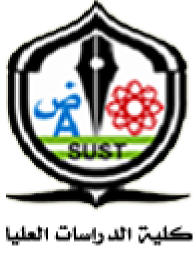

Sudan University of Science and Technology

College of Graduate Studies

Phytochemical Screening and Isolation of Caffeine from *Gora* (*Cola acuminate*)

مسح فايتوكيميائي وعزل الكافيين من القورا(جوز الزنج)

Atheists submitted in partial fulfillment for the requirements of the master degree in chemistry

By

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الاستفتاح

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ تَعَالَىٰ:

﴿ ٱقْرَأْ بِٱسْمِ رَبِّكَ ٱلَّذِى خَلَقَ ۝١ خَلَقَ ٱلْإِنسَـٰنَ مِنْ عَلَقٍ ۝٢ ٱقْرَأْ وَرَبُّكَ ٱلْأَكْرَمُ ۝٣ ٱلَّذِى عَلَّمَ بِٱلْقَلَمِ ۝٤ عَلَّمَ ٱلْإِنسَـٰنَ مَا لَمْ يَعْلَمْ ۝٥ ﴾

صدق الله العظيم

سورة العلق الآيات من 1-5

Acknowledgement

Thanks to Allah the most gracious, for giving me the Determination to complete this master

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I also extend my thanks to everyone who helped me collect information,and to the laboratory supervisors at the University of Sudan.

I also thanks my family and friends are greatly for support me and help for theirs.

Dedication

I dedicate this research to

My dad,

Mother,

And friends

Abstract

At the present research Gora *(Cola acuminate*) was studied. Firstly , the phytochemical screening was carried out to investigate the different natural compounds present, the results obtained showed that the extract of *cola acuminate* was Contains phenols, Flavonoids, Steroids, glycosides, Anthraquinones, Tannins, Saponins, Alkaloids and volatile oil, then caffeine was extracted from *Cola acuminate* and characterized using Infrared spectroscopy and high performance liquid chromatography and the yield of the extracted caffeine was59.3 mg /kg . The chemical structure of caffeine was confirmed by comparing it with infrared spectra of stander caffeine, the Infrared spectrum of the extracted caffeine showed peaks at : 3109.64 cm^{-1} due to N-H stretching vibration, 2952.42 cm^{-1} refer to C-H bond stretching vibration , 1703.01 cm^{-1} due to C=O stretching vibration , 1546.76 cm^{-1} due to C=C , 1232.73 cm^{-1} refer to C-N and 1655.97 cm^{-1} refer to C=N stretching vibration, after that caffeine was also confirmed using High performance liquid chromatography technique and the result showed that the active ingredient was caffeine by comparing the retention time for standard caffeine with extract caffeine (2.125min and 2.121min respectively), also the antimicrobial activity test was studied against some bacteria and one fungi, the results of antimicrobial activity indicated that the extract of *cola acuminate* had activity against four type of bacterial ,the inhbition zone of *Bacillus subtilis* (B.s) was: 16 mm , the inhbition zone of *Staphylococcus aureus* (s.a): 13mm , the inhbition zone *Escherichia coli*(E.c) :16mm , the inhbition zone *Pseudomonas aeruginosa* (p.s) : 16mm,the extract was also showed anti fungal activity against *Candida albicans*(c.a) and the inhbition zone was 13mm.

المستخلص

في هذا البحث تم دراسة الفورا (جوز الزنج). أولاً تم إجراء مسح فايتو كيميائي للتحقق من المركبات الطبيعية الموجودة. أظهرت النتائج المتحصل عليها أن مستخلص جوز الزنج يحتوي على فيتول، فلافنويد، ستيرودات، جليكوسيدات، انثراكيتون وتانين، صابونين، قلويدات وزيت طيار. بعد ذلك تم استخلاص الكافيين من جوز الزنج وتشخيصه باستخدام مطياف الأشعة تحت الحمراء وكروماتوغرافيا السائل عالية الكفاءة وكان ناتج الكافيين المتحلص عليه من المستخلص 59.3 مجم/كجم.

تم تأكيد التركيب الكيميائي للكافيين بمقارنته مع طيف الأشعة تحت الحمراء للكافيين القياسي، طيف الأشعة تحت الحمراء للكافيين آظهر القمم عن:

3109.64 سم$^{-1}$ وتشير إلى اهتزاز الشد ل N-H ، 1703.01 سم$^{-1}$ تشير إلى C=O اهتزاز التمدد، 2952.42 سم$^{-1}$ C-H، 1546.76 سم$^{-1}$ تشير إلى C=C ، 1232.73 سم$^{-1}$ تشير إلى C-N و 1655.97 سم$^{-1}$ تشير إلى C=N.

بعد ذلك تم التأكيد من الكفايين أيضاً "باستخدام تقنية كروماتجرافيا عالية الكفاءة وآظهرت النتيجة أن المادة الفعالة هو الكافيين من خلال مقارنة وقت الاحتفاظ للكافيين القياسي بمستخلص الكافيين (2.125 دقيقة و 2.121 دقيقة على التوالي)، كما تمت دراسة اختبار النشاط المضاد للميكروبات ضد بعض البكتريا وقطر واحد، أشارت نتائج النشاطية المضادة للميكروبات إلى أن مستخلص جوز الزنج له نشاط ضد أربعة أنواع من البكتريا، كانت منطقة تثبيط العضوية الرقيقة : 16مم ، منطقة تثبيط المكورة العنقودية الزهبية: 13مم ، منطقة التثبيط للعصيات القولونية: 16مم ومنطقة التثبيط الزائفة الزنجارية: 16مم، كما أظهر المستخلص نشاطاً مضاداً للفطريات ضد فطر المبيضة البيضاء منطقة التثبيط: 13مم.

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List of Abbreviation

The abridgement	
IR	Infra red spectroscopy
HPLC	High Performance Liquid Chromatography
B.s	*Bacillus subtilis*
s.a	*Staphylococcus aureus*
E.c	*Escherichia coli*
p.s	*Pseudomonas aeruginosa*
ca	*Candida albicans*

Chapter one

Introduction and literature review

Introduction and literature review

1. General introduction

Natural product is a chemical compound or substance produced by a living organism found in nature. in the broadest sense natural products include any substance produced by life Natural products can also be prepared by chemical synthesis and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets (Cragg et al , 2013) . the term natural product has also been extended for commercial purposes to refer to cosmetics dietary supplements, and foods produced from natural sources without added artificial ingredients (Nature Publishing Group ,2007) , (Grasseni, 2016).

1.1 Classification

Natural products may be classified according to their biological function, biosynthetic pathway, or sources (Banerjee et al,2015).

1.1.1 Biological Function

Natural products are often divided into two major classes, the primary and secondary metabolites. Primary metabolites have an intrinsic function that is essential to the survival of the organism that produces them. Secondary metabolites in contrast have an extrinsic function that mainly affects other organisms. Secondary metabolites are not essential to survival but do increase the competitiveness of the organism within its environment. Because of their ability to modulate biochemical and signal transduction pathways some secondary metabolites have useful medicinal properties (Anulika et al ,2016).

1.1.1.1 Primary metabolites

Primary metabolites are components of basic metabolic pathways that are required for life .they are associated with essential cellular functions such as nutrient assimilation, energy production, and growth/development they have a wide species distribution that span many phyla and frequently more than one kingdom . Primary metabolites include carbohydrates, lipids, amino acids, and nucleic acids (Kliebenstein, 2004),(Karlovsky, 2008) which are the basic building blocks of life .

1.1.1.2 Secondary metabolites

Secondary in contrast to primary metabolites are dispensable and not absolutely required for survival. Furthermore, secondary metabolites typically have narrow species distribution. Secondary metabolites have a broad range of functions. these include pheromones that act as social signaling molecules with other individuals of the same species, communication molecules that attract and activate symbiotic organisms, agents that solubilize and transport nutrients (siderophores etc.), and competitive weapons that are used against competitors, prey, and predators (Demain and Fang,2000). for many other secondary metabolites, the function is unknown. One hypothesis is that they confer a competitive advantage to the organism that produces them.

1.1.2 Biosynthetic

The biosynthetic pathways leading to the major classes of natural products are described below (Anulika et al, 2016).

Photosynthesis or gluconeogenesis → monosaccharides → polysaccharides (cel lulose, chitin, glycogen etc.)

