ISOLATION OF ANTIFUNGAL SECONDARY METABOLITES FROM *Phytolloca dodecandra, Basella alba* AND *Lippia javanica* AGAINST MAIZE FUNGAL PATHOGENS

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any institution.

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SM11/3322/12

RECOMMENDATION

This thesis has been prepared under our supervision as per the Egerton University regulations with our approval.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Hezekiah K. Mutai, my wife Gladys, children, siblings, and friends for their moral, emotional, and financial support.

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ABSTRACT

Maize is an important cereal crop and a staple food in most parts of Africa. Food insecurity is one of the major challenges in Sub-Saharan Africa. The food situation is worsened by fungal infestation of maize crops in the fields. The most prevalent fungi on maize crops are *Fusarium moniliforme* and *Fusarium graminearum* that cause red ear rot, stalk rot and Grey leaf spot (GLS) respectively. The development of antifungal resistance and side effects associated with synthetic pesticides has triggered intense research efforts towards natural antifungal agents such as essential oils and the non-volatiles because of their reported efficacy and safety. In an effort to search for new antifungal agents, selected plants; *Phytolloca dodecandra, Basella alba* and *Lippia javanica* were screened for bioactivity against maize pathogens; *F. moniliforme* and *F. graminearum*. The research involved isolation of secondary metabolites from the selected plants and extraction of essential oil from *L. javanica*. The essential oil was extracted by hydro-distillation and its chemical compositions determined by Gas Chromatography-Mass Spectrometry. Monoterpenes which accounted for 43.48% dominated the oil while the Sesquiterpenes constituted 8.70% respectively. The paper-disc diffusion-inhibition test method was used to screen for antifungal activity of both volatile and non-volatile secondary metabolites. Potato Dextrose Agar (PDA) was used as the culturing media for the fungi. The oil showed the highest activity of 25.00mm at 0.87 g/mL as compared to that of the Nystatin16.00mm, which was the positive control. The antifungal activity of the oil was observed to be reducing with time. On the other hand, the non-volatile secondary metabolites showed no activity against the selected phytopathogenic fungi. Bioassay guide column fractionation was done on the ethyl acetate extracts for the plants *P. dodecandra* and *B. alba*. From the two plants, one compound was isolated from each. Compound 16 was isolated from *P. dodecandra* and compound 17 isolated from *B. alba.* The compounds did not show any observable activity against the selected fungal pathogens.

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LIST OF ABBREVIATIONS AND ACRONYMS

COSY	Correlation Spectroscopy
DMSO	Dimethyl sulphoxide
ENDURE	European Network for the Durable Exploitation of Crop Protection Strategies
FAO	Food and Agriculture Organization
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
MIC	Minimum Inhibitory Concentration
NMR	Nuclear magnetic resonance spectroscopy
PTLC	Preparative Thin Layer Chromatography
TLC	Thin layer chromatography

CHAPTER ONE
INTRODUCTION

1.1 Background information

Maize (*zea mays* L.) is a widely cultivated crop in Kenya. It is the staple food to a large Kenyan population. This is because it has high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and storage characteristics (Muthomi *et al*., 2010). However, its cultivation is faced with a major setback of fungal diseases due to pythopathogens that are all over the ecosystem (Wagacha & Muthomi, 2008). Maize is vulnerable to degradation by plant pathogenic fungi, right from planting and through growing. Plant fungi are the causal agents of the most detrimental diseases in plants, including economically important crops, provoking considerable yield losses worldwide (Zaki *et al*., 2012). The annual maize losses all over the world because of diseases have been estimated at 25,000 million US dollars; of this, a major part is due to fungal pathogens carried through seed (Mohana & Raveesha, 2003).

Most fungi are aerobic and are found almost everywhere in the ecosystem in extremely small quantities due to the minute sizes of their spores. They proliferate into huge colonies under favorable conditions and their populations become exceedingly high. Fungal diseases destroy maize during growth and early development. These fungi are also referred to as 'field fungi'. *Cercospora zea-maydis* is a common example causing grey leaf spot (GLS) disease on growing maize. Grey leaf spot is a fungal disease that can greatly lower maize yields since it reduces the photosynthetic leaf area (Kinyua *et al*., 2010). *Fusarium* species *(F. moniliforme, F. graminearum)* are also of economic importance as they are responsible for red ear rot and stalk rot.

Though farmers are constantly advised to use synthetic pesticides, their potential hazards for mammals have increased. This is due to concern by consumers over pesticide residues in processed cereal products, the occurrence of pesticide-resistant insect and pathogen strains. Also, the ecological consequences, increasing cost of application and the precautions necessary to handle the fungicides (Ashouri & Shayesteh, 2010).Because of this, there is an increasing interest to obtain alternative antimicrobial agents from natural sources for use in crop protection. Recent research has shown that some plants produce essential oils

containing compounds likeneral, myrecene and geraniol which are able to inhibit the microbial growth (Matasyoh *et al.*, 2007).

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Varaprasad *et al*., 2009). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Arokiyaraj *et al*., 2008). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trials. These bioactive compounds are naturally produced in the plants as secondary metabolites; the principal groups with antifungal activity are terpenes, tannins, flavonoids, essential oil, alkaloids, lecithin and polypeptides (Castillo, *et al*., 2012). Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Mohana & Raveesha, 2003). Traditional medicinal plants provide a readily available rich source of active antimicrobial agents. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions (Tasleem *et al*., 2009).

1.2 Statement of the problem

Fungal pathogens on maize crops have caused several maize diseases and consequently a deficit in food supply. Therefore, it challenges the food supply because majority of the Kenyan population entirely depend on maize as the staple food. Despite tremendous improvements in the use of conventional methods of fungal control, maize production remains highly dependent on new control methods and Neem pesticides. The existing synthetic fungicides are expensive to small scale farmers and are non-biodegradable, thus stay longer in the ecosystem affecting the non-target organisms. In addition, synthetic drugs have been reported to have developed some serious problems such as drug resistance. It has also been reported that maize grains has the pesticides residues that create long term effects on humans. Moreover, because of the farmers' poor application methods, the control approaches are ineffective and less helpful in disease managements. Therefore, there is a need to develop a control strategy that uses cheap, eco-friendly agents that will be used in controlling the pathogens.

1.3 Objectives

1.3.1 General objective

To determine the antifungal activity of the crude extracts and pure compounds from *P. dodecandra, B. alba* and *L. javanica* leaves against maize pathogens; *F. moniliforme,* and *F. graminearum.*

1.3.2 Specific objectives

1. To isolate secondary metabolites from *P. dodecandra, B. alba* and determine their antifungal activities against *F. moniliforme, F. graminearum.*
2. To screen and carry out bioassay guided fractionation of crude extracts against *F. moniliforme, F. graminearum.*
3. To determine the antifungal activity of essential oil of *L. javanica* and characterize its compounds.
4. To elucidate the structures of the bio-active secondary metabolites isolated *P. dodecandra, B. alba*.

1.4 Hypotheses

1. The isolated secondary metabolites from the plants selected have no significant antifungal activity.
2. Bioassay fractionation of antifungal secondary metabolites cannot be carried out.
3. The crude essential oil will not exhibit significant antifungal activity against the selected fungal pathogens.
4. The spectroscopic data obtained will not provide the requisite data for structure elucidation of bioactive secondary metabolites isolated from the plants.

1.5 Justification

The continuous drop in maize production in Kenya has been attributed mainly to diseases caused by fungal pathogens. Though farmers are constantly encouraged to use conventional methods of applying fungicides, their inappropriate use have been found to possess adverse effects on ecosystems and a possible carcinogenic risk than insecticides and herbicides together. Moreover, resistance by fungi to fungicides has rendered certain fungicides ineffective. To minimize the yield reduction due to pythopathogens infestation and the

consequent diseases, it is important to incorporate strategies that are economical and environmentally friendly in managing these diseases. Thus, there is an increasing interest to obtain alternative active antifungal agents from natural sources for use in crop protection systems. It is therefore, imperative in crop management and protection to use plant based compounds to replace the conventional methods being used. The most prevalent way of achieving this is by screening and evaluating alternative antimicrobial activities of secondary metabolites from plants-based sources to which development of pythopathogens' resistance has not been reported. Compared to synthetic fungicides; botanicals are advantageous owing to their local accessibility and availability, relatively inexpensive, readily biodegradable, less toxic and less prone to resistance by fungal species.

CHAPTER TWO
LITERATURE REVIEW

2.1 Fungi

Fungi are spore-forming, non-chlorophytic, eukaryotic (cells having true nuclei) organisms and most of the true fungi are filamentous and branched. Reproduction of fungi is primarily by means of spores that are reproductive bodies that consist of one or a few cells (McDonald, 1997). Spores are produced by sexual and asexual reproduction. Sexual reproduction involves the union of two compatible nuclei as produced by meiosis. For many phytopathogenic fungi the sexual cycle occurs only once during each growing season. Most of the over 100,000 species of fungi are saprophytes. However, over 20,000 species of fungi are parasites and cause disease in crops and plants (Wagacha *et al.*, 2013). Fungi, depending on type, can grow under a wide range of environments. These fungi are found everywhere in soil, plant debris or other rotting vegetative material. Since fungi do not make their own food, they absorb food from organic matter they live on which include crops and causes them to rot. Fungi are of importance to the agricultural sector due to their infestation and consequent diseases on crops. Fungal contamination of crops causes considerable economic losses due to direct damage, discoloration, off-odors, taints, off-flavors, reduced yields, and loss of nutritive value (Agustin *et al.*, 2007).

Fungal parasites are by far the most prevalent plant pathogenic organisms. Phytopathogenic fungi affecting maize crop are a major threat to food production. Worldwide, this has led to important economic losses, particularly over the past few decades as agricultural production has intensified. Fungal infestation in crops can start and continue from planting to harvesting and are responsible for production of toxic secondary metabolites that are responsible for food spoilage (Kumari, 2010). It also creates a potential health hazard for humans. To curb the problems, farmers have increased their dependence on agrochemicals. However, the intensive use of these synthetic compounds has led to the emergence of pathogen resistance strains of fungi, residue of these compounds in foods and severe negative ecological impacts. There are also a number of plant diseases for which chemical solutions are ineffective or non-existent. Moreover, there is an increasing demand by consumers for pesticide-free food. Thus, control with natural botanicals has emerged as a promising alternative to chemical pesticides for more rational and safe crop management.

2.2 Maize field fungi

Fusarium species are ubiquitous in soils. They are commonly considered as field fungi invading more than 50% of maize crops in the field (Fandohan *et al*., 2004)). The general conditions needed for *Fusarium* field fungi to proliferate are high humidity (>70%), oxygen, and temperatures that fluctuate between hot days and cool nights. They infect maize crop *via* several pathways as shown in figure 1.

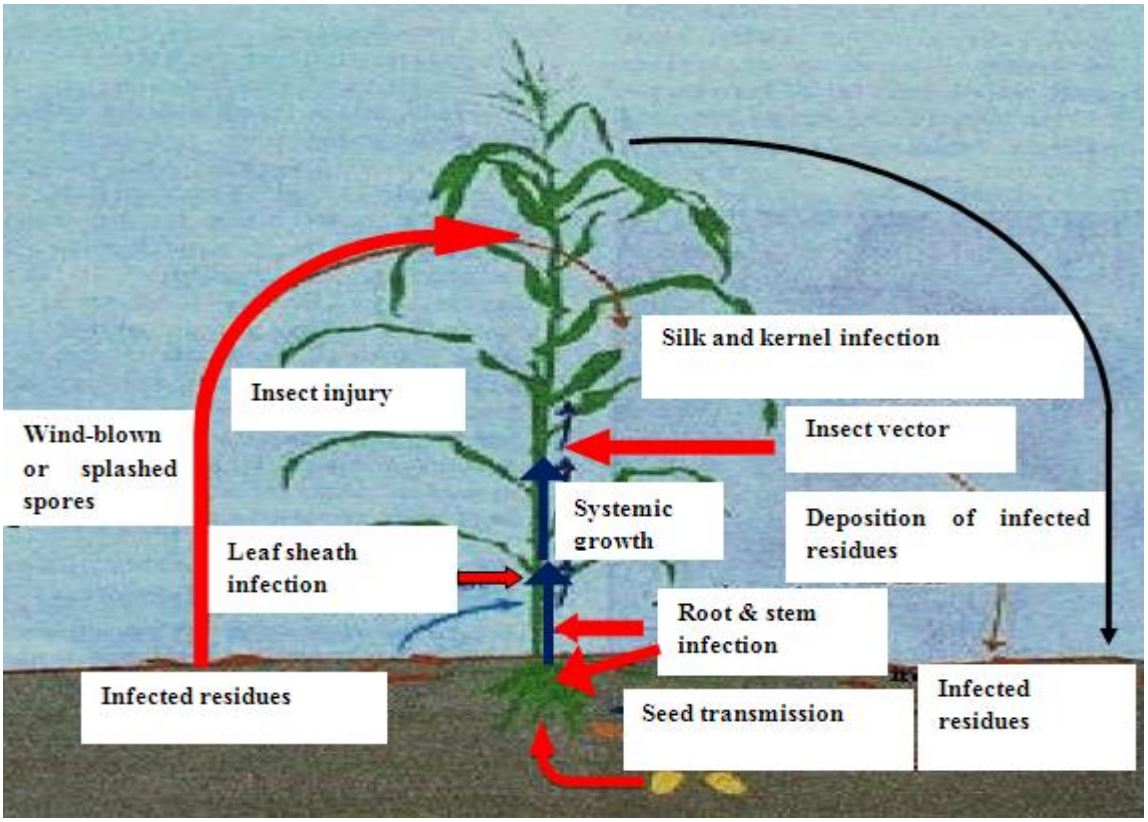


Figure 1: Fusarium cycle on maize showing various infection pathways (Munkvold & Carlton, 1997).

The major *Fusarium* species which affect maize crops in the field are *F. graminearum* and *F. moniliforme*. Each of these causes different diseases on the leaves, stalk and roots. There are a number of pathways by which *Fusarium* species may infect kernels, resulting either in kernel rot or symptomless infection. Insect activity has long been associated with *Fusarium* infection of maize kernels and stalks (Kinyua *et al*., 2010).

Injuries to plants caused by insects such as the European corn borer (ECB) (*Ostrinianubilalis*) are often the initial infection sites for *Fusarium* species (Munkvold *et al*.,

1997). Figure 1 show the *Fusarium* fungal cycle in the field. Maize infections by different fungal species result in huge yield reduction leading to deficit in food supply. Kenya used to be self-sufficient in maize production when there was surplus in yields. Recent agricultural researches show that there is a serious decline in production of maize; this situation has greatly affected both rural and urban households in equal measures (Muthomi *et al*., 2010). Reduction in yields has heavily and negatively affected the larger poor families economically. *Fusarium* infestation reduces germination rates, seedling vigor, and crop development (ENDURE, 2010).

2.2.1 *Fusarium graminearum*

Fusarium graminearum causes red ear rot, kernel rot and Gibberella stalk rot (Munkvold *et al*., 1997). Ear and kernel rot affect growth, quality and the development of the infected maize crops (CIMMYT, 2004). This disease is of serious concern to maize producers in several countries of Asia, Africa, and throughout the America. *Fusarium graminearum* infects maize at all stages of plant development, either via infected seeds, the silk channel or wounds, causing crop rot during both the germination and growth stages (Munkvold & Carlton, 1997). Symptomless infection can exist throughout the plant in leaves, stems, roots, and the presence of the fungus is in many cases ignored because it does not cause visible damage to the plant (Munkvold *et al*, 1997). In red ear rot, infection starts at the tip of the ear and moves toward the base (Varga*et al.*, 2009) as shown in Plate 1. The cycle of infection of all the fungal pythopathogens are illustrated in figure 2.

Plate 1: Red ear rot infection (Munkvold & Carlton, 1997).

2.2.2 *Fusarium moniliforme*

It is the causal agent of ear rot and stalk rot diseases of the maize crop. It differs from *F. graminearum* only by the fact that it does not cause the kernel rot. The infection can occur through the silks, through holes and fissures in the pericarp or at points where the pericarp is torn by the emerging seedling (Antonio *et al*., 2003). The ear rot is a common disease in hail-damaged corn. *Fusarium moniliforme* may infect any part of the ear and take advantage of wounds created by insects or hail.

2.2.3 The Grey Leaf Spot

The GLS is caused by *Fusarium* species. Symptoms of the GLS are similar to those of red ear rot but GLS is characteristic of large grey to reddish or yellow lesions that extend down the leaf veins (Crous *et al*., 2006). The tissue within the “spot" begins to die as spot size increases into longer, narrower leaf lesions as shown in plate 2.

Plate 2: Leaf showing Grey Leaf Spot (Desjardins *et al*., 2002)

Although initially brownish and yellow, the characteristic grey color that follows is due to the production of grey fungal spores (conidia) on the lesion surface (Neves *et al*., 2015). Maize GLS mature lesions are easily diagnosed and distinguishable from those of other diseases. They also have brown rectangular and vein limited shape. Secondary and tertiary leaf veins limit the width of the lesion and sometime individual lesions can combine to blight entire leaves (Benson *et al*., 2015).

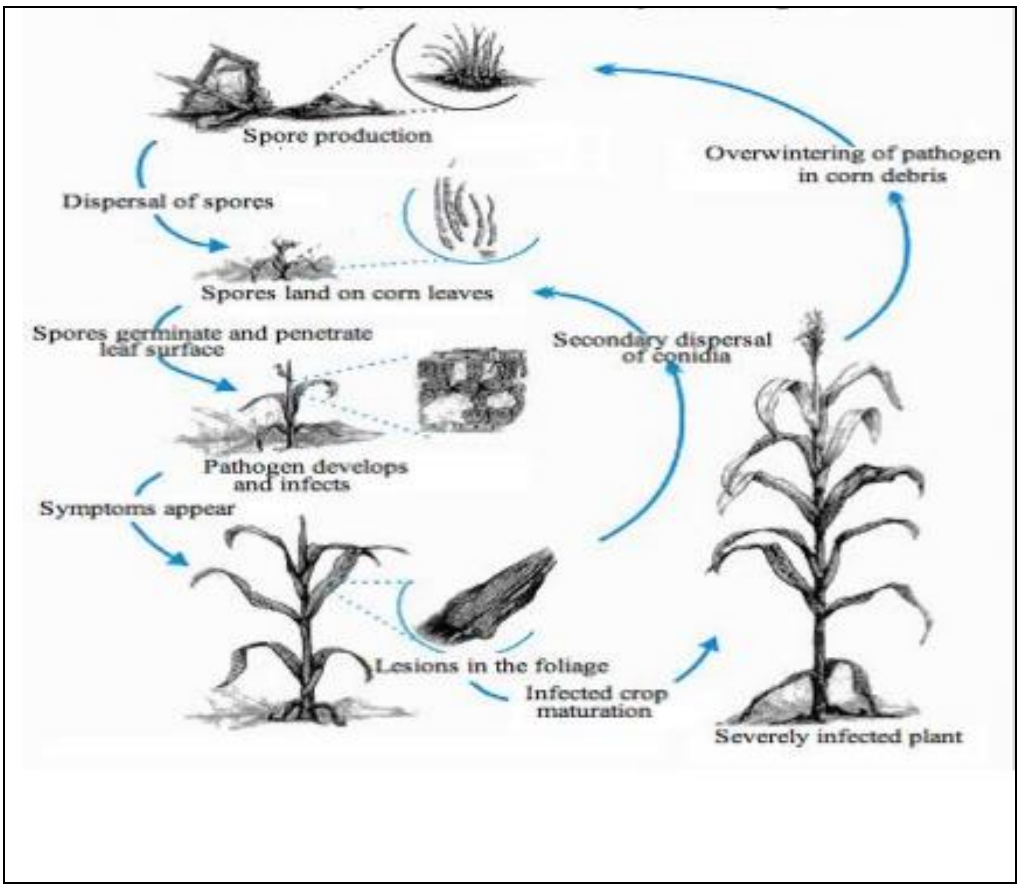


Figure 2: Grey Leaf Spot infection cycle(Youssef, 2009)

2.3 Plants as sources of fungicides

The use of plants, plant materials or crude plant extracts (botanical insecticides) for the protection of crops and stored products from insect pests is probably as old as crop protection itself (Isman, 2008). For example, according to Isman and Machial (2006), USA imported 6700 tons of *Derris elliptica* roots to be used as insecticides from Southeast Asia in 1947. This reflects that the plant-based products have been in use for crop protection. From the academic point of view, plants represent a vast storehouse of potentially useful natural products, and indeed, many laboratories worldwide have screened thousands of species of higher plants not only in search of pharmaceuticals, but also for pest control products. According to Tripathi and Sundararaj (2015), studies have pointed to numerous plant species possessing potential pest-controlling properties under laboratory conditions,

but the step from the laboratory to the field eliminates many contenders, even when judged only on their efficacy against pests under realistic field conditions.

Unfortunately, efficacy against pests is only one of a number of important criteria that need to be met for a plant extract or derivative to move successfully toward commercialization and use (Stevenson *et al*., 2014). Apart from efficacy and spectrum-of-action, biological criteria include favorable toxicology and minimal environmental impact (*i.e.*, vertebrate selectivity; selectivity favoring natural enemies and pollinators; rapid environmental degradation) (Isman, 2008). For example, Neem insecticides meet these criteria admirably, yet the commercialization of Neem for use in North America and Europe has taken many years, and the costs have run into millions of US dollars. Obviously, it is not enough to have an efficacious product that is relatively safe to the user and the environment (Montesinos, 2003). However, other considerations must be satisfied. These 'other' considerations constitute barriers to the commercialization of other botanical insecticides. These factors include the following as suggested by Gardener & Fravel (2002):

(i) The relative scarcity or availability of the natural resource;
(ii) Standardization of extracts and quality control based on active ingredients;
(iii) Special problems in regulatory approval of botanicals to be used in crop protections.

2.4 Antifungal compounds

Antifungal compounds are specific agents of chemical origin that controls fungal infestation and fungal diseases by inhibiting, repelling, or killing the fungus causing the disease (Dubey *et al*., 2010). These compounds are economically important to the agricultural sector often in controlling fungal diseases, which are a common occurrence on crops, causing significant economic impact on yield and quality (Hill*et al*. (2008).

Antifungal compounds are sub-divided into two broad groups; those which are synthesized and are commonly known as fungicides. These are used as a formulated product consisting of an active ingredients plus inert ingredients that improve the performance of the product (Tasleem *et al.*, 2009). According to Matasyoh *et al*., (2007), their actions are highly specific and are widely being used. Nonetheless, environmental pollution due to their slow biodegradation, phytotoxicity, carcinogenicity and toxic residue in agricultural products is an important drawback. Another group of antifungal compounds are those from plants and

are referred to as the botanicals. Many of the plant materials used in traditional medicine offer a rich source of antimicrobial agents. Medicinal plant extracts are promising as alternative control means because of their anti-microbial activity, nonphytotoxicity, systemicity as well as biodegradability as opposed to those of synthetic origin (Castillo *et al*,. 2012). Plants produce a great deal of secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs, many of them with antifungal activity (Varaprasad *et al*, 2009). Well-known examples of these compounds include; essential oils, terpenoids, saponins, phenolic compounds, alkaloids, peptides and proteins (Tasleem *et al*, 2011). The plant parts normally used are the roots, berries, leaves, stems andstem bark.

2.4.1 *Phytollocadodecandra*

Endod (*Phytolloca dodecandra*L.'Herit) is a member of the phytolaccaceae family and it is widely distributed in South America, Africa and Asia. It is an indigenous plant to Ethiopia; the name Endod is the Ethiopian name of the soapberry plant *P. dodecandra* (Plate 3).

Plate 3: *Phytolloca dodecandra* plant

It is a perennial climbing plant growing rapidly in an altitude of 1600-3000 meters above sea level and produce fruits twice in a year from December to February and June to July (Kassa *et al.*, 2004). The family phytolaccaceae is important phytochemically because of the frequent presence of triterpenoids, terpenoids and saponins which are very active classes of

natural products. Numerous diverse compounds and extracts containing activity inhibitory to fungi have been reported. The plant parts normally used are the roots, the berries, the leaves and the stems. The parts that are constantly used are shown in plate 3.

Figure 3 shows the compous that have been previusly isolated from *P. dodecandra*. Phytolaccoside B (**1**), 3-O-β-D-xylopiranosylphytolaccagenin, a monodesmoside triterpenoid glycoside isolated from berries of *Phytolacca tetramera* showed antifungal activity in agar dilution assays, inhibiting human opportunistic and pathogenic fungi with minimum inhibitory concentrations (MICs) between 74-188μM (Andrea *et al.*, 2008). Also oleonolic acid(**2**), limonene(**3**), and luteolin(**4**) have been shown to be active against fungi.

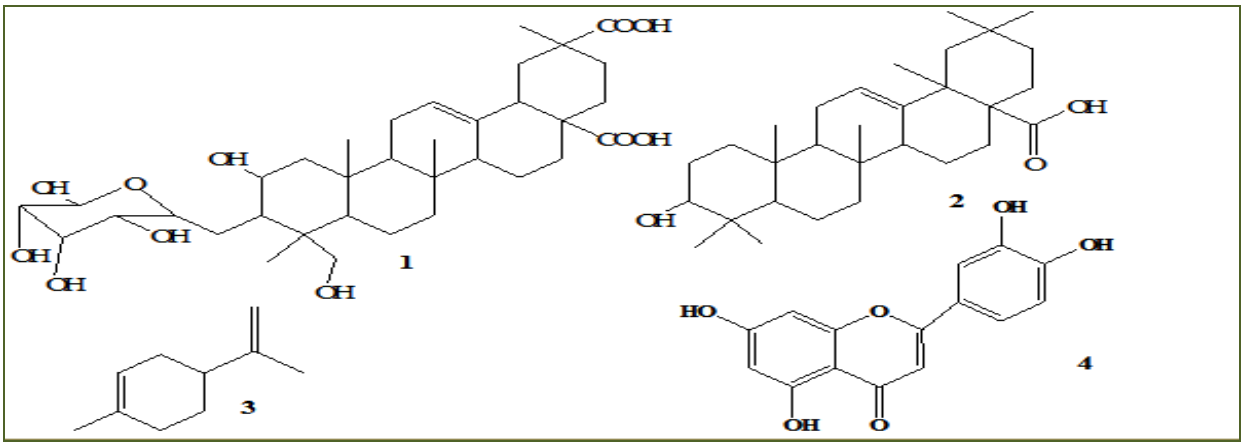


Figure 3: Compounds that have been isolated from *P. dodecandra*

2.4.2 *Basella alba*

The plant *B. alba* belong to the family Chenopodiaceae and sub-family Basellaceae. The genus Basella (Chenopodiaceae) contain about nine species distributed in India, tropical Africa and South-East Asia. The sub-family contains two plants spp; *Basella rubra* Linn and *B. alba* Linn (Satya and Sumeru, 2010). *Basella alba* is the green variety while *B. rubra* has red stalk and lightly coloured reddish purple on the undersides of its leaves (Ogunbusola *et al.*, 2012). The plant is known to be extremely heat tolerant. Its leaves are thick, semi-succulent, heart-shaped having a mild flavor and mucilaginous texture (Reshmi *et al.*, 2012). The aerial part of the plant (leaves, stem shown in plate 4) serves as an edible plant (vegetable) in many parts of the world (Vijender *et al.*, 2010). Numerous diverse compounds and extracts from the Basella plants have been reported to exhibit activity

inhibitory to different microorganisms. Most of these inhibitors are flavonoids, phenylpropanoid glycosides, and triterpenoids all of which have been isolated from the majority of plants in the family Basellaceae to which *B. alba*belong.

Plate 4: *Basella alba* plant

The sub-family Basellaceae is important phytochemically because of the frequent presence of β Betacyanin (**5**), cirsimatic **(6),** and myrcene (**7)** which have shown activity against some fungi. These compounds have been indentified before and isolated in good yield from the leaves of wild plants, *B. rubra* Linn (Satya & Sumeru, 2010). Based on chemotaxonomic approach, it is known that plants from the same family contain the same classes of compounds.

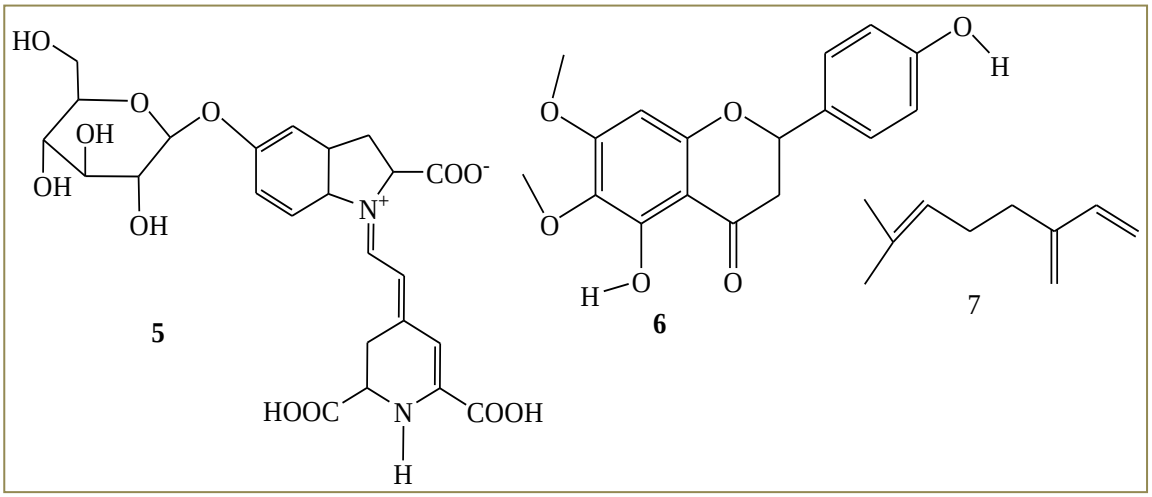


Figure 4: Compounds that have been isolated from *B. alba*

2.4.3 *Lippia javanica*

Lippia javanica belong to the Verbenaceae family. The genus *Lippia* includes approximately 200 different species; they vary from herbs and shrubs to small trees (Ombito *et al*., 2014). *Lippia javanica* is an erect, multi-stemmed woody shrub that can grow to a height of 1 – 2 m. The leaves are hairy with noticeable veins and are highly aromatic, giving off a strong lemon-like smell when crushed (shown in plate 5). *Lippia javanica* is widely distributed throughout larger parts of South Africa, it grows from the eastern cape province and its distributions extends to the tropical African countries which include Mozambique, Swaziland, Botswana, Zambia, Tanzania and Kenya (Ludere*et al*. , 2013). Traditionally, leaves of *L. javanica* are used in making herbal preparations (De Wet *et al*., 2010). An infusion made from the leaves is used for treatment of patients with fever, can also be used as a decongestant for colds and coughs (York*et al*., 2011). In the treatment of malaria, a decoction of boiled leaves is taken and the whole body bathed in the same fluid. Mashed leaves can also be applied on cut wounds, or soaked in water and the juice drunk for the treatment of tapeworm and for indigestion problems (Maroyi, 2013). The oil of Lippia has a lemon-yellow color and it smells like a bruised orange.

