UNIVERSITY OF EDUCATION, WINNEBA

Extraction, biological evaluation and GC-MS characterization of ethyl acetate extract of the stem bark of *newbouldia laevis*

CHURCHLIFE AGAVA AGBETUM

MASTER OF PHILOSOPHY

2025

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EXTRACTION, BIOLOGICAL EVALUATION AND GC-MS CHARACTERIZATION OF ETHYL ACETATE EXTRACT OF THE STEM BARK OF *NEWBOULDIA LAEVIS*

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(220018112)

A thesis in the Department of Chemistry Education, Faculty of Science Education, submitted to the School of Graduate Studies in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (Chemistry Education) in the University of Education, Winneba

SEPTEMBER, 2025

DECLARATION

Student's Declaration

I, Churchlife Agava Agbetum, declare that this thesis, except quotations and references contained in published works, which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

Signature:…….

Date:…….

Supervisors Declaration

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis as laid down by the University of Education, Winneba.

Name of Supervisor: Dr. Alhassan Mahama

Signature:……..

Date:…..…….....

Name of Co-supervisor: Prof. Emmanuel Kyame Oppong

Signature:…….

Date:…….

DEDICATION

This research is dedicated to my husband, Samuel Y. Xenyo, and my sons, Jerry-Sam Etriakor Xenyo and Godslife Senyo Xenyo.

ACKNOWLEDGEMENTS

I am very grateful to the Almighty God for all the blessings I have received and His providence in carrying out this research work. My effort would not have been feasible without the assistance and constructive criticisms of other people, and for that I am immensely thankful. As a result, I would like to express my gratitude to Dr. Alhassan Mahama for his essential advice and recommendations, as well as for taking the time out of his hectic schedule to supervise this thesis and make the necessary changes. May God richly bless you. It is impossible for me to adequately thank Prof. E. K. Oppong, Prof. Ruby Hanson, Prof. Arkoful, and Dr. Boniface Yaayin for all of their contributions to the accomplishment of this endeavour.

My special appreciation goes to Dr. Clement Okraku Tettey, the Chairman of the Basic and Biomedical Research Department at the University of Health and Allied Science-Ho, for all of your assistance. Additionally, I want to thank Mr. Neglo David, a laboratory technician at the University of Health and Allied Sciences - Ho, for his outstanding efforts.

I also want to express my gratitude to my parents, Mr. and Mrs. Agbetum, my grandparents, Mr. Nicholas Kutor, and all of my siblings for their encouragement and support. I finally want to appreciate Mr. Samuel Y. Xenyo and Mr. Felix Nelson Akompi for their meaningful contributions and support. Having all of my classmates as role models was also enjoyable.

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ABBREVIATIONS

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FTIR	Fourier-transform infrared spectroscopy
GC-MS	Gas Chromatography- Mass Spectrometry
HLPC	High Liquid Performance Chromatography
IR	Infra-Red
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibition Concentration
NLE	*Newbouldia laevis* extract
NMR	Nuclear magnetic resonance
UV-VIS	Ultra-Violet Visible Spectroscopy

ABSTRACT

The increasing resistance to conventional antibiotics and the demand for effective natural remedies have prompted the exploration of medicinal plants for bioactive compounds. This study aimed to extract and characterize secondary metabolites from the stem bark of *Newbouldia laevis* and evaluate their antimicrobial and antioxidant activities. The stem bark was collected from Dambai-Zongo in the Oti Region of Ghana and extracted using ethyl acetate via maceration. The crude extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, which identified various compounds, including fatty acids (linoleic acid, octadecanoic acid), sterols (stigmasterol, stigmast-5-en-3-ol), triterpenoids (olean-12-en-3-ol), and esters. The antimicrobial activities of the extract were evaluated using the Kirby-Bauer agar diffusion method and broth microdilution assays against bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*, as well as fungal strains like *Candida albicans*. The extract demonstrated significant antimicrobial activity, particularly against *S. aureus*, with zones of inhibition ranging from 8.33 to 19.33 mm. Minimum Inhibitory Concentrations (MICs) ranged from 0.078 to 25 mg/mL, confirming the extract's bactericidal and fungicidal effects. Antioxidant activity was assessed using DPPH and ABTS scavenging assays. The extract exhibited notable free radical scavenging potential, with IC_{50} values of 0.322 mg/mL and 0.329 mg/mL, respectively, comparable to the standard vitamin C. These findings validate the traditional use of *Newbouldia laevis* in treating microbial infections and oxidative stress-related conditions. The presence of bioactive compounds highlights their potential for pharmaceutical applications, particularly in developing natural antibiotics and antioxidants. Further studies are recommended to evaluate the pharmacokinetics, toxicity, and clinical efficacy of the identified compounds to facilitate their development into therapeutic agents.

CHAPTER ONE

INTRODUCTION

1.0 Overview

The first chapter gives a general introduction to the study. It includes the background to the study, the statement of the problem, the purpose of the study, the objectives of the study, including the significance of the study. This chapter also looks at the organization of this study.

1.1 Background to the Study

Newbouldia laevis, a member of the family Bignoniaceae, is popularly known as the fence tree or the African border tree (Bosha et al., 2018). The local Ghanaian names of the medicinal plant in Ewe, Ga, Twi, and Fante are *aviati*, *hiatso*, *sasanemasa*, and *esisimansa*, respectively (Woode et al., 2008; Bosha et al., 2018).

Traditional medicine has been practiced as long as there have been humans (Yuan et al., 2016). As an alternative to Western or Orthodox medicine, herbalism is also referred to as plant medicine. According to estimates, almost two-thirds of the world's population, particularly in developing countries, receive their healthcare mostly through traditional medicine. Traditional medicine is still practiced for disease management and treatment on the African continent. It is related to sociocultural and socioeconomic lifestyles, an insufficiency of access to primary healthcare, and an insufficiency of qualified medical personnel. These are common challenges in most African nations (Anaduaka, 2013).

In fact, anthraquinones, flavonoids, glycosides, and other active substances can all be found in plants (Anaduaka, 2013). This brings to the fore ethnobotany or the traditional

knowledge of the local culture and people concerning *Newbouldia laevis* in Dambai of Oti Region.

According to data from the World Health Organization (WHO), traditional medicine serves the primary healthcare needs of at least, if not more than 80 % of the entire world's population (Sasidharan et al., 2011). Since the dawn of time, human societies have always had a close relationship with their environment and have used natural resources to make food and medicine. The ability to comprehend and use plants to produce food and medicine has been made feasible through trial and error (Jamshidi-Kia et al., 2017). There are numerous compounds that plants have which can be used to treat diseases (Duraipandiyan et al., 2006).

Similarly, regardless of the underlying philosophical premise throughout history, the employment of plants in all the major areas of medicine serves as an example of the universal function that plants play in the treatment of diseases today. Oftentimes, the details of the manner and time plants used for medicine were initially employed in prehistory are lost (Bosha et al., 2018).

In light of the information mentioned above, Bosha et al. (2018) cited the World Health Organization (WHO) for acknowledging the importance of plant medicine in primary healthcare, particularly in developing nations like Ghana, and for encouraging member countries to enact nationwide policies for appropriate identification and proper use of plant medicines.

Humans resorted to ethnopharmacognosy due to the emergence of bad effects and resistance of microbes to synthetically produced drugs. They discovered literally thousands of plant-derived phytochemicals to be safer, more efficient alternatives with

fewer side effects (Sasidharan et al., 2011). The abundant useful bioactivity properties of medicinal plants, including antibacterial, anticancer, antioxidant, and anti-inflammatory effects, have been extensively investigated (Dar et al., 2023, p. 189). However, there is global evidence to indicate the pharmacological impact of medicinal plants through the collaborative mechanism. In recent times, there has been an awakening of interest in remedies that are based on herbal medicine. For fruitful results concerning herbal medicine research, collaboration among ethnopharmacologists, chemists, as well as ethnobotanists cannot be overemphasized (Saad et al., 2017).

In Ghana and other parts of Africa, there are vast expanses with ecological zones occupied by numerous plant species that have found utility for humans either directly or indirectly (Anaduaka, 2013). The therapeutic benefits of these numerous herbs cannot be overstated, according to some researchers like Yuguda (2023), considering verbal traditions and folk wisdom from the bygone days that have remained to resound the curative properties of theses botanical extracts.

Newbouldia laevis is included in the number of medicinal plants whose therapeutic worth withstood the passage of time (Onyekachi, 2021). Despite the fact that the activity of the leaf extract against diabetes, indicated that a comparative study of the leaves and stem ethyl acetate extracts on some microbes has not been considered Anaduaka (2013). Hence, the present study is designed to tackle some of these concerns.

Natural products, including extracts from plants as purified compounds or extracts that have been standardized, give unending chances for the latest drug discoveries because of the incomparable availability of chemical variety (Sasidharan et al., 2011). The primary sources of pharmaceuticals and industrial raw materials are these natural

products (Elshafie et al., 2023). They come from animals, plants, or microorganisms. These natural products are either parts of the organisms or the secondary metabolites produced by the organisms (Akompi, 2022; Stone & Williams, 1992).

The significance of principal metabolites of plants in basic biological life processes such as respiration, reproduction as well as growth and so on, has been clarified by present-day chemistry. They consist of elements that make up procedures like "glycolysis, the Krebs or citric acid cycle, photosynthesis, and related pathways. Small molecules including sugars, amino acids, tricarboxylic acids, or Krebs cycle intermediates, proteins, nucleic acids, and polysaccharides, are examples of primary metabolites" (Hussein & El-Anssary, 2018, p. 11).

"Secondary metabolites are substances manufactured by plants that make them competitive in their own environment. Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways" (Hussein & El-Anssary, 2018, p. 12). The search for new drugs, a complex activity requiring several processes of both artificial and natural products with the hope of discovering new products or, even better, new approaches for the treatment of disease, is an ongoing process involving the latest technologies that improve the design of drugs (Thomford et al., 2018).

Phytochemical screening research work on the extract from the foliage of *N. laevis* to ascertain the chemical components that it is made of, "revealed the presence of alkaloids, tannins, saponins, terpenes, flavonoids, cardiac glycosides and anthraquinones" (Bwegne, et al., 2022, p.121).

Secretions of many secondary metabolites are harvested by humans to enhance their health in the fields of immunomodulators, antitumor agents, enzyme inhibitors, antibiotics, and plant and animal growth promoters (Thirumurugan et al., 2018). "Unlike primary metabolites, the absence of secondary metabolites does not result in immediate death, but rather in a long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps with no significant change" (Kathirvel, 2021, p. 2). It must also be noted that secondary metabolites are also used to "widen the pyramid of healthy nutrition (pigments and nutraceuticals), enhancing agricultural productivity (pesticides, insecticides, effectors of ecological competition, symbiosis and pheromones), and hence impacting the economics of our society in a certain positive way" (Thirumurugan et al., 2018, p. 4). The role of secondary metabolites in the survival of the human race through extraction, isolation and characterization cannot be over-emphasized.

This research work looked at: "Extraction, Isolation and Characterization of Active Secondary Metabolites from *Newbouldia l*aevis."

1.2 Statement of the Problem

A number of current orthodox medications used in the treatment of illnesses are no longer effective and are becoming very costly. Therefore, it is necessary to uncover more significant therapeutic plants, which typically entails separating, evaluating and characterizing the recognized active metabolites (Appiah, 2013). Medicinal plants, such as the leaves or barks of plants, and *Newbouldia laevis,* is no exception.

Scholars such as Ensminger (1994), Okeke (2003), Parekh, Karathia and Chanda (2006) reported that plants have potency as biochemicals and have constituents of phytomedicine since ancient times; from them, man can obtain an astonishing range of

therapeutic chemicals. These natural constituents can be obtained from the root, leaves as well as the bark and flowers and other active parts of the plant. Many research works made use of the roots, rhizome, and other underground parts and compared their antimicrobial activities with the parts of the plant above the ground (Ncube et al., 2008; Das et al., 2010).