- Acetate pathway → fatty acids and polyketides
- Shikimate pathway → aromatic amino acids and phenylpropanoids

- Mevalonate pathway and methyletrythritol phosphate pathway → terpenoids and steroids
- Amino acids → alkaloids

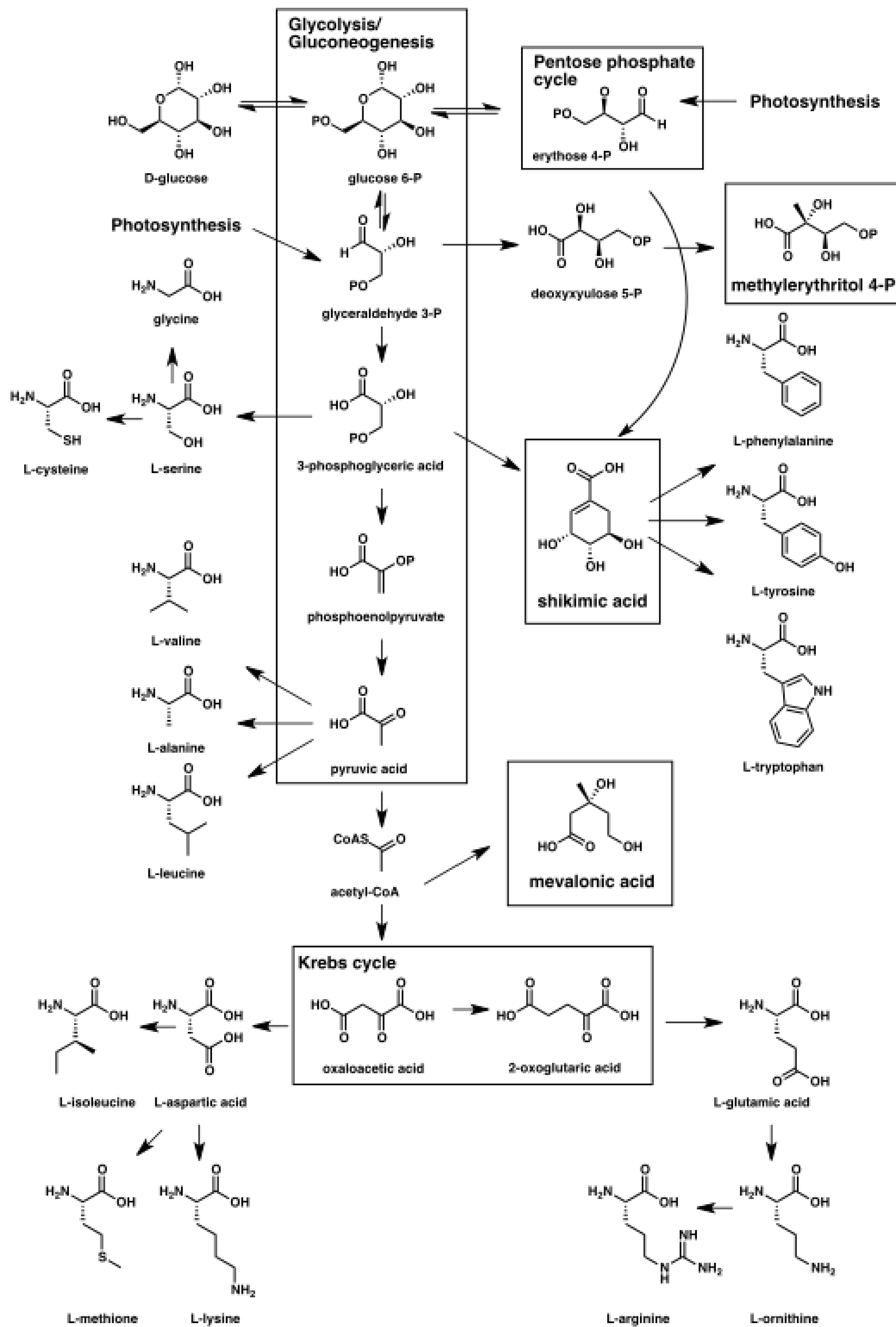


Figure (1-1): biosynthetic pathway of natural products

1.1.2.1 Carbohydrates

A carbohydr is a biomolecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, the empirical formula Cm(H2O)n . not all carbohydrates conform to this precise stoichiometric definition (e.g., uronic acids, deoxy-sugars such as fucose), nor are all chemicals that do conform to this definition automatically classified as carbohydrates,the group includes sugars, starch, and cellulose. The saccharides are divided into four chemical groups: monosaccharides, disaccharides, carbohydrates, are commonly referred to as sugars (Flitsch and Ulijn, 2003).

$n\ CO_2 + n\ H_2O + energy \rightarrow C_nH_{2n}O_n + n\ O_2$

1.1.2.2 Lipids

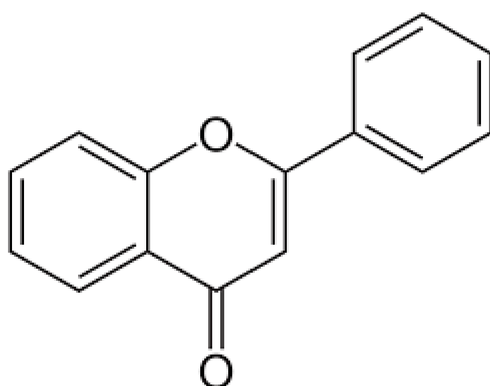
Lipids constitute a group of naturally occurring molecules they represent a large number of important everyday products (e.g ,fats, soaps, and waxes) mono, di, and tri glycerides; phospholipids and fat-soluble vitamins A, D, E, and K The main biological functions of lipids include energy storage, signaling, and acting as structural components of cell membranes.

1.1.2.3 Phenolic compounds

Most of the phenolic compounds are derived from the amino acid phenylalanine Indeed, phenylalanine can be considered the branch point between primary and secondary metabolites. In the steps of the shikimic acid pathway the removal of the amine function by the enzyme phenylalanine ammonia lyase (PAL) produces the phenylpropanoid compound cinnamic acid Thus the two amino acids phenylalanine and tyrosine appear as the precursors in the phenylpropanoid biosynthesis Furthermore these phenylpropanoids are then used to elaborate the major phenolic classes including the flavonoids, coumarins, tannins, anthraquinones, anthocyanins, lignin and lignin's.

1.1.2.3.1 Flavonoids

Flavonoids are ubiquitous in nature and play various roles. Many of these phenolic compounds have antimicrobial activity and are considered to be antioxidants. An important flavonoid found in red wine is known as resveratrol the compound that may be responsible for explaining the "French paradox," as it is believed to lower the risk of heart attacks by inhibiting platelet Aggregation and blood clots In the case of resveratrol, there is no middle oxygenated ring, which is a typical feature of flavone structures In fact, flavonoids are classified into different groups based on the degree of oxidation of the 3-C bridge This classification results in structures
belonging to the following: anthocyanins, flavones, flavonols, and isoflavones



figure(1.1) : the flavonoids structure

1.1.2.3.2Tannins

the definition of tannins as a mixture of 'flavolanes of varying structure' 11 at best only covers some tannins and does not include the hydrolysable tannins which form a considerable portion of the tannins , Tannins are polyphenolic secondary metabolites of higher plants corresponding polyphenolic natural products have not yet been isolated from lower plants such as algae or from the

animal kingdom .the polyphenolic structure of the secondary metabolites from higher plants is a necessary but not sufficient requirement for membership of the tannin class when the structural characteristics of the currently known tannins are analyzed the relatively low occurrence of C- and/or Oglycosidic derivatives of Gallic acid is noteworthy the characterized tannin structures show that, apart from the galloyl glycosides, the galloyl residues can be linked to each other or to other residues through their aromatic carbon and/or phenolic oxygen atoms by these and similar couplings of two or more natural products to each other, nature provides a nearly inexhaustible store of highly diverse structures it should be mentioned, however, that not all tannins must necessarily contain a galloyl unit or derivative (Khanbabaee and Ree 2001).

1.1.2.3.3 Anthraquinones

Anthraquinone, also called anthracenedione or dioxoanthracene, is an aromatic organic compound with formula $C_2H_8O_2$. Isomers include various quinone derivatives. The term anthraquinone, refers to the isomer, 9,10-anthraquinone (IUPAC: 9,10-dioxoanthracene) wherein the keto groups are located on the central ring. It is a building block of many dyes and is used in bleaching pulp for papermaking. It is a yellow, highly crystalline solid, poorly soluble in water but soluble in hot organic solvents. It is completely insoluble in ethanol near room temperature but 2.25 g will dissolve in 100 g of boiling ethanol (Vogel and wei,2005) **.**

1.1.2.4 Alkaloids

Naturally occurring alkaloids all contain at least one basic nitrogen atom and usually this atom is located in a cyclic ring system Alkaloid compounds were among the first natural products to be isolated Two striking examples include strychnine and morphine and both compounds originate from plant sources Strychnine is an insole alkaloid and is highly toxic it has been used as a pesticide causing muscular convulsions leading to death through asphyxia The

compound is isolated from the seeds of the plant *Strychnos nux-vomica*. it should be noted that many other alkaloids are produced in nature by a large variety of organisms, including bacteria, fungi, plants, and animals Due to the basic nature of the nitrogen in their alkaloid skeleton they can be isolated and purified from crude extracts using a combination of acid-base extraction against immiscible solvents such as dichloromethane (DCM) Alkaloids are often divided into the following major groups :

i. Alkaloids containing nitrogen in the heterocyclic and originating from amino acids for example, atropine, nicotine and morphine .

ii. "Proto alkaloids" that originate from amino acids for example, mescaline, adrenaline, and ephedrine.

iii. Polyamine alkaloids.

iv. Peptide and cyclopeptide alkaloids.

v. Pseudo alkaloids, which do not originate from amino acid

1.1.2.5 carotenoids

Carotenoids are terpenoids belonging to a very large family of organic pigmented compounds. they are tetraterpenoids terpenoids of eight isoprene units possessing 40 carbons within the molecular skeleton Carotenoids are of importance to both the plant and the host microorganism Carotenoids are typically found in photosynthetic plants or fungi, algae, and animal products they are also found in eggs, animal tissues, fruits, and many vegetables Carotenoids are involved in the photosynthetic process and offer protection against photo damage. they provide the yellow, orange, and red colors to their respective fruits and vegetables Carotenoids are one of the two key pigments that contribute to the skin yellowness of humans they are also recognized as valuable nutritional compounds to the human body. they are essential for enhancing the immune system, possess photo protection ability, and may support reproductive health

1.1.2.6 saponins

Saponins, glycosides widely distributed in the plant kingdom include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains their structural diversity is reflected in their physicochemical and biological properties which are exploited in a number of traditional (as soaps, fish poison, and molluscicides) and industrial applications (Güçlü and Mazza , 2007) while plant extracts containing saponins have been widely used in food and other industrial applications mainly as surface active and foaming agents (San Martín and Briones, 1999) saponins in foods have traditionally been considered as “ant nutritional factors” and in some cases have limited their use due to their bitter taste (Rideout et al ,1991) therefore most of the earlier research on processing of saponins targeted their removal to facilitate human consumption .

