DETERMINATION OF CAFFEINE CONTENT IN TWO VARIETIES OF KOLANUT

BY

SADO PEDRO

PSC1707381

DEPARTMENT OF CHEMISTRY,
FACULTY OF PHYSICAL SCIENCE
UNIVERSITY OF BENIN,
BENIN CITY

JULY 2021.

DETERMINATION OF CAFFEINE CONTENT IN TWO VARIETIES OF KOLANUT

BY

SADO PEDRO

PSC1707381

A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE [B.Sc] IN CHEMISTRY

JULY 2021.

CERTIFICATION

This is to certify that this project research was carried out by SADO PEDRO with the Matriculation PSC1707381 under the supervision of Dr. OSARO IYAKOWA in the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City.

--------------------------- -----------------------

Dr. OSARO IYAKOWA **Date**

[Project supervisor]

-------------------------- -----------------------

Prof. JULIUS.U.IYASE **Date**

[Head of department]

----------------------------- -----------------------

SADO PEDRO **Date**

400l student, Chemistry Department.

DEDICATION

This project research is dedicated to God almighty who in his infinite mercies saw me through my entire time in the University of Benin, my family and loving supervisor.

ACKNOWLEDGMENT

I am grateful to God almighty for seeing me through the success of this project. My special thanks goes to my supervisor Dr. Osaro Iyekowa who not only acted as an academic guide but also in loco parentis during the course of carrying out this project and the head of department. Mrs. Usena Sado my loving mother whose prayers and words of encouragement was always that stitch in time, I LOVE MUMMY. To Gideon and franklyn. I could never have asked for better siblings. Words fail me, in expressing my gratitude to you Obi Sylvia Kelechi,I remain grateful and indebted to you for a thousand years.

TABLE OF CONTENTS

ABSTRACT

This study is aimed at determining the Caffeine content in two varieties of kolanuts,cola acuminata (red kola)and cola nitida(white kola). The Samples were obtained from New Benin market,Edo state. The extraction of Caffeine was done using solvent extraction method and infra-red spectroscopy was used to detect the functional groups present in the Caffeine isolated. The percentage yield for the cola nitida was 0.04% while that of cola acuminata was 0.06%. the I.R band observed at $1646.24cm^{-1}$ (C=C) stretch of Alkene, $1700.93cm^{-1}$ (C=O) stretch of amide and $3437.36cm^{-1}$ (N-H) stretch of amine among others for the cola nitida (white kolanuts) and $1647.25cm^{-1}$ (C=C) stretch of alkenes,1700.54(C=0) stretch of amide and $3442.11cm^{-1}$ (N-H) stretch of amine among others for the Cola Acuminata (red kolanuts). The values of these bands suggest the presence of amine groups which confirms the presence of alkaloids (Caffeine). From this research, the caffeine content for the Samples was determined and found that the red kolanuts (cola acuminata) contains more caffeine compared to the white kolanuts (cola nitida).

CHAPTER ONE

1.0 INTRODUCTION

Caffeine (1,3,5-trimethylxanthine) is an alkaloid that belongs to the methylxanthine family which is found in leaves, seeds, or fruits of over 63 different plant species (Okoli, *et al*., 2012).It has specific relationship with theobromine and theophylline.

The most important known sources of caffeine are coffee (Coffea spp.), tea (Camellia sinensis), guarana (Paulliniacupana), mate (Ilex paraguariensis), Cola nuts (Cola vera), and cocoa (Theobroma cacao). Reports show that the percentage composition of caffeine in some of the natural product varies – the highest amounts are found in guarana (4– 7%), followed by tea leaves (3.5 %), mate tea leaves (0.89–1.73 %), coffee beans (1.1–2.2 %), cola nuts (1.5 %), and cocoa beans (0.03 %) (Clifford and Ramirez-Martinez, 1990; Komes, *et al*., 2009).

Some beverages and soft drinks are produced from raw materials containing caffeine. There are some products which are sold in market that are scientifically proven to contain caffeine in them. Examples include Nescafe, Milo, Green tea, Bitter kola and Kola nitida. The presence of caffeine in these products contributes to their daily intake by human as a psychoactive substance.

The structure of caffeine is as shown below

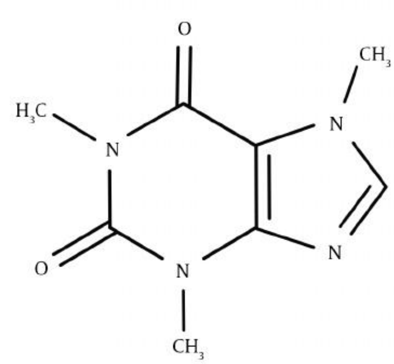


Figure 1.1: Structure of caffeine

Caffeine is a stimulant that may cause irritability and insomnia, and a major raw material in the Pharmaceutical Industries for production of drugs and it is obtained using decaffeination processes such as solvent extraction, Swiss water process and others (Abdul *et al*., 2006).

It is used as a stimulant of the Central Nervous System, and also as a diuretic although its action as a diuretic is weaker than that of theophylline. It is also used in so called caffeine tablet to produce prolong wakefulness and also used in the popup kola beverages because of the mild "Pick up" that it affords(Rubin,2008).

Caffeine prevent sleep and tiredness and allows for a rapid and clearer flow of thought (Aeschbachar *et al*., 1975). Caffeine is used to restore mental alertness when unusual weakness or drowsiness occurs (Ivy *et al*., 1979). Global consumption of caffeine had been estimated at 120,000 tons per annum making it the world's most popular psychoactive substance (Reeling, 1999).

The world's primary source of caffeine is the bean of the Coffee plant, from which Coffee is brewed. Caffeine content in Coffee varies widely depending on the variety of Structural Form ula of Caffeine (1, 3, 5, trimethyl xanthine) and the method of preparation used, but in general one-serving of Coffee ranges from about 40mg for a single shot of expression to about 100mg for strong drip of Coffee. Generally, dark roasted Coffee has less caffeine than lighter roasts since the roasting process reduces caffeine content of the bean (Peker *et al*., 1992).

Tea is another common source of caffeine in many cultures. Tea generally contains less caffeine per serving than Coffee, usually about half as much depending on the strength of the brew, though certain type of Tea such as black and Oolong, contains more caffeine than most other Tea. Tea contains small amounts of theobromine and slightly higher level of theophylline than Coffee (Adulmumin *et al*., 2006).

Kola –nut is mentioned as a major source of Caffeine and Nigeria is recorded as the highest producer of kola –nut with over 130,000 tones production per year, but 70% of it consumed in Nigeria and other neighboring countries (Reeling, 1999).

Analysis showed the various chemical component of Kola-nut as caffeine, theobromine, theophylline, catechine, epicatechine, D-catechine, pheriolics, phlobaphens, betaine, starch, fat, thiamine, riboflavin, niacin, ascorbic acid, sugar, gum, cellulose, water, calcium, potassium, iron, beta-carotene and tannic acid (Reeling, 1999).

1.1.2 BACKGROUND OF PROBLEM

Medically, kolanut was found to have a marked stimulating effect on the human consciousness.In some people consumption gives rise to euphoric state because of it caffeine content.Although kolanut may relieve some migraine headache.The phenolics content in kolanut also provide antioxidant activity.

1.1.3 STATEMENT OF PROBLEM

A lot of people consume kolanut indiscriminately without knowing the content of caffeine in the kolanut , as it is a stimulant. The medicinal significance is under utilized by most people.

1.1.4 JUSTIFICATION / RELEVANCE OF THE RESEARCH WORK

Some beverages and soft drinks are produced from raw materials containing caffeine. The presence of caffeine in products such as kola nut and coffee contribute to daily intake by humans as a psycho active substance .Caffeine in kola nut also help in alertness and focus, energise the body ,ease fatigue ,elevate mood and contribute to performance of athletes. Kolanut is also used in treating whooping cough and asthma, for the caffeine act as bronchodilator. Some people abuse kola nut by taking excessive amount of it and this can result to stained teeth.

1.1.5 SCOPE OF WORK

The scope of the work covers the determination of caffeine content in two varieties of kola nut : • Cola nitida (Vent.), C. nut acuminata

AIMS AND OBJECTIVES

AIM

- To extract caffeine from two varieties of kolanut

To achieve the aim above the following objectives were set;

- To collect dry and pulverize the kolanuts
- To extract the caffeine using a combination of basic extraction method and solvent extraction method
- To detect the functional group of the caffeine Infared Spectroscopy

1.2 HISTORY OF USE

Humans have consumed caffeine since the Stone Age.(Escohotado *et al.,* 1999) Early peoples found that chewing the seeds, bark, or leaves of certain plants had the effects of easing fatigue, stimulating awareness, and elevating mood. Only much later was it found that the effect of caffeine was increased by steeping such plants in hot water. Many cultures have legends that attribute the discovery of such plants to people living many thousands of years ago.

According to one popular Mongolian legend, the Emperor of China Shennong, reputed to have reigned in about 3,000 BC, accidentally discovered that when some leaves fell into boiling water, a fragrant and restorative drink resulted.(Chow p. 2104) Shennong is also mentioned in Lu Yu's Cha Jing, a famous early work on the subject of tea.(Yu, Lu., 1995)

The early history of coffee is obscure, but a popular myth traces its discovery to Ethiopia, where Coffea arabica originates. According to this myth, a goatherder named Kaldi observed goats that became elated and sleepless at night after browsing on coffee shrubs and, upon trying the berries that the goats had been eating, experienced the same vitality. The earliest literary mention of coffee may be a reference to Bunchum in the works of the 9th century Persian physician al-Razi. In 1587, Malaye Jaziri compiled a work tracing the history and legal controversies of coffee, entitled "Umdat al safwa fi hill al-qahwa". In this work, Jaziri recorded that one Sheikh, Jamal-al-Din al-Dhabhani, mufti of Aden, was the first to adopt the use of coffee in 1454, and that in the 15th century the Sufis of Yemen routinely used coffee to stay awake during prayers.

