ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF CHEMISTRY

PHYTOCHEMIAL INVESTIGATIONS AND BIOLOGICAL ACTIVITIES OF SMOKE SAMPLES COLLECTED FROM *OTOSTEGIA INTEGRIFOLIA, SILENE MACROSOLEN,* AND *ECHINOPS KEBERICHO* PLANTS

BY

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Advisor: Dr. Estifanos Ele

A Thesis Submitted to the Department of Chemistry Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Chemistry.

August, 2020
Addis Ababa

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August, 2020

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DECLARATION

I, the undersigned, declare that this Thesis entitled PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL ACTIVIITIES OF SMOKE COLLECTED FROM *OTOSTEGIA INTEGRIFOLIA* (ABYYSINIAN ROSE), *SILENE MACROSOLEN* (WOGERT) AND *ECHINOPS KEBERICHO* (KEBERICHO) PLANTS is my original work and has not been presented for any degree in any other university before and all sources of materials used for this thesis have been appropriately acknowledged.

Name: Tadesse Zewdu

Signature: __________

Date: _________________

This thesis has been submitted for examination with my approval as a university advisor.

Name: Estifanos Ele (PhD)

Signature: ___________________

Date and place of submission: Department of Chemistry Addis Ababa University, August 2020

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List of Abbreviations

AA	Ascorbic acid
ANOVA	Analysis of variance
BHA	Butylated Hydroxy Anisole
DCM	Dichloromethane
FRAP	Ferric reducing antioxidant power
GC-MS	GAS chromatograph mass spectrometry
HAT	Hydrogen atom transfer
NDGA	Nordihydroguaretic Acid
IPM	pest management programme
PG	Propyl Gallate
Ppm	Parts per million
SET	Single electron transfer
SD	Standard deviation
TBHQ	Tertiary Butyl Hydroquinone
TCA	Tirchloric acetic acid
UV	Ultraviolet visible

Abstract

PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL ACTIVITY OF SMOKE COLLECTED FROM *OTOSTEGIA INTEGRIFOLIA* (ABYSSINIAN ROSE), *SILENE MACROSOLEN (*WOGERT*)* AND *ECHINOPS KEBERICHO (*KEBERICHO) PLANTS

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The essential oils and smoke collected from leaves of *Otostegia integrifolia*, *S. macrosolen* and *E.kebericho* were investigated using gas chromatography mass spectrometry (GC-MS). Several compounds were identified in the essential oil of *Otostegia integrifolia*: They were Terpenoids (51.51%) which are monoterpene (12.12%), sesqui terpene (36.36%), di terpene (3.03%) and phenolic compounds (13.60%). From smoke trapped in hexane a group of compound such as phenolic (43.73%), alkane (21.88%), terpene (9.3%) were identified. In smoke trapped by methanol, alkenes (62.50%), and phenolic compounds were detected. From smoke of *S. macrosolen* trapped in hexane and methanol phenolic compounds 54.00% and 70.37% respectively were identified. From smoke of *E.kebericho* trapped in hexane and methanol phenolic compound 57.89% and 65.20% respectively were identified. The antioxidant activities were evaluated using reducing power (FRAP), by UV-Vis absorption spectroscopy. The results revealed that the *Silene macrosolon* exhibited the highest anti-oxidant activity compared to *Echinops kebercho* and *Otostegia integrifolia*. The antioxidant concentration in Hexane trapped smoke of all sample under this study was negligible. The insecticidal activity tests (both repellent and mortality) were conducted using cockroach as a test insect. The repellant activity of insect was tested by *O. integrifolia* leaves extract and, the recorded results of repellency at 60 minutes were 100% by hexane and 91.47% by methanol trapped smokes. The maximum mortality of cockroach at the maximum time by *E. kebericho, S. macrosolen* and *O. integrifolia* smoke extract were 100.00 , 40.00 , 13.2% respectively.

Keywords: Essential oil; *Otostegia integrifolia, Silene macrosolon*, *Echinops kebericho* and antioxidant, mortality rate

CHAPTER ONE

1. Introduction

1.1 Background

Plants provide important lifesaving medicines which are in use all over the world. Among estimated 250,000-400,000 plant species, only 6% have been studied for biological activity and 15% have been investigated phytochemically (Chaudhary et al., 2012). This indicates that there is a need for increased investigation of plant origin drugs. Investigating traditional medicinal plants use has also been used as a basis for the discovery of new lead compounds that are used for the development of modern drugs. About 95% of traditional medicine preparations in Ethiopia are mentioned to be of plant origin (Demissew S , 1998).

Otostegia integrifolia, (Abyssinian rose) whose family name is Lamiaceae, is found in large amount in Ethiopia, Eritrea and Yemen countries (Sabald et al, 2006). *Otostegia integrifolia* is a short tree which grows 2-4 m. *O. integrifolia* is used to repel fly at home. This plant has insecticidal properties (Tesso et al 2004). The roots are used for treating lung diseases (Fichtl and Adi, 1994).

Echinops kebericho (kebericho) which belongs to the family Asteraceae is a common medicinal plant in Ethiopia. Traditionally, this plant has been used in treating many diseases caused by microorganism or bacterial infection such as headache, malaria, and cough (Toma et al, 2015) and to repel insects

S. macrosolen (Wogert) which belongs to the family Caryophyllaceae mostly found in Debark, Wollo and entirely Ethiopian market for the purpose of medicinal application especially used for treatment of malaria (Abera T, 2019). The plants of the *O. integrifolia* is used to repel insects at homes and the flavor is used as perfume. The ceramic jars that are used to store alcohol and milk are being sterilized using smokes of *O. integrifolia* plant. Ethiopian mothers also use to smoke the house after giving birth.

O. integrifolia, S. macrosolen and E. kebericho burnt to fumigate homes as they have insecticidal activities. The present study is on the preliminary phyotochemical analysis, insecticidal activity and antioxidant activities of *O. integrifolia S. macrosolen* and *E. kebericho* plants.

1.2. Statement of the Problem

Herbal medical practitioners may not understand the scientific rationale behind healing power of plants they use, but they know from their day to day activities, cultures, trends of elders about the healing potential of medicinal plants. Since we have a better understanding today how human beings interact with nature, thus we are in a good position to investigate the healing power of medicinal plants. Medicinal plants mainly contain mixture of different chemical compounds that may act individually or in synergy to improve health. Therefore this research work is to investigate the chemistry of the plant parts and their chemical constituents including their biological activities.

1.3. Objectives of the Study

1.3.1 General Objective

The main objective of this research work is:

To investige the chemical compositions and biological activities of traditionally plants that are currently in use among Ethipians.

1.3.2 .Specific Objectives

The Specific objectives of this study are:

- to extract traditionally useful part of *O. integrifolia* using hydrodistilation
- to trap smoke generated by burning parts of *O. integrifolia*,

  S. macrosolen and *E. kebericho*
- to analylze the extracts by GC-MS for their phytochemical composition.
- to test the antioxidant activities of the plants extract.
- to test the pesticidal activities of the plants extract.

1.4. Significance of the Study

It has been claimed that more than 80% of the Ethiopian population rely on medicinal plants for treating various illnesses. Prior to this work, there are no research outputs that focus on the chemical compositions and biological activities of smokes of the plant species. This research finding will give an insight about the chemical composition of the plants parts that are used by human and their biological activities.

CHAPTER TWO

2. Literature Review

2.1. Medicinal Plants

The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal values. These medicinal plants are considered as a rich resource of ingredients which can be used in drug development and synthesis. Traditional plant remedies are the most important source of therapeutics for nearly 80% of the developing world population. The same is true in Ethiopia where medicinal plants play a significant role in supporting the country's primary healthcare system. About 95% the traditional medicine preparations in Ethiopia are describe to be of plant origin, investigating the traditional knowledge on medicinal plant use has also been used as a basis for the discovery of new compounds that are used for the development of modern drugs.

2.2. Phytochemicals of medicinal plants

Phytochemicals (from the Greek word phyto, meaning plant) are chemical compounds which provide health for humans and that protect plants from disease and damage (Gibsol et al 1998). These phytochemicals are located in different parts of the plants; a root, stem, bark, leave etc. Phytochemicals are of different types in their molecular structures and functional groups. They range from macro to micro molecules and non-polar to polar compounds. They are characterized by their respective physical and chemical properties.

Phytochemicals are isolated and characterized from different natural sources through different spectroscopic and chromatographic techniques.

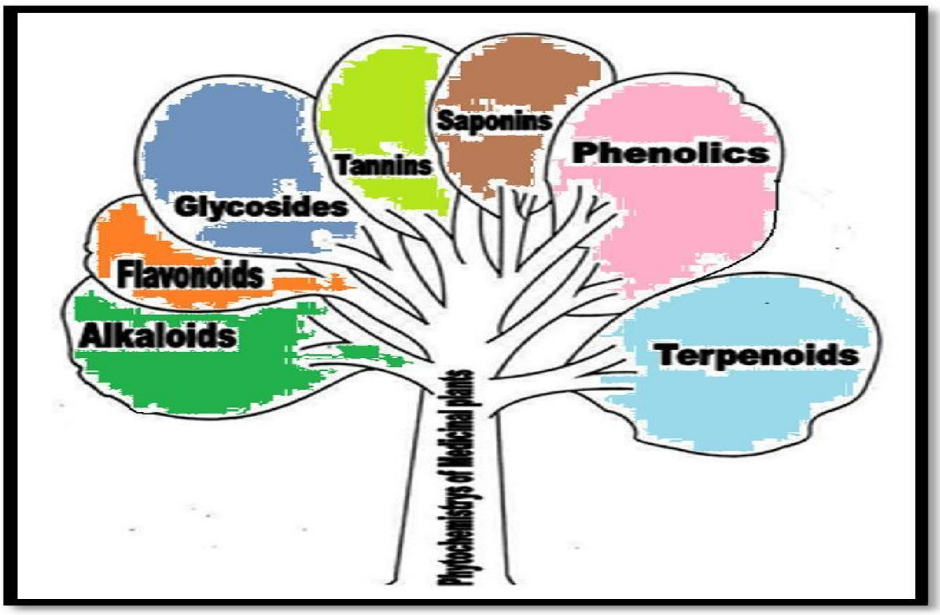


Figure 1. Phytochemicals of medicinal plants

2.2.1. The family Lamiaceae

The Lamiaceae is a commonly encountered family which is sub-shrubs or less often trees. When crushed they usually emit, mostly pleasant odours. Their stems are usually square and they contain abundant flowers which are quite attractive. Many members of the family are used as medicinal herbs, as sources of volatile oils and in some cases for the preparation of constituents of the volatile oils such as menthol (Evans, 2002; Sebald, 2006). One of the plants under this study belongs to this family.

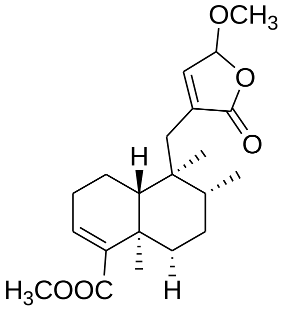
2.2.1.1. Biological activity of the genus *otostegia*

The *O.integrifolia* has insecticidal properties (Karunamoorthi *et al*., 2009). The roots are used for treating lung diseases (Tesso and Konig, 2004). The leaves are used for the treatment of malaria (Giday et al., 2007), whereas the leaves and steam part are used for treatment of stomachache (Teklehaymanot et al., 2007). *O. persica* is traditionally used for the treatment of malaria, fever and diabetes (Ayatollahi et al., 2009). In Saudi Arabia infusion of the flowering branches of *O. fruticosa* is used as a remedy for sun-stroke (Rahman et al., 2004), while in Eritrea the leaf and stem of *O. fruticosa* is used for arthritis, tonsillitis and gynaecological problems (Andemariam, 2010).

2.2.1.2. Phytochemistry of the genus *otostegia*

Chemical compounds such as terpenes, flavonoids, iridoids are constituents of the genus *Otostegia*. Terpenes are the main constituent: labdane diterpenes otostegin A (1), otostegin B (2) (Al-Musayeib et al. 2000), whereas the aerial part of *O. persica* yielded clerodane type (3-5) and the tetracyclic diterpene, limbatenolide C (6) (Ayatollahi et al., 2009). From the leaf essential oil of *O. fruticosa*, thymol (7) and γ-terpinene (8) were isolated (Aboutabl et al., 1995). The flavonoids were isolated isolated from *O. persica*. The compound kaempferol (9) and quercetin (10) were isolated from the active methanolic extract, whereas 3,7-dihydroxy-4'',6,8-trimethoxyflavone (11) (Yassa et al., 2005). Iridoids are monoterpene lactones, which can occur as glycoside, with one or more sugar molecule attached or as aglycones (no sugar attachment). The iridoid glucoside 8-O-acetylharpagide (12) was isolated from the aerial parts of *O. fruticosa* (Al-Musayeib et al, 2000)

1 2



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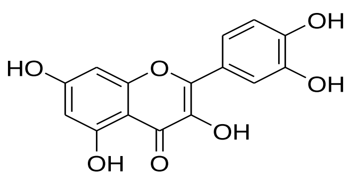
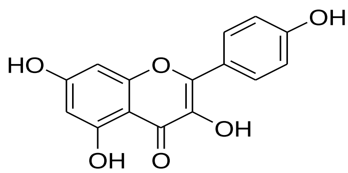
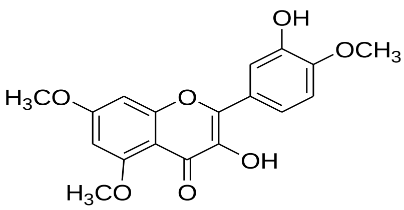






9 10 11

12

Figure 2. Phytochemicals of the genus *otostegia*

2.2.1.3. Phytochemistry of *O. integrifolia*

From the dried leaves of the plant 40 compounds have been detected by hydrodistilation. The distillate was shown to be composed of monoterpenes, sesquiterpenes, diterpenes and their derivatives. *trans*-Sabinol (13), β-cyclocitral (14), dihydroedulan (15), theaspirane (16) and (+)-axinyssene (17) (prenylbisabolane diterpenes) were the major components. The prefuranic and furanic labdane diterpenoids otostegindiol (18) and preotostegindiol (19) along with pentatriacontane and stigmasterol have also been isolated from the dried chloroform extract of the leaves of *O. integrifolia* (Tesso et al 2004).

13 14 15 16

17 18 19

Figure 3**.** Compounds isolated from *O. integrifolia*

2.2.2. The family Asteraceae

The Asteraceae is known by the aggregated flowers often occurring at the ends of branches or stems. The technical term for such a group of flowers is capitulum. Aggregation of flowers occurs on usually flat surface called receptacle (Chadwick et al., 2013).

2.2.2.1. Biological activity of the *genus Echinops*

The root of *Echinops* are used to treat ailments that are caused by infection such as, fever, toothache and earache *E. kebericho* in Ethiopia has been used as a mosquito repellant (Mesfin et al.)

2.2.2.2. Phytochemistry of genus *Echinops*

The genus has compound such as phenolic, thiophenes and terpenoids; sesqui- and triterpenoids. Most terpenoids exist in various forms including lactones, esters, and sterols along with their glycosides (Arroo et al., 1997)

2.2.2.3. Phytochemistry of the *E. kebericho*

The principal components of this plant are monoterpenes oxygenated, sesquiterpenes , Sesquiterpene hydrocarbons ,sesquiterpenes oxygenated, sesquiterpene lactones, aliphatic hydrocarbons, Aromatic hydrocarbons, carotenoid-derived hydrocarbons oxygenated structures (Chaudhuri et al, 1987).