1.2 Cola acuminate

Cola acuminate is a species in the genus *Cola* of the family Malvaceae, native to tropical Africa It is generally known for its fruit the cola nut originally used to impart the cola flavor in manufactured beverages (Göhre et al, 2016).

Figure (1-2): shape cola acuminate

1.2.1 General description

The *cola acuminate* is a caffeine-containing nut of evergreen trees of the genus *Cola* primarily of the species *Cola acuminate* and *Cola nitida. (* Burdock et al, 2009) *Cola acuminate* an evergreen tree about 20 meters in height has long ovoid leaves pointed at both the ends with a leathery texture the trees have yellow flowers with purple spots and star-shaped fruit Inside the fruit about a dozen round or square seeds develop in a white seed-shell the nut's aroma is sweet and rose-like. the first taste is bitter but it sweetens upon chewing the nut can be boiled to extract the caffeine cola nuts contain about 2–4% caffeine and the bromine as well as tannins, alkaloids, saponins, and flavonoids (Burdock et al ,2009).

1.2.2 History

Human use of the cola acuminate like the coffee berry and tea leaf appears to have ancient origins it is chewed in many West African cultures in both private and social settings as a source of mental stimulation (Burdock et al ,2009) *cola acuminate* are an important part of the traditional spiritual practice of culture and religion in West Africa particularly Niger, Nigeria, Sierra Leone and Liberia (Philips,2004) The 1970s hit "Goro City" by Manu Dibango highlights the significance of *cola acuminate* (called "goro" in the Hausa language) , (Bascom ,1964) to the capital of Niger, Niamey *Cola acuminate* are used as a religious object and sacred offering during prayers, ancestor veneration, and significant life events, such as naming ceremonies, weddings, and funerals they are also used in a traditional divination system called Obi divination for this use only Cola acuminate divided into four lobes are suitable they are cast upon a special wooden board and the resulting patterns are read by a trained diviner (Onaolapo and Onaolapo, 2019).

1.2.3 Cultivation

They were used as a form of currency in such West African groups as the Manlike and Barbara of Mali and Senegal they are still used as such today in certain situations such as in negotiation over bride prices or as a form of a respect or host gift to the elders of a village should one move to a village or enter a business arrangement with the village originally a tree of tropical rainforest it needs a hot humid climate but can withstand a dry season on sites with a high ground water level It may be cultivated in drier areas where groundwater is available *C. nitida* is a shade bearer but develops a better spreading crown which yields more fruits in open places though it is a lowland forest tree it has been found at altitudes over 300 m on deep rich soils under heavy and evenly distributed rainfall regular weeding is necessary which can be performed manually or through the use of herbicides Some irrigation can be provided to the plants but it is important to remove the water through an effective drainage system as excess water may prove to be detrimental for the growth of the plant when not grown in adequate shade the cola acuminate plant responds well to fertilizers usually the plants need to be provided with windbreaks to protect them from strong gales.

cola acuminate can be harvested mechanically or by hand by plucking them at the tree branch Nigeria produces 52.4% of worldwide production followed by the Ivory Coast and Cameroon When kept in a cool, dry place, *cola acuminate* can be stored for a long time (Benjamin et al, 1991).

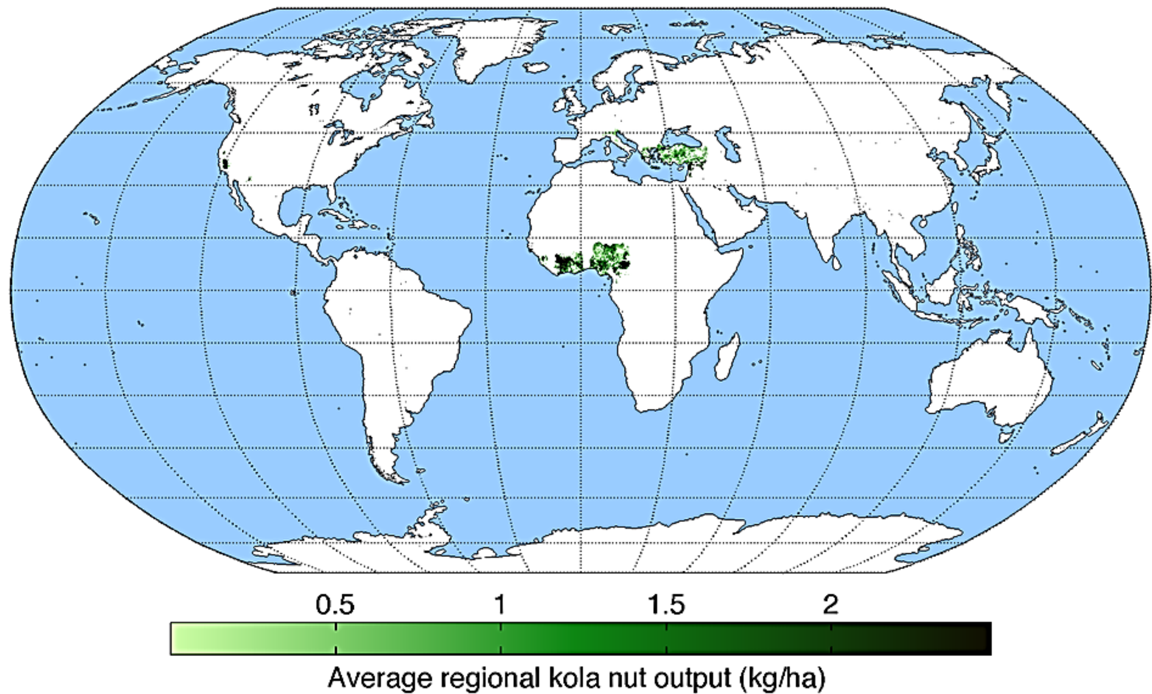


Figure (1-3): place cultivation *cola acuminate*

1.2 .4 Chemical composition

Preliminary studies of photochemical in cola acuminate indicate the presence of various constituents (Burdock et al ,2009).

- caffeine (2–3.5%)
- theobromine (1.0–2.5%)
- theophylline
- polyphenols
- phlobaphens (*kola red*)
- epicatechin
- D-catechin
- tannic acid
- sugar
- cellulose
- water

1.2.5 Uses

Several possible benefits of the cola acuminate and its products include the following:

1.2.5.1 Treats various diseases

Phragmanthera incana found in *cola acuminate* and Cocoa trees helps to prevent the urge of lipid peroxidation in the tissues of tat. yet, the antioxidant activities being the part of mechanisms of the Phragmanthera incana extracts assist in the protection of organs. the leaves of *cola acuminate* trees also provide antioxidant properties. the use of Phragmanthera incana leaves as nutraceuticals helps to manage the oxidative stress that is related to diabetes and other conditions. it could effectively treat the health ailments such as inflammation, hypertension, diabetes, cancer, insomnia etc. due to this reason (Ogunmefun et al,2015).

1.2.5.2 Aid to digestion

cola acuminate powder and extract may help digestion They are thought to promote the production of gastric acid which increases digestive enzyme effectiveness in the stomach.

1.2.5.3Antibacterial benefits

The use of *cola acuminate* extract might stop the growth of harmful bacteria There are several health conditions that might be improved by consumption of the *cola acuminate* (Muhammad and Fatima ,2014).

1.2.5.5 Slow metabolism

People with a slow metabolism could benefit from using products that contain *cola acuminate* Conditions that may affect a person's metabolism include low testosterone, Graves' disease, and cushing syndrome .

1.2.5.6 Increase the sex

70% ethanol extract of *cola acuminate* seeds was prepared and used for treating male Wistar rats (n=8 /group); two doses of cola acuminate (200 and 400 mg/kg body weight) were used for the treatment group, while distilled water was administered to the control group. All the treatments were orally administered daily for 28 days. On day 28, mounting frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) were quantified during sexual behavior tests. At termination, body and organ weights, gastric ulceration and caudal epididymal sperm counts were determined. Serum was collected for determination of testosterone levels. Both doses (200 and 400 mg/kg) showed marked aphrodisiac activity with significantly increased sexual behavior parameters compared to controls. However lower dose of *Cola acuminate* was more effective than the higher dose. Testosterone levels were higher in both treatment groups compared to controls. Sperm counts were similar to controls however testes weights were higher in cola acuminate treated rats compared to controls Thus these results show that cola acuminate enhances sexual activity in normal male rats(Ralebona et al,2012) .

