Cola rostrata K. SCHUM (STERCULIACEAE); PHYTOCHEMISTRY, ANTIOXIDANT, AND ANTIBACTERIAL POTENTIALS

BY

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CERTIFICATION

This is to certify that this study was successfully carried out by **OGEDENGBE FRANCESS ESOSA** in the department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

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DEDICATION

This project is dedicated to God Almighty for his grace, wisdom, and guidance throughout my journey in pharmacy school. I also dedicate this work to my beloved parents for their love, prayers, and constant support that have been my greatest motivation.

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ABSTRACT

Species belonging to the *Cola* genus (Malvaceae) are common across tropical regions of Africa and are well known for their use in traditional medicine, particularly for managing infections, inflammatory conditions, and disorders linked to oxidative stress. Several species are also valued in folk practices for their stimulating, antiemetic, and antiproliferative effects. This study investigates the phytochemical profile, as well as the antioxidant and antimicrobial activities, of the 70% ethanolic stem bark extract of *Cola rostrata*. The plant material was collected from Amapu-Igbengwo village, Umuakpara, Abia State, Nigeria. It was air-dried, pulverized, and extracted using 70% ethanol by maceration. Identification and quantification studies were achieved via high-performance liquid chromatography (HPLC) on a C-12 column with a water–acetonitrile gradient, using 280 nm as detection wavelength. Gas chromatography-mass spectrometry (GC-MS) analysis employed a DB-5MS column with helium as a carrier gas and compound identification was supported by NIST library matching. Antioxidant capacity was evaluated using DPPH, ABTS, FRAP, and TAC assays. Antibacterial activity was determined through agar well diffusion and dilution techniques against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis,*, and *Enterobacter cloacae.* The mode of antibacterial action was determined by calculating the MBC/MIC ratio for susceptible isolates.

HPLC analysis detected nineteen compounds, with prominent representatives being Narigenin (33.61 µg/mL), Tannin (26.11 µg/mL), and Catechin (23.34 µg/mL). The GC–MS identified twenty five bioactive constituents, including 9-octadecenoic acid (Z)-, methyl ester (14.10%), n-hexadecanoic acid (11.17%), and octadecanoic acid (8.31%). Antioxidant assays demonstrated potent activity, with DPPH and ABTS showing high radical-scavenging efficiency with percentage inhibition ranging from (90.18 ± 0.43)% to (82.95 ± 3.20)% and (92.46 ± 0.48)% to (89.20 ± 0.35)% respectively, moderate ferric-reducing power (51.49 ±

0.45)% to (38.15 ± 2.04)% and strong total antioxidant capacity (TAC) from, (93.89 ± 0.84)% to (90.57 ± 0.83)%. Antibacterial evaluation showed selective inhibition zones ranging from 20-13 mm, with pronounced effects against *Staphylococcus aureus.* Minimum inhibitory concentrations (MICs) for *Staphylococcus aureus* and *Escherichia coli* were 25mg/mL and 50mg/mL, while minimum bactericidal concentrations (MBCs) were 50mg/mL and 100mg/mL respectively. The mode of antibacterial action assessed for susceptible organisms, *Staphylococcus aureus* and *Escherichia coli*, was bactericidal.

Overall, the results demonstrate that the stem bark of *Cola rostrata* exhibits strong antioxidant and antibacterial activities. These outcomes support its traditional medicinal applications and underscore its promise as a natural source of bioactive therapeutic compounds.

Keywords: *Cola rostrata*, stem bark, HPLC, GC–MS, antioxidant, antibacterial, phytochemicals.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants have long formed the foundation of healthcare, serving as primary therapeutic agents in various cultures for centuries (Fabricant and Farnsworth, 2001). Although notable progress has been made in modern synthetic drug development, herbal medicine still occupies an essential place in global healthcare. According to the World Health Organization (2013), nearly 80% of the world's population depends on it for primary health care. The plants contain a variety of bioactive compounds responsible for their therapeutic effects, including alkaloids, terpenoids, glycosides, flavonoids and fatty acids (Cowan, 1999). Through research, herbal medicine will continue to be modernized by standardizing its use and developing new methods of extraction (Ekor, 2014; Calixto, 2000).

The *Cola* genus of the family, Malvaceae (formerly Sterculiaceae), is made up of over 125 species distributed across tropical Africa, where they play economic and medicinal roles (Burkill, 1997; Tsopgni *et al.,* 2019). Several well-known species, like *Cola nitida*, *Cola acuminata*, and *Cola gigantea*, produce kola nuts rich in caffeine and theobromine, both of which are natural stimulant compounds (Burdock, 2022). The genus displays a wide range of medicinal potential, with different species used in traditional medicine for treating ailments like infections, inflammation, fever, and fatigue (Burkill, 1997; Adesanwo *et al.,* 2017). Species closely related to *Cola rostrata* include *Cola lateritia*, *Cola pachycarpa* and *Cola lepidota*, all of which are native to the humid lowland rainforests of West and Central Africa (Tsopgni *et al*., 2019; Burkill, 1997).

Cola rostrata K. Schum., commonly known as "monkey cola" and locally referred to as "Achicha" in Igbo, and sometimes "Ndiya" by the Efik, is an evergreen tree native to the humid lowland rainforests of southeastern Nigeria, Gabon and Cameroon (Mukah *et al.,* 2023; Tsopgni *et al.,* 2019). Ethnomedicinally, various parts of the plant are utilized in traditional medicine. Reports indicate that *C. rostrata* is used as a stimulant, antiemetic, and remedy for dysentery, pain, diabetes, erectile dysfunction, and oral infections (Asogwa *et al.,* 2022). It is claimed to be used in treating fever and inflammation traditionally (Odion *et al.*, 2019; Ajayi *et al.*, 2023). The fruit pulp is edible and is eaten as a good source of essential vitamins and minerals, reflecting the nutritional and therapeutic value of the plant (Mukah *et al.*, 2023).

Phytochemical studies on *C. rostrata* have confirmed the occurence of tannins, alkaloids, saponins, flavonoids, terpenoids, and fatty acids, compounds known for diverse bioactive properties (Essien *et al.*, 2015; Odion *et al.*, 2019; Ajayi *et al.*, 2023). Odion *et al.* (2019) observed that the root bark extract and its fractions showed potent antioxidant and cytotoxic activities, with notable flavonoid and phenolic content, while Ajayi *et al.* (2023) identified numerous bioactive compounds, including fatty acids and alkaloids, through GC–MS analysis. Similarly, Mukah *et al*. (2023) documented the nutritional and phytochemical profile of the fruit pulp, revealing that *C. rostrata* is a significant source of vitamins A, C, E, and essential minerals.

Despite these findings, previous research has focused on the leaves, fruit and root bark, with limited information available on its the stem bark. The stem bark of *C. rostrata* is morphologically rough and brown, turning reddish-brown internally. That of other Cola species have been observed to contain phenolic compounds and lignified tissues associated with defensive and therapeutic secondary metabolites (Burkill, 1997; Pandey *et al.,* 2016).

Assuming similar structures share similar phytochemical compositions, it is safe to hypothesize that the stem bark of *C. rostrata* may possess potent antioxidant and antimicrobial properties. *C. acuminata* and *C. nitida* extracts have shown some antimicrobial activities as well as antioxidant properties (Adesanwo *et al.*, 2017). Building on these findings, Ajayi *et al.* (2023) demonstrated that the leaf and fruit epicarp of *C. rostrata* exert cytotoxic effects on MRC5-SV2 and HeLa cells by inducing reactive oxygen species (ROS) production and disrupting mitochondrial membrane potential.

The pharmacological activities of *C. rostrata* can be attributed to its rich phytochemical content. Flavonoids and phenolic compounds may serve as natural antioxidants capable of donating hydrogen or electrons to neutralize free radicals (Pietta, 2000; Panche *et al.,* 2016). Alkaloids may contribute to analgesic, antimicrobial, and anti-inflammatory effects, while tannins display strong astringent and wound-healing properties (Haslam, 1996). Triterpenoids also identified in *C. rostrata,* play critical roles in modulating inflammation and protecting against oxidative damage (Dzubak *et al.*, 2006)

To validate the traditional uses of *Cola rostrata* and fill the knowledge gap regarding the stem bark, this study employed High-Performance Liquid Chromatography (HPLC) and Gas Chromatography–Mass Spectrometry (GC–MS) to characterize its phytochemical constituents, as well as 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging assay, Ferric Reducing Antioxidant Power assay and Total Antioxidant Capacity (TAC) assays to evaluate its antioxidant potential. The antibacterial potential of the extract was evaluated through susceptibility testing, as well as by determining its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

1.1 LITERATURE REVIEW

The *Cola* genus encompasses over a hundred species native to tropical Africa, known for both economic and therapeutic significance (Burkill, 1997). While *C. nitida* and *C. acuminata* are widely cultivated for kola nuts containing caffeine, other species like *C. gigantea*, *C. pachycarpa*, and *C. rostrata* are valued for their medicinal roles (Tsopgni *et al.,* 2019; Agyare *et al.,* 2012). Extracts of some *Cola* species have demonstrated pharmacological activities, including antioxidant, anti-inflammatory and antimicrobial effects (Adesanwo *et al.*, 2017; Dewole *et al.*, 2013). *C. rostrata* has been reported to be used among many traditional remedies for ailments such as dysentery, fever, inflammation, diabetes, erectile dysfunction, and for pain relief (Asogwa *et al.,* 2022). Mukah *et al.* (2023) state that the fruit pulp is consumed as food quite evidently due to its high vitamin content. The outer layer of the fruit (epicarp) has been reported to contain polyphenols like tannins and anti-nutritional compounds such as, hydrocyanic acid, phytic acid, and oxalic acid. Additionally, extracts from this part of the fruit have shown inhibitory effects against the growth of *Escherichia coli*, β-haemolytic *Streptococcus*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Ajayi *et al.,* 2023). Alkaloids, flavonoids, tannins, phenolics, triterpenoids, and saponins were among diverse metabolite classes found during phytochemical analyses (Essien *et al.*, 2015; Odion *et al.,* 2019).

HPLC and GC–MS studies have identified compounds such as catechin, narigenin, tannins, ammodendrine, palmitic acid, and oleic acids, each contributing to the pharmacologic profile of the plant (Ajayi *et al.*, 2023). Odion *et al*. (2019) reported strong radical scavenging and cytotoxic effects from *C. rostrata* root bark extracts, suggesting antioxidant mechanisms which may be tied to its phenolic constituents. Similarly, Ajayi *et al.* (2023) demonstrated that the ethanol extract of the fruit epicarp and leaves induced ROS-mediated cytotoxicity, while Essien *et al.* (2015) demonstrated antioxidant activity associated with its seed and fruit

pulp. Despite growing evidence of the pharmacological potential of *Cola rostrata*, studies on the stem bark remain scarce. Most research have focused on the root, leaves, and fruit, resulting in limited insight into the chemical constituents and biological properties of the stem bark. This study therefore provides new data on the phytochemistry, antioxidant, and antibacterial potential of *C. rostrata* stem bark.

1.1.1 Plant description

Cola rostrata K. Schum. (Malvaceae) is a flowering plant native to the tropical rainforests in southeastern Nigeria, southern Cameroon, and Gabon (Mukah *et al.,* 2023; Tsopgni *et al.,* 2019). It is a small evergreen tree measuring about 10-15 m in height. The leaves are simple with alternate arrangement; they are elliptic to oblong in shape, measuring 5-15 cm in length and 2-7 cm in width (Burkill, 1997). It produces creamy yellow flowers with five petals that are borne in axillary or terminal cymes, followed by large woody fruits bearing seeds with edible arils that are food for both humans and some primates such as monkeys and baboons. The seeds are large, rough, and brown, typically not eaten. The root bark serves as a major organ for nutrient transport and storage of secondary metabolites within the plant system. Morphologically the root exhibits a woody fibrous texture with a reddish-brown hue. Besides its structural role, the root bark had previously been indicated as a rich source of bioactive compounds, including flavonoids, alkaloids, phenolics, tannins, triterpenoids, and fatty acids (Odion *et al.*, 2019; Tsopgni *et al.*, 2019).