Towards the close of the 16th century, the use of coffee was recorded by a European resident in Egypt, and about this time it came into general use in the Near East. The appreciation of coffee as a beverage in Europe, where it was first known as "Arabian wine," dates from the 17th century. During this time "coffee houses" were established, the first being opened in Constantinople and Venice. In Britain, the first coffee houses were opened in London in 1652, at St Michael's Alley, Cornhill. They soon became popular throughout Western Europe, and played a significant role in social relations in the 17th and 18th centuries(Coffee, 1911)

The kola nut, like the coffee berry and tea leaf, appears to have ancient origins. It is chewed in many West African cultures, individually or in a social setting, to restore vitality and ease hunger pangs. In 1911, kola became the focus of one of the earliest documented health scares when the US government seized 40 barrels and 20 kegs of Coca-Cola syrup in Chattanooga, Tennessee, alleging that the caffeine in its drink was "injurious to health". (Benjamin *et al., 1991* Jan) On March 13, 1911, the government initiated The United States vs. Forty Barrels and Twenty Kegs of Coca-Cola, hoping to force Coca-Cola to remove caffeine from its formula by making exaggerated claims, such as that the excessive use of Coca-Cola at one girls' school led to "wild nocturnal freaks, violations of college rules and female proprieties, and even immoralities(Weinberg and Bealer, 2001)

Although the judge ruled in favor of Coca-Cola, two bills were introduced to the U.S. House of Representatives in 1912 to amend the Pure Food and Drug Act, adding caffeine to the list of "habit-forming" and "deleterious" substances which must be listed on a product's label.

The earliest evidence of cocoa use comes from residue found in an ancient Mayan pot dated to 600 BC. In the New World, chocolate was consumed in a bitter and spicy drink called xocoatl, often seasoned with vanilla, chile pepper, and achiote. Xocoatl was believed to fight fatigue, a belief that is probably attributable to the theobromine and caffeine content. Chocolate was an important luxury good throughout pre-Columbian Mesoamerica, and cocoa beans were often used as currency.

Chocolate was introduced to Europe by the Spaniards and became a popular beverage by 1700. They also introduced the cacao tree into the West Indies and the Philippines. It was used in alchemical processes, where it was known as Black Bean.

The first coffee house in Europe was opened Paris in the 1800s by an French-Armenian named Pascal. Armenian merchants played in role in the more modern history of coffee and this is the reason why the coffee growing region in is named the Armenia Region of Columbia.In 1819, the German chemist Friedrich Ferdinand Runge isolated relatively pure caffeine for the first time. According to a legend, he did this at the behest of Johann Wolfgang von Goethe(Weinberg and Bealer, 2001)

Today, global consumption of caffeine has been estimated at 120,000 tons per annum()making it the world's most popular psychoactive substance. This number equates to one serving of a caffeinic beverage for every person, per day. In North America, 90% of adults consume some amount of caffeine daily.

1.2 LITERATURE REVIEW

Kola is common name for a genus of about 125 species of evergreen trees (trees that certain foliage throughout the year). It is a native to tropical areas of the world. Kola trees are best known for their seeds or nuts which are rich in caffeine and used in the manufacturing of carbonated soft drinks known as kola beverages. (Kola Microsoft Encarta, 2009)

Kola trees belong to the cacao family sterculiaceae. The main species grown for their seed production are classified as kola nitida and kola acuminate. (Cobley and Steele, 1976)

They are classified into these groups on the basis of the amount of cotyledons they have: kola nitida is dicotyledonous while kola acuminate has more than two cotyledons. There are two varieties of kola nitida which are rubra and alba. (Annon, 1953)

Economically, the most important kola species are those cultivated in tropical; countries for their caffeine –rich nuts. Harvested by hand, the brown nuts, which resemble chestnuts and have an aroma. Like that of nutmeg are separated from the follicles and sun-dried, after which they are ready for shipment. Kola forms a part of social and religious customs in West Africa. Kola is one of the major sources of caffeine. Humans have consumed caffeine since the Stone Age. (Escohotado *et al.,* 1999)

Early peoples found that chewing the seeds, bark, or leaves of certain plants had the effects of easing fatigue, stimulating awareness, and elevating one's mood. Only much later was it found that the effect of caffeine was increased by steeping such plants in hot water. Global consumption of caffeine has been estimated at 120,000 tones per year, making it the world's most popular psychoactive substance. This amounts to one serving of a caffeinated beverage for every person every day. Caffeine is a central nervous system and metabolic stimulant, (Nehlig, *et al*., 1992) and is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Caffeine and other methylxanthine derivatives are also used on newborns to treat apnea and correct irregular heartbeats. Caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses.(Bolton and Sanford, 1981) Once inside the body, it has a complex chemistry, and acts through several mechanisms as described below. Many cultures have legends that attribute the discovery of such plants to people living many thousands of years ago. Muslims consider kola nuts to be sacred and incorporate them in ceremonial and social occasions. When chewed kola nuts taste bitter initially but leave a sweet, lingering aftertaste.

1.2 CLASSIFICATION

Kolanut (or cola) is a genus of tree native to the tropical rainforests of Africa, scientifically classified in the family Malvaceae, subfamily Sterculioideae. The term "kolanut" is used to refer to the cotyledons of some species of cola. Cola comprises of about twenty-five species, with a good number of them non-edible (Lowor *et al., 2010*)

The most commonly used cola species are C. verticillata (Thonn.); Stapf, C. acuminata (Pal. de Beauv.) Schott and Endl., and C. nitida (Vent.) Schott and Endl., with the latter two having the greatest economic importance (Lovejoy, 1980).

The nuts of a small number of kolanut species, including C. nitida and C. acuminata are edible; most species produce seed that is hard and inedible. Some kola species are poly-cotyledons, such as C. acuminata. The seeds of edible species are ovoid or ellipsoid in shape, getting up to 5cm in length and 3cm in width. Most of the seed consists of cotyledons to which the minute embryo is attached. In C. nitida there are two cotyledons and the seeds readily split into half whilst in C. acuminata, there are three or four cotyledons (sometimes as many as six), with the seed splitting into a corresponding number of pieces. C. nitida was originally distributed along the West Coast of Africa, including Sierra Leone and the Republic of Benin, with Ghana and Cote D'Ivoire having the highest distribution (Opeke, 1992).

A- Shape of *C. nitida* fruit

B- Shape of *C. acuminata* fruit

D- *C. nitida* nuts aspect

E- *C. acuminata* nuts aspect

G- *C. nitida* variety and cotyledon number

H- *C. acuminata* variety and cotyledon number

Plate 1.1: Varieties of Kola Nut

1.2.1 Botanical characteristics of Cola seeds

The Cola genus comprises about 140 species and the most commonly consumed are C. acuminata and C. nitida. (Bruneton, 1998) Other species frequently used in commerce include C. verticillata and C. anomala (Blumenthal *et al., 2000*). The early records did not distinguish between the two commercial species, C. nitida and C. acuminata.

The major centres for C. nitida were Sierra Leone, Benin, Ghana and Ivory Coast. By the middle of the 20th century the cultivation of the species had spread westwards to the southern border of Senegal and Gambia, eastwards into Zaire and also overseas to the Caribbean islands, especially Jamaica. C. acuminata has its original area of distribution stretching from Nigeria to Gabon and it has been extensively planted in other parts of West Africa (Adeyeye, and Ayejuyo, 1994). Synonyms of Cola (according to Seitz, 1992):

- Cola nitida (Vent.) Schott et Endl.: C. vera K. Schum., Sterculia nitida Vent., C. acuminata var latifolia Schum.
- Cola acuminata (P. Beauv.) Schott et Endl.: Sterculia acuminata P. Beauv., C. pseudoacuminata Engl.

1.2.3 TAXONOMY

The genus *Cola*

Cola, a tropical African genus of the family Sterculiaceae, comprises about one hundred and twenty five species. *Cola* species are evergreen, mostly small or moderately sized trees although a few grow to 25 metres. A number of species are widely cultivated in tropicalcountries, especially in Africa. The most commonly used are *C. verticillata* (Thonn.) Stapf, *C.acuminata* (Pal. de Beauv.) Schott and Endl. and *C. nitida* (Vent.) Schott and Endl., with the latter two having the greatest economic importance (Lovejoy, 1980).

The following description of the genus is given in (Opeke, 1992):

The leaves of *Cola* species are simple, entire and narrowed or rounded towards the base. The arrangement of the leaves on the stem is alternate in some species and verticiliate, in whorls of 3 or 4, in others.The flowers of both *C. nitida* and *C. acuminata* have a white or coloured perianth. Typically, trees bare two types of flowers; male, with anthers fused into a single column or hermaphrodite with one or two rings of anthers at the base of the superior ovary. After fertilisation, the ovary divides forming separate fruiting carpels or follicles, usually five to ten in number. Fruits are sessile, placed at the end of a short peduncle, from which they radiate in star-shaped fashion. As the fruit increases in weight, the stem hangs vertically and the follicles are borne horizontally or ascending in recurred fashion, containing one to ten seeds. The nuts of a small number of *Cola* species, including *C. nitida* and *C. acuminata,* are good to eat though most species produce seed that is hard and inedible. Some *Cola* species arepolycotyledonous, e.g. *C. acuminata.* The seed of the edible species is ovoid or ellipsoid, or angular by compression, varying in size up to *5* cm long and 3 cm in wide. Most of the seedconsists of cotyledons to which the minute embryo is attached. In *C. nitida* there are two cotyledons and the seeds readily split into half whilst in *C. acuminata,* where there are three or four cotyledons, sometimes as many as six, the seed splits into a corresponding number of pieces (Irvine, 1956; Keay, 1958; Russell, 1955).

1.3 Botanical history of Cola species

Kola nuts were widely used in West and Central Africa long before the arrival of Europeanvoyagers (Russell, 1955). Leo Africanus referred to a bitter nut with the name '*goro'* which he encountered during a visit to western Sudan in 1556. This is the name that is used to refer to kola in Nigeria. However, the first definite description of kola nuts was made by Edouado Lopez, a Portuguese traveler, who saw seeds with four cotyledons in 1593 (Chevalier and Perrot, 1911).

In 1805 Palisot de Beauvois published an account of specimens that he had collected during a visit in 1786 to parts of what is now Nigeria. Among the species he described was the local kola tree, named by him as *Sterculia acuminata* (Russell, 1955). In the same year Ventenat described a species he was sent from Mauritius as *Sterculia nitida*. Both species subsequently became part of the genus *Cola* when Schott and Endlicher created it in 1932.

According to Russell (1955), the systematics of kola species was in a state of "indescribableconfusion" by the beginning of the Twentieth Century as a result of a profusion of new species, named on the basis of very meagre evidence. It was not until the French botanists Auguste Chevalier and Emile Perrot's (1911) taxonomic account that clarity was restored. Chevalier created the subgenus Eucola to contain the five species of edible kola nut: *C. nitida* (important for trade), *C. acuminata* (important for socio-cultural values), *C. ballayi, C.verticillata* and *C. sphaerocarpa.* The latter three species are not known to be cultivated, but their seeds are sometimes use to adulterate the produce of the commercial species when it is scarce.