1.2.5.7 Use in Ethno medicine

cola acuminate has a bitter taste and high caffeine content (Benjamin et al 1991) It is used traditionally as a caffeine stimulant and it is chewed in many West African cultures individually or in a group setting and is often used ceremonially, be it at weddings, child naming, funeral, the presentation of tribal chiefs or guests chewing cola nut can ease hunger cramps, stimulates digestion and is also used for euphoric qualities. its effects are comparable to other xanthenes containing herbs like cocoa and tea (Endrini et al ,2011) nonetheless, the effects are distinctively different as it produces a stronger state of euphoria and well-being (Benjamin et al 1991) It is thought to enhance

alertness and physical energy, elevate mood, increase tactile sensitivity, and suppress appetite. it may also increase body temperature, blood pressure, and res purgatory rate tt has been used as a common additive to American and European soft drinks the plant produces fruit pods containing seeds that are used to treat poisoning digestive disorders and asthma (Odebunmi et al ,2009) Small doses are used to treat migraine, motion and morning sickness In addition, it has been used to relieve inflammation disorders such as rheumatism and gout and has been administered to treat pneumonia and typhoid fever when great nervous irritability was present. *cola acuminate* is also used to treat diarrhea and has been used as a diuretic (Lowe et al ,2014).

1.2.6.8 Other Biological Activity Properties of *C. acuminate*

The effects of fresh *cola acuminate* extracts on female Swiss Webster mice on post natal development and their of spring was investigated and it was found that mice whose mothers were exposed to the *cola acuminate* extract showed a decline in the rate of post natal body weight gain but they experienced eye opening and hair gain relatively faster than their respective controls (Mensah et al,2008) implying *cola acuminate* affects the uteri development and the effects seem permanent. *cola acuminate* was also shown to exhibit a depressive effect on biphasic locomotors activity in male mice models at high concentrations (10 mg/kg) results obtained from limited human trials infer that *cola acuminate* may have some weight loss properties, positive chronoscopic and weak diuretic properties. animal studies show analeptic and biolytic (fat-burning) properties (Lowe et al,2014). more recent research shows that it stains the cytoplasm of various rat tissues which shows that it can be a suitable alternative for histological staining and so be more environmentally friendly and cost effective than synthetic dyes (Ajarem,1990).

1.2 .6 side effects of cola acuminate

The risk of side effects vary and increase, depending on how much of the nut is eaten Possible side effects of the *cola acuminate* include:

1.2.6.1 Increased blood pressure:

Aqueous extract of *Cola acuminate* designated as AECON was prepared using cold maceration. The extract (25 mg/kg BW, 50 mg/kg BW, 100 mg/kg BW) was administered to the rats for 30 days for hematological and biochemical study. Distilled (0.5 ml) served as the control. Red Blood Cell (RBC) and Total White Blood Cell (TWBC) counts were determined using haemocytometer. Differential leukocyte count was done using the Schilling method Activities of plasma Alanine Aminotransferease (ALT) and Aspartame Aminotransferase (AST) as well as total protein, creatinine and albumin levels were determined by spectrophotometer Data were analyzed using ANOVA at p Data were analyzed using ANOVA at $p<0.05$. Treatment of rats with 25 mg/kg BW, 50 mg/kg BW and 100 mg/kg BW of AECON caused significant increase in mono cyte values relative to the control, while 100 mg/kg BW produced significant increase in eosinophil value relative to the control In addition, 50 mg/kg BW of AECON caused significant decrease in albumin and total protein levels relation to their controls these findings indicate that *Cola acuminate* extract caused deleterious effect on the blood chemistry in male albino rats (Oyedeji et al,2013).

1.2.6.2 Effect on the stomach

Cola acuminate was investigated for possible harmful effect on the morphology of the stomach considering its wide consumption and documented antioxidant properties Twenty-five Adult male Wistar rats with average weight of 167.6 g and randomly divided into five groups A, B, C, D and E each

containing five animals. Care of the animal according to the Rules and Guidelines of the animal Right Committee of the Obafemi Awolowo University, Ile-Ife, Nigeria was adopted the rats in group A (control) were given distilled water while animals in experimental groups B, C, D and E were each given 600 mg/kg body weight of crude extract of *Cola acuminate* by oral intubation for consecutive three, five, seven and nine days respectively and sacrificed The stomach was excised, quickly fixed in 10% formal saline and processed histologically, using routine haematoxylin and eosin (H and E) stain. The stained sections were subjected to morphometrics analysis at a magnification of sign 40 using the eye piece micrometer procedure. the result revealed a significant reduction in the epithelia thickness of the experimental animals, (Groups A=218.40 µm ± 144.61 vs. B=117.00 µm ± 34.88, C=124.80 µm ± 87.01, D=96.60 µm ± 60.04 and E=108.57 µm ± 122.16) (t=3.04, 2.48, 3.57 and 2.58 respectively, $p < 0.05$). The thickness of the lamina propria and submucosa was not significant in all cases of the experimental when compared with the control animals (Groups A=109.20 µm ± 58.52 vs. B=111.80 µm ± 61.18, C=111.80 µm ± 69.45, D=137.80 µm ± 34.88 and E=155.10 µm ± 90.54) (t=0.14, 0.13, 1.88, 1.90 respectively, $p>0.05$) and (A=148.20 µm ± 50.56 vs. B=109.20 µm ± 22.27, C=117.00 µm ± 11.07, D=124.80 µm ± 71.67, E=162.86 µm ± 112.35) (t=1.58, 1.35, 0.60, and 0.46 respectively, $p<0.05$). the thickness of the muscularis mucosa and muscularis externa were significantly increased by the extract, (Groups A=140.40 µm ± 95.84 vs. B=358.80 µm ± 323.07, C=260.00 µm ± 32.89, D=306.80 µm ± 148.90, E=374.83 µm ± 175.44) (t=7.16, 6.36, 3.83, and 2.89 respectively, $p<0.05$) and (140.4 µm ± 47.94 vs. B=358.80 µm ±161.53, C=260.00 µm ± 16.44, D=306.80 µm ± 74.44, E=374.83 µm ± 87.72) (t=2.90, 5.22, 4.20, and 5.22 respectively, $p<0.05$). it is therefore evident that the consumption of *cola acuminate* leads to a reduction in the epithelia thickness and a significant increase in the thicknesses of muscularis mucosa and the muscularis externa and however this is as a result of

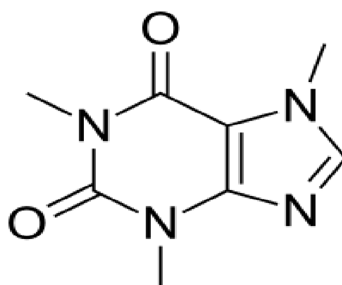
increase in the secretomotor activity of the stomach when *cola acuminate* is ingested (Ojo et al 2010).

1.3 Caffeine

Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class (Nehlig et al ,2016) it is the world's most widely consumed psychoactive drug (Burchfield ,1997) unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world. there are several known mechanisms of action to explain the effects of caffeine. the most prominent is that it reversibly blocks the action of adenosine on its receptor and consequently prevents the onset of drowsiness induced by adenosine. Caffeine also stimulates certain portions of the autonomic nervous system. Caffeine is a bitter, white crystalline Purina, a methylxanthine alkaloid, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It is found in the seeds, nuts, or leaves of a number of plants native to Africa, East Asia and South America (Caballero et al ,2015) and helps to protect them against predator insects and to prevent germination of nearby seeds The most well-known source of caffeine is the coffee bean, a misnomer for the seed of Coffee plants. Beverages containing caffeine are ingested to relieve or prevent drowsiness and to improve performance. to make these drinks, caffeine is extracted by steeping the plant product in water, a process called infusion. Caffeine-containing drinks, such as coffee, tea, and cola, are very popular; as of 2014, 85% of American adults consumed some form of caffeine daily, consuming 164 mg on average (Mitchell et al ,2014) Caffeine can have both positive and negative health effects It can treat and prevent the premature infant breathing disorders bronchopulmonary dysplasia of prematurity and apnea of prematurity. Caffeine citrate is on the WHO Model List of Essential Medicines(Gautier et al ,2014) It may confer a modest protective effect against

some diseases (Belitz et al ,2009) including Parkinson's disease(Qi and Li, 2014) Some people experience sleep disruption or anxiety if they consume caffeine, but others show little disturbance. Evidence of a risk during pregnancy is equivocal; some authorities recommend that pregnant women limit caffeine to the equivalent of two cups of coffee per day or less. (Huang et al,2016), (American College of Obstetricians and Gynecologists, 2010) Caffeine can produce a mild form of drug dependence – associated with withdrawal symptoms such as sleepiness, headache, and irritability – when an individual stops using caffeine after repeated daily intake (Malenka et al,2009) , (Gorsane et al, 2014) (Juliano and Griffiths,2004) tolerance to the autonomic effects of increased blood pressure and heart rate, and increased urine output, develops with chronic use (i.e., these symptoms become less pronounced or do not occur following consistent use

(Robertson and Wade,1972).



