Phytochemicals Analysis, Antibacterial and Antioxidant Activity Evaluation of Bay Leaf *(Laurus nobilis*) Extract

Rebecca Beyene

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Specialization in Applied Microbiology)

Office of Graduate Students

Adama Science and Technology University

July, 2022

Adama, Ethiopia

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Advisors: Dr. Teshome Geremew

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Declaration

I hereby declare that this Master Thesis entitled "**Phytochemicals Analysis, Antibacterial and Antioxidant Activity Evaluation of Bay Leaf *(Laurus nobilis*) Extract**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Recommendation

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Phytochemicals Analysis, Antibacterial and Antioxidant Activity Evaluation of Bay Leaf *(Laurus nobilis*) Extract**" prepared under our guidance by Rebecca Beyene submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Applied Microbiology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
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Co-advisor	Signature	Date

Approval page

we, the advisors of the thesis entitled "**Phytochemicals Analysis, Antibacterial and Antioxidant Activity Evaluation of Bay Leaf *(Laurus nobilis*) Extract**" and developed by Rebecca Beyene hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Rebecca Beyene have read and evaluated the thesis entitled "**Phytochemicals Analysis, Antibacterial and Antioxidant Activity Evaluation of Bay Leaf *(Laurus nobilis*) Extract**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

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Final approval and acceptance of the thesis proposal is contingent upon the submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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LIST OF ACRONYMS/ABBRIVATIONS

ASTM- American Society for Testing and Materials

CC-Column Chromatography

DPPH- 2,2-diphenyl-1-picrylhydrazyl

EO- Essential oil

FT IR- Fourier-transform Infrared Spectroscopy

GC-MS- Gas Chromatography and Mass Spectrometry

HPLC- High Pressure Liquid Chromatography

MBC-Minimum Bactericidal Concentration

MHA- Muller Hinton Agar

MIC-Minimum Inhibitory Concentration

NMA- National Metrology Agency

NMR- Nuclear Magnetic Resonance

TLC- Thin Layer Chromatography

UV- Ultra Violet

ABSTRACT

Bay Leaf (*Laurus nobilis*) *is an evergreen perennial shrub that belongs to the laurel family (Lauraceae). L. nobilis is found effective against many infections from fungi, viruses, bacteria, and protozoa. The aim of this study was to determine the antibacterial and antioxidant activities of Bay Leaf (Laurus nobilis) extract against selected human pathogenic bacteria and to analyze its chemical composition. The plant leaf was collected from Addis Ababa around Piassa area. The leaf was then washed, air dried and the powder was extracted by using solvents like n hexane, methanol, chloroform and ethyl acetate. The ethyl acetate extract was subjected to column chromatography over silica gel (60-120 mesh). The structures of these compounds were characterized using* ^{1}*H-NMR,* ^{13}*C-NMR and DEPT-135. The essential oil of L. nobilis leaf was hydrodistilled by using a Clevenger apparatus. The crude extract, isolated compounds and essential oil of the plant were tested against four Gram negative (P. aeruginosa (ATCC 27853), E. coli (ATCC 25922), S. typhimurium (ATCC 13311), K. pneumoniae (ATCC 700603)) and two Gram positive (S. aureus (ATCC 25923) and E. faecalis (ATCC 29212)) bacterial strains and also the samples were assessed for radical scavenging activity using DPPH assay. Antibacterial activities of the plant extracts were assessed using the agar disk diffusion method. The MIC and MBC test was performed for the bacteria which show the positive result in disk diffusion assay. Two compounds were furnished namely compound* ***1*** *and compound* ***2****. These compounds are flavonoids. The extracted oil was analyzed by GC- MS and revealed presence of a total of 48 chemical components, accounting for 91.4 % of the total compositions. The major constitutes were; 1,8-cineole (26.3%),α-terpinyl acetate (17.1%), Methylisoleugenol (9.1%), α.-Terpineol (5.2%), and Linalool (4.6 %). These results showed the crude extract inhibited the growth of the test organisms in the order of magnitude of concentration from 25 mg/mL to 200 mg/mL. The maximum inhibition zone was recorded against E. faecalis (13.33± 1.52 mm), E. coli (14.33± 1.53mm) and S. typhimurium (16.00± 1,00mm).The highest activity of the isolated compound* ***1****, compound* ***2*** *and essential oil exhibited maximum inhibition against S.aureus* (*13.0±1.73mm*),*E. coli (12.0±1.0 mm) and P. aeruginosa & K. pneumonia (12.33±0.58mm) respectively. Among the studied crude samples methanol extract of (1000 µg/mL) with % inhibition and* IC_{50} *of 94.56 and 0.02, respectively had better radical scavenging activity among the investigated samples. In the case of isolated compounds, compound* ***2*** *showed radical scavenging activity % inhibition at 77.75 and its* IC_{50} *value was 15.45. The finding of this study showed that bay leaf has considerable antimicrobial, antioxidant properties, which should be further examined with particular attention to the mechanisms of antimicrobial activity.*

Key words/Phrases*:* Antimicrobial activity, Antioxidant activity, Bay leaf, Essential oil, Phytochemical

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

The development of drug resistance furthermore as well as lack of availabilities of certain antibiotics has led to the search of the latest antimicrobial agents mainly among plant extracts with the goal to find new chemical structures (Benharref *et al*., 2006). Current research on natural molecules and products primarily focuses on plants since they are locally available, less expensive, and might be selected based on their ethno-medicinal uses (Arora & Kaur, 2007). Natural products perform various functions, and have interesting and useful biological activities. There are over 35,000 plant species getting utilized in various human cultures around the world for medicinal purpose (Veeresham, 2012).

For decades, plants are mainstay of traditional medical practice and have remained an inestimable source of natural health products for humans, particularly within the previous few decades, with more thorough researches having being allotted to explore natural therapies (Sanjoy & Yogeshwer, 2012). The employment of herbs within the treatment of diseases has become widespread and is increasingly achieving popularity worldwide not only because of their continuous usage in developing countries for primary health care of the poor, but also in societies where conventional medicine is prevalent in their health care system (Ekor, 2014).

Extracts isolated from medicinal plants exhibit various biological activities such as antimicrobial, anti-inflammatory, and antioxidant activities (Mehta *et al*., 2001). The antimicrobial compounds from medicinal plants may inhibit the expansion of bacteria, fungi, viruses, and protozoa by different mechanisms than those of presently used antimicrobials and should have a big clinical value within the treatment of resistant microbial strains (Shankar *et al*., 2010).

A number of those active compounds show both intrinsic antibacterial activity and antibiotic resistance-modifying activities and a few of them, while not effective as antibiotics by themselves, when combined with antibiotics, can help overcome antibiotic resistance in bacteria. Chemically complex compounds have great therapeutic potential as they show fewer side effects compared to synthetic drugs and also low chances of developing resistance (Ody, 2017; Ruddaraju *et al* ., 2020). Bacteria may develop

resistance to medicinal plants treatment if just one active ingredient with a particular target is involved, a condition like an antibiotic (Reker *et al*., 2014). However, since the literature on bacteria developing resistance plants is proscribed then further research on resistance mechanisms is required (Almabruk *et al*.,, 2018).

Furthermore, the effectiveness of medicinal plant extracts to inhibit bacteria growth is additionally associated with the synergistic effect between the active compounds of the extracts (Wagner & Ulrich-Merzenich, 2009). The synergism action come from different effects, namely the emergence of multi-target mechanisms, the existence of compounds capable of suppressing bacterial resistance mechanisms, pharmacokinetic or physicochemical effects leading to enhanced bioavailability, solubility and resorption rate, neutralization of adverse effects and reduction of toxicity (Vaou *et al*.,2021).

Bay leaf *(Laurus nobilis)* is an evergreen perennial herb that belongs to the laurel family (Lauraceae). It is vital ingredient in cooking and in many traditional practices (Parthasarathy *et al*., 2008). The herb is found effective against many infections from fungi, viruses, bacteria, and protozoa. It's also helpful in inhibiting growth of carcinogenic cells. It has been used as a herbal remedy for a variety of illnesses, including rheumatism, sprains, dyspepsia, earaches, and to increase perspiration (Fang *et al*., 2005). Bay juice is an efficient medication for sore eyes and night blindness, which is usually caused by deficit of vitamin A. Bay seeds are mucilaginous and relieve indigestion, sore throat, constipation, and diarrhea (Batool *et al.,* 2020). Additionally, its essential oil is employed by the cosmetic industry in creams, perfumes, and soaps (Sharmeen *et al*., 2021).

Phytochemical studies on Bay Leaves and its fruits have indicated various secondary metabolites including alkaloids, flavnols (kaempferol, myricetin and quercitin,flavones (apigenin and luteolin), glycosylated flavonoids, sesquiterpene lactones, monoterpene and germacrane alcohols (Fukuyama *et al.*,2011).

1.2 Statement of the problem

Microbial resistance to classical antibiotics and its rapid progression have raised serious concern within the treatment of infectious diseases (Khameneh *et al*., 2019). Antibiotic resistant microorganisms can increase mortality rates because they will survive and recover through their ability to accumulate and transmit resistance after exposure to antibiotic drugs, which are one of the therapies to infectious diseases (Reygaert, 2018). Antibiotic resistant bacteria threaten the antibiotic effectiveness and limit the therapeutic options even

for common infections (Boucher *et al*., 2009). The decline in research and development of the latest antibacterial agents, which are able to inhibit antibiotic resistant disease-causing microorganisms like enteric pathogens, *Pseudomonas aeruginosa*, *Streptococcus pneumonia, S. aureus* and *Mycobacterium tuberculosis* aggravates the emerging antibiotic resistance (Lowy, 2003). Phytochemicals have exerted potential antibacterial activities against sensitive and resistant pathogens via different mechanisms of action (Khameneh *et al*., 2016).

In Ethiopia there isn't any published research paper on the phytochemical analysis, antibacterial and antioxidant activity of *Laurus nobilis*. The plant was selected for this study based on ethnomedical information by which local communities use it and their genera are rich source of variety classes of secondary metabolites with different pharmacological properties. Since *L. nobilis* utilized in the society this paper aims to study those properties of the plant which have grown in Ethiopia and fulfill this gap.

1.3 Objective of the Study

1.3.1 General Objective

The general objective of this study was to analyze the phytochemical composition and to evaluate the antibacterial and antioxidant activities of Bay Leaf (*Laurus nobilis*) extract against selected human pathogenic bacteria.

1.3.2 Specific Objective

- To extract compounds from the leaf of *Laurus nobilis* using suitable extraction solvents.
- To assess phytochemical constituents in the extracts of *Laurus nobilis*.
- To elucidate the structure of isolated compounds based on spectroscopic methods (NMR).
- To extract essential oil from leaf of *Laurus nobilis* by using hydro-distillation method.
- To test in vitro antibacterial activity of extracts, isolated compounds and essential oil from leaf extracts of *Laurus nobilis* by disk diffusion method against selected pathogens.
- To assess the potency of the extracted components though MIC and MBC.
- To evaluate the antioxidant activity of extracts, isolated compounds and essential oil from *Laurus nobilis*.

1.4 Scope of the Study

The sampling area for this study was Addis Ababa around piassa area. The study was conducted in Adama Science and Technology University and Adama public health research and referral laboratory center. This research mainly focused on antibacterial and antioxidant activity of *Laurus nobilis* on bacteria which cause a severe illness to human beings. Currently, there are so many bacteria that have the ability to resist the antibiotic drugs but by using the natural medicinal plants they may be inhibited.

1.5 Significance of the Study

The main significance of this study is to provide information on the antibacterial and antioxidant activity of *Laurus nobilis* which found in Ethiopia and also about the secondary metabolites which is found in this plant. The other significance which this research expected to give scientific explanation for traditional use claims of this medicinal plant in Ethiopia. Moreover, the study provides baseline information for future pharmacological studies and the researchers who are interested to conduct further work on the area.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 The Concept of Medicinal Plants

A medicinal plant is any plant which, in one or more of its organs, contains substances which will be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora *et al*.,2013). Variety of plants are employed in traditional medicine for several years. Some do seem to work although there might not be sufficient scientific data (double-blind trials, for example) to verify their efficacy **(**Evans, 2008). Such plants should qualify as medicinal plants. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to clarify whole plants or parts of plants which have medicinal properties (Sofowora , 2008).

Medicinal plants are a resource for healing in local communities around the world for thousands of years. Still it remains of contemporary importance as a primary healthcare mode for about 85% of the world's population (Pešić, 2015), and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer & Brönstrup, 2014). Often, a single medicinal plant can have multiple uses, and sometimes different parts of the same plant may be used for the treatment of more than one disease condition (Dawurung *et al*., 2021). Other times, the same plant could be used as an ingredient in herbal preparations for a synergistic effect (Zhou *et al*., 2016). This is made possible due to the range of phytochemicals that are present in medicinal plants along with their diversities of bioactivities (Vickers *et al*., 2001).

Plants are thought to be a rich source of bioactive compounds and could serve as a substitute for other sources of insect repellents (Narendiran *et al*., 2016). Plant-derived secondary metabolites, or phytochemicals, have notable pharmacological properties including anti-oxidative, anti-allergic, antibacterial, hypoglycemic, and anti-carcinogenic properties (Bamola *et al*., 2018). These secondary metabolites protect the cells from the damage caused by unstable molecules known as free radicals (Harini & Nithyalakshmi, 2017). Utilizing natural antibacterial substances, particularly those derived from plants, for food preservation is gaining popularity. Therefore, it is necessary to look for plants with medicinal properties (Chavan, 2016).

2.2 Techniques of isolation and purification of bioactive molecules from plants

Purification and isolation of bioactive compounds from plants might be a method that has undergone new development in recent years (Altemimi *et al*., 2015). This contemporary technique offers the ability to parallel the event and availability of many advanced bioassays on the one hand, and provided precise techniques of isolation, separation, and purification on the alternative (Altemimi *et al*., 2015). The goal when trying to seek out bioactive compounds is to find an appropriate method that may screen the source material for bioactivity like antioxidant, antibacterial, or cytotoxicity, combined with simplicity, specificity and speed (Mulinacci *et al*., 2014).