Plate 5:*Lippia javanica* plant

Numerous monoterpenoids have been identified from *L. javanica* (Angela *et al*., 2012). Different compounds, myrcenone (**8**), 6-methoxyluteolin 4'-methyl ether (**9**), also 4-ethyl-nonacosane (**10**), and four flavanones, apigenin (**11**), Caryophyllene (**12**), dihydro-targetone (**13**), *p*-cymene (**14**), myrcene (**15**) have been isolated from chromatographic separation of the *L. javanica*ethanolic extract (Silva *et al*., 2008).

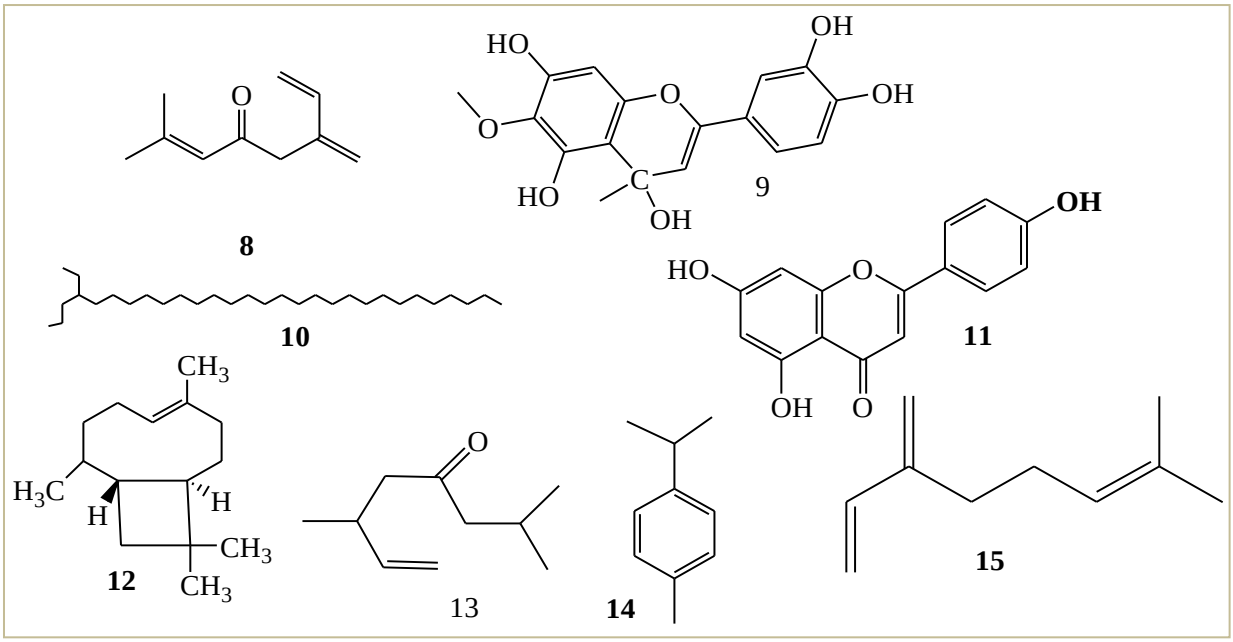


Figure 5: Compounds that have been isolated from *L. javanica*

2.5 Essential oils

Plants have an almost limitless ability to synthesize aromatic substances of different functional groups, most of which are phenols or their oxygen-substituted derivatives (Tasleem *et al*., 2009). Phenolics are the most common and widely distributed compounds in plant kingdom. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some plants used for their odors, pigment, and flavors were found to be endowed with medicinal properties (Hill*et al*., 2008). It is known that plants synthesize a variety of groups of bioactive compounds in tissues as secondary metabolites that have antifungal activity to stop or inhibit the development of mycelia growth, inhibition of germination or reduce sporulation of fungal pathogens (Viljoen *et al*., 2005). Each of these groups present variable mechanisms of action, for example, the toxicity of poly-phenols in microorganisms is attributed to enzyme inhibition by oxidation of compounds (Castillo *et al.,* 2012). Some of the herbs and spices used by humans to season food yield useful medicinal compounds (Hadacek and Greger, 2000). Many plant essential oils and their volatile constituents have been reported to possess potent anti-microbial activities (Sirirat *et al*., 2009). A pure compound piperitenone has been isolated from *L. javanica* essential oil and found to have potential activity against fungi

(Viljoen *et al*., 2005). Perhaps the most attractive aspect of using essential oils and/or their constituents as crop protectants (and in other contexts for pest management) is their favorable mammalian toxicity (Isman, 2008).

According to Moreira *et al*. (2005) plant spices, formulations and volatile oils prevent the increase of bacteria, mildews and ferments. Especially, it is important to prevent the bacteria that cause spoilages in food and affect the human health negatively through spices instead of synthetic protectors. The antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, crop managements, pharmaceuticals, alternative medicine and natural therapies (Viljoen *et al*., 2005). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that in order to find active compounds, a systematic study of medicinal plants is very important (Regnault-Roger *et al*., 2012)

2.6 Control of fungal pythopathogens in maize crop

To design and develop a working strategy for the reduction and control of fungal infestations and mycotoxins, understanding their fungal sources, growth and development is pre-requisite. The growth of fungi in crops and other agricultural commodities is the main cause of maize diseases and infestation (Viljoen *et al*., 2005). Many factors have been reported to contribute to fungal formation and development in crops. These factors are; plant susceptibility to fungal infestation, suitability of fungal substrate, temperate climate, moisture content and physical damages of seeds due to insects and pests (Odendo *et al*, 2003).

The use of biological compounds extracted from plants may be an alternative to conventionally used fungicides in crop management, due to bioactive chemicals such as flavonoids, phenols, tannins, alkaloids, quinones, saponins and sterols (Amini *et al.*, 2012). The practical use of natural compounds as control agents is receiving increased attention and this is partly due to their non-toxicity to humans, their systemicity and biodegradability. Volatile compounds from plants, especially essential oils have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematocidal activity (Nuzhat & Vidyasagar, 2014). Prevention of crop infestation can be done to avoid treatment of diseases. This is more effective and more economical.

2.6.1 Primary prevention

This step involves prevention before the fungal infestation. It is the most important and effective method for reducing fungal growth and associated infections. According to FAO(2009), several practices are recommended to keep the conditions unfavorable for any fungal growth in maize fields. These include:

i. Development of fungal resistant varieties of seeds and growing plants;
ii. Using fungicides and preservatives against fungal growth in seeds during planting;
iii. Control insect infestation in the farms with approved insecticides before planting.

Seed treatment is the safest and the cheapest way of controlling seed-borne fungal diseases and to prevent bio-deterioration of grains (Pawar, 2011). Crop rotation can also help in preventing fungal attack on maize crops including rotation with non-host crops to reduce pathogen load. According to ENDURE (2010), the main inoculum sources for red and pink ear rots of maize are crop residues of previous diseased crops. Therefore, there is high risk of ear rot when maize is grown in monoculture. But in areas where monoculture is practiced, crop residue management is highly recommended. This means residues must be completely removed.

Timing planting dates to minimize exposure to high temperatures and/or drought stress during the period of seed germination could be an important precaution in the prevention of both GLS and ear rot infections (Wagacha *et al.*, 2013).Many maize diseases develop best when moisture is abundant during the growing season. Rain or heavy dew is necessary for spores of disease-producing fungi to germinate and to penetrate the plant. Temperature and moisture of both soil and air may determine the development of corn diseases (ENDURE, 2010).

According to Blandino *et al*. (2012) crop residue management recommends three removal methods that involves; first, the physical removal or the use of specially designed biological crop residue treatments. Secondly, there is microbial decomposition of crop residues is a natural process that can be supported by adding stimulating nutrients or selected micro-organisms. Thirdly, it involves the use of a cultivator it is possible to mix mulched maize residues into the ground to accelerate decomposition. According to Castillo *et al.,* 2012,

mechanical cutting of plant residues (chopping) before ploughing is recommended to minimize infection and to promote rotting.

2.6.2 Secondary prevention

This level of prevention will only be required if the invasion of some fungi begins in crops at early phase. This is mostly shown by disease symptoms on the leaves and stalks of the crops (ENDURE, 2010). The existing toxigenic fungi should be eliminated or its growth stopped to prevent further deterioration of crops and their spread to clean crops. This is also called treatment of the infected crops at early stages. According to KEPHIS (2006), several measures are suggested as follows:

i. Stopping growth of fungi by application of approved fungicides before significant damage has occurred can limit ear infection;
ii. Removal of and destroying the infected crops (by burying deep in the ground or burning);
iii. Removal of lower leaves (if they are heavily attacked) is also useful in reducing disease spread.

2.6.3 Tertiary prevention

This preventive measure will be required once fungi heavily infest the crops, where the primary and secondary preventions methods would not be applicable. The crops will show the advanced characteristic fungal diseases on the leaves and the stalks. Therefore, it is not possible to completely stop toxic fungi and reduce their toxin formation. However, some measures as suggested by ENDURE (2010) should be done to prevent the transfer thereby preventing attacks on a subsequent season's crop. Only a few practices are recommended:

i. Complete destruction of the contaminated crops;
ii. Destroy diseased plant remains (e.g. by ploughing soon after harvesting dry maize to bury the residues in order to reduce the survival of the fungi.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of plant materials and acquisition of pathogens

3.1.1 Collection of plant material

Plant materials of *P. dodecandra, B. alba* and *L. javanica* were collected from botanical garden of Egerton university. Voucher specimens were deposited at the Department of Biological Sciences, Egerton University, where a taxonomist identified them. The *P. dodecandra* and *B. alba* materials were taken to the biotechnology laboratory, Egerton University where they were dried under the shade for three weeks while those of *L. javanica* were taken to biotechnology laboratory for extraction of essential oil.

3.1.2 Acquisition of pathogens

The pure cultures of *F. moniliforme* and *F. graminearum* were obtained from the Department of Biological Sciences of Egerton University.

3.2 Extraction and analysis of essential oil

3.2.1 Distillation of essential oil

The fresh leaves of *L. javanica* were cut into small pieces, weighed and boiled with 500mL of distilled water in a modified Clevenger apparatus until oil distillation ceased after 4h. The essential oil in the distillate was dried over anhydrous Na_2SO_4, put into a vial, and refrigerated at 4^{o}C.

3.2.2 Essential oil chemical composition determination and spectroscopic analysis

The essential oil was analyzed by use of an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS (‘Restek’) (30m x 0.25mm, 0.25µm film thickness) fused-silica capillary column. Briefly, the following protocol was applied. The essential oil was diluted in methyl-t-butyl ether (MTBE) (1:100). The carrier gas used was helium (at 0.8mL/min). Sample was injected in the split mode at a ratio of 1:10 – 1:100. The injector was kept at 250^{o}C and the transfer line at 280^{o}C. The column was maintained at 50^{o}C for 2 min and then programmed to 260^{o}C at 5^{o}C/min and held for 10 min at 260^{o}C. The MS was operated in the electron

impact ionization (EI) mode at 70eV, in *m/z* range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature and supplemented by Wiley 7N.l, HPCH 1607.L and FLAVORS.L GC-MS libraries. The relative proportions of the essential oil constituents are expressed as percentages obtained by peak area normalization, all relative response factors being taken as one. A summary of the extraction is shown as per the flow diagram in figure 3.

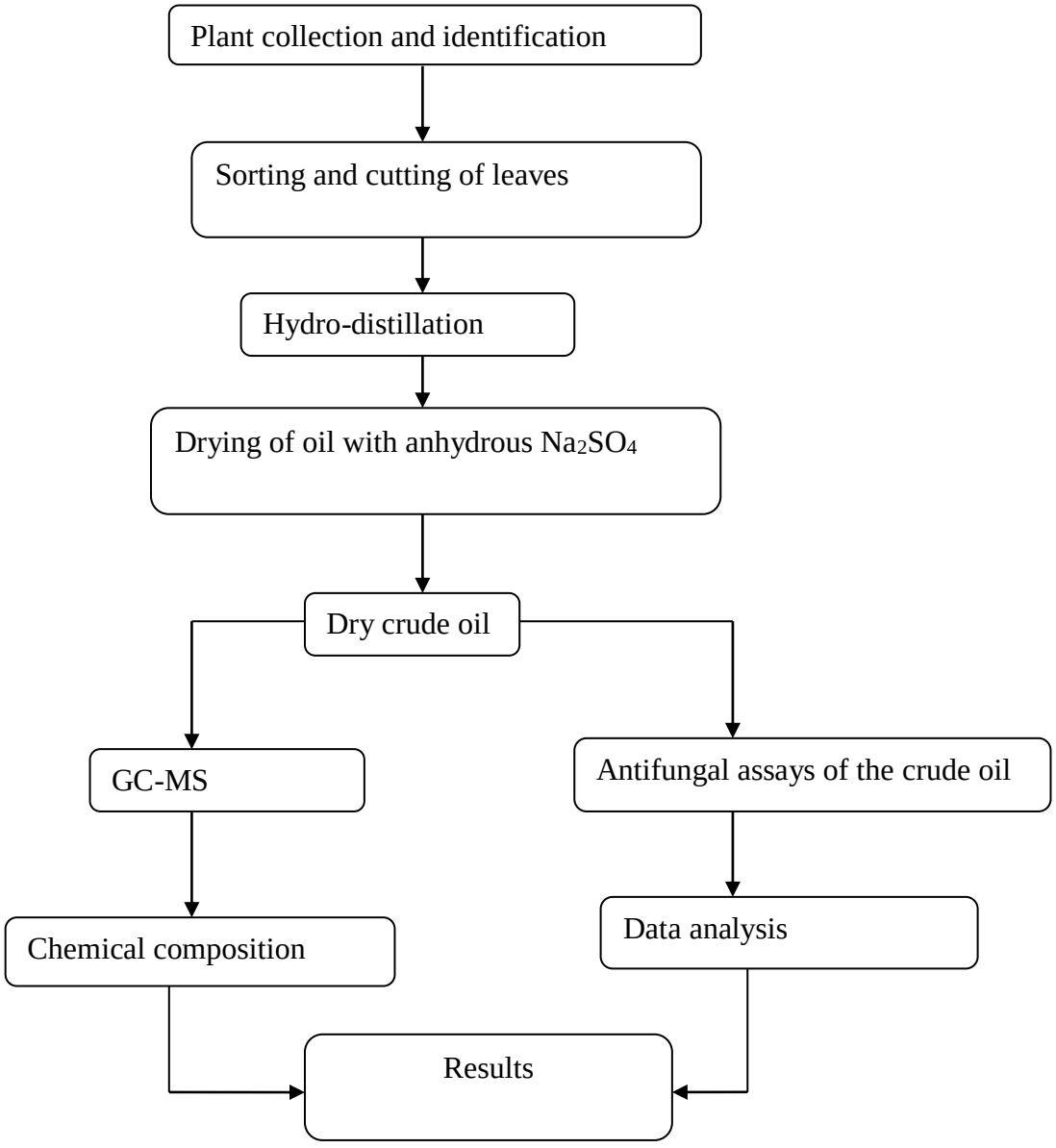


Figure 6: A flow chart showing the summary of isolation and analysis of essential oil

3.3 Extraction, isolation and structure elucidation of non-volatiles

Extraction of secondary metabolites from *P. dodecandra* and *B. alba* was done using solvents of different polarities. These solvents included methanol, ethyl acetate and hexane. The solvents obtained were of GPR grade and were distilled before use. The procedure of the extraction, isolation and structure elucidation of the compounds obtained from the two plants is summarized in figure 4.

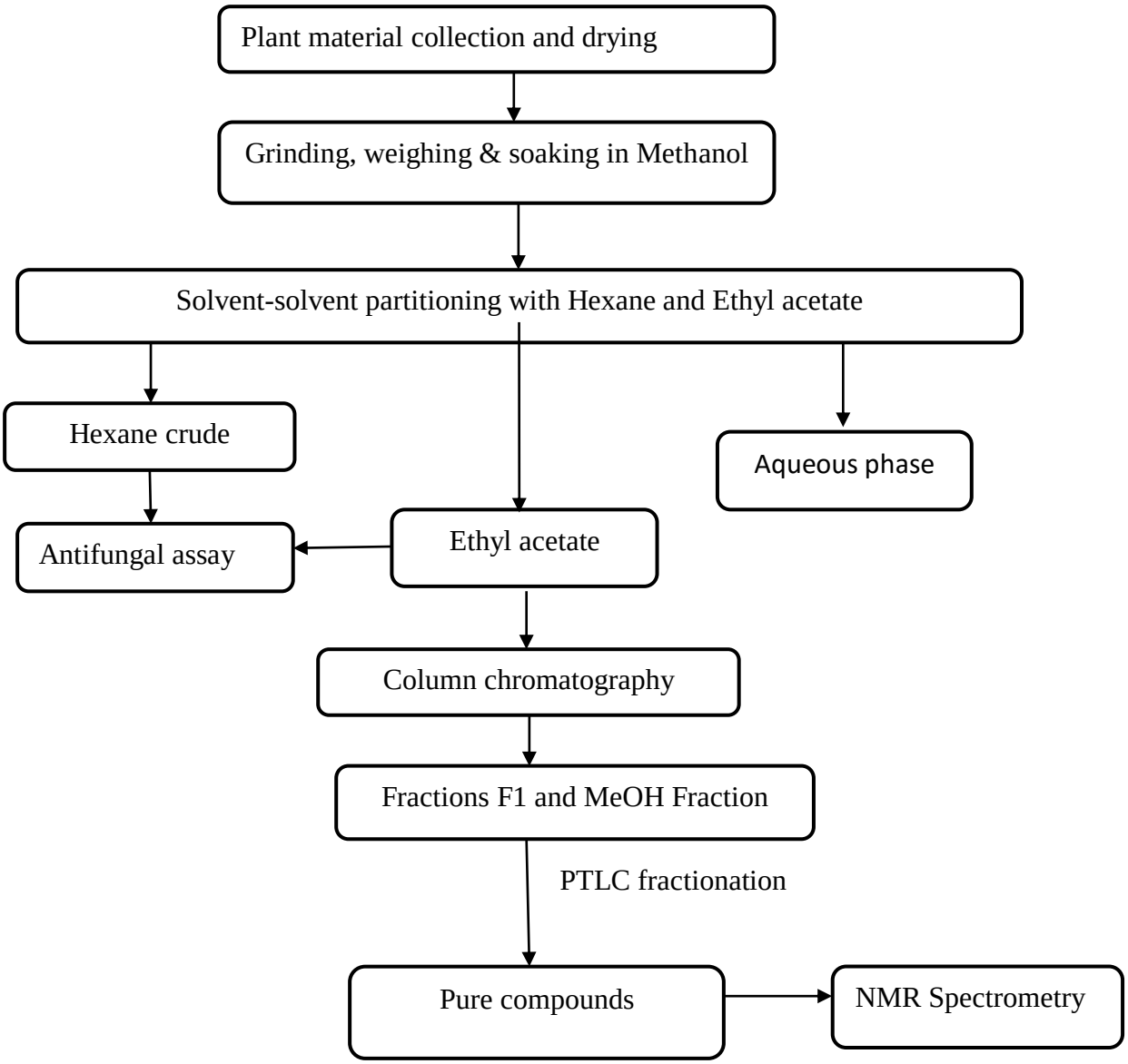


Figure 7: A summary of isolation and structure elucidation of non-volatiles

3.3.1 Extraction of phytochemicals

The leaves obtained from *P. dodecandra* and *B. alba* were differently dried and continuously turned during drying. It was done to enable uniform drying and to avoid rotting. The materials were separately ground to a fine powder using a Thomas-Wiley mill model 4. The ground materials weighing 500g were respectively soaked in methanol at room temperature for 24 hours with periodical shaking. The contents were filtered through Whitman no. 1 filter paper and the filtrate evaporated to dryness in vacuum at 40°C using Buchi Rota vapor R-205 rotary evaporator. The methanol crude extracts weighing 30.5g and 34.0g obtained from *P. dodecandra* and *B. alba* were left to completely dry in a fume hood.

3.3.2 Solvent partitioning of crude methanol extract

The crude methanol extracts of *P. dodecandra* and *B. alba* weighing 28.6g and 31.5g respectively were individually subjected to liquid-liquid fractionation by suspending them in distilled water and sequentially extracting with hexane and ethyl acetate. This liquid-liquid extraction process was done using a 500mL separatory funnel. After making sure the stopcock at the bottom was closed (in the horizontal position), the aqueous mixture was poured into the separatory funnel and 50mL hexane was added. The separatory funnel was then stoppered, and with one hand gripping the top of the funnel so that a finger holds in the stopcock, the separatory funnel was tipped upside down and gently swirled to thoroughly mix the two layers.

The stopcock was occasionally opened with one hand to relieve pressure that usually builds up from the vapor pressure of the solvent. Thorough mixing was important because the two solutions must be in contact with each other to allow the solute to be extracted into the second layer. After the mixing, the funnel was suspended in a retort stand to completely resolve the mixture into two layers. The lower aqueous layer was drained into a clean conical flask by slowly opening the stopcock. Just as the interface between the two layers was entering the stopcock, the stopcock was closed. The hexane was then drained out into a separate beaker. The extraction was exhaustively done until the hexane layer becomes clear and an assumption was made that there was no more extraction from aqueous layer to hexane. Due to the presence of water droplets in the hexane phase, some anhydrous sodium sulphate was added to remove the droplets. From the hexane extract, the solvent was

removed in a rotary evaporator at 40°C and residue weighing 21g (hexane extract) was dried completely in a fume hood. The same procedure above was repeated for ethyl acetate solvent. The ethyl acetate extract obtained was then subjected to extensive TLC analysis and column chromatography.

3.3.3 Thin layer chromatography (TLC)

The hexane extract was not subjected to TLC because it carries fats and sugars. Thus, TLC was used to analyze the ethyl acetate extracts having the ultimate goal of finding the best solvent system for resolution of the extracts. Several different combinations of solvents were tested; it was found that 6:4 (ethyl acetate: hexane mixture) gave a better profiling for *P. dodecandra* ethyl acetate extract and that of *B. alba* was 6:3 (ethyl acetate: hexane mixtures). Briefly, the extracts were re-dissolved in ethyl acetate. Samples were individually spotted on a TLC plate with the dimensions of 1cm x 5cm. A line was drawn with a pencil 1 cm from the bottom along the short (1cm) side of the plate; this was done carefully in order not to disturb the silica gel. Then with a small capillary tube, spotting of the samples was done along the drawn line carefully making sure that the capillary touch the surface of the plate quickly and lightly to avoid overloading of spots. The spotted plates were developed inside a 100mL glass beaker pre-saturated with the corresponding solvent system and covered with aluminum foil. The set up was left to stand undisturbed and the solvent front was allowed to move up the plate until it was approximately 1cm from the top. The developed chromatograms were removed; solvent front drawn with a pencil and visualization was done by illumination under UV lamp (Uvitec-LF-204.LS) at 254nm and 365nm. The visible spots were circled with a pencil.

3.3.4 Column chromatography

After getting the solvent system with the best resolution with the TLC, the samples were prepared for column chromatography. Approximately 5g of the samples were separately re-dissolved in the solvent system before they were individually packed in a chromatographic column. The columns were clamped vertically; cotton wool was used as a plug to support the adsorbent and half-filled with the solvent system to be used for the separation. A long glass rod was used to place the wool at the bottom of the column. The wool was compressed enough to support the column packing yet loose enough that the solvent flow will not be

hindered. The silica gel was weighed out in a conical flask, and enough solvent was added with stirring to form slurry. All of the air bubbles were removed from the slurry before filling the column. With the aid of a 100mL glass funnel, the slurry was added to the column. As soon as the column begins to build, the stopcock is opened. This allowed the excess solvent to drain, and helped to settle the silica gel. The solvent must not be allowed to drop below the silica gel level, as this could introduce air bubbles that would disrupt the continuity of the packing and lead to a loss in or poor resolution.

The sample was then added to the top of the column, as a neat liquid dissolved in a minimum amount of the solvent used to pack the column. The sample was carefully added down the side of the column so as not to disrupt the silica gel surface. Solvent is drawn from the bottom of the column until the level of the liquid is just above the level of the silica gel. Fresh solvent was carefully added to the column, and the stopcock opened so that the solvent was continuously flowing through the column. Fractions of a standard equal volume were collected and TLC done on each fraction. Fraction with nearly the same TLC patterns was pooled. *Phytolloca dodecandra* sample gave three fractions while that of *B. alba* gave four fractions.

3.3.5 Purification of compounds

The fractions collected from the column were subjected to purification by preparative thin-layer chromatography. The plates were made by preparing slurry from silica gel 'G' (for TLC, containing 13% calcium sulphate) using distilled water and evenly distributing on a 20cm × 20cm glass plates. They were then left overnight to dry on the bench before completely drying them in the oven for three hours. After complete drying, a line was carefully and slightly drawn in about 1cm from the bottom of the plate. The fraction sample which was re-dissolved in the solvent system was streaked along the marked line. Streaking was done with care in order not to overload the streaks. The streaked plates were then developed in a chromatographic tank and left until the solvent front was about one inch from the top. The plates were removed from the tank, visualize under UV light and the bands lightly marked with a pencil. Using the edge of a spatula, the bands were scraped off onto a lengthwise folded piece of clean, white paper. The scrapings were placed in a conical

flask, extracted with ethyl acetate and the compound dried in a rotary evaporator. TLC was used to follow the separation and to check for purity.

3.3.6 Nuclear magnetic resonance (NMR) spectroscopy

The ^{1}H, HSQC, COSY and HMBC NMR spectra were recorded on the Bruker Advance 500 MHz NMR spectrometer at the Technical University of Berlin, Germany. All the readings were done in Deuterated chloroform and chemical shifts assigned by comparison with the residue proton and carbon resonance of the solvent. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts were given as δ (ppm). The structures were elucidated using ACD NMR manager program to obtain the chemical shifts of proton.

3.3.7 Two dimensional NMR spectroscopy

The off- diagonal elements were used to identify the spin – spin coupling interactions in the ^{1}H –^{1}H COSY (Correlation spectroscopy). The proton-carbon connectivity, up to three bonds away, was identified using ^{1}H–^{13}C HMBC (Heteronuclear Multiple Bond Correlation) spectrum. The ^{1}H–^{13}C HSQC spectrum (Heteronuclear Single Quantum Coherence) was used to determine the connectivity of hydrogen to their respective carbon atoms. The APT (Attached proton test) spectrum was used to identify the resonances of quaternary, methines, methylene and methyl carbon atoms.

3.3.8 Mass spectrometry

The mass spectra of the compounds were recorded on Finnigan Triple Stage Quadrupol Spectrometer (TSQ-70) with electro spray ionization (ESI) Method. The Thermo Xcalibur Qual computer software was used in analysis of the mass chromatograms.

3.4 Screening for antifungal activity

3.4.1 Antifungal assays

The paper disc diffusion inhibition test was used to screen for antifungal activity of both volatile and non-volatile secondary metabolites as described by Souza *et al*. (2005). The method inhibits both the growth of the fungi and the developments of hyphae. The Potato Dextrose Agar (PDA) was used in the culture of fungi. The medium was prepared by weighing the quantities recommended by the manufacturer and dissolving in recommended

quantities of distilled water. The media was then sterilized using an autoclave set at 121^0C for 15 minutes. It was then allowed to cool to about 40°C and poured onto sterile Petri dishes then allowed to cool completely on a clean bench. Each plate was seeded with 0.1 mL fungi suspension. The discs loaded with the extracts were then placed onto the center of the seeded plates. The fungal cultures were left to grow at 25°C

During the incubation period, diameters of the inhibition zones were continuously measured and recorded for 14 days. Zones of inhibition were recorded in mm as described by Badria and Elgayyar, (2000). Negative control plates had discs with sterile dimethyl sulfoxide (DMSO). During the incubation period, diameters of the inhibition zones were continuously recorded for 14 days. Nystatin was used as the reference standard. The essential oil from *L. javanica* was selected based on the antifungal spectrum and larger size of inhibition zones observed. The data collected from the observations of antifungal activity were analyzed using the SPSS software.

3.4.2 Determination of the Minimum Inhibitory Concentration

Essential oil was evaluated for the Minimum inhibitory concentration (MIC). Using the method described in section 4.1 above, serial dilutions of the essential oil was done using dimethyl sulfoxide (DMSO) which was also used as the negative control. The oil was diluted to the following serial geometric dilutions: 50%, 25%, 12.5%, 6.25%, 3.13%, and 1.56%. The resultant minimum inhibition zone was used to determine the range for MIC analyses. In all cases, the culture plates were kept at 25^oC for the entire experimental period. The lowest concentration able to induce inhibition will be considered as the MIC. The experiment was done in three replicates.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Structure elucidation of isolated compounds

A compound was isolated from each of the plants. These are **16** from *P. dodecandra* and **17** from *B. alba* respectively.

4.1.1 Structure elucidation of compound 16

From the ethyl acetate extract of *P. dodecandr*a, compound (**16**) was obtained. This compound was obtained as dark mass of 67 mg. It was observed as a dark spot and a purple spot under 254 nm and 365 nm respectively under UV irradiation on TLC. The 1D and 2D NMR spectral data of compounds **16** is summarized in table 1.

Compound **16** had twenty-five carbon atoms, forty-two hydrogen atoms, three oxygen atoms and hence the molecular formula $C_{25}H_{42}O_3$. The ^{13}C NMR (appendix 2) and DEPT (appendix 3) spectra of compound **16** confirmed the presence of twenty-five carbon atoms, consisting of three quaternary, seven methine, eleven methylene, three methyl and one methoxy carbon atoms. The chemical shifts of the three quaternary carbons were observed to occur at δ 156.3, 143.0 and 41.4. The chemical shifts of the eight-methine carbons were observed to occur at δ 130.37, 127.78, 113.93, 113.93, 69.06, 50.2, 3102 and 27.98. The chemical shifts of the eight-methylene carbons were observed to occur at δ 73.46, 68.87, 44.79, 37.1, 31.93, 30.04, 29.37, and 22.69. The methoxy carbon was observed to be having a chemical shift of δ 59.3. The chemical shifts occurring at δ59.28 was assigned to methoxycarbonyl carbons (C-1”). Chemical shifts of the quaternary carbons (C-7, C-8, and C-11) were observed to occur at δ 41.43, 143, and 156.33 respectively.

The HSQC spectrum (appendix 4) of compound **16** showed correlations of carbon atoms and the protons directly attached to them. There was a correlation between C-1 and the protons absorbing at δ 3.55 and 3.59. There were correlations between C-2 and the protons absorbing at δ 2.75 and δ 2.9. The protons absorbing at δ 1.28 and δ 1.04 correlated with C-3 and C-4 respectively. The pairs of proton observed to be occurring at δ 0.9, δ 1.27 and δ 3.32, δ 3.39 were observed to be correlating with C-5 and C-6 respectively.

