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PROXIMATE AND PHYTOCHEMICAL CONSTITUENTS OF ETHANOLIC EXTRACT OF SEEDS OF COLA ACUMINATA AND COLA NITIDA

Igwe Esther Osaro[1], Imo Chinedu[2], Yakubu Ojochenemi E[3]
Federal University Wukari, Nigeria
esther2017.osaro@gmail.com

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Abstract

This study evaluated the proximate and phytochemical constituents of ethanolic seeds extracts of Cola nitida and Cola acuminata. The seeds of C. acuminata and C. nitida were purchased and air-dried at room temperature. The dried seeds were pulverized to ground sample. The sample was weighed and used for the proximate and phytochemical analysis. The qualitative phytochemical screening revealed the presence of secondary metabolites: alkaloid, flavonoids, tannins, phenols, Cardiac glycoside, terpenes (tri), steroids and saponins, but the absence of anthraquinones. The seed of C. nitida was higher in alkaloids, saponins, phenols, tannins, steroids, triterpenes and C-glycosides than the seed of C. acuminata, while the seed of C. acuminata was higher in flavonoids and carbohydrates than the seed of C. nitida. There was no statistical significant difference ($p>0.05$) between the levels of alkaloids, saponins, phenols and tannins in seed of C. nitida and seed of C. acuminata.

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The difference between the levels of flavonoids, carbohydrates, steroids, triterpenes and C-glycosides in seed of C. nitida and seed of C. acuminata was statistically significant ($p<0.05$). The seed of C. nitida was higher in moisture, ash, lipid and crude protein than the seed of C. acuminata, while the seed of C. acuminata was higher in crude fibre and carbohydrate than the seed of C. nitida. There was no statistically significant difference ($p>0.05$) between the levels of moisture, ash, lipid, crude fibre and crude protein in seed of C. nitida and seed of C. acuminata. The difference between the level of carbohydrate in seed of C. nitida and seed of C. acuminata was statistically significant ($p<0.05$).

The result shows that seeds of C. acuminata and C. nitida possess an appreciable level of phytochemicals. It could be a good raw material for the production of some medicinal drugs and can be used in folk medicine for the treatment of some diseases.

Keywords: Cola acuminata, Cola nitida, Phytochemical, Proximate.

INTRODUCTION

Plants are a necessity to human existence. Plants provide foods and are used as major raw materials for industrial products; they provide oxygen, biofuels, dyes, perfumes, pesticides, drugs etc. The use of plants in traditional medicinal practice has a long-drawn history and remains the mainstay of primary health care in most of the third world countries, especially those living in the rural areas. Most medicinal plants have been reported to play a vital role in the discovery of drugs and are essential for human to treat different ailments (Imo, 2017). Traditional medicines are used by about 60% of the world population: in both developing and developed countries where modern medicines are predominantly used (Ogbonnia *et al*., 2011). The medicinal value of plants lies in some chemical substances (phytochemicals) that produce definite physiological effects on humans and animals. Some of the medicinal plants include garlic, ginger, pepper, egg plants, *Cola nitida*, and *Cola acuminata.* A number of these plant materials have antimicrobial and antioxidant properties with tremendous therapeutic potentials (Moon *et al*., 2010).

There are different species of kola, the most common of which are cultivated in Nigeria (*Cola nitida* and *Cola accuminata*). *C. acuminata* (P.Beauverd) Schott and Endl and *C. nitida*

