Chapter One

1. Introduction

Sudan is a large African country and is rich in adiversity of forest resources which constitute a base for substantial contributions to health and economic development. Ethnobotany is defined as the study of how people of a particular culture make use of indigenous plants, and how they classify, identify and relate to these plants(Abdulrahaman *et al.*, 2006). Plants are, continuously, in contact with different microorganisms, including viruses, bacteria and fungi. In order to protect themselves, plants synthesize secondary metabolites, known as phytoalexins(Edeoga *et al.*, 2005). The medicinal value of these plants lies in the produced secondary metabolites(active substances) that produce a definite physiological action on the humans(Edeoga *et al.*, 2005). These active substances (natural products) perform various functions and many of them have, interesting and useful, biological activities(Mueller-Harvey *et al.*, 1987). Plants are important source of, potentially, useful structures for the development of new chemotherapeutic agents(Mahesh and Satish, 2008). They are effective in the treatment of infectious diseases and, simultaneously, mitigating many of the side effects that are, often, associated with synthetic antimicrobials(Iwu *et al.*, 1999). The expanding bacterial resistance to antibiotics has become a growing concern worldwide(Al Akeel *et al.*, 2014).

The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents(Ivanova *et al.*, 2005). Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability(Vinary, 2010).

Acacia(Fabacae) has a wide range of ecological amplitudes and is distributed in many regions all over the world. This genus includes more than 1350 species(Seigler, 2003). One of the few strongly gregarious sahelian tree species is *Acacia seyal*, which is native to Egypt, Eritrea, Ethiopia, Ghana, Iran, Israel, Kenya, Malawi, Mali, Mozambique, Namibia,

Niger, Nigeria, Saudi Arabia, Senegal, Sudan, Syrian Arab Republic, Tanzania, Uganda, Yemen, Republic of Zambia, Zimbabwe(Kamali and Mohammed*, 2007). It is known locally in Sudan as *Talh. Acacia* trees have been identified as potentially suitable protein supplements(Topps, 1992). *Acacia* leaf supplements improve diet intake, digestibility and animal performance(Norton, 1994). An important group of these allelochemicals found in tropical browse species are polyphenols, especially tannins (proanthocyanidins) or condensed tannins and hydrolysable tannins(Topps, 1992).

Currently, the main function of *Acacia* is for traditional fuel wood and for structural wood(Chang and Tung, 2013) and(Wu *et al*., 2008), which is produces a hard, dark wood, called shittim wood, with interlocked, irregular and coarse textured grain. *A. seyal* produces good, dense firewood that is used widely throughout its range. The smoke is pleasantly fragrant and the wood burns rather quickly, the smoke of *A. seyal*'s wood is said to be insect-repellent. In Sudan it is used to make a fragrant fire over which women perfume themselves, additionally, roots of *A. seyal* are used for making staves, the bark of *A. seyal* is used for making rope and the fibre has promising technological characteristics for use as particle board(Kimaro *et al*., 2011).

The wood of *A. seyal* is pale yellow to medium brown, with localized pinkish-brown patches and some dark mahogany-red heartwood in larger or older individuals. *A. seyal* wood has potential in rural areas as timber. *A. seyal,* also produces a gum which, in spite of being of an inferior quality than that of *A. senegal,* is still marketed in Sudan sizable amounts. The gum is edible when fresh, with a slightly acidic taste. *Talh* gum is attractive because of its clarity and solubility, gum is mixed with soot and powdered Nubian sandstone for black and red ink(Kimaro *et al*., 2011). Phytochemically *A. seyal* was characterized with high contents of proteins, phenolics, flavonoids and anthocyanins. The bark contains 18-30 % tannins and is a source of red dye(Orwa *et al*., 2012). The bark of *A. seyal* is the most valuable part of *A. seyal*. It is, extensively, used for feeding cattle, goats and sheep during the dry season. In human medicine *A. seyal* leaves,

gum and bark are used in phytotherapy for haemorrhage, colds, diarrhoea, gastro-intestinal disorders, jaundice, biliary diseases, syphilis, and headaches and as emollient, astringent, for burns and ophtalmia(Orwa *et al.*, 2012). Combretaceae is known for medical uses in Africa and Asia. *Combretum* spp, are widely used in folk medicine for the treatment of hepatitis, malaria, respiratory tract infections, cancer, bilharzias, tuberculosis, bacterial and fungal infections and parasitic diseases(Adnyana *et al.*, 2001). In medical preparations leaves, wood and bark of *Combretum* spp, are used predominantly(McGaw *et al.*, 2001; Mariod *et al*., 2006).

Terminalia laxiflora is tree of 12 m in height and nearly 1m width with the usual crooked bole, dark grey, deeply fissured and scaly bark, the wood is fire resistant because of its thick corky bark, *T. laxiflora* commonly knowns as *Sobag*e in Sudan. It is also known that this plant synthesizes derivatives useful for the maintenance of health in human and animals(Srivastaraj and Vietineyer, 1996). Chemical analysis of different plant parts of *C. hartmannianum*, *A. seyal*, and *T.laxiflora* revealed an abundance of tannins, flavonoides and saponines, in which the antimicrobial activity resides(Omer and El Nima, 1999), *in vitro* studies revealed a high antimicrobial activity residing in the bark and leaves of the plant against pathogens(Mbwambo *et al.,* 2007).

The chemistry of *Combretum* species has been studies by a number of researchers. To date around 185 metabolites were reported from the genus *combretum*.These include: stilbenes, phenantherenes, terpenoids, cycloarenoids, macro lactones and flavonoid(Pettit *et al*., 1995). Several unusual compounds have also been isolated from *Combretum* species, for example, 9, 10-dihydrophenanthrenes and a substituted bibenzyl from *C. molle*(Rogers and Verotta, 1996). Several compounds of interest such as flavonoids, stilbenes, cyclobutanes and triterpenoids have been isolated from *Combretum erythrophyllum*(Martini *et al*., 2004).

Acacia seyal, Combretum hartmannianum and *Terminalia Laxiflora* are known locally in Sudan as *Talh, Habeel* and *Sobag*, respectively. The dried wood of these trees is well known and used for special fragrance and some

medicinal uses. The fermented wood *Nikhra* originates from the trees of *A. seyal, C. hartmannianum* and *T. laxiflora* which grow in different Sudan states. *Nikhra* is the Sudanese word for the fermented heartwood of these trees. Afew hundred species produce *Nikhra*, of which, probably, fermented wood of only a few species is well known and used by Sudanese women as fragrance. Additonally, there is little or no scientific information on the ethnobotanical uses of *C. hartmannianum*, *A. seyal*, and *T. laxiflora* as cosmetic in Sudan.

Chromatoghraphic analysis method of testing requires an analytical component, a gas chromatograph, coupled with a detection component, a mass spectrometer. Compounds that cannot be rendered volatile for GC analysis, such as very polar, ionic or large compounds, can often be analysed by high-performance liquid chromatography(HPLC). This technique is suitable for the analysis of a much broader range of compounds. In contrast to GC, the temperature is kept around room temperature, which enables the analysis of thermolabile compounds(Van der Doelen *et al.*, 1998).

Nikhra extracts are composed of complex mixture of compounds, which need to be purified and isolated in order to identify the employing different technique such as thin layer chromatography(TLC), high performance liquid chromatography(HPLC) with mass spectrometric(MS), ultraviolet(UV), diode- array(DAD), and GC/MS. GC/MS is the most frequently used technique for analyzing essential oil composition(Baharum *et al.*, 2010).

1.1: Research Objectives

The main aim of this study is to extract and detect and identify compounds responsible for fragrances in *Nikhra* fractions from *A. seyal, C. hartmannianum* and *T. laxflora* that could be used safely as body perfumes or creams.

This aim could be fulfilled following these objectives:-

- Ethnobotanical survey study of the plants used in Khartoum states.
- To prepare methanolic extraction of *Nikhra* of *A. seyal, C. hartmannianum* and *T. laxflora*.

- To fractionate the methanolic extracts using solvents of increasing polarity.
- To asses biological activity of the fractions employing antimicrobial and antioxidant activities and toxicity assaying
- To detect organoleptically thefractions accumulating the strongest sweet fragrance, color and texture.
- To analyse fragrant and active fractions using chromatographic and spectroscopic analysis with the aim of identification of compounds in them.

Chapter Two

2. Literature Review

2.1: Medicinal plants

According to the World Health Organization, "a medicinal plant" is any plant which in one or more of its organs 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(leaves, flowers, seeds , roots, barks, stems, etc) of a plant used for preparing medicines. Furthermore, the World Health Organization, defines medicinal plant as herbal preparations produced by subjecting plant materials to purification extraction, fractionation, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Aromatic plants have a pleasant, characteristic fragrant smell. The fragrance of these plants is carried in the essential oil fraction. Many aromatic plants are spices, spices defined are as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or in ground forms that contributes relish or piguancy of foods and beverages(Chandarana *et al*., 2005).

The smoke produced by burning the wood of *A. seyal* acts as a fumigant against insects and lice. It is used in the Sudan to make a fragrant fire over which women perfume themselves(Orwa *et al*., 2012). The resinous heartwood of *A. seyal, C. hartmannianum* and *T. laxiflora*, trees are usually used in Sudanese fragrances.

2.2: Plants polyphenols structural classification

Polyphenols have been, comprehensively, defined by Quideau *et al* (2011) as plants secondary metabolites derived exclusively from the shikimate

derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression(Fig 2.1). In excess of 8000 phenol structures have been reported and they are, widely, dispersed throughout the plant kingdom – many occur in food. Phenolics range from simple, low molecular weight, single aromatic – ring compounds to the large and complex tannins and derived polyphenols. They can be classified by the number and arrangement of their carbon atoms and are, commonly, found conjugated to sugars and organic acids. Phenolics occurring in, healthy, plant tissue can be classified into two groups, the flavonoids and the non-flavonoids(Crozier *et al.*, 2006). Polyphenols comprise a large class of compounds including all molecules with more than one hydroxyl group on an aromatic ring. Typical polyphenols are phenolic acids, stilbenes, lignans and flavonoids.

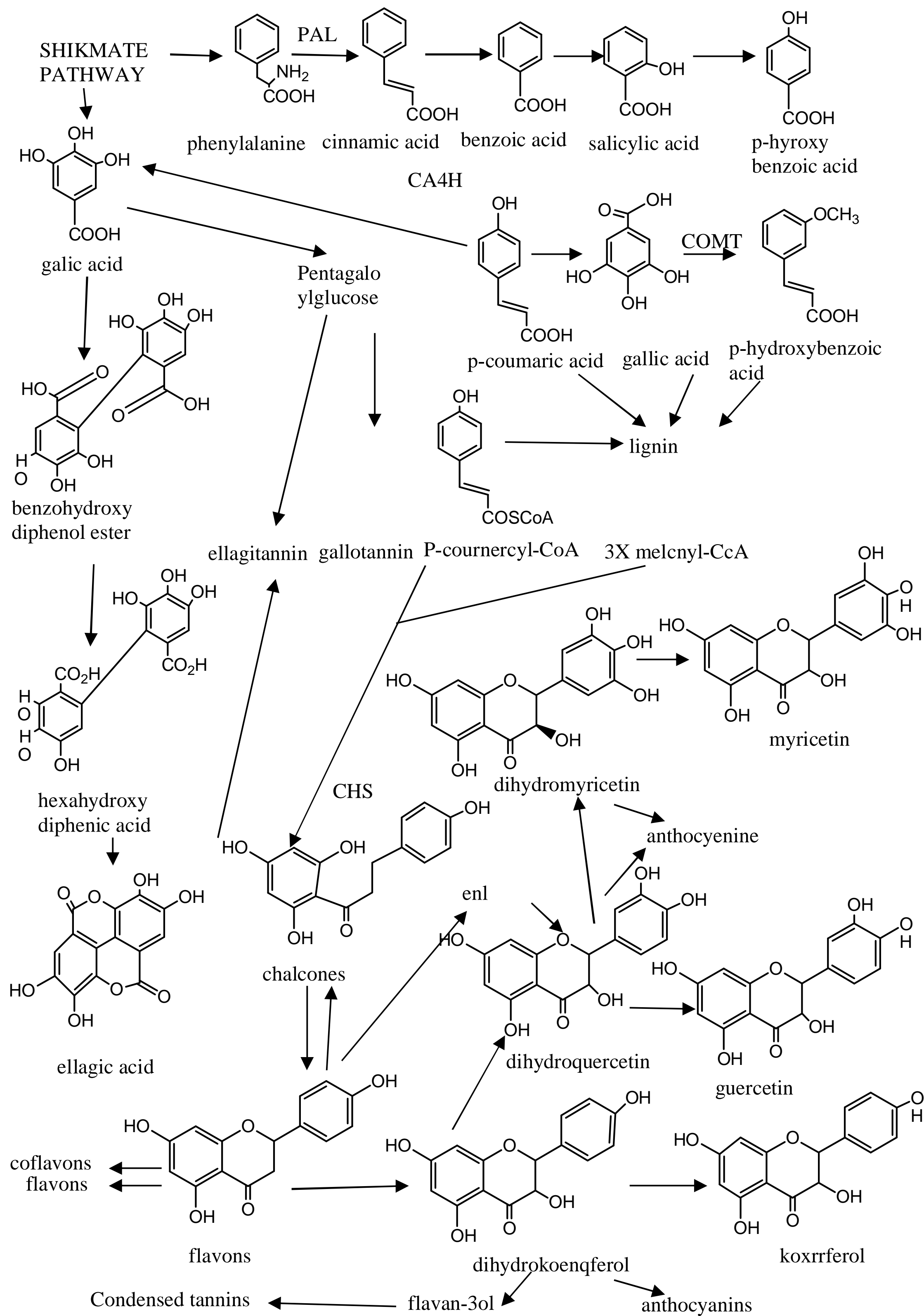


Fig.2.1. Scheam of Biosynthesis of polyphenols

2.2.1: Phenolic acids

Phenolic acids are one of the other main phenolic classes within the plant kingdom and occur in the form of esters, glycosides or amides, but rarely in free form. Variation in phenolic acids is in the number and location of hydroxyl groups on the aromatic ring(Pereira *et al.*, 2009). Phenolic acids have two parent structures: hydroxycinnamic and hydroxybenzoic acid. Hydroxycinnamic acid derivatives include ferulic, caffeic, *p*-coumaric and sinapic acids, while hydroxybenzoic acid derivatives consist of gallic, vanillic, syringic and protocatechuic acids. Phenolic acids (mainly ellagic acid) are the bioactive phytochemicals, which are effective antioxidant, antibacterial and antifungal(Cowan, 1999). Ellagic acid is a naturally occurring polyphenol found in the plant foods in the forms of hydrolyable tannin called ellagitannins; it resulted when Hexa Hydroxy Diphenolic acid(HHDP) group was cleaved from the tannin molecule(http ://www.ellagic-research.org/summary. htm 2010). The plants produce ellagic acid and convert it to ellagitannins glucosides readily hydrolyzed to regenerate ellagic acid when the plant is eaten(http://en.Wikipedia. org/wiki/Ellagic acid 2010). Another major class of phenolic compounds is the cell wall phenolics. They are insoluble and found in complexes with other types of cell component.The two main groups of cell wall phenolics are lignins and hydroxycinnamic acids(Baucher *et al.*, 1998; Vanholme *et al.*, 2010). These compounds play a critical role in the cell wall during plant growth by protecting against stresses such as infection, wounding and UV radiation(Naczk and Shahidi, 2004).

2.2.2: Flavonoids

The flavonoids are, by far, the largest class of polyphenols and consist of at least 9000 identified compounds with many more being discovered(Williams and Grayer, 2004). The subclasses consist of chalcones, flavones, flavanones, flavonols, isoflavones, anthocyanins, flavanols and flavans(Fig 2.2)and within each of these classes, individual compounds are characterized by specific hydroxylation and conjugation patterns(Beecher, 2003). The most common classes are the flavones,

flavonols, flavanones, catechins, isoflavones and anthocyanidins, which account for around 80% of flavonoids. Flavonoids are present in nature as glycosides or other conjugates, with the exception flavanols; this contributes to the complexity and the large number of individual molecules that have been identified isoflavones, anthocyanins, flavanols and flavans. These compounds can be found naturally in the free state or as glycosides; substitutions by hydroxyl, methyl, methoxyl and isopentenyl are also common. Chalcones have been isolated from a number of plants, from the roots, heartwood, flowers, leaves and seeds. Additionally; chalcones have a cytotoxic effect leading to anticancer activity(Elias *et al*., 1999). Flavanols are the least oxidized polyphenols with the C ring fully saturated, and often have a number of hydroxyl functional groups. Flavanols are a class of flavonoids that can be found naturally in either the aglycone form or as glycosides. When proanthocyanidins are heated in acid, they separate to form anthocyanidins, the aglycone form of anthocyanins. Catechins are also found in many types of fruit, but green tea and chocolate are the richest sources, by far(Manach *et al.*, 2004). They are found in high concentrations in citrus fruits, such as grapefruit, oranges, and lemons, as well as some aromatic herbs such as mint. Studies show that while flavanones are found in nature as glycosides but when ingested, they are absorbed as aglycones in the colon(Manach *et al.,* 2004). Flavanones are also found in nature as prenylated derivatives, and have been characterized as a potent phytoestrogens and have been shown to have anti-cancer activity(Cos *et al.*, 2003). The compounds are commonly found in parsley, celery, capsicum pepper, millet and wheat. Flavonols are the most widespread flavonoids in foods, mostly in the form of quercetin and kaempferol; the richest sources are onions, dark greens, berries, and tea(Manach *et al.,* 2004). They are often found naturally as glycosides with glucose or rhamnose moieties. These compounds accumulate in the skin and leaves because their biosynthesis is stimulated by light. Because of this, concentration of flavonols can differ between pieces of fruit on the same branch, depending on exposure to sunlight. Structurally, flavonols are not very different from flavones but with the addition of a hydroxyl at position 3 to increase the oxidation state(Manach *et al.*, 2004). Anthocyanins are flavonoids with the highest oxidation state and are pigments often found in the epidermal tissue of fruits

and flowers, giving them red, blue or purple colors(Welch *et al.,* 2008). Their color depends on pH, red color in acidic conditions progressing to blue as pH moves higher. They are easily degraded by light, pH, and oxygen in the aglycone form but are stabilized as glycosides or other complexations(Manach *et al*., 2004). Anthocyanins can be found in grains, root vegetables(Manach *et al.,* 2004), fruits and flowers but the highest concentration is found in berries and their subsequent juices(Beecher, 2003; Manach *et al*., 2004). Phenylproanoids are natural products derived from amino acid L-phenylalanine via de-amination by L-phenylalanine ammonialyase (PAL) L-phenylalanine are derived from amino acid the enzyme phenylalanine ammonia–lyase (PAL), catalyzes the gateway metabolic step from primary metabolism into phenylpropanoid metabolism, the de-amination of phenylalanine to produce cimamic acid. Cimamic acid is further modified by the action of hydroxylases and O-methyltransferases and most phenylpropanoid compounds are derived from such hydroxycimamic acid(Robards and Antolovich, 1997). The biosynthesis of flavonoids involves the central intermediate *p*-comaroyl CoA and three malony CoA units to elongate the side chain of the original phenylpropanoid unit closure of ring A produces the chalcone structure and subsequent reaction closes the ring B. All flavonoids share abasic C6-C3-C6 phenylbenzopyran blackbone. The position of the phenyl ring relative to the benzopyran moiety allows abroad separation of these compounds into flavonoids(2-phenylbenzopyrans). Division into further groups is made on the basis of the flavonoids are flavons(with a C2-C3 double bond and a C4-oxo function), flavonols(flavons with a 3-OH group) and flavanones(flavones analogues but with a C2-C3single bond), and abundant isoflavonoids include isoflavones(the analogue of flavones). 4-arylcoumarin(a neoflavonoid with a C3-C4 double bond and it's reduced from 3, 4-dihydro-4-arylcomarin, are the major neoflavonoids. Other natural compounds, such as chalcones and aurones also possess the C6-C3-C6 blackbone(Fig 2.1) and are henceforth included in the general group of flavonoids(Robards and Antolovich, 1997). They share a common framework consisting of two aromatic rings (A and B) that are, generally, bound together by three carbon atoms that form an oxygenated heterocycle(ring C), with the exception of chalcones which maintain an open bridge structure(Robards and Antolovich, 1997).

The ring structure is described for the chalcone and flavones. Chalcones, or 1, 3-diaryl-2-propen-1-ones, are polyphenols with only two aromatic rings(A and B) with a three-carbon bridge and are an intermediate in the biosynthetic formation of flavonoids(Welch *et al.*, 2008).

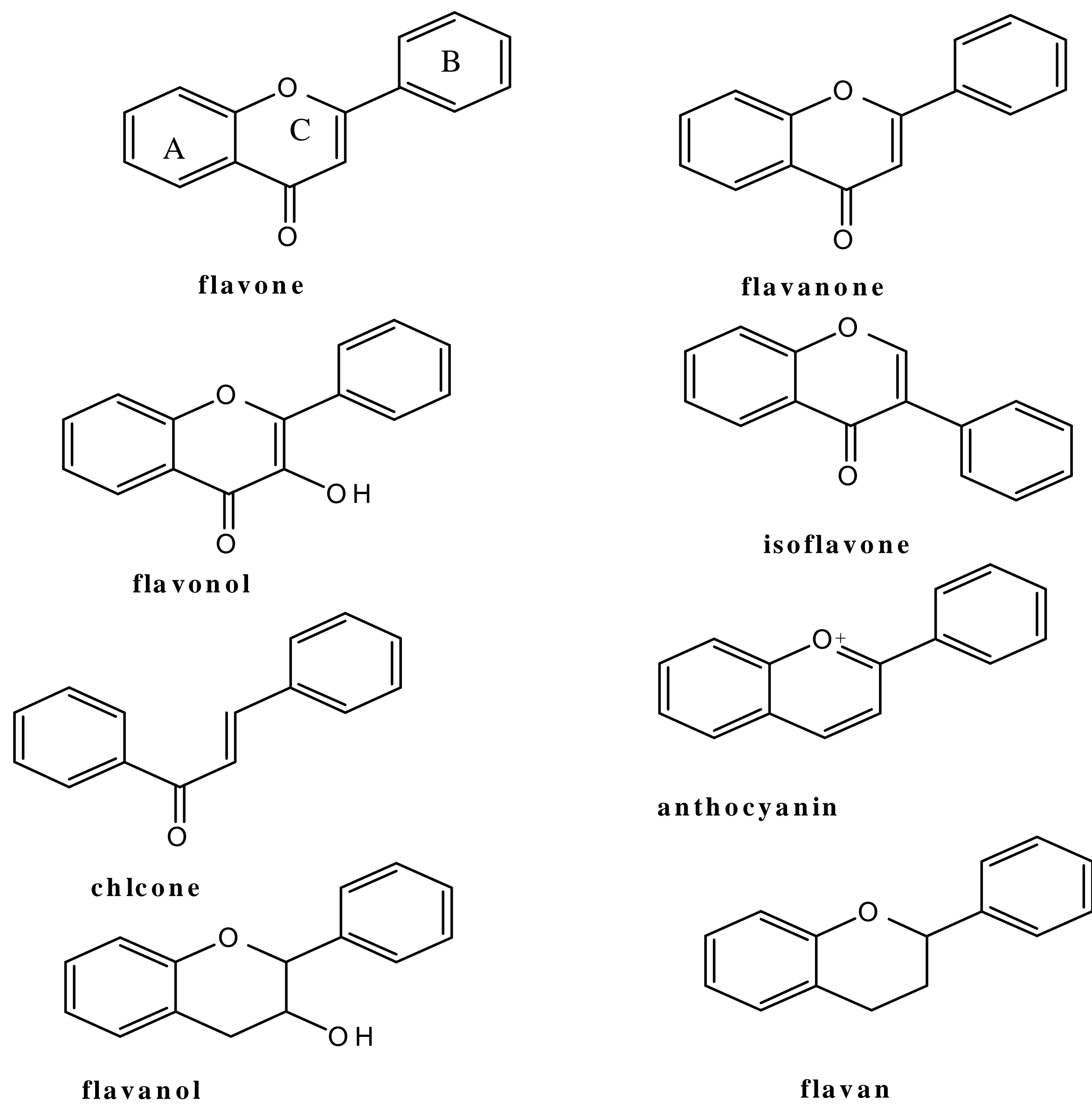


Fig.2.2: Examples of the 8 classes of flavonoids: chalcones, flavones, flavanones, flavonols, isoflavones, anthocyanins, flavanols and flavans.

2.2.3: Stilbenes

There are two major groups of the stibenes, resveratrol (stilbenes), and the phenanthrenes, together with respective dihydro derivatives are characteristics of Combretaceae family(Flores *et al*., 1987). The stibenes are often in plants that are not, routinely, consumed for food or in the nonedible tissue(Cassidy *et al.*, 2000), and are usually assumed that the resistance of these woods to fungal attack is due to presence of these phenolic materials. Stilbenoids are widely distributed in higher plants, as dimeric, trimeric and polymeric stilbenes, the so-called viniferins. Among monomeric stilbenes, trans-resveratrol has been identified as the major active ompound, and most of the studies in the literature about the physiological activity have focused on it; however, there are also some studies of the 3β-glucoside of transresveratrol, the so-called piceid or polydatin, and the viniferins(Cassidy *et al.*, 2000). Phenanthrenes are rather uncommon class on aromatic metabolites, where as presumably formed by oxidative coupling of the aromatic rings of stilbene precursors. Besides these stilbene derived compounds. Phenanthrenes are most likely originated from diterpenoid precursors(Cassidy *et al.*, 2000). Biosynthesis of dihydro phenanthrenes is similar to that of stilbenes, but appears to involve dihydrocinnamic acids and the enzyme bibenzl synthase, where as the biosynthesis of phenanthrenes involves the corresponding unsaturated acids(Flores *et al*., 1987). The phenanthrenes are classified into three major groups: Mnphenanthrenes, phenanthrenes and triphenanthrenes. Large number of biological activities of differently substituted phenanthrene has been reported to occur in plants and has been demonstrated to possess various active compounds; phenanthrenes have been studied for their cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, antiplatelet aggregation, antiallergic activities and phytotoxicity.

2.2.4: Lignans and tannins

The lignans are a group of chemical compounds found in plants. Plant lignansare polyphenolic substancesderivedfrom phenylalanine via dimerizati on of substituted cinnamic alcohols known as monolignols. Many natural

products, known as phenylpropanoids, are built up of C_6C_3 units, derived from cinnamyl units just as terpenechemistry builds on isoprene units. Some examplesoflignansare pinoresinol, podophyllotoxin,and steganacin(https://en .wiktionary.org/wiki/neolignane). When a part of the human diet, some plant lignansaremetabolizedbyintestinalbacteriatomammalianlignans enterodiol an d enterolactone.lignans that can be metabolized to mammalian lignans are pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, hydroxymataires inol, syringaresinol andsesamin.Lignansareoneofthemajorclassesof phytoestr ogens, which are estrogen-like chemicals and also act as antioxidants.The other classes of phytoestrogens are isoflavones and coumestans(Borriello *et al.*, 1985; Heinonen *et al.*, 2001). Lignans are high in antioxidants,highinfiberandhaveantiestrogeniceffects(http://www.ncbi.nlm.n ih.gov/pubmed/23885993).

The enterolignans, enterodiol and enterolactone, are formed by the action of intestinal bacteria on lignan precursors found in plants(Lampe, 2003). Lignan precursors that have been identified in the human diet include pinoresinol, lariciresinol and secoisolariciresinol. Secoisolariciresinol was among the first lignan precursors identified in the human diet and are, therefore, the most extensively studied. Lignan precursors are found in a wide variety of foods, including flaxseeds, sesame seeds, legumes, whole grains, fruit, and vegetables. While most research on phytoestrogen-rich diets has focused on soy isoflavones, lignans are the principal source of dietary phytoestrogens in typical western diets(de Kleijn *et al.*, 2002; Valsta *et al.*, 2003). Tannins commonly known as tannic acid are complex water-soluble polyphenolic compounds with a molecular weight of more than 500 *m/z*. They contain, sufficient, hydroxyl and carboxyl to effectively form strong complexes with protein and other macromolecules under a particular environment. They have the ability to precipitate proteins from aqueous solution and are present in many plant foods. They have a great diversity and in browse plants influence the digestibility of available proteins. They occur almost in vascular plants. Tannins act as chemical defense mechanism in plants against pathogens, herbivores and hostile environmental conditions; they can exert detrimental effects in a multitude of ways(Clausen *et al.*, 1990). Tannins are known as anti-nutritional protein binding secondary plant compounds, which reduce availability of dietary proteins in the digestive

system. Tannins are divided into two major structural classes, hydrolysable tannins and condensed tannins. Although they differ biosynthetically and chemically, they are both phenolics and can precipitate proteins(Rickard, 1986).

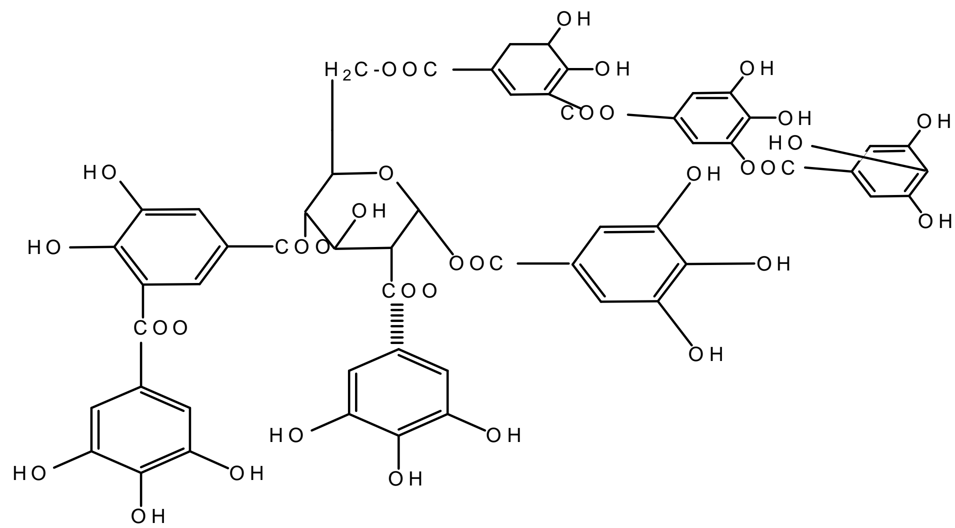
2.2.4.1: Hydrolysable tannins

Hydrolysable tannins are synthesized by a wide, variety of plants and trees(Kumar and Vaithiyanathan, 1990), and several of these have been used as animal feeds(Le Houérou, 1983). They are composed of gallic acid or condensation products of ellagic acid esters with the hydroxyl groups of glucose(Dalzell and Kerven, 1998). The hydroxyl groups of these carbohydrates are partially or totally, esterified with phenolic groups like gallic acid or ellagic acid. Hydrolysable tannins are, usually, present in low amounts in plants. Hydrolysable tannins are also hydrolyzed by hot water or enzymes(Kumar and Vaithiyanathan, 1990). They are also hydrolyzed by mild acids or mild bases to yield carbohydrate and phenolic acids. Hydrolysable tannins are more likely to react with the extracting solvent than condensed tannins. For example, methanol cleaves the depside bonds in gallotannins at neutral pH and room temperature(Pretsch *et al.*, 2009), but acidified methanol ($pH<3$) will not cleave these bonds. Large and complex tannins are, easily, degraded into smaller tannins by water or dilute acids, especially, at elevated temperatures in just 30 minutes(Beasley *et al.*, 1977; Okuda *et al.*, 1993; Okuda, 2005). Water at 60 C is likely to liberate gallic acid from the anomeric C_1 position of glucose Fig.2.3(Pretsch *et al.*, 2009).

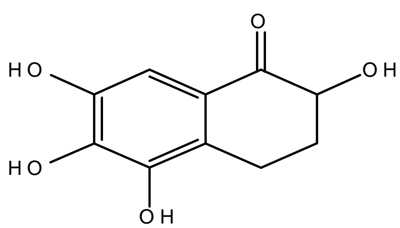
2.2.4.2 Condensed tannins

Proanthocyanidins (PAs) are more often called condensed tannins due to their condensed chemical structure. Condensed tannins are complex phenolic compounds which are found in a variety of browse sources(Kumar and Vaithiyanathan, 1990), including leaves and pods(Silanikove *et al.*, 1996). They are, more widely, distributed in browse species Table(2.1) than hydrolysable tannins and are considered to be more active in precipitating

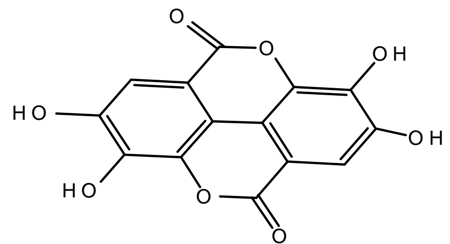
proteins. They are derived from the condensation of flavonoid precursors without participation of enzymes(Schofield *et al*., 2001).



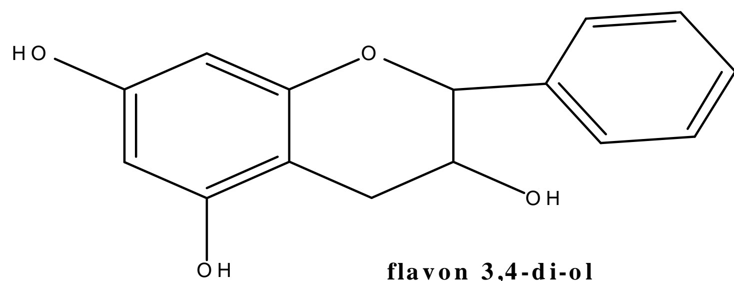
tannic acid (gallotannin)



gallic acid



ellagic acid



flavon 3,4-di-ol

Fig.2.3: Tannins (Hydrolysable, Condensed) in *Acacia* spp

Table 2.1 Proanthocyanidins of some *Acacia* species

Species	Condensed tannins	Reference
Acacia currassavica	4.27 %	(Balogun *et al*., 1998)
Acacia angustissima	15.3 g/kg DM	(Larbi *et al*., 1998)
Acacia boliviana	11.35 Au550nm / g sample	(Maasdorp *et al*., 1999)
Acacia karoo	2.01 A550, g sample	(Dube *et al*., 2001)
Acacia Nilotica	19.0 A550, g sample	(Dube *et al.,* 2001)
Acacia tortilis	47.1 A550, g sample	(Dube *et al*., 2001)
Acacia senegal	04.0 A550, g sample	(Dube *et al*., 2001)
Acacia erioloba	36.1 A550 , g sample	(Dube *et al*., 2001)
Acacia albida	36.1 A550 , g sample	(Dube *et al*., 2001)

Depending on their chemical structure and degree of polymerization, PAs may or may not be soluble in aqueous organic solvents. They bind to proteins and are considered as anti-nutritional compounds.Their concentration and chemical composition changes with physical maturity of the plants. The reactivity of PAs with molecules of biological significance has important nutritional and physiological consequences. Their multiple phenolic hydroxyl groups lead to the formation of complexes with proteins(Hagerman *et al*., 1998; Harborne and Williams, 2000), with metal ions(Santos-Buelga and Scalbert, 2000; van Acker *et al*., 1998), and with other macromolecules like polysaccharides(Mueller-Harvey *et al.,* 1987). Oxidative coupling between flavanol monomers occurs most commonly between positions 4 and 8, but may also involve positions 4 and 6 of the monomer(Fig.2.3) and other positions too. Although variations in the stereochemistry at these positions do occur in natural tannins, observations on model compounds suggest that these variations have, relatively, little effect on most of the reactions used for tannin assays(Schofield *et al*., 2001).

2.3: Plants secondary metabolites and their therapeutic action

Through past, plants have provided human with a sources of food, dyes, perfume, gum, fiber, resin and many other useful products. Nowadays, ethno-pharmacologists are paying more attention and interest to investigate bioactive and phytochemical properties of medicinal plants to treat a variety of diseases. Several medicinal plants have a main therapeutic role and could be used as a natural medicinal source to treat a variety of diseases(Gupta and Sharma, 2006). Many studies have been reported that plants possess various bioactivities(Alam *et al*., 2009; Yadav *et al*., 2011). Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agents. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, food supplements, and folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs(Das *et al*., 2010). Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to

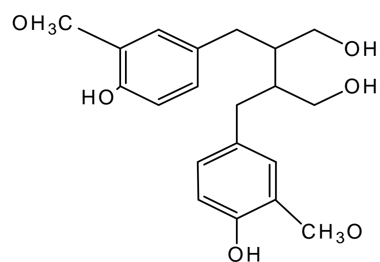
produce a large number of secondary metabolites(Castello *et al.*, 2002). Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance of higher plants for specific diseases(Fyhrquist *et al*., 2002; Ertürk *et al.,* 2006).

Secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids have been found to have medicinal properties(Joglekar *et al.*, 2012). Their function in plants is now attracting attention as some appear to have a key role in protecting plants from herbivores and microbial infection, as attractants for pollinators and seed-dispersing animals, as allelopathic agents, UV protectants and signal molecules in the formation of nitrogen-fixing root nodules in legumes. This compelled the scientists to search out new drugs from plant origin(Fabricant and Farnsworth, 2001; Khoobchandani *et al.*, 2010) identified 122 compounds used in mainstream medicine which were derived from “ethnomedical” plant sources, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.

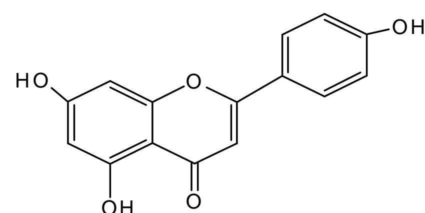
2.4: Combretaceae spp. polyphenols

Combretaceae family is rich in wide variety of free radical scavenging molecules, such as phenolic compounds e.g. (phenolic acids, flavonoids, quinines, coumarins, lignin’s, stilbenes, tannins Fig.2.4), nitrogen containing compounds(alkaloids and amines), vitamins, terpenoids(including carotenoids) and some other endogenous metabolites, which are rich in(Zheng and Wang, 2001), it is rich in ellagitannins(Yoshida *et al.*, 2010), which are toxic to filamentous fungi, yeasts and bactria(Scalbert, 1991). Ellagic acid is a potent anti-carcinogenic /anti-mutagenic phenol compound; it shows several biological properties, such as radical scavenging, cancerchemopreventive, antibacterial, anti-viral, antiplasmodial, anti-inflammatory, antimutagenic and cytoprotective properties(Teel, 1986;Maasdorp *et al.*, 1999).

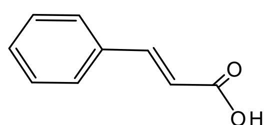
Leaf extracts of *C. erythrophyllum* yield seven flavonoids by bioassay-guided fractionation. Four of these compounds were identified as flavonols and three were identified as flavones using Nuclear Magnetic Resonance(NMR) and Mass Spectroscopy(MS). Six of these flavonoids are reported for the first time for the Combretaceae such akaempferol, rhamnocitrin, rhamnazin, quercetin-5, 3'-dimethylether, genkwanin and 5-hydroxy-4',7-dimethoxyflavone(Martini *et al.,* 2004).



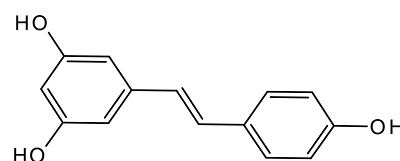
secoisolaricresinol lignan *C.micranthum*



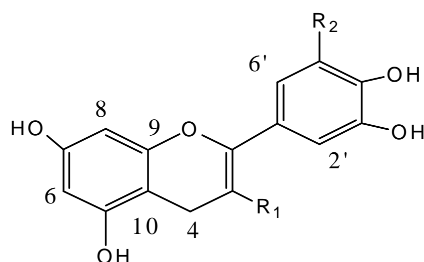
apigenin flavonoid *C.micranthum*



cinnamic acid phenolic acid *C.micranthum*



resveratrol stilbene *C.micranthum*



1(-)-epigallocatechin *C.micranthum*
2(-)-epicatechin:R1=OH,R2=H *C.micranthum*
3(-)-3',4',5',5,7-pentahydroxyflavan: R1=H,R2=OH *C.micranthum*
4(-)-3',4',5,7-tetrahydroxyflavan:R1, R2=H *C.micranthum*

Fig.2.4: Polyphenols in Combretaceae

2.5: Analysis of polyphenols

Chemical procedures are used to detect the presence of total phenolics, while spectrophotometric and chromatographic techniques are utilized to identify and quantify individual phenolic compounds(Khoddami *et al.*, 2013). Thus there is great scope for developing quantification methods based on the type of phenolic group(Liu *et al.*, 2008). High performance liquid chromatography(HPLC) and gas chromatography(GC), or their combinations, with mass spectrometry are the most, commonly, applied methods to quantify phenolic compounds(Naczk and Shahidi, 2004).

2.5.1: Spectrophotometric analysis

Spectrophotometry is one of the, relatively, simple techniques for quantification of plant phenolics. The Folin-Denis and Folin-Ciocalteu methods were the two, widely, used specrophotometric assays to measure total phenolics in plant materials for many years(Lapornik *et al.*, 2005; Naczk and Shahidi, 2006). Both methods are based on a chemical reduction involving reagents containing tungsten and molybdenum(Stalikas, 2007). The products of this reduction in the presence of phenolic compounds have a blue color with a broad light absorption spectrum (around 760 nm). The reagents for both methods do not react specifically with only phenols but also with other substances like ascorbic acid, aromatic amines and sugars(Box, 1983). Total phenolic quantification, total flavonoids, proanthocyanidin (condensed tannin) and hydrolysable tannin can also be estimated by colorimetric methods. Methanolic or ethanolic extracts of plant phenols mixed with $AlCl_3$ allow measurement of total flavonoids in the range 410–423 nm(Huang *et al.*, 2009).Vanillin and dimethylaminocinnamaldehyde (DMCA) assays are used to determine the level of proanthocyanidins(Naczk and Shahidi, 2004). These methods can provide information about the degree of polymerization and the hydroxylation pattern and stereochemistry of flavan-3-ol subunits(Abeynayake *et al.*, 2011; Hartzfeld *et al.*, 2002). Catechin is usually used as a standard in the vanillin method and as a result may lead to the over-estimation of proanthocyanidins(Khoddami *et al.,* 2013).

2.5.2: Chromatographic analysis

2.5.2.1: Gas chromatography

Gas chromatography(GC) is another applied technique for the separation, identification and quantification of phenolic compounds such as phenolic acids(Martin *et al.*, 2012), condensed tannins(Shadkami *et al.*, 2009), and flavonoids(Proestos *et al.,* 2006). The major concerns of GC analysis, that are not applicable to HPLC techniques, are the derivatization and volatility of phenolic compounds. With GC, quantification of phenolics from food matrices may involve clean-up steps such as lipid removal from the extract, release of phenolics from the glycoside and ester bonds in enzymatic(Liggins *et al.*, 1998), alkaline(Siess *et al.*, 1996), and acidic(Wang *et al.,* 2000).

The characterization of phenolics *T. chebula* fruits was done using liquid chromatography coupled with Quadrapole mass spectroscopy in ESI negative mode structural characterization was carried out by MS/MS fragmentation. Analysis of the fractions obtained by liquid chromatography coupled to positive electrosprayionizationtandemmassspectrometry(LCESI-MS/MS) afforded six known polyphenols in *T.chebula*3,3'diOmethylellagic acid); 3,7,8-tri-O-methylellagic acid; Progallin A;3,4-O-Trimethyl-4'-O-β-Dglucopyranosylellagic acid; Punicalagin and, Punicalin(Adiko *et al.*, 2013).

2.5.2.2: High performance liquid chromatography(HPLC)

HPLC is the preferred technique for both separation and quantification of phenolic compounds(Naczk and Shahidi, 2004). Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors(Stalikas, 2007). In general, purified phenolics are applied to an HPLC instrument utilizing a reversed phase C18 column(RP-C18), photo diode array detector(PDA) and acidified polar organic solvents(Ignat *et al.*, 2011).

2.5.2.3: Thin-layer chromatography (TLC)

Thin-layer chromatography(TLC) is a partitioning technique employed to separate phenolics in foods(Naczk and Shahidi, 2004). TLC is a more powerful technique especially in crude plant extracts. Phenolics in crude plant extracts can be separated by a number of TLC techniques, which are cheap and provide for multiple detection on the same TLC plate in a short analysis time, indicated that a silica gel TLC-based video imaging method is a valuable complementary fingerprint technique to identify phenolic acids and flavonoids fractions from different sage species(Ignat *et al.*, 2011; Sajewicz *et al.,* 2012). Thin layer chromatography(TLC) and high performance liquid chromatography(HPLC) are useful tools to screen samples for the different types of tannins, hydrolysable or condensed tannins(Mueller-Harvey, 2001).

Tannins were extracted from *A. mangium* bark using water in presence of three different concentration basic reagent of NaOH(5%,10% and 15%) and were characterized by FT-IR spectrometry(Bharudin *et al.*, 2013).

2.6: Biological activity of polyphenols

2.6.1: Antioxidant Capacity

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, the so-called oxidants, and their elimination by protective mechanisms, referred to as antioxidative systems. This imbalance leads to damage of important biomolecules and organs with potential impact on the whole organism(Ďuračková, 2010). In a biological system, an antioxidant can be defined as ''any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate(Halliwell and Gutteridge, 1995). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials(Khalaf *et al.*, 2008). Flavonoids and phenolic compounds are widely distributed in plants that have been reported to exert multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory, and

anticarcinogenic(Miller, 1996). Further investigation on the isolation and identification of antioxidant components in the plant may lead to chemical entities with the potential for clinical use(Al-Fartosy, 2011). Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing, free radical, induced tissue injury. Generally, there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive attention has been directed towards the identification of plants with antioxidant ability(Vinary, 2010). Oxygen is a highly reactive atom that is capable of becoming part of, potentially, damaging molecules commonly called "free radicals"(Gupta and Sharma, 2006). Free radicals or other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to radiation, ozone, cigarette smoking, air pollutants and industrial chemicals(Bagchi and Puri, 1998). A free radical is a molecule with one or more unpaired electrons in its outer orbital, which makes this species very unstable and tending to react with other molecules to pair this electron and thereby generate more stable species(Guetens *et al.,* 2002). The over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress(Diplock *et al.,* 1994).

Free radicals cause oxidative damage to nucleic acids, proteins, and lipids and this oxidation of biological macromolecules has now been strongly associated with the development of many physiological diseases: Alzheimer's, Parkinson's, diabetes, atherosclerosis, and carcinogenesis(Kitts *et al.,* 1999). The attack of free radicals against the body is known as oxidative stress and while the human body does generate its own enzymatic antioxidants, such as superoxide dismutase, catalase, and peroxidase, it does not provide enough protection against oxidative stress. Many studies have shown that consuming proper quantities of antioxidants can slow oxidative stress and, subsequently, prevent the diseases that may develop from excessive oxidation(Scalbert *et al.*, 2005).

Polyphenolic compounds function as effective antioxidants by quenching the free radicals of biological systems with their phenolic ring and multiple hydroxyl moieties; phenolic activity covers a wide range of reactive oxygen, nitrogen, and chlorine species such as superoxide, hydroxyl radical, peroxyl radicals, hypochlorous acid, and peroxynitrous acid. Polyphenols can also chelate metal ions leading to a decrease in metal ion prooxidant activity(Boersma *et al.,* 1999). Phenolic acids, as well as other polyphenols, can act as antioxidants by a number of pathways, in which the most significant is free-radical scavenging(Manach *et al*., 2004).

In general, antioxidant systems either prevent reactive species from being formed, or remove them before they can damage vital components of the cell. Reactive oxygen species produced human body's use oxygen, such as in respiration and some cell-mediated immune functions and include hydrogen peroxide(H_2O_2), hypochlorous acid(HOCl), and free radicals such as the hydroxyl radical(·OH) and the superoxide anion(O^{2-}). The hydroxyl radical is, particularly, unstable and will react rapidly and non-specifically with most biological molecules. Free radicals or reactive oxygen species(ROS) are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution, pesticides, etc. When the generation of these free radicals or ROS go beyond the antioxidant capacity of a biological system, it gives rise to oxidative stress(Zima *et al.,* 2001). Oxidative stress plays a role in heart diseases, malaria, neurodegenerative diseases, cancer, AIDS and in the aging process(Tharanathan, 2003).

Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial or anti-viral activities to a greater or lesser extent. Numerous types of bioactive compounds have been isolated from plant source of which some are currently in preclinical/clinical trials(Owen *et al*., 2000).

2.6.2: Anti-inflammatory activity

Many medicinal plants containing polyphenolic compounds with antioxidant activity tend to exhibit high anti-inflammatory activity in cell

screen assays, such as herbal teas high in catechins or berries with high concentrations of anthocyanins(Surh *et al.*, 2001; Bora and Sharma, 2009). Inflammation is important in the pathophysiology of numerous human disorders. Accumulating evidence demonstrates that *atherosclerosis* is an inflammatory disease not, necessarily, augmented by cholesterol but rather inflammatory mechanisms(Meng, 2006). Rheumatoid arthritis is another inflammatory disorder that affects approximately 1.0% of the population; in the past, treatment for rheumatoid arthritis consisted of only treating the symptoms but now includes anti-inflammatory medications to achieve partial or even, total, remission(Ruderman, 2005).

Chronic inflammation leads to the development and progression of several cancers such as gastric and colon cancer, largely, due to the pro-growth environment generated by activated, inflammatory, cells(van Kempen *et al.*, 2006). Green tea polyphenols, as an example, were found to have chemopreventive activities in numerous studies utilizing an anti-inflammatory mechanism(Yang *et al.*, 2000; Surh *et al.,* 2001). In fact, the efficacy of anti-inflammatory drugs in chemoprevention argues for anti-inflammatory therapies at the earliest stages of cancer progression(van Kempen *et al.*, 2006).

2.6.3: Glucose-lowering activity

Polyphenolic compounds have effects on a number of diseases but one that is of growing interest is the treatment of *diabetes mellitus*, a disease that affects as many as 180 million worldwide; this number is expected to double by the year 2030(Dembinska-Kiec *et al.*, 2008; Mathers *et al.*, 2008). Diabetes is caused by higher than normal levels of blood glucose because the body cannot produce enough insulin or effectively use the insulin it does produce; there are three types of diabetes, type 1 or juvenile diabetes, type 2(the most common form) and gestational diabetes(Mathers *et al.*, 2008). Due to the prevalence of this disease in low and middle income countries, which account for 80% of diabetes deaths(Mathers *et al.*, 2008), traditional medicines are essential for the treatment of diabetes worldwide, but these herbal formulas often have mechanisms of action that are complex or even

contradictory(Hui *et al.*, 2009). Diabetes is associated with oxidative stress due to hyperglycemia and hyperlipidemia and the depletion of antioxidant concentration in the plasma is well documented. Therefore, increasing antioxidants in the diet, of which polyphenols form a considerable part, reduce the risk of contracting diabetes or ameliorating the negative side effects once the disease has developed(Dembinska-Kiec *et al.*, 2008). Resveratrol, anthocyanins and other condensed tannins have all demonstrated antihyperglycemic activity, whether by reducing obesity or other proposed mechanisms(Tsuda *et al.*, 2003; Chi *et al.*, 2007). Three theaflavins, from black tea or fermented *Camellia sinensis*, as well as two more flavonoids from *Artemisia dranunculus* L., exhibit glucose-lowering activity via down regulation of hepatic gluconeogenesis(Govorko *et al.*, 2007; Cameron *et al.*, 2008). This is assayed by decreased mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK) which is a key enzyme in hepatic gluconeogenesis and its activity is closely correlated with hepatic glucose output(Hanson and Reshef, 1997). The role of polyphenols in the treatment of diabetes is important and beginning to gather more interest when investigating full phytochemical potential of traditional medicines.

2.7: Antimicrobial agent of plants orgin

Clinical microbiologists have two reasons to be interested in the two topic of antimicrobial plant extracts, first, it is very likely that those phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed, second, the public is becoming increasingly aware of problems with the over- description and misue of antibiotics. Plants have an almost limitless ability to synthesize different types of secondary metabolites. Useful antimicrobial phytochemicals can be divided into several categories these include: simple phenols and phenolic acids e.g Cinnamic and caffeic acids which are effective against viruses, bactria and fungi(Cowan, 1999).

Quinones are aromatic ring compounds with two ketenes substitutions. In addition to providing source of stable free radicals, quinonses are known to complex, irreversibly, with nucleophilic amino acids in protein and loss of

function. For that reason, the range of quinones antimicrobial effects is great(Kazmi *et al.,* 1994).

Flavonoids are known to be synthesized by plants in response to microbial infections; it should not be surprising that they have been invitro to be effective antimicrobial substances against awide array of microorganisms. Their activity is probably due to their ability to complex with extra- cellular and soluble proteins and complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes(Fessenden and Fessenden, 1983). Catechins, and the most reduced from of the C_3 unit in flavonoids compounds, deserve special mention. These flavnoids have been extensively researched as compounds of antimicrobial activity(Kaul *et al.*, 1985). More than one study has reported that flavones derivatives are inhibitory to respiratory viruses(Cowan, 1999).

Flavonoids lacking hydroxyl groups on their B-rings are more active against microorganisms than are those with the 2OH groups, this finding supports the idea that their microbial target is the membrane. Lipophilic compounds would be more disruptive of this structure. However, several authors have also found the opposite effect, the more hydroxylation, the greater the antimicrobial activity(Cowan, 1999).Tannin is a general descriptive name for a group of polymeric phnolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Thus their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport protein etc.(Haslam, 1996). Coumarins are phenolic substances made of fused benzene and a-pyrone rings; their fame has come, mainly, from their antithrombotic, anti-inflammatory, and vasodilatory activities. Several other Coumarins have antimicrobial properties(Thastrup *et al.,* 1985). Alkaloids are heterocyclic nitrogen compounds. They have been found to have amaltimicrobial effects against *Giardia* and *Entamoeba* species. *Berberine* is an important representative of the alkaloid group. It is, potentially, effective trypanosomes and plasmodia.The mechanism of action of, highly, aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA. Peptides which are inhibitory to

microogganisms are often positively charged and contain disulfide bonds. Their mechanisms of action mybe the foundation of ion channels in the microbial membrane or competitive inhibition of adhesion of miprotiens to host polysaccharide receptors. Antimicrobial activity of C. *mole* was reported by(Asres *et al.*, 2006). The highest antibacterial action of the acetone extract was against the Gram negative organisms *Escherichia coli* and *Shiglla* spp with an MIC of 50mg/ml. The activity of the extract against these bactria was comparable to that of ciprofloxacin when assessed by the disc diffusion technique. Among the fungal strains tested *C. albicans* showed high susceptibility to the extract and growth was, completely, inhibited at aconcentration of 400µg/ml. At the same concentration, the acetone extract and the standard antifungal drug griseofulvin produced comparable zones of inhabitation on *C. albicans*. Studies on the mode of action of the extract indicated that it was bactericidal and fungicidal. The antimicrobial activity of the extract was attributed to the high amount of hydrolysable tannins present in the bark of the plant. Preliminary studies with *C.erythrophyllum* showed antimicrobial activity against gram positive and Gram negative bacteria. Seven antimicrobial flavonoids were subsequently isolated by bioassay-guided fractionation, i.e. spigenin, genkwanin, 5-hydrxy-7, 4-dimethoxyflavone, rhamocitrin, kaempferol, quercetin, 5,3-dimethylether and rhamnazin. All compounds have good activity against *Vibrio cholera* and *Enterococcus faecalis*, with MIC values in the range of 25-50µg/ml. Rhamnocitrin quercetin-5, 3- dimethylether also inhibited *Micrococcus luteus* and Shigella sonei at 25µg/ml(Martini *et al.*, 2004).

The spread of multidrug-resistant(MDR) strains of bacteria necessitates the discovery of new classes of antibacterial compounds that inhibit these resistance mechanisms(Gibbons, 2005). Hence, there is an urgent need for the development and discovery of new antibacterial agents, there the attention has now been shifted from synthetic to natural products, and there is an antiquity for their antiseptic properties, and it is well–established and proved that they display pharmacological activities with smooth action, better tolerance and few allergic reactions. In this instance, the plant kingdom is, undoubtedly, a valuable source of new bioactive compounds(Bayoud *et al.*, 2007). These antimicrobial compounds

from plants may inhibit bacteria by different mechanisms than the presently used antibiotics and may have clinical value in treatment of resistant microbial strains(Eloff, 1999).

2.8: Combretaceae secondary metablites and their biological siginificance

There is a large variation in the chemical composition and antimicrobial activity among different genera and species the combretaceae. Several species of combretaceae used in traditional medicine, in West Africa, has been investigated for their antifungal activity against the pathogenic fungi. Phytochemistry screening revealed that these plants are particularly rich in tannins, and saponins, which might be responsible for their anti-fungal activity(Baba-Moussa *et al.,* 1999). Stilbenes aglycone are common in heardwood, living tissue often contents small amount of stilbenes glycoside. To date around 185 metabolites were reported from the genus combretum including stilbenes phenantherenes, terpenoids, cycloarenoids, macrolactones and flavonoids(Ogan,1972; Jossang *et al*., 1996;Mencherini *et al*., 2007)

Traditional healers throughout Southern Africa employ species of Combretaceae for many medicinal purposes ranging from bacterial, fungal, viral and parasitic infections(Asres *et al*., 2001; Ojewole, 2008). Some species from the Combretaceae family have been reported to contain antioxidant activities. 24 African *Combretum* species have antioxidant potential(Masoko *et al*., 2007).