2.2.3. The family Caryophyllaceae

This family consists mainly of herbaceous plants and, more rarely, small shrubs or subshrubs. The flowers have free petals, with each petal consisting of a usually visible limb (Popov *et al.* 1999)

2.2.3.1. Biological activity of the *genus* S*ilene*

Zibareva et al, was reported that the ecdysteroid-containing extract of *S. viridiflora* exerted antitumor activity. Popov *et al.* studied the effects of the polysaccharides from plants and callus of *S. vulgaris* (silenans) on uptake capacity and myeloperoxidase. Methanol extracts from three *Silene* species from Iran (*S. gynodioca*, *S. spergulifolia* and *S. swertiifolia*) were screened for their possible antioxidant activities by three complementary test systems, namely DPPH free radical-scavenging, metal chelating activity and β-carotene/linoleic acid oxidation (Karamian, R, 2013).

2.2.3. 2. Phytochemistry of the *genus* S*ilene*

Phytochemical investigations of the genus *Silene* have led to the isolation of several phytoecdysteroids, triterpene saponins, terpenoids, benzenoids, flavonoids, anthocyanidins, *N*-containing compounds, sterols, and vitamins (Popov *et al 1999*)

2.3. Antioxidants

Antioxidants are substances that reduce the number of oxidation-initiating free radicals, which helps to prevent or delay oxidation reactions, such as lipid oxidation. Antioxidant compounds generally possess one or more hydroxylated aromatic or phenolic rings, which contribute to their antioxidant activity. Phenolic antioxidants can quench and terminate the free radicals without being transformed to new free radicals in the system. The hydroxyl groups on the phenolic ring contribute to the antioxidant function by donating electrons to eliminate free radicals in a system. As the phenolic radical intermediates are relatively stable due to resonance occurring on the phenolic ring, phenolic compounds may also suppress reactive oxygen and nitrogen species formation by deactivating related enzymes and chelating free radical-producing metal ions. The number of free hydroxyl groups on phenolic rings is correlated to the antioxidant activity of a phenolic compound. Antioxidants are classified into two according to their sources that possess the ability to reduce the rate of oxidation reactions.

a) Natural antioxidants:are compounds that are produced by natural organisms. They include,Tocopherols, Nordihydroguaretic Acid (NDGA), Sesamol and Gossypol

b) Synthetic antioxidants: are compounds that are synthesized in the lab. They include Butylated hydroxy Anisole (BHA), Butylated hydroxy Toluene (BHT), Propyl Gallate (PG) and Tertiary Butyl hydroquinone (TBHQ) (Kemp, et al 1991). Many scientists have concerns about safety of synthetic antioxidants because they have recently been shown to cause health problems such as liver damage, due to their toxicity and carcinogenicity. Therefore, the development of safer antioxidants from natural sources has increased.

***2.3.1.* Plant origin antioxidants**

Plants are an important source of antioxidants. Many of these medicinal plants are indeed good sources of phytochemicals that possess antioxidant activities. Some typical examples of common ingredients that have been used in ethnic foods are tamarind, cardamom, lemon grass, and galangal basil. These spices or herbs have been shown to contain antioxidants. In nature there are a wide variety of naturally occurring antioxidants which are different in there composition, physical and chemical properties, mechanism and site of action Halliwell, B. (1996). There is a long list of antioxidant plants of which, some have been discussed in Table.1 (Javanmardi, et al 2003).

Table 1. Plants used as the source of reducing power

NO	Scientific name	Common name	Family	Partes of plant
1	*Azadirachtaindica*	Neam	Meliaceae	Leaf
2	*Beta vuulgaris*	Beet root	Amaranthaceae	Root
3	*Camellia sinensis*	Green tea	Theaceae	Green tea
4	*Daucuscarota*	Carrot	Apiaceae	Root

5	*Zingiber officinale*	Ginger	Zigiberaceae	Rhizome
6	*Solanum tuberosum*	Potato	Solanaceae	Tuber

2.3.2. Antioxidant capacity assays activity

Antioxidants can deactivate radicals by two major mechanisms, HAT and SET.

HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation (AH, any H donor).

$$X^{\bullet} + AH \longrightarrow XH + A^{\bullet}$$

Scheme 1: HAT mechanism of antioxidant action

The presence of reducing agents, including metals, is a complication in HAT assays and can lead to erroneously high apparent reactivity (Wright, et al. 2001).

SET-based methods refer to the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals (Wright, et al. 2001).

$$R^{\bullet} + ArOH \longrightarrow R^{-} + ArOH^{+\bullet}$$

Scheme 2: SET mechanism of antioxidant action

2.3.3. Application of antioxidants

Antioxidants have wide application as these are used as additives substances in fats and oils and in sustenance handling businesses to forestall nourishment deterioration. These are added to nourishment which contains unsaturated fats to make them last more and to keep them from turning malodorous under oxidative anxiety, and plant extract (tea and grape seed) contain cell antioxidants segments accordingly giving antioxidant properties to the compound.

2.4. Insecticides

Insecticides are chemicals that control insects. These agents may negatively affect people's health and natural environment when used in inappropriate amount. They have different mode of actions. Not all insecticides are equally effective to different pests. This makes some insecticides more specific to certain species of insects and of course some have broad spectrum. Besides, frequent use of the same chemicals can lead to development of resistance of insects to these insecticides (Ishan Y. 2018)

2.4.1. Plant origin insecticides

Historically the plant materials have been in use longer than any other group (Kemp, et al 1991). Pyrethrum is now the only classical botanical of significance in use. Natural insecticides include *Azadirachta indica* (Margosa/Neem), *Boenighausenia albiflora, Peganum harmala,* Derris (rotenone) and Chrysanthemum (pyrethrum). Some newer plant-derived insecticides that have come into use are referred to as florals or scented plant chemicals and include, among others, limonene, cinnamaldehyde and eugenol. In addition, there is azadirachtin from the neem.

2.4.2. Application of insecticide

Pesticides are the biological substances that are used to control insects and pathogens such as viruses, bacteria, fungi, protozoa and mites. Insecticides are type of pesticide that specifically target and kill insects. Some insecticides have been shown to have no negative impacts on plants, mammals, birds, fish or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall integrated pest management programme (IPM) (Ishan Y, 2018).

CHAPTER THREE

3. Experimental

3.1. Materials and Methods

3.1.1. Materials

Equipment /Apparatus/ used to conduct this study were Suction flasks, Rubber tube, stove, flask, rotary evaporator (buchi Rota Vapor R-200, switzerland) , Oven, balance, UV-Visible spectrometer (Model UV-T60 spectrophotometer), GC-MS (Model: GC-7820A, Agilent

Technologies; Detector-5977EMSD, USA) funnel, Tong, Measuring cylinder, Beakers, Stopper and Test tubes. Micropipettes Bunsen burner, centrifuge and petridish

3.1.2. Chemicals and Reagents

All chemicals, reagents and solvents such as ascorbic acid (standard)**,** phosphate buffer solution (NaH_2PO_4, and Na_2HPO_4), potassium ferricyanide, trichloric acetic acid, iron (III). chloride, hydrochloric acid. sodium hydroxide sodium sulphate and hexane, methanol, dichloromethane, used in this research are analytical grade.

3.1.3. Plant Material

The plant materials *O. integrifolia*, *S. macrosolen*, *E. kebericho* used in this study was collected Merkato.

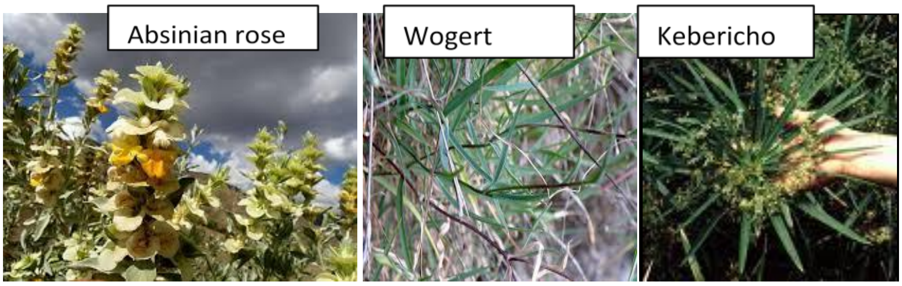


Figure 4. *O. integrifolia S. macrosolon* and *E. kebericho* Plants

3.2. Methods of extraction

3.2.1. Essential oils extraction

Essential oil of the Abyssinian rose was obtained by hydrodistilation method. A fresh leaves (1500g) was divided in to three portions and transferred to a round bottomed flask containing water, 2000 mL and the apparatus was connected to the heater. Hydrodistilation was continued for 4 hrs after initial boiling. The yields are reported as v/*w*, (mL/kg) .

Figure 5. Hydrodistilation set up

3.2.2. Smoke Collection

The plant materials were allowed to dry first and 300 g was weighed to collect smokes for analysis. The plant material was allowed to burn using an electrical stove. Especial air inlet was designed to let air in to facilitate continuous burring of the plant materials. The smokes were trapped by using the inverted funnel fitted with a rubber tube which in turn is connected to a sunction flask containing methanol (100 mL) which sequentially connected to another sunction flask containing 100 mL of hexane as shown in Figure 6 below. The side arm of the second sunction flak containing hexane was connected to a water aspirator so as to create a small amount of vacuum that pulls smoke all the way to the flasks.

Figure 6. Set up of smoke trapping system

The different solvent fractions were concentrated under reduced pressure using rotary evaporator (Buchi Rota Vapor R-200, Switzerland) at 30-35^{o}c and stored in oven at 4^{o} C.

Figure 7. Rotary evaporator

3.3. Sample preparation

3.3.1. Standards Preparation

A 0.0176 mg/mL ascorbic acid standard solution was prepared as follow: A 0.0088 g ascorbic acid was dissolved with distilled water in 500 mL volumetric flask as a stock solution. From this (0.0176 mg/mL concentration of stock), 1 mL, 5 mL, 10 mL, 20 mL, 40 mL and 60 mL were taken and diluted to 100 mL with distilled water to get solution with concentration of, 0.176 ,0.88 , 1.76, 3.52 ,7.04 and 10.56 μg/mL respectively.

3.3.2. Reagent Preparation for Antioxidant Test

To prepare, 2 M stock solution 34.386 g of NaH_2PO_4 was dissolved in 100 mL of water. Similarly a 2M stock solution of Na_2HPO_4 was prepared by dissolving 27.218gram of Na_2HPO_4 in 100 mL distilled water. Using the standard reaction for the preparation of phosphate buffer solution, 38.1 mL NaH_2PO_4 and 61.9 mL Na_2HPO_4 stock solutions were mixed. Finally, the pH of phosphate buffer solution was adjusted using HCl and NaOH. Then a standard solution of 1% W/V potassium hexancyano ferrate was prepared by adding 1 gram of K_3FeCN_6 in 100 mL volumetric flask, then distilled water was added till the mark. 10 % W/V trichroacetic acid was prepared by dissolving 10 gram trichloroacetic acid in 100 mL distilled water. 0.1 % ferric chloride solution was freshly prepared by dissolving 1 gram $FeCl_3$ with 100 mL distilled water.

3.3.3. Sample Preparation for Antioxidant Tests

Samples were prepared following the procedure designed by our group. A 0.3 g smoke trap in methanol was dissolved in 2 mL methanol, then from this 0.5 mL was taken and added to 48 mL of methanol, and mixed with 0.2 M buffer solution of pH = 6.6 and 2.5 mL of 1% of potassium ferric cyanide. Then the mixture was heated by using ware bath at 50 ^{0}C for 20 minutes. Then 2.5 mL TCA (10%) was added to the mixture prepared above and centrifuged at 300 rpm for 10 min. Then the supernatant part (2.5 mL) was taken and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). The mixture was mixed well and allowed to stand for about 10 min at room temperature. Three different concentrations were prepared by taking 500, 1000 and 1500 µL from the stoke solution and diluted with distilled water to get 4 mL final solution. The reaction mixtures were kept for 10 min at room temperature and analyzed by UV-Vis spectrophotometer. Measurement was taken at 700 nm. Distilled water was used as a blank solution. The concentration of the antioxidants was obtained from the calibration curve constructed using ascorbic acid.

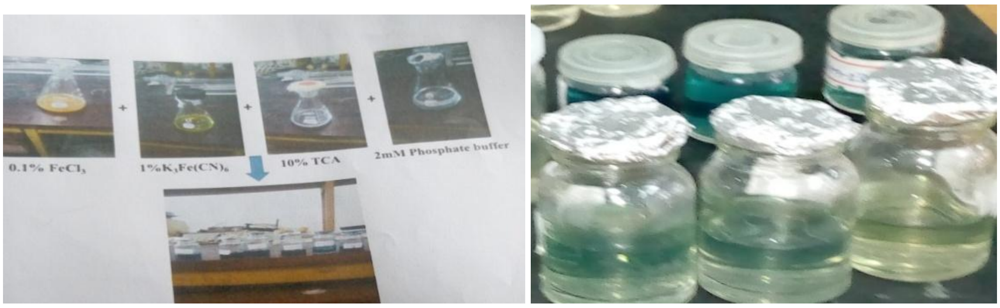


Figure 8**.** Preparation of sample for antioxidant tests

3.3.4. Sample Preparation for GC-MS analysis

The essential oils extracted were dried using sodium sulphate. The samples were diluted using dichloromethane to get a final concentration of 20 ppm.

For the smoke trapped in methanol and hexane, 0.2 mg of each sample was dissolved in 10 mL methanol or hexane to get 0.02 mg/mL final concentrations. All the samples were analyzed right after reparation to avoid unnecessary evaporations of solvents

3.4. Insecticidal Test

3.4.1. Preparation of Samples for repellent Tests

The smoke extracts of the samples of *O .integrifolia* leaves were compared with control samples during the test for repellency. The insecticidal (repellent) was conducted using Hexane and Methanol trapped smoke. In each case 10 mg of the sample was placed on one side of the set up and control (water) was placed on the other side. For one round experiment

10 cockroaches were released from the middle to the horizontal tube. The behaviors of the insects were followed carefully over 60 min by recording data for every 10 min. Experiments were conducted in triplicates and mean value was used for the analysis.

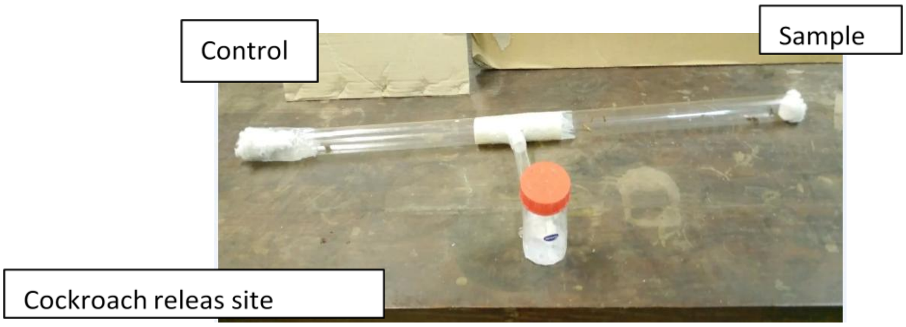


Figure 9. The T-tube for insecticidal activity test

3.4.2. Preparation of sample for mortality tests

The smoke extracts of the samples were compared with the control to check the mortality rate of the experimental insects. Petri-dishes were lined with a filter paper and 1 mL of sample was added. Using a 1.5 g solid sample, a stoke solution of 20 % was prepared and then the stock solution was diluted with water to get 1.25, 2.5, 5.0 and 10.0 % solutions. Five experimental insect were placed in a Petri dish and then the mortality rate data was recorded every three hours from 3 to 48 hours.

Figure 10. Mortality test using Petri-dish

CHAPTER FOUR

4. Results and Discussion

In this research three plant species which their parts are used traditionally for medicinal purposes have been investigated. Essential oil of one of the plants has been extracted by using hydro distillation technique since the plants are used in the form of smoke, smoke of each plant species was trapped by using two different solvents with different polarities. Results and their implications are discussed below.