1.4 *Cola nitida* (Vent.) Schott and Endlicher

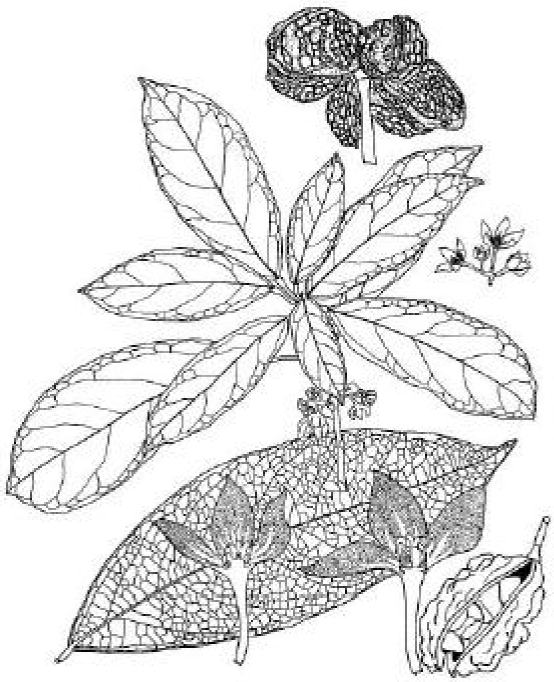
Synonyms

Sterculia nitida Ventenat

Cola vera Schumann

Cola acuminata (Beauvour) Schott and Endlicher

Cola acuminata Engler



e 1: *Cola nitida* (Vent.) Schott & Endl. (Purseglove, 1968).

Plate 1.2: cola nitida

Plate 1.3: Cola nitida

Description

Cola nitida is a medium sized (<25m) evergreen forest tree. The bole is usually unbranched reaching to 8-20 m in height and sometimes attaining 24 m. The trunk may grow to 50cm in diameter with, in old trees, narrow buttresses extending to about 1 metre.

The bark of the tree is grey with longitudinal fissures (FAO, 1995; Keay, 1958; Russell, 1955)

The dark green leathery leaves are usually sparse and confined to the tips of branches. The leaves are simple up to 33 cm long and 13 cm in width with apex abruptly and shortly acuminate with long petioles often swollen at the top (Fig lB). Measurements of two hundred leaves from various sources gave the following mean dimensions: lamina length 16.3 cm, width 5.6 cm, petiole length 4.1 cm (Russell, 1955)

Reproductive ecology

The flowers have a faint smell and are borne in inflorescences of axillary cymes, nonverticilate, flowers male or hermaphrodite, apetalous, cream or white coloured usually with a small dark red markings within. There is however, great variation in the proportion of male and female flowers between racemes and from tree to tree. Typically the hermaphrodite flower has a five lobed calyx, five curved fleshy stigmas lying over the ovaries which in turn rest on a plate-like structure round the edges of which are two rows of ten rudimentary stamens. In the male flowers the anthers develop fully and yield yellowish, slightly sticky pollen; the gynoecium is absent (Keay *et al*., 1960).

The number of carpels may vary from one to six. The green, warty carpels are arranged as a star and are borne on a short, pendant peduncle (1-10 cm), and are up to l3 cm long and 7 cm wide, usually horizontal or in a recurved position. Each carpel contains 4-10 seeds, usually with two cotyledons, arranged in two rows, surrounded by a thin but tough membranous white skin. The nuts are generally pink or red depending on the variety though sometimes they are white (FAO, 1982; Keay *et al*., 1960; Russell, 1955; Voelcker, 1935).

Species and cotyledon colour within C. nitida

Chevalier and Perrot (1911) recognised a number of sub-species within *Cola nitida*.. In their publication the sub-species were described as *alba, rubra, mixta* and *pallida* all of which are cultivated in Nigeria. The main difference between these sub-species lies in the colour of the seed. The sub-species *alba* is distinguished by the absence of red coloration in the flower. Chevalier further observed that *C. rubra* trees produce only red nuts; *C. alba,* pink and white, and *C. pallida* trees producing small pale pink or white and pink nuts mixed and that the colour of the nuts of any one variety may alter from year to year.

Russell (1955) believed that the criteria that Chevalier's used to differentiate sub-species within *Cola nitida* were too slight to justify their formation. Two distinctive kinds of *Cola nitida* were therefore described merely as 'white flowered' and 'red flowered'. Chamney

(1927, cited in (Voelcker, 1935)), in his publication of *Distribution of white and pink seeds of kola nuts plants in Ghana* concluded that cotyledon colour is a function of age. However,

Voelcker (1935) summaris2ed his findings from the experiments at Moor Plantations in Ibadan, Nigeria as below:

- The colour of the cotyledon of *C. nitida,* may be red, white or intermediate shades of pink. The red is the most common; white is rare in Nigeria.
- The colour of the nuts may vary from follicle to follicle, from tree to tree, and, on the same tree, from year to year.
- The colour has been shown to be determined by the genetic constitution of the tree on which the flowers are borne and of the tree, which yields the fertilising pollen.
- Trees grown from white nuts produce only white nuts when the flowers were self fertilised.
- Trees grown from white nuts produce red, pink or white when the flowers are fertilised by pollen from trees grown from red nuts.
- Crossing flowers from trees grown red nuts with white pollen gave red and white fruits.

- Voelcker proposed that his findings indicated that the inheritance of cotyledon colour is purely Mendelian, and is determined by the interaction of a number of genes.

*1.*5 Cola *acuminata* (Beauvoir) Schott and Endlicher

Synonyms

Sterculia acuminata Beauvoir

Cola pseudoacuminata Engler

Listed by Hutchinson and Dalziel (1958).

Description

Cola acuminata is a slender tree which can grow up to a height of 30 m, but usually 10 - 20 m with diameter of about 30 cm. The trunk commonly branches close to the base with the leaves almost reaching the ground. The branches are many, frequently divided, slender and crooked, markedly ascending. The bark of the old trees is rather rough and corky, grey in colour and often split into squares. On the larger branches the bark is smooth, corky warts and an olive colour are frequent and are distinctive characters of this species when present.

The foliage is simple, sparse and confined to the tips of the branches. They are very dark green, rather fleshy, often curled with lateral veins, which is not prominent. In shape they are elliptic or slightly obovate, rounded at the base and with long petiole which is as long as one metre. The leaf is not flat but often curved, keeled at the mid-rib and twisted at the tip. Russell (1955) gave mean dimensions of a large number of leaves from different: lamina length 16.3 cm, maximum width 5.6 cm, petiole length 4.1 cm.

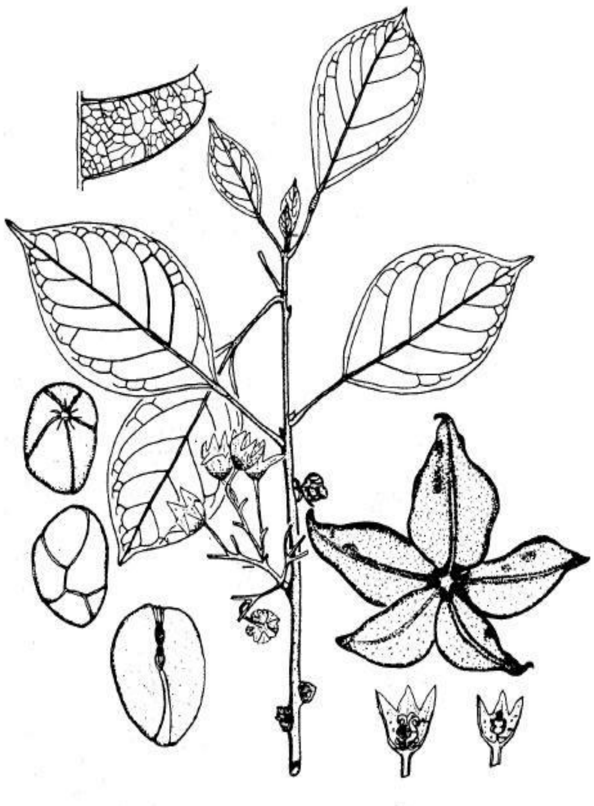


Figure 2: ***Cola acuminata*** **(P. Beauv.) Schott & Endl. (Nkongmeneck, 1985).**

Plate 1.14: ***Cola acuminata***

Reproductive ecology

The inflorescence has several to many flowers, which are not arranged in whorls. The perianth is joined for nearly half its length and often forms an open cup up to 2.5 cm, with white or cream colour and small dark-red coloration at the base of segment inside, being most marked along the veins. Both hermaphrodite and male flowers are found on a single tree, with male flowers being smaller. The perianth is glabrous inside or with scattered stellate hairs. The anthers are borne on a short, but distinct column (Keay, 1958; Opeke, 1992; Purseglove, 1968; Russell, 1955).

The fruits are borne on young branches and form a star-shaped cluster of pods, usually numbering five, with each carpel bearing 4-10 seeds (Fig. 3C). Most of the fruits are harvested from April to June and hence making it markedly different from *C. nitida.* The carpels are sessile, brownish in colour, straight or slightly curved with a prominent straight point or tip, the whole up to 20.3 cm long and 6.4 cm wide, the surface is rough to the touch, russet or olive-brown.

The seeds in each carpel are arranged in two rows covered with a thin white skin. The seeds are commonly pink or red, but occasionally white, up to 4 cm long and 2.5 cm wide (Fig. 3D). The cotyledons are distinctively three to five and even six, pink, red or sometimes white in colour and more viscid than those of *C. nitida* (Keay *et al*., 1960; Purseglove, 1968; Russell, 1955).

Table 1: Key distinctions between *C. nitida and Cola acuminata*

Tree parts	*Cola nitida*	*Cola acuminata*
Leaves	Leaves abruptly acuminate, flat, with nerves prominent,	Leaves remain acuminate curved and twisted.
Fruits	Fruits curved, with prominent keel extended to form a curved beak, rugose or tuberculate, green, smooth to the touch.	Fruits straight or slightly curved not rugose or tuberculate, resset, rough. to the touch.
Seeds	Seeds with two cotyledons.	Seeds with more than three cotyledons, usually four or more.
Germination	The two cotyledons remain closed, and the plumule arises outside them	Four cotyledons spread open and the plumule grows up between them.
Harvesting season	October to December.	April to June.

1.6 DISTRIBUTION

Cola nitida was originally distributed along the west coast of Africa from Sierra Leone to the Republic of Benin with the highest frequency and variability occurring in the forest areas of Côte d'Ivoire and Ghana (Opeke, 1992). Chevalier and Perrott (1911) and Warburg (1902) both quoted in (Opeke, 1992) stated that cultivation of *C. nitida* was carried eastwards through Nigeria towards Cameroon and the Congo around 1900, and spread westwards as far as Senegal (Opeke, 1992). *C. nitida* is planted through Senegal, Guinea, Liberia, Côte d'Ivoire, and Ghana towards the western part of Nigeria (Voelcker, 1935).