In contrast to humid and semi-humid regions, phosphorus and nitrogen ranking in leaves are often inversely connected with the average yearly temperature and not related to yearly precipitation. This is one of the examples of how temperature and geography affect the design of nutrient levels in plants (He et al., 2014). *Newbouldia laevis* is known to be a vital therapeutic plant that has been used extensively in Africa, but little work has been done on its chemical composition, especially in the Oti Region of Ghana.

The scientific processing of parts of plants to unearth novel therapeutic compounds involves laboratory procedures which are systematic. This involves the collection of plants from the environment or from local herbalists of places where the plants are found (Parekh, Karathia & Chanda, 2006). Owing to the versatile applications of plant-derived substances, the need to carry out work on the characterization of bioactive components of the stem bark or leaves of *Newbouldia laevis* becomes imperative. Even so, there seems to be a paucity of academic literature on this area of study in Ghana, especially in Dambai of the Oti Region. It is against this backdrop that this study seeks to extract and characterize the active secondary metabolites from *Newbouldia l*aevis.

1.3 Purpose of the Study

In this study, the chemical composition and the bioactivity of the stem bark of *Newbouldia laevis* will be evaluated.

1.4 Research Objectives

This research work seeks to carry out the following objectives:

1. Extract the secondary metabolites from the stem bark of the *N. laevis* plant materials;
2. Anti-microbial and antioxidant assay and analysis of the extract;
3. GC-MS profiling and analysis of the extract.

1.5 Significance of the Study

Active secondary metabolites from the extracts can serve as a hit to the development of new drugs. The study will also add knowledge to the use of *N. laevis* in its application as a medicinal herb and serve as the basis for further research. In addition, the outcome of this study has great implications for the pharmaceutical industry.

1.6 Organization of the Study

Five chapters make up the structure of the thesis. The background, problem statement, purpose, objectives, significance, and study organization were all covered in chapter one.

The Second Chapter examined the study's supporting literature. Several researchers' perspectives on the description of the *Newbouldia laevis* plant; scientific classification of *Newbouldia laevis*; extraction and analysis of its chemical components; identification and characterization; and common health and medical uses of *Newbouldia laevis* in West Africa, among other sub-topics, were presented in this chapter.

The methodology is covered in Chapter Three. It involves the collection and preparation of samples, the bioactivity screening and characterization of active secondary

metabolites from *Newbouldia l*aevis would be done. The Fourth Chapter took care of the analysis and discussion of the results obtained from the research. Finally, the Fifth Chapter gives a summary, conclusions and recommendations for further study.

CHAPTER TWO

LITERATURE REVIEW

2.0 Overview

This chapter reviews literature related to the study. The opinions, ideas, and research works of other researchers and authors who have studied and written on *Newbouldia laevis*. Further reviews are presented on the general extraction of natural products, identification and characterization of secondary metabolites. The literature review was therefore done in the following thematic areas:

- Description of the *Newbouldia laevis* plant;
- Traditional uses of *Newbouldia laevis;*
- Phytochemical review of *Newbouldia laevis*;
- Extraction methods of Natural Products; and
- Characterization of Natural Products.

2.1 Description of the *Newbouldia Laevis* plant

Newbouldia laevis is a medicinal plant and may grow to the height of 7 to 20 meters. It may have varied heights according to the geographical area where it is located. It has an upright stem with not many branches that bear green, glistening foliage. The bloom of the plant smells good and are of purple shade (Okagu et al., 2022).

Newbouldia laevis, a popular African plant which is popularly known as border or fence tree. In Ghana, the local names in Ewe, Twi, Ga and Fante are *aviati, sasamansa, hiatso, esisimansa* respectively (Woode et al., 2008).

Furthermore, it is scientifically classified as a middle-sized angiosperm and placed in the family of plants called Bignoniaceae. It is a plant that is photophilic, matures quickly

and is resistant to conditions of drought. Though it normally tends to be shrubbery, it may be of the height of 2 to 3 meters and has multiple branches. At the mature stage, it is able to attain the height of roughly 7-15 meters (Habu & Ibeh, 2015).

The seeds of the tree are obtained during wind dispersion of a mature fruit that gets ripped open; therefore, one needs to observe closely to detect when the fruits are ripped to collect the seeds when the fruits begin to split open. The seeds are stored under moisture-free but warm conditions to preserve their shelf life. It is propagated by nursing the seeds in a nursery. It usually germinates within 8 days with a germination percentage of 36 %. It can be propagated vegetatively by using cuttings of the stem, which, within a fortnight of planting, begin to sprout. The plant produces flowers after five years when seed is used and within a year when stem cuttings are used (Obum-Nnadi et al., 2020).

2.1.1 Scientific Classification of *Newbouldia laevis*

Etymologically, Merriam-Webster dictionary, indicated that "the term taxonomy was developed from two Greek words, *taxis*, meaning arrangement, and *nomia*, meaning distribution or method" (Popescu et al., 2018, p. 1). Taxonomy helps to put living things into various groups. It can be seen as a discipline of science that basically identifies living things, describes them, groups and names them (Haider, 2018).

In 1735, the scientist Linnaeus, came up with an order of ranking that puts every single living thing into various subsets. This method of classification made living things of a particular subset bear close resemblance and look different from those of another subset. The Linnaeus classification puts plants into their subsets based on sexual reproduction organs. This order of ranking living things is still accepted today (Rouhan & Gaudeul, 2021). This method ranks living things from the wider set to the smallest

set. The groups are "kingdom, phylum or division, class, order, family, genus and species" (Manimekalai & Vijaya, 2014, p.65).

Newbouldia laevis is classified among plants that belong to the family known as Bignoniaceae (Anaduaka, 2013). This shrub is classified as: "Kingdom: Plantae; Sub-kingdom: Viridaeplantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Lamiales; Family: Bignoniaceae; Genus: Newbouldia; Species laevis" (Achaw & Salis, 2013, p.7). Figure 2.1 shows the *Newbouldia laevis* tree at various stages.

Newbouldia laevis at early stages

Newbouldia laevis at flowering stage

Newbouldia laevis at fruit stage

Newbouldia laevis fully grown used as fence

Figure 1: Images showing the various stages of the *Newbouldia laevis* tree

Source: https://tropical.theferns.info/image.php?id=Newbouldia+laevis. Retrieved on January 10, 2023.

2.2 Traditional Uses of *Newbouldia laevis* plant

Herbs have been made use of in indigenous treatment of diseases. This herbalism is not within the parameters of western medicine. Research has estimated that nearly 80 % of people around the globe of which majority belong to the third world countries depend on herbs as the number one means of treating ailment. The use of indigenous medicine cannot be done away with in Africa due to economic hardships and challenges

associated with the regular health care system (Ekor, 2014). “Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins, and tannins, etc., which possess medical properties that are harnessed for the treatment of different diseases” (Ajuru et al., 2017, p. 198).

The therapeutic properties of herbs and spices are due to the presence of secondary metabolites they produce. Research has shown that these herbs and spices contain high levels of antioxidants. Notably, the antioxidant activities of the secondary metabolites are of tremendous health benefits in stopping cardiovascular diseases and cancer (Rubio et al., 2013). It is also employed in the treatment of worms, malaria, sexually transmitted diseases, and in the reduction of dental care (Eyong et al., 2015, p. 605). Based on the views expressed by researchers above, it is logical to conclude that almost every part of *Newbouldia laevis* is useful for medicine.

In Ghana, it is also believed that the leaves have been used in treating stomach pains, pelvic pains, rheumatic swelling and hemorrhoids. Again, the liquid stock obtained from the foliage is employed as an eye lotion to address trachoma, conjunctivitis, and eye sore. The chewed foliage is also administered to snake bites (Boakye-Gyasi et al., 2013). People in certain parts of Nigeria apply the therapeutic plant in the management of wounds that are infected with bacteria and eye challenges (Akerele et al., 2011). When the leaves of *N. laevis* prepared in palm soup are taken by pregnant women, it helps labour to progress with ease. The postnatal use also induces the mammary glands to supply sufficient quantity of breastmilk. Also, the foliage of the plant is converted to ash and mixed with table salt is a treatment for gastric ulcers. It should be noted that every part of the plant is nutritious and is used to feed animals to improve their appetite (Dassekpo et al., 2020).

It has been scientifically proven that *N. laevis* has therapeutic values "ranging from anti-inflammatory, antioxidant, antimicrobial, antifungi, analgesic and wound healing properties" (Obum-Nnadi et al., 2020, p.60). Additionally, it has been reported to be helpful for chest discomfort, epilepsy, earaches, aching feet, and convulsions in youngsters. In particular, it has been noted that stem bark combined with red pepper and clay can effectively treat a variety of illnesses, including bone lesions, fever, cough, and pneumonia (Rashed, 2021). "*Newbouldia laevis* is widely used in trado-medical (modern traditional medical) practice for the management of diseases and conditions like skin infection, tooth and stomach aches, pains, diabetes, hypertension, tumor, malaria and sickle cell anaemia "(Okagu et al., 2022, p. 52). Figure 2.2 shows a summary of ethno medicinal usage of *N. Levis*

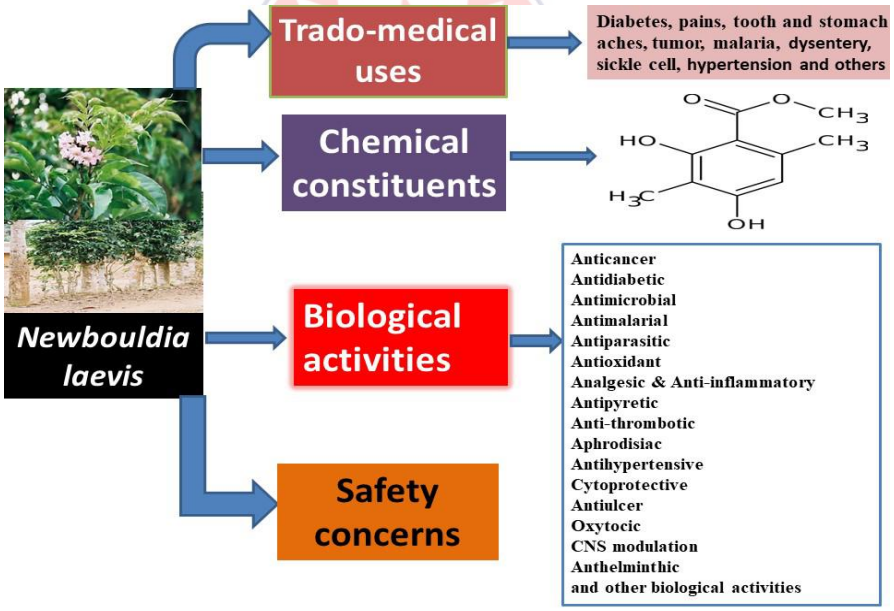


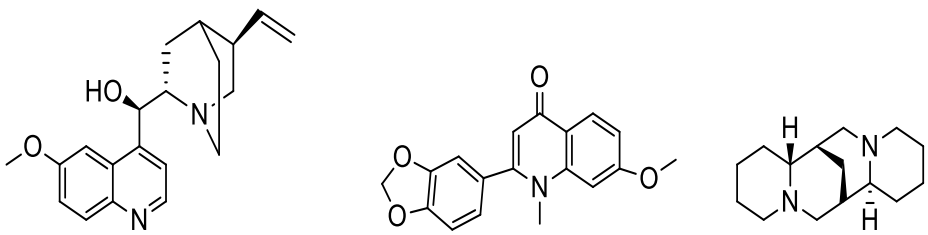
Figure 2: Common health and medical uses of Newbouldia laevis in West Africa

Source: Okagu et al. (2022). *Pharmaceutical Sciences, 28*(1), 51-75.

2.3 Phytochemical review of *Newbouldia laevis*

"Several classes of compounds have been identified in different parts of the plant, which include phenolics, glycosides, anthraquinones, volatile oils, tannins, steroids, alkaloids,

flavonoids, and terpenoids" (Okagu et al., 2022, p. 51). Significant quantities of alkaloids such as quinine (2.1), lunamarine (2.2), and sparteine (2.3) were recorded by GC-FID from roots of N. laevis (Forghe & Nna, 2020). Structures of the above-mentioned compounds are shown in Fig. 2.3.

