

**CHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITIES OF
ESSENTIAL OILS OF *EUCALYPTUS GLOBULUS* LEAVES**

M.Sc. THESIS

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**Chemical Profile and Antimicrobial Activities of Essential
Oils of *Eucalyptus globulus* Leaves**

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**In Partial Fulfillment of the Requirements for the MASTER OF SCIENCE
DEGREE IN BIOTECHNOLOGY**

By

Yemane Werede

October 2016

Haramaya University, Ethiopia

DEDICATION

I dedicate this thesis to my family for their love and sacrifice throughout my education for the success of my life

STATEMENT OF THE AUTHOR

First, I declare that this thesis entitled **Chemical Profile and Antimicrobial Activities of Essential Oils of *Eucalyptus globulus* Leaves** is the result of my own research except as cited in the references. All sources of materials used for this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at the Haramaya University and can be deposited at the University library to be made available to borrowers under the rules of the library.

I assert that this thesis is not submitted to any other University or institution anywhere for the award of any academic degree.

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BIOGRAPHICAL SKETCH

Mr Yemane was born in Abiy-Adi wereda, Central Zone, Tigray Regional State, from his father Werede Hailu and his mother Mulu Kahsay in May, 1986. He attended his elementary school education at Guya Primary School. He also attended his high school at Mekelle Secondary School and preparatory school education at Abiy-Adi senior Secondary School, respectively. After completing his preparatory school education in 2006, he joined Bahir Dar University in 2007 and graduated with Bachelor of Education (B.Ed.) degree in Biology in 2009. Soon after graduation, he was employed by Ministry of Education in Addis Ababa and served as biology teacher for about 6 years in Addis Ababa. Then he joined Haramaya University for his graduate studies in the summer in-service program in 2016 to specialize in biotechnology.

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LIST OF ABBREVIATION AND ACRONMYS

DMSO	Dimethyl Sulfide
DNA	Doxy ribose Nucleic Acid
EO	Essential Oil
EPHI	Ethiopian Public Health Institute
FDA	Food and Drug Authority
GC	Gas Chromatography
HMT	Hydrocarbon Mono Terpene
IGE	Immunoglobulin E
HST	Hydrocarbon SesquiTerpene
MS	Mass Spectrometer
OC	Oligo-carragenans
OMT	Oxygenated Mono Terpene
OST	Oxygenated SesquiTerpene
PPCS	Polyphenolic compounds
RNA	Ribose Nucleic Acid
RTI	Respiratory Tract Infection
TC	Total Composition
TLC	Thin Layer Chromatography
UNESCO	United Nations Educational, Scientific and Cultural Organization
UTI	Urinary Tract Infection
UVS	Ultra Violate spectroscopy
WHO	World Health Organization

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Chemical Profile and Antimicrobial Activities of Essential Oils of *Eucalyptus globulus* Leaves

ABSTRACT

Eucalyptus globulus is one of the abundant tree species in Ethiopia known for medicinal values of its essential oils. The essential oil composition of a given species may vary with environmental factors. In this study, leaf of essential oil compositions and their antimicrobial activities were analyzed from *E. globulus* sampled from Haramaya University (HU) and Entoto Park (EP). Essential oils were extracted by steam distillation method and their compositions were analyzed using GC. Antimicrobial activities of the essential oils at different concentration levels were assessed with streptomycin and dimethyl sulfoxide used as positive and negative controls, respectively. The results showed that the essential oils from HU and EP samples contained 20 and 17 terpene compounds, respectively. Chemical profile of essential oils contained Terpene, Oxygenated monoterpene, Hydrocarbon monoterpene, Oxygenated sesquiterpene and Hydrocarbon sesquiterpene ranging from 86.67-87.01%, 8.01-8.32%, 1.57-1.97%, and 1.04 -2.64%, respectively. The extracted essential oils were also evaluated for their antibacterial activities by papering disc diffusion method against four bacterial species of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. EPEO extracts of the plant were shown to have higher antibacterial activities with maximum zone of inhibition (14.43 ± 0.15 mm) against *S. aureus* and minimum zone of inhibition (7.77 ± 0.29 mm) against *P. aeruginosa* than HUEO extract with the maximum zone of inhibition (14.23 ± 0.11 mm) against *S. aureus* while minimum zone of inhibition (6.47 ± 0.21 mm) against *P. aeruginosa* compared with that of Streptomycin against bacteria as references antibiotics.

Keywords: Essential oil, *Eucalyptus globulus*, Terpene, Monoterpene, Sesquiterpene, antimicrobial activity

1. INTRODUCTION

Eucalyptus plant belongs to the *Myrtaceae* (*Myrtle*) family. *Eucalyptus* is a tall evergreen tree native to Australia, nowadays found around the world. It now grows in almost all tropical and subtropical areas and is cultivated in many other climates. *Eucalyptus* was introduced to Ethiopia in either 1894 or 1895, either by Emperor Menelik II. Menelik II endorsed its planting around his new capital city of Addis Ababa because of the massive deforestation around the city for firewood (Pankhurst, 1968).

Plantations of *eucalyptus* spread from the capital to other growing urban centers and later expanded throughout the country. Pankhurst(1968) reports that the most common species found in Addis Ababa in the mid-1960s was *Eucalyptus globules*, although he also found *Eucalyptus melliodora* and *Eucalyptus rostrata* in significant numbers. *Eucalyptus globulus* is an extremely adaptable species which grows well on a variety of soils and in a variety of climates. In Ethiopia it succeeds everywhere in the highlands, and does best at altitudes from 1800-2600m on loamy soils. *Eucalyptus camaldulensis* grows well over a wide range of soils and is more suited to lower elevations (Husnia, 1985).

The products of this tree have multifold uses i.e. medicinal as well as industries, but due to harmful side effects of synthetic products and the fact that plants are easily accessible, interest in drugs of herbal origin has significantly increased. Scientific research is being conducted all over the world to determine whether plants that are traditionally used to treat various diseases are actually appropriate for their intended use. *Eucalyptus* leaf extract, a natural food additive, is commercially available as steam distillate, or an ethanol extract of *Eucalyptus globulus* leaf (Usman *et al.*, 2009).

Much research has been conducted on the medicinal properties of *Eucalyptus*. Of the different species, *E. globulus* has been the most widely studied. Several hundred species of *Eucalyptus* contain volatile oils which are classified into three types; medicinal, perfumery and industrial. Some studies have demonstrated that leaf extract and essential oil of *Eucalyptus* (Boland *et al.*, 1991).

E. globulus is the one of the most important species of Eucalyptus. Within species the quantity of essential oil and the specific compounds in the essential oil and extracts of dry and fresh leaves, buds, mature fruit, and bark vary with the origin of the tree and the age of the leaves. The essential oil in the leaves is commonly used for medicinal purposes. The pharmacological activity of eucalyptus is related to the content of several classes of secondary metabolites. The important compounds found in the leaves, buds, branches and bark of *E. globulus* include such as essential oils, terpinene and terpineol (Freire *et al.*, 2011).

Eucalyptus is known to be a rich source of secondary compounds with a variety of biological activities. The essential oil of *Eucalyptus globulus* contains 1,8-cineole as its major constituent that varies between 70-95%, however, some studies have shown as little as 4% in its oil. The use of *eucalyptus* oils, mostly obtained from the leaves, is grouped as medicinal, industrial, aromatic and flavoring, depending on their chemical composition. Essential oils are volatile aromatic products that can be carried by steam and are extracted by steam distillation (Tkaczyk *et al.*, 2006). *Eucalyptus* oil is sold world-wide. Most of the oil sold in Europe and North America is used in products'. Medicinal *eucalyptus* oil produced from *Eucalyptus globulus* is widely used for the relief of cold and influenza symptoms. It is a unique natural product having antiseptic properties and the power to clear the nasal passages and bronchial tubes making it easier to breathe (Frette *et al.*, 2011).

A popular new use is to vaporize it in saunas. It is an excellent rub for muscular aches and pains and it has been widely used for many years by sportsmen to help keep muscles trim and supple. A use which is gaining widespread acceptance is the practice of adding *eucalyptus* oil to the laundry wash for cleaning and freshening clothes, which utilizes its cleaning, deodorizing and antiseptic properties (Sporer *et al.*, 2011). Medicinal *eucalyptus* oil is used extensively as a raw material and active ingredient in cough lozenges inhalation sprays and drops, gargles, mouth washes, toothpastes, balms and ointments, liniments and soaps. *Eucalyptus* oil is also used in antiseptics and germicidal disinfectants because of its pleasant odor and its effectiveness in killing bacteria (Loo *et al.*, 2010).

In Ethiopia, the increased demand for wood, particularly fuel wood, has led to a rapid expansion of plantations of fast-growing species of *Eucalyptus globulus*, and more than 127,000 ha of land have been planted in the last decades and usually harvested at the age of 5 - 7 years (Kidanu, 2004). The *eucalyptus* species growing in Ethiopia have been the subject of only botanical studies. The essential oils of some of these *eucalyptus* species found in the central parts of the country were studied (Nyssen, 2005) but to the best of our knowledge, no reports are available on the constituents of the *eucalyptus* species grown in the eastern and central Ethiopia. Extraction and selling of the *eucalyptus* oil is not also widely known in most regions of the country. It is therefore necessary to investigate the chemical composition of the essential oils of these plants and correlate the economic potential of the essential oils with the volatile oil constituents. The leaves may contain essential oils of medicinal importance, but, variation in the contents and composition of its oils may occur due to different soil natures and climatic conditions.

Though many studies have shown the antimicrobial properties of essential oils from *E. globulus*, efficacy may vary based on origin of the plant as essential composition can vary with geographical location. The study hypothesized that essential oil from leaves of Haramaya University vary in its composition and antimicrobial effect from that of Entoto Park. With this premise this study was designed to analyze essential oil composition of *E. globulus* leaves obtained from Haramaya University and Entoto Park, and compare their antimicrobial effects on selected pathogenic bacteria with the following general and specific objectives.

General Objective of the Study was:

- To characterize the chemical profile of essential oils of *Eucalyptus globulus* leaves and evaluate their antimicrobial activities *in vitro*

Specific objectives of the study were:

- To extract and analyze the essential oil composition of *Eucalyptus globulus* leaves
- To test the antimicrobial activities of essential oils of *Eucalyptus globulus* leaves against selected bacterial pathogens

2. LITERATURE REVIEW

2.1. Botanical Description and Ecology of *Eucalyptus globulus*

Eucalyptus is a diverse genus of trees, the member of which are dominant tree flora of Australia. There are almost 600 species of *Eucalyptus*, mostly native to Australia. They can be found in almost every part that continent, adapted to all of Australia's climate condition; in fact, no other continent is so characterized by a single genus of tree as Australia is by *Eucalyptus*. All *Eucalyptus* species are evergreen, although some species have some deciduous attributes (Wordiq, 2005). *Eucalyptus globulus* ssp. *globulus* (*nechbahirzaf* in local Amharic language) belongs to the family Myrtaceae. The genus *Eucalyptus* was described and named in 1788 by the French botanist Heritier. The flowers of the various *Eucalyptus* species are protected by an operculum, hence the generic name, which comes from the Greek words 'emu' (well), and 'calypsos' (covered), (Boland *et al.*, 1985). The species is adapted to subtropical climates with winter rainfall, such as the Mediterranean region, and to cool zones of tropical mountains, but it is not hardy in warm, temperate climates. The major successes of *E. globulus* have been attained largely in mild, temperate climates and at high elevations in cool, tropical climate (Attendee *et al.*, 1995). *Eucalyptus* is one of the world's most widely planted genera. *Eucalyptus globulus* Labill, commonly referred to as Tasmanian Blue Gum, is a fast growing, evergreen tree, bearing pendant leaves, native to Tasmania and south-east Australia (Adebayo *et al.*, 2009).

2.2. Essential Oils

In the early study, the term "essential oil" was defined as volatile oils obtained by steam distillation of plants. This definition is clearly intended to make a distinction between "fatty oils" and the oils which are easily volatile. Gradually, with the advancement of science came improvements in the methods of preparing the oils and parallel with this development a better knowledge of the constituents was gained. Essential oils are concentrated volatile aromatic compounds produced by plants - the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant life. Essential oils are frequently referred to as the "life force" of plants.

Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from part of plants. These oils have potent antimicrobial factors, having wide range of therapeutic constituents, flavor and odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics (Rao and Pandey, 2007).

Essential oils are extracted from oil 'sacs' in flowers, leaves, stems, seeds, wood, roots, and bark. They differ significantly from the well-known vegetable, nut and seed oils, which are made up of various fatty acids (essential oils are not). Essential oils are used by the plants in somewhat the same way they are by humans - they fight infection, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. Despite their foliar origins, however, essential oils have a similar structure to some compounds found in blood and tissues, allowing them to be compatible with our own physiology (Rao and Pandey, 2007).

2.3. Beneficial Uses of Essential oils of *Eucalyptus globulus*

Essential oils are natural products composed mainly of terpenes and terpene-derivatives in addition to some other non-terpene components (Edris, 2007). The leaves of *E. globulus* contain up to 3.5 % w/w essential oil (Bekele *et al.*, 2010). 1, 8-cineole (eucalyptol) is the principal constituent found in Eucalyptus. However, other chemo types such as α -phellandrene, ρ cymene, γ -terpinene, ethanone, spathulenol, among others have been documented (Chalchat *et al.*, 2001). Composition pattern of essential oils reflects their nutritional, cosmetic, pharmaceutical or medicinal values. The major component essential oil of the leaves of *E. globulus* was 1,8-cineole (85.8%), while α -pinene (7.2%) and β -myrcene (1.5%) were minor main components. Other compounds identified in the oil obtained were β -pinene, limonene, α -phellandrene, γ -terpinene, linalool, pinocarveol terpinen-4-ol, and α -terpineol (Vratnica *et al.*, 2011).

Leaves of *E. globulus* contain essential oils which are mainly terpenes and terpenoids showing antioxidant, anti-inflammatory and antimicrobial properties (Singh *et al.*, 2009). Essential oils of *E. globulus* are constituted mainly by the monoterpenes 1, 8-cineole (eucalyptol, 60%), α -

pinene (30%) and D-limonene (5%) and the sesquiterpene aromadendrene (Ammone *et al.*, 1985). Eucalyptol has antifungal, antibacterial and antiviral activity in vitro (Morcia *et al.*, 2012). In addition, eucalyptol and aromadendrene have synergistic effects with regard to antioxidant and antimicrobial properties (Mulyaningsih *et al.*, 2010). Moreover, it has been determined that *E. globulus* leaves contain several polyphenolic compounds such as ellagic acid, gallic acid, caffeic acid, chlorogenic acid, luteolin, rutin and quercetin in free or conjugated forms which have antioxidant and antimicrobial properties (Almeida *et al.*, 2009).

The essential oil of *Eucalyptus* used in medicine is obtained by aqueous distillation of the fresh leaves. It is a colorless or straw-colored fluid when properly prepared, with a characteristic odor and taste, soluble in its own weight of alcohol. The most important constituent is Eucalyptol, present in *E. globulus* up to 70 per cent of its volume.

2.4. Medicinal and Pharmaceutical Uses of Leaves of *Eucalyptus globulus*

Since the beginning of human civilization, compounds of plant origin have been used by mankind for their therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007).

According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.). Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced

for immediate consumption or as a basis for herbal products. Medicinal plants contain inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.*, 2008).

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fueled by the rising costs of prescription drugs in the maintenance of personal health and wellbeing and the bio prospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2005). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998) (Adesokan *et al.*, 2008).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering with the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way, the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So, the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important (Nascimento *et al.*, 2000).

The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the photochemical in them (Adesokant *et al.*, 2008). Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenyl propanic derivatives, in which the chemical and structural differences between compounds are minimal.

They can be essentially classified into: volatile fraction and non-volatile residue. Volatile fraction of essential oil constitutes 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters. Nonvolatile residue comprises 1–10% of the oil, and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids (Rao and Pandey, 2007).

Many common essential oils have medicinal properties that have been applied in medicine since ancient times and are still widely used today. For example, many essential oils have antiseptic properties, though some are stronger than the other. In addition, many have an uplifting effect on the mind, though different essential oils have different properties (Rao and Pandey, 2007). The most effective way to use most essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oils include body oils, compresses, cosmetic lotions, baths, hair rinses, inhalation by steam, perfumes and room sprays. Essential oils are very potent - some will cause skin irritation or have other harmful effects if not used properly. Unless specifically noted, it is best to dilute all essential oils in a carrier of base oil like Almond, Jojoba or Apricot Kernel before applying to the skin - appropriate dilution is usually only 1 - 10% essential oil in carrier. For inhalation, a diffuser or oil lamp is effective for releasing essential oils into your environment - a very pleasant way of creating a particular atmosphere (Rao and Pandey, 2007).

Medicinal and pharmaceuticals properties of the plant extracts are also exploited in folk phototherapy and aromatherapy. Aromatherapy is the therapeutic use of fragrances or mere volatile (essential) oils by inhalation to cure or prevent diseases and infections (Jayer *et al.*, 1993). Study on the biological activities of essential oils extracted from plants is generating great interest among researchers. Ant diabetic, antioxidants, antimicrobial, and insecticidal activities of essential oil extracts from leaf of *E. globulus* have been established (Mohamed *et al.*, 2008). Below are some uses of essential oil extracts from *E. globulus*.

2.4.1. Antioxidants

Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species or free radicals. These free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis, and cell growth in living systems (Packer *et al.*, 2008). An imbalance in the rate of production of free radicals or removal by the antioxidant defense mechanisms leads to a phenomenon, referred to as oxidative stress. In disease conditions such as diabetes, cancer and cardiovascular diseases, an aggravated imbalance could occur in deleterious oxidation of biomolecules resulting in cell or tissue damage (Jung *et al.*, 2009). Due to characteristics adverse effects of synthetic antioxidants there is need to explore phytotherapies to develop viable alternatives (Wang, 2001). Antioxidant activities of *E. globulus* leaf essential oil as well as some of its main constituents have been reported (Candan, 2010). However, it has also been established that the composition pattern of essential oil is affected by factors such as geographical location which consequently influence their biological activities (Usman *et al.*, 2011).

2.4.2. Air Fresheners

The treatment of anxiety or minor medical conditions included rubbing pleasant smelling natural oils into the skin or breathing in their smell. It is the use of aromatic essential oils to benefit the body – in emotional and physical health and beauty. Science has discovered that our sense of smell plays a significant role in our overall health. Most of *eucalyptus* oils are in aroma lamps, electric room diffusers, and spray mists. To make a simple mist spray, dilute 50 to 100 drops or so of essential oils in 4 fluid ounces (120ml) of pure water. Spray to refresh and cleanse the air (Rao and Pandey, 2007).

2.4.3. Antiseptic

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antibiotic-resistant strains. Moreover, they are also known to be active against fungi and yeasts (*Candida*). The most common sources of essential oils used as antiseptics are: Cinnamon, Thyme; Clover; *Eucalyptus*; Culin savory; Lavender. Citral, geraniol, linalool and thymol are much more potent than phenol. The medicinal *Eucalyptus* oil is probably the most

powerful antiseptic of its class, especially when it is old, as ozone is formed in it on exposure to the air. It has been decided to be of disinfectant action, destroying the lower forms of life. Internally, it has the typical actions of a volatile oil in a marked degree (Kokate *et al.*, 1999).

2.4.4. Anti-malarial

Its antiseptic properties confer some anti-malarial action, though it cannot take the place of Cinchona (Kumar and Laxmidhar,2011). *Eucalyptus globulus* oil significant action against Plasmodium species. It is popularly used anti-malarial plants in Brazil (Njoroge *et al.*, 2006).

2.4.5. Anthelmintic action

For some years *Eucalyptus*-chloroform was employed as one of the remedies in the tropics worm, due to the presence of Phytochemical constituents such as Borneo, cineol, linalool, gernayl acetate, saffrol, antheol due to which it exhibit anthelmintic action of different intestinal worms(Kumar and Laxmidhar,2011).

2.4.6. Treatment against Urethra Truck Infection and Respiratory Truck Infection

Eucalyptus globulus has been used in traditional medicine in the treatment of bronchitis, asthma and other respiratory disease. Cutaneous application of essential oils of *eucalyptus globulus* to mice suppressed the cellular inflammation of skin. This suggests that essential oils using in aromatherapy massage may suppress inflammatory symptoms related with neutrophil accumulation and edema. An emulsion made by shaking up equal parts of the oil and powdered gum-arabic with water has been used as a urethral injection, and has also been given internally in drachm doses in pulmonary tuberculosis and other microbes diseases of the lungs, bronchitis and Spasmodic action In croup and spasmodic throat troubles, the oil may be freely applied externally (Dixit *et al.*, 2012).

2.4.7. Anti-inflammatory

1,8-cineole, major constituent present in volatile oil of *Eucalyptus globulus* is a strong inhibitor of cytokines, that might be suitable for long term treatment of airway inflammation

in bronchial asthma and other steroid- sensitive dish (Nagpal *et al.*, 2010). Using acetic acid-induced writhes in mice and hot plate thermal stimulation in rats, it has proved that the essential oil of *Eucalyptus globulus* induced analgesic effects in both models, suggesting peripheral and central actions.

In addition, essential oil extracts from the *Eucalyptus globulus* produced anti-inflammatory effects, as demonstrated by inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan, and vascular permeability induced by carrageenan and histamine (Silva *et al.*, 2003). *E. globulus* oil has the anti-inflammatory effect on chronic bronchitis induced by lipopolysaccharide in rats and the inhibition effect on hypersecretion of airway mucins (Lux *et al.*, 2004). *E. globulus* extracts significantly inhibited the enhanced production of NO induced by LPS and IFN-gamma in a dose-dependent manner. It is well known that nitric oxide (NO) plays an important role in the pathogenesis of inflammatory diseases. *Eucalyptus globulus* have been used in traditional medicine in the treatment of bronchitis, asthma and other respiratory diseases (Vigo *et al.*, 2004). Cutaneous application of essential oils of *Eucalyptus globulus* to mice suppressed the cellular inflammation of skin. This suggests that essential oils using in aromatherapy massage may suppresses the inflammatory symptoms related with neutrophil accumulation and edema (Maruyama *et al.*, 2005).

2.4.8. Treatment against parasitic infection

Essential oils are used by the plants in somewhat the same way they are by humans-they fight infection, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. Despite their foliar origins however, essential oils have a similar structure to some compounds found in blood and tissues, allowing them to be compatible with our own physiology. In veterinary practice, Eucalyptus oil is administered to horses in influenza, to dogs in distemper, to all animals in septicemia. It is also used against parasitic skin affections (Dixit *et al.*, 2012).

2.4.9. Antimicrobials

Many studies deal with the antibacterial effects of plants or plant extracts. For *Eucalyptus globulus* leaf extracts a high inhibition activity on bacterial growth has been described.

Although these studies found no correlation between the amount of 1, 8-cineole content and the antibacterial activity, it seems to be the main active compound in the leaves (Cumana *et al.*, 2002).

Dried residue of methadone extract of *Eucalyptus globulus* leaves showed antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* with minimum inhibitory concentration of 5, 10, 10, 1.25 mg/ml respectively. Phloroglucinol- sesquiterpene coupled compounds showed potent antibacterial activity and inhibitory effect of glucosyl transferase. Ethanol extract of *Eucalyptus globulus* also active against the reference strains of *Staphylococcus aureus*, *Saturiahortensis L.*, *Teucrium polium L* (Mansouri *et al.*, 1999). Most concentrations of the extracts of the *Eucalyptus globulus* showed a high antibacterial activity against *Pseudomonas aeruginosa*. Methanol-dichloromethane extract of *Eucalyptus globulus*, significantly inhibited the growth of six Gram-positive bacteria (Takarada *et al.*, 2004).

Freshly prepared camphor oil from *Eucalyptus globulus* with or without glycerol dilutions gave complete cure of human facial demodicidosis with concentrations of 100%, 75% and 50% 40%. *Eucalyptus globulus* leaf extracts and oil showed antifungal property as they progressively inhibited the growth of *Malassezia furfur* on Sabouraud's dextrose agar medium (Vijayakumar *et al.*, 2006). The volatile oil extracted from the leaves of *Eucalyptus citriodora* showed a wide spectrum of antifungal activity. *Eucalyptus* oil, camphor and menthol and thymol oil are the most efficacious component against the fungal pathogens (Ramsewak *et al.*, 2003).

Eucalyptus grand exhibited significant inhibitory effects on Epstein-Barr virus (EBV) activation induced by TPA. Twelve euglobals from *Eucalyptus globulus* and their twenty-six related compounds were examined for their inhibitory effects on Epstein-Barr virus activation by a short-term *in vitro* assay. The results showed that most of the euglobals having monoterpene structures, and euglobal-III had strong inhibitory activities. *Eucalyptus globulus* oil has antiviral activity against herpes simplex virus (herpes simplex virus-1 and virus -2) (Schnitzler *et al.*, 2001).

2.5. Detrimental Effects of Essential Oils of *Eucalyptus globulus*

The most effective way to use most essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oils include body oils, compresses, cosmetic lotions, baths, hair rinses, inhalation by steam, perfumes and room sprays. Essential oils are very potent - some will cause skin irritation or have other harmful effects if not used properly. In large doses, it acts as an irritant to the kidneys, by which it is largely excreted, and as a marked nervous depressant ultimately arresting respiration by its action on the medullary Centre (Dixit *et al.*, 2012).

