
Proteins	Melon's test	+	++	++	++
	Biuret's test	_	_	-	-

Key: (-) Absent, (+) Present in low, (++) Present in moderate, and (+++) present in high concentration

Qualitative analysis of the coconut water from palms growing in both sandy and loam soils for both polar and non polar extracts revealed the presence of saponins, phenols, resins, alkaloids, glycosides and acidic compounds. Flavonoids were present only in the ethanol extract [Table 2].

Table 2: Qualitative phytochemical screening of coconut water in palms growing on loam and sand soil using ethanolic and n-hexane extracts

Phytochemical	Test	Ethanolic extract		n-hexane extract	
		Loam soil	Sand soil	Loam soil	Sand soil

Flavonoids	Ammonia test	-	-	-	-
	Pew's test	-	-	-	-
	Shinoda test	+	+	-	-
	NaOH test	-	-	-	-
Tannins	Gelatin test	-	-	+	+
Phenols	Phenol test	+	+	+	+
Saponins	Foam test	+	+	+	+
	Emulsion test	+	+	+	+
Alkaloids	Picric acid test	-	-	-	-
	Iodine test	+	+	+	+
Glycosides	Keller-Killian test	+	+	+	+
	Conc H_2SO_4 test	-	-	-	-
	Molisch's test	+	+	+	+
Acidic compounds	Litmus test	+	+	+	+

Key: (+) phytochemical present (-) phytochemical absent

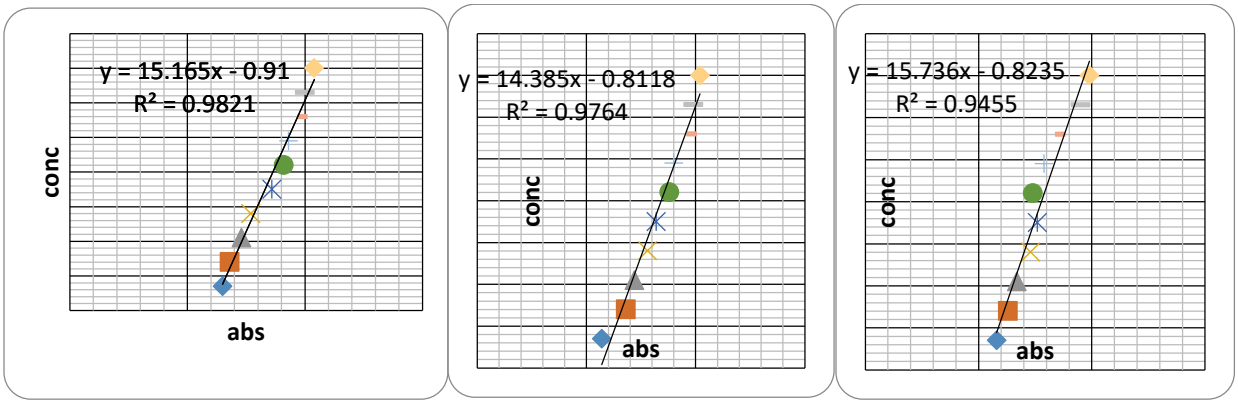
There was a significant difference in lipid total carbohydrates between coconut of water from palm growing from sand and loam soils ($p<0.05$). Quantitative analysis of sugars; glucose, fructose and sucrose in coconut water from palm growing in loam and sand soils revealed substantial amount of these sugars [Table 3].

Table 3: Concentration (mg/mL) of glucose, fructose and sucrose of coconut water from palms growing in sand and loam soils

	Sandy soil palms			Loam soil			
Coconut	Glucose	Fructose	Sucrose	Coconut	Glucose	Fructose	Sucrose
S01	0.39±0.05	0.22±0.04	0.39±0.02	L01	0.09±0.02	0.07±0.03	0.14±0.11
S02	0.35±0.06	0.36±0.05	0.42±0.09	L02	0.07±0.05	0.06±0.04	0.14±0.07
S03	0.38±0.07	0.39±0.03	0.56±0.06	L03	0.46±0.14	0.23±0.05	0.11±0.08
S04	0.69±0.02	0.41±0.03	0.51±0.02	L04	0.18±0.05	0.09±0.02	0.14±0.10
S05	0.57±0.08	0.65±0.10	0.42±0.06	L05	0.08±0.04	0.08±0.00	0.14±0.06
S06	0.42±0.06	0.35±0.13	0.25±0.12	L06	0.16±0.06	0.06±0.05	0.13±0.10
S07	0.52±0.05	0.65±0.06	0.40±0.05	L07	0.08±0.01	0.03±0.01	0.14±0.11
S08	0.60±0.04	0.37±0.06	0.41±0.02	L08	0.07±0.04	0.04±0.00	0.37±0.05
S09	0.58±0.07	0.51±0.08	0.43±0.06	L09	0.06±0.01	0.32±0.04	0.13±0.04
S10	0.50±0.05	0.44±0.08	0.45±0.06	L10	0.35±0.04	0.03±0.00	0.27±0.05
S11	0.46±0.14	0.32±0.06	0.25±0.12	L11	0.16±0.02	0.22±0.06	0.25±0.03
S12	0.28±0.63	0.40±0.06	0.39±0.02	L12	0.37±0.02	0.26±0.05	0.14±0.11

Each data is the mean of three replicates ±Standard Error (SE)

The sucrose and fructose concentration from palm growing in sand and loam soil differed significantly ($p<0.05$). However, the concentration of glucose in the coconut water was not affected by soil type ($p>0.05$).



