ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVIITIES OF SMOKE COLLECTED FROM *AZADIRACHTA INDICA (NEEM) AND OLEA EUROPAEA* (OLIVE) LEAVES

BY: Kibrom Teshome

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

BY: Kibrom Teshome

July, 2020

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DECLARATION

I, the undersigned, declare that this Thesis entitled "CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVIITIES OF SMOKE COLLECTED FROM *AZADIRACHTA INDICA* (NEEM) AND *OLEA EUROPAEA* (OLIVE) LEAVES "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: Kibrom Teshome

Signature: ________________________________

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

Name: Estifanose Ele (PhD)

Signature: _________________________________

Place and date of submission: Addis Ababa University, Department of Chemistry

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LIST OF ABBREVATIONS

DCM	Dichloride methane
TCA	Trichloro acetic acid
FRAP	Ferric Reducing Antioxidant Power
WHO	World Health Organization
NIST	National Institute of and Standard and Technology
GC-MS	Gas Chromatography Mass Spectroscopy
UV	Ultra-Violent
BHA	Butylated Hydroxy anisole
BHT	Butylated Hydroxy toluene
ppm	Part Per Million

ABSTRACT

This study aimed at the analysis of essential oil and smoke from neem and olive leaves for their biological (antioxidants and insecticidal) activities. Essential oil was extracted using hydrodistillation technique, and the smoke was collected using lab designed set of apparatus. The chemical compositions of the oils and smoke were analyzed by Gas Chromatography Mass Spectrometry (GC-MS). The test for antioxidant activities was performed using UV-Visible spectroscopy. The antioxidant concentration of the sample was determined by using a calibration curve constructed from standard ascorbic acid, and the insecticidal activity test (both repellent and mortality) was conducted using cockroaches.

The measured average antioxidant concentrations for neem smoke trapped in methanol was 19.65% for 152.20 µg/mL, and for olive smoke trapped in methanol was 4.91% for 152.20 µg/mL. While, the antioxidant concentration test in the neem and olive smoke trapped in hexane was found to be negligible. The time course insecticidal (insect repellent) activities were performed at 10, 20, 30, 40, 50, and 60 minutes using 15 mg of the smoke trapped in hexane and methanol against cockroaches.

For the neem hexane trapped smoke sample the minimum % repellent was 20 % at 10 minutes, and the maximum was 81.24 % at 60 min, and for olive hexane trapped smoke was 14.16 % at 10 min and 77.96 % at 60 minutes. The neem methanol trapped smoke showed 42.92 % at 10 min, and 100 % at 60 min. The olive methanol trapped smoke showed 33.33 % at 10 min, and 92.10 % at 60 minutes. The time course insecticidal (insect mortality) activities were performed at 3, 6, 9, 12, 24, and 36 hours using 1.25%, 2.5%, 5%, 10% samples concentration.

The minimum and maximum % mortality of neem hexane trapped smoke was 6.66 % at 1.25% concentration in 3 h, and 80 % at 10 % concentration in 36 h, For olive hexane trapped smoke the minimum rate was 6.66 % at 1.25% in 3 h, while the maximum was 60 % at 10 % over 36 h. For neem methanol trapped smoke the minimum was 20% at 1.25% concentration in 3 h and the maximum was 100 % at 5 and 10% concentrations in 6 h, for the olive methanol trapped smoke the minimum was 20 % at 1.25 % concentration at 3 h while, the maximum rate was 100% at 10 % concentration in 24 hours.

The major compounds detected in the neem hexane trapped smoke were Methyl Hexadecanoate (12.11%), Methyl stearate (4.88%). The major compounds detected in olive hexane trapped smoke were Methyl octadec-9-enoate (E)- (29.66%), Methyl (9Z,12Z) – octadeca-9,12-dienoate (15.35%), Methyl Hexadecanoate (13.54%), The methanol trapped smoke of the neem showed 3,5-ditert butyl phenol (29.35%), Methyl Hexadecanoate (12.93%), 1,2-15,16-Diepoxy hexa-decane (7.92%) as major compounds, and the olive methanol trapped smoke showed 2,6-ditert-butyl phenol (38.27%), Methyl Hexadecanoate (8.70%), (E) -Tetradec-2-ene (8.45%), as major compounds. From the leaves the essential oil of neem extract showed Methyl tetracosanoate (3.72%), 2,4-Bis (dimethyl benzyl)-6-t-butyl phenol (3.39%), Glabrol (2.79 %), as major compounds, and the olive essential oil extract were Benzene acetaldehyde (3.24%), N-desmethyltapentadol (1.90%), 1-(2,6,6-trimethyl cyclohexa-1,3-dien-2-en-1-one (1.88%) as major compounds.

Key word: Biological activities, antioxidant, insecticidal, bioassay, neem, and olive leaves.

1. INTRODUCTION

Plants are in use as sources of medicinal health care since the ancient time. The World Health Organization (WHO) estimated that 80% of the world uses traditional medicines which are produced mainly from natural products (animals and plants) for daily health care requirements. There are about, 35,000-70,000 species of plants that are used for medicinal purposes globally according to WHO. The medicinal plants are the major constitute of traditional system of medicine hence, many countries should give emphasis to develop and utilize local medications. Ethiopia is sixth center of biodiversity in the world that contains diversified flora and fauna. There are more than 6,500 species of plants of which 12% are endemic and about 887 species are used as medicinal plants. Almost 80% of the Ethiopian people depend on traditional medicine of which 95% of them are plant origin [1].

Plants and animals produce primary and secondary metabolites. The primary metabolites are like (proteins, carbohydrates, lipids, nucleic acid), and secondary metabolites are terpenoids, alkaloids, steroids, phenols, etc. which, are potential sources of bioactive compounds [2].

Bioactive compounds are highly beneficial for the treatment and preventions of diseases like cardiovascular and cancer. The protective abilities of bioactive compounds are associated to their antioxidant, insecticides, antimicrobial, antiviral or anti-carcinogenic effects. Plant extracts (polyphenols) are also protective ingredients in food, pharmaceutical and cosmetics industries as food additives, preservatives and dietary supplements instead of synthetic chemicals [3].

The neem tree commonly referred to *Azadirachta Indica* is from the meliaceae family. The neem tree can reach a height of 30 meters with a trunk girth of 2.5 meters. Its deep root system is well adapted to retrieving water and nutrients from the soil. The neem tree survives in hot, dry climates, even the shade temperatures often reach 50 degrees Celsius and annual rainfall ranges from 400 to 1,200 millimeters [4].

Olive tree is an evergreen tree, and the fruitiest tree in the world. It is believed to be originated from Mediterranean region. The plant is associated with human religious, social, culture, medicinal, and nutritional needs. The olive plant extract contains phenols, phenolic acids, catechol, tyrosol, and inhibited oxidative deterioration of refined vegetable oils. The olive leaves also, contain phenolic compounds which are used as antioxidant compounds [5].

Both neem and olive plants uses for antioxidant and insecticidal activities. Antioxidants are compounds that reduce free radical activity, and protect the components of cells, and biomolecules from oxidation by scavenging an electron from reactive oxygen species (ROS) such as superoxide, peroxy, and hydroxyl radicals. Many recent studies reported that people can reduce DNA damages, which cause risk for cancer and cardiovascular diseases, significantly by eating more fruits, vegetables, and other foods from plants that contain phytochemicals [6].

Insecticides are compounds that protect insects from attacking animals and plants, and it can be natural or synthetic. The compounds fall into several classifications including repellents, feeding deterrents, toxins, and growth regulators [7].

The objective of this work was to investigate biological activities (antioxidant & insecticidal) of the plants *Azadirachta indica* (neem) and *Olea eurpoaea* (olive) leaves. Investigation was made on the essential oils and smokes collected by burning the plant parts.

2. LITERATURE REVIEW

2.1. Medicinal Plants

Medicinal plants are important traditional medicinal systems in the world. Ethno pharmacological surveys are rational for the selection and scientific investigation of medicinal plants, since a huge number of people use an indigenous remedy effectively over extended periods of time. People uses many wild species of plants as food, medicine, cosmetics, clothing, perfume, shelter, fiber, fuel, religious, and other applications.

Developing countries use traditional medicinal plants as primary sources of healthcare. Ethiopia is a huge potential of medicinal plants, and their uses that provide a wide contribution to the treatment of human and livestock aliments. About 80% of the human population and 90% of livestock depend on traditional medicine for primary health care services, and most of these come from plants. As a result there are high expectation of enormous traditional knowledge, and uses of medicinal plant species [8, 9].

2.2. Phytochemicals of medicinal plants

Phytochemicals are chemical compounds of the plant origin; they are essential and have protective or disease preventive properties [10]. They act as natural defense system for host plant and provide color, aroma and flavor. The phytochemicals play a substantial role in the prevention from microbial, insecticidal or herbivorous. They contain secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, phenols, and glycosides which are antimicrobial, anthelmintic, antidiarrheal activities while, saponins and polypeptides are considered to have anticancer and antiviral activities respectively [11,12].