The stem bark of *Cola rostrata* is rough and brown externally, becoming reddish-brown on the inner surface when freshly cut, a characteristic feature of several other *Cola* species (Burkill, 1997; Mukah *et al.*, 2023). It plays a key role in the structural support, nutrient conduction, and defence mechanisms of the plant. The rough brown outer surface protects the plant from environmental stress, insect predation, and microbial attack. Although detailed studies on the stem bark are limited, phytochemical investigations of other parts of the plant

including the root bark and whole plant extracts have identified similar classes of bioactive metabolites, known for their antioxidant and cytotoxic activities (Odion *et al.*, 2019; Ajayi *et al.*, 2023). This evidence suggests that *Cola rostrata* stem bark, like other parts, may serve as a reservoir of pharmacologically active compounds and provide biological rationale for its use in traditional practices in managing inflammatory and oxidative stress-related conditions.

1.1.2 Botanical classification

Table 1.1

Kingdom	Plantae
phylum	Streptophyta
Class	Equisetopida
Subclass	Magnolidae
Order	Malvales
Family	Malvaceae
Genus	*Cola*
Species	*Cola rostrata*

Source: (Royal Botanic Gardens, Kew, 2024).

1.1.3 Ethnomedicinal uses

Diverse ailments such as dysentery, headache, fever, cough, and malaria symptoms, as well as for their stimulant and aphrodisiac effects (Burkill, 1997; Adesanwo *et al.,* 2017). *Cola rostrata* K. Schum. has been reported in local ethnomedicinal practices across southeastern Nigeria and Cameroon for the relief of pain, management of diabetes, treatment of erectile dysfunction, maintenance of dental hygiene, and as an antiemetic remedy (Asogwa *et al.,* 2022). These uses underscore its potential within traditional healing systems, although scientific validation remains limited. Locally, the bark and root of *Cola rostrata* are used in folk medicine for the treatment of fever, inflammation, and microbial infections in southeastern Nigeria (Odion *et al.*, 2019). Such preparations are typically taken as decoctions or herbal teas in traditional practice. In addition, various parts of Cola species (leaves, bark, fruit) are prepared as infusions or tonics, this is consistent with the stimulant and tonic uses of kola nuts in the genus (Essien *et al.,* 2015; Mukah *et al.*, 2023).

1.1.4 Pharmacological benefits

Studies on *Cola rostrata* and related species state that the bark contains a wide range of bioactive compounds with therapeutic relevance. These activities may be attributed to secondary metabolites like flavonoids, saponins, alkaloid, triterpenoids, and fatty acids, which are common in *Cola* species and have been reported to exert antimicrobial, antioxidant, anti-inflammatory, and cytotoxic effects (Odion et al., 2019; Ajayi *et al.*, 2023; Adesanwo *et al.,* 2017).

Antimicrobial activity: The presence of tannins, flavonoids, and alkaloids in *C. rostrata* suggests possible antimicrobial potential, as these classes of compounds are known to inhibit bacterial and fungal growth (Odion *et al.*, 2019; Mukah *et al.,* 2023). Similar findings have been observed in *C. acuminata* seed extract and *C. nitida,* which were

active against *Staphylococcus aureus* and *Candida albicans* (Mboto & Udoh 2014; Adesanwo *et al.,* 2017). This suggests that *C. rostrata* may also play a role in managing microbial infections affecting the skin, respiratory tract, and gastrointestinal system.

Antioxidant properties: Phytochemical studies established that *C. rostrata* comprised both flavonoids and polyphenols, characterized by radical scavenging effects (Odion *et al.*, 2019; Ajayi *et al.*, 2023). These compounds neutralize the generation of free radicals and thereby safeguard biological tissues from suffering oxidative damage leading to chronic diseases such as cardiovascular and neurodegenerative diseases.

Cytotoxic and Anticancer Activity: The ethanol extract from the fruit epicarp of C. rostrata has been reported to induce cytotoxicity in the Hela human cervical cancer cell line and MRC5-SV2, a transformed human lung cell line (Ajayi et al., 2023). The epicarp of the fruit demonstrated almost three to four times higher potency of cytotoxicity than the leaf extract against the two cell lines.

Antiproliferative and Apoptotic: Moderate antiproliferative and pro-apoptotic effects were observed in the MCF-7 human breast adenocarcinoma cell line mediated by the methanol extract and solvent fractions of Cola rostrata root bark (Odion et al., 2019).

Antidiabetic potential: Root bark extracts of *C. rostrata* have been known to possess antioxidant activities coupled with very high phenolic content, thus indicating an excellent potential for glycemic control caused by the modulation of oxidative stress (Odion et al., 2019).

1.1.5 Phytochemicals

Phytochemicals are substances created by plants primarily for their adaptation and defence. They are not involved with plant growth or reproduction, yet they serve various ecological

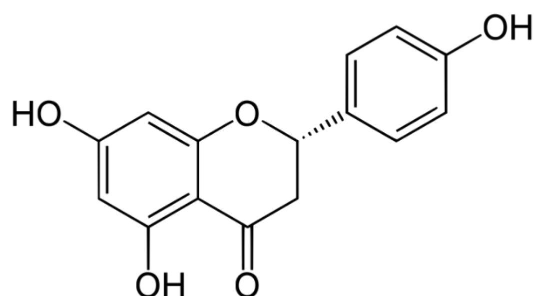
roles such as protecting plants against pathogens, herbivores, and oxidative damage. These substances are categorized into systematically grouped classes of compounds based on either their chemical structure or biosynthetic pathway. These groups include alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, and glycosides. Phytochemical studies carried out on *Cola rostrata* confirmed the existence of various classes of bioactive secondary metabolites and some of these compounds were identified quantitatively in different parts of the plant, particularly the seed, root, and fruit extracts (Odion *et al.*, 2019; Essien *et al.*, 2015; Ajayi *et al.,* 2023; Mukah *et al.*, 2023). Flavonoids and phenolic compounds reported in *C. rostrata* have antioxidant and radical-scavenging properties, while alkaloids and saponins may contribute to its antimicrobial and anti-inflammatory actions. Triterpenoids and steroids exert membrane-stabilizing and cytotoxic properties, while fatty acids including palmitic acid and oleic acid primarily exhibit antioxidant and anti-inflammatory activities. Together, these phytochemicals help contribute to the therapeutic efficacy of this plant, supporting its ethnomedicinal use in infections, inflammation, pain, and oxidative stress-related disorders. Although related direct reports on stem bark are still limited, the reoccurrence of the same phytochemical classes in studies on other parts suggests that stem bark may also harbour similar metabolites with possible pharmacological significance.

Flavonoids

Flavonoids are a broad class of naturally occurring polyphenols characterized by a 15-carbon framework arranged in a C6–C3–C6 structure, with two aromatic rings connected through a heterocyclic pyran ring. Flavonoids are widely distributed and naturally occur in fruits, leaves, bark, and flowers of higher plants (Panche *et al.,* 2016). Pharmacological activities attributed to flavonoids include antioxidant activity, anti-inflammatory action, antimicrobial

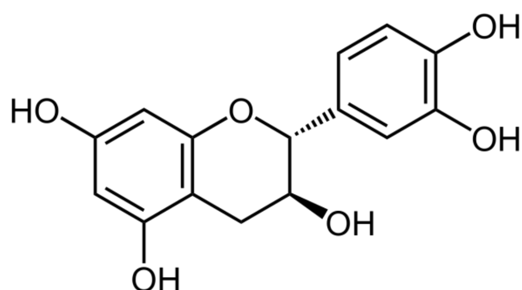
activity, anticancer activity, antiviral action, and cardioprotective effects. The antioxidant capacity via hydrogen atom donation or electron donation for neutralizing free radicals builds a protective wall against oxidative damage to biological membranes and macromolecules. Quercetin, kaempferol, luteolin, apigenin, catechin, and flavone are common examples. In pharmaceutical sciences, flavonoids are applied in the form of natural antioxidants in drug forms and the bioactive molecule to prevent and control the incidence of cardiovascular disorders and cancers (Panche *et al.,* 2016; Pietta, 2000).

A) Naringenin



(2*S*)-5,7-Dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one

a) Catechin



(2*R*,3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol

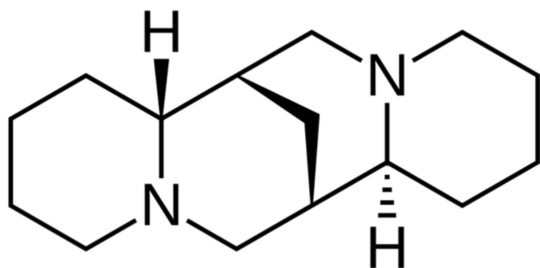
Phenolic compounds

Phenolic compounds are secondary metabolites that have hydroxyl groups linked directly to an aromatic ring and also found as simple phenols to complex polyphenols, occupying a pervasive position in nature i.e. fruits, seeds, bark, and leaves of plants. The compounds are known to possess very strong antioxidant potentials which also chelate metal ions in addition to scavenging reactive oxygen species thereby mitigating oxidative damage to cellular components. In addition, they display anti-inflammatory, antimicrobial, and anticancer activities combined with flavonoids for a synergistic effect. Popular examples are gallic acid, ferulic acid, caffeic acid, and catechol ((Dai & Mumper, 2010; Kruk *et al.*, 2022).

Alkaloids

Alkaloids constitute naturally occurring nitrogenous organic compounds derived from amino acids with considerable physiological and pharmacological activities. Alkaloids possess a broad spectrum of bioactivities, including analgesic, antimalarial, antihypertensive, and antimicrobial activities. Well-known examples of alkaloids include morphine (analgesic), quinine (antimalarial), caffeine (stimulant), and atropine (anticholinergic agent). Their pharmacological potency has made them indispensable in drug development and clinical therapeutics, especially for pain management, cardiovascular regulation, and infectious disease treatment. Besides that, the structures of alkaloids are complicated, making it important for synthetic and medicinal chemistry research (Dewick, 2009; Roberts & Wink, 1998).

A) Spartein



(1*S*,2*R*,9*S*,10*S*)-7,15-diazatetracyclo[7.7.1.0^{2,7}.0^{10,15}]heptadecane

Tannins

Tannins are high molecular weight polyphenolic compounds capable of precipitating proteins and alkaloids because of their multiple hydroxyl groups. They are found primarily in bark, seeds, and leaves. Tannins contribute to the astringency and protection of a variety of plants. Because of their strong astringent, antimicrobial, antioxidant, and wound-healing properties, they may find application in pharmacology in the treatment of diarrhea, skin infections, and ulcers. Some examples of tannins include tannic acid and ellagic acid, and those based on catechin are known as condensed tannins. Their protein-binding and complexing ability is the basis of their biological and therapeutic activities, as well as their industrial application in tanning and dyeing (Haslam, 1996).

Triterpenoids

Triterpenoids belong to a subgroup of terpenes consisting of six isoprene units ($C_{30}H_{48}$), which form the carbons of many steroidal and saponin backbones. Triterpenoids abound in plant resins, bark, and cuticular waxes. These compounds show wide-ranging biological activities of pharmacological interest. Anti-Inflammatory, antiviral, hepatoprotective and anticancer effects are some examples. Their mechanism of action may include modifying the

concentration of inflammatory mediators, antioxidant activity, and apoptosis induction in tumor cells. Some examples include oleanolic acid, ursolic acid, lupeol, and β-amyrin. In modern medicine, triterpenoids are employed in the development of hepatoprotective and anti-inflammatory drugs and serve as biochemical precursors in steroidal drug synthesis (Dzubak *et al.,* 2006).

Fatty acid and fatty acid esters

Fatty acids are carboxylic acids with long hydrocarbon chains, while fatty acid esters are formed through the esterification of these acids with alcohols. These compounds are essential components of plant lipids, oils, and waxes, performing functions like reserve energy and membrane integrity. In *Cola rostrata*, methyl palmitate, methyl oleate, palmitic acid, and oleic acid have been identified by GC-MS as major fatty acids and fatty acid esters (Ajayi *et al*., 2023). Fatty acids pharmacologically exhibit antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities. Unsaturated fatty acids such as oleic acid maintain fluidity of the cell membrane and regulate inflammatory pathways. Such compounds are widely included in topical formulations, nutraceuticals, and lipid-based drug delivery systems for improved bioavailability and therapeutic stability (Ajayi *et al*., 2023).