the chemical structure of caffeine

1.3.1 Chemistry

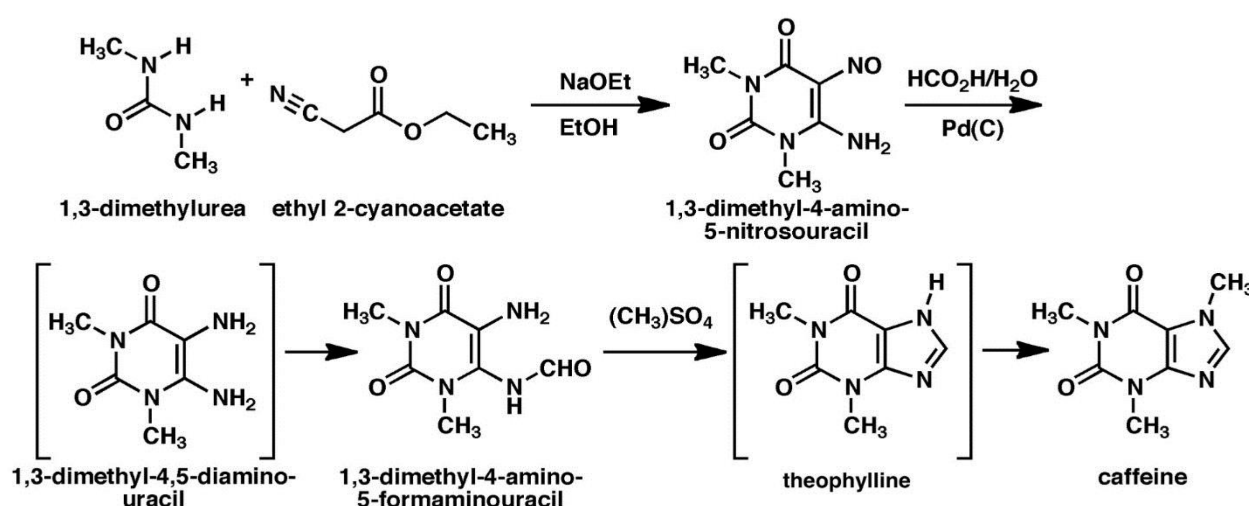
Pure anhydrous caffeine is a bitter-tasting, white, odorless powder with a melting point of 235–238 °C (Lipton atc al,2017), (Cláudio et al, 2014),(Caffeine is moderately soluble in water at room temperature (2 g/100 mL), but very soluble in boiling water (66 g/100 mL).[50] It is also moderately soluble in ethanol (1.5 g/100 mL) (Mufakkar et al ,2014),(Nehlig et al,1992) it

is weakly basic (pK_a of conjugate acid = ~0.6) requiring strong acid to protonate it Caffeine does not contain any stereogenic centers and hence is classified as an achiral molecule (The xanthine core of caffeine contains two fused rings, a pyrimidinedione and imidazole. the pyrimidinedione in turn contains two amide functional groups that exist predominantly ina zwitterionic resonance the location from which the nitrogen atoms are double bonded to their adjacent amide carbons atoms. Hence all six of the atoms within the pyrimidinedione ring system are sp^2 hybridized and planar. Therefore, the fused 5,6 ring core of caffeine contains a total of ten pi electrons and hence according to Hückel's rule is aromatic· (Keskineva , 2014).

1.3.2 Synthesis

The biosynthesis of caffeine is an example of convergent evolution among different species (Denoeud et al ,2014) ,(Williams et al,1989).

Caffeine may be synthesized in the lab starting with dimethylurea and melodic (Wilson and Kaspar,2017),(Zajac et al ,2003) Commercial supplies of caffeine are not usually manufactured synthetically because the chemical is readily



available as a byproduct of decaffeination(Hak,2015) .

1.3.3 Decaffeination

Extraction of caffeine from coffee, to produce caffeine and decaffeinated coffee, can be performed using a number of solvents.

Benzene, chloroform, trichloroethylene, and dichloromethane have all been used over the years but for reasons of safety, environmental impact, cost, and flavor, they have been superseded by the following main methods:

i- **Water extraction:** Coffee beans are soaked in water. The water, which contains many other compounds in addition to caffeine and contributes to the flavor of coffee, is then passed through activated charcoal, which removes the caffeine. The water can then be put back with the beans and evaporated dry, leaving decaffeinated coffee with its original flavor. Coffee manufacturers recover the caffeine and resell it for use in soft drinks and over-the-counter caffeine tablets.

ii- **Supercritical carbon dioxide extraction:** Supercritical carbon dioxide is an excellent no polar solvent for caffeine, and is safer than the organic solvents that are otherwise used. The extraction process is simple: CO 2 is forced through the green coffee beans at temperatures above 31.1 °C and pressures above73 atm. Under these conditions, CO_2 is in a "supercritical" state: It has gas like properties that allow it to penetrate deep into the beans but also liquid-like properties that dissolve 97–99% of the caffeine. The caffeine-laden CO_2 is then sprayed with high-pressure water to remove the caffeine. The caffeine can then be isolated by charcoal adsorption (as above) or by distillation, recrystallization, or reverse osmosis .

iii- **Extraction by organic solvents:** Certain organic solvents such as ethyl acetate present much less health and environmental hazard than chlorinated and aromatic organic solvents used formerly. Another method is to use triglyceride oils obtained from spent coffee grounds.

"Decaffeinated" coffees do in fact contain caffeine in many cases – some commercially available decaffeinated coffee products contain considerable levels. One study found that decaffeinated coffee contained 10 mg of caffeine per cup, compared to approximately 85 mg of caffeine per cup for regular coffee (McCusker et al ,2006).

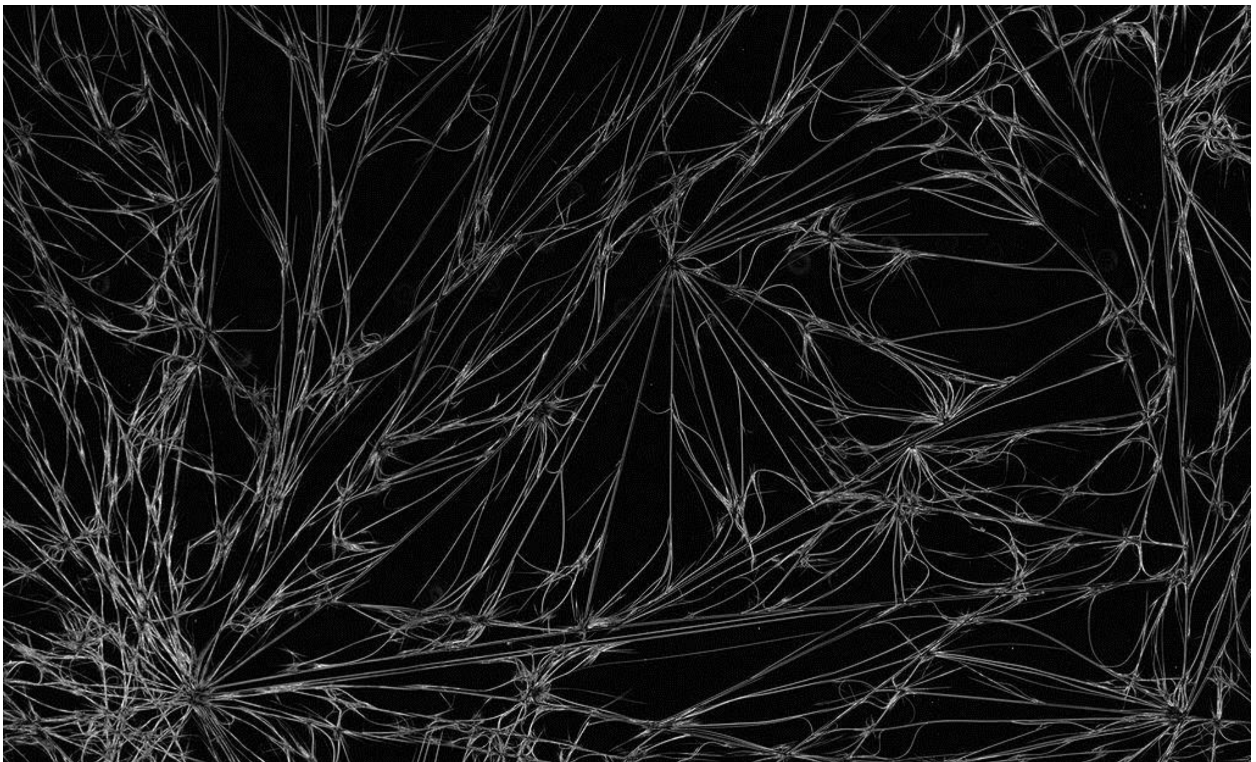


Figure (1-4): Fibrous crystals of purified caffeine. Dark-field microscopy image

1.3.4 Natural occurrence

Around sixty plant species are known to contain caffeine Common sources are the "beans" (seeds) of the two cultivated coffee plants, Coffee Arabica and Coffee (the quantity varies, but 1.3% is a typical value); in the leaves of the tea plant; and in *cola acuminate* . other sources include yaupon holly leaves, South American holly yerba mate leaves, seeds from Amazonian maple Guarani berries, and Amazonian holly Guayas leaves. Temperate

climates around the world have produced unrelated caffeine-containing plants Caffeine in plants acts as a natural pesticide: it can paralyze and kill predator insects feeding on the plant (Müller and Jacobson, 2011). high caffeine levels are found in coffee seedlings when they are developing foliage and lack mechanical protection (Hemingway and Laks,2012) In addition, high caffeine levels are found in the surrounding soil of coffee seedlings, which inhibits seed germination of nearby coffee seedlings, thus giving seedlings with the highest caffeine levels fewer competitors for existing resources for survival Caffeine is stored in tea leaves in two places. firstly, in the cell vacuoles where it is complexes with polyphones. This caffeine probably is released into the mouth parts of insects, to discourage herbivore. Secondly, around the vascular bundles, where it probably inhibits pathogenic fungi from entering and colonizing the vascular bundles (Nathanson,1984) Caffeine in nectar may improve the reproductive success of the pollen producing plants by enhancing the reward memory of pollinators such as honey bees (Frischknecht et al, 1986) the differing perceptions in the effects of ingesting beverages made from various plants containing caffeine could be explained by the fact that these beverages also contain varying mixtures of other methylxanthine alkaloids,includingthe cardiac stimulants theophylline and the bromine, and polyphones that can form insoluble complexes with caffeine (Baumann and Gabriel,1984).