Table 1: ^{1}H NMR and ^{13}C NMR spectral data of compound **16**

Carbon	$\delta^{13}C$ (ppm)	DEPT	δ ^{1}H/HSQC (ppm)	HMBC	COSY
1	73.46	CH_2	3.55, 3.59		22,4,14
2	44.79	CH_2	2.75, 2.9		
3	27.98	CH	1.28	6,7	5,16
4	24.34	CH_3	1.04		5,18
5	22.69	CH_2	0.9, 1.27		18
6	50.02	CH	3.32, 3.39		
7	41.43	Q	-		22,4,14
8	143	Q	-		
9	130.37	CH	7.14		
10	114	CH	6.83	7,813,16,	6,2’
11	156.33	Q	-		2,2’
12	113.93	CH	6.84		22,4,14
13	127.78	CH	7.14		
14	27.09	CH_3	2.01	6	6,2’
15	28.41	CH_3		1	2,2’
16	29.37	CH_2	1.27	1	
18	31.02	CH	1.64	6,15	
19	30.04	CH_2		1	1
20	31.93	CH_2	1.28	2,6,11	
21	22.93	CH_3		1,1”	1’
22	14.12	CH_3	0.92		5,14,16,
1’	68.87	CH_2	4.16		13,9
1”	59.28	CH_3	3.42		13,9
2’	69.06	CH	3.65	8,11,12,13	12,10,11

There was a correlation between C-9 and a proton which was observed to resonate at δ 7.14, while a proton resonating at δ 6.83 was observed to correlate with C-10. Carbons C-12 and

C-13 were also observed to be correlating with protons resonating at δ 6.84 and δ 7.14 respectively. There were correlations that were observed between C-14 and a proton resonating at δ 2.01 while C-16 correlated with a proton resonating at δ 1.27 respectively.

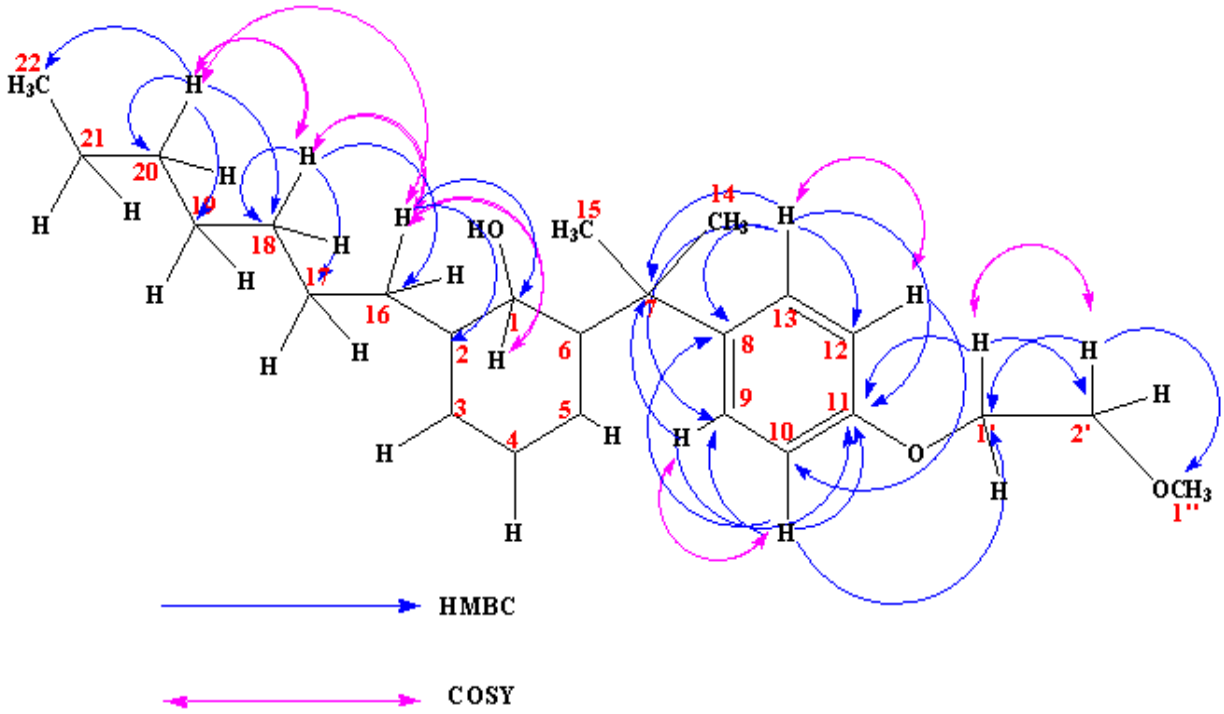


Figure 7: Structure of compound **16** showing COSY and HMBC

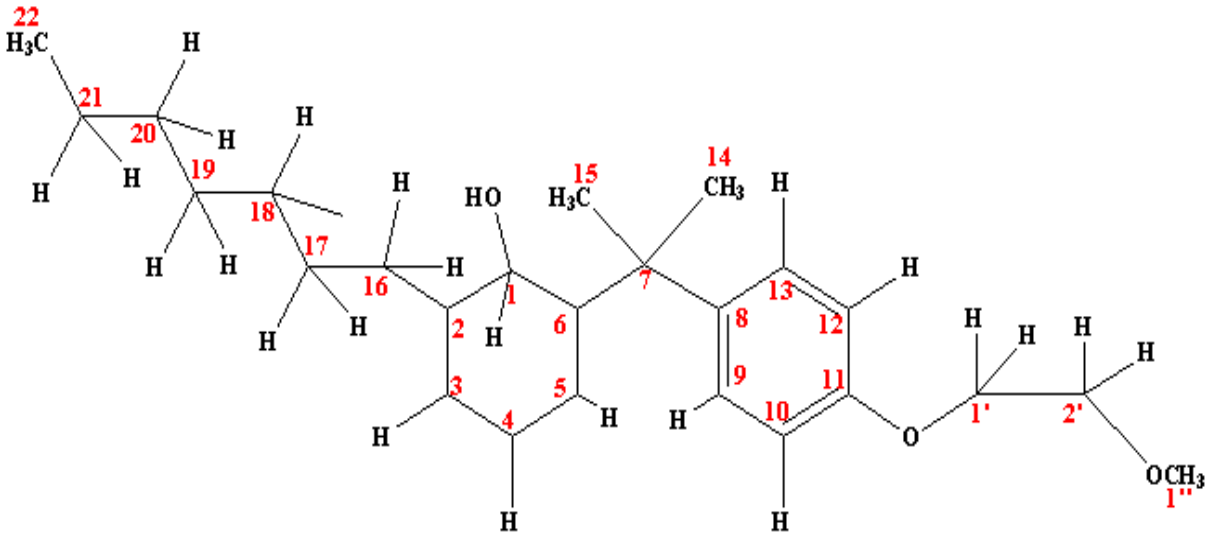


Figure 8: Structure of compound 16

The ^{1}H NMR spectrums (appendix 1) of compound **16** revealed the presence of four aromatic protons; the two doublets resonating at δ 6.84 and 7.14. There was also an observed singlet at δ 3.42 which for the methoxy group.

The proton resonating at δ 1.64 was observed to be correlating with C-18. From the appendix 1, it can also be seen that C-20 and C-22 correlated with the protons resonating at δ 1.28 and δ 0.92 respectively. The carbon C-1' and C-2' correlated with the protons resonating at δ 4.16 and t δ 3.65 respectively. The methoxy carbon absorbing at δ 59.28 (C-1") correlated with a proton resonating at δ 3.42.

The ^{1}H-^{1}H COSY (appendix 5) and TOCSY (appendix 6) correlation that correspond to compound 16 were also determined. From the TOCSY spectrum, there were correlations between the protons attached to C-1 and those attached to C-4, C-14, and C-22. The coupling constant for peaks absorbing at δ 3.59 (H bonded to C-1) and δ 1.04 (H bonded to C-4) and δ 0.89 (H bonded to C-22) were calculated to be 2.53 Hz and 2.69 Hz respectively. The proton resonating at δ 1.28 (H bonded to C-3) correlated with the protons resonating at δ 0.9 (H bonded to C-5) and 1.27 (H bonded to C-16). The coupling constants were calculated to be 0.38 Hz and 0.01 Hz respectively. The proton resonating at δ 1.04 (H bonded to C-4) showed correlated with protons resonating at δ 1.64 (H bonded to C-18), the calculated coupling constants was found to be 0.60 Hz. Proton. The aromatic proton resonating δ 6.83 (H bonded to C-10) correlated with the protons resonating at δ 2.75 (H bonded to C-2) and δ 3.39 (H bonded to C-6).

From the HMBC spectrum (appendix 7), the proton attached to C-3 resonating at 1.28 showed correlations with C-6 and C-7 respectively. The proton bonded to C-10 resonating at 6.83 showed correlations with C-7, C-8, C-13, and C-16. The proton attached to C-14 showed correlations with C-6. There were correlations observed between C-1 and the proton resonating at δ 1.27 (attached to C-16). Proton resonating at δ 1.64 showed correlation with C-6 and C-15. The proton resonating at δ 3.65 (attached to C-2') showed correlations with C-8, C-11, C-12, and C-13. The calculated coupling constants were found to be 4.08 Hz and 3.41 Hz respectively. Based on the 1D and 2D NMR information, the proposed structure for compound **16**is shown in figure 5

Compounds **16** had a molecular mass of 390.599 calculated for $C_{25}H_{42}O_3$. The compound was analyzed at wavelength range of 200-600 nm and therefore, only the M peak was recorded. This corresponded with the retention time at 9.1 minutes. The positive electron impact mass spectrometry (EIMS) for compounds **16** revealed a peak at *m/z* 391.13 representing the molecular ion $[M + H]^+$ corresponding to molecular formula $C_{25}H_{42}O_3$ and *m/z* 413.14 representing $[M + Na]^+$ and corresponding to$C_{25}H_{42}O_3$. The mass spectrum for compound **16** is figure 7.

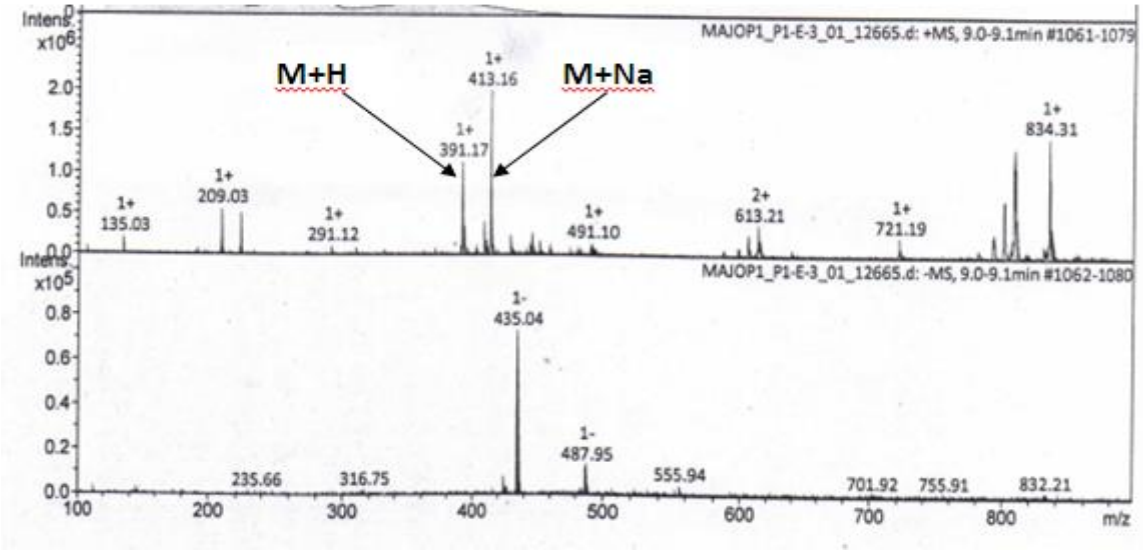


Figure 8: Mass spectrum of compound 16

4.1.2 Structure elucidation of compound 17

This compound was obtained as a yellow jelly like mass. It was observed as a bright yellow spot under UV irradiation with a wavelength of 365 nm on a TLC. The NMR data showed the presence of eight carbon atoms, 16 hydrogen atoms, 2 oxygen atoms and hence the molecular formula $C_8H_{16}O_2$. The 1D and NMR spectral data of compound **17** is given in Table 2.

The C^{13} NMR (appendix 9) and DEPT (appendix 10) spectra of compound 17 confirmed the presence of eight carbons consisting of 1 carbonyl carbon, 6 methelene carbons and 1 methine carbon. The chemical shift of the carbonyl carbon occurred at δ174.7. The chemical shifts for the methelene carbons were observed to be occurring at δ24.1, 25, 29.2, 29.0, 31.8, and 22.6. The methyne carbon atom was observed at δ14.4. The ^1H NMR (appendix 8) of compound 17 revealed the presence of methene protons and methine protons.

The chemical shifts observed at δ174.5 was assigned to carboxylic carbon atom(C-1), while the chemical shifts occurring at δ34.1, 25, 29.2, 29.0, 31.8, and 22.5 were assigned to methelene carbons (C-2, C-3, C-4, C-5, C-6, and C-7) respectively. Methine carbon (C-8) was assigned the chemical shift, which was observed at δ14.4.

Table 2: ^{1}H NMR and ^{13}C NMR spectral data of compound **17**

Carbon	**^{13}C(δ)**	**^{1}H(δ)**	**DEPT**	**COSY**	**HMBC**	**^{13}C(δ)**
1	174.5	-	C	-	-	178.5
2	34.13	2.18	CH_2	2,5	1,4,5	33.83
3	24.95	1.49	CH_2	4,6	1,2,5,	24.53
4	29.19	1.24	CH_2	5,9,10		29.01
5	29.01	1.23	CH_2	5,10		28.86
6	31.76	1.90	CH_2			31.57
7	22.55	0.83	CH_2	4,6		22.57
8	14.40	0.83	CH_3	4,6		13.79

HSQC spectrum (appendix 11) showed that the protons absorbing at δ0.83, 0.83, 1.49, 1.23, 1.24 and δ2.18 correlated with carbon atoms C-1, C-2, C-3, C-4, C-5, C-6, C-7 and C-8. The proton- proton COSY (appendix 12) gave information on protons, which are attached to adjacent carbon atoms. The spectrum showed correlations between protons H-2 and H-3 absorbing at δ2.18 and δ1.90 respectively. Proton H-4 absorbing at δ1.24 correlated with H-6 and H-7 absorbing at δ1.49 and δ0.83 respectively. There was also proton correlation between H-5 absorbing at δ 1.23 with both H-2 and H-1 absorbing at δ 0.83.

The proton-carbon HMBC spectrum (appendix 13) showed correlation spectrum representing proton correlations with carbon atoms, which are two bonds or three bonds away. This helps in identification of carbon atoms, which are next to each other and those that are two bonds away from each other. The proton H-2 resonating at δ 2.18 correlated

with carbons C-1, C-4, and C-5. In addition, the proton oscillating at δ 1.49 correlated with the C-1, C-2, and C-5.

The high resolution positive electron impact mass spectrometry (HREIMS) of this compound at 1-16 minutes retention time showed a molecular ion peak at *m/z* 309.21 ($[2M+Na-2H]^+$) (calculated for ($2[C_8H_{16}O_2]$ + Na-2H); *m/z* 309). The mass spectrum of the compound is shown in figures 20. The compound was identified as with molecular ion peak m/z 309.21 ($[2M+Na - 2H]^+$) (calculated for $[C_8H_{16}O_2]$ (m/z 144.211) using high resolution positive electron impact mass spectrometry (HREIMS)

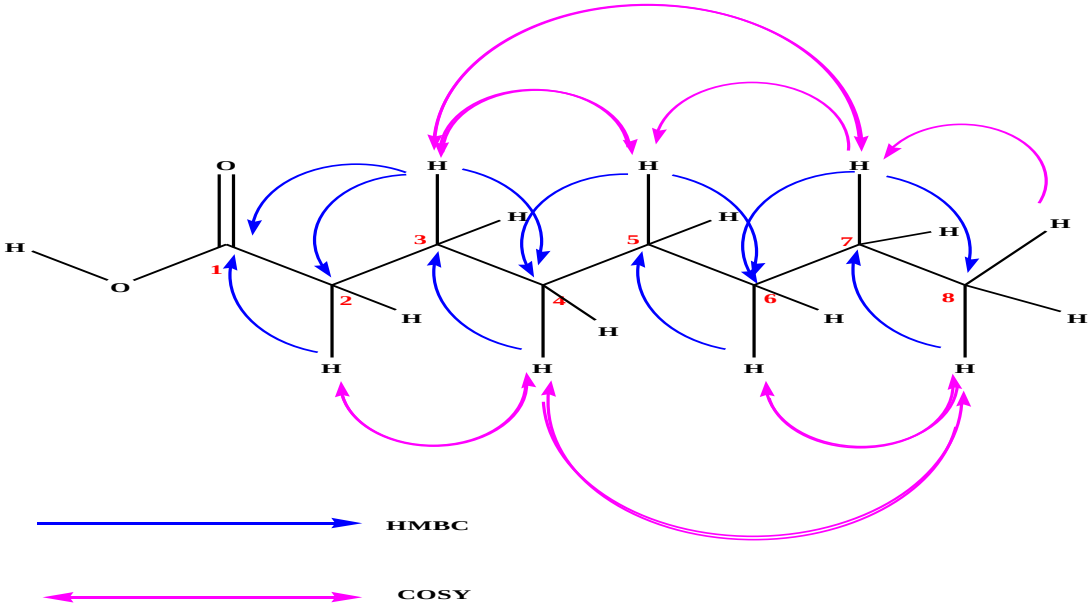


Figure 9: Structure of compound **17** showing COSY and HMBC

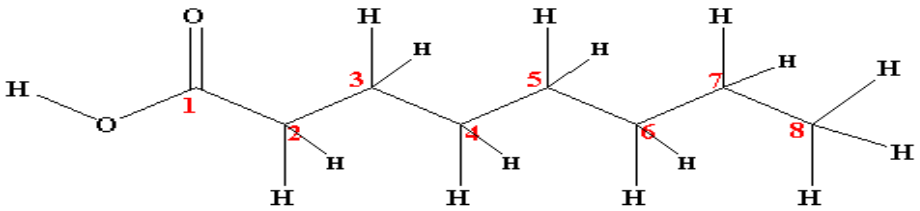


Figure 10: Structure of compound **17**.

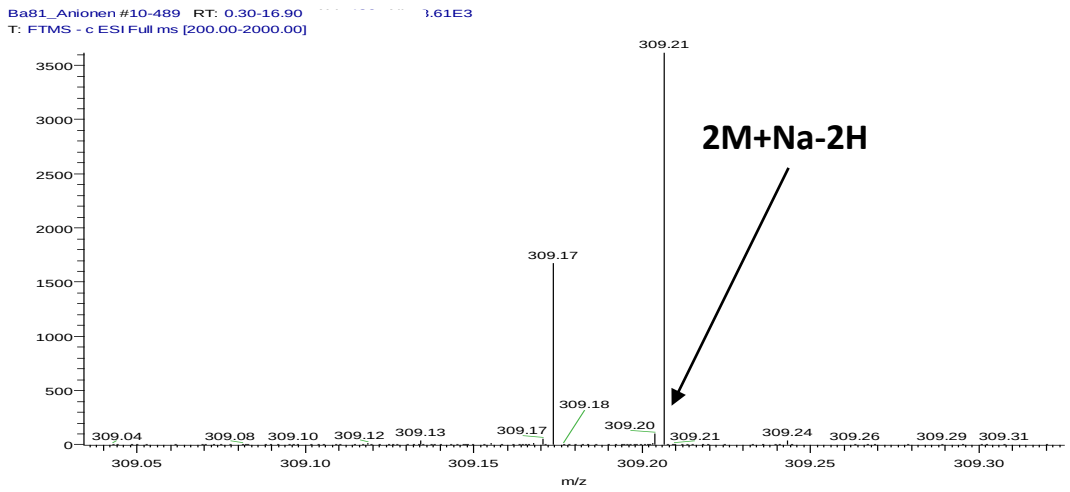


Figure 11: Mass spectrum of compound **17**.

4.2 Antifungal assays of *Lippia javanica* oil

Lippia javanica leaves gave a percentage yield of 0.3% v/w essential oil. The density was found to be 0.87g/mL. Antifungal activity of the oil was tested against the *F. moniliforme* and *F. graminearum*. At 100% oil concentration, there was no observable activity of the oil against *F. moniliforme*. It was concluded that the oil has no activity against *F. moniliforme* and no further assays were done against it. The oil was observed to be active against *F. graminearum* and the observations made were tabulated in the Table 3.

Activity of the oil against *F. graminearum* was determined by measuring the diameters of inhibition zones. Activity of the oil against the fungi was observed to be concentration dependent. It was observed that the zones of inhibition reduced with the reduction in percentage concentration of the oil. The results tabulated demonstrate that essential oils from *L. javanica* interfere with the growth of *F. graminearum*. The bioassays were carried out at concentrations of 0.87, 0.65, 0.43, 0.22, 0.11, 0.054, and 0.027mg/mL (essential oil/mL). For positive control in antifungal assays, commercial Nystatin at a concentration of 100µg/disc was tested. In general, the oil is active against the *F. graminearum.* From the data in Table 3, the oil showed the variation of inhibition effects on the visible growth of the fungi with the variation of concentration in comparison with control. The maximum antifungal activity was recorded from the concentration of 0.87g/mL, and the least activity was recorded for the least concentration of 0.027g/mL.

After the third day (appendix 16), the inhibition zone for the 0.87g/mL concentration was larger (25.00mm) as compared to that of the Nystatin (16.00mm). The activity of Nystatin was comparable to the oil concentration of 0.435g/mL (16.00mm). It was also observed that the inhibition zones reduced with increasing time. It implies that the activity of both the oil concentration was reducing as time increases. By the seventh day, the inhibition zone of the 0.87g/mL concentration had reduced to 16.00 mm; while that of Nystatin was at 12.33mm. Comparing the inhibition of Nystatin to that of 0.435g/mL concentration of the oil that had the same concentration with Nystatin after three days; their values are 12.33 and 6.00 mm, respectively.

The observations point out the fact that the activity of the oil reduces faster as compared to that of Nystatin. The inhibition zones reduced with time, and during the fourteenth day, it had reduced to 6.67, 6.33, 2.33, 2.00, 0.33, 0.00mm and 0.00 for the 8.7, 6.5, 4.4, 2.2, 1.2, 0.54, 0.27mg/disc respectively. The inhibition zone of the positive control after fourteen days had reduced to 10.67mm. The data obtained showed that the potency of the oil reduces with time. Comparing the inhibition zones of the oil to that of the positive control after 7 days, 100% concentration of oil showed almost equal inhibition zone with that of Nystatin. During the 14^{th} day, the inhibition of the oil had greatly reduced to 6.67mm while that of Nystatin had reduced only to 10.67mm. Though there was reduction in activity for both the positive control and the oil, the observations showed that the activity of the oil reduced greatly when compared to Nystatin. This was shown by the reduction in inhibition zones. According to Birkett *et al*., (2011), the volatility nature of the essential oil is characteristic feature for easy oxidation. Therefore, the oil loses its activity with time.

From the analysis that was done using the SPSS (appendix 14) the variation in concentration shows the significant variation in the level of activity. The mean activity was done at 95% Confidence Interval. From the results, concentration of 87mg/mL registered the highest activity while the least activity was observed when concentration of 2.72mg/mL (the least concentration used). Also, the highest concentration showed the slightest standard deviation in activity as compared to recorded activity from other observations.

From the SPSS POST-HOC analysis (appendix 16), it was noted that the activity of the essential oil showed big difference in the mean activity exhibited by the oil. Moreover, the

variation in the activity showed significant mean difference. From the third day, it was observed that the mean difference in activity of the positive control and 87mg/mL is +9.00. This mean difference was comparable to that between the highest concentration (87mg/mL) and 43.5mg/mL. It can also be noted that there was an increase in the significant mean differences in activity as the concentration reduces. The highest difference in the observed activity was when the concentration was the least (2.72mg/mL). Similarly, the highest mean difference of the observed activity was between the highest and the least concentrations (between 87mg/mL and 2.72mg/mL). On day four, there was a slight variation in the mean activity.

On the fifth day (Appendix 16), there was a reduction in the mean activity. The reduction was attributed to several factors that include reduction in potency due to aerial oxidation and volatility of the essential oil. The mean difference remain relatively constant. Comparing the highest concentration (87mg/mL) and that of the standard, the mean difference in activity is given to be 8.0, which reduced from 9.0 as seen in day 3. The activity observed was comparable to those observed in day 3. The highest activity was seen on concentration 87mg/mL and the least was seen on concentration 2.72mg/mL. the activity gradually reduced with the reduction in concentration. The trend was comparable to that seen in appendix 16, 17 and 18.

The activity of Nystatin is slightly stable when compared to that of the oil. The reduction in inhibition zone could be attributed to instability of the oil. These observations are in agreement with the work done by Viljoen *et al* (2005) where concentration was compared to time in killing *Klebsiella pneumonia.* They found out that efficacy of *L. Javanica* oil showed a killing rate of *Klebsiella pneumonia* within 30 min at 100% concentration tested. For the lowest concentration of 0.25%, it took 8 h before a bactericidal effect could be observed.

The MIC was calculated from the least concentration used (3.125%) and it was obtained to be 27.19mg/mL. When based on GC-MS results obtained from the oil analysis, it is possible that the antifungal activity was attributed to synergism action of the major and the minor components of the oil whose antimicrobial properties have been shown in previous studies. Synergistic combinations reduces the dose of potentially polluting substance and also

reduces the risks of fungi developing resistance (Tripathi *et al*., 2009).

The antifungal mechanisms of the chemical constituents of essential oils are unknown but may be related to their general properties of destroying the development of fungi's cells wall and cells membranes (Isman and Machial, 2006).In addition, the mode of antimicrobial action of the oil may also be due to the inhibition of respiration (Koschier *et al* 2001). Considering that the oil is majorly constituted of Monoterpenes, Tripathi *et al* (2009) argued that the structural modifications common to natural monoterpenoids may lead to improved biological activity. In addition, their biological activities are related to position and nature of the functional groups and molecular configurations of the oil constituents rather than its volatility and molecular size(Kumbhar & Dewang, 2001). The other speculation on the enhanced efficacy of the oil is due to differential permeability as a result of molecular actions which have been prompted by adhesive activities of the oil molecules (Lukwa, 1994). However, according to (Bakkali *et al*., 2008), it is possible that the activity of the major components is modulated by other minor molecules in the mixture.

Table 3: Antifungal activity of *L. javanica* oil against *F. gramenearum*

Days	Essential oil concentration (g/mL) *10^{-2}								
	87	**65.25**	**43.5**	**21.75**	**10.88**	**5.44**	**2.72**	**Nystatin**	**Control**
3	25.00±1.00	20.33±1.53	16.00±2.00	12.17±1.26	8.33±2.31	5.67±2.52	3.33±1.53	16.00±1.00	0.00±00
4	23.00±1.00	17.00±2.65	14.17±2.08	10.00±2.60	5.33±1.53	4.67±2.89	2.33±1.15	15.33±0.75	0.00±00
5	22.00±1.00	15.83±2.25	10.33±1.53	8.33±1.61	4.00±1.00	2.00±1.00	0.67±0.58	14.00±0.69	0.00±00
6	19.00±1.00	13.67±2.08	7.67±0.58	5.17±0.58	3.17±1.04	1.67±3.21	0.33±0.58	13.33±0.68	0.00±00
7	16.00±1.00	12.33±2.08	6.00±1.00	6.00±1.00	2.83±1.04	1.33±0.58	0.17±0.29	12.33±0.66	0.00±00
8	13.33±0.58	10.67±1.53	5.00±1.00	5.00±1.00	2.33±0.58	0.67±0.58	0.00±00	11.00±0.54	0.00±00
9	10.67±0.58	8.67±0.58	4.67±1.15	4.67±1.15	1.50±0.50	0.67±0.58	0.00±00	11.00±0.54	0.00±00
10	9.33±0.58	6.33±0.58	4.00±1.00	4.00±1.00	1.00±00	0.50±0.50	0.00±00	11.00±0.54	0.00±00
11	8.00±1.15	5.00±1.00	3.67±0.58	3.67±0.58	0.67±0.58	0.50±0.50	0.00±00	11.00±0.54	0.00±00
12	7.67±0.58	4.00±3.33	3.00±1.00	3.00±1.00	0.33±0.58	0.33±0.29	0.00±00	11.00±0.54	0.00±00
13	7.33±1.53	3.33±.058	2.67±1.53	2.67±1.53	0.33±0.58	0.17±0.29	0.00±00	11.00±0.54	0.00±00
14	7.000±1.73	2.67±.058	2.33±1.15	2.33±1.15	0.33±0.58	0.00±00	0.00±00	10.67±0.49	0.00±00

4.3 Determination of the chemical composition of the oil extract

The chemical composition of *L. javanica* essential oil was determined using the GC-MS. The chemical constituents of the oil were identification by comparing the electron impact mass spectrum of the compounds in the oil and those in the Wiley7N.l, FLAVORS.L and HPCH1607.L computer library databases. The essential oil composition of *L. javanica* and the relative amounts of the components are summarized in Table 4

Table 4: Major constituents of *L. javanicca* essential oil

Compound Number	R.T (min)	Compound Name	% Concentration	Detection Method
18	8.45	β – Myrcene	3.13	GC/MS
19	9.18	Menthatriene	0.51	GC/MS
20	10.12	Tagetone	2.85	GC/MS
21	11.23	Bicyclo[3.1.0]hexane,6-methylene-	0.44	GC/MS
22	11.46	Linalool	4.43	GC/MS
23	12.67	Camphor	0.90	GC/MS
24	13.11	Artemisia Ketone A21	49.52	GC/MS
25	15.21	Phenol, M-tert butyl	8.73	GC/MS
26	16.28	Isopiperitenone	2.13	GC/MS
27	20.06	Z-Caryophellene	1.99	GC/MS
28	20.91	4,7,10-Cycloundecatriene, 1,1,4,8-tetramethyl-, cis, cis, cis-	0.92	GC/MS
29	24.05	Caryophellene oxide	1.31	GC/MS

A total of 22 components were identified accounting for 76.85% of the oil composition. The oil is characterized by Monoterpenes as shown by the high percentage of Artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%),

Targetone (2.85%) and Isopiperitenone (2.13%). Monoterpenoids has a great variety of structures and are the most common representations in all the essential oils from aromatic plants (Tripathi *et al*., 2009). The high percentages of monoterpenes proved that the *L. javanica* essential oil clearly belongs to the monoterpenoid chemotype. In contrast, the content of Sesquiterpenes constituted only 6.06% of the total oil composition. Main representatives of Sesquiterpenes were trans-caryophyllene (27) (2.0%) and caryophyllene oxide (29) (1.3%). According to Viljoen *et al*., (2005), there has been a lot of research on chemical constituents of the essential oil from the *Lippia* species. The results showed great variations due to ecological variations and population or chemotypic races. These monoterpenes are characterized by low molecular weight terpenes mainly the monoterpenes (C10) and Sesquiterpenes (C15).