Southern Nigeria is considered the center of occurrence of *C. acuminata,* with its original area of distribution stretching from Nigeria to Gabon. *C. acuminata* also occurred spontaneously in the mountainous areas of Angola, Zaïre and Cameroon, and it has long been in cultivation on the islands of Principe and São Tomé (Opeke, 1992). It has also been cultivated in Angola, Fernando Po and Tanzania. (Fereday *et al*., 1997).

West Africans took the seed with them when they traveled, even in the days of the Slave Trade. Kola has therefore been extensively cultivated in tropical South and Central America and the West Indies. It has also spread eastwards to Mauritius and Malaysia (FAO, 1982;

Purseglove, 1968; Russell, 1955). Seeds were distributed from Kew Royal Botanic Gardens for planting in Calcutta, Singapore, Sri Lanka, Java, the West Indies and United States of America (Moloney, 1887).

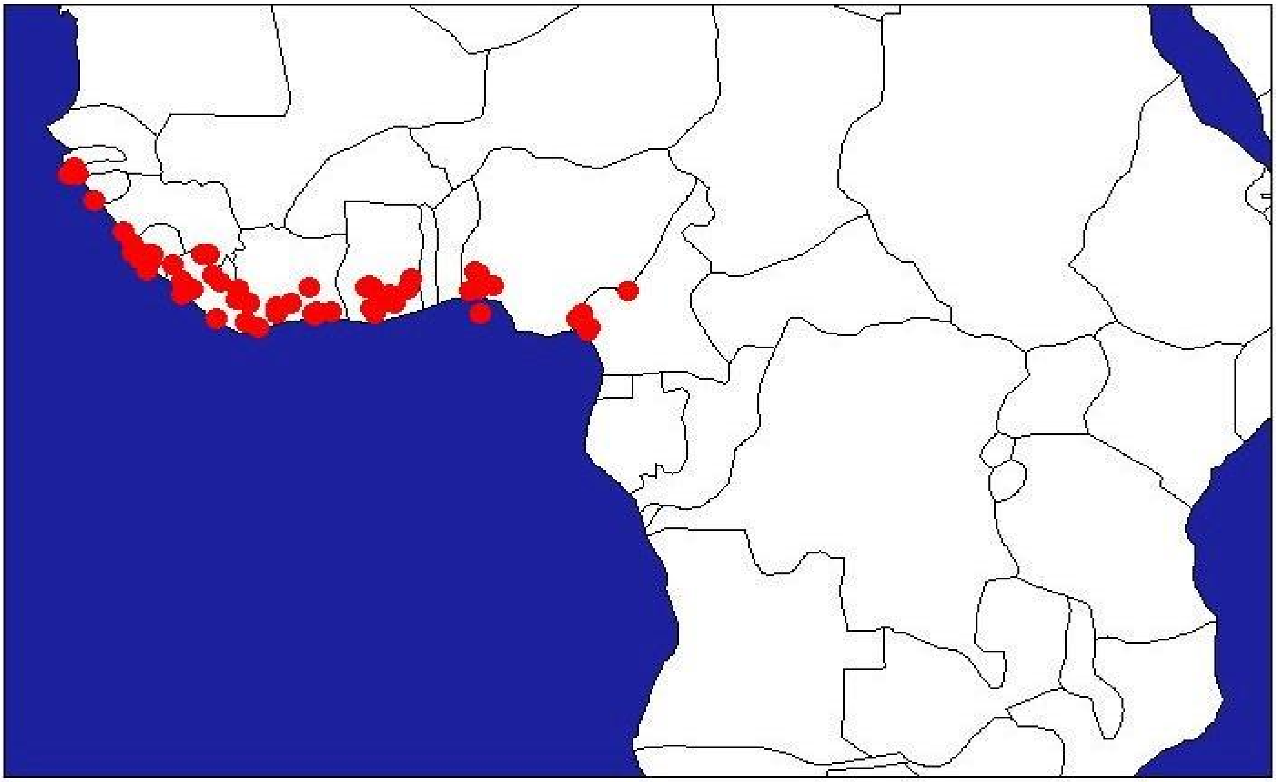


Figure 1.3: Collection locations of 83 herbarium specimens of *C. acuminata* from Meise, Wageningen, Kew and Missouri herbaria. Specimens taken from cultivated trees have not been plotted.

Kola nuts are widely cultivated in West Africa because they contain two alkaloids, caffeine and theobromine, which are powerful stimulants that counteract fatigue, suppress thirst and hunger, and are believed to enhance intellectual activity (Nickalls, 1986; Sundstrom, 1966). Due to their unique bitter taste, kola nuts are effective for refreshing the mouth, and the twigs are used as **"**chewing sticks" to clean the teeth and gums (Lewis and P.F., 1985). Kola nuts are also used as a source of alkaloids in pharmaceutical preparations (Opeke, 1992).

Large quantities of the nuts are exported to Europe and North America, where they are used chiefly for flavouring cola drinks such as Coca-Cola, which are refreshing or stimulating substitutes for tea or coffee (Irvine, 1956). Beverages such as kola wine, kola cocoa and kola chocolate – a type of chocolate containing cacao and kola powder in cocoa butterfat (Opeke, 1992) – and one interesting sounding concoction called "Burroughs and Wellcomes Forced March Tabloid" were once tried in Britain, but they were short lived (Tindall, 1998).

1.7 Medicinal uses

Traditionally, the leaves, twigs, flowers, fruits follicles, and the bark of both C. *nitida* and C. *acuminata* were used to prepare a tonic as a remedy for dysentery, coughs, diarrhœa, vomiting (Ayensu, 1978) and chest complaints (Irvine, 1961). The nuts have considerable potential for the development of new pharmaceuticals and foods (Fereday *et al*., 1997). Extracts of *C. nitida* bark have been tested on various pathogenic bacteria (*Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* , beta-haemolytic streptococci, *Escherichia coli* and *Neisseria gonorrhoeae*) (Ebana *et al*., 1991). All the extracts showed inhibitory activity against these organisms. Benie *et al.* (1987) report that stem bark extracts of *C. nitida* inhibited the release of luteinizing hormone (LH) from rat pituitary cells and may therefore regulate gonadotropin release. This has potential to be used as a natural fertility regulator.

1.7.1 Socio-cultural values and uses

Chewing of kola nuts is a widespread habit in the Sub-Saharan countries of Africa, especially in northern Nigeria and Sudan. Kola chewing plays a similar social role to tea and coffee drinking or cigarette smoking in Western countries (Purseglove, 1968; Rosengarten, 1984;

Russell, 1955). *C. acuminata* is widely used ceremonially and socially by the people of West and Central Africa. At a birth a kola tree may be planted for the new-born child. The child remains the lifelong owner of the tree. A kola tree is also often planted at the head of a grave as part of local death rites (Tindall, 1998).

Russell (1955) described cultural uses of kola in the Yorubaland of western Nigeria. He reported that the seed is normally kept in the house and an offering of kola forms part of the greeting to an honoured guest. The older the kola the more highly it is regarded, and white and pink nuts are kept for particularly favoured guests. The gift of kola and especially the splitting and sharing of kola nuts between two or more people signifies a special bond of friendship. Similarly, the sharing of kola nuts is a necessary prerequisite to business dealings that involve a strict etiquette in presenting, dividing, and eating of the fruits. Proposals of marriage may be made by a young man's presentation of kola nuts to the prospective bride's father and her acceptance or refusal may be conveyed by a reciprocal gift of nuts, with the meaning depending upon the quality and colour. Kola nuts presented by the bride's family signify fertility, productivity, prosperity, contentment and desire for the union (Johnson and Johnson, 1976; Sundstrom, 1966).

Kola figures prominently in religion and magic. It is used in divination and to learn the mind or intent of a god for healing the sick or against barrenness. In some areas it is a component of an oath-taking process. The possession and use of kola nuts may be a symbol of wealth and prestige (Hauenstein, 1974; Lovejoy, 1980).

1.7.2 Pharmacological Effects of C. acuminata

Anti-Cancer Potential of C. acuminate Extract Being the leading cause of death in Jamaica and the third leading cause of death worldwide, cancer is a disease of major concern and the search for compounds that can combat these deadly diseases is ongoing. Presently, 60% of chemotherapeutic drugs are from natural origin (Cragg and Newman, 2005), however more often than not they are associated with insufferable side effects which make the hunt for other molecular entities or plant extracts an ongoing one. Because of its abundant enthomedicinal practices, the bissy but plant seemed ideal to be screened for anti-cancer potential even though the plant has no traditional use in that arena. Research from our lab showed that of the five solvents used (hexane, dichloromethane, acetone, methanol and water), the hexane bissy nut extract was most effective as it (160 µg/ml) killed 100% of breast (BC) and prostate (DU145 and PC) cancer cell lines (Lowe *et al.,* 2012)

1.7.3 Anti-Microbial Properties of C. acuminata Microbial

Infections are of major importance to the medical arena since they are responsible for 90% of infections found in health care services. The occurrence of MDR bacterial strains seems to be the major cause of treatment failure (Koyama, 2006) Not only do they warrant concern in the medical arena but to food preservation as well. Presently there is growing global demand for consumers to reduce chemical preservation that can be unfavorable to human health (Smid and Gorris, 1999).

1.8 BIOACTIVE COMPOSITION OF KOLANUTS

The analysis of the two predominant species k. acuminata and k. nitida showed that crude protein range from 3.9-6.7%. kola contains between 1.0 and 1.2 caffeine. Kola contains a glycoside kolanine, 9% protein, 2% fat, 74% carbohydrate on fresh bases.

