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Phytochemical Study of Underutilized Leaves of *Cola acuminata* and *C. nitida*

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Abstract: Human dependence on natural crude drugs as remedies increases day after day and there are still very many plants/ plant parts whose medicinal values are yet to be established. Research is still ongoing to bridge the gap by extracting the active ingredients in plants for preparation of useful drugs by pharmaceutical industries. Interestingly, the medicinal values of the nuts, barks and roots of the two species of *Cola* in the present study have been reported by some researchers. However, there is no information regarding the phytochemistry of the leaves of this species. Therefore, the present study is the first to provide information on the phytochemistry of leaves of *Cola acuminata* and *C. nitida*. The Phytochemistry of the leaves was carried out using standard procedures. The results of the study revealed the presence of useful phytochemicals. Averages of 70.03 ± 23.34(mgQE/g), 22.96 ± 7.65(mgGAE/g), 13.44 ± 4.48(mgTAE/g), 1.01 ± 0.34(mg/g), and 0.16 ± 0.05(mg/g) were the quantities of flavonoids, phenols, tannins, alkanoids and saponins in the leaf of *C.nitida* respectively. Whereas, the leaf of *C. acuminata* contained 26.71 ± 12.24 (mgQE/g), 23.52 ± 7.84(mgGAE/g), 15.32 ± 5.11(mgTAE/g), 1.23 ± 0.41(mg/g) and 0.22 ± 0.07(mg/g) of flavonoids, phenols, tannins, alkanoids and saponins respectively. The relevance of the phytochemicals was discussed with a view to increasing the utility of the leaves by humans.

Keywords: underutilized, leaves, phytochemicals

Introduction

Cola acuminata is a tree that grows up to 20m tall, usually less in cultivation. The bole commonly branches close to the ground. It is claimed in Nigeria that the root has aphrodisiac property. It is used in the Borno area as a stimulant. The leaves, not specifically reported to contain any active principle, are also said to be taken in Borno as a stimulant (Burkill, 2000). The fruit is composed of up to 5 carpels, to 20cm long by 6cm broad, with as many as 14 seed covered with a white skin. The number of cotyledons is usually 3-5. The seeds are generally red or pink, but occasionally white. The latter are preferred, commanding a higher price and have special significance for ritual. A tree may have nuts of different colours, but self-pollinated white bearing trees produce only white nuts.

The bark shows a strong presence of alkaloid. In Congo and Ivory Coast, Preparations are recommended for abdominal troubles generally, especially of women, in palm-wine or pulped in water taken by draught or in enema as an ecbolic. Bark has use in bronchial affection as an expectorant and with a little salt and seed of *Xylopia aethiopica* (Annonaceae) to relieve cough and colic. It is said to cause release of bile. A decoction has healing property in cleansing sores and maturating furuncles and buboes. The bark mixed with bark of *Khaya* (Meliaceae) is said to produce a brown dye (Irvine, 1961 and Gill, 1992).

Like its counterpart, *C. nitida*, it has much importance in social life. The tree is respected. Some races plant a seed to commemorate a social event, birth, marriage, etc. Other races consider this impious, and the person doing so will die when that tree flowers. The stock of trees is maintained by the transplanting of naturally

produced seedlings. The nut has many religions and magical uses. It is commonly an offering to the spirits and genies, and a masticatory during initiations, and a stimulant tonic dances (Burkill, 2000). Yoruba invoke it in an Odu incantation to enable one to wage a successful fight.

Caffeine content of the nuts is 1-2$^{1/2}$%. *Theobromine* is recorded as 0.0023%, *tannin* 1.618% with a considerable amount of fructose. Two phenolic substances are recorded, *kolatin* and *kolatein*, and other substances. Caffeine is an excitant of the central nervous system, a mental, muscular, respiratory and cardiac stimulant. The nuts are chewed for their stimulant property, though excessive intake can be dangerous. A red pigment, known as "Cola Red" is present in the seed. This is thought to be a part of the tannin content (Burkill, 2000).