(Vent.) A. Chev. (Kolanut) are seeds of a tropical tree that both belong to the plant family Sterculiaceae (Adisa *et al*., 2010). The genus *Cola* is indigenous to West Africa and consists of about 100–125 species of trees, shrubs and herbs that thrive in the wet and dry forests of Africa (Atiba *et al*., 2021; Lateef, 2023; Nyadanu *et al*., 2023). The most popular among the members of the genus are *Cola nitida, Cola acuminata* and *Cola verticillata* (Adenuga *et al*., 2012); however, *C. acuminata* and *C. nitida* have been the most studied species (Ekalu and Habila, 2020). *C. acuminata* bears seeds containing 3–6 cotyledons; *C. nitida* produces seeds with 2 cotyledons only (Adeleye *et al*., 2015). Other morphological differences between the two species have been described (Adeleye *et al*., 2015). Three subspecies (alba, ballayi and astrophora) in *C. acuminata* and one subspecies (vera) in *C. nitida* was reported by Adeleye *et al.* (2015), while albida was recognized as a subspecies of *C. nitida.* The plants are grown purposely for their nuts which are widely consumed in West Africa as stimulants and also used in traditional ceremonies (Ekalu and Habila, 2020). Kolanut is depicted as an object of life that brings peace and progress in African society (Odo *et al.,* 2023) and traded across countries (Margaret *et al*., 2022). In an Igbo ceremony for example, the nut is presented to welcome visitors and used to symbolize peace and goodwill (Kanu, 2020). It is also traditionally chewed before meals to aid digestion and also to assist combat possible harmful effect from impure drinking water (Okeke *et al*., 2015).

Kolanut contains kolanin (Lowe *et al.,* 2014). Several researches stated that caffeine is one of the most important components of Kolanut, which is mainly responsible for its physiological activities (Burdock, 2005; Umoren *et al*., 2009). The percentage of caffeine may differ from one study to another and the reason may be due to the methods of preparation used and other factors such as the duration of the experimentation period and plant collection process, in addition to geographic location or habitat (Salahdeen *et al.,* 2014). The nature of the climate affects the concentrations of active substances, especially alkalis and phenolic compounds present in plants (Arogba, 1999; Salahdeen *et al*., 2015). Kola nut is used in many industries for example oil spices, soft drinks, Cola drinks, sweeteners, and in caramel and chocolate (Dah-Nouvlessovnon *et al*., 2015). Kolanut has been used in folk medicine as an aphrodisiac, an appetite suppressant, nausea, migraine, indigestion treatment, and in some cases, it is used to control vomiting in pregnant women (Esimone *et al.,* 2017).

Plant compounds known as phytochemicals which are not edible have curative or disease-preventive effects. Although plants create these substances to defend themselves, research has shown that many phytochemicals can also shield people from disease (Ejimofor *et al.*, 2023). Understanding the chemical components of plants is desirable since it is useful for synthesizing more complicated chemicals. According to Dahanukar *et al.* (2000), phytochemical analysis is the study of organic compounds that build up in plants as a result of their chemical makeup and other biological and natural processes. A chemical method of determining and quantifying the nutritional content of a feed, proximate analysis, also known as Weende analysis, reports the moisture, ash (minerals), crude fibre, crude fat, crude protein and carbohydrate (nitrogen free extract) contained in a diet as a percentage of dry weight. The proximate analyses provide an overview of the sample's nutritional makeup, which is briefly supplemented by information on the antinutrient and mineral content (Ejimofor *et al.*, 2023). The results of a proximate analysis may be used to determine the amount of moisture, ash, volatile matter, fixed carbon, and other substances. Ash, the inorganic residue that remains after water and organic materials have been heatedly removed from the meal, serves as a gauge for the overall quantity of minerals present.

METHODS

Plants Materials

The seeds of *Cola nitida* and *Cola acuminata* were obtained from new market in Wukari, Taraba State, Nigeria. The seeds were cleaned, chopped and air-dried separately at room temperature and pulverized using mortar and pestle.

Preparation of *Cola nitida* and *Cola acuminata* ethanolic extract

The pulverized seeds were extracted with 70% ethanol with occasional shaking, using cold maceration techniques for two days. The samples were filtered using Whatman No 1 filter paper. The filtrates were concentrated using water bath (HH-N420 thermostatic water cabinet search tech instrument) at 65 ^{0}C. The extracts were stored in a refrigerator prior to experiment.

Phytochemical screening of plant extracts

Qualitative phytochemical screening

Phytochemical screening of the seeds extract was carried out to qualitatively determine the presence or absence of the following secondary metabolites: Alkaloids, Tannins, Flavonoids, Saponins, Anthraquinones, Phenolic Compounds, Carbohydrates, Steroids, Terpenoids and Cardiac glycosides using the method outlined by Sofowora (1993) and Trease and Evans (1999).