Previous researchers have reported a number of secondary metabolites from the kola nut, including caffeine, theobromin, catechin, epicatechin, procyanidins and proanthocyanidins.6–8 Aliphatic and heterocyclic amines in different species of cola have been reported as well,9 and quinic, tannic and chlorogenic acids have been found as present in the kola nut.10 Caffeine was the major alkaloid identified in Cola seeds and was considered as one of the signature compounds due to its concentration range

Knebel and Higler showed that fresh kola nut contained a glucoside named “kolanin”, reporting that it is readily hydrolysed or split into glucose and caffeine in ripe or dried fruit.7

Alkaloids

These are organic nitrogenous compounds that have complex molecular structures of good pharmacological activity thus, bitter in taste and mostly basic in nature (El-Olemyl *et al*., 1994). These chemicals comprise up largest single class of secondary plant substances which contain one or more nitrogen atoms usually in combination as part of cyclic system. Alkaloids do not have an exact nomenclature but they are named as proto alkaloids, when they are without heterocyclic ring in their structures, pseudo alkaloids are those alkaloids with and without heterocyclic rings that are not derived from amino acids and the carbon skeleton is soprenoid, and true alkaloids, which are those that have heterocyclic rings in their structure. True alkaloids and photo alkaloids almost always have amino acids as their distal biosynthetic precursors and acetate is also incorporated in their structure. Alkaloids accumulate in actively growing tissues, epidermal and hypodermal cells, vascular sheath and latex vessels (Evans and Trease, 1999). Alkaloids are usually colourless, often optically active substance, most are crystalline but few are liquid at room temperature e.g. nicotine. The alkaloid quinine for example is one of the prodominant bitter substances known, and is significantly bitter at molar concentration of 1 X 105 . The most common precursors of alkaloids are amino acids. Many alkaloids are terpenoids in nature and some (e.g Solanine, the steroidal alkaloid of the potato) are the best considered from the biosynthetic point of view as modified terpenoids. Others are aromatic compounds e.g. colchicines. Alkaloids are rich in the angiosperms families and are generally absent or infrequent in the gymnosperms, ferns and lower plants (Evans and Trease, 1999).

Saponins are one of the groups of glycosides found in many plant species with known foaming properties when mixed with water, allowing the formation of small stable bubbles. The amount of foam created by the crushed plant samples shaken with water in a jar is a good indication of the amount of saponins present. Saponins are normally broken down in the digestive system and are toxic when absorbed into the blood stream. They are used in modern times in the manufacture of fire extinguisher foam, tooth paste, shampoos, liquid soap and cosmetics. It is also used to increase the foaming of beer soft drink. As glycosides they are hydrolysed by acids to give an agylcone (sapogenin) and various sugar and related uronin acids. The steroidal saponin and pertocyclicterpenoids have a glycosydial linkage at -C3 and have a common biogenetic origin through malvalonic acid and isoprenoid unit (Evans and Trease, 1999).

Glycosides are non-reducing substances, which on hydrolysis with reagents or enzymes yield one or more reducing sugars among the products of hydrolysis. The non-sugar part of the molecule is called the agylcone or genin, and the sugar component, the glycone. The usual linkage between the sugar and agylcone is an oxygen linkage, connecting the reducing group of a sugar and an alcoholic or phenolic hydroxyl group of the agylcone. Such glycosides, sometimes called O-glycosides, are the most numerous ones found in nature. Other glycosides however occur, e.g. S-glycosides and N-glycosides in S-glycosides, e.g. Sinirin, where the sugar is linked to the thiol group of the agylcone. In n-glycosides (e.g. streptidine moiety of streptomycin and glucosamine), the sugar is linked to the amino group of the agylcone. There are also c-glycosides (e.g. barbalion) in which the sugar is linked to the agylcone by a carbon to carbon bond. All naturally occurring glycosides are of the β-type, although the α-linkage is found in some carbohydrates such as sucrose, glycogen and starch (Evans and Trease, 1999). Glycosides occur widely in nature and occur in low concentration in nearly all plants. They occur not only in angiosperms but also in lower plants e.g. in streptomyces species. Glycosides are found in all parts of the plant, in roots, bark, leaves, flowers, fruits and seeds. Much plant pigments responsible for the colour of flowers and fruits are glycosides. Glycoside formation may well be a method of storing certain organic compounds e.g. phenols. It was also suggested that, some glycosides have a role of defence against the invasion of the tissue by micro-organisms subsequent to wounding, since many agylcones are aseptic and hence bactericidal in character. Plant glycosides that are currently used in medicine, though not larger in number, are important drugs. Glycosides of medicinal plants may be used as cardial stimulants (e.g. digitoxin and quabian or laxatives) Sinnosides and barbaloin or local irritants e.g. sinigrin or analgesics (silicon) and against capillary frugility (hesperidin) (Evans and Trease, 1999).

1.9 CAFFEINE

Caffeine is a bitter, white crystalline xanthine alkaloid and a psychoactive stimulant drug. Caffeine was discovered by a German chemist, Friedrich Ferdinand Runge. He coined the term kaffein, a chemical compound in coffee (the German word for which is Kaffee), which in English became caffeine (and changed to Koffein in German).9 Caffeine belongs to the family of heterocyclic compounds known as purines.

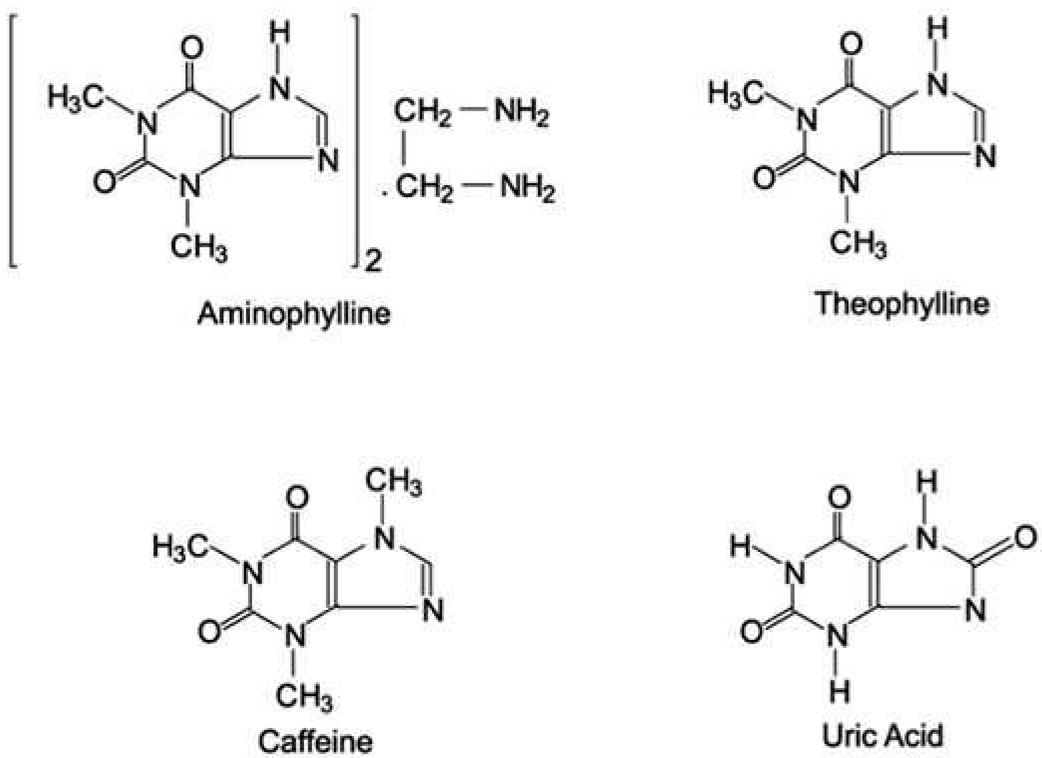


Figure 1.4: Structure of derivative of compounds of purine

The structure of caffeine and other purine derivative can be shown in the figure above . It has the systematic name 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6- dione; it is also known as 1,3,7-trimethylxanthine, and 1,3,7-trimethyl2,6-dioxopurine.

Caffeine was discovered in coffee in 1820. In 1838, it was established that theine discovered in tea in 1827 is identical to caffeine.10 The exact composition of cola nitida have been shown to include xathine alkaloid (caffeine, theophylline, theobronine) tamin, betaine and kolanine.11 The bitter taste is due to the presence of tamin and betaine while the reddish stain extracted is the kolatine and kolanine content. It occurs in the fruit and bark of a number of plants; like tea leaves, coffee, cocoa, kola nuts, beans and mate-leave. Its molecular formula is $C_8H_{10}O_2N_4$ with 28.85% nitrogen content.

1.9.1 PROPERTIES OF CAFFEINE IUPAC

Name 1,3,7-trimethyl- 1H-purine- 2,6(3H,7H)-dione Other names 1,3,7-trimethylxanthine, trimethylxanthine, methyltheobromine, 7-methyltheophylline, theine, mateine, guaranine

Properties

Molecular formula $C_8H_{10}N_4O_2$

Molar mass 194.19 g/mol

Exact mass 194.080376 u

Appearance Odorless, white needles or powder

Density 1.23 g/cm^3 ,

solid Melting point 227–228 °C (anhydrous); 234– 235 °C (monohydrate)

Boiling point 178 °C subl. S

olubility in water 2.17 g/100 ml (25 °C) 18.0 g/100 ml (80 °C) 67.0 g/100 ml (100 °C) Acidity (pKa) −0.13–1.22[1] Dipole moment 3.64 D (calculated) 1.4.2

SOLUBILITY OF CAFFEINE

Caffeine is not a highly water soluble substance and, therefore, has a moderately slow release from chewing gum. Caffeine is 2.1% soluble in water at room temperature, 15% soluble in water at 80oC, and 40% soluble in boiling water.

1.9.2 CLASSIFICATION OF CAFFEINE

Caffeine can be classified as an alkaloid, a term used for substances produced as end products of nitrogen metabolism in some plants. The chemical formula is $C_8H_{10}N_4O_2$. Caffeine has a molar mass of 194.19 grams (6.85 ounces). It is soluble in water and in many organic solvents, and it appears in pure form as white crystals.

1.9.3 HEALTH USES OF CAFFEINE

The precise amount of caffeine necessary to produce effects varies from person to person depending on body size and degree of tolerance to caffeine. The uses of caffeine can be seen both from a moderate intake view and an over use view.

1.9.4 OVERUSE

An acute overdose of caffeine, usually in excess of about 300 milligrams, dependent on body weight and level of caffeine tolerance, can result in a state of central nervous system over-stimulation called caffeine intoxication. The symptoms of caffeine intoxication are not unlike overdoses of other stimulants. It may include restlessness, fidgetiness, nervousness, excitement, euphoria, insomnia, flushing of the face, increased urination, gastrointestinal disturbance, muscle twitching, a rambling flow of thought and speech, irritability, irregular or rapid heartbeat, and psychomotor agitation. (Kamijo *et al*., 1999)

1.9.5 Molecular interaction with electromagnetic radiation.

Molecules have electromagnetic fields derived from their electrons and nuclei. We saw earlier that plane-polarized light interacts by being rotated by an enantiomer. As seen below, energy varies across the spectrum and matches that required for various interactions.