Table 1: Some medicinal plants used for different purposes in Ethiopia [13, 14.18.15]

#	Scientific name	Family name	Local name	Part	Mode of smoke	Treatments
1	*Allium staivvm.L*	Alliaceae	Nech shunkurt	. Root . Seed	. Inhalation . Oral	. Evil eye . Antibiotic
2	*Carissa spinarum L.*	Apocynaceae	Agam	Root	. Inhalation. . Fumigation . Fumigation	. Evil aye . Bleeding after delivery . Insect repellent
3	*Clausena ansata*	Ruteceae	Limch	. All part . Leaf	. Topical . Skin	. Exorcism . Skin rash
4	*Cordial aficana lam*	Boraginacea	Wanza	. Leaf / . Root	. Topical . Oral	. Nightmare, Liver disease, Amoebiasis
5	*Enchinops kebericho*	Asteraceae	Kebercho (O&A)	. Root . Shrub/root . Root . Root	. Inhalation, Smoke, /Nasally/Orally . Oral . Nasally, oral	. Evil aye . Febrile illness . Snake repellent . Internal Parasite . Febrile
6	*Ficus vasta forssk*	Moraceae	Shoal	Bark	. Inhalation . Anal	. Epilepsy . Hemorrhoid
7	*Ruta chalepensil L*	Rutaceae	Tenadam	Leaf	. Inhalation . Orally	. Evil aye . Deficient disorder . Common cold
8	*Crton macrostachyus*	Cuphorbiaceae	Bisana	. Root, Leaf . Leaf . Leaf, bark . Leaf	. Topical, and inhalation . Smoke, Skin . Nasal . Dermal	. Psychosis, . For good smell materials . Antifungal, Malaria. . Headache & Ring Worm
9	*Phytolacca*	Phytolacceae	Endod	. Root, Leaf . Root	. Skin . Inhalation, . Skin/ Orally	. Itching skin . Gonorrhea . Rabies
10	*Vernonia odoensis*	Asteraceae	Este mussie	Root Leaf	Oral	. Psychosis, Malaria . Tooth infection
11	*Vernanine amygdalina*	Asteraceae	Girawa	Root	Inhalation	Evil eye
12	*Otostegia integrifolia*	Lamiaceae	Tunjut (T)	. Leaf/bark . Root, Leaf, Herb	Inhalation, oral	. Psychosis Mallari Abagorba
13	*Aloe megalacantha*	Aloaceae	Ere (T)	. Bark, leaf juice, Herb	. Inhalational, oral . Skin,	. Evil eye . wound . Insect repellent
14	*Gymnosporia senegalensis*	Celastraceae	Kombolcha (O)	All parts	Oral	Evil eye
15	*Brachiaria brizontha*	Asteraceae	Maas'uwa (D)	All parts	Topical	Evil eye
16	*Silene macrosolen*	Caryophyllaceae	Wegert	Root	Inhalation	Evil eye
17	*Sida tenuicarpa Vollesen*	Malvaceae	Chifrig	Root, and Leaf	Topical, oral Skin	. Evil spirit . Foot wound

18	*Osyris quadripartita Decn.*	Santalaceae	Waatoo (Af)	Root	Inhalation	Evil sprite
19	*Plectranthus edulis Vatke*	Lamiaceae	Dinicha Oromo (O)	Leaf, root	Oral	Epilepsy
20	*Nigella sativa L*	Ranunculaceae	Tiqur-azmud	Seed	Skin	. Skin fungus . Antioxidant
21	*Ocimum lamiifolium*	Lamiaceae	Yemich-medhani	. Leaf . Leaf	. Skin . Nasal	. Mich . Headache
22	*Stereospermum kunthianum .C*	Bignoniaceae	Washinte	Stem bark	Oral	Snake and scorpion bites
23	*Azadirachta indica A. Juss.*	Meliaceae	Neem/kinin	Leaf, tree	Fumigation	Antioxidant, anti-malaria,
24	*Olea europaea subsp.*	Oleaceae	Weyra	. Leaf . Oil . Tree	. Inhalation . Smoking, and Fumigant	. Psychiatric disease . Body bath . Insect repellent

Local name of plants in Amharic (A), Oromigna (O), Tigrigna (T), Afar (Af), Debub H (D)

2.3. Plant derived smokes

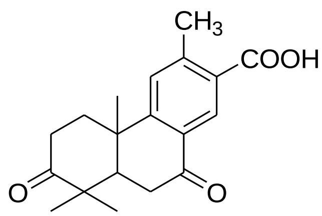
The use of smokes of medicinal plants has started from Mesopotamia, dated about 2600 BC; are still in use today for the treatment ailments ranging from coughs and colds to parasitic infections and inflammations [16]. In addition to its medicinal values, plant derived smoke has multiple uses, including air purification, ceremonial, flavoring [17].

Fumigation is commonly used mode processing plants parts for their use as medicine and insect repellents in Ethiopia. Mostly women use smoke as cosmetics to keep their skin clean and healthy [18]. There are different plant materials that are in use in the form of smoke to disinfect, heal and fragrance. This thesis focused on investigation of chemical compositions of smoke trapped from neem and olive leaves.

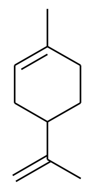
2.4. Chemistry of the Neem plant

Neem commonly known as *Azadirachta indica* is recently grow in about 72 worldwide countries, mostly in the tropical and subtropical countries. Neem has more than 135 unique bioactive compounds, which have been isolated from different parts of the plant. These compounds are categorized into isoprenoids including diterpenoids and triterpenoids containing liminoids, azadirone and its derivatives, genudin and its derivatives and csecomeliacins such as nimbin, salannin and azadirachtin. And non-isoprenoids including proteins (amino acids), carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, tannins, aliphatic compounds and phenolic acids [19,20].

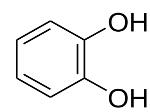
Terpenes are a unique group of hydrocarbon based natural products whose, structure is derived from isoprene. Terpenes are thus classified by the number of 5 carbon units they contain: Hemiterpenes C5, Monoterpene C10, sesquiterpene C15, Diterpene C20, Sesterterpene C25, Triterpene C30, and, tetratrepene C40.



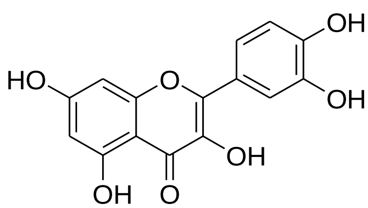
Margolonone (**1**)



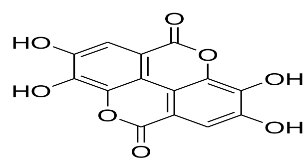
Limonene (**2**)



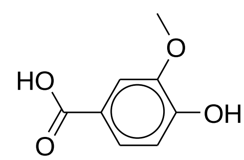
Phenol (catechol) (**3**)



Flavonoid (quercetin) (**4**)



Tannis (Ellagic acid) (**5**)

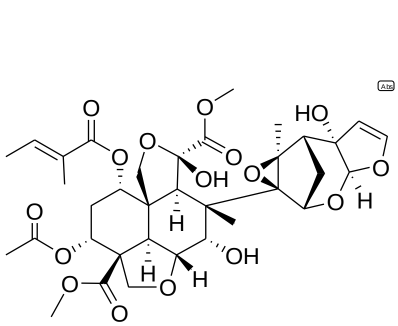


Phenolic acid (Vanillic acid) (**6**)

Figure 1 **:** Some bioactive compounds that are isolated from the neem plant [20, 21]

Table 2: Compounds that are isolated from neem plant, their sources, and biological activities and source [20, 21].

Compounds	**Source**	**Biological activity**	**Compounds**
Azadirachtin	Seed oil	Antimalarial	**7**
Nimbidin	Seed oil	Antigastric, antibacterial	**8**
Nimbin	Seed oil	Spermicidal	**9**
Margolone	Bark	Antibacterial	**10**
Epicatechin	Bark	Immunomodulatory	**11**
Catechin	Bark	Immunomodulatory	**12**
Cyclic trisulphide	Leaf	Antifungal	**13**
Gallic acid	Bark	Anti-inflammatory	**14**



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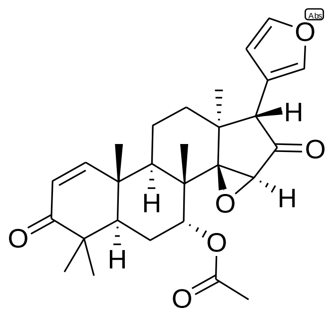

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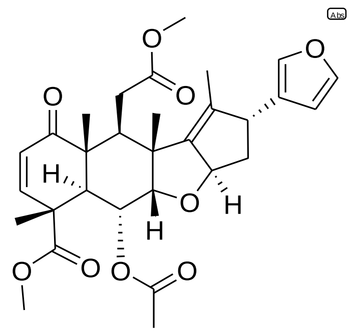

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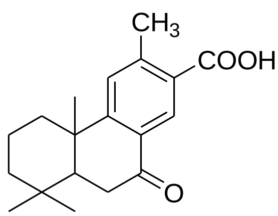

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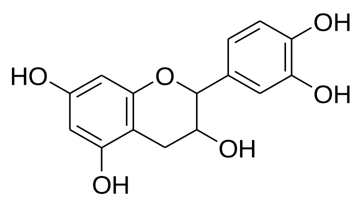

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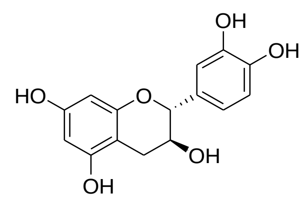

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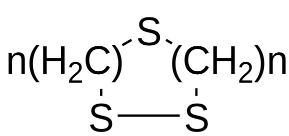

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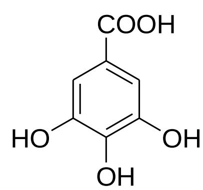

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Figure 2 : The bioactive compounds isolated from neem [21].

The most active compounds like alkaloids, ketones, phenolic compounds, carotenoids, flavonoids, steroids are effective against microorganism and ectoparacites including, bacteria, fungi, viruses, ticks, antimalarial, anticancer, antioxidant, antidiabetic, antifeedant, antitumor, antiseptic, antiviral, contraceptive, cosmetics, as oral deodorant, toothache reliever and tooth cleaners, insecticides, wound healing effects. Neem tree also used in agriculture as pesticides and fertilizer, medicine for diabetes, aids, cancer, heart disease, and, allergies. Generally, neem tree is one of the medicinal plants widely used in Ethiopia [22].