The Combretaceae has yielded mainly pentacyclic triterpenoids varying from oleanoic and ursanoic acids to friedelins, cycloartanes and dammaranes. Arjunolic acid and its glycosides have been isolated from *C. molle* and *T. arjuna*(Kumar and Prabhakar, 1987). Sericic acid and sericoside have been found from the roots of *T. sericea*(Eldeen *et al*., 2006). Friedelin, epifriedelin and betulinic acid from the bark of *C. imberbe* and an oleanene-based pentacyclic triterpene(imberbic acid) and its glycosides have been reported(Rogers, 1989; Angeh *et al*., 2007). Other oleanene-type pentacylic triterpenoids bearing 29-carboxy and 1-hydroxy substituents have been

isolated from *C. molle, C. edwardsii, C. eleagnoides, C. apiculatum, C. kraussi, C. padoides* and Anogeissus leiocarpus(Rogers and Verotta, 1996; Katerere *et al.,* 2003; Angeh *et al.*, 2007). These compounds demonstrate the close chemotaxonomic relationships among the species and also between African and South American *Combretum* species(Facundo *et al.*, 1993). Cycloartane-type triterpenoids have been isolated from *C. erythrophyllum*(Rogers and Verotta, 1996), and *C. quadrangulare* (Banskota *et al.,* 2000), while acidic dammarane arabinofuranosides have been reported from *C. rotundifolium*(Facundo *et al.,* 1993). One compound in particular was isolated, simultaneously, from both plant species, thus cementing their close evolutionary relationships. These compounds have good activity against *Mycobacterium fortuitum,* which is being further investigated(Katerere *et al.*, 2003).

C. imberbe have four known triterpenoids, 1α,3^{β}-dihydroxy-12-oleanen-29-oic, 1-hydroxy-12-olean-30-oic acid and 1,3,24-trihydroxyl-12-olean-29-oic acid, a new pentacyclic triterpenoid (1α,23-dihydroxy-12-oleanen-29-oic acid-3^{β}-O-2,4-di-acetyl-L-rhamnopyranoside) has been isolated through a bioassay-guided procedure from the leaves of *C. imberbe*(Angeh *et al*., 2007).

2.8.1: *Combretum* spp. secondary metabolites and its biological siginificance

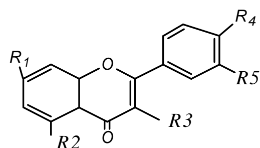
Combretum spp are widely used in folk medicine for the treatment of hepatitis malaria respiratory track infections, cancer, bilharzias, tuberculosis, HIV infection and parasitic diseases(Adnyana *et al*., 2001; Asres *et al.*, 2001). In medical preparations leaves and bark of *Combretum* spp. are predominant(McGaw *et al*., 2001).The chemistry of *Combretum* species has been studied by number of researchers to date around 144 metabolites were reported from the genus *Combretum* including stilbenes, phenantherenes, triterpenoids, cycloarenoids, macro lactones and flavonoids(Pettit *et al.*, 1995), summarized some of the important biologically active metabolites isolated from this genus. Among the metabolites of combretum stilbenes are the most important. Stilbenes have been reported to interact with microtubule

formation by binding to tubulin, the major structural component of microtubules, and to cause mitotic arrests, that inhibit the growth of cancer cells. Microtubules are among the most strategic sub cellular targets of anticancer chemotherapeutics(Pettit *et al.*, 1987). One of the most active stilbenes isolated is combretastatin A4, which is in very late stages of clinical trials. Combretastatin A4 was isolated from *Combretum caffrum*(Pettit *et al.*, 1995). Furthermore, combrestatins led to the discovery of a potent cancer cell growth inhibitor designated phenstatin benzophenone derivative(Miura *et al.*, 1999; Pettit *et al.*, 1999), studied the antioxidative and prooxidative effects of stilbenes. Their study showed that phenolic stibenes, resveratrol and diethylstilboestrol have a strong antioxidant activity that inhibits lipid peroxidation and that only resveratrol acted as a prooxidant of DNA. In terms of their biological activity they are not as important as the stilbenes(Letcher *et al.*, 1972). Amongst *Combretum* species, *Combretum woodie* has shown significant antioxidant and anti-inflammatory potential(Eloff *et al.*, 2011). In the present study, *C. hartmannianum* demonstrated strong antiproliferative and antiangiogenic activities. It is reported that *C. hartmannianum* has strong capability to inhibit tyrosine kinase(Ali *et al.*, 2002). Tyrosine kinase is an important cellular signaling protein which has essential and critical role in several biological activities including cell proliferation and angiogenesis(Ali *et al.,* 2002).

The leaves of *C. hartmannianum* were used as an antipyretic, diuretic and for various diseases such as yellow fever, hepatic disorder(von Maydell, 1986). Extracts of different parts of C. *hartmannianum* possessed significant activity against the chloroquine-sensitive *P. falciparum* strain(NF54) with an IC_{50} values of 0.2 μ/ml (bark), 0.4 μ/ml(stem) and 4.3μ/ml(leaves). More interestingly, the extracts of the leaves of *C. hartmannianum* totally inhibited the enzyme HIV-1 reverse transcriptase(HIV-1 RT) at aconcentration of 66 μ/ml(Ali *et al.,* 2002). Several plants of the genus *Combretum* have been reported for their biological activities. Antibacterial activity of different extracts (ethanol, chloroform and water) of *C. micranthum* was noted against *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* species, *Streptococcus* species, *Proteus vulgaris, Klebsiella* species, *Sarcina lutea, Micrococcus luteus* and *Bacillus subtilis*(de Morais Lima *et al.*, 2012).

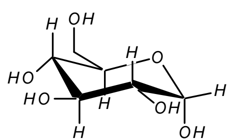
Antifungal activity against *C.albicans*, antiviral activity against *Herpes simplex 1* and *Herpes simplex 2*, antimalarial activity against *Plasmodium falciparum* and antidiabetic activity was also reported(Masoko and Eloff, 2008). *C. molle* has also demonstrated antibacterial, antifungal, anthelmintic, antiasthmatic and antitussive activities(Asres *et al.*, 2006), *C. molle* is widely used in African traditional medicine for the treatment of various ailments and diseases. Various parts such as leaves, roots and stem bark of *C. molle* are, predominantly, used(Rogers and Verotta, 1996)

Extracts of *C. erythrophyllum* obtained with different solvents (acetone, hexane, chloroform, carbon tetrachloride and butanol) have shown antibacterial activity at different doses against *Escherichia coli, P. aeruginosa, S. aureus* and *Enterococcus faecalis*(Martini and Eloff, 1998). Moreover, in studies evaluating antifungal activity, extracts obtained with different solvents (acetone, hexane, dichloromethane and methanol) were active against the following species: *C.albicans, Cryptococcus neoformans, Aspergillus fumigatus, Sporothrix schenckii* and, Microsporum canis(Masoko and Eloff, 2008). Existing phytochemical investigations indicated the presence of triterpenoids from *C. molle, C. nigricans Lepr., C. quadrangulare, C. petrophilum Retief, C. edwardsii Exell, C. elaeagnoides Klotzsch., C.nelsonii Dümmer, C. bracteatum(Laws.) Engl. et Diels, C. laxum Jacq., C. micranthum, C. imberbe, C. padoides Engl.* And *Diels, C. leprosum Mart., C. sundaicum M iquel, C. oliviforme Chao, C. Zeyheri Sond., C. vendee A.E.van Wyk., C. erythrophyllum, C. coccineum (Sonn.) Lam.* and *C. rotundifolium Rich*(Ahmed *et al.*, 2004). Other classes of compounds isolated from *Combretum* include, flavonoids from *C. quadrangulare, C. micranthum, C. erythrophyllum, C. apiculatum Sond., C. yannanense Exll , C. lanceolatum Pohl.,* and *C.leprosum*(Mabry *et al.*, 1987; George *et al.*, 2001).

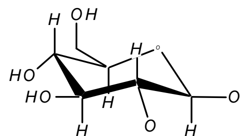


compound	R1	R2	R3	R4	R5
5-hydroxy-7,4-dimethoxyflavone	**OMe**	**OH**	**H**	**OMe**	**H**
rhamnocitrin	**OMe**	**OH**	**OH**	**OH**	**H**
kaempferol	**OH**	**OH**	**OH**	**OH**	**H**
quercetin 5,3-dimethylether	**OH**	**OMe**	**OH**	**OH**	**OMe**
rhamnazin	**OMe**	**OH**	**OH**	**OH**	**OMe**

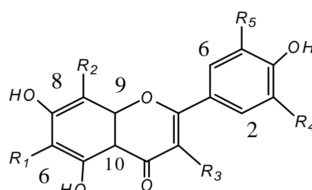
C.erythrophllum



C-glycoside



O-glycoside

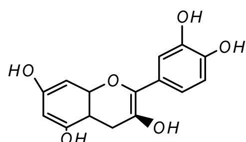


vitexin:R1,R3,R4,R5=H,R2=C-glycoside *C.micranthum*

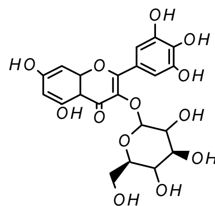
isovittexin:R1=C-glycoside R2,R3,R4,R5=H *C.micranthum*

homoorientin:R1=C-glycoside,R2,R3,R5=H,R4=OH *C.micranthum*

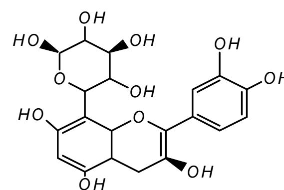
myricetin-3-O-glycoside:R1,R2=H,R3=O-glycoside ,R4,R5=OH *C.micranthum*



Catechin *C.micranthum*

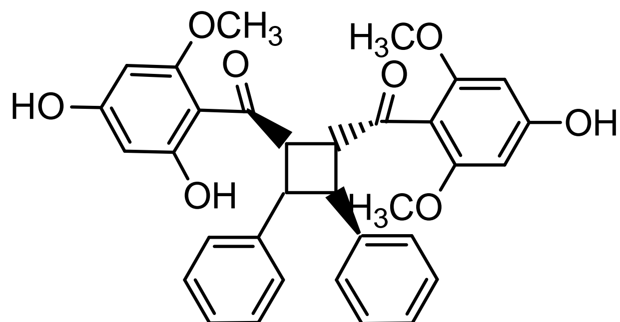


myricetin-3-O-glucoside *C.micranthum*

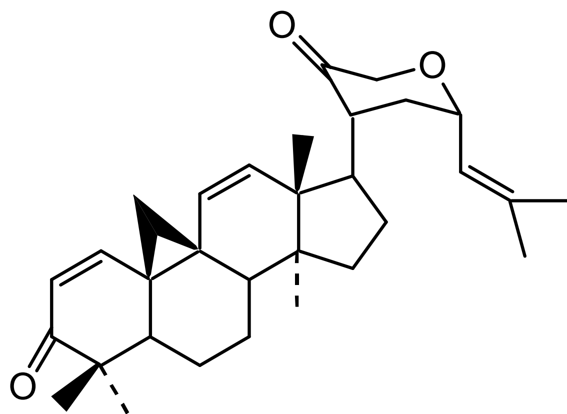


vitexin isovitexin-6-glucoside *C.micranthum*

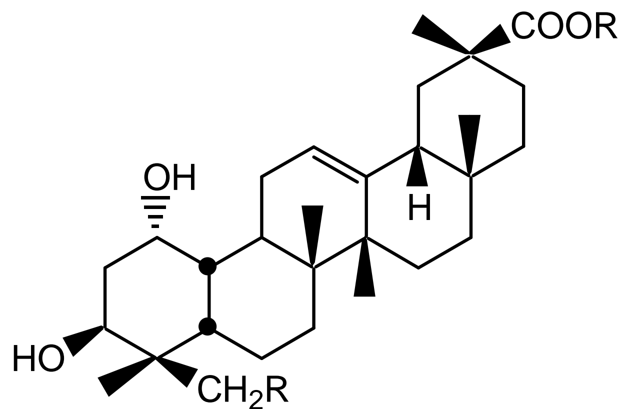
Fig.2.5: Flavonoids reported in *Combretum* spp



cyclobutanedimmer *C.apiculatum* and *C.albopunctatum*

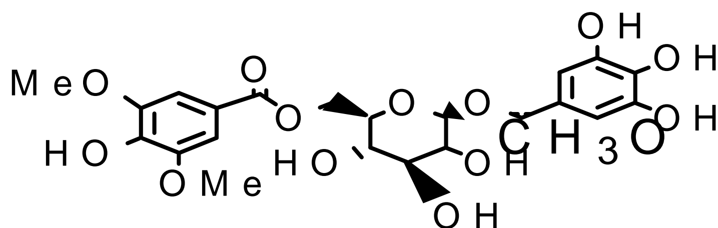


cycoartane dienone lactone *C.erythrophyllum*

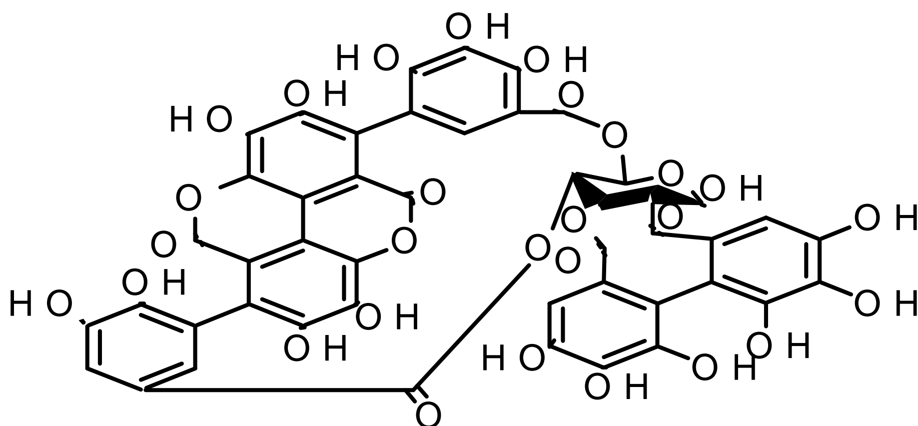


Pentacyclic tritepenes C. imberbe

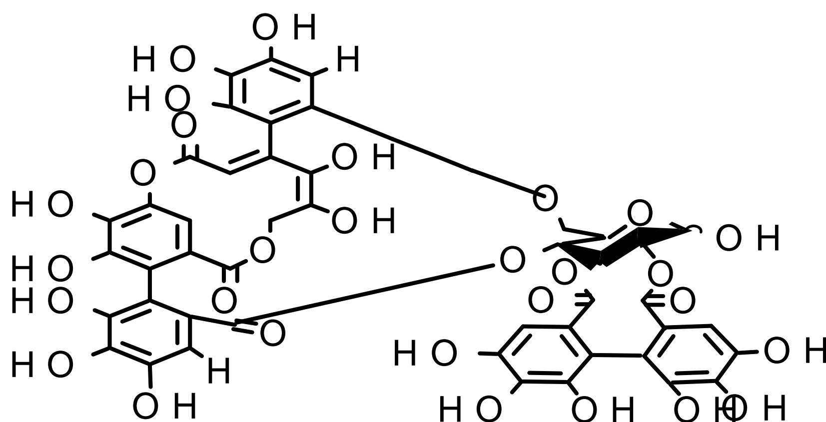
Fig.2.6: Terpenoids reported in *Combretum* spp



O-Galloyl-6-O-(4-hydroxy-3,5-dimethoxy) benzoyl-Bita-D-glucose *C.micranthum*



Punicalgin *C.apiculatum*



pugicalagin *T.ivoiriensis*

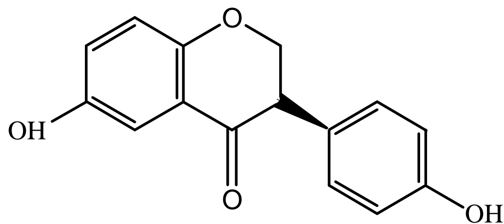
Fig.2.7: Tannins reported in *Combretum* spp

2.8.2: *Terminalia* secondary metabolites and their biological siginificance

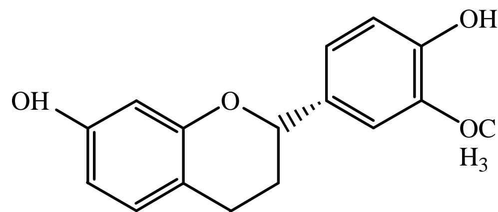
Plants of the genus *Terminalia* are known as a rich source of secondary metabolites, such as pentacyclic triterpenes and their glycoside derivatives, flavonoids, Fig. 2.8 and Fig.2.9 tannins Fig. 2.10 and other aromatic compounds, some of which with antibacterial, antifungal, anticancer and hepatoprotective activities(Garcez *et al.*, 2003).

Phytochemical analyses of *T. laxiflora* root and bark extract revealed to the presence of tannins alkaloids, saponins, flavonoids and cardiac glycosides. Have shown that some of these phytochemicals have antimicrobial- related composition such as tannins (an aromatic substance), alkaloids (serves as defense against microbial predations) and flavonoids having antimicrobial medicinal properties(Lai and Roy, 2004)

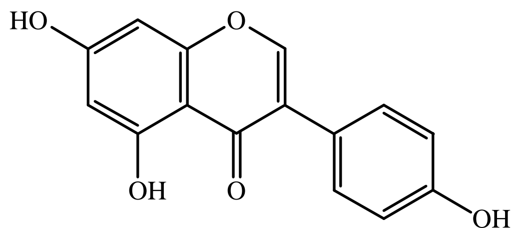
T.laxiflora leaves methanol 80% extract showed IC_{50}= 1851µg/mL and also inhibited completely the development of the HSV-1 induced cytopathic effect at concentration of 238 µg/mL while the extract had no effect on bacterial strains(Bag *et al.,* 2012).



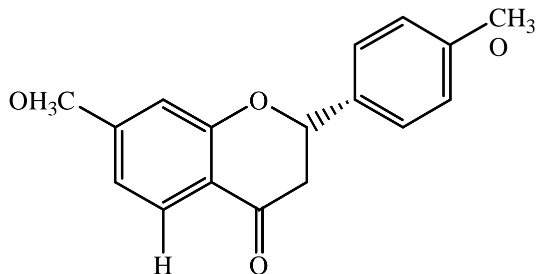
Liquiritigenin
T. fagifolia



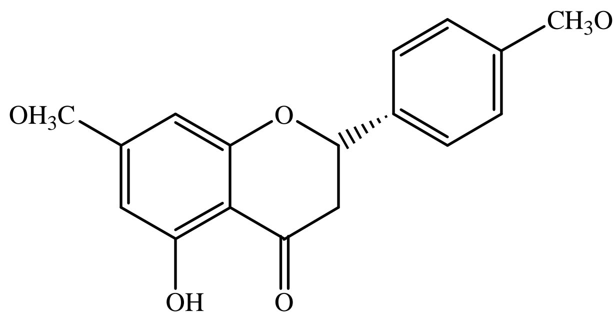
7,4' dihydroxy-3'-methoxy falvan
T. fagifolia and *T.aregentena*



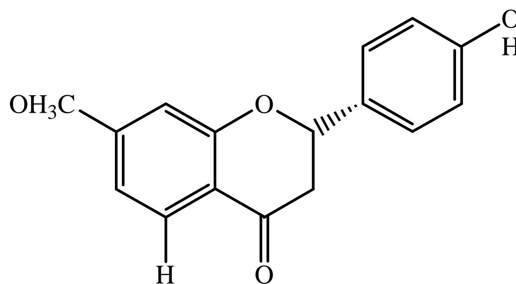
Genistien
T. arjuna



Liquiritigenin-4',7-dimethyl ether
(7,4'-dimethoxy flavan)
T. fagifolia

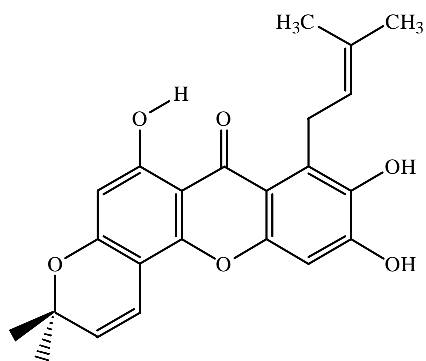


Narigenin-4',7dimethyl ether
T. fagifolia

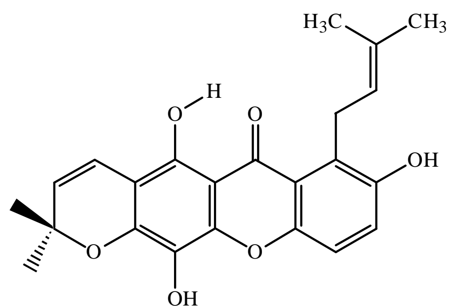


liquiritigenin-7-methyl ether
T.fagifolia

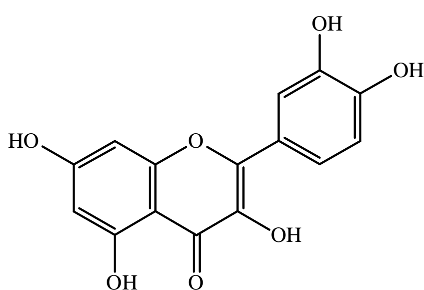
Fig.2.8: Flavonoids reported in *Terminalia* spp



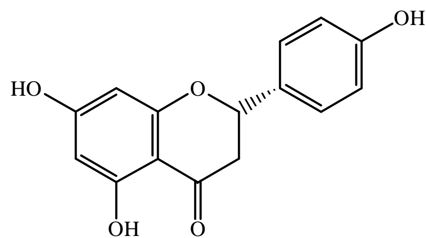
Termicalcicolanone B
T. Calcicola



Termicalcicolanone A
T. calcicola

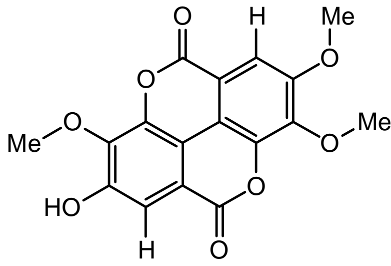


Quercetin
T. arjuna

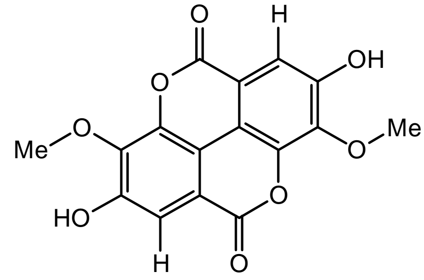


Naringenin
T. fagifolia

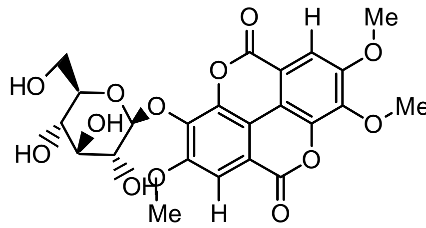
Fig.2.9: Flavonoids reported in *Terminalia* spp



2-Hydroxy 3-7-8-trimethoxychromeno{5,4,3-cde} chromene-5-10-dione*T.ivoiriensis*



3,3'-di-Omethylellagic acid
T.ivoiriensis



3,3',4-O-trimethyl-4'-O-B-D-glucopyranosylellagic acid
T.ivoiriensis

Fig.2.10: Tannins reported in *Terminilia* spp

2.9: *Acacia* secondary metabolites and its biological siginificance

Some parts of *Acacia* species contain high levels of tannins that may hamper protein digestibility and animal performance(Carter *et al.,* 1994). It should be noted that the measurement and nutritional interpretation of phenolics and tannins content in *Acacia* species are particularly difficult(Mlambo *et al.*, 2007). *Acacia nilotica* foliage and pods contain alkaloids and saponins that may have antinutritional effects(Cheema *et al.*, 2011). *Acacia* species is considered as a rich source of gallic and ellagic acid. It is a medicinally and economically important plant. Most of the *Acacias* are of medicinal and health benefits to human being. For example, *Acacia nilotica* pods are used in treatment of wound (pods), malaria, sore throat (aerial part) and toothache(Sodipo *et al.*, 2000; Malviya *et al.,* 2011). *A. laeta* shows a high content of carbohydrates. *A. nilotica* and *A. seyal* are characterized with higher contents of phenolics, anthocyanins, flavonoids, saponins and proteins than *A. laeta*(Choi *et al.,* 2005).

There are publications regarding *Acacia* extracts on free radical inhibition and antioxidant(Chang and Tung, 2009). Different parts(leaves, flowers and pods) of *Acacia* species(*A. nilotica, A. seyal* and *A. laeta*) were evaluated. On the basis of these results, total antioxidant capacity, DPPH free radical scavenging activity and reducing power of the methanolic extracts of studied parts were evaluated *A. nilotica* and *A. seyal* extracts showed less inhibitory concentration 50 (IC_{50}) compared to *A. laeta* extracts which means that these two species have the strongest radical scavenging activity whereas *A. laeta* extracts have a lower radical scavenging activity. A positive correlation between saponins and flavonoids with total antioxidant capacity and DPPH radical scavenging activity was observed(Abdel-Farid *et al*., 2014).

2.10: Descrption of *C.hartmannunum* and *T.laxiflora*

C. hartmannianum a shrub up to 4 m, as a tree under favorable conditions 10 m high known locally in Sudan as *Habeel*.Leaves alternate, shining light green when young, typically rust-colored(von Maydell, 1986).

T. laxiflora is a species of plant in the Combretaceae family, the germination of this species varies from 0-70% under the same condition. The tree is of 12m in height and nearly 1m width with the usual crooked bole, dark grey, deeply fissured and scaly bark(Batawila *et al.*, 2005).

Botanical classification of *C. hartmannunum* and *T. laxiflora*

Kingdom	plantea; plants
Subkingdom	Trachebionta-----vascular plants
Super division	Spermato phyla ----------seed plants
Division	Mangnoliophyta---- flowering plants
Subclass	*Rosidae*
Order	*Myrtales*
Family	Comberaceae
Genus	*Combretum*
Species	*hartmannianum*
S.N	*Combretum hartmannianum*
Vernacular	*Habeel*
S.N	*Terminalia laxiflora*
Vernacular	*Sobage*

A B

Fig.2.11: *Combretum hartmannianum,* (*Habeel*), (A) Tree of *Combretum hartmannianum* (*Habeel*), (B) Fermented (*Nikhra*) and non fermented wood of *Combretum hartmannianum,* (*Habeel*).

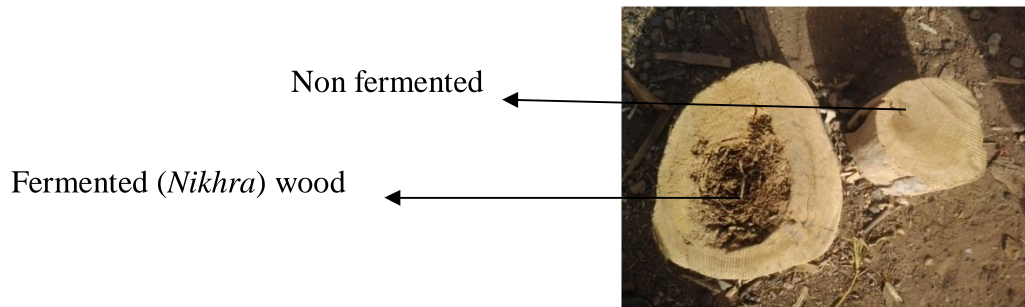


Fig.2.12: *Terminalia laxiflora* (*Sobage*) Fermented (*Nikhra*) and non fermented wood of *Terminalia laxiflora* (*Sobage*)

2.11: Description of *A. seyal*

Acacia seyal is small to medium-sized tree, growing to 17 m tall and 60 cm in diameter at breast height; crown is umbrella shaped, resembling that of *A.tortilis* and *A.characteristic* feature of the tree is its rust –coloured powdery bark; *A.seyal* or *A.fistula* has whitish bark. Large, straight spines occur on the branches, and smaller. Leaves bipinnate, dark green, 4-12 pairs of pinnae, 10-12 pairs of leaflets each 1-2x4-12 mm. Flower clustered in shining, yellow, globose heads.1.5 cm diameter, on stems 3cm long(Orwa *et al.*, 2012).

Botanical classification of *A. seyal*

Kingdom	plantea; plants
Subkingdom	*Trachebionta*-----vascular plants
Super division	*Spermato phyla* ----------seed plants
Division	*Mangnoliophyta*---- flowering plants
Class	*Mangnolipsida-dicotyledons*
Subclass	*Rosidae*
Order	*Myrtales*
Family	Fabaceae
Genus	*Acacia*
Species	*seyal*
S.N	*Acacia seyal* or *Acacia fistula*
Vernacular	*Talh*
Vernacular	Sufar

Non fermented

Fermented
(*Nikhra*) wood

A

B

Fig.2.13. ***Acacia seyal*** **(*Talh*)** ***Acacia seyal*****, (A) Tree of** ***Acacia seyal*** **(*Talh*) (B) Fermented wood (*Nikhra*) and non fermented wood of** ***Acacia seyal***

2.12: Traditional medicinal uses of the plants studied

T. laxiflora has a variety of medicinal applications across the areas of occurrence. The stem are used as chewing stick in Nigeria, and as gastric stimulant to prevent and cure diarrhoea in infants and children. It also aids digestion and relieves constipation in adults. The leaves and the bark of the root are used as anti-dysentery while the stem bark for the treatment of tuberculin cough and the yellow pulp of root and the black leaves are used as dye. The scented heart wood is used as perfume called "amu" and the root bark is used to treat wound and strains. The macerated stem bark serves as antiseptic to wash mouth in order to resist gingivitis and thrush serves as wound dressing, diuretic management, pile and yaws treatment(Ivory coast), anti-skin inflammation, sores and ulcers treatment(Sierra Leone), eye lotion(Gambia), hair perfume, severe jaundice and chewing stick(Cameroon) across other African countries(Abbiw, 1990; Daniel, 1990; Batawila *et al.,* 2005). The barks decoction of *T. laxiflora* is used for malaria(Doka and Yagi, 2009). The root bark of *T. laxiflora* is used traditionally as gastric stimulant to prevent and cure diarrhea in infants and children, aids digestion and relieves constipation in adults. As quality antibiotics are rarely possessed, human pathogens are fast developing resistance to synthetic drugs yet medicinal plants are scantily validated(Daniel, 1990). And for treatment of diarrhea and gonorrhea(Mbwambo *et al.*, 2007). *Acacia* is used as chewing sticks with an antimicrobial activity against *Streptococcus facials*, also shows some cholesterol-lowering and anti diabetic properties(Muazu and Kaita, 2008).

2.12.1: Smoke or scent (*Bakhour*)

Bakhour is a typical Sudanese traditional, authentic, cosmetic that is used in many different patterns. It is a sweet scented smoke that evolves when burning special types of wood that are primarily having lovely scent and moreover they are treated by adding a very sophisticated combination of

perfumes and powdered musk with caramelized sugar added afterwards to fix all the scents on the wood. Such woods are like *Talh*, *Sobage*. *Bakhour* is used, primarily, as a perfume for married women. Women also have their clothes scented with *Bakhour*; they also use it just like an air freshener to give a very lovely, scent to their houses. Sudanese all the time ignite *Bakhour* in all occasions that's why *Bakhour* is perceived, always, as asign of happiness, brides (and wealthy women) normally use a special type of *Bakhour* for their bodies, clothes, and bedrooms. *Bakhour* of other types of wood are used as insect (flies and mosquitoes) repellents, especially, in autumn.

2.12.2: Smoke (*Dokhan*)

Dokhan is a traditional process used by Sudanese married women in which woman wraps her entire body in a blanket and sits on a hole in the ground. The hole contains half burning *Talh* or *Habeel* or *Sobag* woods or their *Nikhra* or the two together with intensive smoke as an aroma that gives the skin a breathtaking glow. Sudanese women also use *Dokhan* to make their own perfume, rarely single female use it for medical purpose(Mariod *et al*., 2014).

2.13: Fermented wood "*Nikhra*"

The dried fermented wood of *Acacia seyal, Combretum hartmannianum* and *Terminalia laxiflora* is well known used for special fragrance and some medicinal uses. *Nikhra* is the Sudanese words for (fermented wood) of the heartwood of which grow in different Sudan states. A few hundred species produce *Nikhra* resin, of which only a few species is well known and used by Sudanese women as fragrance(Mariod *et al*., 2014).

2.13.1: Wood fermentation by fungi

Wood decay is caused by *Serpula lacrymans* a wood-decay fungus that digests moist wood, causing it to rot. Some species of wood-decay fungi attack dead wood, such as brown rot, and some, such as *Armillaria*(honey fungus), are parasitic and colonize living trees. Fungi that not only grow on

wood but, actually, cause it to decay are called lignicolous fungi.Various lignicolous fungi consume wood in various ways; for example, some attack the carbohydrates in wood and some others decay lignin(Vane *et al.*, 2005).

2.13.1.1: Classification of wood fermenting fungi

Wood-decaying fungi can be classified according to the type of decay that they cause. The best-known types are brown rot, soft rot, and white rot(Vane *et al.*, 2005). Each produce different enzymes, can degrade different plant materials, and can colonise different environmental niches. The residual products of decomposition from fungal action have variable pH, solubility and redox potentials. Over time this residue becomes incorporated in the soil and sediment, then having a noticeable effect on the environment of that area(Vane *et al.*, 2005).

Chapter Three

3. Materials and Methods

3.1: Ethnobotanical Study of The plants studied

3.1.1: Study area

This study was conducted in Khartoum State, Sudan, with emphasis on Khartoum, Khartoum North and Omdurman localities. Wood tree species were *Combretum hartmannianum*(*Habeel*), *Acacia seyal*(*Talh*), and *Terminalia laxiflora*(*Sobage*). The users of these wood trees as *Bakhour* and *Dokhan* were the real targets of this study. Information gathered inclouded: knowledge of the common name, uses, how to use, knowlege *Nikhra*, method of separation, the part used for separation, which of *Nikhra C. hartmannianum*(*Habeel*), *A. seyal*(*Talh*), and *T. laxiflora*(*Sobage*) you prefer to use for fragrance, which *Nikhra* of *C. hartmannianum*(*Habeel*), *A. seyal* (*Talh*), and *T. laxiflora* (*Sobage*) you use have health problems, which of *Nikhra* of *C. hartmannianum*(*Habeel*), *A. seyal*(*Talh*), and *T. laxiflora* (*Sobage*) have strong fragrance in body after use, which of *Nikhra* of *C. hartmannianum*(*Habeel*), *A. seyal*(*Talh*), and *T. laxiflora*(*Sobage*) last longer in body after use, which of *Nikhra* have strong fragrance, which of *Nikhra* of ethyl acetate fractions of *A. seyal* (*Talh*), *T. laxiflora* (*Sobage*) and *C. hartmannianum* (*Habeel*) have strong fragrance , which of *Nikhra* of ethyl acetate fractions of *A. seyal*(*Talh*), and *T. laxiflora*(*Sobage*) *C. hartmannianum*(*Habeel*) last long in the body after use. Field trips were made to the study areas. The respondents to the questionnaire include sellers of fragrance, business women, and housewives. These categories of people use the trees as cosmetic, medical treatment, fuel, fodder and other purposes. A total of 100 questionnaires were distributed and conducted from April to June, 2011. Frequency of consumption of each tree species was determined as a percentage of the number of respondents using it in cosmetic, medicinal treatment, fuel, fodder and other purposes in relation to the total number of respondents that used cosmetic, treatment, fuel, fodder and other purposes.

3.2: Collection of plant materials

Fermented hardwood *"Nikhra"* of *C. hartmannianum*, *A. seyal* and *T. laxiflora* were collected in March 2011 from Kordofan state, Sudan. They were, carefully, washed, oven-dried for 1 h at 50°C and put in the shade in an aerated place till complete drying, then were ground into a fine powder.

3.2.1: Plant materials preparation and extraction

100 g of ground powder of each plant was extracted using methanol and a soxhlet apparatus Fig.3.1. Percentage of yield was calculated from the dry extract powder. The methanolic extract was fractionated, sequentially, using solvents of increasing polarity namely petroleum ether, chloroform, ethyl acetate and aqueous. Fractions were dried using an evaporator and stored at 4C for further analysis(Fyhrquist *et al.*, 2002).

3.3: Treatment of the ethyl acetate fraction with 2%NaCl

30 mls of 2% NaCl were added to one gram of fine powder of the ethyl acetate fraction in a separatory funnel for extraction of tannin three times. Extraction showed two layers, the upper one was ethyl acetate extract and the lower layer was the aqueous. The two extracts obtained were evaporated to dryness by air and the powder stored at 4C for TLC analysis.

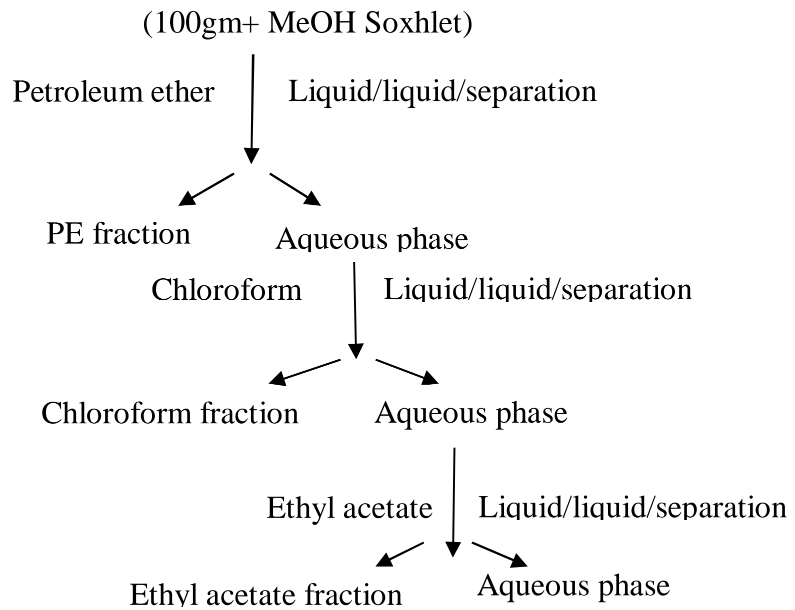


Fig.3.1: Scheam of extraction and fractionation of fermented wood "*Nikhra*" of *A. seyal T. laxiflora* and *C. hartmannianum*

3.4: Antimicrobial activity

The fractions of C. *hartmannianum*, *A. seyal* and *T. laxiflora Nikhra* were tested for their antimicrobial activity using cup-plate agar diffusion method(Kavanagh, 2014) with minor modifcations. Plants fractions were tested against the following human pathogens; *Salmonella Typhi*(ATCC 25921), *Escherichia coli*(ATCC 25922), *Staphylococcus aureus*(ATCC 27853), *Aspergillus flavus(*ATCC 97638), *Aspergillus niger*(ATCC 97638) at the Aromatic Plants department, National Center for Research, Khartoum, Sudan.

3.4.1: Preparation of the test organisms

3.4.1.1: Preparation of bacterial suspensions

One ml aliquot of a, 24 hours, broth culture of test organisms was, aseptically, distributed onto nutrient agar slope and was incubated at 37C for 24 hours. The bacterial broth was harvested and washed off with sterile normal saline, and was, finally, suspended in 100 ml of normal saline to produce a suspension containing about(10^8 –10^9) colony forming units per ml. The suspension was stored in a refrigerator at 4C. Serial dilutions of the stock suspension were made with sterile normal saline in tubes and 0.02 ml volumes (one drop) of the appropriate dilution were transferred on to the surface of dried nutrient agar plates. The plates were allowed to stand for 2 hours at room temperatures for the drops to dry, and then was incubated at 37C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop(0.02) was multiplied by 50 and the dilution factor to give the viable count of stock suspension was expressed as the number of colony forming unit C.F.U/ ml of suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained (constant) so that suspensions with very close viable counts would be obtained.

3.4.1.2: Preparation of fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar, incubated at 25 C for 4 days. The fungal growth was harvested and washed

with sterile normal saline and finally suspended in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

3.4.2: Agar diffusion method

3.4.2.1: Screening for antibacterial activity

0.2ml of the standardized bacterial stock suspension was mixed with 20 ml of sterile nutrient agar and poured into a sterile petri dish; the agar was left at room temperature to dry. Four cups(10 mm in diameter) were cut using a cork borer and agar discs were removed. Cups were filled with 0.1ml of the different fractions in aspirate petri dish(aqueous, ethyl acetate, chloroform and petroleum ether fractions), three replicates for each fraction for each testing organisms(*Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*). The concentration used from each fraction is 2.5mg and 5mg of the fraction in 1ml of the solvent, the fractions were left to diffuse for two hours, and the plates were incubated in the upright position at 37C for 18 hours. After incubation the diameter of the inhibition zone was measured the mean value was taken. Positive control for each solvent was carried out to know the activity of the different solvents.

3.4.2.2: Screening for antifungal activity

Fungal cultures (*Aspergillus flavus*, *Aspergillus niger, Candida albicans*) were prepared in sabouraud dextrose agar, incubated at 25C for 4 days. Growing fungi were harvested and washed with sterile normal saline and, finally, suspended in 100 ml of sterile normal saline, and the suspension was stored (4C) for further use. The cup plate agar diffusion method was followed in the same way as in the antibacterial activity. After 48-72 hours the inhibition zone was measured for each fraction and mean values were calculated.

3.4.2.3: Minimum inhibitory concentration (MIC)

To quantify the activity of the active fractions modified serial dilution method was used to determine inhibitory concentration (Abdallah *et al.,* 2009). Plates were prepared in series of increasing concentration of the agent (plant fractions), in the order: 0.1, 0.05, 0.025, 0.0125, 0.0062 and

0.0031mg/ml. The bottom of each plate was marked into four segments, in the case of standard bacteria, and two segments in the case of fungi. The organism to be tested is grown in broth over-night, and diluted in broth to contain 10 per ml of the diluted culture is spotted with standard loop that derives 0.1ml into the surface of each segment and then incubated at 37C for 18 hours for bacteria and at 25C for 2-3days for the fungi.

3.5: Free radical scavenging assay (Antioxidant)

Radical scavenging assay is a spectrophotometric test using a methanolic solution of the stable free radical 2, 2-diphenylpicrylhydrazyl (DPPH) as a reagent. DPPH method based on increase in alcoholic DPPH solution by reduction of stable DPPH nitrogen radicals in presence of H binding antioxidants. The hydrogen atoms or electrons donation ability of the corresponding fractions and some pure compounds was measured from the bleaching of purple coloured of DPPH methanolic solution, whereas, the DPPH solution in a dark violet coloured and has a strong absorption range at 517 nm. It loses its color when transformed to DPPH-H and the absorption level decreases. This decrease in absorption shows the cytochiometric decrease in DPPH.

3.5.1: Free radical scavenging procedure

In the experiment 10 µl from the fractions(5mg/ml) were added to 90 µl of the 300 µM DPPH solution and placed in a 96-well microtiter plate. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. The mixture was incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the remaining DPPH was read against a blank at 517 nm using multiplate reader spectrophotometer. Propylgallate was used as the positive control, where as DMSO as a negative. All tests and analyses were carried out in triplicate(Chun *et al*., 2005). The inhibition of free- radical DPPH in percent (%) or the capacity to scavenging the DPPH radical(radical scavenging activity) was expressed as EC_{50} value(mg ml-1) (the concentration demanded to inhibition the 50% of DPPH radical scavenging activity(Luís *et al.,* 2009); calculated using the following equation:

[(A control –A sample)/ A control] x100

A= Absorbance

3.6: Brine shrimp lethality assay (Toxicity)

The *Nikhra* fractions of *A. seyal, T. laxiflora* and *C. hartmannianum* were tested for their cytotoxicity using the brine shrimp *Artemia salina* standard method as described by(Meyer *et al*., 1982) with a minor modification. This is a rapid, inexpensive, general bioassay, has been developed for screening and monitoring of physiologically active natural products.

3.6.1: Materials used in toxicity assay

The materials and reagents used for cytotoxicity includes, test sample, *A.salina* (shrimp eggs), sea salt (38 g/L of D/W, Ph 7.4), hatching tray with perforated partition, lamp to attract brine- shrimp larvae, micro pipette (5, 50, 500 µl), vial tray, 9 vial samples and organic solvent (methanol). The eggs of brine- shrimp *A. saline* are readily available as fish food in pet shops. They are stored at a low temperature(4C), and remain viable for years. Half hatching tray (a rectangular dish 22x32cm) was filled with filtered brine solution, then(50 mg) eggs of brine were sprinkled and incubated at 37C. When the eggs were placed in artificial seawater they hatch within 48 h, providing large numbers of larvae. They can be used for 48-72 hours after the initiation of hatching, and after 72 hours they should be discarded.

3.6.2: Toxicity assessing procedure

A rectangular dish(22x32cm) was divided into two unequal halves with plastic divider of 2 mm artificial seawater(2with several holes and filled with 28g sea salt/L, Sigma). Approximately 50 mg eggs(*Artemia saline* Sera Heidelberg Germany) were sprinkled in the larger compartment, which was darkened, while the smaller compartment was illuminated. 0.5 ml of 100, 1000 and 10,000 ppm concentrations of the extract prepared in respective solvent (methanol) 20 mg of extracts were dissolved in 2 ml of 5, 50, 500 µl, the concentrations were (10,100 and 1000 µl/ml respectively) was poured

in vials (3vials/concentrations) and kept at room temperature to evaporate methanol. After 24 hours, phototropic nauplii(brine- shrimp larvae) were collected by a Pasteur pipette from the lightened side, and 10 shrimps were transferred to each vial. The vials were placed under the illumination at room temperature, and the volumes were made up to 5ml with sea water , and then incubated at(25 -27Ċ) for 24 hours under illumination. Other vials were supplemented with solvent and reference cytotoxic drugs(Etoposide) as negative and positive control respectively. The numbers of survivors were counted after 24 hours. The data were analyzed with Finney computer program and the lethal concentrations 50%(LD_{50}) were determined.

3.7: Total phenolic content (TPC)

The concentration of phenolics in plant fractions was determined using spectrophotometric method(Singleton *et al*., 1999). Samples solutions of the fractions in the concentration of 1 mg/ml were used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of samples solutions of fractions, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were, thereafter, incubated in a thermostat at45C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration curve was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration curve; then the content of phenolics in fractions was expressed in terms of gallic acid equivalent (mg of GAE/g of fraction).

3.8: Phytochemical screening

Phytochemical examinations were carried out for all the fractions using different standard methods.

3.8.1: Alkaloids

The presence of alkaloid was determined using the Mayer and Dragendorff tests as described by(Harbone, 2001), 0.2 g of each fraction was added to 20 ml of dilute sulphuric acid in methanol and the mixture was heated in water bath for 5 min. The mixture was filtered(vacuum pump) and the filtrates were treated with 2 drops of Mayer`s(Potassium mercuric iodide solution) and Dragendorff's(Potassium bismuth iodide solution) reagents in test-tubes. Development of creamy and an orange color indicated appositive result.

3.8.2: Flavonoids

3.8.2.1: Ammonium test

The presence of flavonoids in the samples was determined using(Sofowora, 1982; Harbone, 2001), 10 ml of ethyl acetate was added to 0.2 g of each fraction and heated in a water bath for 5 min. The mixture was cooled filtered and the filtrates used for the test. About 4.0 ml of filtrate was shaken with 1.0 ml of diluted ammonia solution. The layers were allowed to separate and the yellow color in the ammonical layer (bottom layer) indicates the presence of flavonoids.

3.8.2.2: Shinoda test

Small pieces of magnesium ribbon followed by few drops of concentrated hydrochloric acid were added to a small amount of fraction of the plant material. Immediate development of a pink scarlet or crimson red color was taken as an indication of the presence of flavonoids(Harbone, 2001).

3.9: Saponins

The froth test and emulsion test as described by(Harbone, 2001) were used to determine the presence of saponins. 20 ml of water was added to 0.25 g of the fraction in 100 ml beaker and boiled, filtered and then the filtrates used for the tests:

3.9.1: Froth test

5 ml of the filtrate was diluted with 20 ml of water and shaken vigorously. A stable froth(foam) up on standing indicates the presence of saponins(Sofowora, 1982)

3.9.2: Emulsion test

2 drops of olive oil was added to the frothing solution and shaken vigorously the formation of emulsion indicates the presences of saponins.

3.10: Steroids / Terpenoids

3.10.1: Liebermann-Burchardt test

One ml of fraction of each sample was boiled with 2–3 ml of acetic anhydride, and then cooled; 1 to 2 drops of concentrated sulfuric acid were added, carefully, by the wall of the tube. Dark green coloration of the solution indicates the presence of steroids and dark pink or red coloration in the interface indicates the presence of terpenoids.

3.10.2: Salkowski test

One ml of fraction of each sample was boiled with 2 ml chloroform, then cooled, and 1 to 2 drops of, concentrated, sulfuric acid were added, carefully, through the wall of the tube. The tube was shaken well and allowed to stand for some time, red color appears at the lower layer indicates the presence of steroids and formation of yellow colored lower layer indicates the presence of triterpenoids.

3.11: Cardiac glycosides

About 5 ml of the fraction was mixed with 2 ml of glacial acetic acid containing one drop ferric chloride solution. To this, one ml of concentrated sulphuric acid was slowly under layed to the sample mixture. A positive test result was confirmed by the presence of a brown ring at the interface(Edeoga *et al*., 2005).

3.12: Tannins

3.12.1: Ferric chloride test

About 0.5 g of the fraction was dissolved in 5 to 10 ml of distilled water and filtered. A few drops of a 10% ferric chloride solution were added to the filtrate. A greenish black colour or a precipitate was taken as an indication of the presence of tannins(Harbone, 2001).

3.12.2: Alkaline reagent test

Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

3.13: Chemical group tests

Nikhra fractions were tested for some of functional groups as follows :

3.13.1: Phenolic group

To test for the presence of phenolic groups, 3 to 5 drops of 1M NaOH (aq) were added to 2 ml of the sample. Solubility of the sample was an indication of presence of phenolic groups(Engel *et al*., 1990).

3.13.2: Carboxylic acid group

To test for the presence of carboxylic acid groups, 3-5 drops of 1M $NaHCO_3$ (aq) were added to 2 ml of sample fraction. Solubility and effervescence of the sample was a confirmation of a presence of carboxylic groups(Engel *et al*., 1990).

3.13.3: Potassium permanganate test for unsaturation or hydroxyl group

To test for the presence of double and/or triple bonds or OH groups, 3-5 drops of 1 M potassium permanganate was added dropwise and shaken. Decolourization of potassium permanganate was a confirmation of a positive test(Furniss, 1989).

3.14. Chromatographical and spectroscopical analysis

3.14.1 Thin layer chromatography(TLC)

Aluminum silica gel plates $60F_{254}$ Merck5554 and pre-coated TLC plates SII, NP-18W/UV254(Macherey/Nagel) were used as stationary phases in carrying out TLC of the different plants fractions. Standard chromatograms were prepared by applying 20µl of dissolved fractions(5mg/ml) to a silica gel plate and developing it in solvent systems depending on the type of extract, in case petroleum ether fraction solvent systems be 8:2 petroleum ether: ethyl acetate and fraction of ethyl acetate solvent systems be 4:4:1Touene: ethyl acetate: Formic acid. Chromatograms were detected under UV light(254 and 366 nm), and sprayed with diagnostic reagents which include: Vanillin - HCL Reagent, Vanillin - H_2SO_4 Reagent and Natural Product(polyethlenglycol)(NP/PEG) Reagent(NPR). The retention factor value (Rf) of the visible bands were marked under daylight. Rf = distance moved by analyte (compound) distance moved by solvent.

3.14.1.1. TLC diagnostic reagents

- ***Natural products (polyethlenglycol) Reagent (NPR)***

Plates were sprayed with 1% methanolic diphenyl boric acid(NP), followed by 5% ethanolic polyethylenglycol- 4000(10ml and 8ml, respectively).

- *Vanillin H_2SO_4*

0.5g vanillin was dissolved in a ready prepared. Mixture of 85 ml MeOH, 10 ml acetic acid and 2.5 ml conc. H_2SO_4 sprayed TLC plates were examined after heating to 120°Ċ.

- *Vanillin HCL*

0.5g vanillin was dissolved in 50 ml 37% hydrochloric acid. Vanillin HCL sprayed TLC plates were examined after dry at room temperature. Catechins show red spots.

- *Vanillin H_3PO_4*

One gram vanillin was dissolved in 100 ml 50% aqueous phosphoric acid. Vanillin H_3PO_4 sprayed TLC plates were examined after dry at room temperature and heated for 10-20 min at 120°C. Steroids show red spots.

3.15 Gas Chromatography/ mass spectrometry (GC/MS)

3.15.1 Gas chromatography/ mass spectrometry (GC/MS) (Polyphenols):

The GC-MS analysis of petroleum ether fractions(*Nikhra* of *A. seyal, T. laxiflora* and *C. hartmannianum*) was carried out using a GCMS-QP2010Plus gas chromatography(Company, town, country) equipped and coupled to a mass detector Turbo mass gold –Perkin Elmer Turbo mass 5.1 spectrometer with an Elite – 1(100% Dimethyl poly siloxane), 30m x 0.25mm ID x µm of capillary column. The instrument was set to an initial temperature of 35C, and maintained at this temperature for 2 min. At the end of this period(4-61 min) the oven temperature rose up to 280C, at the rate of an increase of 5C/min, and maintained for 9 min. Injection port temperature was ensured as 250C and helium flow rate was one ml/min. The ionization voltage was 70e V. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 35-450(*m/z*) with in a time range of 4-61 min. Interpretation on Mass-Spectrum GC-MS was conducted using the database of National Institute Standard and Technology(NIST) have more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3.15.2: Gas chromatography/mass spectrometry(GC/MS) of(Terpenoids):

The GC-MS analysis was performed on HP(5890A, II MSD) gas chromatograph, connected with MS system (HP 5971 A): dimethylsiloxane DB-5 fused silica capillary column(30 m x 0.25 mm, 0.1 m film thickness); carrier gas: helium 9 ml/min; injector temperature 250C; detectortemperature 200 C; column temperature 50 -180 C at 4 C/min, then 180-250 C at 20 C/min. Mass spectrometer operating conditions 70 eV

ionization energy. Identification of individual components was based on their mass spectral fragmentation using two computer library MS searches(Wiley Mass Spectral Database 229), retention Indices and comparison with literature data. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

Chapter Four

4. Results and Discussion

4.1: Ethnobotanical study of the plants studied

This study reports for the first time an ethnobotanical, complete, survey among Sudanese women in Khartoum state covering the traditional medical and cosmetic uses of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal* wood and fermented hart wood.

The questionnaire results Table(4.1) revealed the most common names used in three localities(Khartoum, Khartoum North and Omdurman) for *Acacia Seyal* was *Talh*(81%), while 19% was Makntosh. For *Combretum hartmannianum* was *Habeel*(100%) and *Terminalia laxiflora* was *Sobag*(80%), 12% was *Darot* and 8% was *Kolit*, common names of the fermented wood of *A. sayal, C. hartmannianum* and *T. laxiflora* used in the three localities were *Nikhra*(80%), *Nukhara*(10%) and *Guur*(10%). The 100 respondents of the analyzed questionnaire showed that 73% of the respondents that used *A. seyal*, *C. hartmannianu* and *T. laxiflora* were married females, while 27% were single females Table(4.2). The educational background, age and job were not influencing factors because women aged 20-80 years from different education back ;grounds and job levels traditionally used these trees fermented wood for *Dokhan* and other benefits Table(4.2). *A. seyal* was mainly used for *Dokhan* purpose by 68% of the respondents, while *C. hartmannianum* was used by 20% and *T. laxiflora* was used by 25%. *A. seyal* used for *Bakhour* by 22% of the respondent and only 1% of the respondent used *C. hartmannianum* as *Bakhour* while 50% used *T. laxiflora* as *Bakhour*. This is because *Bakhour* depend on fragrance of fermented wood and *C. hartmannianum* is not an aromatic plant Table(4.2). The results showed other uses of *A. seyal*, *C. hartmannianum* and *T. laxiflora* wood for fighting mosquitoes, and as fuel, fodder and other purposes Table(4.2). 57, 58, and 46% of the questioned women knew about *Nikhra* of *A. seyal*, *C. hartmannianum* and *T. laxiflora,* respectively, Table(4.2). This may also be due to lack of knowledge because(27%) of respondent are single and married respondent use these tree for medical and

cosmetics or *Dokhan* purposes.The terminology is not famous in Khartoum but rather in the west Sudan.

4.1.1: Organoleptic survey of wood fragrance

Organoleptic survey of fragrance in different extracts and fractions of *Acacia sayal*, *Combretum hartmannianum* and *Terminalia laxiflora Nikhra* showed that petroleum ether and ethyl acetate fractions were the most fragrance among extracts and fractions of three plants Table(4.6). Questionnaires revealed that 31-50 years age women prefered petroleum ether and ethyl acetate fractions following by 20-30 years age and lastly 51-80 years age women Fig.1 (Appendex). Questionnaires revealed that *A.seyal* fragrance is preferred(53%) followed by *T. laxiflora(*47%) Fig 2 (Appendex). *C. hartmannianum* is not an aromatic plant it was mostly used for treatment health problems(89%) following by *A. seyal*(9%) and *T. laxiflora*(2%) Table 4.6; Fig 3(Appendex).

4.2: Physical properties of fermented and non fermented crude wood extracts

Generally *Nikhra* from *Acacia sayal*, *Combretum hartmannianum* and *Terminalia laxiflora* was obtained as scratches from fermented heartwood.

The physical properties of methanolic extracts of fermented and non fermented heartwood of *A. seyal, C. hartmannianum* and *T. laxiflora* are presented in Table 4.3. The methanolic extracts of fermented *A. seyal, C. hartmannianum* and *T. laxiflora* yield 2.99, 3.10, and 3.64%, respectively, while the methanolic extracts of non fermented *A. seyal, C. hartmannianum* and *T. laxiflora* yield 0.64, 0.92, and 2.27 % respectively. All methanolic extracts were powders in form and fragrant, having dark and faint brown colors Tables(4.3, 4.4) showed that the percentage yield of methanolic extracts of fermented wood were more than nonfermented this might be due to the solubitity of compounds in feremented wood in solvents. The yield percent was also found to be related directly to the softness of fermented wood of *T. laxiflora*(softness) yield percentage was 3.64% followed by *C. hartmannianum* 3.10% lastly *A .seyal* 2.99% which was hardest, extracts percentage of *T. laxiflora* of non fermented heartwood the most one 2.27%

following by *C. hartmannianum* 0.92% lastly *A. seyal* 0.64%. Fractions(petroleum ether, ethyl acetate, chloroform and aqueous) of fermented and non fermented wood of *T. laxiflora* (0.39,1.68, 0.16, 1.71), (0.20, 1.31, 0.05,0.71) % *C. hartmannianum*(0.36, 0.35, 0.49,1.27), (0.27, 0.21, 0.09, 0.35)% and *A. seyal*(0 .21, 0.97, 0.92, 0.89), (0.06, 0.36, 0.05, 0.17)% respectively Table 4.5, fractions percentage of fermented plants more than nonfermented, these due to fermentation make wood plants soft so compounds in these plants be available to extract by solvents.