1.4 Phytochemical screening

It refers to the extraction, screening and identification of the medicinally active substances found in plants Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds (Pendyala et al , 2017). Photochemical screening was performed to identify photochemical in the chloroform and hexane, Methanol, Acetone, extracts of plant leaves or seed were used in the study in this present

work (Karandeet al,2016) , the photochemical were detected by color tests. the photochemical analysis of the plant is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases It is expected that the important photochemical properties recognized by our said study will be very useful in the curing of various diseases of this region (Awoyinka et al ,2007) .

1.5 Objectives of the study

The aims of this research are to :

- ❖ Phytochemical screening for extract of *cola acuminate*
- ❖ Isolation of caffeine form *cola acuminate* and characterize using Infrared spectroscoby (IR).
- ❖ Identification of caffeine using High performance liquid chromatography(HPLC).
- ❖ Test the antimicrobial activity of *cola acuminate* extract .

Chapter two

Material and methods

2.1 Materials

The *cola acuminate* was obtained from market in Khartoum on the 4th of August , 2019 the *cola acuminate* was cured by the traditional method and crushed by blender.

2.1.1 Chemicals

- Hydrochloric acid (35%, LOBA Chemie, India)
- Sulfuric acid (98%, LOBA Chemie, India)
- Potassium iodide (99.5%, LOBA Chemie, India)
- Ferric chloride (99%, LOBA Chemie, India)
- Glacial Acetic Acid (99.5%, LOBA Chemie, India)
- Ammonia solution (25% , LOBA Chemie , India)
- Chloroform (99% , LOBA Chemie, India)
- Petroleum ether (99%, LOBA Chemie, India)
- Toluene (99.5, LOBA Chemie, India)
- Ethanol(94.8% , LOBA Chemie, India)
- Methanol (99.8%, LOBA Chemie, India)
- Distilled water

2.1.2 Reagents

- Dragendoff's reagent
- Wagner's reagent

2.1.3 Equipments

- hot plate stirrer
- water bath
- infrared spectrum

- High performance liquid chromatography

2.1.4 Apparatus

Beaker - test tube – rod glass – volumetric flask – conical flask –funnel –fitter paper _Cylinder.

2.1.5 Infrared Insturment (IR)

infrared spectrum was recorded by thermo Mattson IR300FTIR spectrophotometer the spectrum was obtained by the disc technique KBr (4000--400).

2.1.6 Chromatography

Mobile phase : Methanol and water (40:60 v/v).

That solution was mixed with methanol with ratio 1:1

Column temperature25 ºC

Flow rate : 1 ml/min

Injection : 20 µl

Detector RID-10A :272 nm

2.1.7 Preparation of reagents

2.1.7.1 Dragendoff's reagents

1.7gram of Bismuth sub-nitrate was added to 20ml of Glacial Acetic Acid then 80ml of distilled water was added . After that 10 ml of Potassium iodide(50%) was added .The solution was transferred into volumetric flask (100ml) . then 20ml of Glacial Acetic Acid was added to the volumetric flask and dilute with water to the mark.

2.1.7.2 Wagner's Reagent:

2.5 gram iodine was dissolved in 12.5 g of potassium iodide . 250 ml of distilled Water was added to produce solution.

2.2 Methods

2-2.1 Preparation of Plant Materials

The seeds were shade dried at room temperature for month and then Grinded to powder. 200gram of powder seed was extracted by maceration of 500 ml in water .

2.3 Phytochemical screening

2.3.1 Test of Alkaloids

70ml of hydrochloric acid 10% was added to 4g of sample in conical flasks and was boiled for 10 minutes then was filtered and allowed to cool. The filtrates were poured into four labeled test tubes. Then added two drops of Dragendoff's, Wagner's reagents were added to each test tube separately.

2.3.2 Test of Saponins

4gram of sample was dissolved in distilled water and heated for 4 minutes. The mixture was filtered then lefted for 15 minutes allowed to cool and it was shaken continuously for 2 minutes to production of froth.

2.3.3 Test for Tannins:

1gram of sample was heated with 20ml of water for 5 minutes in appropriately labeled test-tubes .the solution was allowed to cool and then filtered. 1ml of filtrate was diluted with 5ml distilled water in a test tube and two drops of ferric chloride(0.1%) was added .

2.3.4 Test of Anthraquinones:

1gram of sample was mixed with 10ml of ferric chloride solution mixed and 5ml of Hydrochloric acid. Then mixture was heated in a water bath for 15 minutes, filtered and allowed to cool. The filtrate was extracted with chloroform and shaken gently. The clear layer at transferred into test tube, then 2ml of ammonia solution was added.

2.3.5 Test for Steroids

Salvoski test: 1 ml of sample was dissolved in 1 ml of chloroform and 1ml of concentrated sulfuric acid.

2.3.6 Test for Flavonoids

5 ml of Dilute ammonia was added to 5ml of sample then 2ml of concentrated sulfuric acid was added .

2.3.7 Test for phenols

Three drops of ferric chloride solution was added to 1 ml of sample .

2.3.8 Test for glycosides

1 ml of ferric chloride (5%) was added to 1ml of sample then 1ml of acetic acid was added to the mixture and two drops of sulfuric acid was added.

2.3.9 Test for volatile oil

Mixed 1 ml of sample with dilute Hydrochloric acid .

2.4 Extraction of caffeine

2.4.1 method for caffeine extraction

The *cola acuminate* was crush into small and the powder. 50g of *cola acuminate* powder was introduced put into Soxhlet apparatus then 500 ml of chloroform was added into the Soxhlet apparatus after prescribe time the mixture was collected and filtrated it by using filter paper f then the liquid mixture was place into evaporator apparatus to recover the solvent The sample was collected and put into Beaker Finally.

2.4.2 Purification of caffeine

The crude caffeine was transferred to clean beaker then 5ml of toluene was added and heated on water bath to dissolve the caffeine. Beaker was removed from the heating source then 10ml of petroleum ether was added , caffeine was allowed to crystalline The product was collected washed with 1ml of petroleum ether.

2.4.3 Preparation of KBr pellet for IR analysis

The powder sample of potassium bromide was grinded to reduce the particle size to less than 5 mm diameter spatula full was Added in agate mortar and grinded it to fine powder ,then 0.2 g of sample was mixed with the potassium bromide powder, the mixture was grinded for 5 minutes. After that the die-set was assembled as shown in the drawling When assembled , the died was added the powder to the 7mm collar Put the died together with the powder into the Qwik Handi-Presse. Pressed the powder for 2 minutes to form a pellet. then the die set was Disassembled and the 7mm collar was take out and Put the collar together with the pellet into the sample holder in the spectrometer.

2.4.4 Preparation sample for High Performance Liquid Chromatography HPLC)

0.05 gram of sample was transferred into volumetric flask(100ml) and the volume was completed by water to the mark The solution was filtered by filter paper, and 20 µl was injected into the column .caffeine elution was carried out with mobile phase consist 40:60 methanol /water at 25 C° and a flow rate 1m/min, the retention time of caffeine was monitored using the refractive index detector (RID-10A) .The retention time obtained was compared to that determined standard caffeine , the concentration of caffeine in the sample, was determined by the following .Prepared a standard caffeine solution ,The standard caffeine was Prepared by dissolving 0.1 g of standard in distilled water, and The solution was filtered by filter paper and transferred into volumetric flask(25ml) .20 µl from the this solution was injected into the

column , and the retention time and peak area of standard was recorded was computed the mass of analyze in unknown sample.

2.5 Antimicrobial activity

2.5.1 Preparation of bacterial suspensions:

One ml of aliquots broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Pitts et al ,2003). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained

2.5.2 Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, The suspension were stored in the refrigerator until used.