The chemotype of the oil was identified as Artemisia ketone. Oil composition varies according to isolation method (Tripathi *et al*., 2009). The chemical profile of the essential oil products differs not only in the number of molecules but also in the stereo chemical types of molecules extracted(Koul, Walia and Dhaliwal, 2008). The extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage (Masotti *et al*., 2003). The method used for extraction can also affect the quantity and concentration of the oil constituents. Steam distillation is the procedure most frequently used to isolate essential oils by Clevenger-type apparatus. However, when distillation is used, it may influence the composition of the oil extracted, because saponification, isomerization, and other reaction may occur under distillation conditions (Tripathi *et al*., 2009).

According to Viljoen *et al*, (2005), there has been a lot of research in chemical constituents of the essential oil from the *Lippia* species. The results showed great variations due to ecological and geographical variations. Certain studies have mentioned that *Lippia javanica* displays chemical variation but most of these studies have mentioned myrcenone as a major component (Stafford *et al*, 2008; Chagonda *et al*., 2000). According to Chagonda *et al*. (2000), *L. javanica* samples collected from three locations in Zimbabwe showed high amounts of limonene. Viljoen*et al.,* (2005) showed greater variations of the major oil constituent among the samples collected from different locations in Mozambique. Myrcenone, myrcene and α-phellandrene that were observed to be major compounds in five samples, while in one sample it was not detected in appreciable quantities.

Compound (**18**) had a retention time of 8.45 and identified as β– Myrcene. It constituted

3.13% of the total oil. The compound has a molecular weight of 136. Through the analysis of the MS spectrum, it showed the presence of a peak at *m/z* 136,there was also peaks observed at m/z 93, m/z 69 and m/z 41 which corresponds to $[C_7H_9]^+$, $[C_5H_9]^+$, and $[C_3H_5]^+$ respectively.

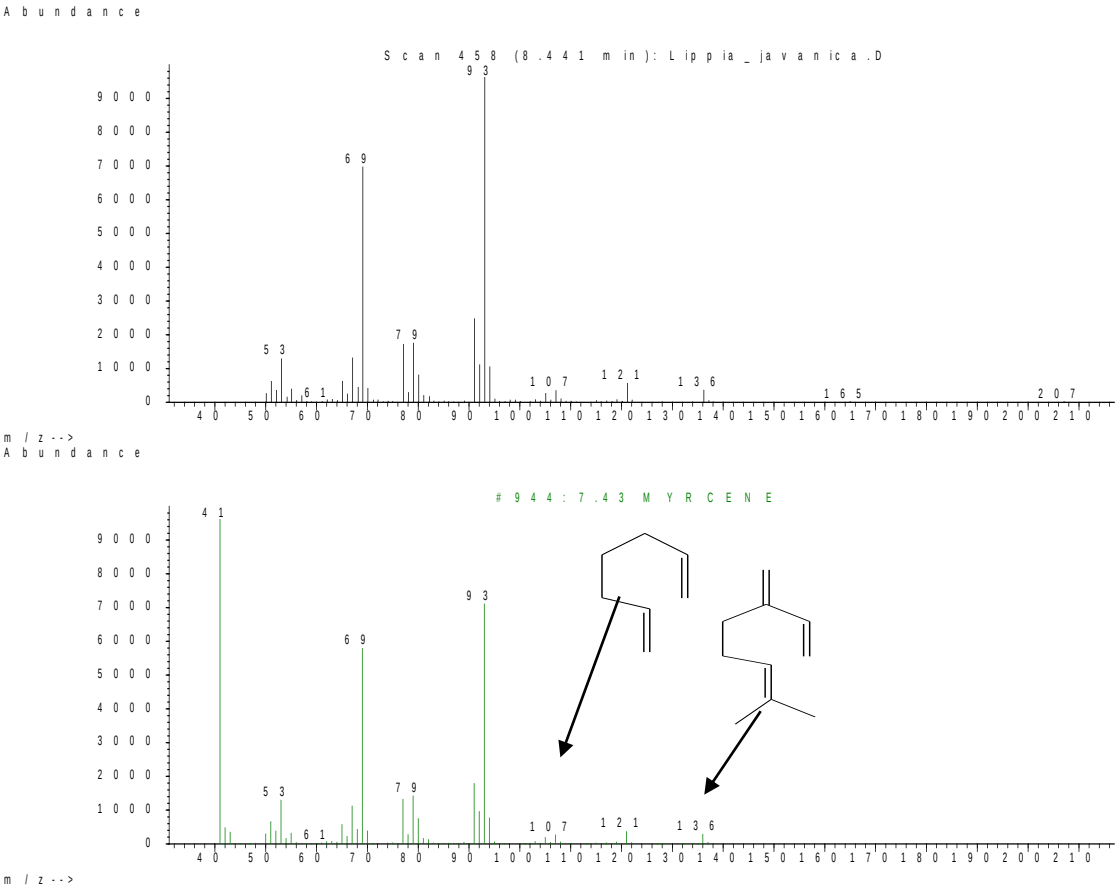


Figure 12: Mass spectra of compound 18

Abad *et al*., (2007) reported that the compound was a major constituent in the oil extracted from the plant *Haplophyllum tuberculatum* (Forsskal). This oil affected the mycelia growth of *Curvularia lunata* and *Fusarium oxysporium*. In the essential oil extracted from *H. hyssopifolium*, Myrcene concentration was reported to be (3.8%) (Cakir *et al*., 2004). This oil was found to be active against five agricultural *Fusarium* fungal pathogenic species (*F. oxysporum, F.culmorum, F. sambucinum, F. solani and F. acuminatum*), which originates from the soil (Cakir *et al*., 2004). Therefore, even though its activity against *F. graminearum* is not in the literature, its effects may not be ruled out due to its observed activity against some *Fusarium* pathogens.

Compound **(19)** had a retention time 9.18 and was identified as Menthatriene with a molecular weight of 134. It constituted 0.51% of the total oil. The GC-MS spectrum of the

oil showed a peak at *m/z* 134 which correspond to $[C_{10}H_{14}]^+$. There were also other peaks at *m/z* 119, *m/z* 105, *m/z* 91, *m/z* 77, *m/z* 65, *m/z* 51 and *m/z* 41 which correspond to $[C_9H_{11}]^+$, $[C_8H_9]^+$, $[C_7H_{14}]^+$, $[C_7H_7]^+$, $[C_6H_5]^+$, $[C_5H_5]^+$, $[C_3H_5]^+$ respectively.

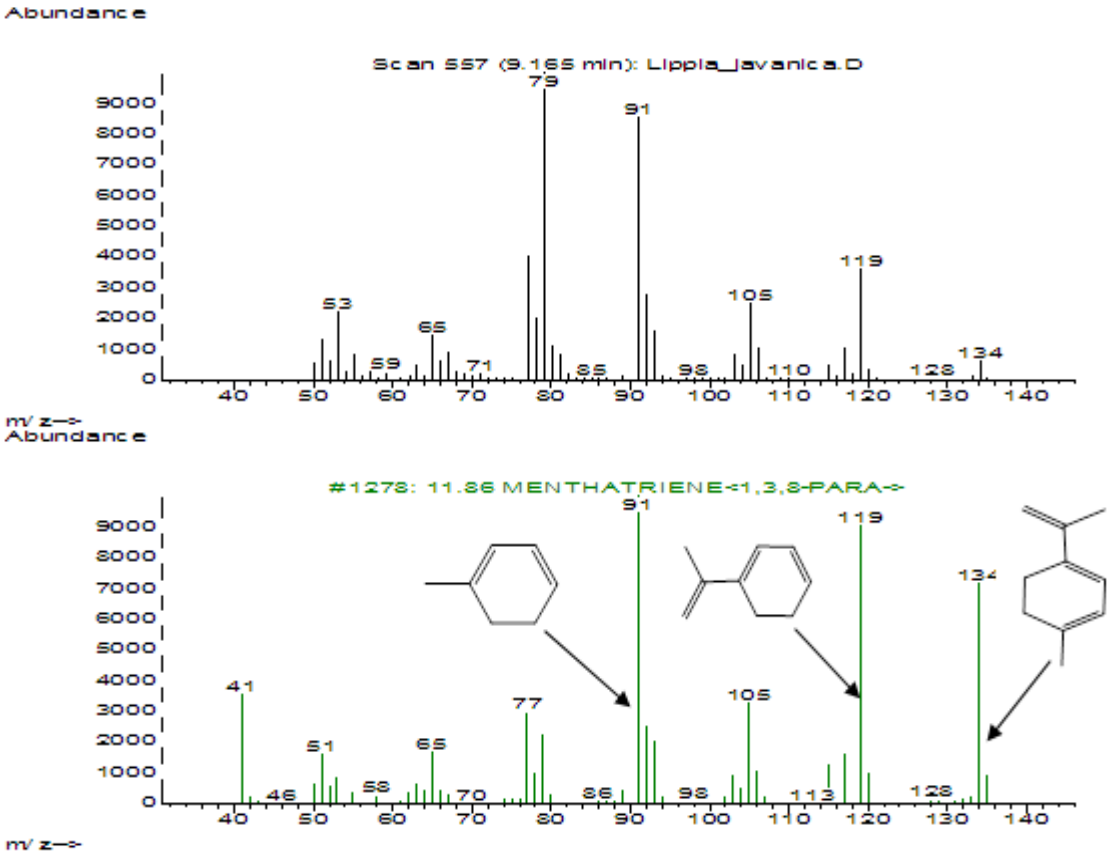


Figure 13: Mass spectra of compound 19

Menthatriene has been reported to be minor component in the essential oil extracted from *Teucrium montanum* (Vukovic *et al*., 2007). The oil showed great antimicrobial activity and greater activity against *Fusarium oxysporum*. However, there is no literature on it being tested against *F. graminearum*. Its concentration in the oil was small but its activity against *F. graminearum* may not be ruled out. Individual antifungal activity of Menthatriene has not been documented.

Compound **(20)** had a retention time of 10.12 and identified to be Tagetone. Its molecular weight is 154 and it constituted 2.85% of the total oil. The GC-MS spectrum showed a peak at *m/z*97, *m/z* 85, *m/z* 69 *m/z* 57 and *m/z* 41which corresponds the fragments $[C_8H_{13}]^+$, $[C_6H_{13}]^+$, $[C_5H_9]^+$, $[C_4H_{11}]^+$, and $[C_3H_5]^+$ respectively.

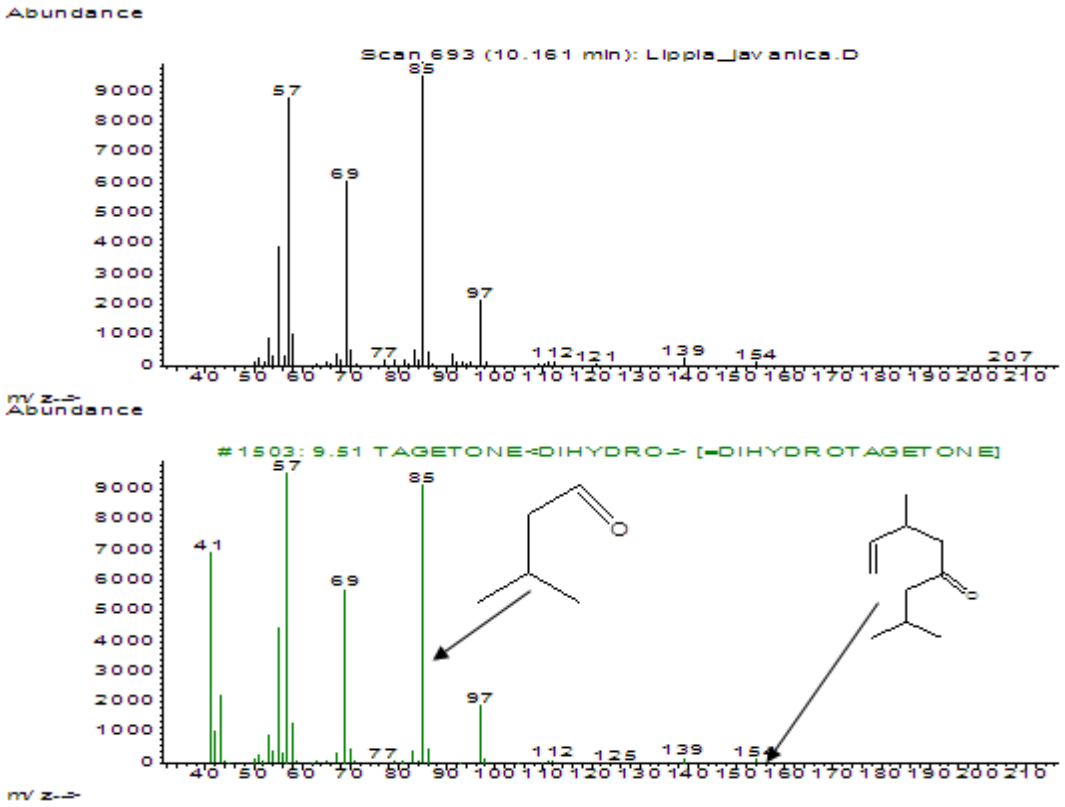


Figure 14: Mass spectra of compound **20**.

According to (Osee *et al*., 2004), they reported that Tagetone was one of the major component in essential oil extracted from *L. javanica* and *Tagetes minuta* plants obtained from the Eastern Cape province of south Africa. It further reported that the oils showed remarkable activity against Fungi and other microbes with that of *L. javanica* showing more activity. Activity of the oils increased with concentration. The *L. javanica* essential oil having shown activity against fungi before might be due to the presence of Tagetone. However, no test of oil has been done before on any of *Fusarium* species. The activity it shown by the oil against *F. graminearum* species might be attributed to the presence of Tagetone.

Compound (**21**) had a retention time of 11.23 and a percentage of 0.44% of the total oil. Its percentage is very minimal (below 0.5%) and thus will not be discussed in details. Compound (**22**) was found to be having a retention time of 11.46 and a percentage of 4.43% of the total oil. It was identified as Linalool. Through the analysis of MS spectrum, a peak at *m/z* 136 was observed. The peak corresponded to $[C_{10}H_{18}O]^+$, which is the formula mass of the compound. There were also other peak at m/z, 121, m/z 93, m/z 80, m/z 71, m/z

55, m/z 43 which corresponds to $[C_8H_8O]^+$, $[C_6H_5O]^+$,, $[C_5H_4O]^+$, $[C_4H_8O]^+$, $[C_3H_3O]^+$, $[C_3H_7]^+$ respectively.

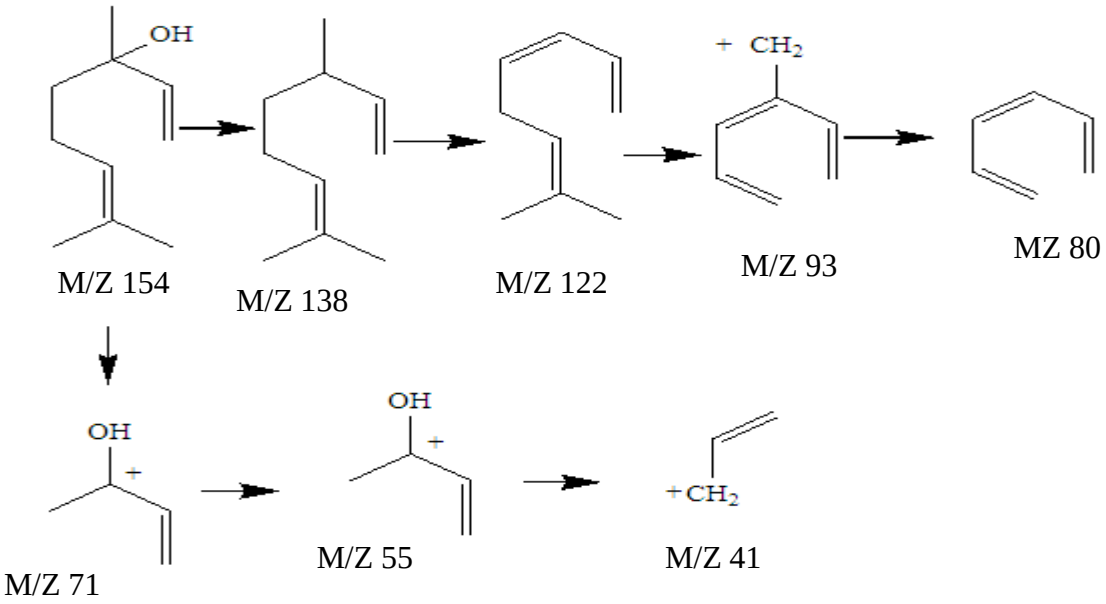


Figure 15: The fragmentation patern of compound **22**

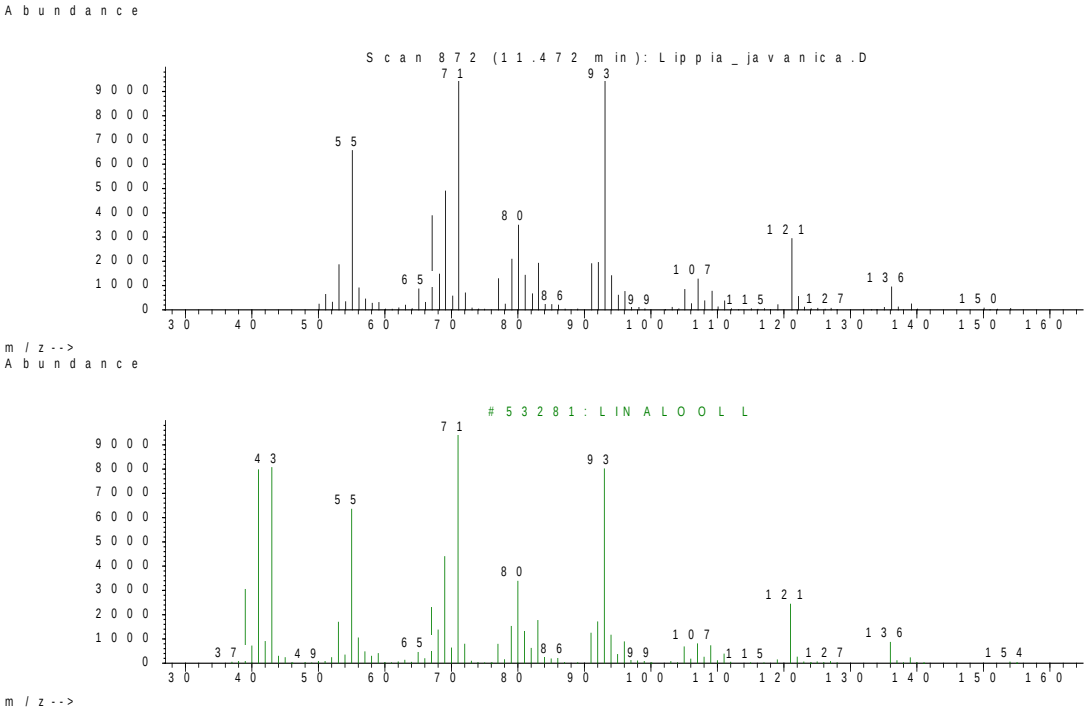


Figure 16: Mass spectra of compound **22**

Compound Compound (**23)** has a retention time of 12.67 and identified as camphor. It has a molecular weight of 152 amu and its percentage of 0.9% of the total oil was observed. The

GC-MS showed a peak at 152 which correspond to $[C_{10}H_{16}O]$. From the spectra, it showed the presence of small peaks at *m/z* 108 that corresponded to $[C_7H_7O]^+$. Other peak at *m/z* 95, 81, 69, 55 and 41 which were corresponding to $[C_6H_7O]^+$, $[C_5H_9]^+$, $[C_4H_7]^+$ and $[C_3H_5]^+$ respectively.

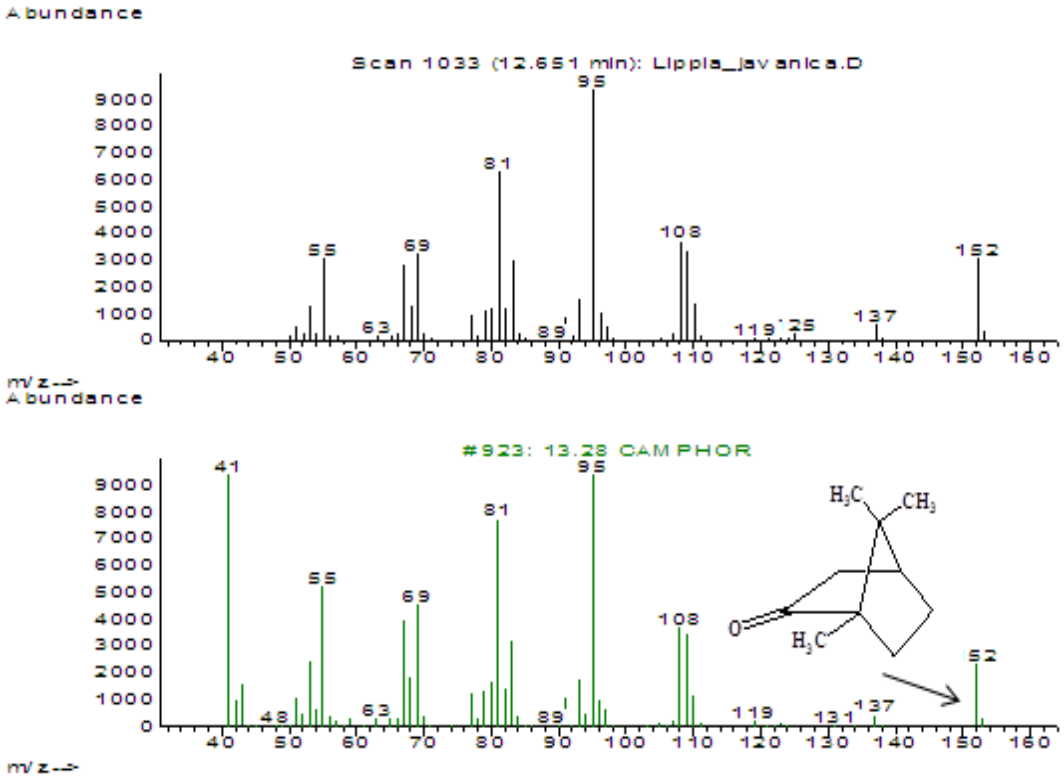


Figure 17: Mass spectra of compound **23**.

Camphor is described as a very volatile compound and is the main common constituent of essential oil isolated from *Artemisia asiatica* which demonstrated activity against the fungi growth at broad spectrum and other fungi completely inhibited (Alvarez-Castellanos *et al*., 2001). When camphor was obtained commercially and tested for antifungal activity, it showed less activity compared to oil from *Artemisia asiatica* (Kordali *et al*., 2005). In addition, its activity against limited fungi species was observed as compared to the essential oil in which whose percentage component was higher.

Compound **(24)** had a retention time of 13.11 and identified as derivative of Artemisia ketone. It had the highest percentage of 49.52% of the total oil components. Its molecular mass is 152amu. The GC-MS showed a peak at *m/z* 152 and a strong peak at *m/z* 85 which correspond to $[C_{10}H_{16}O]^+$, $[C_5H_9O]^+$

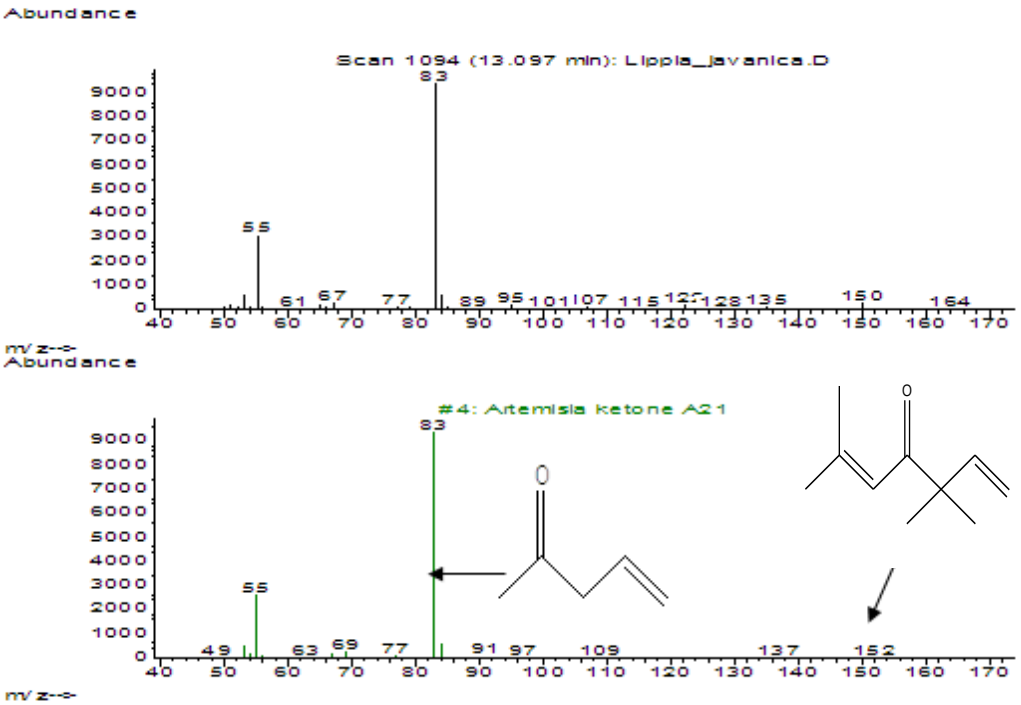


Figure 18: Mass spectra of compound **24**

Compound (**26**) has a retention time of 16.28 and identified as Isopiperitone. Its molecular weight is 50. It had the percentage of 2.13% of the total oil components. The GC-MS showed a peaks at *m/z* 150, *m/z* 135 and a strong peak at *m/z* 82 which correspond to $[C_{10}H_{16}O]^+$, $[C_5H_9O]^+$

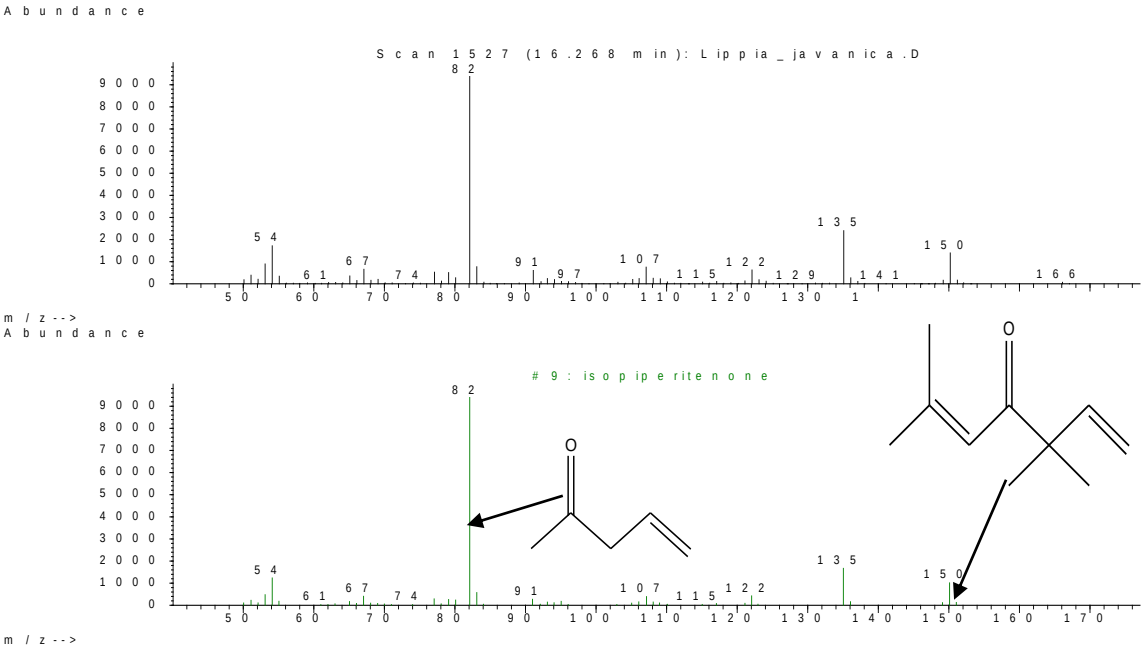
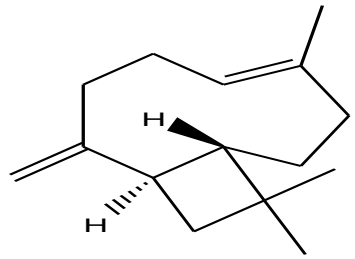


Figure 19: Mass spectra of compound **26**

The constituent *trans*-Caryophyllene (**27**) occurring at retention time 20.06 minutes and comprising of 2.0 % concentration of the total oil with a molecular mass of 204 was identified with the help of the library database. The oil spectra and the spectra of the oil in the library database showed five major peaks (Fig. 19). The peak occurring at *m/z* 161 resulted from the detachment of the isopropyl radical leaving the fragment $[C_{12}H_{17}]^+$ while the peak *m/z* 133 was attributed to the loss of pentyl unit leaving the fragment $[C_{10}H_{13}]^+$. The fragmentation peak at 69 was attributed to the fragment $[C_5H_9]^+$. Earlier reports according to El-Shiekh *et al*., (2012), show that *trans*-Caryophyllene is an active constituent with their findings indicating that the *trans*-caryophyllene isolated from the essential oil of *Croton sonderianus*, had larvicidal activity against *Ae. aegypti* with LC_{50} value of 104 ppm.



27

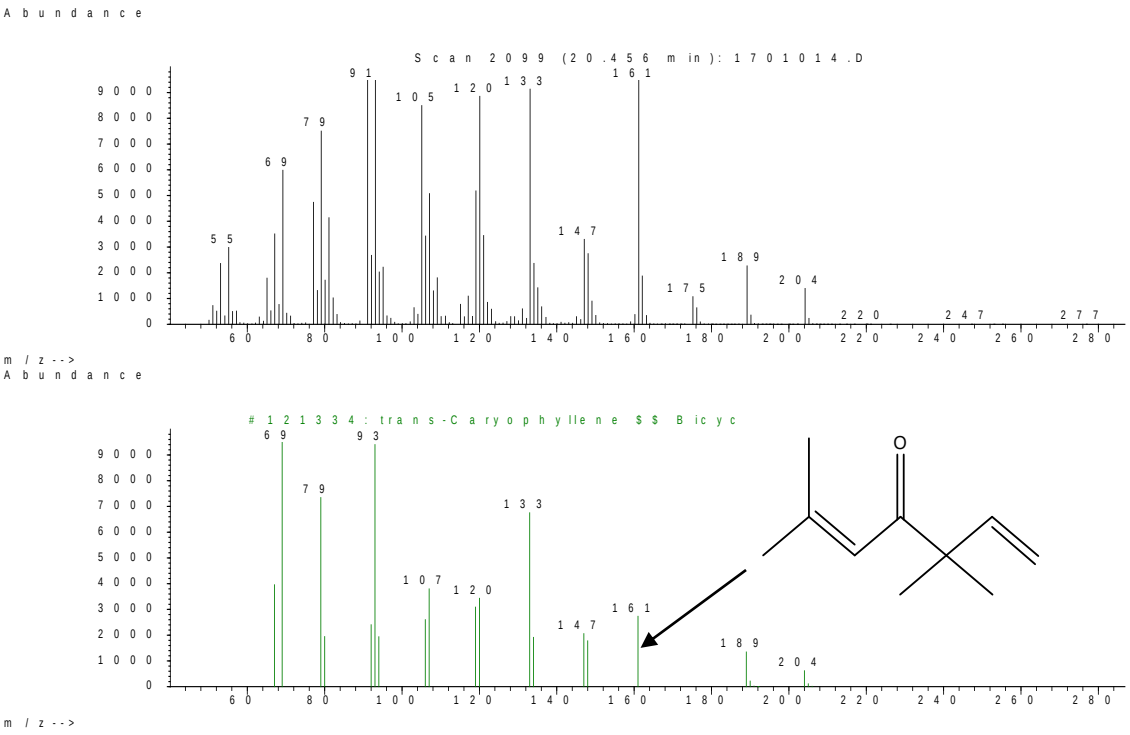


Figure 20: Mass spectra of compound **27**

Compound (**29**) has a retention time of 24.05 and identified as caryophyllene oxide. It had

the percentage of 1.31% of the total oil components. The GC-MS showed a peak at *m/z*152 and a strong peak at *m/z* 85 which correspond to $[C_{10}H_{16}O]^+$, $[C_5H_9O]^+$ respectively.