ANOVA analysis results of speices VS solvents, speices VS fermented and non fermented wood and species VS solvents VS fermented and non fermented wood of *T. laxiflora, C. hartmannianum* and *A. seyal* are high signivigant when compere *f* ratio with *f* table and showed *f* ratio was higher than *f* tablulated Table 4 (Appendex).

This study confirms that *A. seyal* is still a major source of cosmetics souna *Dokhan* and *C. hartmannianum* is used for health purposes while *T. laxiflora* is not well known in Khartoum State. Thus, traditional medicine remains the most popular medicine in solving health problems. The abundance of information on the traditional uses of trees is in danger of disappearing since the knowledge of how to use plants is mostly passed down orally and even to date is poorly documented(Sofowora, 1982), moreover, the most serious threat to local plant knowledge, appears to be a cultural change, particularly the influence of modernization and the western world view(Voeks and Leony, 2004), which has contributed to undermining traditional values among the young(Giday *et al.,* 2003).

Table 4.1: Percentage of common name of plants studied and common name of fermented wood of plants study in Khartoum State locality

Locality	Common name of non fermented wood (%)						Common name of fermented wood (%)		
	T. laxiflora			*C. hartmannianum*	*A. seyal*				
	Sobage	*Kolit*	*Darot*	*Habeel*	*Talh*	*Makntosh*	*Nikhra*	*Nikhara*	*Guur*
Khartoum	23	5	10	23	23	10	23	3	1
Khartoum North	40	2	2	57	40	1	25	2	3
Omdurman	17	1	0	20	18	8	32	5	6
Total	80	8	12	100	81	19	80	10	10

Table 4.2: Uses, knowing of *T. laxiflora* (A), *C. hartmannianum* (B) and *A. sayal* (C) fermented woo questionnaires

Plant	Mode of use (%)						Percentage of respondents theyknow *Nikhra*	Frequency % of different Resp									
								Social status		Age			Level of education				J
	D	U	B	F	MR	O	Yes	Single	Married	20-30	31-50	51-80	1	2	3	4	S fr
A	25	10	50	1	12	2	46	27	73	32	58	10	1	19	18	62	
B	20	50	1	1	28	0	58										
C	68	6	22	1	3	0	57										

D=*Dokhan* U=Fuel B=*Bakhour* F= Fooder MR= Mosquto repellent O= Other use

1-Illiterate 2-Primary 3-Secondary 4- University

Table 4.3: Yielded and physical properties of methanolic extracts of fermented wood "*Nikhra*" of *T. laxiflora, C. hartmannianum* and *A. seyal.*

Scientifice name	Physical properties of methanolic extracts			
	Yielded %	Color	Oder	Consistency
A. seyal	2.99	Dark Brown	fragrant	Powder
C. hartmannianum	3.10	Faint Brown	fragrant	Powder
T. laxiflora	3.64	Faint Brown	fragrant	Powder

Table4.4: Yield of methanolic extracts of fermented wood "*Nikhra*" and non fermented of *T. laxiflora, C. hartmannianum* and *A. seyal.*

Fermented and non fermented	**Fermented**	**Non fermented**
Speices		
A. seyal	2.99±0.01	0.64±0.01
C. hartmannianum	3.10±0.01	0.92±0.01
T. laxiflora	3.64±0.01	2.27±0.01
Fermented and non Fermented means	3.24±0.01	1.28±0.01

Table4.5: Yield of fractions of fermented and non fermented wood of "*Nikhra*" of *T. laxiflora, C. hartmannianum* and *A. seyal.*

Solvents	*A. seyal*		*C. hartmannianum*		*T. laxiflora*	
	Means Fermented	Means Non fermented	Means Fermented	Means Non Fermented	Means Fermented	Means Non Fermented
Petroleum ether	0.31±0.01	0.06±0.01	0.36±0.01	0.27±0.01	0.39±0.01	0.20±0.01
Ethylacetate	0.97±0.01	0.36±0.01	0.35±0.01	0.21±0.01	1.68±0.01	1.31±0.01
Chloroform	0.92±0.01	0.05±0.01	0.49±0.01	0.09±0.01	0.16±0.01	0.05±0.01
Aqueous	0.89±0.01	0.17±0.01	1.27±0.01	0.35±0.01	1.71±0.01	0.71±0.01

Table 4.6: Organoleptic survey of fragrance in different fractions of *A. sayal*, *C. hartmannian*
fermented wood "*Nikhra*"

Scientifice name	%Health problem	% of strength of fragrance of fermented wood	% of strength fragrance of frac fermented wood			
			M	**P**	**C**	**E**
***A. seyal* (A)**	9	53	9	26	7	51
***C. hartmannianum* (C)**	89	0	6	28	3	59
***T. laxiflora* (T)**	2	47	9	31	5	49

M=Methanol P= Petroleumether C= Chloroloform E= Ethyl acetate A= Aqueous

4.4: Antimicrobial activities

4.4.1: Antibactrial activities

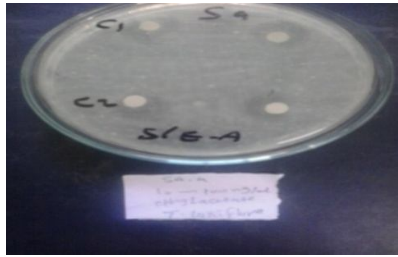
The antimicrobial activity of fractions of three medicinal plant species were assessed(Table 4.7; Fig.4.1), displays that the ethyl acetate fraction of *Combretum hartmannianum, Terminalia laxiflora* and *Acacia seyal* showed high significant($p<0.05$) antimicrobial activity against *S. typhi*, *E.coli* and *S. aureus* with inhibition zone sizes ≥ 15 mm at two concentratons 2.5mg/1ml and 5mg/1ml as follows(18, 15 and 15mm) (17,18 and 15mm) (17, 15 and 15mm) respectively at 2.5mg/1ml, and(18, 16 and 15mm) (17, 18 and 15mm) (17, 18 and 15mm) at 5mg/1ml, (Table 4.7; Fig.4.1). Although *C. hartmannianum* and *T. laxiflora* belonged to the same family but their fractions (aqueous, chloroform, peterolum ether) exhibited different level of activites antibactrial activities. Aqueous phases of *Nikhra* of *A. seyal, C. hartmannianum* and *T. laxiflora* showed no antibacterial activity at a concentratons 2.5mg/1ml and 5mg/1ml. On the other hand, all chloroform and petroleum ether fractions of *Nikhra* of *C. hartmannianum, T. laxiflora* and *A. seyal* showed weak antibacterial activity (Table 4.7; Fig.4.1).

Similar which were observed in the root and the leaf methanolic extracts of *T.glaucescens* with high activity (20, 25 and 32) mm against *(E. coli*, *S. typhi* and *S. aureus*) respectively at 100 mg/ml(Ayepola, 2009). The effect of different *acacia* spp, including methanolic extracts of bark of *A. Senegal,* pod of *Acacia nilotica* and bark of *Acacia catechu* whole plant of *Acacia jacquemontii,* gram positive(*E.coli, S. typhi*) (0,0),(5,10), (0,5), (0,0) mm gram negative(*S. aureus*) (10,0,0,0) mm respectively at 5 mg /disc(Saini *et al.*, 2008). Acetone extracts of *C.molle, C. fragrans* and *C. micranthum* inhibition zone(15, 12, 8) mm respectively against *E. coli*(Eloff *et al.*, 2008).

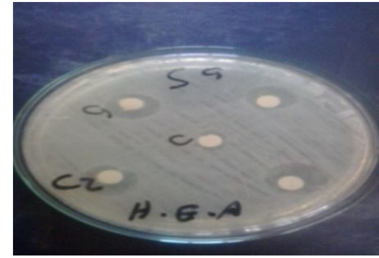
Table 4.7: Inhibition zones of bacteria (mm) of *Nikhr* fractions of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Fractions	fractions concentrations	*S. typhi*	*E.coli*	*S. aureus*
	2.5mg/1ml			
Aqueous *C. hartmannianum*		-	-	-
Ethyl acetate *C. hartmannianum*		18	15	15
Chloroform *C. hartmannianum*		-	-	-
Petroleum ether *C.*		-	-	-
Aqueous *T.laxiflora*		-	-	-
Ethyl acetate *T. laxiflora*		17	18	15
Chloroform *T.laxiflora*		13	-	-
Petroleum ether *T.laxiflora*		10	-	-
Aqueous of *A.seyal*		-	-	-
Ethyl acetate *of A.seyal*		17	15	15
Chloroform *A. seyal*		14	13	13
Petroleum ether *of A. seyal*		-	-	-
Aqueous *C. hartmannianum*	5mg/1ml	-	-	-
Ethyl acetate *C. hartmannianum*		18	16	15
Chloroform *C.hartmannianum*		12	15	13
Petroleum ether *C.*		-	-	-
Aqueous *T. laxiflora*		-	-	-
Ethyl acetate *T.laxiflora*		17	18	15
Chloroform *T. laxiflora*		13	-	-
Petroleum ether *T. laxiflora*		12	-	-
Aqueous *A.seyal*		-	-	-
Ethyl acetate *A. seyal*		17	18	15
Chloroform *A. seyal*		14	13	13
Petroleum ether *A. seyal*		-	-	-

-= no inhibition, *S. aureus = Staphylococcus aureus, E. coli= Escherichia coli, S. typhi = Salmonella Typhi*. Control= organic solvent (petroleum ether and methanol) (1:2), MIZD mm >18mm: Sensitive, MIZD mm 14-18mm: Intermediate, MIZD mm <14mm: Resistant.



T. laxiflora



C. hartmannianum

A. seyal

Fig.4.1: Activity of the ethyl acetate fractions against *S. aureus* at a concentrations C_1=2.5mg/ml, C_2=5mg/ml C=Control (organic solvents)

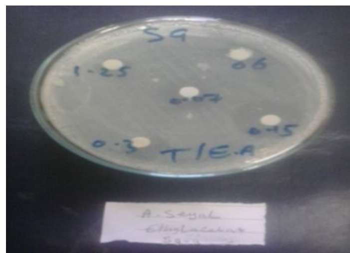
4.4.1.1: Minimum inhibitory concentration of bacteria (MIC)

The MIC of the most active ethyl acetate fraction of *Combretum hartmannianum*, was recorded at five concentrations and found tobe 0.04mg/ml, 0.04 mg/ml and 0.07 mg/ml against *S. aureus*, *S. typhi* and *E.coli,* respectiviley. Similar results were recorded for *C. glutinosum* crude extract against *S. aureus* ATCC 6538(Sore *et al*., 2012) with MIC1.41mg/ml. *Terminalia laxiflora* MIC was recorded at five concentrations and were found tobe 0.04mg/ml, 0.005mg/ml and 1.25mg/ml against *S. aureus*, *S. typhi* and *E. coli,* respectiviley and *A. seyal* of ethyl acetate fraction at five concentrations was recorded and found 0.005mg/ml, 0.04mg/ml and 0.15mg/ml against *S. aureus*, *S. typhi* and *E.coli* respectiviley(Table 4.8; Fig 4.2, 4.3,4.4). Samilar achiving results were recorded for *A. albida, A. senegal* and *A. tortilis* extracts against(*S. typhi* and *E. coli*) were(0.50, 2.0, 0.25) mg/ml and(0, 0, 2.0) mg/ml respectively(Kubmarawa *et al.*, 2007). Acetone extracts of *C.molle, C. fragrans* and *C. micranthum* MIC(0.625, 0.625, 2.50) mg/ml respectively against *E.coli*(Eloff *et al*., 2008).

Table 4.8: (MIC) of bacteria in of ethyl acetate fractions of *"Nikhra"* from *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Ethyl acetate fractions	concentrations	*S. typhi*	*E. coli*	*S. aureus*
C. hartmannianum	1.25	15	16	14
	0.6	15	15	21
	0.3	14	15	22
	0.15	16	12	24
	0.07	15	9	24
	0.04	12	-	13
	0.02	13	-	14
	0.01	13	-	14
	0.005	12	-	13
T. laxiflora	1.25	15	14	14
	0.6	15	15	15
	0.3	19	19	22
	0.15	14	14	25
	0.07	15	15	19
	0.04	15	-	13
	0.02	13	-	14
	0.01	12	-	14
	0.005	11	-	15
A. seyal	1.25	15	17	14
	0.6	17	20	15
	0.3	15	15	14
	0.15	15	13	19
	0.07	13	16	15
	0.04	12	-	13
	0.02	14	-	14
	0.01	13	-	13
	0.005	13	-	12

S. aureus =Staphylococcus aureus *E. coli=Escherichia coli* *S. typhi =Salmonella typh*



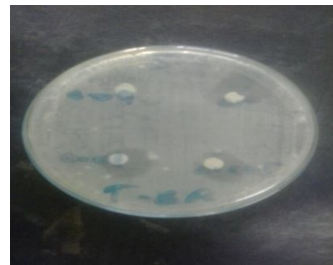


Fig.4.2. ***A. seyal*** **/ethyl acetate/*S. typhi***

1

2

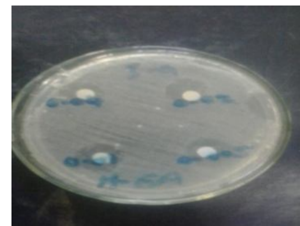
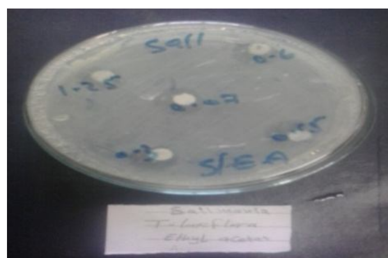


Fig.4.3.*C. hartmannianum* **/ethyl acetate/ 1- *S. aureus***
2- *S. typhi*



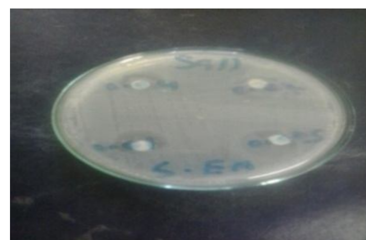


Fig.4.4.*T. laxiflora* **/ethyl acetate / *S. typhi***

All MIC valu of (1.25, 0.6, 0.3, 0.15, 0.07, 0.04, 0.02, 0.01, 0.005)

4.4.2 Antifungal activities

Chloroform fractions of *Nikhra* of *Combretum hartmannianum*, *Terminalia laxiflora* showed segnificant activity against *C. albicans* with inhibition zones 18-19 mm at concentration of 1mg/ml. At concentration of 5mg/ml chloroform fractions of *"Nikhra"* of *C. hartmannianum* and *T. laxiflora* possessed lower activity against *C. albicans* with inhibition zones of 15-16 mm(Table 4.10; Fig 4.4 and 4.5). The Chloroform fraction of *A. seyal* had intermediate activity against *C. albicans* with inhibition zones of 14-15 mm(Table 4.9; Fig.4.5).

Aqueous extracts of *Nikhra* of *C. hartmannianum*, *T. laxiflora* and *A. seyal* have no activity against *C. albicans.* The ethyl acetate fractions of *Nikhra* of *C. hartmannianum*, *T. laxiflora* and *A. seyal* showed weak activity with inhibition zones of 11-14 mm against *C. albicans* at two concentrations 1 and 5 mg/ml used in this study Table(4.9).

In the same manner the petroleum ether fraction of *A. seyal* have no activity against *C. albicans* at two concentrations of 1 and 5 mg/ml , ethyl acetate and chloroform fractions of *Nikhra* of *A. seyal* possessed low activity against *C. albicans* inhibition zones(13-15) mm Table(4.9).

The chloroform fraction of *A. seyal* was, significantly active against *A. flavus* and *A. niger* with inhibition zones 20mm at(100 mg/ml) and at concentration of 10 mg/ml inhibition zones of chloroform fraction of *A. seyal*(16,18) mg/ml for *A. flavus* and *A. niger* respectively(Table 4.10; Fig.4.6). Methanolic extract of *A. catechu* and *A. nilotica* showed very high antimicrobial activity against *C. albicans* and *A. niger* and *Microsporum canis*). This is due to the presence of hydrophilic components such as polyphenols, polysaccharides and tannins present in one or more parts of these plants. The hexane extracts of these species also showed significant activity(Saini *et al.,* 2008).

The chloroform fraction of *T. laxiflora* at a concentration of 100mg/ml was active against *A. flavus* and *A. niger*(18mm). The chloroform fraction of *C. hartmannianum* showed fungical activity against *A. flavus* and *A. niger* with inhibition zones 21 mm and 20 mm respectively, at concentrations of 100 mg/ml. At the concentration of 10 mg/ml the inhibition zones of chloroform fraction of *C. hartmannianum* were 15 and16 mm against *A. flavus* and *A. niger* respectively Table(4.10). The ethyl acetate fraction of *C. hartmannianum*

showed intermediate activity with inhibition zones of 15 mm against *A. flavus* and *A. niger* at concentration of 10 mg/ml, and at concentration of 100 mg/ml the inhibition zones with16 mm against *A. flavus* and *A. niger*. The ethyl acetate fraction of *T. laxiflora* was active against *A. flavus* at two concentrations of 10 and100 mg/ml with inhibition zone of 15 mm but it was not active against *A .niger*(Table 4.10; Fig.4.6, 4.7).

The petroleum ether fraction of *T. laxiflora* showed no significant activity against *A. flavus* and *A. niger* with inhibition zone of 11 mm at 10mg/ml but at 100mg/ml concentration posscssed inhibitory zone of 14mm. The petroleum ether fraction of *C. hartmannianum* had no antifungal activity at the two concentrations 10 and 100 mg/ml Table(4.10).

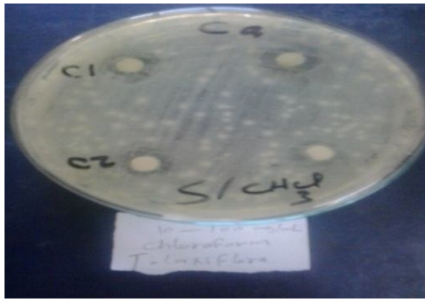
The petroleum ether fraction of *A. seyal* possessed moderate activity against *A. flavus* and *A. niger* with inhibition zones 14 and 16 mm respectively at two concentrations(10,100) mg/ml. The petroleum ether fraction of *A. seyal* showed no activity against *A. flavus* wih inhibition zones 10 and11 mm at two concentrations (10,100) mg/ml respectively Table (4.10).

The aqueous fractions of *Nikhra* of *C. hartmannianum*, *T. laxiflora* and *A. seyal* showed no antifungical activity of both concentrations, Table (4.10). Saini *et al*., (2008) found no effect of different *acacia* spp. including methanolic extracts of bark of *A. Senegal,* pod of *A. nilotica* and bark of *A. catechu* whole plant of *A. jacquemontii* against *A. niger* at 5mg/disc (Saini *et al*., 2008). Similar activities were observed in both the root and the leaf extracts of *T. glaucescens* with a high activity of 30mm against the *C.albicans* at 100 mg/ml(Ayepola, 2009).

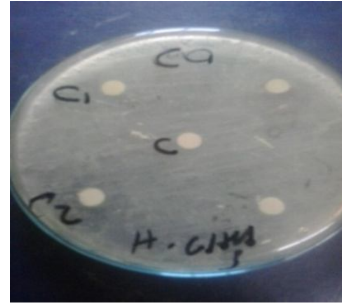
Table 4.9: Inhibition zones of *C. albicans* "*Nikhra*" of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Extracts	Extracts Concentrations	*C. albicans*
	1mg/1ml	
Aqueous *C. hartmannianum*		-
Ethyl acetate *C. hartmannianum*		14
Chloroform *C. hartmannianum*		18
Petroleum ether *C. hartmannianum*		16
Aqueous *T.laxiflora*		-
Ethyl acetate *T.laxiflora*		11
Chloroform *T.laxiflora*		19
Petroleum ether *T.laxiflora*		15
Aqueous of *A.seyal*		-
Ethyl acetate *of A. seyal*		13
Chloroform *A.seyal*		14
Petroleum ether *of A. seyal*		-
Aqueous *C.hartmannianum*	5mg/1ml	-
Ethyl acetate *C.hartmannianum*		12
Chloroform *C.hartmannianum*		16
Petroleum ether *C.hartmannianum*		11
Aqueous *T. laxiflora*		-
Ethyl acetate *T. laxiflora*		12
Chloroform *T. laxiflora*		15
Petroleum ether *T. laxiflora*		13
Aqueous *A. seyal*		-
Ethyl acetate *A. seyal*		14
Chloroform *A.seyal*		15
Petroleum ether *A.seyal*		-

-=no inhibition Control=organic solvent (petroleum ether and (ethyl acetate with methanol (1:2)) MIZD mm :>18mm :Sensitive MIZD mm :14-18mm:Intermediate MIZD mm :<14mm :Resistant



T. laxiflora



C. hartmannianum

A. seyal

Fig.4.5. Antifungal activity of chloroform fractions against *C. albicans*

Table4.10: Inhibition zones of fungi "*Nikhra*" of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Extracts	xtracts concentrations 10 mg/1ml	*A. flavus*	*A. niger*
Aqueous *C.hartmannianum*		-	-
Ethyl acetate *C.hartmannianum*		15	15
Chloroform *C.hartmannianum*		15	16
Petroleum ether		-	-
Aqueous *T. laxiflora*		-	-
Ethyl acetate *T. laxiflora*		15	7
Chloroform *T. laxiflora*		15	13
Petroleum ether *T. laxiflora*		11	11
Aqueous of *A. seyal*		-	-
Ethyl acetate *of A. seyal*		14	16
Chloroform *A. seyal*		16	18
Petroleum ether *of. A seyal*		10	14
Aqueous *C. hartmannianum*	100 mg/1ml	-	-
Ethyl acetate *C. hartmannianum*		16	16
Chloroform *C. hartmannianum*		21	20
Petroleum ether *C.*		-	-
Aqueous *T. laxiflora*		-	-
Ethyl acetate *T. laxiflora*		15	9
Chloroform *T. laxiflora*		18	18
Petroleum ether *T. laxiflora*		14	14
Aqueous *A. seyal*		-	-
Ethyl acetate *A. seyal*		16	21
Chloroform *A. seyal*		20	20
Petroleum ether *A. seyal*		11	16

-=no inhibition Control=organic solvent (petroleum ether and methanol (1:2) MIZD mm >18mm Sensitive MIZD mm :14-18mm Intermediate MIZD mm <14mm Resistant

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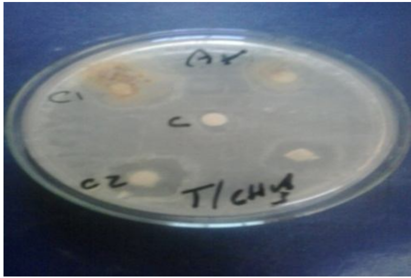

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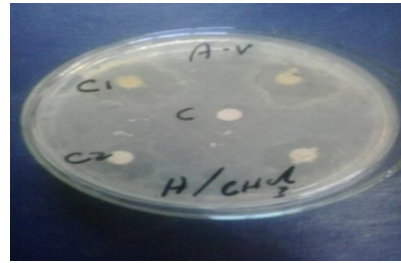


Fig.4.6.1- ***A. seyal*** **/chloroform /** ***A. niger*** **2-** ***C. hartmannianum*** **/chloroform /** ***A.flavus*****C_1=10mg/ml, C_2=100mg/ml C= Control (organic solvents)**

Fig.4.7.1- ***A. seyal*** **/ethyl acetate/** ***A.flavus C. hartmannianum*** **/ethyl acetate /** ***A.flavus*** **(C_1=10mg/ml, C_2=100mg/mlC=Control (organic solvents)**

4.4.2.1: Minimum inhibitory concentration of antifungal activities

The MIC of chloroform fractions of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal* against *A. flavus* and *A. niger* were recorded at different concentrations *C. hartmannianum*(0.3, 0.04 mg/ml) respectively, *T. laxiflora* both *A. flavus* and *A. niger* was 0.6 mg/ml and *A. sayal* both *A. flavus* and *A. niger* 0.3 mg/ml(Table 4.11; Fig 4.8). The MIC of the chloroform fractions of *C. hartmannianum, A. seyal* and *T. laxiflora* against *C. albicans* were recorded at concentrations 0.07 mg/ml, 0.15 mg/ml and 0.15 mg/ml(Table 4.12; Fig 4.9, 4.10), respectively. The MIC of petroleum ether, aqueous and ethanol of *T. laxiflora* fractions were not active againest *A. flavus*(Daniel, 1990)

Table 4.11: Minimum inhibitory concentrations of Antifungal activities by mg/ml of chlorform fractions of fermented wood *"Nikhra"* of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Chloroform fractions	Extracts Concentrations	*A.flavus*	*A.niger*
C. hartmannianum	5	16	15
	2.5	15	17
	1.25	15	16
	0.6	16	15
	0.3	17	14
	0.15	-	14
	0.07	-	14
	0.04	-	14
	0.02	-	15
T. laxiflora	5	14	16
	2.5	14	17
	1.25	15	15
	0.6	15	14
	0.3	14	15
	0.15	14	-
	0.07	15	-
	0.04	13	-
	0.02	14	-
A. seyal	5	15	14
	2.5	15	13
	1.25	14	14
	0.6	15	15
	0.3	15	13
	0.15	13	-
	0.07	14	-
	0.04	13	-
	0.02	14	-

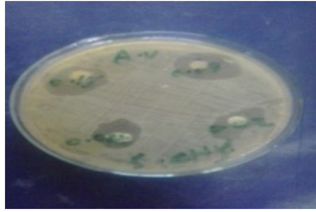
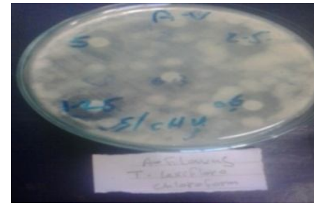
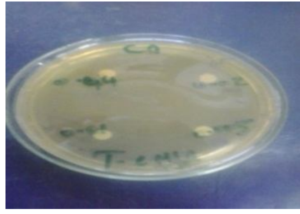



Fig.4.8. ***T. laxiflora*****/chloroform /*****A. flavus*** **MIC (0.5, 2.5, 1.25, 0.6, 0.3, 0.15, 0.07, 0.04, 0.02)**

Table 4.12: Minimum inhibition concentration of *C. albicans* by (mg/ml) in chloroform fractions of fermented wood *"Nikhra"* of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Chloroform fractions	Fractions concentrations (mg/ml)	Measurement of inhibition zones diameter (mm) of (MIZD)
C. hartmannianum	0.6	13
	0.3	12
	0.15	13
	0.07	10
	0.03	12
	0.02	-
	0.01	-
	0.005	-
T. laxiflora	0.6	10
	0.3	9
	0.15	8
	0.07	-
	0.03	-
	0.02	-
	0.01	-
	0.005	-
A. seyal	0.6	14
	0.3	13
	0.15	12
	0.07	13
	0.03	14
	0.02	-
	0.01	-
	0.005	-



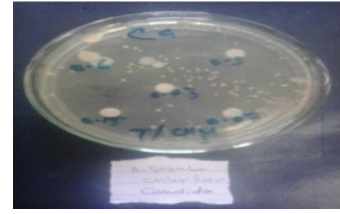


Fig.4.9. ***A. seyal*** **/chloroform /** ***C. albicans*** **MIC (0.6, 0.3, 0.15, 0.07, 0.030.04, 0.02, 0.1, 0.005)**

Fig. 4.10.*T.laxiflora* **/chloroform /** ***C. albicans*** **MIC (0.6, 0.3, 0.15, 0.07, 0.03)**

4.5: Antioxidant activity

When the *Nikhra"* fractions of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal* tested for their antioxidant potential using DPPH assay, the ethylacetate fractions were most active among other fractions (91+0.02> 90+0.01 > 89+0.01%, respectively Table(4.13). This could be attributed mainly to presence of polyphenols e.g flavonoids(Estevinho *et al.*, 2008).

The IC_{50} values of scavenging DPPH radicals for fractions of *A. seya*l *T. laxiflora* and *C. hartmannianum* on DPPH radical were in the following order: ethyl acetate < chloroform < petroleum ether < aqueous, (0.482±0.073, 0.496±0.102, 0.831±0.208 and 0.921±0.073) mg/ml, (ethyl acetate < aqueous < chloroform), (0.347±0.026, 0.463±0.487, 2.771±0.118), (Petroleum ether < Chloroform< Ethyl acetate < Aqueous), (0.366±0.071, 0.413±0.073, 0.460±0.026 and 3.219±0.095) respectively Table(4.13). This study revealed that the ethyl acetate fractions of *A. seya*l, *T. laxiflora* and *C. hartmannianum* have prominent antioxidant activity Table(4.13). These phenolics are responsible for this hight antiradicalactivities. The highest antioxidant activity of the three plants studied was found in the ethyl acetate extracts which showed the highest phenolic content of 404.96-594.60 mg GAE/g, a significant relationship between antioxidant capacity and total phenolic content was found, indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants(Dudonne *et al.*, 2009).

Different parts of *A. seyal* extracts showed high DPPH radical scavenging activity of 66.67% for pod, and 66.27% for leaves(Abdel-Farid *et al.,* 2014). Extracts of *C. hartmannianum* bark demonstrated potent antioxidant effect with IC_{50} range from 0.94–2.24 mg/ml(Hassan *et al.*, 2014). The antioxidant activity of the extracts measured by DPPH free radical showed high reduction of 50% DPPH in *C. hartmannianum* leaves extract followed by Guiera senegalensis roots and Guiera senegalensis leaves(Mariod *et al.*, 2006)

Table 4.13: Antioxidant activity of *Nikhra* fractions of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Plant extracts	RSA±SD (DPPH)	IC_{50} ±SD mg/ml (DPPH)
Petroleum ether *A. seyal*	78±0.01	0.831±0.208
Chloroform *A. seyal*	86±0.03	0.496±0.102
Ethyl acetate *A. seyal*	91±0.02	0.482±0.073
Aqueous *A. seyal*	89±0.03	0.921±0.073
***T. laxiflora* Petroleum ether**	44±0.24	-
***T. laxiflora* Chloroform**	90±0.01	2.771±0.118
***T. laxiflora* Ethyl acetate**	90±0.01	0.347±0.026
Aqueous *T. laxiflora*	89±0.07	0.463±0.487
***C. hartmannianum* Petroleum ether**	84±0.01	0.413±0.073
***C. hartmannianum* Chloroform**	71±0.23	0.366±0.071
***C. hartmannianum* Ethyl acetate**	89±0.01	0.460±0.026
***C. hartmannianum* Aqueous**	52±0.18	3.219±0.095
Propyl gallate (control)	91±0.03	0.0312±0.053

4.6: Brine shrimp toxicity bioassay

In vitro toxicity of *Nikhra* of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal* fractions against the brine shrimp *(Artemia salina*) are presented in Table (4.14). All fractions proved to be non toxic against *A. salina* expect ethyl acetate and chloroform fractions of *A. seyal* and chloroform fractions of *C. hartmannianum* which possessed slight toxicity.

Nguta and Mbaria, (2013) reported that *A. seyal* root methanolic extracts was considered to be non toxic. *In vitro* toxicity of extracts of the roots and stem bark of *T. brownii*(Combretaceae) against the brine shrimp *(Artemia salina*) larvae with LC_{50} values ranging from 113.75– 4356.76 and 36.12–1458.81 µg/ml, respectively. The genus *Terminalia*, which are known to contain cytotoxic compounds such as hydrolysable tannins(Mbwambo *et al.*, 2007).

Table4.14: LD_{50} bioassay of *Nikhra* fractions of *C. hartmannianum*, *A. seyal* and *T. laxiflora* against the brine shrimp (*Artemia salina*).

Extracts	LC50 (mg/ml)	Toxicity
A. seyal petroleum ether	386.3872	No
A. seyal chloroform	43.3315	slight
A. seyal ethyl acetate	27.2092	slight
*A. seya*l aqueous	67.7551	No
T. laxiflora petroleum ether	168.9470	No
T. laxiflora chloroform	242.3399	No
T. laxiflora ethyl acetate	78.6234	No
T. laxiflora aqueous	126.8730	No
C. hartmannianum petroleum ether	115.5829	No
C. hartmannianum chloroform	44.3499	slight
C. hartmannianum ethyl acetate	55.4412	No
C. hartmannianum aqueous	59.5492	No

Control = 7.4625, ≤ 7.4625 toxic ≥7.4625 not toxic, LD_{50}=Lethal Dose

4.7: Total phenolic content of plants studied fractions (TPC)

Phenolic content of fractions of the *Nikhra* of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal* was determined by Folin−Ciocalteu method. The results of this colorimetric method, expressed as mg gallic acid equivalents are shown in Table(4.15). *T. laxiflora* fractions presented the highest phenolic content which were reported as 747.05> 594.60> 506.56> 382.35mg GAE/g for aqueous, ethylacetate, chloroform and petroleum ether fractions, respectively. *C. hartmannianum* fractions presented the second highest phenolic contents which were reported as 473.52>460.39>404.96>363.38 mg GAE/g, chloroform, petroleum ether, ethylacetate and aqueous fractions respectively. The *A. seyal* fractions presented moderate phenolic contents which were reported as 461.85>424.65>410.79>175.93mg GAE/g, chloroform, ethylacetate, aqueous and petroleum ether, fractions respectively, chloroform fraction of *A. seyal* gave the highest phenolic content of 461.85mg GAE/g followed by ethyl acetate 424.65, aqueous 410.79 and petroleum ether175.93 fractions mg GAE/g. Abdel-Farid *et al.*, (2014) reported a high content of phenolics for the methanolic extracts of *A. seyal* pods and leaves. Aqueous phase of *T. laxiflora* gave the highest phenolic content of 747.05 mg GAE/g followed by ethyl acetate 594.60, petroleum ether 382.35 and chloroform 206.56 mg GAE/g respectively, the total phenolic compounds of fractions of the root, stem and leave of *T.glaucescens* 96.50±0.25 mg GAE/g(Aberoumand and Deokule, 2008). Finally chloroform fractions of C. *hartmannianum* gave high phenolic content of 473.52 mg GAE/g followed by petroleum ether 460.39, ethyl acetate 404.96 and aqueous, 363.38 mg GAE/g respectively.

Table4.15: Total phenol content (TPC) of *Nikhra* fractions of *C. hartmannianum*, *A. seyal* and *T. laxiflora*.

Samples Fractions	TPC(mgGAE/g extract)
A. seyal **petroleum ether**	175.93
A. seyal **chloroform**	461.85
A. seyal **ethyl acetate**	424.65
A. seyal **aqueous**	410.79
T. laxiflora **petroleum ether**	382.35
T. laxiflora **chloroform**	506.56
T. laxiflora **ethyl acetate**	594.60
T. laxiflora **aqueous**	747.05
C. hartmannianum **petroleum ether**	460.39
C. hartmannianum **chloroform**	473.52
C. hartmannianum **ethyl acetate**	404.96
C. hartmannianum **aqueous**	363.38

4.8: Phytochemical test

Arrays of phytochemicals were detected in the fractions of *Nikhra* of *Combretum hartmannianum*, *Terminalia laxiflora* and *Acacia sayal*. These phytochemicals include: flavonoids, saponins, cardiac glycosides, steroids, tannins and terpenoids. The qualitative screening of the phytochemical compounds in *Nikhra* of *C. hartmannianum, T. laxiflora* and *A. seyal* revealed the presence of flavonoids, cardiac glycosides, tannin alkaloids, saponins and phenolic compounds Tables(4.16, 17, 18). Triterpenoid / steroid are also present in all sample fractions Tables(4.16, 17, 18), except in chloroform fraction of *T. laxiflora* Table(4.17), and ethyl acetate, chloroform and petroleum ether fractions of *C. hartmannianum* Table(4.16).

Acacia nilotica and *Acacia seyal* were subjected to phytochemical screening, which indicated the presence of tannins, saponins, coumarins and flavonoids. However, negative results were recorded for alkaloids, triterpnoids, steroids and anthraquinones(Jacknoon *et al.*, 2012). Phytochemical studies carried out in the genus *Combretum* have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans and non-protein amino acids(Pietrovski *et al.*, 2006). Phytochemical analysis of the fraction of *Terminallia laxiflora* revealed the presence of carbohydrates, tannins, flavonoids, alkaloids, triterpenes and chromatographic separation of the fraction of *T. laxiflora* leaves resulted in the isolation and identification of β-sitosterol, m-gallate, gallic acid, ellagic acid and five flavonoids, quercetin, vitexin, iso vitexin, quercetin 3-O-α-rhamnoside and rutin(Bag *et al.*, 2012).. These results agreed with our results because phytochemical compounds in *Nikhra* of *C. hartmannianum, T. laxiflora* and *A. seyal* presence in same genus *Combretum* and *Acacia*.

Table 4.16: Phytochemical screening of *Nikhra* frations of *C. hartmannianum*.

Fractions of *C. hartmannianum*	**Aqueous**	**Ethyl acetate**	**Chloroform**	**Petroleum ether**
Test	Result and Observation	Result and Observation	Result and Observation	Result and Observation
Alkaloids (Mayer's Reagent)	+ Creamy	+ Creamy	+ Creamy	+ Creamy
Alkaloids (Dragndorff's Reagent)	+++ Reddish brown	+++ Reddish brown	++ Reddish brown	+ Reddish brown
Triterpenoid/ Steroid (Salkowski-Liberman)	T++ Reddish brown ring in the interface	T- Dark black	T- Dark black	+++T Reddish brown ring in the interface
cardiac glycosides	+ a brown ring	+ a brown ring	+ a brown ring	+ a brown ring
Flavonoids (Harbone and Sofowora Methods) Ammonium test	+++++ Yellow color obtained in the ammonical layer (lower layer)	++++++ Yellow color obtained in the ammonical layer (lower layer)	+++++ Yellow color obtained in the ammonical layer (lower layer)	++ Yellow color obtained in the ammonical layer (lower layer)
Saponin (Froth test)	+++ Stable persistent froth(thick layer)	+ Stable persistent froth(thick layer)	++ Stable persistent froth(thick layer)	+ Stable persistent froth(thick layer)
Saponin test (Froth test)	+++ Formation of emulsion	+ Formation of emulsion	++ Formation of emulsion	- Do not be emulsified
Tannin (Harbone, Braemer's methods)	++++++++ Dark green solution	+++++++ Dark green solution	+++++++ Dark green solution	+++++ Dark green solution

±: Positive or negative result ±T: Positive or negative triterpenoid (presence or absent of the phytochemical).

Table 4.17: Physiochemical screening of *Nikhra* fractions of. *T. laxiflora*.

Fractions of *T.laxiflora*	**Aqueous *T.laxiflora***	**Ethyl acetate *T. laxiflora,***	**Chloroform *T. laxiflora***	**Petroleum ether *T. laxiflora,***
Test	Result and observation	Result and observation	Result and observation	Result and observation
Alkaloids test (Mayer's Reagent)	+ Creamy	+ Creamy	+++ Dense Creamy	+++ Dense Creamy
Alkaloids test (Dragndorff's Reagent)	++ Reddish brown ring in the interface	+++ Reddish brown ring in the interface	+ Reddish brown ring in the interface	+ Reddish brown ring in the interface
Triterpenoid / Steroid (Salkowski-Liberman)	T+ Reddish brown ring in the interface	T++ Reddish brown ring in the interface	T- Dark black	T+++ Reddish brown ring in the interface
cardiac glycosides	+ a brown ring	+ a brown ring	+ a brown ring	+ a brown ring
Flavonoids test (Harbone and Sofowora Methods -Ammonium test)	++ Yellow color obtained in the ammonical layer (lower layer)	+++++ Yellow color obtained in the ammonical layer (lower layer)	+++ Yellow color obtained in the ammonical layer (lower layer)	++ Yellow color obtained in the ammonical layer (lower layer)
Saponin test (Froth test)	++ Stable persistent Froth (layer)	++ Stable persistent Froth (layer)	+ Stable persistent Froth (layer)	+ Stable persistent Froth (layer)
Saponin test-Emulsion test	++ Formation of emulsion	++ Formation of emulsion	- Formation of emulsion	- Do not be emulsified
Tannin test (Harbone, Braemer's methods)	++++++++ Dark green solution	++++++++ Dark green solution	+++++ Dark green solution	++++ Dark green solution

±: Positive or negative result ±T: Positive or negative triterpenoid (presence or absent of the phytochemical).

Table 4.18: Phytochemical screening of *Nikhra* fractions of *A. seyal*.

Fractions of *A.seyal*	Aqueous *A.seyal*	Ethyl acetate *A. seyal*	Chloroform *A.seyal*	Petroleum ether *A. seyal*
Test	Result and observation	Result and observation	Result and observation	Result and observation
Alkaloids test (Mayer'sreagent)	+ Creamy	++ Dense Creamy	+ Creamy	+++ Dense Creamy
Alkaloids test (Dragndorff's Reagent)	Dense Creamy Reddish brown ring in the interface	++ Reddish brown ring in the interface	+ Reddish brownring in the interface	+ Reddish brown ringin the interface
Triterpenoid / Steroid (Salkowski-Liberman)	T++ Reddish brown ring in the interface	T++ Reddish brown ring in the interface	T +++ Reddish brown ring in the interface	T+++ Reddish brown ring in the interface
cardiac glycosides	+ a brown ring	+ a brown ring	+ a brown ring	+ a brown ring
Flavonoids test (Harbone and Sofowora Methods) -Ammonium test)	+++++ Yellow color obtained in the ammonical layer (lower layer)	+++ Yellow color obtained in the ammonical layer (lower layer)	++++ Yellow color obtained in the ammonical layer (lower layer)	++++ Yellow color obtained in the ammonical layer (lower layer)
Saponin test (Froth test)	+ Stable persistent froth(thick	+ Stable persistent froth(thick layer)	+ Stable persistent froth(thick layer)	+ Stable persistent froth(thick layer)
Saponin test-Emulsion test	- Do not be emulsified	- Do not be emulsified	- Do not be emulsified	+ Formation of Emulsion
Tannin test (Harbone, Braemer's methods)	+++++++ Dark green solution	+++++++ Dark green solution	++++++ Dark green solution	+++++ Dark green solution

±: Positive or negative result ±T: Positive or negative triterpenoid (presence or absent of the phytochemical).

4.9: Chromatography analysis of the bioactive fractions of fermented (F) and non fermented wood of *A. seyal, T. laxiflora* and *C. hartmannianum*.

4.9.1 Thin layer chromatography

The presence of flavonoids was confirmed by their color change from quenching fluorescence(254nm) to yellow or orange color for flavonoid and prominent blue color in case of flavonoidal acids or other phenolic acids(366 nm) after spraying with Natural Product Reagent(NPR). Polyphenols were mainly accumulated in the ethyl acetate fractionas has been detected using NPR. The selection of suitable stationary phase and solvents depends on the class of phenols to be examined(Robards and Antolovich, 1997). Best separation was obtained using NP-TLC(Merck). Fluorescence behavior of flavonoids in response to NPR is structure dependent. Flavonoids e.g. quercetin and myrecitin develops orange color and those of kaempferol and isorhamntin yellow to green colors. Flavones glycosides of luteolin develops orange colors and those apigenin yellow to green(Wagner and Bladt, 1996).

Flavonoids, phenolic acids and phenolic were detected in the ethyl acetate fraction of *Nikhra* of *T. laxiflora*(A), *C. hartmannianum*(B) and *A. seyal*(C), using Natural Product Reagent(NPR) Fig. 4.11.

Table 4.19: TLC Profile of the ethyl acetate fraction of *Nikhra* for *T. laxiflora* (A), *C. hartmannianum* (B) and *A. seyal* (C).

Spot No	R_f value	UV at 366nm	Reaction to diagnostic NPR	Expected Metabolite
AI	0.92	Yellow	Blue	Phenolic acid
A2	0.84	Yellow	Pale Yellow	Flavanoid
A3	0.77	Fluorescent	Pale Yellow	Flavanoid
A4	0.62	Pale Yellow	Pale Yellow	Flavanoid
A5	0.55	Brown	Pale Yellow	Flavanoid
A6	0.45	Brown	Pale Yellow	Flavanoid
A7	0.33	Brown	Green	Flavanoid
B1	0.92	Yellow	Green	Flavanoid
B2	0.82	Yellow	Pale Yellow	Flavanoid
B3	0.72	Brown	Pale Yellow	Flavanoid
B4	0.66	Brown	Pale Yellow	Flavanoid
C1	0.92	Yellow	Fluorescent Blue	Phenolic acid
C2	0.83	Fluorescent	Fluorescent Blue	Phenolic acid
C3	0.68	Green	Pale Yellow	Flavanoid
C4	0.62	Green	Pale Yellow	Flavanoid
C5	0.55	Green	Pale Yellow	Flavanoid
C6	0.45	Green	Orange	Flavanoid
C7	0.40	Fluorescent	Orange	Flavanoid
C8	0.30	Yellow	Yellow	Flavanoid

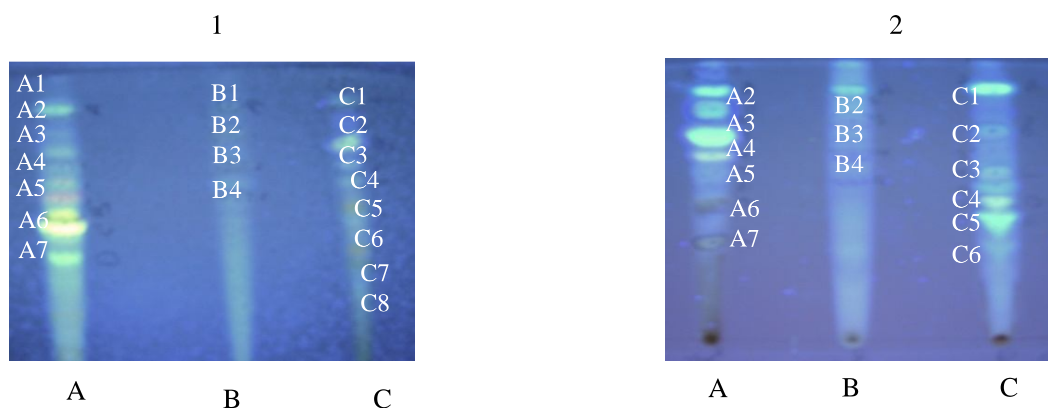


Fig.4.11: NP TLC chromatogram of *T. laxiflora* (A) *C. hartmannianum* (B), and *A. seyal* (C) of the ethyl acetate 1- at UV 366 nm 2- after spraying with NPR at UV 366 nm.

According to the questionnaire results, targeted fragrance of the plants studied was mainly accumulated in the petroleum ether and, ethyl acetate fractions. Tannins were among the metabolites expected to be contribute to these fragrance in the ethyl acetate fractions, removal of tannins using 2% NaCL reduced the fragrance in the ethyl acetate fractions proving them to be responsible for those fragrances. Comparision TLC of the ethyl acetate fractions of *T. laxiflora*(A) *C. hartmannianum*(B), and *A. seyal*(C); ethyl acetate fractions of *T. laxiflora*(a_x) *C. hartmannianum*(b_x), and *A. seyal*(c_x) after treatment with 2% NaCL and aqueous phase of ethyl acetate fractions *T. laxiflora*(a_{xx}), *A. seyal*(c_{xx}) and *C. hartmannianum*(b_{xx}), are presented in(Table 4.20; Fig 4.12), respectively. Compound spots of ethyl acetate fractions of *T. laxiflora*(A), *C. hartmannianum*(B) and *A. seyal*(C); ethyl acetate fractions of *T. laxiflora*(a_x) *C. hartmannianum*(b_x), treatment with 2% NaCL and aqueous phase of ethyl acetate fractions *T. laxiflora*(a_{xx}), *A. seyal*(c_{xx}) and *C. hartmannianum*(b_{xx}) were(A1, A2, A3, A4, A5, A6 ,A7, A9, A10, a1, a2, a3, a4, a5, a6,a7, a8, a9, a10, a11, a22, a33,a 44 and a55), (C1,C2,C3,C4,c1,c3,c4,c11,c33) and(B1,B2, B3, b1,b2,b3 and b11) with Rf values (0.95, 0.86, 0.77, 0. 36, 0.62, 0.55, 0.46, 0.30, 0.28, 0.95, 0.86, 0.77, 0.62, 0.55, 0.46, 0.40,0, 36, 0. 30, 0.28, 0.26, 0.95, 0.86,0.77, 0.62 and 0.55) (0.87,0.750.55,0. 51, 0.87 and 0.55) and(0.95, 0.88, 0.76, 0.95, 0.88, 0.76 and 0.95) respectively(Table 4.20; Fig 4.12), were proved to be polyphenols after spraying with NPR, which contributed to the fragrances of these fractions.

TLC of the ethyl acetate fractions of *T. laxiflora*(A) *C. hartmannianum*(B), and *A. seyal*(C) stock, ethyl acetate fractions of *T. laxiflora*(a_x) *C. hartmannianum*(b_x), and *A. seyal*(c_x) after treatment with 2% NaCl and aqueous part of ethyl acetate fractions *T. laxiflora* (a_{xx}), *A. seyal*(c_{xx}) and *C. hartmannianum*(b_{xx}), showed a positive reaction under UV at 366 nm and these compounds under UV at 366 nm and 254 nm are polyphenolics.

Similarly compounds of fraction of *Nikhra* of *A. seyal*(C), *A. seyal*(c_x) treated by sodium chloride 2% and aqueous phase of ethyl acetate fraction of *A. seyal*(c_{xx}) and *C. hartmannianum*(B), *C. hartmannianum*(b_x) treated by sodium chloride 2% and aqueous phase of *C. hartmannianum*(b_{xx}) showed positive reaction under UV at 366 nm after spraying with NPR these compounds are polyphenols(Table4.20;Fig4.12).

Table 4.20: NP TLC profile of ethyl acetate fraction of fermented wood "*Nikhra*" of *T. laxiflora* ethyl acetate (A) fraction, ethyl acetate treated with NaCl 2%(a_x) and aqueous phase of ethyl acetate of *T. laxiflora* (a_{xx}), *C. hartmannianum*(B), *C. hartmannianum*(b_x) treated by sodium chloride 2% and aqueous phase of *C. hartmannianum*(b_{xx}) and *A. seyal*(C), *A. seyal*(c_x) treated by sodium chloride 2% and aqueous phase of ethyl acetate fraction of *A. seyal*(c_{xx}) at 366nm

Spot No.			R_f vValue 366nm			UV reaction (366nm)		
A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
AI	BI	CI	0.95	0.87	0.95	Fluorescent Blue	Fluorescent Blue	Fluorescent Blue
A2	B2	C2	0.86	0.75	0.88	Fluorescent light Green	Fluorescent Blue	Pale Blue
A3	B3	C3	0.77	0.55	0.76	Fluorescent light Blue	Fluorescent Blue	Pale Blue
A4	-	C4	0.62	0.51	0.95	whitish	-	Fluorescent Blue
A5	-	-	0.55	-	-	Fluorescent light Purple	-	-
A6	-	-	0.46	-	-	Fluorescent light Blue	-	-
A7	-	-	0.40	-	-	Fluorescent light Purple	-	-
A8	-	-	0. 36	-	-	Fluorescent Dark Orange	-	-
A9	-	-	0.30	-	-	Fluorescent light Blue	-	-
A10	-	-	-	-	-	Fluorescent light Yellow	-	-
a1	b1	c1	0.95	-	-	Fluorescent Blue	Fluorescent Blue	Pale Blue
a2	b2	c3	0.86	-	-	Fluorescent light Green	Fluorescent Blue	Pale Blue
a3	b3	c4	0.77	-	-	Fluorescent light Blue	Fluorescent Blue	Pale Blue
a4			0.62	-	-	Fluorescent whitish	-	-
a5	-		0.55	-	-	Fluorescent light Purple	-	-
a6	-	-	0.46	-	-	Fluorescent light Blue	-	-
a7	-	-	0.40	-	-	Fluorescent light Purple	-	-
a8	-	-	0. 36	-	-	Fluorescent Dark Orange	-	-
a9	-	-	0.30	-	-	Fluorescent light Blue	-	-
a10	-	-	0.28	-	-	Fluorescent light Yellow	-	-
a11	b11	c11	0.95	-	-	Fluorescent Dark Blue	Fluorescent Blue	Blue
a22	-	-	0.86	-	-	Fluorescent Dark Green	-	Fluorescent Blue
a33	-	c33	0.77	-	-	Fluorescent Dark Blue	-	-
a44	-	-	0.62	-	-	Fluorescent Dark Purple	-	-
a55	-	-	0.55	-	-	Fluorescent Dark Purple	-	-
a66	-	-	0.46	-	-	Dark Blue	-	-

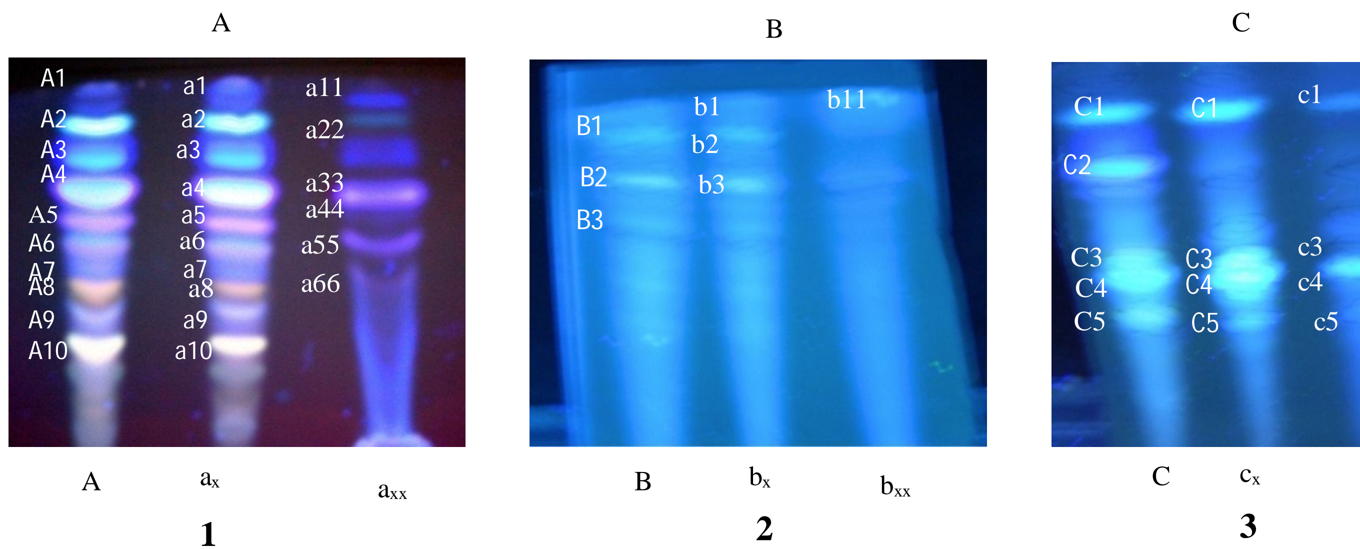


Fig 4.12. NP: TLC. chromatogram of 1- *T. laxiflora* of ethyl acetate(A), ethyl aceta Aqueous phase ethyl acetate(a_{xx}) at 366nm 2- *C. hartmannianum* ethyl acetate(B), e %(b_x) and aqueous phase of ethyl acetate(b_{xx}), 3- *A. seyal* ethyl acetate(C), ethyl ac and Aqueous of ethyl acetate (c_{xx}), at 366nm .A= *T.* B= *C. hartmannianum*, T. *laxiflo*

Vanillin H_2SO_4 is a universal reagent that detects components of essential oils, terpenoids, phenols etc., typical pink to purple colors were developed upon spraying with vanillin H_2SO_4 (heat 110C). All phenolic at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm (Wagner and Bladt, 1996). After spraying the ethyl acetate fractions and petroleum ether of fermented (F) and non fermented wood of *A. seyal, T. laxiflora* and *C. hartmannianum* by vanillin H_2SO_4, they showed typical red and Purple zones of phenolic (Tables 4.21, 4.22; Fig 4.13, 4.14). Accordingly compounds spots (C1, C2, C3, C4, C5, C6, C7), (c1, c2, c3, c4, c5, c6, c7) and (B1, B2), (C3,C6, C7) R f values (0.92, 0.86, 0.71, 0.64, 0.57, 0.50, 0.36) (0.92, 0.86, 0.71, 0.64,0.57, 0.50, 0.36) and (0.84,0.81), (0.70, 0.20, 0.16) respectively were expected to be phenolic.

Vanillin HCL is specific reagent that detects components of catechin. All catechin at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm (Wagner and Bladt, 1996). After spraying the ethyl acetate and petroleum ether fractions of fermented (F) and non fermented wood of *A. seyal, T.laxiflora* and *C. hartmannianum* by vanillin HCL, they showed typical red to Red zones of catechin (Table 4.21, 4.22; Fig 4.14, 4.15), accordingly compounds spots (C5, C6, C7), (c5, c6, c7) and (B2) with R f values (0.57, 0.50, 0.37), (0.57, 0.50, 0.37) and (0.81) respectively were expected to be catechin. Lignans are formed by oxidative coupling of p-hydroxyphenylpropeue units, often linked by an oxygen bridge. They are found in fruits, foliage, heartwood and roots. All lignans at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm(Wagner and Bladt, 1996). After spraying the petroleum ether and ethyl acetate fractions of fermented. (F) and non fermented wood of *A. seyal, T. laxiflora* and *C. hartmannianum* by vanillin H_3PO_4, they showed typical red to blue-violet and brown zones of lignans (Tables 4.21, 4.22; Fig 4.13, 4.14), accordingly compounds spots (C6, C7), (c6, c7) and (A1, A2, A3), (B1, B2, B3, B4), (C3, C6, C7) with R f values (0.50, 0.36), (0.50, 0.36) and (0.88, 0.78, 0.67), (0.84, 0.81, 0.67, 0.59), (0.70, 0.20, 0.16) respectively were expected to be lignans.

Table 4.21: TLC profile of the ethyl acetate fractions of fermented (F) and non fermented wood of *A. seyal, T. laxiflora* and *C. hartmannianum* sprayed NPR, Vanillin H_2SO_4, Vanillin HCL, and Vanillin H_3PO.