1.1.6 Harvesting and Collection

Plant collection and processing have a major impact on their phytochemical composition, purity, and biological activity. Before embarking on the collection of *Cola rostrata,* the plant must be accurately identified by a qualified botanist, and a voucher specimen should be submitted to a recognized herbarium. Harvesting should be carried out using clean, corrosion free tools, and the bark should be removed in a manner that allows the parent tree to survive and regenerate, aligning with sustainable harvesting practices. After removal, the material must be cleaned of debris, shaded-dried to protect heat-sensitive and volatile constituents, and

then pulverized using a sterile mechanical grinder. Shade drying is critical, as exposure to sunlight or high heat may degrade thermolabile compounds such as flavonoids and polyphenols. Sustainability considerations such as selective bark removal, harvesting from mature plants, and protecting wild populations from over-exploitation are essential for conservation and maintaining consistent phytochemical profiles across batches (Pandey *et al.*, 2011)

1.2 EXTRACTION AND SOLVENT

Extraction holds great significance in phytochemicals and pharmacological studies as it separates bioactive compounds from plant matrices while preserving their structure and integrity. Extraction is affected by several factors, including type of solvent used, time of extraction, temperature, and the solvent to plant ratio (Harborne, 1998; Sasidharan *et al.,* 2011; Azwanida, 2015).

There are many useful methods of extraction for plant compounds. Maceration consists of soaking plant material in a solvent at ambient temperature for at least 24 h to a couple of days, providing enough time for the solvent to penetrate the tissues and dissolve the desired compounds. Other techniques considered are Soxhlet extraction, which consists of the continuous refluxing of the solvent over the plant material; percolation, wherein the solvent is slowly passed through the plant matrix; modern approaches like microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE).

Among the techniques mentioned previously, maceration is easy, inexpensive, and mainly applicable to thermolabile compounds that can be destroyed at high temperatures (Harborne, 1998; Sasidharan *et al.*, 2011). This method was adopted in this study because of its simplicity, low cost, and ability to extract thermolabile substances that would be degraded by the application of high temperature.

The choice of solvent is equally very important. Between the multiple solvents used in natural product research, ethanol is the most utilized of the solvents applied in phytochemical and pharmacological studies for its capability to extract a wide range of bioactive compounds. The amphiphilic nature of ethanol enables it to dissolve polar and moderately nonpolar compounds, which includes flavonoids, phenolics, alkaloids, glycosides, tannins, terpenoids, and saponins (Azwanida, 2015; Do *et al*., 2014). The use of ethanol as a solvent in herbal extraction dates back centuries, and it remains the preferred solvent in modern phytochemical analysis due to its safety, efficiency, and its capability to dissolve a wide range of phytochemicals (Handa *et al.,* 2008). Ethanol is recognized by the World Health Organization (WHO) as a low-toxicity solvent considered safe for the preparation of medicinal plant extracts, a class 3 solvent (WHO, 2011).

Ethanol (C_2H_5OH) contains a hydroxyl (-OH) and an ethyl ($-C_2H_5$) group, which give it an amphiphilic character. The hydroxyl group is polar and can form hydrogen bonds with polar molecules, making it effective at dissolving hydrophilic phytochemicals and the ethyl group is nonpolar, allowing ethanol to also solubilize moderately nonpolar compounds like some alkaloids, terpenoids, and sterols. Water (H_2O) is highly polar due to its two hydrogen atoms bonded to oxygen, creating a bent structure with a strong dipole moment. When mixed with ethanol, (e.g. 50 to 80% hydroalcoholic mixtures), the resulting solvent enhancing the extraction of highly polar metabolites while maintaining solubility for moderately polar compounds. Water in the mixture also promotes swelling of plant tissues and improves solvent penetration, facilitating efficient extraction of bioactive secondary metabolites (Sasidharan *et al*., 2011). For this study, 70% ethanol (v/v) was employed, striking an optimal balance. It is polar enough to extract highly polar compounds while still capable of dissolving moderately polar and nonpolar metabolites.

1.3 CHROMATOGRAPHY

Chromatography is an essential analytical technique for the separation, identification, and quantification of components in complex mixtures based on differential distribution between two phases: one stationary and one mobile (Harborne, 1998). Chromatography works on the principle that sample molecules migrate through the stationary phase as they are carried along by the mobile phase. Each compound interacts differently with these phases depending on polarity, molecular size, and affinity. This behaviour results in the separation of component compounds present within a substance. Those compounds having higher affinity for the stationary phase will move slower while those with a greater solubility in the mobile phase will migrate faster (Skoog *et al,* 2018). Thus, chromatography has become one of the major techniques in natural products research for qualitative and quantitative analyses of phytochemicals. This analytical method plays an important role in pharmacognosy, pharmaceutical quality control, and metabolomic profiling, as it enables scientists to characterize bioactive compounds responsible for therapeutic effects (Sasidharan, 2011).

High-performance liquid chromatography, or better known as HPLC, was the method used in this study to separate non-volatile and thermally unstable compounds under high pressure through a liquid mobile phase. It operates on principles of adsorption, partition, or ion-exchange chromatography, depending on the stationary phase. The detector records eluting compounds as peaks on a chromatogram, from which retention time and concentration are determined (Skoog *et al.,* 2018).

Gas Chromatography–Mass Spectrometry (GC–MS) integrates the separation efficiency of gas chromatography with the molecular identification strengths of mass spectrometry. The method is based on differences in volatility and thermal stability. compounds are vaporized and carried by an inert gas through a column which contains the stationary phase, where

separation occurs according to boiling point and polarity. The separated compounds are then ionized and detected by the mass spectrometer, producing a characteristic fragmentation pattern (Adams, 2007). GC–MS is particularly suited for volatile and semi-volatile phytochemicals, including fatty acids, esters, alcohols, terpenes, and hydrocarbons. It is one of the most powerful tools for plant metabolite profiling and chemotaxonomic studies (Harborne, 1998; Sasidharan, 2011)

1.4 ANTIOXIDANT ASSAY

Free radicals are unstable molecules with one or more unpaired electrons in their outermost orbit and are likely to react with nearby cellular components like lipids, proteins, and nucleic acids (Apel & Hirt, 2004). They are produced in biological systems as by-products of normal processes such as cellular respiration and immune defence, and from external factors such as pollution, radiation, and toxins (Valk*o et al.*, 2007). Common examples include reactive oxygen species (ROS) as well as reactive nitrogen species (RNS). Excessive accumulation of these radicals leads to oxidative stress which disrupts cellular homeostasis and contributes to aging, inflammation, and chronic diseases including cancer, diabetes, and neurodegenerative disorders (Pham-Huy *et al*, 2008). Antioxidants, both enzymatic and non-enzymatic, counteract these free radicals by donating electrons or hydrogen atoms to neutralize them, thereby preventing oxidative damage and maintaining physiological balance (Shahidi and Ambigaipalan, 2015).

Several plants have the ability to scavenge free radicals and reactive oxygen species, thereby protecting biological systems from damage due to oxidative stress. They produce secondary metabolites such as phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, and vitamins to be naturally protective to their cells from oxidative stress. During photosynthesis and respiration, reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals are produced by plants, which can potentially damage

proteins, lipids, and DNA (Apel & Hirt, 2004; Foyer & Noctor, 2005). In turn, plants prepare enzymatic and non-enzymatic antioxidants that scavenge the free radicals and stabilize reactive intermediates to maintain redox balance (Gill & Tuteja, 2010; Shahidi & Ambigaipalan, 2015). This property is evaluated via antioxidant assays, which determine free radical scavenging, reducing, or total antioxidant capacities of plant extracts and bioactive molecules. These assays operate mainly through the hydrogen atom transfer (HAT) or single electron transfer (SET) mechanisms, which represent different aspects of antioxidant function (Prior *et al.*, 2005). Among the very mostly used methods, DPPH, ABTS, FRAP, and TAC assay were applied for this study.

The DPPH radical scavenging assay evaluates the ability of a substance to reduce the stable purple DPPH radical to its yellow hydrazine form, with the color change detected spectrophotometrically at 517 nm. (Brand-Williams *et al.,* 1995). Likewise, the ABTS assay determines antioxidant activity by measuring the reduction in color of the blue green $ABTS^{\bullet+}$ radical cation, which has an absorption maximum at 734 nm (Re *et al.*, 1999; Pellegrini *et al.,* 1999). Ferric Reducing Antioxidant Power (FRAP) assay determines the ability of antioxidants to convert ferrous (Fe^{3+}) to ferrous (Fe^{2+}) ions, where TPTZ generates a blue Fe^{2+}–TPTZ measurable at 593 nm (Benzie & Strain, 1996). The Phosphomolybdenum Total Antioxidant Capacity (TAC) assay measures the total antioxidant capacity through the reduction of molybdenum (VI) to molybdenum (V) which forms a green phosphate/molybdenum complex measurable at 695 nm (Prieto *et al.*, 1999). Collectively, these assays offer complementary insights into the antioxidant mechanisms of the plant extract.

1.5 ANTIBACTERIAL ACTIVITY

Plants have been recognized as sources of secondary metabolites rich in antibacterial properties. These bioactive compounds including flavonoids, tannins, alkaloids, terpenoids, saponins, and phenolic acids, can inhibit the growth of pathogenic microorganisms by different mechanisms (Cowan, 1999; Cushnie & Lamb, 2011). Common antibacterial mechanisms include disruption of bacterial cell walls or membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and chelation of metal ions required for microbial growth (Ríos & Recio, 2005; Nazzaro *et al*., 2013).

Differences in cell wall composition often cause plant extracts to exhibit varying antibacterial effects on Gram-positive and Gram-negative bacteria. Gram-positive species usually show greater susceptibility because their peptidoglycan layer is thick but less complex, while Gram-negative bacteria contain an outer membrane that restricts some phytochemicals (Nazzaro *et al.*, 2013).

In vitro tests are usually used for evaluating antibacterial activity, such as the well diffusion method, disk diffusion assay, agar dilution, and broth microdilution techniques (Afolayan & Meyer, 1997). This includes determining key parameters such as the inhibition zone, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). This provides standards for measuring the effectiveness of plant extracts and comparing them to other studies. In general, the wide array of plant secondary metabolites and their multiple modes of antibacterial action position medicinal plants as valuable candidates for developing natural antimicrobial agents, especially in light of increasing antibiotic resistance.

1.6 JUSTIFICATION OF STUDY

This study was conducted in response to the limited research on the ethanolic extract of *Cola rostrata* stem bark. Investigations into the phytochemical composition and biological

activities of *Cola rostrata* stem bark are still scarce. The use of 70% ethanol in this study facilitates the effective extraction of both polar and moderately non-polar bioactive compounds.

1.7 AIM AND OBJECTIVES OF STUDY

This research aims to quantitatively and qualitatively determine the phytochemical constituents, antioxidant activities, and antibacterial capacities of the stem bark extract of *Cola rostrata.*

Specifically, the study has the following objectives: to collect and identify the stem bark of *Cola rostrata*; to prepare and extract the stem bark of *Cola rostrata*; to determine and quantify the phytoconstituents using HPLC; to identify the phytocompounds using GCMS; to assess the antioxidant potentials using DPPH, ABTS, FRAP, and TAC methods; to prepare and collect the selected bacteria isolate; and to evaluate the susceptibility, MIC and MBC assay for the extract.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION, IDENTIFICATION AND PREPARATION

The stem bark of *Cola rostrata* was collected from Amapu-Igbengwo village in Umuakpara, Osisioma Local Government, Abia State, Nigeria (Lat: 5°10’43.7” N Long: 7°18’57.8” E) by Mrs. Onyekachi-Chigbu, Agnes Chinyere. The plant was identified and authenticated by Mr. A. Ozioko of the International Center for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria and the voucher number of the plant (CEDD/B9/010) was deposited.