2.5.3 Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (Kiehlbauch et al, 200). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculums was allowed to dry for 5 minutes. Sterilized filter paper discs (Whitman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Chapter three

Results and discussion

3.1 phytochemical screening

The photochemical screening of the water extract of cola acuminate seed was positive test for alkaloids, saponins, flavonoid, tannins ,anthraquinones, cardenolides, phenolic compound and glycosides the results was show in tables (3.1)

Table (3.1): result of photochemical screening

The test	The results	The observable
4g of sample +70ml of Hcl +two drops of Dragendoff's	Appearing a orange precipitate	The sample contains Alkaloids
4g of sample +70ml of Hcl +two drops of Wagner reagent	Appearing brownish solution	
4g of sample + distilled water and shaken continuously for 2 minutes	production of froth	The sample contains Saponins
1g of sample +20ml of distilled water and heated +drops of 0.1% ferric chloride solution	blue-green color precipitate	The sample contains Tannins
1ml sample +10ml of ferric chloride solution +5ml Hcl+ chloroform +2ml of ammonia solution	Appearing a delicate pink rose color	The sample contains Anthraquinones

1ml of sample +1mlchoroform +1ml H_2SO_4	. Formation of Bluish red	The sample contains steroids .
1ml of sample +5ml of Dilute ammonia+2ml 0f H_2SO_4	Appearing A yellow coloration	The sample contains flavonoids
1ml of sample + drops of $FeCl_3$ solution	. Appearing blue green color	The sample contains phenols
1ml of sample +1 ml $FeCl_3$(5%), +1ml acetic acid + drops of H_2SO_4	Appearing Greenish blue color	The sample contains glycosides
1ml of sample +1ml of HCL	white precipitate	The sample contains volatile oil

3.2 Isolation and purification of caffeine

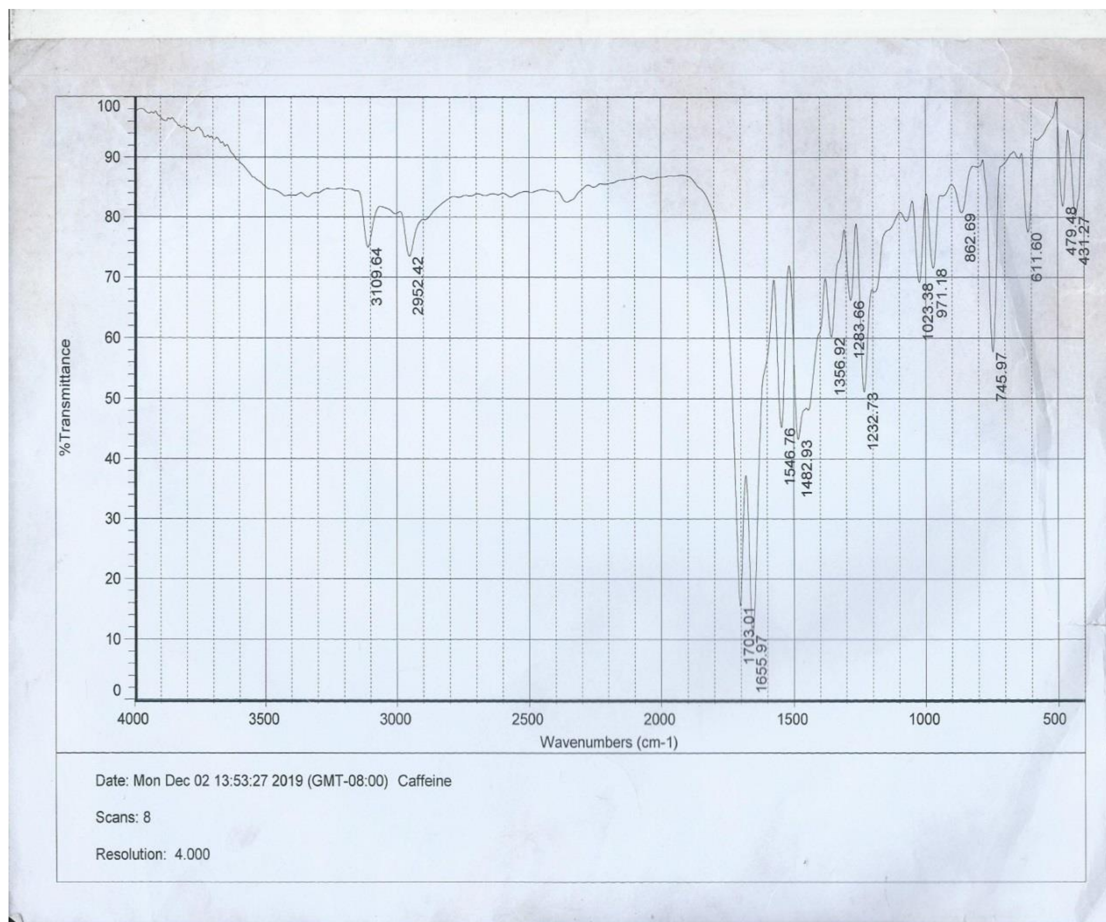
Isolatied and purified the caffeine by toluene and petrorum ether .

3.3 IR characterization of the isolated caffeine

The IR spectrum of caffeine showed peaks at : 3109.64cm-1 refer to N-H stretching vibration 2952.42 cm-1 due to C-H bond stretching vibration , 1703.01 refer to C=O (Amide) stretching vibration , 1546.76 due to C=C , 1232.73 refer to C-N and 1655.97 refer to C=N.

Table (3-2): IR spectrum data of caffeine

Literature value cm-1	Type of frequency bond	position
3500----3100	N-H stretching vibration	3109.64
3000-----2850	C-H stretching vibration	2952.42
1725----1700	C=O stretching vibration	1703.01
1600----1470	C=C stretching vibration	1546.76
1350---1000	C-N stretching vibration	1232.73
1690----1640	C=N stretching vibration	1655.97



Figure(3 -1) appeared IR spectrum

3.4 Result of High Performance Liquid Chromatography (HPLC)

In High Performance Liquid Chromatography, appeared one peak and it indicates that the sample contains one component. The retention time for the sample was compared with that of standard caffeine retention time. It was found that the retention times of the both sample and standard are closed, which indicating the tested sample is caffeine (2.125 and 2.121min respectively). The yield of obtained caffeine in the extract was59.3 mg |kg from Table (3-2) and figures (3-3) in the below shows the results of HPLC:

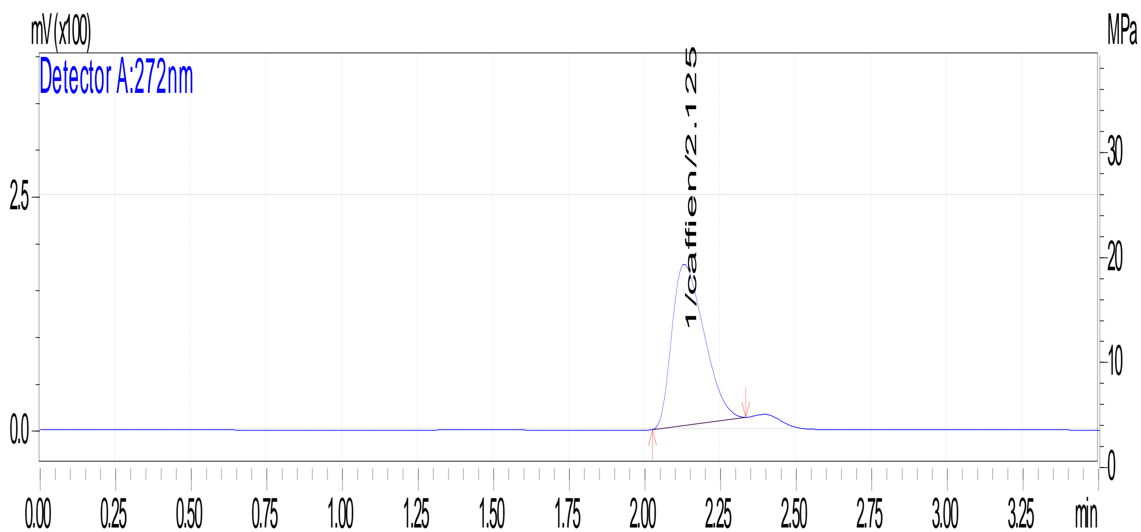


Figure (3-2): Characteristic chromatograms of caffeine: chromatogram of stand

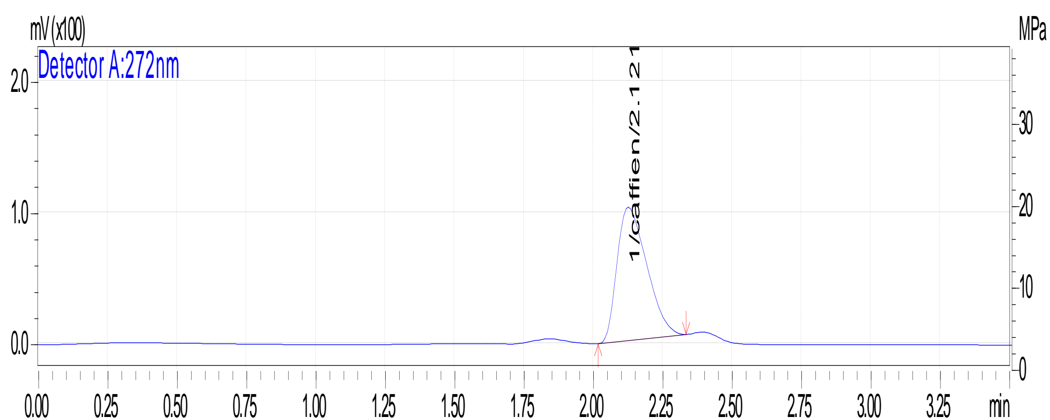


Figure (3-3): Characteristic chromatograms of caffeine: chromatogram of sample extract

Table (3.3): ID# compound name: caffeine

Title	Ret Time	Area	Height	plate	units	conc
8..1caf20ppm2.Icd	2.125	1280523	172398	1615.391	mg|L	20.000
8..1cafsam2.Icd	2.121	759275	101848	1586.235	mglL	11.859
Average	2.123	1019899	137123	1600.813		15.929
%RSD	0.144	36.139	36.381	1.288		36.136
maximum	2.125	1280523	172398	1615.391		20.000
minimum	2.121	75975	101848	1586.235		11.859
Standard deviation	0.003	368578	49886	20.616		5.757

3.5 Evaluation of antimicrobial activities

A test was performed a antibacterial activities for cola acuminate extract to four type of bacterial and one fungi . The extract has activity against antimicrobial the activity of *Bacillus subtilis (B.s)* was 16mm ,the activity of *Staphylococcus aureus(s.a)* was 13mm ,activity of *Escherichia coli(E.c)* was16mm activity of *Pseudomonas aeruginosa* (p.s) was 16mm. the activity of fungal *Candida albicans(c.a)* was 13mm .from table (3-4) and figures (3-4),(3-5),(3-6),(3-7),(3-8) in the below shows the results of antimicrobial activities.