The compound Azadirachtin (**7**) is one a powerful insect repellent at low concentrations. Neem repels a wide variety of pests including, beet armyworm, aphids, the cabbage worm, white flies, mites, beetles, moth larvae, mushroom flies, leaf miners, caterpillars [23].

Neem plant used in the activation of antioxidative enzyme, that play a role in the control of problem caused by free radicals/reactive oxygen species. Most plant fruits, seeds, oil, leave, bark, and roots show an important role diseases prevention due to the rich source of antioxidant [24].

2.5. Chemistry of the olive plant

Olive tree, known as *Olea europaea* which belongs to the Oleaceae family have higher contents of bioactive compounds; including phenolic compounds, with diverse biological properties, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, in addition to regulating blood pressure, and cholesterol levels in animals. Thus, a previous study verified the effective antioxidant activity of olive leaf extract as a potential natural functional ingredient in food products [25].

Olive leaf is one of the potent sources of plant polyphenols having antioxidant, antimicrobial, antiviral properties due to its rich phenolic content. The most abundant phenolic component gives the bitter taste to olive and olive oil. Olive is rich in antioxidant compounds, especially tocopherols hydroxytyrosol, caffeic, protocatechuic, ferulic, syringic, and vanillic acids [26].

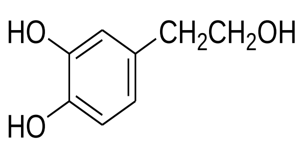
2.5.1 Phenolic compounds in the olive leaf and their antioxidants capacity

There are five groups of phenolic compounds present in olive leaves, namely oleuropeosides (oleuropein and verbasscoside), flavones (luteolin-7glucoside, apigenin-7-glucoside, diosmetin-7-, luteolin, and diosmetin), flavonols (rutin), flavan-3-ols (catechin), and substituted phenols (tyrosol, hydroxrosol, vanillin, vanillic acid, and caffeic acid). The antioxidant activity of flavonoids present in olive leaves are influenced by the present of functional group in their structure, mainly the B-ring catechol, the 3-hydroxyl group and the 2, 3-double bond conjugated [27].

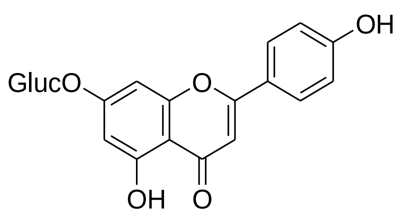
Table 3: The amounts of phenolic, and antioxidants extracted from olive leaves [28, 29].

Name of compounds	Amount in %	Antioxidant (TEAC) (mmol/L)
Olueropein	24.54	0.88 ± 0.09
Hydroxytyrosol	1.46	1.57 ± 0.12
Apigenin-7-glucoside	1.37	0.42 ± 0.03
Luteolin-7-glucoside	1.38	0.71 ± 0.04
Verbascoside	-	1.02 ± 0.07

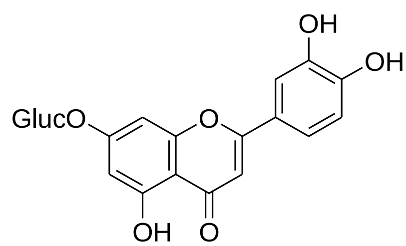
Antioxidant capacity of each individual phenolic compound based on their relative radical scavenging ability was found to be "Rutin > luteolin > olive leaf extract = hydroxytyrosol > caffeic acid > verbascoside > oleuropein > luteolin-7-glucoside-vannillic acid > apignin-7glucoside > tyrosol". Hydroxytyrosol a phenolic alcohol and one of the derivatives of oleuropein have demonstrated a good antioxidant property [29].



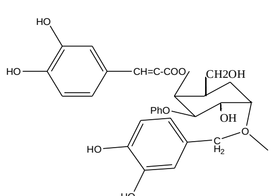
Hydroxtyrosol (**15**)



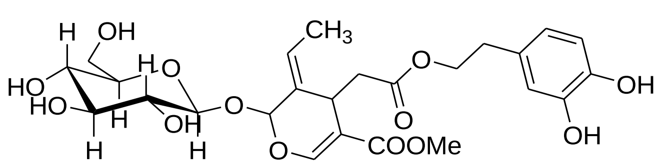
Apigenin-7-glucoside (**16**)



Luteolin-7-glucoside (**17**)



Verbasecoside (**18**)



Oleuropein (**19**)

Figure 3: Some of polyphenolic (antioxidant) compounds extracted from olive plant [30].

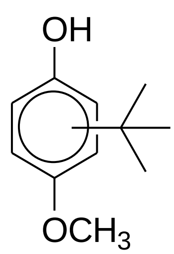
2.5.2 Oleuropein (19) and Its Properties

Oleuropein is the major phenolic compound in olive leaves. The Oleuropein is an ester of 2-(3, 4-dihydroxyphenyl) ethanol (hydroxytyrosol) with elenolic acid glucoside. Oleuropein has many benefits for health, including antioxidant, antimicrobial, anticancer, anti-inflammatory. Oleuropein uses as antioxidant at both prevention and intervention levels [31].

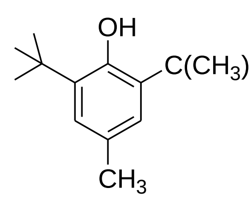
2.6. Antioxidant

2.6.1. Antioxidant Compounds

Antioxidants are compounds which are present at low concentrations compared to the oxidizable substrates that significantly delay or inhibit oxidization of those substrates. Antioxidants are mainly classified as; primary (chain breaking) and secondary (preventing). Antioxidants deactivate radicals by two major mechanisms, Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). Antioxidants that work with HAT mechanism quench free radicals simply by donating hydrogen atom while those of which work with SET mechanism transfer one electron to reduce any compound, such as metal ions, butylated hydroxyl toluene (BHT), and butylated hydroxyl anisole (BHA), most of them used in many foods additives, oils and fats [32].



Butylated hydroxyl anisole (BHA) (**20**)



Butylated hydroxyl tolune (BHT) (**21**)

Figure 4: The structures of some synthetic antioxidants

2.6.2. Free Radicals: - Free radicals are unstable and high energy atoms with an extra unpaired electron, produced unwanted metabolic by-products [33].

Free radicals in the body attack the nearby tissues by oxidizing membrane lipids, cellular proteins, DNA and causes complete shutdown of cellular activities such as respiration. Furthermore, the interaction of oxygen free radicals with members of lipidic portion of body leads us to formation of new radicals such as hydro peroxides, superoxide, lipoid oxides and hydroxyl radical whose type may interact with biological systems in a citotoxic manner [34].

DNA damage has been implicated as a cause of cancer prevention of oxidative stress caused by ROS and RNS has important implications for the prevention and treatment of diseases [35].

They interfere with the oxidation of lipids and other molecules by rapid donation to radicals (R):

$$R^{\cdot} + POH \rightarrow RH + PO^{\cdot} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

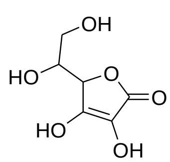
The phenoxy radical intermediates ($PO^{\cdot}$) are relatively stable due to resonance and therefore a new chain reaction is not easily initiated. Moreover, the phenoxy radical intermediates also act as terminators of propagation by reacting with other free radicals:

$$PO^{\cdot} + R^{\cdot} \rightarrow POR \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

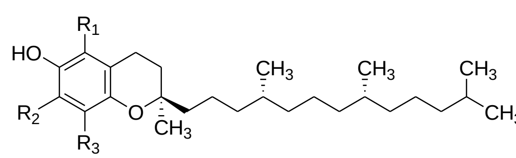
Phenolic compounds possess ideal structure for free radical scavenging activities because they have: (1) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; (2) extended conjugated aromatic system to delocalize an unpaired electron [36].

2.6.3 Compositions of Antioxidant compounds

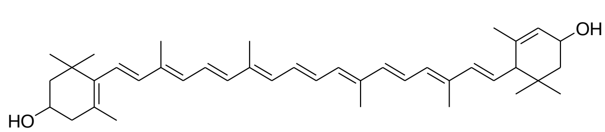
The most common antioxidants extracts or exists in plants are vitamin (C, A and E), Carotenoids, and phenolic compounds [37].



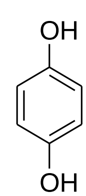
Vitamin C (ascorbic acid) (**22**)



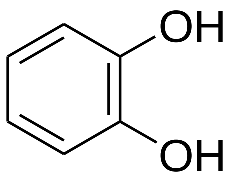
Vitamin E (Tocopherols) (**23**)



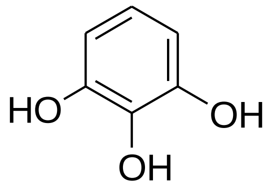
Carotenoid (Lutein) (24)



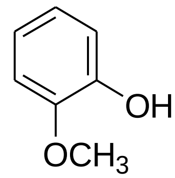
Hydroxy phenol (**25**)



Catechol (**26**)



Pyrogallol (**27**)



hydroxy anisole (**28**)

Figure 5: Representative of antioxidant compounds [37, 38].

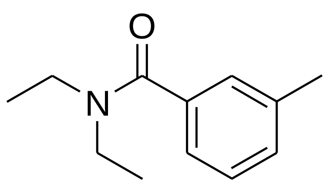
Table 4: Some plants commonly uses as the potential source of antioxidant [39].