2.2 EXTRACTION OF PLANT MATERIAL

The stem bark was washed to remove sand and dirt. It was then air dried for two weeks and pulverized using a clean dry mechanical grinder before placing in an airtight container till required. 400g of powdered bark was macerated with 2x2.5L Ethanol 70% (v/v) for a period of 72 hours. The filtrate was decanted and filtered with a size 1 filter paper. The filtrate was evaporated with a rotary evaporator at 60°C to reduce its volume, then transferred into a beaker and concentrated to dryness using a water bath at 60°C. The concentrated extract was stored in a refrigerator at a temperature of 4°C until use.

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High performance liquid chromatography analysis was carried out using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The analysis employed a C-12 normal phase column (Phenomenex, Gemini 5 µmm, 200 mm × 4.8 mm). The mobile phase was composed of two solvents: solvent A, which was deionized water acidified with acetic acid to pH 2.8, and solvent B, which was acetonitrile. The flow rate was maintained at 0.8 mL/min. Prior to each injection, the column was re-equilibrated with 5% solvent B for 20 minutes. The column temperature was

maintained at 38 °C, the injection volume was 20 µL, and detection was carried out at 280 nm. Identification and quantification of the standard compounds were performed by comparing their retention times and peak areas with those of reference standards using an external calibration curve (Odion et al., 2025).

2.4 GAS CHROMATOGRAPHY MASS SPECTROMETRY

2.4.1 Sample preparation

High performance liquid chromatography analysis was carried out using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The analysis employed a C-12 normal phase column (Phenomenex, Gemini 5 µmm, 200 mm × 4.8 mm). The mobile phase was composed of two solvents: solvent A, which was deionized water acidified with acetic acid to pH 2.8, and solvent B, which was acetonitrile. The flow rate was maintained at 0.8 mL/min. Prior to each injection, the column was re-equilibrated with 5% solvent B for 20 minutes. The column temperature was maintained at 38 °C, the injection volume was 20 µL, and detection was carried out at 280 nm. Identification and quantification of the standard compounds were performed by comparing their retention times and peak areas with those of reference standards using an external calibration curve (Odion et al., 2025).

2.4.2 Procedure for GC-MS analysis

One microlitre (1 µL) of the cleaned extract was injected into the inlet of an Agilent 6890N Gas Chromatograph equipped with an autosampler and coupled to an Agilent mass spectrometric detector. The sample was introduced in pulsed splitless mode onto a DB-5MS fused-silica capillary column (30 m × 0.25 mm × 0.15 µm for length, internal diameter, and film thickness, respectively). Helium was used as the carrier gas, with the head pressure set at

20 psi to maintain a constant flow rate of 1 mL/min. The oven temperature was initially held at 55 °C for 0.4 minutes, increased to 200 °C at 25 °C/min, then to 280 °C at 8 °C/min, and finally to 300 °C at 25 °C/min, where it was held for 2 minutes. Mass spectrometric detection was carried out in electron ionization (EI) mode, with the ion source temperature set at 250 °C and the interface maintained at 300 °C. The system operated at a pressure of 16.2 psia with an out time of 1.8 minutes. Compound identification was achieved by comparing retention times and mass spectra with reference standards and the NIST II library, ensuring accurate and reliable identification (Odion et al., 2025).

2.5 DETERMINATION OF IN VITRO ANTIOXIDANT ACTIVITY

2.5.1 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of the ethanolic extract of *Cola rostrata* stem bark was evaluated using a modified version of the method described by Brand-Williams *et al*. (1995). In this assay, 0.5 mL of a 0.3 mM DPPH solution prepared in methanol was mixed with 2 mL of the extract at varying concentrations ranging from 0.2 to 1.0 mg/mL. The mixtures were thoroughly shaken and incubated for 15 minutes at room temperature in the dark to allow the reaction to occur, after which absorbance was measured at 517 nm. All analyses were conducted in triplicate to ensure accuracy. Ascorbic acid, prepared in similar concentrations, served as the reference standard, while a blank consisting of 0.5 mL of 0.3 mM DPPH and 2 mL of methanol was treated identically to the test samples.

The free radical scavenging activity was determined using the formula below:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$,

Where; A_0 was the absorbance of DPPH radical + Methanol.

A_1 was the absorbance of DPPH radical + sample extract or standard.

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted following a modified procedure described by Benzie and Strain (1996). In this method, 1 mL of the extract, prepared in concentrations ranging from 0.2 to 1 mg/mL, was added to 1.5 mL of freshly prepared FRAP reagent composed of 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$). The reaction mixtures were incubated at 37°C for 30 minutes, after which the increase in absorbance was recorded at 593 nm.

2.5.3 Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the extract was evaluated using the phosphomolybdenum assay described by Prieto *et al.* (1999). This method is based on the ability of antioxidant constituents in the sample to reduce molybdenum (VI) to molybdenum (V), producing a green phosphate–molybdenum (V) complex under acidic conditions. In this procedure, 3 mL of the extract (0.2–1 mg/mL) was combined with 1 mL of the molybdate reagent, and the reaction mixture was incubated at 95 °C for 90 minutes. After heating, the tubes were cooled at room temperature for 20–30 minutes, and absorbance was then measured at 695 nm. Ascorbic acid, prepared similarly, was used as the standard for comparison.

2.5.4 Determination of the scavenging activity of ABTS.+ free radical

The ABTS radical scavenging activity was assessed using a method described by Pellegrini *et al.* (1999). In this assay, 0.9 mL of freshly prepared ABTS reagent was combined with 0.1 mL of the sample extract, and the mixture was incubated at room temperature for 6 minutes. After incubation, the decrease in absorbance was measured at 734 nm. The percentage inhibition of the $ABTS^{+}$ radical was then calculated using the formula:

$$\text{Scavenging activity for } ABTS^{\cdot +} (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\ \%$$

2.6 ANTIBACTERIAL ACTIVITY

2.6.1 Specimen collection

The microorganisms used for this study were selected bacterial isolates obtained from the University of Benin teaching hospital, Benin city, Edo state, Nigeria. The strains used in this study include; *Escherichia coli, Staphylcoccus aureus, Bacillus subtilis, Enterobacter cloacae, Pseudomonas aeruginosa and Klebsiella pneumonia.*

2.6.2 Preparation of test bacteria

All test microorganisms were stored in 20% glycerol Tryptic Soy Broth and frozen until required. For use, they were sub-cultured from the stock onto sterile nutrient agar plates and incubated overnight at 37°C. Following incubation, uniform colonies from the overnight plates were suspended in sterile broth for 12 hours and standardized to a 0.5 McFarland turbidity, yielding an inoculum of roughly 10^5 CFU/mL. The inoculum was then diluted 1:100 to achieve a final concentration of approximately 10^6 CFU/mL.

2.6.3 Antimicrobial susceptibility tests

An antimicrobial susceptibility assay was conducted to assess the antibacterial effects of the ethanolic extract of *Cola rostrata.* The test was carried out using the agar the well diffusion method (Murray *et al.,* 2009) with some modifications.

A concentration of 100mg/mL of the extract was prepared. Mueller Hinton agar was prepared and sterilized at 121°C for 15mins using autoclave following the manufacturer's instructions. Upon sterilization the agar was allowed to cool and 30ml was dispensed into six sterile petri dishes and allowed to set. The agar plates were dried upon solidification with a hot air oven at 50°C for 5mins to remove excess moisture from the surface and labelled using a marker.

A standardized suspension of each bacterial isolate was prepared in 1 mL sterile water and a sterile inoculating wire loop was used to streak corresponding plates with each bacterial

isolate. Using a sterile cork borer of 8mm in diameter, a well was cut on the agar plates and the base was sealed with molten Muller Hinton agar.

0.2 mL of the extract was introduced into the well on each plate using a calibrated micropipette with a rubber teat. The plates were allowed to stand for 30mins and for 24 h at 37°C. After incubation, the inhibition zone diameter of the extract was measured using a meter rule and the results were recorded

2.6.5 Determination of minimum inhibitory concentration

The agar dilution method of Afolayan and Meyer (1997) was used for the determination of minimum inhibitory concentration (MIC) of the ethanolic extract of *Cola rostrata.*

Five sterile petri dishes were used and labelled 200mg, 100mg 50mg 25mg and 12.5mg. The agar was prepared following the manufacturer's instructions and placed in a water bath at 50°C. The stock solution of the extract was filtered and incorporated into the molten agar at different volumes to obtain a range of concentrations; 12.5mg, 25mg, 50mg, 100mg and 200 mg. 1 mL of prepared 12.5 mg was introduced into a universal bottle containing 9 mL of Meuller Hinton molten agar and mixed gently. The mixture was poured into the petri dish labelled 12.5 mg. The same procedure was repeated for each agar plates respectively and the plated were dried in the oven at 50°C for 5 min.

A marker was used to divide the base of each plate into 4 sections and each section labelled to represent three micro-organisms and a control. A sterile inoculating loop was used to streak each section with its corresponding label. The plates were incubated at 37°C for 24 h. After incubation, they were observed for growth, and the results were recorded.

2.6.6. Determination of minimum bactericidal concentration

After determining the MIC, plates showing no visible bacterial growth were swabbed and streaked onto fresh Mueller–Hinton agar plates containing 100 mg, 50 mg, and 25 mg of the test extract. All plates were incubated at 37 °C for 18–24 hours. The minimum bactericidal concentration (MBC) was defined as the lowest extract concentration that completely prevented bacterial growth. (Aiwonegbe *et al.*, 2025).

2.7.6. Determination of mode of action

The bacteriostatic of bactericidal effect of the ethanolic stem bark extract was assessed using the MBC/MIC ratio derived from the determined MIC and MBC values. For each organism with measurable MIC and MBC results, the ratio was obtained by dividing the MBC (mg/mL) by the MIC (mg/mL). MBC/MIC values ≤ 4 indicated a bactericidal effect and MBC/MIC > 4 indicated a bacteriostatic effect.

2.7 Statistical analysis

Data from antioxidant assays were expressed as mean ± standard deviation. Antimicrobial data was analysed using paired t-test to compare extract activity with a control. A significance level of $p<0.05$ was applied.

CHAPTER THREE

3.0 RESULTS

3.1 HIGH PERFOEMANCE LIQUID CHROMAROGRAPHY (HPLC)

Figure 1 shows the HPLC chromatogram of the stem bark extract of *Cola rostrata*. It displays several clear peaks showing the different compounds contained in the extract. Some peaks are higher, suggesting larger amounts of the corresponding compound.

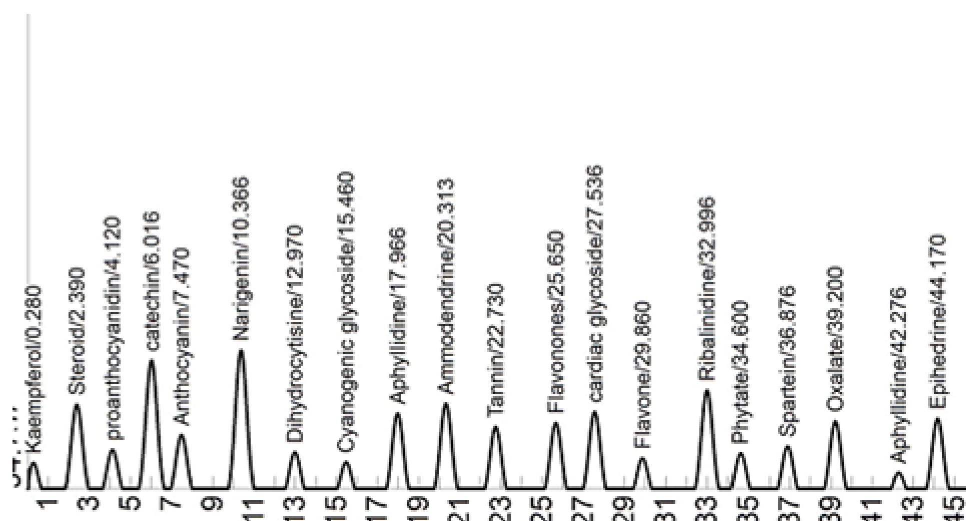


Figure 1: Chromatogram of the stem bark extract of *Cola rostrata*

Table 2 shows the diverse profile of secondary metabolite revealed, their retention times, peak area and varying concentrations in the extract. Notable amounts of flavonoids such as catechin, kaempferol, flavones, and flavonones, as well as tannins, steroids, proanthocyanidins, and cardiac glycosides can be seen, suggesting strong antioxidant and potential pharmacological properties. The presence of alkaloids like aphyllidine, sparteine, and ephedrine further supports the therapeutic potential.