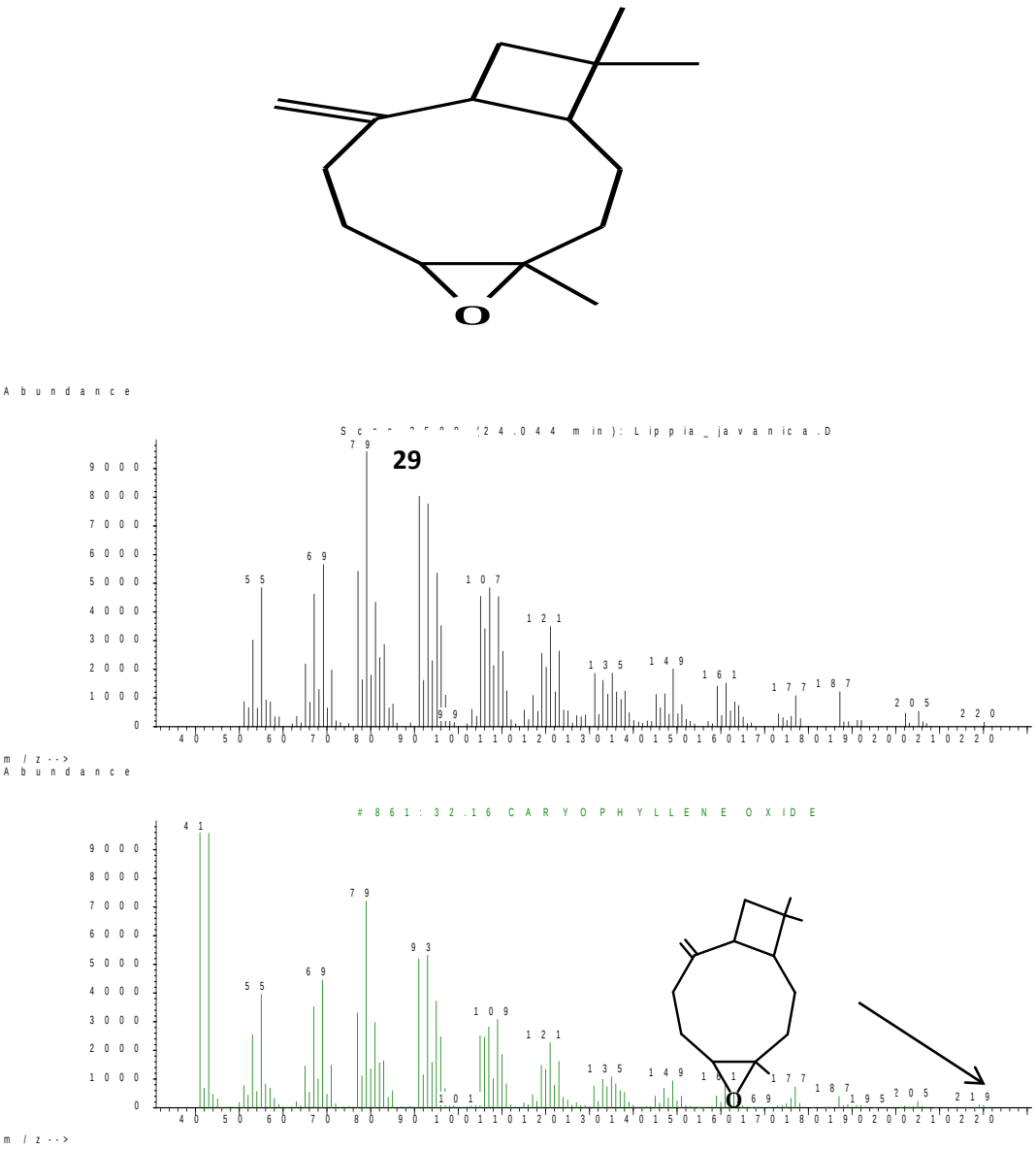


Figure 21: Mass spectra of compound **29**

Caryophyllene oxide is a Sesquiterpenoid with a molecular formula of $C_{15}H_{24}O$. According to (Cakir *et al*., 2004), β-caryophyllene oxide exhibited a pronounced inhibition effect (range 33–85%) on the growth of all agricultural pathogenic fungi. In addition, according to Oztürk *et al.,* (2009), Caryophellene oxide possesses larvicidal activities against mosquito parasite *Anthropophagus.* It also possesses repellency activity against *An. Gambiae* (Omolo *et al*., 2004) and acaricidal activity (Birkett *et al*., 2011).

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The non-volatiles of both *P. dodecandra* and *B. alba* did not show any visible activity against the fungal *Fusarium* species. The secondary metabolites isolated were successfully identified through analysis of their NMR and MS data as well as comparison with literature data. Therefore, the compounds isolated indicated that the two plants contains secondary metabolites

The oil from *L. javanica* was found to be dominated by monoterpenes, which accounted for 52.17% of the oil. These monoterpenes are characterized by low molecular weight terpenes mainly the monoterpenes (C10) and Sesquiterpenes (C15). Monoterpenes in the oil were characterized by a high percentage of Artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%), Targetone(2.85%) and Isopiperitenone(2.13%). Sesquiterpenes which included constituted Z-caryophyllene, and Caryophellene oxide constituted only 17.39% of the total oil.

Recent research has demonstrated capacity of the *L. javanica* oil to inhibit the growth of *F. graminearum* fungi. The essential oils completely stopped the growth of the fungal species. Initially, their inhibition effect was stronger than that of Nystatin. But the inhibition decreased with increasing numbers of days. This showed that the essential oil is unstable and can undergo photo-oxidation and other transformation which affects the activity. Therefore, it can be suggested that the essential oils of *L. javanica* may be used as antifungal agents to protect maize crops against root rot, stem rot and leaf lesions which are associated with *F. graminearum* fungi.

Despite this most promising property, problems related to the volatility of the oil, poor water solubility and aptitude for easy aerial oxidation on exposure have to be resolved before they are incorporated and used as crop protecting tool.

5.2 Recommendations

In relation to this research, the following recommendations were made;

1. More advanced methods be applied in purifying compound 16 such as HPLC, be used to purify. In addition, more assays to be done on the other maize microorganisms to be done to determine its activity.

2. Toxicity tests to be carried out for the essential oil.
3. Further studies need to be conducted to evaluate activity of the oil against other wide range of phyto-pathogenic fungi.
4. Isolation of compounds in essential oil to be done and each tested for their antifungal activities.
5. Formulations to improve potency and stability of the active but volatile oil components should be evaluated.
6. Evaluation of the cytotoxicity on non-target organisms of these compounds can also be done.

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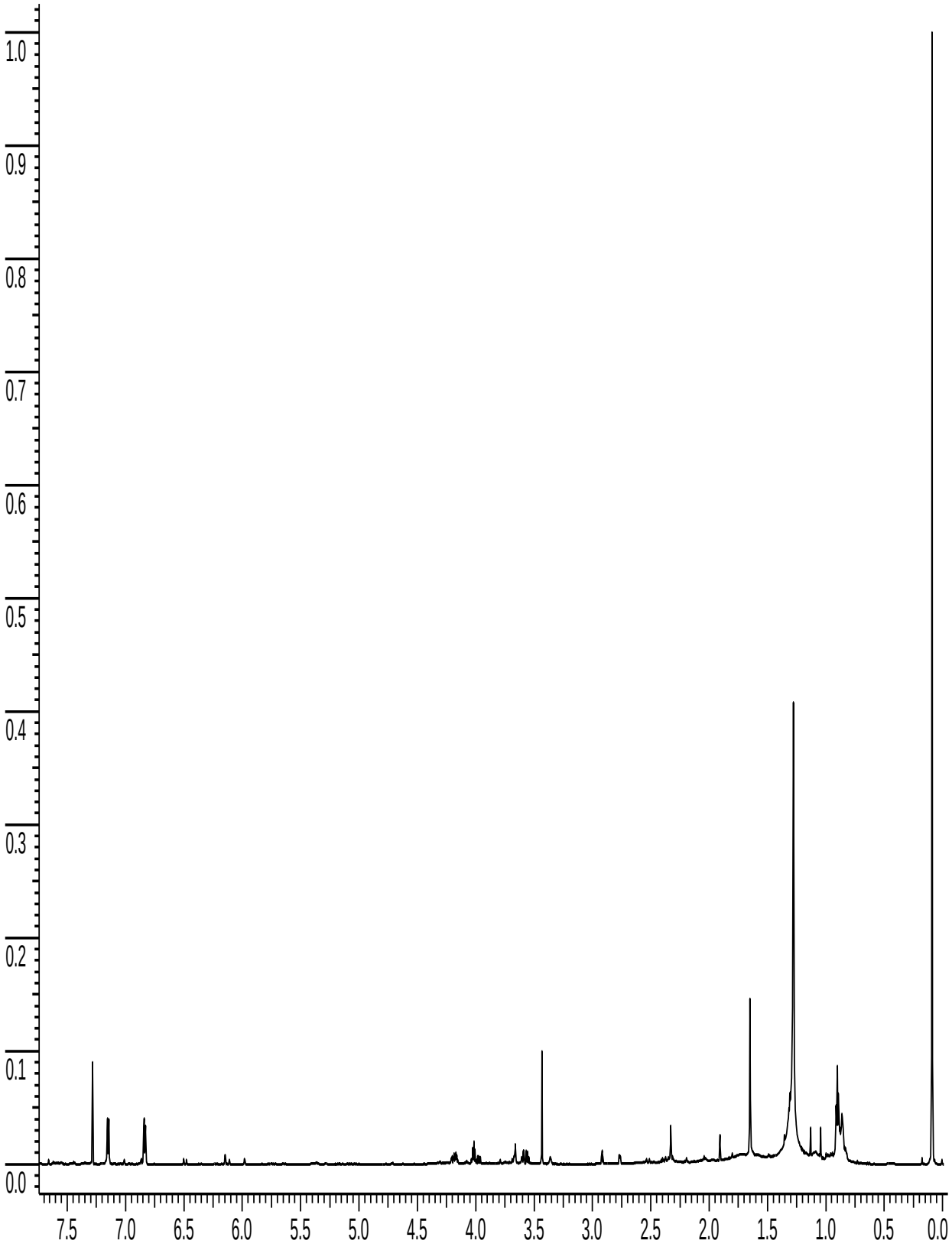
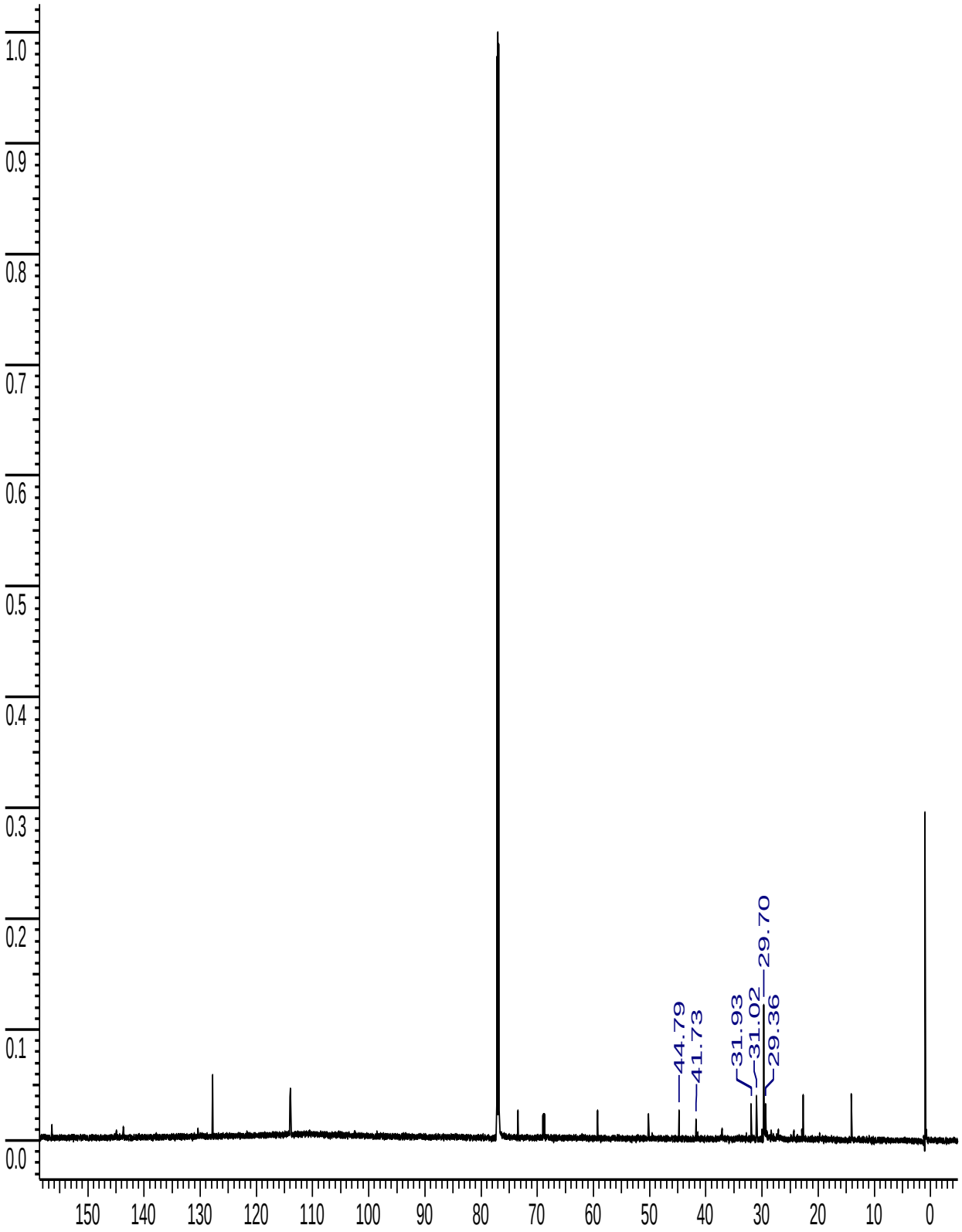
APPENDICES

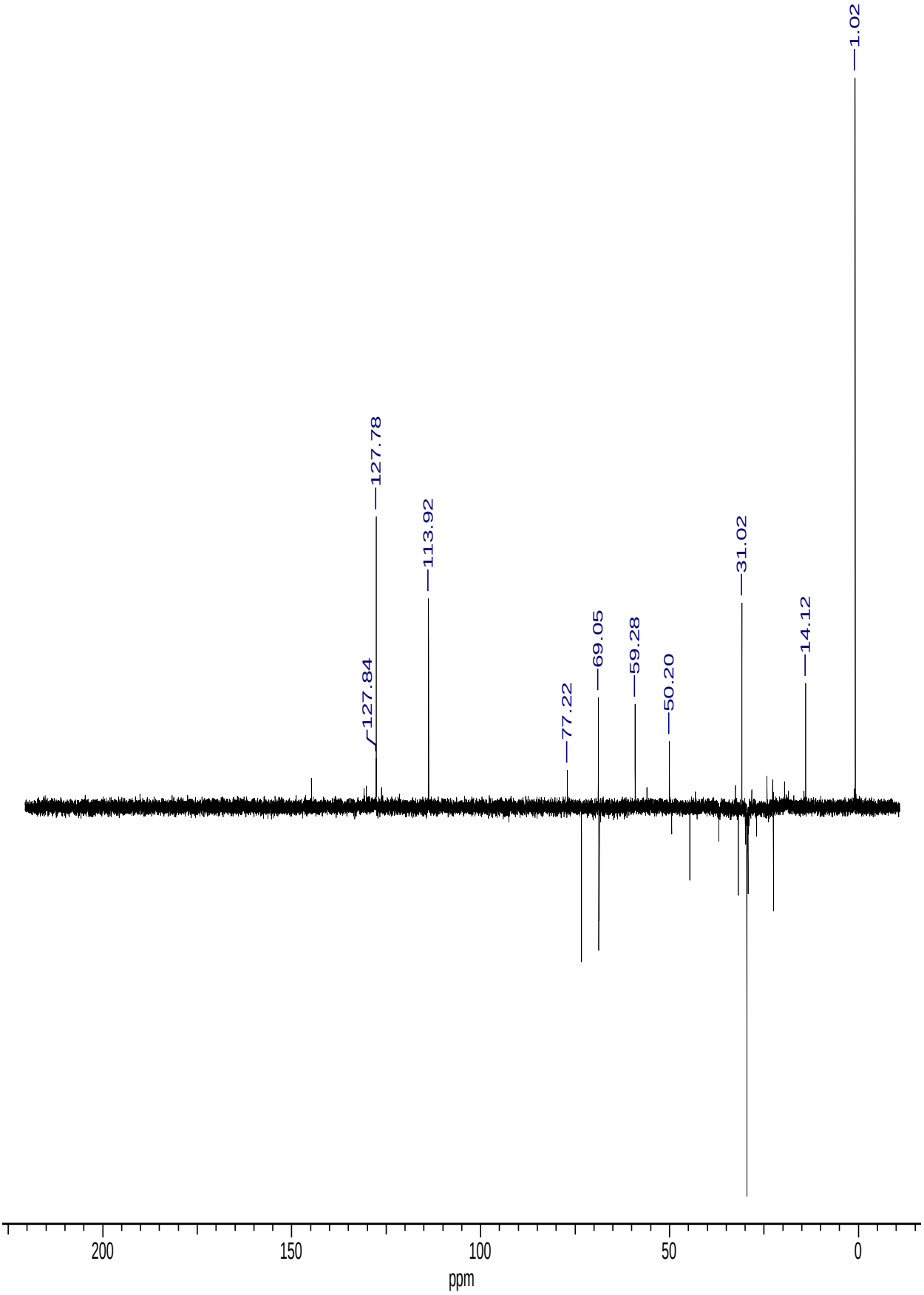
Appendix 1: ^{1}H NMR spectrum for compound 16



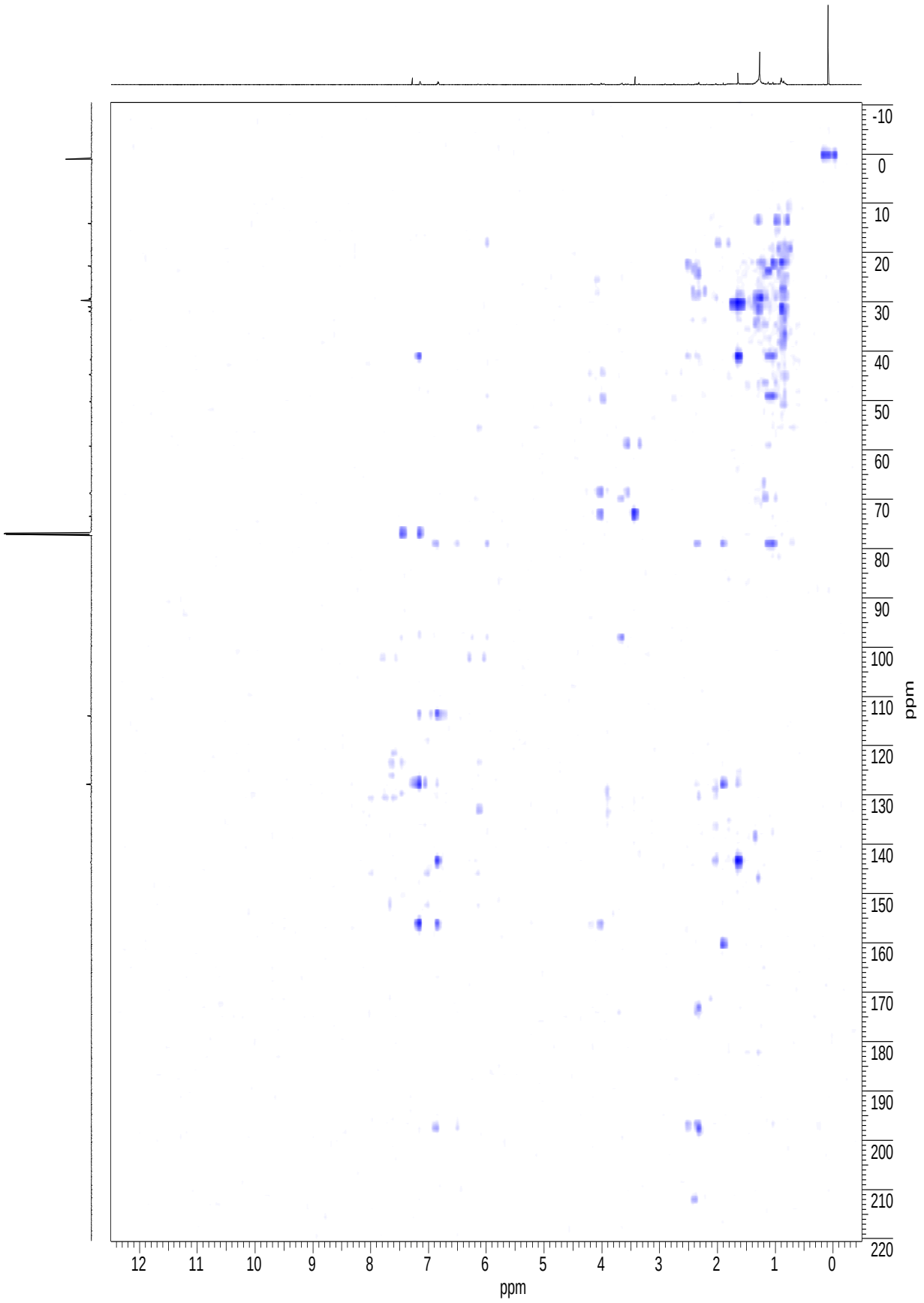
Appendix 2: ^{13}C NMR spectrum for compound 16



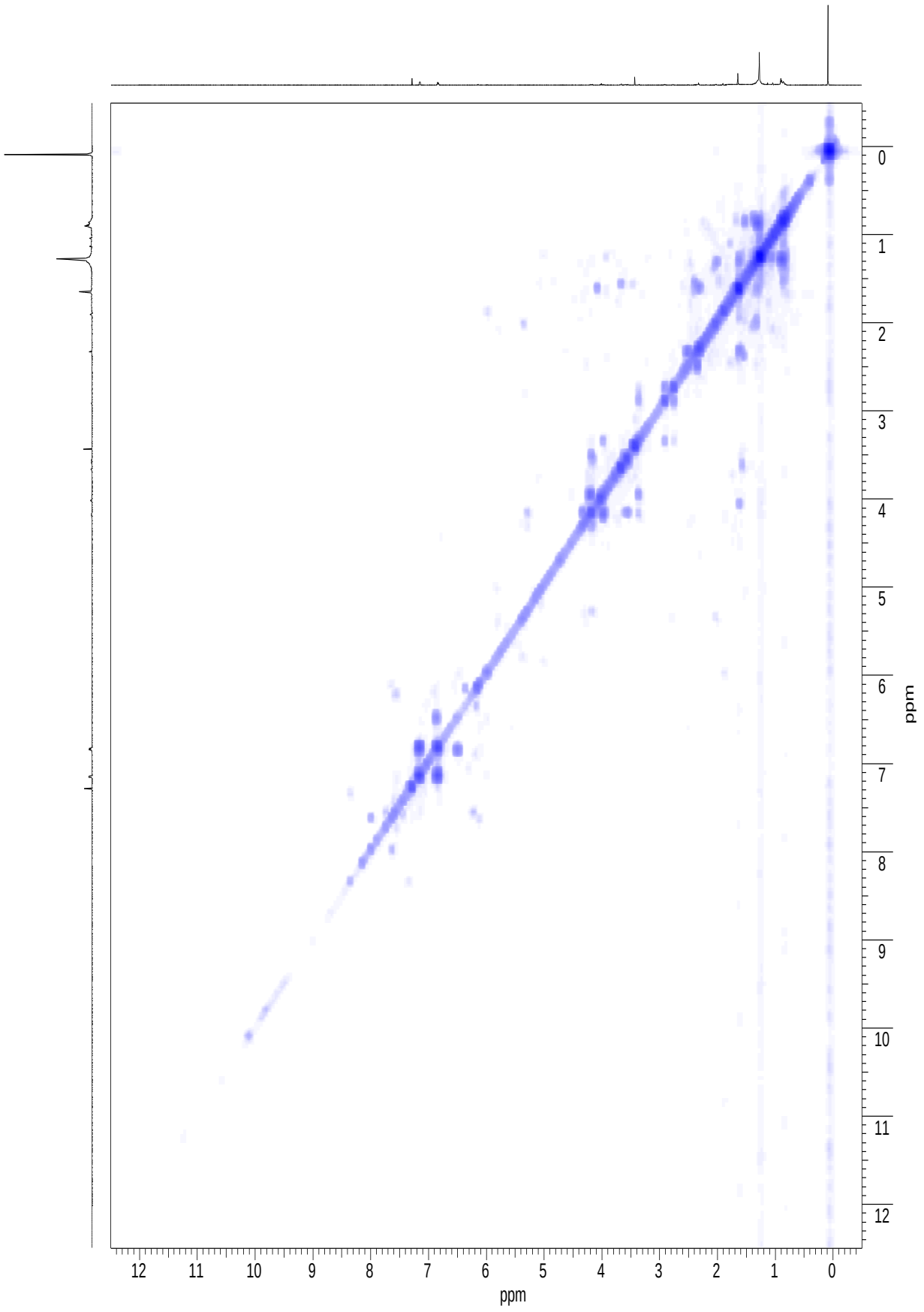
Appendix 3: DEPT spectrum for compound 16



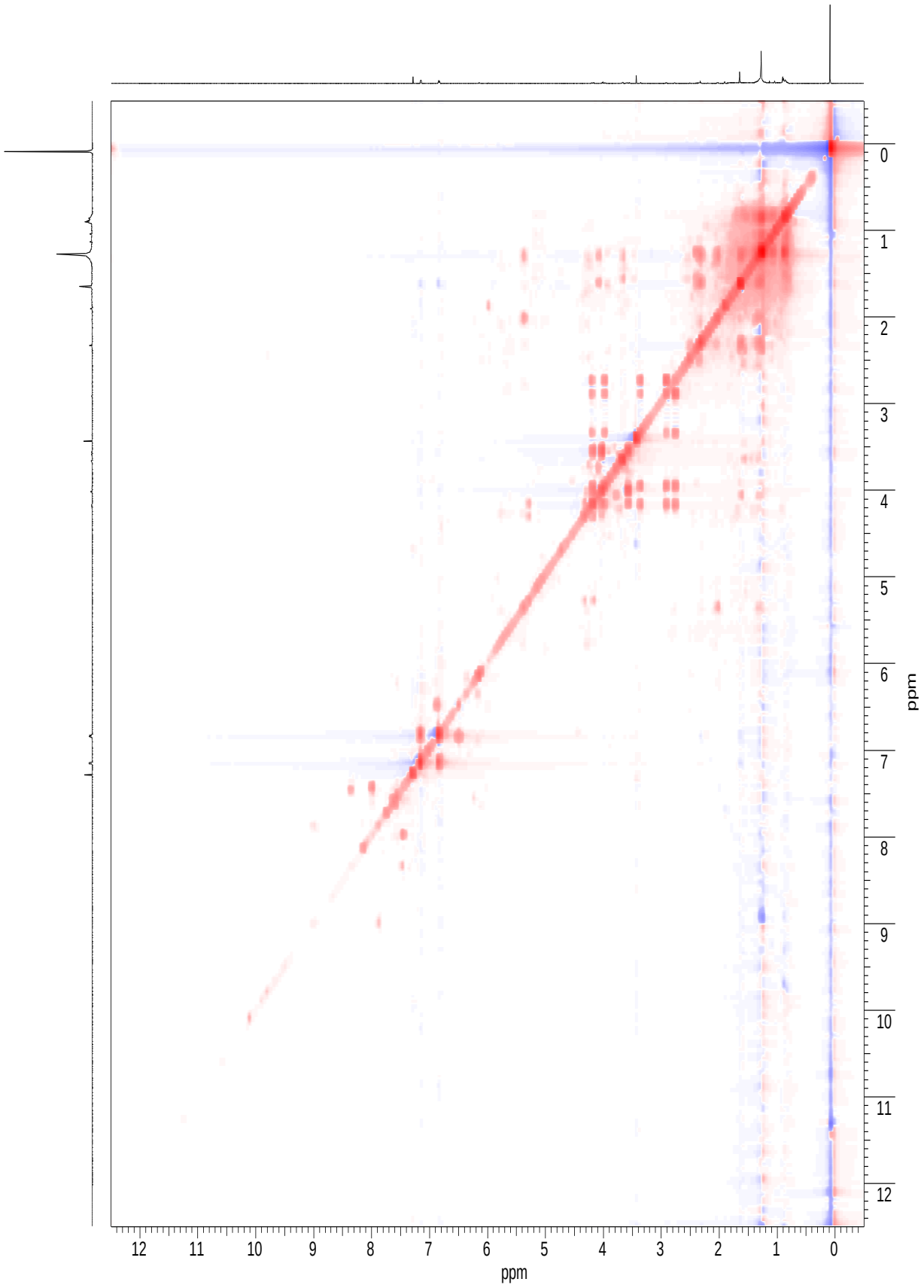
Appendix 4: HSQC spectrum for compound 16