4.1. The amount essential oil obtained and smoke trapped

Table 2**.**The amount of essential oil obtained by hydro-distillation and smoke trapped.

Plants	Techniques	Solvent	Yields		
			Leaves	Leaves	Root
O. integrifolia	Hydrodistillation	Water	13.5 mL/kg		
	Smoke trapping	Methanol		12.90 mg/mL	
	Smoke trapping	Hexane		12.03 mg/mL	
S. macrosolon	Smoke trapping	Methanol			7.670 mg/mL
	Smoke trapping	Hexane			3.980 mg/mL
E. kebercho	Smoke trapping	Methanol			6.57 mg/mL
	Smoke trapping	Hexane			3.206 mg/Ml

4.2. Calibration Curve

Aqueous solutions of ascorbic acid standard at different concentrations were used to construct the calibration curve and the absorbance of ascorbic acid was recorded. A graph of absorbance *versus* concentration was drawn in which a straight line with an equation, $y = 0.0475x + 0.2144$ and a linear regression coefficient (R^2) of 0.9946 was obtained (Figure 11). Thus, the antioxidant activities *of O. integrifolia, S. macrosolon a*nd *E. kebercho* relate to their reducing ability of the ferric cyanide complex to the ferrous were measured based on their concentrations.

Table 3. Absorbance vs. concentration of Ascorbic acid

Concentration µg/mL	Average absorbance at 200 nm mean± standard devotion
0.176	0.24±0.005
0.88	0.26±0.0104
1.76	0.29±0.0087
3.52	0.36±0.0128
7.04	0.55±0.0128
10.56	0.72±0.013

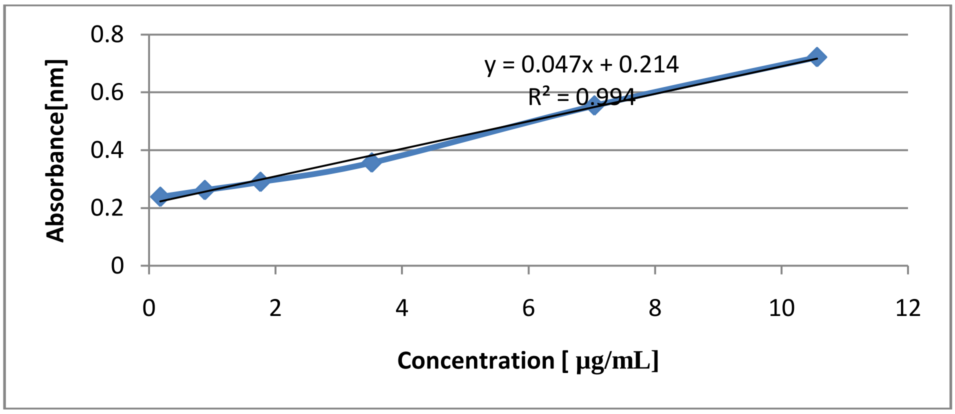


Figure 11. Linear regression relationships between absorbance and AA concentration

Where y = absorbance obtained at 200 nm and x = concentration of ascorbic acid. For the samples analyzed absorbance was measured at 700 nm and the concentrations were calculated based on the calibration curve. Concentrations of the antioxidants were compared with that of the ascorbic acid.

4.3. Antioxidant result

Table 4. Average absorbance *vs.* antioxidant concentration of plants

Species	Average absorbance (700nm)			Conc. calculated (µ g / mL)		
	A_1	A_2	A_3	C_1	C_2	C_3
O.integrifolia	0.76±0.19	0.58±0.18	0.36±1.23	11.50±0.19	7.66±0.13	3.06±0.23
S.macrosolen	2.31±0.32	1.33± 0.12	1.03±0.24	43.97± 0.32	23.46±0.11	17.11±0.24
E. kebericho	2.26±0.29	1.36±0.29	0.75±0.07	43.04±0.18	24.22±0.24	10.53±0.24

Where $A_{1=}$ average absorbance of the stoke solution

A_2 = average absorbance first diluted concentration

A_3 = average absorbance second diluted concentration

C_1, C_2 , C_3 = concentration calculated from standard calibration curve.

A calibration curve was constructed to determine the antioxidant activity using the standards.

$y = bx + c$ ------------------ (1)

Where y is the absorbance, x is the concentration of the standard in µg/mol, c is the y-intercept and b is the slope, then the concentration of the samples in (µg/mol) is obtained from the equation of the calibration curves as:

$x = y\text{-}c / b$ ----------------------- (2)

Table 5. Concentration of sample vs calculated antioxidant concentration

Sample extracted	Concentration (µg/mL)	Average absorbance at 700 nm	Cal. Concentration of Antioxidant (µg/mL)	% cal. Concentration of antioxidant
Otostegia integrifolia	76.10	0.36 ±1.23	3.60± 0.23	4.73
	152.19	0.58 ± 0.18	7.66±0.13	5.03
	228.29	0.76 ± 0.19	11.51±0.19	5.04
Silene macrosolon	76.10	1.03 ±0.34	17.11± 0.24	11.24
	152.19	1.33 ± 0.12	23.46± 0.11	15.41
	228.29	2.31±0.32	43.97±0.32	19.26
Echinops kebercho	76.19	0.75±0.07	10.55±0.24	13.86
	152.19	1.36± 0.29	24.22±0.24	15.91
	228.29	2.26 ± 0.29	43.38± 0.18	19.00

As the Table indicates, the percent of antioxidant increase when the concentration of sample and absorbance increases. The maximum concentration of antioxidant compounds was measured for *Silene macrosolon* which is 43.97 ± 0.32 µg/mL and the minimum concentration measured was 3.60 ± 0.23 for *Otostegia integrifolia*,

The percent antioxidant nature of the two plants, *Silene macrosolon* and *Echinops kebercho* are almost similar. However, the level of antioxidant of *Otostegia integrifolia* is three to four folds less that the two. From the result above one can conclude that *Otostegia integrifolia* use as antioxidant is not scientifically supported.

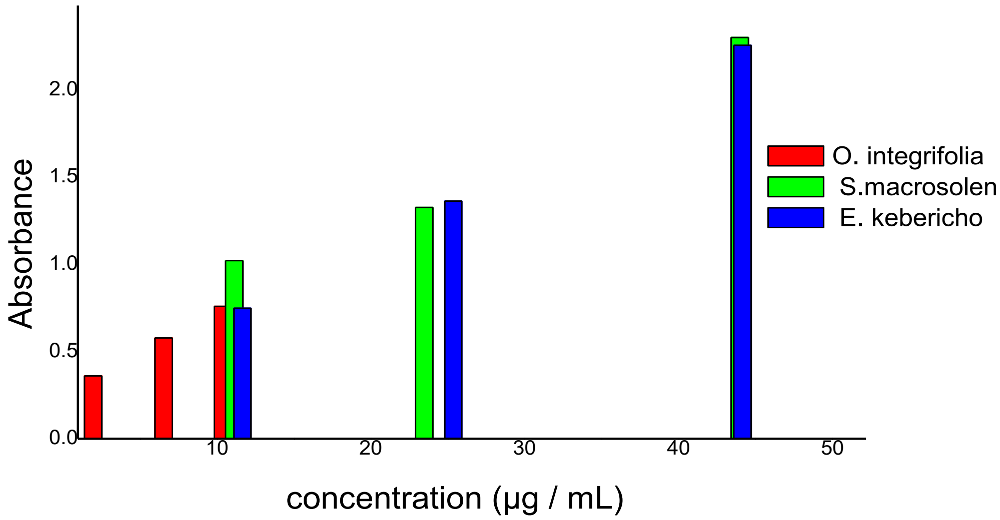


Figure 12.The concentration of antioxidant compounds

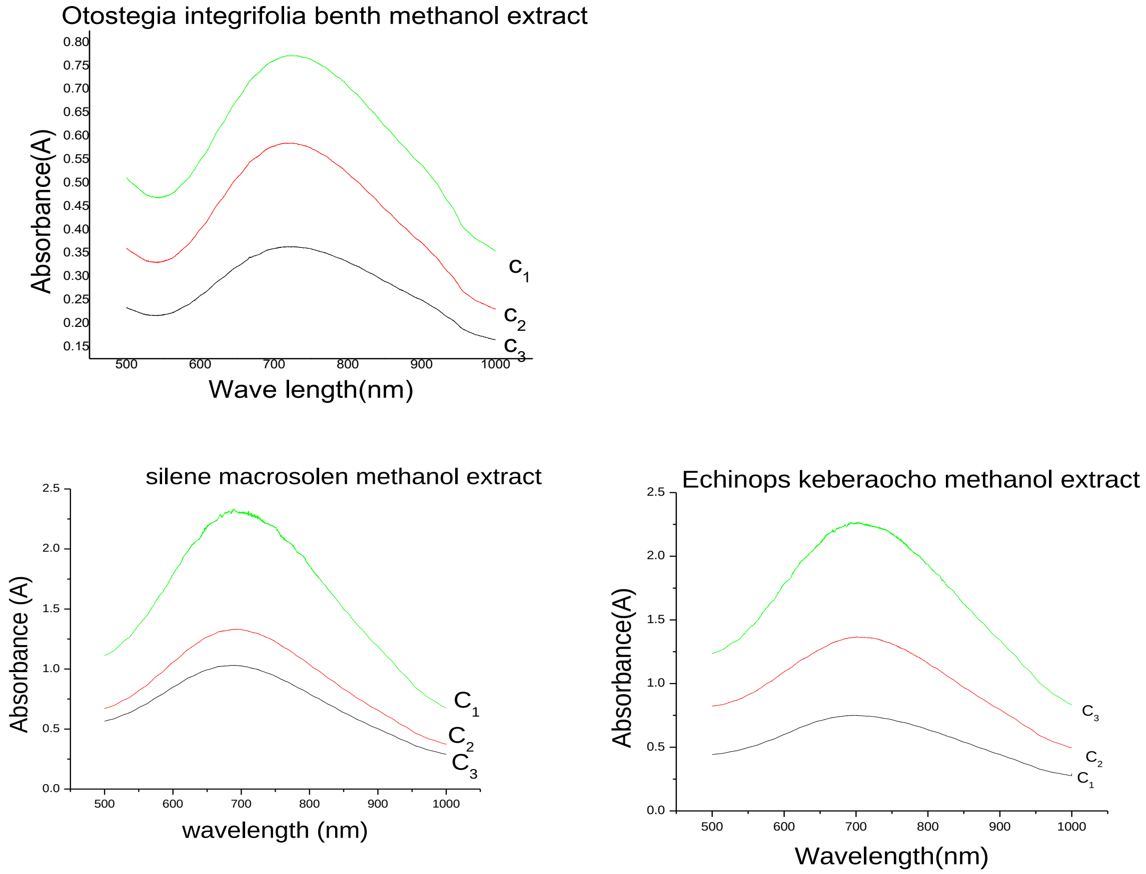


Figure 13.UV- Vis Absorbance samples trapped in methanol

Figure 13 shows that absorbance increases as concentration of the samples increase.

$K[Fe[III][CN]_6]_3$ + Ph-OH → $K_X(Fe_n^{3+}[Fe^{2+}(CN)_6]^{3+})$ + $Ph\text{-}O^-$ + H^+

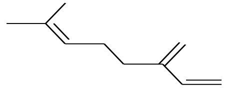
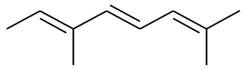
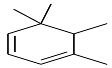
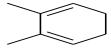
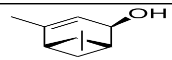
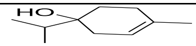
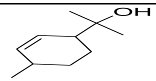
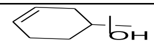
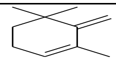
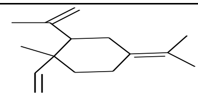
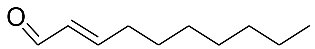
4.4. Characterization of samples using GC-MS

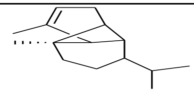
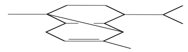
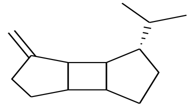
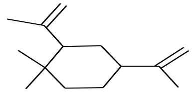
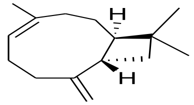
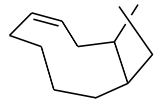
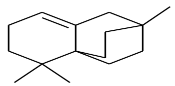
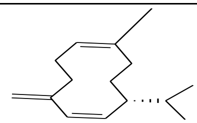
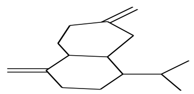
GC-MS, was used to identify the type and compounds in the essential oil and smoke samples. Compounds with the quality greater than 80% were subjected to analysis with the help of NIST Library 2014 mass spectral .Based on the information from the GC chromatogram, retention index was calculated for all the compounds by comparing with series of hydrocarbons analyzed using the same conditions.

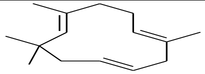
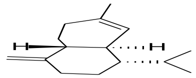
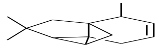
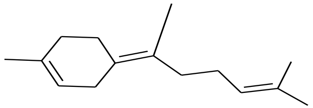
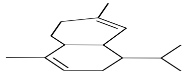
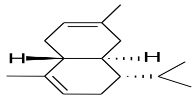
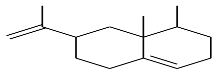
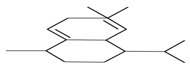
$$I=100n + 100\left(\frac{Rt(unknown) - Rt(n)}{Rt(n+1) - Rt(n)}\right)$$

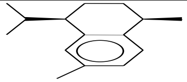
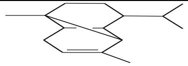
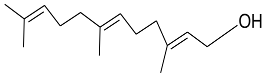
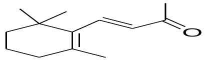
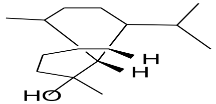
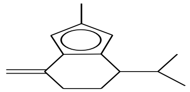
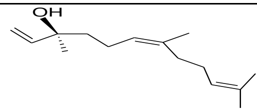
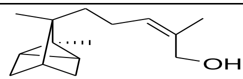
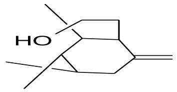
I is the retention index of the analyte, n is the number of carbon atom in the smaller alkane, Rt(unknown) the relation time of analyte , $Rt(n+1)$ and $Rt(n)$ the retention time of the reference n-Alkane

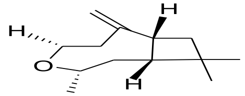
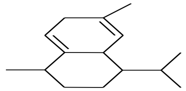
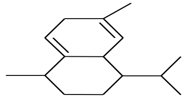
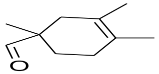
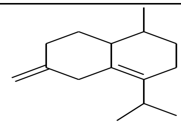
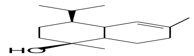
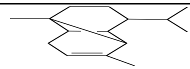
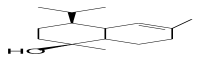
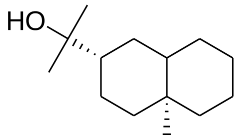
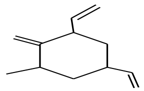
Table 6. GC-MS analysis result of essential oil *O. integrifolia*