Quantitative phytochemical screening

Phytochemical screening of the seeds extract was carried out to quatitatively determine the presence or absence of the following secondary metabolites: Alkaloids by Sharief *et al.* (2014), Tannins by Marinova *et al.* (2005), Flavonoids by Trease and Evans (1999), Saponins by Makkar *et al.* (2007), Phenols by Trease and Evans (1999), Triterpenes by Trease and Evans (1999), Steroids by Trease and Evans (1999), Saponins by Trease and Evans (1999), Carbohydrates by Trease and Evans (1999), Cardiac glycosides by Trease and Evans (1999).

Proximate analyses

Proximate analyses of the pulverized sample was carried out to determine moisture, ash (minerals), crude fibre, crude fat, crude protein and carbohydrate (nitrogen free extract) using the method outlined by Association of Official Analytical Chemists (AOAC) guidelines (2003).

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 23. The results were expressed as mean ± standard deviation in all parameters and the statistical difference was determined using standard student-T-distribution-test ($p<0.05$)

RESULTS

Table 1: Qualitative phytochemical screening results of seeds of *Cola nitida* and *Cola acuminata*

Phytoconstituents	Test	*Cola nitida*	*Cola acuminata*
Alkaloids	Dragendorff test	+	+
Cardiac Glycosides	Keller-Kiliani test	+	+
Saponins	Frothing test	+	+
Phenolic Compounds	Lead acetate test	+	+
Tannins	Ferric Chloride test	+	+
Steriods	Salkowiski test	+	+
Carbohydrates	Molisch test	+	+
Flavonoids	Shinoda test	+	+
Terpeniods	Liebermann Burchard	+	+
Anthraquinones	Bontragers test	-	-

The seed of *Cola nitida* and *Cola acuminata* showed presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpeniods while Anthraquinones showed absence for *Cola nitida* and *Cola acuminata.*

Legend: - = Absent, + = Present

Table 2: Quantitative phytochemical composition of seeds of *Cola nitida* and *Cola acuminata* (%)

Constituents	*Cola nitida* seed	*Cola acuminata* seed
Alkaloids	1.93 ± 0.04^{a}	1.74 ± 0.05^{a}
Flavonoids	1.21 ± 0.04^{a}	1.49 ± 0.06^{b}
Saponins	1.68 ± 0.09^{a}	1.59 ± 0.02^{a}
Phenols	2.68 ± 0.02^{a}	2.43 ± 0.02^{a}
Tannins	3.97 ± 0.09^{a}	3.69 ± 0.13^{a}
Carbohydrates	13.84 ± 0.05^{a}	15.51 ± 0.02^{b}
Steroids	14.96 ± 0.02^{a}	1.41 ± 0.01^{b}
Triterpenes	15.56 ± 0.01^{a}	1.63 ± 0.04^{b}
C-glycosides	30.63 ± 0.04^{a}	3.28 ± 0.03^{b}

Values are mean ± standard deviation of duplicate determinations. Mean in the same row, having different letters of the alphabet are statistically significant ($p<0.05$).

The seed of *Cola nitida* is higher in alkaloids, saponins, phenols, tannins, steroids, triterpenes and C-glycosides than the seed of *Cola ac*

.uminata, while the seed of *Cola acuminata* is higher in flavonoids and carbohydrates than the seed of *Cola nitida*. There is no statistically significant difference ($p>0.05$) between the levels of alkaloids, saponins, phenols and tannins in seed of *Cola nitida* and seed of *Cola acuminata*. The difference between the levels of flavonoids, carbohydrates, steroids, triterpenes and C-glycosides in seed of *Cola nitida* and seed of *Cola acuminata* is statistically significant ($p<0.05$).

Table 3: Proximate composition of seeds of *Cola* nitida and *Cola acuminata* (%)

Sample	*Cola nitida* seed	*Cola acuminata* seed
Moisture	8.90 ± 0.14^{a}	7.10 ± 0.28^{a}
Ash	6.10 ± 0.14^{a}	4.75 ± 0.35^{a}
Lipid	1.85 ± 0.92^{a}	0.75 ± 0.35^{a}
Crude fibre	4.65 ± 0.49^{a}	5.50 ± 0.71^{a}
Crude protein	16.09 ± 1.29^{a}	14.09 ± 0.13^{a}
Carbohydrate	62.41 ± 0.13^{a}	67.81 ± 0.41^{b}

Values are mean ± standard deviation of duplicate determinations. Mean in the same row, having different letters of the alphabet are statistically significant ($p<0.05$).