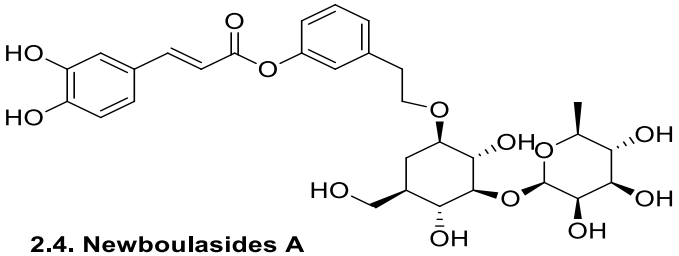


2.1 Quinine 2.2 Lunamarine 2.3 Sparteine

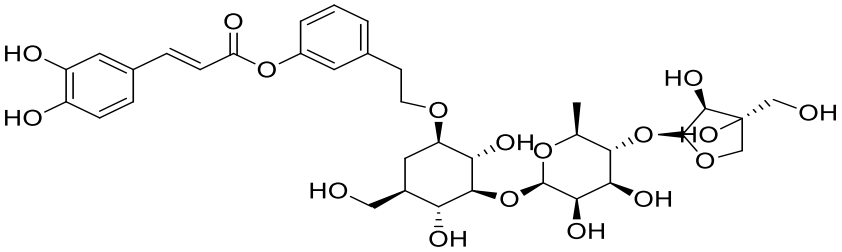
Figure 3: Structures of compounds from the root of *N laevis* mentioned in the text

It has been reported that various portions of the plant were harnessed for secondary metabolites which were detected to contain therapeutic properties like "antioxidant, antimalarial, trypanocidal, antimicrobial, anthelmintic, analgesic, anti-inflammatory, antidiabetic, antiarthritic, anti-thrombotic, cytoprotective, anti-hypertensive, central nervous system modulatory, male reproduction enhancing and oxytocic properties" (Okagu et al., 2022 p. 52).

Ethanol extract of the leaves of *Newbouldia laevis* produced two novel caffeic acid glycosides, that is, Newboulasides A (2.4) and B (2.5). These two compounds were obtained from the extract after a series of purification and spectroscopic analysis (Mbagwu et al., 2020). Structures of the above-mentioned new compound are shown in Fig. 4



2.4. Newboulasides A



2.5. Newboulasides B

Figure 4: Structures the novel compounds from the leaves of *N. laevis*

When methanol was used to extract the bark of the root of *N. laevis*, 10 secondary metabolites were isolated and identified as "chrysoeriol(2.6), newbouldiaquinone (2.7), 2-acetylfuro-1,4-naphthoquinone(2.8), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (2.9), lapachol (2.10), beta-sitosterol-3-O-beta-D-glucopyranoside (2.11), oleanolic acid (2.12), canthic acid (2.13) newbouldiamide (2.14) and 2-(4-hydroxyphenyl)-ethyltrioctanoate (2.15), were tested for in vitro antimicrobial activity" (Kuete et al., 2007, p.552).

Figure 5 shows the structures of the compounds mentioned in the text.

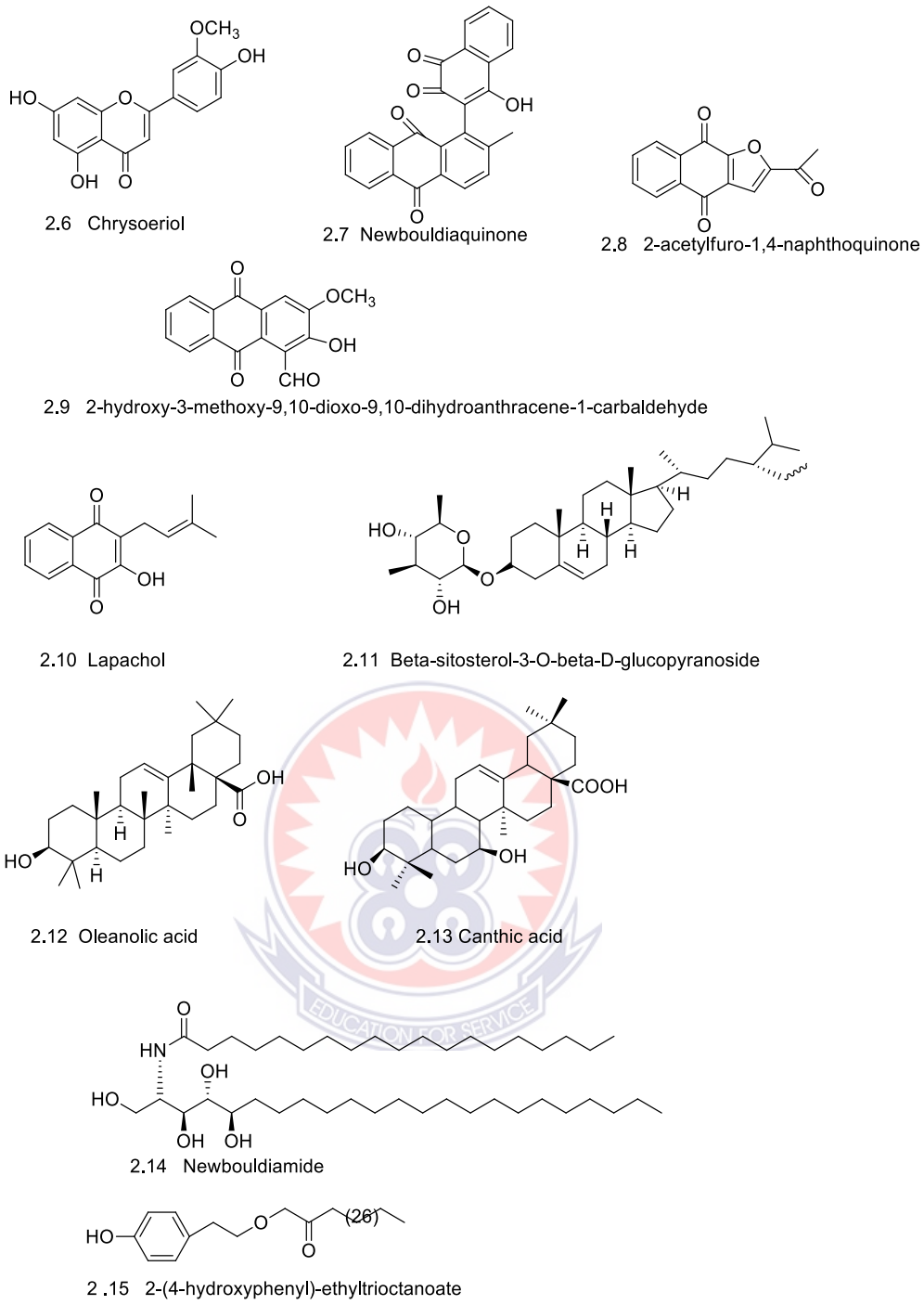


Figure 5: Structures of compounds from the root bark of *N. laevis*

2.4 Extraction Methods of Natural Products

The first and most important step in the examination of medicinal plants is extraction since it is vital to separate and characterize the desired chemical components from the plant materials (Sasidharan et. al., 2011). Even after significant advancements in

extraction and separation methods, isolating natural compounds remains a difficult undertaking. However, isolated, highly pure compounds will be required in order to obtain the complete chemical structures of compounds, including their stereochemistry. The purification step is essential to excluding chemicals that may interfere in the biological activity of the compound. Furthermore, isolated chemicals with verified purity serve as the basis for a major portion of the reference standards for quality control of herbal medicinal plants and herbal medications. The identification, collection, and processing of the biological material typically by drying are the first steps in the traditional method of natural product isolation. This is followed by extraction using various solvents ranging in polarity from low to high (Bucar et. al., 2013).

> Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency (Zhang et al., 2018, pp. 1-2).

Low toxicity, ease of vaporization at low temperatures, and the inability to agglomerate or break down during the extraction process are all necessary characteristics of an effective solvent. The most frequently utilized solvents include acetone, chloroform, methylene chloride, ethanol, methanol, and diethyl ether, or a combination of several of them. Chloroform, methylene chloride, and diethyl ether could be selected as suitable solvents for components that are moderately polar to non-polar; nevertheless, ethanol, methanol, and acetone are typically utilized for the extraction of compounds with greater polar qualities (Zhang et. al., 2015).

The most commonly used extraction methods include: microwave-assisted extraction, Soxhlet extraction and maceration method.

a. Microwave assisted extraction:

The microwave aided extraction technique is a productive and contemporary technology with a variety of advantages. Benefits include lower costs, quicker extraction times, less solvent consumption, less energy utilization, and fewer CO_2 emissions (Akhtar et al., 2019). In microwave assisted extraction (MAE), the desired elements are extracted from the sample by heating the solvents in contact with it. This approach saves a great deal of time and effort because it allows us to separate the analytes in a matter of minutes. This approach of subjecting plant material to microwave irradiation enhances the recovery of secondary metabolites during extraction. Utilizing microwave heating for plant matrices regarding extraction of plant samples, is heavily influenced by the characteristics of the matrix and solvent. The chosen solvent typically has a high dielectric constant, which allows it to absorb microwave energy very strongly (Veggi et al., 2012).

To stop the breakdown of thermolabile compounds, it is especially helpful to heat only the sample matrix in certain situations, releasing the solutes into a cool solvent. When heat is applied to the plant material, it forces the liquid in the material to evaporate, which may cause the plasma membranes and/or cell walls to burst. This action increases the rate of diffusion of metabolites into the solvent for efficient extraction of from the cytoplasm. (Destandau et al., 2013).

b. Soxhlet extraction

Continuous hot extraction is another name for the Soxhlet extraction technique. The equipment for the extraction process is composed of glass. The various components of

the extractor are “round-bottomed flask, extraction chamber, siphon tube, and condenser at the top”. The pulverized sample is placed in a perforated bag. The bag is actually made up of a very strong filter paper. The solvent is added into the flat-bottomed flask. Heat is then applied to the bottom of the flask which causes the solvent to evaporate and condense in the extraction chamber to extract the metabolite from the plant material. Consequently, when the solvent level in the flask reaches the brim, the solvent containing the metabolite is siphoned back into the round bottomed flask to repeat the whole process until all of the metabolites are efficiently extracted (Abubakar & Haque, 2020, p.4). However, this process is not appropriate for metabolites that degenerate with heat. The advantage of this method is that a small amount of solvent is required to extract a lot of plant materials. It is also suitable for samples that can withstand heat (Zhang et al., 2018).

c. Maceration extraction method

It is an extraction technique that uses pulverized material, which could be of the stem, foliage or even the root. This material is transferred into a container, followed by the addition of the solvent or menstruum until the plant sample is completely covered. The container is tightly covered or sealed. The content is agitated from time to time to enhance efficient extraction. The next stage involves separating the content from the marc. The separation can be done using filtration. This method of maceration is good for substances that degenerate in the presence of heat, thus thermolabile substances. The filtrate from the extraction process is then concentrated (Abubakar & Haque, 2020).

After extraction processes, to acquire the active fraction or pure natural products, additional refinement and isolation techniques are required due to the complex and

diverse nature of secondary metabolites present in extracts that are obtained through the aforementioned extraction procedures. The difference in the physical or chemical nature of each unique natural substance determines the method of separation. Chromatographic technique is the primary technique used to separate pure natural compounds from a complicated mixture, particularly column chromatography (Zhang et. al, 2018).

2.5 Characterization of Natural Product

The identification of compounds from extracts of medicinal plants uses a variety of techniques. Among the methods employed are "infrared (IR), mass spectrometry (MS), ultraviolet (UV) and nuclear magnetic resonance (NMR) spectroscopy" (Rehman et. al., 2020, p.8). It includes finding the functional group, finding several bonds and rings, arranging the hydrogen and carbon, and fully elucidating the structure. The fundamental principle of spectroscopy involves exposing electromagnetic radiation to an organic material, which then absorbs part of the light according to its various vibrational modes. A spectrum can be created by measuring the quantity of electromagnetic energy absorbed. The spectra are particular to bonds in a compound. These spectra can be used to determine the natural compound's structure (Boughendjioua & Boughendjioua, 2017).