Spot	R_f value		UV Reaction		Reaction to diagnostic reagents				Expected metabolite			
No	254	366 nm	254 nm	366 nm	NPR366	Van	an HCL	Van H_3PO_4	NPR366 nm	Van	an HCL	Van H_3PO_4
A1	0.71	-	Quenching	-	Blue	-	-	-	Phenolic acid	-	-	-
A2	0. 68	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
A3	0.37	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
a3	0.37	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
B1	0.64	0.64	Quenching	Blue	-		-	-	Phenolic	-	-	-
B2	0.52	0.50	Quenching	Yellow	-	-	-	-	Phenolic	-	-	-
C1	0.92	-	Quenching	-	-	Purple	Yellow	-	-	Terpernoid	Catechin	-
C2	0.86	-	Quenching	-	Blue	-	-	-	Phenolic acid	-	-	-
C3	0.71	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
C4	0.64	0.64	Quenching	Blue	Yellow	-	-	-	Flavanoid	-	-	-
C5	0.57	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
C6	0.50	-	Quenching	-	Orange	-	-	Red	Flavanoid	-	-	Lignan
C7	0.36	0.36	Quenching	Yellow	Yellow	Purple	Red	Red	Flavanoid	Terpernoid	Catechin	Lignan
c1	0.92	0.64	Quenching	Blue	-	Purple	Yellow	-	-	Terpernoid	Catechin	--
c2	0.86	-	Quenching	-	Yellow	Red	Red	-	Flavanoid	Terpernoid	Catechin	-
c3	0.71	-	Quenching	-	Yellow	-	-	-	Flavanoid	-	-	-
c4	0.64	-	Quenching	-	-	Red	Yellow	-	-	Terpernoid	Catechin	-
c5	0.57	-	Quenching	-	-	Purple	-	-	-	Terpernoid	-	-
c6	0.50	-	Quenching	-	-	Purple	-	Red	-	Terpernoid	-	Lignan
c7	0.36	-	Quenching	-	-	Purple	-	Red	-	Terpernoid	-	Lignan

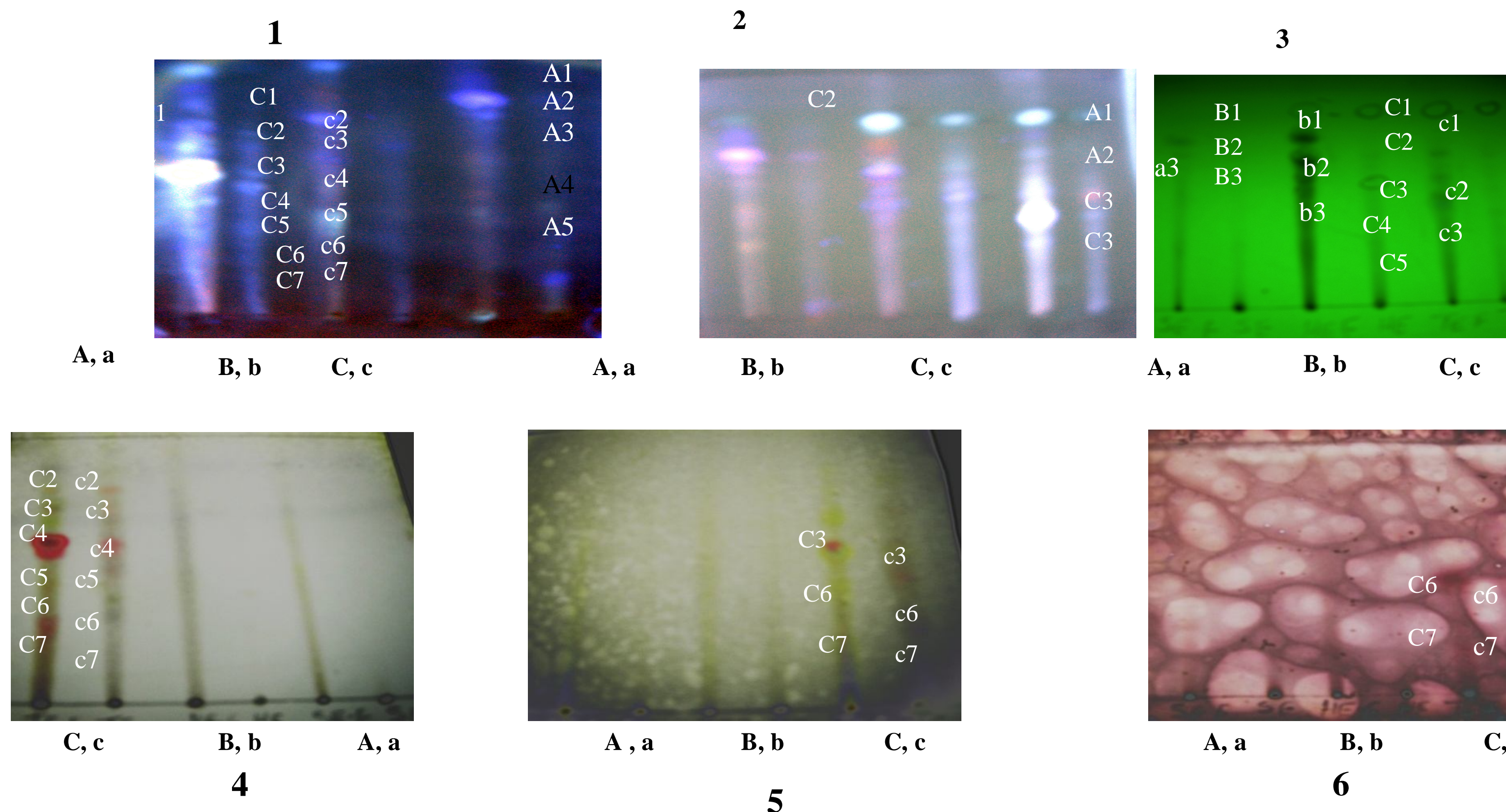


Fig.4.13: NP TLC of the ethyl acetate fractions of fermented and non fermented wood "*Nikhra*" of *hartmannianum* (B,b), and *A. seyal* (C,c), at UV 1-254nm 2-366nm spraying with 3- NPR 4- Vanilli HCL 6- Vanillin H_3PO_4 A= *T. laxiflora*,B= *C. hartmannianum*, C= *A. seyal.*

Table 4.22: TLC profile petroleum ether fractions of fermented (F) and non fermented wood of
and *C. hartmannianum* spray Vanillin H_2SO_4, Vanillin HCL, and Vanillin H_3PO_4

Spot No	Rf vValue		UV Reaction		Reaction to diagnostic reagents				Ex	
	254	366	254 nm	366 nm	NPR366	Van	Van	Van	NPR366 nm	Va
A1	0.88	-	Quenching	Yellow	Yellow	-	-	Red	-	
A2	0.78	-	Quenching	Blue	Blue	-	-	Red	Phenolic	
A3	0.67	-	Quenching	Blue	Blue	-	-	Red	Phenolic	
A4	0.13	-	Quenching	Blue	Blue	-	-	-	Phenolic	
B1	0.84	0.64	Quenching	Yellow	Yellow	Yellow	-	Red	Phenolic acid	
B2	0.81	0.55	Quenching	Yellow	-	purple	Red	Red	-	T
B3	0.67	0.45	Quenching	Blue	Blue	-	-	Red	Phenolic acid	
B4	0.59	-	Quenching	Blue	Blue	-	-	Red	Flavanoid	
B5	0.38	0.23	Quenching	Yellow	Yellow	-	-	-	Flavanoid	
B6	0.30	0.21	Quenching	Yellow	Yellow	-	-	-	Flavanoid	
B7	0.24	0.16	Quenching	Blue	Yellow	-	-	-	Flavanoid	
B8	0.18	0.09	Quenching	Blue	Yellow	-	-	-	-	
CI	0.84	0.84	Quenching	Blue	-	-	-	-	Phenolic	
C2	0.81	0.81	Quenching	Blue	-	-	-	-	Phenolic	
C3	0.70	-	Quenching	Blue	Blue	Yellow	-	Red	Phenolic acid	T
C4	0.59	-	Quenching	Blue	-	-	-	-	-	
C5	0.50	0.50	Quenching	Blue	-	-	-	-	-	
C6	0.20	-	Quenching	Blue	Yellow	purple	-	Blue	Phenolic acid	T
C7	0.61	-	Quenching	Blue	Yellow	Yellow	-	Red	Phenolic acid	
c3	0.70	0.64	Quenching	Blue	-	Yellow	-	-	Phenolic acid	
c6	0.20	0.55	Quenching	Yellow	-	-	-	-	Flavanoid	
c7	0.16	0.16	Quenching	Blue	-	-	-	-	Phenolic acid	

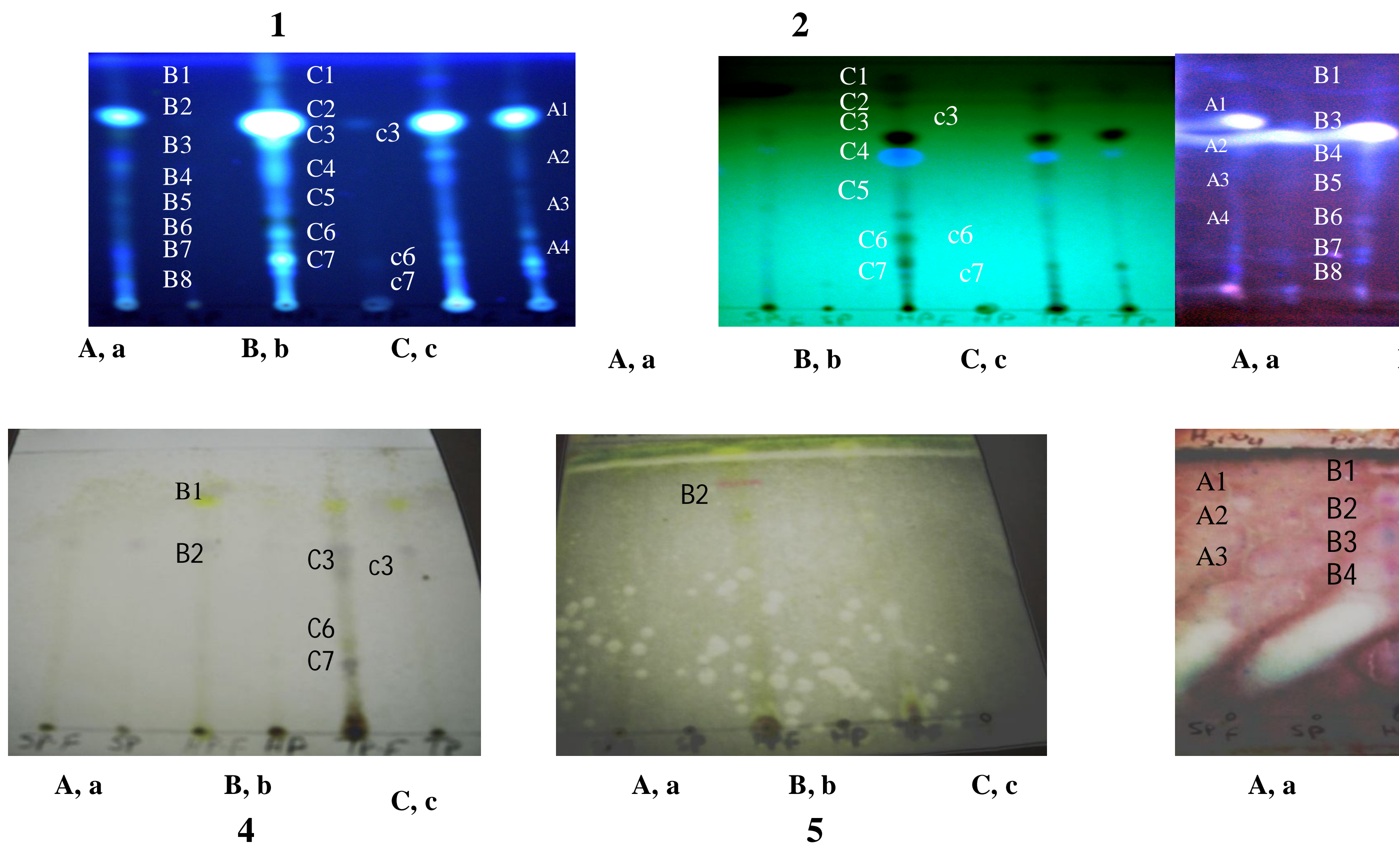


Fig.4.14: NP TLC petroleum ether extract of fermented and non fermented of *T. laxiflora* (A, a), *C. hartmannian* (C, c) respectively at UV 1-254nm 2-366nm spraying with 3- NPR 4- Vanillin H_2SO_4 5- Vanillin HCL 6- V

4.9.2: Gas chromatography mass spectrometry (GC/MS) of petroleum ether fractions.

The chemical compsititon of *Nikhra* petroleum ether fractions of *T. laxiflora, A. seyal* and *C. hartmannianum* were analyzed by GC/MS. The compounds identified by matching their fragmentation patterns in mass spectra with those stores in NIST library with the help of HPCHEM software published mass spectra. The details are summarized in Tables(4.23, 4.24).

Petroleum ether fractions were divided into two types of compounds classes aromatic and non aromatic and hence compounds were classified to phenolics and terpenoids compounds by GC/MS. Fragrant aromatics or terpenoids were targeted in this part of study by http://research.easybib.com.

In all petroleum ether fractions Area% represent the concentrations of corresponding compound, main fragrance aromatic phenolics and terponoids compounds in the petroleum ether fractions of *T. laxiflora, A. seyal* and *C. hartmannianum* "*Nikhra*" are presented in Tables(4.23, 4.24) respectively.

Main fragrance aromatic compounds (phenolics) in the petroleum ether fractions of *T. laxiflora,* was Lup-20(29)-en-3-ol, acetate, (3β) which represntding 15.71% and Tetracosamethyl-cyclododecasiloxane repeated in different concentrations the hightest one was(3.02%) total compounds(34.56%) (Table 4.23; Fig 4.29, 4.33) and main terponoids compounds was Eicosamethylcyclodecasiloxane(2.69%) total fragrance aromatic compounds(10.08%) (Table 4.24; Fig. 4.48).

Main fragrance a romatics compounds (Phenolics) in the petroleum ether fractions of *A.seyal* was Petadecanoic acid(5.64%) Fig(4.12) and Tetracosamethyl-cyclododecasiloxane(4.17%) total fragrance aromatic compounds(44.57%) (Table 4.23; Fig 4.37), and main terponoids compounds was Octadecanoic acid(2.52) % total fragrance aromatic compounds(11.87%) (Table 4.24; Fig 4.35).

Main fragrance aromatics compounds in the petroleum ether fractions of *C. hartmannianum* was 2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran(7.73%) total fragrance aromatic compounds(11.85%), (Table4.23; Fig 4.7), and main terponoids compounds was Tetracosamethylcyclododecasiloxane(2.36%) total fragrance aromatic compounds(7.54%) (Table 4.24; Fig 4.26).

GC/MS analysis of *T. laxiflora* and *A. seyal* were showed them to have the same aromatic fragrance which is Tetracosamethyl-cyclododecasiloxane. Terpenoids GC/MS profil were different in three plants to studies.

We observed that petroleum ether fractions of *A.seyal* and *T. laxiflora* have many fragrance aromatics compounds in high concentrations opposite of the petroleum ether fractions of *C. hartmannianum* have only two fragrance aromatics compounds in low concentrations this same which questionnaires proved.

Table4.23: Chemical composition of fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *T. laxiflora*, *A.seyal* and *C. hartmannianum* fermented wood "*Nikhra*"

Science name	Peak	t_R(min)	Area	Mol Weight	Structure assigned (MS data comparison NIST27)
T. laxiflora	1	15.217	0.32	138	1,2,3-Trichloro-4-nitrobenzene
	2	15.442	0.19	117	7-amino-4-chloro-3-methoxyisocoumarin
	3	15.558	0.81	185	1,2-dimethoxy-3,4,5-trichlorobenzene
	6	17.767	0.50	209	Benzene,1,2,4-tetrachloro-3,6-dimethoxy-5-nitro-
	7	18.125	2.04	218	1-bromo-3,6-di(tbutyl)naphthalene
	8	18.692	0.23	129	Benzenamine,2,3,4,5-tetrachloro-4-methoxy-(CAS)
	13	21.908	0.26	110	Tetracosamethylcyclododecasiloxane
	17	24.100	0.29	117	Cyclononasiloxane,octadecamethyl-
	18	25.167	0.32	130	7-Phenyl-4trans-heptenone-
	20	17.767	0.42	209	Eicosaethylcyclodecasiloxane
	21	28.358	0.45	73.05	Cyclodecasiloxane,eicosamethyl-
	23	29.700	1.94	248	Stigmast-5-en-3-olm(3.beta)-(cas)24.Beta-ethyl-5.Delta-
	25	30.392	0.51	137	Cyclononasiloxane,octadecasiloxayclononamethyl-
	26	31.192	2.97	170	Tetracosamethyl-cyclododecasiloxane
	27	31.192	2.25	171	Tetracosamethyl-cyclododecasiloxane
	28	32.325	0.69	206	Cyclononasiloxane,eicosamethyl-
	29	33.467	15.71	268	Lup-20(29)-en-3-ol,acetate,(3.beta)-
	30	34.467	0.63	162	Cyclononasiloxane,eicosamethyl-
	32	37.108	0.45	134	Tetracosamethylcyclododecasiloxane
	33	40.483	3.02	131	Tetracosamethyl-cyclododecasiloxane
	34	40.483	0.68	131	Tetracosamethyl-cyclododecasiloxane
	Total		34.56		
A.seyal	1	13.024	0.73	130	Cyclononasiloxane,tetradecamethyl-
	2	13.225	0.37	78	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-
	3	13.550	0.3	118	Phenol,3,5-bis(1,1-dimethyl)-
	5	14.725	0.39	58	Hexadecane

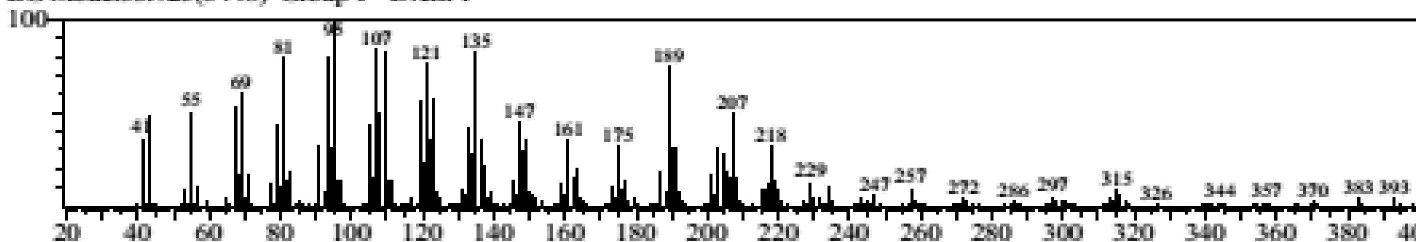
	7		2.700	192	Benzene,1,2,4,5-tetrachloro-3,6-di
	9	17.625	1.19	189	Octadecamethylcyclononasiloxane
	11	19.724	0.87	165	Eicosamethylcyclodecasiloxane
	12	19.858	5.64	178	Petadecanoic acid
	15	21.909	0.99	186	Tetracosamethylcyclododecasiloxa
	19	23.134	1.91		Hexadecanamide
	21	24.097	1.77	247	Cyclononasiloxane,octadecamethy
	22	24.953	0.57	156	Gingerol
	25	26.260	2.83	251	1H-Purin-6-amine,[(2-fluoropheny
	26	28.367	2.45	213	1H-Purin-6-amine,[(2-fluoropheny
	28	30.398	3.11	203	Cyclononasiloxane,octadecamethy
	29	30.811	2.47	235	E,E,Z-1,3,12-Nonadecariene-5,14-
	32	32.334	3.39	199	Cyclodecasiloxane,eicosamethyl-
	33	32.626	0.87	256	Squalene
	34	34.475	3.85	188	Cyclodecasiloxane,eicosamethyl-
	35	37.11	4.00	190	Tetracosamethyl-cyclododecasilox
	37	40.498	4.17	234	Tetracosamethyl-cyclododecasilox
	Total		44.57		
C. hartmannianum	4	22.717	2.22	123	Octadecanoic acid
	6	26.724	1.90	87	Octadecanal
	7	27.758	7.73	204	2-tert-Butyl-5-(hydroxtmethyl)-4-f
	Total		11.85		

Table4.24: Chemical composition of fragrant aromatic compounds (Terpenoids) in the petroleum *T. laxiflora*, *A. seyal* and *C. hartmannianum* fermented wood "*Nikhra*"

Science name	Peak #	t_R(min)	Area%	Mol Weight (*m/z*)	Structure assigned (MS d NIST27)
T. laxiflora					
	7	7.942	0.30	36	o-Xylene
	9	9.545	1.45	71	Alpha-pinene,(-)
	10	10.162	0.19	44	Camphene
	11	11.259	0.29	47	Beta.-Phellandrene
	14	13.636	0.1	42	dl-Limonene
	16	19.335	0.14	39	Benzenepropanal
	20	29.142	0.11	63	Calarne
	22	34.992	0.16	113	1,2,3-Trichloro-4-nitrobenzene
	23	35.437	0.19	92	Deuterio-.alpha.-2,4,5-Trichlo
	24	35.764	0.56	139	1,2-dimethoxy-3,4,5-trichlorob
	26	39.239	0.26	111	5,7-DECADIEN-3-IN, 2,9-Dil
	28	39.859	1.26	222	1-bromo-3,6-di(t-butyl)naphth
	35	42.639	0.19	259	Hexadecamethylcyclooctasilox
	38	43.429	1.21	247	Octadecanoic acid
	39	43.684	0.98	218	Octadecanamide
	48	47.625	2.69	271	Eicosamethylcyclodecasiloxan
	Total		10.08		

A. seyal	6	7.942	0.39	40	o-Xylene
	8	9.550	2.04	69	Alpha-pinene,(-)
	9	10.167	0.24	44	Camphene
	10	11.258	0.39	47	beta.-Phellandren
	13	13.625	0.23	43	dl-Limonene
	14	13.733	0.10	47	1,8-Cineole
	21	31.108	0.10	49	Zingiberene
	23	34.183	0.30	55	Heptadecane
	24	35.933	0.25	74	methyl2-(4-methoxy-phenoxy)
	27	39.525	1.02	216	Octadecamethylcyclononasilox
	35	43.442	2.52	242	Octadecanoic acid
	36	43.692	1.82	213	Octadecanamide
	43	46.000	0.58	288	Eicosamethylcyclodecasiloxan
	44	46.217	0.47	184	Octadecanamide
	47	48.283	1.42	155	Tetracosamethyl-cyclododecas
	Total		11.87		
C. hartmannianum					
	6	7.942	0.43	38	o-Xylene
	8	9.550	2.31	70	Alpha-pinene,(-)
	9	10.167	0.29	44	Camphene
	10	11.258	0.45	46	Beta.-Phellandrene
	13	13.633	0.26	42	dl-Limonene
	20	40.383	1.44	188	Tetracosamethylcyclododecasi
	26	43.425	2.36	238	Octadecanoic acid
	Total		7.54		

<< Target >>
Line#:29 R.Time:33.467(Scan#:3417) MassPeaks:268
RawMode:Single 33.467(3417) BasePeak:95.10(223450)
BG Mode:33.725(3448) Group 1 - Event 1



Hit#:1 Entry:138955 Library:NIST147.LIB
SI:89 Formula:C32H52O2 CAS:1617-68-1 MolWeight:468 RetIndex:0
CompName:Lup-20(29)-en-3-ol, acetate, (3.beta.)- $$ Lup-20(29)-en-3.beta.-ol, acetate $$ Lupenyl acetate $$ Lupeol acetate $$ Lup

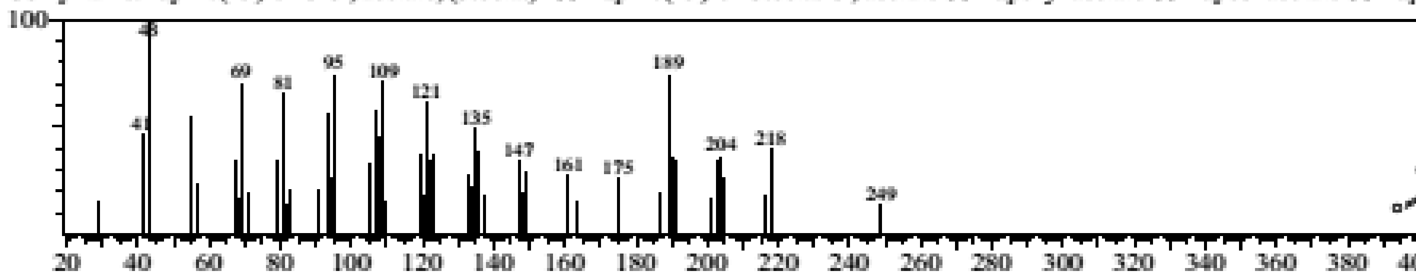
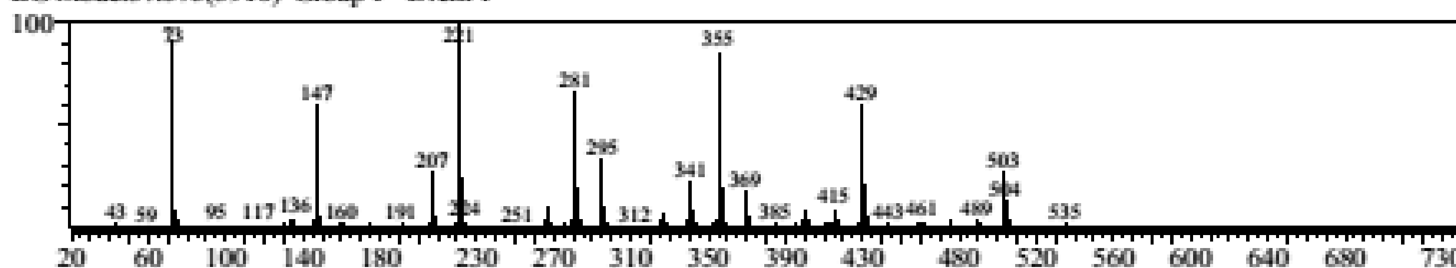


Fig.4.29. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *T. laxiflora*

(Lup-20(29)-en-3-ol, acetate,(3.beta-)

<<< Target >>
Line#:33 R.Time:37.317(Scan#:3879) MassPeaks:244
RawMode:Single 37.317(3879) BasePeak:221.00(240455)
BG Mode:37.575(3910) Group 1 - Event 1



Hit#:1 Entry:147059 Library:NIST147.LIB
SI:84 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosar

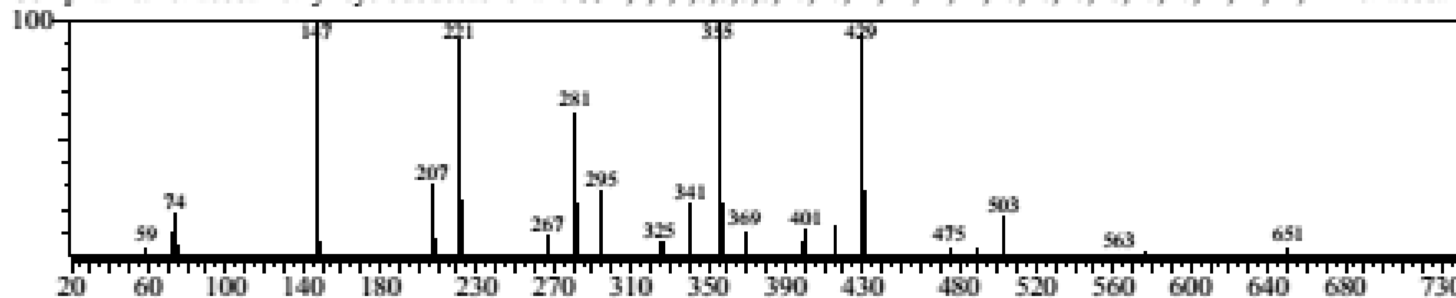
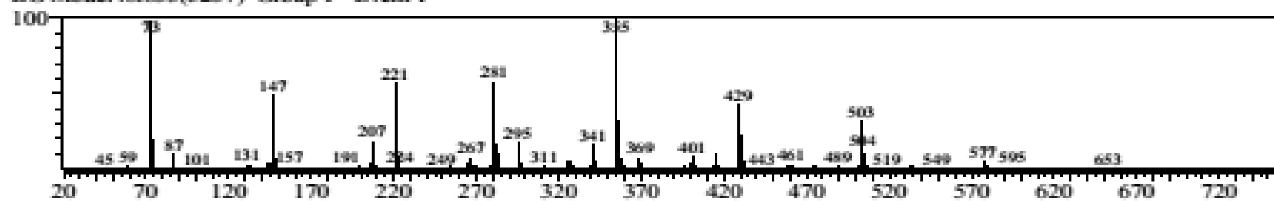


Fig.4.33. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *T. laxiflora Nikhra* (T cyclododecasiloxane).

<< Target >>
Line#:48 R.Time:48.517(Scan#:5223) MassPeaks:271
RawMode:Single 48.517(5223) BasePeak:355.00(320996)
BG Mode:48.633(5237) Group 1 - Event 1



Hit#:1 Entry:335376 Library:WILEY7.LIB
SI:83 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$

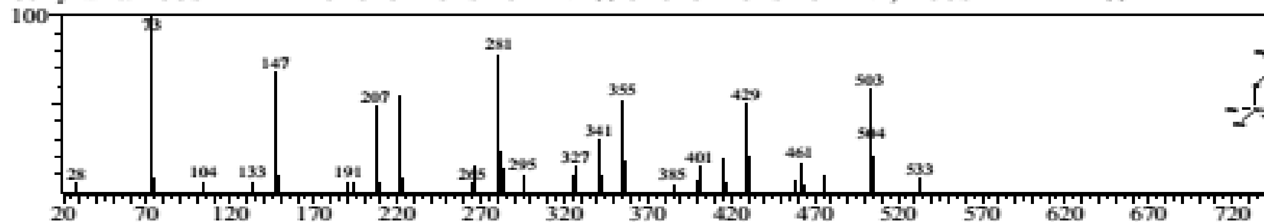
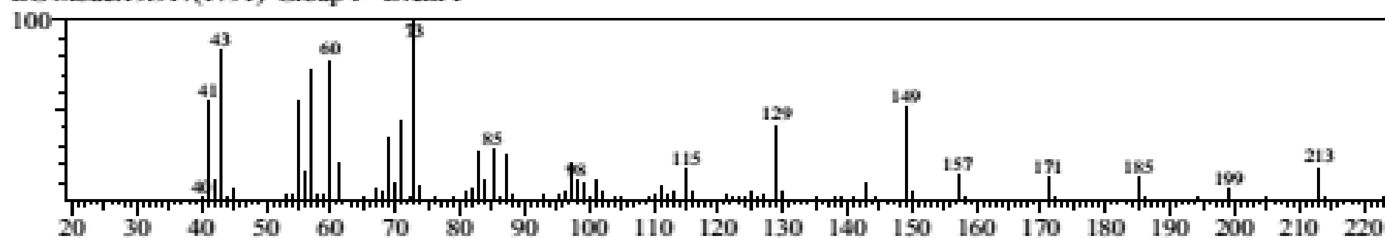


Fig.4.48. Fragrant aromatic compounds (terpenoids) in the petroleum ether fractions

***Nikhra* (Eicosamethylcyclodecasiloxane).**

<< Target >>
Line#:12 R.Time:19.858(Scan#:1784) MassPeaks:178
RawMode:Single 19.858(1784) BasePeak:73.00(925714)
BG Mode:19.917(1791) Group 1 - Event 1



Hit#:1 Entry:20335 Library:NIST27.LIB
SI:91 Formula:C15H30O2 CAS:1002-84-2 MolWeight:242 RetIndex:0
CompName:Pentadecanoic acid

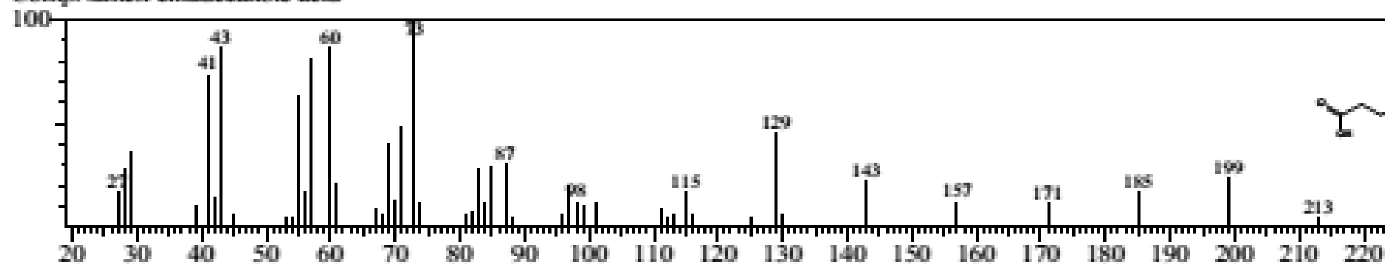
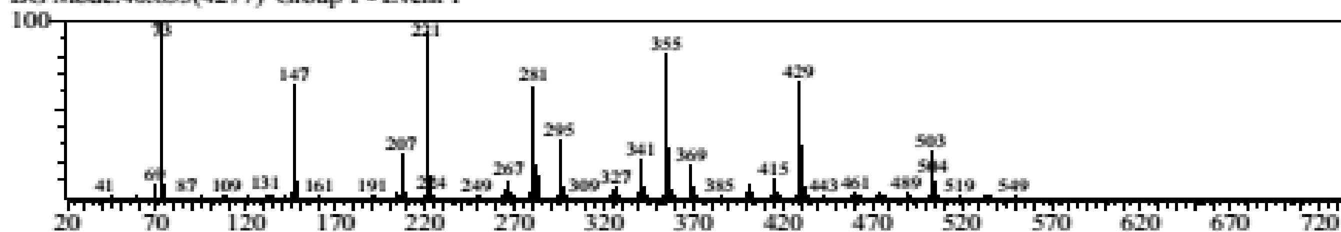


Fig.4.12. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *A.seyal Nikhra* (Pet

<< Target >>
Line#:37 R.Time:40.492(Scan#:4260) MassPeaks:234
RawMode:Single 40.492(4260) BasePeak:73.00(468891)
BG Mode:40.633(4277) Group 1 - Event 1



Hit#:1 Entry:147059 Library:NIST147.LIB
SI:85 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracos

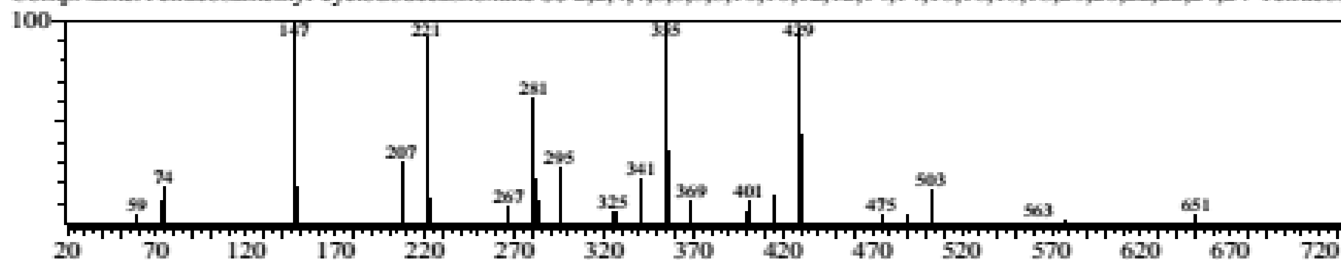
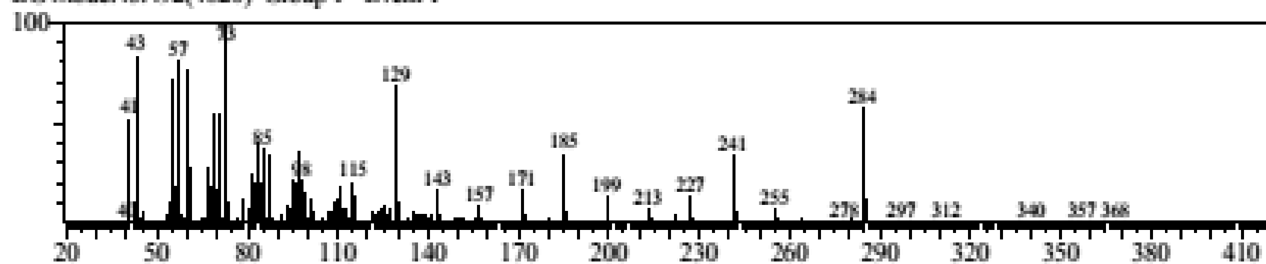


Fig.4.37. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *A.sey*

***Nikhra* (Tetracosamethyl-cyclododecasiloxane).**

<< Target >>
Line#:35 R.Time:43.442(Scan#:4614) MassPeaks:242
RawMode:Single 43.442(4614) BasePeak:73.05(457506)
BG Mode:43.492(4620) Group 1 - Event 1



Hit#:1 Entry:22966 Library:NIST27.LIB
SI:89 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid

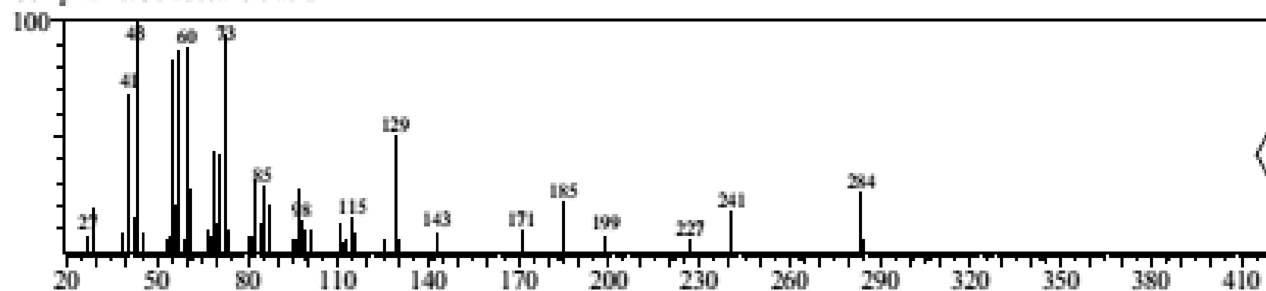
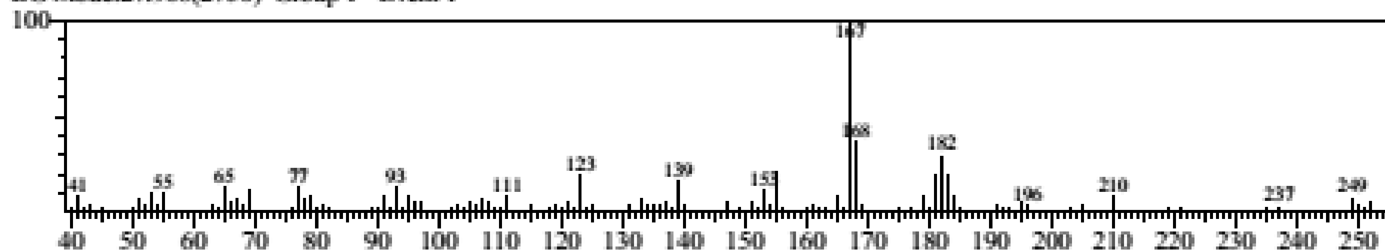


Fig.4.35. Fragrant aromatic compounds (terpenoids) in the petroleum ether fractions of *A.sey*

***Nikhra* (Octadecanoic acid).**

<< Target >>
Line#:7 R.Time:27.758(Scan#:2732) MassPeaks:204
RawMode:Single 27.758(2732) BasePeak:167.10(132405)
BG Mode:27.908(2750) Group 1 - Event 1



Hit#:1 Entry:73402 Library:WILEY7.LIB
SI:61 Formula:C10 H14 O3 CAS:0-00-0 MolWeight:182 RetIndex:0
CompName:2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran $$

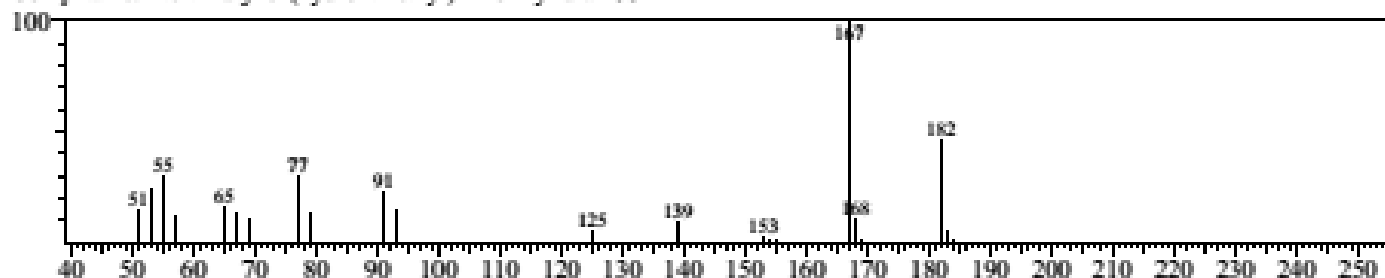
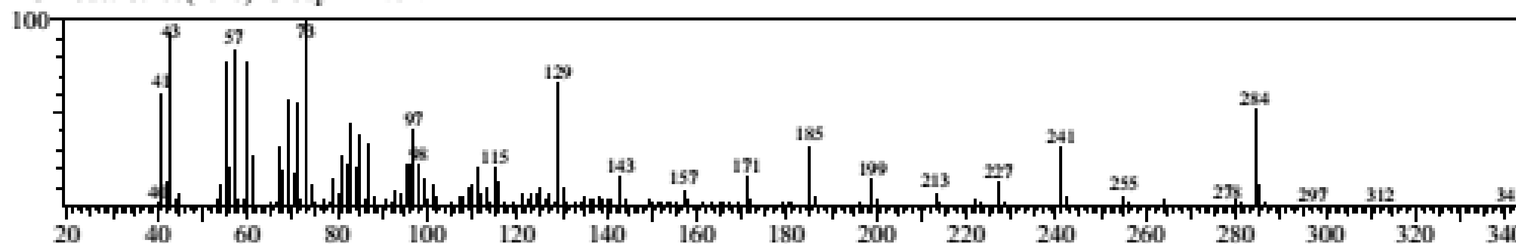


Fig.4.7. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *C. hartmann*

***Nikhra* (2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran).**

<< Target >>
Line#:26 R.Time:43.425(Scan#:4612) MassPeaks:238
RawMode:Single 43.425(4612) BasePeak:73.05(390489)
BG Mode:43.467(4617) Group 1 - Event 1



Hit#:1 Entry:22966 Library:NIST27.LIB
SI:89 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid

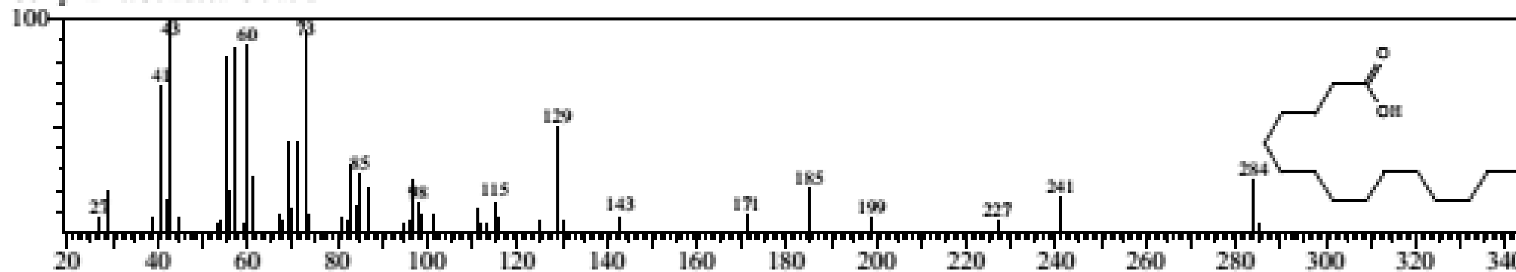


Fig.4.26. Fragrant aromatic compounds (terpenoids) in the petroleum ether fractions of *C. hartmar* *Nikhra* (Octadeca noic acid)

CONCOLUSIONS AND RECOMMENDATIONS

CONCOLUSIONS

This study concludes the following:

- Ethnobotanical study conducted on Khartoum, Khartoum North and Omdurman localities Khartoum State - Sudan recorded the common names used for these plants as *Talh* and *Makntosh* for *A . seyal, Habeel* for *C. hartmannianum,* while *T. laxiflora* is known as *Sobage , Darot* and *Kolit*.
- The common names for fermented wood of *A. seyal, C. hartmannianum* and *T. laxiflora* used is *Nikhra, Nukhara* and. 57% of the questioned women in Khartoum states knew about *Nikhra* of *A. seyal*, *C. hartmannianum* and *T. laxiflora*.
- The ethnobotanical use of fermented wood of *A. seyal*, and *T. laxiflora Nikhra* as cosmetic in (*Dokhan*, *Bakhour*) were 90% for *A. seyal* and 75% for *T. laxiflora* while that of *C. hartmannianum* was (21%). *C. hartmannianum* is mainly used for health problems (89%).
- Organoliptic survey of fragrance in different fractions of the plants studied was ensured stable and strong fragrances; these fragrances were mainly accumulated in the petroleum ether and the ethyl acetate fractions.
- Bactericidal activities (MIC) of the ethyl acetate fractions of *C. hartmannianum T. laxiflora* and *A. seyal* were (0.005-1.25) mg/ml against *S. aureus,* S. *typhi* and *E.coli.* Fungicidal activites (MIC) of the chloroform fractions of *C. hartmannianum, A.seyal* and *T. laxiflora* were (0.040-0.60) mg/ml against *A. flavus, A. niger* and the MIC of the chloroform fractions of *C. hartmannianum, A. seyal* and *T. laxiflora* against *C. albicans* were recorded at concentrations 0.07-0.15 mg/ml.
- The highest antioxidant activity was accumulated in the ethyl acetate fractions of *A. seyal, T. laxiflora* and *C. hartmannianum* with an IC_{50} of (0.482±0.073, 0.347±0.026, 0.460±0.026) mg/ml respectively. The ethyl

acetate fractions of *A. seyal, T. laxiflora* and *C. hartmannianum* also showed the highest total phenol compounds content 424.65, 506.56, 404.96 mg GAE/g respectively which was reflected in their antioxidant activity. *T. laxiflora* fractions presented the highest phenolic content with a range of (747.05-382.35) mg GAE/g while *C. hartmannianum* fractions presented the second highest phenolic contents with a range of 473.52-363.38 mg GAE/g and *A. seyal* fractions presented moderate phenolic content with a range of 175.93−461.85mg GAE/g.

- All fractions were quite safe in brine shrimp lethality assay and some fractions of *A. seyal* and C. *hartmannianum* showed very slight toxicity.
- Polyphenolics and terpenoids were expected to be responsible for the fragrances in the petroleum ether and ethyl acetate fractions. Removal of polyphenols as tannins with the aids of 2% NaCl reduced the fragrance in ethyl acetate fractions proving the fact of their contribution to fragrance by using (TLC).
- *Nikhra* fragrance was stronger than non fermented wood was proved by TLC. Fragrance in the petroleum ether and, ethyl acetate fractions which have different scents were proved to be polyphenols by TLC after spraying with NPR, specific reagent for detects components: catechin (van HCL), terponoids (van H_2SO_4) and lignans (van H_3PO_4).
- GC/MS analysis of the petroleum ether fractions revealed that the total fragrant compounds, phenolics and terponoids, for *A. seyal* was 56.44%, total fragrant compounds phenolics and terponoids for *T. laxiflora* (44.64%), while those of *C. hartmannianum* which is mostly used for treatment health problems were (19.39%).

RECOMMENDATIONS

- Polyphenols in the fragrant ethyl acetate fractions of fermented wood of *T. laxiflora, A. seyal* and *C. hartmannianum* should be identified using Tandem Mass Spectrometry.
- Fungi which naturally induced ferementation of plants wood should be identified and recorded for commercial use
- Up grade the production of most fragrant fractions namely petroleum ether and ethyl acetate of fermented wood of *T. laxiflora, A.seyal* and *C. hartmannianum* from lab scale to a pilot scale and hence commercialized.
- Micropropagation of these fragrent plants to maintain their production.

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Appendexes

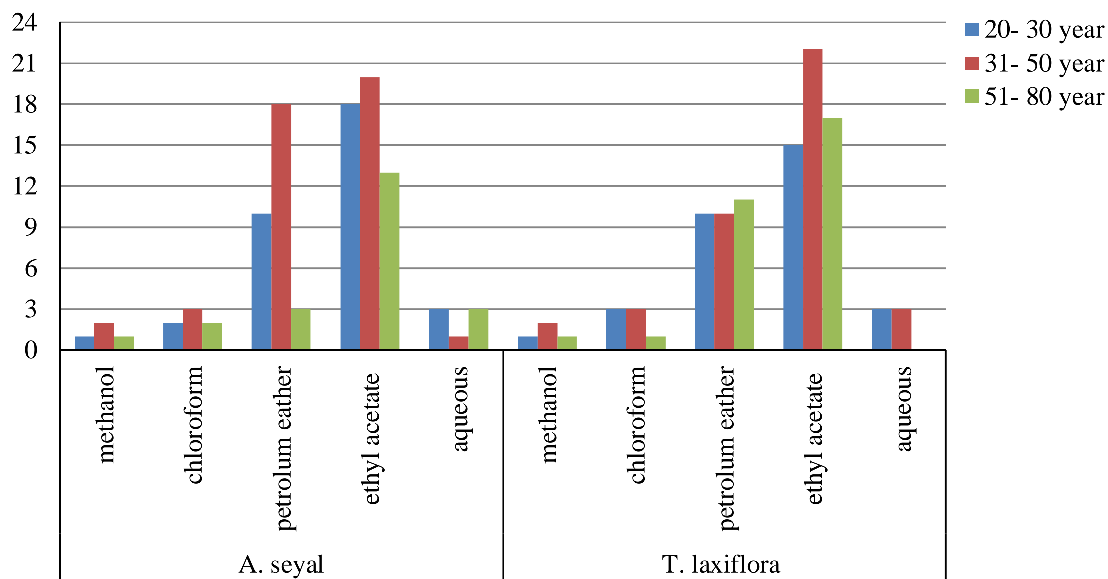

Fig.1: Age of questionnaires against perfering fragrance fractions of the *A.seyal* and *T. laxiflora*

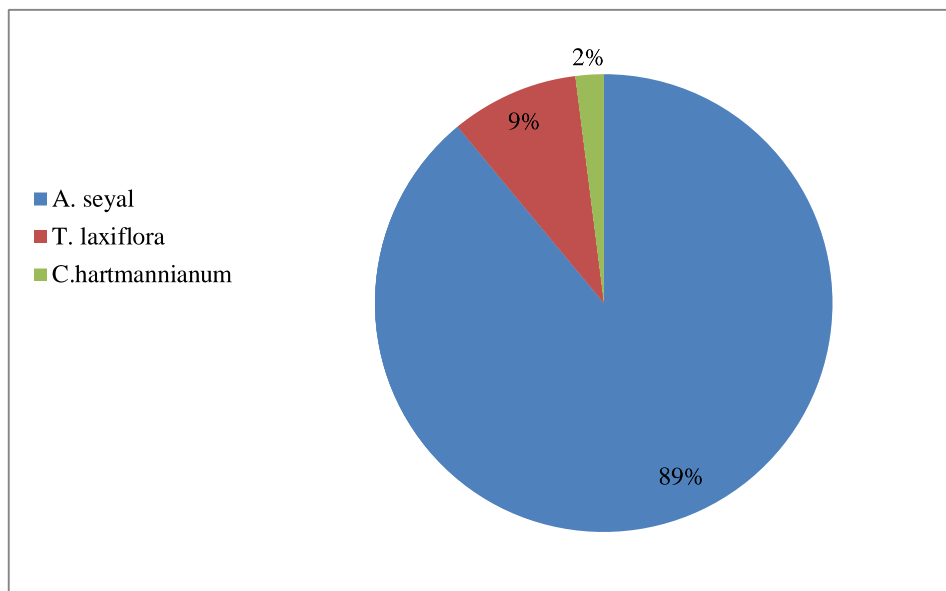

Fig.2: Percentage of usage *T. laxiflora, C. hartmannianum*and *A. seyal* by respondents for fragrance purposes

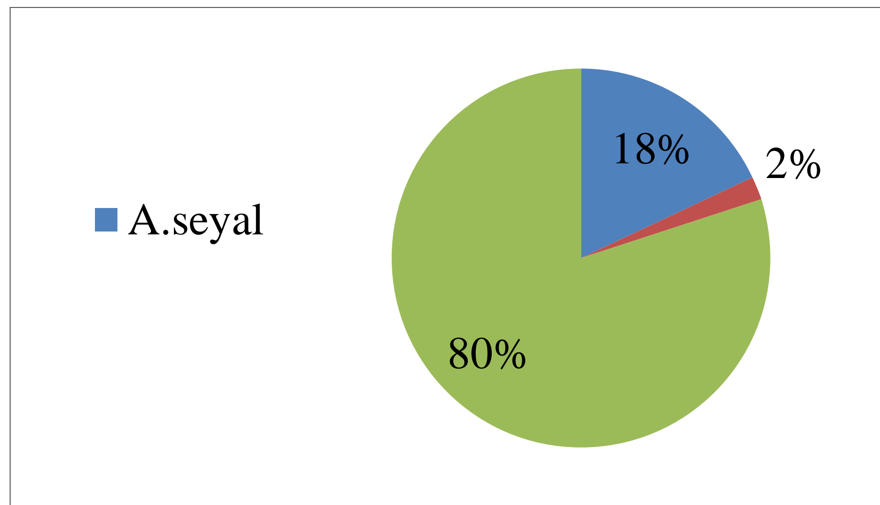

Figure 3: Percentage of respondents using *T. laxiflora, C. hartmannianum* and *A. seyal* for health problems

Table.1. ANOVA species VS solvents, species VS fractions of fermented and non fermented wood and species VS solvents VS fractions of fermented and non fermented wood of "*Nikhra*" of *T. laxiflora, C.hartmannianum* and *A.seyal.*

SOURCE	D. F.	f ratio	f table		M S
			f t0.05	f t. 0.01	
Species	2	9769.023	3.15	4.98	2.769301**
Solvent	4	60601.56	2.53	3.65	17.1792**
Fermentation	1	2795.36	4.00	7.08	0.792423**
Species x solvent	8	7355.719	2.10	2.82	2.085183**
Species x fermentation	2	34379.35	3.15	4.98	9.745781**
Solvent x fermentation	4	5603.762	2.53	3.65	1.588542**
Species x solvent x fermentation	8	8996.297	2.10	2.82	2.55025**
Error	60				0.000283
Total	89				

GC/MS structures of fragrant phenolic aromatic compounds in the petroleum ether fractions of *Aseyal, C. hartmannianum* and *T. laxiflora* fermented wood "*Nikhra*"

T.laxiflora

Library
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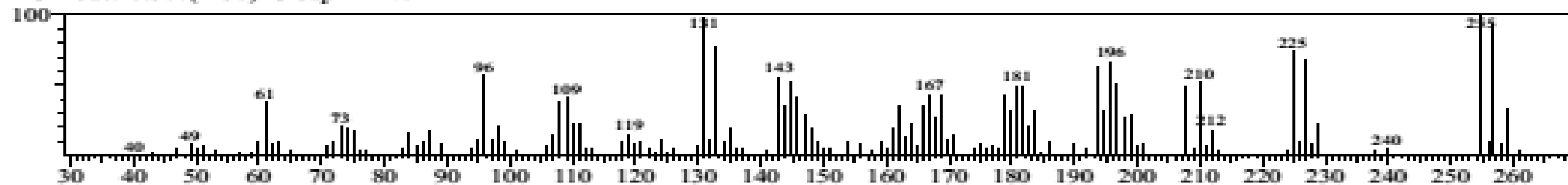

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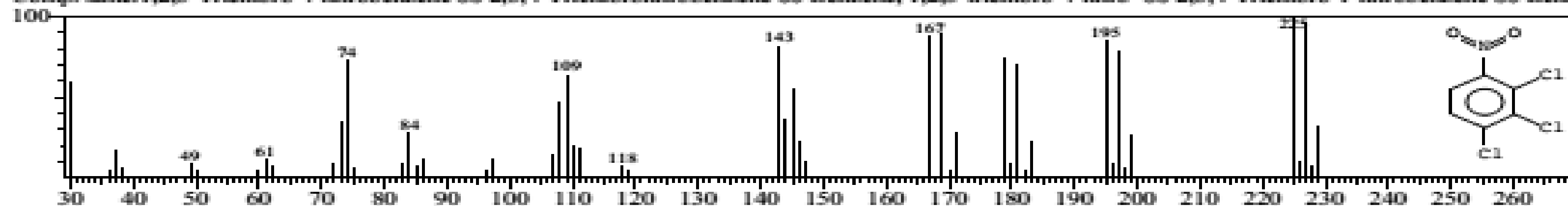

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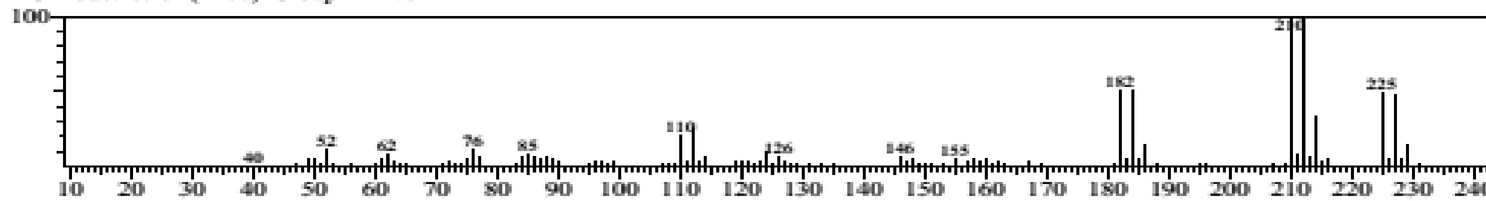