Number	Name of plant	Common English name	Family	Local name	Plant part used
1	*Allium cepa*	Onion	Amaryllidacea	Key shunkurt	Bulp
2	*Aloe vera*	Aloe vera	Xanthorovhoeaceae	Ret	Leaf
3	*Azadirachta inidica*	Neem	Maliacea	Neem/kinin	Leaf
4	*Beta vuulgaris*	Beet root	Amaranthaceae	Key sir	Root
5	*Camellia sinersis*	Green tea	Theaceal	shay kittel	Green tea
6	*Cinnamomum tamala*	Tejpat	Lauranceae	Kerfa	Tejpat
7	*Curcum longa*	Turmeric	Lingiberaceae	Erd	Turmeric
8	*Daucuscarota*	Carrot	Apiaceae	Carrot	Root
9	*Emblica officinalis*	Amla	Euphorbiaceae	Machchew	Fruit
10	*Ocimum sanctum*	Tulsi	Lamiaceae	Damakesse	Leaf
11	*Zingiber officinale*	Ginger	Zigiberaceae	Zinjibele	Rhizome
12	*Solanum nigrum*	Blacknightshade	Soanaceae	Tikur awitt	Leaf
13	*Solanum tuberosum*	Potato	Soanacea	Dinich	Tuber
14	*Terminalia bellarica*	Behda	Combretaceae	Weyefba	Fruit

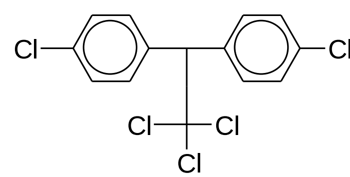
The medicinal plants are an important source of antioxidants which increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The secondary metabolites like phenolic and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots, and barks [39].

2.7. Insecticidal

Insecticides contain essential compounds preventing attack from phytophagous insects. The Insecticides are categorized as repellents, feeding deterrents, toxins, and growth regulators. Most can be grouped into five major chemical categories: (1) nitrogen containing compounds (primarily alkaloids), (2) terpenoids, (3) phenolic, (4) proteinase inhibitors, and (5) growth regulators. The gold-standard synthetic repellents DDT (Dichloro diphenyl trichloro ethane) and DEET (N, N-diethyl-3-methylbenzamide), are highly conserved across insect species.



N-N-Diethyl- m-toluamide (DEET) (**29**)



Dichloro diphenyl trichloro ethane (DDT) (**30**)

Figure 6: The structures of some synthetic insecticidal

Plant-based repellents are still extensively used in traditional way throughout rural communities as it is the only means of protection from mosquito in developing countries [40]. Plant-based repellents are safer than DEET since they are natural and less side effects, better for the environment than synthetic molecules, and simply have been used for a long time with tested safety record [41].

In Ethiopia, wogert, (Silene macroserene), kebericho (Echinops kebericho), tinjut (Ostostegia-integrifolia), and woira (Oleaeuropaea) and, neem have been shown to have repellent effects against insects is under laboratory conditions [42].

Table 5: Neem extract tested for Insect repellence.

The percentage repellency and EPI value of treated (cockroaches) with different concentrations of the power of *Azadirachta indica* for 1, 6, & 24 hours [43].

PLANT NAME	TREATMENT	TIME					
		1 Hours		6 Hour		24 Hour	
		EPI	PC	EPI	PC	EPI	PC
Neem	0d.5 gm (25%)	-0.38	70%	-0.52%	77%	-0.46	74%
	1.0 gm (50%)	-0.57	79%	-0.60	81%	-0.71	86%
	1.5 gm (75%)	-0.71	86%	-0.73	87%	-0.80	90%
	2.0 gm (100%)	-0.77	98%	-0.80	90%	-0.85	93%
	Control (biscuit)	0	0%	0	0%	0	0%

*PC = percentage of repellency *EPI = excess proportion index (EPI) < 1 Repellency, EPI > 1. Attractant, EPI = 1, Neutral

Table 5: Shows the repellency of Periplanata Americana (cockroaches), treated with different concentrations of the powder of *Azadirachta indica*. The highest repellency was recorded with the highest repellency dose of (2.0 g) neem powder.

Table 6: Mean percentage mortality of P. Americana (Cockroaches) treated with different concentrations of the powder of *Azadirachata indica* for 3 days [44].

Treatment	Mean Percentage Mortality For 3 Days		
	Day 1	Day 2	Day 3
0.5g (25%)	0.00	6.67	6.67
1.0g (50%)	8.33	33.34	33.34
1.5g (75%)	0.00	0.00a	33.34
2.0g (100%)	66.67	83.34	83.34
Control (biscuit)	0.00	0.00	0.00

The maximum mortality of cockroaches recorded at a maximum concentration was 83.34%.

2.8.Extraction of Essential oil

Essential oils contain volatile substances that are isolated by a physical method or process from plants. The essential oils are so termed perceived to represent the very essence of odor and flavor. The essential oils are of a great benefit to help protect our bodies and homes from pathogens. The immune system needs support and required endorsement of essential oils. Essential oil can be extracted using a variety of methods, depending on the plant material to be distilled, and the desired product. The essential oils can be extracted by steam distillation and hydrodistillation, microwave extraction, soxhlet extraction, supercritical fluid extraction method, solvent extraction, and cold pressed expression methods [45].

2.8.1. Steam distillation and hydrodistillation

Steam distillation method is used for the extraction of essential oil from the plant materials. It is a special type of distillation process for temperature sensitive materials like oils, resins, hydrocarbons, in which they are insoluble in water and may decompose at their boiling point [46]. Hydro distillation process involves that the plant materials to be extracted are completely immersed in water, and boiled by applying heat. The boiling water would carry the less volatile materials with it and condensed in a condenser attached to the round bottomed flask. It's recommended that the process is performed under atmospheric pressure at the boiling temperature about 100 °C [47].

2.8.2. Microwave assisted extraction (MAE)

Microwaves generate heat by interacting with polar compounds such as water and some organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms that allow the extraction of essential oils. The transfers of heat and mass are in the same direction in a MAE, which generates a synergistic effect to accelerate extraction and improve extraction yield. The application of MAE provides many advantages, such as increasing the extract yield in addition to avoid decomposition of the components [48, 49].

2.8.3. Soxhlet extraction

This method integrates the advantages of the reflux extraction and percolation, which utilizes the principle of reflux and siphoning to continuously extract the herb with fresh solvent. This extraction is an automatic continuous extraction method with high extraction efficiency, requires less time, and solvent consumption than the other. The high temperature and long extraction time in the Soxhlet extraction will increase the possibilities of thermal degradation [50].

2.8.4. Supercritical fluid extraction (SFE)

It has similar solubility to liquid and similar diffusivity to gas, and can dissolve a wide variety of natural products. Their solvating properties dramatically changed near their critical points due to small pressure and temperature changes. Supercritical carbon dioxide was widely used in SFE because of its attractive merits such as low critical temperature (31 °C), selectivity, inertness, low cost, non-toxicity, and capability to extract thermally labile compounds. The low polarity makes it ideal for the extraction of non-polar natural products such as lipid and volatile oil [51].

2.8.5. Solvent extraction

The hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing resin or a combination of wax, and essential oils remains. The principle of solid liquid extraction is when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent. The extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent and this takes place in a concentration gradient. It is one of the methods used in the preparation of oils from flowers, in which flower petals are mixed with volatile solvents such as a petroleum ether or benzene until all the volatile oils dissolve in the solvent, and the solvent is evaporated at a low pressure [52].

2.8.6. Cold pressing

This technique is an extraction without heating. This method is used to extract acid oils such as orange and lemon, grapefruit and mandarin. The principle of this mechanical process is based on machine squeezing the citrus at room temperature for the release of essential oils. One way is to puncture the oil glands and then the fruit to remove oil from the glands and wash with a little spray of water. It is important to note that oils extracted using this method have a relatively short shelf life [53].

3. OBJECTIVE OF THE WORK

3.1. General objective

The main objective of this study is:-

To extract, analyze and conduct biological activities of essential oils and smokes collected from plant materials that are used locally as fragrances and for disease control.

3.2. Specific objectives

- extract essential oils from the neem and olive leaves by hydro distillation.
- collect smoke from the neem and olive leaves by burning the plant materials and trapping the smokes using hexane and methanol solvents.
- analyze the extracts by GC-MS.
- test the antioxidant activities of the extracts using UV-visible spectroscopy.
- test the insecticidal activities (repellent and mortality) rates of the smokes on a selected insect.

3.3. Significance of the study

This study provide scientific information concerning smoke as traditional medicine, and it also led to the discovery of potential bioactive compounds from smoked medicinal plants which can help as antioxidant, and insecticidal activities.

4. EXPERIMENTAL PART

4.1. Materials and Methods

4.1.1. Plant materials

The neem leaf (mogersa, kinin) and olive leaf (weyra local name) used in this study were collected from Addis Ababa area. The plant materials were allowed to dry under shade and burned to collect smoke. Essential oils from both plants were extracted using Clevenger apparatus.

Figure 7: Photo of a) neem leaf b) olive leaf

4.1.2. Equipment's /Apparatus/ and Chemicals used

The equipment used to conduct this study was hydrodistillation set up, locally manufactured electrical stove, Petridish, insect repellent device set up, Suction flasks (250 mL), Rubber tube, funnel, different size Erlenmeyer flask, rotary evaporator, Spatula, Watch glass, oven, What-man number 1 filter paper, digital balance, UV-Visible spectra meter (model), thermometer, Gas chromatography-Mass spectrometer (GC-MS) (Model: GC-7820A, Agilent Technologies; Detector-5977EMSD, USA) Column: DB-1701, (30 m × 0.250 mm), and 0.25 micrometer particle size), tong, measuring cylinder, heating gun.

All chemicals, reagents, and solvents used in the experiment are hexane, methanol, Dichloro methane (DCM), ethyl acetate, ascorbic acid (standard)**,** buffer solution of (NaH_2PO_4, and Na_2HPO_4), 1% of potassium ferric cyanide, 10% of trichloro acetic acid (TCA), and 0.1% of ferric chloride ($FeCl_3$).