2.5.1 Infrared (IR) Spectroscopy

Infrared spectroscopy involves exposing an organic compound to Infrared radiation. The technique is used to evaluate the functional groups that are present in a chemical compound. Understanding a compound's functional group can assist define its physical and chemical characteristics? Through this method, single, double, and multiple bonds are also detected (Abubakar & Haque, 2020).

One of the most widely used technique for functional groups determination is Fourier transform infrared spectroscopy (FR-IR). The frequencies at which IR light is absorbed by a sample are correlated with the vibrations of particular chemical bonds inside the molecule. The amount of energy absorbed at each frequency (or wavelength) can be found by examining the transmitted light, which produces a spectrum of transmittance with respect to incident frequency of infrared light. A typical conventional infrared equipment typically records spectra between 4000 cm^{-1} and 400cm^{-1}. A sample molecule's unique transmittance (or absorption) patterns in the IR spectrum aid in determining if a certain functional group is present in the sample or not (Nalla et al., 2018). The unknown functional groups in a sample can be identified by comparing its infrared spectrum to a database or library of spectra of recognized compounds. This method of using computerized spectrum data bases and digitized spectra is used in research and has important applications in a number of areas, including molecular biology, biochemistry, medicinal chemistry, toxicity assessment, biochemistry, and chemical nomenclature (Boughendjioua & Boughendjioua, 2017).

2.5.2 Mass Spectroscopy

At the age of almost a century, Mass spectroscopy started to play a crucial role in the discovery of natural products. McLafferty, Budzikiewicz, Djerassi, Hanus, and numerous others utilized electron ionization as the principal method of ion formation for mass analysis. More recently, quick characterization of natural products has been made possible by the precise molecular formula determination made possible by the combination of precise mass analyzers and soft ionization techniques with high resolution. (Přichystal et al., 2016). Nonetheless, the chemical characteristics of natural products offer a useful set of compounds that require analysis and testing using mass spectrometry methods. The molecular structures of numerous natural products have

been made known by the use of molecular formulae in conjunction with fragmentation pattern. (Jarmusch & Cooks, 2014). An organic molecule is first subjected to an electron bombardment, which transforms it into extremely energetic charged ions. A 70-eV electron ionization energy is used to detect the signal. Additionally, sample spectra are detected and recorded as a percentage peak. The Molecular weight and relative molecular mass are used to identify compounds.by plotting the mass of the fragmented ions against their charges of the individual ions (Abubakar & Haque, 2020). Major technical advancements in mass spectrometry (MS) have led to improvements in ionization methods as well as mass resolution and accuracy. Mass spectrometers have been gradually modified for routine use, with simpler instructions for instrument operation and maintenance, thereby opening up this analytical tool to a wider community of scientists. The technique has been adopted for routine laboratory use by natural product scientists due to its simplicity, sensitivity, and durability, as well as the relatively affordable instrumentation prices (Bouslimani et al., 2014).

2.5.3 Gas Chromatography

Gas chromatography (GC) is a common and frequently used analytical technique. It functions in identifying the chemical constituents of a sample. It also separates the constituents and gives the proportions in which they occur. The substance for analysis is turned into vapour for the success of the process of analysis. For that matter, the components should be stable to heat due to the high temperature exposure, to prevent degradation in the system. The mobile phase that transports the sample of vapour is a carrier gas as the name GC suggests. The carrier gas conveys the analyte through the system of the instrument without reacting with the analyte nor damaging parts of the instrument. The GC technique has a challenge in establishing accurately the structure of the separated compounds. A system for detection is therefore required. For this

reason, it is mostly hyphenated to the mass spectrometer to acquire the fingerprint of the various compounds (Stashenko & Martínez, 2014).

2.5.4 Ultraviolet Spectroscopy

An essential modern and progressing spectroscopic tool that has been used in sample analysis in the pharmaceutical industry for the past three decades is the UV spectroscopy. It operates by quantifying the monochromatic light taken by absorption. This occurs with compounds that are colourless within the wavelength of 200 nm to 400 nm which is also the path of near UV (Shinde et al., 2020). It is used to analyse both organic and inorganic samples. “It is used to determine identity, strength, quality and purity of several compounds” (Sudharshan & Swetha, 2023, p. 1344).

Actually, the operation is about absorption of light by the sample. The energy excites the electrons in the compound thus electrons are made to move to the excite state (Verma & Mishira, 2018). The method determines if the system has conjugated pi electrons or not. Beer- Lambert's law can be used to quantify the absorbance at a particular wavelength in order to determine the concentration of the analyte solution. It helps when analytes such as phytochemicals are Characterized using the optical properties of the substance (Banu & Cathrine, 2015).

2.5.5 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and indispensable tool for determining structure. It is based on the fact that distinct nuclei within a molecule will resonate at different frequencies because they are not experiencing the same magnetic field. The nuclei in the sample absorb and release radio waves based on which other atoms or groups are connected to them. A detector picks up this wave and shows the energy absorbed as a spectrum in proportion to a property called chemical shift

(Baranac-Stojanovic, 2014). This method focuses more on the physical characteristics of the bioactive molecule, such as the quantity and distribution of carbon atoms, the presence of hydrogen atoms, protons, and carbon isotopes. It also explained the arrangement of atoms within molecules (Abubakar & Haque, 2020). Hydrogen-1 and Carbon-13 NMR spectroscopy is routinely used for the structure elucidation of newly synthesized natural products. While only about 10% of carbon atoms are isotopes of carbon-13, the majority of hydrogen atoms are hydrogen-1 isotopes. This implies that results from hydrogen-1 spectra are more distinct and clearer. Furthermore, structure elucidation can be accomplished through the use of ^{19}F, ^{15}N, and ^{31}P NMR spstroscopy (Holzgrabe, 2010). Numerous uses for two-dimensional NMR spectroscopy exist, such as molecule identification and structural clarification. By expanding the peaks into a second dimension, 2D NMR can be used to solve the issue of overlapping resonances in metabolomics. Potentially more metabolites can be detected and identified using 2D-NMR's higher resolution than with 1D-NMR. "Diffusion Ordered Spectroscopy (DOSY) and two-dimensional J-resolved NMR spectroscopy (J-Res) are examples of other 2D NMR experiments that have also been used in several NMR-based metabolomics studies" (Emwas et al., 2019, p. 12).

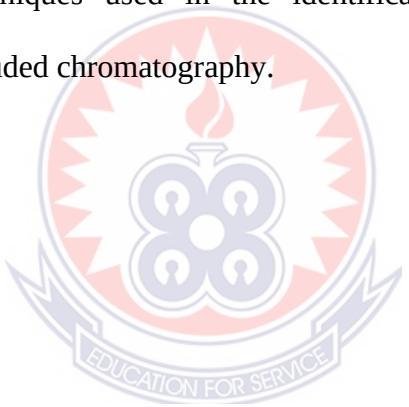
2.6 Summary of Literature Review

This study's literature review covered the following thematic areas: Description of the Newbouldia laevis plant; scientific classification of *Newbouldia laevis*; Extraction and analysis of its chemical components; Identification and characterization; and Common health and medical uses of *Newbouldia laevis* in West Africa.

This review primarily focused on the search for new bioactive compounds in the face of the increasing emergence of novel diseases and drug-resistant microorganisms. It has

been revealed in this review that bioactive compounds are obtained either as primary or secondary metabolites. Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separation and determination still create problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound(s).

Extraction protocols of natural products depending on the source and nature of the target compounds were not left out in this review. Ethyl acetate has been noted to be one of widely used solvent for the extraction of natural products. Finally, this review considered some techniques used in the identification and characterization of compounds which included chromatography.



CHAPTER THREE

METHODOLOGY

3.0 Overview

This section of the research's primary focus is on the study area, steps and techniques employed to carry out the investigation. The following subheadings make up its division: materials, sample collection and pretreatment, maceration, extraction, phytochemical component screening. Also, a gas chromatography examination was performed to pinpoint the precise components that were present in the extracts. Furthermore, the antimicrobial activity test, list of test species of organisms utilized, microwell dilution, determination of minimum bactericidal (MBC) and minimum fungicidal concentration, and free radical scavenging activity were other subdivisions under which the bioactivity test was conducted.

3.1 The Sample Collection Area

The samples were picked for the study from Dambai in the of Oti Region of Ghana. "Dambai is situated in the area where the Moist Semi- Deciduous Forest and the Northern Savannah meet. Savannah grassland, which makes up almost 75% of the municipality, is distinguished by short, drought-resistant trees, such as shea, dawadawa, and others. It lies between latitudes 8º 3′ 54″N and longitudes 0º 10′ 49″E. Dambai is within the Krachi East Municipality of the Oti Region and bounded to the North by the Volta River" (Kombonah ,2025, p.3).

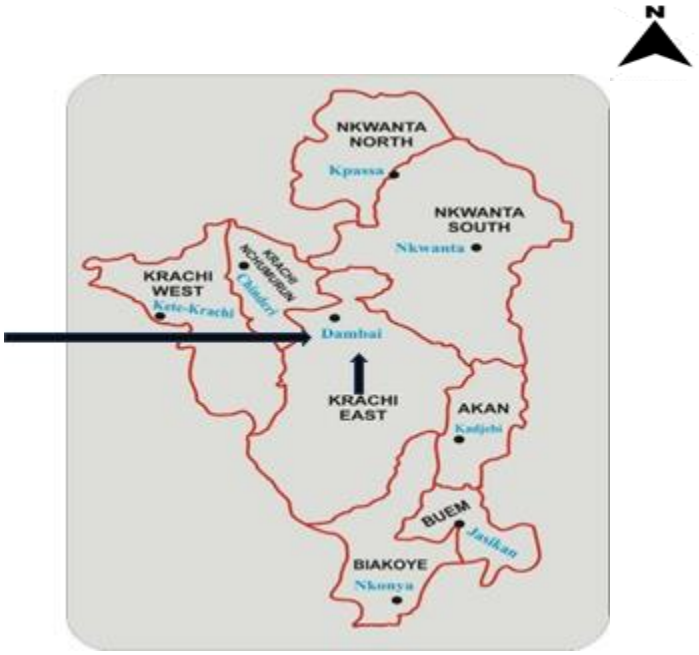


Figure 6: Map of Krachi-East Municipality in Oti Region showing Dambai

Source: https://www.bing.com/images/search?q=Map%20of%20Oti%20Region&first1

3.2 Materials

The bark of the plant (*Newbouldia laevis*) was collected in June 2022 from Dambai-Zongo of the Krachi-East Municipality in the Oti Region with the following coordinates 8°03’58.2”N 0°10’46.1”E, Ghana. The botanical authentication of the plant sample was done by Mr. David Yentumi Affram, a botanist in the Department of Science, Dambai College of Education. The plant samples were air-dried and ground into powder using an electric blender.

Reagents and chemicals used in this study were purchased from Merck, Ghana and all organic solvents were redistilled and dried according to standard procedures before being used. Petri-dish™ and Whatman No 41 filter papers were purchased from the market. in Ghana. Analytical balance, Winchester bottles, beakers were obtained from the University of Education, Winneba laboratory.

GC-MS data were recorded on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column. He (2 mL min^{-1}) was used as a carrier gas and ethyl acetate was used to dissolve the sample. The injector was kept at 250 ºC and the transfer line at 280 ºC. The column temperature was held at 50 ºC for 2 min, and then ramped to 280 ºC at 20 ºC min^{-1} where it was held for 15 min. The MS was operated in the EI mode at 70 eV.