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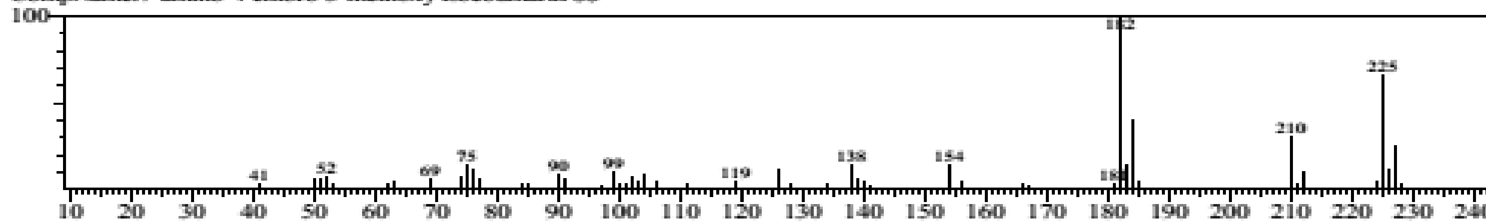

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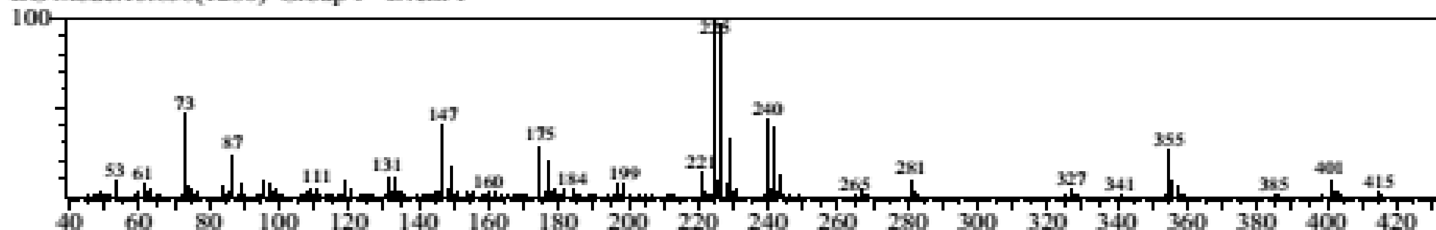

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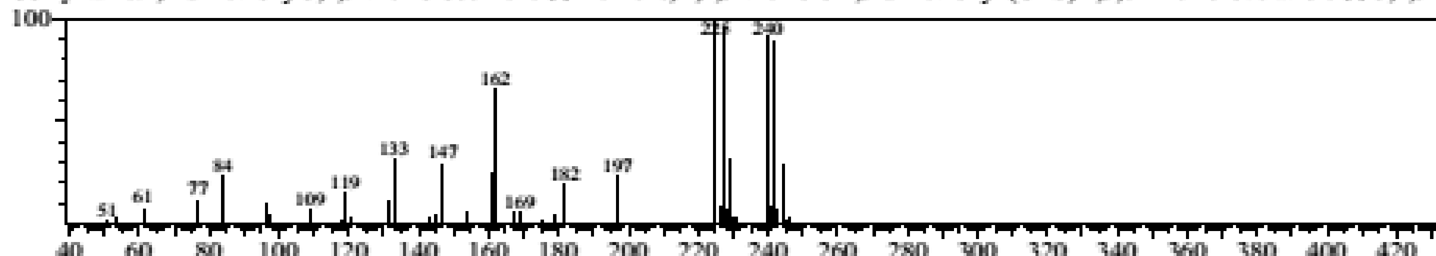

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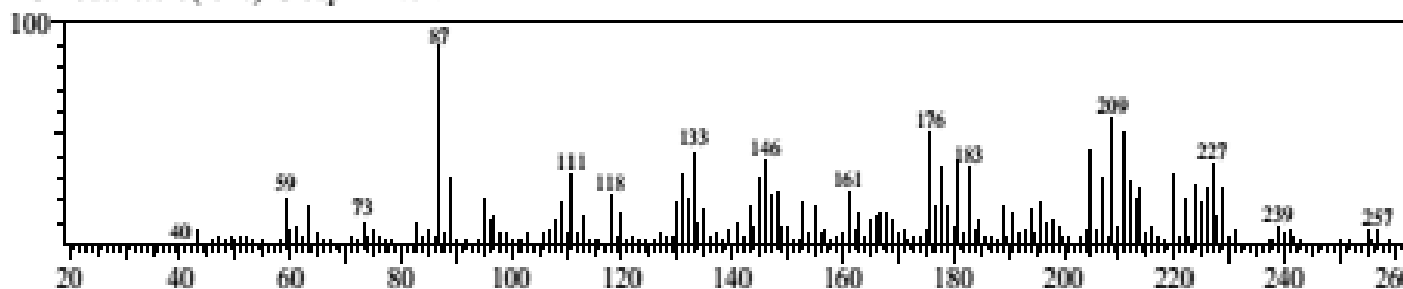

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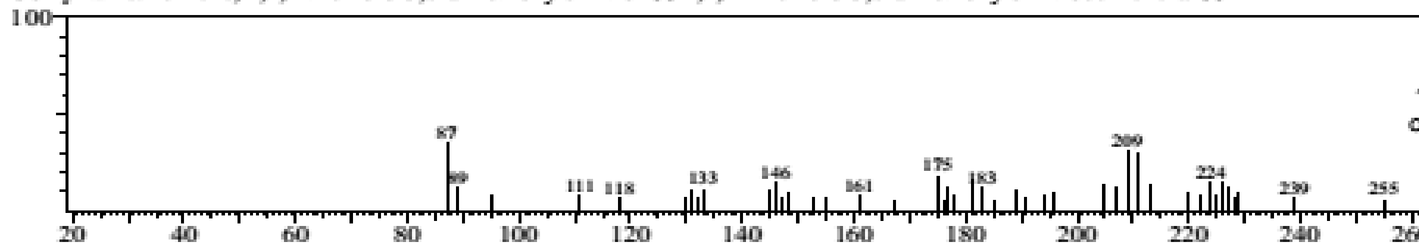

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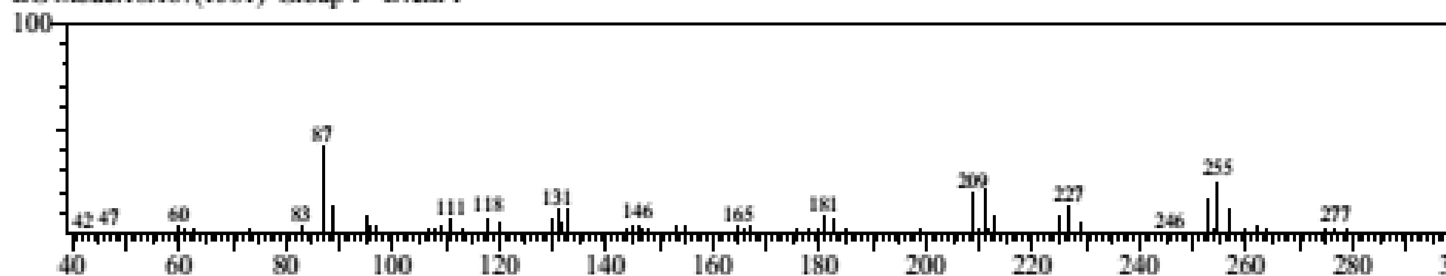
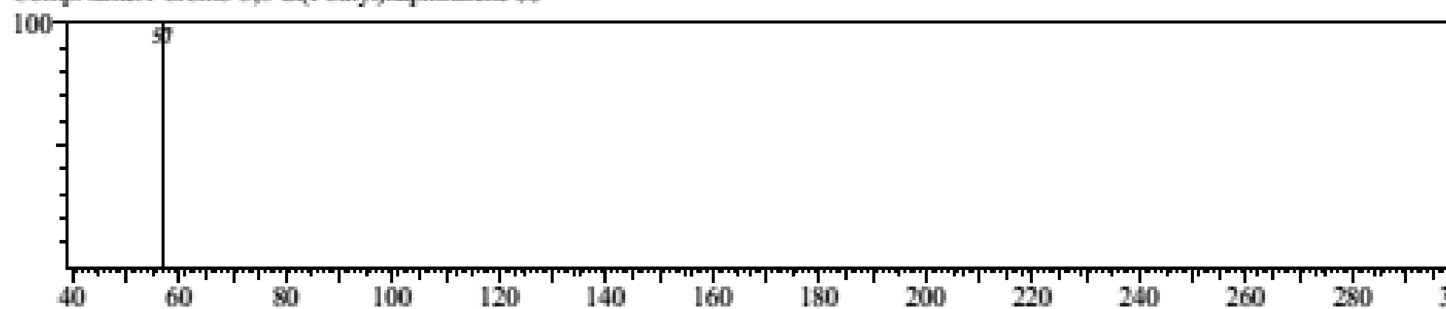
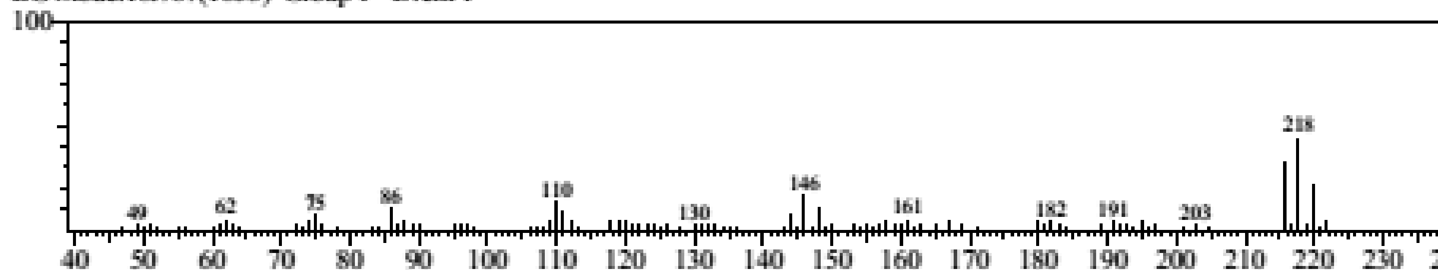


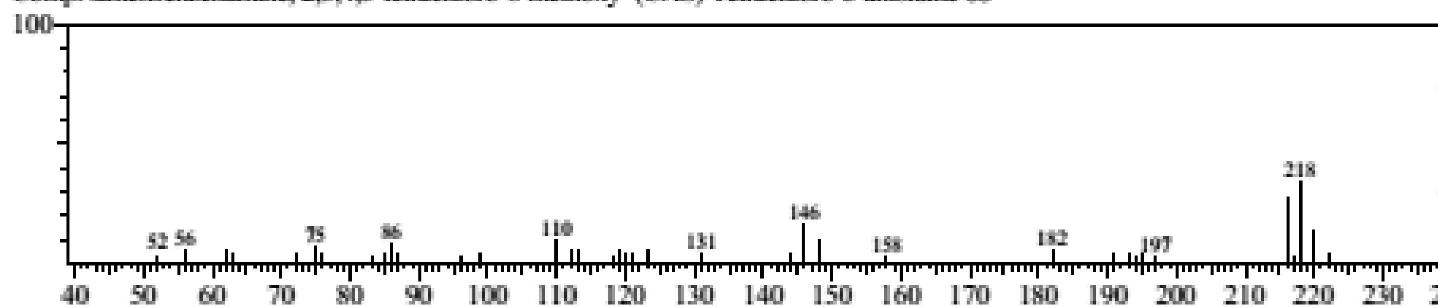
Hit#:1 Entry:228279 Library:WILEY7.LIB
SI:59 Formula:C18 H23 BR CAS:10239-76-6 MolWeight:318 RetIndex:0
CompName:1-bromo-3,6-di(t-butyl)naphthalene $$



<< Target >>
Line#:8 R.Time:18.692(Scan#:1644) MassPeaks:129
RawMode:Single 18.692(1644) BasePeak:245.80(79643)
BG Mode:18.767(1653) Group 1 - Event 1



Hit#:1 Entry:166748 Library:WILEY7.LIB
SI:88 Formula:C7 H5 CL4 N O CAS:42138-72-7 MolWeight:259 RetIndex:0
CompName:Benzenamine, 2,3,4,5-tetrachloro-6-methoxy- (CAS) Tetrachloro-o-anisidine $$



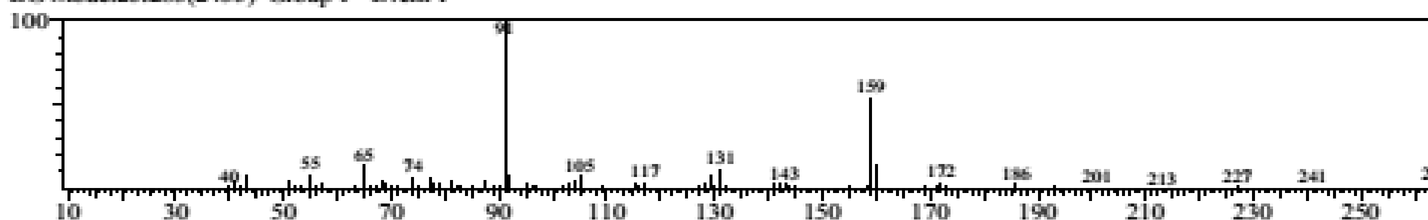
<< Target >>
Line#:13 R.Time:21.908(Scan#:2030) MassPeaks:110
RawMode:Single 21.908(2030) BasePeak:73.05(128085)
BG Mode:21.958(2036) Group 1 - Event 1

Hit#:1 Entry:557317 Library:WILEY7.LIB
SI:86 Formula:C24 H72 O12 SI12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:TETRACOSAMETHYLCYCLODODECASILOXANE $$ Cyclododecasiloxane, tetracosamethyl- (CAS)

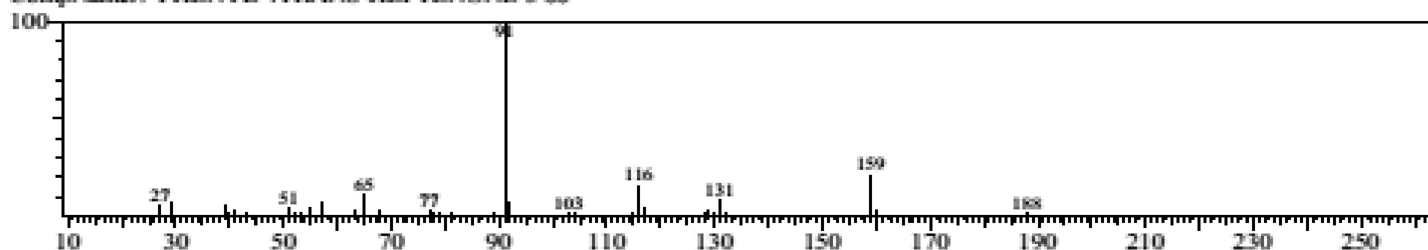
<<< Target >>
Line#:17 R.Time:24.100(Scan#:2293) MassPeaks:117
RawMode:Single 24.100(2293) BasePeak:73.05(148900)
BG Mode:24.208(2306) Group 1 - Event 1

Hit#:1 Entry:146019 Library:NIST147.LIB
SI:89 Formula:C18H54O9Si9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:Cyclononasiloxane, octadecamethyl- $$ Octadeamethyl-cyclononasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,

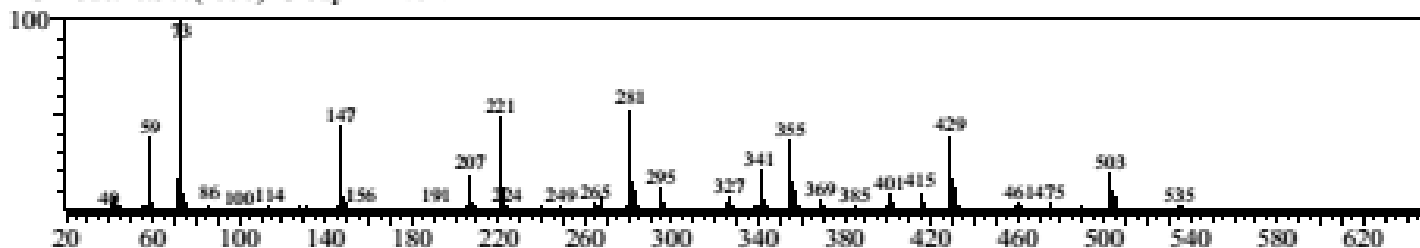
<< Target >>
Line#:18 R.Time:25.167(Scan#:2421) MassPeaks:130
RawMode:Single 25.167(2421) BasePeak:91.05(179402)
BG Mode:25.283(2435) Group 1 - Event 1



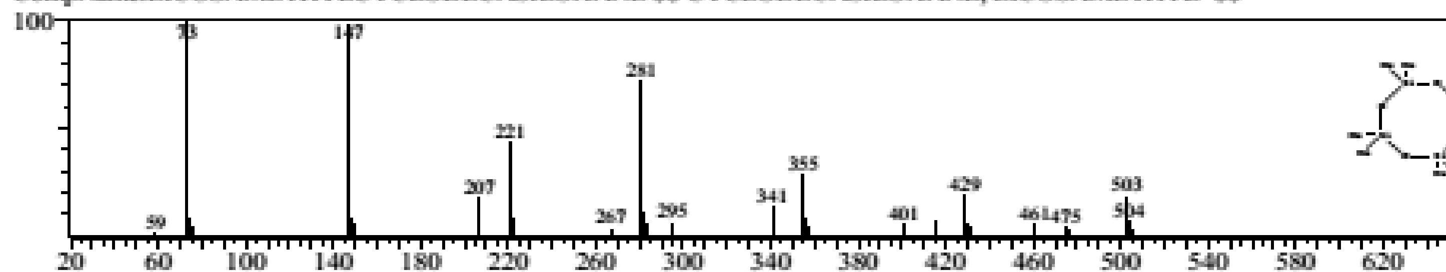
Hit#:1 Entry:80740 Library:WILEY7.LIB
SI:79 Formula:C13 H16 O CAS:0-00-0 MolWeight:188 RetIndex:0
CompName:7-PHENYL-4TRANS-HEPTENONE-3 $$



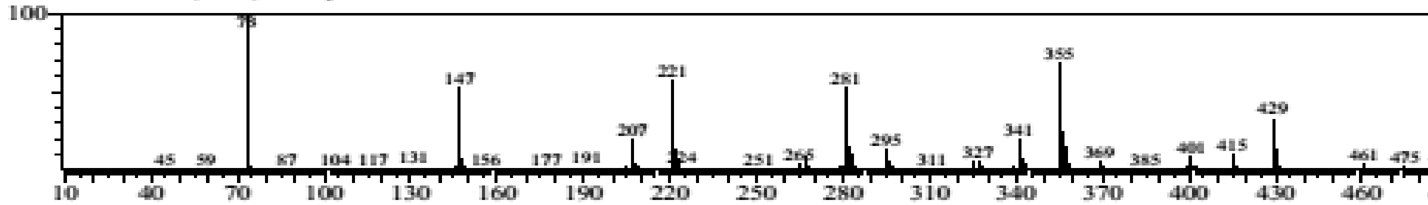
<< Target >>
Line#:20 R.Time:26.250(Scan#:2551) MassPeaks:152
RawMode:Single 26.250(2551) BasePeak:73.05(153505)
BG Mode:26.300(2557) Group 1 - Event 1



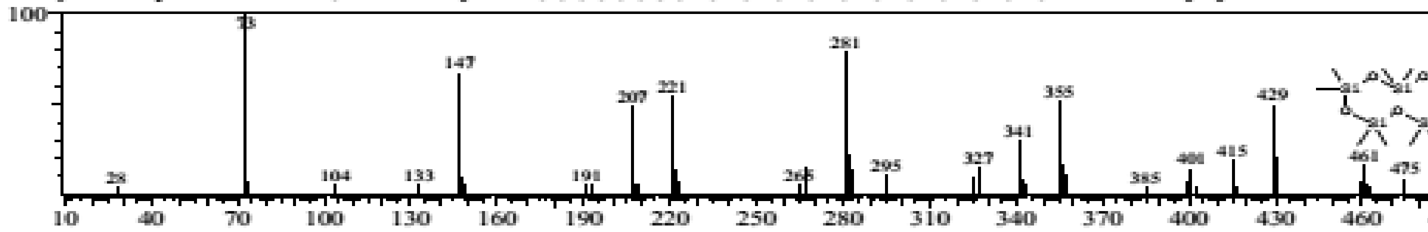
Hit#:1 Entry:335377 Library:WILEY7.LIB
SI:82 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



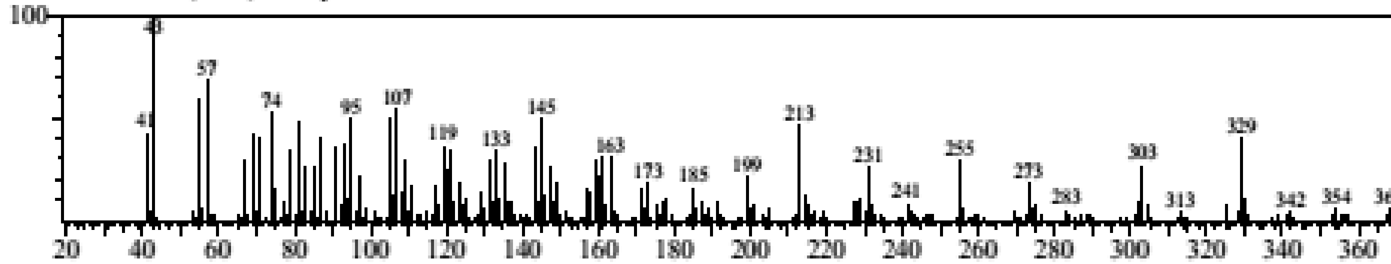
<< Target >>
Line#:21   R.Time:28.358(Scan#:2804) MassPeaks:142
RawMode:Single 28.358(2804) BasePeak:73.05(176182)
BG Mode:28.417(2811) Group 1 - Event 1



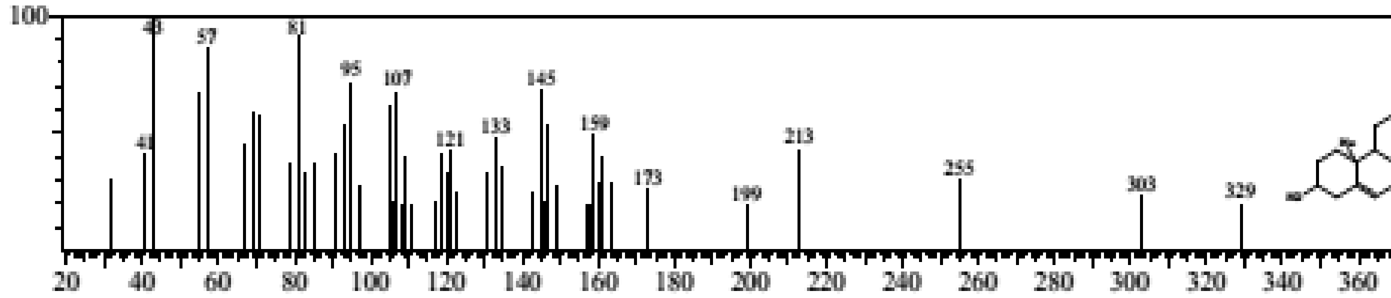
Hit#:1 Entry:146629 Library:NIST147.LIB
SI:85 Formula:C20H60O10Si10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:Cyclodecasiloxane, eicosamethyl- SS 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane #



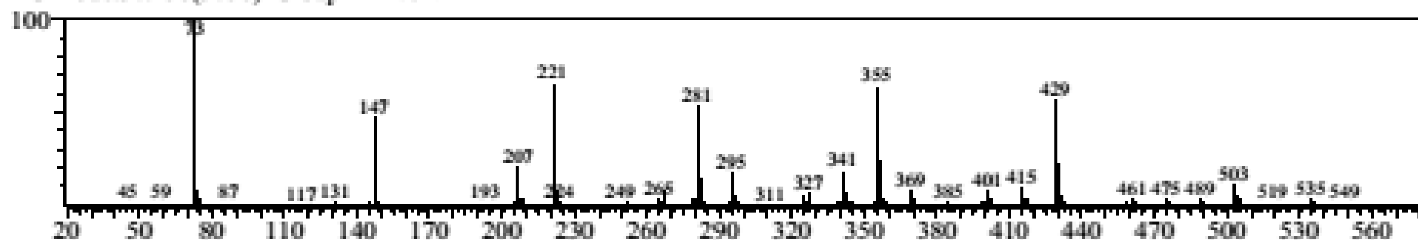
<<< Target >>
Line#:23 R.Time:29.700(Scan#:2965) MassPeaks:248
RawMode:Single 29.700(2965) BasePeak:43.05(58196)
BG Mode:29.875(2986) Group 1 - Event 1



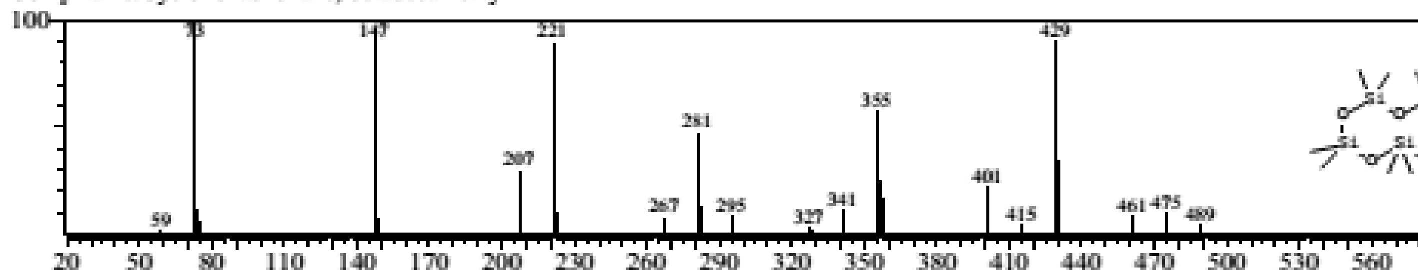
Hit#:1 Entry:291300 Library:WILEY7.LIB
SI:87 Formula:C29 H50 O CAS:83-46-5 MolWeight:414 RetIndex:0
CompName:Stigmast-5-en-3-ol, (3.beta.)- (CAS) 24.BETA.-ETHYL-5.DELTA.-CHOLESTEN-3.BETA.-OL $$ SKF 14463 $$ Rhamno



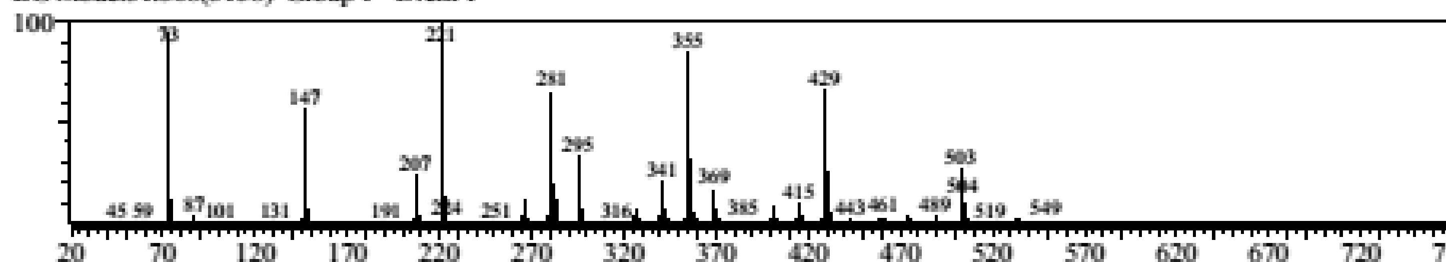
<< Target >>
Line#:25 R.Time:30.392(Scan#:3048) MassPeaks:137
RawMode:Single 30.392(3048) BasePeak:73.00(205080)
BG Mode:30.458(3056) Group 1 - Event 1



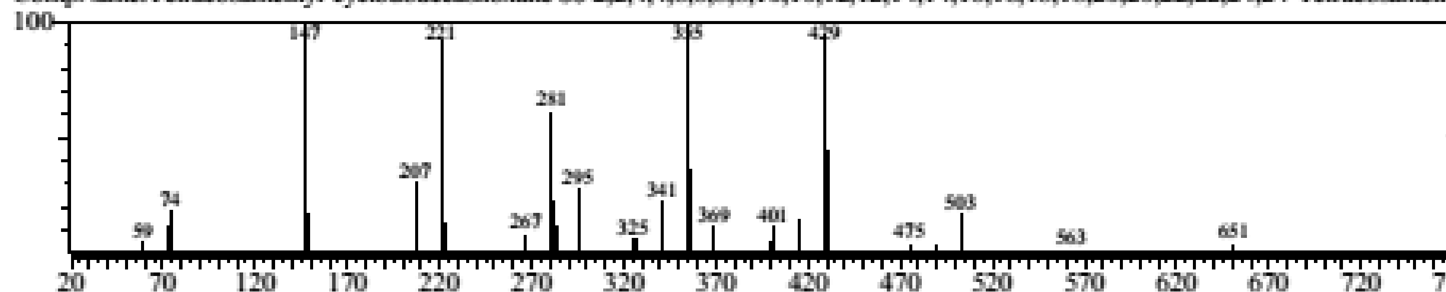
Hit#:1 Entry:27682 Library:NIST27.LIB
SI:85 Formula:C18H54O9Si9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:Cyclononasiloxane, octadecamethyl-



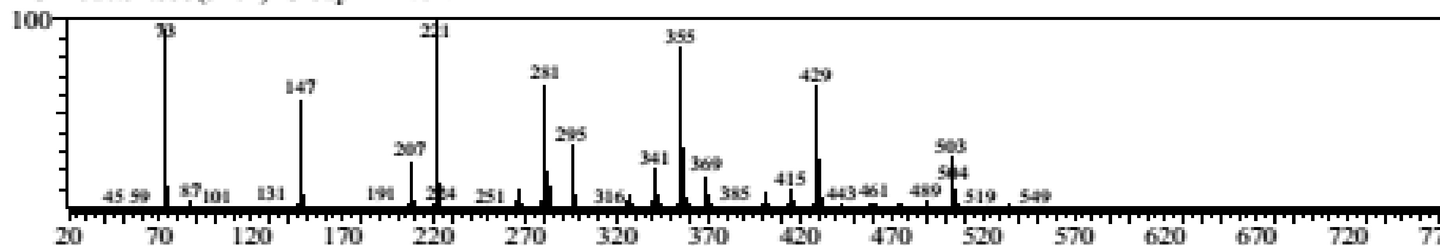
<< Target >>
Line#:26   R.Time:31.192(Scan#:3144) MassPeaks:170
RawMode:Single 31.192(3144) BasePeak:221.00(378959)
BG Mode:31.308(3158) Group 1 - Event 1



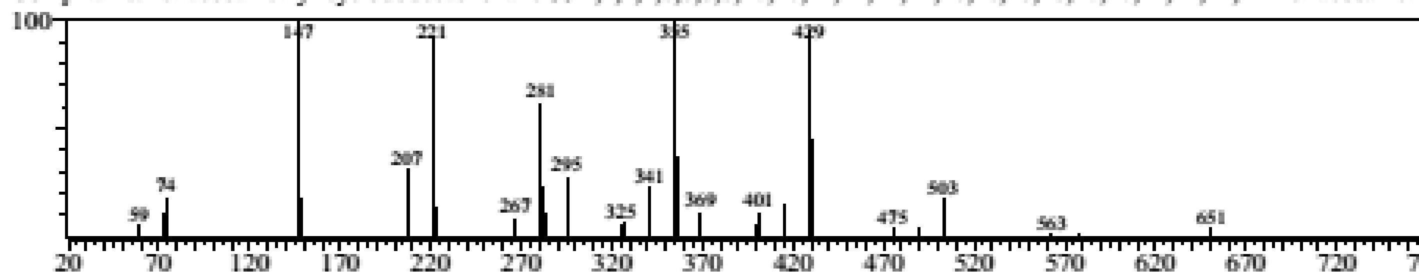
Hit#:1 Entry:147059 Library:NIST147.LIB
SI:85 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosameth



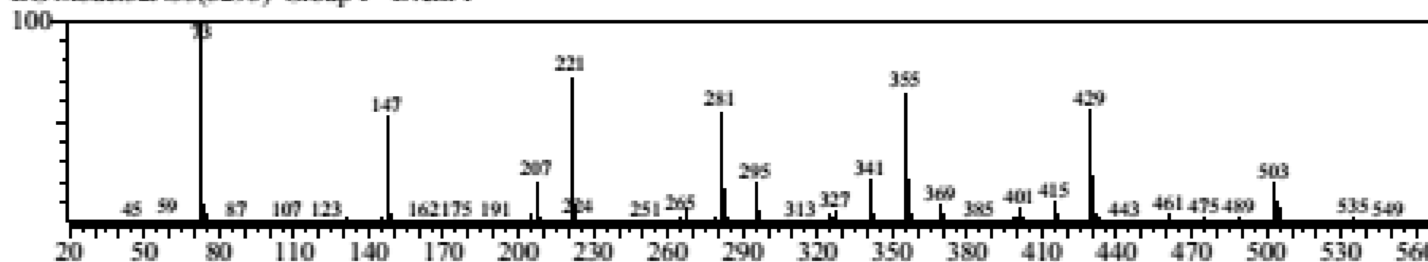
<< Target >>
Line#:27 R.Time:31.192(Scan#:3144) MassPeaks:171
RawMode:Single 31.192(3144) BasePeak:221.00(392629)
BG Mode:31.333(3161) Group 1 - Event 1



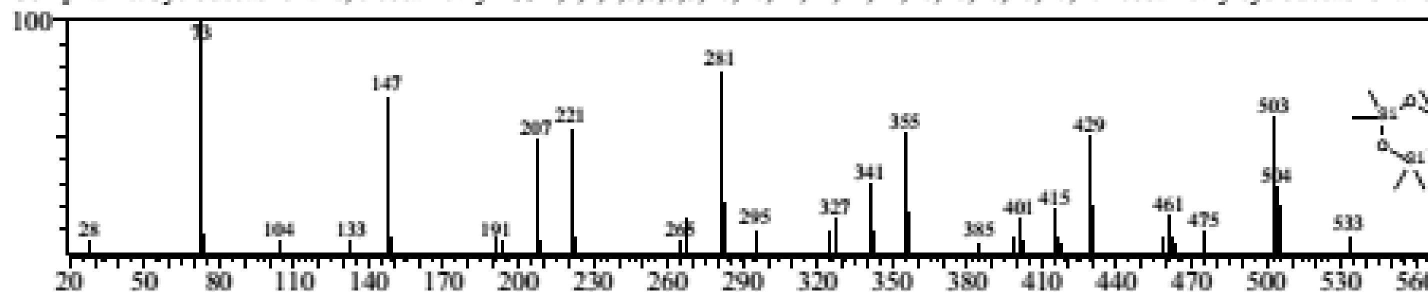
Hit#:1 Entry:147059 Library:NIST147.LIB
SI:85 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosamet



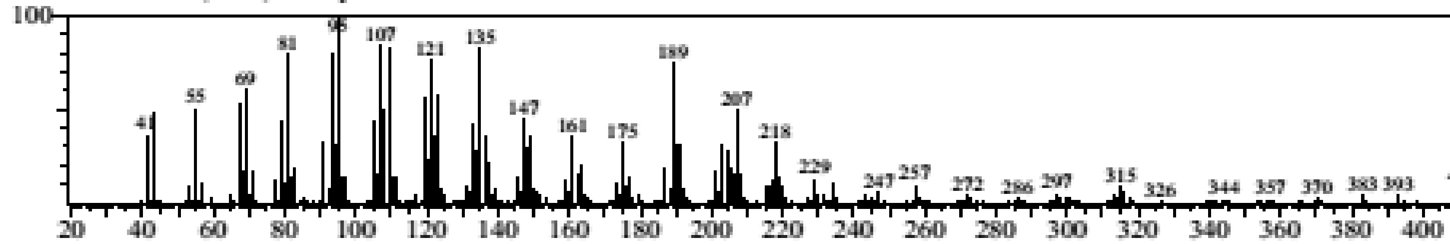
<<< Target >>
Line#:28 R.Time:32.325(Scan#:3280) MassPeaks:206
RawMode:Single 32.325(3280) BasePeak:73.05(214937)
BG Mode:32.433(3293) Group 1 - Event 1



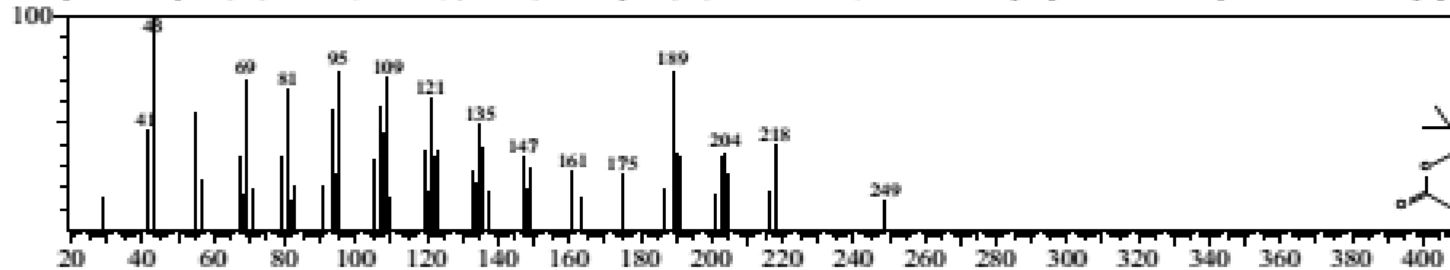
Hit#:1 Entry:146629 Library:NIST147.LIB
SI:86 Formula:C20H60O10Si10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:Cyclodecasiloxane, eicosamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane



<< Target >>
Line#:29 R.Time:33.467(Scan#:3417) MassPeaks:268
RawMode:Single 33.467(3417) BasePeak:95.10(223450)
BG Mode:33.725(3448) Group 1 - Event 1



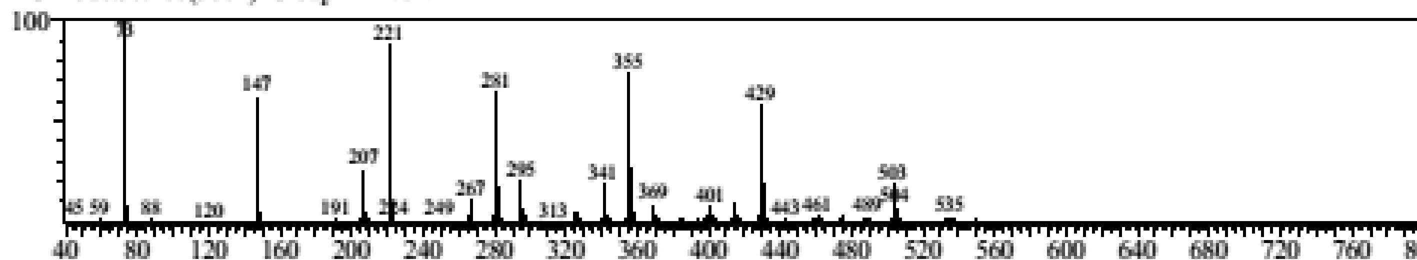
Hit#:1 Entry:138955 Library:NIST147.LIB
SI:89 Formula:C32H52O2 CAS:1617-68-1 MolWeight:468 RetIndex:0
CompName:Lup-20(29)-en-3-ol, acetate, (3.beta.)- $$ Lup-20(29)-en-3.beta.-ol, acetate $$ Lupenyl acetate $$ Lupeol acetate $$ Lupey



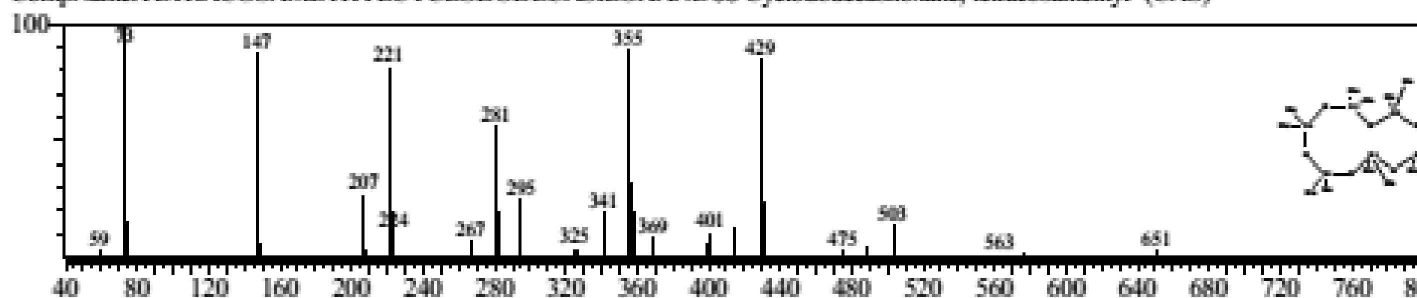
<< Target >>
Line#:30 R.Time:34.467(Scan#:3537) MassPeaks:162
RawMode:Single 34.467(3537) BasePeak:73.05(185719)
BG Mode:34.600(3553) Group 1 - Event 1

Hit#:1 Entry:146629 Library:NIST147.LIB
SI:85 Formula:C20H60O10Si10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:Cyclodecasiloxane, eicosamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane # $$

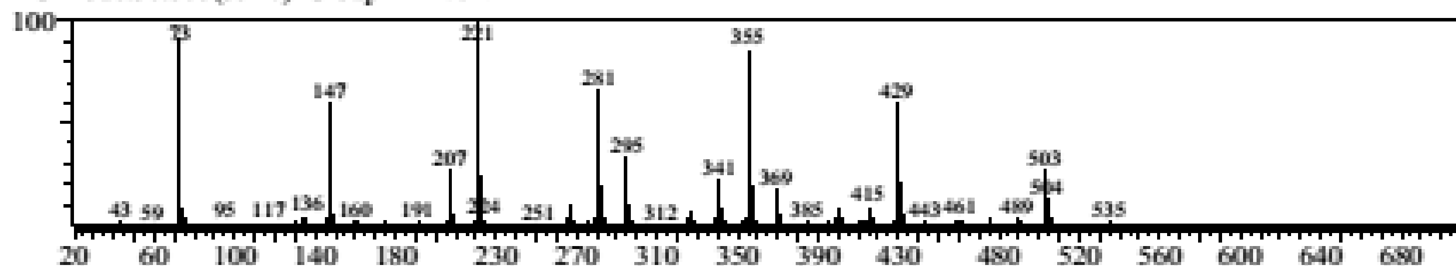
<< Target >>
Line#:32 R.Time:37.108(Scan#:3854) MassPeaks:134
RawMode:Single 37.108(3854) BasePeak:73.05(81324)
BG Mode:37.167(3861) Group 1 - Event 1



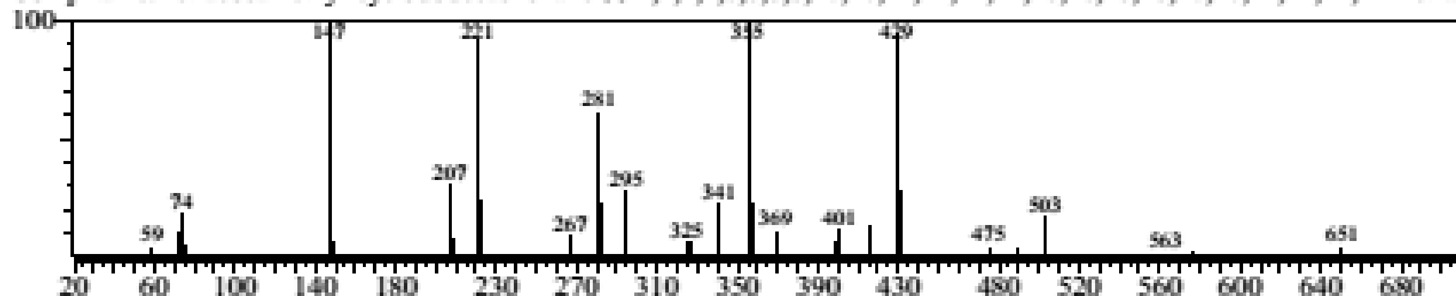
Hit#:1 Entry:337318 Library:WILEY7.LIB
SI:85 Formula:C24 H72 O12 SI12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:TETRACOSAMETHYLCYCLODODECASILOXANE $$ Cyclododecasiloxane, tetracosamethyl- (CAS)



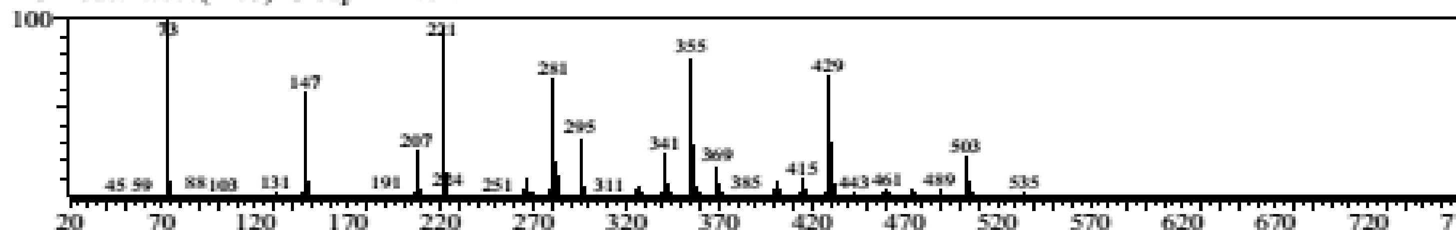
<< Target >>
Line#:33 R.Time:37.317(Scan#:3879) MassPeaks:244
RawMode:Single 37.317(3879) BasePeak:221.00(240455)
BG Mode:37.575(3910) Group 1 - Event 1



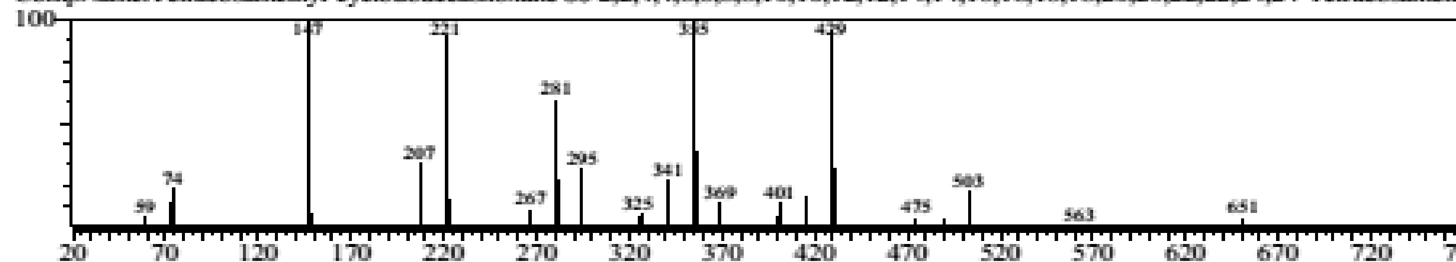
Hit#:1 Entry:147059 Library:NIST147.LIB
SI:84 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetra



<< Target >>
Line#:34 R.Time:40.483(Scan#:4259) MassPeaks:131
RawMode:Single 40.483(4259) BasePeak:73.05(106219)
BG Mode:40.600(4273) Group 1 - Event 1



Hit#:1 Entry:147059 Library:NIST147.LIB
SI:86 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosameth



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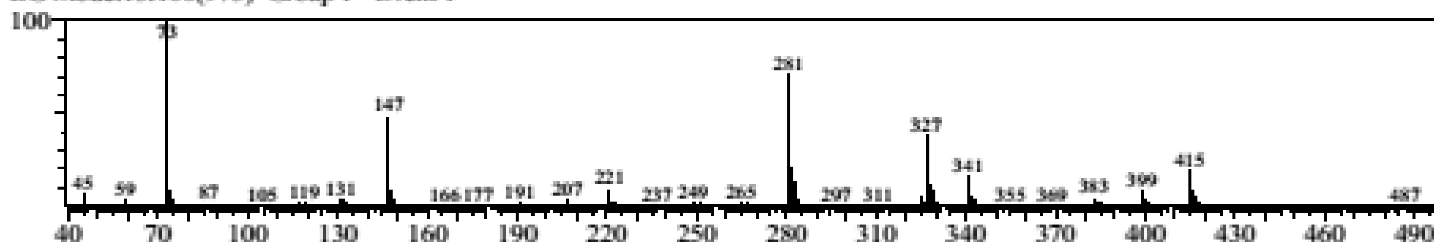
<< Target >>

Line#:1 R.Time:13.042(Scan#:966) MassPeaks:130

RawMode:Single 13.042(966) BasePeak:73.05(468720)

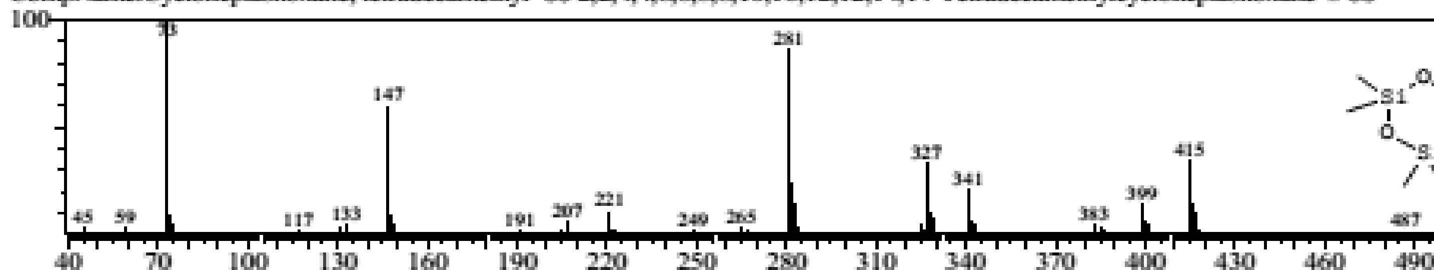
BG Mode:13.100(973) Group 1 - Event 1



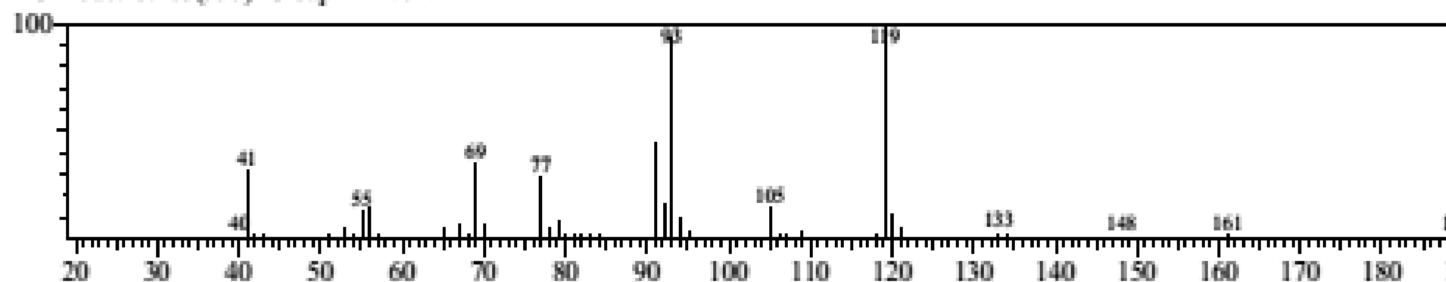
Hit#:1 Entry:142207 Library:NIST147.LIB

SI:92 Formula:C14H42O7Si7 CAS:107-50-6 MolWeight:518 RetIndex:0

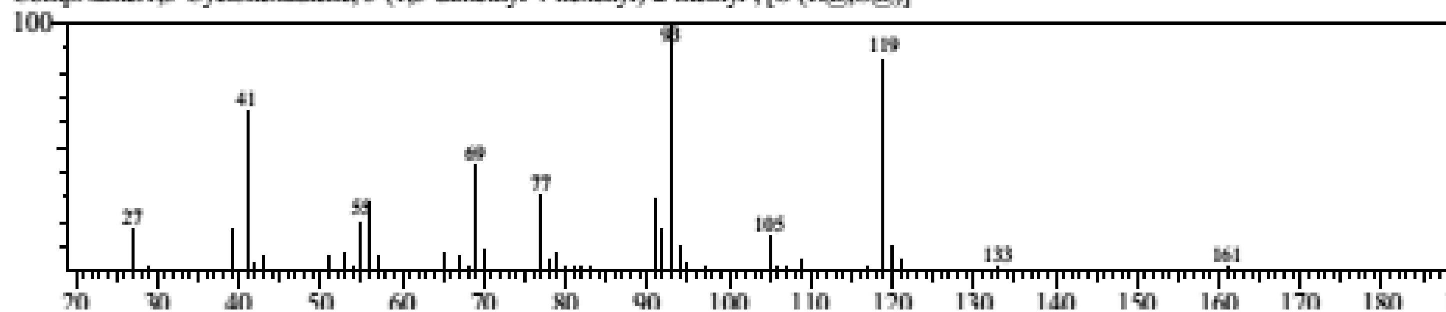
CompName:Cycloheptasiloxane, tetradecamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14-Tetradecamethylcycloheptasiloxane # $$



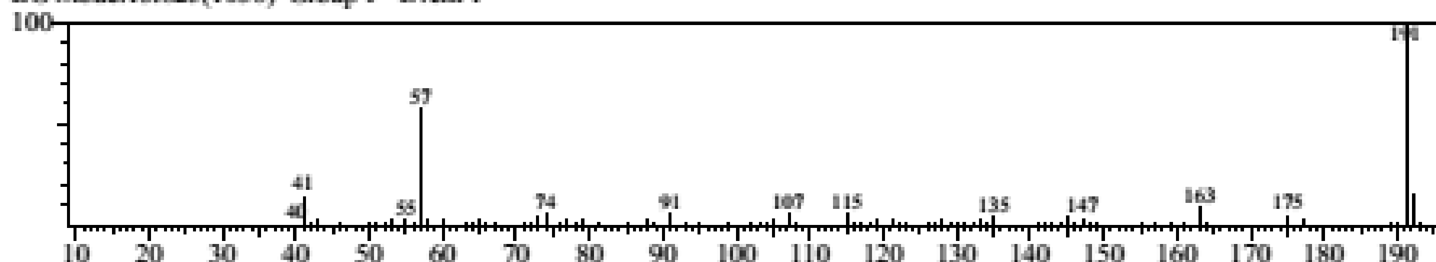
<< Target >>
Line#:2 R.Time:13.225(Scan#:988) MassPeaks:78
RawMode:Single 13.225(988) BasePeak:119.10(249688)
BG Mode:13.283(995) Group 1 - Event 1



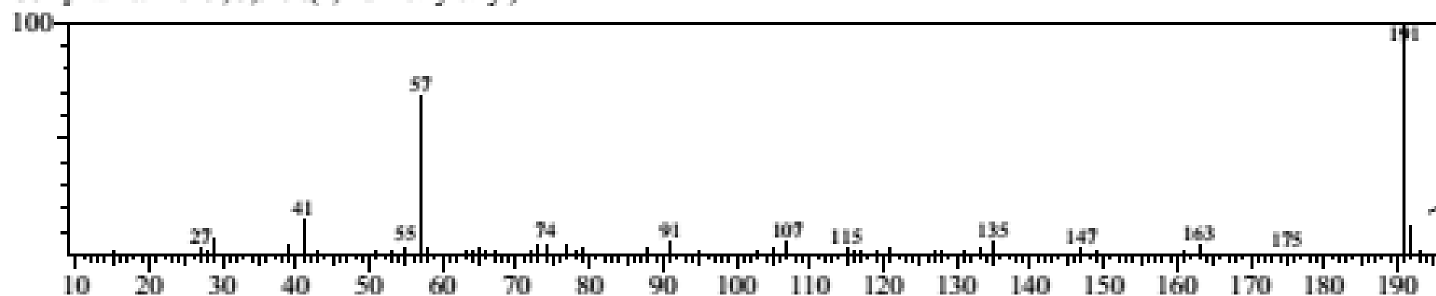
Hit#:1 Entry:16827 Library:NIST27.LIB
SI:93 Formula:C15H24 CAS:495-60-3 MolWeight:204 RetIndex:0
CompName:1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R@,S@)]-



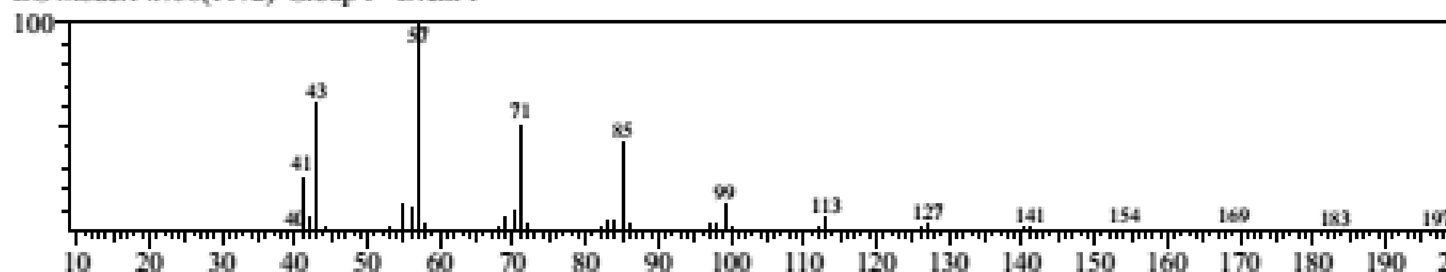
<< Target >>
Line#:3 R.Time:13.550(Scan#:1027) MassPeaks:118
RawMode:Single 13.550(1027) BasePeak:191.10(235775)
BG Mode:13.625(1036) Group 1 - Event 1