4.1.3. Essential oils extraction

Essential oil of the neem was obtained by hydro distillation. The fresh leaves (1 kg) of neem were inserted in to a distillation flask containing distilled water (2 L). The flask was attached to a Clevenger apparatus, which was attached to a condenser. Hydrodistillation continued at for 4 hrs after initial boiling. The essential oil was separated from the aqueous phase, and the essential oils were stored at 4 °C in refrigerator until it was analyzed by GC-MS. Following the same procedure, 1 kg fresh leaves of olive was used for extraction of the essential oil. The yields (v/w, mL/kg) were calculated using the amount of essential oil (mL) or mass (gram) obtained relative to the fresh weight (kg) of the sample used.

Figure 8: Hydrodistillation set up.

4.1.4. Smoke collection

The neem and olive leaves were chipped off separately into small pieces and air dried at room temperature. The dried sample plant materials (300 g) was burned by an electrical stove smoker with a small opening underneath which is designed for the inlet of air to keep the plant material burning and increase the amount of smoke entering in to methanol and hexane solvents. The smoke produced from the burning plant material was collected using the inverted funnel fitted with a heat resistance rubber tube. Then, smoke flows from the smoker through the heat resistance rubber tube, and, the other end of heat resistance rubber tube was fitted with a glass tube which passed through the hole of stopper (cork) and placed below the surface of 100 mL methanol in 250 mL suction flask. The stopper was chosen so that the outer diameter of the stopper fits the inner diameter of the mouth of the flask. The arm of the flask was connected to the second suction flask by rubber tube which was fitted into glass tube on the other end. Glass tube was passed through the opening of stopper on the mouth of the flask and placed below the surface of 100 mL hexane in 250 mL suction flask. The advantage of the second suction flask (100 mL hexane in it) was to condensate smoke which was not trapped by methanol in the first flask. A water aspirator is attached to the side arm of the second flask [54]. The vacuum generated by the aspirator was used to draw the smoke from the smoker into solvents (Methanol & Hexane), as shown in Figure 9. The smoke extracted was concentrated using a rotary evaporator and analyzed.

Figure 9: Smoke trapping and extraction system.

4.2. Gas chromatography mass spectroscopy (GC-MS)

The essential oils and smoke were analyzed by gas chromatography (Agilent technologies 7820A GC system coupled with Agilent technologies 5977E MSD, USA). The chromatographic capillary column (DB-1701) 30 m long and 0.25 mm in internal diameter was used. Ultra-high pure (99.999%) helium gas, as the carrier gas, was used at a constant flow mode. An Agilent 7820A auto sampler was used to inject 1 µL of the sample with a splitless injection mode into the inlet heated to 275 ºC. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min, and then, the temperature was increased at a rate of 10 ºC/min until the column temperature was reached 200 ºC, and then heated at the rate of 3 ºC/min till the temperature reached 240 ºC. No mass spectra were collected during the first 4 minute of the solvent delay. The transfer line, the ion source and ion filter temperature were 280 ºC, 230 ºC and 150 ºC respectively. The parameters, such as the quality, and probability values of peaks identifications was made through a library search using NIST 2014.

4.3. GC-MS sample preparation

4.3.1. Smoke sample preparation

A 0.2 mg of sample of hexane extracted smoke was dissolved in DCM solvent to form 10 mL solution with a concentration of 0.02 mg/ml (20 ppm). The smoke trapped in methanol was also prepared in similar way.

4.3.2. Essential oils sample preparation

5 µl of essential oil was added in to DCM solvent to form 25 mL solution (200 ppm). 0.1 mL solution of 200 ppm was added to 0.9 mL DCM solvent to form 1 mL (20 ppm) solution. One µL solution was analyzed by GC-MS.

4.4. Antioxidant activity determination

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The presences of antioxidant in the extracts result into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} in to Fe^{2+}, there by changing the solution into various shades from green to blue, depending on the reducing power of the compounds. Strong reducing agents, however, formed Perl's Prussian blue color and absorbed at 700 nm. The higher the absorbance of the reaction mixture, the higher would be the reducing power. It has been reported that the reducing power of substances is probably because of their hydrogen-donating ability. The donors react with free radicals to convert them into more stable products, and then terminate the free radical chain reactions [55].

The reducing ability assay is based on the principle that substances, which have reduction potential, react with potassium ferric cyanide ($K_3Fe^{3+}(CN)_6$) to form potassium Ferro cyanide ($K_4Fe^{2+}(CN)_6$), which then reacts with ferric chloride to form a ferric-ferrous complex that has an absorption maximum at 700 nm.

Potassium ferric cyanide + ferric chloride $\xrightarrow{\text{antioxidant}}$ potassium ferric cyanide + ferrous chloride

$K[Fe(III)(CN_6)]^{3+}$ + Ph-OH → $Kx(Fe_n^{3+}[Fe^{+2}(CN)_6])$ + Ph-O• + H^+ .... (1)

(Yellowish color) (Color of essential oil) (Greenish-blue color)

$K_3Fe^{3+}(CN)_6$ (aq)+Iron chelator [red] (aq)→$K_4Fe^{2+}(CN)_6$ (aq)+ Iron chelator [ox] (aq)…..(2)

$K_4Fe^{2+}(CN)_6(aq) + FeCl_3(aq) \rightarrow KFe^{3} + Fe^{2+}(CN)_6(aq) + 3KCl\ (aq)$ …………………(3) [56].

The antioxidant activities of the smoke extract was performed by UV-Visible analysis using the standard ascorbic acid solution and prepared solutions.

4.4.1. Preparation of reagents

A 200 mM sodium phosphate buffer solution (NaH_2PO_4, and Na_2HPO_4) prepared as, 34.836 g Na_2HPO_4 was dissolved in 100 mL distilled water to gives 2 M solution, and 27.218 g of NaH_2PO_4 was also dissolved in 100 mL of distilled water gives 2 M solution. The two solutions were mixed up in the ratio of 38.1 mL of Na_2HPO_4 and 61.9 mL of NaH_2PO_4. The PH of prepared solution has been checked using pH meter at room temperature (adjust if it is not pH = 6.6), the combined stock solution (2 M) was diluted to 1 litter with distilled water to obtain a 0.2 M solution. 1% potassium ferric cyanide was prepared by dissolving 1gram of potassium ferric cyanide in100 mL of distilled water. 10% TCA was prepared by dissolving 10 grams of TCA in 100 mL, and 0.1% $FeCl_3$ was prepared by dissolving 0.05 g of $FeCl_3$ in 50 mL.

4.4.2. Preparation of stock solution

The stock solution was prepared by dissolving 0.0088 grams of ascorbic acid in 500 mL to get a concentration 17.6 µg/mL. The remaining concentrations (0.176 µg/mL, 0.88 µg/mL, 1.76 µg/mL, 3.52 µg/mL, 7.04, 10.56 µg/mL) were prepared from the stock solution by a serial dilution method. The calibration curve was drawn from these concentrations, and the reducing power of the extract and smoke of a different concentration would be determined according to [57].

4.4.3. Samples preparation and UV analysis

The samples were prepared by dissolving 0.3 gram of smoke trapped in methanol in 2 mL of methanol solvent, then from the prepared sample solution 0.5 mL was dissolved in to 48 mL of methanol, and mixed with 2.5 mL of 200 mM or (0.2 M) sodium phosphate buffer of pH = 6.6 and 2.5 mL of 1% of potassium ferric cyanide [$K_3Fe\ (CN)_6$]. Shaked well using vortex shaker and the mixture were incubated in water bath at 50 ^{0}C for 20 minutes. Then 2.5 mL 10%TCA added to the mixture and centrifuged at 300 rpm for 10 minutes. Then 2.5 mL upper layer was taken and mixed with 2.5 mL of distilled and 0.5 mL of 1%$FeCl_3$ mixed well and let to stand for

10 minutes at room temperature. Then samples of 500 µL, 1000 µL, 1500 µL were diluted with a solvent (distilled water) 3.5 mL, 3 mL, & 2.5 mL respectively to form 4 mL volume solution. The prepared solution was added to cuvettes for UV measurement. The antioxidant activity of the samples was observed at 700 nm against the blank solution as control. The concentration of the sample complex was calculated by stating equivalent to regression equation of ascorbic acid calibration curve.

4.5. Insecticidal (repellents) tests

The insecticidal (repellent) activity was conducted using Hexane and Methanol trapped smoke. In each case 15 mg of the sample was placed on one side of the set up and control (water or solvent) 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.

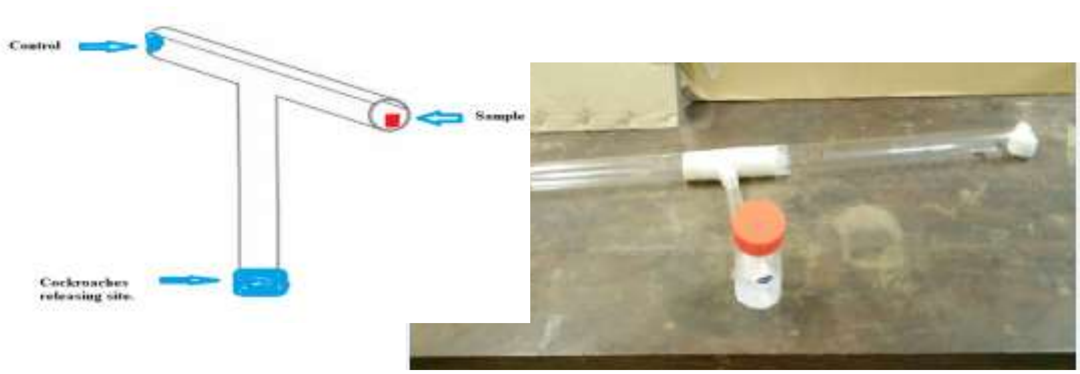


Figure 10: A set up to test repellent activity of the smoke samples

According to Abbot's formula's, and the device set up, the % insect repellent calculated as:

$$\text{Repellent (\%)} = \frac{\text{(\# of cockroach in the control side (CC)} - \text{(\# cockroach sample side (CS))}}{\text{Total \# of cockroaches in the control and sample sides}} \times 100$$

$$\text{Repellent (\%)} = (\#\,CC - \#\,CS) \times 100 \,/\, (\#\,CS + \#\,CC)$$

4.6. Insecticidal (mortality) tests

4.6.1. Bioassays of extracts of neem and olive leave smokes against cockroaches

Bioassay methods that are commonly employed for insecticide toxicity evaluation are topical application, potter’s tower method, and injection method, dipping method, contact or residual method, and film method. Film method is the technique used for this experiment.