The seed of *Cola nitida* is higher in moisture, ash, lipid and crude protein than the seed of *Cola acuminata*, while the seed of *Cola acuminata* is higher in crude fibre and carbohydrate than the seed of *Cola nitida*. There is no statistically significant difference ($p>0.05$) between the levels of moisture, ash, lipid, crude fibre and crude protein in seed of *Cola nitida* and seed of *Cola acuminata*. The difference between the level of carbohydrate in seed of *Cola nitida* and seed of *Cola acuminata* is statistically significant ($p<0.05$).

DISCUSSION

Using herbs and other plants as medicine is common among rural residents of developing and poor countries. Traditional plant treatments have been used to treat and prevent illnesses in humans for thousands of years, suggesting that they are the ancestors of today's pharmaceuticals (Apiamu *et al*., 2018; Attah *et al.,* 2022; Khan *et al*., 2023). Even in regions where modern treatment is the norm, including the poor and industrialized nations, a good number of people still use traditional medicine (Himal *et al*., 2008; Apiamu *et al*., 2018; Attah *et al*., 2022). The seeds of both species are edible. *Cola acuminata* has a very high cultural value in Southeastern Nigeria. They are used in every traditional rituals and ceremonies. *Cola nitida* seeds, on the other hand, are predominantly taken by the Northerners (Okeke *et al*., 2015). To this end, this study examined the proximate and phytochemical constituents of ethanolic extract of seed of *Cola acuminata* and *Cola nitida*. It has been established that phytochemicals have important roles in human health by preventing disease and promoting recovery (Oguntibeju, 2018; Evuen *et al*., 2022).

The results of qualitative phytochemical screening of *C. acuminata* and *C. nitida* seeds extract is as shown in Table 1 and Table 2 respectively. Alkaloids were found in seeds extracts of *C. acuminata* and *C. nitida* according to phytochemical screening of the leaves. Alkaloids are core framework in many pharmaceuticals and are documented to be bioactive agents used as anti-inflammatory substances (Heinrich *et al*., 2021). Alkaloids are known to improve immune functions, nutrition, and physical performance, being present in daily foods, beverages, and supplements (Ibe-diala and Igwe, 2022). The presence of alkaloids in many plants is responsible for their anti-malaria action (Uzoekwe and Ezenwajiugo 2023). Qiu *et al.* (2014) have shown that alkaloids have a wide range of pharmacological activities. Hence, the presence of alkaloids in *C. acuminata* and *C. nitida* could account for their use as antimicrobial agents. The alkaloids level in seed extracts of *C. nitida* shows that the sample may also be used as a Central Nervous System (CNS) stimulant and as a powerful pain reliever (Stray, 1998).

Cardiac glycosides are steroids that can have a specific and significant effect on the heart muscle (Kumavath *et al.,* 2021). A very small amount can have a positive simulation on a sick heart. The seeds of *C. nitida* may help in relieving cardiovascular pains when consumed. These chemicals are most beneficial in the treatment of congestive heart failure (Kumavath *et al.,* 2021). They raise the force of heart contraction without increasing the

amount of oxygen consumed. As a result, the myocardium becomes a more efficient pump capable of meeting the needs of the circulatory system.

Tannins are the most abundant secondary metabolites generated by plants, accounting for 5 to 10% of the dry weight of tree leaves (Okunade *et al*., 2019). The presence of tannins in seed of *C. acuminata* and *C. nitida* suggests that it could be effective in the fight against insect herbivores. Tannins are aromatic compounds containing phenolic groups. They are one of the principal active ingredients found in plant-based medicines possessing antiviral, antibacterial, and antitumor activities and also promote tissue regeneration in case of superficial burn injury (Mohammad and Fatima, 2014). Tannin is higher in *C. nitida* than in *C. acuminata*. Consequently, *Cola nitida* may serve a better potential regarding to tannins as a major active ingredient in drug production compared to *C. acuminata* (Khanbabaee and vanRee, 2001).