In vitro methods are usually more desirable than in vivo assays because animal experiments are expensive, take longer time, and are liable to ethical controversies (Ammar *et al*., 2017). There are some factors that make it impossible to search out final procedures or protocols to isolate and characterize certain bioactive molecules. This might flow from different parts (tissues) in a plant, many of which might produce quite different compounds, additionally to the various chemical structures and physicochemical properties of the bioactive phytochemicals (Sarajlija *et al*., 2012).

Both the selection and therefore the collection of plant materials are considered primary steps to isolate and characterize a bioactive phytochemical. The next step involves a retrieval of ethno-botanical information to discern possible bioactive molecules. Extracts can then be made with various solvents to isolate and purify the active compounds that are responsible for the bioactivity (Ammar *et al*., 2017). Column chromatographic techniques can be used for the isolation and purification of the bioactive compounds. Developed instruments such as High Pressure Liquid Chromatography (HPLC) accelerate the method of purification of the bioactive molecule (Cavaliere *et al*., 2018). Different types of spectroscopic techniques like UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy can identify the purified compounds (Popova *et al*., 2009).

2.3 Bay Leaf (*Laurus nobilis*)

2.3.1 Distribution and traditional uses of *Laurus nobilis*

Laurus nobilis is a small tree, having alternate, narrowly oblong-lanceolate leaves. The flowers are small and four lobed; the male has 8-12 stamens and female 2-4 staminodes. The ripe fruit is 10-15 mm, ovoid and black when ripe. The smooth bark may be olive green or reddish-blue. The plant is hardly multi-branched, usually grows to a height of 20-30 feet in many warm regions of world (Said & Hussein, 2014). One of the most well-known plants from the Lauraceae family is *Laurus nobilis*, which is additionally referred to as Bay or laurel leaves. Bay is one among the foremost frequently used cooking spices for flavoring meat products, fishes and soups. It is a native plant in the Southern Mediterranean area, found in warm climate regions, but it is used as ornamental plant in Europe and USA (Fang *et al.*,2005) . In addition, it is commercially grown in, Algeria, Morocco, Portugal, Spain, Italy, France, Turkey and Mexico (Ivanoi'c *et al.*, 2010).

Traditionally, the dry *Laurus nobilis* and their infusions are used as herbal medicine against number of diseases such as rheumatism, sprains, indigestion, earaches, and to enhance perspiration (Fang *et al.*, 2005). Leaves and fruits of *Laurus nobilis* are used as astringent, diaphoretic, stimulant and emetic as well as emmenagogue, abortifacient and insect repellent. Additionally because it is an aromatic plant, its essential oil is added in the cosmetic products like soaps, creams and perfumes (Kaurinovic *et al.*, 2010).

Figure 1: Picture of *Laurus nobilis* (Picture taken by Rebecca Beyene; Piassa, Addis Ababa, Ethiopia .06/11/2021G.C)

2.3.2 Antibacterial activity of *Laurus nobilis*

Interestingly, there is a worldwide concern about the use of antibiotics to treat bacterial infections may result within the increase and spread of organisms resistant to broad-spectrum antibiotics opening ways to use plants as natural sources for novel antibacterial agents with identical activity (Carocho & Ferreira, 2013). Natural medicinal plants, as *L. nobilis*, are rich sources of bioactive compounds. Thus, the biological properties of Bay extracts and its essential oil are documented, specifically their antimicrobial, anti-fungi and antioxidant effect.

A previous study agar well diffusion assay was performed in order to check the antibacterial activity of spices against *E.coli* and Bacillus species with the relation to different concentration of the extracts. All extracts of Coriander and Bay Leaves were more practical against *E.coli* and Bacillus species. The aqueous extracts of coriander and Bay Leaves were more effective against Bacillus species than *E.coli* because they depicted higher zones of inhibition against Bacillus species. In comparison to both spices, *L. nobilis* showed a wider zone of Inhibition against both the bacteria compared to coriander. The results suggested that these spices have inhibitory facts against bacteria and might be used for various purposes within the field of medication for the welfare of the mankind. These spices can be used for the treatment of infectious diseases and also help in the development of recent drugs, thus providing a good source of herbal medication (Debjani *et al.*, 2015).

2.3.3 Antioxidant Activity of *Laurus nobilis*

Assessments of antioxidant properties of natural compounds are important due to their uses in medicine, food and cosmetics (HalliwSánchez-Moreno, 2002; Liu, 2003). In living systems various metabolic processes and environmental stresses generate various reactive species. These are free radicals and mainly reactive oxygen species (ROS). Increased level of ROS can damage structure of biomolecules and modify their functions and cause cellular dysfunction and even cell death. The cumulative effect of increased ROS can increase oxidative stress in systemic level and it is manifested within the kind of a spread of health problems like cancer, age related disease and cardiovascular diseases (Noguchi & Niki, 2000; Grune *et al*., 2001).

Cellular ROS are regulated by interplay of complex antioxidant machineries in living systems. Nature has bestowed living systems with numerous antioxidant molecules. These natural antioxidants are known to reduce the adverse effects of free radicals in living

system. Many of these naturally occurring antioxidants are now isolated, fully characterized, and available for various applications. The chemical structures of these pure compounds are very diverse in nature. Thus, it is expected that the correlation between structure and antioxidant properties will be a difficult preposition. Many of these natural antioxidants are actively considered as prophylactic and therapeutic agents for possible applications for radiation countermeasures (Weiss & Landauer, 2003), combating cancers and aged related disease.

The antioxidant activity could also be analyzed by different methods both in vitro and in vivo. Among in vitro assays, the DPPH•-based method is perhaps the foremost popular one due to its simplicity, speed, and low cost (Alam *et al*., 2013). DPPH• (1,1-diphenyl-2-picrylhydrazyl) could be stable free radical which will be reduced by transferring a hydrogen from other compounds.

The DPPH antioxidant assay relies on the ability of DPPH to decolorize within the presence of antioxidants. The odd electron within the DPPH radical is responsible for the deep purple color. When DPPH accept an electron from antioxidant compound, the DPPH decolorize (Kumarasamy *et al*., 2007).

Several previous studies assessed the quantitative antioxidant activity of various extracts of Bay Leaves by using different models; all of them indicated that Bay Leaves extracts provide both In vitro and In vivo antioxidant effect (Kaurinovic *et al*., 2010; Conforti *et al*., 2016; Kivrak *et al*., 2017).

2.3.4 Chemical composition of *Laurus nobilis*

Laurus nobilis encompasses a sharp and bitter taste. The difference in fragrance and aroma is because of the presence of essential oils in leaves and other parts of the plant. It has flavonoids, tannins, eugenol, citric acid, carbohydrate, steroids, alkaloids, triterpenoids, and essential oils. Antioxidant properties were discovered with in the extract of *Laurus nobilis* to have phenolic compounds. Each of those chemical constituents varies depending on the type of species. Tanine is a liquid glycoside derived from polypeptide and ester polymer that may be hydrolyzed by the secretion of bile (3, 4, 5–trinidrokside benzoic acid) and glucose (Sumono, 2008). Tanine or tanat acid isolated from some part of plants. It is a cream-colored powder, aromatic, with astringent taste (Sumono, 2008). Tanine is used as an astringent for the gastrointestinal tract or skin and can cause precipitation of the

cell membrane protein. It also has a little penetration activity, so it can influence the permeability of the cell membrane.

Laurus nobilis has traces of fats; (that is, a low amount is present) so it has low caloric value. It is also known as a good and main source of vitamin A and many minerals. One ounce of *Laurus nobilis* gives 54 calories, 1–1.2 g protein, 12–13 g carbohydrates, a trace of fat, 1–1.5 mg of iron (Fe), 51–53 mg of calcium (Ca), 2000–3000 IU of vitamin A, 14–15 mg of vitamin C, and a small amount of potassium. Bay seeds are rich in dietary fibers. *Laurus nobilis*). The essential oils in leaves vary from 0.8% to 3% and dry bay fruits from 0.6% to 10% (Batool *et al*., 2020).

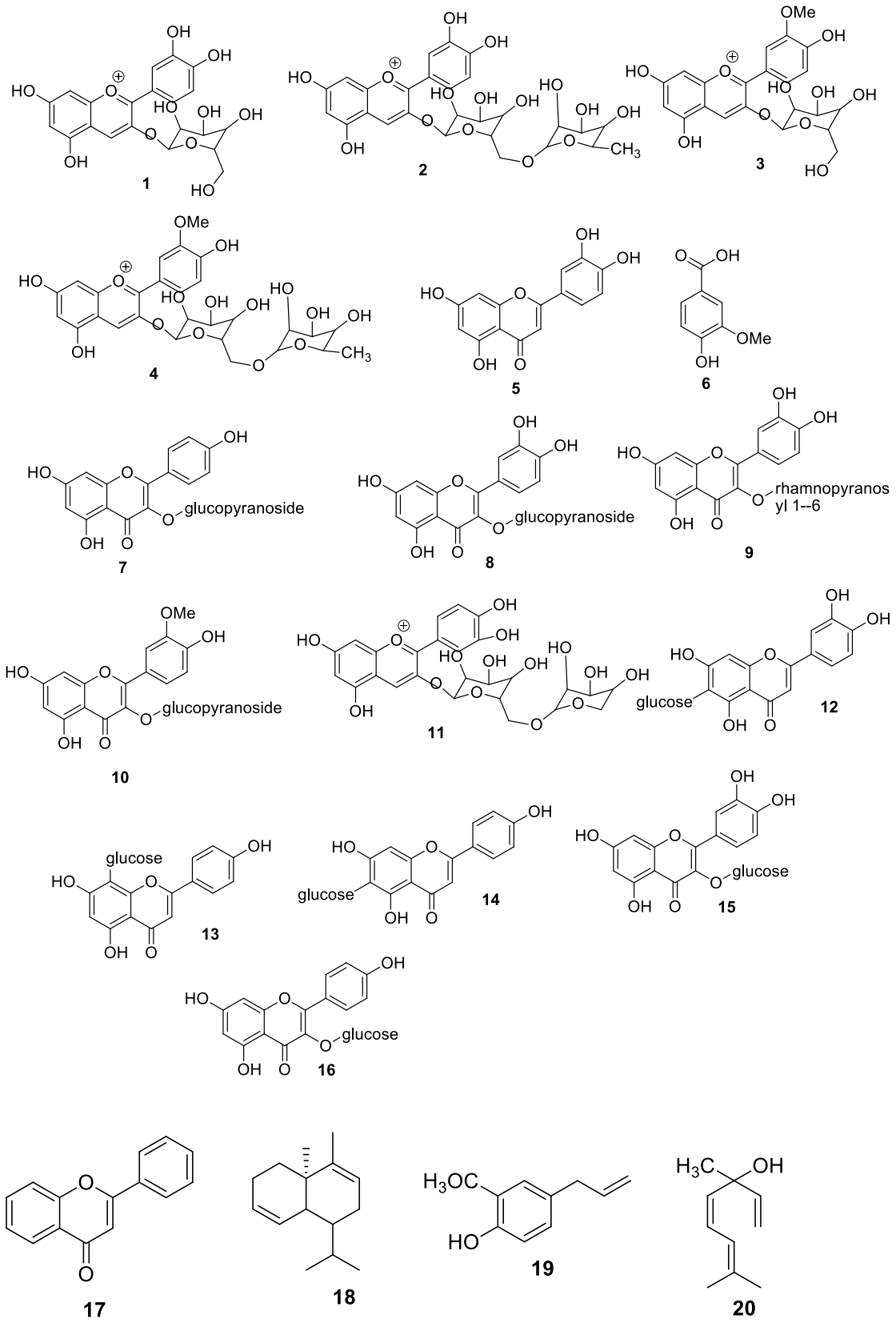

Figure 2: Structures of the flavonoids isolated from *Laurus nobilis* (Dmitry *et al*., 2019)

2.3.5 Essential oil of *Laurus nobilis*

Essential oils (EOs) are concentrated aromatic hydrophobic oily volatile liquids characterized by a strong odor and produced by all plant organs. The most effective extraction method to use depends on the ease of evaporating (volatility) and the hydrophilicity or hydrophobicity (polarity) of the desired components. However, the three most commonly applied techniques to extract EOs are Soxhlet, hydro-distillation, and SFE. The extraction method chosen significantly affects the chemical composition of Eos (Abu-Dahab *et al*., 2014).

The laurel EO yield and composition were shown to be influenced by various factors, such as growth environment, harvest season, plant parts, extraction method and others. The EO content (yield) of the laurel fruits varied within a large range, 0.60–4.30% (Zolfaghari *et al*., 2013; Abu-Dahab *et al*.,2014), and the EO content of the leaves also varied widely, from 0.5 to 4 (El *et al*., 2014; Bahmanzadegan *et al*., 2015; Goudjil *et al*., 2015; Caputo *et al*., 2017;Tanab *et al*., 2018).

The main compounds of the fruit EOs in previous studies were 1,8-cineole (8.10–48.0%), α-terpinyl acetate (3.67–10.4%), sabinene (4.49–11.4%), α-phellandrene, eugenol, methyl eugenol, α-pinene; β- ocimene, β-pinene, etc, (3.91–12.8%) (Said *et al*., 2014).

The leaf EO was found to be rich in 1,8-cineole (30–70%), linalool (0.9–26.9%), α-terpinyl acetate (4.50– 25.7%), α-pinene, β-pinene, sabinene etc., (3.91–12.8%) (Zolfaghari *et al*., 2013; Said *et al*., 2014). The terpineol, terpineol-4, etc. (Ivanovich *et al*.,2010; Moghtader & Salari, 2012; Bahmanzadegan *et al*., 2015; Fernandez-Andrade *et al*., 2016; Caputo *et al*., 2017). The growing interest in natural products, such as EOs, and the inclusion of plant extracts in various cosmetic products is a prerequisite for an in-depth analysis of the chemical composition of laurel genotypes from various regions. According to some studies (Bras *et al*., 2018; Sarkic & Stappen, 2018), certain EO constituents may cause allergic reactions when included in cosmetic products.