3.5.1. Antihistaminic

Hexane extract of leaves, ethanol extract of fruits and leaves of *Eucalyptus globulus* inhibited IgE dependent histamine release from RBL-2H3 cells 42 (Nagpal *et al.*, 2010).

3.5.2. Stimulant

Eucalyptus oil is used as a stimulant and antiseptic gargle. Locally applied, it impairs sensibility. It increases cardiac action (Kokate *et al.*, 1999).

3. MATERIALS AND METHODS

3.1 Description of Study Area

Fresh leaves of *Eucalyptus globulus* used in this study were collected from Haramaya University (Eastern Ethiopia), which is located at altitudinal range of 1400 to 2340 meters above sea level. It has an annual average rainfall and temperature of 1372mm and 19°C, respectively. Other source of leaf sample was Entoto Natural Park (Central Ethiopia) located between 2,600 and 3100 meters above sea level. Entoto has an annual average rainfall and temperature of 1200mm and 14°C, respectively. The identity of these plant species was verified by comparing their characteristics with those of the voucher specimens available at the National Herbarium, Addis Ababa University.

3.2 Source of the Test Bacterial Pathogens

Pure cultures of the test bacterial pathogens were obtained from pure culture collections of *Ethiopian Public Health Institute* (EPHI). The pure cultures of the strains were originally isolated from human skin. These included *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 35659 and *Streptococcus pyogenes* ATCC 19615.

3.3. Extraction and Analysis of Essential Oils of *Eucalyptus globulus*

Steam distillation method was used for essential oil extraction. For this, a leaf (500g) was mixed with 300mL of distilled water in 1L distillation flask and steam distilled using an apparatus of Clevenger type for 3 hr., which was then essential oils extract and analyzed in chemistry laboratory of Chemistry Department, Ambo University. The distillation flask was placed in heating mantle and allowed to boil the sample up with the addition of boiling chips until the distillation was completed. The distillate was then collected in receiver apparatus (500mL beaker). The extracted fractions of plant parts exhibited two distinct layers an upper oily layer and the lower aqueous layer.

The essential oil was separated from aqueous portion by extracting twice with drop of chloroform using a separatory funnel. The chloroform (lower) layer was slowly drowned off

until only the water layer remains. After filtration, the solvent was eliminated in rotary evaporator at 40°C under reduced pressure. The collected oil was dried over anhydrous Na₂SO₄ in order to remove water traces and concentrated under vacuum. Thereafter, the yield was weighed and stored in clean brown glass bottles at 4°C in refrigerator until used for antimicrobial screening (Usman *et al.*, 2009).

The essential oil from leaves of *E. globulus* was analyzed using GC which diluted samples (essential oil as external concentration and chloroform is internal concentration as standard compound) (1/100 in chloroform, v/v) of 1.0 µl was injected through auto sampler and analyzed with GC in single replication. The temperature of the injector was fixed to 260 °C and the detector (FID) to 270 °C. Carrier gas was helium (1mL/min) with 69.8 kPa and a split ratio of 100:1. The interface temperature was 280 °C. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 40 to 600 m/z at 0.5 s and ion source temperature was set at 230 °C. The percentage of each constituent in the oil was determined based on GC peak areas. The quantification of essential oil components, expressed in relative percentage composition on the total area of identified compound by chromatogram, was carried out by peak area normalization measurements. The constituents of essential oil were identified by their retention index, and MS Library search (NIST 08) with authentic compounds.

Determination of Extract Yield

The percentage yield of the essential oil was determined gravimetrically using the volume of the extract (x) and the weight of wet leaves sample material (y) as follows

$$\text{Percentage yield} = x/y * 100$$

Table1. The yield of the essential oils from *Eucalyptus globulus* Entoto Park and Haramaya University

	Entoto Park(sample material)	Haramaya University (sample material)
Weight of leaves(g)	500	500
Volume of extract oil(ml)	1.19	1.15
Percentage of yield(% ml/g)	0.24	0.23

3.4. Media Preparatio

Thirty nine grams of Mueller-Hinton agar (MHA) was suspended in 1000 ml of sterile distilled water and then sterilized by autoclaving at 120°C for 15 minutes and 15 psi pressure. Immediately after autoclaving, the medium was allowed to cool at 48 to 50°C in a water bath and poured aseptically into a sterile Petri dish in the biological safety hood at room temperature until it solidified.

3.5. Experimental Design and Data Analysis

The research was designed based on the laboratory chemical analysis in a commercial Chemical relational database (CRD). The treatments were include two area plant extracts and three test pathogens in three replications (2x3x4).Streptomycin dimethyl sulfoxide were used as negative and positive control group for each test respectively.

Data were analyzed using computer software SPSS version 20. Results of the study were expressed as mean \pm standard error (M \pm SEM). Statistical significant differences values were determined using one way analysis of variance (ANOVA).

3.6. Preparation of Inoculum for Antimicrobial Activity Test

Four well isolated bacterial strains (i.e. *S. aureus*, *P. aeruginosa*, *Proteus mirabilis* and *S. pyogenes*) were transferred from agar slants separately to appropriate liquid media. Inoculum from the resulting stock cultures were streaked on MHA plates and incubated for 24 h at 37°C in an incubator. Well separated bacterial colonies of *S. aureus*, *P. aeruginosa*, *Proteus mirabilis* and *S. pyogenes*, appearing on the plates were then used in the actual preparation of the respective inoculum. The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oil. Each suspension of the tested microorganism (1×10^4 CFU/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with undiluted *E. globulus* essential oil (10 μ l and 20 μ l) and placed on the incubated plates. The plates were kept at room temperature for 30 min and then incubated at 37°C for 24 hrs. The results reading was carried out by measuring the diameters of the inhibition zones, clear growth in mm. Streptomycin was used for comparison under the

same conditions as in the essential oil experiment. All tests of the inhibitory activity were carried out in duplicates and the developing inhibition zones were compared with that of reference discs.

3.7. Antibacterial Activities of Essential Oils

The essential oils extracted by steam distillation were evaluated *in vitro* for antimicrobial activities by using the paper disc diffusion method against the selected four intestinal bacterial species: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Proteus mirabilis* (Takarada *et al.*, 2004). The bacterial cultures were inoculated into the Mueller Hinton Agar (MHA) (and incubated at 37°C). The bacteria were obtained from Ethiopian Public Health Institution (EPHI). The antimicrobial activity tests were conducted in Plant Pathology Laboratory, School of Plant Sciences Haramaya University. Streptomycin was used as standard drug against the selected bacteria. Streptomycin and DMSO were used as positive and negative controls, respectively. The antibacterial activities of the essential oil extracts plants were tested using the disc diffusion method. The dried surface of a Mueller-Hinton agar plate was aseptically inoculated with bacterial suspension by swabbing over the entire agar surface in the safety hood. This procedure was repeated by swabbing two more times, rotating the plate approximately 90° and 60° each time to ensure an even distribution of inoculums. As final step, the edge of the agar was swabbed. After inoculation, the lids of the plates were left partly open for 3-5 minutes to allow surface moisture to be absorbed before applying the respective plant extract-impregnated discs, DMSO and the standard drug (Adesokant *et al.*, 2008).

3.8. Testing for Antimicrobial Activity

Filter paper discs of 6 mm diameter placed in a beaker were sterilized in an oven at 180 °C for one hour. Then, aliquots containing 10 and 20 µg/mL of the essential oil extracts as well as the standard drugs were pipetted to the discs in three replications. The paper discs impregnated with samples were then transferred with sterile forceps to media seeded with bacterial strains as described above. The anti-microbial activities of the essential oil extracts were evaluated by measuring the zone of inhibitions against the test bacteria after an incubation period of 24 hrs

and compared with those of commercial standard drugs (Roomiani *et al.*, 2013). The experiment was repeated three times and the results were expressed as mean values of zone of bacterial growth inhibition by each essential oil of *Eucalyptus globulus* leaves.

4. Results and Discussion

4.1. Characterization of Chemical Profile of the *E. globulus* leaf Essential Oils

The extraction yields of essential oils of the leaves of *E. globulus* collected from Haramaya University and Entoto Park were 0.23% and 0.24%, respectively. The yield variability in the total essential oil of *E. globulus* species growing in Ethiopia can be attributed to the climatic conditions, ecological, soil conditions, age of plant and the season of harvest (Husnia, 1985).

Table2. Chemical Composition of *E. globulus* leaf Essential Oil Extracts.

S.No	Chemical Components	Plant Sample							
		Haramaya University				Entoto park			
		Retention Time (min)	Cal. RI	Reported RI	Composition (%)	Retention Time (min)	Cal. RI	Reported RI	Composition (%)
1	Limonene	5.05	967	1031	2.35	5.01	957	1031	2.96
2	α -Terpinolene	5.93	975	1070	1.24	6.53	972	1070	0.90
3	Camphene	6.20	997	932	0.76	6.95	996	932	0.87
4	cis-Sabinol	6.56	1006	955	14.31	7.18	1047	955	12.10
5	α -terpinol acetate	6.91	1011		0.56	7.23	1067		0.36
6	α -Pinene	7.07	1018	922	2.15	7.73	1086	922	2.50
7	1,8-Cineole	7.29	1067	1016	68.25	9.27	1104	1016	71.30
8	cis-Carveol	7.55	1087	1229	0.78	9.35	1120	1229	0.48
9	Globulol	7.75	1109	1553	1.33	10.58	1138	1553	1.60
10	Aromadendrene	8.05	1117	1449	0.35	-	-	1449	-
11	Pulegone	8.70	1128	-	0.89	10.84	1183	-	1.00
12	γ -Terpinene	9.27	1137	1042	0.79	11.15	1216	1042	0.37
13	β -pinene	10.31	1165	958	1.12	12.04	1236	958	0.42
14	4-terpineol	10.56	1184	1159	1.62	12.06	1263	1159	2.13
15	β -myrcene	10.83	1197	974	0.34	12.34	1284	974	0.30
16	α -Fenchene	11.15	1206	1098	0.42	-	-	1098	-
17	p-Cymen	12.03	1219	1026	0.25	12.44	1306	1026	0.23
18	Spahulenol	12.35	1248	1546	0.24	12.50	1328	1546	0.37
19	Caryophyllene oxide	12.44	1273	-	0.82	-		-	-
20	tr-p-Mentha-1,7-8dien-2-ol	13.24	1289	-	0.63	12.77	1361	-	0.14
Total Content (%)					99.20	98.03			

Where, RI =Retention Index, Cal. =Calculated

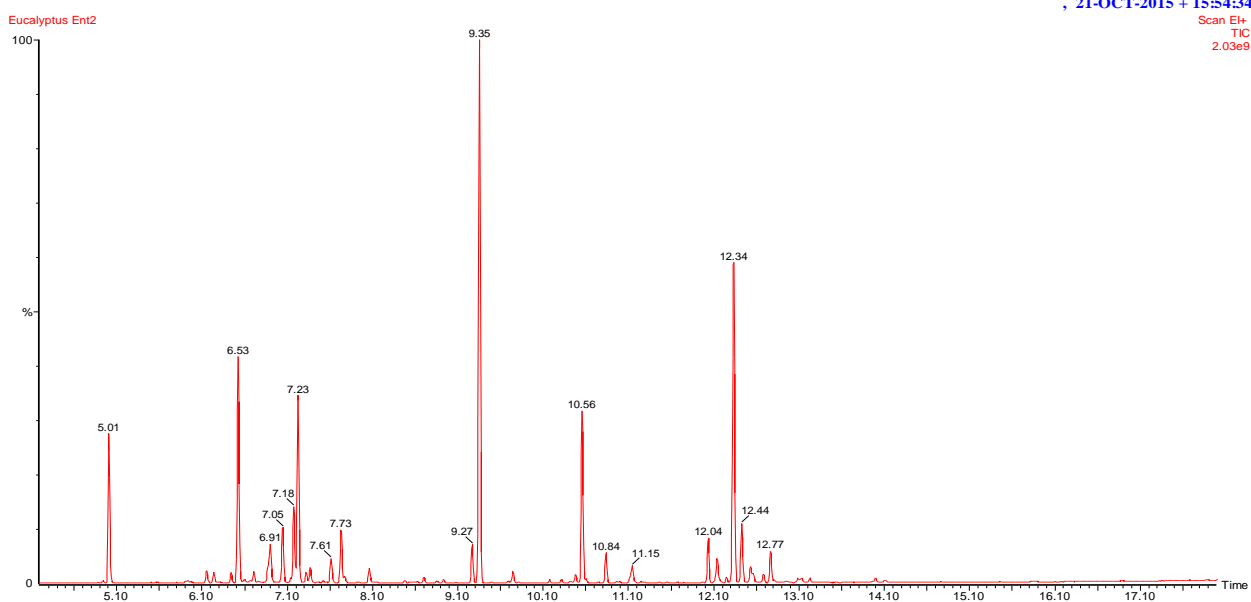


Figure 1: GC-MS Chromatogram of *E. globules* essential oils collected from Entoto Park.

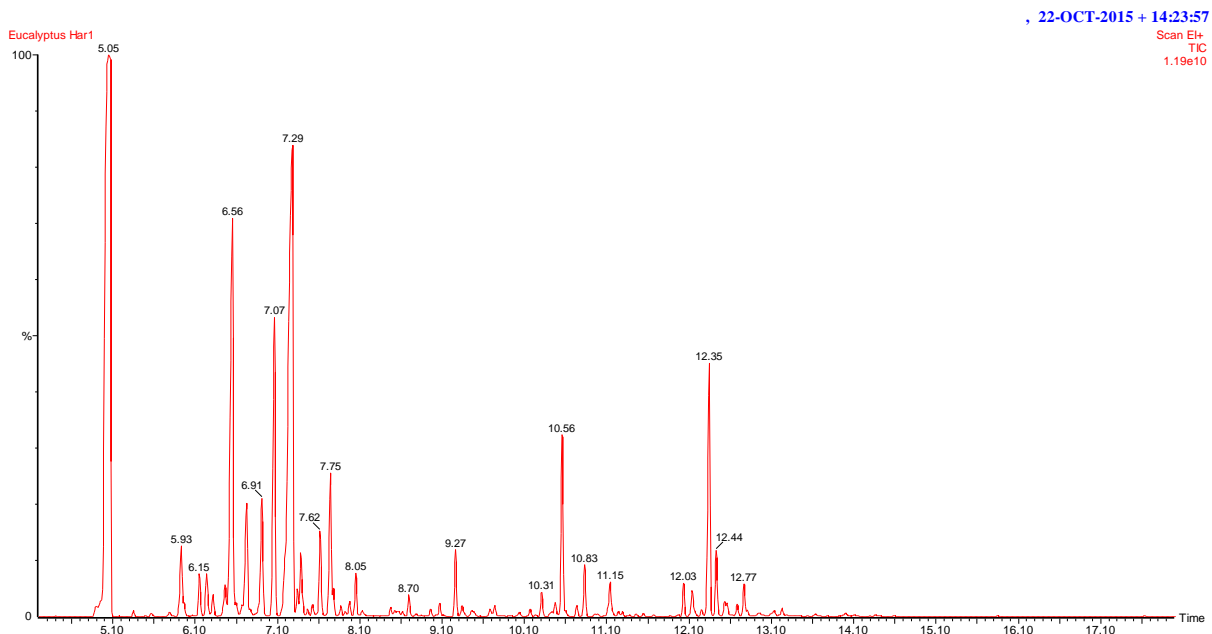


Figure2: GC-MS of Chromatogram of *E. globules* essential oils collected from Haramaya University.

Determination of the chemical composition of the extracted oils from two different *Eucalyptus globulus* was carried out by gas chromatography-mass spectroscopy. It is well-known that *eucalyptus* plants produce terpenoidal hydrocarbons and essential oils (*eucalyptus* oils). The GC/MS total ion chromatogram of the *eucalyptus* product was obtained under the conditions described depending on their chemical composition above, and the obtained results are seen in (Table 2).

Analysis of essential oil composition from leaves of *E. globulus* obtained from Haramaya University (HU) and Entoto Park resulted in 20 and 17 compounds, respectively (Table 2). Variation in chemical profile of the essential oils of leaves from both sampling sites suggests the impact of environment. Though the age of plants at harvest was assumed to be at mature stage, it is also possible that variation in composition could be due to age. Plant genotypes, developmental stages and biotic and abiotic factors influence the chemical profile of a given species ((Peñuelas and Louisa, 2001; Mark *et al.*, 1988; Eckey-Kaltenbach *et al.*, 1994; Hajer *et al.*, 2011). Of the essential oil components of HU collected samples, 92.37 % and 6.83% were monoterpenes and sesquiterpenes, respectively. On the other hand, monoterpenes and sesquiterpenes respectively constituted 93.37 % and 4.66% of the essential oils of samples from Entoto Park.

Based on the peak area or peak height, concentration of all the identified compounds was calculated by the softcopy results of GC/MS Turbo Mass using peak area normalization measurements. The main constituents of the essential oil in *E. globulus* leaves collected from Haramaya University were these of 1, 8- cineole (68.25%), cis-Sabinol (14.31%), Limonene (2.35%), α -Terpinolene (1.24%), and α -pinene (2.15%). Similarly, 1, 8- cineole (71.30%), cis-Sabinol (12.10%), Limonene (2.96%), α -Terpinolene (0.90%), and α -pinene (2.50%) were found to be dominant in samples from Entoto Park. This result is similar with previous work of (Abraha *et al.*, 2012) who reported *E. globulus* leaf essential oil to have 1,8-cineole (66.28-75.36%), *cis*-sabinol (15.92-21.33%), α -terpineolacetate (2.70-3.39%), α -terpineol (1.5-2.26%), aromadendrene (0.69 - 2.85%), globulol (0.82 - 1.43%), β -pinen (0.96 - 1.24%), β -myrcene (0.66 - 1.00%), 4-terpineol (0.46 - 0.52%) and camphene (0.16 - 0.27%) as the main leaf oil component.

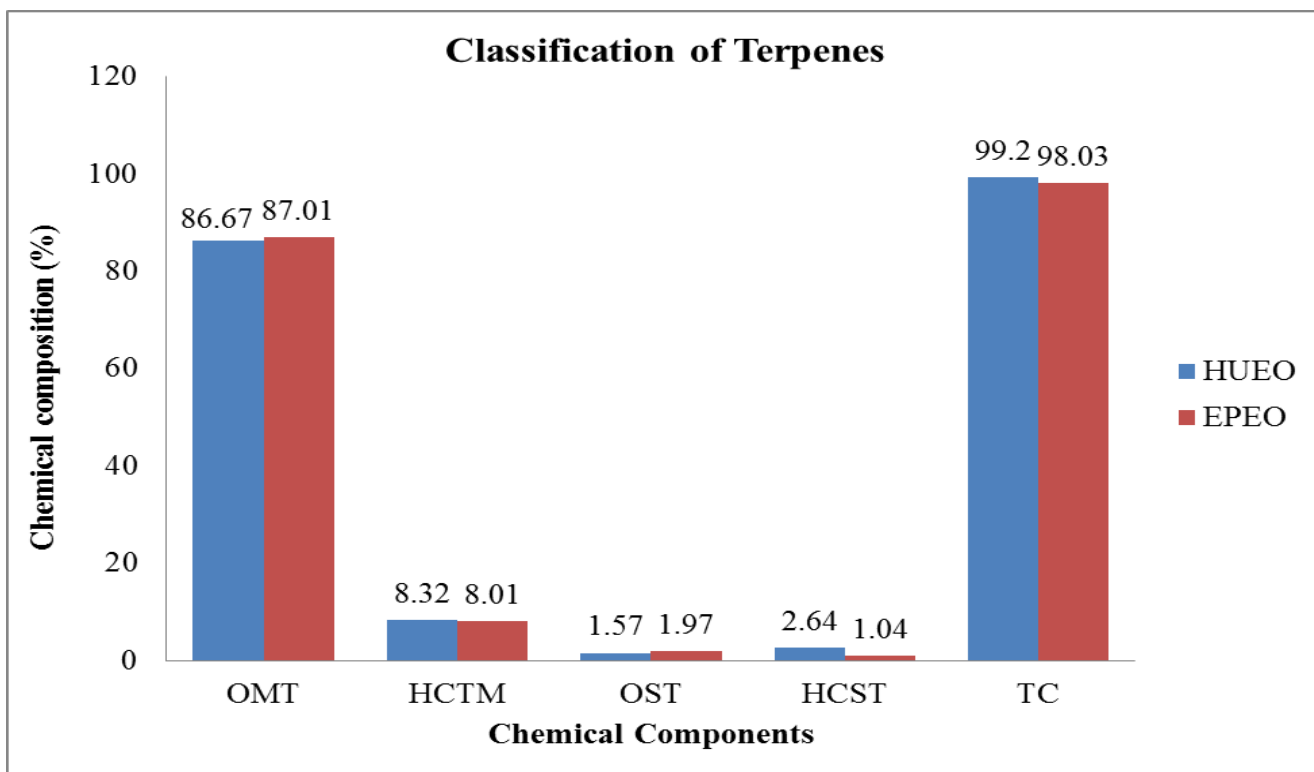


Figure 3: Monoterpene and sesquiterpene proportions of essential oils of HU and EP
 KEY: EPEO=Entoto Park Essential Oil, HUEO=Haramaya University Essential Oil,
 OMT=Oxygenatedmonoterpenes,HCTM=Hydrocarbonmonoterpenes,HCST
 =Hydrocarbonsesquiterpene, OST=OxygenatedSesquiterpenes, TC= Total Composition

The essential oil consisted mainly of oxygenated monoterpenes, hydrocarbon monoterpenes and oxygenated sesquiterpenes. Of these, 1, 8-cineole, cis-sabinol and terpinen-4-ol were the main oxygenated monoterpenes, while α -pinene; β -pinene, p-Cymene α -terpineolacetate, α -terpinolene and γ -Terpinene and limonene are the main hydrocarbon monoterpenes. On the other hand, globulol and Spahulenol were the main oxygenated sesquiterpene (Vratnica *et al.*, 2011).

Based on the results shown in figure3, *E. globulus* leaves essential oils were contained more oxygenated terpene derivatives mainly oxygenated monoterpenes (86.20-87.01%) compared to monoterpenes hydrocarbons (8.01-8.32%). however, Sesquiterpenes hydrocarbons (1.04-2.64%) species were also detected in a higher percentage than oxygenated Sesquiterpenes (1.57-1.97%) of HUEO and EPEO in agreement with reported (Abraha *et al.*, 2012).

4.2. Antibacterial Activities of *Eucalyptus globulus* leaf Essential Oil Extracts

Compared to the positive control, leaf essential oils of *E. globulus* obtained both from Entoto Park and Haramaya University showed significant inhibitory activities in all tested bacterial species and the effects were concentration dependent (Table 3). As can be seen from the table below, the essential oil extracts of the plant leaves from Entoto Park showed more effective inhibitory activities at 10 μ g/mL concentration with maximum zone of inhibition of 10.17 \pm 0.40mm against *S. pyogenes* and minimum inhibitory activity with a zone of inhibition of 7.5 \pm 0.28mm against *P. mirabilis* than the essential oil extracts of the plant leaves from Haramaya University.

At the concentration of 10 μ g/mL, oil extracts from HU plant leaves resulted in a maximum zone of inhibition of 10.0 \pm 0.12mm against *S. aureus* and a minimum zone of inhibition of 6.47 \pm 0.21mm against *P. aeruginosa*. On the other hand, essential oil extracts of leaves from Entoto park plants at 20 μ g/mL concentration indicated higher inhibitory activities with a maximum zone of inhibition of 14.43 \pm 0.15mm against *S. aureus* and a minimum zone of inhibition of 12.2 \pm 0.84mm against *P. aeruginosa* than essential oil extracts from HU plant leaves showing a maximum zone of inhibition of 14.23 \pm 0.11mm against *S. aureus* and a minimum zone of inhibition of 11.83 \pm 0.31mm against *P. aeruginosa*. *S. pyogenes* was more affected by EP essential oil at 10 μ g/mL concentration while *S. aureus* was more affected by HU essential oil at 10 μ g/mL concentration; and *S. aureus* was more affected by both HU and EP essential oil extracts at 20 μ g/mL concentration. The essential oil extracts of plant leaves from EP at 20 μ g/mL concentration and with the maximum zone of inhibition of 14.43 \pm 0.15mm is par with streptomycin zone of inhibition (17.20 \pm 0.09mm) against *S. aureus* (Sporer *et al.*, 2011).