Film method is the technique involves insecticide solution is usually deposited on glass surface such as Petri dish, flask, vial, wide mouth jar etc. In this approach Petri dishes (90 mm diameter) were lined with a filter paper and sample solutions (1 mL) was applied and allowed to dry. Samples were dissolved in appropriate solvents, methanol for the methanol extract, and DCM for the hexane extract. Using a 1.5 g solid sample, 20% stock solutions were prepared and diluted to get 10%, 5%, 2.5%, and 1.25% solutions and controls were prepared using blank solvents. After leaving for overnight for solvents to vaporize, 5 cockroaches were placed in a Petri dish. Experiments were conducted in a time course fashion to follow the mortality rate. Data was collected at 3, 6, 9, 12, 24 and 36 hours. All the measurements were done in triplicate and results were recorded as mean, and standard deviation. [58].

Figure 11: Mortality rate tests

5. RESULTS AND DISCUSSION

5.1. The percentage yield of the essential oil, and smoke extracts

The percentage yield of the extract was calculated from the relationship between the mass/volume of the extract and the mass or volume of the raw material used for the extraction. The % yield = (mass of the extract ×100) / mass of the sample, which is reported in the table below.

Table 7: Amount of essential oils, and smoke obtained from the extracts

No.	Extract Species	Volume obtained (mL)	Mass obtained (g)	Yield (%)
1	Neem essential oil	3	2.7	0.27
2	Olive essential oil	2	1.8	0.18
3	Neem methanol trapped smoke	-	5.5	1.83
4	Neem hexane trapped smoke	-	3.2	1.07
5	Olive methanol trapped smoke	-	6.1	2.33
6	Olive hexane trapped smoke	-	3.8	1.27

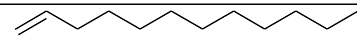
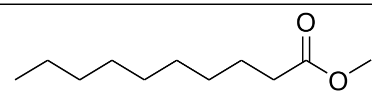
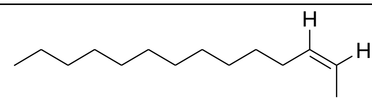
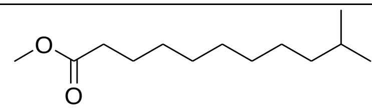
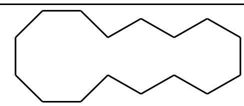
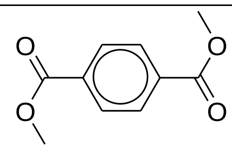
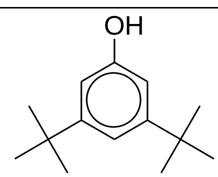
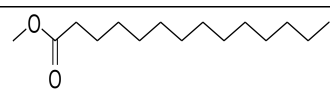
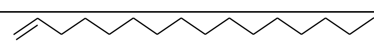
The amount of sample used for the essential oil extraction was 1 kg, and for the smoke extraction was 300 g. As it can be seen from the table above, the amount of smoke extracted in methanol is high in yield compared to the extract in hexane. The olive methanol smoke gave 2.33 % while that of neem was 1.83%. Relatively the amount of smoke in both solvents is higher than the essential oil extracted from the two plants. The extracts yield between 0.01 to 10% categorized as standard essential oils extract.

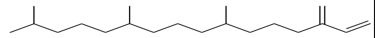
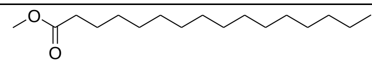
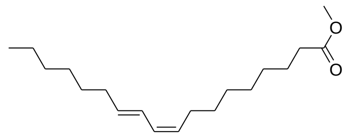
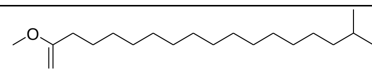
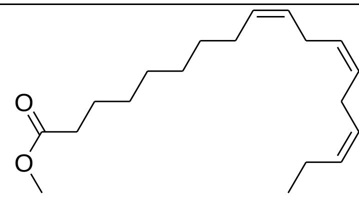
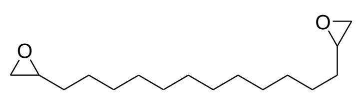
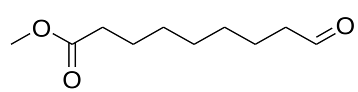
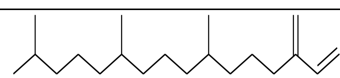
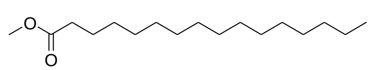
5.2.GC-MS analysis results

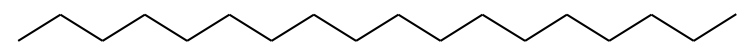
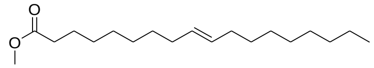
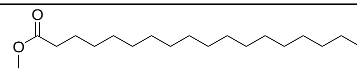
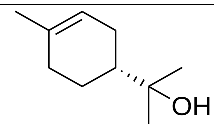
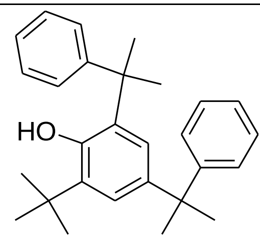
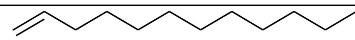
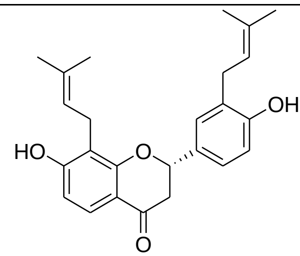
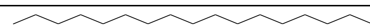
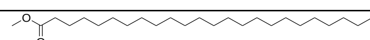
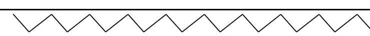
5.2.1. Characterization of essential oils and smokes

From GC-MS Analysis the result identified in terms of the total number of compounds and compounds with qualities ≥ 70% subjected to analysis with the help of NIST library 2014 mass spectral library. The following table shows GC-MS analysis results for the essential oils and smokes collected from neem and olive leaves.

Table 8: GC-MS analysis results of neem leaves essential oil and smokes (trapped by hexane and methanol).

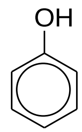
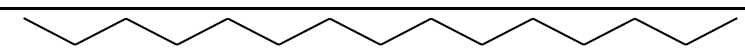
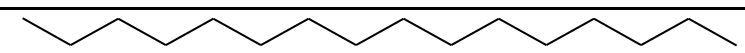
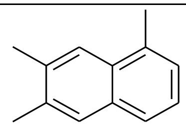
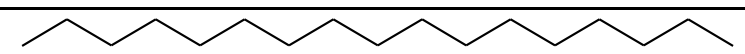
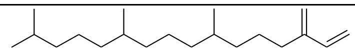
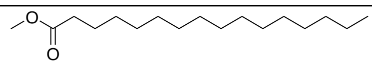
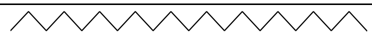
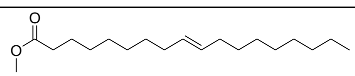
Sample name	Chromatogram Peak #	Name	Structure	Molecular Formula	MW	Retention time (t_R)	Area%	Retention index (RI)	Quality (%)
Neem leaves smoke trapped by methanol (NM)	1	1-Dodecene		$C_{12}H_{24}$	168.32	8.89	5.64	1092.22	96
	2	Methyl decanoate		$C_{11}H_{22}O_2$	186.29	11.57	3.96	1286.38	97
	3	(E)-2-Tetradecene		$C_{14}H_{28}$	196.37	11.68	6.51	1496.12	98
	4	Methyl, 10-methylundecanoate		$C_{13}H_{26}O_2$	214.34	14.10	2.28	1484.53	96
	5	Cyclohexadecane		$C_{16}H_{32}$	224.42	14.18	4.59	1649.10	94
	6	Dimethyl benzene-1,4-dicarboxylate		$C_{10}H_{10}O_4$	194.18	14.94	1.75	1081.56	97
	7	**3**,5-Di-t-butylphenol		$C_{14}H_{22}O$	206.32	15.11	29.35	14688.91	87
	8	Methyl tetradecanoate		$C_{15}H_{30}O_2$	242.40	16.39	1.0934	1686.95	97
	9	Cetene		$C_{16}H_{32}$	224.42	16.44	1.61	1745.30	99