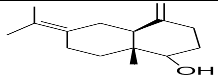
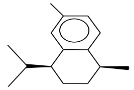
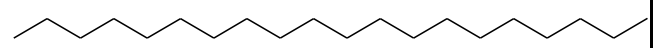
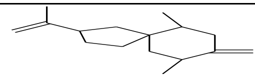
PK	RT	Area pct	Name of the compound	Formula	Structure	Q	I
1	6.18	0.10	Myrcene	$C_{10}H_{16}$		90	1019.74
2	7.77	0.13	2,6-dimethyl-2,4,6-Octatriene	$C_{10}H_{16}$		96	1124.66
3	8.53	2.48	1,5,5,6-tetramethyl-1,3-Cyclohexadiene	$C_{10}H_{16}$		81	1175.33
4	9.41	0.04	2,3-Dimethyl-cyclohexa-1,3-diene	C_8H_{12}		92	1234.00
5	9.78	0.08	Verbenol	$C_{10}H_{16}O$		95	1258.67
6	9.93	0.20	Terpinen-4-ol	$C_{10}H_{18}O$		96	1268.66
7	10.17	0.05	p-Mentha-1,5-dien-8-ol	$C_{10}H_{16}O$		80	1284.66
8	10.37	0.15	Terpineol	$C_{10}H_{18}O$		91	1298.00
9	11.03	0.08	2,6,10,10-tetramethyl-1-Oxaspiro[4.5]dec-6-ene	$C_{15}H_{22}O$		91	1348.46
10	11.15	0.05	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$		94	1357.69
11	11.32	0.66	Elemene isomer	$C_{15}H_{24}$		87	1370.76
12	11.47	0.01	(E)-2-Decenal,	$C_{10}H_{18}O$		89	1382.30

13	11.69	0.02	alpha a.-ylangene	$C_{15}H_{24}$		99	1399.23
14	11.76	0.35	alpha.-Copaene	$C_{15}H_{24}$		99	1401.58
15	11.99	0.36	beta.-Bourbonene	$C_{15}H_{24}$		99	1419.84
16	12.18	0.25	1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- cyclohexane	$C_{15}H_{24}$		96	1434.92
17	12.38	0.08	4,11,11-trimethyl- 8-methylene-,[1R-(1R*,4Z,9S*)]- Bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$		99	1450.07
18	12.68	7.21	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	$C_{15}H_{24}$		60	1474.60
19	12.80	0.11	1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalene	$C_{15}H_{24}$		81	1484.41
20	12.87	0.64	(S,1Z,6Z)-8-Isopropyl-1-methyl-5- methylenecyclodeca-1,6-diene	$C_{15}H_{24}$		99	1489.68
21	13.05	0.65	Decahydro-1,6-bis(methylene)-4-(1-methylethyl)-(4.alpha.,4a.alpha.,8a.alpha.)- Naphthalene	$C_{15}H_{28}$		99	1502.50

22	13.14	2.16	Humulene	$C_{15}H_{28}$		81	1510.00
23	13.23	1.36	1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha)- Naphthalene	$C_{15}H_{24}$		94	1517.50
24	13.38	0.91	gamma.-neoclovene	$C_{15}H_{24}$		91	1530.00
25	13.58	0.34	(Z)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)cyclohex-1- ene	$C_{15}H_{24}$		95	1546.66
26	13.68	0.69	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- Naphthalene,	$C_{15}H_{24}$		95	1554.09
27	13.77	0.86	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-naphthalene,	$C_{15}H_{24}$		94	1562.50
28	13.86	0.08	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-naphthalene,	C15H24		93	1570.00
29	13.98	0.09	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- naphthalene	$C_{15}H_{24}$		96	1575.00

30	14.09	0.05	cis-Calamenene	C15H22		95	1589.17
31	14.22	0.17	Copaene	C15H24		90	1600.00
32	14.45	0.03	3,7,11-trimethyl-, (Z,E)-2,6,10-Dodecatrien-1-ol,	C15H26O		87	1619.88
33	14.49	0.34	2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butene	C13H20O		95	1623.48
34	14.56	0.10	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethylocta hydro- 1H-cyclopenta-[1,3]cyclopropa[1,2]benzen-3-ol	$C_{15}H_{26}0$		98	1629.57
35	14.77	0.04	4-Isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene	$C_{15}H_{20}$		84	1647.82
36	14.91	0.33	Nerolidol	$C_{15}H_{26}O$		83	1660.00
37	15.43	0.20	cis,alpha.- Santalol	$C_{15}H_{22}O$		83	1705.50
38	15.49	0.32	1,7- trimethyl- 4-methylene-, [1ar-(1a.alpha, 4 .a.alpha -7.beta.,7a.beta.,7b.alpha.)]-Cycloprop[e]azule-7-ol, decahydro1	$C_{15}H_{24}O$		99	1711.00

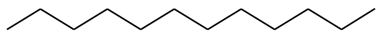
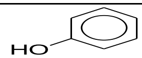
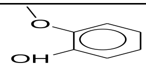
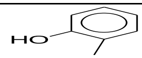
39	15.61	1.25	Caryophyllene oxide	$C_{15}H_{24}O$		94	1722.02
40	15.73	0.23	1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4- (1-methylethyl)- naphthalene,	$C_{15}H_{24}$		95	1733.03
41	15.79	0.16	6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)- cyclohexene	$C_{15}H_{28}$		93	1738.53
42	15.91	0.29	3,4-dimethyl-3-cyclohexen-1-carboxaldehyde	$C_{10}H_{16}O$		97	1749.54
43	16.01	0.50	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	$C_{15}H_{24}$		95	1758.72
44	16.06	0.38	tau.-Muurolol	$C_{15}H_{24}$		97	1763.30
45	16.15	0.54	Copaene	$C_{15}H_{24}$		98	1771.56
46	16.29	0.54	tau.-Muurolol	$C_{15}H_{24}$		95	1784.40
47	16.39	1.26	decahydro-.alpha,alpha.,4a-trimethyl-8-methylene-, [2R- (2.alpha , 4a.alpha.,8a.beta.)]-2-naphthalenemethanol	$C_{15}H_{26}O$		99	1793.58
48	16.81	0.28	1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)- Cyclohexane,	$C_{12}H_{18}$		90	1828.69

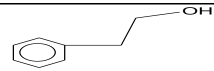
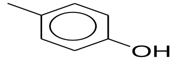
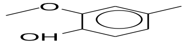
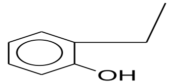
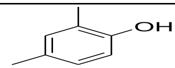
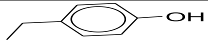
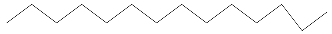
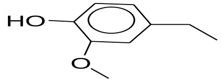
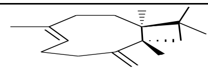
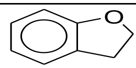
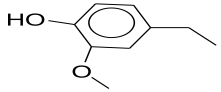
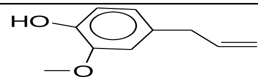
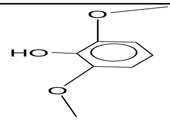
49	16.99	0.17	Eudesma-4(15),7-dien-1.beta. –ol	$C_{15}H_{24}$		83	1843.44
50	17.16	0.29	(1S,3aR,4R,8R,8aS)-1-Isopropyl-3a-methyl-7-methylenedecahydro-4,8-epithioazulene	$C_{15}H_{26}$		93	1857.38
51	17.58	0.09	Eicosane	$C_{20}H_{42}$		97	1891.80
52	17.87	0.10	Solavetivone	$C_{15}H_{24}$		86	1914.07
53	18.92	0.09	Heneicosane	$C_{21}H_{44}$		93	1991.85
54	20.47	0.16	Hexacosane	$C_{26}H_{54}$		99	2094.11

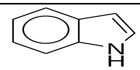
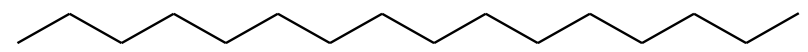
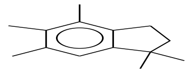
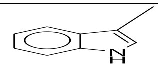
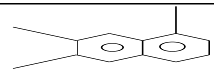
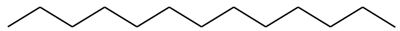
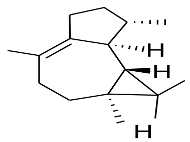
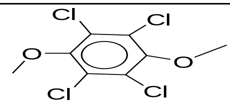
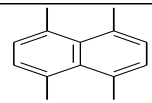
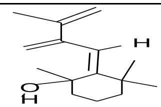
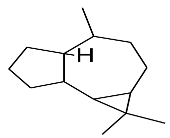
Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retention index, Q = quality

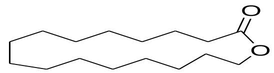
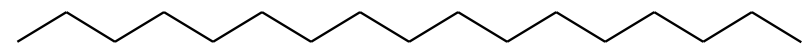
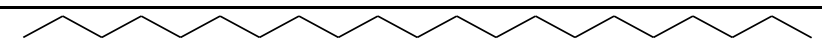
Most compound that are identified in the essential oil of *Otostegia integrifolia* leaves were Terpenoids; which are monoterpene sequiterpene , diterpene and phenolic compounds. All together 54 compounds with quality greater than 80% were identified. The minimum area percent was 0.01 and the maximum was 7.21%.

Table 7. GC-MS analysis of smoke of *O. integrifolia* trapped in hexane

PK	RT	Area pct	Name of the compound	Formula	Structure	Q	I
1	8.88	0.76	Dodecane	$C_{12}H_{26}$		87	1193.42
2	9.11	3.40	Phenol	C_6H_6O		94	1212.14
3	9.40	2.03	2-Methoxy- phenol	C_7H_8O2		97	1232.86
4	9.82	0.87	2-methyl- Phenol	C_7H_8O		97	1261.11

5	10.05	1.00	2-Phenylethanol	$C_8H_{10}O$		81	1279.29
6	10.34	2.78	p-Cresol	C_7H_8O		97	1300.00
7	10.88	0.879	Creosol	$C_8H_{10}O_2$		96	1336.92
8	10.92	0.51	2-Ethylphenol	$C_8H_{10}O$		64	1340.00
9	11.01	1.07	2,4-dimethyl phenol	$C_8H_{10}O$		96	1346.92
10	11.57	2.32	4-ethyl- Phenol	$C_8H_{10}O$		94	1386.92
11	11.65	0.39	Tetradecane	$C_{14}H_{30}$		95	1396.15
12	12.04	1.94	4-ethyl-2-methoxy- Phenol,	$C_9H_{12}O_2$		81	1423.44
13	12.64	1.09	Caryophyllene	$C_{15}H_{24}$		99	1470.31
14	12.75	1.86	2,3-Dihydro- Benzofuran	C_8H_8O		87	1478.91
15	12.81	1.43	2-Methoxy-4-vinylphenol	$C_{10}H_{12}O_2$		93	1483.59
16	13.13	0.95	Eugenol	$C_9H_{10}O_2$		96	1509.17
17	13.51	2.71	2,6-Dimethoxy- Phenol	$C_8H_{10}O_2$		97	1540.83

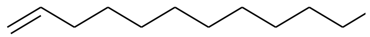
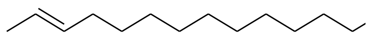
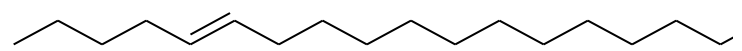
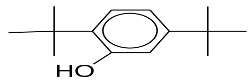
18	13.56	1.89	Indole	C_9H_7N		97	1537.50
19	14.14	0.84	Hexadecane	$C_{16}H_{34}$		86	1593.33
20	14.21	1.09	1,1,4,5,6-Pentamethyl-2,3-dihydro-1H-indene	$C_{14}H_{20}$		96	1599.17
21	14.50	1.85	3-Methylindole	$C_{14}H_9N$		93	1624.35
22	15.56	2.05	1,6,7-Trimethylnaphthalene	$C_{13}H_{14}$		98	1717.43
23	16.38	0.71	Tridecane	$C_{13}H_{28}$		89	1792.66
24	17.16	4.93	1a,2,3,5,6,7,7a,7boctahydro1,1,4,7tetra mthyl,[1aR(1a.alpha.,7.alpha.,7a.beta.,7 b.alpha.)]-1H.cycloprop[e]azulene	$C_{15}H_{24}$		86	1845.45
25	17.27	0.75	1,2,4,5-Tetrachloro-3,6- benzene	$C_8H_6Cl_4O_2$		95	1852.59
26	17.34	1.60	1,4,5,8-Tetramethylnaphthalene	$C_{14}H_{16}$		95	1857.14
27	17.79	0.94	1,3,3-trimethyl2[3methyl-2-methylene-3butenylidene]-,[Z]- cyclohexanol	$C_{15}H_{24}0$		83	1908.14
28	19.86	1.73	1,1,7tetramethyl[1aR(1a.alpha.,4.beta-4-a.beta.,7.beta.,7a.beta.,7b.alpha.)]-1Hcycloprop[e]azulene,decahydro	$C_{15}H_{24}$		86	2054.60

29	20.02	1.84	(8Z)-Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$		99	2065.13
30	20.46	0.34	Heptadecane	$C_{17}H_{34}$		94	2094.08
31	22.16	0.55	Heneicosane	$C_{21}H_{44}$		89	2193.60

Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retention index Q =Quality

A total of 31 compounds were identified from GCMS data. Most of the compounds detected were phenolic and there were significant number of non phenolic aromatic compounds. Terpenoids and straight chain alkanes were also part of the identified compounds.

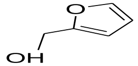
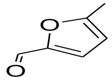
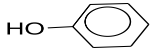
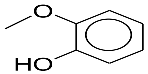
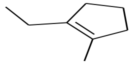
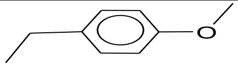
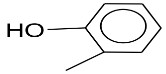
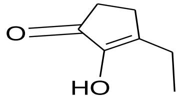
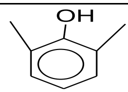
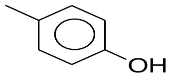
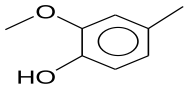
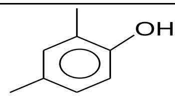
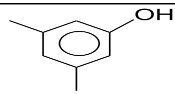
Table 8. GC-MS analysis of smoke of *O. integrifolia* trapped in Methanol

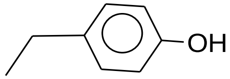
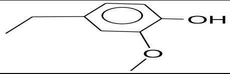
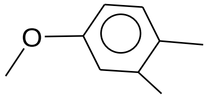
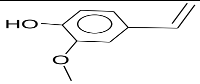
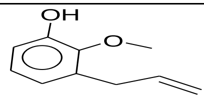
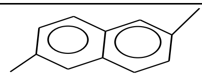
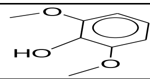
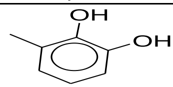
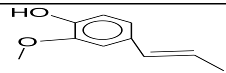
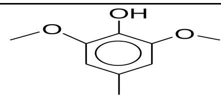
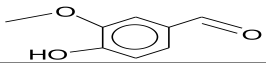
PK	RT	Area pct	Name of the compound.	Formula	Structure	Q	I
1	8.89	11.90	1-Dodecene	$C_{12}H_{24}$		96	1197.35
2	11.68	13.735	(E)-2-Tetradecene	$C_{14}H_{28}$		95	1396.32
3	14.18	9.15	(E)-5-Octadecene	$C_{18}H_{36}$		99	1596.67
4	15.11	65.21	2,6-bis(1,1-Dimethylethyl) phenol	$C_{14}H_{22}O$		87	1677.39

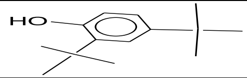
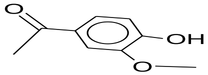
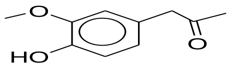
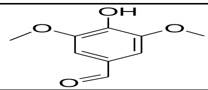
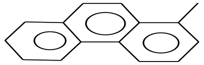
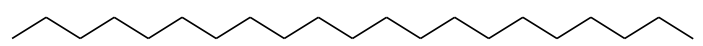
Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retention index Q = Quality

The compounds found in smoke of *Otostegia integrifolia* trapped in methanol were alkenes and phenolic. The number of trapped compounds was less than that trapped in hexane.