3.3 Sample collection and pretreatment

The stem bark samples of the full grown *Newbouldia laevis* plant were collected at settlement from Dambai-Zongo of the Krachi-East Municipality in the Oti Region, Ghana. Cutlass was used to harvest the bark of the stem of only one plant. The stem bark of the *Newbouldia laevis* were washed with water and dried under shade for four weeks and then pulverized by pounding with mortar and pestle. Figure 7 shows pictures of the stages of harvesting and shade drying of the stem bark of *N. laevis.*

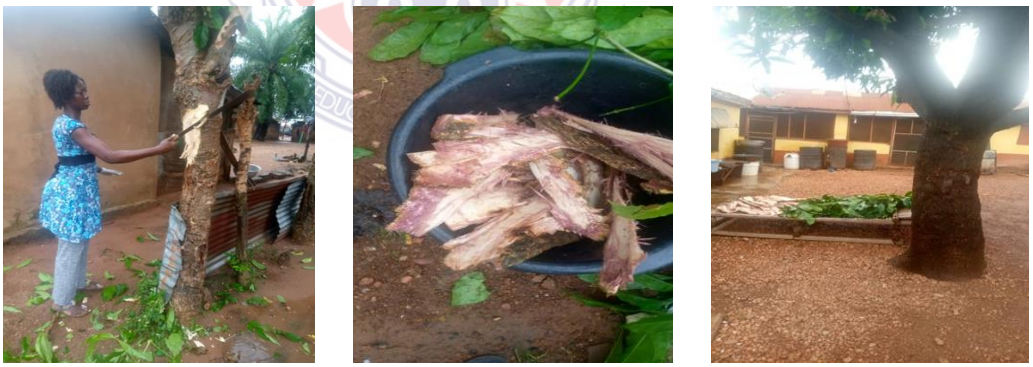


Figure 7: Images showing the stages of harvesting and shade drying N. laevis stem bark

3.4 Methodology

3.4.1 Extraction by Maceration

The air-dried samples were crushed and blended into powder using a mortar and pestle. The dry mass obtained was 500 g.

Maceration method was employed for the extraction process. A total of 200 g of the powdered sample was placed in a flat-bottom flask, followed by the addition of 500 mL of pure ethyl acetate. The flask's mouth was sealed to prevent solvent evaporation, and the mixture was left to stand for 24 hours. Afterward, the contents were filtered using Whatman™ No. 41 filter paper. This procedure was repeated twice, and the combined filtrates were concentrated using a rotary evaporator, figure 8 below. The crude extract obtained weighed 18.33 g, yielding 9.17 % of the product. Figure 8 shows the stages of the work up in the laboratory.

Figure 8: Images of the stages of filtration and rotary evaporation

3.5 Determination of Antioxidant Activities

3.5.1 ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic) acid) Scavenging Activity

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid) free radical scavenging activity of the extract was examined according to the method previously described (Sebastian et al., 2014; Re et al., 1999) with slight modification. The ABTS scavenging activity was determined by mixing 10 mL each of ABTS and 2.4 mM potassium persulphate to generate the ABTS free radical. This stock solution was

further diluted in 50 mL of methanol as a working solution. A 150 µL of the solution was added to 50 µL of the prepared extract concentrations (0.0625- 2.0 mg/mL), vortexed and incubated at 30 ^{o}C for 30 minutes. The absorbance of each concentration was recorded at 734 nm using the UV spectrophotometer (Jenway, Bibby Scientific Ltd, Staff, UK) for control ABTS (Ao) and the test samples (A). The experimental protocol was repeated for ascorbic acid with a concentration range of (0.0625- 2.0 mg/mL). The experimental procedure was performed in triplicate. The free radical scavenging activity for the extract against ABTS was therefore evaluated by inputting data into the relation:

$$\% \text{ ABTS scavenging activity} = [\frac{(\text{Ao}) - (\text{A})}{(\text{Ao})}] \times 100$$

The IC_{50} (mg/mL) of the extract was further deduced by employing the Microsoft excel using Nonlinear regression (curve fit) with the Log (Inhibitor) vs response (three parameters).

3.5.2 DPPH (1,1-diphenylpicrylhydrazyl) free radical scavenging Potential Assay Activity

The DPPH (1,1-diphenylpicrylhydrazyl) free radical scavenging activity of the isolated compound was examined according to the method previously described (Gyamfi et al., 2006) with slight modification. A 10 mL of 0.1 mM solution of DPPH was prepared by dissolving 394 µg in 10 mL of ethanol as a working solution. An aliquot (0.5 mL) of the freshly prepared solution was added to 1 mL of the extract with concentrations of (0.0625- 2.0 mg/mL) mg/mL. The mixtures were vortexed and incubated at 30 ^{o}C for 30 minutes. The absorbances were recorded at 517 nm using the UV spectrophotometer (Jenway, Bibby Scientific Ltd, Staff, UK). The procedure was repeated for the reference standard (ascorbic acid) with concentrations of (0.0625- 2.0

mg/mL). The experimental procedure was performed in triplicates. The free radical scavenging activity for the compound against DPPH was evaluated by inputting the measured absorbance data into the relation below:

$$\% \text{ Scavenging activity} = [\frac{(\text{Ao}) - (\text{A})}{(\text{Ao})}] \times 100$$

where A is the absorbance of the extract and A_O is the absorbance of DPPH solution. The IC_{50} (mg/mL) of the extract was further deduced by employing the Microsoft excel using Nonlinear regression (curve fit) with the Log (Inhibitor) vs response (three parameters).

3.6 Test Organisms Used

The test microorganisms involving *Staphylococcus aureus* (NCTC 29212), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae (NCTC 13440)*, *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (ATCC14028), *Candida albicans* (ATCC 90028), *Aspegillus niger* (clinical) and *Phytophthora megakarya* (clinical) used in this study were obtained from the Microbiology Laboratory, Department of Basic Sciences, School of Basic and Biomedical Sciences, UHAS on the basis of its implication in most infections.

3.6.1 Microbial Culture Preparation

Pure microbial cultures of both standard and clinical strains were sub-cultured onto nutrient agar and potato dextrose agar, respectively, and incubated at 37 ^{0}C overnight. Next, pure isolates of the cultures were inoculated into physiological saline and using a spectrophotometer, a desired standard cell density of 1.8 x 10^8 CFU were obtained using 0.5 McFarland turbidity standard prepared by adding 0.05 mL of 1.175 % of Barium chloride dihydrate with 9.95 mL of 1 % Sulphuric acid.

3.7 Antimicrobial Activity Determination

The antimicrobial activity of the extracts were determined using both the Kirby-Bauer agar well diffusion method (Neglo et al., 2022; Eloff, 1998) and the broth micro-dilution method (Bonifácio et al., 2019).

3.7.1 Well Diffusion Method

20 mL of sterile Muller-Hinton agar was poured and allowed to set, then, inoculated with a drop of the test microorganisms of 1x 10^6 colony forming units (CFU)/mL. Each of the strains were cultured overnight at 37°C in Muller-Hinton broth with further dilution to 0.5 McFarland standards with saline and then inoculated on Muller-Hinton agar. Five wells were bored in each plate using a cork borer (No.3, 5 mm). Three of these wells were filled with 70 ul of 100 mg/mL of the extract (*N. laevis*). 20% DMSO was used as a negative control. Whereas, Tetracycline (30µg/disc) and Nystatin (100 µg/disc) were used as positive control for the bacteria and *fungi* inoculum respectively. Each of the extracts were then allowed to diffuse for 15 mins at room temperature after which they are incubated at 37 ^{O}C for 24/48 h and zones of inhibitions recorded. The procedure was performed in triplicates.

3.7.2 Broth Dilution Method

The Minimum inhibitory concentrations, MIC of the test extracts were carried out by micro broth dilution method using the 96 well microtiter plates per the protocol previously reported with slight modification (Kachkoul et al., 2021; Clinical and Laboratory Standards Institute, 2018; Bonifácio et al., 2011 Eloff, 1998). A 100 mg/mL stock solution of the extract prepared in DMSO. A two-fold serial dilution of this stock was prepared until 10 different concentrations were obtained. An aliquot of 100 µL of double strength Mueller Hinton broth (Oxoid Limited, United Kingdom) was dispensed

into each 96-well plate (Citotest Labware Manufacturing Co. Ltd, Jiangsu, China) and mixed with 100 uL of the extract to prepare well concentrations ranging from 50.0 –0.1 mg/mL. The wells 11 and 12 served as both positive control (Broth + organism only) and negative control (Broth with no organism) respectively for each microbial strain on each column. This was followed by the addition of 100 µL of each of the 0.5 McFarland standardized, test organisms after which the plates were subjected to incubation at 37 ^{o}C for 24-48 hours for bacterial and fungal strains respectively. The MIC values were then evaluated by visual analysis by adding tetrazolium chloride (TTC), 0.1% (w/v) dye after 10 minutes and the minimum inhibitory concentrations (MIC) recorded as the least concentration which did not change colour from Colourless/light yellow to red/pink.

3.7.3 Determination of Minimum Bactericidal (MBC) and Fungicidal Concentration (MFC)

In order to confirm if the extract would be able to kill the microbial cells (bacterial-/fungicidal effect), the MBC and MFC were determined. Aliquots from each well from susceptibility testing assays were transferred to plates containing Nutrient agar and then incubated for 24 - 48 hours at 37 ^{o}C. The plates were then checked for the presence or absence of growth in the Nutrient agar or Sabouraud Dextrose Agar (SDA) (Nester et al., 2004).

3.8 Data Analysis

Microsoft Excel Version 2010 was used for the data collection and calculation of mean and SEM (Standard Error of Mean) estimates in the tables. Mean+SEM and IC_{50} values were compared with the standard.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Overview

This chapter presents the findings obtained from the study and also the discussion based on the results.

4.1 PERCENTAGE YIELD OF EXTRACT

200 g of the powdered stem bark of the *Newboudia laevis* gave 18.33g of the crude extract from cold maceration using ethyl acetate as solvent. This represents a percentage yield of 9.17 %. The ethyl acetate solvent is less toxic and environmentally friendly. It has medium polarity can extract a range of compounds (Piotrowski & Kubica ,2021).

4.2 ANTIMICROBIAL ACTIVITY DETERMINATION

4.2.1 Results and Discussion of antimicrobial study

Table 1 presents the antimicrobial activity of *Newbouldia laevis* against eight microorganisms, measured by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC).

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Organism	MIC/mg/ml	MBC, MFC/ mg/ml	MBC,MFC/ MIC	Activity
E.coli	12.5	25	2^{bc}	Bactericidal
K.pneumonia	3.125	3.125	1^{bc}	Bactericidal
S.aureus	25	25	1^{bc}	Bactericidal
S.typhi	12.5	25	2^{bc}	Bactericidal
P.aeroginosa	12.5	12.5	1^{bc}	Bactericidal
C.albicans	25	25	1^{fc}	Fungicidal
A.niger	0.078	1.56	20^{fs}	Fungistatic
P.megakarya	0.625	6.25	10^{fc}	Fungistatic

The extract was potent against all eight tested organisms at significant concentrations. This included five strains of both Gram-positive and Gram-negative bacteria and three fungal strains. The extract exhibited impressive bactericidal effects against all of the six tested bacteria. Fungistatic activity against *A. niger* and *P. megakarya*. was also demonstrated by the sample but fungicidal against *C. albicans.* An MBC/MIC ratio ≤ 4 indicates a bactericidal/fungicidal effect, whereas a ratio > 4 suggests a bacteriostatic/fungistatic effect (García-Vela et al., 2024). An antibacterial agent is considered bactericidal if it kills bacteria or bacteriostatic if it inhibits their growth (Ishak et al., 2025).

The extract exhibited strong antibacterial activity, mainly against *K. pneumonia, S. aureus*, and *P. aeruginosa*, which all have an MBC/MIC ratio of 1 indicating a strong

bactericidal effect. However, the lowest MIC of 3.125 mg/mL among these three was against *K. pneumonia*. This means that the extract was more potent against *K. pneumonia* than the other two. The bactericidal nature of the extract is also evident against *E. coli* and *S. typhii* with MBC/MIC ratios of 2 and MIC values of 12.5 mg/mL. This suggests that *Newbouldia laevis* has significant potential as an antibacterial agent, particularly against Gram-negative pathogens.