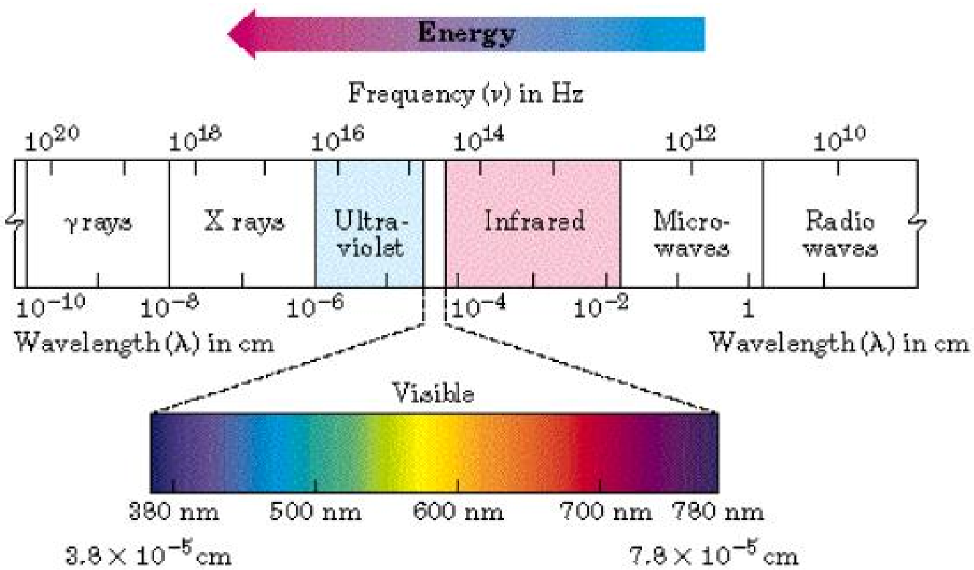


Figure 1.5: Electromagnetic Spectrum

Energy increases going to the left. The electromagnetic radiation interacts with the electromagnetic fields of the electrons to raise their energy levels from one state to the next. The nature of that interaction depends on the energy available. Ultraviolet and visible have sufficient energy to effect electronic transitions. Infrared has sufficient energy only to effect transitions between vibrational energy states. Microwave has only enough energy to effect transitions between rotationally energy states. Thus the radiation absorbed tells us different information. Radio waves have insufficient energy to effect molecules but affect nuclear spin energy states found in magnetic fields. This latter interaction is most important because it is used in Nuclear Magnetic Resonance spectroscopy.

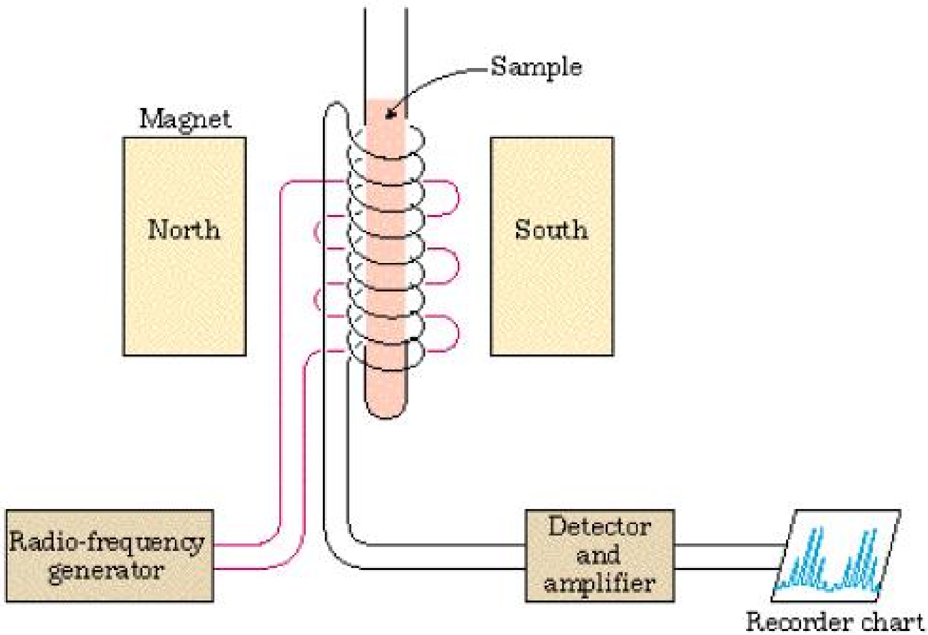


Figure 1.: Schematic of an NMR spectrometer

A. All nuclei with unpaired protons or neutrons are magnetically active- they have a magnetic field arising from the unpaired nuclear particle. Of greatest interest to an organic chemist is hydrogen (including deuterium) and carbon (the ^{13}C isotope not the ^{12}C isotope which has 13 12 paired neutrons and protons).

B. Placed in an external magnetic field this magnetic field of the nucleus has two stable states, alignment with or against the applied field, which are of slightly different energies (aligned against is higher). The greater the applied field the greater this difference (this is a crucial fact).

a. Internal (in the molecule) factors which affect (add to or subtract from) the applied magnetic field so as to put the individual nucleus in a different magnetic environment from that felt by another nucleus create differences in the nuclei.

b. Higher applied magnetic fields will create larger absolute numerical values of the differences between energy states and allow easier distinction between two different nuclei (better resolution)

Infrared Spectroscopy

We mentioned in the chapter's beginning that infrared radiation was of the right energy level to cause increases in the vibrational energy states of organic molecules. The energy needed to change vibrational states depends on bond strength and the mass of the atoms bonded. Thus different bonds absorb different IR wavelengths and these are indicative of what functionality (bonds) are in the molecule. Each functionality can vibrate in several different ways and thus absorb several wavelengths but usually we use only one as diagnostic.

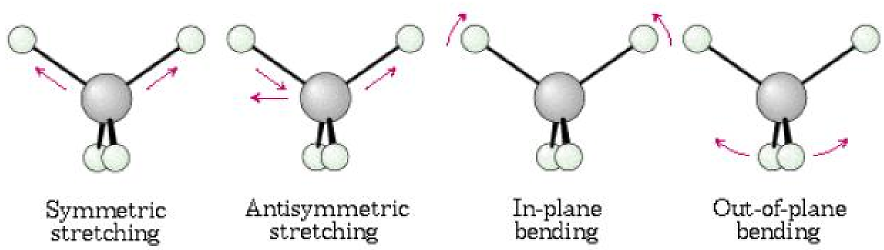


Figure 1.7: Infrared Spectroscopy

Mass spectrometry Mass spectra give us information on the molecular weight, the molecular formula (at high resolution), and what substructures are present. How does it work?

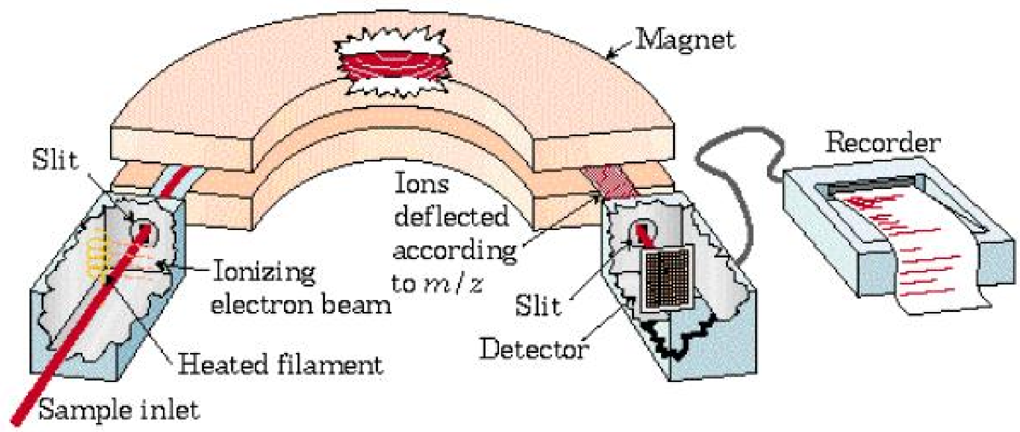


Figure 1.8: Mass spectrometry

An electron is ejected from the molecules by the ionizing electro on beam and passed through an electrical field to accelerate them to a uniform velocity. These ions are then passed through a magnetic field. Moving charges are deflected by a magnetic field, with low mass ions being deflected more than heavy mass ions (the force is constant because they all have a single charge but momentum is greater for the heavier ions). These ion positions are recorded as they strike the detector and a spectrum with mass on the x-axis is recorded.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

1. Beakers (500ml and 250ml)
2. Erlenmeyer Flask
3. Separatory Funnel
4. Stirrer
5. Retort Stand / Clamp
6. Funnel
7. Filter Paper
8. Cola nitida
9. Cola acuminate

2.2 REAGENTS

1. Na_2CO_3 Sodiumtrioxocarbonate(IV)
2. Na_2SO_4 Sodiumtetraoxosulphate(VI)
3. Dichloromethane CH_2Cl_2
4. Distilled Water

2.3 EQUIPMENT

1. Electric blender
2. Weighing machine
3. Heating Source

2.4 METHODS

2.4.1 collection of sample : The sample was obtained from New Benin Market, Benin city Edo state, Nigeria. The sample was identified by Prof. J.F. Bamidele ,a taxonomist in the department of Plant Biology and Biotechnonlogy, University of Benin, Benin city Nigeria.

2.5 PROCEEDURE

The Cola nitida and Cola acuminate were grinded with an electric Blender.
The Caffeine was then be extracted easily by using the gravimetric method. This method involves extracting the caffeine from the samples using Dichloromethane in a liquid-liquid extraction. After this, the sample was sent for IR analysis.

2.5.1 EXTRACTION OF CAFFEINE:

Distilled Water was heated to boil, then 50g of the Cola nitida powder was added, then left to boil for 10minutes. After this, the mixture was filtered and the filtrate allowed to cool. 5g of Na_2CO_3 was added to the filtrate to convert the tannins to water soluble salts. The solution was then transferred to a Separatory Funnel for liquid-liquid extraction.

This procedure was repeated for both Cola acuminate

2.5.2 LIQUID- LIQUID SEPARATION

The extracted liquid was transferred into a separating funnel, then 20ml of Dichloromethane (CH_2Cl_2) was added to the liquid extract in the separating funnel. The separating funnel was agitated gently so as to mix the two layers together. After a little while, the mixture separated into two layers. The lower layer containing the Dichloromethane and Caffeine was collected in a beaker. This process was repeated two more times with 20ml each of CH_2Cl_2 to completely extract the Caffeine. Na_2SO_4 was added to dry any water for a few minutes, then the solution was decanted to remove the

Na_2SO_4. The beaker was heated to evaporate the CH_2Cl_2. This whole procedure was repeated for the and Cola acuminate

CHAPTER THREE

RESULT AND DISCUSSION

3.1 RESULTS

The sample analyzed are as follows;

Figure 3.1.Cola acuminata (Red cola) **Figure 3.2. Cola nitida (white cola)**

The amount of caffeine extracted from these samples using Dichloromethane via liquid-liquid separation was weighed and recorded. The results obtained is given in table 3.1.1 below.