(a) **(b)** **(c)**

Figure1: Standard curves for (a) glucose, (b) fructose and (c) galactose

The n-hexane, ethyl acetate extracts and plain coconut water from palms growing in sand and loam soils were tested against *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans* with tetracycline, gentamycin, streptomycin and fluconazole as standard drugs respectively and distilled sterilized water as the negative control [Table 4].

Table 4: Antimicrobial activity of coconut water from coconut palms growing in sandy soils

Microorganism	Zone of inhibition (Diameter in mm ±SE)						
	Sand soil palms		Loam soil palms		Controls		
	n-Hexane	Ethyl acetate	Hexane	Ethyl acetate	Plain c. water	Distilled water	Standard drugs
P. aeruginosa	7.13±0.12	7.27±0.06	6.97±0.06	7.10±0.10	0.00±0.00	0.00±0.00	1.20±0.00
S. aureus	7.17±0.15	7.13±0.06	7.00±0.17	7.03±0.06	0.00±0.00	0.00±0.00	1.07±0.06
E. coli	7.00±0.10	7.20±0.01	6.97±0.06	7.10±0.10	0.00±0.00	0.00±0.00	1.10±0.10
Candida	6.30±0.10	6.03±0.21	6.13±0.15	6.10±0.17	0.00±0.00	0.00±0.00	1.20±0.00

Each data is the mean of three replicates ±Standard Error (SE)

Plain coconut water and distilled water did not interfere with the growth kinetics of any of the tested microorganisms. The n-hexane and ethyl acetate extracts showed inhibition of the tested microorganisms [Table 4]. The antimicrobial activity of the extracts differed with soil types ($p<0.05$). No significant difference in zone of inhibition between n-hexane and ethyl acetate extracts ($p>0.05$). However, significant differences were observed when comparing the hexane and ethyl acetate extracts with the standard drugs ($p<0.05$).

Figure 2: Plates showing inhibition of different micro-organism by water from coconut

DISCUSSION

Coconut water is the liquid endosperm of the coconut fruit. The study revealed the presence of macronutrients; carbohydrates, proteins, fats and oils in varying concentration depending on environmental conditions of growth. On exposure to air for long, it loses its organoleptic and nutritional characteristics and begins to ferment losing its worth and credibility (Adolf et al., 2011) thus it's consumed when still fresh.

The present study carried out on coconut water using ethanol and hexane extracts revealed the presence of phytochemicals; glycosides, alkaloids, flavonoids, tannins, phenols, saponins and acidic compounds. Phenols and polyphenols contribute to the antioxidant properties. They also possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Bergsson, 2001). Tannins and resins are employed as astringent both in gastro-intestinal tract and on skin abrasions. Glycosides have the ability to lower blood pressure (Delanty and Dichter, 2000). Flavonoids activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Portillo *et al.,* 1998). Saponins are known to produce inhibitory effect on inflammation (Thormer et al., 1987) while alkaloids are known for their cytotoxicity (Trease and Evans 1983), analgesic, antispasmodic and antibacterial (Rice et al., 1995).

The presence of oil in coconut water makes it a good source of lipids. The various fatty acids may be responsible for the health benefits of coconut water. Lauric acid, a medium chain fatty acid, considered responsible for many health benefits (Enig, 1999) may be present in coconut water. It is converted to monolaurin in the human body which has

antifungal, antibacterial, antiprotozoal and antiviral properties (Fife, 2000). The antifungal and antibacterial properties of coconut water observed may be due to presence of such fatty acids along side other phytochemicals.

The coconut water extracts from palm growing in sand and loam soils exhibited nominal antibacterial and antifungal activity against the test microorganisms though to levels of no significant difference between them with plain coconut water showing no antimicrobial activity. However, nominal susceptibility of fungal and bacterial species to coconut water extracts could be associated with the presence of super-molecular complexes of its cell wall including chitin, which may be difficult to digest, thus imposing resistance to prospective drugs (Marcilla et al., 1991).

It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among the species. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobial have enormous therapeutic potential as they can serve the purpose with lesser side effects hat are often associated with synthetic antimicrobials (Iwu *et al.,* 1999). In this context, coconut water is a prime candidature for the development of nutraceutical compounds

CONCLUSION

The presence of macronutrients; carbohydrates, proteins, fats and oils in coconut water indicate that it can be used in the manufacture of energy drinks. The presence of phytochemical; saponins, glycosides, flavonoids, phenols, acidic compounds, alkaloids, tannins and resins in coconut water suggests its use in development of therapeutic agents. The fact that the different extracts from coconut water portrayed potency against the test microbes justifies the rationale for use of coconut water for treatment of related ailments. The study unveils rich resources of bioactive compounds present in coconut water from palms growing in the coast region of Kenya and lays crucial needs for isolation and development of pharmaceuticals from the extracts.

RECOMMENDATION

Although the chemical composition of coconut water is well studied, it is recommendable that; research should intensified to quantify and compare the phytochemical composition of coconut water and how these concentrations are affected by both environmental and chemical factors. Studies need to be done to identify the best combination of factors that gives the highest composition of secondary metabolites of medicinal value and nutrients in the coconut water. Breeding studies should be carried out to produce coconut water rich in specific chemical compounds.

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