The laurel EOs have demonstrated antimicrobial (El *et al*., 2014; Fernandez-Andrade *et al*., 2016; Goudjil *et al*., 2015), antioxidant (Ozek,2012; Caputo *et al*., 2017; Politeo *et al*., 2017; Tanab *et al*., 2018 ; Derwich *et al*., 2019), and pharmacological properties (Snuossi *et al*., 2016; Caputo *et al*., 2017). Because of its biological activity, *Laurus nobilis* EO could be considered a natural supplement or antioxidant in cosmetics (Georgiev and Stoyanova, 2006; Vasundhara *et al*., 2016) and medicine (Zolfaghari *et al*., 2013).

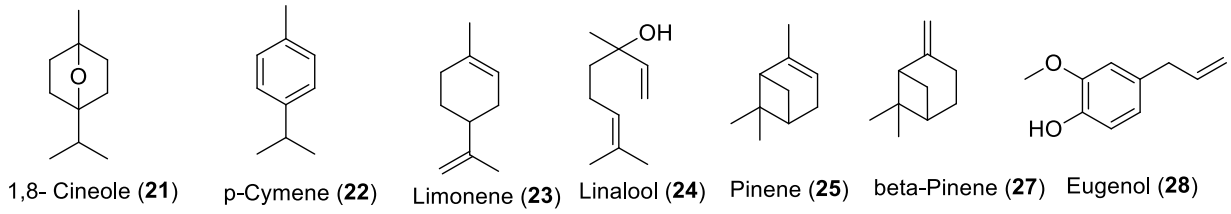

Figure 3: Structures of compounds present in *Laurus nobilis* essential oil (Chahal *et al*., 2017)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

The plant sample used for this study was collected from Addis Ababa. Addis Ababa is geographically located at the heart of Ethiopia, $9^{o}2'$N latitude and $38^{o}45'$E longitude. Its average altitude is 2,400 meter above sea level, with the highest elevations at Entoto Hill to the north reaching 3,200 meters. This makes Addis Ababa one of the high-altitude capital cities of the world. Addis Ababa has sub-tropical highland climate with a constant moderate temperature of roughly 23°C average high and 11°C average low throughout the year. The average annual rainfall is about 1,200 mm, out of which close to 80% falls during the main rainy season (NMA, 2017).

3.2 Research design

The *Laurus nobilis* leaves were collected and washed by using distilled water then left to dry at shade place. Phytochemical screening tests were conducted using the methanol, chloroform and ethyl acetate extracts following standard protocol. The antibacterial assay was done using disk diffusion method and the antioxidant activities of the extracts, isolated compounds and essential oils of *Laurus nobilis* were conducted DPPH assay method. Isolation of secondary metabolites from the ethyl acetate extract of *Laurus nobilis* was conducted by using silica gel column chromatographic technique and the obtained pure fractions were submitted NMR spectroscopy for further analysis.

3.3 Study samples and sampling techniques

3.3.1 Collection and authentication of selected plant sample

The *Laurus nobilis* leaf was collected from Addis Ababa city around Piassa area and brought to Adama Science and Technology University. A voucher specimen of the plant was collected and pressed on newspaper separately for taxonomic identification. The taxonomic identification was carried out at Department of Plant Biology and Biodiversity Management ,Addis Ababa University by plant taxonomists.

3.3.2 Chemicals, culture media, apparatuses and instruments

3.3.2.1 Chemicals and media

TLC visualizing reagents, analytical grade organic solvents (methanol, n-hexane, dichloromethane, ethyl acetate, chloroform, acetone DMSO, silica gel 60-120 mesh ASTM and culture media (MHA), Nutrient broth, Nutrient agar were used.

3.3.2.2 Apparatuses and instruments

Electric grinder, shaker, rotary evaporator, CC, TLC sheet (pre-coated aluminum sheet silica gel 60 F254), TLC chamber, capillary tube, UV- lamp at 254 and 365 nm, Clevenger apparatus, NMR spectroscopy, GC-MS, Petri plate, incubator, autoclave and Falcon tubes.

3.3.3 Preparation of plant extract

In this study, the solid liquid extraction technique was employed as a general extraction method. Total extraction of plant material was prepared by mixing the dried and grounded *Laurus nobilis* with organic solvents. Briefly, the air dried and ground plant material (0.5 kg) was extracted with methanol (2.5 L) and macerated at room temperature by placing on a shaker for 24 h and filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator. The dry out of methanol extract was suspended and consecutively partitioned with equal volumes of n-hexane, chloroform ($CHCl_3$) and ethyl acetate (EtOAc) twice. Each solvent fraction was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator.

3.3.4 Phytochemical screening

The preliminary screening of phytochemical constituents of crude extract of the *Laurus nobilis* was done for the investigation of secondary metabolites compounds such as alkaloids, flavonoids, saponins, tannins, phenols, terpinoid and steroid by using the standard procedures.

3.3.4.1 Test for steroid

Two mL of acetic anhydride was added to the *Laurus nobilis* extracts which was dissolved in appropriate solvent with two mL of sulphuric acid. A color changes from violet to green indicates the presence of steroids (Auwal *et al*., 2014).

3.3.4.2 Test for terpenoid

Five mL of the extract was mixed with 2mL of chloroform. Then 3mL of concentrated sulphuric acid was added to form a layer (Salkowski test). A reddish brown coloration at the interface indicated the presence of terpenoids (Samkeliso *et al*., 2018).

3.3.4.3 Test for saponins

When a few drops of distilled water were added to 1 ml of extract and the mixture was violently agitated, the presence of saponins was determined by the existence of persistent foam (Onuminya *et al*., 2017).

3.3.4.4 Test for flavonoids

Three mL of Laurus *nobilis* crude extract and fractions were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids (Edema & Alaga, 2012).

3.3.4.5 Test for tannins

The leaf extract was boiled in 20 mL of water in a test tube. The solution was filtered and a few drops of 0.1% Iron III chloride were added. The appearance of green-blackish color indicates the presence of tannins (Auwal *et al*., 2014).

3.3.4.6 Test for alkaloids

One mL of 1% HCl was added to 3mL of the leaf extract. The mixture was heated for 20 min, cooled and filtered. Then 1 mL of the filtrate was tested with 0.5 mL Mayer's, reagent (Potassium Mercuric Iodide). Formation of a yellow color precipitate indicates the presence of alkaloids (Joshi, 2013).

3.3.4.7 Test for phenol

The leaf extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols (Kiliobas *et al.*, 2019).

3.3.5 Isolation of compounds

The ethyl acetate extract (15 g) was adsorbed with silica gel (15g) and subjected to silica gel column chromatography (150 g silica gel). Elution of the extract was done with increasing gradient of solvent systems (increasing gradient of ethyl acetate in n-hexane followed by dichloromethane in ethyl acetate finally methanol in dichloromethane). Silica gel was used as the stationary phase while varying solvent combinations as the mobile

phase. Each fraction was collected and labeled and the purity of all fractions was analyzed with TLC. Further purification of isolated compounds was done using repeated column chromatography. The pure compounds were submitted for NMR analysis and biological activity tests to elucidate their structures and examine their biological activities, respectively.

Table 1: Summary of fraction of mixed extracts

Solvent System	Ratio	Fractions	Solvent System	Ratio	Fractions
n hexane	10	F1-F4	EtOAc	10	F95-F104
n hexane: EtOAc	9.5:0.5	F5	DCM	10	F105-F115
"	9:01	F6	DCM:MeOH	9:01	F116-F173
"	8.5:1.5	F7-F24	"	8.5:1.5	F174-F185
"	8:02	F25-F31	"	8:02	F186-F211
"	7:03	F32-F41	"	7.5:2.5	F212-F246
"	6:04	F42-F57	"	6:04	F247-F257
"	5:05	F58-F64	"	5:05	F258-F271
"	4:06	F65-F67	"	4:06	F272-F275
"	3:07	F68-F79	"	2:08	F276-F281
"	2:08	F80-F86	MeOH	10	F282-F285
"	1:09	F87-F94			

Fraction 172 and 168 (eluted with 10% MeOH in dichloromethane) afforded compound **1** (17 mg) and compound **2** (20 mg) respectively, both compounds had yellowish color.

3.3.6 Extraction of the essential oil

Laurus nobilis (60 g) was powdered using an electrical grinder and extracted using hydro-distillation for 2 h in a Clevenger apparatus. The essential oil was collected,dried over anhydrous Na_2SO_4, filtered, concentrated using rotary evaporator and kept in a refrigerator at -4 °C until further analysis.

3.3.7 GC-MS analysis of essential oil extract

GC-MS analysis of essential oil was performed by a GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP-5MS column (30 m × 250 μm internal diameter (i.d.) and 0.25 μm). The GC-MS method

reported by (Hanuš *et al*., 2008) was used for essential oil analysis. Helium was used as a carrier gas (flow rate 1 mL/ min). The initial oven temperature was 100°C for 2 min and raised up from 100 to 280ºC at the speed of 10ºC/min (inlet 250 °C; detector 280° C; split less injection/purge time 1.0min), solvent delay 4.00 min. Mass spectra was recorded in electron-impact mode, with ionization energy of mode at 70 eV, scanning the 33-550 m/z range. The volatile compounds in the oils were identified by comparing the mass spectra of the compounds in the oils with those in the database of NIST11 GC-MS libraries.

3.3.8 Antibacterial assay of *Laurus nobilis* extracts

Antibacterial assay of *Laurus nobilis* extract was performed by disk diffusion method in Mueller Hinton Agar (MHA) plates. Different concentrations of the plant extract, isolated compound and essential oil were prepared (Balouiri *et al*., 2016).

3.3.8.1 Preparation of plant extracts

Laurus nobilis leaf extracts (0.4g) with 0.4g was dissolved in 1 mL DMSO. Thus 400 mg/mL of stock was obtained as a standard concentration13.6µg, 10 µg and 0.0455µg of compound **1**, compound **2** and essential oil were taken and dissolved in 1 mL DMSO respectively.

3.3.8.2 Preparation of inoculum and McFarland standard

Bacterial cultures (*S. aureus (ATCC25923), E. faecalis (ATCC29212), P. aeruginosa (ATCC27853), E. coli (ATCC25922), S. typhimurium (ATCC13311) and K. pneumonia (ATCC700603))* grown in the blood agar media at 37°C for 24 h were adjusted 0.5 McFarland which is approximately 1.5 $\times 10^8$ colony forming units (CFU/mL) (Andrews, 2002). The 0.5 McFarland standard was prepared by mixing 9.95mLof 1% H_2SO_4 in distilled water and 0.05mL of 1% $BaCl_2$ in distilled water in order to estimate bacterial concentration (Saeed &Tariq, 2007). The preparation was kept in flask and used for contrast of bacterial suspension.

3.4 Plant extracts activity assay

3.4.1 Paper disk diffusion assay

Two hundred microliter of a culture suspension of testing bacteria (approximately 1.5 $\times 10^8$ CFU/mL) was spread on Muller Hinton Agar (MHA) medium for bacteria. Sterilized filter paper disks (6mm in diameter) soaked in 20mg/mL of plant extract soaked filter paper disks were placed on the agar plates which was inoculated with the tested bacteria.

Tobramycin (10µg/mL) standard antibiotic disk was used as a positive control and 10% Dimethyl Sulfoxide-soaked filter paper disks were used as the negative control. The plates were subsequently incubated at 37°C for 24h. After incubation the growth inhibition zone was quantified by measuring the diameter of the zone of inhibition in mm. The results were expressed as mean value ± standard deviation.

3.4.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC)

3.4.2.1 Determination of MIC against the test pathogens

Plant extracts that showed antimicrobial activity with disk diffusion were selected for determination of MIC using broth dilution method. Two fold serial dilutions were used to dilute the initial concentration of the crude extract (5mg/mL), for the isolated compounds 1mg/mL and for the essential oil 0.91mg/mL were taken by transferring 1mL from the plant extract (stock) solution into 1mL of sterile nutrient broth kept in a falcon tubes and mixing the contents thoroughly and then serially diluting in other 4 tubes. The serial dilutions for the crude gave concentrations of 2.5 mg/mL, 1.25mg/mL,0.62mg/mL and 0.31mg/mL (Hadia *et al*., 2012).

After obtaining different concentrations of the extract, each concentration was inoculated with tested bacterial cultures (0.02mL), which grown overnight on blood agar media and adjusted to 0.5McFarland standard, equivalent to 1.5 x 10^8 CFU/mL and incubated at 37ºC for 24h. (Hadia *et al*., 2012). The growth of the bacterial test pathogens was assessed by comparing turbidity or cloudiness of the nutrient broth which contain plant extract and bacterial strains with the negative control. The lowest concentration, at which there will be no turbidity was considered as the Minimum Inhibitory Concentration (MIC) value of the extract (Abou-Elkhair *et al*., 2010; Radojević *et al*., 2012).

3.4.2.2 Determination of MBC against test pathogen

Loopful of the broth was taken from the test tubes in which growth was not visually detected and streaked on nutrient agar (Muller Hinton Agar). The plates were incubated at 37 °C for 24 h and examined for bacterial growth. MBC was taken as the concentration of plant extract that did not exhibiting any bacterial growth on the freshly inoculated agar plates.

3.4.3 Antioxidant assay of *Laurus nobilis* extract

Antioxidant activity of dried *Laurus nobilis* extracts and isolated compounds was analyzed qualitatively by using 2, 2, diphenyl-1-picrylhydrazyl (DPPH). DPPH is a free radical of violet color. The antioxidants in the sample scavenge the free radicals and turn it into yellow color. The change of color from violet to yellow is proportional to the radical scavenging activity (Elmatas *et al*., 2006).