Table 2: HPLC analysis of the stem bark extract of *Cola rostrata* extract

S/N	Compounds	Retention Time (min)	Area	Concentration (µg/mL)
1	Kaempferol	0.280	3426.8154	7.2795
2	Steroids	2.390	12252.8106	14.9590
3	Proanthocyanidin	4.120	6344.5478	8.1619
4	Catechin	6.016	18153.9630	23.3441
5	Anthocyanin	7.470	8442.8722	3.8970
6	Narigenin	10.366	19598.0759	33.6159
7	Dihydrocytisinine	12.970	6238.6258	7.5115
8	Cyanogenic glycoside	15.460	4967.5726	7.8325
9	Aphyllidine	17.966	11339.3364	12.7741
10	Ammodendrine	20.313	12756.4948	22.3551

11	Tannins	22.730	9573.3711	26.1172
12	Flavonones	25.650	10008.8176	12.8703
13	Cardiac glycoside	27.536	11458.0104	7.9331
14	Flavone	29.860	5478.6156	7.0480
15	Ribalinidine	32.996	14337.0482	0.0000
16	Phytate	34.600	6059.7940	8.1449
17	Spartein	36.876	6988.5601	11.9872
18	Oxalate	39.200	10234.8247	3.2216
19	Ephedrine	44.170	10509.6912	7.2765

3.2 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Figure 2 shows the chromatogram of the GC-MS analysis of the ethanolic extract of *Cola rostrata* stem bark. Each peak represents a distinct compound contained in the extract, with higher peaks or larger area peaks indicating the relative abundance of those compounds in the extract.

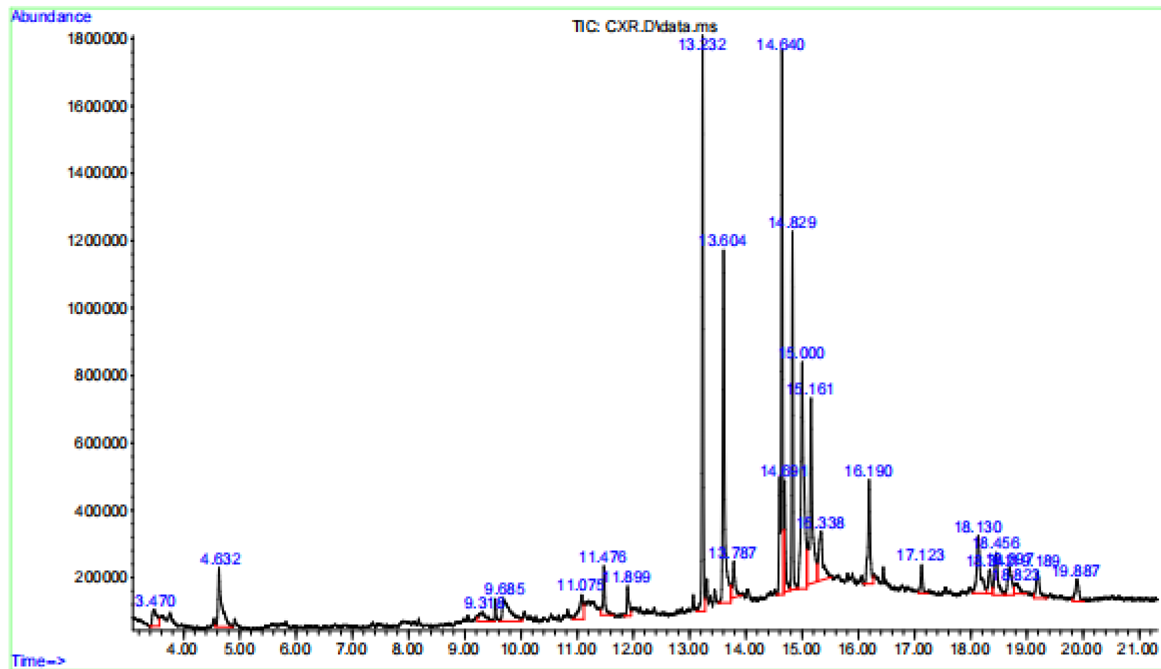


Figure 2: Chromatogram of GC-MS analysis of stem bark extract of *Cola rostrata*

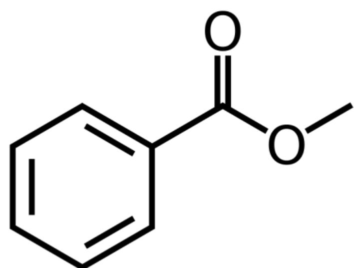
Table 3 shows the different constituents identified by the GC-MS analysis of *Cola rostrata* extract. The retention time indicates volatility, the lower the retention time, the more volatile the compound. The percentage area represents the relative area under each compounds chromatographic peak (fig2). This estimates the relative abundance of each compound in the extract. The molecular formular and weight confirms identity and classification of the compounds present.

Table 3: GC-MS analysis of stembark extract of *Cola rostrata*

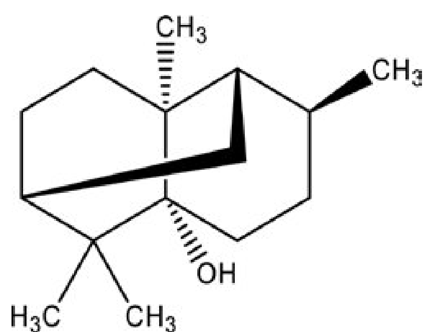
S/N	Compounds	Retention Time (min)	Area (%)	Molecular formular	Molecular weight
1	Thiodiglycol	3.470	1.243	$C_4H_{10}O_2S$	122.19
2	Benzoic acid, methyl ester	4.632	3.265	$C_8H_8O_2$	136.15
3	4-Methylphthalic anhydride	9.318	1.002	$C_9H_6O_3$	162.14
4	3-(2-Pyridyl)-5-hydroxymethylisoxazoline	9.685	2.744	$C_9H_{10}N_2O_2$	178.19
5	Patchouli alcohol	11.075	1.593	$C_{15}H_{26}O$	222.37
6	Methyl tetradecanoate	11.476	1.566	$C_{15}H_{30}O_2$	242.40
7	Tetradecanoic acid	11.899	1.019	$C_{14}H_{28}O_2$	228.37
8	Hexadecanoic acid, methyl ester	13.232	11.696	$C_{17}H_{34}O_2$	270.45
9	n-Hexadecanoic acid	13.604	11.170	$C_{16}H_{32}O_2$	256.42

10	Hexadecanoic acid, ethyl ester	13.787	1.437	$C_{18}H_{36}O_2$	284.48
11	9-Octadecenoic acid (Z)-, methyl ester	14.640	14.104	$C_{19}H_{36}O_2$	296.49
12	6-Octadecenoic acid, methyl ester, (Z)	14.691	2.661	$C_{19}H_{36}O_2$	296.49
13	Methyl stearate	14.829	7.179	$C_{19}H_{38}O_2$	298.51
14	9-Octadecenoic acid	15.00	10.893	$C_{18}H_{34}O_2$	282.47
15	Octadecanoic acid	15.161	8.307	$C_{18}H_{36}O_2$	284.48
16	6-Octadecenoic acid, (Z)-	15.338	3.309	$C_{18}H_{34}O_2$	282.47
17	Glycidyl palmitate	16.190	3.731	$C_{19}H_{36}O_3$	312.49
18	1-Heptadecene	17.123	0.883	$C_{17}H_{34}$	238.46
19	cis-9-Hexadecenal	18.130	3.445	$C_{16}H_{30}O$	238.41
20	Eicosane	18.342	1.103	$C_{20}H_{42}$	282.56
21	cis-Vaccenic acid	18.456	2.317	$C_{18}H_{34}O_2$	282.47
22	Oleic Acid	18.697	1.788	$C_{18}H_{34}O_2$	282.47
23	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	18.823	0.776	$C_{21}H_{40}O_4$	356.55
24	Bis(2-ethylhexyl) phthalate	19.189	1.519	$C_{24}H_{38}O_4$	390.56
25	Eicosane	19.887	1.248	$C_{20}H_{42}$	282.56

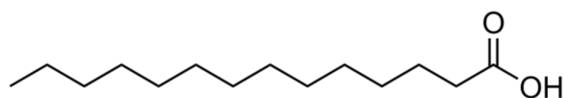
Some representative structures from GC-MS analysis



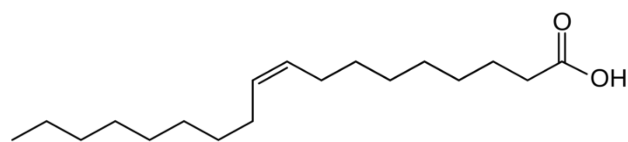
Benzoic acid methyl ester



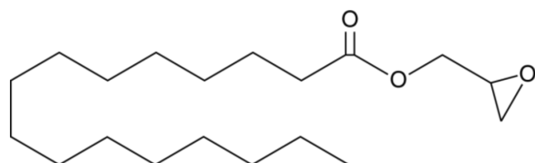
Patchouli alcohol



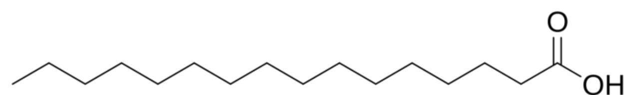
Tetradecanoic acid



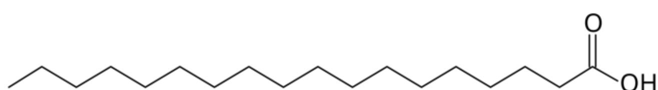
Oleic acid



Glycidyl palmitate



Hexadecenoic acid



Octadecanoic acid

3.3 IN VITRO ANTIOXIDANT ACTIVITY

3.3.1 DPPH radical scavenging activity

Figure 3 shows the result of the DPPH radical neutralizing activity of the *Cola rostrata* stem bark extract, alongside the reference compound, ascorbic acid. The percentage inhibition of determined at concentrations of 0.2 to 1.0 mg/mL, remained high, ranging between (90.18 ± 0.44) % and (82.95 ± 3.20) % and (97.37 ± 0.43) % to (95.2 ± 0.46) % respectively.

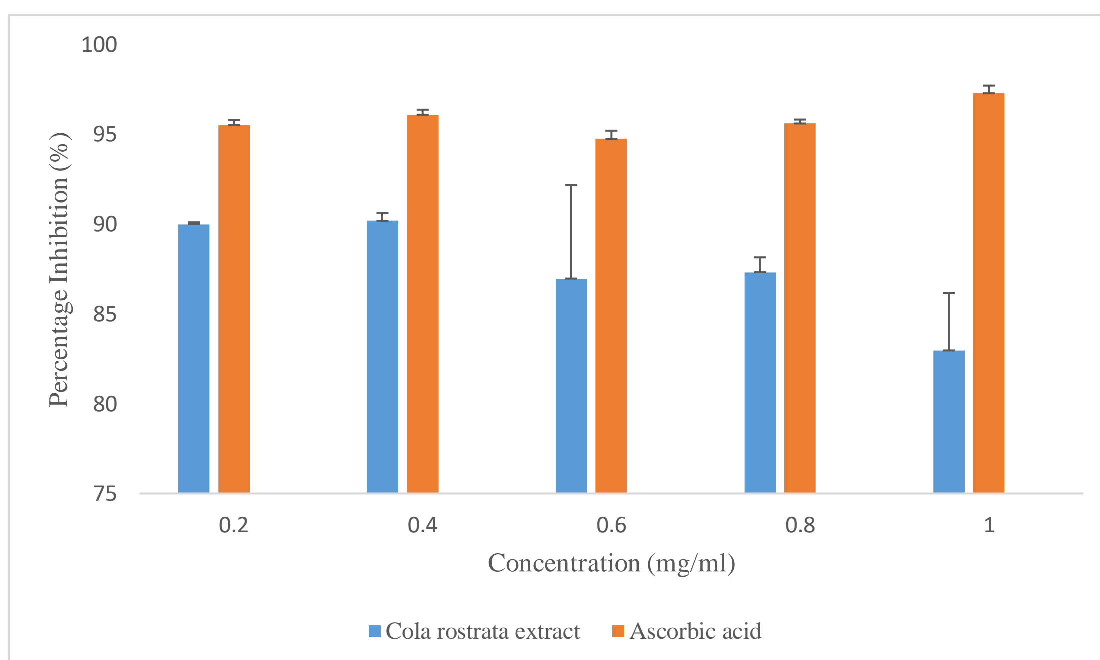


Figure 3: DPPH radical scavenging activity of Cola rostrata stem bark extract and ascorbic acid.