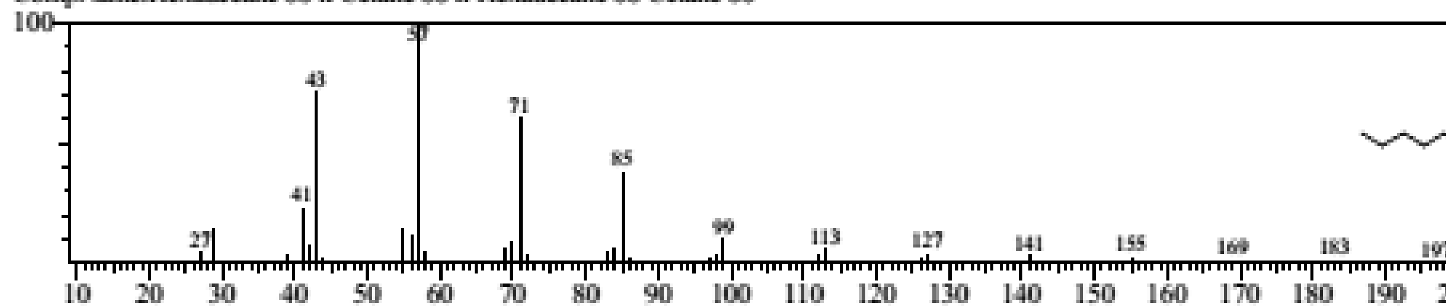
Hit#:1 Entry:17012 Library:NIST27.LIB
SI:91 Formula:C14H22O CAS:1138-52-9 MolWeight:206 RetIndex:0
CompName:Phenol, 3,5-bis(1,1-dimethylethyl)-



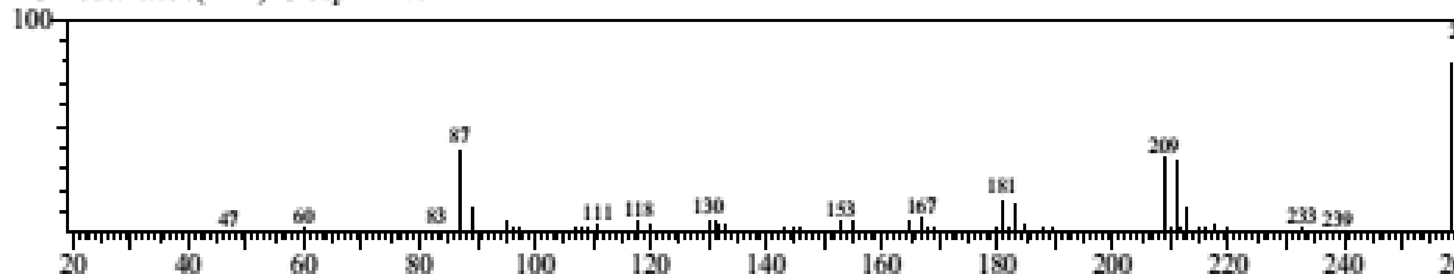
<< Target >>
Line#:5 R.Time:14.725(Scan#:1168) MassPeaks:58
RawMode:Single 14.725(1168) BasePeak:57.05(371780)
BG Mode:14.758(1172) Group 1 - Event 1



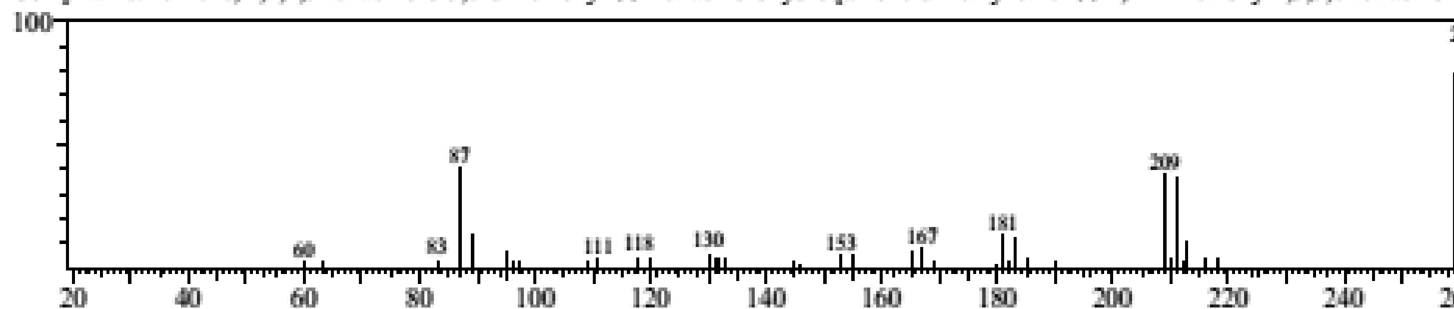
Hit#:1 Entry:55060 Library:NIST147.LIB
SI:97 Formula:C16H34 CAS:544-76-3 MolWeight:226 RetIndex:0
CompName:Hexadecane $$ n-Cetane $$ n-Hexadecane $$ Cetane $$



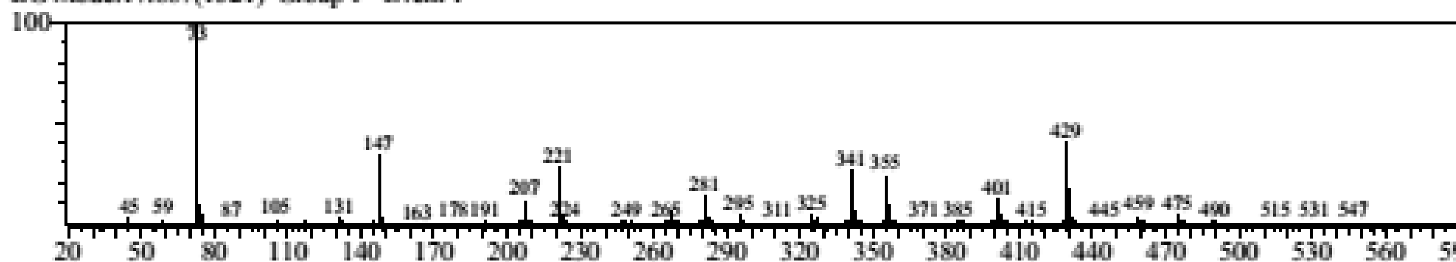
<< Target >>
Line#:7 R.Time:16.700(Scan#:1405) MassPeaks:192
RawMode:Single 16.700(1405) BasePeak:260.85(1064314)
BG Mode:16.750(1411) Group 1 - Event 1



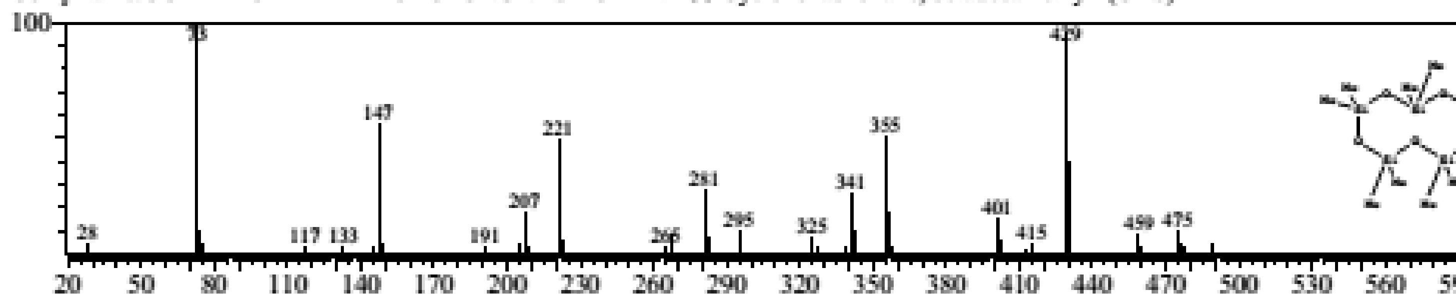
Hit#:1 Entry:80175 Library:NIST147.LIB
SI:96 Formula:C8H6Cl4O2 CAS:944-78-5 MolWeight:274 RetIndex:0
CompName:Benzene, 1,2,4,5-tetrachloro-3,6-dimethoxy- $$ Tetrachlorohydroquinone dimethyl ether $$ 1,4-Dimethoxy-2,3,5,6-tetrachlor



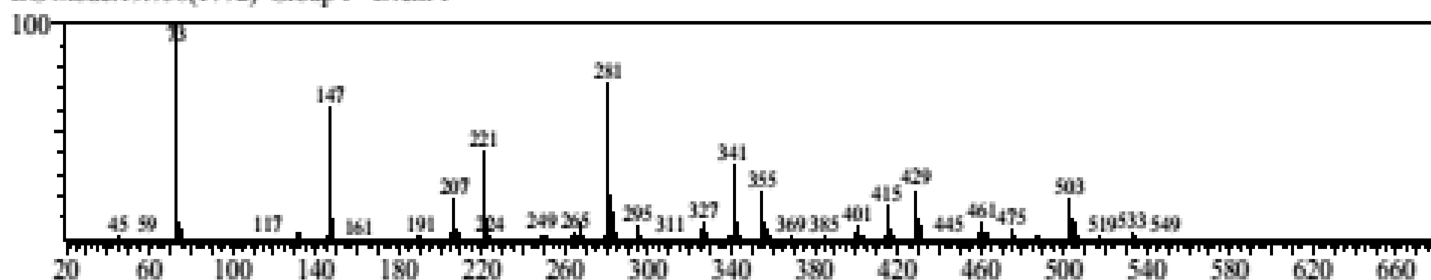
<< Target >>
Line#:9 R.Time:17.625(Scan#:1516) MassPeaks:189
RawMode:Single 17.625(1516) BasePeak:73.00(894033)
BG Mode:17.667(1521) Group 1 - Event 1



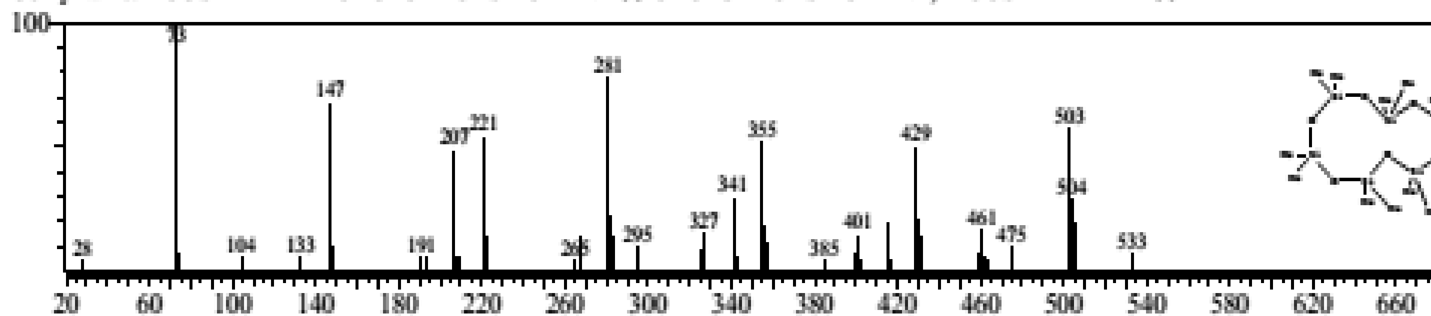
Hit#:1 Entry:332919 Library:WILEY7.LIB
SI:85 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)



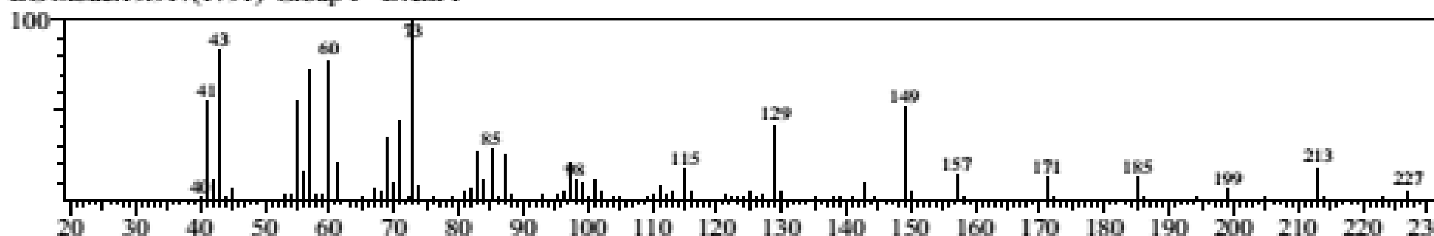
<< Target >>
Line#:11 R.Time:19.725(Scan#:1768) MassPeaks:165
RawMode:Single 19.725(1768) BasePeak:73.00(510730)
BG Mode:19.758(1772) Group 1 - Event 1



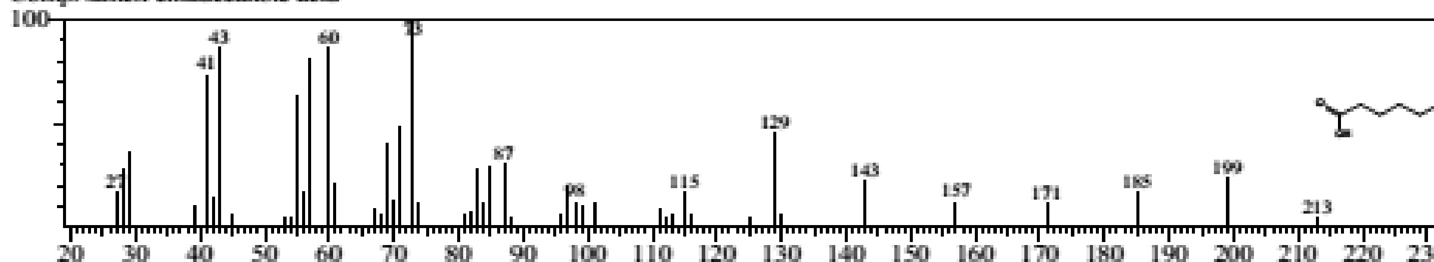
Hit#:1 Entry:335376 Library:WILEY7.LIB
SI:88 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



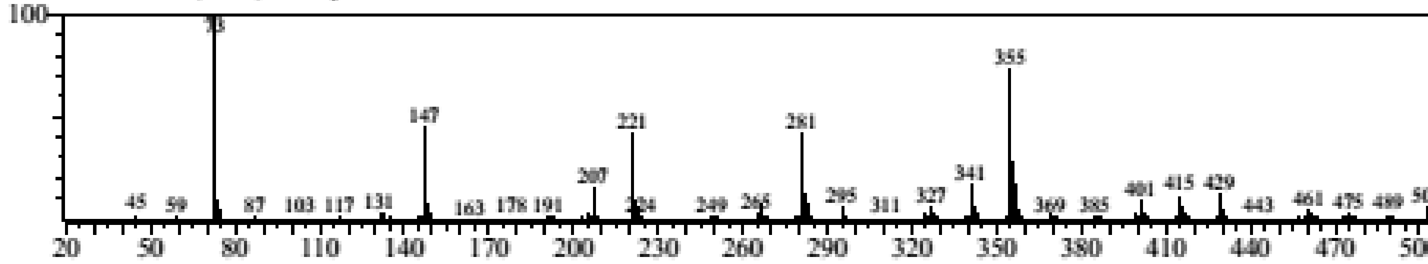
<< Target >>
Line#:12 R.Time:19.858(Scan#:1784) MassPeaks:178
RawMode:Single 19.858(1784) BasePeak:73.00(925714)
BG Mode:19.917(1791) Group 1 - Event 1



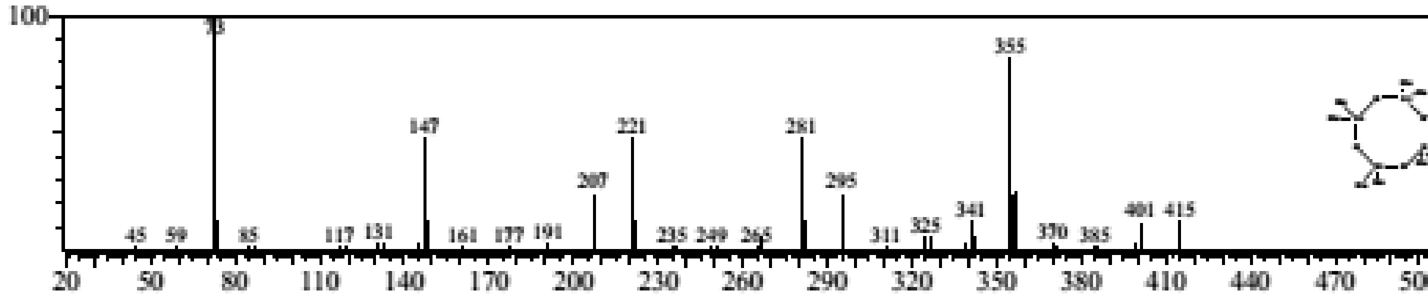
Hit#:1 Entry:20335 Library:NIST27.LIB
SI:91 Formula:C15H30O2 CAS:1002-84-2 MolWeight:242 RetIndex:0
CompName:Pentadecanoic acid



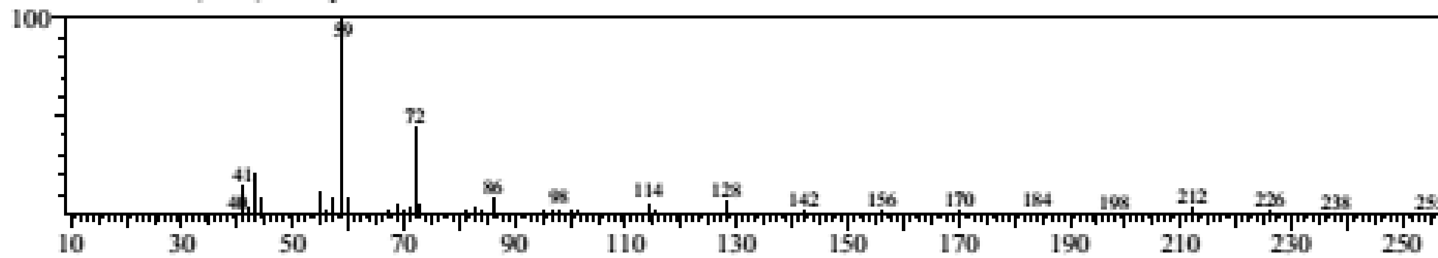
<< Target >>
Line#:15 R.Time:21.908(Scan#:2030) MassPeaks:186
RawMode:Single 21.908(2030) BasePeak:73.05(562734)
BG Mode:21.950(2035) Group 1 - Event 1



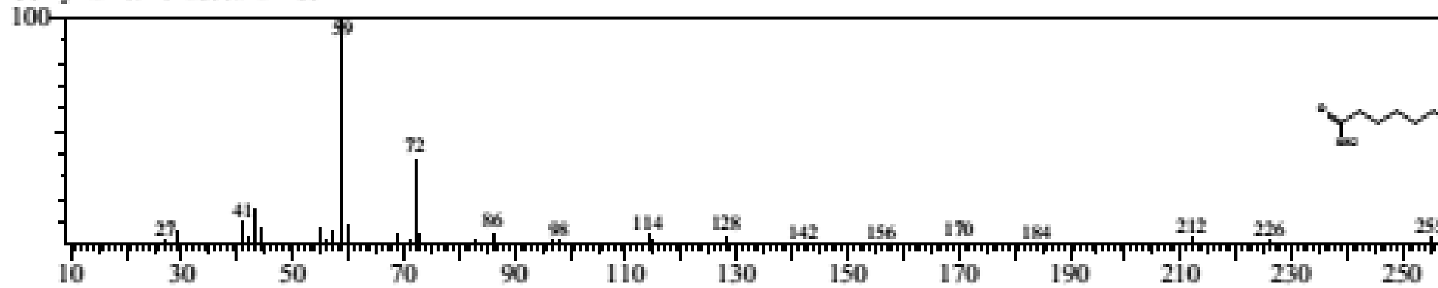
Hit#:1 Entry:337317 Library:WILEY7.LIB
SI:85 Formula:C24 H72 O12 SI12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:TETRACOSAMETHYLCYCLODODECASILOXANE $$ Cyclododecasiloxane, tetracosamethyl- (CAS)



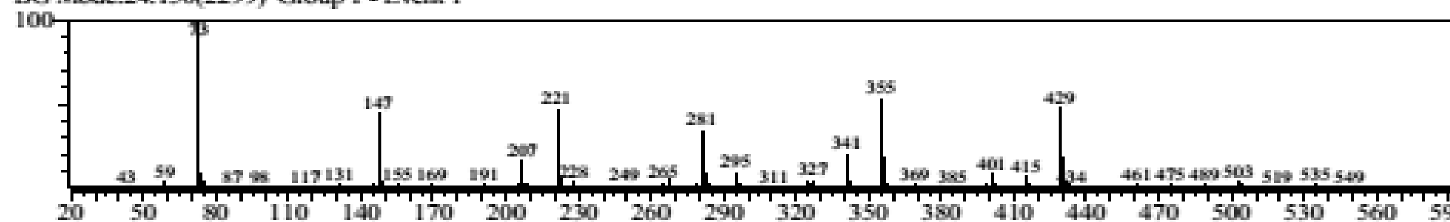
<< Target >>
Line#:19 R.Time:23.133(Scan#:2177) MassPeaks:165
RawMode:Single 23.133(2177) BasePeak:59.05(1548908)
BG Mode:23.192(2184) Group 1 - Event 1



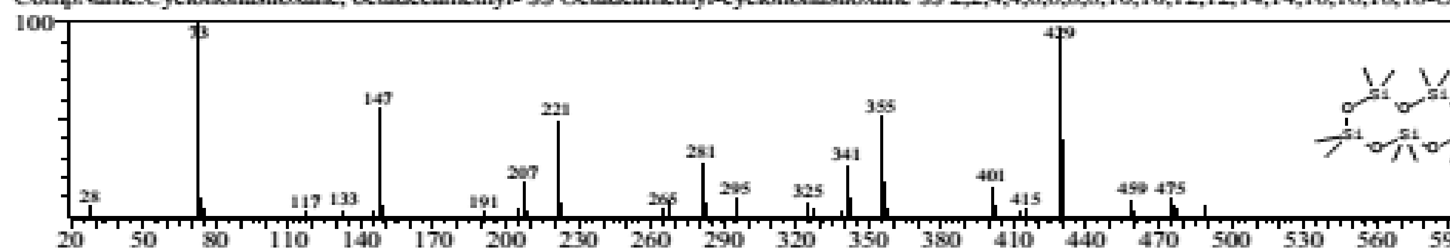
Hit#:1 Entry:21213 Library:NIST27.LIB
SI:94 Formula:C16H33NO CAS:629-54-9 MolWeight:255 RetIndex:0
CompName:Hexadecanamide



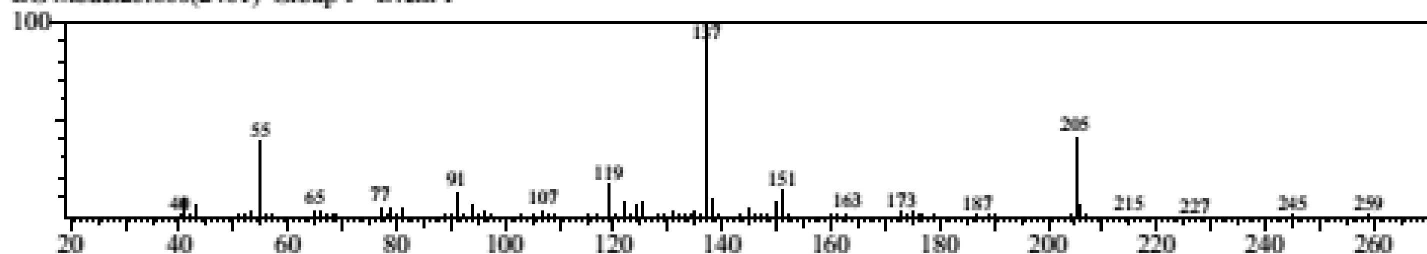
<< Target >>
Line#:21 R.Time:24.100(Scan#:2293) MassPeaks:247
RawMode:Single 24.100(2293) BasePeak:73.00(622396)
BG Mode:24.150(2299) Group 1 - Event 1



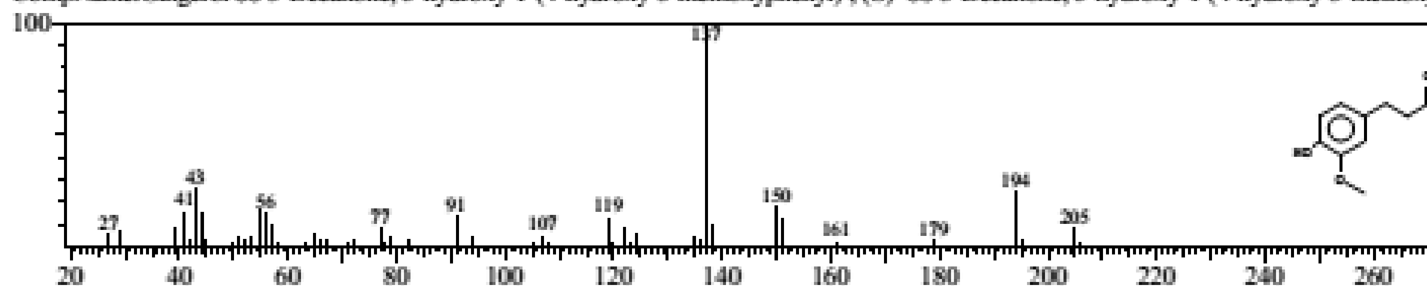
Hit#:1 Entry:146019 Library:NIST147.LIB
SI:88 Formula:C18H54O9Si9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:Cyclononasiloxane, octadecamethyl- $$ Octadeamethyl-cyclononasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18-O



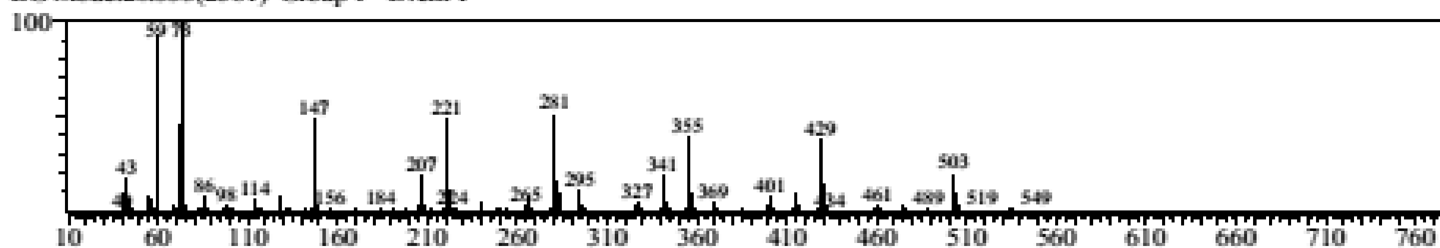
<< Target >>
Line#:22 R.Time:24.950(Scan#:2395) MassPeaks:156
RawMode:Single 24.950(2395) BasePeak:137.10(389037)
BG Mode:25.000(2401) Group 1 - Event 1



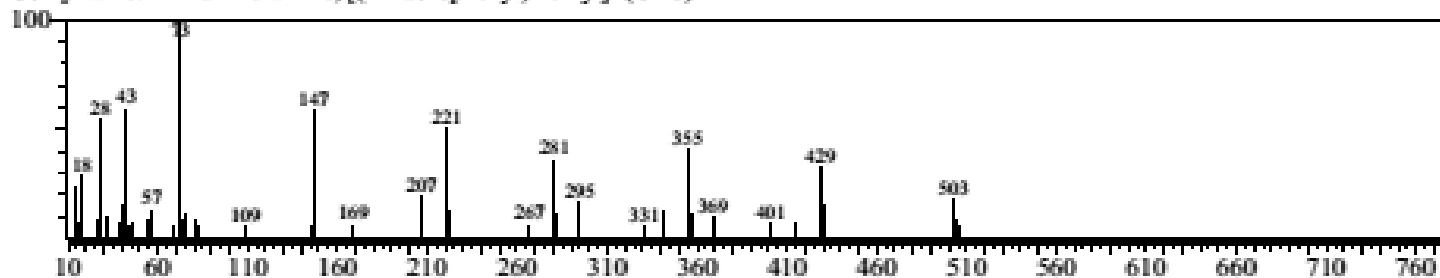
Hit#:1 Entry:90795 Library:NIST147.LIB
SI:74 Formula:C17H26O4 CAS:23513-14-6 MolWeight:294 RetIndex:0
CompName:Gingerol $$ 3-Decanone, 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-, (S)- $$ 3-Decanone, 5-hydroxy-1-(4-hydroxy-3-methox



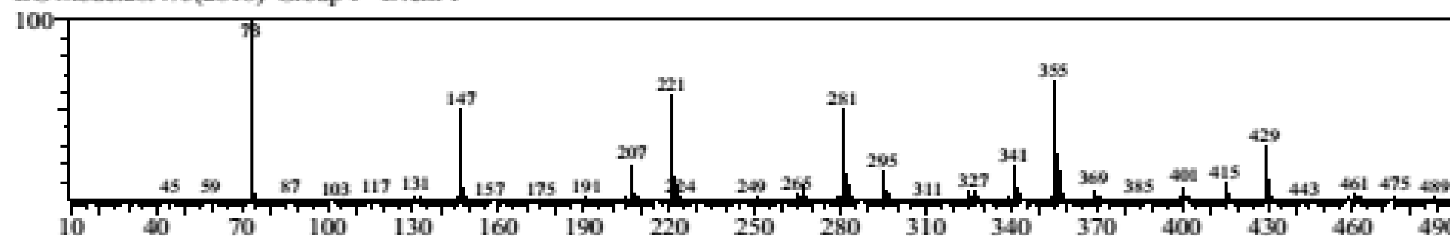
<< Target >>
Line#:25 R.Time:26.258(Scan#:2552) MassPeaks:251
RawMode:Single 26.258(2552) BasePeak:73.05(733056)
BG Mode:26.333(2561) Group 1 - Event 1



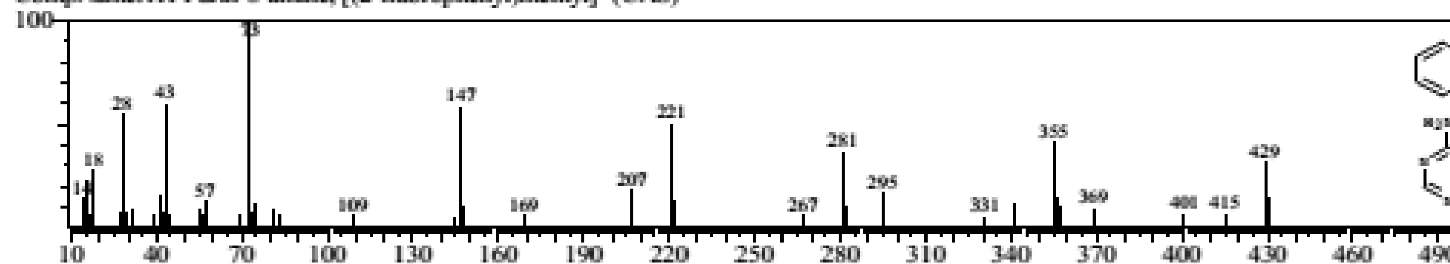
Hit#:1 Entry:148639 Library:WILEY7.LIB
SI:76 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



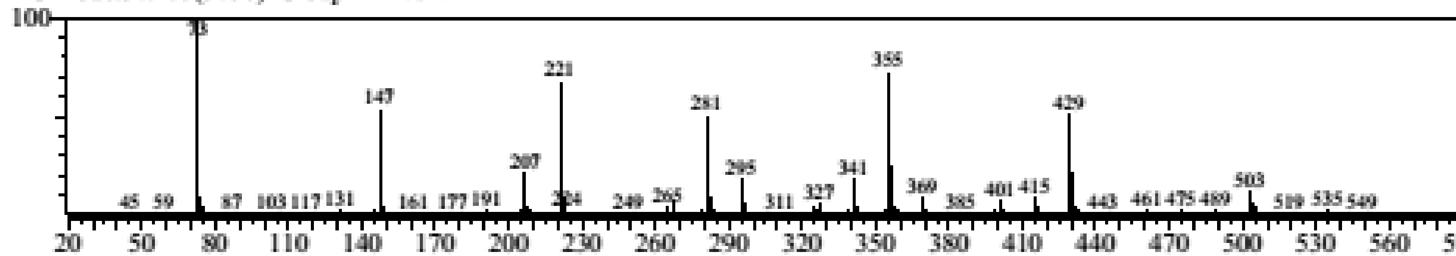
<<Target>>
Line#:26 R.Time:28.367(Scan#:2805) MassPeaks:213
RawMode:Single 28.367(2805) BasePeak:73.05(835525)
BG Mode:28.475(2818) Group 1 - Event 1



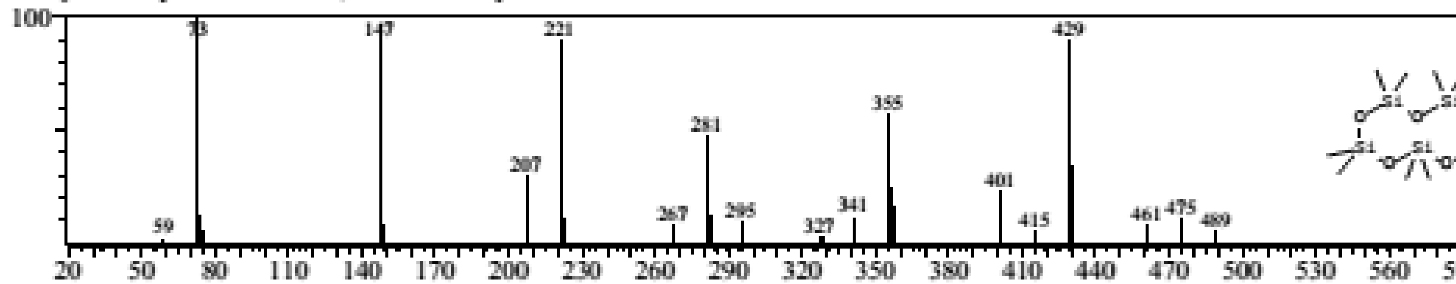
Hit#:1 Entry:148639 Library:WILEY7.LIB
SI:85 Formula:C12 H10 F N5 CAS:74421-44-6 MolWeight:243 RetIndex:0
CompName:1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)



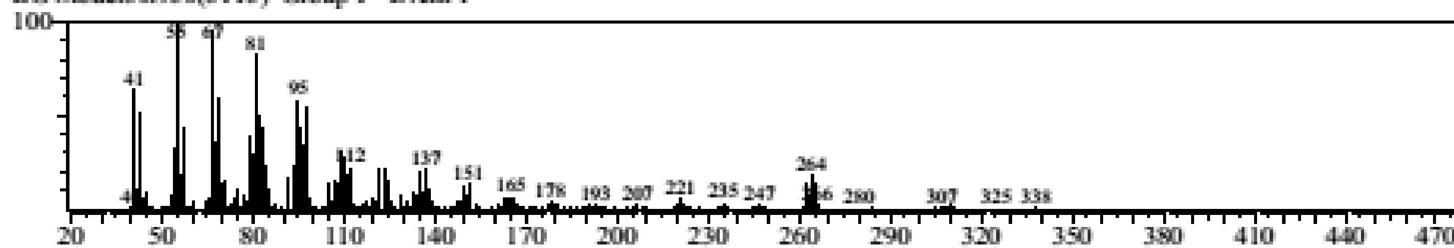
<< Target >>
Line#:28 R.Time:30.400(Scan#:3049) MassPeaks:203
RawMode:Single 30.400(3049) BasePeak:73.00(891953)
BG Mode:30.475(3058) Group 1 - Event 1



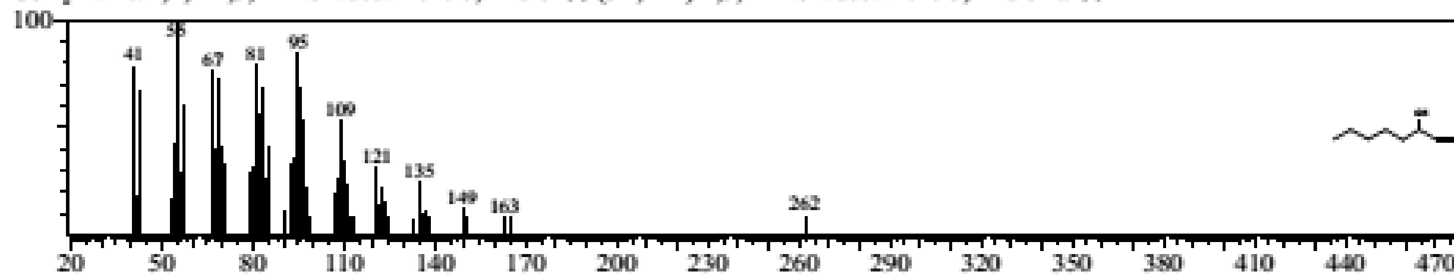
Hit#:1 Entry:27682 Library:NIST27.LIB
SI:85 Formula:C18H54O9Si9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:Cyclononasiloxane, octadecamethyl-



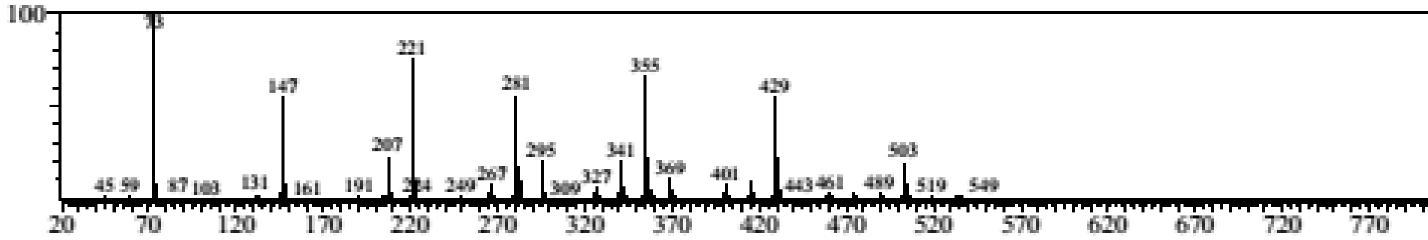
<< Target >>
Line#:29   R.Time:30.808(Scan#:3098) MassPeaks:235
RawMode:Single 30.808(3098) BasePeak:55.05(191753)
BG Mode:30.950(3115) Group 1 - Event 1



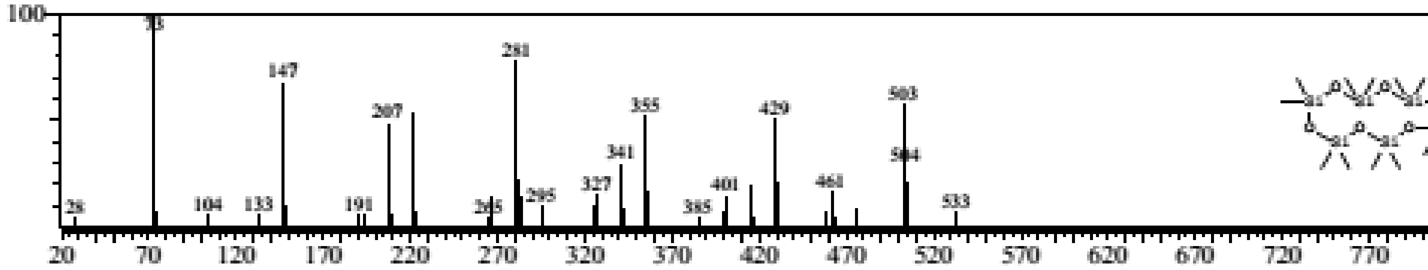
Hit#:1 Entry:90935 Library:NIST147.LIB
SI:88 Formula:C19H34O2 CAS:0-00-0 MolWeight:294 RetIndex:0
CompName:E,E,Z-1,3,12-Nonadecatriene-5,14-diol $$ (3E,12Z)-1,3,12-Nonadecatriene-5,14-diol # $$



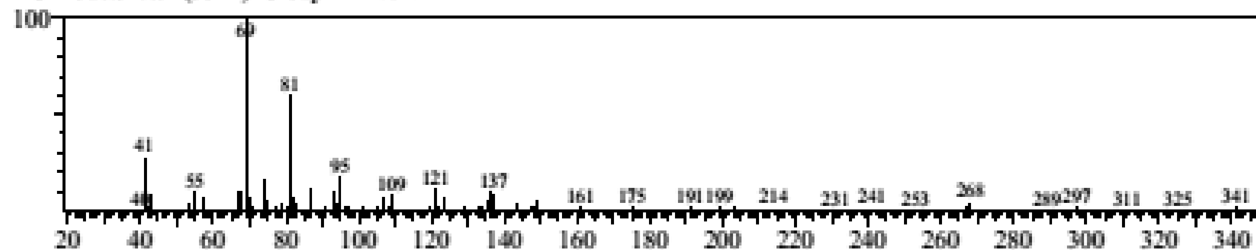
<<Target>>
Line#:32 R.Time:32.333(Scan#:3281) MassPeaks:199
RawMode:Single 32.333(3281) BasePeak:73.05(869771)
BG Mode:32.433(3293) Group 1 - Event 1



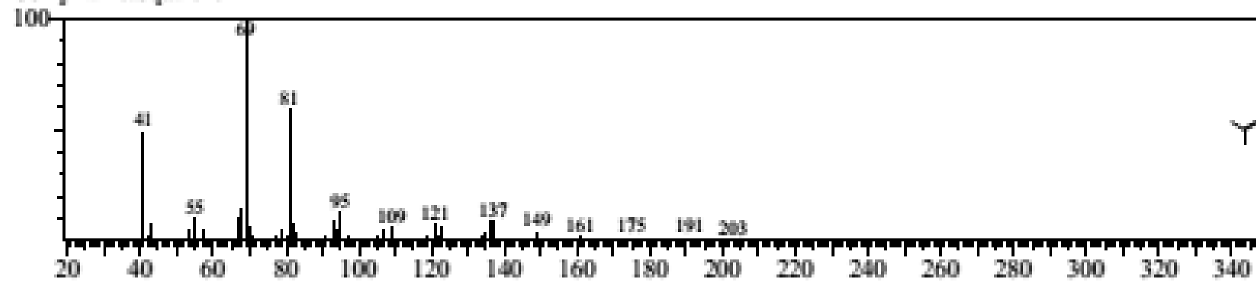
Hit#:1 Entry:146629 Library:NIST147.LIB
SI:85 Formula:C20H60O10Si10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:Cyclodecasiloxane, eicosamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane # $$



<< Target >>
Line#:33 R.Time:32.625(Scan#:3316) MassPeaks:256
RawMode:Single 32.625(3316) BasePeak:69.05(418498)
BG Mode:32.692(3324) Group 1 - Event 1



Hit#:1 Entry:26737 Library:NIST27.LIB
SI:90 Formula:C30H50 CAS:7683-64-9 MolWeight:410 RetIndex:0
CompName:Squalene

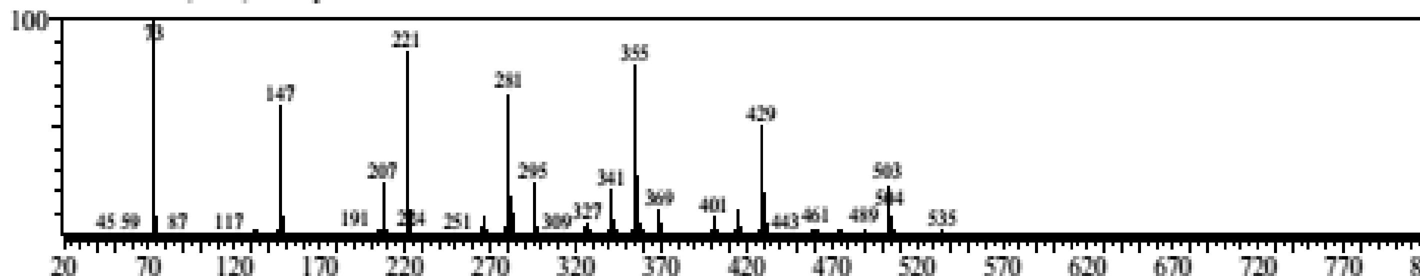


<< Target >>

Line#:34 R.Time:34.475(Scan#:3538) MassPeaks:188

RawMode:Single 34.475(3538) BasePeak:73.05(762000)

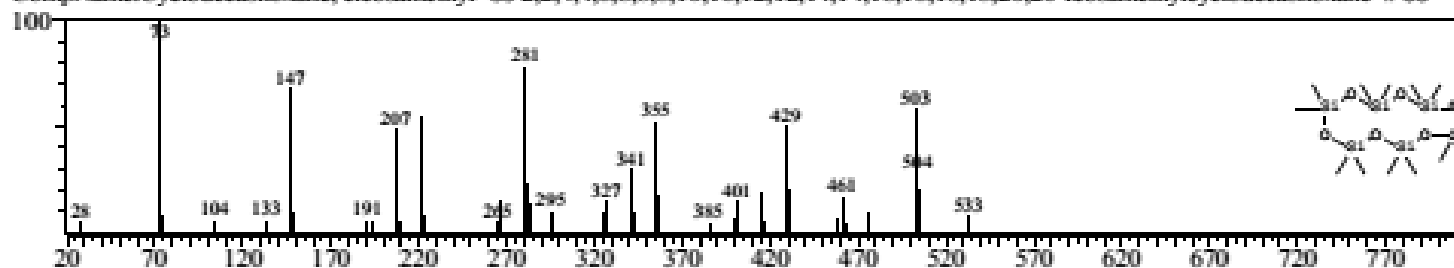
BG Mode:34.592(3552) Group 1 - Event 1



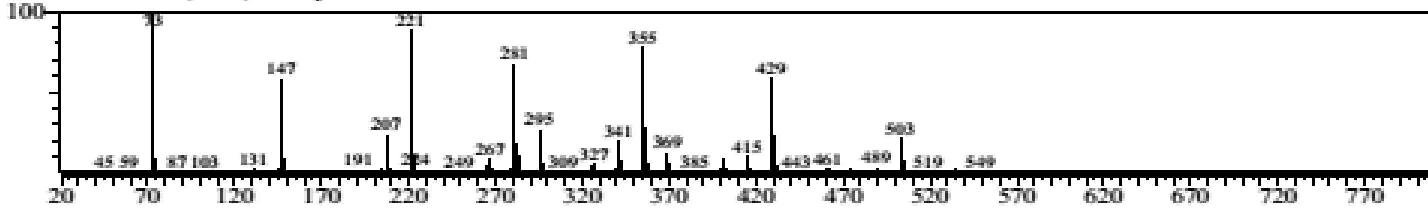
Hit#:1 Entry:146629 Library:NIST147.LIB

SI:86 Formula:C20H60O10Si10 CAS:18772-36-6 MolWeight:740 RetIndex:0

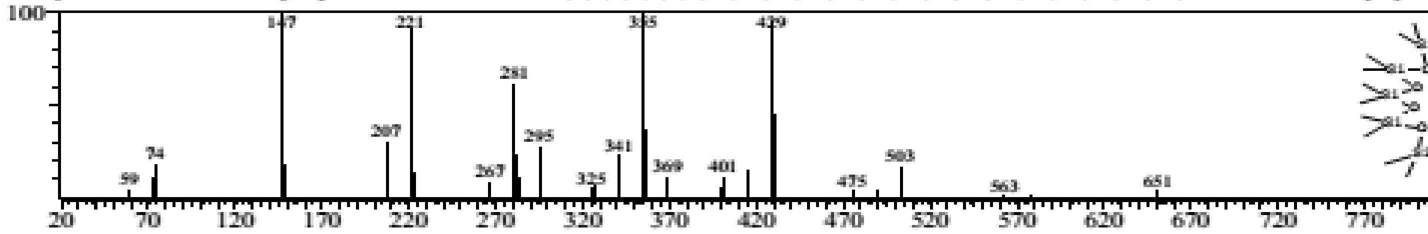
CompName:Cyclodecasiloxane, eicosamethyl- $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-Icosamethylcyclodecasiloxane # $$



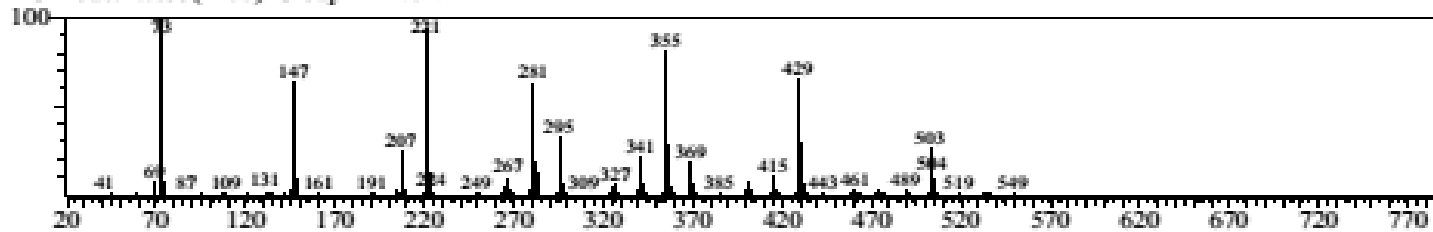
<< Target >>
Line#:35 R.Time:37.117(Scan#:3855) MassPeaks:190
RawMode:Single 37.117(3855) BasePeak:73.05(609397)
BG Mode:37.233(3869) Group 1 - Event 1



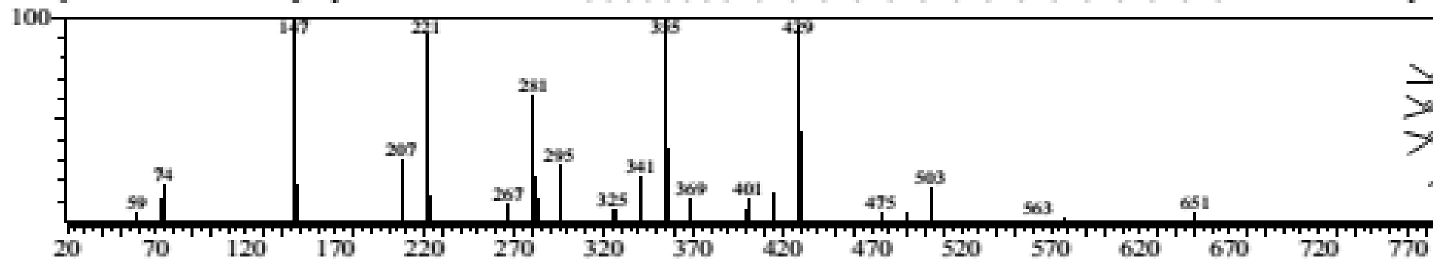
Hit#:1 Entry:147059 Library:NIST147.LIB
SI:85 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosamethylcycl



<< Target >>
Line#:37 R.Time:40.492(Scan#:4260) MassPeaks:234
RawMode:Single 40.492(4260) BasePeak:73.00(468891)
BG Mode:40.633(4277) Group 1 - Event 1

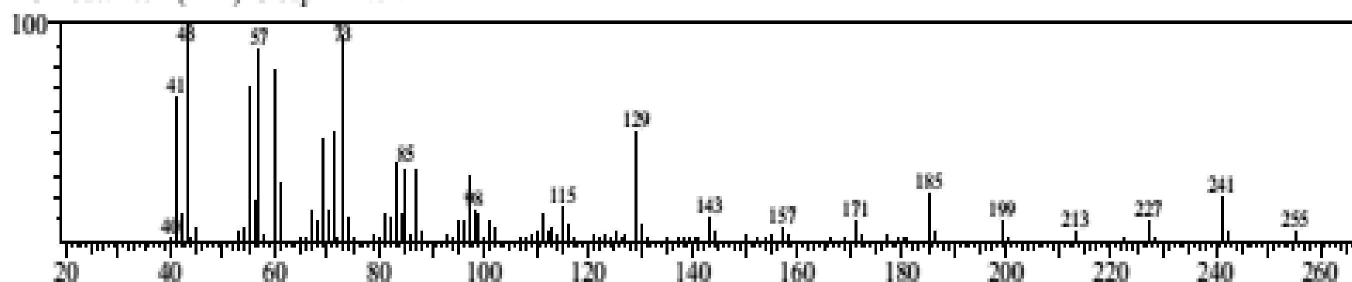


Hit#:1 Entry:147059 Library:NIST147.LIB
SI:85 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosamethylc

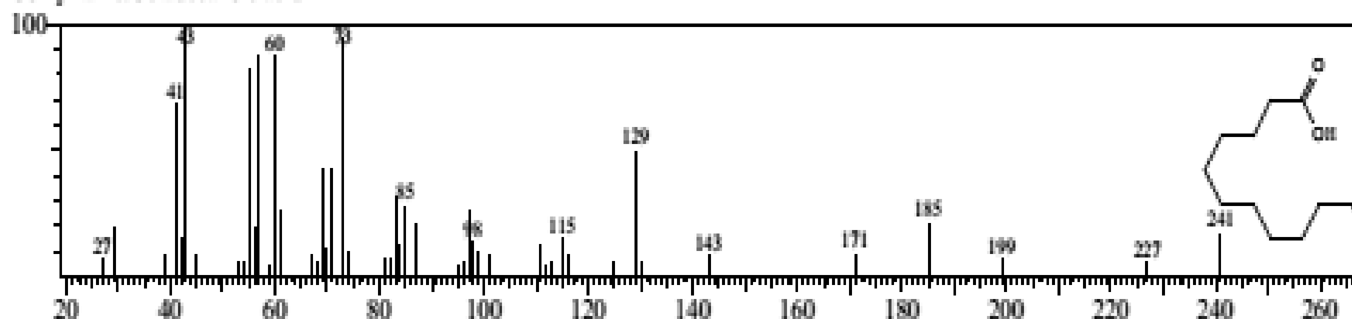


C. hartmannianum

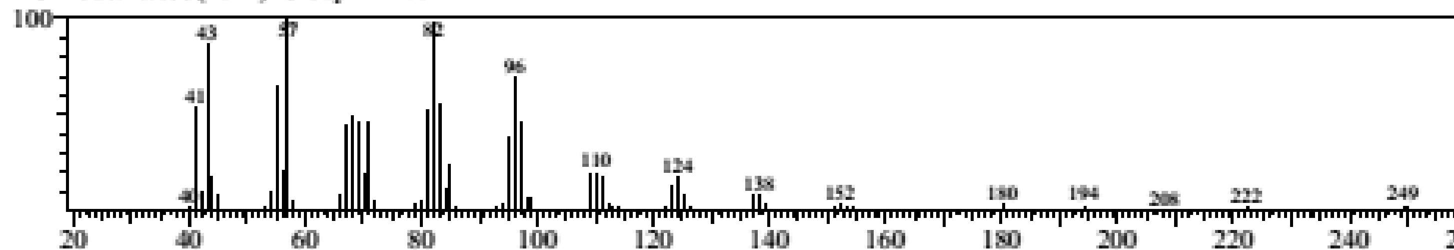
<< Target >>
Line#:4 R.Time:22.717(Scan#:2127) MassPeaks:123
RawMode:Single 22.717(2127) BasePeak:43.05(49630)
BG Mode:22.842(2142) Group 1 - Event 1



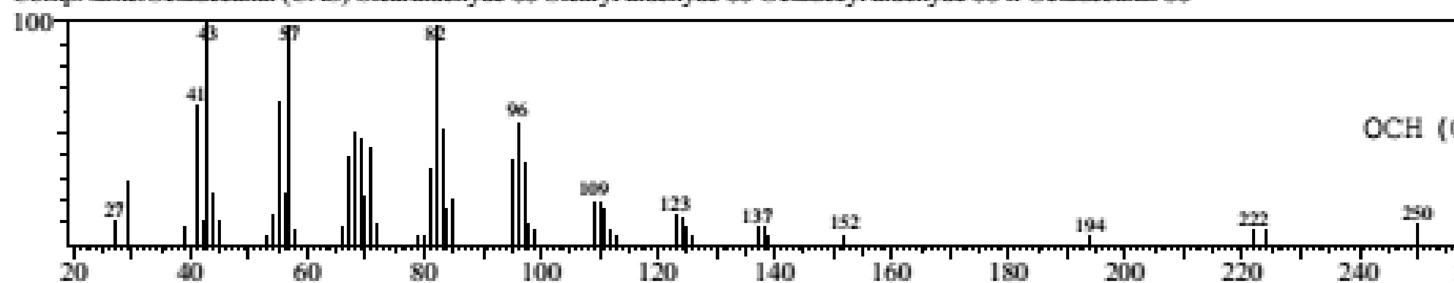
Hit#:1 Entry:22966 Library:NIST27.LIB
SI:95 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid



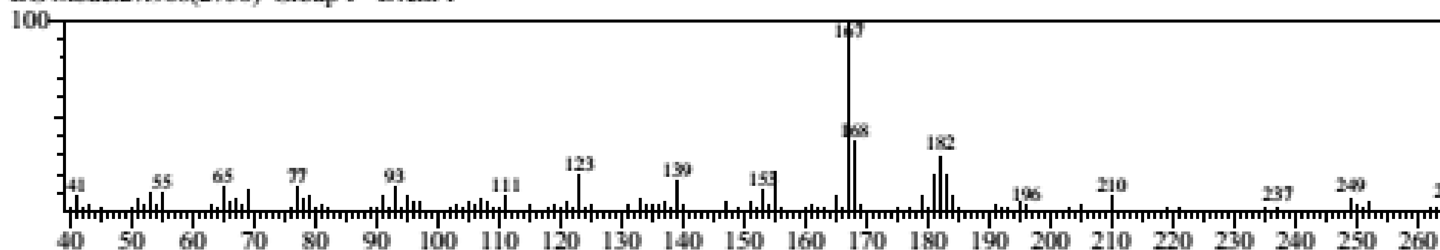
<< Target >>
Line#:6 R.Time:26.742(Scan#:2610) MassPeaks:87
RawMode:Single 26.742(2610) BasePeak:57.05(47886)
BG Mode:26.833(2621) Group 1 - Event 1