Table 3.1: Amount Of Caffeine Extracted

Sample name	Mass of sample used (Grams)	Amount of caffeine obtained (Mg)
Cola nitida (white cola)	50	20
Cola acuminata (Red cola)	50	30

From the results obtained, it was found , Cola nitida (white cola) contained 20mg that the while of Cola acuminata (Red cola) contained 30mg

The amount of Caffeine in Cola acuminata (Red cola) was found to be higher than, Cola nitida (white cola).

It should be noted that these results are not standard as the amount of Caffeine in a product varies from type of product to the method of preparation.

The values gotten could be considered low and safe if we consider the U.S. Food and drugs Administration classification of caffeine consumption. Under this classification, caffeine intake of 130-300 mg/day is low/moderate, above 400 mg/day is high.

It is difficult to calculate the exact amount of caffeine consumed by individuals because the amount of product used per cup is usually not weighed/measured but dispensed by means of teaspoons.

The amount of caffeine needed to produce effects varies from persons to persons according to the body weight and individual sensitivity. Children for instance, due to their smaller weight, may manifest hyperactivity and other side effects if they regularly consume beverages with moderate caffeine content which are considered safe for adults (Benjamin, Rogers and Rosenbaum, 1991; www.kidshealth.org). Therefore, people who need caffeine restriction (pregnant women, people suffering from hypertension, diabetes, open angle glaucoma, insomnia, etc.) Should choose products with low caffeine content and avoid regular consumption.

Caffeine is an addictive substance, so regular consumption of products that contains even moderate amounts of caffeine may lead to addiction to the products with withdrawal reactions like mood changes and flu-like Symptoms. These include headaches, fatigue, irritability, difficulty in concentrating, depression, nausea/vomiting and muscle ache or stiffness (Juliano and Griffiths, 2004). These reactions may lead to increased intake of caffeine with increased adverse effects.

3.2 IR ANALYSIS RESULT

The IR analysis was done to determine the Functional group present in the extracted Alkaloid. It was done using a FTIR System, Spectrum BX. The results obtained is given below.

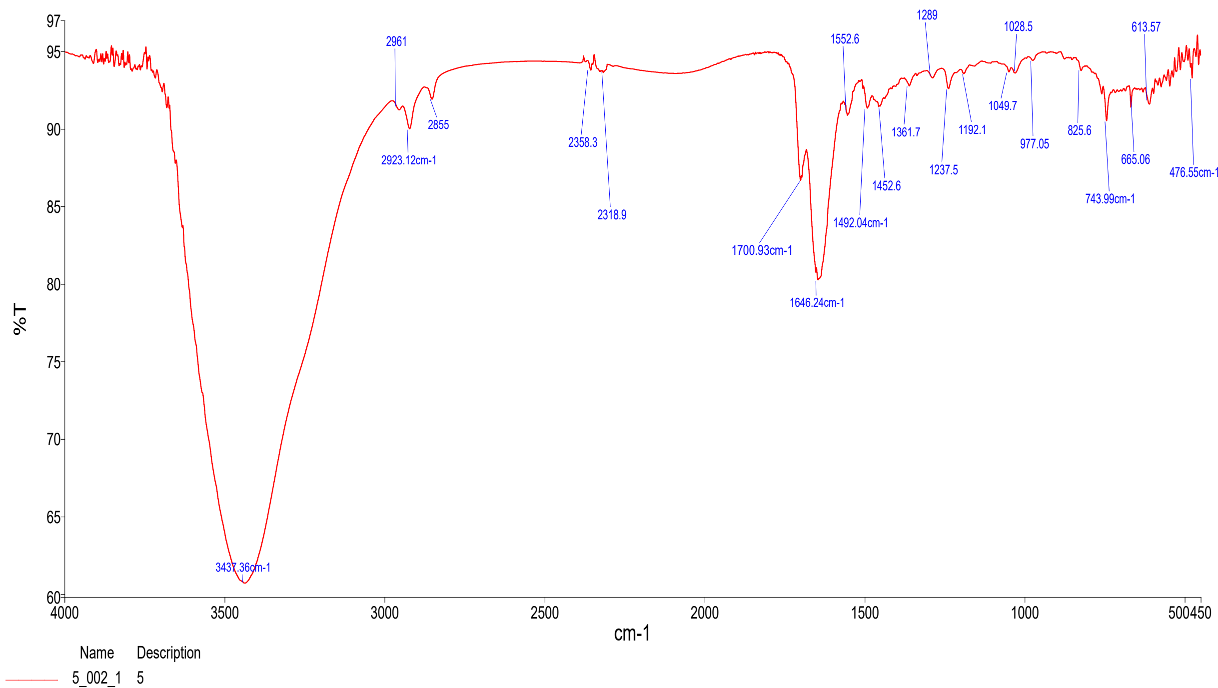


FIG SPECTRA SHEET Cola nitida (white cola)

TABLE 3.2 : IR ABSORPTION BANDS OF FUNCTIONAL GROUP DETECTED IN Cola nitida (white cola)

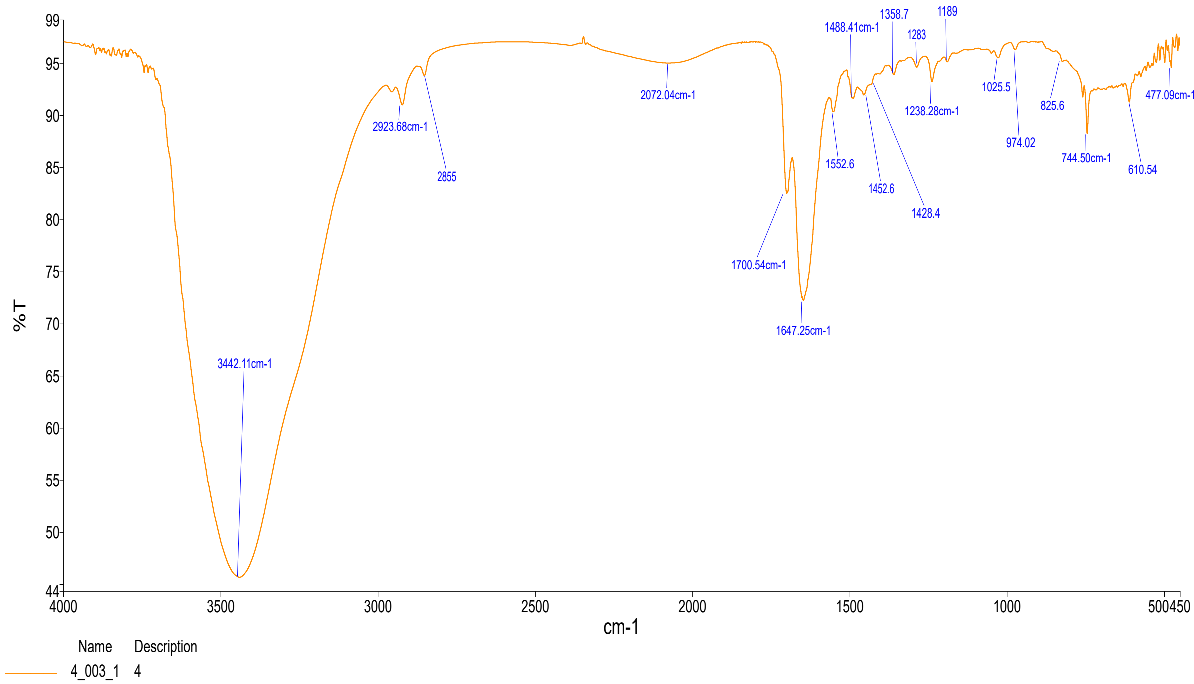
S/N	Peak(cm^{-1})	Appearance	Bond	Functional Group
1	3437.36	MEDIUM	N-H Stretch (1-band or 2 if H-bonded)	AMINES, SECONDARY AMINES (R2NH)
2	2923.12	MEDIUM,STRONG	C-H stretch of C-H	Alkyl groups CH3, CH2,CH
3	2855	MEDIUM STRONG	C-H stretch of C-H	Alkyl groups CH3, CH2,CH
4	1700.93	VERY STRONG	C=O stretch	Amide RCONH2
5	1646.24	QUITE VARIABLE	C=C stretch	Alkenes
6	1492.04	VARIABLE 2 or 3 BANDS	C=C stretch	Arenes
7	1237.5	STRONG	C-O	Acids,Esters,Anhydride
8	743.99	BOTH STRONG	C-H BEND	Mono-substituted benzene
9	665.02	Strong	C-Br	Halogen
10	476.55	(strong)Hidden in the fingerprint region	C-I	Halogen compound

FIG SPECTRA SHEET Cola acuminata (Red cola)

S/N	Peak(cm^{-1})	Appearance	Bond	Functional Group
1	3442.11	MEDIUM	N-H Stretch (1-band or 2 if H-bonded)	Amines ,secondary amines RNH2
2	2923.68	MEDIUM, STRONG	C-H Stretch of C-H	Alkyl Group CH3, CH2, CH
3	2885	MEDIUM, STRONG	C-H Stretch of C-H	Alkyl Group CH3, CH2, CH
4	1700.54	VERY STRONG	C=O Stretch	Amides, RCONH2
5	1647.25	QUITE VARIABLE	C=C Stretch	Alkenes
6	1552.6	VARIABLE (2 or 3 bands)	C=C Stretch	Arenes
7	1488.41	VARIABLE (2 or 3 bands)	C=C Stretch	Arenes
8	1238.28	STRONG	C=O Stretch	Acid, Esters ,

				Anhydride
9	744.50	BOTH STRONG	C-H Bend	Mono- substituted benzene
10	610.54	STRONG	C-Br	Halogen

TABLE: IR ABSORPTION BANDS OF FUNCTIONAL GROUP DETECTED IN Cola acuminata (Red cola)



PERCENTAGE YIELD OF CAFFEINE

The percentage yield of crude caffeine-extract obtained from this process is calculated as follows;

$$= \frac{\text{Mass of crude caffeine extract}}{\text{Mass of Kolanut}} \times 100$$

Therefore % yield of Cola nitida = $\underline{0.02g}$ X 100 = 0.04%

50g

% yield of Cola acuminata = $\frac{0.03g}{50g}$ X 100 = 0.06%

DISCUSSION

From the FT-IR analysis of the caffeine fraction of Cola nitida (white cola),the I.R band were observed at 1646.24 cm^{-1}(c=c)stretch of Alkene;1700.93 cm^{-1}(c=o)stretch of Amide RCONH2; 2855 cm^{-1}(C-H)stretch of Alkyl groups;3437.36 cm^{-1}(N-H)stretch of Amines. The broad band observed at 3437.36 cm^{-1} does not have 2 or 3 band (not H-banded) and hence will likely be a tertiary Amine which are present in most Alkaloid of heterocyclic origin.The sharp peak at 3437.36 cm^{-1}suggests that the isolate is rich in Alkaloid.