Saponins are low molecular weight secondary plant constituents containing either a tetracyclic steroidal or a pentacyclic triterpenoid aglycone with one or more sugar chains (Vincken, 2007). Salawu *et al.* (2021) have shown that saponins possess antioxidant, antitumor, and anti-mutagenic activities and may also reduce the incidence of human cancers by inhibiting the growth of cancer cells. The saponins are also responsible for lowering cancer risks by lowering the blood cholesterol levels (Imo *et al.,* 2015). The saponin content of the kolanuts ranged between 1.59% for *C. acuminata* and 1.68% for *C. nitida*. Interestingly, toxicological studies of saponin using relevant experimental models have established that even at a higher concentration of 3.5%, saponin was safe and did not cause any systemic side effects (Khatuntseva *et al*., 2012). Thus, it can be deduced from the above that the levels of saponin in both kolanuts are safe for human consumption. It is possible that the saponins present in *C. acuminata* and *C. nitida* may contribute to the above stated activities.

Flavonoids have been reported to exert wide range of biological activities. These include: anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumour, treatment of neurodegenerative diseases and vasodilatory action (Tsuchiya, 2010). Flavonoids are known antioxidants in plants, hence, *C. acuminata* may have a greater antioxidant potential in comparison with *C. nitida* owing to its higher flavonoids constituent. Flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclooxygenase and lipoxygenase enzyme activities (Imo *et al*., 2015). Flavonoids wage war

against free radicals to arrest oxidative stress in the body. It may also regulate the cellular activity for effective metabolism (Ullah *et al.,* 2020).

A growing interest exists in the phenol contents of plants owing to their roles against pathogenic organisms and in the scavenging of free radicals. Phenols were found to be 2.68% in *C. nitida* and 2.43% in *C. acuminata*. Phenols are known antioxidants in plants and humans. Hence, *C. nitida* may have a greater antioxidant potential in comparison with *C. acuminata* owing to its higher constituent of phenols.

Carbohydrates were found to be among the abundant phytoconstituents in both *C. acuminata* and *C. nitida* evaluated in this study. It ranged from 13.84 to 15.51% with *C. nitida* having the lowest amount and *C. acuminata* having the highest amount. Carbohydrates such as glucose provide energy to cells in the body, especially the brain, which solely depends on glucose for energy (Evuen *et al*., 2022). Therefore, the high carbohydrate contents observed in the kolanuts indicate that they may be good sources of fuel and energy for the body's daily activities though the latter is more preferred.

Steroids and triterpenes possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity. *C. nitida* contains chemical constituents having more triterpenes and steroidal structure than *C. acuminata*. Therefore, *C. nitida* may prove to be anti-inflammatory agents by modern clinical and pre-clinical studies (Snehal and Jignasha, 2015). *C. nitida* may be useful in therapeutic roles and may also prove to be of great importance in the field of drug research (Uttu *et al*., 2021).

Proximate composition of plant materials gives information of the level of acceptance of the materials in general nutrition (Imo *et al.,* 2018). It is also known as Weende analysis. The moisture content values are 8.90% in *C. nitida* and 7.10% in *C. acuminata*. These are lower than those reported by Dewole *et al.* (2013) with values of 9.73% in *C. acuminata* and 9.81% in *C. nitida*. The variation in moisture contents observed from the different study may be related to loss of weight during storage and transportation or season of evaluation since the studied samples were not obtained directly from the same tree. It may also be due to the location, soil, variety, maturity and the cultural practices adopted during planting (Adeleke and Abiodun, 2010). No doubt the low moisture contents of the samples are

good for their long preservation as this would prevent early decay or deterioration of the kolanuts.

The protein contents ranged between 14.07% in *C. acuminata* and 16.09% in *C. nitida* (Table 3). These values are higher than those reported by other authors including 10.06% in *C. nitida*, 10.64% in *C. acuminata* and also 15.4% and 13.38% in *C. nitida* and *C. acuminata* reported by Atanda *et al.* (2011) and Dewole *et al.* (2013) respectively. The importance of protein in the human body cannot be over-emphasized. It is a source of amino acids in the diet of man and an essential food content without which our bodies would be unable to repair, regulate or protect itself. Essential body processes such as water balancing; nutrient transport, enzyme catalysis and muscle contractions require protein to function properly, while it is also required for the synthesis of enzymes and hormones. It also aids in the formation of antibodies that enable the body to fight infection (Brosnan, 2006). The protein level in this study may aid or stabilize the above biochemical processes required by the body.