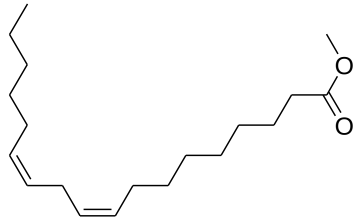
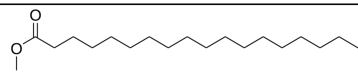
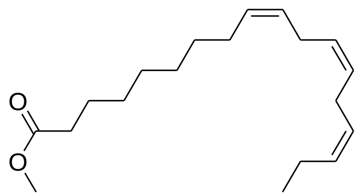
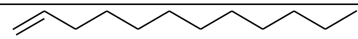
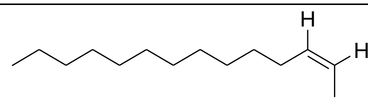
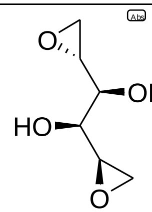
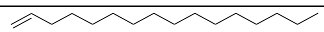
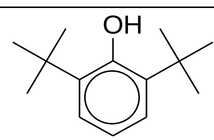
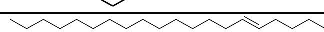
	10	Neophytadiene		C20H_{38}	278.52	17.11	2.86	1980.06	99
	11	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	12.93	1912.21	99
	12	Methyl (9*E*,11Z)-octadeca-9,11-dienoate		$C_{19}H_{34}O$	294.47	22.10	7.14	2119.73	98
	13	Heptadecanoic acid, 16-methyl-, methyl		$C_{19}H_{38}O_2$	298.50	22.27	4.57	2126.03	98
	14	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate		$C_{19}H_{32}O_2$	298.50	22.40	5.59	2131.57	99
	15	1,2-15,16-Diepoxyhexadecane		$C_{16}H_{30}O_2$	254.41	22.66	7.92	1680.09	86
Neem leaves smoke trapped by Hexane (NH)	1	Methyl 9-oxononanoate		$C_{10}H_{18}O_3$	186.25	14.30	0.33	-	70
	2	Neophytadiene		$C_{20}H_{38}$	278.52	17.11	0.3237	1882.03	99
	3	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	12.13	1912.26	99

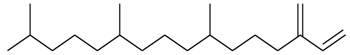
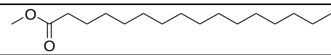
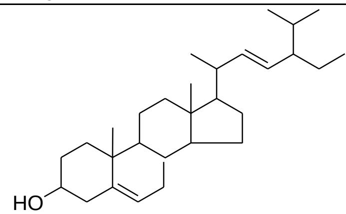
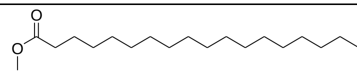
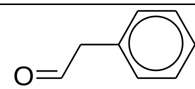
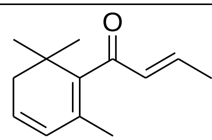
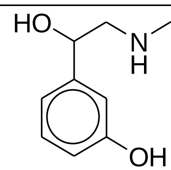
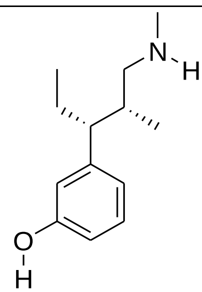
	4	Octadecane		$C_{18}H_{38}$	254.49	20.33	0.62	2014.73	89
	5	Methyl octade-9-enoate		$C_{19}H_{36}O_2$	296.49	22.01	0.59	2116.32	99
	6	Methyl stearate		$C_{19}H_{38}0_2$	298.50	22.27	4.88	2059.79	99
Neem Essential oil	1	Alpha.-Terpineol (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol)		$C_{10}H_{18}O$	154.25	10.37	0.29	-	93
	2	2,4-Bis(dimethylbenzyl)-6-t-butylphenol		$C_{28}H_{34}O$	386.65	14.63	3.40	-	99
	3	1-Docosene		$C_{12}H_{24}$	168.32	19.01	0.47	1725.22	99
	4	Glabrol		$C_{25}H_{28}0_4$	392.50	22.71	2.78	-	95
	5	Heptadecane		$C_{17}H_{36}$	240.47	26.90	2.60	2266.36	95
	6	Tetracosanoic acid, methyl ester		$C_{25}H_{50}O_2$	382.66	27.58	3.72	-	96
	7	Eicosane		$C_{20}H_{42}$	282.54	28.43	2.23	2026.16	92

Similarly the following table contains GC-MS data of the olive leaves sample

Table 9 GC-MS analysis results of olive leaves essential oils and smokes (trapped by hexane and methanol).

Sample name	Chromatogram Peak#	Name	Structure	Molecular Formula	MW	Retention time (t_R)	Area %	Retention index (RI)	Quality (%)
Olive leaves smoke trapped by Hexane (OH)	1	Phenol		C_6H_6O	94.11	9.12	1.82	-	90
	2	Pentadecane		$C_{15}H_{32}$	212.41	12.94	1.27	1548.36	96
	3	Hexadecane		$C_{16}H_{34}$	226.44	14.15	1.71	1647.96	96
	4	Naphthalene, 1,6,7-trimethyl-		$C_{13}H_{14}$	170.25	15.25	1.42	1525.54	98
	5	Heptadecane		$C_{17}H_{36}$	240.47	15.30	1.43	2954.43	97
	6	**Neophytadiene** (7,11,15-trimethyl-3-methyl idenehexadec-1-ene)		$C_{20}H_{38}$	278.52	17.13	6.41	1980.77	94
	7	Nonadecane		C_{19}H40	268.52	17.60	1.31	1944.42	97
	8	Methyl **hexadecanoate**		$C_{17}H_{34}O_2$	270.45	19.00	13.54	1913.24	99
	9	Heneicosane		$C_{21}H_{44}$	296.57	20.48	1.30		98
	10	Methyl octadec-9-enoate		$C_{19}H_{36}O_2$	296.49	22.04	29.66	21175.51	99

	11	Methyl (9Z,12Z)-octadeca-9,12-dienoate		$C_{19}H_{34}O_2$	294.47	22.13	15.35	2120.99	99
	12	Methyl stearate		$C_{19}H_{38}O_2$	298.50	22.30	9.52	2127.51	99
	13	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate		$C_{19}H_{32}O_2$	298.50	22.43	2.98	2132.80	99
Olive leaves smoke trapped by Methanol (OM)	1	1-Dodecene		$C_{12}H_{24}$	168.32	8.89	6.79	1250.02	96
	2	(E)-2-Tetradecene		$C_{14}H_{28}$	196.37	11.68	8.45	1449.52	98
	3	1,2:5,6-Dianhydromannitol		$C_6H_{10}O_4$	146.14	12.00	4.60	-	90
	4	Cetene		$C_{16}H_{32}$	224.42	14.18	5.72	1649.73	95
	5	Phenol, 2,6-bis(1,1-dimethylethyl)-		$C_{14}H_{22}O$	206.32	15.11	38.27	1578.59	87
	6	(E) - 5-Eicosene		$C_{20}H_{40}$	280.53	16.44	1.89	1956.60	99

	7	Neophytadiene		$C_{20}H_{38}$	278.52	17.11	5.85	1980.02	98
	8	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	8.7054	1912.19	98
	9	Stigma sterol		$C_{29}H_{48}O$	412.69	21.13	5.84	-	96
	10	Methyl stearate		$C_{19}H_{38}O_2$	298.50	22.27	2.68	2126.30	96
Olive Essential Oil	1	Benzene acetaldehyde		C_8H_8O	120.15	8.69	3.24		80
	2	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-		$C_{13}H_{18}O$	190.28	13.08	1.81	1447.92	80
	3	Dl-Phenylephrine		$C_9H_{13}NO_2$	167.20	16.30	1.04	-	72
	4	N-Desmethyltapentadol		$C_{13}H_{21}NO$	207.31	16.59	1.90	1573.19	72

From the above tables, phenolic compounds appeared in neem and olive methanol trapped smoke. The leaves hexane trapped smoke mainly contain esters and alkanes as major compounds which are consistent with the absence of antioxidant activities of the extracts.

Table 10 below shows the classes of compounds that were identifies by GC-MS in the extracts.

Table 10: Classification of the compounds

Sample extracted	Total number of Compounds	Number of Compounds ≥70% quality	Major compound	Number of compounds In (%)	Area (%)
Neem essential oil	15	7	Phenolic Alkane Ester Alkene Terpene	28.57 14.28 14.28 14.28 14.28	40.42 30.28 24.33 3.07 1.90
Olive essential oil	34	4	Phenolic Terpene / ketone Aromatic	50 25 25	36.80 22.65 40.55
Neem methanol Trapped smoke	17	15	Phenolic Ester Alkene Terpene	6.67 53.33 20 7.7	34.74 30.74 16.29 3.96
Neem hexane Trapped	9	6	Ester Alkane Terpene	66.67 16.67 16.67	98.19 3.39 1.75
Olive methanol Trapped Smoke	12	10	Phenolic Alkene Ester Terpene Alcohol	10 40 20 10 10	43.10 25.73 12.62 6.60 6.58
Olive hexane Trapped smoke	18	13	Ester Alkane Terpene Phenol	38.46 38.46 7.69 7.69	81.0 8.00 7.31 2.08

Area % of the compounds ≥ 70 % quality was calculated as:

$$\text{Area \%} = \frac{\text{Total area \% of the same functional group compounds in the extract}}{\text{Total area \% of the compounds in the extract}} \times 100 \quad \text{........ (1)}$$

The area % of the major compounds (≥ 70 % quality) of the neem and olive leaves essential oils were dominantly phenolic with 40.42 % and 36.80 % respectively. While the neem and olive smoke trapped in hexane contain ester 98.19 % and 81.00 % respectively. When it comes to the smokes trapped in methanol, the major compounds were phenolic with 34.74 % in neem and 43.10 % in olive extract.

5.2.3. The major compounds identified from GC-MS result

The top major compounds of both neem and olive essential oils, and smoke trapped in methanol were mainly phenolic compounds. While the neem and olive smoke trapped in hexane were fatty acid esters.