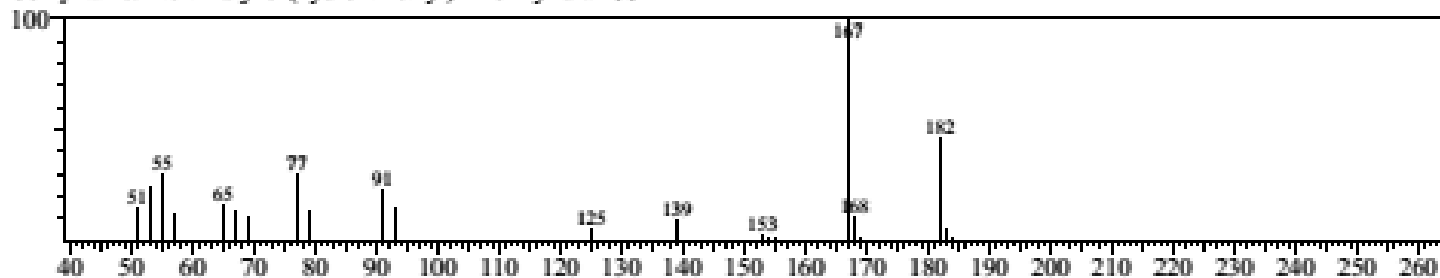
Hit#:1 Entry:178149 Library:WILEY7.LIB
SI:96 Formula:C18 H36 O CAS:638-66-4 MolWeight:268 RetIndex:0
CompName:Octadecanal (CAS) Stearaldehyde $$ Stearyl aldehyde $$ Octadecyl aldehyde $$ n-Octadecanal $$



<< Target >>
Line#:7   R.Time:27.758(Scan#:2732) MassPeaks:204
RawMode:Single 27.758(2732) BasePeak:167.10(132405)
BG Mode:27.908(2750) Group 1 - Event 1

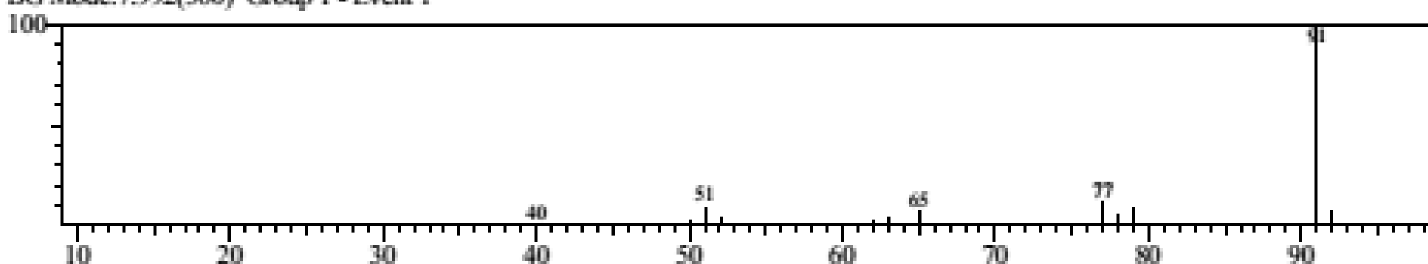


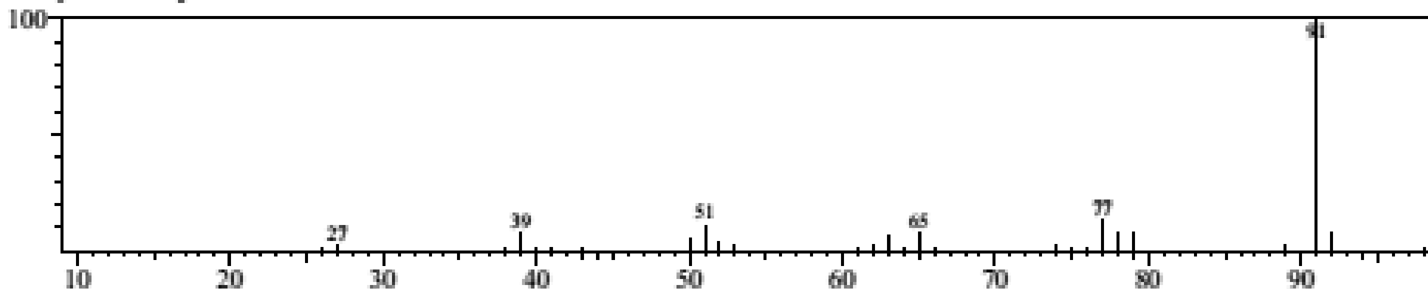
Hit#:1 Entry:73402 Library:WILEY7.LIB
SI:61 Formula:C10 H14 O3 CAS:0-00-0 MolWeight:182 RetIndex:0
CompName:2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran $$



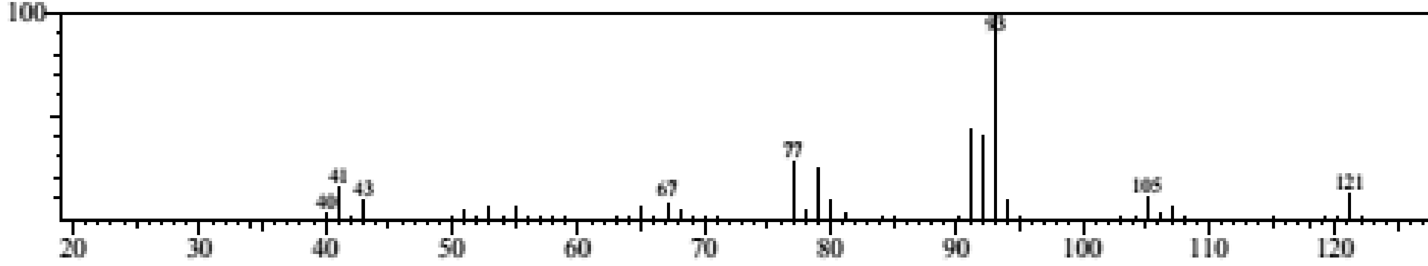
GC/MS structures of fragrant aromatic compounds (Terpenoids) in the petroleum ether fractions of *Aseyal, C. ha* ***laxiflora*** **fermented wood "*Nikhra*"**

T. laxiflora

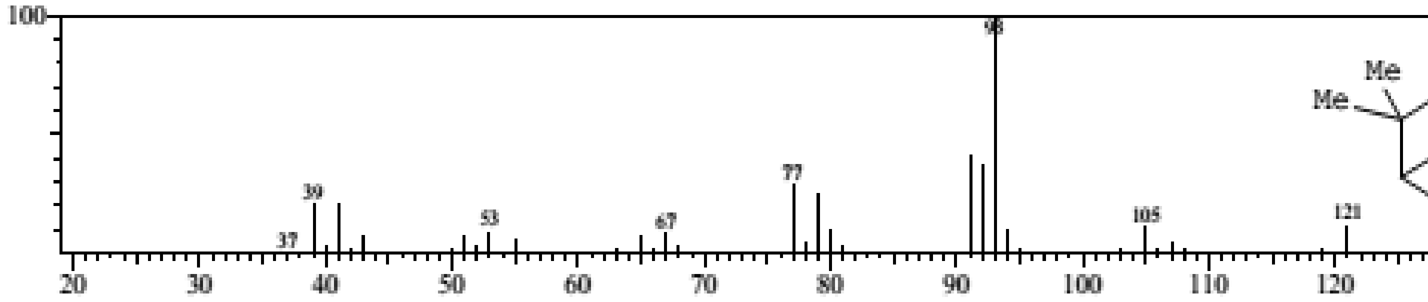




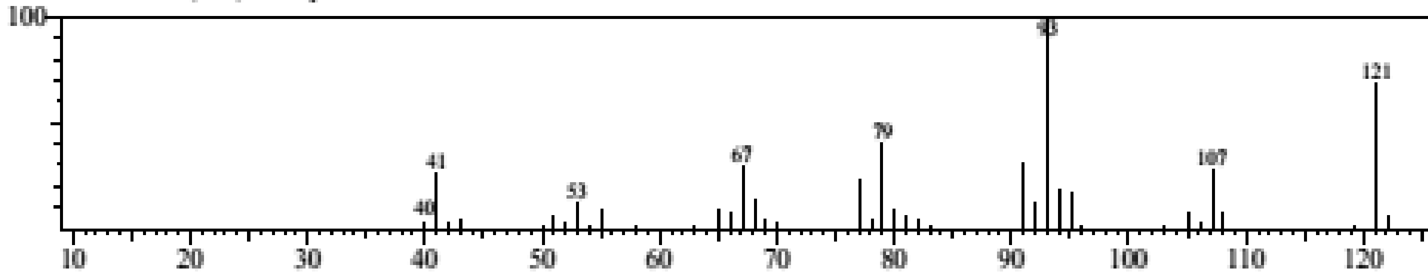
<< Target >>
Line#:9 R.Time:9.542(Scan#:546) MassPeaks:71
RawMode:Single 9.542(546) BasePeak:93.10(1079940)
BG Mode:9.608(554) Group 1 - Event 1



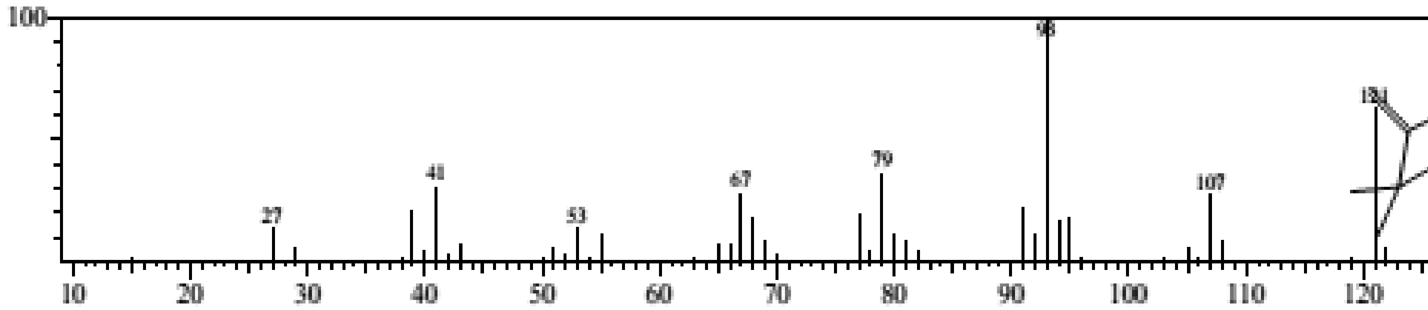
Hit#:1 Entry:26447 Library:WILEY7.LIB
SI:98 Formula:C10 H16 CAS:80-56-8 MolWeight:136 RetIndex:0
CompName:.ALPHA.-PINENE, (-)- $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- (CAS) Pinene $$ 2-Pinene $$ .alpha.-Pinene $$ 2,6,6-Tri



<< Target >>
Line#:10 R.Time:10.167(Scan#:621) MassPeaks:44
RawMode:Single 10.167(621) BasePeak:93.10(100260)
BG Mode:10.242(630) Group 1 - Event 1



Hit#:1 Entry:6316 Library:NIST27.LIB
SI:97 Formula:C10H16 CAS:79-92-5 MolWeight:136 RetIndex:0
CompName:Camphene

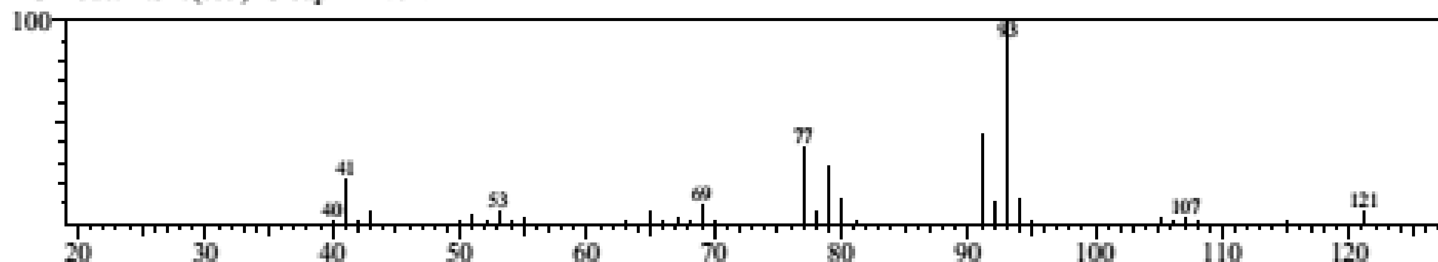


<< Target >>

Line#:11 R.Time:11.258(Scan#:752) MassPeaks:47

RawMode:Single 11.258(752) BasePeak:93.10(227884)

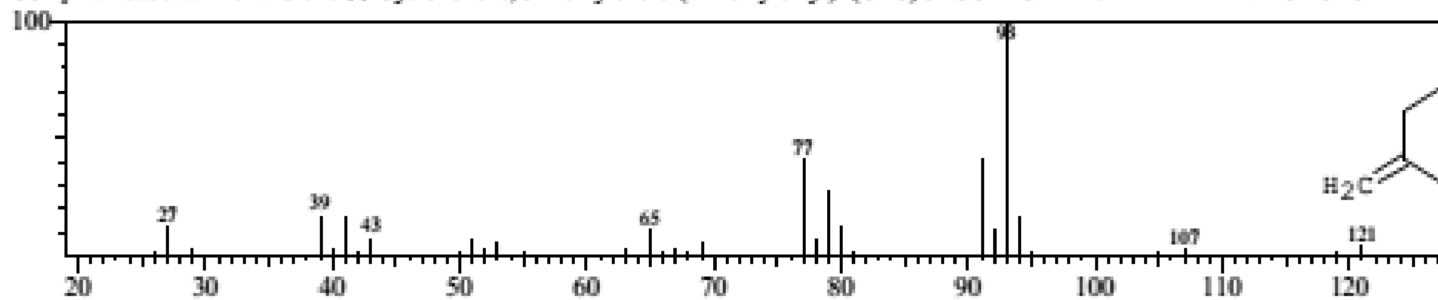
BG Mode:11.317(759) Group 1 - Event 1



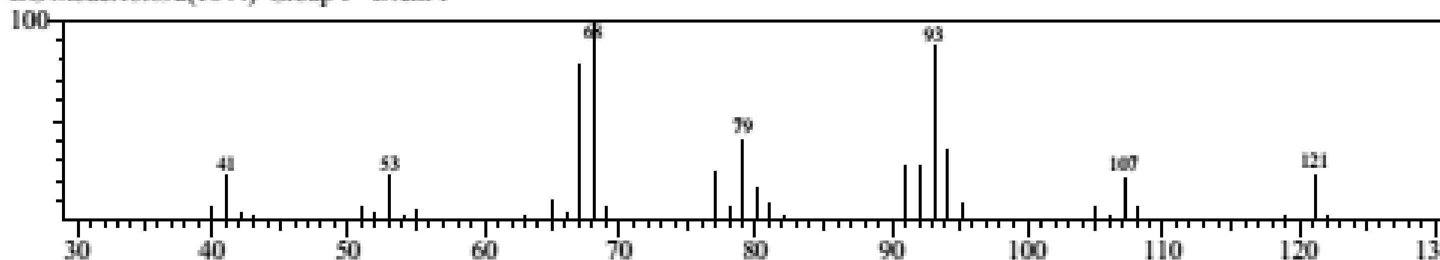
Hit#:1 Entry:26356 Library:WILEY7.LIB

SI:97 Formula:C10 H16 CAS:555-10-2 MolWeight:136 RetIndex:0

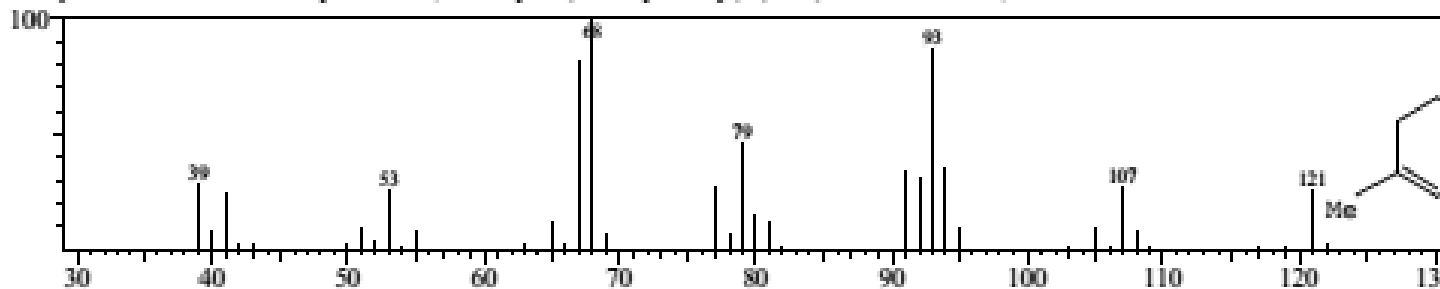
CompName:.beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- (CAS) 3-ISOPROPYL-6-METHYLENE-CYCLOHEXE



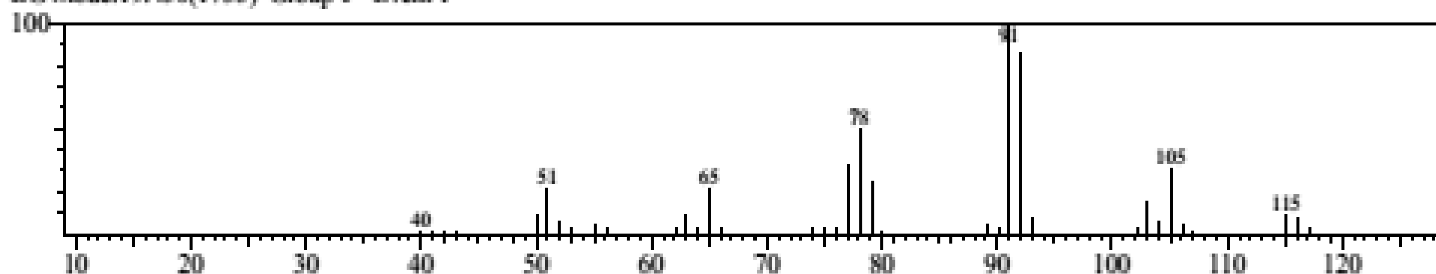
<< Target >>
Line#:14 R.Time:13.633(Scan#:1037) MassPeaks:42
RawMode:Single 13.633(1037) BasePeak:68.05(75154)
BG Mode:13.692(1044) Group 1 - Event 1



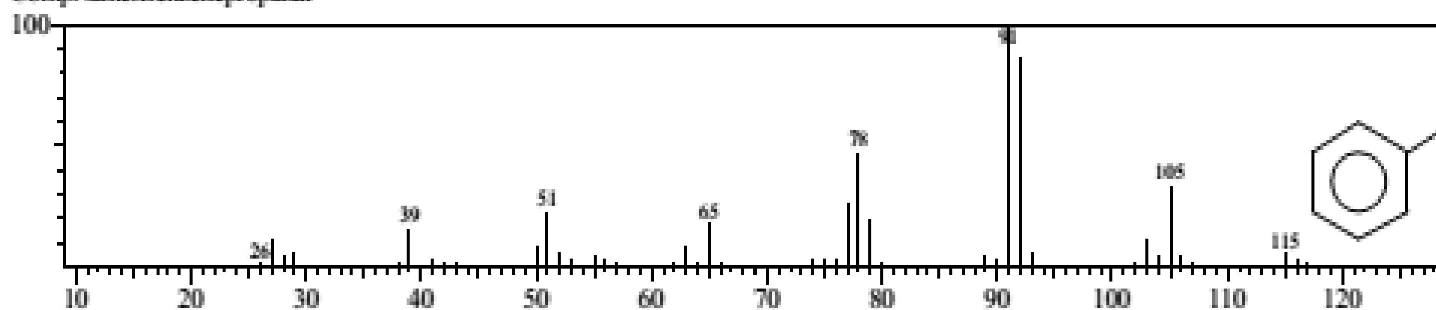
Hit#:1 Entry:26305 Library:WILEY7.LIB
SI:98 Formula:C10 H16 CAS:138-86-3 MolWeight:136 RetIndex:0
CompName:dl-Limonene $$ Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS) 1-P-MENTHA-1,8-DIENE $$ Limonene $Cinen $$ Nesol $$



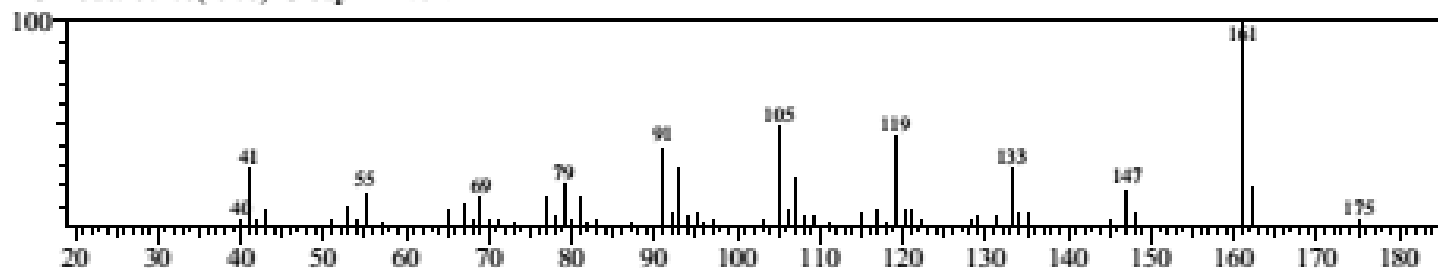
<<< Target >>
Line#:16 R.Time:19.333(Scan#:1721) MassPeaks:39
RawMode:Single 19.333(1721) BasePeak:91.05(57588)
BG Mode:19.450(1735) Group 1 - Event 1



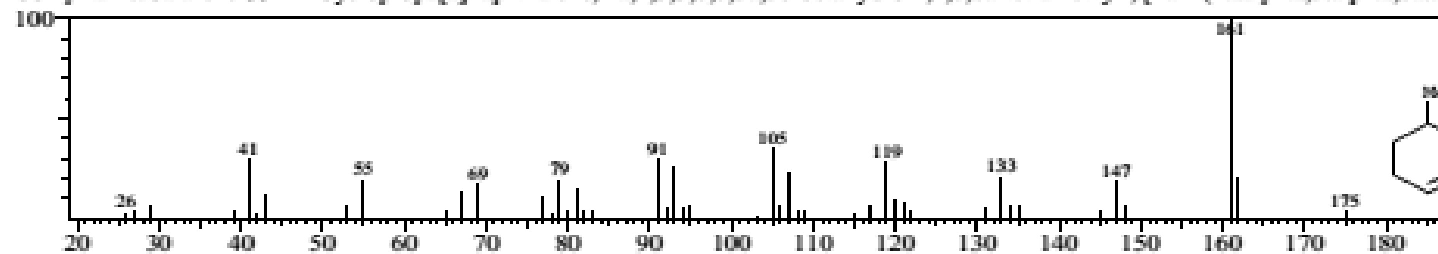
Hit#:1 Entry:5868 Library:NIST27.LIB
SI:97 Formula:C9H10O CAS:104-53-0 MolWeight:134 RetIndex:0
CompName:Benzenepropanal



<< Target >>
Line#:20 R.Time:29.142(Scan#:2898) MassPeaks:63
RawMode:Single 29.142(2898) BasePeak:161.15(40463)
BG Mode:29.208(2906) Group 1 - Event 1



Hit#:1 Entry:101030 Library:WILEY7.LIB
SI:93 Formula:C15 H24 CAS:17334-55-3 MolWeight:204 RetIndex:0
CompName:Calarene $$ 1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.a

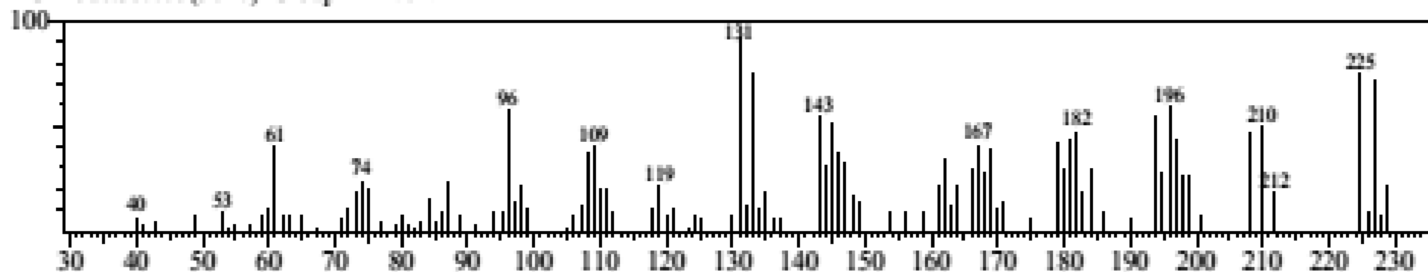


<< Target >>

Line#:22 R.Time:34.992(Scan#:3600) MassPeaks:113

RawMode:Single 34.992(3600) BasePeak:256.90(16553)

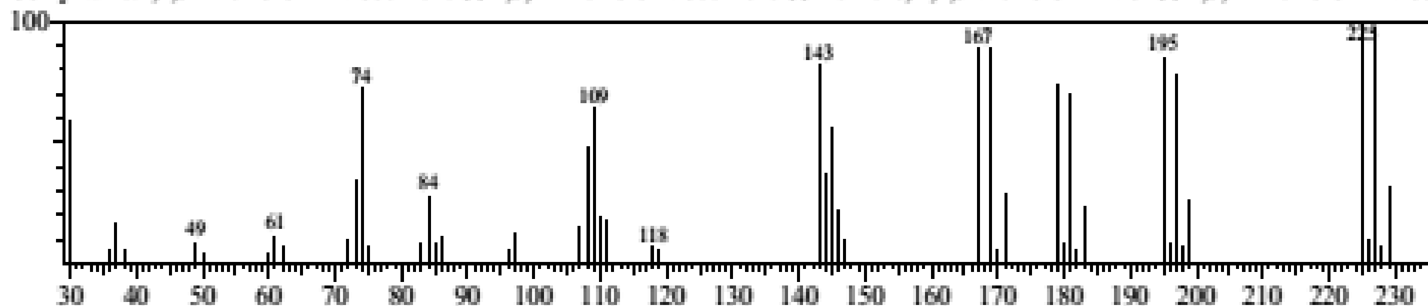
BG Mode:35.075(3610) Group 1 - Event 1



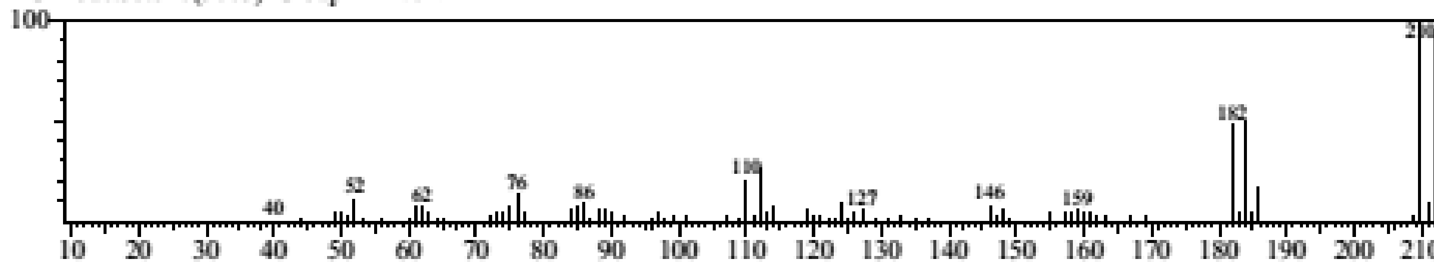
Hit#:1 Entry:53835 Library:NIST147.LIB

SI:61 Formula:C6H2Cl3NO2 CAS:17700-09-3 MolWeight:225 RetIndex:0

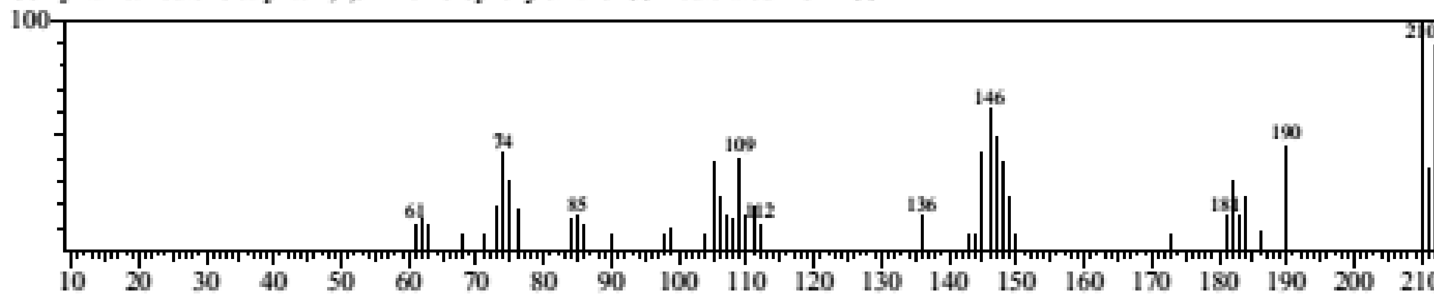
CompName:1,2,3-Trichloro-4-nitrobenzene $$ 2,3,4-Trichloronitrobenzene $$ Benzene, 1,2,3-trichloro-4-nitro- $$ 2,3,4-Trichloro-1-nitrob



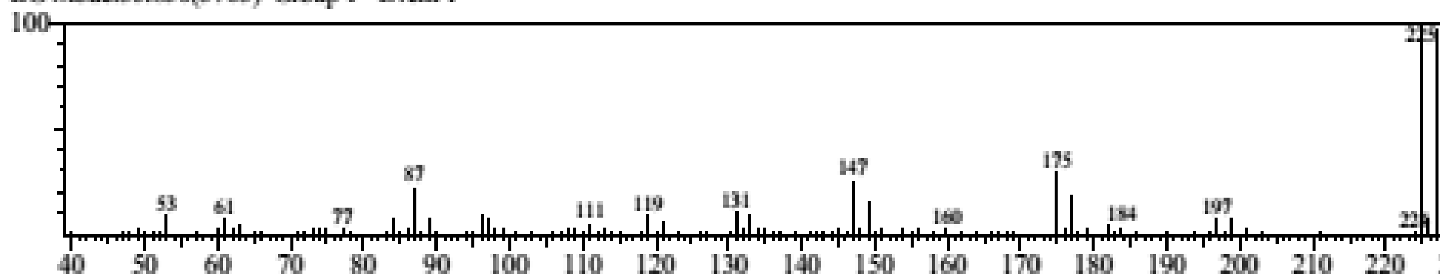
<<Target>>
Line#:23   R.Time:35.433(Scan#:3653) MassPeaks:92
RawMode:Single 35.433(3653) BasePeak:209.90(48091)
BG Mode:35.517(3663) Group 1 - Event 1



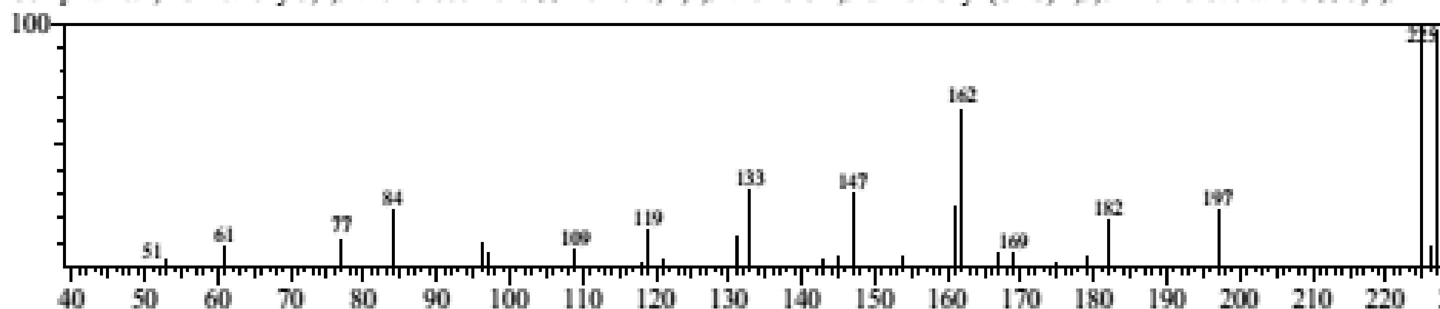
Hit#:1 Entry:124934 Library:WILEY7.LIB
SI:61 Formula:C8 H6 D CL3 O CAS:0-00-0 MolWeight:224 RetIndex:0
CompName:Deuterio-.alpha.-2,4,5-Trichlorophenylethanol $$ Deuterated-TCPE $$



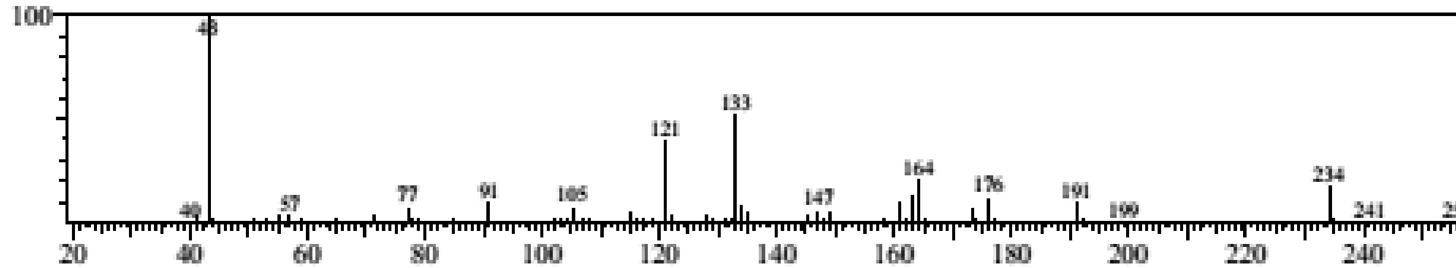
<< Target >>
Line#:24 R.Time:35.767(Scan#:3693) MassPeaks:139
RawMode:Single 35.767(3693) BasePeak:224.90(178031)
BG Mode:35.850(3703) Group 1 - Event 1



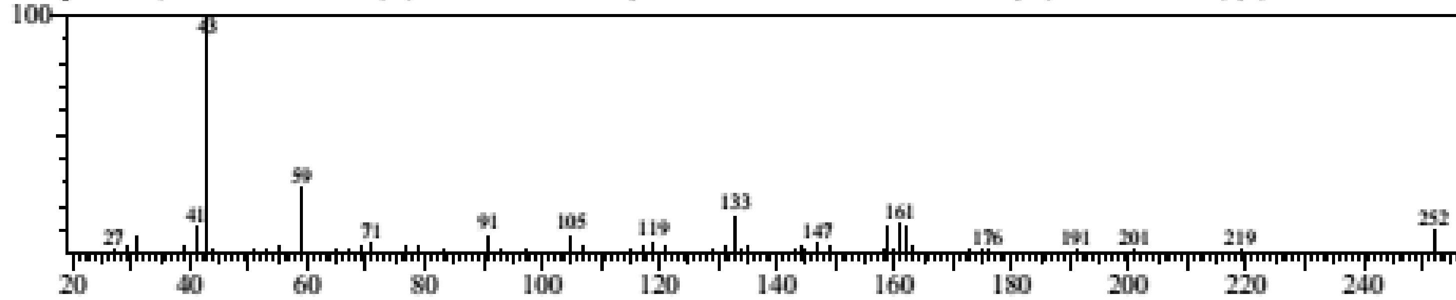
Hit#:1 Entry:144643 Library:WILEY7.LIB
SI:74 Formula:C8 H7 CL3 O2 CAS:16766-29-3 MolWeight:240 RetIndex:0
CompName:1,2-dimethoxy-3,4,5-trichlorobenzene $$ Benzene, 1,2,3-trichloro-4,5-dimethoxy- (CAS) 4,5,6-Trichloroveratrole $$ 3,4,5-Tri



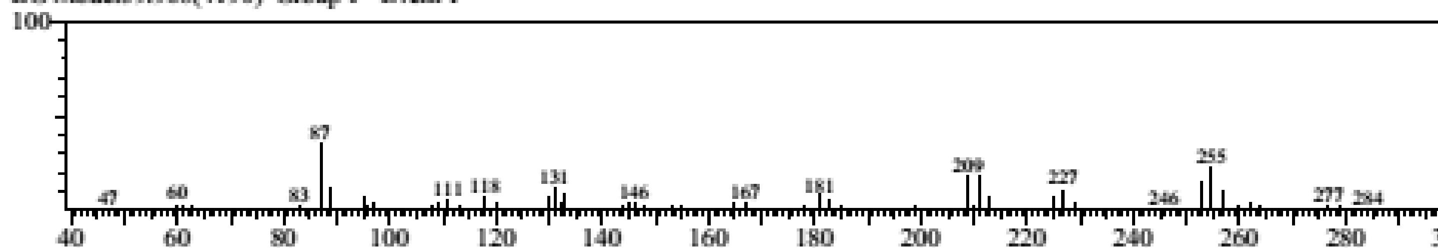
<< Target >>
Line#:26 R.Time:39.242(Scan#:4110) MassPeaks:111
RawMode:Single 39.242(4110) BasePeak:43.05(200462)
BG Mode:39.300(4117) Group 1 - Event 1



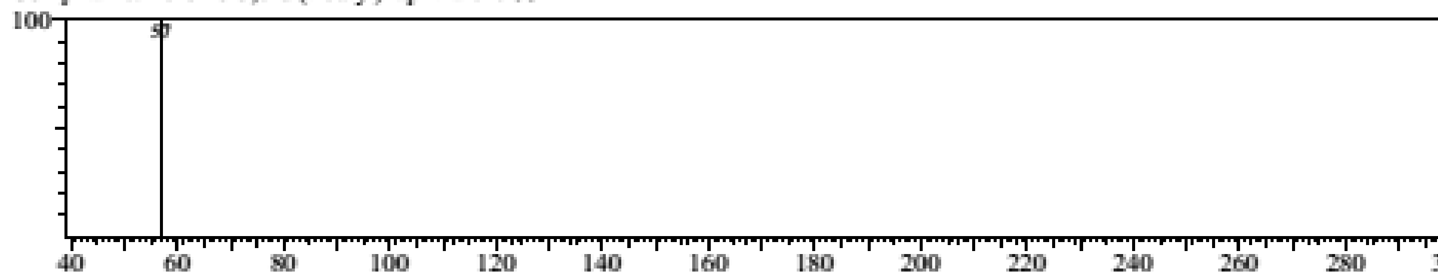
Hit#:1 Entry:159450 Library:WILEY7.LIB
SI:72 Formula:C15 H24 O3 CAS:57956-69-1 MolWeight:252 RetIndex:0
CompName:5,7-DECADIEN-3-IN, 2,9-DIHYDROXY-5-(1-HYDROXY-1-METHYLETHYL)-2,9-DIMETHYL-, (Z,E $$



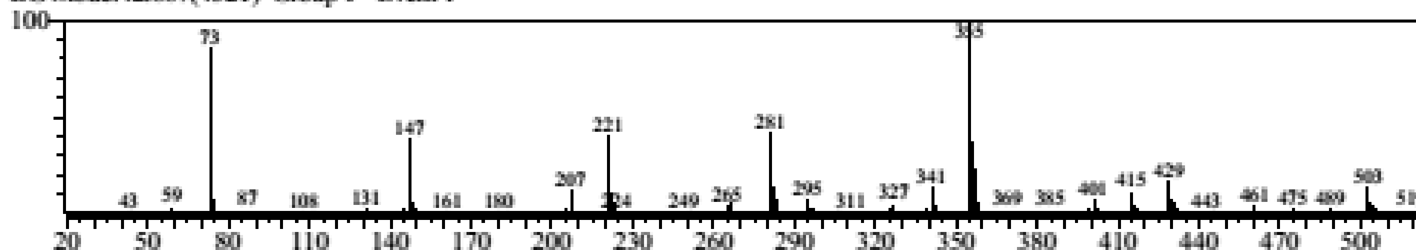
<< Target >>
Line#:28 R.Time:39.858(Scan#:4184) MassPeaks:222
RawMode:Single 39.858(4184) BasePeak:304.80(506589)
BG Mode:39.908(4190) Group 1 - Event 1



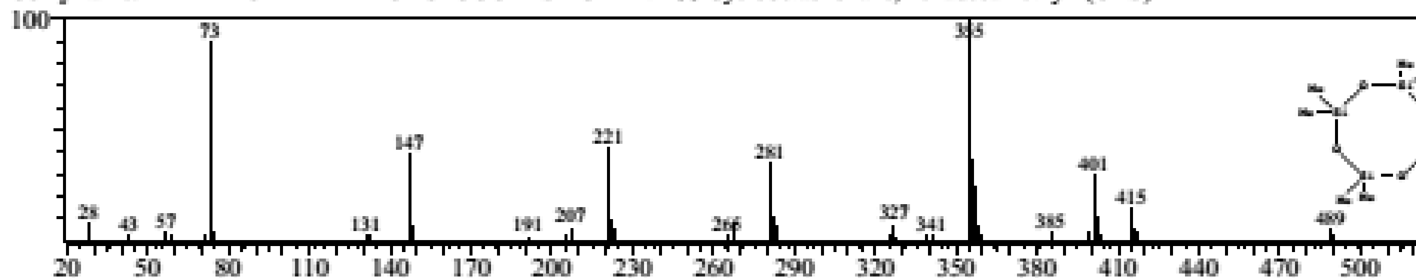
Hit#:1 Entry:228279 Library:WILEY7.LIB
SI:61 Formula:C18 H23 BR CAS:10239-76-6 MolWeight:318 RetIndex:0
CompName:1-bromo-3,6-di(t-butyl)naphthalene $$



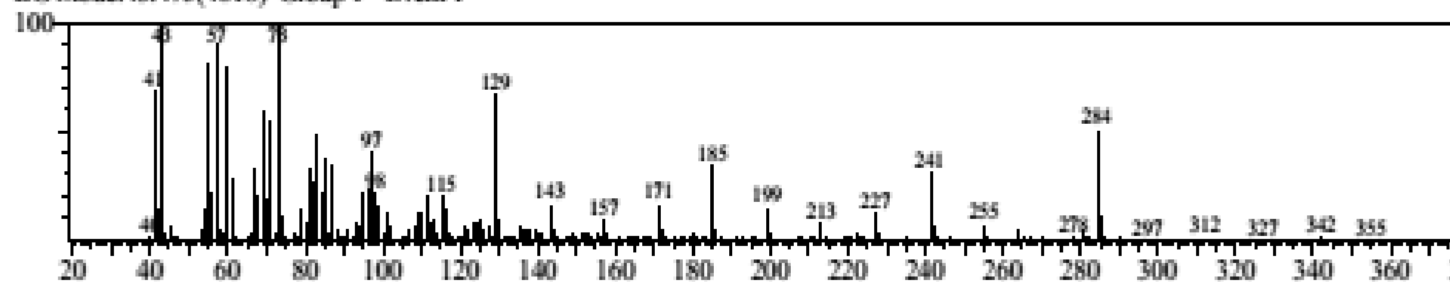
<< Target >>
Line#:35 R.Time:42.642(Scan#:4518) MassPeaks:259
RawMode:Single 42.642(4518) BasePeak:355.00(404379)
BG Mode:42.667(4521) Group 1 - Event 1



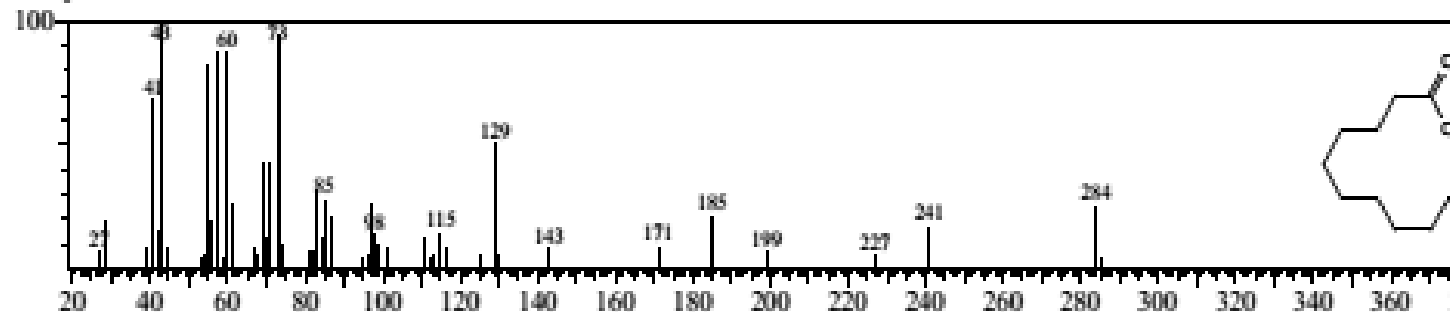
Hit#:1 Entry:328298 Library:WILEY7.LIB
SI:86 Formula:C16 H48 O8 SI8 CAS:556-68-3 MolWeight:592 RetIndex:0
CompName:HEXADECAMETHYLCYCLOOCTASILOXANE $$ Cyclooctasiloxane, hexadecamethyl- (CAS)



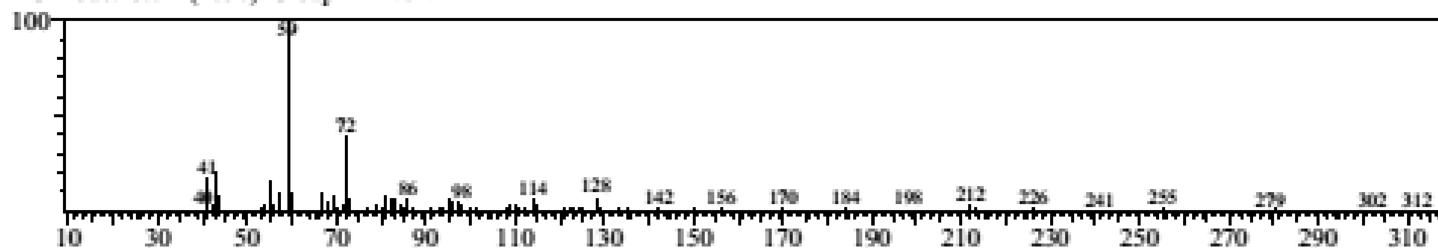
<< Target >>
Line#:38 R.Time:43.433(Scan#:4613) MassPeaks:247
RawMode:Single 43.433(4613) BasePeak:73.05(307913)
BG Mode:43.475(4618) Group 1 - Event 1



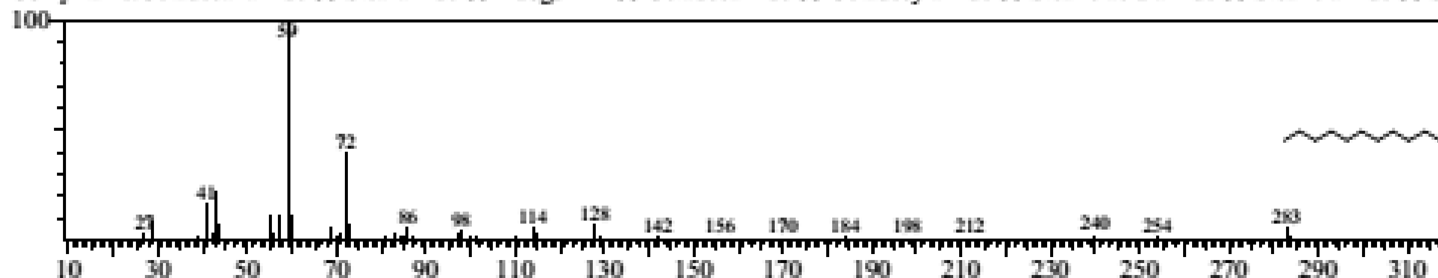
Hit#:1 Entry:22966 Library:NIST27.LIB
SI:89 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid



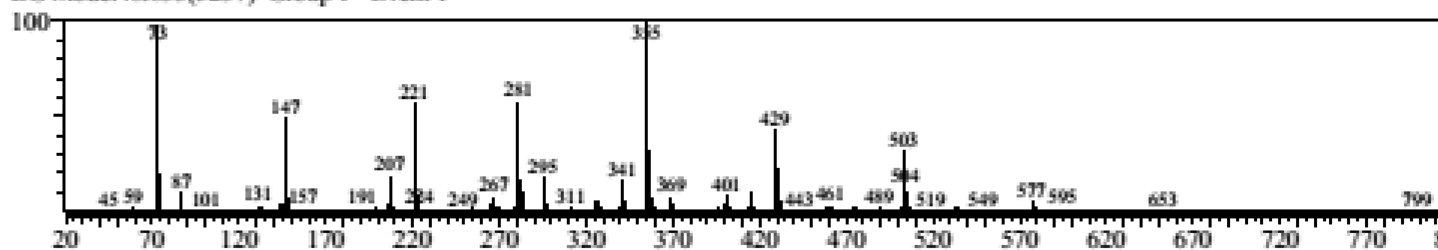
<< Target >>
Line#:39 R.Time:43.683(Scan#:4643) MassPeaks:218
RawMode:Single 43.683(4643) BasePeak:59.05(789295)
BG Mode:43.742(4650) Group 1 - Event 1



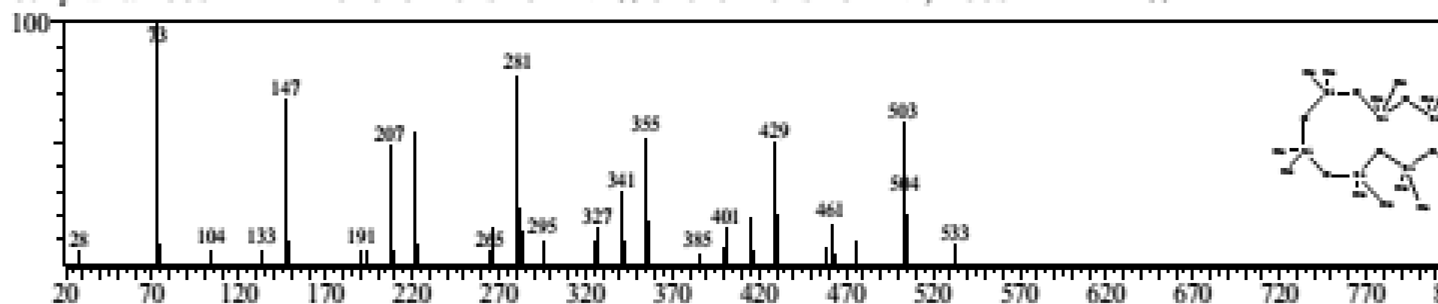
Hit#:1 Entry:85274 Library:NIST147.LIB
SI:89 Formula:C18H37NO CAS:124-26-5 MolWeight:283 RetIndex:0
CompName:Octadecanamide $$ Stearamide $$ Adogen 42 $$ Octadecamide $$ Octadecylamide $$ Stearic acid amide $$ Stearic amide $$ S



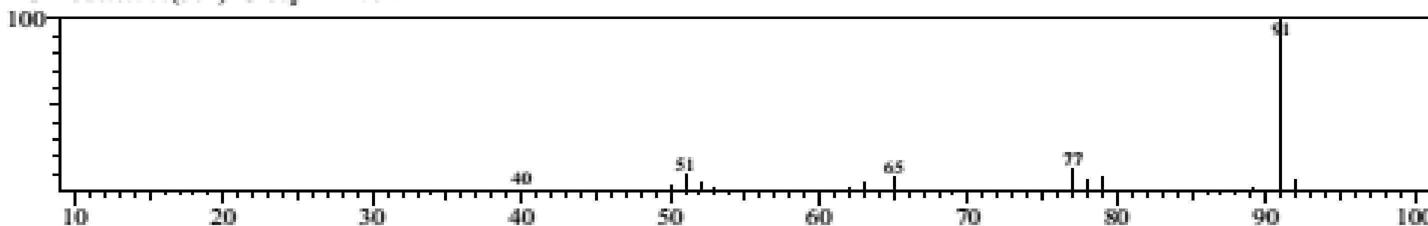
<< Target >>
Line#:48 R.Time:48.517(Scan#:5223) MassPeaks:271
RawMode:Single 48.517(5223) BasePeak:355.00(320996)
BG Mode:48.633(5237) Group 1 - Event 1



Hit#:1 Entry:335376 Library:WILEY7.LIB
SI:83 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



Aseyal



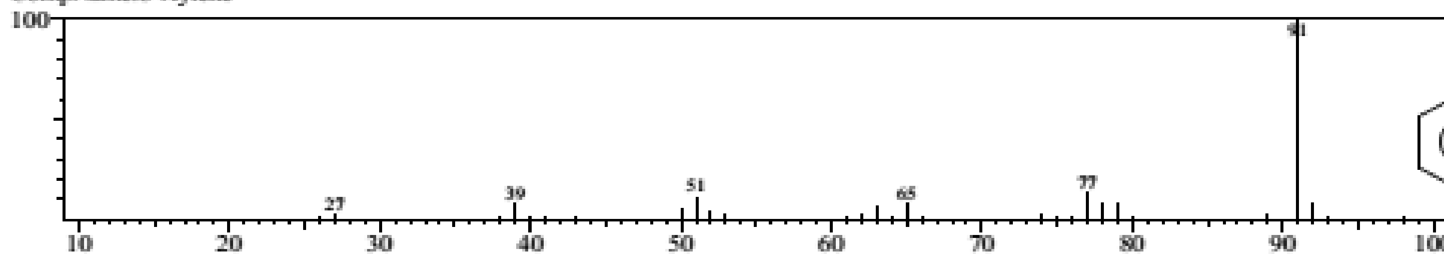
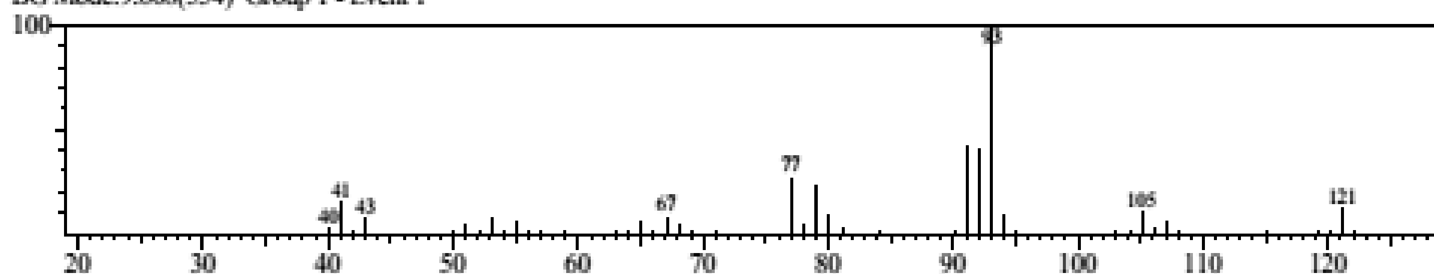
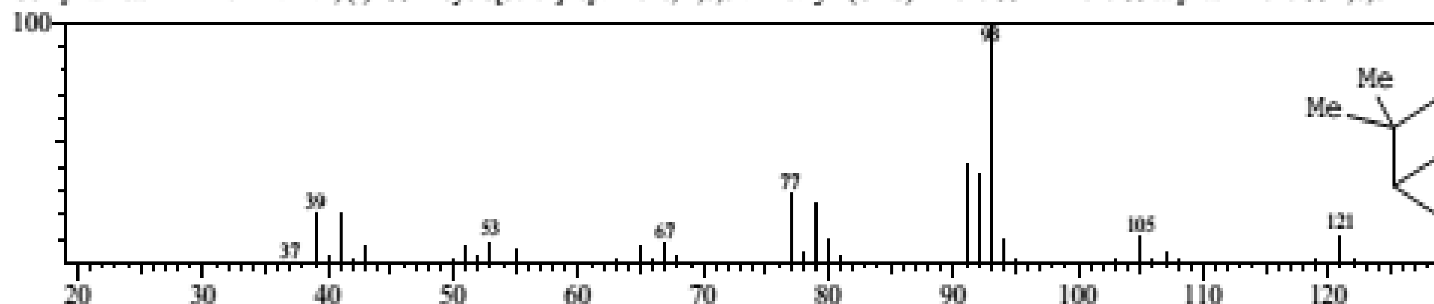


Fig. 4.175 Peak No.6 GC/MS date (R_t), molecular weight (*m/z*), GC/MS date (*m/z*) and assigned of the fermented wood "Nikhra" of *seyal* of petrolum ether extract (Terpenoids compounds)



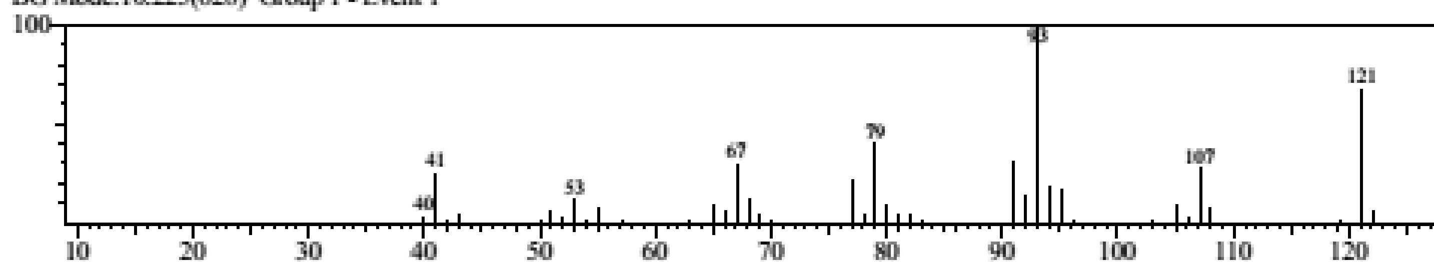


<< Target >>

Line#:9 R.Time:10.167(Scan#:621) MassPeaks:44

RawMode:Single 10.167(621) BasePeak:93.10(99030)