3.3.2 Ferric reducing antioxidant power assay (FRAP)

Figure 4 illustrates the ferric reducing antioxidant power (FRAP) of the *Cola rostrata* stem bark extract at varying concentrations. The average absorbance value of the extract along side that of the control was used to calculate the percentage antioxidantat each concentration as seen below.

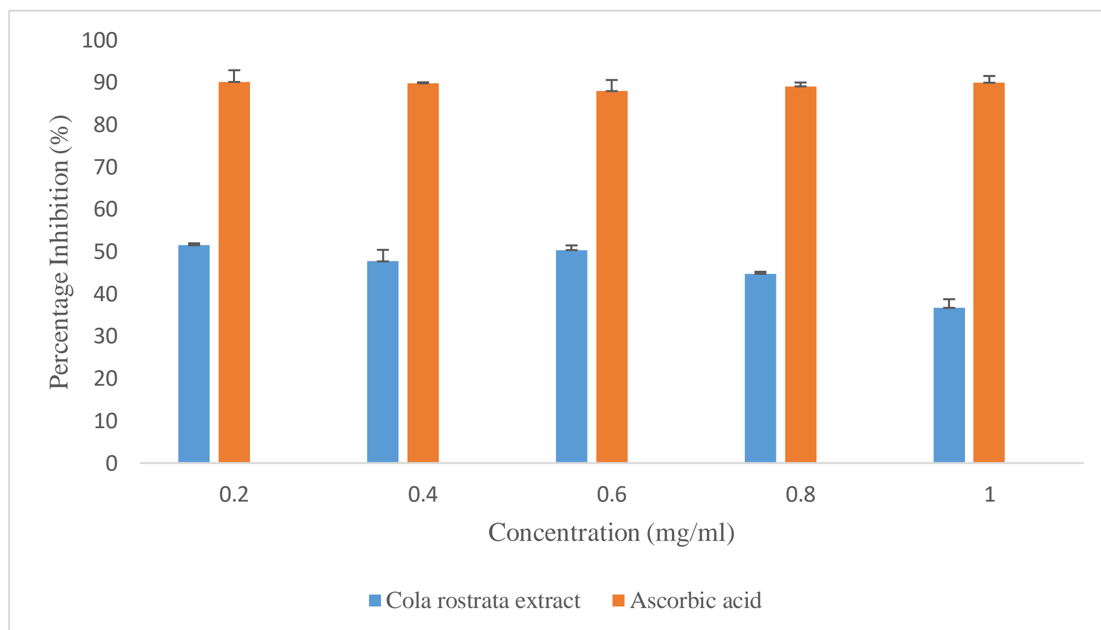


Figure 4: FRAP analysis of Cola rostrata stem bark and ascorbic acid

3.3.3 Total antioxidant capacity assay (TAC)

Figure 5 shows the total antioxidant capacity (TAC) of the *Cola rostrata* stem bark extract compared with ascorbic acid at different concentrations. The percentage antioxidant activity of the extract calculated, ranged from 90.57% to 93.89%, increasing as concentration goes higher. Ascorbic acid showed constant percentage inhibition across all tested concentrations.

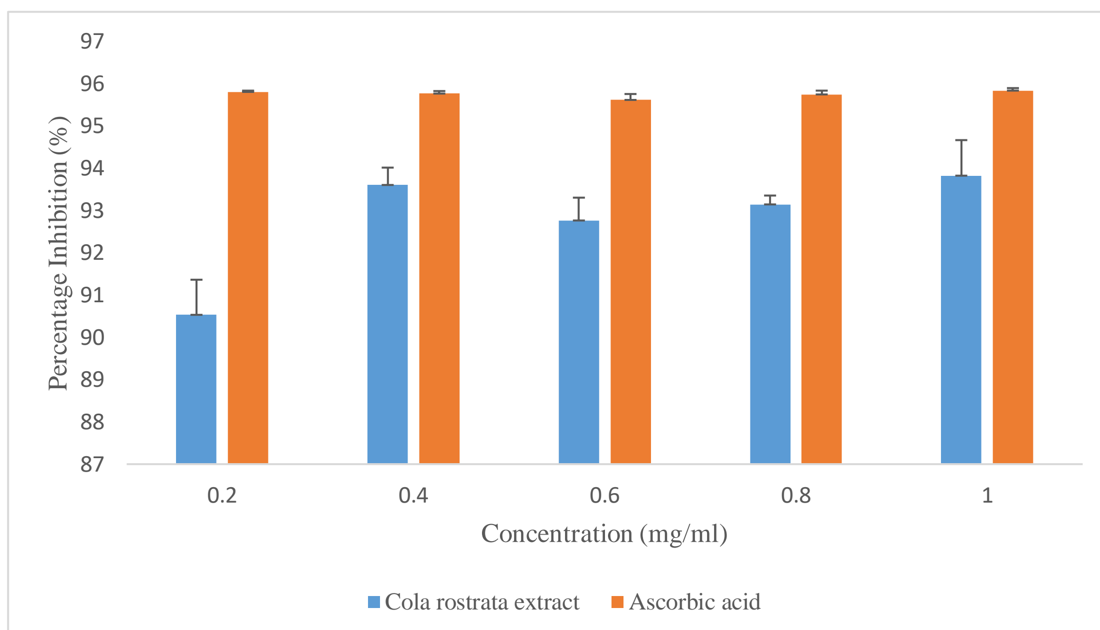


Figure 5: Total antioxidant capacity of *Cola rostrata* stem bark and ascorbic acid

3.3.4 ABTS radical scavenging activity

Figure 6 depicts the ABTS radical scavenging activity of the *Cola rostrata* stem bark extract. The results show that the extract effectively reduced the ABTS radical at lower concentrations. Although the percentage inhibition values decreased slightly as the concentration increased, the extract consistently maintained high antioxidant activity when compared with the standard. Inhibition values ranged from (89.36 ± 0.36) % to (92.46 ± 0.49) % indicating strong and relatively stable antioxidant potential of the extract.

ABTS analysis of *Cola rostrata* stem bark

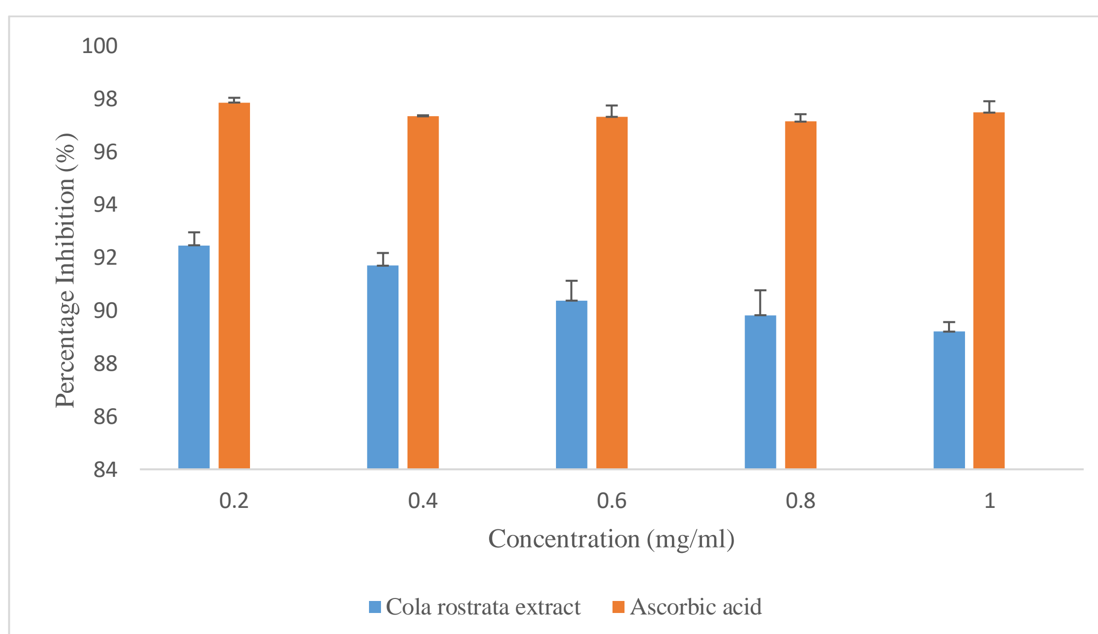


Figure 6: ABTS analysis of *Cola rostrata* stem bark and ascorbic acid

3.4 ANTIMICROBIAL ACTIVITY

3.4.1 Determintion of inhibitory zones

Table 4 shows the antibacterial activity of Cola rostrata extract compared with a standard antibacterial agent, indicating their zones of inhibition across different bacterial strains. The extract was most effective against *Staphylococcus aureus* with a 20 mm zone of inhibition and moderately effective against *Escherichia coli* and *Bacillus subtilis* with zones of inhibition of 13mm each.

Table 4: Antibacterial activity of stem bark extract of *Cola rostrata* extract measured in millimeter

S/N	Bacteria	Extract (100mg/mL)	Ciprofloxacin (1.5 mg/mL)
1	*Klebsiella pneumonia*	No zone	20
2	*Escherichia coli*	13	20
3	*Pseudomonas aeruginosa*	No zone	20
4	*Bacillus subtilis*	13	34
5	*Staphylococcus aureus*	20	40
6	*Enterobacter clocae*	No zone	36

The antibacterial activity of 100mg/mL crude *Cola rostrata* extract was compared to ciprofloxacin 1.5mg/mL using a paired t-test. The analysis yielded a p-value of 0.071064 showing no statistically significant difference between the two treatments ($p > 0.05$). This suggests that under the conditions tested, the extract did not show a significantly different antibacterial effect compared to ciprofloxacin.

3.4.2 minimum inhibitory concentration (MIC)

MIC analysis revealed that growth of *E. coli* was inhibited at 25 mg/mL, *S. aureus* at 50 mg/mL, and B. subtilis at 200 mg/mL. These results indicate that the extract was more effective against *E. coli* and *S. aureus* compared to *B. subtilis*, suggesting a relatively broad antibacterial potential but stronger activity toward Gram-positive *S. aureus*.

Table 5: Minimum Inhibitory Concentration of stem bark of *Cola rostrata* extract

S/N	Bacteria	200 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL
1	*Escherichia coli*	NG	NG	NG	NG	G
2	*Bacillus subtilis*	NG	G	G	G	G
3	*Staphylococcus aureus*	NG	NG	NG	G	G

3.4.3 minimum bactericidal concentration (MBC)

Table 6 shows the bactericidal potential of the *Cola rostrata* stem bark extract. *Escherichia coli* showed no growth up to 50 mg/mL, with growth observed at 25 mg/mL, indicating a minimum bactericidal concentration (MBC) of 50 mg/mL. *Staphylococcus aureus* was completely inhibited only at 100 mg/mL, suggesting that higher concentrations are required for complete bacterial killing. These findings demonstrate that while the extract possesses bactericidal activity, relatively high concentrations are necessary to achieve total elimination of the test organisms.