Cola nitida on the other hand, is a shrub or tree that grows up to 18m tall, usually less, slightly buttressed to 1m high with a short bole above to 2m girth, bearing a crown of slender ascending branches. It is a shrub of lowland rain- forest, indigenous to Guinea and Ghana but introduced into central Africa. Cultivation has assumed much importance in Nigeria. The bark is used in Liberia in infusion to relieve the pain of fire-burns. In Gabon an infusion of powdered bark is taken to relieve colic, to promote release of bile, and with a little salt and some corns of *Xylopia aethiopica* (Annonaceae) for coughs. In Ghana the insoluble part from leaf-ash is used in making snuff. The small roots are a common item of market trade in Senegal, sold as toothbrushes (Burkill, 2000). The kernels have place in all social ceremonies, baptisms, marriages, funerals and fetish sacrifices. In Mali, the fruit is a part of prescription, known as saraka issued by soothsayers. Fang of Gabon use pieces of the bark along with egg in acts of magic (Irvine, 1961).

Of the four *Cola spp* of major interest**,** *C. acuminata, C.anomala*, *C. nitida and C. verticillata. C. nitida* is the most important**.** The kernels are valued as a masticatory conferring tonic, stimulant, invigorant and excitant symptoms. They produce a capacity for sustained physical effort and for annulling pangs of hunger. Gut secretions and peristaltic movement are increased as is blood pressure. Flavour of the nut is bitter. The principle stimulant is caffeine, 1.5 to 2.5%. The cultivation of this species has shown up varieties, red-seeded and white seeded but with little difference in caffeine content between them. But the white-seeded form seems perhaps on eye-appeal, to be given favour, and is accorded the title of 'The Chief's or King's Kola. Other substances present are *theobromine***,** 0.023%, *tannins*, 1.618%, *fructose*, a phenolic substance, *kolatin*, and a glycoside, *kolanin*. The caffeine is not free but is bonded to the tannin in a complex but having the same effect as free caffeine. The seed kernels are official in the British Pharmaceutical Codex and the U.S. National Formulary. Besides used as a masticatory, the kernels have use as a flavouring in the burgeoning 'coke' drinks industry. Medicine-men in Ivory Coast prepare the kernels in decoctions, enemas or injections as an aphrodisiac, or in cases of difficult labour as an ecbolic. In Nigeria, it is prescribed as a restorative to stimulate the nervous system and to counteract strain and depression.

According to Adewole *et. al*. (2013), Kola contains about two percent caffeine and is chewed by many people as a stimulant. It is used in the manufacture of dyes. It is also used in the manufacture of the cola group beverages-coca-cola, Pepsi cola and kola (Javies, 2002). Plants have been used since antiquity for medicinal purposes by diverse peoples and cultures throughout the world. Indeed, the recorded use of natural products as a source of relief from illness dates back at least four thousand years and it can be assumed that unrecorded practices are as old as mankind (Christophersen *et. al*., 1991). The use of plants for medicinal purposes continues to this day, usually in the form of traditional medicine, which is now recognized by the World Health Organization (WHO, 2005) as a building block for primary health care (Akerele, 1988; WHO, 2005). The vibrant healing power of herbs had been recognized since creation and hence botanical medicine is one of the oldest practiced professions by mankind (Van Wyk and Gericke, 2000; Iwu, 1993). Hamburger and Hostettmann (1991) reported that 25% of prescribed drugs today are of plants origin. Well-known examples of drugs with plant origins includes aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine and vinblastine, as well as several plant steroidal2sapogenins which serve as semi-synthetic precursors to the steroidal drugs. The study of plants of

medicinal importance in the first years of the nineteenth century led to the isolation in crystalline form of such complex substances as Strychnine ($C_{21}H_{22}O_2N_2$), Quinine ($C_{20}H_{24}O_2N_2$) and Morphine ($C_{17}H_{19}O_3N$) which have physiological actions in man and animals (Farnsworth and Bingel, 1997). Herbal medicine has for too long been neglected in favor of synthetic drugs of which its misuse or abuse and cases of side effects have become a social evil (Farnsworth, 1990).