Table 9. GC-MS analysis of smoke of *S. macrosolen* trapped in Hexane

PK	RT	Area pct	Name of the compound	Formula	Structure	Q	I
1	6.07	1.02	2-Furanmethanol	$C_5H_6O_2$		94	1012.34
2	7.59	1.77	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$		93	1111.26
3	9.10	1.16	Phenol	C_6H_6O		94	1248.61
4	9.38	2.76	2-Methoxyphenol	$C_7H_8O_2$		96	1231.25
5	9.54	0.63	2- Ethyl -2-methyl cyclopentene	C_8H_{14}		76	1242.36
6	9.72	0.41	1-Ethenyl-4-methoxy- benzene	$C_8H_{10}O$		96	1254.86
7	9.82	0.78	2-MethylPhenol	C_7H_8O		97	1261.81
8	9.96	0.30	3-ethyl-2-hydroxy-2-cyclopenten-1-one	$C_7H_{10}O_2$		97	1271.53
9	10.05	0.68	2,6-Dimethyl- Phenol	$C_8H_{10}O$		86	1277.78
10	10.34	1.65	p-Cresol	C_7H_8O		97	1297.92
11	10.86	1.42	Creosol	$C_8H_{10}O_2$		96	1335.76
12	10.99	0.69	2,4-DimethylPhenol	$C_8H_{10}O$		96	1345.26
13	11.50	0.43	3,5-DimethylPhenol	$C_8H_{10}O$			1382.48

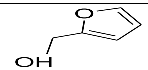
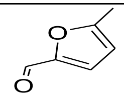
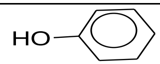
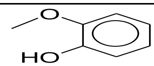
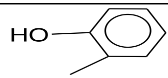
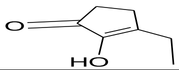
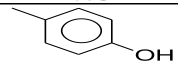
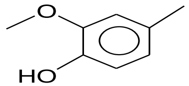
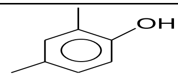
14	11.56	0.65	4-EthylPhenol	$C_8H_{10}O$		93	1386.86
15	11.67	0.33	(Z)-7-Hexadecene	$C_{16}H_{32}$	$CH_3(CH_2)_{12}(CH)_2CH_3$	91	1394.89
16	12.03	1.63	4-Ethyl-2-methoxyphenol	$C_9H_{12}O_2$		90	1422.66
17	12.58	0.33	3,4-Dimethylanisole	$C_9H_{12}O$		83	1465.63
18	12.79	2.04	2-Methoxy-4-vinylphenol	$C_9H_{12}O_2$		95	1482.03
19	12.91	0.60	Pentadecane	$C_{15}H_{32}$	$CH_3(CH_2)_{13}CH_3$	96	1491.41
20	12.95	0.21	1-Heneicosyl formate	$C_{22}H_{44}O_2$		95	1494.53
21	13.11	0.61	2-Methoxy-3-(2-propenyl)phenol	$C_{10}H_{12}O_2$		98	1507.50
22	13.23	0.63	2,6-Dimethylnaphthalene	$C_{12}H_{12}$		96	1517.50
23	13.49	4.31	2,6-Dimethoxyphenol	$C_8H_{10}O_3$		96	1539.17
24	13.84	0.62	3-Methyl-1,2-benzenediol	$C_7H_8O_2$		81	1568.33
25	14.48	1.48	Transisoeugenol	$C_{10}H_{12}O_2$		98	1617.39
26	14.60	1.34	3,5-Dimethoxy-4-hydroxytoluene	$C_9H_{12}O_3$		97	1633.64
27	14.76	1.37	Vanillin	$C_8H_8O_3$		90	1646.96

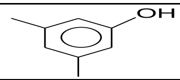
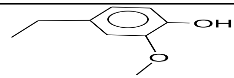
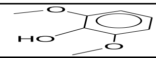
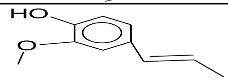
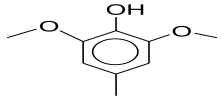
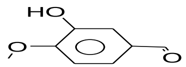
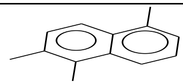
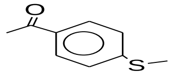
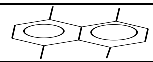
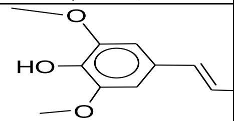
28	15.11	0.43	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$		87	1677.39
29	15.75	0.61	Apocynin	$C_9H_{10}O_3$		91	1734.86
31	16.37	1.37	1-(4-hydroxy-3- methoxyphenyl)-2-propanone	$C_{10}H_{12}O_3$		94	1791.74
32	17.56	0.72	Tetradecane	$C_{14}H_{30}$		95	1890.16
33	17.63	0.29	1-Octadecene	$C_{18}H_{36}$		99	1895.90
34	18.36	0.46	4-Hydroxy-3,5-dimethoxy- benzaldehyde	$C_9H_{10}O_4$		95	1950.37
35	18.95	1.98	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		98	1994.07
36	20.29	0.41	1-Methylphenanthrene	$C_{15}H_{12}$		95	2082.89
37	20.44	0.54	Heneicosane	$C_{21}H_{44}$		95	2092.76
38	21.11	0.54	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		99	2132.55
39	22.14	0.47	Heptadecane	$C_{17}H_{36}$		95	2192.44
40	22.24	0.73	Behenic alcohol	$C_{22}H_{46}O$	$C_{22}H_{46}O$	94	2196.26

Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retention index, Q = quality

Among the detected compounds major part is account for phenolic compounds. This might have something to do with the antioxidant activity of the plant extract as phenolic compounds are antioxidant compounds.

Table 10. GC-MS analysis of smoke of *S. macrosolen* trapped in Methanol

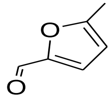
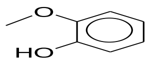
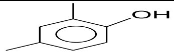
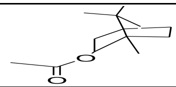
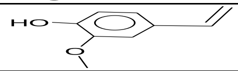
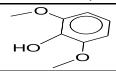
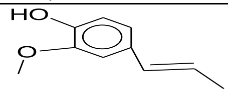
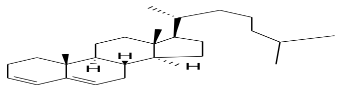
PK	RT	Area pct	Name of the compound	Formula	Structure	Q	I
1	6.08	2.27	2-Furanmethanol	$C_5H_6O_2$		95	1013.64
2	7.59	3.92	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$		93	1017.95
3	9.11	2.61	Phenol	C_6H_6O		95	1212.50
4	9.38	6.13	2-methoxyphenol	$C_7H_8O_2$		95	1231.25
5	9.82	1.11	2-Methylphenol	C_7H_8O		97	1261.81
6	9.96	0.49	3-Ethyl-2-hydroxy-2-cyclopenten-1- one	$C_7H_{10}O_2$		96	1271.53
7	10.34	2.83	p-Cresol	C_7H_8O		97	1297.92
8	10.86	2.26	Creosol	$C_8H_{10}O_2$		97	1335.77
9	10.99	1.48	2,4-Dimethylphenol	$C_8H_{10}O$		96	1345.26

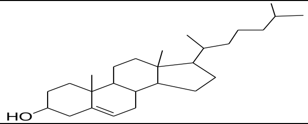
10	11.50	0.56	3,5-Dimethylphenol	$C_8H_{10}O$		96	1382.48
11	11.56	1.65	4-Ethylphenol	$C_8H_{10}O$		94	1386.86
12	12.03	2.69	4-Ethyl-2-methoxyphenol	$C_9H_{12}O_2$		90	1422.66
13	13.49	6.41	2,6-DimethoxyPhenol	$C_8H_{10}O_3$		96	1539.17
14	14.48	1.68	trans-Isoeugenol	$C_{10}H_{12}O_2$		98	1622.61
15	14.64	1.62	3,5-Dimethoxy-4-hydroxytoluene	$C_9H_{12}O_3$		97	1636.52
16	14.76	1.83	3-Hydroxy-4-methoxy-benzaldehyde	$C_8H_8O_3$		93	1646.96
17	15.22	3.08	1,6,7-TrimethylNaphthalene	$C_{13}H_{14}$		98	1689.96
18	15.76	0.88	1-[4-(methylthio)phenyl]-ethanone	$C_9H_{10}OS$		81	1735.78
19	16.69	1.40	1,4,5,8- Tetramethylnaphthalene	$C_{14}H_{16}$		96	1818.85
20	17.89	1.25	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl) phenol	$C_{11}H_{14}O_3$		96	19155.5
21	22.24	0.73	1-Nonadecene	$C_{19}H_{38}$		96	2099.07

Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retetion index, Q = quality

The major parts of compounds trapped in Methanol were phenolic. That is exactly why higher amount of antioxidant compounds was measured in the sample. It is a good indication that the plant part can be used as antioxidant agent. The cultural use of the plant for medicinal purpose can be supported scientifically when it comes to the antioxidant activity.

Table 11. GC-MS analysis of smoke of *E. kebericho* trapped in Hexane

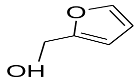
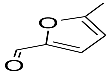
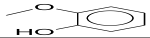
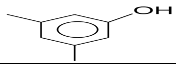
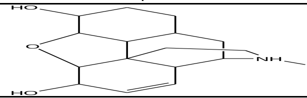
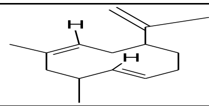
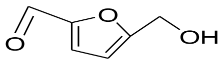
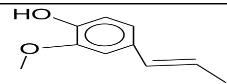
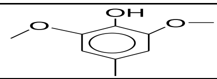
PK	RT	Area pc	Name of the compound	Formula	Structure	Q	I
1	7.58	0.60	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$		81	1110.59
2	9.39	1.10	2-Methoxyphenol	$C_7H_8O_2$		95	1234.94
3	11.00	0.34	2,4-Dimethylphenol	$C_8H_{10}O$		96	1345.98
4	11.33	0.26	Bornyl acetate	$C_{12}H_{20}O_2$		92	1368.08
5	12.80	0.47	2-Methoxy-4-vinylphenol	$C_9H_{12}O_2$		93	1482.17
6	13.49	1.74	2,6-DimethoxyPhenol	$C_8H_{10}O_3$		96	1538.66
7	14.48	1.77	trans-Isoeugenol	$C_{10}H_{12}O_2$		95	1622.61
8	14.78	1.17	Cholesta-3,5-diene	$C_{27}H_{44}$		95	1650.45

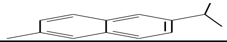
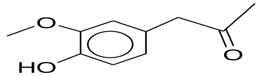
9	15.71	6.10	(3.alpha.)- Cholest-5-en-3-ol	$C_{27}H_{46}O$		90	1729.56

Area pct = percentage area, Pk = peak number, RT=Relative Time (minute), I = retention index, Q = quality

Only nine compounds were detected in the sample of which most were phenolic and few terpnoides. The presence of phenolic compounds is an asset to use the plant for medicinal purposes such as skincare.

Table 12. GC-MS analysis of smoke of *E. kebericho* trapped in Methanol

PK	RT	Area pct	Name of the compound	Formula	Structure	Q	I
1	6.07	0.53	2-Furanmethanol	$C_5H_6O_2$		96	1012.33
2	7.57	1.01	5-Methyl-2-furancarboxaldehyde	$C_6H_6O_2$		94	1109.33
3	9.39	0.92	2-Methoxyphenol	$C_7H_8O_2$		95	1231.94
4	11.51	0.06	3,5-Dimethylphenol	$C_8H_{10}O$		93	1383.21
5	11.91	0.19	Modephene	$C_{17}H_{19}O_3$		99	1413.28
6	12.16	0.17	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	$C_{15}H_{24}$		94	1432.81
7	13.31	0.08	5-Hydroxymethylfurfural	$C_6H_6O_3$		60	1524.17
8	14.48	0.29	trans-Isoeugenol	$C_{10}H_{12}O_2$		98	1622.61
9	14.64	0.09	3,5-Dimethoxy-4-hydroxytoluene	$C_9H_{12}O_5$		96	1636.52

10	15.78	0.14	2-Isopropyl-7-methylnaphthalene	$C_{14}H_{16}$		91	1737.61
11	16.38	0.11	1-(4-Hydroxy-3-methoxyphenyl)-2-propanone	$C_{10}H_{12}O_3$		93	1792.66

A rea pct = percentage area, Pk = peak number, RT=Relative Time(minute),, I = retention index , Q =Quality

The profiles of compounds identified in both samples were identical. Hence, it is advisable to use the plant parts as a whole for smoking purposes instead of going into extraction which is time and resource demanding.

Table 13. Compounds that are identified using GC-MS

Sample	Total compounds	≥80% Q	Major compound	Percent compound
O. integrifolia oil	142	54	Terpenoid Phenolic	51.39 13.64
O. integrifolia hexane	63	31	Phenolic Alkane Terpene	43.75 21.88 9.3
O. integrifolia methanol	10	4	Alkene Phenol	62.5 25
S. macrosolen hexane	103	40	Phenolic Aromatic Alkane	54 10 10
S. macrosolen methanol	50	21	Phenolic Aromatic	70.37 11.11
E. kebericho hexane	40	9	Phenolic Terpenoid Ketone	57.89 14.04 15.78
E. kebericho methanol	64	11	Phenolic Aldehydes Aromatic Terpene	65.2 5.20 15.68 13.04

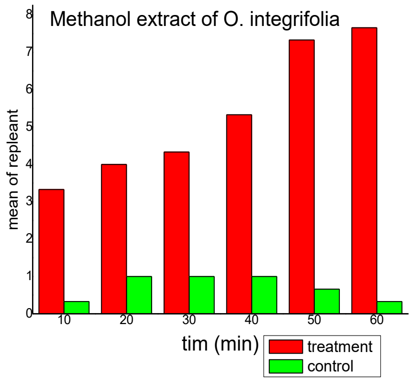
Among the compounds detected by GCMS, phenolic compounds are the major. It is surprising that in *E. kebericho* and *S. macrosolen* extracts, the amount of phenolic compounds were higher in Methanolic extract unlike *O. integrifolia* where the concentration of phenolic compounds was higher in Hexane.

4.4.1. Repellent activities due to smoke extract from *O. integrifolia* leaves

The insecticidal activity of O. integrifolia was tested using smoke trapped in methanol and hexane solvents. Insect (cockroach) replant activities were evaluated using as described in the experimental section and the result was recorded at every 10 min from 10 min to 60 min. All measurements were in triplicate and the results were recorded as mean ± standard deviation (SD)

Table 14. Average repellent activities due to smoke extracts obtained from *O. integrifolia* leaves

No	Solvent	concentration	Average Repellant					
			Before heating			After heating		
			10 min	20 min	30 min	40 min	50 min	60 min
1	Hexane	10mg	4.33±0.57	4.66± 0.57	4.66± 0.57	5.66± 0.57	7.66± 0.57	8.66 ± 0.57
2	Control		0.66± 0.57	0.66± 0.57	0.66± 0.57	0.33± 0.57	0.33± 0.57	0
3	methanol	10mg	3.33± 0.57	4 ± 0.57	4.33± 0.57	5.33± 0.57	7.33± 0.57	7.66 ± 0.57
4	Control		0.66± 0.57	1 ± 0	1 ± 0	1 ± 0	0.66± 0.57	0.33 ± 0.57



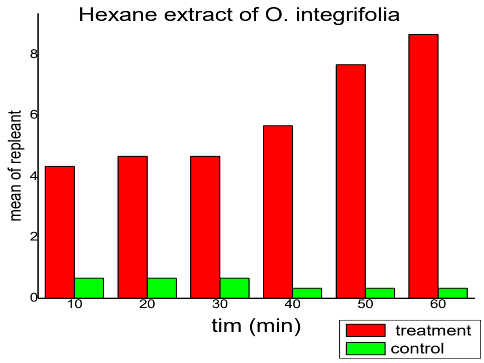


Figure 14. Mean repellent activities of smokes

As the Table 14 and Figure 14 show, the plant extract showed a higher replant activities compared to the control and the repelling activity increases when the time increase in both smoke samples (Hexane and Methanol extracts). In the first 30 min the variation in repellent values was less. That could be due to different factors such as insect movement from the release area, and level of exposure. However, ones the samples were heated, the values becoming different as time goes by. The maximum repellent value was recorded for sample trapped in hexane at 60 min. This shows that the plant extract has repelling ability when compared to the control.