The three crude extracts (methanol, chloroform and ethyl acetate) were dissolved in four vials containing methanol to give 1000, 500, 250 and 125 µg/mL. For the isolated compounds 1 mg of the sample was dissolved in the 1mL of methanol then 100, 50, 25, 12.5 µg/mL were obtained. Ascorbic acid was used as a positive control. Sample free DPPH solution in methanol was used as negative control.

The solution of DPPH (0.04 mg/mL) in methanol was prepared, and 4 mL of this solution was mixed with 1 mL of extract solution at various concentrations immediately and then incubated for 30 min at room temperature. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage (IP) of free radical and was calculated using the formula:

$$IP\ (\%) = ([A_{control} - A_{test})/A_{control}]) \times 100\%$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the tested extracts), and A_{test} is the absorbance of the test extract (Suprava *et al*., 2012). Scavenging activity of the plant extracts was also estimated based on the percentage of the DPPH reduction by calculating the IC_{50} values (concentration in µg/mL that caused 50% inhibition of DPPH radicals) using a non-linear regression analysis.

3.5 Method of data analysis

The collected data for the antibacterial activity of the extracts, isolated compounds and essential oil against the tested bacteria were analyzed by using SPSS software, version 22. Results was presented using mean value and standard deviation

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Phytochemical screening

The result of phytochemicals screening of the methanol extract was positive for all phytochemical tests and revealed the presence of secondary metabolites such as, tannins, flavonoids, saponins, alkaloids, phenol, terpenoids, and steroids. However, the phenol and tannin constituent and alkaloids were absent in chloroform and ethyl acetate extracts, respectively (Table 2). Accordingly a study done by *Eliya et al., 2021* shows the ethanol extract of *L.nobilis* contain active compounds such as tannins, flavonoids, saponins, alkaloids, and essential oils, which is consistent with this study finding (Eliya *et al*., 2021). This study results were also compatible with research conducted by Wilapangga & Syaputra , 2018 and Algabri *et al*., 2018 who reported the presence of bioactive secondary metabolites in the leaf extracts of *L.nobilis*.

Furthermore, a study conducted by Onuminya *et al*., 2017 showed that, the methanol extract of *L.nobilis* contains active compounds similar to those reported in the current study except tannin. Variation in the composition of the secondary metabolites might be due to the difference in their genotypes, climatic conditions in the respective locality where the plants were grown, and also to the plant parts processed and extracted (Onuminya *et al*., 2017, Dewijanti *et al*., 2019).

Table 2: Phytochemical constituents of the leaf extracts from *Laurus nobilis*

Phytochemical Screening	Extracts		
	Methanol	Chloroform	Ethyl acetate
Alkaloids	+	+	_
Flavonoids	+	+	+
Saponnins	+	+	+
Phenol	+	_	+
Tannin	+	_	+
Terpenoids	+	+	+
Sterioid	+	+	+

Key: (+) presences (-) absences

4.2 Characterization of compounds

In the course of this work, two compounds (Compound **1** and compound **2**) were isolated from the ethyl acetate leaf extracts of *Laurus nobilis*. The detailed characterizations of these compounds are presented below. Compound **1**and Compound **2** were both obtained as yellow solids with R_f value of 0.53 and 0.58 respectively (10% MeOH in Dichloromethane as eluent).

4.2.1 Compound 1

Compound **1** (17mg) was obtained as a yellow solid with an R_f value of 0.53 (DCM/MeOH (9:1)) solvent system as eluent after column chromatography. The ^{1}H-NMR spectrum of compound **1** showed a singlet at 12.6 due to the chelated hydroxy group. The proton signals observed in the aromatic region at δ 7.75 (d, *J* = 7.1 Hz, 1H), 6.89 (d, *J* = 7.4 Hz, 1H), and 6.43 (d, *J* = 10.6 Hz, 1H) are ascribed to H-6', H-2', and H-5', respectively. The two doublets observed at δ 6.68 (d, *J* = 11.3 Hz, 1H, H-8) and 6.43 (d, *J* = 10.6 Hz, 1H, H-6) are characteristic of A-ring type in a flavonoid. The two doublets at δ 5.31 (d, *J* = 21.1 Hz, 1H) and 4.71 (d, *J* = 13.9 Hz, 1H) are assigned for the anomeric protons of the glucose, H-1''and rhamnose H-1''' moieties, respectively. The latter signal along with the multiplet observed at δ 1.17 (m, 3H) for a secondary methyl group was evident for the presence of rhamnopyranose moiety. The spectrum also exhibited a series of peaks in the region between 4.00-3.00 accounting for the presence of sugar moieties. The signal at δ 5.7, 5.0, 4.8 and 4.71 are due to protons on the oxygen atoms of hydroxyl groups in the structure of the compound.

The proton decoupled ^{13}C-NMR together with the DEPT-135 spectrum of compound **1** displayed ten quaternary, one methyl, one methylene and fifteen methine carbons. The downfield signal at *δ* 178.2 (C-4) suggested the presence of an *α*, *β*-unsaturated carbonyl group. Other signals in the ^{13}C-NMR spectrum were observed at *δ* 156.7 (C-5), 160.4 (C-7), 157.0 (C-2), 164.75 (C-9), 156.2 (C-4'), 144.93 (C-3'), 137.19 (C-3), 131.06 (C-6'), 121.0 (C-1'), 118.42 (C-2'), 115.85 (C-5'), 104.55 (C-10), 95.5 (C-6) and 94.6 (C-8). A series of signals in the region were δ 79.56 to 63.08 combined with the signals at *δ* 102.2 (C-1''), 99.2 (C-1''') and 17.88 (C-6''') are evident for the presence of two sugar units. The methyl signal at *δ* 17.88 further supports the presence of a rhamnose group in compound **1**. The above spectral data indicated that compound **1**is quercetin-3-O-rutinoside.

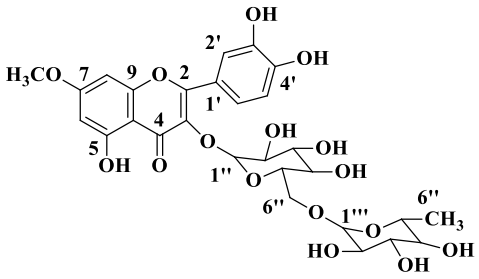

Figure 4: Chemical structure of Compound **1**, quercetin-3-O-rutinoside

Table 3: ^{1}H and ^{13}C NMR spectral data of quercetin-3-O-rutinoside

Position	Compound 1		quercetin-3-O-rutinoside (Wang *et al.*,2017)	
	^{1}H	^{13}C	^{1}H	^{13}C
2		157	7.54 (1H, s)	161.12
3		137.2	9.22(1H, br s)	133.24
4		178.2	9.72 (1H, br s)	177.23
5	12.6	156.7	12.60 (1H, s)	156.32
6	6.43 (d, *J* = 10.6 Hz, 1H)		7.55 (1H, s)	98.58
7		160.4	10.87 (1H, br s)	164
8	6.68 (d, *J* = 11.3 Hz, 1H)	94.6	6.40 (1H, s)	93.49
9		164.8		156.45
10		104.5		103.86
1’		121		121.47
2’		118.4		116.18
3’		144.9		144.65
4’		156.2		148.3
5’		115.9		115.12
6’		131.1		121.08
1’’	5.31 (d, *J* = 21.1 Hz, 1H)	102.2	5.34 (1H, s)	101.12
2’’		79.6-63.1	5.33 (1H, br s)	73.96
3’’			5.11 (1H, br s)	76.4

4''				69.94
5''				75.84
6''				
1'''	4.71 (d, *J* = 13.9 Hz, 1H)	99.2	4.39 (1H, s)	100.62
2'''		40.4-31.1		70.28
3'''				71.79
4'''				70.5
5'''	0.93 (d, 3H)		0.98 (3H, d)	68.14
6'''	1.17 (m, 3H)	17.9		17.58

4.2.2 Compound 2

Compound **2** (20 mg) was obtained as a yellow solid with an R_f value of 0.58 (DCM/MeOH (9:1)) solvent system as eluent after column chromatography. The ^{1}H-NMR spectrum of compound **2** showed a singlet at 12.7 due to the chelated hydroxy group. The proton signals in the aromatic region were observed at δ 7.8 (d, 1H), 7.0 (d, 1H), and 6.8 (d, 1H). The two doublets observed at δ 6.5 (d, 1H, H-8) and 6.3 (d, 1H, H-6) are characteristic of the A-ring type in a flavonoid. The two doublets at δ 6.0 (d, 1H) and 4.9 (d,1H) are assigned for the anomeric protons of the glucose, H-1''and rhamnose H-1''' moieties, respectively. The latter signal along with the multiplet observed at δ 1.2 (m, 3H) for a secondary methyl group was evident for the presence of rhamnopyranose moiety. The spectrum also exhibited a series of peaks in the region between 3.9-3.2 accounting for the presence of sugar moieties. The signal at δ 5.9 is due to protons on the oxygen atoms of hydroxyl groups in the structure of the compound.

The ^{13}C-NMR together with the DEPT-135 spectrum of compound **2** displayed ten quaternary, one methyl, one methylene, and fifteen methine carbons. Other signals in the ^{13}C-NMR spectrum were observed at *δ* 156.7 (C-5), 164.3 (C-7), 162.3 (C-2), 157.1 (C-9), 156.2 (C-4'), 115.4 (C-3'), 101.8 (C-3), 144.4 (C-6'), 118.4 (C-1'), 115.4 (C-2'), 114.6 (C-5'), 114.4 (C-10), 98.7 (C-6) and 95.3 (C-8). A series of signals in the region between δ 79.56 to 63.08 combined with the signals at *δ* 98.8 (C-1''), 101.8 (C-1''') and 16.9 (C-6''') are evident for the presence of two sugar units. The methyl signal at *δ* 16.9 further supports the presence of a rhamnose group in compound **2**.

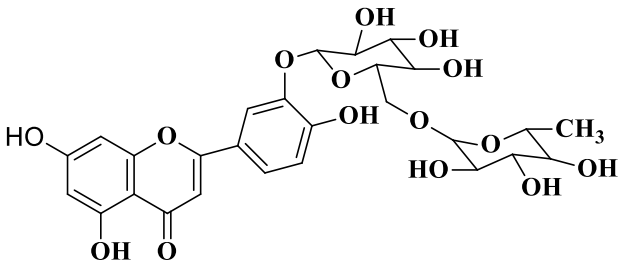

Figure 5: Proposed chemical structure of Compound **2**

4.3 Essential oil of *Laurus nobilis*

The chemical composition of the EOs from *L.nobilis* leaves were listed in Table 4. The *L.nobilis* EO was light yellow and had a specific odor. GC-MS analysis of essential oils from leaves of *L.nobilis* revealed a total of 48 chemical components, accounting for 91.4 % of the total compositions, the major constitutes were 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- (2-Hydroxy-1,8-cineole) (26.3%), 3-Cyclohexene-1-methanol, .α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate) (17.1%), Benzene, 1,2-dimethoxy-4-(2-propenyl)-(Methylisoleugenol) (9.1%), α.-Terpineol [.α.,α.,4-trimethyl-3-cyclohexene-1-methanol](5.2%), and 1,6-Octadien-3-ol, 3,7-dimethyl- -(Linalool) (4.6 %) (Appendex 7-11).The remaining constituents ranged from 0.3 to 3.5%. In general the plant is composed of monoterpenes and sesquiterpenes or their derivatives.

The comparison with most recent literature concerning the chemical composition of the essential oil of *L. nobilis* from other areas, showed substantial differences: 1,8-cineole percentage found (26.3%) is lower than the values recorded in other studies: Turkey 44.97% (Ekren *et al*., 2013), Tunisia 56.0% (Snuossi *et al*., 2016), Cyprus 58.59% (Yalcin *et al*., 2007), Morocco 52.43% (Derwich *et al.*, 2009) and Algerian essential oil (34.62%) (Jemâa *et al*.,2012). In our sample, the amount of Linalool was 4.6 but in essential oil analyzed by Derwick and co-workers linalool was not detected (Derwich *et al.*, 2009). The amount of α-terpinyl acetate was (17.1%) which is greater than the one found by Hafize *et al*.,2019 ,α-terpinyl acetate (14.4%).the result was comparable to the values reported previously (Jemâa *et al*., 2012).

This result is compatible from the essential oil found in Georgia. The major ones in the Georgian laurel EO were: 1,8- cineole (29.2%), α-terpinyl acetate (22.6%), sabinene (12.2%), methyleugenol (8.1%), α-pinene (5.5%), linalool (3.7%), and β-pinene (3.7%).

Some of the differences in the chemical composition of the laurel leaf EOs may be due to environmental and genetic factors (Galina *et al*., 2020).

The chemical composition of *L. nobilis* volatile oil from different locations has been studied by different researchers. In all studies, 1,8-cineole was the major component with percentages ranging between 26.70% and 68.48% (Ozcan & Chalchat, 2005). Moreover, α-terpinyl acetate with percentages ranging between 0.65-25.70% (Sangun *et al*., 2007; Sellami *et al*., 2011) and terpinen-4-ol with percentages ranging between 1.50-4.56% (Lira *et al*., 2009; Sellami *et al*., 2011) was found as major components. According to the result, the average amount of 1, 8-cineole and α-terpinyl acetate was found to be compatible with amounts reported by previous researches.