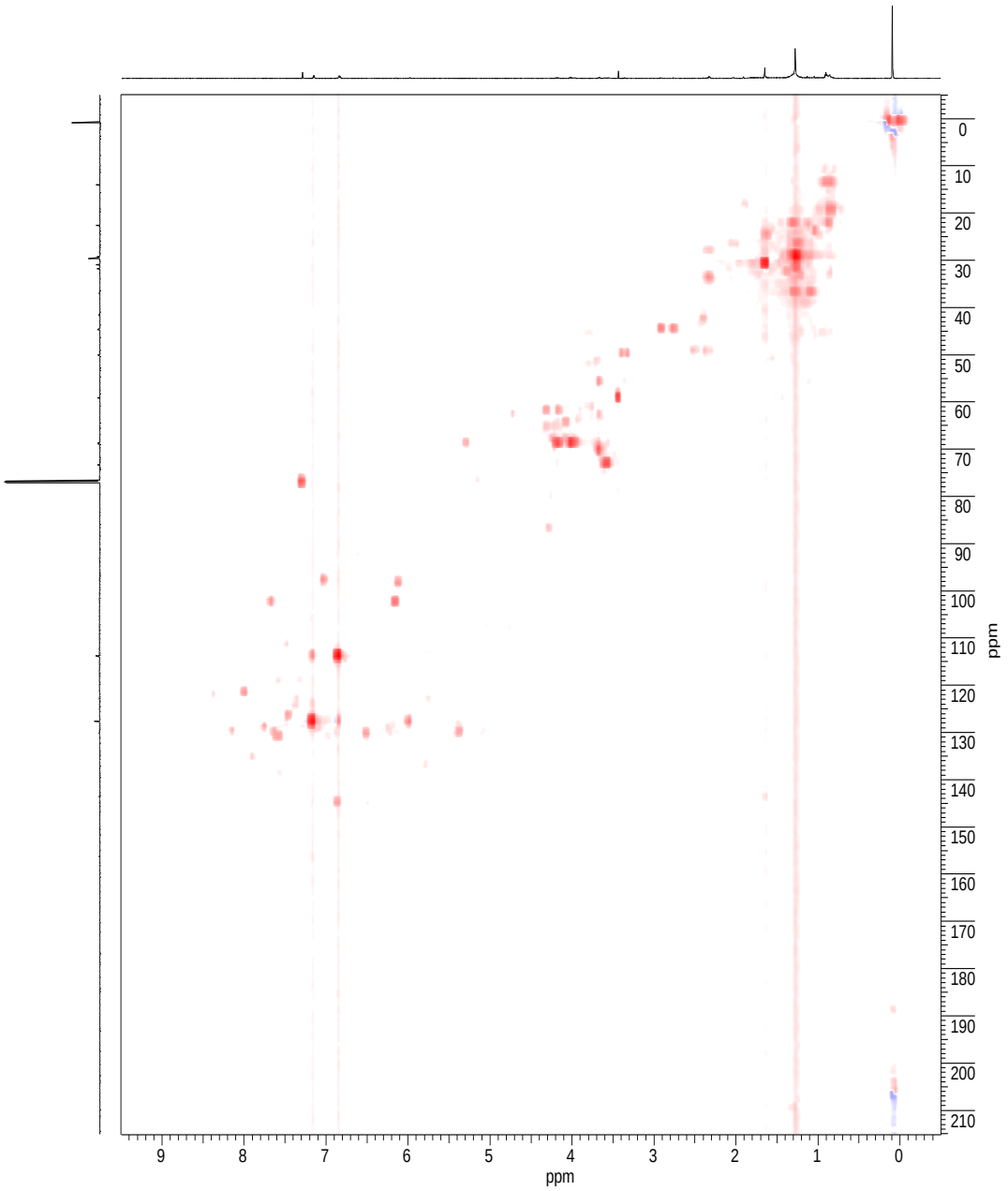
Appendix 5: COSY spectrum for compound 16



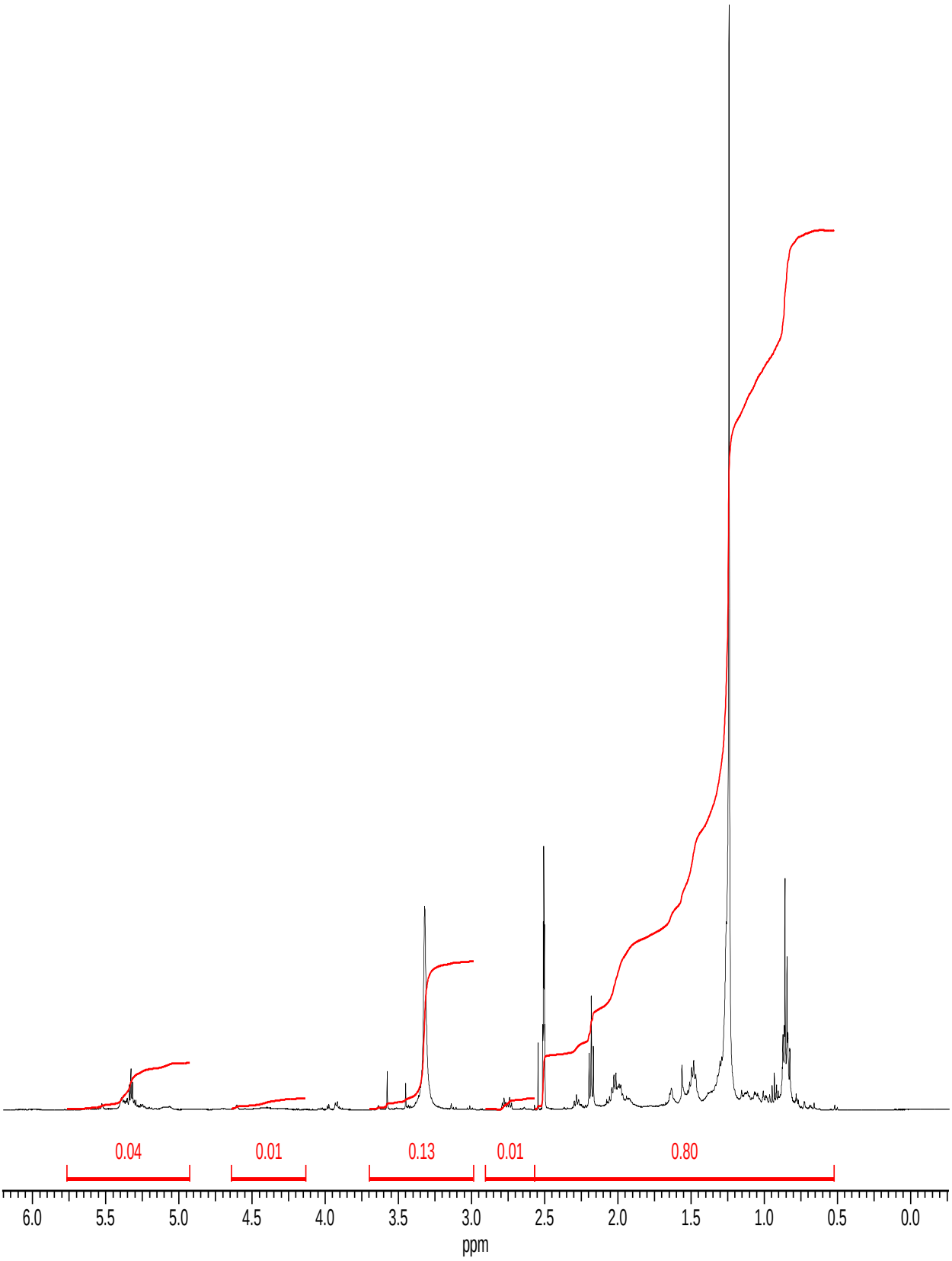
Appendix 6: TCOSY spectrum for compound 16



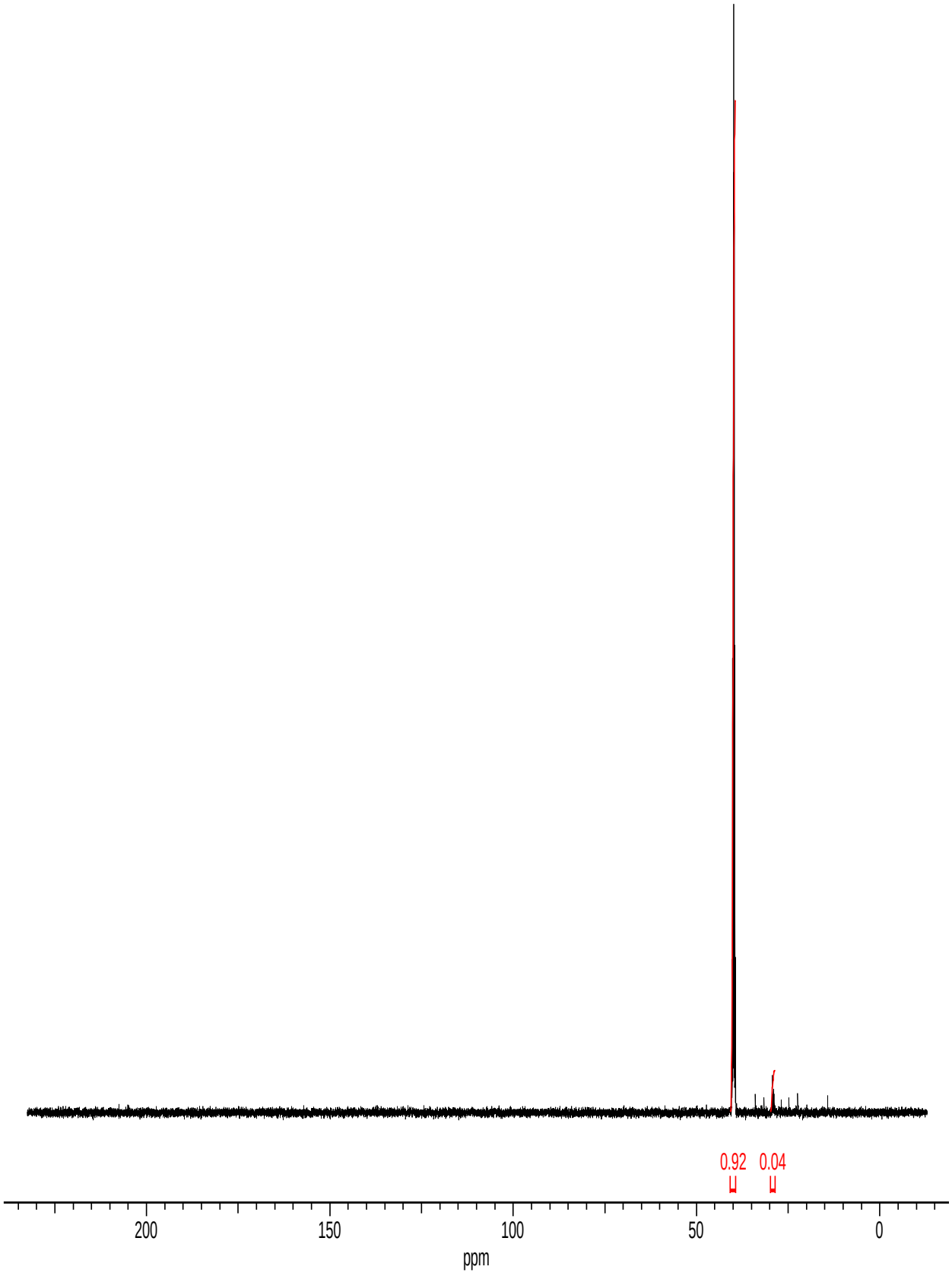
Appendix 7: HMBC spectrum for compound 16



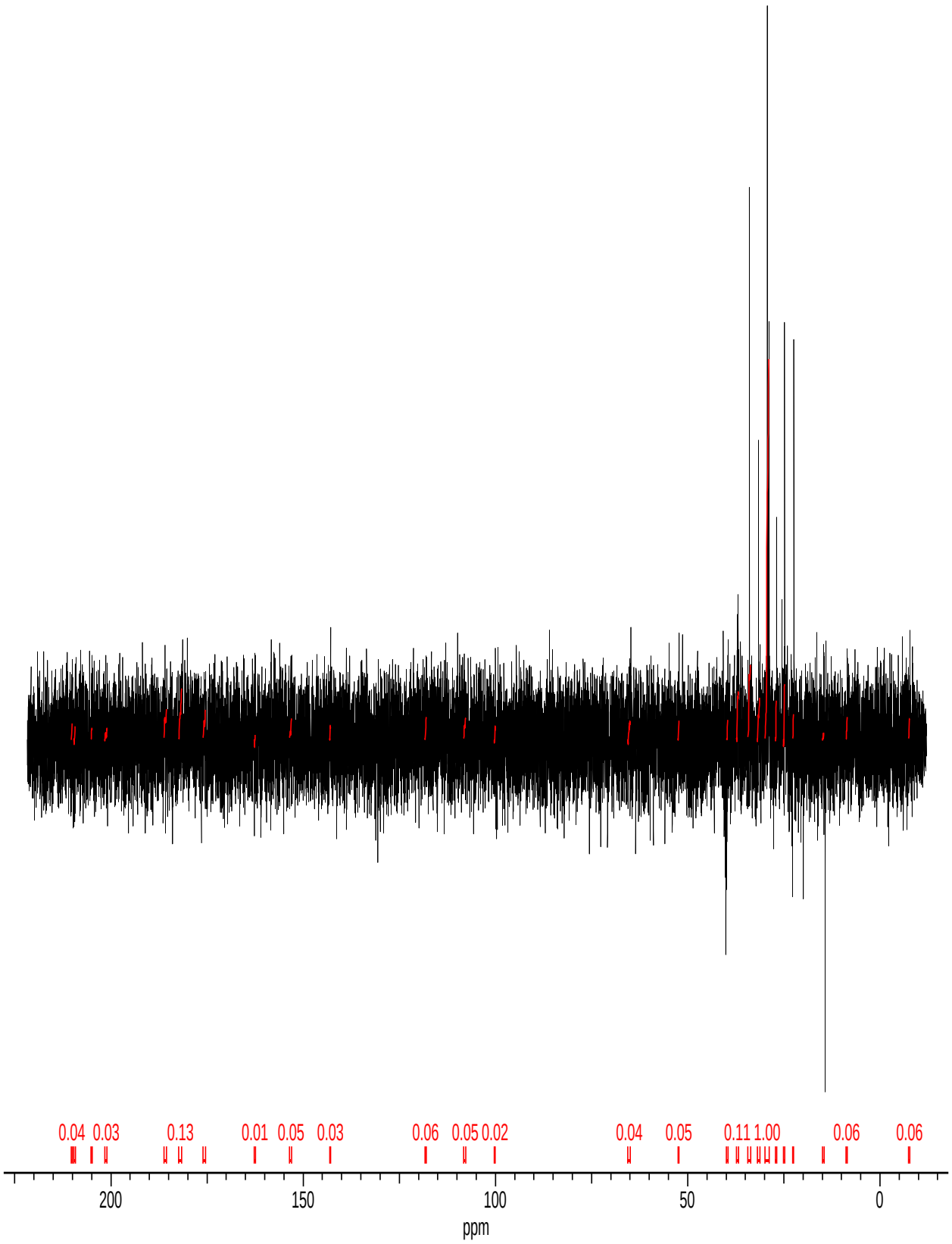
Appendix 8: **1H spectrum for compound 17**



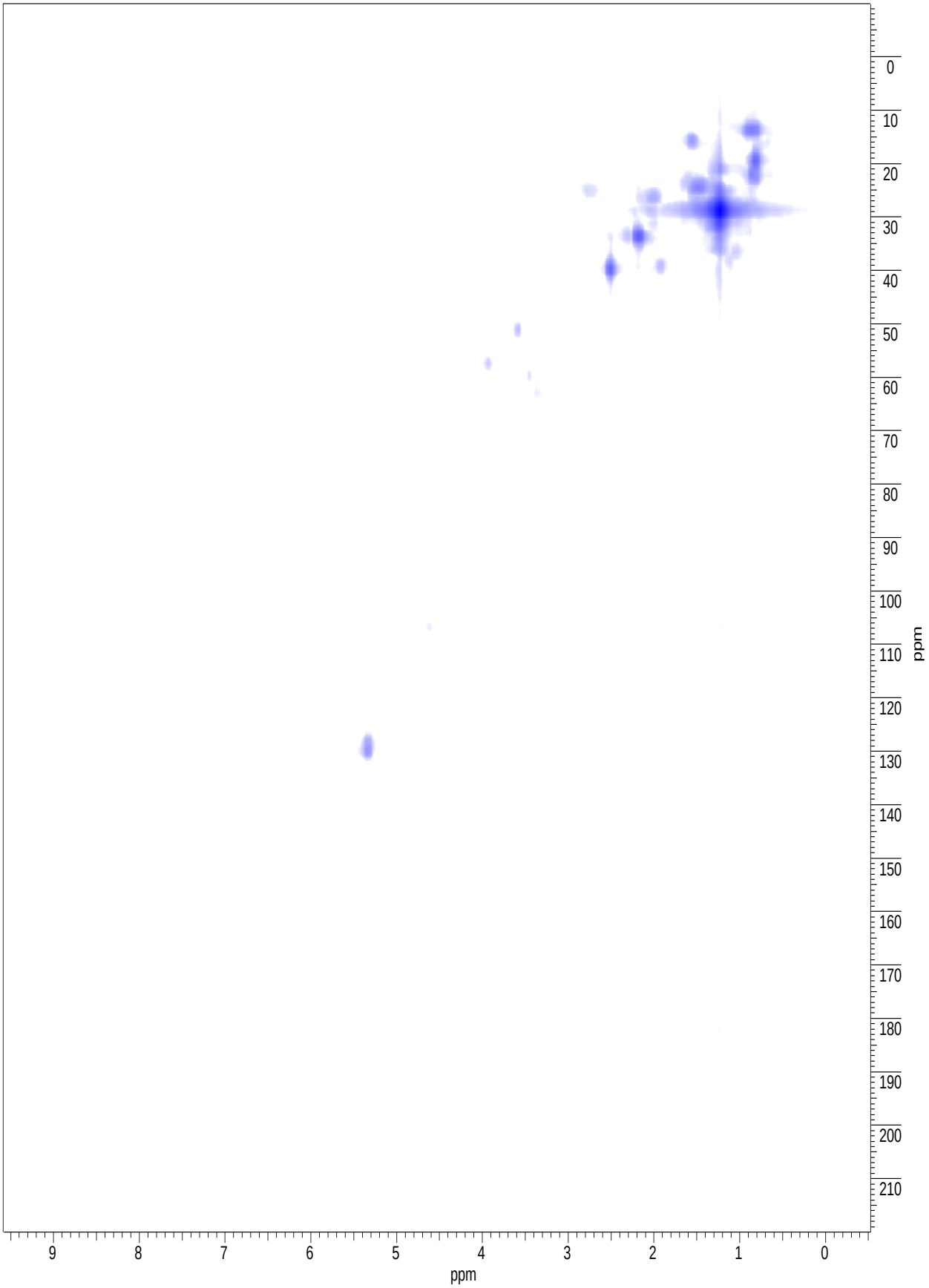
Appendix 9: ^{13}C spectrum for compound 17



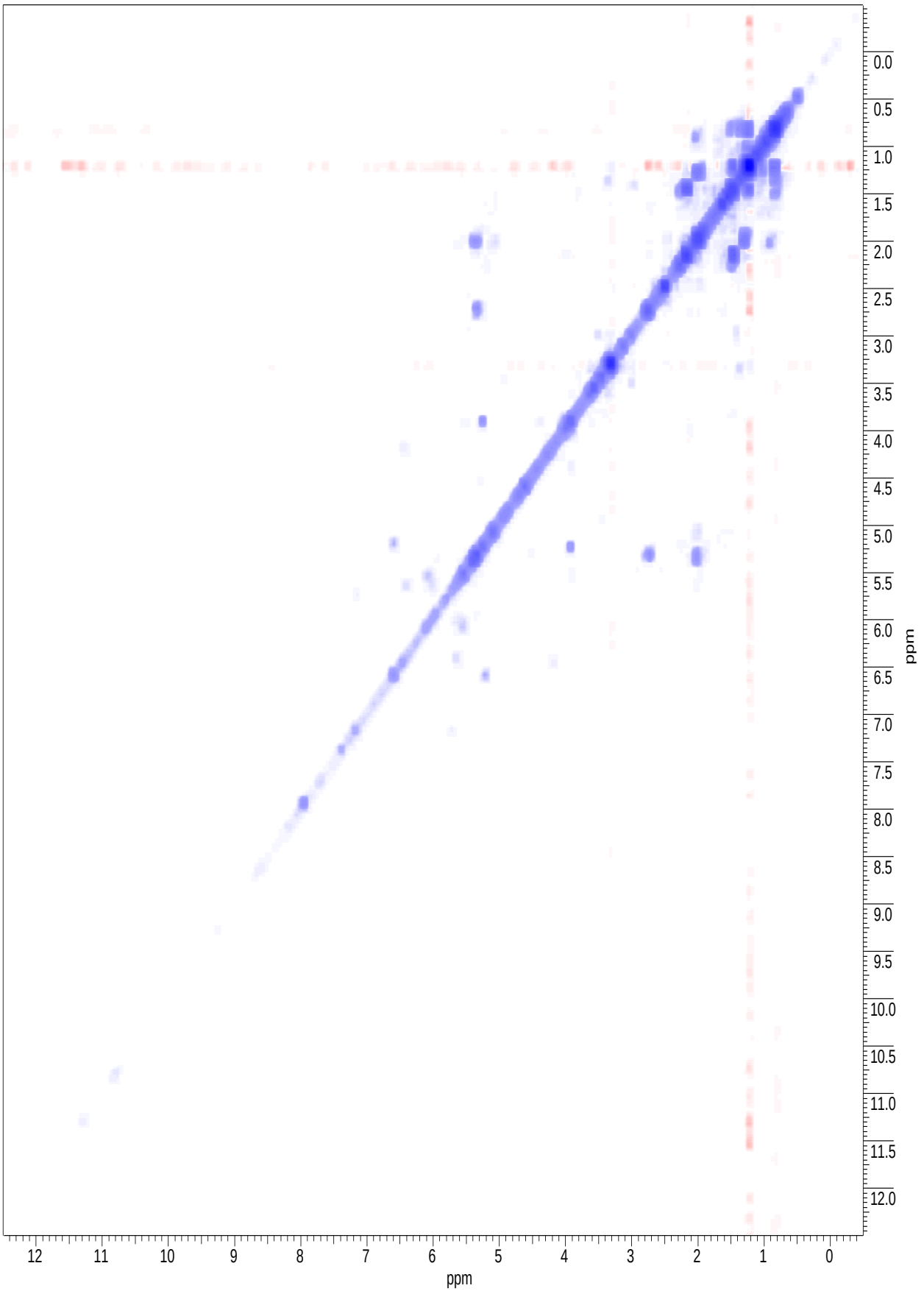
Appendix 10: DEPT spectrum for compound 17



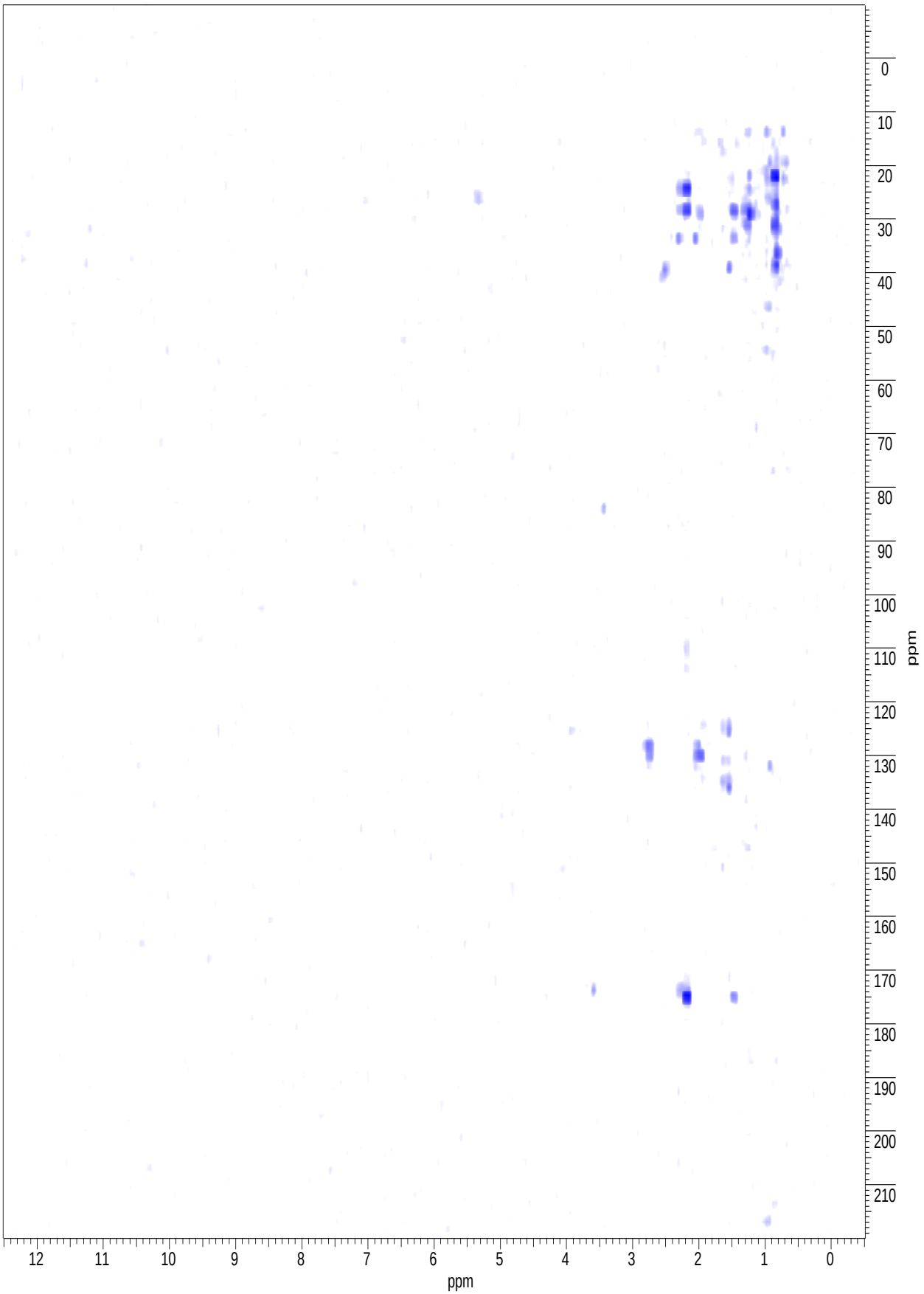
Appendix 11: HSQC spectrum for compound 17



Appendix 12: COSY spectrum for compound 17



Appendix 13: HMBC spectrum for compound 17



Appendix 14: One-way ANOVA results for the differences in activity with the variation of time (days) at specific concentration levels

Conc. level: 87.00 (g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	25.0000	0.102	0.057	24.7516	25.2484
Day 4	3	23.0000	0.095	0.053	22.7516	23.2484
Day 5	3	22.0000	0.112	0.056	21.7516	22.2484
Day 6	3	19.0000	0.100	0.058	18.7516	19.2484
Day 7	3	16.0000	0.083	0.056	15.7516	16.2484
Day 8	3	13.3300	0.085	0.056	13.0816	13.5784
Day 9	3	10.6700	0.079	0.052	10.4216	10.9184
Day 10	3	9.3300	0.072	0.055	9.0816	9.5784
Day 11	3	8.0000	0.089	0.056	7.7516	8.2484
Day 12	3	7.6700	0.077	0.055	7.4216	7.9184
Day 13	3	7.3300	0.060	0.052	7.0816	7.5784
Day 14	3	7.0000	0.062	0.016	6.7516	7.2484
Total	36	14.0275	6.533	1.088	11.8170	16.2380

Calculated F – Ratio $_{(11, 24)}$ = 13578.97, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 65.25(g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	20.3300	0.099	0.054	20.0816	20.5784
Day 4	3	17.0000	0.092	0.050	16.7516	17.2484
Day 5	3	15.8300	0.109	0.053	15.5816	16.0784
Day 6	3	13.6700	0.097	0.055	13.4216	13.9184
Day 7	3	12.3300	0.080	0.053	12.0816	12.5784
Day 8	3	10.6700	0.082	0.053	10.4216	10.9184
Day 9	3	8.6700	0.076	0.049	8.4216	8.9184
Day 10	3	6.3300	0.069	0.052	6.0816	6.5784
Day 11	3	5.0000	0.086	0.053	4.7516	5.2484
Day 12	3	4.0000	0.074	0.052	3.7516	4.2484
Day 13	3	3.3300	0.057	0.049	3.0816	3.5784
Day 14	3	2.6700	0.059	0.013	2.4216	2.9184
Total	36	9.9858	6.530	1.085	8.0457	11.9259

Calculated F – Ratio $_{(11, 24)}$ = 10459.03, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 43.50 (g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	16.0000	0.090	0.037	15.7516	16.2484
Day 4	3	14.1700	0.083	0.033	13.9216	14.4184
Day 5	3	10.3300	0.100	0.036	10.0816	10.5784
Day 6	3	7.6700	0.088	0.038	7.4216	7.9184
Day 7	3	6.0000	0.071	0.036	5.7516	6.2484
Day 8	3	5.0000	0.073	0.036	4.7516	5.2484
Day 9	3	4.6700	0.067	0.032	4.4216	4.9184
Day 10	3	4.0000	0.060	0.035	3.7516	4.2484
Day 11	3	3.6700	0.077	0.036	3.4216	3.9184
Day 12	3	3.0000	0.065	0.035	2.7516	3.2484
Day 13	3	2.6700	0.048	0.032	2.4216	2.9184
Day 14	3	2.3300	0.050	0.004	2.0816	2.5784
Total	36	6.6258	6.521	1.068	5.1254	8.1263

Calculated F – Ratio $_{(11, 24)}$ = 6254.79, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 21.75(g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	12.1700	0.114	0.077	11.9216	12.4184
Day 4	3	10.0000	0.107	0.073	9.7516	10.2484
Day 5	3	8.3300	0.124	0.076	8.0816	8.5784
Day 6	3	5.1700	0.112	0.078	4.9216	5.4184
Day 7	3	6.0000	0.095	0.076	5.7516	6.2484
Day 8	3	5.0000	0.097	0.076	4.7516	5.2484
Day 9	3	4.6700	0.091	0.072	4.4216	4.9184
Day 10	3	4.0000	0.084	0.075	3.7516	4.2484
Day 11	3	3.6700	0.101	0.076	3.4216	3.9184
Day 12	3	3.0000	0.089	0.075	2.7516	3.2484
Day 13	3	2.6700	0.072	0.072	2.4216	2.9184
Day 14	3	2.3300	0.074	0.036	2.0816	2.5784
Total	36	5.5842	6.545	1.108	4.5742	6.5941

Calculated F – Ratio $_{(11, 24)}$ = 2832.74, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 10.88(g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	8.3300	0.105	0.060	8.0816	8.5784
Day 4	3	5.3300	0.098	0.056	5.0816	5.5784
Day 5	3	4.0000	0.115	0.059	3.7516	4.2484
Day 6	3	3.1700	0.103	0.061	2.9216	3.4184
Day 7	3	2.8300	0.086	0.059	2.5816	3.0784
Day 8	3	2.3300	0.088	0.059	2.0816	2.5784
Day 9	3	1.5000	0.082	0.055	1.2516	1.7484
Day 10	3	1.0000	0.075	0.058	.7516	1.2484
Day 11	3	.6700	0.092	0.059	.4216	.9184
Day 12	3	.3300	0.080	0.058	.0816	.5784
Day 13	3	.3300	0.063	0.055	.0816	.5784
Day 14	3	.3300	0.065	0.019	.0816	.5784
Total	36	2.5125	2.536	1.091	1.7099	3.3151