Table 15. Percent repellent activities due to *O. integrifolia* smoke extracts

No	Solvent	Amount of sample	Percent of repellant					
			Before heating			After heating		
			10 min	20 min	30 min	40 min	50 min	60 min
1	Hexane	10 mg	73.55±11.5	75.19±11.5	75.19±11.5	88.98±11.54	91.74±11.5	100.00± 0
2	Methanol	10 mg	66.92± 0	60.00±0	62.48±11.5	68.40±11.54	83.48±11.5	91.74±11.5

Repellent (%)= (#of cockroach in the control side (CC) – (# cockroach sample side (CS)) x100

Total # of cockroaches in the control and sample sides

4.4.2. Mortality activities of *O. integrifolia* leaves smoke

The insecticidal activities of smoke samples obtained from *O. integrifolia* were determined by using smoke trapped in methanol and hexane. The result was recorded at 3, 6, 9, 12, 24, 36 and 48 hrs. All measurements were in triplicate and the result was recorded as mean ± standard deviation (SD).

Table 16. Insect mortality rate due to *O. integrifolia* leaf smoke

No	Solvent	Concentration of sample	Average Mortality (h)						
			3h	6h	9h	12h	24h	36h	48h
1	hexane	1.25%	0	0	0.66±0.57	0.66 ± 0.57	0.66±0.57	1.00 ± 0	4.33 ± 0.57
2		2.5%	0	0	0.66±0.57	0.66 ± 0.57	0.66±0.57	1.00 ± 0	5.00 ± 0
3		5%	0	0	0.66±0.57	0.66 ± 0.57	1.00± 0	2.00 ± 0	5.00 ± 0
4		10%	1.00	1.33± 0.57	1.66± 0.57	1.66 ± 0.57	2.00 ± 0	3.00 ± 0	5.00 ± 0
5	Control	DCM (1mL)	0	0	0	0	0	0	0
6	methanol	1.25%	0	0	0.33± 0.57	0.33± 0.57	0.33± 0.57	0.33 ± 0.57	4.00 ± 0
7		2.5%	0	0	0.33± 0.57	0.33± 0.57	0.33± 0.57	0.33 ± 0.57	4.33± 0
8		5%	0	0	0.33 ± 0.57	0.33± 0.57	1.00 ± 0.	1.00 ± 0	4.66 ± 0
9		10%	0	0	0.66 ± 0.57	0.66 ± 0.57	1.00± 0.57	2.00 ± 0	5.00 ± 0
10	Control	Methanol (1mL)	0	0	0	0	0	0	0

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The insecticidal activities due to the samples have been recorded over 48 hours. For the concentrations 1.25 to 5% in the first 36 hours the mortality rate was not significant as it can be seen from the table above. However, there was a huge lip in the last 24 hours. Despite of observable deaths of the insects, the time span it took was longer to consider the plant extract as an efficient.

Table 17. Correlation between mortality and Time

HEO	*3h*	*6h*	*9h*	*12h*	*24h*	*36h*	*48h*	*MEO*	*3h*	*6h*	*9h*	*12h*	*24h*	*36h*	*48h*
3h	1	1	1	1	0.96	0.98	0.33	3h	1						
6h	1	1	1	1	0.96	0.87	0.33	6h	-	1					
9h	1	1	1	1	0.96	0.87	O.33	9h	-	-	1	1	0.58	0.92	0.78
12h	1	1	1	1	0.96	0.87	0.33	12h	-	-	1	1	0.58	0.92	0.78
24h	0.96	0.96	0.96	0.96	1	0.96	0.44	24h	-	-	0.58	0.58	1	0.92	0.89
36h	0.87	0.87	0.87	0.87	0.96	1	0.52	36h	-	-	0.92	0.92	0.85	1	0.93
48h	0.33	0.33	0.33	0.33	0.44	0.52	1	48h	-	-	0.78	0.78	0.89	0.93	1

MEO = methanol extracted of *E. kebericho* HEO = hexane extracted *E.kebericho*

Table 18. Correlation between mortality and concentration

HEO	*1.25%*	*2.5 %*	*5%*	*10%*	*MEO*	*1.25%*	*2.5 %*	*5%*	*10%*
1.25%	1				*1.25%*	1			
2.5 %	0.99	1			*2.5 %*	0.99	1		
5%	0.98	0.97	1		*5%*	0.98	0.98	1	
10%	0.96	0.95	0.99	1	*10%*	0.94	0.94	0.98	1

HEO = Hexane extract of *O. integrifolia* MEO = Methanol extract of *O.integrifolia*

As it can be seen from the table above mortality rate and concentration and time of the sample are strongly correlated. The death rates of the insects do increase with time. The increase in death as time increase is due to exposure time of the insects to the chemical compounds. This gives a good indication that the plant material can be used to control pests.

Table 19. Percent mortality activities due to *O. integrifolia* leaf extract

No	Solvent	Conc.	% Mortality (h)						
			3h	6h	9h	12h	24h	36h	48h
1	Hexane	1.25%	-	-	13.20± 11.54	13.20 ± 11.54	13.20±11.54	20.00 ± 0	86.66±11.54
2		2.5%	-	-	13.20 ± 11.54	13.20 ± 11.54	13.20±11.54	20.00 ± 0	100.00±0
3		5%	-	-	13.20 ± 11.54	13.20 ± 11.54	20.00 ± 0	40,00 ± 0	100.00±0
4		10%	20.00±0	26.66± 11.54	33.20 ± 11.54	33.20 ± 11.54	40.00± 0	60.00 ± 0	100.00 ±0
5	Methanol	1.25%	-	-	6.60 ± 11.54	6.60 ± 11.54	6.60 ± 11.54	6.60±0.57	80.00 ±0
6		2.5%	-	-	6.60 ± 11.54	6. 60 ± 11.54	6.60 ± 11.54	6.60±0.57	86.66±11.54
7		5%	-	-	6.60 ± 11.54	6.60 ± 11.54	20.0 ±11.54	20.00±0	93.20±11.54
8		10%			13.20 ± 11.54	13.20 ± 11.54	20.00 ± 0	40.00±0	100.00±0

$$\text{Mortality (\%)} = \frac{\text{(death due to treatment of sample – death in control) x 100}}{\text{Total number of cockroaches treated}} \quad \ldots\ldots\ldots\ldots\ldots \text{(4)}$$

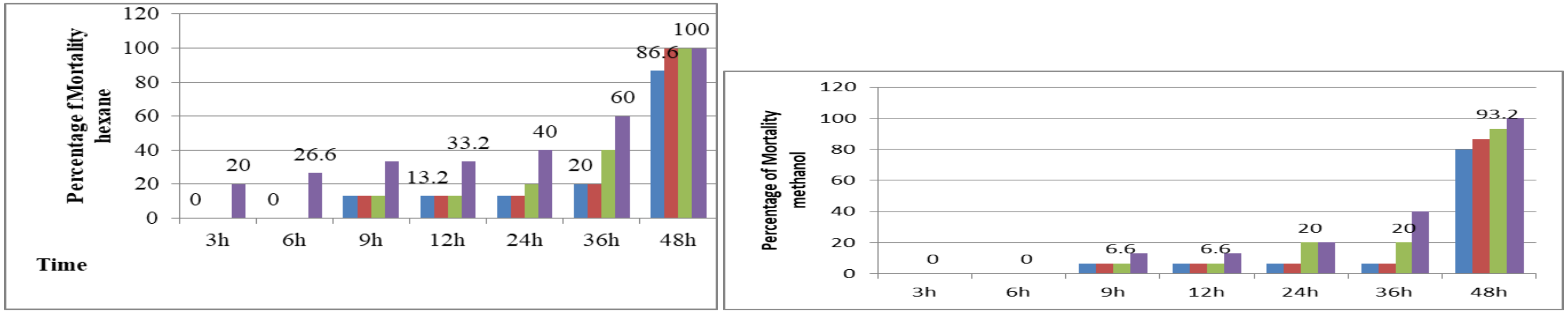


Figure 15. Percent mortality vs time of *O. integrifolia* leaf extarct

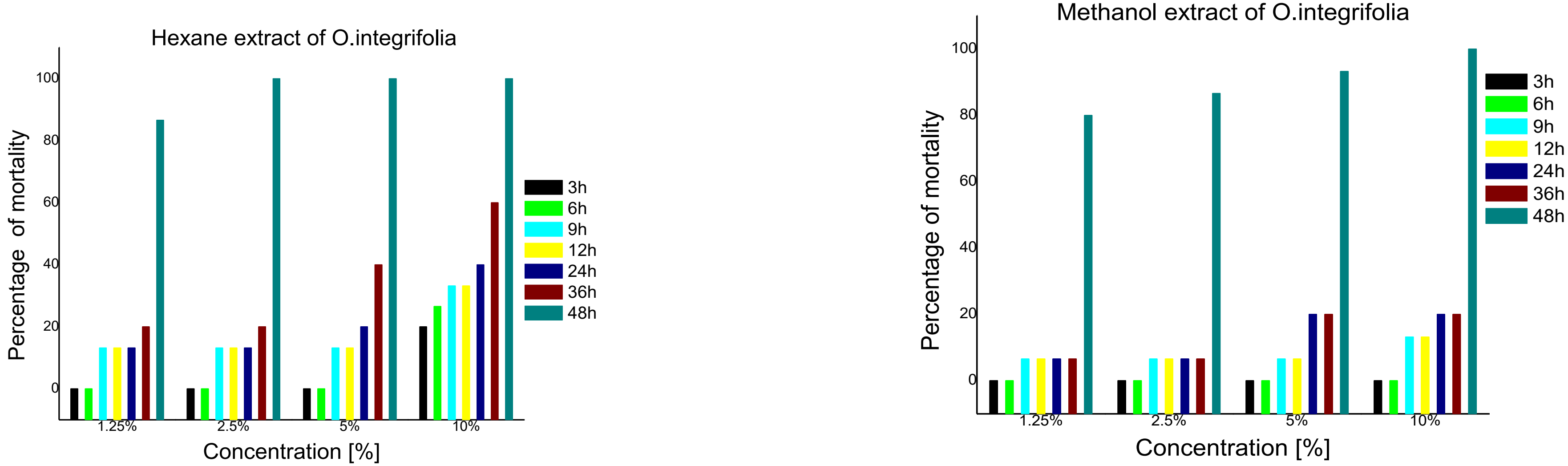


Figure 16. Percent mortality *vs* concentration of *O. integrifolia* leaf extract

4.3.3. Mortality activities of *S. macrosolen* root smoke

The insecticidal activities of *S. macrosolen* samples were obtained by using smoke trapped in methanol and hexane. The result was recorded after 3, 6, 9, 12, 24 and 36h. All measurements were in triplicate and the result was recorded as mean ± standard deviation (SD).

Table 20. Average mortality due to *S. macrosolen* root extracts (smoke)

No	solvent	Concentration of sample	Mean mortality (h)					
			3h	6h	9h	12h,	24h	36h
1	Methanol	1.25%	0	0.33 ± 0.57	0.66 ± 0.57	1.66 ± 0.57	2.00 ± 0	5.00 ± 0
2		2.5%	0	1.33 ± 0.57	1.33 ± 0.57	2.00 ± 0	2.66 ± 0.57	5.00 ± 0
3		5%	0	2.00 ± 0	2.00± 0	3.00 ± 0	3.00 ± 0	5.00 ± 0
4		10%	1.66 ± 0.57	3.00 ± 0	3.00 ± 0	4.00 ± 0	4.00 ± 0	5.00 ± 0
5	Hexane	1.25%	0	0.33	0.66 ± 0.57	1.00 ± 0	2.00 ± 0	4.00 ± 0
6		2.5%	0	0.66 ± 0.57	1.00 ± 0	1.33 ± 0.57	2.00 ± 0	5.00± 0
7		5%	0	1 .00± 0	1.33 ± 0.57	2.00 ± 0	3.00 ± 0	5.00 ± 0
8		10%	1.00 ± 0	2.00± 0	2.66 ± 1.15	3.00 ± 0	4.00 ± 0	5.00 ± 0
9	Control	Methanol	0	0	0	0	0	0
10		DCM	0	0	0	0	0	0

The methanol sample showed better activity compared to sample in hexane. At 6 hour, 5% concentration was killed 2/5 cockroaches while 10% sample concentration had killed 3/5 cockroaches. Hexane sample showed similar effect just after 12 hour. By 36 hour all concentrations of both samples were able to kill more than 4/5 of the experimental insects. From the mortality rate data it can be inferred that the plant extracts can make moderate efficacy towards protecting the insects. Still the effects of the extracts need to be tested in an open air environment instead of a confined area like the one used in this exeperiment.

Table 21. Correlation between mortality and time

MES	*3h*	*6h*	*9h*	*12h*	*24h*	*36h*	*HES*	*3h*	*6h*	*9h*	*12h*	*24h*	*36h*
3h	1						3h	1					
6h	0.79	1					6h	0.92	1				
9h	0.83	0.99	1				9h	0.94	0.99	1			
12h	0.84	0.97	0.98	1			12h	0.88	0.99	0.98	1		
24h	0.86	0.99	0.99	0.97	1		24h	0.87	0.967	0.95	0.98	1	
36h	-	-	-	-	-	1	36h	0.33	0.616	0.57	0.62	0.522	1

MES = methanol extract of *S. macrosolen,* HES = hexane extract of *S. macrosolen*   h = hour

Table 22. Correlation between mortality and concentration

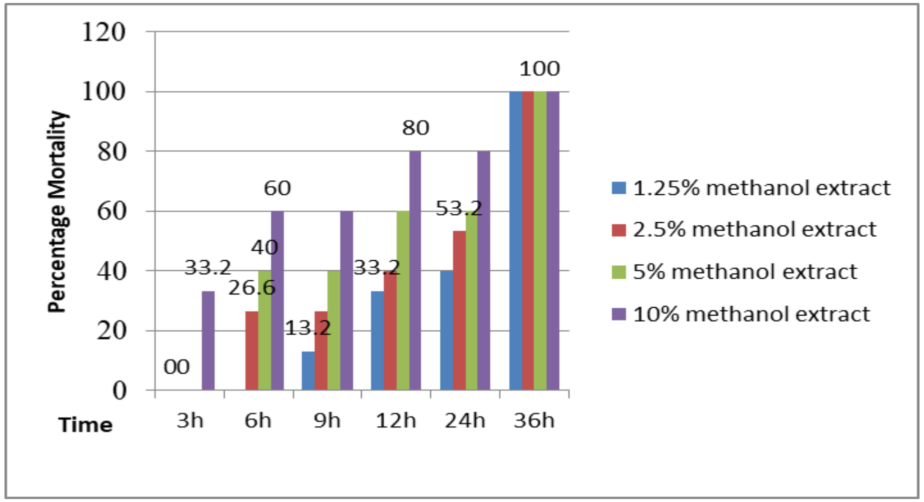
MES	*1.25%*	*2.5%*	5%	*10%*	*HES*	*1.25%*	2.5%	*5 %*	*10%*
1.25%	1				1.25%	1			
2.5%	0.97	1			2.5%	0.99	1		
5%	0.91	0.97	1		5%	0.98	0.97	1	
10%	0.89	0.94	0.98	1	10%	0.94	0.92	0.98	1

MES = methanol extract of *S. macrosolen,* HES = hexane extract of *S. macrosolen*

As it can be seen from the table above mortality rate and concentration of the sample are correlated and the mortality rate and time are also well correlated. Both mortality and concentration, and mortality and time are directly related.