From the FT-IR analysis of the caffeine fraction of Cola acuminata (red cola) the IR bands were observed at1646.24 cm^{-1}(C=C) stretch of alkene ,1700.93 (C=0) stretch of amide RCONH2;2855(C-H) stretch of alkyl groups. The sharp band observed at 3437.36 (cm^{-1}) does not have 2 or 3 band (not H-bonded) and hence will likely be a tertiary amine which are present in most alkaloids of heterocyclic origin. The sharp peak at 3437.36(cm^{-1}) suggests that the isolate is rich in alkaloids

The results from the calculation of percentage yield of the caffeine fraction obtained from the kolanut sample(cola.acuminata and cola.nitida) shows that caffeine is more abundant in red kolanut(cola.acuminata) with a percentage yield of 0.06% compared to white kolanut(cola.nitida) with a percentage yield of 0.04%.

CONCLUSION

This study covers the extraction, isolation, and characterization of caffeine which was obtained from the two varieties of native Kolanut (cola-acuminata and cola nitida). The crude caffeine in the study is adequate and compares very well with properties of caffeine literature. The use of kolanut as a source of caffeine production will diversify the economic use of kolanut other than for consumption as food . (Oluokun and oladokun 1999)

References

A brief History of Drugs: From the Stone age to the Stoned age. Park Street Press. ISBN 0-89281-826-3 8) Psychoanaleptics: psychostimulants, agents used for ADHD and Nootropic (http://en.wikipedia.org/wiki/caffeine) 81 9) Encyclopedia Britannica (1911). “Coffee”.

Abdul M., Kazi F.A., Zainal, A. and Zakir, H. (2006). Determination of Caffeine by solid phase Extraction and High Performance Liquid Chromatography. *Malaysian Journal of Chemistry,* 9,45-51

Seitz, (1992). The Development of Bodily-Kinesthetic Intelligence in Children: Implications for Education and Artistry

Adeyeye, E. and Ayejuyo, O. (1994) Chemical Composition of Cola acuminata and Garcinia kola Seeds Grown in Nigeria. *International Journal of Food* . pp 34-56

Aeschbacher H.V., Graham, R. & Bradbrook, J. (1975). The effect of Caffeine on Barbiturate sleeping Time and Brain Level. *Journal of Pharmacology and Experimental therapeutics*, 196,635

Benie, T., Izzi, A. E., Tahiri, C., Duval, J., Thieulant, M. L., and El Izzi, A. (1987). Natural substances regulating fertility. The effect of extracts of plants from the Ivory Coast pharmacopoeia on LH release by cultured pituitary cells. Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 181, 163-167

Benjamin, LT Jr; Rogers AM, Rosenbaum A (1991). "Coca-Cola, caffeine, and mental deficiency: Harry Hollingworth and the Chattanooga trial of 1911". *J Hist Behav Sci* **27** (1): 42-55.

Blumenthal, M., Goldberg, A. and Brinckmann, J. (2000) Herbal medicine Expanded

Bolton, Ph.D., Sanford (1981). "Caffeine: Psychological Effects, Use and Abuse". Orthomolecular Psychiatry 10 (3): 202–211.

Chevalier, A., and Perrot, A. (1911). "Les Vegetaux utiles de l'Afrique tropicale Francaise," Challamell, Paris.

Chow p. 2014 19-20 (Czech edition); also Arcimovicova p. 9, Evans p. 2 and others

Clifford, M.N and J.R. Ramirez-Martinez. 1990. "Chlorogenicacids and Purine Alkaloids Contents of Mate (Ilex paraguariensis) Leaf and Beverage". Food Chemistry. 35: 13–21

Cobley L.S , Steele W.M. (1976): Beverage mastictory and Drug plants. In an introduction to the Botany of Tropical crops 2nd edition. Longman Grp limited

Coffee". EncyclopÃ¦dia Britannica. (1911).

commission E monographs

Determination and Characterization of Caffeine on Tea, Coffee and soft Drink by solid phase Chromatography (SPE - HPLC). Malysian Journal of Chemistr. 8, 065 – 075.) Escohotado, Antonio; Ken Symington (1999).

Ebana, R. U. B., Madunagu, B. E., Ekpe, E. D., and Otung, I. N. (1991). Microbiological exploitation of cardiac glycosides and alkaloids from Garcinia kola, Borreria ocymoides, Kola nitida and Citrus aurantifolia . Journal of Applied Bacteriology71, 398-401.

Escohotado, Antonio; Ken symington (1999). A brief history on drugs: from the stone age to the stoned age. Park street press.

Escohotado, Antonio; Ken Symington (May 1999). A Brief History of Drugs: From the Stone Age to the Stoned Age. Park Street Press. ISBN 0-89281-826-3.

FAO (1995). "Edible Nuts," Non-Wood Forest Products Series, Number 5, FAO, Rome. (http://www.fao.org/docrep/V8929e/V8929e00.htm

Fereday, N., Gordon, A., and Oji, G. (1997). “Domestic market potential for tree products from farms and rural communities: experience from Cameroon,” NRI Socio-Economic Serices Report No. 13. Natural Resources Institute (NRI), Chatham.

Hauenstein, A. (1974). La noix de cola. Coutumes et rites de quelques ethaies de Cote d'Ivoire. Antropos69, 457-493.

Hutchinson, J., and Dalziel, J. M. (1958). “Flora of West Tropical Africa,

Irvine, F. R. (1956). “Plants of the Gold Coast,” Oxford University Press.

J.L.IVY, D.L.COSTILL, W.J.FINK, and R.W.LOWER. 1979 Human Performance laboratoryBall State University Muncie,Indiana 47306

Jarvis, Gail MAY 21 2002 The Rise and Fall of Cocaine Cola.

Johnson, E. J., and Johnson, T. J. (1976). Economic plants in a rural Nigerian market. Economic Botany30, 375-381.

Keay, R. W. J., Onochie, C. F. A., and Stanfield, D. P. (1960). “Nigerian trees,” Federal Department of Forest Research, Ibadan, Nigeria.

Keay, R. W. J., Onochie, C. F. A., and Stanfield, D. P. (1960). “Nigerian trees,” Federal Department of Forest Research, Ibadan, Nigeria.

Kola Microsoft Encarta 2009 [DVD]Redmond, WA: microsoft coorperation 200

L.K. Opeke, Tropical tree crops (Ibadan: Spectrum, 1992)

Lovejoy, P. E. (1980). Kola in the history of West Africa. Cahier d'Etudes Africaines 77-78, 97-134. Masefield, G. (1

Mc- Graw- Hill Encyclopedia of Science and Technology volume 4 Bor-cle, sixth Edition, Standen A: Edition John wiley and sons, Inc. New York p 129 4 Annon C.B.(1953): kola nuts and kola chocolatee Rev. vol. 8 (185) from Abstract Curri. Sch. Tech. vol. 6 (153)

Moloney, A. (1887). “Sketch of the Forestry of West Africa,” Sampson Low, London.

Nehlig, A; Daval, JL; Debry, G (1992). "Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic, and psychostimulant effects". Brain Res Rev 17 (2): 139–70. doi:10.1016/0165-0173(92)90012-B. PMID 1356551.

Nickalls, R. W. D. (1986). W.F. Daniell (1817 - 1865) and the discovery that cola -nuts contain caffeine. Pharmaceutical Journal 236, 401-402.

Nkongmeneck, B. (1985). Le genre cola au Cameroun. Revues Sciences et Techniques, Série de la Science Ag ronomique 1, 57-70.

Okoli, B.J., K. Abdullahi, O. Myina, and G. Iwu. 2012. “Caffeine Content of Three Nigerian Cola”. Journal of Emerging Trends in Engineering and Applied Science. 3(5): 830-833.

Oluokun,J.A and E.A. Oladokun,1999.The effects of graded levels of brewers spent grains and kolanut pod meal in the performance characteristics and carcass quality of rabbit.Nigerian J.Animal production.26:71-77

Opeke, L. K. (1992). “Tropical tree crops,” Spectrum Books Ltd., Ibadan.

P.E. Lovejoy, Kola in the History of West Africa, Cahier d’Etudes Africaines, 1980, 77-134.

Plantas tóxicas by BRUNETON, J. and a great selection of related books, art and collectibles ... Published by Springer 1998-12-10 (1998).

Purseglove, J. W. (1968). “Tropical crops: Dicotyledons,” Longmans Green & Co Ltd, London.

Reeling, J. (1999). Therapeutic of Kola Nut. Journal of the American Medical Association,5, 6-10Adulmumin, M.D., Kazi F. A., Zainal, A. M. & Zakir, H. (2006).

Rosengarten, F. (1984). “The book of edible nuts,” Walker and Company, New York.

Rubin, R. (2008). New Study, different outcomes on Caffeine, "Pregnancy" Journal of Clinical pharmacology, 41, 35 – 41

Russell, T. A. (1955). The kola of Nigeria and the Cameroons. Tropical Agriculture, Trinidad 32, 210-240

S.T. Lowor, P.C. Aculey, and M.K. Assuah, Analysis of some quality indicators in cured Cola nitida (Vent)., Agriculture and Biology Journal of North America, 1(6), 2010, 1206-1214.

Sundstrom, L. (1966). The cola nut. Functions in West African social life. Studia Ethnographia Upsa liensia 26, 135-146.

Tindall, R. (1998). The culture of Cola: social and economic aspects of a West African domesticate. Ethnobo tanical Leaflets 2 (Winter 1998), 1-3

Tindall, R. (1998). The culture of Cola: social and economic aspects of a West African domesticate. Ethnobo tanical Leaflets 2 (Winter 1998), 1-3

Voelcker, O. J. (1935). Cotyledon colour in kola. Tropical Agriculture 12, 231-234.

Weinberg, BA; BK Bealer (January 2001). The World of Caffeine. Routledge. ISBN 0-415-92722-6.

What's your Poison: Caffeine (http://www.abc.net.com.au/quantum/poison/caffeine/caffeine.htm). Australian Broadcasting Corporation (1997)

Whats your poison: caffeine. Australian Broadcasting Corporation (1997).

Yu, Lu (October 1995). The Classic of Tea: Origins & Rituals. Ecco Pr; Reissue edition. ISBN 0-88001-416-4.