Calculated F – Ratio $_{(11, 24)}$ = 1787.95, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 5.44(g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	5.6700	0.103	0.060	5.4216	5.9184
Day 4	3	4.6700	0.096	0.056	4.4216	4.9184
Day 5	3	2.0000	0.113	0.059	1.7516	2.2484
Day 6	3	1.6700	0.101	0.061	1.4216	1.9184
Day 7	3	1.3300	0.084	0.059	1.0816	1.5784
Day 8	3	.6700	0.086	0.059	.4216	.9184
Day 9	3	.6700	0.080	0.055	.4216	.9184
Day 10	3	.5000	0.073	0.058	.2516	.7484
Day 11	3	.5000	0.090	0.059	.2516	.7484
Day 12	3	.3300	0.078	0.058	.0816	.5784
Day 13	3	.1700	0.061	0.055	-.0784	.4184
Day 14	3	.0000	0.063	0.019	-.2484	.2484
Total	36	1.5150	1.534	1.091	.9158	2.1142

Calculated F – Ratio $_{(11, 24)}$ = 995.69, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Conc. level: 2.72 (g/mL) *10^{-2}

Day	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 3	3	3.3300	0.113	0.070	3.0816	3.5784
Day 4	3	2.3300	0.106	0.066	2.0816	2.5784
Day 5	3	.6700	0.123	0.069	.4216	.9184
Day 6	3	.3300	0.111	0.071	.0816	.5784
Day 7	3	.1700	0.094	0.069	-.0784	.4184
Day 8	3	.0000	0.096	0.069	-.2484	.2484
Day 9	3	.0000	0.090	0.065	-.2484	.2484
Day 10	3	.0000	0.083	0.068	-.2484	.2484
Day 11	3	.0000	0.100	0.069	-.2484	.2484
Day 12	3	.0000	0.088	0.068	-.2484	.2484
Day 13	3	.0000	0.071	0.065	-.2484	.2484
Day 14	3	.0000	0.073	0.029	-.2484	.2484
Total	36	.5692	1.544	1.101	.2079	.9304

Calculated F – Ratio $_{(11, 24)}$ = 360.47, Critical F – Ratio $_{(11, 24)}$ = 2.22, P-Value = 0.000

Appendix 15: One-way ANOVA results for the differences in activity with the variation of concentration levels at specific time duration (days)

Day3

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	25.0000	.08165	.04082	24.8701	25.1299
Conc. 65.25	4	20.4975	.34481	.17240	19.9488	21.0462
Conc. 43.50	4	16.0000	.08165	.04082	15.8701	16.1299
Conc. 21.75	4	12.3775	.42296	.21148	11.7045	13.0505
Conc. 10.88	4	8.4975	.34481	.17240	7.9488	9.0462
Conc. 5.44	4	5.7525	.18410	.09205	5.4596	6.0454
Conc. 2.72	4	3.4975	.34481	.17240	2.9488	4.0462
Conc. Nystatin	4	16.0000	.08165	.04082	15.8701	16.1299
Conc. Control	4	.0000	.00000	.00000	.0000	.0000
Total	36	11.9581	7.86579	1.31096	9.2967	14.6195

Calculated F – Ratio $_{(11, 24)}$ = 4129.494, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day4

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	23.0000	0.075	0.037	22.8701	23.1299
Conc. 65.25	4	17.0000	0.075	0.037	16.8701	17.1299
Conc. 43.50	4	14.3775	0.417	0.208	13.7045	15.0505
Conc. 21.75	4	10.0000	0.075	0.037	9.8701	10.1299
Conc. 10.88	4	5.4975	0.338	0.169	4.9488	6.0462
Conc. 5.44	4	4.7525	0.178	0.089	4.4596	5.0454
Conc. 2.72	4	2.4975	0.338	0.169	1.9488	3.0462
Conc. Nystatin	4	15.4975	0.338	0.169	14.9488	16.0462
Conc. Control	4	.0000	0.000	0.000	.0000	.0000
Total	36	10.2914	7.332	1.219	7.8086	12.7742

Calculated F – Ratio $_{(11, 24)}$ = 3593.514, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day5

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	22.0000	0.074	0.031	21.8701	22.1299
Conc. 65.25	4	15.8725	0.074	0.031	15.6850	16.0600
Conc. 43.50	4	10.4975	0.415	0.202	9.9488	11.0462
Conc. 21.75	4	8.4975	0.074	0.031	7.9488	9.0462
Conc. 10.88	4	4.0000	0.337	0.163	3.8701	4.1299
Conc. 5.44	4	2.0000	0.176	0.082	1.8701	2.1299
Conc. 2.72	4	.7525	0.337	0.163	.4596	1.0454
Conc. Nystatin	4	14.0000	0.337	0.163	13.8701	14.1299
Conc. Control	4	.0000	.0000	.0000	.0000	.0000
Total	36	8.6244	7.330	1.213	6.1560	11.0929

Calculated F – Ratio $_{(11, 24)}$ = 6708.610, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day6

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	19.0000	0.071	0.033	18.8701	19.1299
Conc. 65.25	4	13.7525	0.071	0.033	13.4596	14.0454
Conc. 43.50	4	7.7525	0.412	0.203	7.4596	8.0454
Conc. 21.75	4	5.3775	0.071	0.033	4.7045	6.0505
Conc. 10.88	4	3.3775	0.334	0.164	2.7045	4.0505
Conc. 5.44	4	1.7525	0.173	0.084	1.4596	2.0454
Conc. 2.72	4	.4975	0.334	0.164	-.0512	1.0462
Conc. Nystatin	4	13.4975	0.334	0.164	12.9488	14.0462
Conc. Control	4	.0000	.0000	.0000	.0000	.0000
Total	36	7.2231	3.327	1.215	5.0317	9.4144

Calculated F – Ratio $_{(11, 24)}$ = 2343.028, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day7

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	16.0000	0.069	0.026	15.8701	16.1299
Conc. 65.25	4	12.4975	0.069	0.026	11.9488	13.0462
Conc. 43.50	4	6.0000	0.410	0.197	5.8701	6.1299
Conc. 21.75	4	6.0000	0.069	0.026	5.8701	6.1299
Conc. 10.88	4	2.8725	0.332	0.158	2.6850	3.0600
Conc. 5.44	4	1.4975	0.171	0.077	.9488	2.0462
Conc. 2.72	4	.3775	0.332	0.158	-.2955	1.0505
Conc. Nystatin	4	12.4975	0.332	0.158	11.9488	13.0462
Conc. Control	4	.0000	.0000	.0000	.0000	.0000
Total	36	6.4158	5.325	1.208	4.4985	8.3332

Calculated F – Ratio $_{(11, 24)}$ = 2216.936, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day8

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	13.4975	0.069	0.026	12.9488	14.0462
Conc. 65.25	4	10.7525	0.069	0.026	10.4596	11.0454
Conc. 43.50	4	5.0000	0.41	0.197	4.8701	5.1299
Conc. 21.75	4	5.0000	0.069	0.026	4.8701	5.1299
Conc. 10.88	4	2.4975	0.332	0.158	1.9488	3.0462
Conc. 5.44	4	.7525	0.171	0.077	.4596	1.0454
Conc. 2.72	4	.0000	0.332	0.158	-.1299	.1299
Conc. Nystatin	4	11.0000	0.332	0.158	10.8701	11.1299
Conc. Control	4	.0000	0.003	0.005	.0000	.0000
Total	36	5.3889	5.325	1.208	3.7137	7.0641

Calculated F – Ratio $_{(11, 24)}$ = 2901.835, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day 9

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	10.7525	0.070	0.032	10.4596	11.0454
Conc. 65.25	4	8.7525	0.070	0.032	8.4596	9.0454
Conc. 43.50	4	4.7525	0.412	0.203	4.4596	5.0454
Conc. 21.75	4	4.7525	0.070	0.032	4.4596	5.0454
Conc. 10.88	4	1.6250	0.333	0.164	1.2065	2.0435
Conc. 5.44	4	.7525	0.173	0.084	.4596	1.0454
Conc. 2.72	4	.0000	0.333	0.164	-.1299	.1299
Conc. Nystatin	4	11.0000	0.333	0.164	10.8701	11.1299
Conc. Control	4	.0000	0.000	0.000	.0000	.0000
Total	36	4.7097	7.327	1.214	3.2531	6.1663

Calculated F – Ratio $_{(11, 24)}$ = 2892.774, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day10

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	9.4975	0.340	0.168	8.9488	10.0462
Conc. 65.25	4	6.4975	0.340	0.168	5.9488	7.0462
Conc. 43.50	4	4.0000	0.077	0.036	3.8701	4.1299
Conc. 21.75	4	4.0000	0.077	0.036	3.8701	4.1299
Conc. 10.88	4	1.0000	0.077	0.036	.8701	1.1299
Conc. 5.44	4	.6250	0.258	0.127	.2065	1.0435
Conc. 2.72	4	.0000	0.077	0.036	-.1299	.1299
Conc. Nystatin	4	11.0000	0.077	0.036	10.8701	11.1299
Conc. Control	4	.0000	0.000	0.000	.0000	.0000
Total	36	4.0689	3.968	0.657	2.7249	5.4129

Calculated F – Ratio $_{(11, 24)}$ = 1822.410, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day11

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	8.0000	0.351	0.181	7.8701	8.1299
Conc. 65.25	4	5.0000	0.351	0.181	4.8701	5.1299
Conc. 43.50	4	3.7525	0.088	0.049	3.4596	4.0454
Conc. 21.75	4	3.7525	0.088	0.049	3.4596	4.0454
Conc. 10.88	4	.7525	0.088	0.049	.4596	1.0454
Conc. 5.44	4	.6250	0.269	0.140	.2065	1.0435
Conc. 2.72	4	.0000	0.088	0.049	-.1299	.1299
Conc. Nystatin	4	11.0000	0.088	0.049	10.8701	11.1299
Conc. Control	3	.0000	0.000	0.000	.0000	.0000
Total	35	3.7580	3.979	0.670	2.4907	5.0253

Calculated F – Ratio $_{(11, 24)}$ = 2535.101, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day12

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	7.7525	0.346	0.176	7.4596	8.0454
Conc. 65.25	4	4.0000	0.346	0.176	3.8701	4.1299
Conc. 43.50	4	3.0000	0.083	0.044	2.8701	3.1299
Conc. 21.75	4	3.0000	0.083	0.044	2.8701	3.1299
Conc. 10.88	4	.4975	0.083	0.044	-.0512	1.0462
Conc. 5.44	4	.4975	0.264	0.135	-.0512	1.0462
Conc. 2.72	4	.0000	0.083	0.044	-.1299	.1299
Conc. Nystatin	4	11.0000	0.083	0.044	10.8701	11.1299
Conc. Control	3	.0000	0.000	0.000	.0000	.0000
Total	35	3.3997	3.974	0.665	2.1379	4.6615

Calculated F – Ratio $_{(11, 24)}$ = 1626.210, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day13

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	7.4975	0.341	0.171	6.9488	8.0462
Conc. 65.25	4	3.4975	0.341	0.171	2.9488	4.0462
Conc. 43.50	4	2.7525	0.078	0.039	2.4596	3.0454
Conc. 21.75	4	2.7525	0.078	0.039	2.4596	3.0454
Conc. 10.88	4	.4975	0.078	0.039	-.0512	1.0462
Conc. 5.44	4	.3775	0.259	0.130	-.2955	1.0505
Conc. 2.72	4	.0000	0.078	0.039	-.1299	.1299
Conc. Nystatin	4	11.0000	5.078	2.039	10.8701	11.1299
Conc. Control	3	.0000	.0000	.0000	.0000	.0000
Total	35	3.2429	3.969	0.660	1.9880	4.4977

Calculated F – Ratio $_{(11, 24)}$ = 793.832, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Day14

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Conc. 87.00	4	7.0000	0.357	0.189	6.8701	7.1299
Conc. 65.25	4	2.7525	0.357	0.189	2.4596	3.0454
Conc. 43.50	4	2.4975	0.094	0.057	1.9488	3.0462
Conc. 21.75	4	2.4975	0.094	0.057	1.9488	3.0462
Conc. 10.88	4	.4975	0.094	0.057	-.0512	1.0462
Conc. 5.44	4	.0000	0.275	0.148	-.1299	.1299
Conc. 2.72	4	.0000	0.094	0.057	-.1299	.1299
Conc. Nystatin	4	10.7525	0.094	0.057	10.4596	11.0454
Conc. Control	3	.0000	.0000	.0000	.0000	.0000
Total	35	2.9711	3.985	0.678	1.7466	4.1956

Calculated F – Ratio $_{(11, 24)}$ = 1049.776, Critical F – Ratio $_{(8, 27)}$ = 3.31, P-Value = 0.000

Appendix 16: Mean difference in activity with variation of concentration and time (Days)

Dependent Variable: Day3
LSD

(I) NewConc	(J) NewConc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	4.50250*	.18096	.000	4.1312	4.8738
	Conc. 43.50	9.00000*	.18096	.000	8.6287	9.3713
	Conc. 21.75	12.62250*	.18096	.000	12.2512	12.9938
	Conc. 10.88	16.50250*	.18096	.000	16.1312	16.8738
	Conc. 5.44	19.24750*	.18096	.000	18.8762	19.6188
	Conc. 2.72	21.50250*	.18096	.000	21.1312	21.8738
	Conc. Nystatin	9.00000*	.18096	.000	8.6287	9.3713
	Conc. Control	25.00000*	.18096	.000	24.6287	25.3713
Conc. 65.25	Conc. 87.00	-4.50250*	.18096	.000	-4.8738	-4.1312
	Conc. 43.50	4.49750*	.18096	.000	4.1262	4.8688
	Conc. 21.75	8.12000*	.18096	.000	7.7487	8.4913
	Conc. 10.88	12.00000*	.18096	.000	11.6287	12.3713
	Conc. 5.44	14.74500*	.18096	.000	14.3737	15.1163
	Conc. 2.72	17.00000*	.18096	.000	16.6287	17.3713
	Conc. Nystatin	4.49750*	.18096	.000	4.1262	4.8688
	Conc. Control	20.49750*	.18096	.000	20.1262	20.8688
Conc. 43.50	Conc. 87.00	-9.00000*	.18096	.000	-9.3713	-8.6287
	Conc. 65.25	-4.49750*	.18096	.000	-4.8688	-4.1262
	Conc. 21.75	3.62250*	.18096	.000	3.2512	3.9938
	Conc. 10.88	7.50250*	.18096	.000	7.1312	7.8738
	Conc. 5.44	10.24750*	.18096	.000	9.8762	10.6188
	Conc. 2.72	12.50250*	.18096	.000	12.1312	12.8738
	Conc. Nystatin	.00000	.18096	1.000	-.3713	.3713
	Conc. Control	16.00000*	.18096	.000	15.6287	16.3713
Conc. 21.75	Conc. 87.00	-12.62250*	.18096	.000	-12.9938	-12.2512
	Conc. 65.25	-8.12000*	.18096	.000	-8.4913	-7.7487
	Conc. 43.50	-3.62250*	.18096	.000	-3.9938	-3.2512
	Conc. 10.88	3.88000*	.18096	.000	3.5087	4.2513
	Conc. 5.44	6.62500*	.18096	.000	6.2537	6.9963
	Conc. 2.72	8.88000*	.18096	.000	8.5087	9.2513
	Conc. Nystatin	-3.62250*	.18096	.000	-3.9938	-3.2512
	Conc. Control	12.37750*	.18096	.000	12.0062	12.7488
Conc. 10.88	Conc. 87.00	-16.50250*	.18096	.000	-16.8738	-16.1312
	Conc. 65.25	-12.00000*	.18096	.000	-12.3713	-11.6287
	Conc. 43.50	-7.50250*	.18096	.000	-7.8738	-7.1312
	Conc. 21.75	-3.88000*	.18096	.000	-4.2513	-3.5087
	Conc. 5.44	2.74500*	.18096	.000	2.3737	3.1163
	Conc. 2.72	5.00000*	.18096	.000	4.6287	5.3713
	Conc. Nystatin	-7.50250*	.18096	.000	-7.8738	-7.1312
	Conc. Control	8.49750*	.18096	.000	8.1262	8.8688
Conc. 5.44	Conc. 87.00	-19.24750*	.18096	.000	-19.6188	-18.8762
	Conc. 65.25	-14.74500*	.18096	.000	-15.1163	-14.3737
	Conc. 43.50	-10.24750*	.18096	.000	-10.6188	-9.8762
	Conc. 21.75	-6.62500*	.18096	.000	-6.9963	-6.2537
	Conc. 10.88	-2.74500*	.18096	.000	-3.1163	-2.3737
	Conc. 2.72	2.25500*	.18096	.000	1.8837	2.6263

	Conc. Nystatin	-10.24750*	.18096	.000	-10.6188	-9.8762
	Conc. Control	5.75250*	.18096	.000	5.3812	6.1238
Conc. 2.72	Conc. 87.00	-21.50250*	.18096	.000	-21.8738	-21.1312
	Conc. 65.25	-17.00000*	.18096	.000	-17.3713	-16.6287
	Conc. 43.50	-12.50250*	.18096	.000	-12.8738	-12.1312
	Conc. 21.75	-8.88000*	.18096	.000	-9.2513	-8.5087
	Conc. 10.88	-5.00000*	.18096	.000	-5.3713	-4.6287
	Conc. 5.44	-2.25500*	.18096	.000	-2.6263	-1.8837
	Conc. Nystatin	-12.50250*	.18096	.000	-12.8738	-12.1312
	Conc. Control	3.49750*	.18096	.000	3.1262	3.8688
Conc. Nystatin	Conc. 87.00	-9.00000*	.18096	.000	-9.3713	-8.6287
	Conc. 65.25	-4.49750*	.18096	.000	-4.8688	-4.1262
	Conc. 43.50	.00000	.18096	1.000	-.3713	.3713
	Conc. 21.75	3.62250*	.18096	.000	3.2512	3.9938
	Conc. 10.88	7.50250*	.18096	.000	7.1312	7.8738
	Conc. 5.44	10.24750*	.18096	.000	9.8762	10.6188
	Conc. 2.72	12.50250*	.18096	.000	12.1312	12.8738
	Conc. Control	16.00000*	.18096	.000	15.6287	16.3713
Conc. Control	Conc. 87.00	-25.00000*	.18096	.000	-25.3713	-24.6287
	Conc. 65.25	-20.49750*	.18096	.000	-20.8688	-20.1262
	Conc. 43.50	-16.00000*	.18096	.000	-16.3713	-15.6287
	Conc. 21.75	-12.37750*	.18096	.000	-12.7488	-12.0062
	Conc. 10.88	-8.49750*	.18096	.000	-8.8688	-8.1262
	Conc. 5.44	-5.75250*	.18096	.000	-6.1238	-5.3812
	Conc. 2.72	-3.49750*	.18096	.000	-3.8688	-3.1262
	Conc. Nystatin	-16.00000*	.18096	.000	-16.3713	-15.6287

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day5

LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	6.12750*	.13171	.000	5.8573	6.3977
	Conc. 43.50	11.50250*	.13171	.000	11.2323	11.7727
	Conc. 21.75	13.50250*	.13171	.000	13.2323	13.7727
	Conc. 10.88	18.00000*	.13171	.000	17.7298	18.2702
	Conc. 5.44	20.00000*	.13171	.000	19.7298	20.2702
	Conc. 2.72	21.24750*	.13171	.000	20.9773	21.5177
	Conc. Nystatin	8.00000*	.13171	.000	7.7298	8.2702
	Conc. Control	22.00000*	.13171	.000	21.7298	22.2702
Conc. 65.25	Conc. 87.00	-6.12750*	.13171	.000	-6.3977	-5.8573
	Conc. 43.50	5.37500*	.13171	.000	5.1048	5.6452
	Conc. 21.75	7.37500*	.13171	.000	7.1048	7.6452
	Conc. 10.88	11.87250*	.13171	.000	11.6023	12.1427
	Conc. 5.44	13.87250*	.13171	.000	13.6023	14.1427
	Conc. 2.72	15.12000*	.13171	.000	14.8498	15.3902
	Conc. Nystatin	1.87250*	.13171	.000	1.6023	2.1427
	Conc. Control	15.87250*	.13171	.000	15.6023	16.1427
Conc. 43.50	Conc. 87.00	-11.50250*	.13171	.000	-11.7727	-11.2323
	Conc. 65.25	-5.37500*	.13171	.000	-5.6452	-5.1048
	Conc. 21.75	2.00000*	.13171	.000	1.7298	2.2702
	Conc. 10.88	6.49750*	.13171	.000	6.2273	6.7677
	Conc. 5.44	8.49750*	.13171	.000	8.2273	8.7677
	Conc. 2.72	9.74500*	.13171	.000	9.4748	10.0152
	Conc. Nystatin	-3.50250*	.13171	.000	-3.7727	-3.2323

	Conc. Control	10.49750*	.13171	.000	10.2273	10.7677
Conc. 21.75	Conc. 87.00	-13.50250*	.13171	.000	-13.7727	-13.2323
	Conc. 65.25	-7.37500*	.13171	.000	-7.6452	-7.1048
	Conc. 43.50	-2.00000*	.13171	.000	-2.2702	-1.7298
	Conc. 10.88	4.49750*	.13171	.000	4.2273	4.7677
	Conc. 5.44	6.49750*	.13171	.000	6.2273	6.7677
	Conc. 2.72	7.74500*	.13171	.000	7.4748	8.0152
	Conc. Nystatin	-5.50250*	.13171	.000	-5.7727	-5.2323
	Conc. Control	8.49750*	.13171	.000	8.2273	8.7677
Conc. 10.88	Conc. 87.00	-18.00000*	.13171	.000	-18.2702	-17.7298
	Conc. 65.25	-11.87250*	.13171	.000	-12.1427	-11.6023
	Conc. 43.50	-6.49750*	.13171	.000	-6.7677	-6.2273
	Conc. 21.75	-4.49750*	.13171	.000	-4.7677	-4.2273
	Conc. 5.44	2.00000*	.13171	.000	1.7298	2.2702
	Conc. 2.72	3.24750*	.13171	.000	2.9773	3.5177
	Conc. Nystatin	-10.00000*	.13171	.000	-10.2702	-9.7298
	Conc. Control	4.00000*	.13171	.000	3.7298	4.2702
Conc. 5.44	Conc. 87.00	-20.00000*	.13171	.000	-20.2702	-19.7298
	Conc. 65.25	-13.87250*	.13171	.000	-14.1427	-13.6023
	Conc. 43.50	-8.49750*	.13171	.000	-8.7677	-8.2273
	Conc. 21.75	-6.49750*	.13171	.000	-6.7677	-6.2273
	Conc. 10.88	-2.00000*	.13171	.000	-2.2702	-1.7298
	Conc. 2.72	1.24750*	.13171	.000	.9773	1.5177
	Conc. Nystatin	-12.00000*	.13171	.000	-12.2702	-11.7298
	Conc. Control	2.00000*	.13171	.000	1.7298	2.2702
Conc. 2.72	Conc. 87.00	-21.24750*	.13171	.000	-21.5177	-20.9773
	Conc. 65.25	-15.12000*	.13171	.000	-15.3902	-14.8498
	Conc. 43.50	-9.74500*	.13171	.000	-10.0152	-9.4748
	Conc. 21.75	-7.74500*	.13171	.000	-8.0152	-7.4748
	Conc. 10.88	-3.24750*	.13171	.000	-3.5177	-2.9773
	Conc. 5.44	-1.24750*	.13171	.000	-1.5177	-.9773
	Conc. Nystatin	-13.24750*	.13171	.000	-13.5177	-12.9773
	Conc. Control	.75250*	.13171	.000	.4823	1.0227
Conc. Nystatin	Conc. 87.00	-8.00000*	.13171	.000	-8.2702	-7.7298
	Conc. 65.25	-1.87250*	.13171	.000	-2.1427	-1.6023
	Conc. 43.50	3.50250*	.13171	.000	3.2323	3.7727
	Conc. 21.75	5.50250*	.13171	.000	5.2323	5.7727
	Conc. 10.88	10.00000*	.13171	.000	9.7298	10.2702
	Conc. 5.44	12.00000*	.13171	.000	11.7298	12.2702
	Conc. 2.72	13.24750*	.13171	.000	12.9773	13.5177
	Conc. Control	14.00000*	.13171	.000	13.7298	14.2702
Conc. Control	Conc. 87.00	-22.00000*	.13171	.000	-22.2702	-21.7298
	Conc. 65.25	-15.87250*	.13171	.000	-16.1427	-15.6023
	Conc. 43.50	-10.49750*	.13171	.000	-10.7677	-10.2273
	Conc. 21.75	-8.49750*	.13171	.000	-8.7677	-8.2273
	Conc. 10.88	-4.00000*	.13171	.000	-4.2702	-3.7298
	Conc. 5.44	-2.00000*	.13171	.000	-2.2702	-1.7298
	Conc. 2.72	-.75250*	.13171	.000	-1.0227	-.4823
	Conc. Nystatin	-14.00000*	.13171	.000	-14.2702	-13.7298

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day7

LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	3.50250*	.17787	.000	3.1375	3.8675

	Conc. 43.50	10.00000*	.17787	.000	9.6350	10.3650
	Conc. 21.75	10.00000*	.17787	.000	9.6350	10.3650
	Conc. 10.88	13.12750*	.17787	.000	12.7625	13.4925
	Conc. 5.44	14.50250*	.17787	.000	14.1375	14.8675
	Conc. 2.72	15.62250*	.17787	.000	15.2575	15.9875
	Conc. Nystatin	3.50250*	.17787	.000	3.1375	3.8675
	Conc. Control	16.00000*	.17787	.000	15.6350	16.3650
Conc. 65.25	Conc. 87.00	-3.50250*	.17787	.000	-3.8675	-3.1375
	Conc. 43.50	6.49750*	.17787	.000	6.1325	6.8625
	Conc. 21.75	6.49750*	.17787	.000	6.1325	6.8625
	Conc. 10.88	9.62500*	.17787	.000	9.2600	9.9900
	Conc. 5.44	11.00000*	.17787	.000	10.6350	11.3650
	Conc. 2.72	12.12000*	.17787	.000	11.7550	12.4850
	Conc. Nystatin	.00000	.17787	1.000	-.3650	.3650
	Conc. Control	12.49750*	.17787	.000	12.1325	12.8625
Conc. 43.50	Conc. 87.00	-10.00000*	.17787	.000	-10.3650	-9.6350
	Conc. 65.25	-6.49750*	.17787	.000	-6.8625	-6.1325
	Conc. 21.75	.00000	.17787	1.000	-.3650	.3650
	Conc. 10.88	3.12750*	.17787	.000	2.7625	3.4925
	Conc. 5.44	4.50250*	.17787	.000	4.1375	4.8675
	Conc. 2.72	5.62250*	.17787	.000	5.2575	5.9875
	Conc. Nystatin	-6.49750*	.17787	.000	-6.8625	-6.1325
	Conc. Control	6.00000*	.17787	.000	5.6350	6.3650
Conc. 21.75	Conc. 87.00	-10.00000*	.17787	.000	-10.3650	-9.6350
	Conc. 65.25	-6.49750*	.17787	.000	-6.8625	-6.1325
	Conc. 43.50	.00000	.17787	1.000	-.3650	.3650
	Conc. 10.88	3.12750*	.17787	.000	2.7625	3.4925
	Conc. 5.44	4.50250*	.17787	.000	4.1375	4.8675
	Conc. 2.72	5.62250*	.17787	.000	5.2575	5.9875
	Conc. Nystatin	-6.49750*	.17787	.000	-6.8625	-6.1325
	Conc. Control	6.00000*	.17787	.000	5.6350	6.3650
Conc. 10.88	Conc. 87.00	-13.12750*	.17787	.000	-13.4925	-12.7625
	Conc. 65.25	-9.62500*	.17787	.000	-9.9900	-9.2600
	Conc. 43.50	-3.12750*	.17787	.000	-3.4925	-2.7625
	Conc. 21.75	-3.12750*	.17787	.000	-3.4925	-2.7625
	Conc. 5.44	1.37500*	.17787	.000	1.0100	1.7400
	Conc. 2.72	2.49500*	.17787	.000	2.1300	2.8600
	Conc. Nystatin	-9.62500*	.17787	.000	-9.9900	-9.2600
	Conc. Control	2.87250*	.17787	.000	2.5075	3.2375
Conc. 5.44	Conc. 87.00	-14.50250*	.17787	.000	-14.8675	-14.1375
	Conc. 65.25	-11.00000*	.17787	.000	-11.3650	-10.6350
	Conc. 43.50	-4.50250*	.17787	.000	-4.8675	-4.1375
	Conc. 21.75	-4.50250*	.17787	.000	-4.8675	-4.1375
	Conc. 10.88	-1.37500*	.17787	.000	-1.7400	-1.0100
	Conc. 2.72	1.12000*	.17787	.000	.7550	1.4850
	Conc. Nystatin	-11.00000*	.17787	.000	-11.3650	-10.6350
	Conc. Control	1.49750*	.17787	.000	1.1325	1.8625
Conc. 2.72	Conc. 87.00	-15.62250*	.17787	.000	-15.9875	-15.2575
	Conc. 65.25	-12.12000*	.17787	.000	-12.4850	-11.7550
	Conc. 43.50	-5.62250*	.17787	.000	-5.9875	-5.2575
	Conc. 21.75	-5.62250*	.17787	.000	-5.9875	-5.2575
	Conc. 10.88	-2.49500*	.17787	.000	-2.8600	-2.1300
	Conc. 5.44	-1.12000*	.17787	.000	-1.4850	-.7550
	Conc. Nystatin	-12.12000*	.17787	.000	-12.4850	-11.7550
	Conc. Control	.37750*	.17787	.043	.0125	.7425

Conc. Nystatin	Conc. 87.00	-3.50250*	.17787	.000	-3.8675	-3.1375
	Conc. 65.25	.00000	.17787	1.000	-.3650	.3650
	Conc. 43.50	6.49750*	.17787	.000	6.1325	6.8625
	Conc. 21.75	6.49750*	.17787	.000	6.1325	6.8625
	Conc. 10.88	9.62500*	.17787	.000	9.2600	9.9900
	Conc. 5.44	11.00000*	.17787	.000	10.6350	11.3650
	Conc. 2.72	12.12000*	.17787	.000	11.7550	12.4850
	Conc. Control	12.49750*	.17787	.000	12.1325	12.8625
Conc. Control	Conc. 87.00	-16.00000*	.17787	.000	-16.3650	-15.6350
	Conc. 65.25	-12.49750*	.17787	.000	-12.8625	-12.1325
	Conc. 43.50	-6.00000*	.17787	.000	-6.3650	-5.6350
	Conc. 21.75	-6.00000*	.17787	.000	-6.3650	-5.6350
	Conc. 10.88	-2.87250*	.17787	.000	-3.2375	-2.5075
	Conc. 5.44	-1.49750*	.17787	.000	-1.8625	-1.1325
	Conc. 2.72	-.37750*	.17787	.043	-.7425	-.0125
	Conc. Nystatin	-12.49750*	.17787	.000	-12.8625	-12.1325