Table (3-4): The antimicrobial activity of cola acuminate against the standard organisms

Plant No	Part used	Conc used mglml	Standard tested organisms (mm)				
			(B.s)	*(s.a)*	*(E.c)*	*(p.s)*	*(ca)*
Cola acuminate	seed	100	16	13	16	16	13

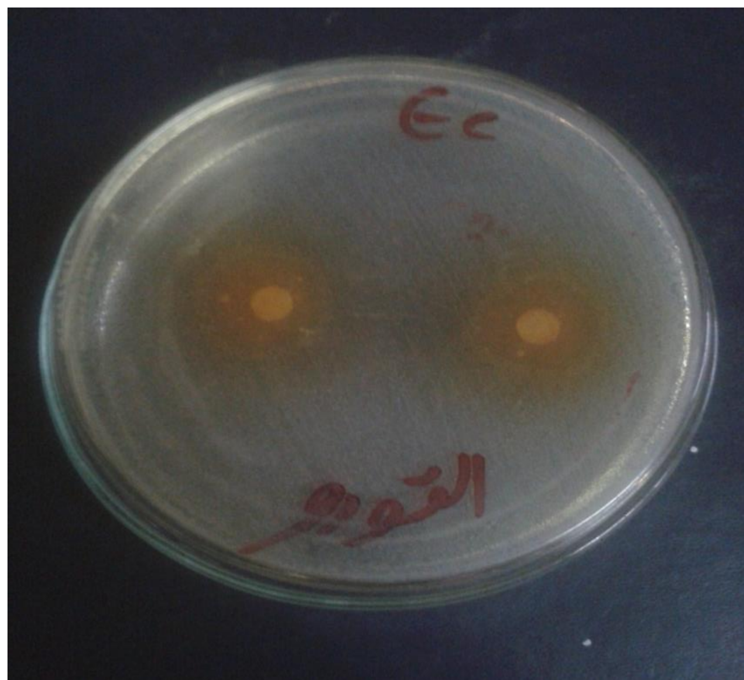


Figure (3-4): inhbition zone *of cola* extract against *Escherichia coli*

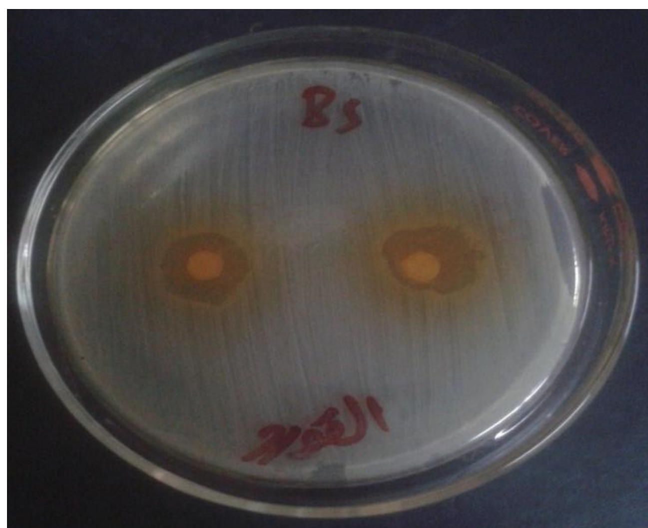


Figure (3-5): inhbition zone of *cola* extract against *Bacillus subtil*

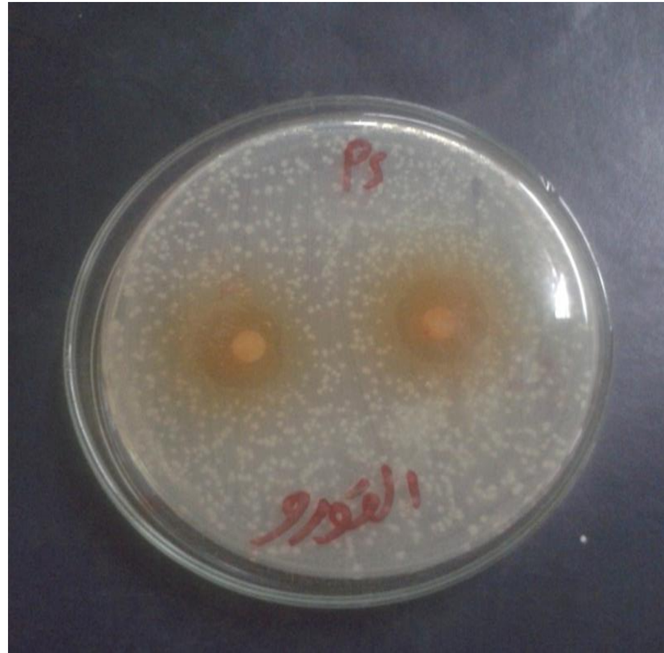
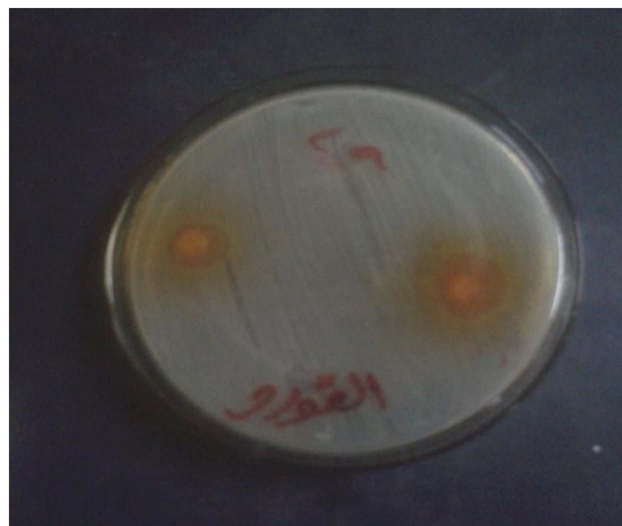


Figure (3-6): inhbition zone of *cola* extract against *Pseudomonas aeruginosa*



Figure(3-7): inhbition zone of *cola* extract against *Staphylococcus aureus*

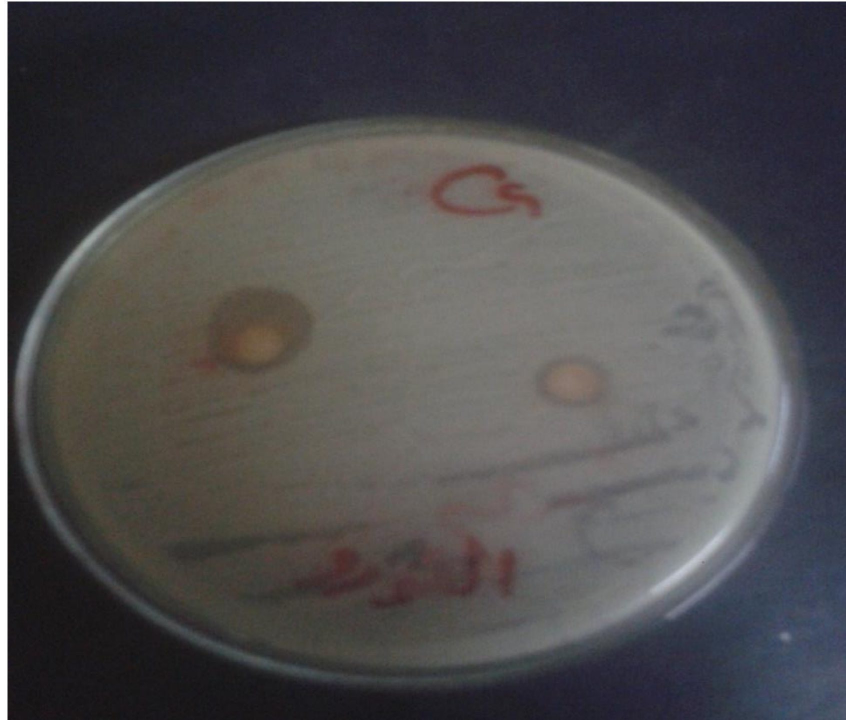


Figure (3-8): inhbition zone of *cola* extract against fungal *Candida albicans*

3.6 Conclusion

The chemical study of *cola acuminate* showed many types of natureal products. Include the phenolic compound, alkaloids, steroids, flavonoides ,sponins, tannins, anthraquinones ,cardenolides ,volatile oil,and glycosides. The caffeine was isolated by chloroform and purified by toluene and petroleum ether. The active material was charactered by IR spectrum. The results showed that many functional group .include carbonyl group, primary amines, alkenes, alkenes and Imines. The active material of *cola acuminate* was subjected to HPLC technique and analyzed it .the result showed that the active ingredient was caffeine by comparing the standard retention time for caffeine with extract caffeine (2.125 and 2.121min respectively). The yield of obtained caffeine in the extract was59.3 mg |kg. Finally the antimicrobial activity results for *cola acuminate* extract found that the extract was active against antimicrobial.

3.7 Recommendation

The study that was conducted on the *cola acuminate* plant showed that it was a source of many natural products. I recommend that researchers in this field separate All natural products, determine the chemical structure by spectroscopic methods and characterization active material (caffeine) using NMR spectroscopy.

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