The crude fibre value is 5.50% in *C. acuminata* and 4.65 % in *C. nitida*. The values for *C. nitida* and *C. acuminata* are lower than those reported by Atanda *et al.* (2011) with values of 10.70% in *C. nitida* and 9.68% in *C. acuminata*, but higher than the ones reported by Ajai *et al.* (2012) with values of 3.38% and 4.25% in *C. acuminata* and *C. nitida* respectively. Fibre is known to increase the bulk of diet content and also enhance the frequent release of bowel content (Imo, 2017). This study has shown positive impact in human health and nutrition since it may reduce certain conditions such as constipation. Dietary fibre and whole grains have health benefits that may include a lower risk of death and lower incidences of coronary heart disease, colon cancer, and type 2 diabetes (Morenga *et al.,* 2019). Adequate caution should be taken in consumption of food materials that are very high in fibre since indigestibility will cause loss of nutrients and utilization of the undigested food materials (Imo *et al.*, 2018).

The lipid contents ranged from 1.85% in *C. nitida* to 0.75% in *C. acuminata*. The values fall below those reported by Atanda *et al.* (2011) for *C. nitida* (11.90%) and *C. acuminata* (10.80%) and also different from those reported by Ajai *et al.* (2012) with values of 0.87% and 0.80% for *C. nitida* and *C. acuminata* respectively. Lipid are known to be good sources of high energy generation compound (Imo *et al.*, 2018), but excess of it leads to excessive weight gain and its consequent deleterious effects. Since the kolanuts serve as snacks and

refreshments, the lipid percentage from this source is small and may not be detrimental to the consumer's health but they may be recommended as part of a weight loss regimen (Evuen *et al*., 2022). Certain hormones of lipid origin are required for regulation of certain human bodily functions (Imo *et al*., 2018), but since the percentage lipid of *C. acuminata* and *C. nitida* is low, those human bodily functions may still be properly regulated. *C. acuminata* and *C. nitida* may provide the required lipids needed for the biosynthesis of such hormones even at low level.

The total ash, which measures the equivalent mineral contents of biological samples, ranged ssbetween 6.10% in *C. nitida* and 4.75% in *C. acuminata*. The values are higher than those of Dewole *et al.* (2013) with values of 2.27% for *C. acuminata* and 2.21% for *C. nitida*, and those of Ajai *et al.* (2012) with values of 2.59% and 2.50% for *C. nitida* and *C. acuminata* respectively, but are a bit comparable with those of Atanda *et al.* (2011) with values of 4.00% for *C. acuminata* and 4.30% for *C. nitida*. The high percentage ash in *Cola nitida* seed indicates that its mineral content may be higher compared to *C. acuminata* seed. A food's ash content is a gauge of its mineral content (Ibe-diala and Igwe, 2022). The high percentage of ash in the seeds of *C. acuminata* and *C. nitida* indicates that there will be a high mineral content in them.

Carbohydrates are the most significant source of food energy, making up between 40 to 80 percent of daily caloric consumption. Carbohydrates play an important role in the human body (Irabor *et al*., 2023). The significant difference in carbohydrate level in seed of *C. acuminata* and *C. nitida* showed that it may contribute in the metabolism of cholesterol and triglycerides, regulate the metabolism of blood sugar and insulin, aid in fermentation and contribute to energy gained for daily activities after consumption. Although there are several categories of carbohydrates, the human diet mostly benefits from one particular subset (Mills *et al*., 2019).

CONCLUSION

This study showed that the proximate and phytochemical constituents were in appreciable amount and it revealed the nutritional, possible medicinal and pharmacological properties of seeds of *Cola nitida* and *Cola acuminata* which make them raw materials in food, beverages and pharmaceutical industries and may aid the catalysis of many processes, as well as in maintenance of immunity and homeostasis.

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