The study showed that the essential oil extracts investigated were active against Gram positive and Gram negative bacteria. The essential oils extracts of *E. globulus* leaves had exhibited effective antimicrobial activities against *P. aeruginosa*, *P. mirabilis*, *S. aureus* and *S. pyogenes* at concentrations of 10 and 20 μ m/ml. The antibacterial activities of the same plant species (*E. globulus*) extracts obtained from two different locations against four bacterial species are summarized in Table-2. Essential oils of *E. globulus* leaves extract from Entoto

Park was produced maximum zone of inhibition ($14.43\pm 0.15\text{mm}$) against *S.pyogenes* while minimum zone of inhibition ($6.57\pm 0.26\text{mm}$) against *P.mirabilis* Essential oil extracts of *E.globulus* leaves from Haramaya University showed maximum zone of inhibition ($14.23\pm 0.11\text{mm}$) against *S. aureus* while the minimum zone of inhibition ($6.47\pm 0.21\text{mm}$) against *P. aeruginosa*. Essential oil extracts of *Eucalyptus globulus* leaves from Haramaya University showed comparatively less antibacterial activities than oil extracts from EP plants. Essential oils of *E. globulus* from Entoto park have shown higher antibacterial properties which constituted mainly the monoterpenes 1, 8-cineole (eucalyptol, 71.30%). It seems to be the main active compound in the leaves in agreement with (Olalekan *et al.*, 2012).

Table3. *In vitro* antimicrobial activities of *E. globulus* leaf essential oil extracts and standard antibiotics against test-bacteria (mean \pm SEM).

Cpds	Zone of Inhibition (mm) of Test Microorganisms							
	Gram(-) Bacteria				Gram(+) Bacteria			
	<i>P. aeruginosa</i>		<i>P. mirabilis</i>		<i>S. aureus</i>		<i>S. pyogenes</i>	
	10 $\mu\text{g/mL}$	20 $\mu\text{g/mL}$	10 $\mu\text{g/mL}$	20 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	20 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	20 $\mu\text{g/ml}$
EPEO	7.77 \pm 0.20 ^{dB}	12.20 \pm 0.84 ^{cA}	7.5 \pm 0.28 ^{aA}	12.23 \pm 0.16 ^c _A	10.01 \pm 0.10 ^{bA}	14.43 \pm 0.15 ^{dA}	10.17 \pm 0.40 ^{bA}	12.43 \pm 0.69 ^{cA}
HUEO	6.47 \pm 0.21 ^{aA}	11.83 \pm 0.31 ^{cA}	6.57 \pm 0.26 ^{aA}	11.87 \pm 0.33 ^c _A	10.00 \pm 0.12 ^{bA}	14.23 \pm 0.11 ^{eA}	9.87 \pm 0.14 ^{bA}	12.57 \pm 0.28 ^{dA}
SM	10.07 \pm 0.29 ^{aC}	15.90 \pm 0.11 ^{cB}	9.73 \pm 0.17 ^{aB}	15.83 \pm 0.21 ^{cB}	13.80 \pm 0.07 ^{bA}	17.20 \pm 0.9 ^{dB}	13.97 \pm 0.04 ^{BB}	16.73 \pm 0.06 ^{dB}
DMSO	-	-	-	-	-	-	-	-

Key: EPEO=Entoto park essential oil, HUEO= Haramaya University essential oil, Cpds= compounds, I=inhibition zone, =No inhibition was observed, DMSO= dimethyl sulfoxide as a negative control, SM= streptomycin as a positive control, SD=standard deviation, values with different superscription on the same row (lower case) and values with different superscription on the same column (upper case) are significantly different ($p < 0.05$).

The antibacterial activities presented in Table3 revealed that Entoto Park essential oil extracts of the Plant showed higher inhibition effect than Haramaya University essential oil extracts against the tested bacteria. The essential oil extracts of the plant were shown to have higher antibacterial activities with maximum zone of inhibition ($14.43\pm 0.15\text{mm}$) against *S. aureus* and minimum zone of inhibition (7.77 ± 0.29 mm) against *P. aeruginosa* by EPEO extract in comparison with the maximum zone of inhibition ($14.23\pm 0.11\text{mm}$) against *S. aureus* while

minimum zone of inhibition (6.47 ± 0.21 mm) against *P. aeruginosa* by HUEO extract were obtained. The commercial standard drug Streptomycin in Table-3 showed maximum zone of inhibition (17.20 ± 0.09 mm) against *S. aureus* and minimum zone of inhibition (11.83 ± 0.31 mm) against *P. aeruginosa*. The essential oils extracts of the plant also showed different antibacterial activities between concentrations for each essential oil type at $10 \mu\text{g/mL}$ concentration. EPEO extracts of the plant exhibited maximum zone of inhibition on gram negative bacteria with 7.77 ± 0.29 mm zone diameter while minimum the zone of inhibition (6.47 ± 0.21 mm) by HUEO against *P. aeruginosa*; and the maximum zone of inhibition on gram positive bacteria with 10.17 ± 0.40 mm against *S. pyogenes* while minimum zone of inhibition (10.0 ± 0.12 mm) against *S. aureus*. The antibacterial activities at $20 \mu\text{g/mL}$ concentration of EPEO extract of the plant exhibited maximum zone of inhibition in gram positive bacteria with (14.43 ± 0.15 mm) against *S. aureus* while minimum zone of inhibition (12.43 ± 0.69 mm) by HUEO against *S. pyogenes* and maximum zone of inhibition in gram negative bacteria with (12.23 ± 0.16 mm) against *P. mirabilis* while minimum zone of inhibition (11.83 ± 0.31 mm) against *P. aeruginosa*.

The antibacterial activities presented in table 3 showed that when the concentration of essential oils increase zone of inhibition also increase. As a result, the commercial standard drug was revealed higher antimicrobial activity in comparison with the plant essential oils extract. DMSO was used as negative control and did not show any inhibition effect against the tested microorganism (Loo *et al.*, 2010). The leaves essential oils extracts of the plant in Table 3 revealed that the presence of different types of bioactive secondary metabolites such as Limonene, α -Terpinolene, cis-Sabinol- α -Pinene and 1, 8-Cineole, were likely to be responsible for antibacterial activity (Olalekan *et al.*, 2012). As indicated from the results presented in Table 3, the antibacterial activity of the plant essential oil extract was more pronounced on the Gram-positive bacteria (*S. aureus* and *S. pyogenes*) than the Gram-negative bacteria (*P. aeruginosa* and *P. mirabilis*). This might be due to the fact that Gram-negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components, which makes their cell wall impermeable to antibacterial chemical (Comparisons of the current finding with the previous study were showed a similar result (Takarada *et al.*, 2004).

5. SUMMARY, CONCLUSIONS AND RECOMMENDATION

5.1. Summary

The results of the present study have revealed that the chemical composition and antibacterial activities of essential oils of *E. globulus* varied greatly depending upon the different leaf of the plants extracted and different type of geographical location. The main constituent of Haramaya University and Entoto Park *E. globulus* oil are 1, 8-cineole (*Eucalyptol*), ranging from 68.25%- 71.10% and cis-sabinol, ranging from 12.10%-14.31%. The slight difference in composition might be attributed due to different agro-climatic conditions and soil composition in the region. Twenty compounds were detected from Haramaya University and seventeen chemical components were identified from Entoto Park *E. globulus* essential oils. The essential oils obtained from Haramaya University and Entoto Park could be used for medicinal application and/or pharmaceutical agents.

The results of the antibacterial activity of essential oils from different areas of Ethiopia may be directly associated with their major constituents or the presence of synergy between the major and minor constituents within the essential oil. The relationship evaluated between the chemical composition of the oil and the antibacterial activity was suggested that the presence of oxygenated monoterpene 1, 8- cineole composition from HU *E. globulus* leaf essential oil extracts as the principal component of the essential oil which is very responsible for the potent antibacterial activities. In general, the strong antibacterial activity was not related only to a high composition of major component such as 1, 8-cineole, but also the presence of other moderate and minor chemical constituents of the oil. The Gram (-) bacteria (*P. aeruginosa* and *P. mirabilis*) was the most resistant to the oils in comparison with the Gram (+) bacteria (*S. pyogenes* and *S. aureus*). Antibacterial characteristics of this tree leaves can be further investigated so as to be used in the treatment of bacterial infections. Thus, *E. globulus* leaves essential oils chemical components can be used against for selected pathogenic and some microorganisms, and may provide better alternatives or supplements to the conventional antibacterial.

5.2. Conclusions

Based on the findings of this investigation the following conclusions are made. First, the chemical composition and antibacterial activity of essential oils of *E.globulus* leaves vary greatly depending upon the plants leaves collected as well as the geographical location of the plants.

Second, *Eucalyptus globulus* leaves possess antibacterial substances which inhibit the test bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aurous*, *proteus mirabilis*, and *Streptococcus pyogenes*). Thus, the essential oil extracts of *Eucalyptus globules* leaves in terms of their antibacterial activities makes them a potential therapeutic plants for treatment of intestinal pathogen diseases.

5.3. Recommendations

Based on this study finding, it is possible to recommend the following points.

- More bioactive compounds which are responsible for the bactericidal activities of the essential oil of this plant leaves should be isolated and characterized using different advanced chromatographic techniques.
- The antibacterial screening was conducted on limited bacterial so the same work should be carried out on large variety of antibacterial strains, so as to have a clear picture of the spectrum of antimicrobial activities of the herb.
- Although, the *in vitro* antimicrobial activities of the essential oil extracts of *E. globulus* showed promising results, further detailed studies are required to investigate their effectiveness *in vivo* prior to use for treatment of intestinal pathogens.
- All experiments in this study were conducted using plants collected the same species from two local area. Therefore, similar work is recommended to be conducted on the same species collected from different localities in the country so as to assure reproducibility of results or have information on the effect of geographical variation on the composition and hence biological activity.

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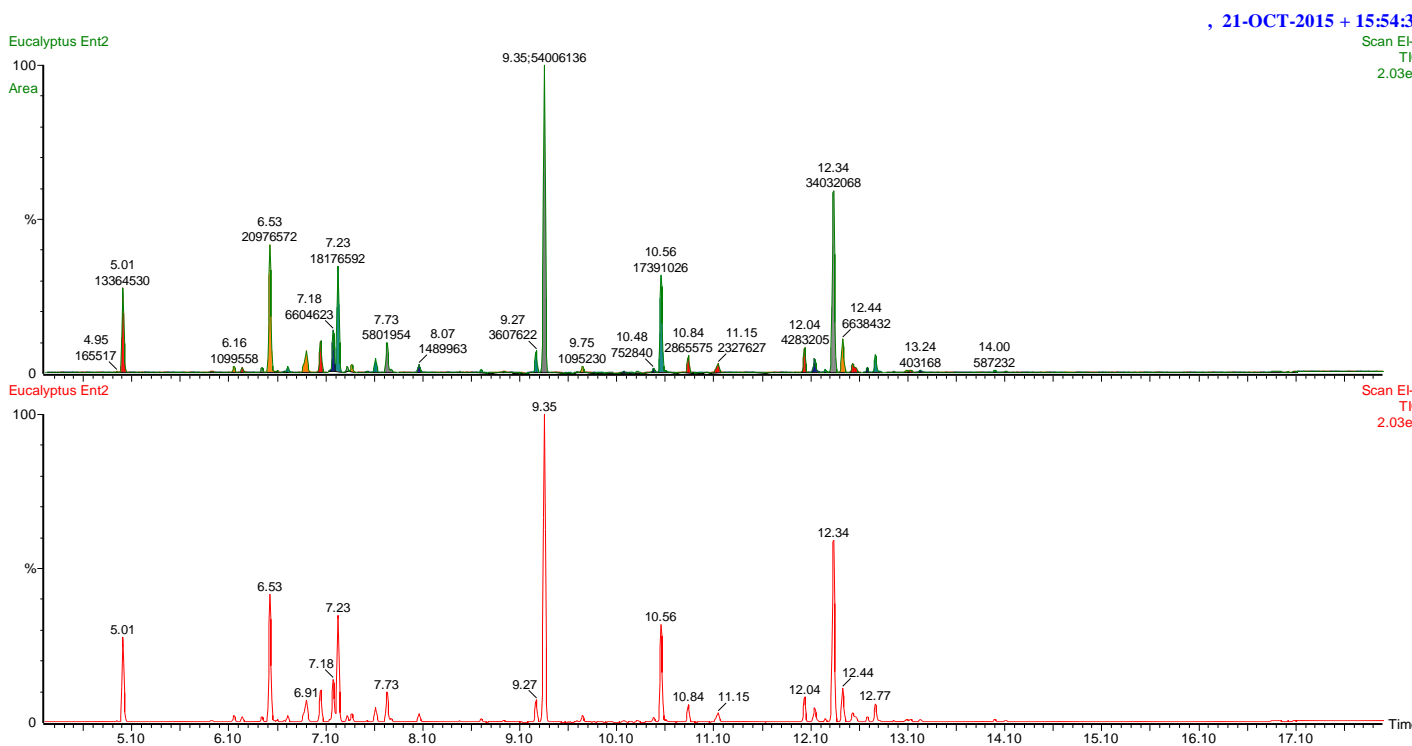
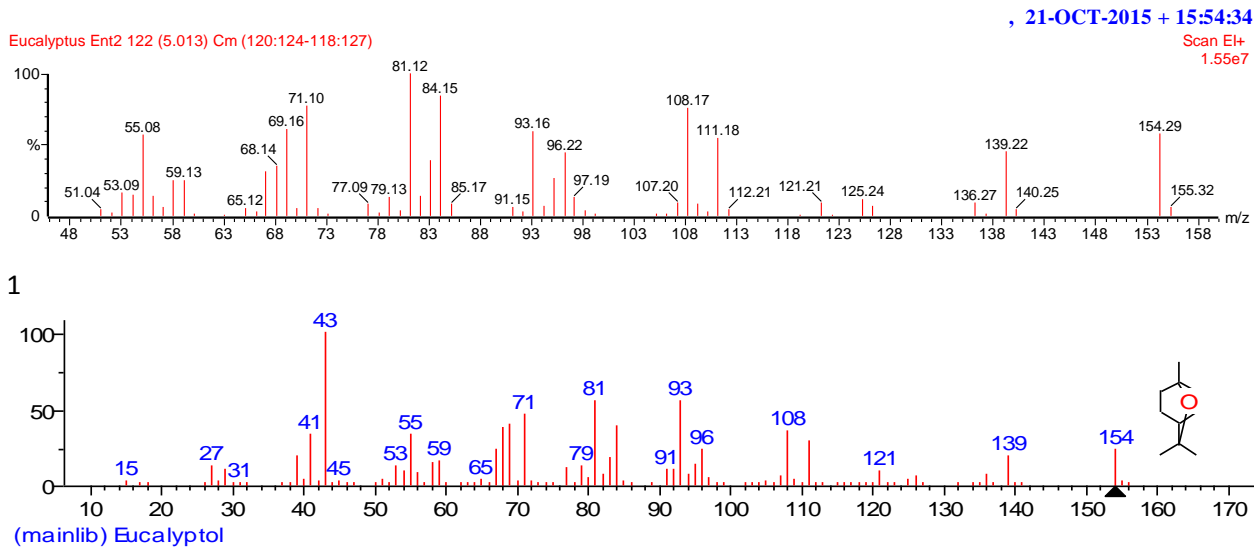
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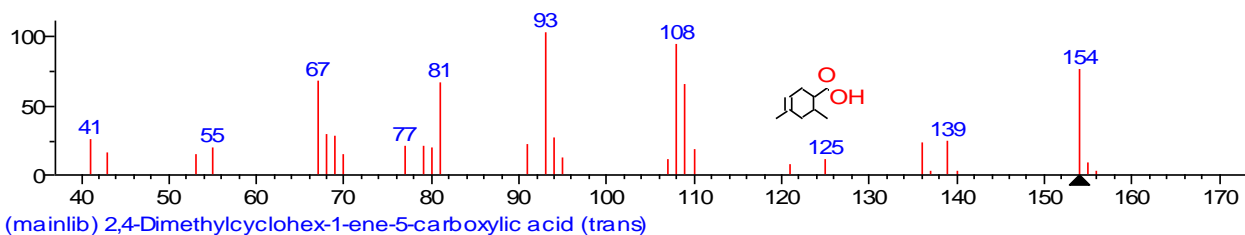
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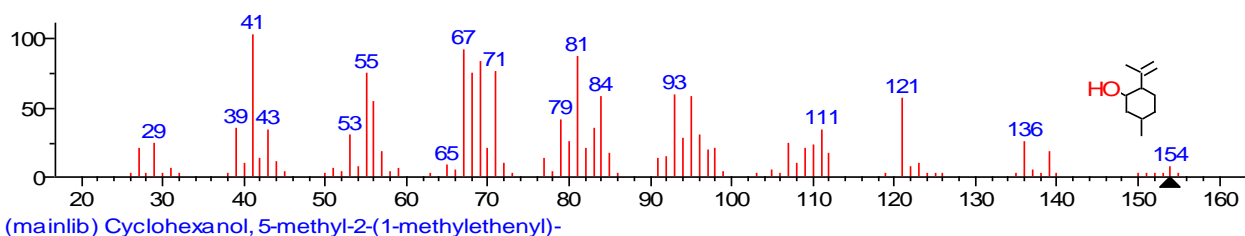
7. APPENDIX

Figure 1: GC-MS Chromatogram of *E. globules* essential oils collected from Entoto Park.

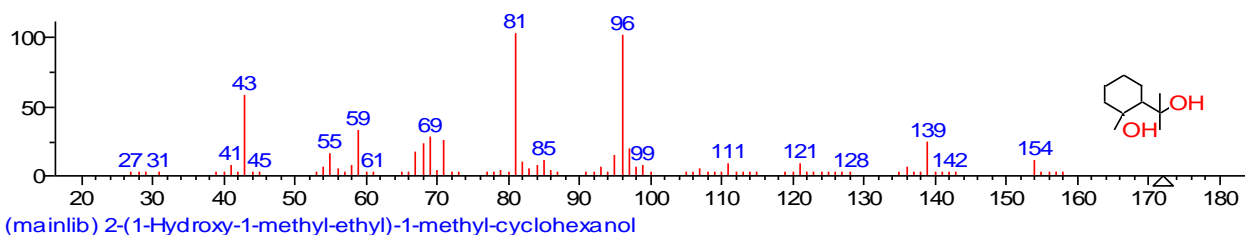
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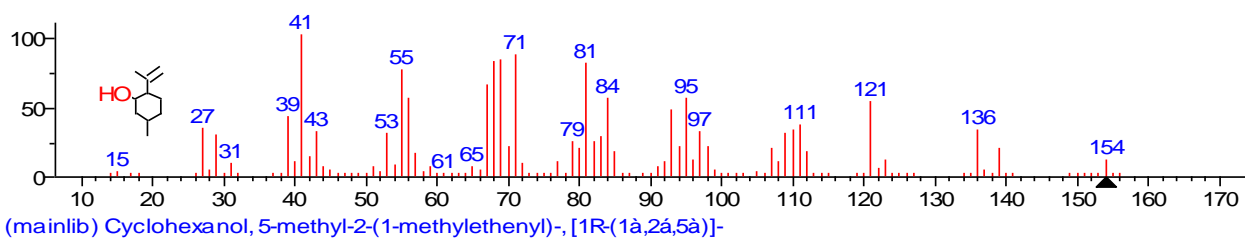
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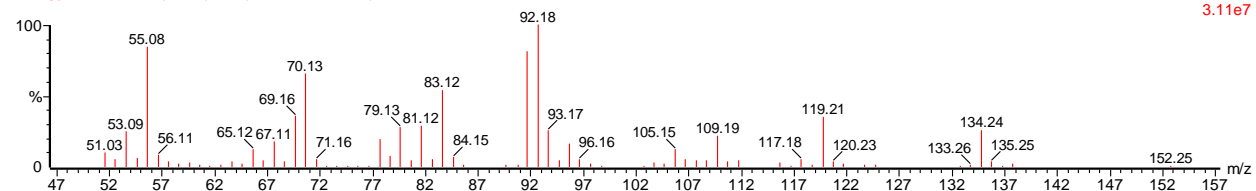


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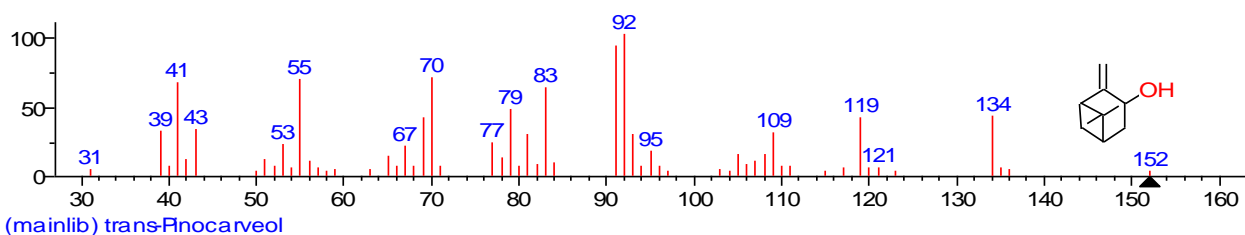


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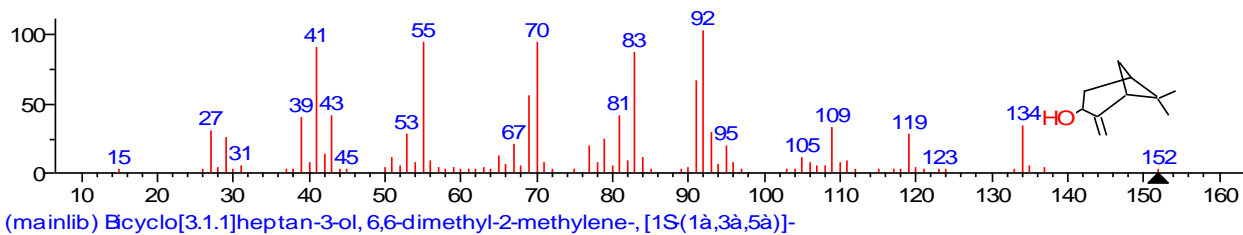
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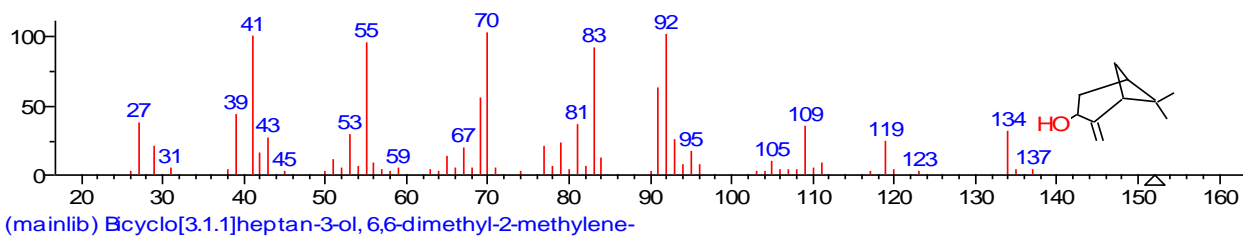
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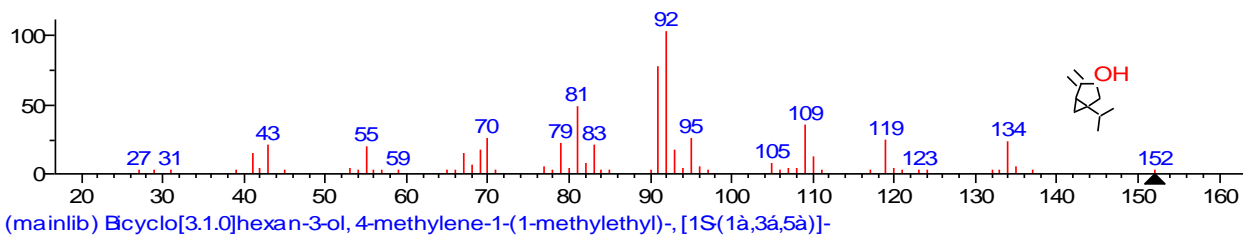
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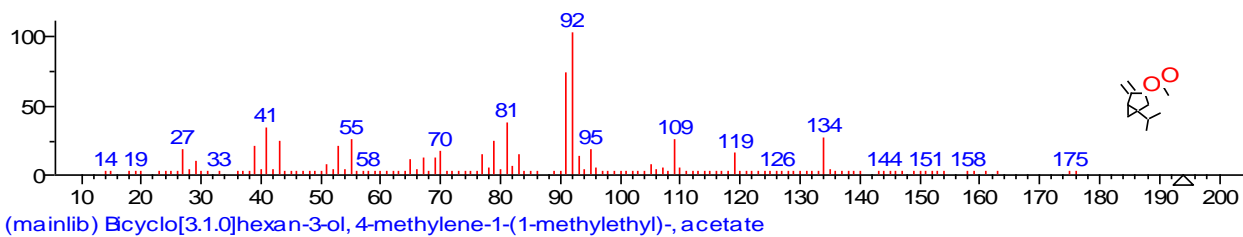
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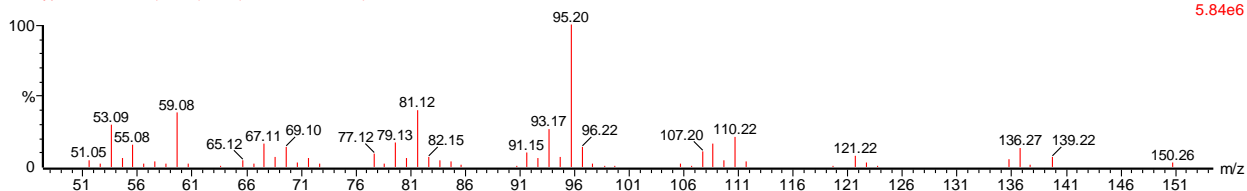