The extract also shows strong fungicidal activity against *C. albicans* with MBC to MIC ratio of 1. The lowest MIC value of 0.078 mg/mL more potent than that reported in literature was recorded against *A. niger* but was fungistatic with an MBC value of 1.56 mg/mL and an MBC to MIC ratio of 20 (Wei et al., 2022). Against *P. megakarya*, the MBC/MIC of 10, indicates a fungistatic effect rather than a fungicidal effect. It can be deduced that while the extract can inhibit the fungal growth, it may not completely eradicate these moulds at the tested concentrations.

4.3 Results and Discussion of ABT and DPPH study

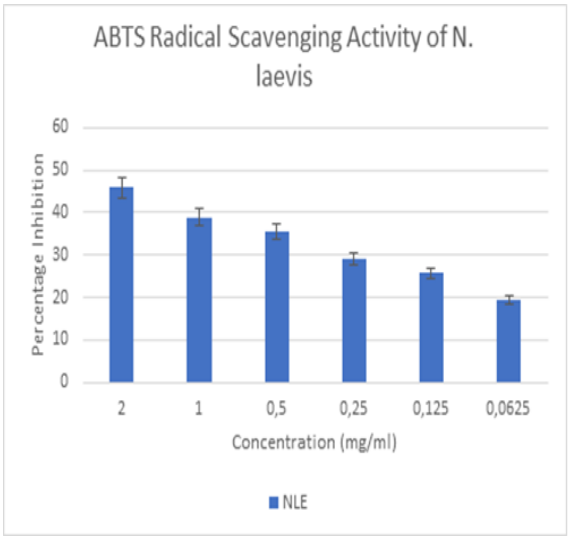
Table 1: Free Radical Savenging Activity of Newbouldia Laevis Extract

ABTS	IC_{50}=0.329mg/mL				DPPH	IC_{50} = 0.322 mg/mL		
Conc. mg/mL	Exp 1	Exp 2	Mean±SD	%ABTS	Expt 1	Expt 2	Mean±SD	%DPPH
2	0.171	0.19	0.1805±0.01	45.7958	0.365	0.395	0.38±0.02	62.78159
1	0.213	0.194	0.2035±0.01	38.88889	0.427	0.415	0.421±0.01	58.76592
0.5	0.225	0.204	0.2145±0.01	35.58559	0.434	0.414	0.424±0.01	58.47209
0.25	0.241	0.231	0.236±0.01	29.12913	0.444	0.441	0.4425±0.01	56.66014
0.125	0.257	0.238	0.2475±0.01	25.67568	0.452	0.459	0.4555±0.01	55.38688
0.0625	0.279	0.257	0.268±0.01	19.51952	0.452	0.467	0.4595±0.01	54.9951

From table 2 above, the ABTS Assay's IC_{50} is 0.329 mg/mL. while that for DPPH is 0.322. The low IC_{50} value indicates that the extract from *Newbouldia laevis* is

comparatively effective in scavenging ABTS radicals and DPPH radicals. The lower the IC_{50} value, the higher the antioxidant activity of sample (Tetteh et al., 2024).

A dose-dependent antioxidant response is demonstrated in both the DPPH and ABTS experiments, where percentage inhibition falls as extract concentration drops. Both assays show the lowest inhibition at 0.0625 mg/mL (19.52% for ABTS, 54.99% for DPPH) and the maximum at 2 mg/mL (45.80% for ABTS, 62.78% for DPPH). The low standard deviations indicate the reproducibility in the experimental runs (Poulos et al., 2020). Figure 9 show the bar chart of the ABTS assay conducted on the extract along Vitamin C as standard control.



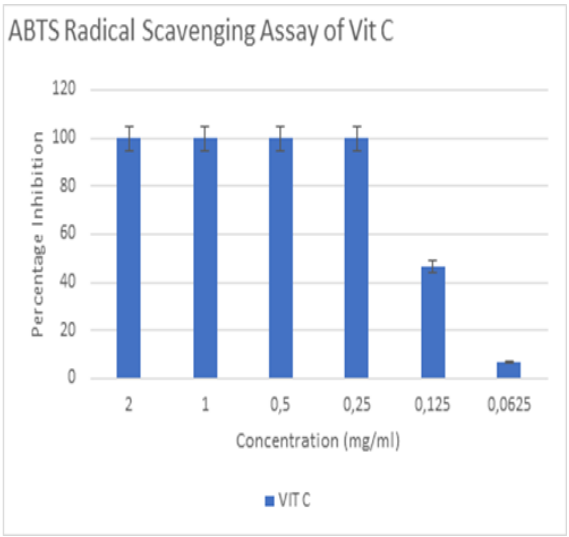


Figure 9: Bar chart showing ABTS scavenging activity of N. laevis and Vitamin C as control

The low IC_{50} value in the ABTS and DPPH assays in an indication of high antioxidant capability for *Newbouldia laevis*. This could be attributed to the fact that secondary metabolites were present in the extract with strong antioxidants capabilities. The DPPH assay primarily evaluates hydrophobic antioxidants, whereas the ABTS assay is frequently thought to be more sensitive to hydrophilic and lipophilic acids. A

comparison of the IC_{50} values of the extract and vitamin C in the ABTS assay is shown in figure 10.

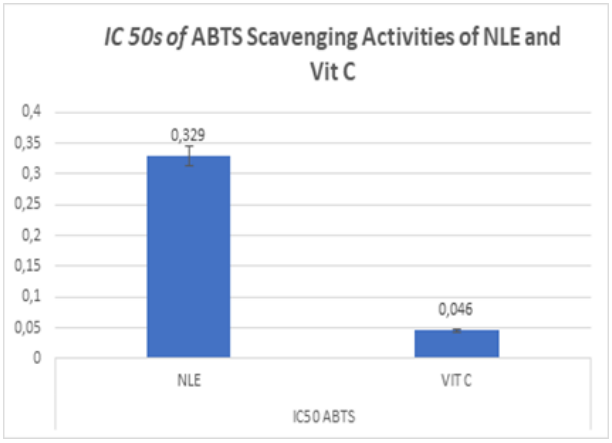


Figure 10: A comparison of the IC_{50} values of the crude extract and the control

Newbouldia laevis is known to contain flavonoids, phenolic acids, tannins, and alkaloids, which contribute to its antioxidant effects (Habu & Ibeh, 2015). Flavonoids and phenolics are particularly effective against ABTS radicals, which may explain the stronger response in that assay. Figure 11 below shows the bar chart of DPPH assay of the extract and vitamin C while figure 12 shows a comparison of the IC_{50} values of the crude extract and the vitamin C in the DPPH assay (Control).

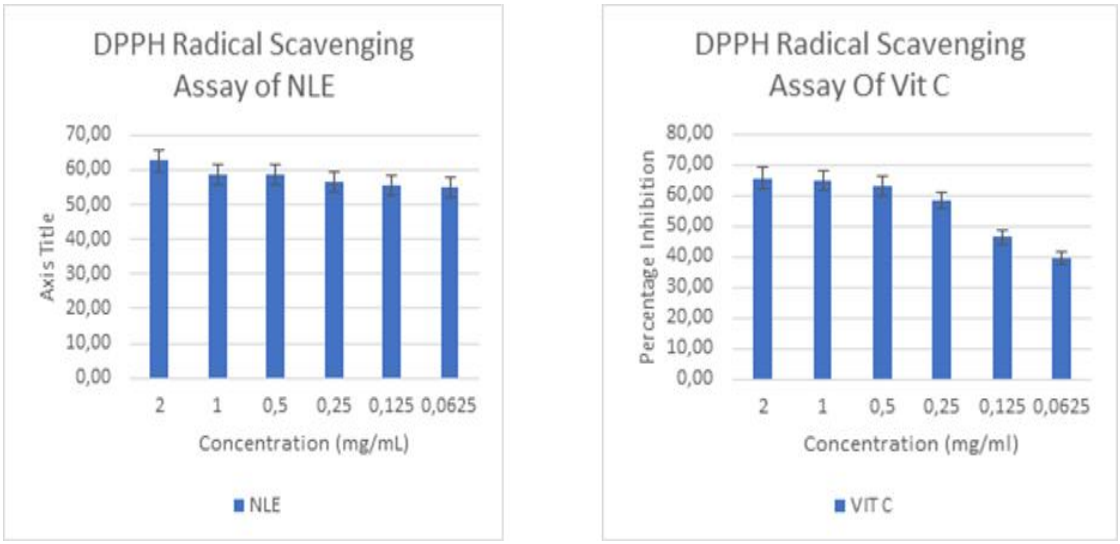


Figure 11: A bar chart showing a comparison of DPPH results of the crude extract and the control Vitamin C

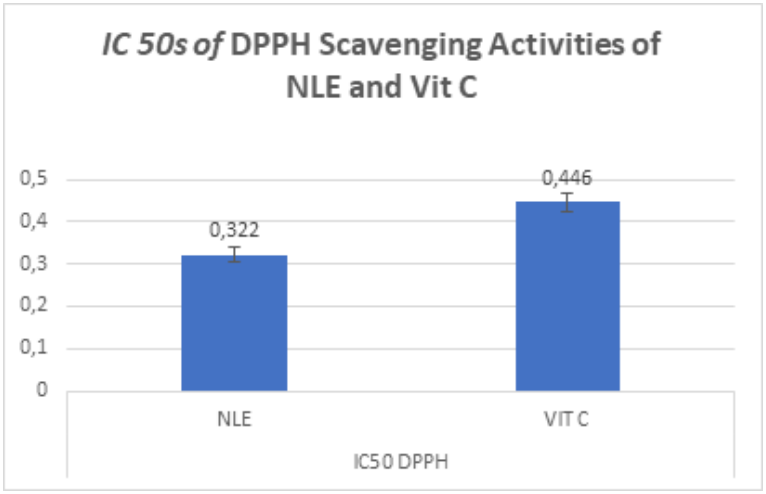


Figure 12: A bar chart showing the comparison of the IC50 values in the DPPH assay of the extract and the Vitamin C as control

The comparison of the IC50 values of the extract and the standard Vitamin C in figure 12 indicates that the *N. laevis* extract performed relatively better than Vitamin C in the DPPH free radicals scavenging activities shown by the lower IC50 value. This explains the high antioxidant activity in its use to treat oxidative stress conditions.

4.4 Phytochemical Screening/GC-MS Analysis

The GC-MS results revealed the presence of several compounds in the ethyl acetate crude extract. The table (3) below shows the compounds identified from the analysis of the GC-MS data. Appendix A and B show the structures of the compounds identified. Look for table 3 below for a list of compounds from the ethyl acetate extract of the stem bark of *Newbouldia laevis.*

Table 2: List of compounds identified from the ethyl acetate extract of the stem bark of Newbouldia laevis

S/N	Retention time (t_R)	Name of Compound	M/z
1	17.1	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278.3
2	18.6	1,2-Benzenedicarboxylic acid, dibutyl ester	278.5
3	22.7	9,12-Octadecadienoic acid (Z,Z)-	280.5
4	23.5	Octadecanoic acid	284.5
5	28.1	Eicosanoic acid	312.5
6	28.6	Hexanedioic acid, bis(2-ethylhexyl) ester	370.6
7	31.5	Bis(2-ethylhexyl) phthalate	390.6
8	35.6	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390.6
9	43.9	Stigmasta-5,22-dien-3-ol	412.7
10	44.9	Stigmast-5-en-3-ol, (3.beta.,24S)-	414.7
11	45.0	Stigmastan-3-ol, (3.beta.)-	416.7
12	45.4	Olean-12-en-3-ol, (3.beta.)-	426.7
13	45.6	3-Oxocholestane	386.7
14	45.7	5,6-Dihydroergosterol	398.7
15	46.8	Cholest-4-en-3-one	384.7
16	49.2	Card-20(22)-enolide, 3,14,16-trihydroxy-, (3.beta.,5.beta.,16.beta.)-	390.5
17	49.6	Androstan-3-one, 17-hydroxy-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-	304.5 324.4
18	52.6	Dodecanoic acid, 4-nitrophenyl ester	

The compounds as identified in the table above can be categorized into fatty acids, sterols and triterpenoids, phthalates and esters.