Table 4: GC-MS analysis result of essential oil of *L.nobilis*

Compound Name	RT	Formula	%
2(10)-Pinene	3.168	C10H16	1.3
2-Oxabicyclo[2.2.2]octane,1,3,3-trimethyl-(2-Hydroxy-1,8-cineole)	3.763	C10H18O	26.3
1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool)	4.392	C10H18O	4.6
α-Terpineol[.α.,α.,4-trimethyl-3-cyclohexene-1-methanol]	5.605	C10H18O	5.2
3-isopropyl-6-methyl-7-oxabicyclo[4.1.0]hept-4-ene	6.685	C10H16O	0.5
1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate	6.87	C12H20O2	2.3
3-Cyclohexene-1-methanol,.α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate)	7.713	C12H20O2	17.1
Eugenol	7.788	C10H12O2	2.3
Copaene	8.089	C15H24	1.3
Benzene, 1,2-dimethoxy-4-(2-propenyl)-	8.325	C11H14O2	9.1
1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	8.702	C15H24	3.5
(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene	9.007	C15H24	0.5
(-)-Germacrene D	9.452	C15H24	1.3
β-Selinene	9.532	C15H24	0.7
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.α.,4a.β.,8a.α.)-	9.839	C15H24	1.6
δ-Cadinene	9.919	C15H24	2.4
3a(1H)-Azulenol,2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-,[3R-(3.α.,3a.α.,8a.α.)]-	9.971	C15H26O	0.3

(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	10.041	C15H26O	0.3
Benzene,1,2,3-trimethoxy-5-(2-propenyl)-(Methylisoleugenol)	10.191	C12H16O3	1.3
α.-Santalol	10.254	C15H24O	0.3
1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.α.,4a.α.,7.α.,7a.β.,7b.α.)]-	10.636	C15H24O	1.8
1,1,7-trimethylspiro[2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene-4,2'-oxirane]	10.722	C15H24O	1.1
1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	10.82	C15H26O	1
γ.-HIMACHALENE	11.178	C15H24	0.6
Guaiol	11.236	C15H26O	0.5
.tau.-Cadinol	11.323	C15H26O	0.9
2-Naphthalenemethanol,decahydro-.α.,.α.,4a,8-tetramethyl-,didehydroderiv.,[2R-(2.α.,4a.α.,8a.β.)]-	11.49	C15H26O	2.5
2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	11.698	C15H24O	0.4
(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	11.866	C15H24O	0.4
Total			91.4

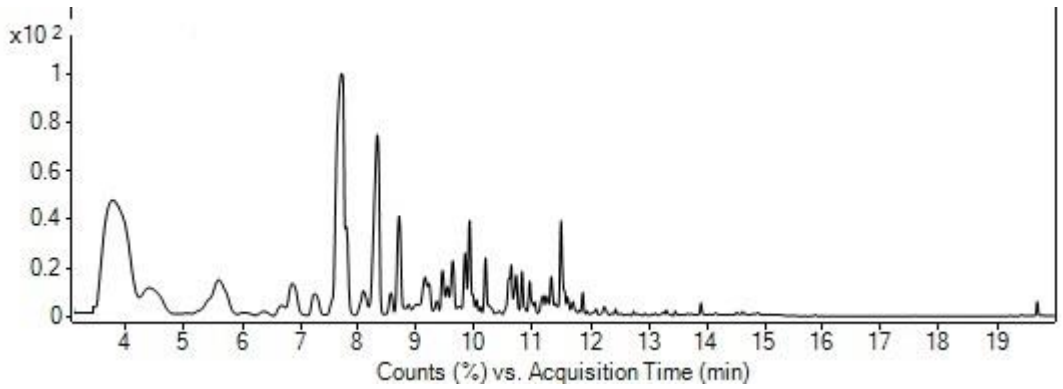

Figure 6: GC of essential oil of *L.nobilis*

4.4 Biological activity of crude extract, isolated compounds and essential oil of *Laurus nobilis*

4.4.1 Antibacterial activity of *L. nobilis*

The extract (methanol, chloroform and ethyl acetate), isolated compounds and essential oil of the *Laurus nobilis* were tested for their antibacterial activity in different concentrations against two Gram positive and four Gram negative compared to Tobramycin as the positive control and DMSO was used as a negative control. Overall antibacterial activities of *L. nobilis* extracts based on the zone of inhibition ranged between 7.00± 0.00mm and 16.0±1.00mm.

Among the extracts the ethyl acetate extract showed maximum zone of inhibition on *S. typhimurim* (16.0±1.00 mm). While, the chloroform extract showed high inhibition on *E. coli* (14.33±1.53mm) and methanol showed high activity on *S. aureus* which is 13.33±1.52 mm at concentration 200mg/mL compared to Tobramycin (17.30±0.50mm, 16.90±0.80mm and 19.16±0.58mm respectively) at a concentration of 10 µg/mL. However, the crude extract of methanol showed no inhibitory activity against *K. pneumonia, S. typhimurium and E. faecalis,* and the chloroform extract didn't inhibit *E. faecalis* at concentration of 25-200mg/mL respectively (Table 4).

In another study the extracts of *L. nobilis* were found to be prominently active against the tested microorganisms (*B. subtilis, S. aureus, S. epidermidis*, *E. coli* and *P. aeruginosa*) at the concentrations less than 5 mg/mL (Ertürk, 2006). In study conducted by Algabri *et al*., 2018, the methanol extract of Bay Leaves has an antibacterial activity against *S. aureus* with zone of inhibition 18 ± 0.8 mm. Whereas, there was no antibacterial activity on other tested bacteria like *P. aeruginosa and E. coli* (Algabri *et al*., 2018). This may support the findings of this study in terms of the antibacterial effect. The difference may be caused by several factors such as the type and origin of the *L. nobilis*, drying and extraction methods and difference in the crude concentration.

Furthermore, compound **1** (6.8 mg/mL) showed strong activity (13.0±1.73 mm) against *S.aureus*, compound **2** (5mg/mL) showed maximum inhibitory activity 12.0±1.0 against *E. coli*, compared to Tobramycin (19.66±1.34 mm and 19.83±1.15mm respectively) at 10 µg/mL concentration. The essential oil of *L.nobilis* shows an antibacterial activity in both Gram positive and negative strains at a concentration of 0.045 mg/mL. Among this maximum zone of inhibition of 12.33±0.58mm inhibitory diameter was measured in *P.*

aeruginosa and *K. pneumonia, the* positive control Tobramycin showed inhibitory zone of inhibition 24.0± 1.73mm and 12.33± 0.58 mm for tested bacteria pathogens respectively. The negative control (DMSO) showed no inhibitory activity against all tested bacteria (Table 7).

The antibacterial investigation of this work revealed the antibacterial activities of the extracts, isolated compounds and essential oil of *L. nobilis* increase with concentration. This observation is also in good agreement with previously reported finding, antibacterial activities were aggravated by increasing the quantity of compounds (Hossein *et al*., 2013), which can be used as an alternative for antibiotics.

There are several different methods for testing the antimicrobial activity of plants and their constituents, which may significantly influence the observed levels of inhibition. Additionally, various other factors, such as seasonality, variability the plant material, and the EO composition within a plant species, may cause differences in the antimicrobial activity outcomes (Fidan *et al*., 2019).

In this study the three extracts of *L.nobilis* exhibit different zone of inhibition against the tested organism (Table 5), this is because the solvents used for the extraction of the plant was different. In this case, they possess a wide variety of bioactive compounds, which exhibit different solubility properties with different solvents, hence screening with different solvent helps identify the most appropriate solvent for biological studies (Abarca-Vargas *et al*., 2016; Bakari *et al*., 2017). The ethyl acetate extract were showed good activity compared to other extracts.

A possible explanation for the antibacterial activity of *Laurus nobilis* EO is the fact that plant EOs disrupt cellular membranes and increase membrane permeability; they may alter membrane embedded proteins and subsequently disrupt membrane transport. It was previously demonstrated that terpenes are the constituents responsible for the antibacterial activity of *Laurus nobilis* EO (Siriken *et al*., 2018). Similar to this study finding, main constituent of *L. nobilis* EO in some previous studies was 1, 8-cineole, that has shown antimicrobial activity against several microorganisms (Baser & Buchbauer, 2015; Caputo *et al*., 2017).

The variation in the result of inhibition zones and MIC values shown by Gram positive and negative bacteria is attributed primarily to the chemical composition and morphology of the bacterial cell membrane. The pronounced antibacterial activity shown by Gram positive

bacteria could be due to the peptidoglycan layer which is permeable to the hydrophobic compounds present in the extract (Ramesh kumar *et al*., 2007). The resistance shown by Gram negative bacteria could be due to the lipopolysaccharide layer present in the cell membrane. The outer membrane of Gram negative bacteria is made up of tough and rigid lipopolysaccharide bilayer, with porins and uptake channels embedded in them, this kind of an arrangement is very efficient in resisting the entry of antibacterial compounds (Delcour, 2009). According to Zgurskaya *et al.*, 2015 the lipopolysaccharide layer is rigid and impermeable to hydrophobic compounds. The porins restrict the entry of hydrophilic compounds while the multidrug transporter pumps help in extruding the bioactive compounds. Hence, together they serve as an excellent permeability barrier to antimicrobial compounds across the cell membrane (Zgurskaya *et al*., 2015).

In the present study, *L. nobilis* extracts showed varied inhibitory activities against bacterial isolates, some isolates were significantly inhibited, while others responded poorly. The comparison of antibacterial activity of the extracts and isolated compounds were done with related work from literature. In the contrast to the previous research works the chloroform, ethyl acetate and compound **2** shows relatively high inhibition zone in Gram negative bacteria. Similar to our findings, essential oil of *L.nobilis* is more effective against Gram negative bacteria than Gram positive bacteria. This resistance is due to bacterial cellular membranes' nature group. Hence, their external structures make them to highly hydrophobic surface (Ouibrahim *et al*., 2013).

In this study the essential oil of *L.nobilis* exhibit greater antibacterial activity against Gram negative bacteria. This finding doesn't go along with the previous report of Fidan *et al*., 2019 that claimed Gram-positive bacteria are generally more susceptible to the action of the oils compared with the Gram-negative ones. In contrary to our current finding EO of *L. nobilis* didn't show inhibitory activity against *P .aurognosa* and *E. coli*. The main EOs constituent of this *L.nobilis* was 1,8-cineole, that has shown good antimicrobial activity against several microorganisms, and this constituent has been also reported by previous studies (Baser & Buchbauer, 2015; Caputo *et al*., 2017). Each EO included a mixture of a number of constituents that may have contributed to the extended spectrum of antimicrobial activity.

Table 5: Antibacterial activities of extracts from *Laurus nobilis*

Extract type	Conc.(mg/mL)	Inhibition Diameter (mm)±SD					
		E. coli	*P. aeruginosia*	*K. pneumonia*	*S. typhimurium*	*E. faecalis*	*S. aureus*
Methanol	200	NI	9.00±0.00	NI	NI	NI	13.67±0.58
	100	NI	8.67±0.58	NI	NI	NI	13.33±1.52
	50	NI	7.00±0.00	NI	NI	NI	12.33±0.58
	25	NI	7.00±0.00	NI	NI	NI	12.67±0.58
Chloroform	200	14.33±1.53	13.0±0.00	14.00±0.00	11.67±1.15	NI	12.33±1.53
	100	14.00±1.00	12.67±0.58	12.67±0.57	11.33±0.58	NI	11.0±1.73
	50	12.67±0.58	12.0±1.0	11.67±1.15	10.33±0.58	NI	10.0±1.73
	25	11.33±0.58	8.0±1.0	6.33±0.58	8.67±1.15	NI	9.0±1.73
Ethyl acetate	200	14.33±0.58	14.0±0.00	13.0±0.00	16.0±1.00	14.67±0.58	13.33±1.53
	100	14.33±0.58	13.0±1.00	13.0±0.00	15.33±1.53	13.67±0.58	13.33±1.53
	50	14.0±1.00	12.33±0.58	11.67±0.58	14.0±1.00	13.0±0.00	8.33±0.58
	25	14.0±1.00	11.67±0.58	10.67±1.15	13.0±1.00	12.33±2.08	7.33±0.58
Tobramycin	10µg/mL	16.90±0.80	20.84±0.80	11.33±0.80	17.30±0.50	13.83±1.15	19.16±0.58
-ve control		6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Key: NI= No Inhibition

Table 6: Antibacterial activities of isolated compounds from *Laurus nobilis*

Active compounds	Conc. (mg/mL)	Inhibition Diameter (mm)±SD					
		E. coli	*P. aeruginosia*	*K. pneumonia*	*S.typhimurium*	*E. faecalis*	*S.aureus*
Compound **1**	6.8	11.0±1.00	9.67±0.58	10.67±1.15	10.67±0.58	NI	13.0±1.73
	3.4	10.33±1.53	8.67±0.58	10.33±0.58	9.33±0.58	NI	11.33±0.58
	1.7	9.0±0.00	8.33±0.58	9.33±1.53	8.33±0.58	NI	10.0±0.00
	0.85	9.0±0.00	7.67±0.58	9.0±1.00	7.67±0.58	NI	8.33±1.53
Compound **2**	5	12.0±1.00	11.67±1.08	11.67±0.57	8.00±0.00	NI	9.0±0.00
	2.5	11.0±0.00	10.67±1.08	11.33±0.57	7.0±0.00	NI	8.0±0.00
	1.25	11.0±0.58	9.67± 1.08	10.67±0.57	7.0±0.00	NI	7.0±0.00
	0.6	11.0±1.00	8.67±0.58	10.67±0.57	7.0±0.00	NI	7.0±0.00
Tobramycin	10 µg/mL	19.83±1.15	18.00±1.26	11.16±1.0	16.33±1.34	10.33±0.58	19.66±1.34
-ve control		6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Key: NI=No Inhibition

Table 7: Antibacterial activities of essential oil from *Laurus nobilis*

Essential oil	Conc. (mg/mL)	Inhibition Diameter (mm)±SD					
		E. coli	*P. aeruginosia*	*K. pneumonia*	*S.typhimurium*	*E. faecalis*	*S.aureus*
	0.045	12.33±1.15	12.33±0.58	12.33±0.58	NI	11.33±1.15	11.67±1.15
	0.022	10.6±0.58	11.67±0.58	10.33±0.58	NI	9.33±1.53	10.67±1.15
	0.011	10.33±0.58	10.0±0.00	10.33±1.15	NI	7.00±0.00	7.0±0.00
	0.005	10.0±0.00	9.0±0.00	9.67±1.52	NI	7.0±0.00	7.0±0.00
Tobramycin	10µg/mL	16.67±1.15	20.0±1.73	12.33±0.58	14.33±1.53	10.67±0.58	20.0±1.00
-ve control		6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Key: NI=No Inhibition

4.4.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that prevents the growth of microorganism after a specific incubation time. The MIC was studied for those bacterial strains which were sensitive to the plant extracts in the disk diffusion assay. The MIC value for the extracts were 2.5 mg/mL for the EtOAc and chloroform extracts (except *P. arognosa* and *S. aureus* which were inhibited at 5mg/mL). For the Methanol extract and isolated compounds the MIC was 1.25 mg/mL and 0.125 mg/mL respectively. Essential oil MIC was 0.11 mg/mL against all tested bacteria except for *S. aureus* (0.22 mg/mL).