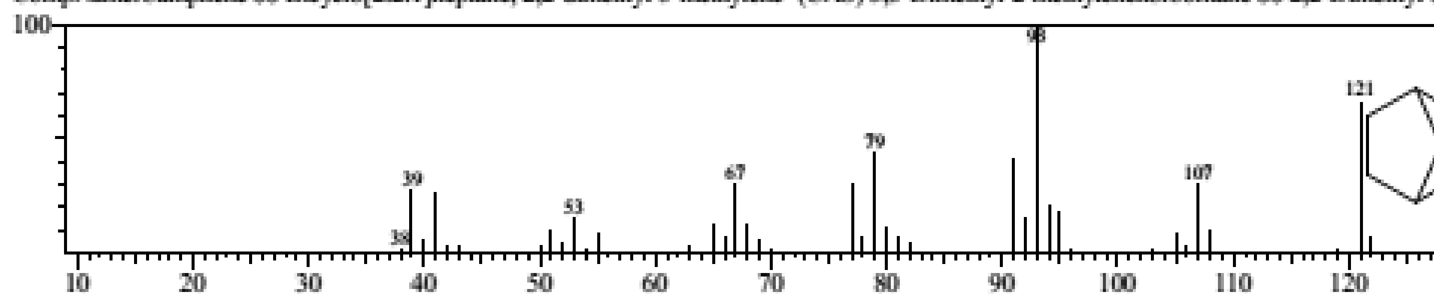
BG Mode:10.225(628) Group 1 - Event 1



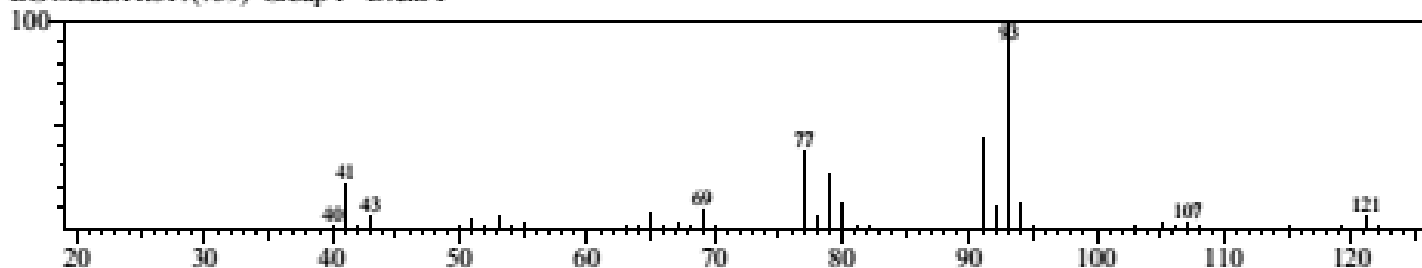
Hit#:1 Entry:26393 Library:WILEY7.LIB

SI:97 Formula:C10 H16 CAS:79-92-5 MolWeight:136 RetIndex:0

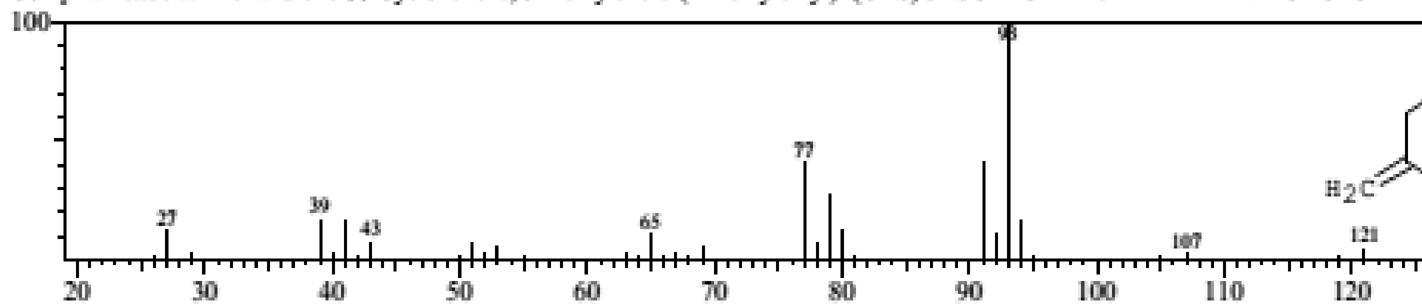
CompName:Camphene $$ Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene- (CAS) 3,3-Dimethyl-2-methylenenorbornane $$ 2,2-Dimethyl-



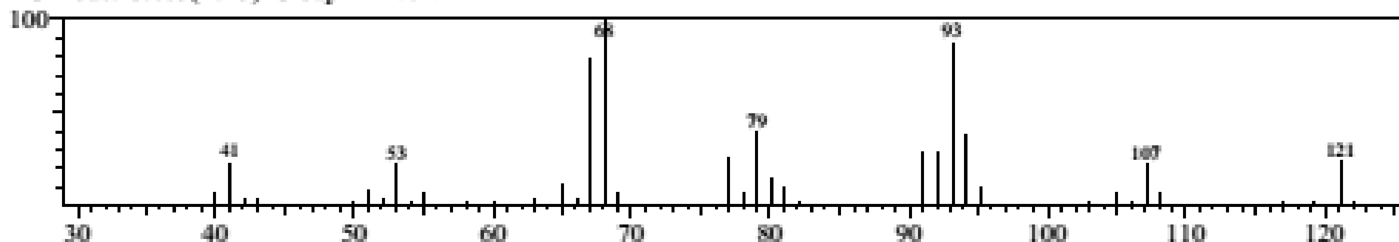
<< Target >>
Line#:10 R.Time:11.258(Scan#:752) MassPeaks:47
RawMode:Single 11.258(752) BasePeak:93.10(231290)
BG Mode:11.317(759) Group 1 - Event 1



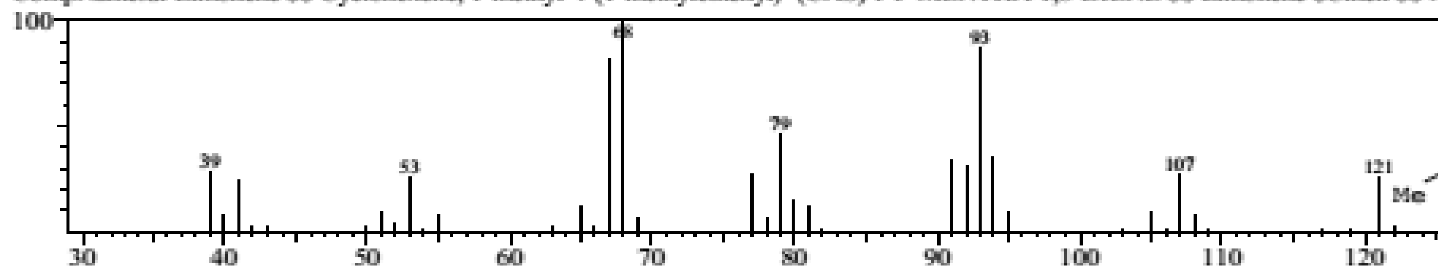
Hit#:1 Entry:26356 Library:WILEY7.LIB
SI:97 Formula:C10 H16 CAS:555-10-2 MolWeight:136 RetIndex:0
CompName:.beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- (CAS) 3-ISOPROPYL-6-METHYLENE-CYCLOHEX



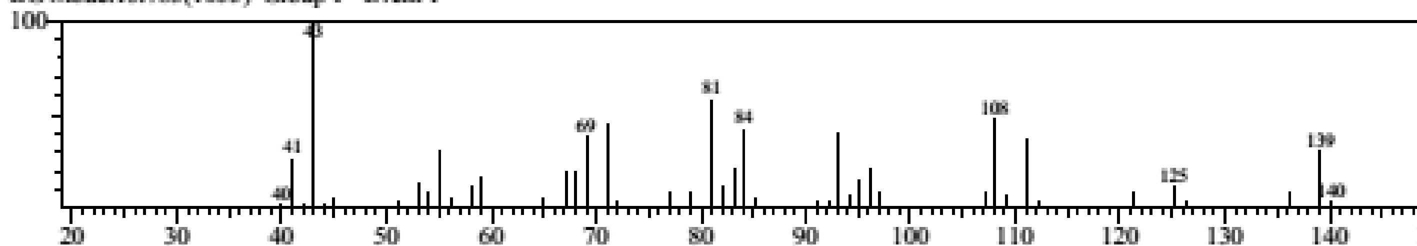
<< Target >>
Line#:13 R.Time:13.625(Scan#:1036) MassPeaks:43
RawMode:Single 13.625(1036) BasePeak:68.05(74162)
BG Mode:13.683(1043) Group 1 - Event 1



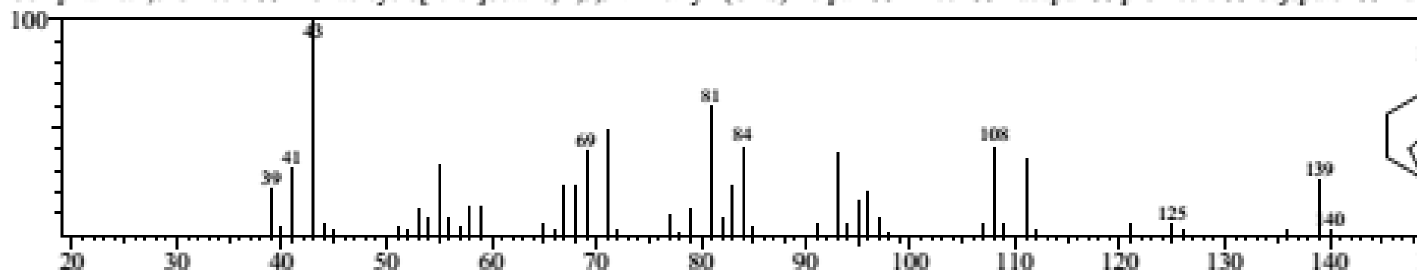
Hit#:1 Entry:26305 Library:WILEY7.LIB
SI:98 Formula:C10 H16 CAS:138-86-3 MolWeight:136 RetIndex:0
CompName:dl-Limonene $$ Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS) 1-P-MENTHA-1,8-DIENE $$ Limonene $Cinen $$ N



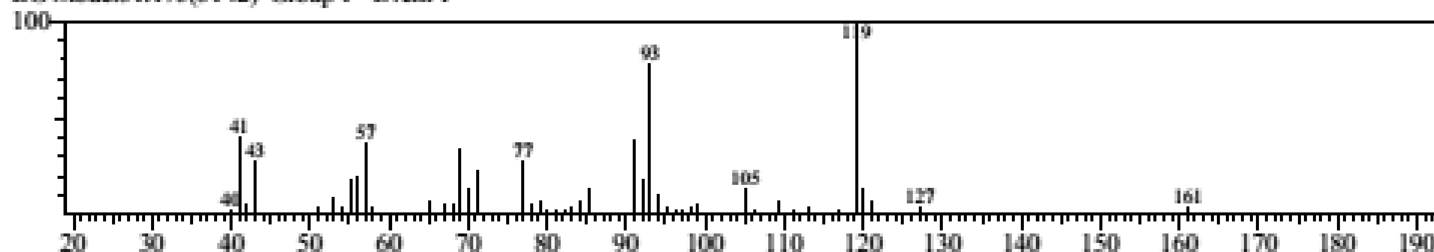
<< Target >>
Line#:14 R.Time:13.733(Scan#:1049) MassPeaks:47
RawMode:Single 13.733(1049) BasePeak:43.00(27456)
BG Mode:13.783(1055) Group 1 - Event 1



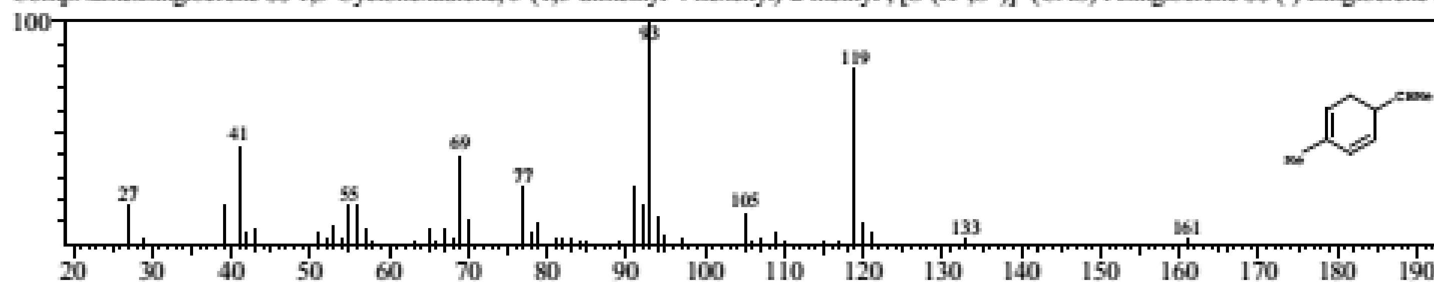
Hit#:1 Entry:43987 Library:WILEY7.LIB
SI:96 Formula:C10 H18 O CAS:470-82-6 MolWeight:154 RetIndex:0
CompName:1,8-Cineole $$ 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- (CAS) Terpan $$ Zineol $$ Eucapur $$ p-Cineole $$ Cajeputol $$ Eu



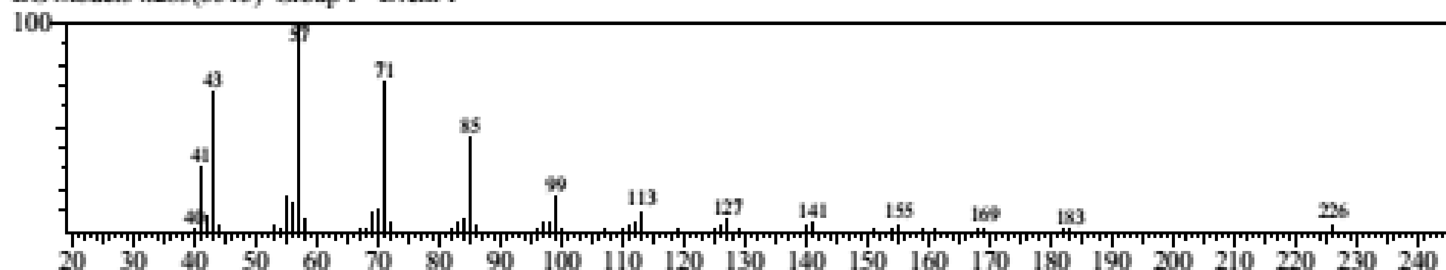
<< Target >>
Line#:21 R.Time:31.108(Scan#:3134) MassPeaks:49
RawMode:Single 31.108(3134) BasePeak:119.15(33140)
BG Mode:31.175(3142) Group 1 - Event 1



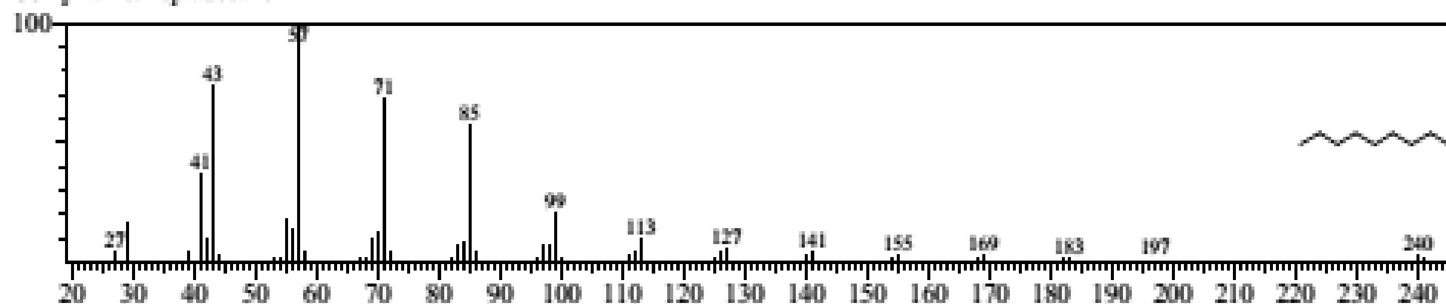
Hit#:1 Entry:100698 Library:WILEY7.LIB
SI:88 Formula:C15 H24 CAS:495-60-3 MolWeight:204 RetIndex:0
CompName:Zingiberene $$ 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- (CAS) l-Zingiberene $$ (-)-Zingiberene



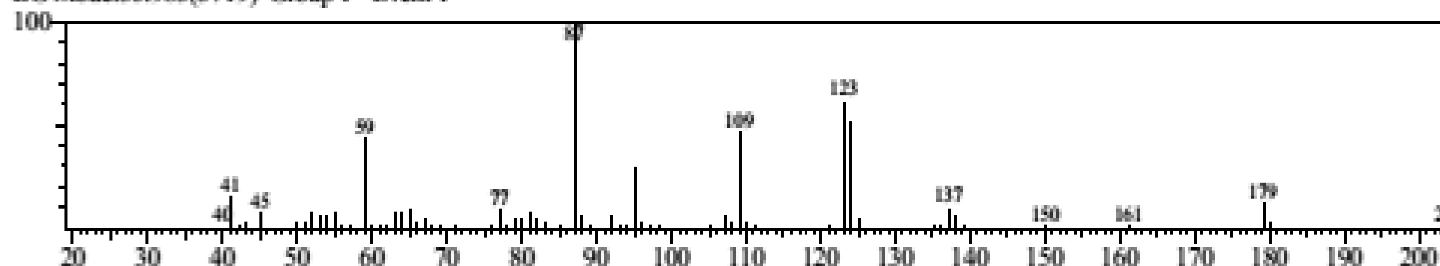
<< Target >>
Line#:23 R.Time:34.183(Scan#:3503) MassPeaks:55
RawMode:Single 34.183(3503) BasePeak:57.10(95397)
BG Mode:34.283(3515) Group 1 - Event 1



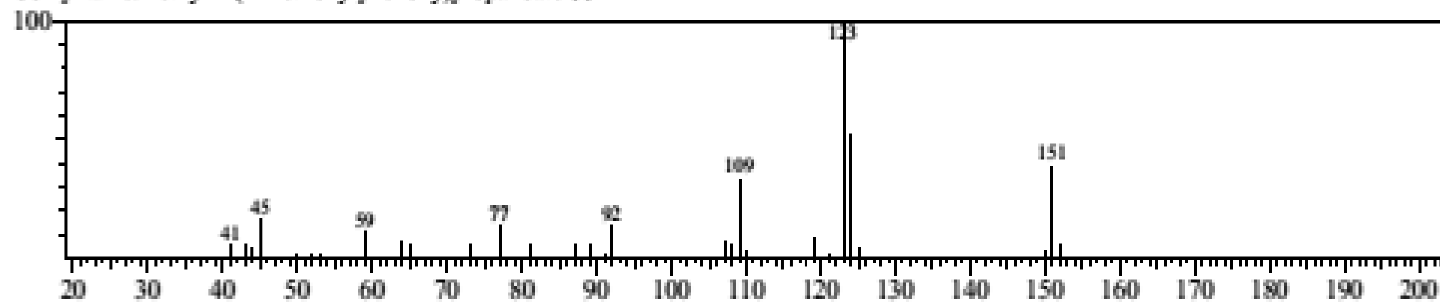
Hit#:1 Entry:20201 Library:NIST27.LIB
SI:96 Formula:C17H36 CAS:629-78-7 MolWeight:240 RetIndex:0
CompName:Heptadecane



<< Target >>
Line#:24 R.Time:35.933(Scan#:3713) MassPeaks:74
RawMode:Single 35.933(3713) BasePeak:87.05(78019)
BG Mode:35.983(3719) Group 1 - Event 1



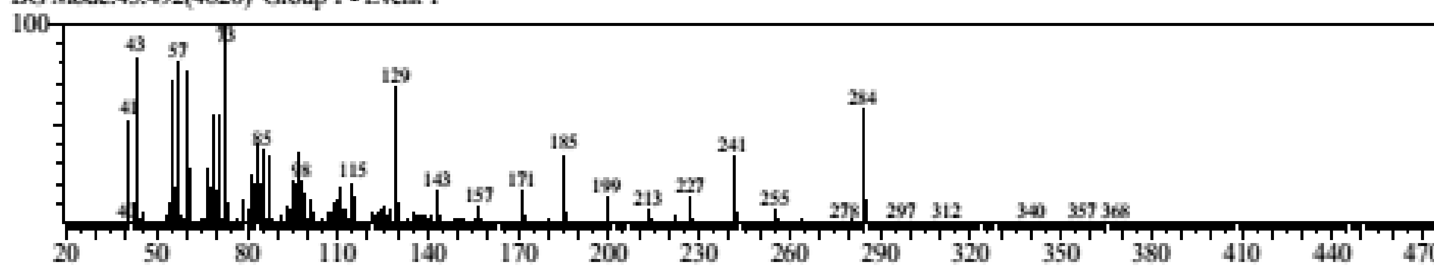
Hit#:1 Entry:107510 Library:WILEY7.LIB
SI:70 Formula:C11 H14 O4 CAS:0-00-0 MolWeight:210 RetIndex:0
CompName:methyl 2-(4-methoxy-phenoxy)propanoate $$



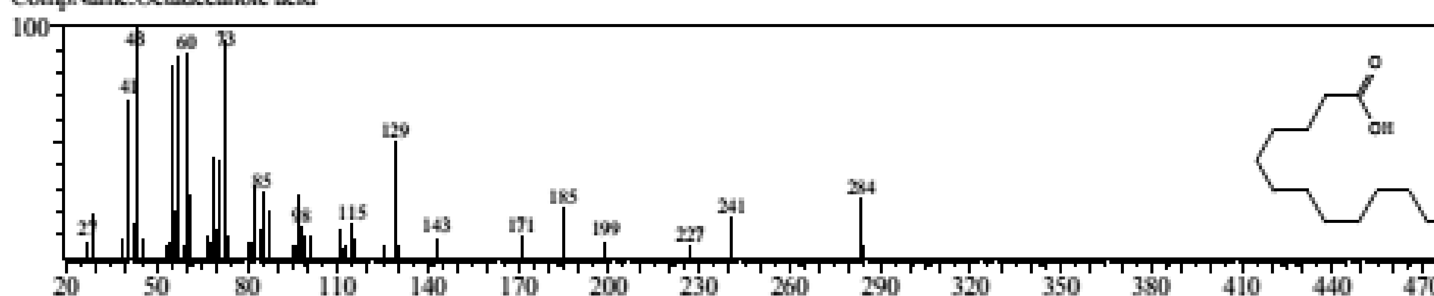
<< Target >>
Line#:27 R.Time:39.525(Scan#:4144) MassPeaks:216
RawMode:Single 39.525(4144) BasePeak:73.05(547656)
BG Mode:39.575(4150) Group 1 - Event 1

Hit#:1 Entry:332919 Library:WILEY7.LIB
SI:89 Formula:C18 H54 O9 SI9 CAS:556-71-8 MolWeight:666 RetIndex:0
CompName:OCTADECAMETHYLCYCLONONASILOXANE $$ Cyclononasiloxane, octadecamethyl- (CAS)

<< Target >>
Line#:35 R.Time:43.442(Scan#:4614) MassPeaks:242
RawMode:Single 43.442(4614) BasePeak:73.05(457506)
BG Mode:43.492(4620) Group 1 - Event 1



Hit#:1 Entry:22966 Library:NIST27.LIB
SI:89 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid

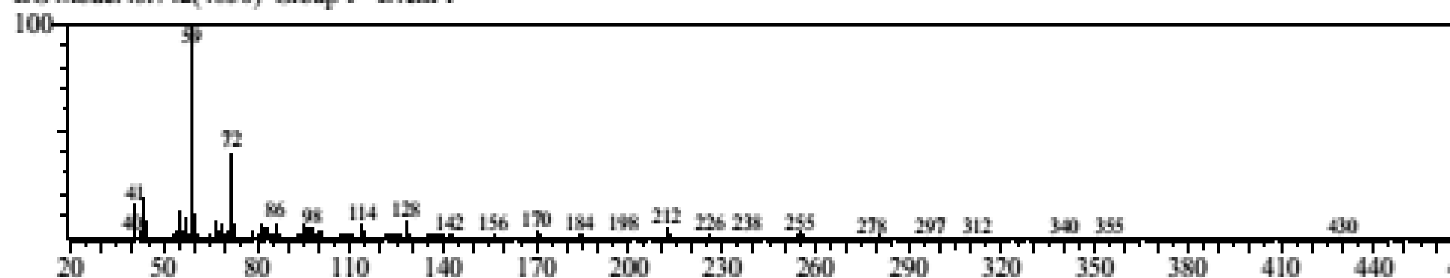


<< Target >>

Line#:36 R.Time:43.692(Scan#:4644) MassPeaks:213

RawMode:Single 43.692(4644) BasePeak:59.05(1177273)

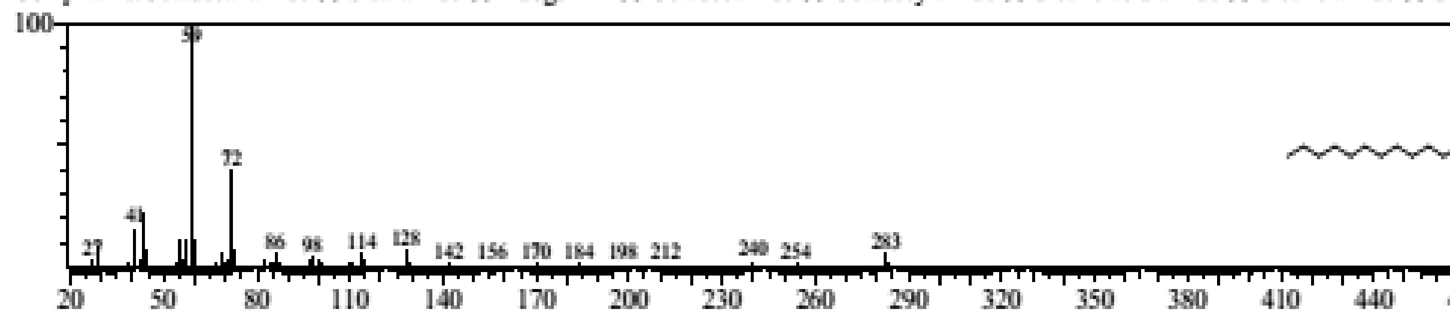
BG Mode:43.742(4650) Group 1 - Event 1



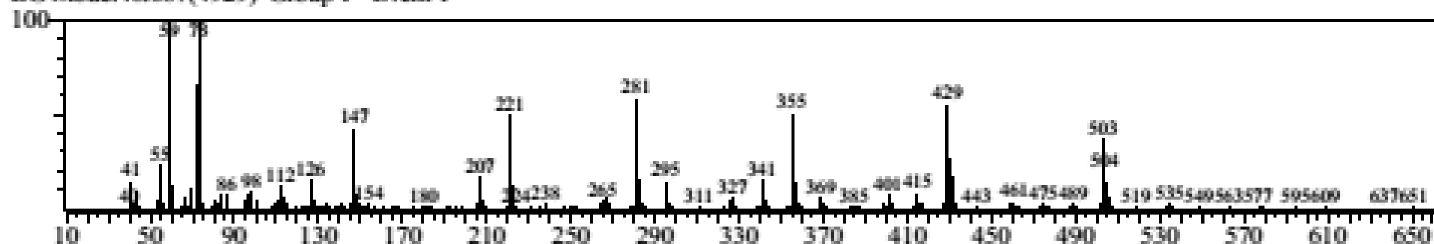
Hit#:1 Entry:85274 Library:NIST147.LIB

SI:91 Formula:C18H37NO CAS:124-26-5 MolWeight:283 RetIndex:0

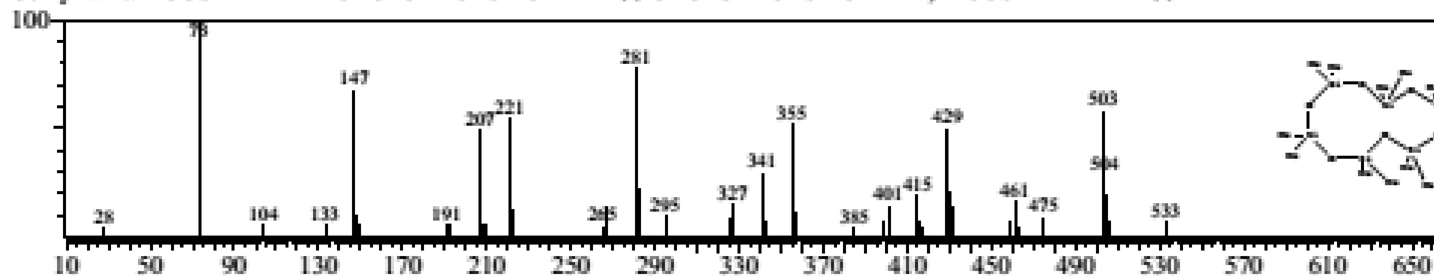
CompName:Octadecanamide $$ Stearamide $$ Adogen 42 $$ Octadecamide $$ Octadecylamide $$ Stearic acid amide $$ Stearic amide $$ St



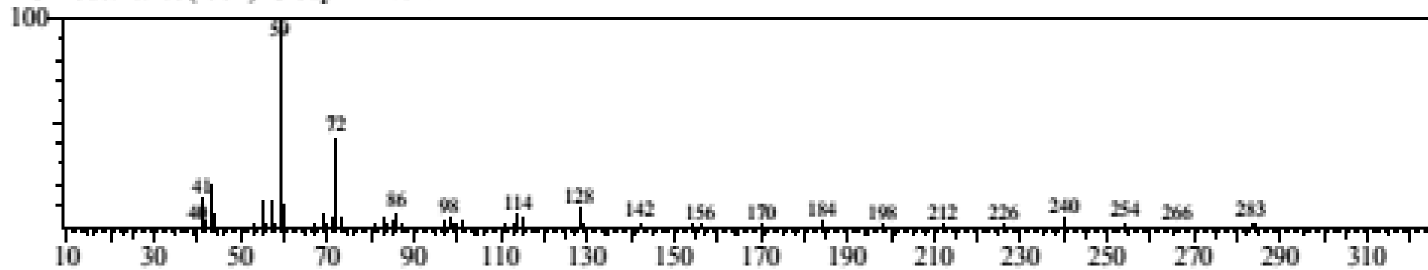
<< Target >>
Line#:43 R.Time:46.000(Scan#:4921) MassPeaks:288
RawMode:Single 46.000(4921) BasePeak:73.05(397472)
BG Mode:46.067(4929) Group 1 - Event 1



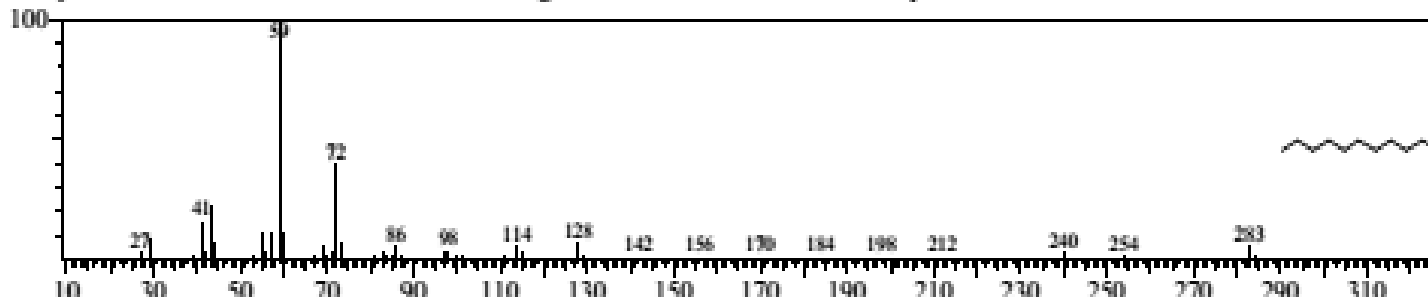
Hit#:1 Entry:335376 Library:WILEY7.LIB
SI:70 Formula:C20 H60 O10 SI10 CAS:18772-36-6 MolWeight:740 RetIndex:0
CompName:EICOSAMETHYLCYCLODECASILOXANE $$ CYCLODECASILOXANE, EICOSAMETHYL- $$



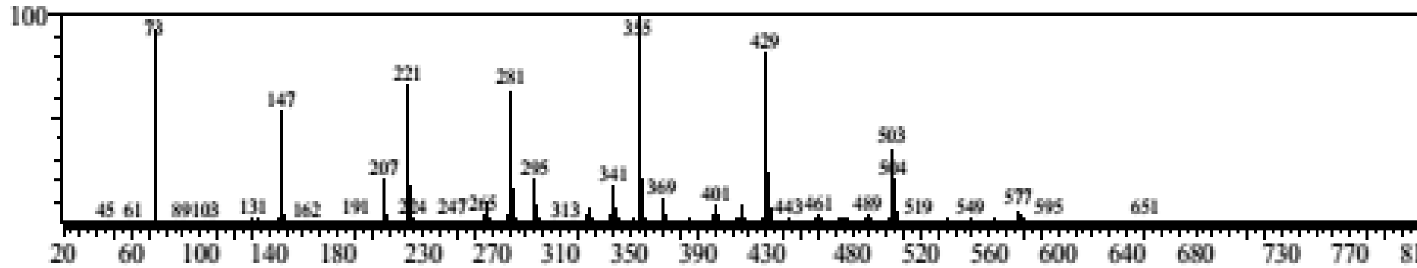
<< Target >>
Line#:44 R.Time:46.217(Scan#:4947) MassPeaks:184
RawMode:Single 46.217(4947) BasePeak:59.05(389122)
BG Mode:46.275(4954) Group 1 - Event 1



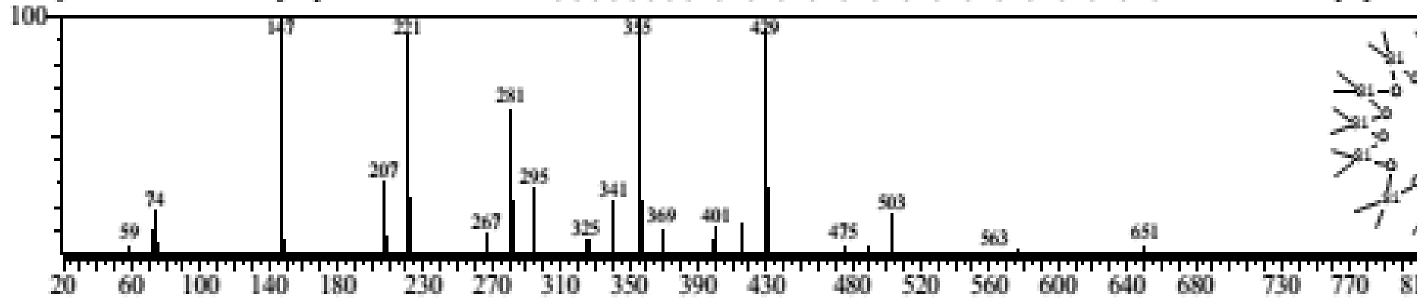
Hit#:1 Entry:85274 Library:NIST147.LIB
SI:95 Formula:C18H37NO CAS:124-26-5 MolWeight:283 RetIndex:0
CompName:Octadecanamide $$ Stearamide $$ Adogen 42 $$ Octadecamide $$ Octadecylamide $$ Stearic acid amide $$ Stearic amide $$



<< Target >>
Line#:47 R.Time:48.283(Scan#:5195) MassPeaks:155
RawMode:Single 48.283(5195) BasePeak:355.00(116662)
BG Mode:48.408(5210) Group 1 - Event 1

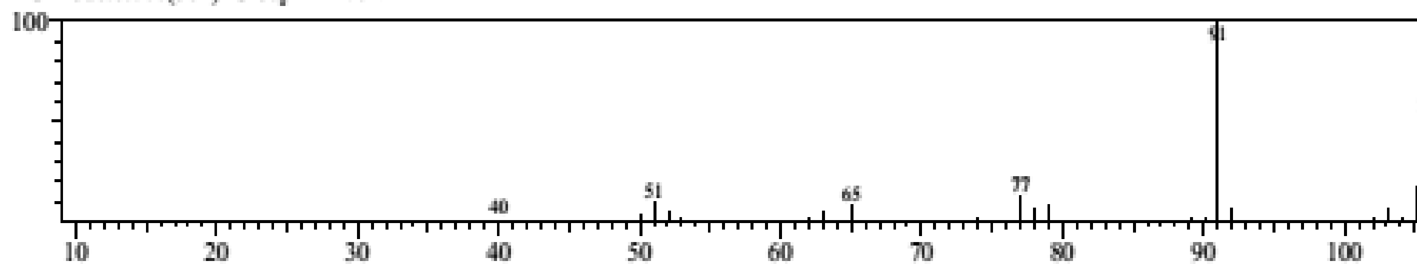


Hit#:1 Entry:147059 Library:NIST147.LIB
SI:84 Formula:C24H72O12Si12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:Tetracosamethyl-cyclododecasiloxane $$ 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20,22,22,24,24-Tetracosamethylcyclod

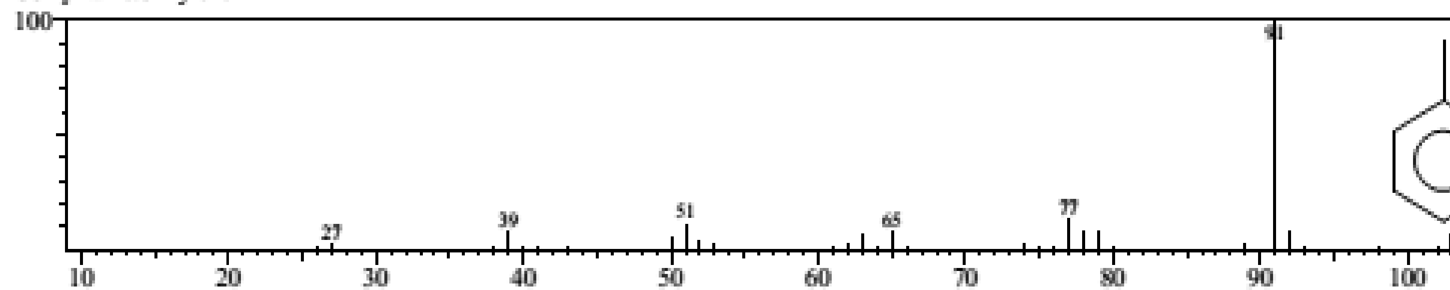


C. hartmannianum

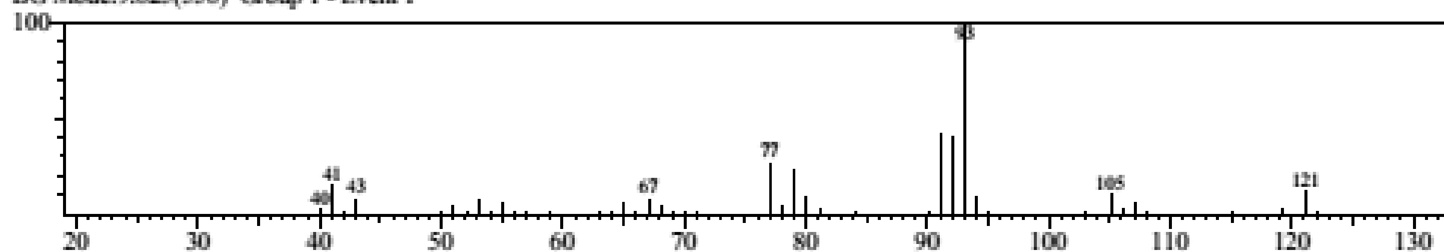
<< Target >>
Line#:6 R.Time:7.942(Scan#:354) MassPeaks:38
RawMode:Single 7.942(354) BasePeak:91.05(370223)
BG Mode:8.000(361) Group 1 - Event 1



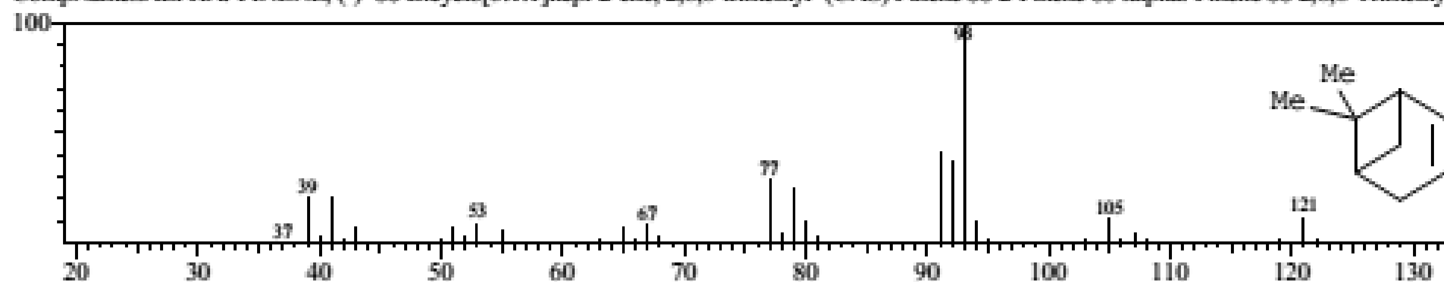
Hit#:1 Entry:2357 Library:NIST27.LIB
SI:98 Formula:C8H10 CAS:95-47-6 MolWeight:106 RetIndex:0
CompName:o-Xylene



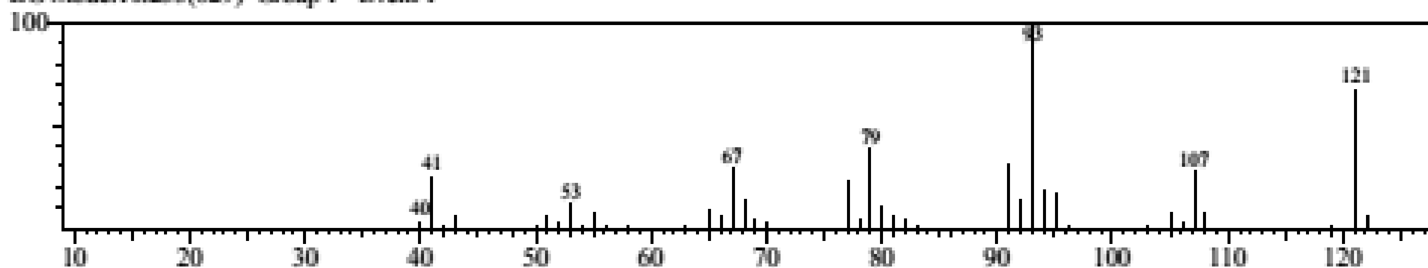
<< Target >>
Line#:8 R.Time:9.550(Scan#:547) MassPeaks:70
RawMode:Single 9.550(547) BasePeak:93.10(1123784)
BG Mode:9.625(556) Group 1 - Event 1



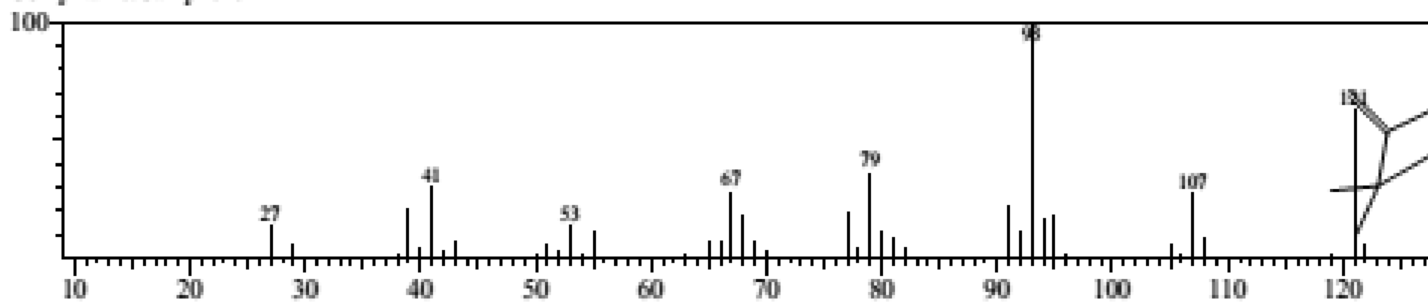
Hit#:1 Entry:26447 Library:WILEY7.LIB
SI:98 Formula:C10 H16 CAS:80-56-8 MolWeight:136 RetIndex:0
CompName:.ALPHA.-PINENE, (-)- $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- (CAS) Pinene $$ 2-Pinene $$ .alpha.-Pinene $$ 2,6,6-Trimethy



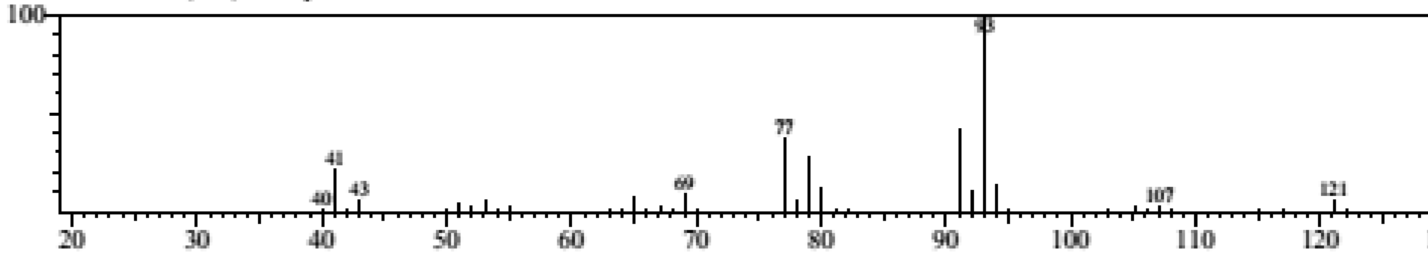
<< Target >>
Line#:9 R.Time:10.167(Scan#:621) MassPeaks:44
RawMode:Single 10.167(621) BasePeak:93.10(104139)
BG Mode:10.233(629) Group 1 - Event 1



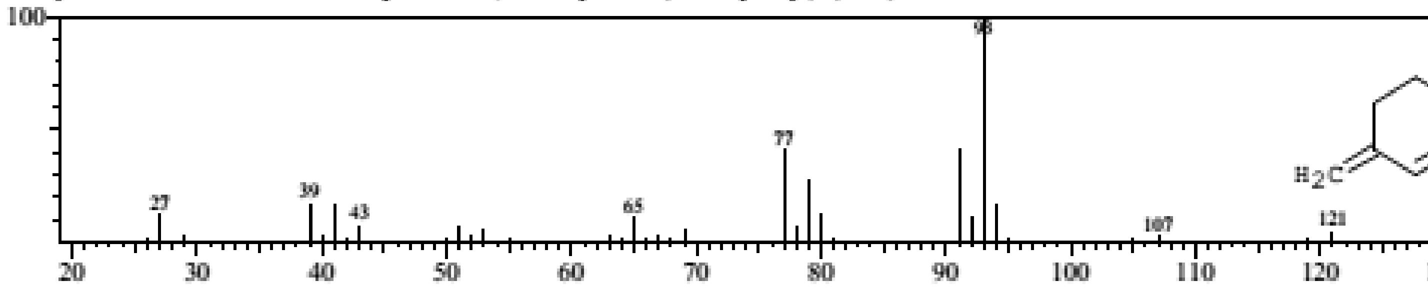
Hit#:1 Entry:6316 Library:NIST27.LIB
SI:97 Formula:C10H16 CAS:79-92-5 MolWeight:136 RetIndex:0
CompName:Camphene



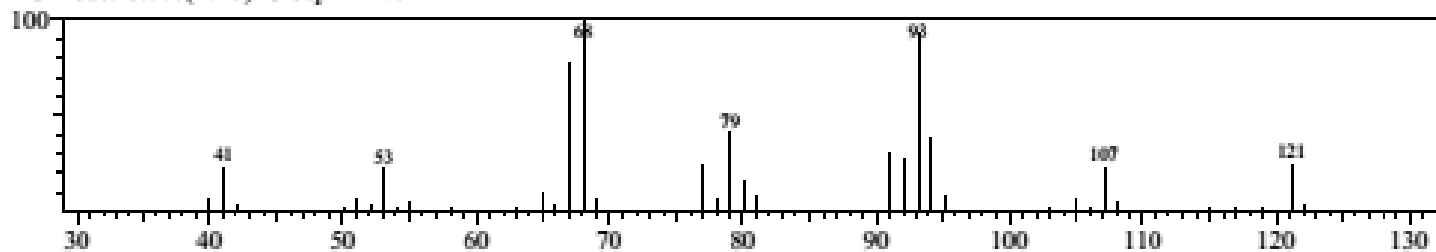
<< Target >>
Line#:10 R.Time:11.258(Scan#:752) MassPeaks:46
RawMode:Single 11.258(752) BasePeak:93.10(233200)
BG Mode:11.317(759) Group 1 - Event 1



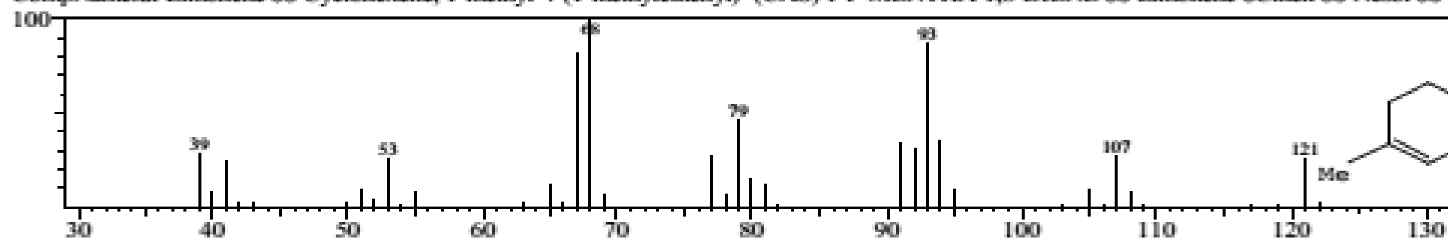
Hit#:1 Entry:26356 Library:WILEY7.LIB
SI:97 Formula:C10 H16 CAS:555-10-2 MolWeight:136 RetIndex:0
CompName:.beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- (CAS) 3-ISOPROPYL-6-METHYLENE-CYCLOHEXEN



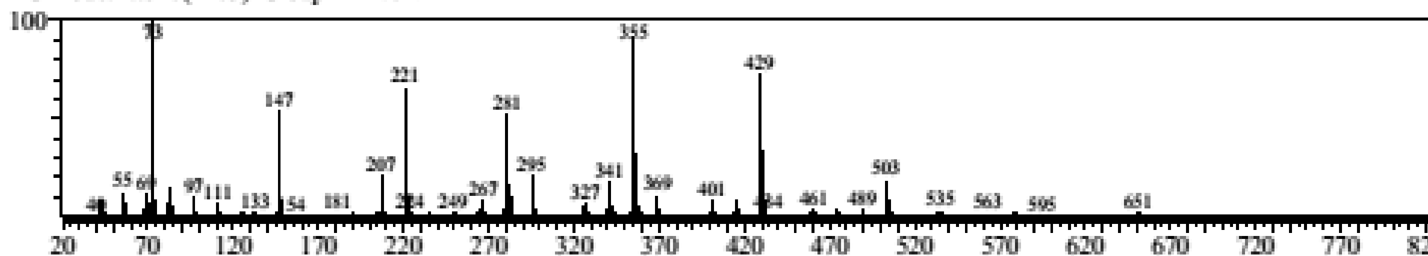
<< Target >>
Line#:13 R.Time:13.633(Scan#:1037) MassPeaks:42
RawMode:Single 13.633(1037) BasePeak:68.05(78781)
BG Mode:13.700(1045) Group 1 - Event 1



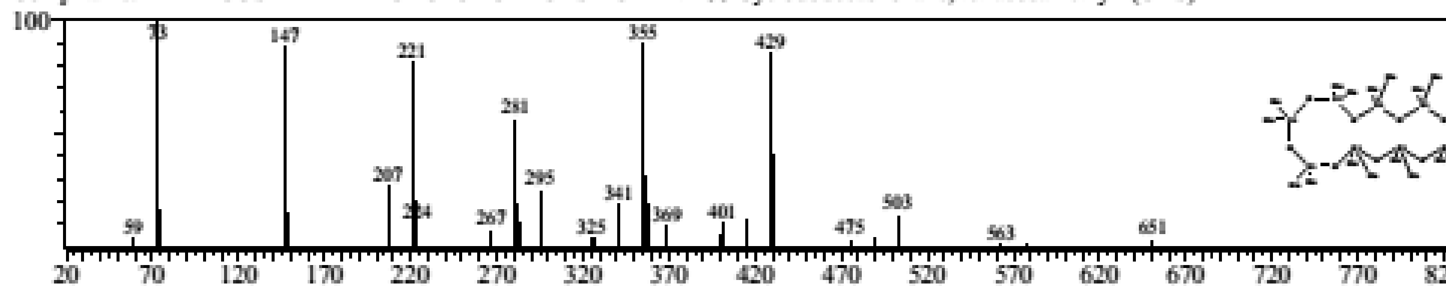
Hit#:1 Entry:26305 Library:WILEY7.LIB
SI:97 Formula:C10 H16 CAS:138-86-3 MolWeight:136 RetIndex:0
CompName:dl-Limonene $$ Cyclohexene, 1-methyl-4-(1-methylethenyl)- (CAS) 1-P-MENTHA-1,8-DIENE $$ Limonene $Cinen $$ Nesol $$



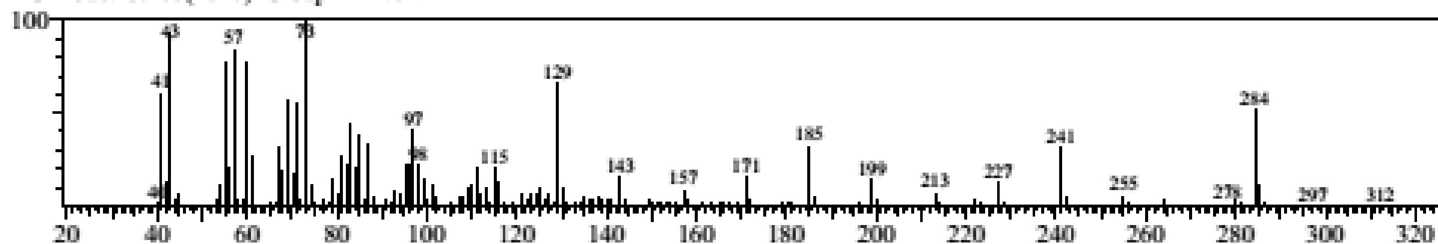
<< Target >>
Line#:20 R.Time:40.383(Scan#:4247) MassPeaks:188
RawMode:Single 40.383(4247) BasePeak:73.05(137423)
BG Mode:40.517(4263) Group 1 - Event 1



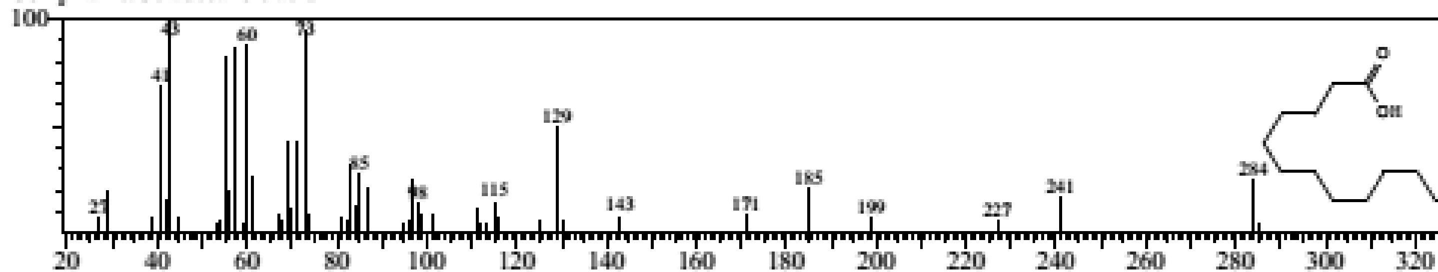
Hit#:1 Entry:337318 Library:WILEY7.LIB
SI:80 Formula:C24 H72 O12 SI12 CAS:18919-94-3 MolWeight:888 RetIndex:0
CompName:TETRACOSAMETHYLCYCLODODECASILOXANE $$ Cyclododecasiloxane, tetracosamethyl- (CAS)



<< Target >>
Line#:26 R.Time:43.425(Scan#:4612) MassPeaks:238
RawMode:Single 43.425(4612) BasePeak:73.05(390489)
BG Mode:43.467(4617) Group 1 - Event 1



Hit#:1 Entry:22966 Library:NIST27.LIB
SI:89 Formula:C18H36O2 CAS:57-11-4 MolWeight:284 RetIndex:0
CompName:Octadecanoic acid



استبيان حول ثلاث نباتات الطلح الصباغ والهبيل

الحاله الاجتماعيه: (1) متزوج (2) غير متزوج

(1) 20 – 31 (2) 30-50 (3) 51-80 العمر :

التعليم: (1) امى (2) ابتدائى (3) ثانوى (4) جامعى

الوظيفة : (1) باعة عطور (2) ربة منزل (3) سيدة اعمال (4) اخرى

السكن : (1) الخرطوم (2) بحرى (3) ام درمان

أيهم تفضل فى الدخان حطب ؟ (1) الطلح (2) الصباغ (3) الهبيل

فى حالة وجود مشكله طبية تستخدم؟ (1) الطلح (2) الصباغ (3) الهبيل

ايهم له قوة عطرية اكبر ؟ (1) الطلح (2) الصباغ (3) الهبيل

ايهم يدوم عطره اكثر مدة ؟ (1) الطلح (2) الصباغ (3) الهبيل

ايهم له قوة عطرية اكبر ؟ (1) مستخلص الطلح بالميثانول (2) مستخلص الطلح بالبتروليوم ايثر
مستخلص الطلح بالايسايل اسيتيت (4) مستخلص الطلح بالكلوروفورم (5) مستخلص الطلح بالماء

ايهم له قوة عطرية اكبر ؟ (1) مستخلص الصباغ بالميثانول (2) مستخلص الصباغ بالبتروليوم ايثر

(3) مستخلص الصباغ بالايسايل اسيت (4)مستخلص الصباغ بالكلوروفورم

(5) مستخلص الصباغ بالماء

ايهم له قوة عطرية اكبر ؟ (1) مستخلص الهبيل بالميثانول (2) مستخلص الهبيل بالبتروليوم ايثر
(3) مستخلص الهبيل بالايسايل اسيتيت (4) مستخلص الهبيل بالكلوروفورم

(5) مستخلص الهبيل بالماء

أيهما اقوى من الناحية العطرية ؟ (1) مستخلص الطلح بالايسايل اسيتيت (2) مستخلص الصباغ بالايسايل اسيت

(3) مستخلص الهبيل بالايسايل اسيتيت

ايهم يدوم عطره اكثر مدة ؟ ؟ (1) مستخلص الطلح بالايسايل اسيت (2) مستخلص الصباغ بالايسايل اسيت (3) مستخلص الهبيل بالايسايل اسيت