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day10

LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	3.00000*	.13749	.000	2.7179	3.2821
	Conc. 43.50	5.49750*	.13749	.000	5.2154	5.7796
	Conc. 21.75	5.49750*	.13749	.000	5.2154	5.7796
	Conc. 10.88	8.49750*	.13749	.000	8.2154	8.7796
	Conc. 5.44	8.87250*	.13749	.000	8.5904	9.1546
	Conc. 2.72	9.49750*	.13749	.000	9.2154	9.7796
	Conc. Nystatin	-1.50250*	.13749	.000	-1.7846	-1.2204
	Conc. Control	9.49750*	.13749	.000	9.2154	9.7796
Conc. 65.25	Conc. 87.00	-3.00000*	.13749	.000	-3.2821	-2.7179
	Conc. 43.50	2.49750*	.13749	.000	2.2154	2.7796
	Conc. 21.75	2.49750*	.13749	.000	2.2154	2.7796
	Conc. 10.88	5.49750*	.13749	.000	5.2154	5.7796
	Conc. 5.44	5.87250*	.13749	.000	5.5904	6.1546
	Conc. 2.72	6.49750*	.13749	.000	6.2154	6.7796
	Conc. Nystatin	-4.50250*	.13749	.000	-4.7846	-4.2204
	Conc. Control	6.49750*	.13749	.000	6.2154	6.7796
Conc. 43.50	Conc. 87.00	-5.49750*	.13749	.000	-5.7796	-5.2154
	Conc. 65.25	-2.49750*	.13749	.000	-2.7796	-2.2154
	Conc. 21.75	.00000	.13749	1.000	-.2821	.2821
	Conc. 10.88	3.00000*	.13749	.000	2.7179	3.2821
	Conc. 5.44	3.37500*	.13749	.000	3.0929	3.6571
	Conc. 2.72	4.00000*	.13749	.000	3.7179	4.2821
	Conc. Nystatin	-7.00000*	.13749	.000	-7.2821	-6.7179
	Conc. Control	4.00000*	.13749	.000	3.7179	4.2821
Conc. 21.75	Conc. 87.00	-5.49750*	.13749	.000	-5.7796	-5.2154
	Conc. 65.25	-2.49750*	.13749	.000	-2.7796	-2.2154
	Conc. 43.50	.00000	.13749	1.000	-.2821	.2821
	Conc. 10.88	3.00000*	.13749	.000	2.7179	3.2821
	Conc. 5.44	3.37500*	.13749	.000	3.0929	3.6571
	Conc. 2.72	4.00000*	.13749	.000	3.7179	4.2821
	Conc. Nystatin	-7.00000*	.13749	.000	-7.2821	-6.7179
	Conc. Control	4.00000*	.13749	.000	3.7179	4.2821
Conc. 10.88	Conc. 87.00	-8.49750*	.13749	.000	-8.7796	-8.2154

	Conc. 65.25	-5.49750*	.13749	.000	-5.7796	-5.2154
	Conc. 43.50	-3.00000*	.13749	.000	-3.2821	-2.7179
	Conc. 21.75	-3.00000*	.13749	.000	-3.2821	-2.7179
	Conc. 5.44	.37500*	.13749	.011	.0929	.6571
	Conc. 2.72	1.00000*	.13749	.000	.7179	1.2821
	Conc. Nystatin	-10.00000*	.13749	.000	-10.2821	-9.7179
	Conc. Control	1.00000*	.13749	.000	.7179	1.2821
Conc. 5.44	Conc. 87.00	-8.87250*	.13749	.000	-9.1546	-8.5904
	Conc. 65.25	-5.87250*	.13749	.000	-6.1546	-5.5904
	Conc. 43.50	-3.37500*	.13749	.000	-3.6571	-3.0929
	Conc. 21.75	-3.37500*	.13749	.000	-3.6571	-3.0929
	Conc. 10.88	-.37500*	.13749	.011	-.6571	-.0929
	Conc. 2.72	.62500*	.13749	.000	.3429	.9071
	Conc. Nystatin	-10.37500*	.13749	.000	-10.6571	-10.0929
	Conc. Control	.62500*	.13749	.000	.3429	.9071
Conc. 2.72	Conc. 87.00	-9.49750*	.13749	.000	-9.7796	-9.2154
	Conc. 65.25	-6.49750*	.13749	.000	-6.7796	-6.2154
	Conc. 43.50	-4.00000*	.13749	.000	-4.2821	-3.7179
	Conc. 21.75	-4.00000*	.13749	.000	-4.2821	-3.7179
	Conc. 10.88	-1.00000*	.13749	.000	-1.2821	-.7179
	Conc. 5.44	-.62500*	.13749	.000	-.9071	-.3429
	Conc. Nystatin	-11.00000*	.13749	.000	-11.2821	-10.7179
	Conc. Control	.00000	.13749	1.000	-.2821	.2821
Conc. Nystatin	Conc. 87.00	1.50250*	.13749	.000	1.2204	1.7846
	Conc. 65.25	4.50250*	.13749	.000	4.2204	4.7846
	Conc. 43.50	7.00000*	.13749	.000	6.7179	7.2821
	Conc. 21.75	7.00000*	.13749	.000	6.7179	7.2821
	Conc. 10.88	10.00000*	.13749	.000	9.7179	10.2821
	Conc. 5.44	10.37500*	.13749	.000	10.0929	10.6571
	Conc. 2.72	11.00000*	.13749	.000	10.7179	11.2821
	Conc. Control	11.00000*	.13749	.000	10.7179	11.2821
Conc. Control	Conc. 87.00	-9.49750*	.13749	.000	-9.7796	-9.2154
	Conc. 65.25	-6.49750*	.13749	.000	-6.7796	-6.2154
	Conc. 43.50	-4.00000*	.13749	.000	-4.2821	-3.7179
	Conc. 21.75	-4.00000*	.13749	.000	-4.2821	-3.7179
	Conc. 10.88	-1.00000*	.13749	.000	-1.2821	-.7179
	Conc. 5.44	-.62500*	.13749	.000	-.9071	-.3429
	Conc. 2.72	.00000	.13749	1.000	-.2821	.2821
	Conc. Nystatin	-11.00000*	.13749	.000	-11.2821	-10.7179

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day6
LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	5.24750*	.19775	.000	4.8417	5.6533
	Conc. 43.50	11.24750*	.19775	.000	10.8417	11.6533
	Conc. 21.75	13.62250*	.19775	.000	13.2167	14.0283
	Conc. 10.88	15.62250*	.19775	.000	15.2167	16.0283
	Conc. 5.44	17.24750*	.19775	.000	16.8417	17.6533
	Conc. 2.72	18.50250*	.19775	.000	18.0967	18.9083
	Conc. Nystatin	5.50250*	.19775	.000	5.0967	5.9083
	Conc. Control	19.00000*	.19775	.000	18.5942	19.4058
Conc. 65.25	Conc. 87.00	-5.24750*	.19775	.000	-5.6533	-4.8417

	Conc. 43.50	6.00000*	.19775	.000	5.5942	6.4058
	Conc. 21.75	8.37500*	.19775	.000	7.9692	8.7808
	Conc. 10.88	10.37500*	.19775	.000	9.9692	10.7808
	Conc. 5.44	12.00000*	.19775	.000	11.5942	12.4058
	Conc. 2.72	13.25500*	.19775	.000	12.8492	13.6608
	Conc. Nystatin	.25500	.19775	.208	-.1508	.6608
	Conc. Control	13.75250*	.19775	.000	13.3467	14.1583
Conc. 43.50	Conc. 87.00	-11.24750*	.19775	.000	-11.6533	-10.8417
	Conc. 65.25	-6.00000*	.19775	.000	-6.4058	-5.5942
	Conc. 21.75	2.37500*	.19775	.000	1.9692	2.7808
	Conc. 10.88	4.37500*	.19775	.000	3.9692	4.7808
	Conc. 5.44	6.00000*	.19775	.000	5.5942	6.4058
	Conc. 2.72	7.25500*	.19775	.000	6.8492	7.6608
	Conc. Nystatin	-5.74500*	.19775	.000	-6.1508	-5.3392
	Conc. Control	7.75250*	.19775	.000	7.3467	8.1583
Conc. 21.75	Conc. 87.00	-13.62250*	.19775	.000	-14.0283	-13.2167
	Conc. 65.25	-8.37500*	.19775	.000	-8.7808	-7.9692
	Conc. 43.50	-2.37500*	.19775	.000	-2.7808	-1.9692
	Conc. 10.88	2.00000*	.19775	.000	1.5942	2.4058
	Conc. 5.44	3.62500*	.19775	.000	3.2192	4.0308
	Conc. 2.72	4.88000*	.19775	.000	4.4742	5.2858
	Conc. Nystatin	-8.12000*	.19775	.000	-8.5258	-7.7142
	Conc. Control	5.37750*	.19775	.000	4.9717	5.7833
Conc. 10.88	Conc. 87.00	-15.62250*	.19775	.000	-16.0283	-15.2167
	Conc. 65.25	-10.37500*	.19775	.000	-10.7808	-9.9692
	Conc. 43.50	-4.37500*	.19775	.000	-4.7808	-3.9692
	Conc. 21.75	-2.00000*	.19775	.000	-2.4058	-1.5942
	Conc. 5.44	1.62500*	.19775	.000	1.2192	2.0308
	Conc. 2.72	2.88000*	.19775	.000	2.4742	3.2858
	Conc. Nystatin	-10.12000*	.19775	.000	-10.5258	-9.7142
	Conc. Control	3.37750*	.19775	.000	2.9717	3.7833
Conc. 5.44	Conc. 87.00	-17.24750*	.19775	.000	-17.6533	-16.8417
	Conc. 65.25	-12.00000*	.19775	.000	-12.4058	-11.5942
	Conc. 43.50	-6.00000*	.19775	.000	-6.4058	-5.5942
	Conc. 21.75	-3.62500*	.19775	.000	-4.0308	-3.2192
	Conc. 10.88	-1.62500*	.19775	.000	-2.0308	-1.2192
	Conc. 2.72	1.25500*	.19775	.000	.8492	1.6608
	Conc. Nystatin	-11.74500*	.19775	.000	-12.1508	-11.3392
	Conc. Control	1.75250*	.19775	.000	1.3467	2.1583
Conc. 2.72	Conc. 87.00	-18.50250*	.19775	.000	-18.9083	-18.0967
	Conc. 65.25	-13.25500*	.19775	.000	-13.6608	-12.8492
	Conc. 43.50	-7.25500*	.19775	.000	-7.6608	-6.8492
	Conc. 21.75	-4.88000*	.19775	.000	-5.2858	-4.4742
	Conc. 10.88	-2.88000*	.19775	.000	-3.2858	-2.4742
	Conc. 5.44	-1.25500*	.19775	.000	-1.6608	-.8492
	Conc. Nystatin	-13.00000*	.19775	.000	-13.4058	-12.5942
	Conc. Control	.49750*	.19775	.018	.0917	.9033
Conc. Nystatin	Conc. 87.00	-5.50250*	.19775	.000	-5.9083	-5.0967
	Conc. 65.25	-.25500	.19775	.208	-.6608	.1508
	Conc. 43.50	5.74500*	.19775	.000	5.3392	6.1508
	Conc. 21.75	8.12000*	.19775	.000	7.7142	8.5258
	Conc. 10.88	10.12000*	.19775	.000	9.7142	10.5258
	Conc. 5.44	11.74500*	.19775	.000	11.3392	12.1508
	Conc. 2.72	13.00000*	.19775	.000	12.5942	13.4058
	Conc. Control	13.49750*	.19775	.000	13.0917	13.9033

Conc. Control	Conc. 87.00	-19.00000*	.19775	.000	-19.4058	-18.5942
	Conc. 65.25	-13.75250*	.19775	.000	-14.1583	-13.3467
	Conc. 43.50	-7.75250*	.19775	.000	-8.1583	-7.3467
	Conc. 21.75	-5.37750*	.19775	.000	-5.7833	-4.9717
	Conc. 10.88	-3.37750*	.19775	.000	-3.7833	-2.9717
	Conc. 5.44	-1.75250*	.19775	.000	-2.1583	-1.3467
	Conc. 2.72	-.49750*	.19775	.018	-.9033	-.0917
	Conc. Nystatin	-13.49750*	.19775	.000	-13.9033	-13.0917

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day9

LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	2.00000*	.11831	.000	1.7572	2.2428
	Conc. 43.50	6.00000*	.11831	.000	5.7572	6.2428
	Conc. 21.75	6.00000*	.11831	.000	5.7572	6.2428
	Conc. 10.88	9.12750*	.11831	.000	8.8847	9.3703
	Conc. 5.44	10.00000*	.11831	.000	9.7572	10.2428
	Conc. 2.72	10.75250*	.11831	.000	10.5097	10.9953
	Conc. Nystatin	-.24750*	.11831	.046	-.4903	-.0047
	Conc. Control	10.75250*	.11831	.000	10.5097	10.9953
Conc. 65.25	Conc. 87.00	-2.00000*	.11831	.000	-2.2428	-1.7572
	Conc. 43.50	4.00000*	.11831	.000	3.7572	4.2428
	Conc. 21.75	4.00000*	.11831	.000	3.7572	4.2428
	Conc. 10.88	7.12750*	.11831	.000	6.8847	7.3703
	Conc. 5.44	8.00000*	.11831	.000	7.7572	8.2428
	Conc. 2.72	8.75250*	.11831	.000	8.5097	8.9953
	Conc. Nystatin	-2.24750*	.11831	.000	-2.4903	-2.0047
	Conc. Control	8.75250*	.11831	.000	8.5097	8.9953
Conc. 43.50	Conc. 87.00	-6.00000*	.11831	.000	-6.2428	-5.7572
	Conc. 65.25	-4.00000*	.11831	.000	-4.2428	-3.7572
	Conc. 21.75	.00000	.11831	1.000	-.2428	.2428
	Conc. 10.88	3.12750*	.11831	.000	2.8847	3.3703
	Conc. 5.44	4.00000*	.11831	.000	3.7572	4.2428
	Conc. 2.72	4.75250*	.11831	.000	4.5097	4.9953
	Conc. Nystatin	-6.24750*	.11831	.000	-6.4903	-6.0047
	Conc. Control	4.75250*	.11831	.000	4.5097	4.9953
Conc. 21.75	Conc. 87.00	-6.00000*	.11831	.000	-6.2428	-5.7572
	Conc. 65.25	-4.00000*	.11831	.000	-4.2428	-3.7572
	Conc. 43.50	.00000	.11831	1.000	-.2428	.2428
	Conc. 10.88	3.12750*	.11831	.000	2.8847	3.3703
	Conc. 5.44	4.00000*	.11831	.000	3.7572	4.2428
	Conc. 2.72	4.75250*	.11831	.000	4.5097	4.9953
	Conc. Nystatin	-6.24750*	.11831	.000	-6.4903	-6.0047
	Conc. Control	4.75250*	.11831	.000	4.5097	4.9953
Conc. 10.88	Conc. 87.00	-9.12750*	.11831	.000	-9.3703	-8.8847
	Conc. 65.25	-7.12750*	.11831	.000	-7.3703	-6.8847
	Conc. 43.50	-3.12750*	.11831	.000	-3.3703	-2.8847
	Conc. 21.75	-3.12750*	.11831	.000	-3.3703	-2.8847
	Conc. 5.44	.87250*	.11831	.000	.6297	1.1153
	Conc. 2.72	1.62500*	.11831	.000	1.3822	1.8678
	Conc. Nystatin	-9.37500*	.11831	.000	-9.6178	-9.1322
	Conc. Control	1.62500*	.11831	.000	1.3822	1.8678
Conc. 5.44	Conc. 87.00	-10.00000*	.11831	.000	-10.2428	-9.7572

	Conc. 65.25	-8.00000*	.11831	.000	-8.2428	-7.7572
	Conc. 43.50	-4.00000*	.11831	.000	-4.2428	-3.7572
	Conc. 21.75	-4.00000*	.11831	.000	-4.2428	-3.7572
	Conc. 10.88	-.87250*	.11831	.000	-1.1153	-.6297
	Conc. 2.72	.75250*	.11831	.000	.5097	.9953
	Conc. Nystatin	-10.24750*	.11831	.000	-10.4903	-10.0047
	Conc. Control	.75250*	.11831	.000	.5097	.9953
Conc. 2.72	Conc. 87.00	-10.75250*	.11831	.000	-10.9953	-10.5097
	Conc. 65.25	-8.75250*	.11831	.000	-8.9953	-8.5097
	Conc. 43.50	-4.75250*	.11831	.000	-4.9953	-4.5097
	Conc. 21.75	-4.75250*	.11831	.000	-4.9953	-4.5097
	Conc. 10.88	-1.62500*	.11831	.000	-1.8678	-1.3822
	Conc. 5.44	-.75250*	.11831	.000	-.9953	-.5097
	Conc. Nystatin	-11.00000*	.11831	.000	-11.2428	-10.7572
	Conc. Control	.00000	.11831	1.000	-.2428	.2428
Conc. Nystatin	Conc. 87.00	.24750*	.11831	.046	.0047	.4903
	Conc. 65.25	2.24750*	.11831	.000	2.0047	2.4903
	Conc. 43.50	6.24750*	.11831	.000	6.0047	6.4903
	Conc. 21.75	6.24750*	.11831	.000	6.0047	6.4903
	Conc. 10.88	9.37500*	.11831	.000	9.1322	9.6178
	Conc. 5.44	10.24750*	.11831	.000	10.0047	10.4903
	Conc. 2.72	11.00000*	.11831	.000	10.7572	11.2428
	Conc. Control	11.00000*	.11831	.000	10.7572	11.2428
Conc. Control	Conc. 87.00	-10.75250*	.11831	.000	-10.9953	-10.5097
	Conc. 65.25	-8.75250*	.11831	.000	-8.9953	-8.5097
	Conc. 43.50	-4.75250*	.11831	.000	-4.9953	-4.5097
	Conc. 21.75	-4.75250*	.11831	.000	-4.9953	-4.5097
	Conc. 10.88	-1.62500*	.11831	.000	-1.8678	-1.3822
	Conc. 5.44	-.75250*	.11831	.000	-.9953	-.5097
	Conc. 2.72	.00000	.11831	1.000	-.2428	.2428
	Conc. Nystatin	-11.00000*	.11831	.000	-11.2428	-10.7572

*. The mean difference is significant at the 0.05 level.

Day12

Dependent Variable: Day12

LSD

		Mean Difference			95% Confidence Interval	
(I) NewConc	(J) NewConc	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	3.75250*	.13265	.000	3.4798	4.0252
	Conc. 43.50	4.75250*	.13265	.000	4.4798	5.0252
	Conc. 21.75	4.75250*	.13265	.000	4.4798	5.0252
	Conc. 10.88	7.25500*	.13265	.000	6.9823	7.5277
	Conc. 5.44	7.25500*	.13265	.000	6.9823	7.5277
	Conc. 2.72	7.75250*	.13265	.000	7.4798	8.0252
	Conc. Nystatin	-3.24750*	.13265	.000	-3.5202	-2.9748
	Conc. Control	7.75250*	.14328	.000	7.4580	8.0470
Conc. 65.25	Conc. 87.00	-3.75250*	.13265	.000	-4.0252	-3.4798
	Conc. 43.50	1.00000*	.13265	.000	.7273	1.2727
	Conc. 21.75	1.00000*	.13265	.000	.7273	1.2727
	Conc. 10.88	3.50250*	.13265	.000	3.2298	3.7752
	Conc. 5.44	3.50250*	.13265	.000	3.2298	3.7752
	Conc. 2.72	4.00000*	.13265	.000	3.7273	4.2727
	Conc. Nystatin	-7.00000*	.13265	.000	-7.2727	-6.7273
	Conc. Control	4.00000*	.14328	.000	3.7055	4.2945
Conc. 43.50	Conc. 87.00	-4.75250*	.13265	.000	-5.0252	-4.4798

	Conc. 65.25	-1.00000*	.13265	.000	-1.2727	-.7273
	Conc. 21.75	.00000	.13265	1.000	-.2727	.2727
	Conc. 10.88	2.50250*	.13265	.000	2.2298	2.7752
	Conc. 5.44	2.50250*	.13265	.000	2.2298	2.7752
	Conc. 2.72	3.00000*	.13265	.000	2.7273	3.2727
	Conc. Nystatin	-8.00000*	.13265	.000	-8.2727	-7.7273
	Conc. Control	3.00000*	.14328	.000	2.7055	3.2945
Conc. 21.75	Conc. 87.00	-4.75250*	.13265	.000	-5.0252	-4.4798
	Conc. 65.25	-1.00000*	.13265	.000	-1.2727	-.7273
	Conc. 43.50	.00000	.13265	1.000	-.2727	.2727
	Conc. 10.88	2.50250*	.13265	.000	2.2298	2.7752
	Conc. 5.44	2.50250*	.13265	.000	2.2298	2.7752
	Conc. 2.72	3.00000*	.13265	.000	2.7273	3.2727
	Conc. Nystatin	-8.00000*	.13265	.000	-8.2727	-7.7273
	Conc. Control	3.00000*	.14328	.000	2.7055	3.2945
Conc. 10.88	Conc. 87.00	-7.25500*	.13265	.000	-7.5277	-6.9823
	Conc. 65.25	-3.50250*	.13265	.000	-3.7752	-3.2298
	Conc. 43.50	-2.50250*	.13265	.000	-2.7752	-2.2298
	Conc. 21.75	-2.50250*	.13265	.000	-2.7752	-2.2298
	Conc. 5.44	.00000	.13265	1.000	-.2727	.2727
	Conc. 2.72	.49750*	.13265	.001	.2248	.7702
	Conc. Nystatin	-10.50250*	.13265	.000	-10.7752	-10.2298
	Conc. Control	.49750*	.14328	.002	.2030	.7920
Conc. 5.44	Conc. 87.00	-7.25500*	.13265	.000	-7.5277	-6.9823
	Conc. 65.25	-3.50250*	.13265	.000	-3.7752	-3.2298
	Conc. 43.50	-2.50250*	.13265	.000	-2.7752	-2.2298
	Conc. 21.75	-2.50250*	.13265	.000	-2.7752	-2.2298
	Conc. 10.88	.00000	.13265	1.000	-.2727	.2727
	Conc. 2.72	.49750*	.13265	.001	.2248	.7702
	Conc. Nystatin	-10.50250*	.13265	.000	-10.7752	-10.2298
	Conc. Control	.49750*	.14328	.002	.2030	.7920
Conc. 2.72	Conc. 87.00	-7.75250*	.13265	.000	-8.0252	-7.4798
	Conc. 65.25	-4.00000*	.13265	.000	-4.2727	-3.7273
	Conc. 43.50	-3.00000*	.13265	.000	-3.2727	-2.7273
	Conc. 21.75	-3.00000*	.13265	.000	-3.2727	-2.7273
	Conc. 10.88	-.49750*	.13265	.001	-.7702	-.2248
	Conc. 5.44	-.49750*	.13265	.001	-.7702	-.2248
	Conc. Nystatin	-11.00000*	.13265	.000	-11.2727	-10.7273
	Conc. Control	.00000	.14328	1.000	-.2945	.2945
Conc. Nystatin	Conc. 87.00	3.24750*	.13265	.000	2.9748	3.5202
	Conc. 65.25	7.00000*	.13265	.000	6.7273	7.2727
	Conc. 43.50	8.00000*	.13265	.000	7.7273	8.2727
	Conc. 21.75	8.00000*	.13265	.000	7.7273	8.2727
	Conc. 10.88	10.50250*	.13265	.000	10.2298	10.7752
	Conc. 5.44	10.50250*	.13265	.000	10.2298	10.7752
	Conc. 2.72	11.00000*	.13265	.000	10.7273	11.2727
	Conc. Control	11.00000*	.14328	.000	10.7055	11.2945
Conc. Control	Conc. 87.00	-7.75250*	.14328	.000	-8.0470	-7.4580
	Conc. 65.25	-4.00000*	.14328	.000	-4.2945	-3.7055
	Conc. 43.50	-3.00000*	.14328	.000	-3.2945	-2.7055
	Conc. 21.75	-3.00000*	.14328	.000	-3.2945	-2.7055
	Conc. 10.88	-.49750*	.14328	.002	-.7920	-.2030
	Conc. 5.44	-.49750*	.14328	.002	-.7920	-.2030
	Conc. 2.72	.00000	.14328	1.000	-.2945	.2945
	Conc. Nystatin	-11.00000*	.14328	.000	-11.2945	-10.7055

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Day14

LSD

(I) NewConc	(J) NewConc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Conc. 87.00	Conc. 65.25	4.24750*	.16013	.000	3.9183	4.5767
	Conc. 43.50	4.50250*	.16013	.000	4.1733	4.8317
	Conc. 21.75	4.50250*	.16013	.000	4.1733	4.8317
	Conc. 10.88	6.50250*	.16013	.000	6.1733	6.8317
	Conc. 5.44	7.00000*	.16013	.000	6.6708	7.3292
	Conc. 2.72	7.00000*	.16013	.000	6.6708	7.3292
	Conc. Nystatin	-3.75250*	.16013	.000	-4.0817	-3.4233
	Conc. Control	7.00000*	.17296	.000	6.6445	7.3555
Conc. 65.25	Conc. 87.00	-4.24750*	.16013	.000	-4.5767	-3.9183
	Conc. 43.50	.25500	.16013	.123	-.0742	.5842
	Conc. 21.75	.25500	.16013	.123	-.0742	.5842
	Conc. 10.88	2.25500*	.16013	.000	1.9258	2.5842
	Conc. 5.44	2.75250*	.16013	.000	2.4233	3.0817
	Conc. 2.72	2.75250*	.16013	.000	2.4233	3.0817
	Conc. Nystatin	-8.00000*	.16013	.000	-8.3292	-7.6708
	Conc. Control	2.75250*	.17296	.000	2.3970	3.1080
Conc. 43.50	Conc. 87.00	-4.50250*	.16013	.000	-4.8317	-4.1733
	Conc. 65.25	-.25500	.16013	.123	-.5842	.0742
	Conc. 21.75	.00000	.16013	1.000	-.3292	.3292
	Conc. 10.88	2.00000*	.16013	.000	1.6708	2.3292
	Conc. 5.44	2.49750*	.16013	.000	2.1683	2.8267
	Conc. 2.72	2.49750*	.16013	.000	2.1683	2.8267
	Conc. Nystatin	-8.25500*	.16013	.000	-8.5842	-7.9258
	Conc. Control	2.49750*	.17296	.000	2.1420	2.8530
Conc. 21.75	Conc. 87.00	-4.50250*	.16013	.000	-4.8317	-4.1733
	Conc. 65.25	-.25500	.16013	.123	-.5842	.0742
	Conc. 43.50	.00000	.16013	1.000	-.3292	.3292
	Conc. 10.88	2.00000*	.16013	.000	1.6708	2.3292
	Conc. 5.44	2.49750*	.16013	.000	2.1683	2.8267
	Conc. 2.72	2.49750*	.16013	.000	2.1683	2.8267
	Conc. Nystatin	-8.25500*	.16013	.000	-8.5842	-7.9258
	Conc. Control	2.49750*	.17296	.000	2.1420	2.8530
Conc. 10.88	Conc. 87.00	-6.50250*	.16013	.000	-6.8317	-6.1733
	Conc. 65.25	-2.25500*	.16013	.000	-2.5842	-1.9258
	Conc. 43.50	-2.00000*	.16013	.000	-2.3292	-1.6708
	Conc. 21.75	-2.00000*	.16013	.000	-2.3292	-1.6708
	Conc. 5.44	.49750*	.16013	.005	.1683	.8267
	Conc. 2.72	.49750*	.16013	.005	.1683	.8267
	Conc. Nystatin	-10.25500*	.16013	.000	-10.5842	-9.9258
	Conc. Control	.49750*	.17296	.008	.1420	.8530
Conc. 5.44	Conc. 87.00	-7.00000*	.16013	.000	-7.3292	-6.6708
	Conc. 65.25	-2.75250*	.16013	.000	-3.0817	-2.4233
	Conc. 43.50	-2.49750*	.16013	.000	-2.8267	-2.1683
	Conc. 21.75	-2.49750*	.16013	.000	-2.8267	-2.1683
	Conc. 10.88	-.49750*	.16013	.005	-.8267	-.1683
	Conc. 2.72	.00000	.16013	1.000	-.3292	.3292
	Conc. Nystatin	-10.75250*	.16013	.000	-11.0817	-10.4233
	Conc. Control	.00000	.17296	1.000	-.3555	.3555
Conc. 2.72	Conc. 87.00	-7.00000*	.16013	.000	-7.3292	-6.6708
	Conc. 65.25	-2.75250*	.16013	.000	-3.0817	-2.4233

	Conc. 43.50	-2.49750*	.16013	.000	-2.8267	-2.1683
	Conc. 21.75	-2.49750*	.16013	.000	-2.8267	-2.1683
	Conc. 10.88	-.49750*	.16013	.005	-.8267	-.1683
	Conc. 5.44	.00000	.16013	1.000	-.3292	.3292
	Conc. Nystatin	-10.75250*	.16013	.000	-11.0817	-10.4233
	Conc. Control	.00000	.17296	1.000	-.3555	.3555
Conc. Nystatin	Conc. 87.00	3.75250*	.16013	.000	3.4233	4.0817
	Conc. 65.25	8.00000*	.16013	.000	7.6708	8.3292
	Conc. 43.50	8.25500*	.16013	.000	7.9258	8.5842
	Conc. 21.75	8.25500*	.16013	.000	7.9258	8.5842
	Conc. 10.88	10.25500*	.16013	.000	9.9258	10.5842
	Conc. 5.44	10.75250*	.16013	.000	10.4233	11.0817
	Conc. 2.72	10.75250*	.16013	.000	10.4233	11.0817
	Conc. Control	10.75250*	.17296	.000	10.3970	11.1080
Conc. Control	Conc. 87.00	-7.00000*	.17296	.000	-7.3555	-6.6445
	Conc. 65.25	-2.75250*	.17296	.000	-3.1080	-2.3970
	Conc. 43.50	-2.49750*	.17296	.000	-2.8530	-2.1420
	Conc. 21.75	-2.49750*	.17296	.000	-2.8530	-2.1420
	Conc. 10.88	-.49750*	.17296	.008	-.8530	-.1420
	Conc. 5.44	.00000	.17296	1.000	-.3555	.3555
	Conc. 2.72	.00000	.17296	1.000	-.3555	.3555
	Conc. Nystatin	-10.75250*	.17296	.000	-11.1080	-10.3970

*. The mean difference is significant at the 0.05 level.