Similarly, according to Durand Dah-Nouvlessounon *et. al*. (2015), In West African's forest areas, *Cola* is perhaps second in importance to the palm tree as an indigenous cash crop. *Cola* nut has been an important article of international trade in many parts of Africa. The nuts of *C. nitida* contain about two percent of caffeine and are chewed by many people as a stimulant. It is a very special and important item used in social and ceremonial activities by Africans. The nuts of cola also have industrial usage for the production of drugs, soft drinks, wines, candies, and beverages such as Coca-Cola and Pepsi-Cola (Jayeola, 2001). It has many pharmacological properties and contains some active principles: it prevents sleep, thirst, and hunger and acts as an antidepressant. The *Cola* nuts are source of antioxidants and contain a wide array of complex secondary plant metabolites such as theobromine, d-catechin, L-epicatechin, and kolatin (Lowor *et. al*., 2010). The use of the plant in the treatment of certain diseases has been reported by several authors (Muhammad and Fatima, 2014).

The two species have corresponding economic and cultural values. They are commonly planted around villages. Preferences are shown. *C. nitida* has a higher caffeine content, and is required for overseas export, but forest people in Nigeria familiar with *C. acuminata*. prefer it. Seeds of the two species are distinguishable on the basis of the number of cotyledons**,** *C. nitida* with 2, *C. acuminata* 3 to 6.

Statement of the Problem and Objective of the Study

The underutilization of the leaves of *Cola acuminata* and *C. nitida* when compared with other parts of the Kola nut tree by humans calls for curiosity. The leaves of the two species are readily available all through the seasons of the year but in spite of this, its level of utility by man is very low. In view of this, scientific information especially in the fields of phytochemistry, pharmacognosy and toxicology about the leaves of these species is therefore imperative.

Materials and Methods

Preparation of Leaf Samples

The leaf samples were prepared following Otoide and Kayode (2011). The leaves were air-dried and ground into powder using a Thomas Willey milling machine. The aqueous extract of each sample was prepared by soaking 100g of dried powdered samples in 200ml of distilled water for 12 hours. The extracts were filtered using Whatman filter paper (No 42-125mm).

Phytochemical Screening

Standard procedures of Sofowora (1993), Trease and Evans (1989), Harborne (1973) and Mohammad *et. al*. (2013) were adopted in the phytochemical screening of the aqueous extract and powdered samples.

Test for Tannins

Dried powdered samples (0.5g) were boiled in 20 ml of water, in a text-tube and then filtered. A few drops of 0.1 % Ferric chloride was added and observed. Brownish-green or blue-black colour indicates the presence of tannins (Trease and Evans, 1989).

Test for Saponins

Powdered samples (2g) were boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was

mixed with three drops of olive oil and shaken vigorously and formation of emulsion was observed for the presence of saponin (Sofowora, 1993).

Test for Flavonoids

Dilute ammonia (5ml) was added to a portion of the aqueous filtrate of each of plant extract followed by addition of 2ml concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids (Trease and Evans, 1989).

Test for Alkaloids

The method of Harbone (1973) was used in this test. The sample (5g) was weighed into a 250ml beaker and 200ml of 10 % acetic acid in ethanol was added and covered, and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, which was the alkaloids, was dried and weighed.

Determination of Total Phenol by Folin-Reagent Method

Total phenol content was determined by Folin-Ciocalteu reagent method with modification. From each crude extracts (1 mg) was dissolved in methanol (1 mL). A total of 10% Folin-Ciocalteu reagent was prepared by adding Folin-Ciocalteu reagent (10 mL) in water (90 mL). Then, 5% Na_2CO_3 (3 g) was prepared by dissolving Na_2CO_3 (3 g) in water (50 mL). Each crude sample (200 µL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all the test tube was kept in a dark place for 5 min. Finally, 5% Na_2CO_3 (1.5 mL) was added to the solution and mixed well by hand. Again all the test tube was kept in the dark for 2 h. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength 750 nm (Mohammad *et. al*., 2013)

Results and Discussion

The results obtained in the present study revealed the occurrences of Flavonoids, Phenols, Tannins, Alkaloids and Saponins in the leaves of *Cola acuminata and Cola nitida*. These have been summarized in Tables 1 and 2

Cola acuminata contained 26.71 ± 12.24mgQE/g, 23.52 ± 7.84GAE/g, 15.32 ± 5.11mgTAE/g, 1.23 ± 0.41mg/g and 0.22 ± 0.07mg/g of Flavonoids, Phenols, Tannins, Alkaloids and Saponins respectively. On the other hand, *Cola nitida* contained 70.03 ± 23.34, 22.96 ± 7.65, 13.44 ± 4.48, 1.01 ± 0.34 and 0.16 ±0.05 of Flavonoids, Phenols, Tannins, Alkaloids and Saponins respectively (Table 2).