Table 6: Minimum Bactericidal Concentration of the stem bark extract of *Cola rostrata*

S/N	Bacteria	100 mg/mL	50 mg/mL	25 mg/mL
1	*Escherichia coli*	NG	NG	G
2	*Staphylococcus aureus*	NG	G	G

CHAPTER FOUR

4.0 DISCUSSION

Previous research on *Cola rostrata* has identified various phytochemicals, including flavonoids, phenols, saponins, steroids, tannins, triterpenoids, and reducing sugars. In this study, phytochemical analysis of the crude ethanolic stem bark extract revealed a broad spectrum of bioactive constituents, such as phenolic compounds, flavonoids, alkaloids, glycosides, tannins, steroids, and fatty acids. These compounds may contribute to the extract's observed antioxidant, antibacterial, and antiproliferative activities.

4.1 HPLC

The HPLC analysis (Table 2) identified nineteen bioactive compounds, which fall into the following classes: flavonoids, alkaloids, glycosides, phenolic compounds, steroids and anti nutrients. Catechin, narigenin, tannins, and ammodendrine occured in relatively higher concentrations compared to other constituents such as anthocyanin, kaempherol, phytate, flavone and ephedrine. These findings agree with prior studies on members of the Cola genus, where flavonoids, tannins, and alkaloids have been consistently reported as major phytochemicals which contribute to multiple therapeutic actions.

Catechin is a flavanol (flavan-3-ol), a polyphenolic compound with strong antioxidant, anti-inflammatory and antiproliferative effects, Its effects are mediated via modulation of signalling pathways (NF-κB, MAPKs, Nrf2, STATs), direct radical scavenging, metal chelation and the inhibition of enzymes involved in pro-oxidant reactions. It impacts inflammation by lowering cytokine production as well as cell cycle and apoptosis in cancer cells. Naringenin (33.61 µg/ml) occurred in the highest concentration as seen from the result (fig 1, table). It is a flavanone found in citrus fruits and some medicinal plants. It has been reported to exhibit antifibrotic activity, preventing fibrosis in vital organs such as the liver,

kidney, and lungs (Yu et al., 2024). It also possesses anti-inflammatory and antioxidant properties, which contribute to its ability to mitigate oxidative stress and suppress inflammatory pathways. Evidence also suggests neuroprotective effects, with potential benefits in models of Alzheimer's disease and age-related cognitive decline (Lai et al., 2025).

Tannins are polyphenolic compounds of two main types: hydrolyzable tannins and condensed tannins which are polymers of flavanols. They are found in many plants (bark, leaves and fruits). They are known for their astringent, antimicrobial, antioxidants and anti-inflammatory properties (Cosme *et al.,* 2025). Tannins exert their biological effects through several mechanisms. They precipitate proteins, tightening mucosal membrane, thereby reducing permeability and providing a protective barrier. They can also bind to microbial membranes and inactivate pathogen enzymes, contributing to their antimicrobial activity. In addition, they scavenge free radicals and chelate metals, thereby limiting oxidative stress. They are known to modulate inflammatory mediators and may inhibit enzymes such as lipoxygenase and cyclooxygenase, reducing inflammatory responses. However, they can interfere with digestive enzymes involved in carbohydrate and protein breakdown, which may reduce nutrient absorption when consumed in excess. Ammodendrine is a lupine-type alkaloid. Although reports on any therapeutic uses are poorly documented, there are published toxicity data for ammodendrine. Both ammodendrine and N-methylammodendrine enantiomers have been tested in mice, with reported LD_{50} values of approximately 94.1 mg/kg for (+)-d-ammodendrine and around 115 mg/kg for (−)-l-ammodendrine, while the N-methyl derivatives exhibited slightly lower LD_{50} values (Lee *et al*., 2005). The mechanism of toxicity of has not been fully elucidated, but it is thought to follow the pattern of piperidine alkaloid toxicity; involving interactions with the nervous system and physiological receptors such as nicotinic acetylcholine receptors, leading to neuromuscular and systemic toxic effects (Green *et al.,* 2013). It has been shown to possess teratogenic (Panter *et al.*, 1998) and neurotoxic

potential. Toxicity studies in humans are limited. Steroids in plants are lipophilic secondary metabolites characterized by a tetracyclic cyclopentanoperhydrophenanthrene structure, occurring mainly as phytosterols and brassinosteroids (Harborne, 1998). They play vital physiological and ecological roles, including maintaining membrane stability, regulating growth and development, and enhancing tolerance to biotic and abiotic stress. Their actions are mediated through several mechanisms: brassinosteroids initiate signalling by binding to the BRI1 receptor, triggering phosphorylation cascades that activate transcription factors such as BZR1 and BES1, which regulate genes involved in cell expansion, vascular differentiation, and stress responses. Phytosterols modulate membrane fluidity and the activity of membrane-bound proteins, while plant steroids overall enhance antioxidant defence by upregulating enzymes like SOD, CAT, and POD, thereby reducing oxidative damage (Clouse & Sasse, 1998; Bajguz & Tretyn, 2003). Collectively, these mechanisms enable steroids to support structural integrity, promote growth, and improve resistance against environmental challenges.

4.2 GC-MS

The ethanolic extract of *Cola rostrata* stem bark was analysed by GC-MS analytical technique which was applied to determine the volatile and semi-volatile components of the extract. These bioactive components contribute to multiple biological effects, including antioxidant, antimicrobial, and anti-inflammatory actions. This technique provides high sensitivity and accuracy in identifying complex mixtures of bioactive compounds. This study revealed a total of twenty-five compounds composed mainly of fatty acids, esters, hydrocarbons, and terpenoid compounds. The major constituents included 9-Octadecenoic acid (Z)-, methyl ester (methyl oleate), hexadecenoic acid, methyl ester (methyl palmitate), n-Hexadecenoic acid (palmitic acid), 9-Octadecenoic acid (oleic acid) and methyl stearate with higher peak areas indicating their relative abundance in the extract. Minor constituents

included terpenoids (patchouli alcohol), hydrocarbons (eicosane), and heterocycles. In contrast to the fatty acid–rich profiles reported for *Cola* species (Chinenye *et al.,* 2022; Mebude & Adeniyi, 2017). While occuring in trace concentration, the detection of thiodiglycol, 4-methylphthalic anhydride, and bis(2-ethylhexyl) phthalate in the extract deviates from the characteristic phytochemical profile commonly reported for the *Cola* genus. Previous GC–MS analyses of *Cola rostrata*, *C. nitida*, and *C. millenii* have consistently shown long-chain fatty acids, esters, hydrocarbons, and terpenoids as the predominant constituents, with no record of sulfur-containing or phthalate derivatives (Ajayi *et al*., 2023; Chinenye *et al.,* 2022; Mebude & Adeniyi, 2017). Phthalic acid derivatives and bis(2-ethylhexyl) phthalate are well-known plasticizers that may leach from laboratory materials or solvent containers and have frequently been identified as contaminants in GC–MS analyses of plant extracts (Santana *et al.,* 2019). The dominance of palmitic and oleic acid derivatives is consistent with previous GC–MS reports on *Cola rostrata* and other *Cola* species where fatty acids represent the major non-polar constituents (Ajayi *et al.,* 2023; Adesanwo *et al.,* 2017). These fatty acids have been associated with antimicrobial and cytotoxic activities and may account for the bioactivities observed in this study. The detection of methyl and ethyl esters may result from derivatization during sample preparation or represent naturally occurring esters, hence, clear methodological descriptions are necessary to distinguish these possibilities. Hexadecanoic acid, methyl ester is a fatty acid methyl ester (FAME) derived from palmitic acid. It is one of the most frequently identified compounds in plant GC–MS analyses, particularly in methanolic or ethanolic extracts where mild derivatization may occur. Methyl palmitate is a fatty acid methyl ester that can be found in various lipid-rich plant tissues. It has been studied for anti-inflammatory and antifibrotic effects, through modulation of signaling pathways such as NF-κB, MAPK, and PI3K/AKT. This suggest that methyl palmitate may serve as a bioactive lipid with potential therapeutic applications in

inflammatory and oxidative stress-related conditions (El-Demerdash, 2011). Another major compound identified was n-hexadecanoic acid, the free fatty acid form of palmitate. It is a saturated fatty acid found in plants. It plays essential roles in maintaining membrane structure, energy storage, and stress tolerance. Palmitic acid exhibit antimicrobial, antioxidant, and anti-inflammatory properties that contribute to the overall bioactivity of plant extracts. Its presence at high abundance in *Cola rostrata* aligns with reports from other *Cola* species, where palmitic acid is a predominant constituent in seed extracts. The simultaneous detection of both palmitic acid and methyl palmitate indicates either partial esterification during extraction or the natural coexistence of free and esterified fatty acids in the extract. Fatty acids and their esters are known to function synergistically in plants, contributing to antioxidant and antimicrobial defense through radical scavenging and membrane-stabilizing activities. Following the palmitic acid derivatives, methyl stearate was identified at a relative abundance of 7.18%. This compound is a fatty acid methyl ester derived from stearic acid. It can occur naturally in plants or be formed during extraction processes involving alcohol solvents through mild esterification. Methyl stearate contributes to the non-polar lipid fraction of plant extracts. It has been reported to possess notable antimicrobial, antioxidant, and anticancer properties, and contributes to the hydrophobic stability of lipid membranes (Adesanwo *et al.,* 2017). Another prominent compound identified was 9-octadecenoic acid (Oleic acid). It is a monounsaturated fatty acid that is crucial for maintaining membrane fluidity and protecting plant cells from oxidative and environmental stresses. It is one of the most biologically significant fatty acids in nature and is commonly found in high amounts in bark and seed extracts. Oleic acid has been linked to a wide range of biological activities, including antioxidant, anti-inflammatory, antidiabetic, and cytoprotective effects. In this study, 9-octadecenoic acid (Z), methyl ester, also known as methyl oleate, was identified as the predominant compound in the Cola rostrata stem bark

extract, accounting for 14.10% of the total composition. It is the methyl ester derivative of oleic acid, an unsaturated fatty acid widely distributed in plants. Methyl oleate is known to exhibit a broad spectrum of biological activities, including anti-inflammatory, antioxidant, antimicrobial, and cytoprotective effects. The high abundance of this compound indicates a lipid-rich extract, consistent with previous GC–MS studies on *Cola* species such as *C. acuminata* and *C. nitida*, where oleic and palmitic acid derivatives were dominant constituents (Adesanwo *et al.*, 2017; Ajayi *et al.,* 2023). The detection of methyl oleate, alongside the free oleic acid, may suggest partial esterification during ethanol extraction or the natural coexistence of both esterified and free lipid forms. This dual occurrence supports the interpretation that unsaturated fatty acids and their esters play a significant role in the observed antioxidant and anti-inflammatory activities of the extract.

Collectively, the predominance of these fatty acids and their methyl esters in the GC–MS profile of *Cola rostrata* stem bark demonstrates that the extract is rich in bioactive lipids. These compounds provide strong chemical evidence supporting the plant's antioxidant, anti-inflammatory, and antimicrobial properties observed in complementary assays. The coexistence of free and esterified fatty acids suggests a dynamic lipid matrix that may enhance radical scavenging, membrane stabilization, and overall biological activity.

4.3 ANTIOXIDANT ASSAY

Antioxidant compounds act through multiple mechanisms. Therefore, the use of a single experimental model may provide a limited evaluation of antioxidant potential. To achieve a more accurate and comprehensive assessment, complementary assays such as, DPPH, ABTS, TAC, and FRAP, were employed in this study to evaluate the antioxidant capacity of *Cola rostrata* stem bark. Each of these assays targets different mechanisms of antioxidant action, including free radical scavenging, electron donation, and overall reducing power, thereby

providing a comprehensive evaluation of the antioxidant potential of the plant extract (Re *et al*., 1999; Benzie & Strain, 1996; Prieto *et al.,* 1999).