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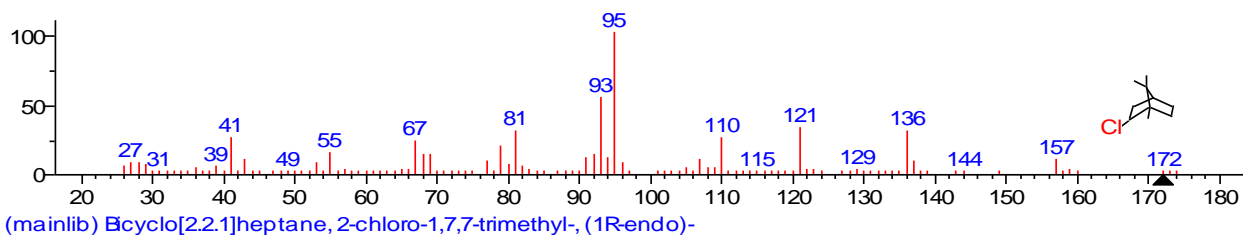


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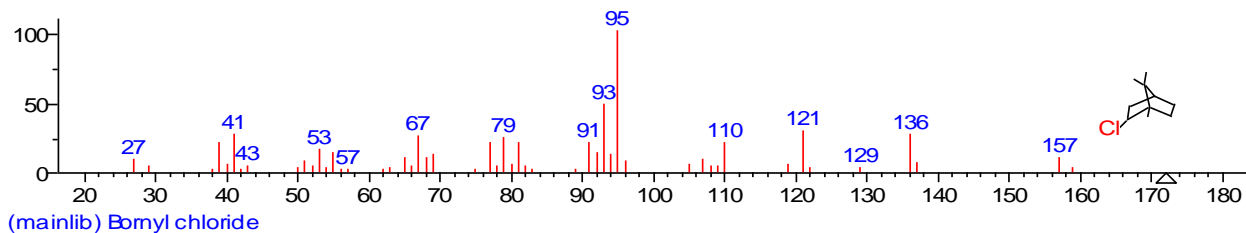
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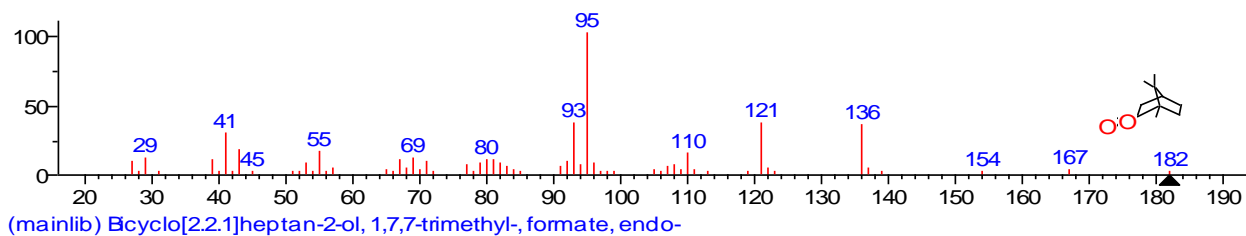
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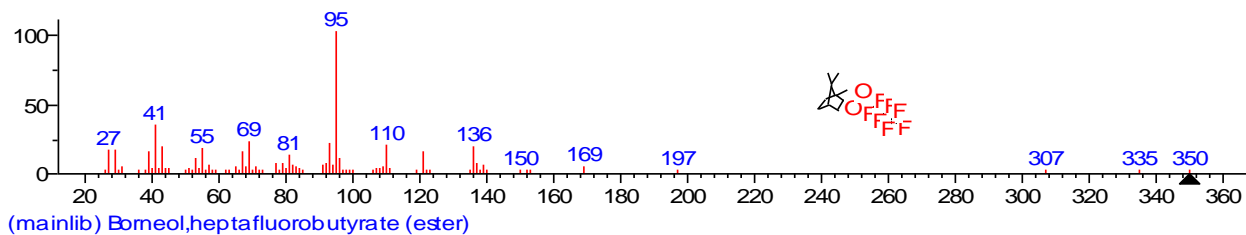
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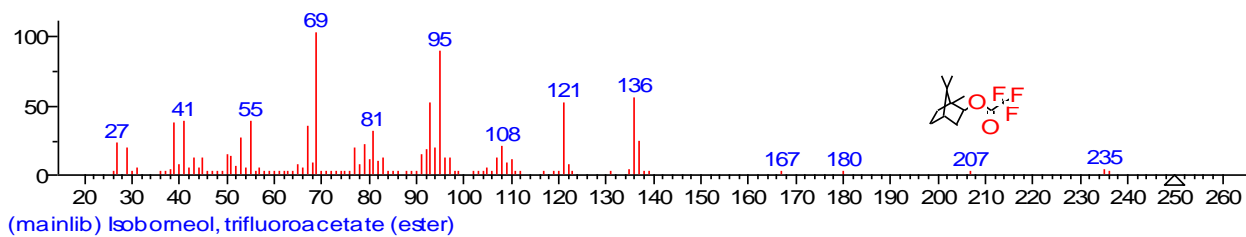
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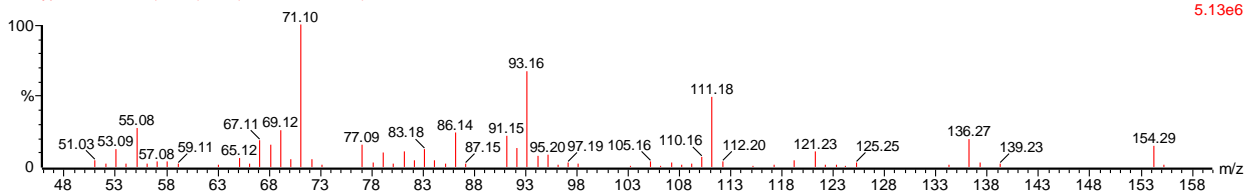


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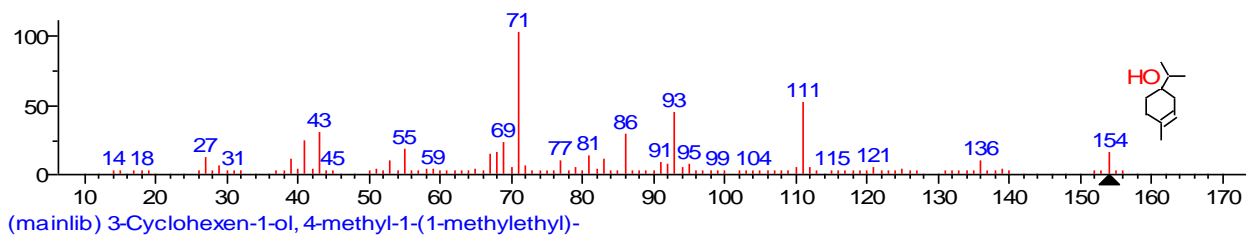


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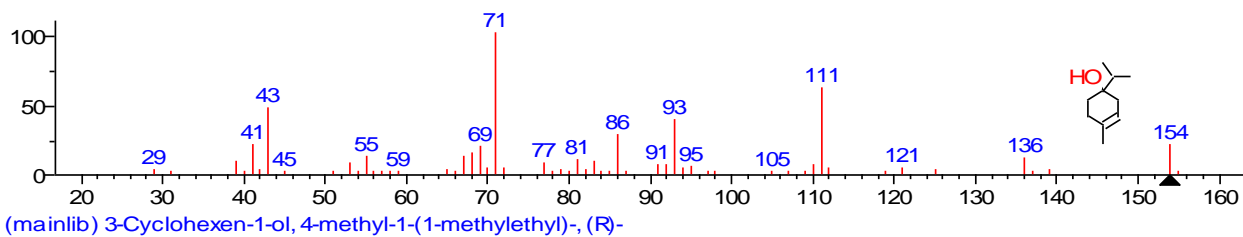
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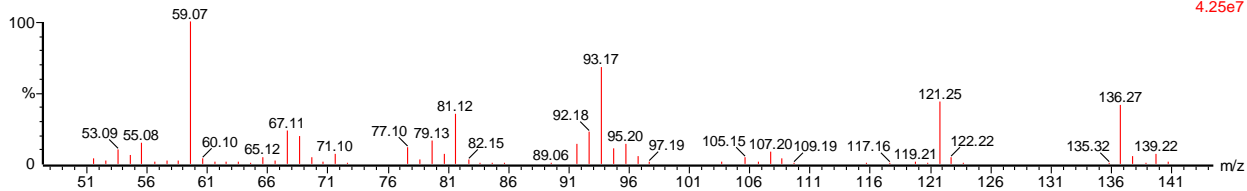


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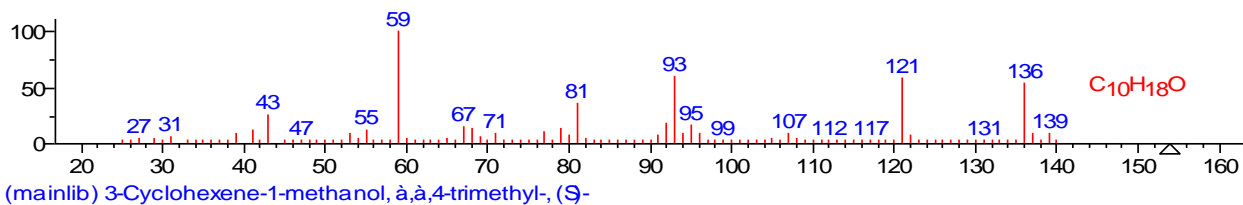


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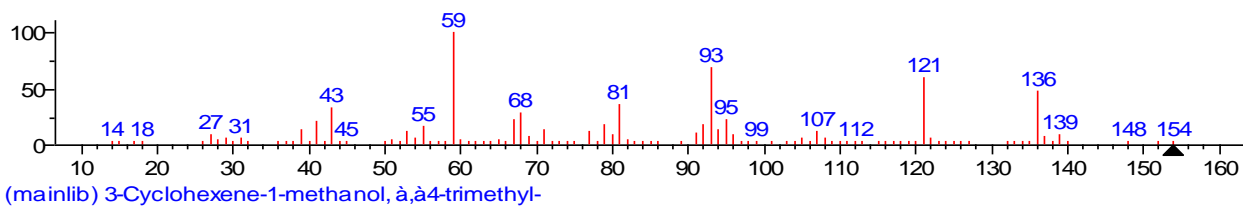
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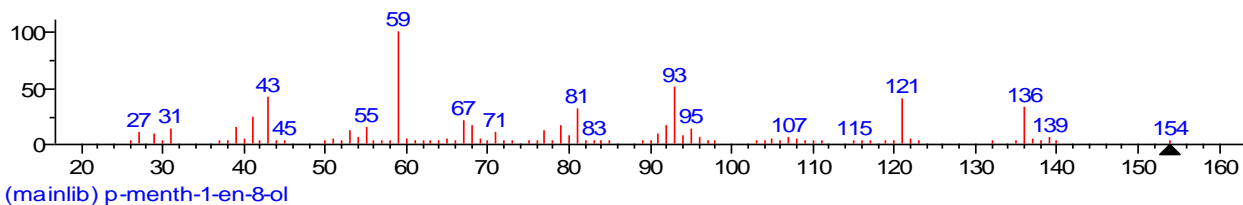
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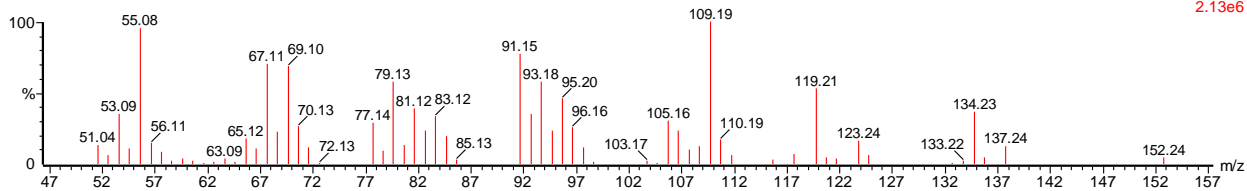


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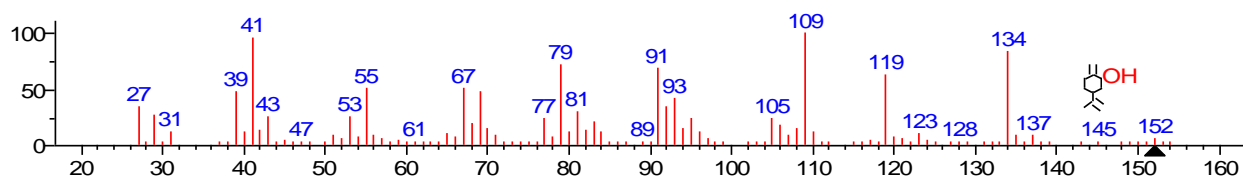


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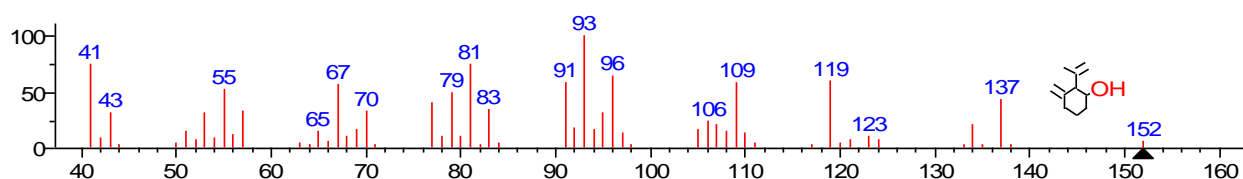
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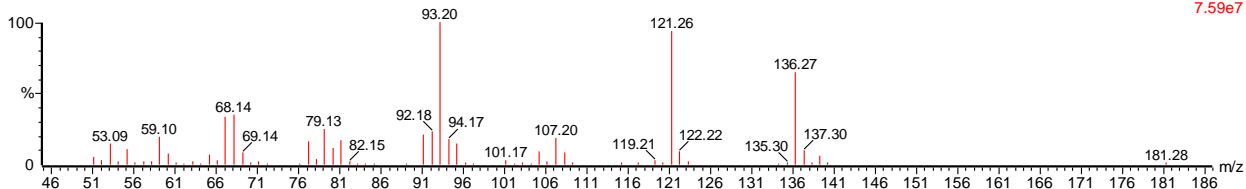
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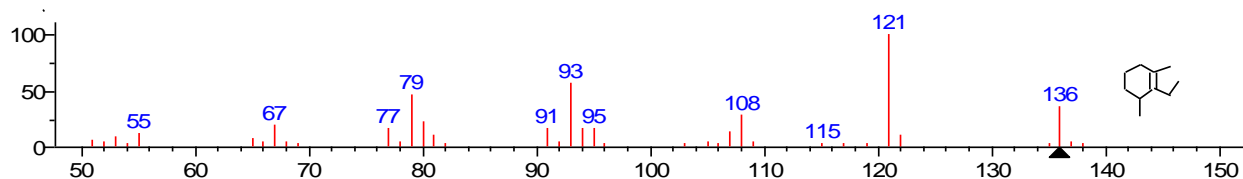
(mainlib) o-Mentha-1(7),8-dien-3-ol

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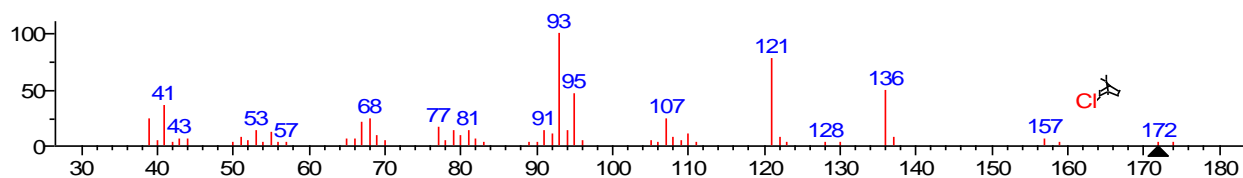
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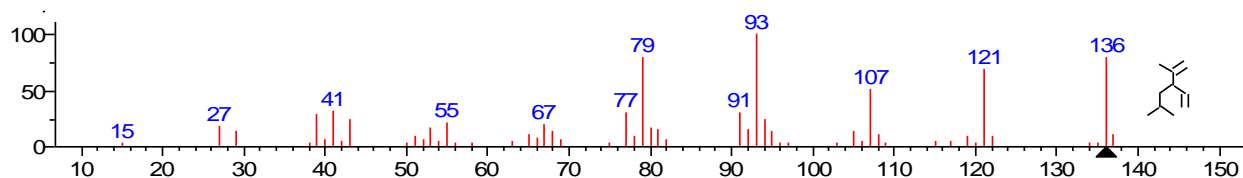
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2

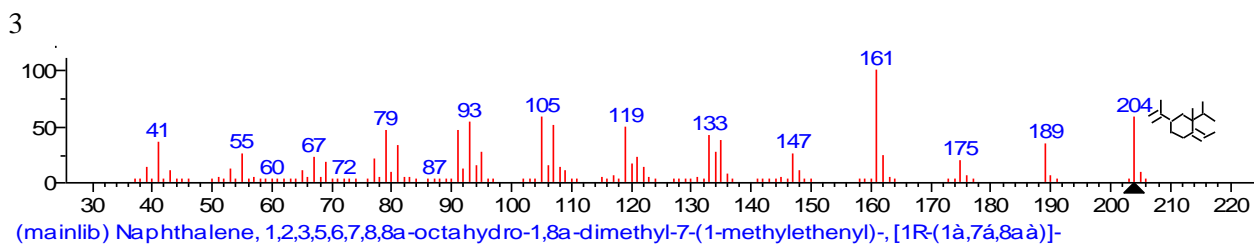
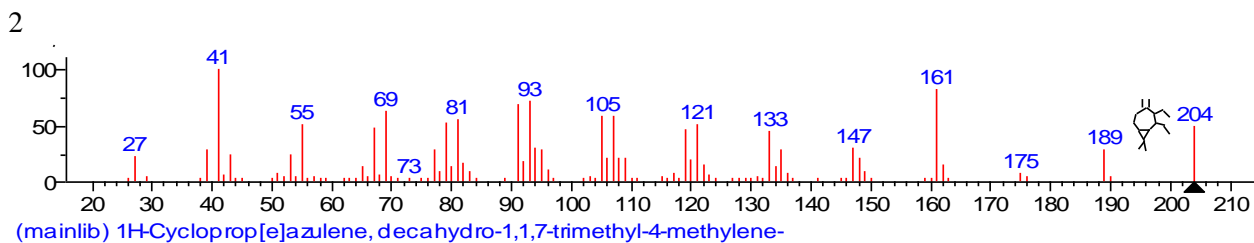
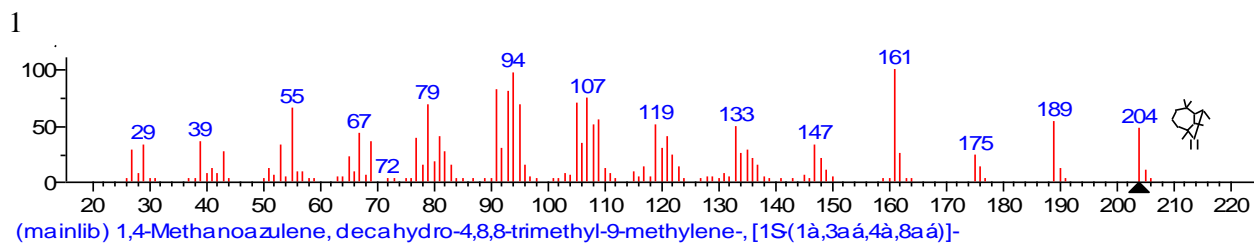
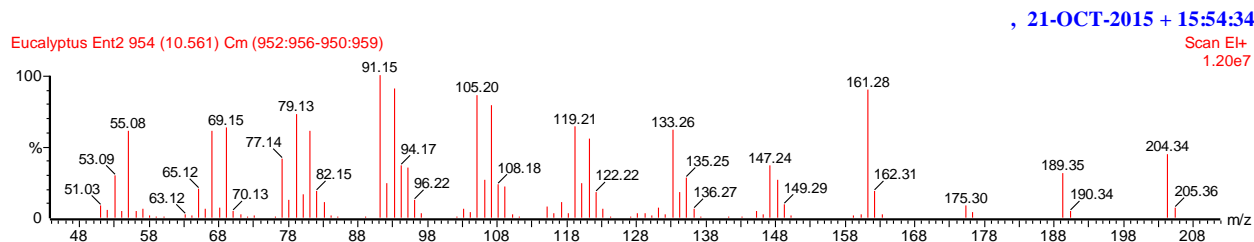
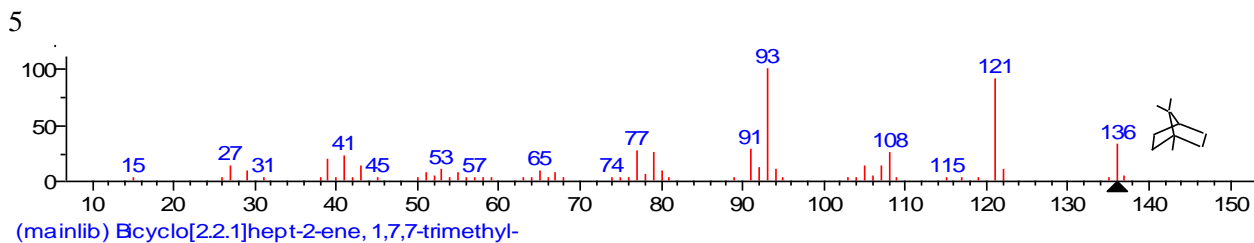
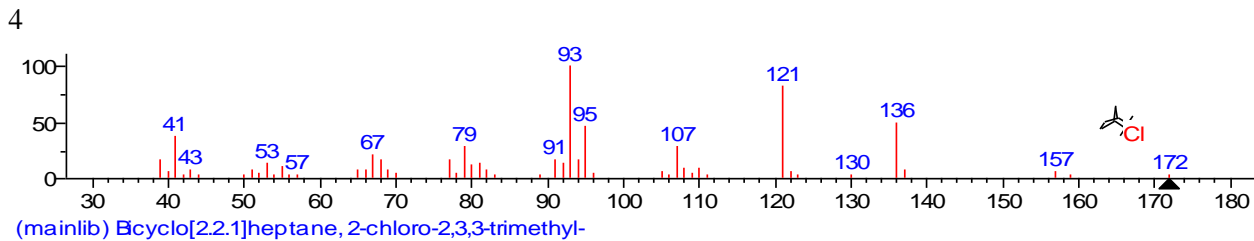


(mainlib) Bicyclo[2.2.1]heptane, 2-chloro-1,7,7-trimethyl-, exo-

3

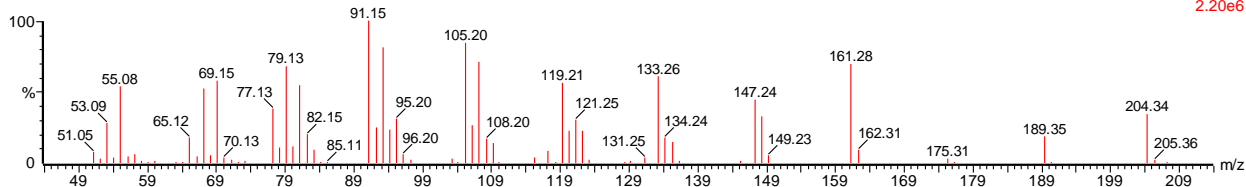


(mainlib) Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans(-)-

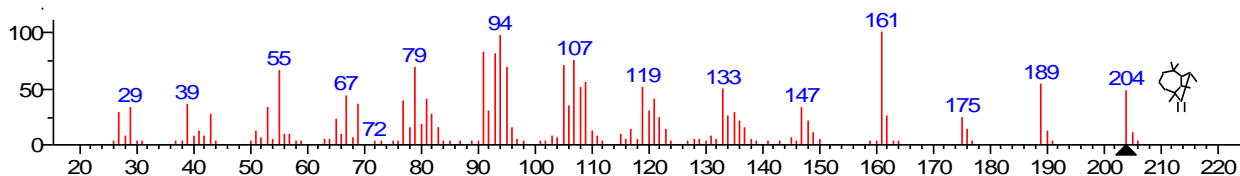


, 21-OCT-2015 + 15:54:34

Eucalyptus Ent2 996 (10.841) Cm (994:998-992:1000)