The leaves of *Cola acuminata* and *Cola nitida* are often neglected by humans when anything has to do with these two species. The immediate points of call are their fruits and barks. Conventional knowledge revealed that the leaves of these two species are readily available while their fruits are seasonal yet, the leaves are often neglected.

Available literatures revealed a lot of documented results of complete researches on *Cola acuminata* and *Cola nitida* in the fields of science, applied science, social science and marketing on theirs seeds, stem, bark and sometimes fruits. Conversely and surprisingly, the leaves of these two species have not received warmth attention of researchers just like the leaves of other plant taxa even in ethnomedicine. This has led to dearth of scientific reports regarding this special organ of these species. Whereas, the most common plant organ where medicinal properties are domiciled is the leaf.

Results obtained in the present study showed that the leaves of these two species of *Cola,* though often neglected in day to day activities by humans are not devoid of phytochemicals and other useful constituents. The occurrences of Flavonoids, Phenols, Tannins, Alkaloids, Saponnins in the leaves of both species are proofs

of therapeutic values inherent in them. This claim is warranted by the assertions of Hill (1952 and Rabiu *et. al.,* 2018) that the medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. The reports of Chong *et. al.* (2012), Diaz *et. al;* (2015) and Adoum (2016) that biologically active compounds like alkaloids, phenolic compounds, saponins, flavonoids and many others with known antioxidant and antibacterial properties, can be of great significance in therapeutic treatment also support the authors claim made above.

It is known that different phytochemicals have a broad range of pharmacological activities. For instance, saponins can be used as an anti-inflammatory agent and in treatment for tuberculosis; steroids were used as allergy, arthritis and coronary failure therapy, control in menstrual cycle and increasing women fertility; alkaloids can increase nutrient absorption and blood circulation, reduce pain and stimulate nerve system as it has narcotic effect; and tannins are reported to possess anti-irritant, anti-secretolytic, anti-phlogistic, antimicrobial and anti-parasitic effects (Ong *et. al., 2004*). Moreover, flavonoids are well documented to have important effects on various biological systems. Flavonoids have been referred as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activities (Cushnie and Lamb, 2005; YIN *et. al*; 2013).

Table1. *Qualitative Composition of the Phytochemicals in the Leaves of the Cola Species*

Species	Phytochemicals				
	Alkaloids(mg/g)	Tannins(mgTAE/g)	Saponin(mg/g)	Flavonoids(mgQE/g)	Phenols(mgGAE/g)
Cola acuminata	+	+	+	+	+
Cola nitida	+	+	+	+	+

Table2. *Quantitative Compositions of the Phytochemicals in the Leaves of the Cola Species*

Species	Phytochemicals				
	Alkaloids(mg/g)	Tannins(mgTAE/g)	Saponin(mg/g)	Flavonoids(mgQE/g)	Phenols(mgGAE/g)
Cola acuminate	1.23 ± 0.41	15.32 ± 5.11	0.22 ± 0.07	26.71 ± 12.24	23.52 ± 7.84
Cola nitida	1.01 ± 0.34	13.44 ± 4.48	0.16 ± 0.05	70.03 ± 23.34	22.96 ± 7.65

It is pertinent to report here that the leaves of the two *Cola spp* in the present study are not been browsed by herbivores. This phenomenon could be attributed to the occurrence of great quantity of tannins in them. This notion is possible since Woodward and Cappock (1995) opined that the presence of tannins, spines and other physical attributes are capable of reducing browse palatability of plants. This might be ecologically advantageous to these species as they will be able to compete for the available resources in their environment.

In conclusion, the negligence of the leaves of these species of *Cola* has contributed greatly to their under-utility in many specific ways. To this end, the authors wished to call for further researches in the fields of Biochemistry, Pharmacology, Pharmacognosy and Pharmacy in order to confirm the novel bioactive compounds derivable from them and which can be precursors for synthesis of useful drugs.

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