The MBC was validated by the absence of bacterial growth in the nutrient agar media streaked with inhibition zones matching to the tested strains' lowest MICs. Ethyl acetate and chloroform extract showed potentially bactericidal activity against the tested pathogenic bacteria with MBC of 5 mg/mL except in the case of chloroform the MBC for *P. arognosa* and *S. aureus* bacteria was 10 mg/mL, while the MBC of methanol extract was 2.5mg/mL against all of the tested bacteria. In the case both isolated compounds show MBC activity at 0.25 mg/mL. The MBC result for the essential oil was 0.22mg/mL except for *S. aureus* which was less sensitive and its minimal bactericidal concentration reached to 0.44 mg/mL.

Table 8: The MIC and MBC value for the extracts

Bacteria	Methanol		Chloroform		Ethyl acetate	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E. coli	_	_	2.5 m	5	2.5	5
P. aeruginosia	1.25	2.5	5	10	2.5	5
K. pneumonia	_	_	2.5	5	2.5	5
S.typhimurium	_	_	2.5	5	2.5	5
E. faecalis	_	_	_	_	2.5	5
S. aureus	1.25	2.5	5	10	2.5	5

Table 9: The MIC and MBC value for the isolated compounds and essential oil

Bacteria	Compound **1**		Compound **2**		Essential oil	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E. coli	0.125	0.25	0.125	0.25	0.11	0.22
P. aeruginosia	0.125	0.25	0.125	0.25	0.11	0.22
K. pneumonia	0.125	0.25	0.125	0.25	0.11	0.22
S.typhimurium	0.125	0.25	0.125	0.25	_	_
E. faecalis	_	_	_	_	0.11	0.22
S. aureus	0.125	0.25	0.125	0.25	0.22	0.44

4.4.3 Antioxidant activity of *Laurus nobilis*

The DPPH radical scavenging activities (%) of crude extracts were 94.75, 89.12, 96.11 for the ethyl acetate, chloroform and methanol extracts respectively. Among the studied crude samples MeOH extract of (1000 µg/mL) has the same radical scavenging activity with the standard ascorbic acid (96.11). For compound **1**, compound **2** and essential oil sample at 100µg/ml concentration free radical scavenging activities were 55.69, 77.75 and 24.40 respectively. The obtained result showed that activity of the extracts and compounds against DPPH radical was dose dependent, in which the DPPH scavenging activity percentage was directly increase with the concentration increases (Elmastas *et al*., 2006).

The IC_{50} value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, Low IC_{50} values correspond to a higher antioxidant capacity (Fausto *et al*., 2020).

Numbers of studies have been done to evaluate the antioxidant properties of different extracts of *L. nobilis* leaves. In this study IC_{50} value for the ethyl acetate, chloroform and methanol extracts were 45.5, 153.2 and 0.02 respectively. Since low IC_{50} values correspond to a higher antioxidant capacity, compare to previous research studies done on the antioxidant activity of ethyl acetate extract of *L.nobilis* the current study result (45.5) is the better one. DPPH free radical inhibition IC_{50} value of ethyl acetate extract of *L. nobilis* leaves was found to be 75.65 µg/mL (Conforti *et al*.,2016). In another study, ethyl acetate extract of *L. nobilis* leaves exhibited an IC_{50} value of 83.24 µg/mL (Kaurinovic *et al*., 2010). More studies in the literature indicated that leaf extracts of *L. nobilis* provide

significant antioxidant effect (Ferreira *et al*., 2006; Dias *et al*., 2014). This paper results are compatible with the literature with respect to antioxidant effect.

Laurus nobilis extract recorded an effective antioxidant capacity, showing that the phenolic compounds present in this extract have a high degree of hydroxylation, which is manifested in the high capacity to donate protons and thus stabilize the DPPH radical. Elmastaş *et al*. (2006) studied the antioxidant activity of ethanolic extracts of Bay Leaves (at 60 µg/ mL) and reported an inhibition of 92% of radical DPPH. This is consistent with the results reported by Muñiz *et al*.,2013, who evaluated an antioxidant activity (94% inhibition DPPH radical) in *L. nobilis* extract, this result is almost comparable to our finding in the ethyl acetate extract (94.66% in the concentration of 500µg/mL).

All tested samples except essential oil samples in the high concentration show color change from purple to yellow. This happens because of the scavenging effect of the extract antioxidants, the color changes from purple to yellow thus cause a proportional decrease of absorbance as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through a donation of hydrogen to form the stable DPPH-H molecule (Wissal *et al*.,2020).

The antioxidant activity of the plant extracts would be expected to relate to the chemical composition (Renuka *et al*., 2014; Archnad *et al*., 2015; Bimal *et al*., 2017). Therefore, there is a certain correlation between the antioxidant activity and the total amount of phenols and flavonoids (Silva *et al*., 2017). Laurel leaves represent an important antioxidant source of natural phenols and flavonoids which are beneficial to human public health. Indeed, the use of these substances may help in reducing chemical products which usually induce certain secondary effects (Amal *et al*., 2018).

According to Ibrahim *et al*., 2020 the strong free radical scavenging capacity of the essential oil of *L. nobilis* may be due to different chemical compounds present in the oil, especially the relatively high percentage of 1.8-cineole (26.3%) and α-terpinyl acetate(17.1%),although other compounds such as Methylisoleugenol (9.1%), α.-Terpineol (5.2%) and Linalool (4.6 %) may be involved.

Furthermore, the antioxidant activity may be altered by synergistic and antagonistic effects between some components of the essential oil (Guenane *et al*., 2016). Our results are in

accordance with those found by other researchers (Santoyo *et al*., 2006; Bounatirou *et al*., 2007; Celikel & Kavas ,2008; Cherrat *et al*., 2014), further confirming the antioxidant capacity of essential oil from *L. nobilis* and its potential as a natural preservative in food and pharmaceutical industries.

It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis (Elmastaşa *et al*., 2006). Oxidative stress is identified as an imbalance between production of reactive species and antioxidant defense activity, and an increase in antioxidants is directly associated with the reduction of degenerative diseases like cancer, diabetes, neurodegenerative and cardiovascular diseases (Carocho & Ferreira, 2013). Based on the data obtained from this study, all crude extracts, isolated compounds and the essential oil are free radical inhibitors or scavengers, which may limit free radical damage occurring in the human body.

Table 10: Antioxidant activities of crude extract from *Laurus nobilis*

		Crude Extracts						Positive control	
		Ethyl acetate		Chloroform		Methanol		Ascorbic Acid	
Conc. (µg/mL)	Control	A	%RSA	A	%RSA	A	%RSA	A	%RSA
1000	1.029	0.054	94.75	0.112	89.12	0.04	96.11	0.04	96.11
500	1.029	0.055	94.66	0.279	72.89	0.049	95.24	0.045	95.63
250	1.029	0.181	82.41	0.428	58.41	0.056	94.56	0.047	95.43
125	1.029	0.358	65.21	0.543	47.23	0.142	86.2	0.05	95.14
IC_{50}		45.5		153.2		0.02			

Table 11: Antioxidant activities of isolated compounds from *Laurus nobilis*

Concentration (µg/ml)	Control	Compounds				Positive control	
		Compound **1**		Compound **2**		Ascorbic Acid	
		A	%RSA	A	%RSA	A	%RSA
100	1.029	0.456	55.69	0.23	77.75	0.085	91.74
50	1.029	0.515	49.95	0.24	76.87	0.168	83.67
25	1.029	0.708	31.2	0.35	65.99	0.22	78.62
12.5	1.029	0.766	25.56	0.67	35.18	0.67	34.89
IC_{50}		50		15.45		0.016	

Table 12: Antioxidant activities of essential oil from *Laurus nobilis*

Concentration (µg/ml)	Control	Essential oil		Positive control	
				Ascorbic Acid	
		A	%RSA	A	%RSA
100	1.275	0.964	24.40	0.028	97.80
50	1.275	1.027	19.45	0.028	97.80
25	1.275	1.059	16.94	0.032	97.49
12.5	1.275	1.086	14.82	0.035	97.25
IC_{50}		339.96			

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Nowadays, it is clear to see that the escape from synthetic drug will further increases the importance of natural products. With this reason, investigation of composition and biological activities of natural substances is important. The air dried *L. nobilis* was extracted by using solvents like methanol, chloroform and ethyl acetate. Qualitative phytochemical screening tests were done on the crude extracts of *Laurus nobilis* leaf and the results showed the presence of alkaloid, phenol, saponins, terpenoids, steriod, flavonoid and tannins. The EtOAc crude extract of the plant subjected to column chromatography and revealed two compounds namely Compound **1** and Compound **2** they were characterized by NMR spectroscopy technique.

GC-MS analysis of essential oils from leaves of *L.nobilis* revealed a total of 48 chemical components, accounting for 91.4 % of the total compositions, the major constitutes were 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- (2-Hydroxy-1,8-cineole) (26.3%), 3-Cyclohexene-1-methanol, .α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate) (17.1%), Benzene, 1,2-dimethoxy-4-(2-propenyl)- (Methylisoleugenol) (9.1%), α.-Terpineol [.α.,α.,4-trimethyl-3-cyclohexene-1-methanol](5.2%), and 1,6-Octadien-3-ol, 3,7-dimethyl--(Linalool) (4.6 %).

The study showed the antibacterial and antioxidant activity of crude extracts, isolated compound and essential oil of *L.nobilis* on the bacterial strains through in vitro appeared interesting and promising. The crude extract of ethyl acetate showed the highest zone of inhibition against *S. thyphimurium* (16.00± 1.00mm) at the concentration of 200 mg/mL compared to the positive control Tobramycin (17.30±0.50mm) at the concentration of 10 µg/mL. From the extracted compounds, compound **1** show better antibacterial activity against *S.aureus* (13.0±1.73mm) at the concentration of 6.8 mg/mL compared to Tobramycin (19.66± 1.34mm) at the concentration of 10 µg/mL. Essential oil of *L.nobilis* showed maximum zone of inhibition against *P. aeruginosa and K. Pnumonia* (12.33±0.58mm) at the concentration of 0.045 mg/mL compare to Tobramycin (24.0±1.73 and 12.33±0.58mm) respectively at the concentration of 10 µg/mL.

The radical scavenging activity of *L. nobilis* crude extracts (methanol, chloroform and ethyl acetate), isolated compounds (compound **1** and compound **2**) and essential oil were 94.75,89.12 and 96.11 for the crude extracts respectively and the positive standard ascorbic acid %RSA was 96.11 at the concentration of 1000µg/mL, for the compound **1** (55.69) , compound **2** (77,75), compared to ascorbic acid (91.74),in the case of essential oil (24.40) compare to ascorbic acid 97.80 at the concentration of 100µg/mL. The findings of this study supports the traditional use of the plant for the treatment of infectious disease caused by Gram positive and Gram negative bacterial strains.

5.2 Recommendation

These observations promote further research work with biological activity (antifungal, anticancer and cytotoxicity) on crude extracts as well as isolated compounds and essential oil. Additional research efforts are also recommended to conduct structure activity relationships. It is recommended that a detail study on 2D, NMR and MS which is essential to fully characterize structures of the isolated compounds. Our findings show isolated compounds from this plant might be used as a lead compounds in the process of bacterial and bioactive drugs.