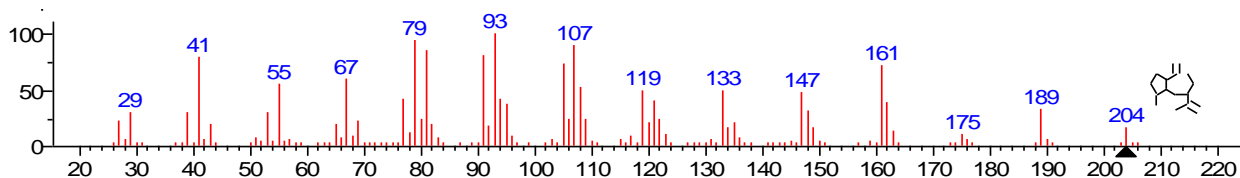
Scan E1+
2.20e6

1



(mainlib) 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1à,3aá,4à,8aá)]-

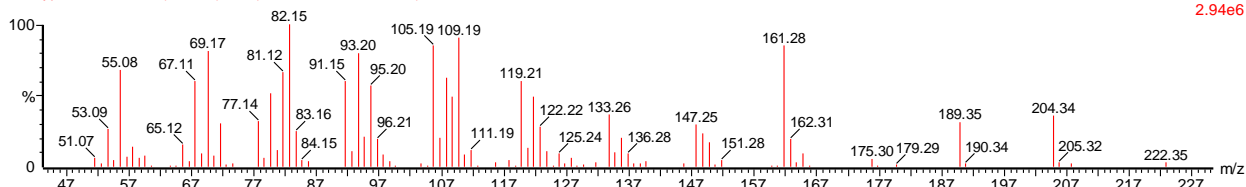
2



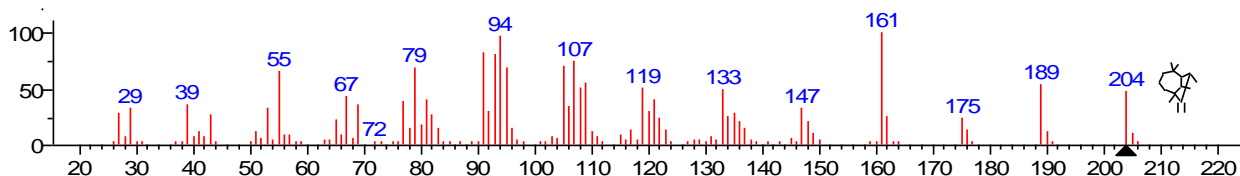
(mainlib) Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-

, 21-OCT-2015 + 15:54:34

Eucalyptus Ent2 1176 (12.042) Cm (1174:1178-1171:1180)

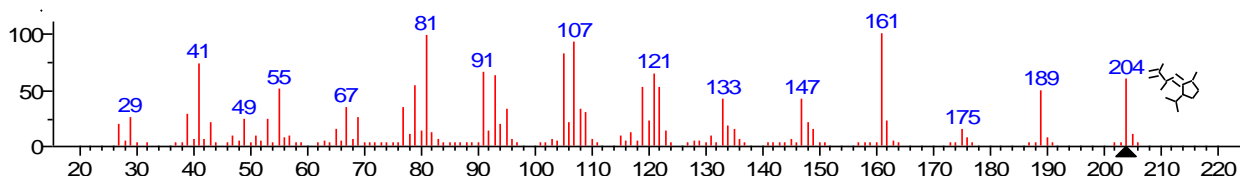
Scan E1+
2.94e6

1



(mainlib) 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1à,3aá,4à,8aá)]-

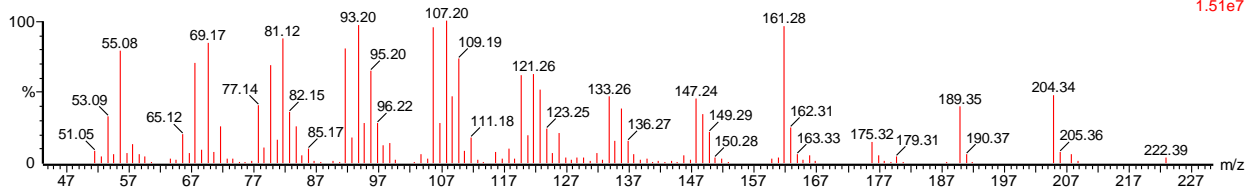
2



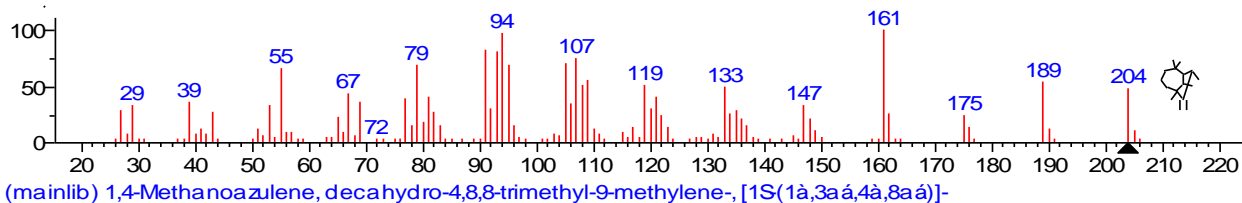
(mainlib) Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-

, 21-OCT-2015 + 15:54:34

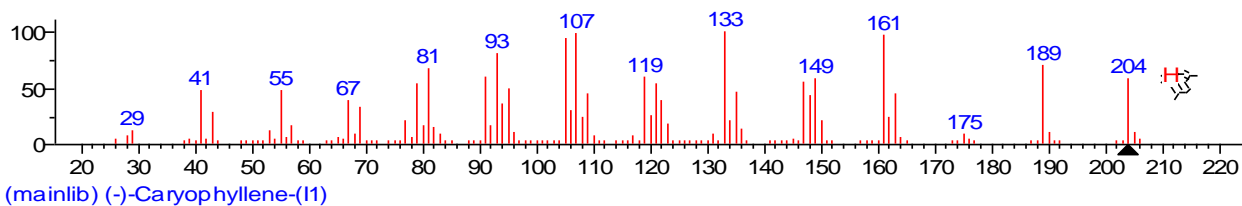
Eucalyptus Ent2 1221 (12.342) Cm (1218:1223-1215:1225)

Scan EI+
1.51e7

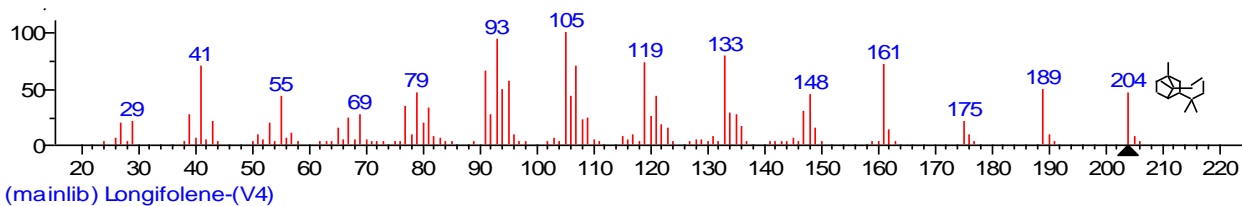
1



2

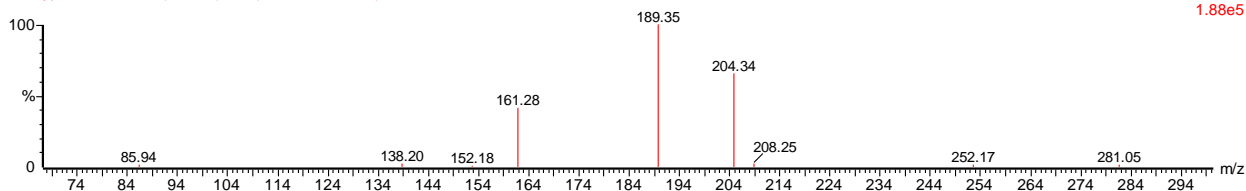


3

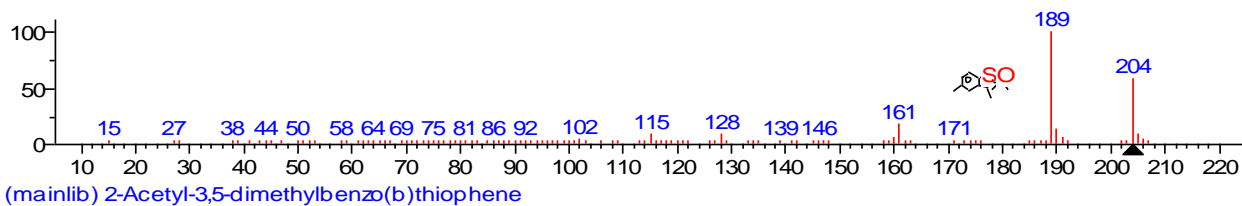


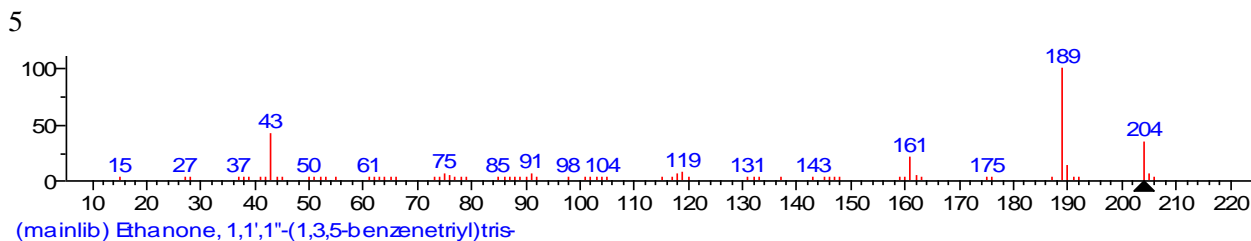
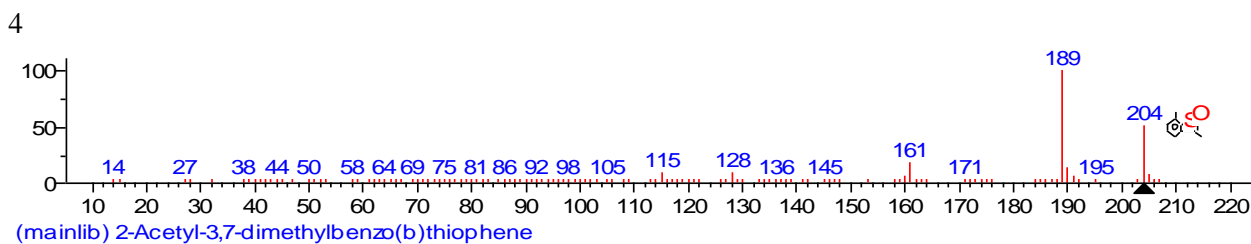
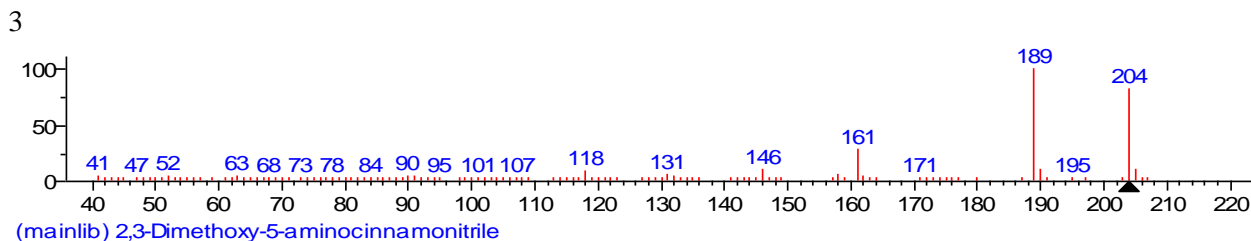
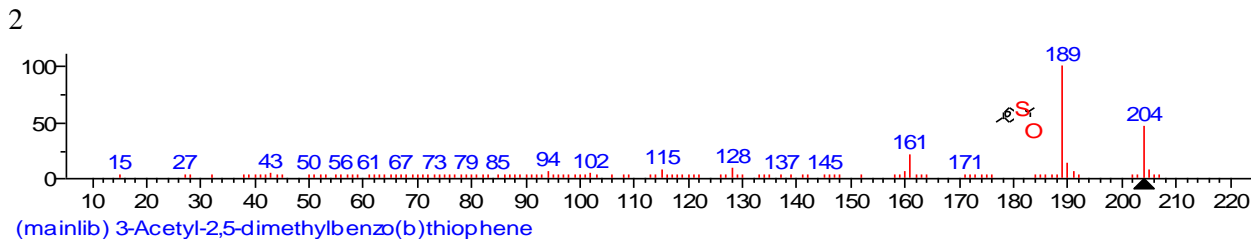
, 21-OCT-2015 + 15:54:34

Eucalyptus Ent2 1232 (12.415) Cm (1232-1230:1240)

Scan EI+
1.88e5

1

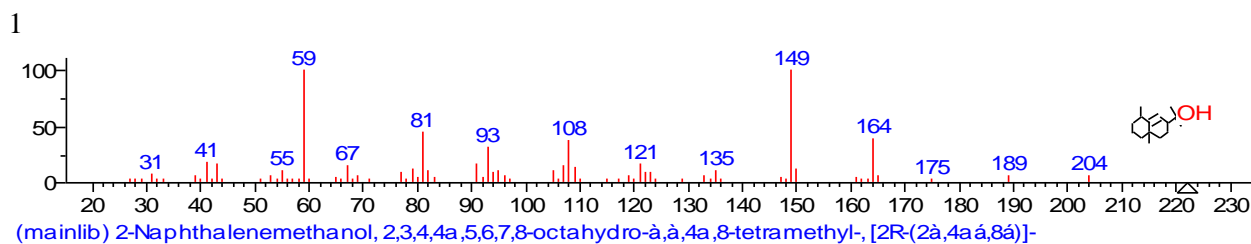
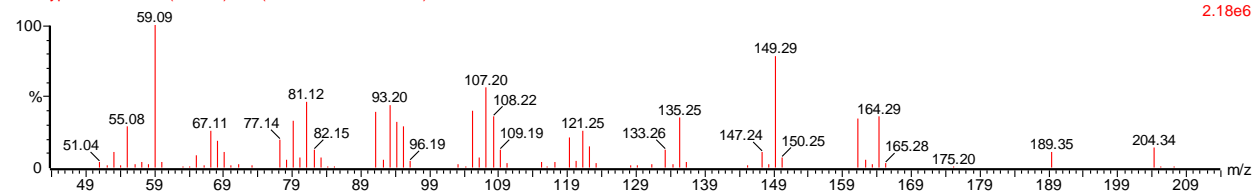


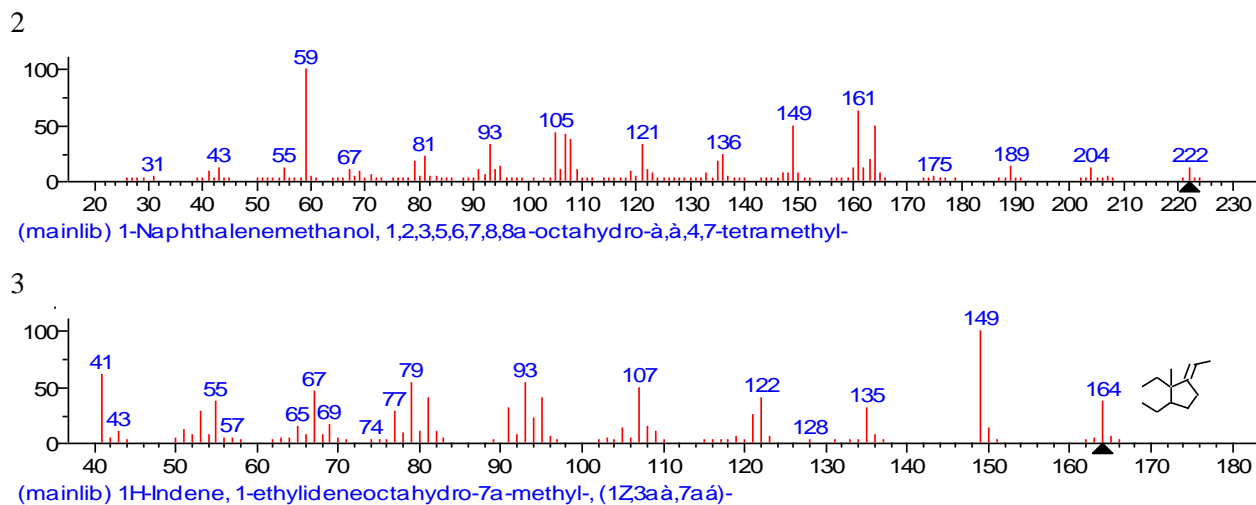


, 21-OCT-2015 + 15:54:34

Eucalyptus Ent2 1285 (12.769) Cm (1283:1288-1282:1290)

Scan EI+
2.18e6





, 22-OCT-2015 + 14:23:57

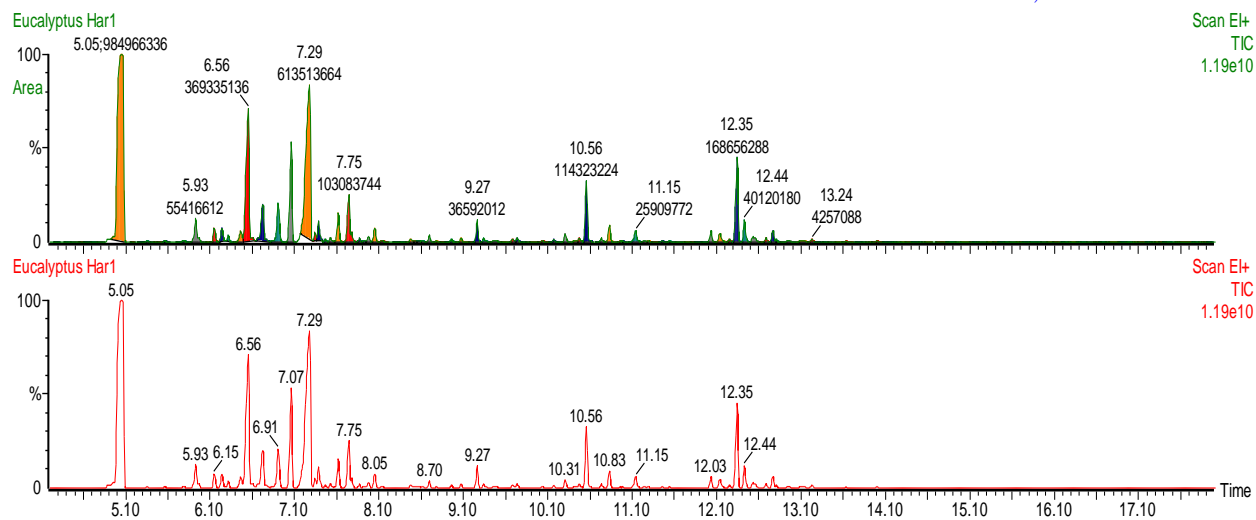
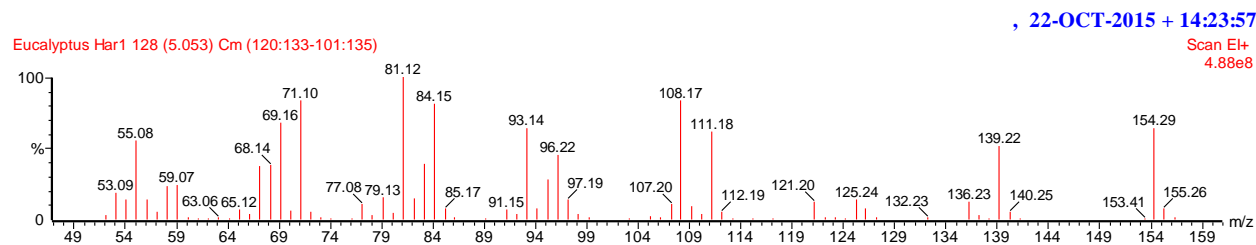
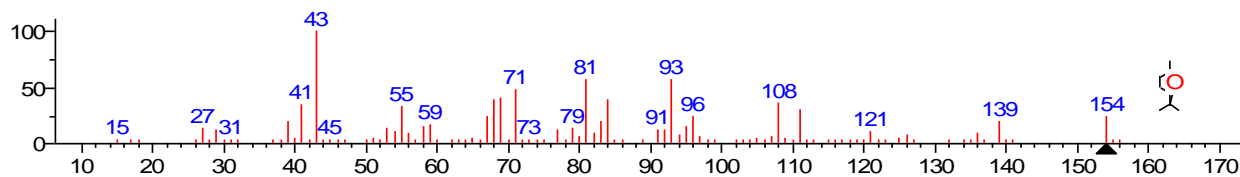


Figure2: GC-MS Chromatogram of *E. globules* essential oils collected from Haramaya University.

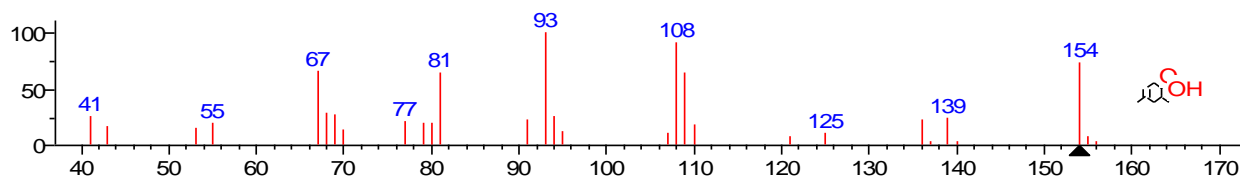


1



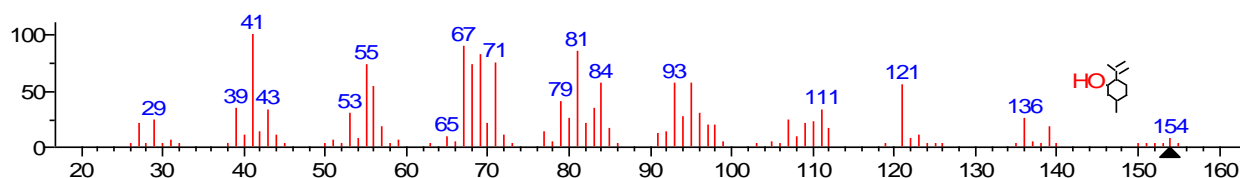
(mainlib) Eucalyptol

2



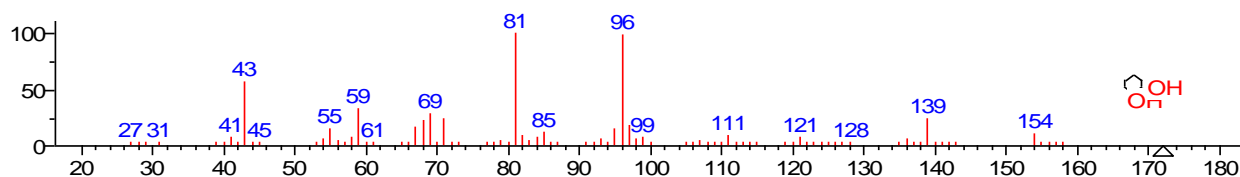
(mainlib) 2,4-Dimethylcyclohex-1-ene-5-carboxylic acid (trans)

3



(mainlib) Cyclohexanol, 5-methyl-2-(1-methylethenyl)-

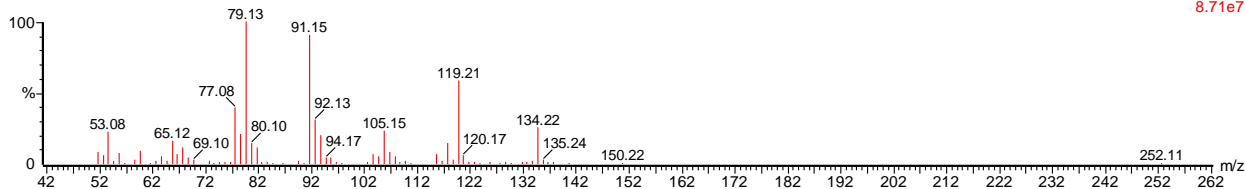
4



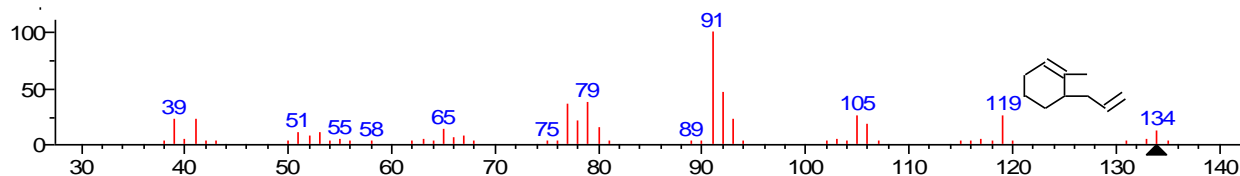
(mainlib) 2-(1-Hydroxy-1-methyl-ethyl)-1-methyl-cyclohexanol

Eucalyptus Har1 260 (5.933) Cm (257:263-252:270)