Table 23. Percent mortality activities due to *S. macrosolen* roots smoke

No	Solvent	Plant part and concentration	% Mortality (h)					
			3h	6h	9h	12h	24h	36h
1	Methanol	1.25%	-	6.60 ± 11.54	13.20 ± 11.54	33.20 ±11.54	40.00±0	100 ± 0
2		2.5%		26.60± 11.54	26.60 ± 11.54	40 00± 0	53.20±11.54	100 ± 0
3		5%	-	40.00 ± 0	40.00 ± 0	60.00 ± 0	60.00 ± 0	100 ± 0
4		10%	33.20 ± 11.54	60.00 ± 0	60.00 ± 0	80'00 ± 0	80.00 ± 0	100 ± 0
	Control	Methanol	0	0	0	0	0	0
5	Hexane	1.25%	0	6.60 ± 0.57	13.20 ± 11.54	20.00 ± 0	40.00 ± 0	80.00± 0
6		2.5%	0	13.20± 11.54	20.00 ± 0	26.60± 0.57	40.00 ± 0	100.00 ± 0
7		5%	0	20 .00± 0	26.60 ± 11.54	40.00 ± 0	60.00 ± 0	100.00 ± 0
8		10%	20.00±0	40.00 ± 0	53.20 ± 20.09	60 .00± 0	80.00 ± 0	100.00 ± 0
10		DCM	0	0	0	0	0	0



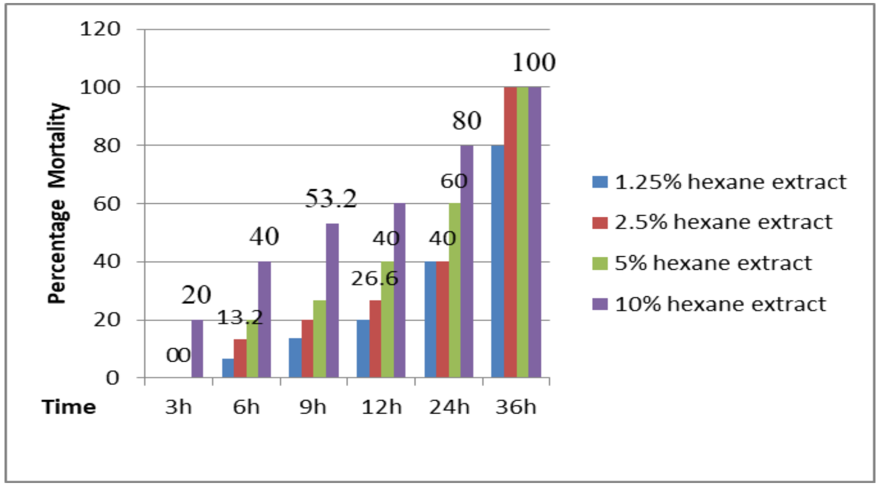
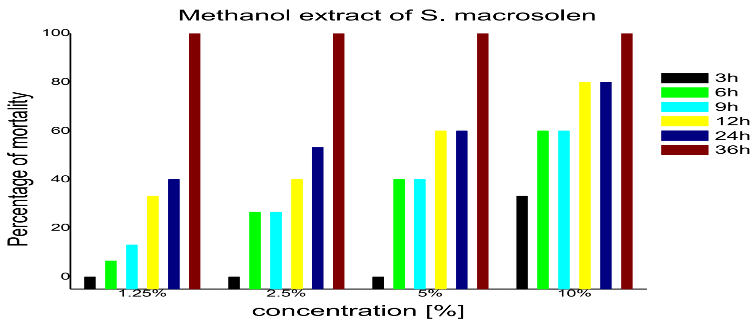


Figure 17. Percent mortality *vs* time of *S. macrosolon* samples



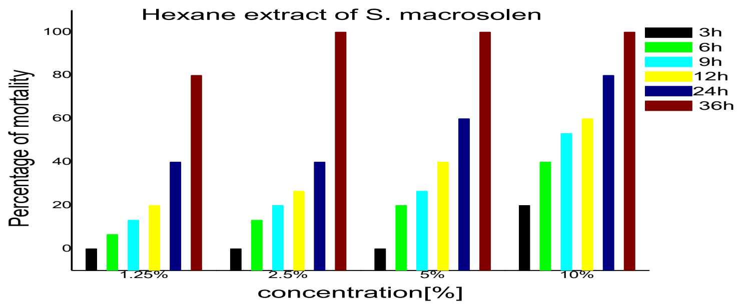


Figure 18. Percent mortality *vs* Concentration of *S. macrosolon* samples

By 36 hour, almost all insects were killed by all concentrations of both samples. This time is a bit higher to consider as optimum time.

4.3.4. Mortality rate due to *E. kebericho* root smoke samples

The insecticidal activities of *E. kebericho* samples were obtained by using smoke trapped in methanol and hexane. The result was recorded after 3, 6, 9, 12, 24 and 36h. All measurements were in triplicate and the result was recorded as mean ± standard deviation (SD).

Table 24. Average mortality due to *E. kebericho* root samples

No	solvent	Concentration of sample		Mean Mortality (h)				
			0h	3h	6h	9h	12h	24
1	Methanol	1.25%	0	1.33 ± 1.53	1.66 ± 1.15	1.66 ± 1.15	2.00 ± 0	5.00 ± 0
2		2.5%	0	1.33 ± 1.53	1.66 ± 1.53	1.66 ± 1.53	3.00 ± 0	5.00 ± 0
3		5%	0	1.66 ± 0.57	1.66 ± 0.57	1.66 ± 0.57	4.33 ± 0.57	5.00 ± 0
4		10%	0	2.66 ± 1.15	2.66 ± 1.15	2.66 ± 0.57	4.66 ± 0.57	5.00 ± 0
5	Hexane	1.25%	0	0	0.33±0.57	0.33 ± 0.57	1.00 ± 0	4.66 ± 0.57
6		2.5%	0	0	0.66 ± 0.57	1 ± 0	1.00 ± 0	5.00 ± 0
7		5%	0	0	1.33 ± 0.57	1.33 ± 0.57	1.33 ± 0.57	5.00 ± 0
8		10%	0	1.00 ± 0	2.33 ± 0.57	2.66 ± 0.57	4.00 ± 0	5.00 ± 0
9	Control	Methanol	0	0	0	0	0	0
10		DCM	0	0	0	0	0	0

By 3 hour the methanol extract with 10% concentration did kill 50% of the test insects. A similar effect was observed in hexane samples by 9 hour. It can be clearly seen from the data that methanol sample shoed better efficacy that the methanol sample. All the concentrations did kill greater than 90% of the test insects by 24 hour.

Table 25. Correlation between mortality and time

MEK	*3h*	*6h*	*9h*	*12h*	*24h*	*HEK*	*3h*	*6h*	*9h*	*12h*	*24h*
3h	1					3h	1				
6h	1	1				6h	0.88	1			
9h	0.96	0.96	1			9h	0.86	0.98	1		
12h	0.77	0.77	0.61	1		12h	0.99	0.92	0.90	1	
24h	-	-	-	-	1	24h	0.33	0.62	0.73	0.38	1

MEK =methanol extract of *E. kebericho*, HEK = hexane extract *E.kebericho*, h=hour

Table 26. Correlation between mortality and concentration

MEK	*1.25%*	*2.5%*	*5%*	*10%*	*HEK*	*1.25%*	*2.5%*	*5%*	*10%*
1.25%	1				1.25%	1			
2.5%	0.95	1			2.5%	0.99	1		
5%	0.81	0.94	1		5%	0.97	0.99	1	
10%	0.79	0.93	0.99	1	10 %	0.836361	0.83	0.86	1

MEK = methanol extract *E. kebericho*, HE -hexane extract *E.kebericho*

As it can be seen from the Table above mortality rate and concentration of the sample are strongly correlated, the mortality rate and time are also correlated well. Both mortality and concentration, and mortality and time are directly related. When compared to the former two plant extracts, *E. kebericho* has shown better effect in killing the pests.

Table 27. Percent mortality due to *E. kebericho* root smoke samples

No	Solvent	Concentration of sample		% Mortality (h)				
			0	3h	6h	9h	12h	24h
1	Methanol	1.25%		26.66± 30.55	33.20 ± 23.09	33.20 ± 23.09	40.00 ± 0	100.00 ± 0
2		2.5%		26.66 ± 30.55	33.20 ± 30.55	33.20 ± 30.55	60.00± 1.73	100.00 ± 0
3		5%		33.20 ± 11.54	33.20± 11.54	33.20±11.54	53.20± 23.09	100.00 ± 0
4		10%		33.20 ± 23.09	53.20 ± 23.09	53.20±11.54	86.66 ± 11.54	100.00 ± 0
	Control	Methanol	-	--	-	-	-	-
5	Hexane extract	1.25%		-	6.60 ± 11.54	6.60 ± 11.54	20.00 ± 0	93.20 ± 11.54
6		2.5%		-	13.20 ± 11.54	20 .00 ± 0	20.00 ± 0	100.00 ± 0
7		5%			26.66 ± 11.54	26.66 ± 11.54	26.666 ±11.54	100.00 ± 0
8		10%		20.00 ± 0	46.66 ±11.54	53.20 ± 11.54	80.00 ± 0	100.00 ± 0
10		DCM	0	0	0	0	0	0

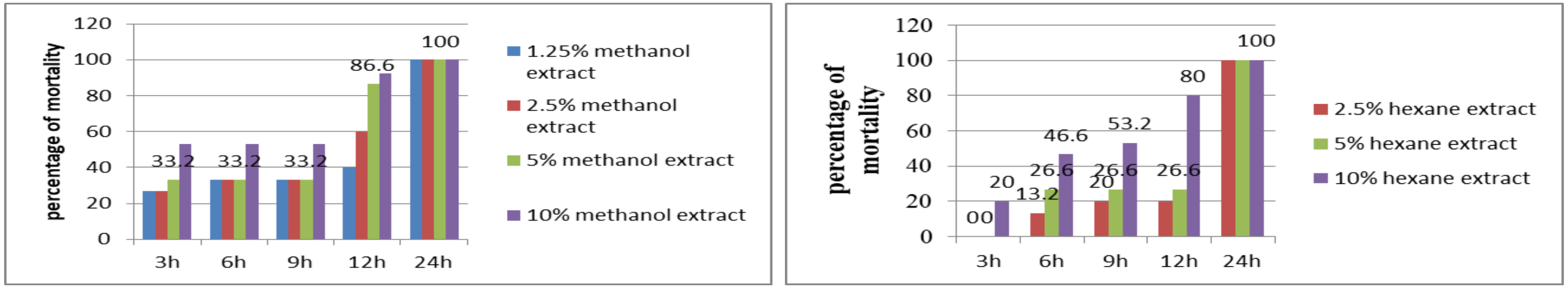


Figure 19**.** Percent mortality *vs* time of *E. kebericho* samples

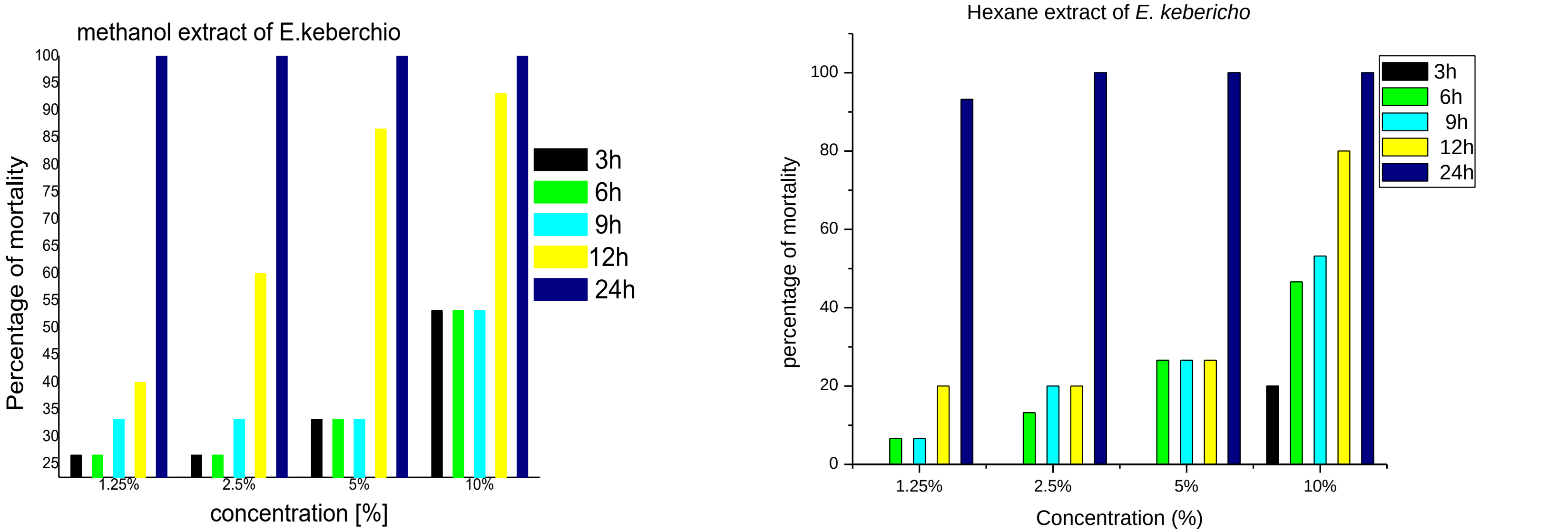


Figure 20**.** Percent mortality *vs* concentration of *E. kebericho* samples

CHAPTER FIVE

5. Conclusion and Recommendations

5.1. Conclusions

From the GC-MS analysis several compounds were identified in the essential oil of *Otostegia integrifolia* of which major components were terpenoids. Phenolic compounds were also among the detected compounds. From smoke of *S. macrosolen* trapped in hexane and methanol, phenolic compounds were the major components. From smoke of *E.kebericho* trapped in hexane and methanol also phenolic compound were the major components. The presence of phenolic compounds was believed to be a reason why all the samples have shown better antioxidant effects. The antioxidant concentration in Hexane trapped smoke of all samples under this study was negligible.

The killing ability of the plant extracts were tested and observed that *E. kebericho* samples showed better efficacy compared to *S. macrosolen* and *O. integrifolia* smoke extracts.

5.2. Recommendations

As this research is the first time attempts, different parts of the plants may be used to see the biological activities.

The repellent test was only done for *O. integrifolia* samples, it is better to do similar or related tests to *S.macrosolen and E.kebericho.*

It is also good if the in-vivo biological tests could be done for the extrats.