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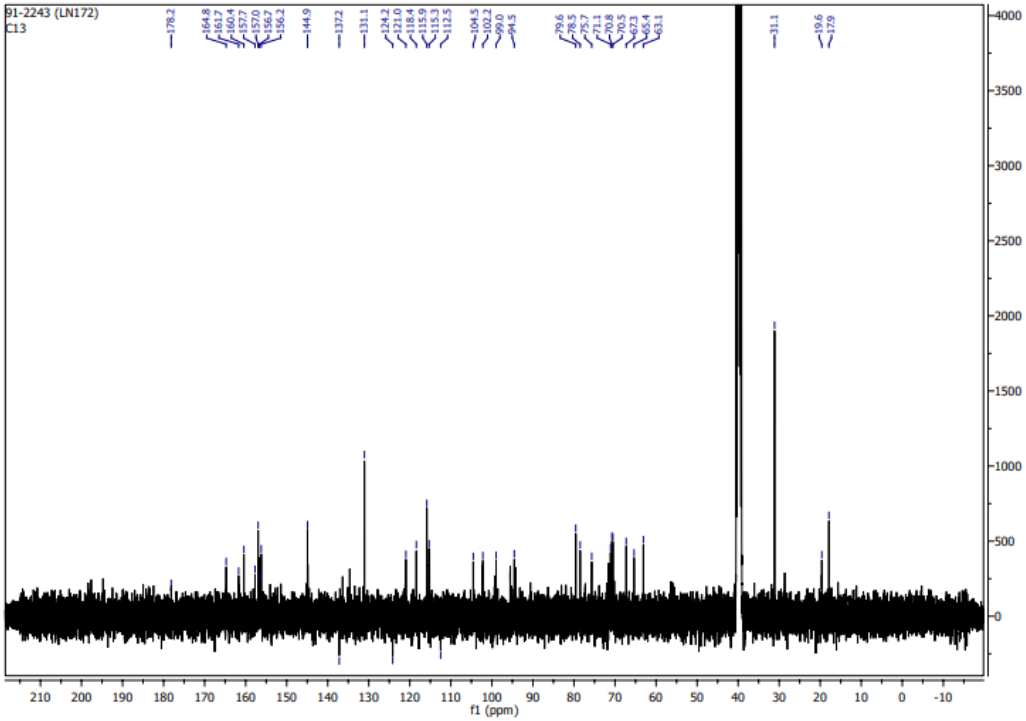
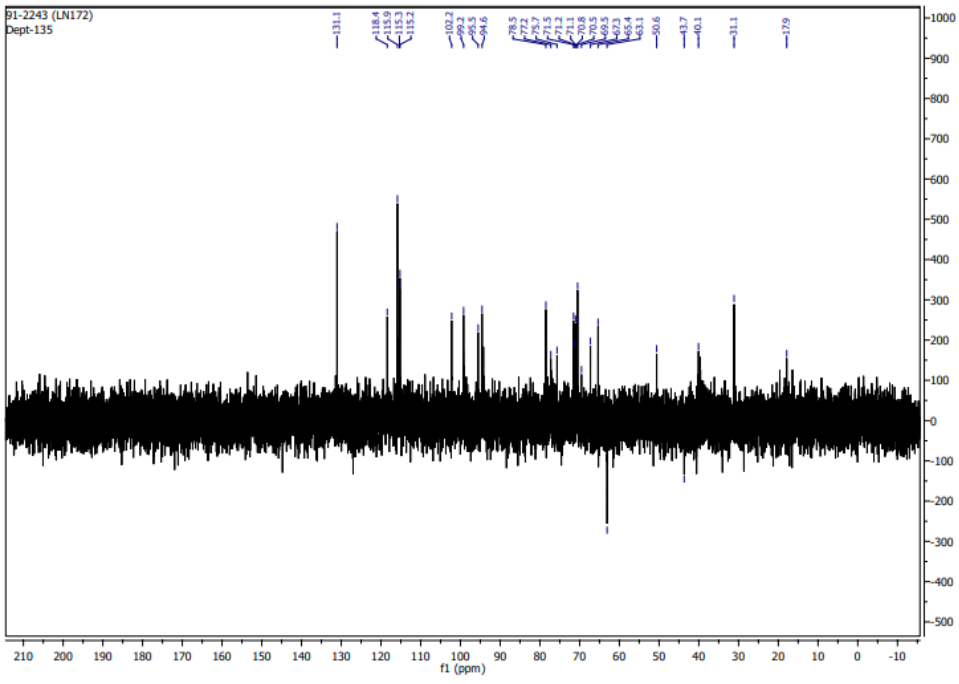
Appendixes

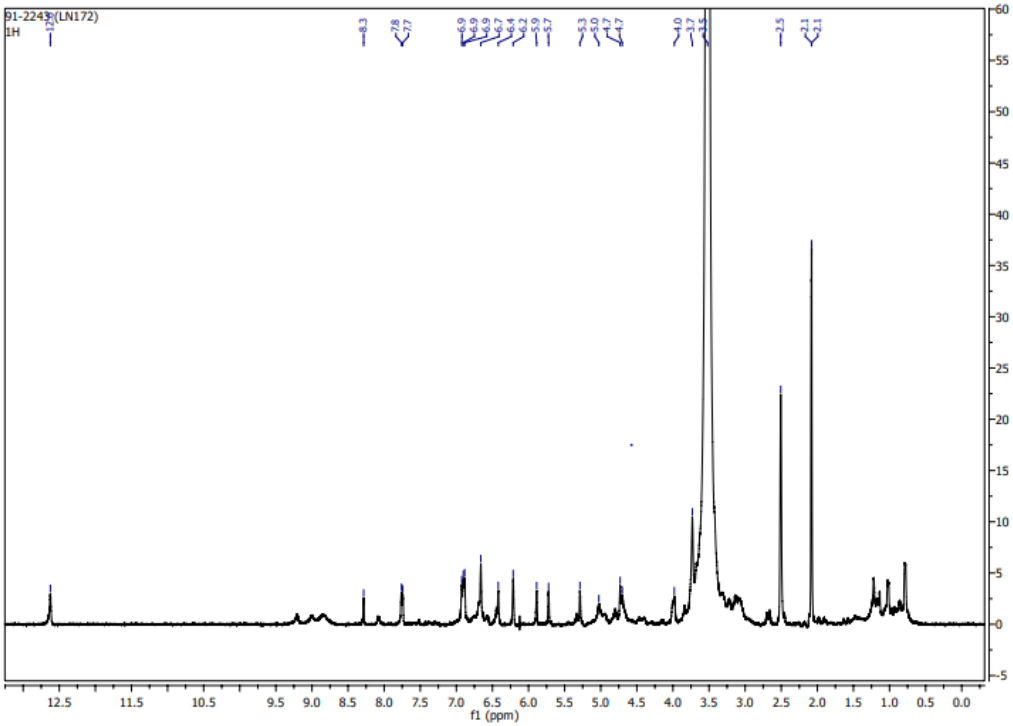
Appendix 1: ^{13}C-NMR spectrum of compound **1**



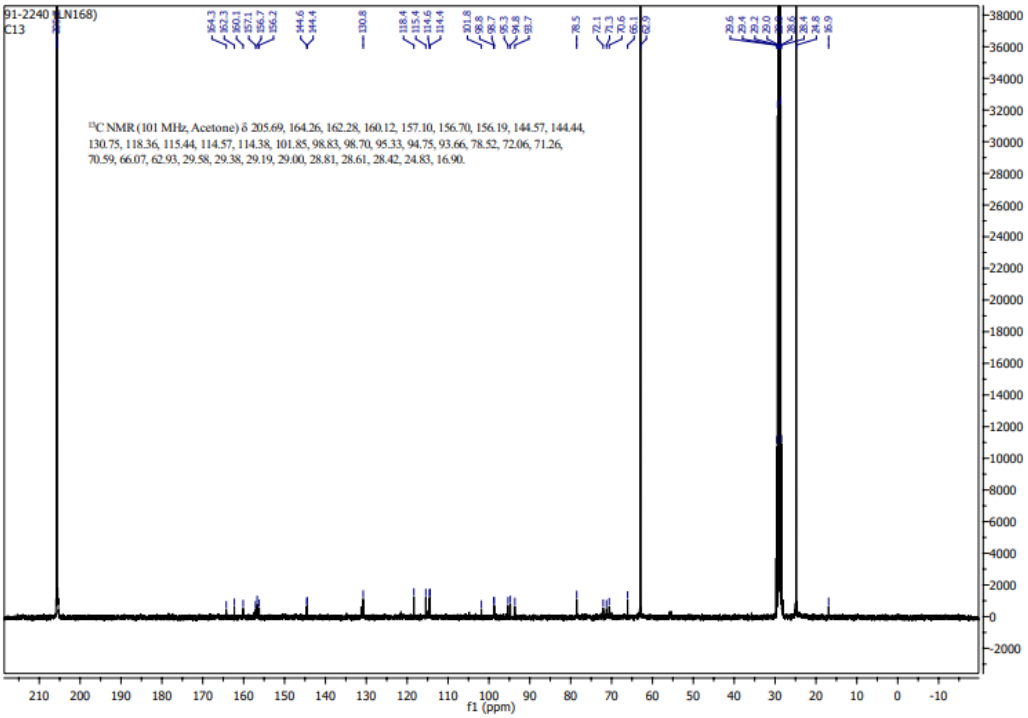
Appendix 2: DEPT-135 spectrum of compound **1**



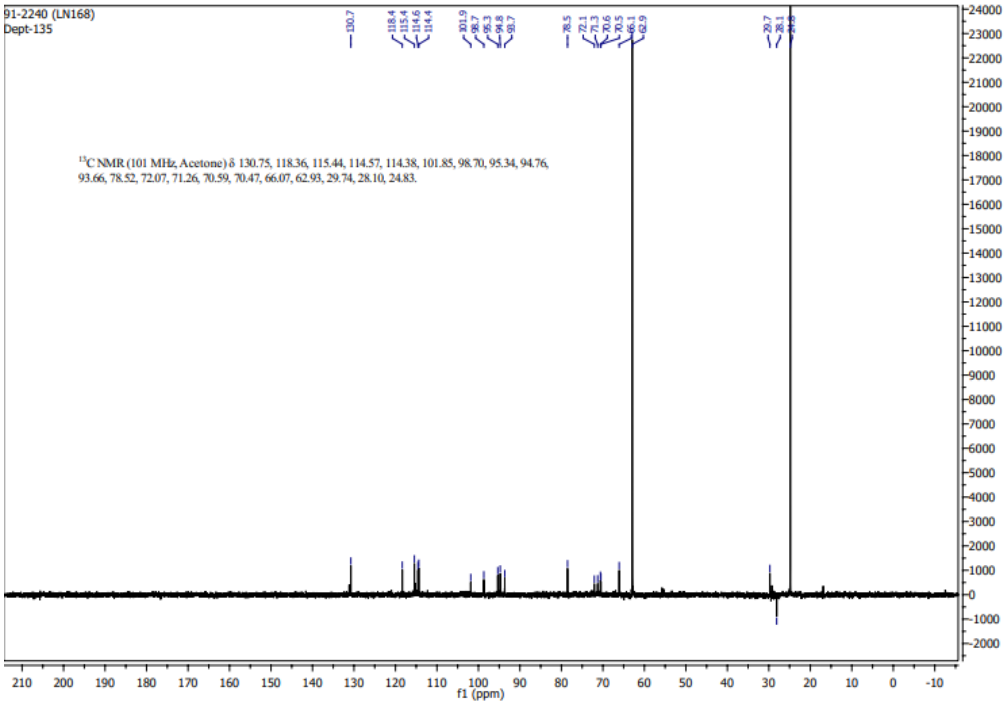
Appendix 3: ^{1}H NMR spectrum of compound **1**



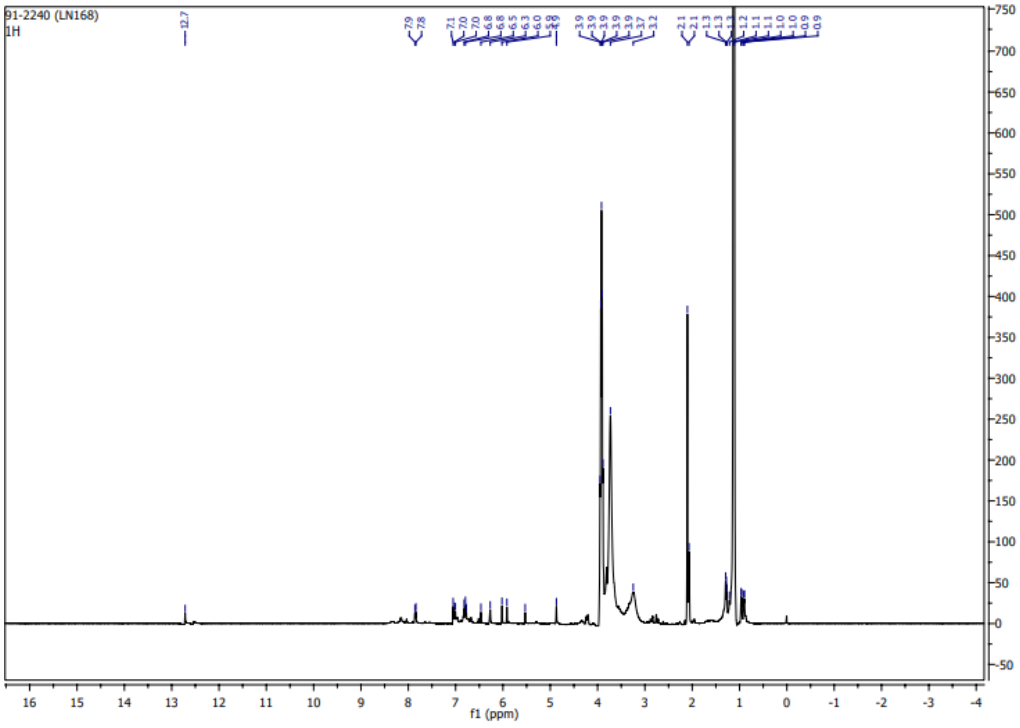
Appendix 4: ^{13}C-NMR spectrum of compound **2**