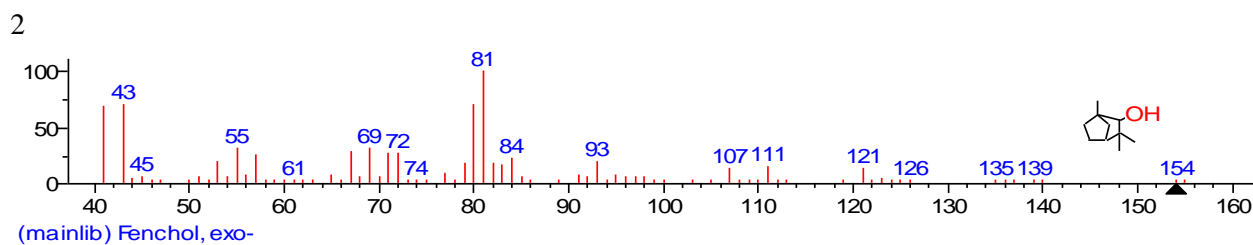
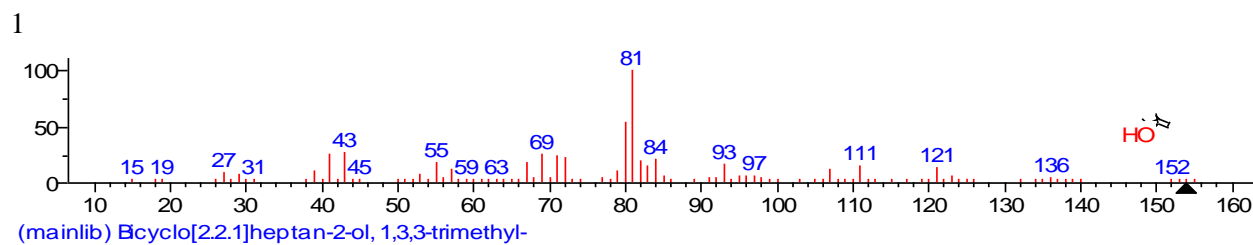
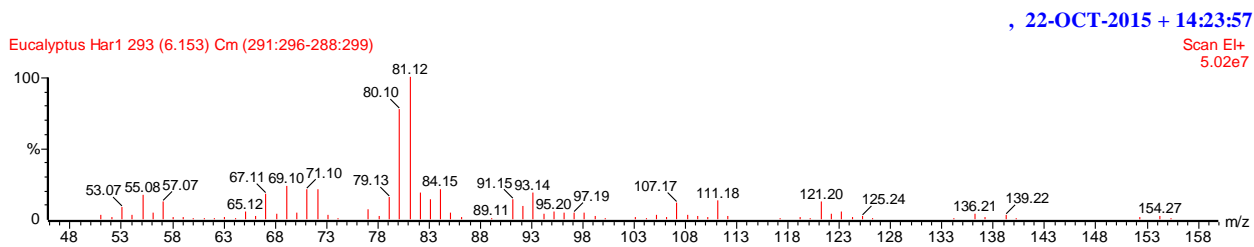
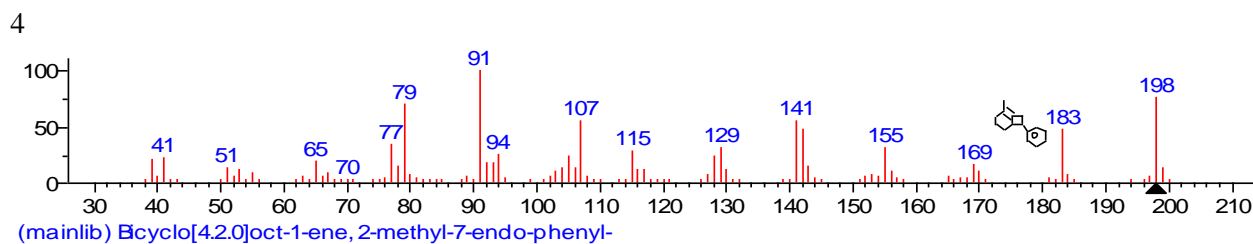
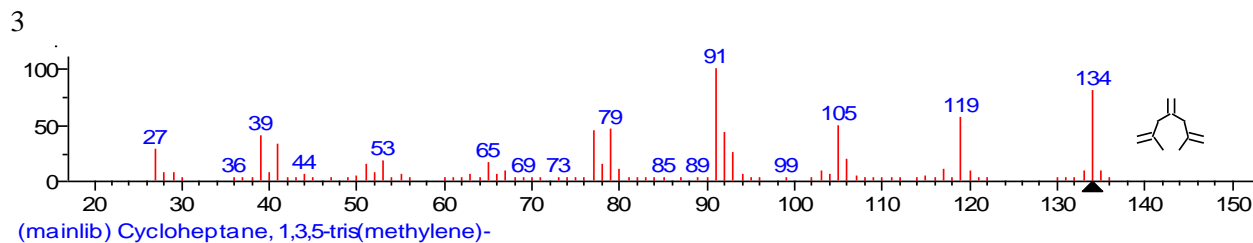
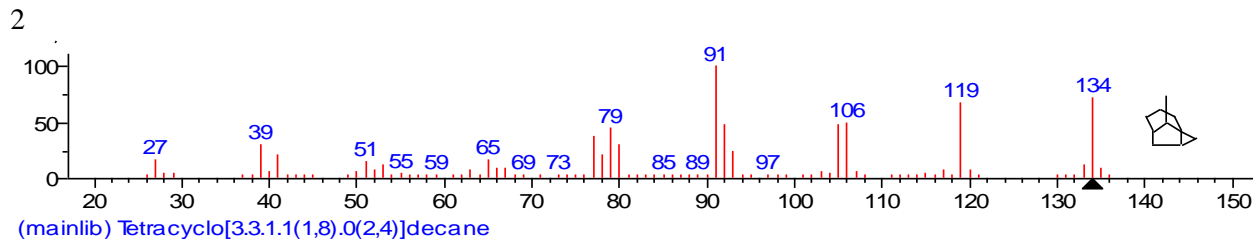
, 22-OCT-2015 + 14:23:57

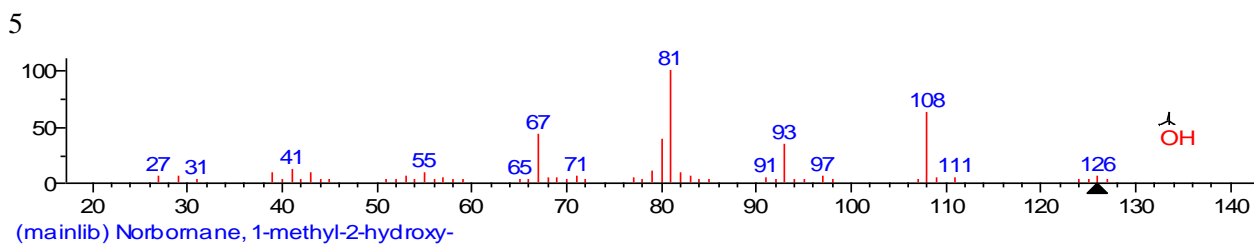
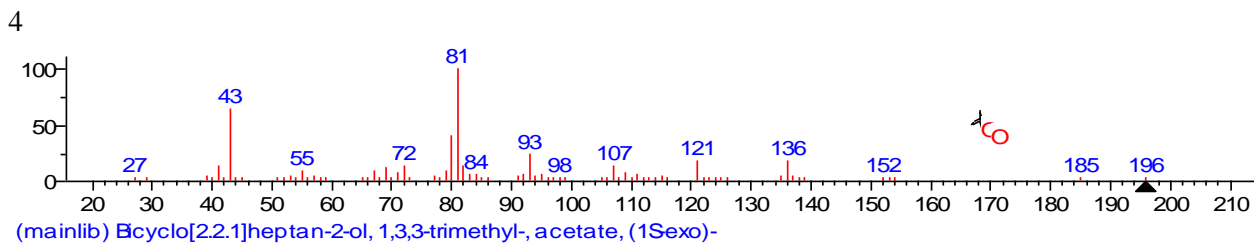
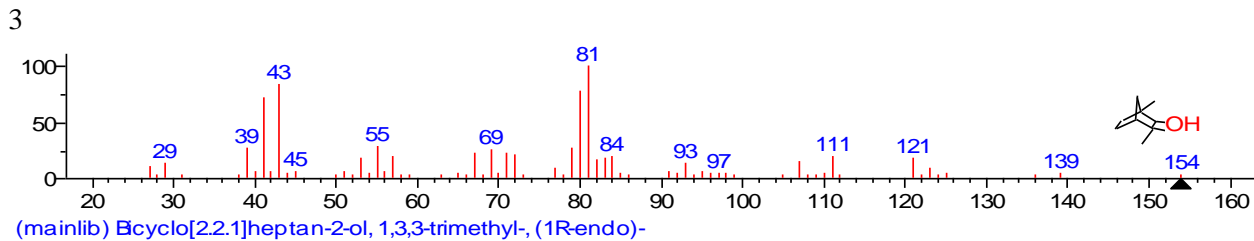
Scan E1+
8.71e7

1



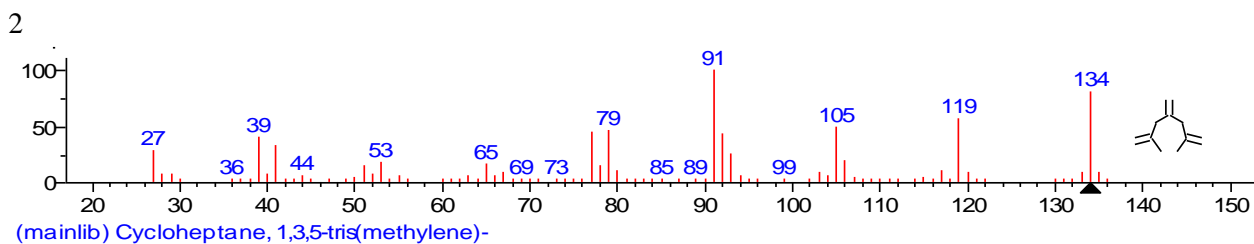
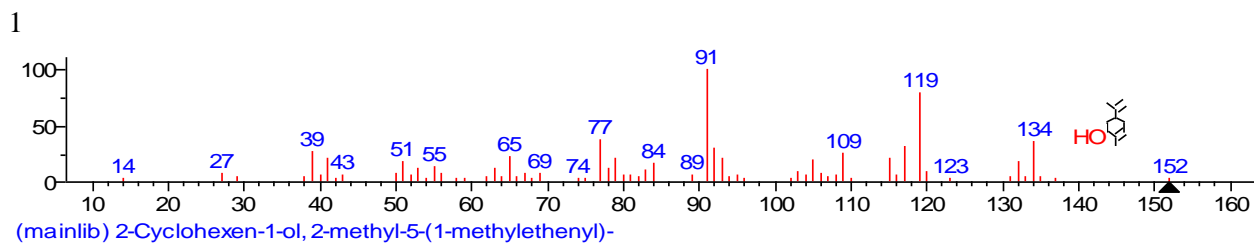
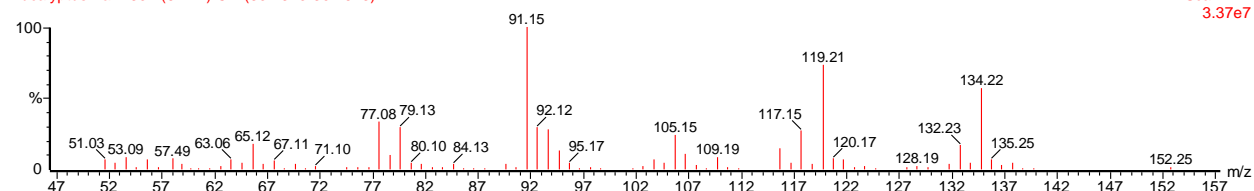
(mainlib) Bicyclo[4.2.0]oct-1-ene, 7-exo-ethenyl-

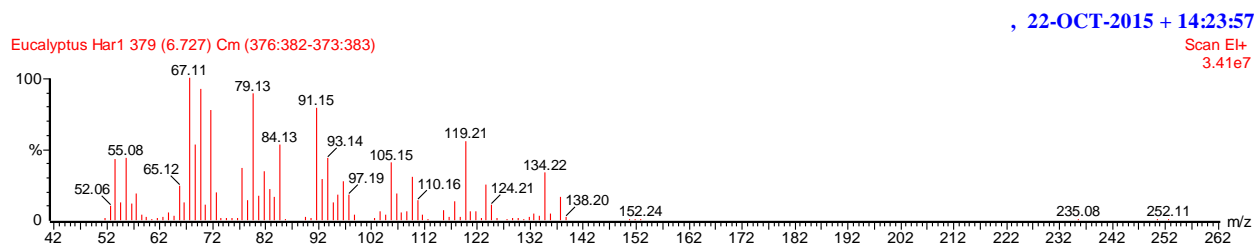
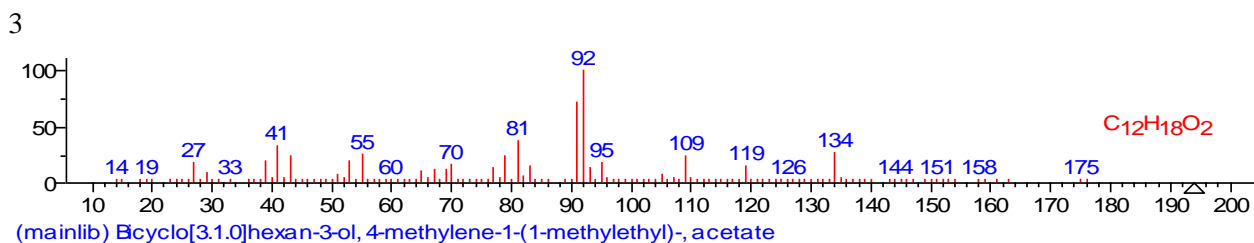
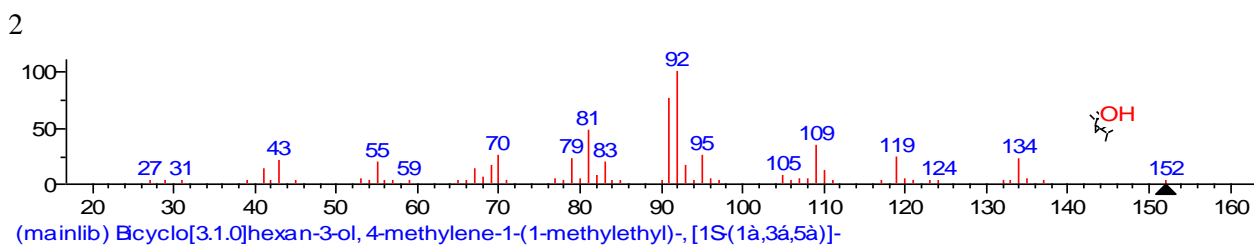
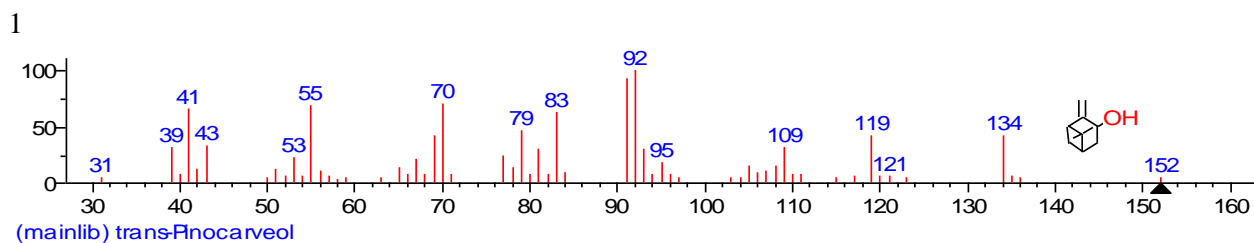
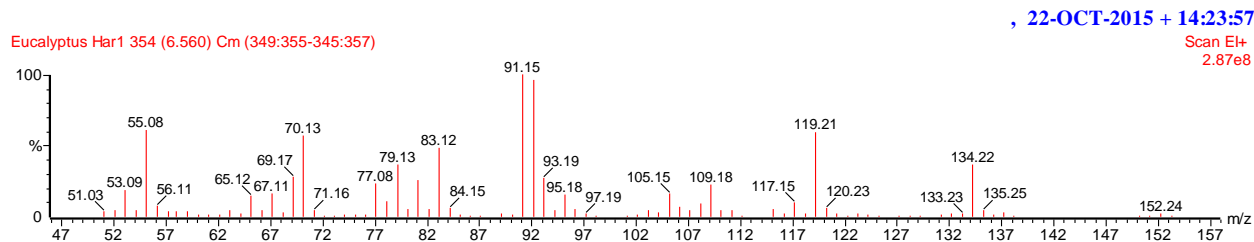
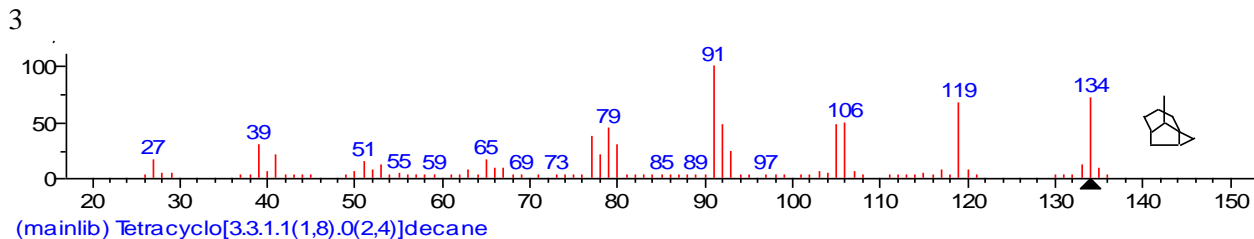


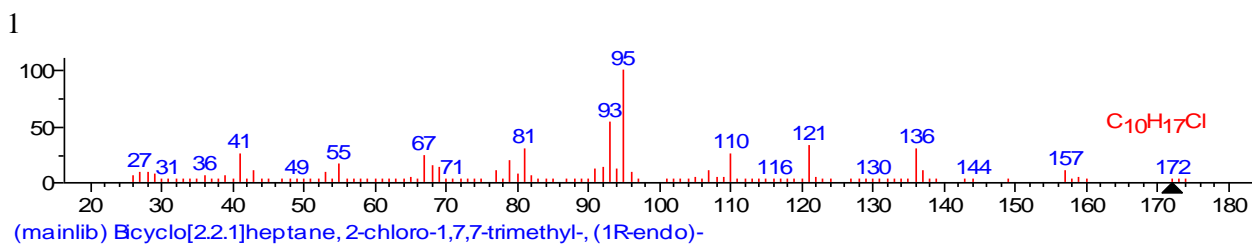
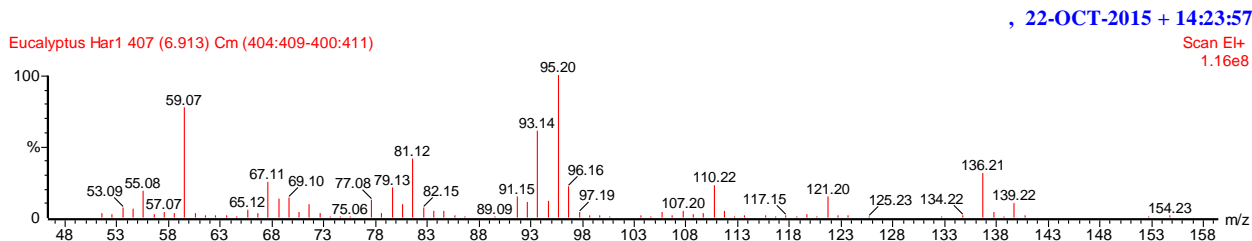
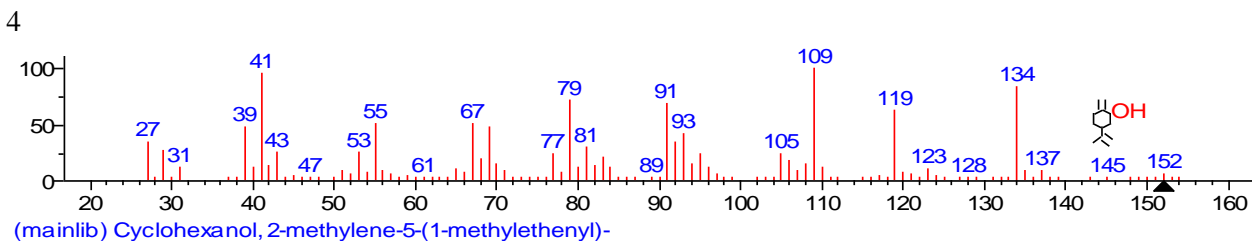
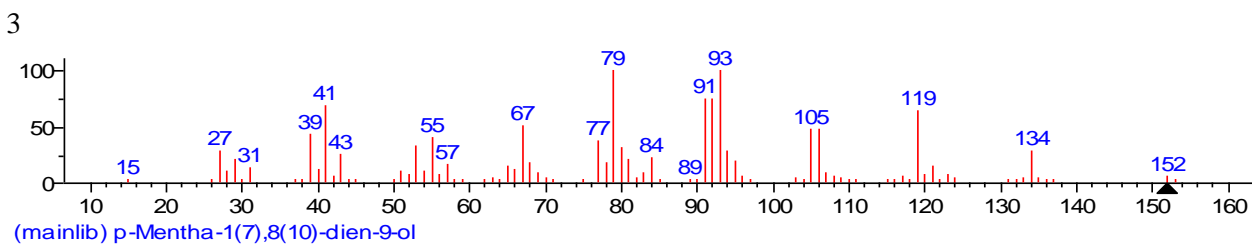
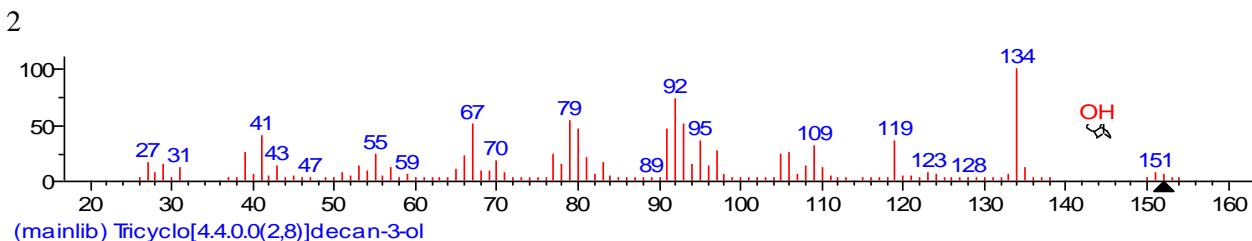
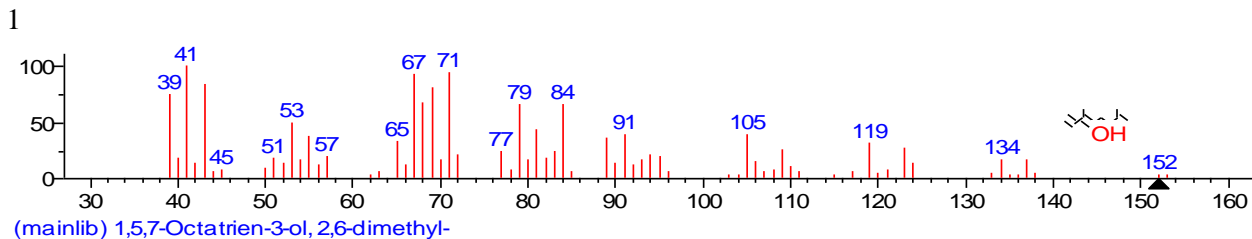