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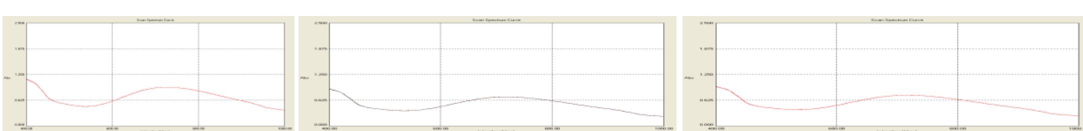
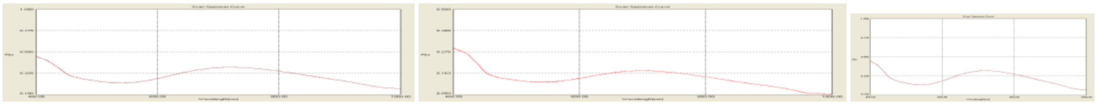
Appendices

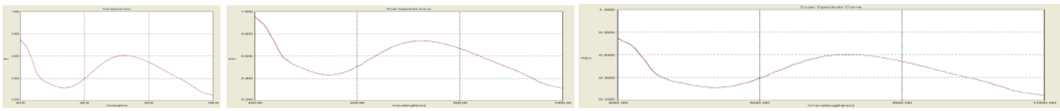
Appendices 1: Result of UV-Vis Analysis of the samples



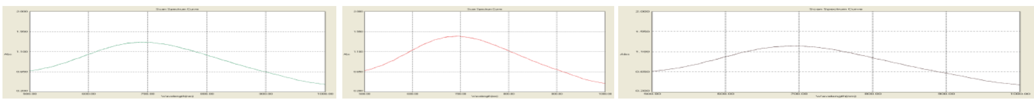
Appendix 1.UV-Vis 0. 761 mg/ mL of *O. integrifolia*



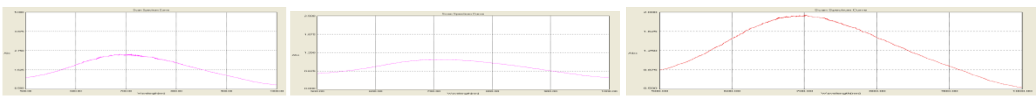
Appendix 2. UV-Vis 0. 1522 mg/mL of *O. integrifolia*



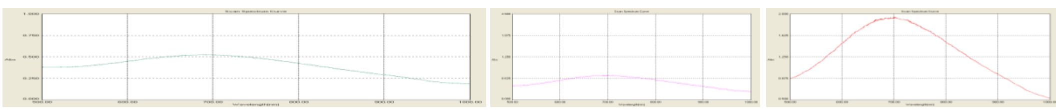
Appendix 3: UV-Vis 0.228.3 mg/mL of *O. integrifolia*



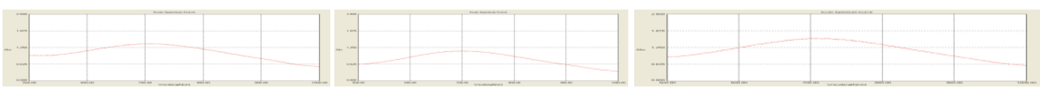
Appendix 4. UV-Vis 0.761 mg/mL of *S. macrosolen*



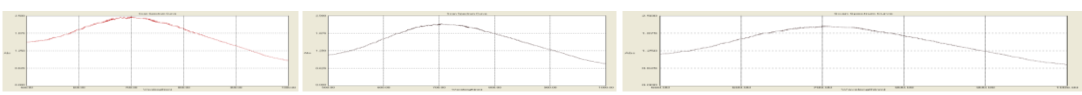
Appendix 5. UV-vis 0. 1522 mg/mL of *S. macrosolen*



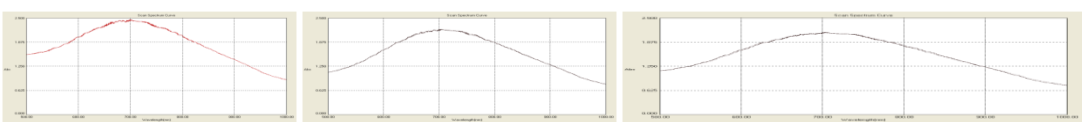
Appendix 6. UV-Vis 0.2283 mg/mL of *S. macrosolen*



Appendix 7. UV-Vis 0.0761 mg/ mL of *E. kebericho*

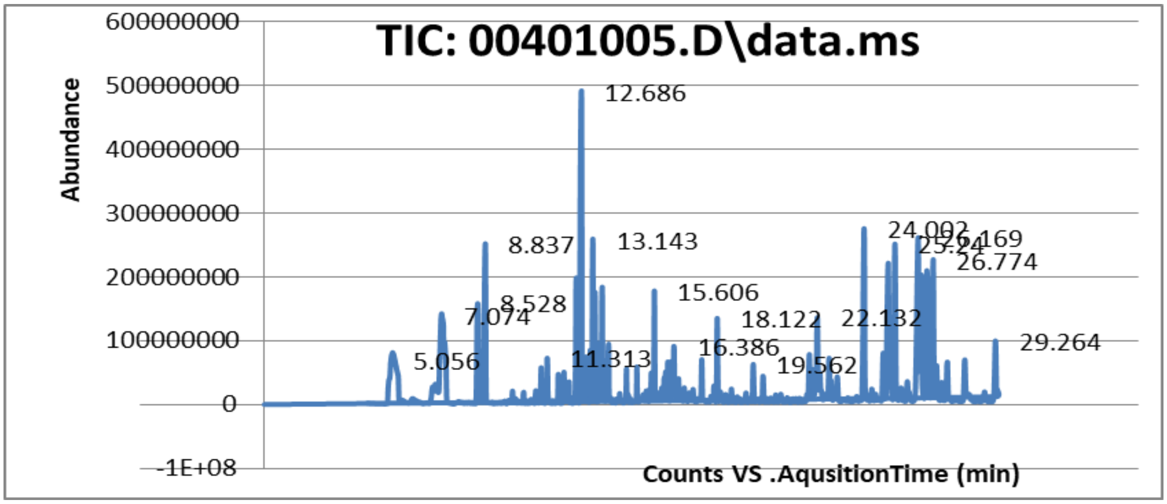


Appendix 8. UV-Vis 0.1522 mg /mL of *E .kebericho*

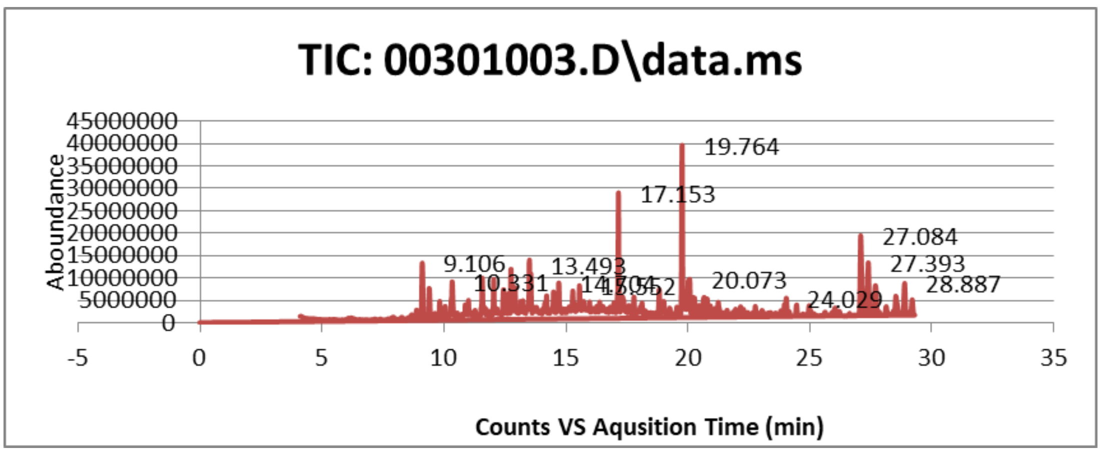


Appendix 9. UV-Vis 0. 2283 mg/mL of *E.kebericho*

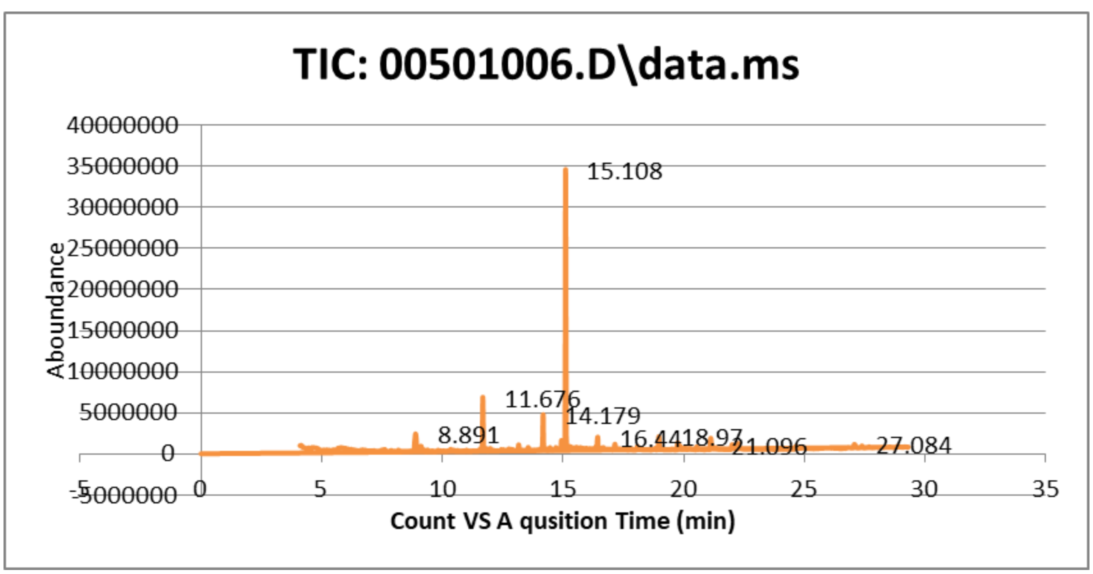
Appendices 2: Result of GC-MS Analysis of the samples



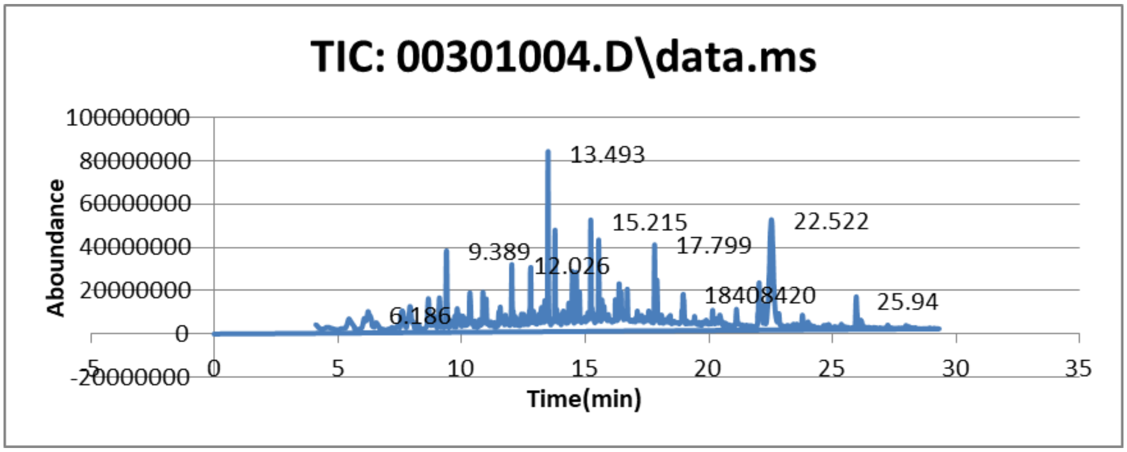
Appendix 10. GC-MS chromatogram of the essential oil of leaf of *O. integrifolia*



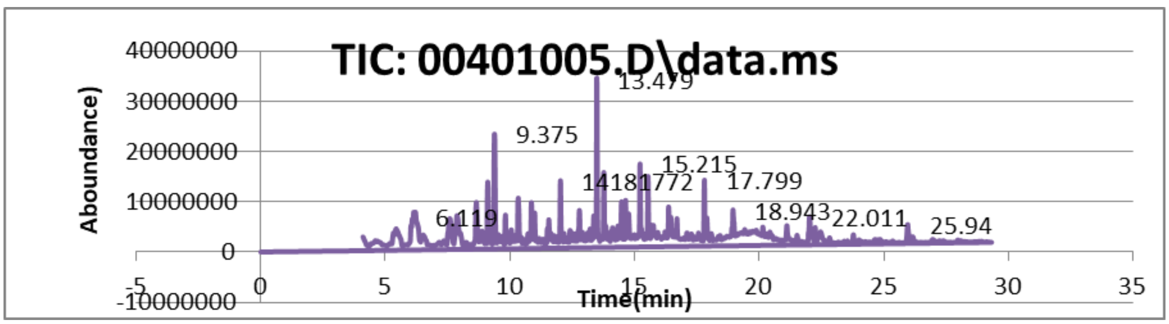
Appendix 11 . GC-MS chromatogram for smoke of *O. integrifolia trapped in hexane*



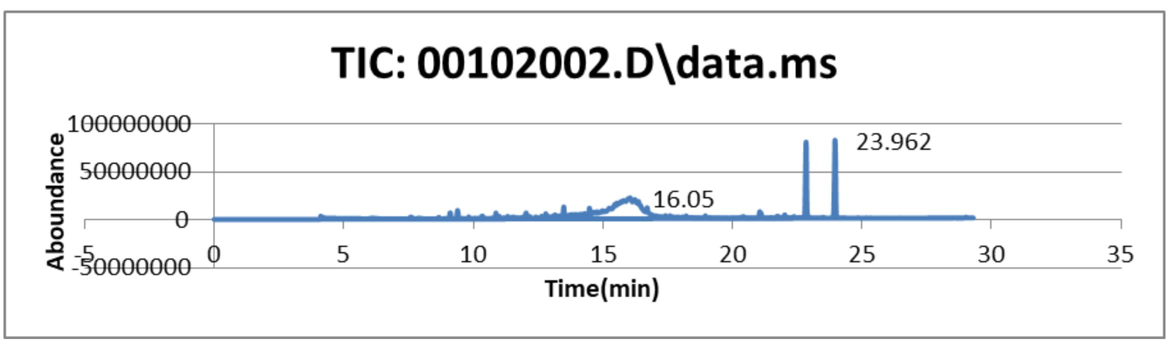
Appendix 12. GC-MS chromatogram for smoke of *O. integrifolia trapped in methanol*



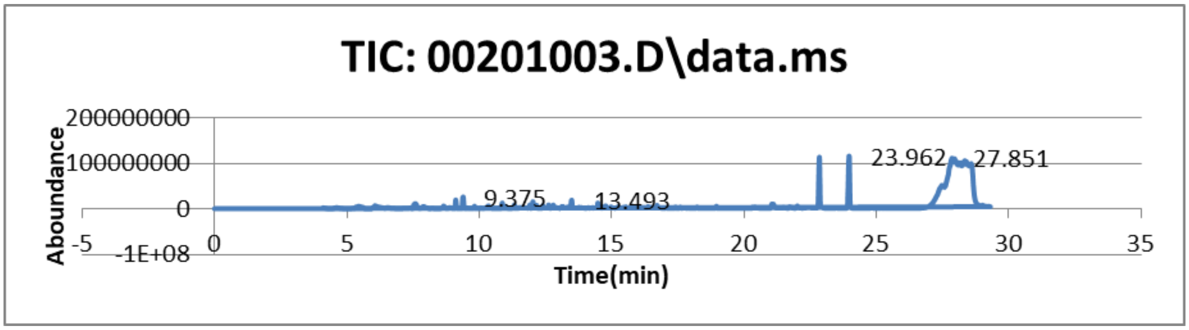
Appendix 13**.** GC-MS chromatogram for smoke of *S. macrosolen* trapped in hexane



Appendix 14. GC-MS chromatogram for smoke of *S. macrosolen trapped in methanol*



Appendix 15 . GC-MS chromatogram for smoke of *E. kebericho* trapped in hexane



Appendix 16 .GC-MS chromatogram for smoke of *E. kebericho* trapped in methanol