Fatty Acids: Linoleic acid (9, 12-Octadecadienoic acid (Z,Z)-, a polyunsaturated omega-6 fatty acid, is well-documented for its antimicrobial properties. Studies revealed that shown that it has significant activity against Gram-positive bacteria, such as *Staphylococcus aureus*, by disrupting the bacterial membrane (Desbois & Smith, 2010). The double bonds in its structure increase membrane fluidity, causing the

content of the cell to leak out and eventual death of cell (Lv et al., 2020). Linoleic acid, one of the most frequent constituents found in *A. Saralicum* has also shown antifungal and antioxidant activities, making it a key compound in antimicrobial formulations (Jalalvand et al., 2019).

Stearic acid (Octadecanoic acid), a saturated long-chain fatty acid, exhibits limited direct antimicrobial effects. However, its synergistic role when combined with other active compounds is noteworthy (Chaidir et al., 2020).

Eicosanic acid (arachidonic acid), a long-chain fatty acid is an endogenous metabolite that has a synergistic role when combined with other unsaturated fatty acids. The alveolar macrophages release Arachidonic acid into the alveolar fluid, exerting antimicrobial action and protecting the lungs from various infective organisms. It is also noteworthy that it activates macrophages and enhances their ability to generate free radicals that are tumoricidal. It has been found that Arachidonic acid is active against gram-positive and gram-negative bacteria and enveloped viruses including influenza. The antimicrobial action is by inducing leakage and lysis of the bacterial cell membrane (Das, 2018).

Sterols and Triterpenoids: Stigmasta-5, 22-dien-3-ol (Stigmasterol), belongs to tetracyclic triterpenes and is an unsaturated phytosterol, one of the most prevalent plant sterols found in vegetable fats and oils derived from numerous plants. Findings indicate strong pharmacological effects, including anti-inflammatory, anti-diabetic, immunomodulatory, anti-parasitic, antifungal, antibacterial, antioxidant, neuroprotective, anticancer, and anti-osteoarthritis effects (Bakrim et al., 2022).

Stigmast-5-en-3-ol, (3.beta. 24S)-. It is a phytosterol with a range of biological activities: it is a strong antidiabetic drug that regulates the transport, inhibits the growth of cancer cells, and modifies the membrane lipid profile (Ahmed et al., 2021).

Olean-12-en-3-ol, (3.beta.)-, an Oleanane triterpenoids like this one, are widely studied for its antimicrobial properties. It is endowed with a range of biological activities: antibacterial, antifungal, antiviral and anti-carcinogenic (Castellano et al., 2022). Oleanolic acid derivatives have therapeutic effect against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* (Zhou et al., 2020). The antimicrobial action is attributed to the inhibition of bacterial enzymes and disruption of membrane (Kim et al., 2015).

5,6-Dihydroergosterol, as a sterol derivative, this compound exhibits antifungal activity by disrupting fungal cell membranes, particularly in pathogenic species like *Candida albicans*. It acts by reducing membrane fluidity, thereby impairing nutrient transport and enzyme activity (Hassan et al., 2022).

Cholest-4-en-3-one, a crucial intermediate in a lot of steroid transformations. According to studies, it works well against keratinization, liver disease, and obesity. An evaluation of cholest-4-en-3-one functional impact in human cells reveals that it is produced by cholesterol oxidation, restraining cell migration. Additionally, Cholest-4-en-3-one can be used as a precursor to create various pharmacological intermediates, including Androst-4-ene-3, 17-dione and Androsta-1,4-diene-3,17-dione, which are important building blocks for the production of anabolic drugs and hormones used in contraception (Wu et al., 2015).

No infornation was found on Androstan-3-one, 17-hydroxy-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-. Meanwhile, its derivative Androstan-3-one,17-hydroxy-2-methyl-,(2.beta.,5.beta,17.beta.)-, a metabolite extracted from African nutmeg, is a plant steroid with antioxidant properties and anti-sickling potentials. The antioxidant protective effect works against free radicals in sickled erythrocytes. The docking studies revealed that it has the "ability to allosterically bind to haemoglobin with good binding affinity and support their ability to with good binding affinity and support their tendency to alter oxygen binding affinity of the haemoglobin to suppress the occurrence of a vaso-occlusive episode by reducing abnormal adherence of sickle erythrocytes to vascular endothelial cells "(Erukainure et al., 2018, pp.464-465).

3-Oxocholestane, also cholestan-3-one, is one of the steroids extracted from the green algae *Ulva fasciata*. The antibacterial activity was determined by the Broth Dilution Methods against clinical Gram-negative and Gram-positive bacteria. The outcomes presented activity against bacteria that are Gram-positive: *S. aureus, Bacillus cereus, and B. subtilis* (Motallebi, 2020).

Stigmastan-3-ol, (3. beta.)- also known as Stigmastan-3-ol, is a phytosterol. The juice of L. siceraria contains stigmastan-3-ol, which lowers serum cholesterol and lowers the death rate from cardiac artery disease and depression. Elevated levels of cholesterol in the plasma, are the main source of hypercholesterolemia, which may be the results of obesity and diabetes. In humans as well as most animals, when HMG-CoA reductase is inhibited, it lowers the level of cholesterol effectively by inducing sterol regulatory element-binding protein-2, which raises the HMG-CoA reductase and LDL receptor (Kanwal et al., 2023).

Phthalates And Esters: 1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester is a secondary metabolite often reported in extracts from fungi and plants. It has antimicrobial, and hypoglycemic effects and acts as an α-glucosidase inhibitor (Adeyemo et al., 2024).

1,2-Benzenedicarboxylic acid, dibutyl ester also Dibutyl phthalate, is a bioactive compound isolated from the stem of the plant *Ipomoea carnea*. It showed activity against *Klebseilla pneumonia*, *Pseudomonas aeruginosa*, *Proteu mirabilis*. It also inhibited the growth of human malaria parasites. (Obi & Okwute, 2023). This confirms the strong antimicrobial activity exhibited by the extract against *K. pneumonia* shown in table 1 and also confirms the use of *N laevis* in the treatment of pneumonia as reported by (Rashed, 2021).

Hexanedioic acid, bis(2-ethylhexyl) ester, a main secondary metabolite in the methanolic flower extract of *Bergenia ciliata*, exerted anti-cancer, anti-inflammatory, diuretic and anti-diabetic effects (Iraqi et al., 2025).

Dodecanoic acid, 4-nitrophenyl esters also known as 4-nitrophenyl laurate are known for their antimicrobial properties. The metabolite identified in the methanolic extract of *Ipomoea pescaprae*, showed antibacterial effect (Sujatha et al., 2020).

Bis(2-ethylhexyl) phthalate is a bioactive metabolite isolated and purified is antimicrobial, antitumor, anti-leukemic and it is able to fight microorganisms that cause diseases. Also, BEHP was investigated for larvicidal activity and displayed very high mortality in *C. quinquefasciatus* larvae when exposed after 72 hours. The mortality was directly proportional the duration of exposure the concentration of dose (Javed et al., 2022). Also, when Bis(2-ethylhexyl) phthalate was extracted from a Culture, it tested

active against a lot of Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus equosemens*. The compound also has strong antifungal effect against *C. albicans*. The cytotoxic activity of the isolated bioactive compound was assessed against a range of human cancer cells. Strong cytotoxicity was demonstrated against human breast and colon cancer cells, although moderate cytotoxicity was shown against human liver cancer cells (El-Sayed, 2012). On the other hand, this supports the strong bactericidal and fungicidal activities of the *N. laevis* extract against *S. aureus* and *C albicans* respectively as shown in Table 1.

1, 4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester also known as di(2-ethylhexyl) terephthalate or bis-2-ethylhexyl terephthalate, production was revealed in the study of extracts from *Ziziphus lotus* leaves. It is a plasticizer that is" used to confer elasticity and flexibility to various fiber and plastic products. Many studies have identified its presence in medicinal plants, which most often exhibited antimicrobial activities" (Ghazi-Yaker et al., 2024, p. 16).

CHAPTER FIVE

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.0 Overview

This chapter summarizes the key findings of the research, draws conclusions based on the analysis of the data, and presents recommendations for future studies and practical applications. This research aimed to extract, isolate, and characterize active secondary metabolites from the stem bark of *Newbouldia laevis* and evaluate their antimicrobial and antioxidant activities. The study also explored the bioactive properties of these metabolites and their potential applications in medicine.

5.1 Summary of Findings

The research began with the collection and pretreatment of *Newbouldia laevis* stem bark from Dambai-Zongo in the Oti Region of Ghana. The bark was subjected to extraction via the maceration method using ethyl acetate, followed by concentration and characterization using chromatographic techniques. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed a range of compounds, including fatty acids, sterols, triterpenoids, phthalates, and esters. The compounds identified were found to have varying degrees of antimicrobial and antioxidant activities.

The antimicrobial activity was assessed using the Kirby-Bauer agar diffusion method and broth microdilution assays. The extracts exhibited significant antimicrobial properties against a range of bacterial and fungal pathogens, with the most notable effects observed against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. However, the extracts showed limited activity against, *Aspergillus niger*, and *Phytophthora megakarya*.

The antioxidant activities of the extracts were measured using DPPH and ABTS scavenging assays. The results showed moderate to strong antioxidant potential, with the ethyl acetate extract exhibiting IC_{50} values comparable to the reference standard, vitamin C. The extract demonstrated effective free radical scavenging abilities, indicating its potential as a natural antioxidant source.

5.2 Conclusions

The study successfully extracted and characterized several bioactive compounds from the stem bark of *Newbouldia laevis*. The identified compounds, including linoleic acid, stigmasterol, and oleanolic acid, have been shown in the literature to possess various biological activities, particularly antimicrobial and antioxidant properties. The findings confirm that *Newbouldia laevis* has considerable medicinal potential, especially for treating microbial infections and mitigating oxidative stress-related diseases.

The antimicrobial activity of the extract indicates that *Newbouldia laevis* could be a valuable source of natural antimicrobial agents, especially considering the growing concern over antibiotic resistance. Additionally, the antioxidant properties of the extract further support its use in traditional medicine, where it is already employed for a variety of health conditions.

5.3 Recommendations

Further Pharmacological Studies

Further studies are recommended to evaluate the pharmacokinetics, bioavailability, and safety profile of the active compounds from *Newbouldia laevis*. Clinical trials and toxicological assessments should be conducted to validate the therapeutic potential of these compounds.

Exploring Synergistic Effects

Since the extract showed better antimicrobial and antioxidant effects when compared to individual compounds, it would be beneficial to explore the synergistic effects of the compounds present in the extract. This can be achieved through combination studies and network pharmacology approaches.

In-depth Toxicological Assessment

Although the study found significant bioactivity, an in-depth toxicological evaluation is needed to confirm the safety of *Newbouldia laevis* extracts, particularly in long-term use. The results should guide safe dosage recommendations for potential therapeutic applications.

Sustainable Harvesting Practices

Given the medicinal value of *Newbouldia laevis*, it is essential to establish sustainable harvesting practices to prevent overexploitation. Further research into the cultivation of this plant could ensure its availability for future medicinal use without depleting natural populations.

Commercial Application Development

The pharmaceutical and cosmetic industries could explore the commercial potential of the active metabolites from *Newbouldia laevis* as natural antioxidants and antimicrobial agents. Development of standardized extracts for use in topical or oral formulations could provide alternatives to synthetic drugs.