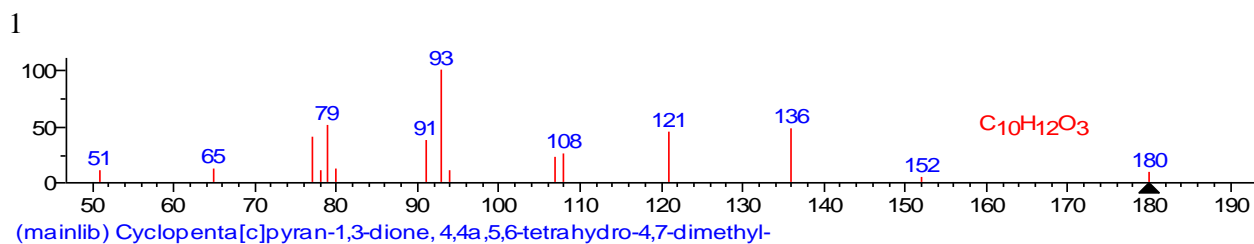
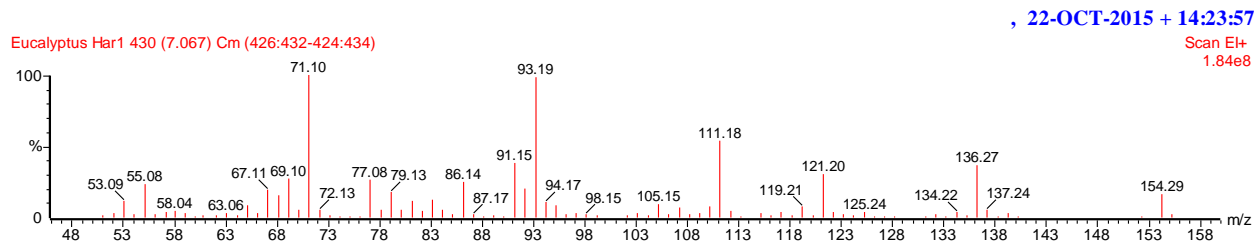
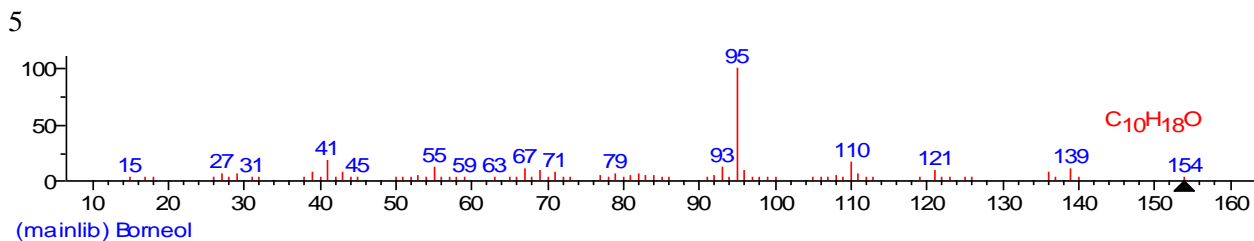
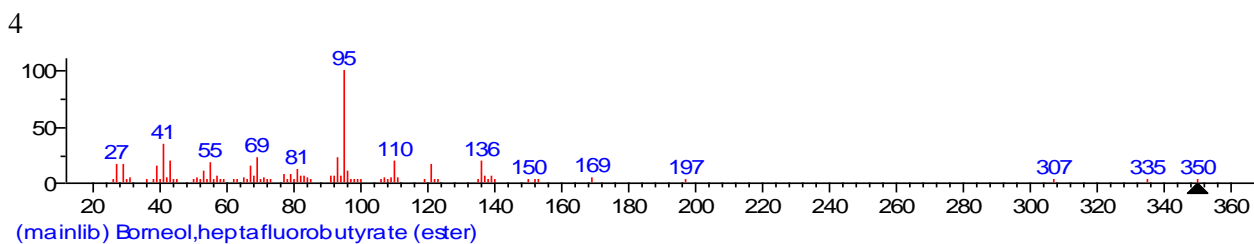
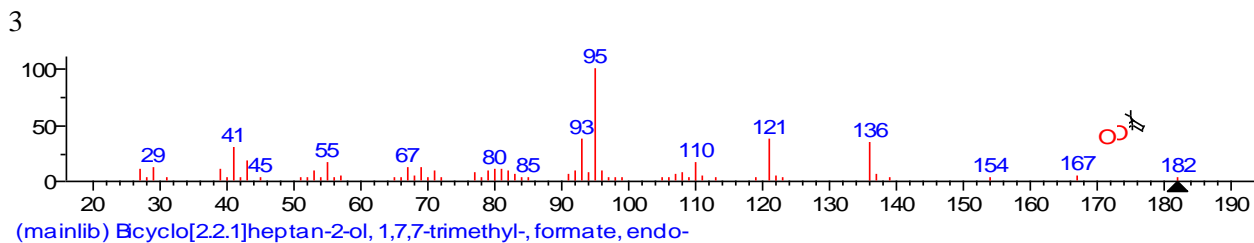
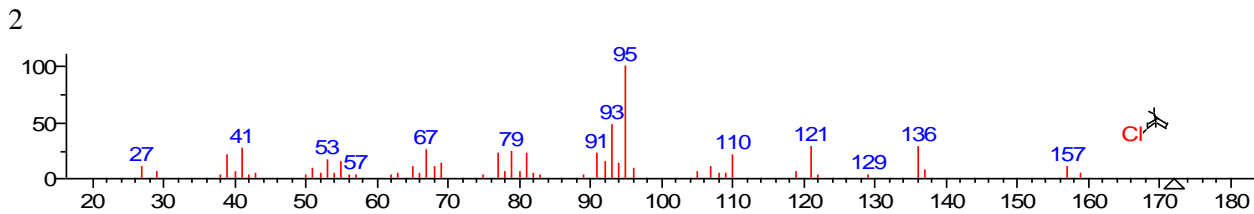
, 22-OCT-2015 + 14:23:57

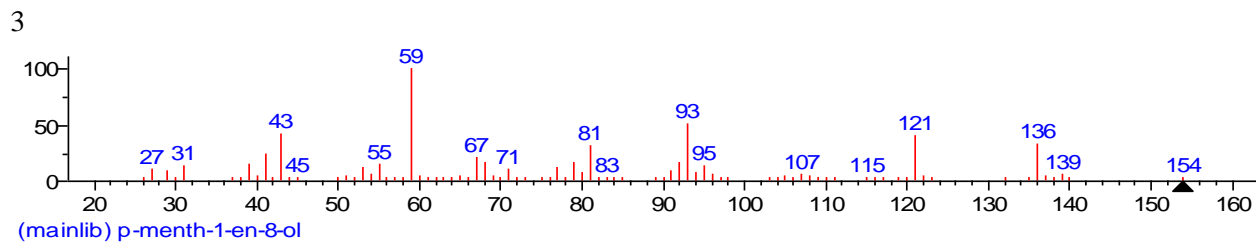
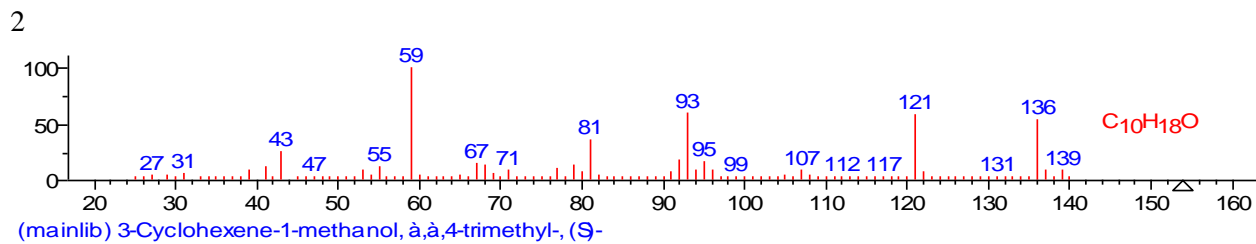
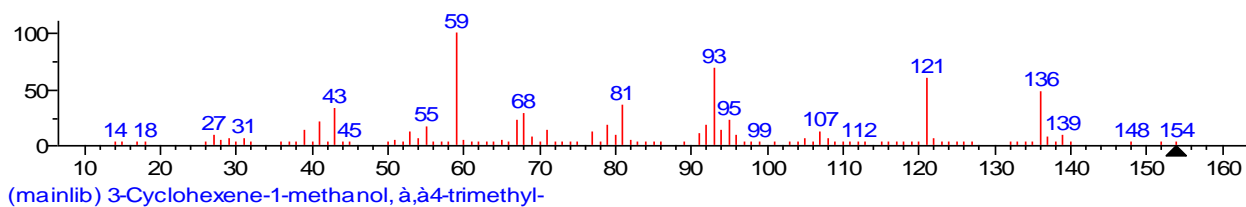
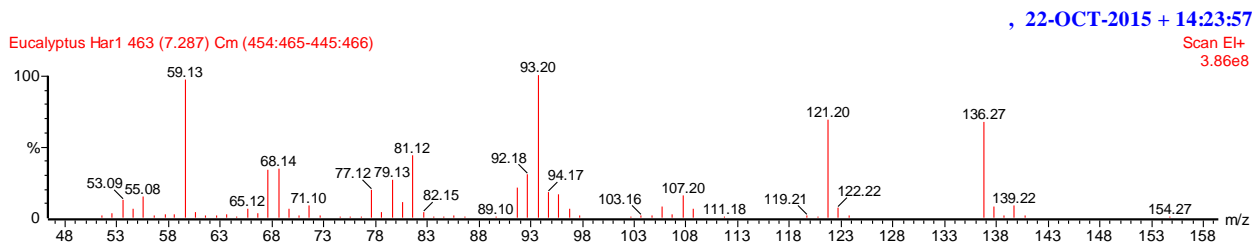
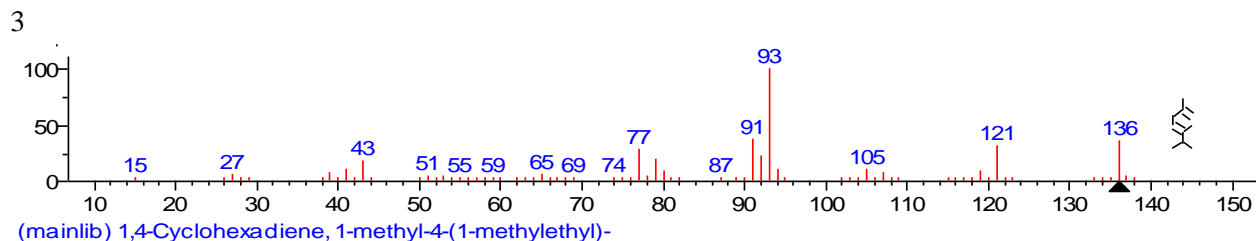
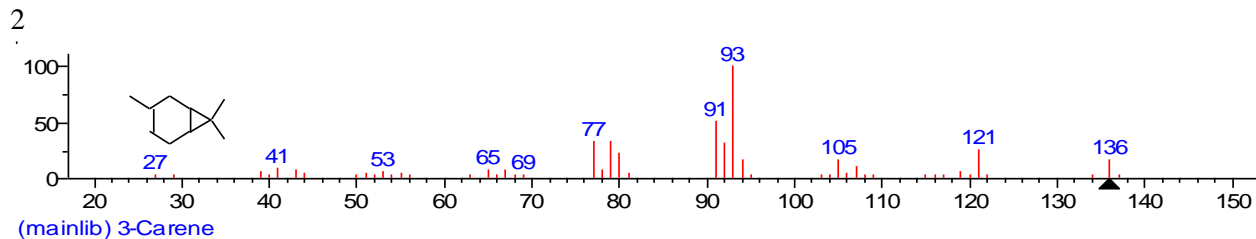
Eucalyptus Har1 307 (6.247) Cm (304:310-302:313)

Scan El+
3.37e7



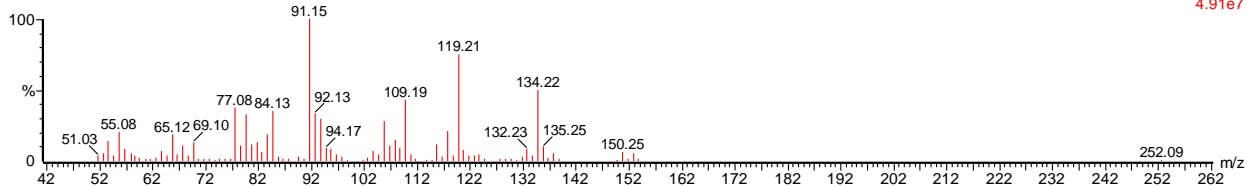




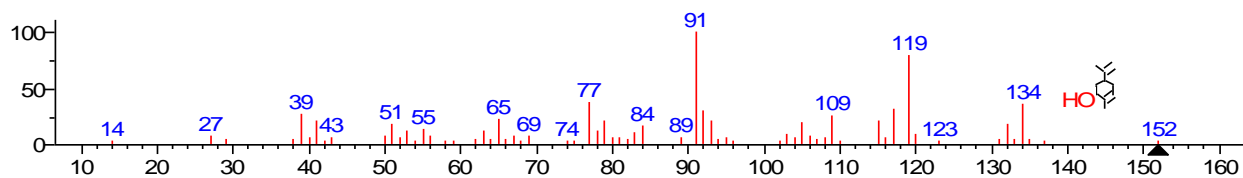


, 22-OCT-2015 + 14:23:57

Eucalyptus Har1 513 (7.620) Cm (511:516-509:518)

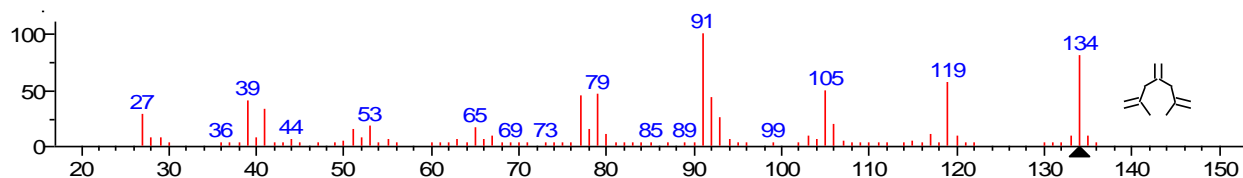
Scan E1+
4.91e7

1



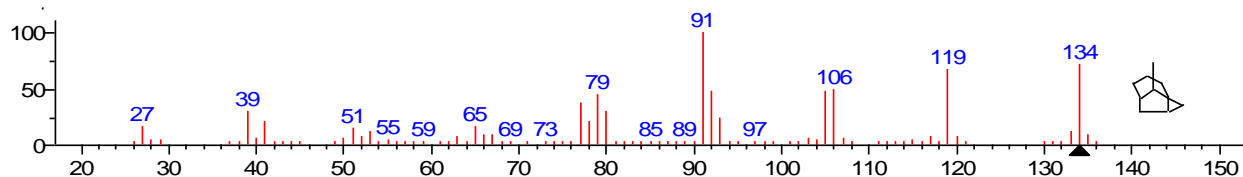
(mainlib) 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-

2



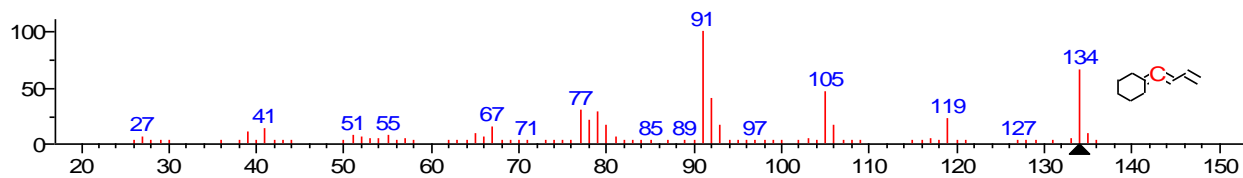
(mainlib) Cycloheptane, 1,3,5-tris(methylene)-

3



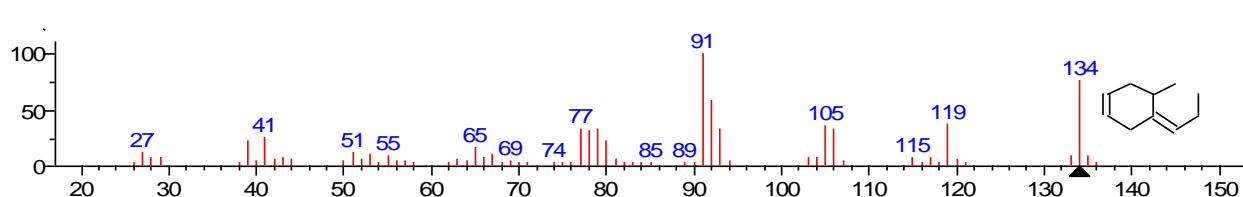
(mainlib) Tetracyclo[3.3.1.1(1,8).0(2,4)]decane

4



(mainlib) Cyclohexane, 1,3-butadienylidene-

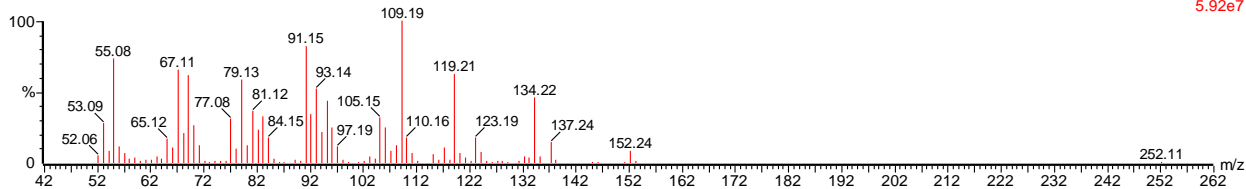
5



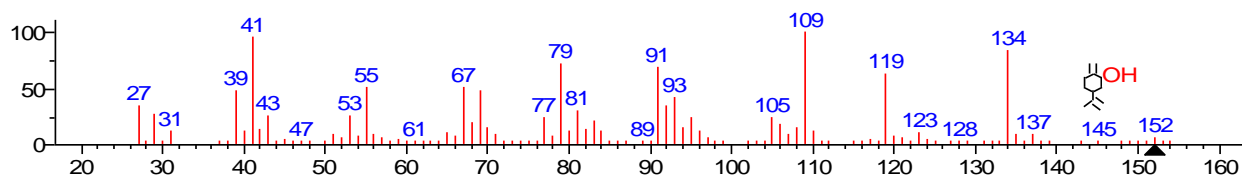
(mainlib) Naphthalene, 1,2,3,5,8,8a-hexahydro-

, 22-OCT-2015 + 14:23:57

Eucalyptus Har1 532 (7.747) Cm (530:534-528:536)

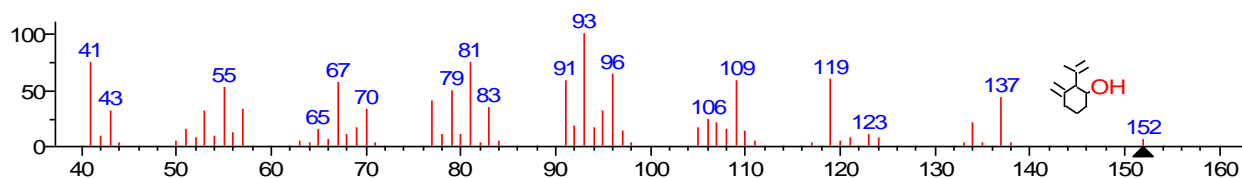
Scan E1+
5.92e7

1



(mainlib) Cyclohexanol, 2-methylene-5-(1-methylethenyl)-

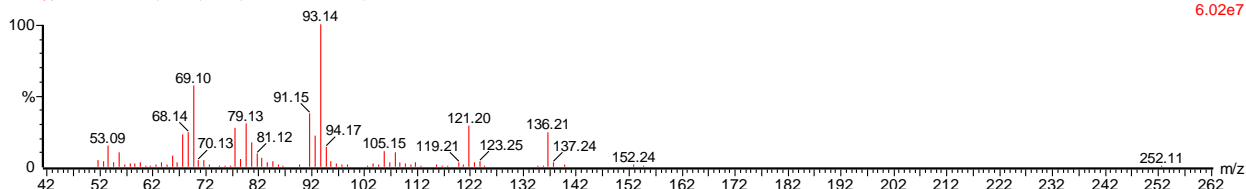
2



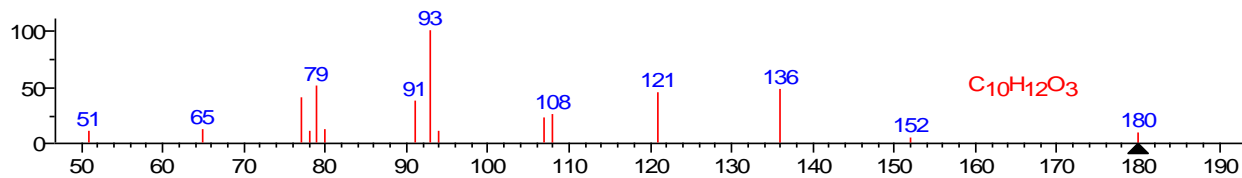
(mainlib) o-Mentha-1(7),8-dien-3-ol

, 22-OCT-2015 + 14:23:57

Eucalyptus Har1 578 (8.054) Cm (576:580-573:584)

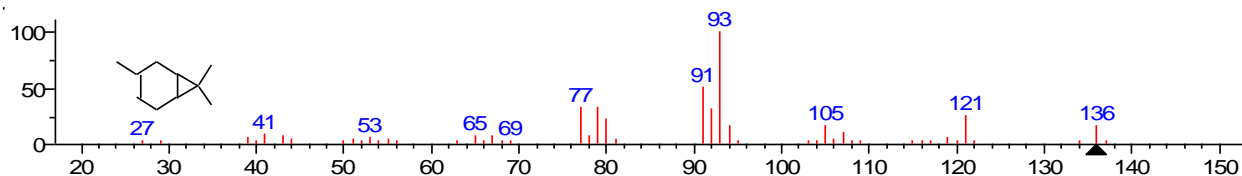
Scan E1+
6.02e7

1

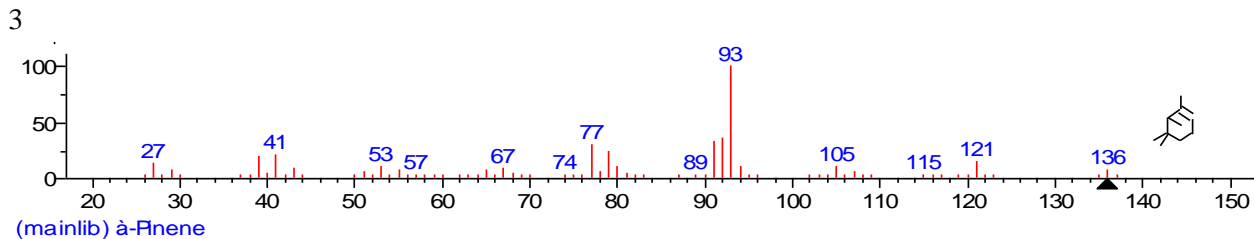


(mainlib) Cyclopenta[c]pyran-1,3-dione, 4,4a,5,6-tetrahydro-4,7-dimethyl-

2

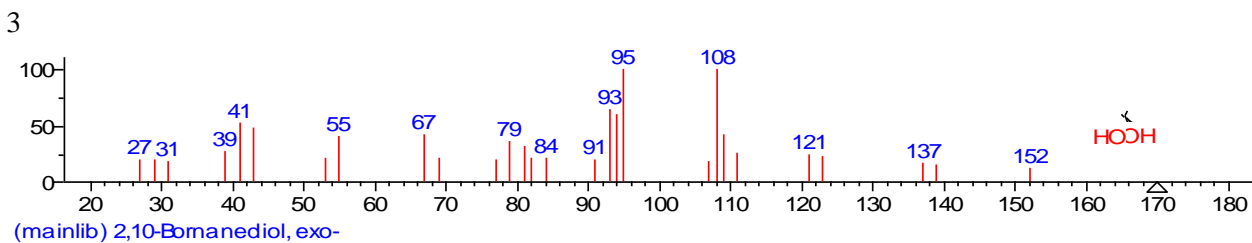
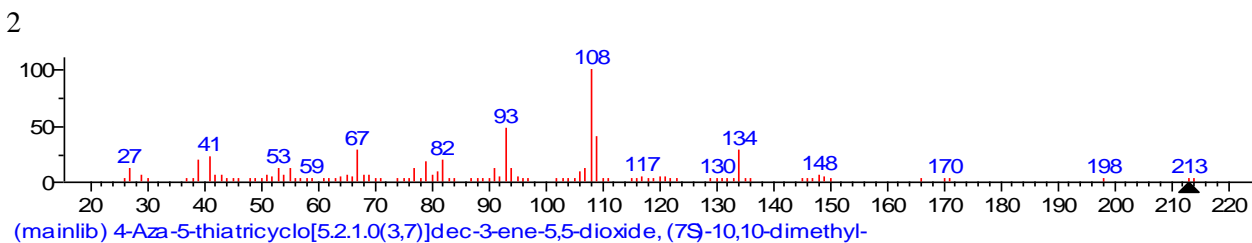
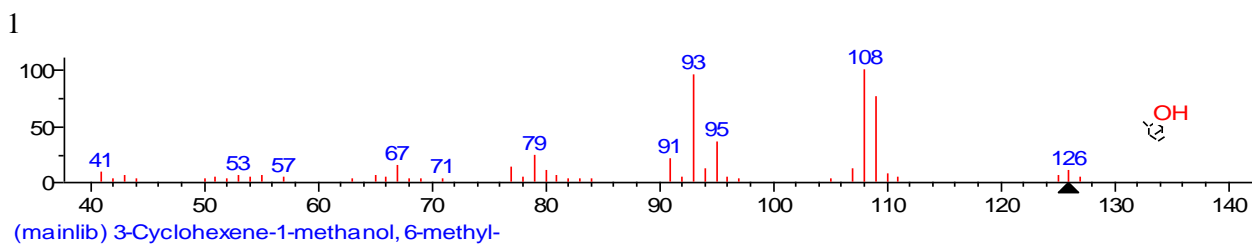
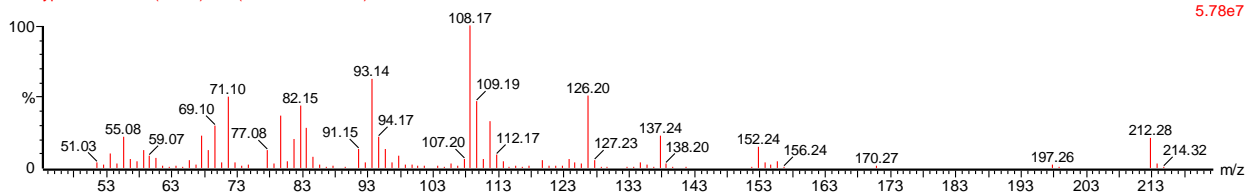