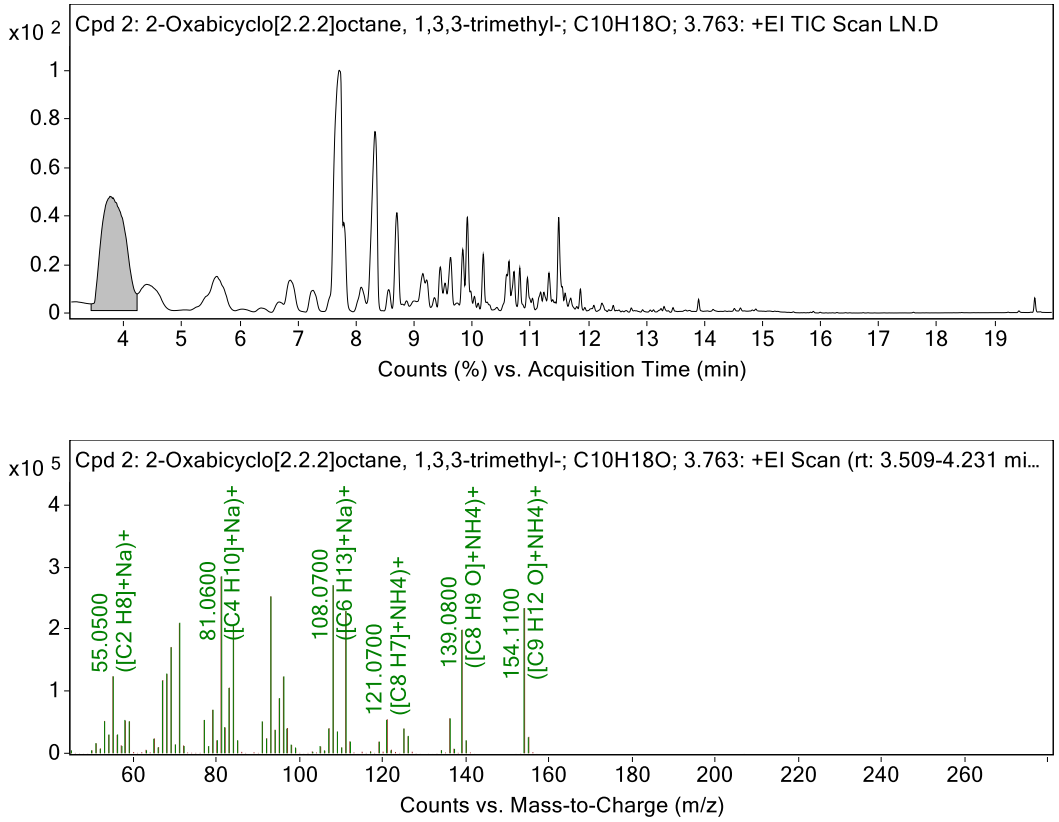
Appendix 5: DEPT-135 spectrum of compound **2**



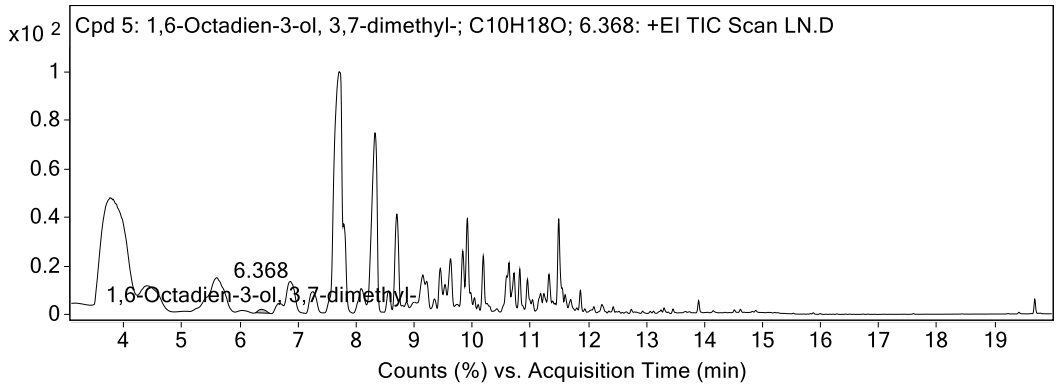
Appendix 6: ^{1}H NMR spectrum of compound **2**

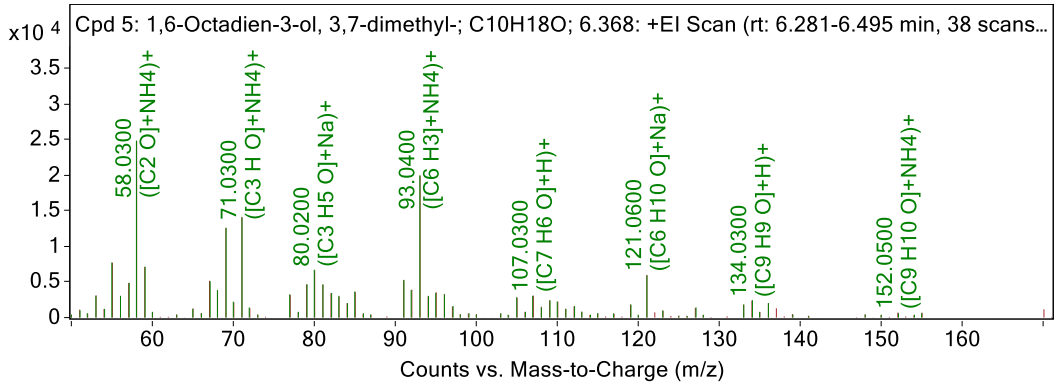


Appendex7: The GC-MS result of 2-Oxabicyclo[2.2.2]octane,1,3,3-trimethyl-(2-Hydroxy-1,8-cineole)

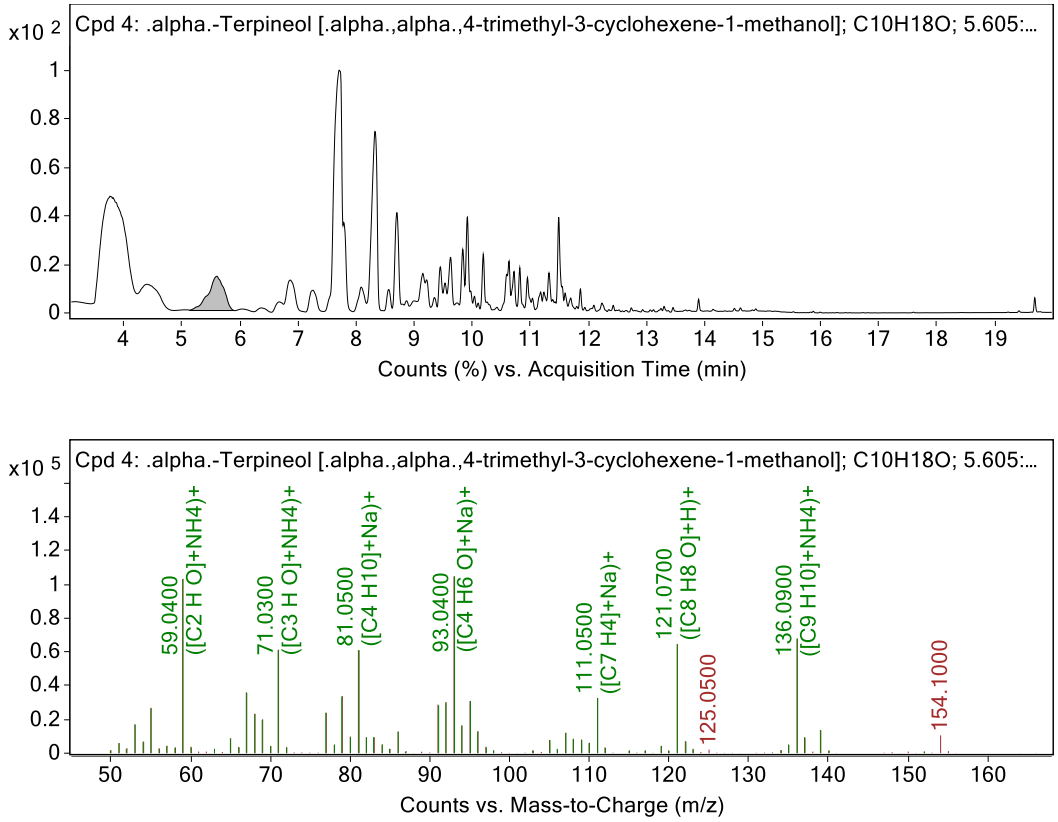


Appendex8: The GC-MS result of 1,6-Octadien-3-ol, 3,7-dimethyl-(Linalool)

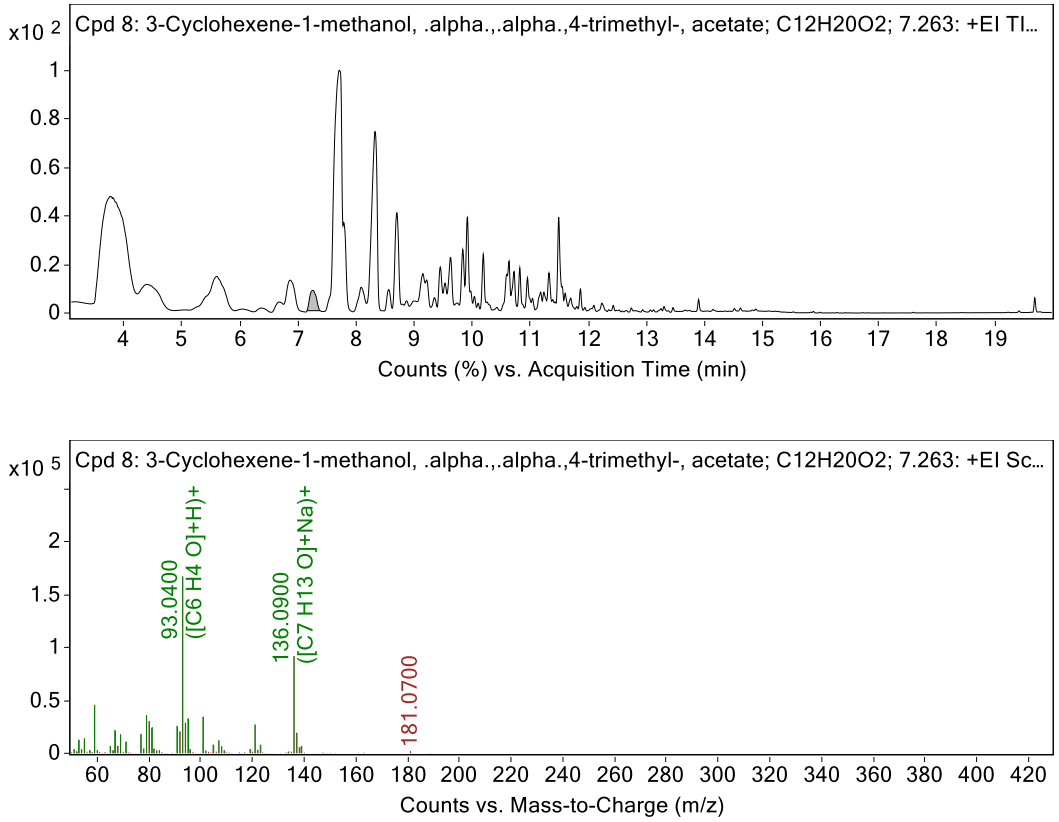




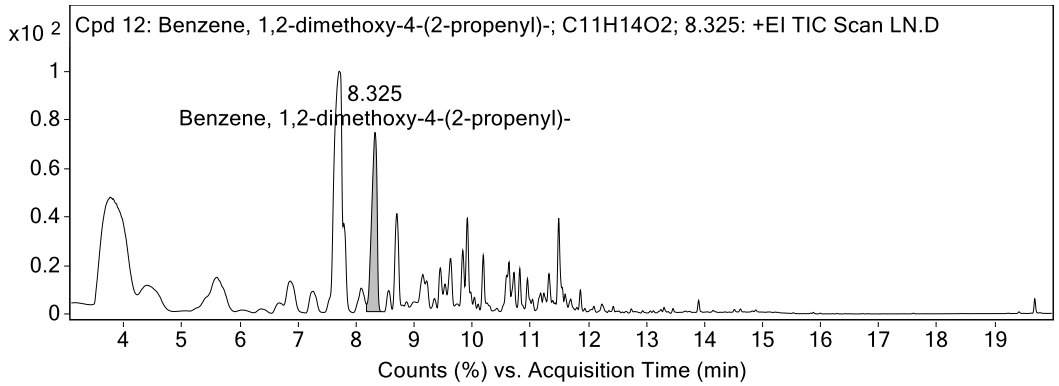
Appendex 9: The GC-MS result of α-Terpineol[.α.,α.,4-trimethyl-3-cyclohexene-1-methanol]

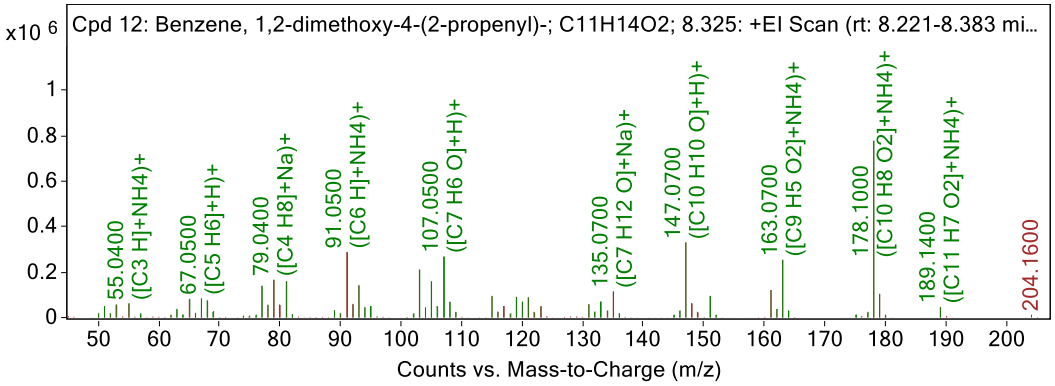


Appendex 10: The GC-MS result of 3-Cyclohexene-1-methanol,.α.,.α.,4-trimethyl-, acetate (α-terpinyl acetate



Appendex 11: The GC-MS result of Benzene, 1,2-dimethoxy-4-(2-propenyl)-




Cpd 12: Benzene, 1,2-dimethoxy-4-(2-propenyl)-; C11H14O2; 8.325: +EI Scan (rt: 8.221-8.383 mi…
x10 6
1
0.8
0.6
0.4
0.2
0
55.0400 ([C3 H]+NH4)+
67.0500 ([C5 H6]+H)+
79.0400 ([C4 H8]+Na)+
91.0500 ([C6 H]+NH4)+
107.0500 ([C7 H6 O]+H)+
135.0700 ([C7 H12 O]+Na)+
147.0700 ([C10 H10 O]+H)+
163.0700 ([C9 H5 O2]+NH4)+
178.1000 ([C10 H8 O2]+NH4)+
189.1400 ([C11 H7 O2]+NH4)+
204.1600
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200
Counts vs. Mass-to-Charge (m/z)