Further Research on Extracting Other Plant Parts

While this study focused on the stem bark, future studies should explore other parts of the plant, such as the leaves and roots, for their potential medicinal properties. These

studies could offer a more comprehensive understanding of the plant's overall therapeutic value in modern medicine

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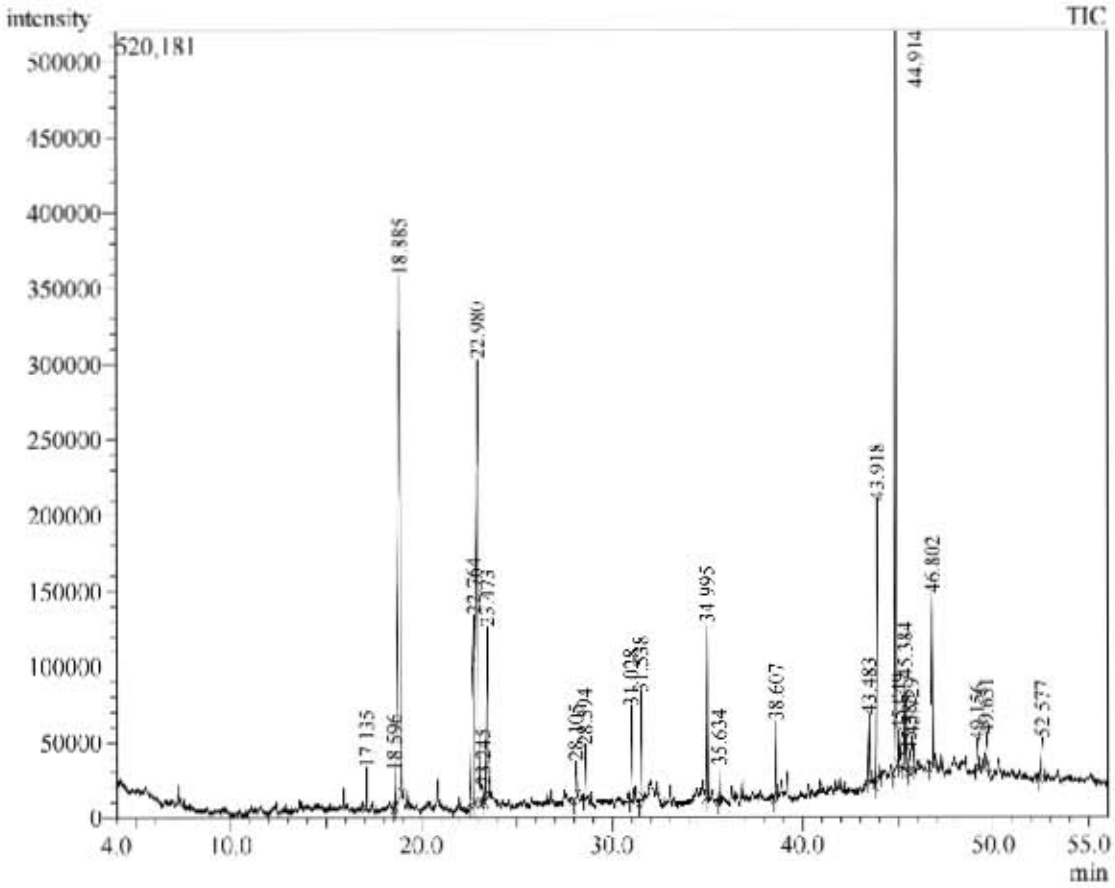
APPENDICES

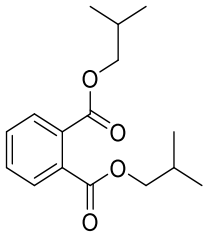
Appendix A: GC-MS Chromatogram of Crude Extract



Appendix B: CHEMDRAW Structure of Compounds

Structures of compounds identified in Table 4.3 page 44 in text

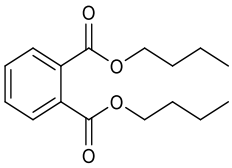
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester



Chemical Formula: $C_{16}H_{22}O_4$
Exact Mass: 278,15
Molecular Weight: 278,35

$t_{R=}$17.1

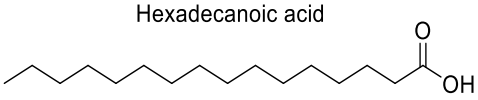
1,2-Benzenedicarboxylic acid, dibutyl ester



Chemical Formula: $C_{16}H_{22}O_4$
Exact Mass: 278,15
Molecular Weight: 278,35

$t_{R=}$18.6

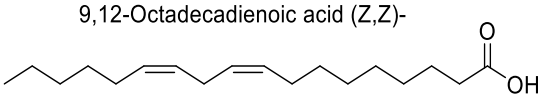
Hexadecanoic acid



Chemical Formula: $C_{16}H_{32}O_2$
Exact Mass: 256,24
Molecular Weight: 256,43

$t_{R=}$18.8

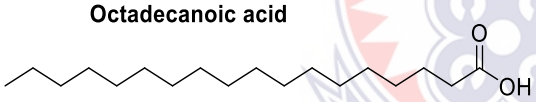
9,12-Octadecadienoic acid (Z,Z)-



Chemical Formula: $C_{18}H_{32}O_2$
Exact Mass: 280,24
Molecular Weight: 280,45

$t_{R=}$22.7

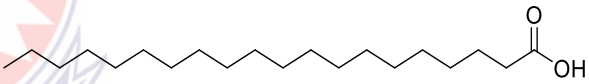
Octadecanoic acid



Chemical Formula: $C_{18}H_{36}O_2$
Exact Mass: 284,27
Molecular Weight: 284,48

$t_{R=}$23.5

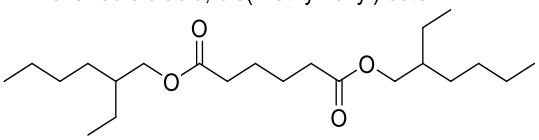
Eicosanoic acid



Chemical Formula: $C_{20}H_{40}O_2$
Exact Mass: 312,30
Molecular Weight: 312,54

$t_{R=}$28.1

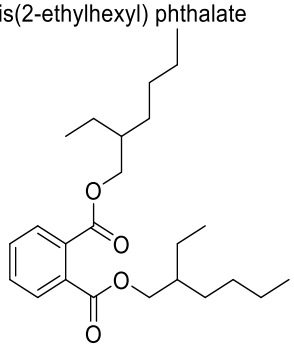
Hexanedioic acid, bis(2-ethylhexyl) ester



Chemical Formula: $C_{22}H_{42}O_4$
Exact Mass: 370,31
Molecular Weight: 370,57

$t_{R=}$28.6

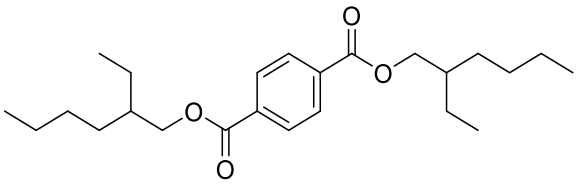
Bis(2-ethylhexyl) phthalate



Chemical Formula: $C_{24}H_{38}O_4$
Exact Mass: 390,28
Molecular Weight: 390,56

$t_{R=}$31.5

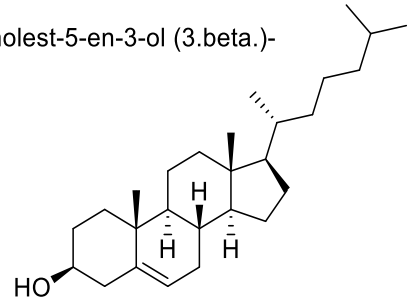
1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester



Chemical Formula: $C_{24}H_{38}O_4$
Exact Mass: 390,28
Molecular Weight: 390,56

$t_{R=}$36.5

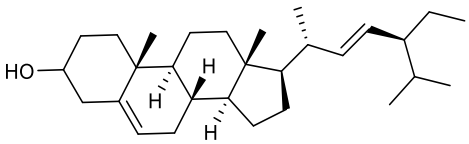
Cholest-5-en-3-ol (3.beta.)-



Chemical Formula: $C_{27}H_{46}O$
Exact Mass: 386,35
Molecular Weight: 386,66

$t_{R=}$43.5

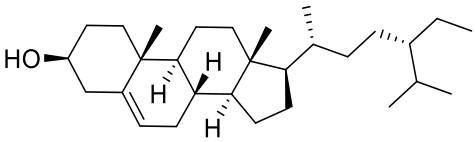
Stigmasta-5,22-dien-3-ol



Chemical Formula: $C_{29}H_{48}O$
Exact Mass: 412,37
Molecular Weight: 412,70

$t_{R=}$43.9

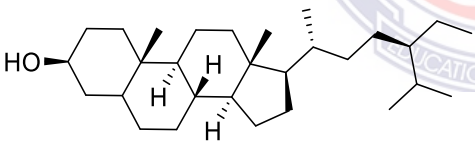
Stigmast-5-en-3-ol, (3.beta.,24S)-



Chemical Formula: $C_{29}H_{50}O$
Exact Mass: 414,39
Molecular Weight: 414,72

$t_{R=}$44.9

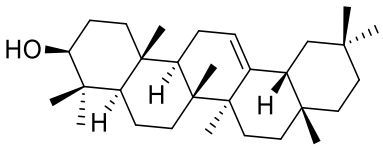
Stigmastan-3-ol, (3.beta.)-



Chemical Formula: $C_{29}H_{52}O$
Exact Mass: 416,40
Molecular Weight: 416,73

$t_{R=}$45.0

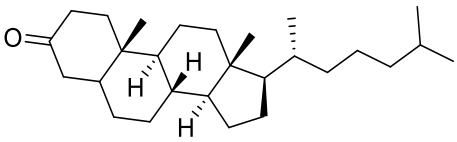
Olean-12-en-3-ol, (3.beta.)-



Chemical Formula: $C_{30}H_{50}O$
Exact Mass: 426,39
Molecular Weight: 426,73

$t_{R=}$45.4

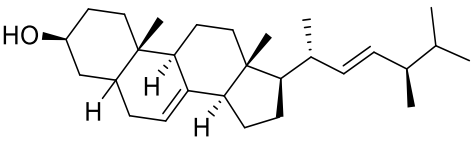
3-Oxocholestane



Chemical Formula: $C_{27}H_{46}O$
Exact Mass: 386,35
Molecular Weight: 386,66

$t_{R=}$45.6

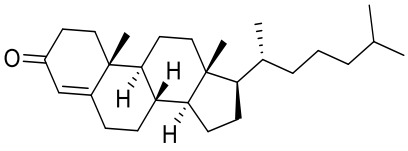
5,6-Dihydroergosterol



Chemical Formula: $C_{28}H_{46}O$
Exact Mass: 398,35
Molecular Weight: 398,68

$t_{R=}$45.7

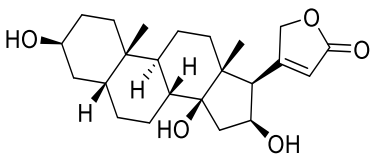
Cholest-4-en-3-one



Chemical Formula: $C_{27}H_{44}O$
Exact Mass: 384,34
Molecular Weight: 384,65

$t_{R=}$46.8

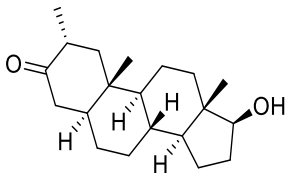
Card-20(22)-enolide, 3,14,16-trihydroxy-, (3.beta.,5.beta.,16.beta.)-



Chemical Formula: $C_{23}H_{34}O_5$
Exact Mass: 390,24
Molecular Weight: 390,52

$t_{R=}$49.2

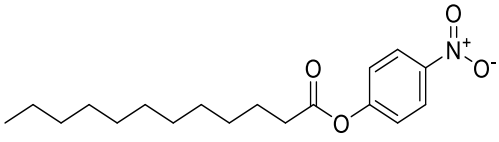
Androstan-3-one, 17-hydroxy-2-methyl-, (2.alpha.,5.alpha.,17.beta.)-



Chemical Formula: $C_{20}H_{32}O_2$
Exact Mass: 304,24
Molecular Weight: 304,47

$t_{R=}$49.6

Dodecanoic acid, 4-nitrophenyl ester



Chemical Formula: $C_{18}H_{27}NO_4$
Exact Mass: 321,19
Molecular Weight: 321,42

$t_{R=}$52.6