(mainlib) 3-Carene



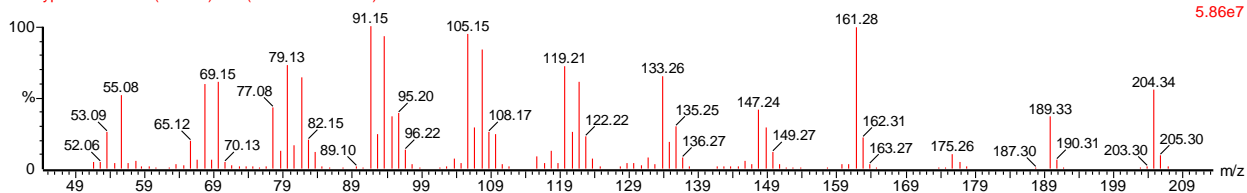
, 22-OCT-2015 + 14:23:57

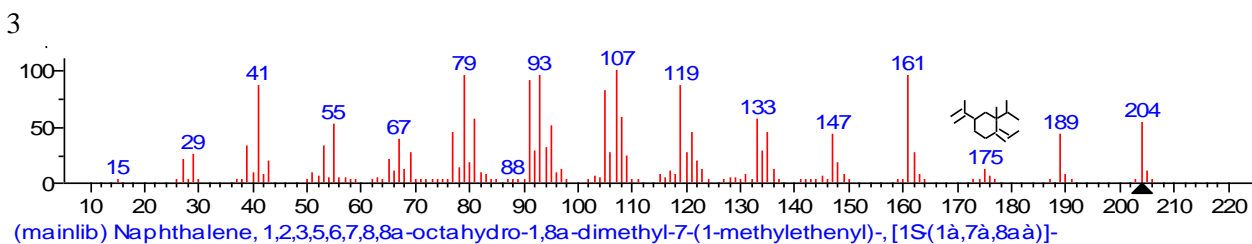
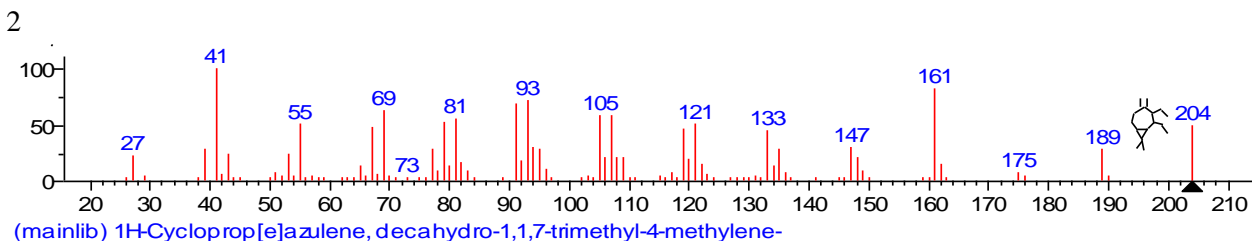
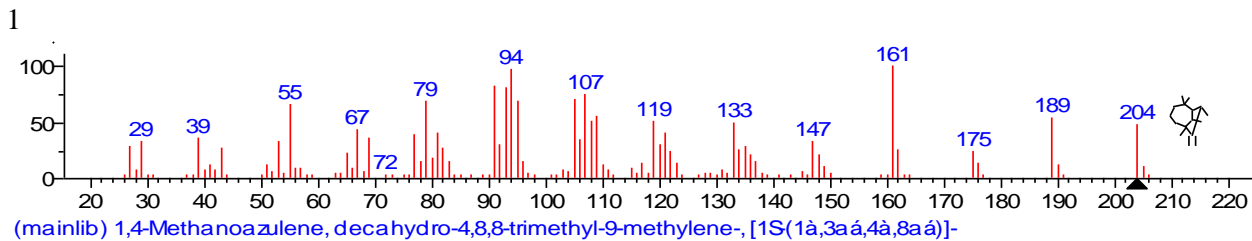
Eucalyptus Har1 760 (9.267) Cm (758:762-755:765)

Scan E1+
5.78e7

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Eucalyptus Har1 954 (10.561) Cm (952:957-949:959)

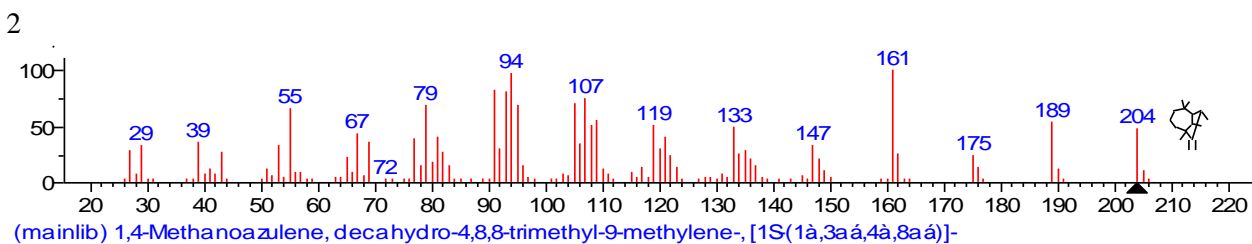
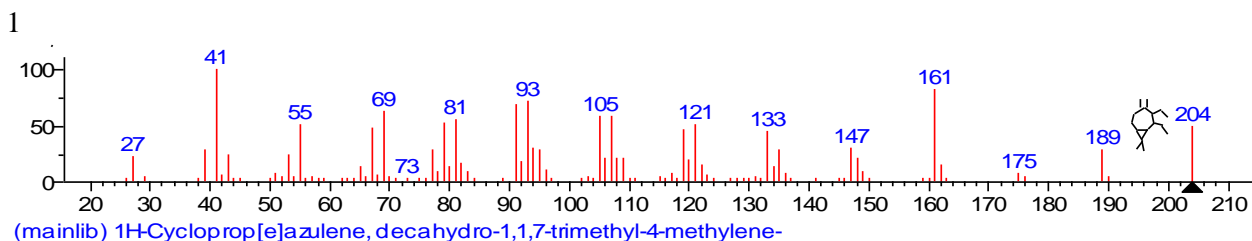
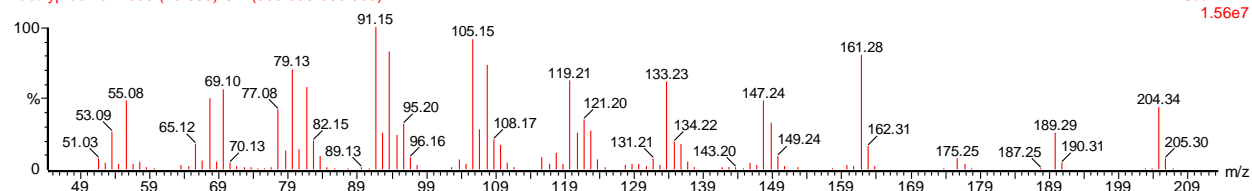
Scan E1+
5.86e7



, 22-OCT-2015 + 14:23:57

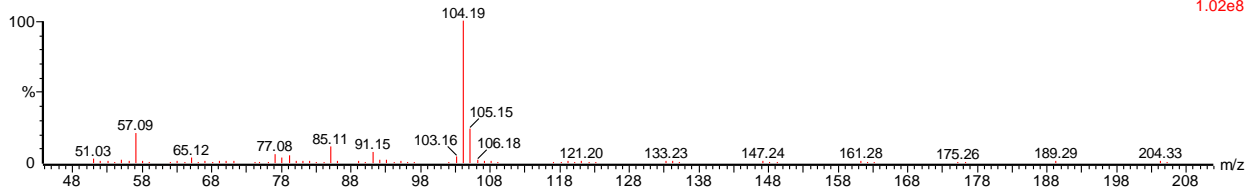
Eucalyptus Har1 995 (10.835) Cm (993:998-990:999)

Scan EI+
1.56e7

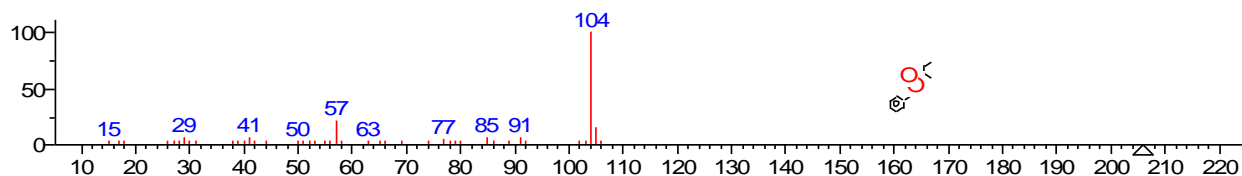


, 22-OCT-2015 + 14:23:57

Eucalyptus Har1 1042 (11.148) Cm (1039:1044-1035:1048)

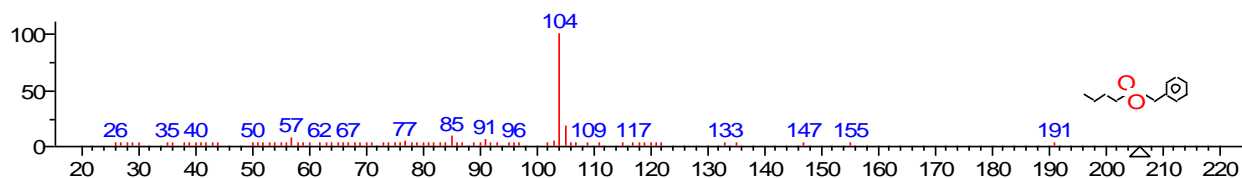
Scan E1+
1.02e8

1



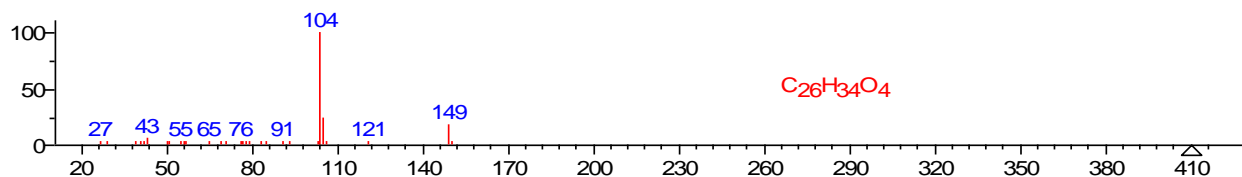
(mainlib) Butanoic acid, 2-methyl-, 2-phenylethyl ester

2



(mainlib) Pentanoic acid, 2-phenylethyl ester

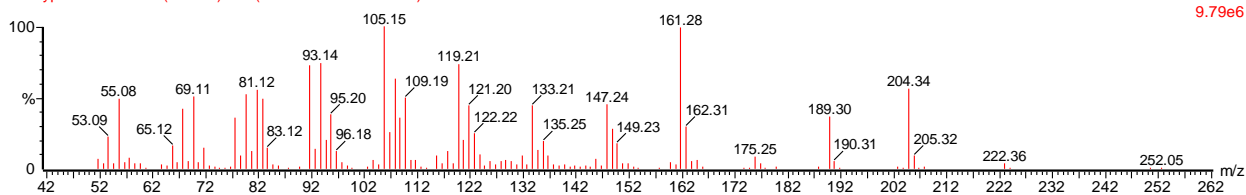
3



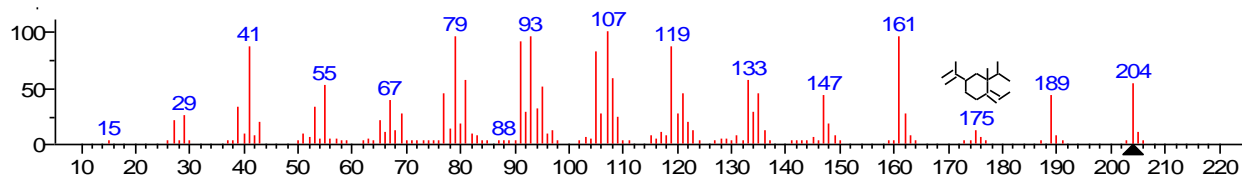
(mainlib) Phthalic acid, decyl 2-phenylethyl ester

, 22-OCT-2015 + 14:23:57

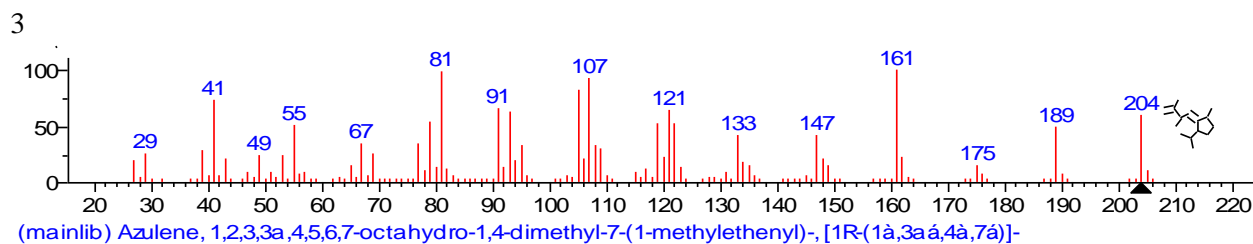
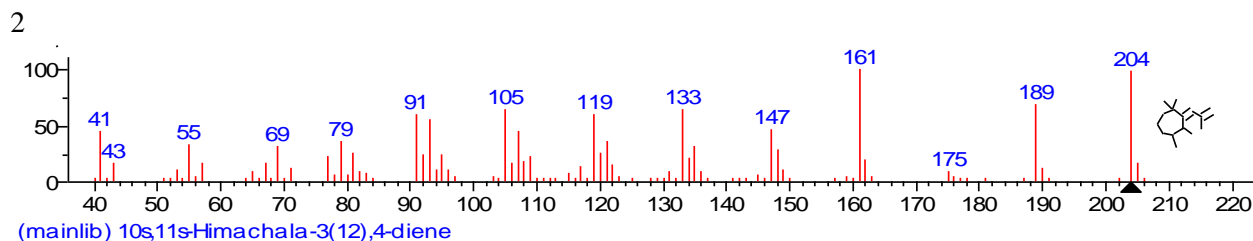
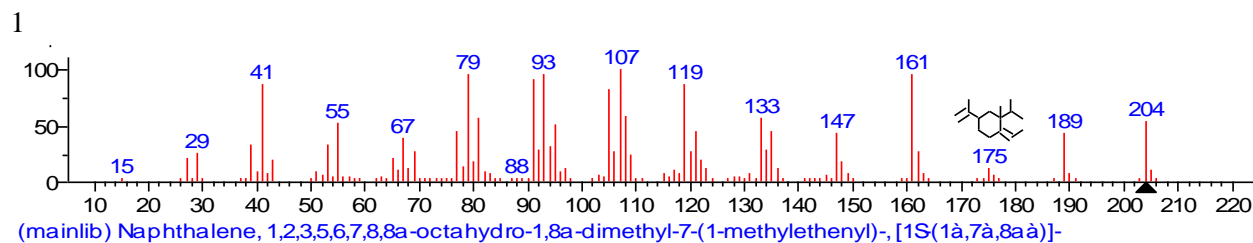
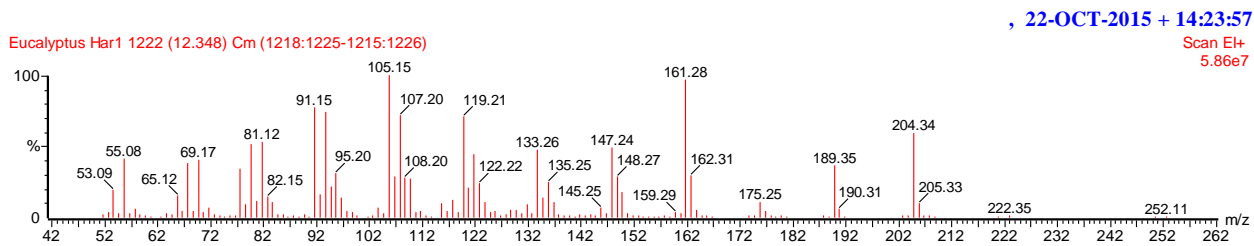
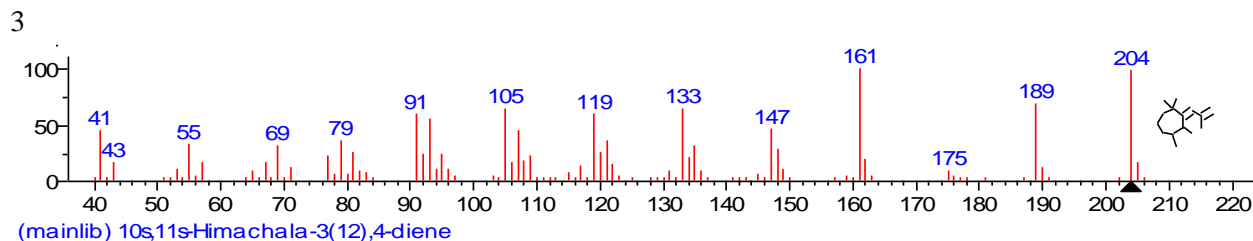
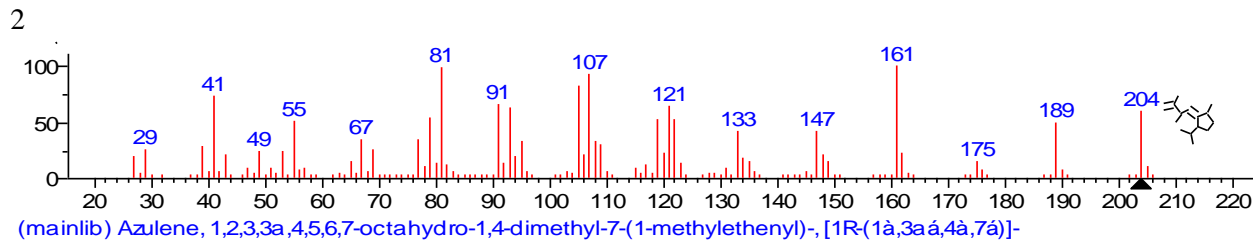
Eucalyptus Har1 1175 (12.035) Cm (1173:1178-1171:1180)

Scan E1+
9.79e6

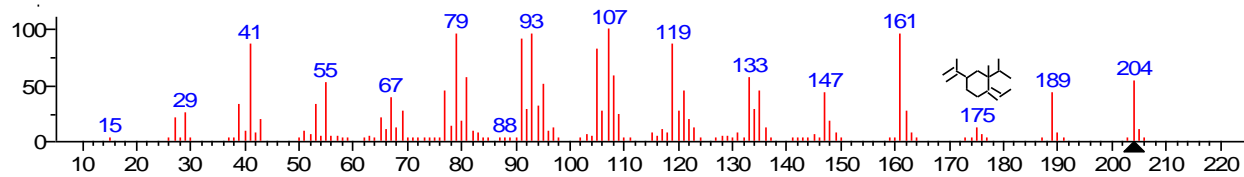
1



(mainlib) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S(1à,7à,8aà)]-



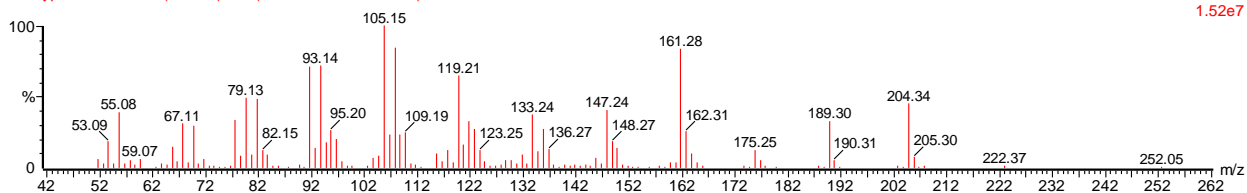
4



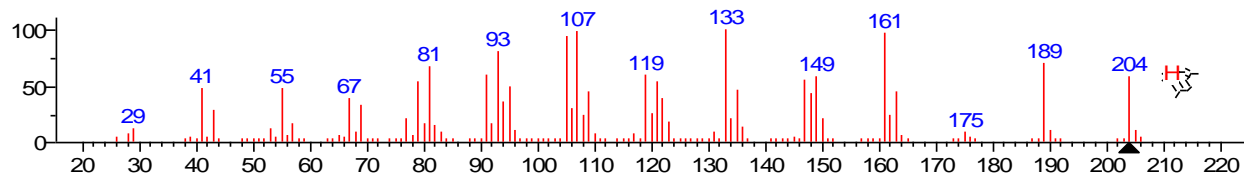
(mainlib) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aà)]-

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Eucalyptus Har1 1235 (12.435) Cm (1232:1238-1231:1240)

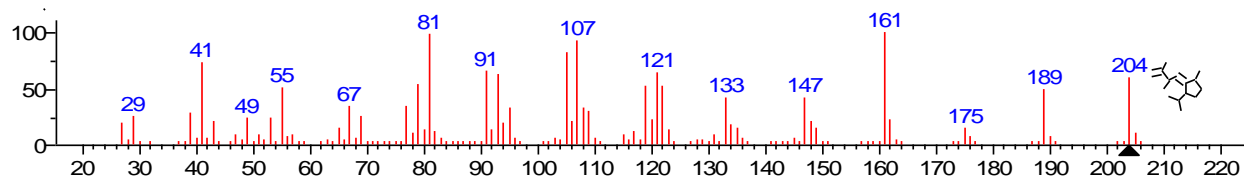
Scan EI+
1.52e7

1



(mainlib) (-)-Caryophyllene-(1)

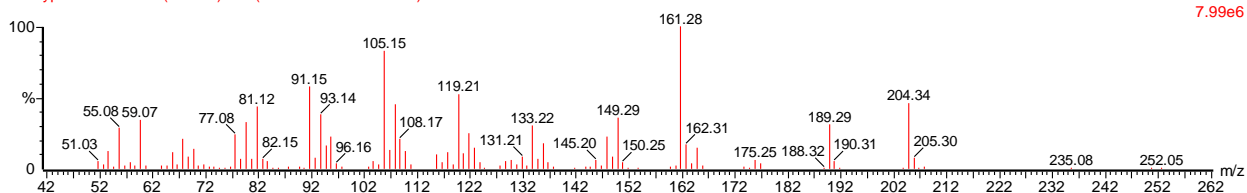
2



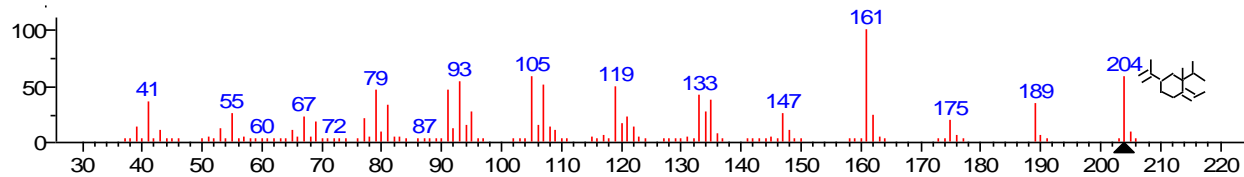
(mainlib) Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-

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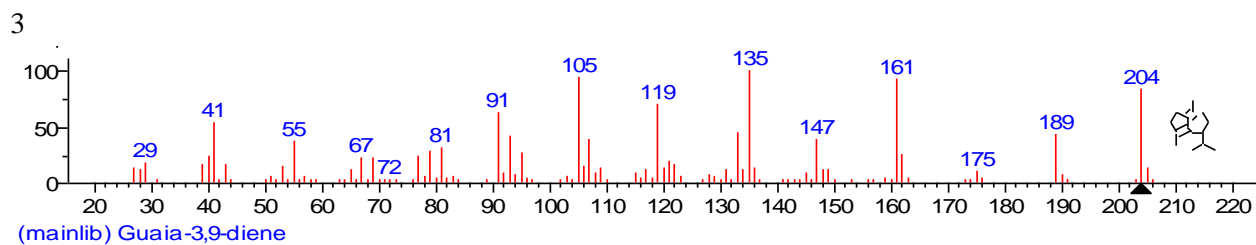
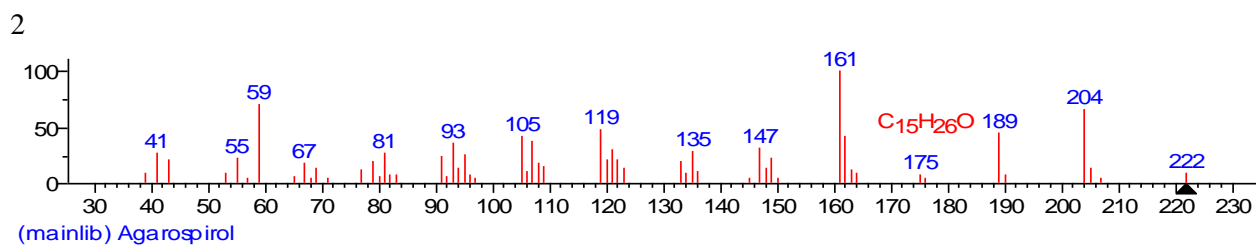
Eucalyptus Har1 1285 (12.769) Cm (1283:1288-1282:1289)

Scan EI+
7.99e6

1



(mainlib) Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1à,7á,8aà)]-



E. globulus from Entoto Park.



E. globulus from Haramaya University.