The antioxidant assays employed in this study provide valuable insight into the ability of the test extract to neutralize reactive oxygen species (ROS) and other free radicals that contribute to oxidative stress. These assays are based on distinct chemical principles that evaluate different mechanisms of antioxidant action. For instance, the DPPH and ABTS assays assess the capacity of compounds to donate hydrogen atoms or electrons to stable radicals, thereby converting them into non-radical, less reactive forms (Brand-Williams *et al.*, 1995; Re *et al.,* 1999). The ferric reducung antioxidant power assay measures the electron-donating potential of the extract to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, indicating its overall reducing capacity (Benzie & Strain, 1996). The total antioxidant capacity (TAC) assay measures the overall reducing power of an extract by assessing its ability to convert molybdenum (VI) [Mo(VI)] to molybdenum (V) [Mo(V)] under acidic conditions, resulting in the formation of a green phosphate/Mo(V) complex that can be quantified spectrophotometrically at 695 nm. The intensity of the resulting color is directly proportional to the extract's total antioxidant capacity. This assay captures the combined activity of both water- and fat-soluble antioxidants, offering a comprehensive evaluation of the extract's total reducing potential (Prieto *et al.*, 1999).

In the DPPH assay, the *Cola rostrata* stem bark extract exhibited strong DPPH radical scavenging activity across all tested concentrations (0.2–1.0 mg/mL), with percentage inhibition values ranging from approximately 90.18% to 82.96%. The result (fig 3) showed notable decline in activity with increasing concentrations, though the higher percentage inhibition (90.18%) was seen at a concentration of 0.4 mg/ml. A slightly similar trend was also observed for the standard, ascorbic acid. Although the percentage inhibition at 0.2, 0.6

and 0.8 mg/ml were constant while higher inhibition occurred at the highest concentration, 1.0 mg/ml. This non-linear trend suggests a possible biphasic antioxidant response, in which increasing extract concentration initially enhances radical scavenging but, beyond a certain point, leads to antioxidant saturation or redox interference among active molecules. Such interactions can slightly reduce overall scavenging efficiency at higher doses. Nevertheless, the consistently high inhibition values confirm the antioxidant potential of the extract. Similar strong concentration-dependent DPPH scavenging trends have been reported in other parts of *C. rostrata*. Odion *et al.* (2019) demonstrated potent DPPH activity in the root bark extract, while Essien *et al.* (2015) recorded significant antioxidant capacity for the fruit and seed extracts. Asogwa *et al.* (2022) further confirmed high radical-scavenging efficiency in methanolic leaf extracts.

The ABTS radical cation assay complements the DPPH test by evaluating both hydrophilic and lipophilic antioxidants. In this method, antioxidants reduce the blue green $ABTS^+$ radical to a colorless form, measurable as a decrease in absorbance (Re *et al.*, 1999). The *C. rostrata* stem bark extract exhibited exceptionally high ABTS radical scavenging activity (fig 6), with inhibition values exceeding 89% at all the concentrations tested. This result implies that the extract contains highly reactive compounds capable of rapidly donating electrons or hydrogen atoms to neutralize free radicals. The same biphasic antioxidant response was also observed. Ascorbic acid showed constant scavenging activity across all concentrations with slightly higher percentage inhibition (97.85%) at the lowest concentration (0.2mg/ml). Although there are few published reports specifically assessing ABTS activity in *C. rostrata*, these results are consistent with its previously established strong antioxidant performance in other assays such as DPPH and FRAP (Essien et al., 2015; Asogwa *et al.*, 2022).

In the Total Antioxidant Capacity (TAC) assay, *Cola rostrata* stem bark extract exhibited stable antioxidant activity. There was a slight decline at 0.6 mg/mL, but the percentage antioxidant activity picked up with subsequent concentrations (0.8 and 1.0 mg/ml) This possible indicates that the total reducing capacity increased with increasing extract concentration. Constant percentage antioxidant activity values across the tested concentrations were observed for ascorbic acid.

Previous studies have reported comparable trends in *C. rostrata* extracts. Odion *et al*. (2019) found high total phenolic and flavonoid contents in the root bark extract, while Essien *et al.* (2015) observed similar antioxidant behavior in fruit and seed extracts. Since these phytochemicals are directly associated with reducing capacity, their presence explains the strong TAC response recorded in this study. These results confirm that *C. rostrata* stem bark possesses robust overall antioxidant potential, aligning with reports in other *Cola* species where high TAC values were attributed to abundant polyphenols and tannins (Adesanwo *et al.*, 2017).

The FRAP assay evaluates the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, forming an intense blue Fe^{2+}–TPTZ complex measurable at 700 nm (Benzie & Strain, 1996). In this study, the *C. rostrata* stem bark extract showed low to moderate FRAP values compared to its DPPH, ABTS, and TAC performances. The lowest concentration (0.2 mg/ml) exhibited the highest reducing potential (51.49%) while subsequent concentrations showed fluctuating and generally lower activities (47.67%, 50.35%, 44.74%, and 36.70%, respectively).

This irregular trend suggests that the reducing capacity of the extract was unstable across the concentration range, possibly due to differential interactions among the phytoconstituents at varying concentrations. While the extract demonstrated appreciable ferric reducing power, its

antioxidant response appeared non-linear, with the most pronounced activity occurring at a lower concentration. Notably, this observation contrasts with several previous studies that reported stronger FRAP activity in *C. rostrata* and related *Cola* species. Essien *et al*. (2015) found measurable but moderate ferric-reducing power in *C. rostrata* fruit and seed extracts, whereas Odion *et al.* (2019) and Asogwa *et al.* (2022) observed comparatively higher reducing capacity in root bark and leaf extracts. This variation may reflect differences in plant part composition and the relative abundance of phenolic compounds.

Collectively, the results from the four antioxidant assays demonstrate that *Cola rostrata* stem bark possesses remarkable antioxidant potential. The consistently high radical-scavenging activities (DPPH and ABTS), coupled with substantial total reducing power (TAC) and moderate FRAP activity, suggest that the extract contains both electron- and hydrogen-donating phytochemicals. These findings are consistent with earlier reports on other *Cola* species and reinforce the pharmacological relevance of *C. rostrata* as a potential natural source of antioxidants.

4.4 ANTIMICROBLAL ASSAY

The antibacterial assay revealed selective inhibition against Gram-positive and Gram-negative bacteria. The extract exhibited highest activity against Staphylococcus aureus with an inhibition zone diameter (IZD) of 20mm and moderate activity against *Escherichia coli* and *Bacillus subtilis* with 13mm IZD each. *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Enterobacter cloacae* showed no susceptibility. This suggests that the extract may be more effective against Gram-positive organisms, consistent with reports that plant-derived polyphenols often target Gram-positive cell walls more easily due to structural differences (Daglia M. 2012). Compared to ciprofloxacin, the extract showed weaker activity

overall, but its effect on *S. aureus* and *E.coli* indicates potential as a natural antibacterial agent.

The MIC results (table 5) showed high sensitivity of *E. coli* a gram-negative bacterium to the extract as it was inhibited at the lowest concentration tested (25 mg/mL) suggesting greater susceptibility. In contrast, B. subtilis required 200 mg/mL for complete inhibition, suggesting a higher tolerance. S. aureus showed growth inhibition at 50 mg/mL . Minimum bactericidal concentration (MBC) assays showed that *E. coli* was completely killed at a concentration of 50mg/mL and S. aureus at a 100mg/mL, indicating that the extract not only inhibits bacterial growth but also possesses bactericidal properties at sufficient concentrations. The mode of antibacterial action, determined using the MBC/MIC ratio for the susceptible organisms, *S. aureus* and *E. coli*, was found to be bactericidal, indicating that the extract was capable of completely eliminating these bacteria.

CHAPTER FIVE

5.0 CONCLUSION

The present study provides comprehensive evidence that the stem bark extract of *Cola rostrata* is a rich source of bioactive phytochemicals with significant antioxidant and antibacterial properties. Chromatographic analyses (HPLC and GC–MS) confirmed the presence of diverse secondary metabolites, such as flavonoids (catechin, kaempferol, naringenin), alkaloids (ammodendrine, sparteine, aphyllidine), tannins, and fatty acids such as palmitic and oleic acids. These compounds are well-known for their free radical scavenging and antimicrobial potentials, supporting the ethnomedicinal and pharmacological benefits of *Cola rostrata*.

Antioxidant assays (DPPH, ABTS, FRAP, and TAC) revealed high free radical neutralizing and total reducing capacities. This suggests that the stem bark of *C. rostrata* can effectively mitigate oxidative stress and may serve as a natural antioxidant source for therapeutic application.

Furthermore, the extract exhibited considerable antibacterial activity, indicating that the synergistic action of its phytochemical constituents may inhibit the growth of pathogenic microorganisms. This reinforces its ethnomedicinal use in treating infections, wounds, and inflammatory conditions.

5.1 LIMITATIONS OF STUDY

The study was limited to in vitro antioxidant and antibacterial assays, in vivo investigations are needed to confirm efficacy and safety within living systems. There was no fractionation, the crude ethanolic extract was analyzed as a whole. Isolation and testing of individual compounds would provide better insight into the specific constituents responsible for its

biological activities. Finally, the safety profile of the extract was not assessed in this study, which is essential before considering therapeutic application.

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APPENDIX A

Table A1: Raw absorbance reading and % inhibition for DPPH radical scavenging assay presented as (mean ± SD, n = 3)

Extract conc(mg /mL)	Absorbance	DPPH percentage inhibition (%)	Ascorbic acid conc (mg/ml)	Absorbance	DPPH percentage inhibition
0.2	0.163 ± 0.002	89.98	0.2	0.073 ± 0.004	95.51
0.4	0.160 ± 0.001	90.18	0.4	0.064 ± 0.005	96.08
0.6	0.212 ± 0.085	86.96	0.6	0.086 ± 0.008	94.73
0.8	0.206 ± 0.013	87.31	0.8	0.072 ± 0.004	95.59
1.0	0.277 ± 0.052	82.96	1.0	0.044 ± 0.007	97.27

Table A2: Raw absorbance reading and % inhibition for FRAP assay presented as (mean ± SD, n = 3)

Extract conc (mg/ml)	Absorbance	FRAP percentage inhibition	Ascorbic conc(mg/ml)	Absorbance	FRAP % Inhibition
0.2	0.692 ± 0.007	51.47	0.2	0.142 ± 0.039	90.06
0.4	0.747 ± 0.038	47.62	0.4	0.146 ± 0.004	89.76
0.6	0.708 ± 0.015	50.35	0.6	0.172 ± 0.038	87.91
0.8	0.788 ± 0.069	44.74	0.8	0.156 ± 0.013	89.03

1.0	0.903 ± 0.029	36.68	1.0	0.144 ± 0.023	89.90

Table A3: Raw absorbance reading and % inhibition for TAC assay presented as (mean ± SD, n = 3)

Concentration (mg/mL)	Absorbance	TAC% inhibition	Ascorbic acid conc	Absorbance	TAC % Inhibition
0.2	1.114 ± 0.093	90.57	0.2	2.449 ± 0.017	95.80
0.4	1.656 ± 0.105	93.66	0.4	2.476 ± 0.032	95.76
0.6	1.456 ± 0.097	92.79	0.6	2.417 ± 0.079	95.66
0.8	1.531 ± 0.047	93.14	0.8	2.464 ± 0.057	95.74
1.0	1.719 ± 0.217	93.89	1.0	2.519 ± 0.037	95.83

Table A4: Raw absorbance reading and % inhibition for ABTS radical scavenging assay presented as (mean ± SD, n = 3)

Extract (mg/ml)	Absorbance	ABTS percentage Inhibition	Ascorbic acid conc	Absorbance	ABTS percentage Inhibition
0.2	0.097 ± 0.006	92.46	0.2	0.028 ± 0.002	97.85
0.4	0.107 ± 0.006	91.69	0.4	0.034 ± 0.001	97.33
0.6	0.124 ± 0.009	90.37	0.6	0.035 ± 0.006	97.30
0.8	0.131 ± 0.012	89.82	0.8	0.037 ± 0.004	97.13
1.0	0.137 ± 0.001	89.36	1.0	0.032 ± 0.006	97.49