Table 11: The major compounds of olive and neem leaves essential oil

Extract	Name of the compounds	Area (%)	Compound type	Compound
Neem essential oil	Methyl tetracosanoate	3.72	Fatty acid ester	31
	2-Tert-Butyl-4,6-bis (1-methyl-1-phenyl ethyl) phenol	3.39	Phenolic	32
	Glabrol (2*S*)-7-hydroxy-2-[4-hydroxy-3-(3-methylbut-2-enyl) phenyl]-8-(3-methylbut-2-enyl)-2,3-dihydro chromen-4-one	2.79	Phenolic	33
Olive essential oil	Benzene acetaldehyde	3.24	Aromatic aldehyde	34
	N-Desmethyltapentadol (3-[(2*R*,3*R*)-2-methyl-1-(methyl amino)pentan-3-yl] phenol)	1.90	Phenolic	35
	1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one	1.88	Ketone	36

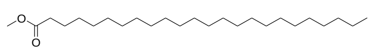
The top major compounds of both neem and olive essential oils were mainly phenolic compounds which were easily decomposed compared to fatty acid ester of smoke. The essential oils extracted contain compounds uses as insecticidal and antioxidant activities (example: 2-Tert-Butyl-4,6-bis (1-methyl-1-phenylethyl) phenol [59].

Table 12: The major compounds of the neem leaves smoke

Extracts	Name of compounds	Area (%)	Compound type	Cpd.
Neem hexane trapped smoke	Methyl hexadecanoate	12.11	Fatty acid ester	37
	Methyl stearate	4.88	Ester (Stearic acid)	38
Neem methanol trapped smoke	3,5-di*tert*-butylphenol	29.35	Phenolic	39
	Methyl hexadecanoate	12.93	Fatty acid ester	37
	1,2-15,16-Diepoxyhexadecane	7.92	Alkane (epoxy)	40

Table 13: The major compounds of olive leaves smoke

Extracted	Name of compounds	Area (%)	Compound type	Cpd.
Olive hexane trapped smoke	Methyl octadec-9-enoate	29.66	Fatty acid ester	41
	Methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate	15.35	Fatty acid ester	42
	methyl hexadecanoate	13.54	Fatty acid ester	37
Olive Methanol trapped smoke	2,6-Di-*tert*-butylphenol	38.27	Phenolic	43
	Methyl hexadecanoate	8.70	Fatty acid ester	37
	(E)- tetradec-2-ene	8.45	Alkene	44



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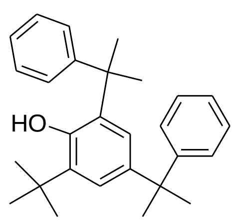

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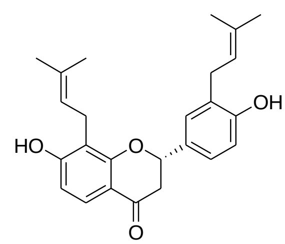

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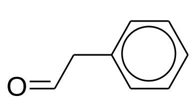

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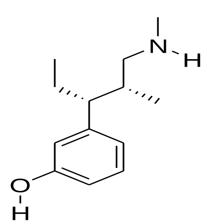

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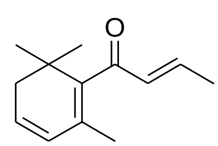

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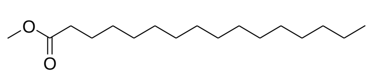

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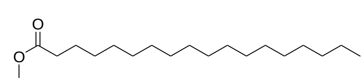

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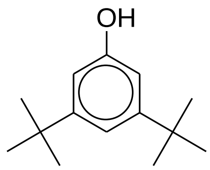

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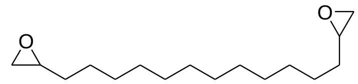

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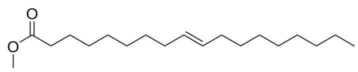

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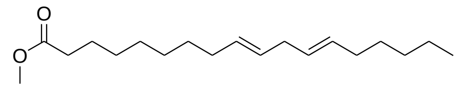

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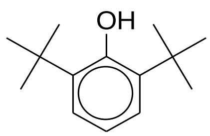

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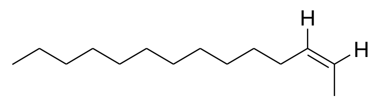

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Figure 12: The structures of the major compound of the extracts

The phenolic compounds 2-Tert-butyl-4,6-bis (1-methyl-1-phenylethyl)phenol and it analog's are good contributor for biological activities as antioxidant, and insecticidal [59]. While, the fatty acid esters reported in table 11, 12, and 13 are used as pesticides [60].

5.3. Antioxidant tests

5.3.1. Concentration and regression curve of ascorbic acid

Table 14: UV-Visible data of ascorbic acid

Sample Standard	Concentration (μ g/mL)	Absorbance
Ascorbic acid	0.176	0.2387 ± 0.0057
	0.88	0.2620 ± 0.0104
	1.76	0.2903 ± 0.0087
	3.52	0.3570 ± 0.0128
	7.04	0.5540 ± 0.0128
	10.56	0.7213 ± 0.0173

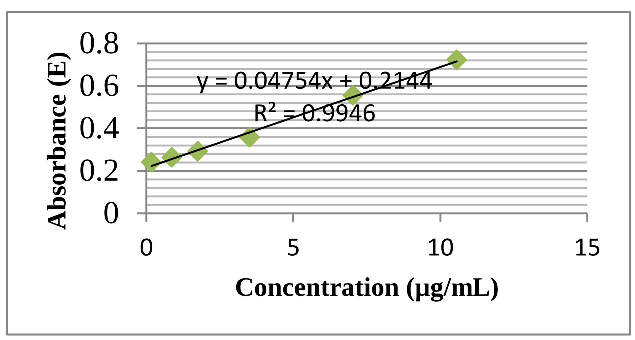


Figure 13: Linear regression curve of ascorbic acid

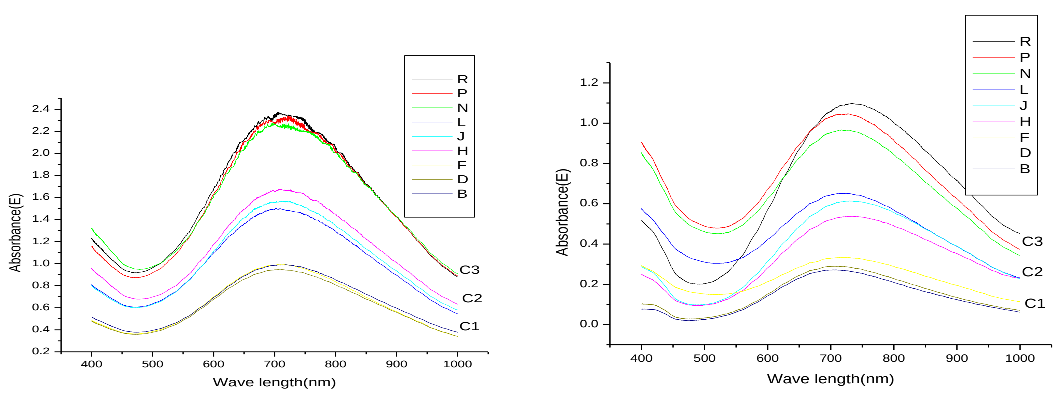
The relationship between concentration (μg/mL) of ascorbic acid and its average absorbance determined at 220 nm was used as positive control or standard calibration curve. The regression equation obtained for ascorbic acid was y = 0.04754x + 0.2144, (R^2 = 0.9946), which is used to calculate the concentration of antioxidant of the samples.

5.3.2. Concentration of antioxidants in the test samples

Table 15: Average absorbance of neem and olive leaves extracts

Sample of Methanol trapped Smoke	Concentration of Samples (µg/mL)	Absorbance Of Sample at 700 nm	Con. of Antioxidant Calculated from standard Calibration Curve (µg/mL)	% of antioxidant extract from the samples (%)	Mean % of antioxidant extract from the samples
Neem Leaf Trapped smoke	76.10	0.973 ± 0.0251	15.97	20.99	19.65 ± 0.84
	152.20	1.567 ± 0.05003	28.48	18.71	
	228.29	2.301 ± 0.0456	43.92	19.24	
Olive Methanol Trapped smoke	76.10	0.296 ± 0.0196	1.718	2.26	4.91 ± 1.81
	152.20	0.583 ± 0.0655	7.76	5.10	
	228.29	1.015 ± 0.0377	16.86	7.38	

The reducing power was determined according to Oyaizu (1984). The absorbance at 700 nm was measured. Increased absorbance of the reaction mixture indicated increased reducing power. or the concentrations of antioxidant compounds. A significant relationship was found between antioxidant potential and total phenolic content, suggesting that phenolic compounds are the major contributors to the antioxidant activity of neem and olive leaves. The antioxidants concentrations obtained were 19.65 ± 0.84 for the neem smoke trapped in methanol 4.91 ± 1.81 for olive smoke trapped in methanol. The results show that the antioxidant compounds in neem sample is in a higher concentration compared to that of olive. This value is consistent with the literature data that promote the medicinal values of the neem plant



a) b)

Figure 14 : Absorbance of a) Neem methanol smoke b) Olive methanol smoke

The antioxidant activity has direct relation with the concentration of the samples (Figure 14).

5.4. Insect repellent test result of solvent trapped smoke

The percentage repellent was calculated as:

$$\% \text{ repellent} = \frac{(\#\text{ of cockroaches in the control side} - \#\text{ cockroaches in the sample side}) \times 100}{\text{Total }\#\text{ of cockroaches in the control and sample}}$$

% repellent = (# CC - # CS) x 100 / (# CC + # CS)

For example: repellent (%) of olive methanol trapped smoke at 20 minutes calculated as;

Repellent (%) = (4.66 – 2.00) x 100 / 6.66 = 3 9.94%

Based on this, % repellent was calculated and shown in the table below.

Table 16: Insect (cockroaches) repellent test using neem and olive leaves smokes

Sample extracted	Time (min)	# Cockroaches move to control (C)	# cockroaches move to sample (S)	# cockroaches repellent (C-S)	# cockroaches treated (C+S)	#cockroaches un- treated 10 - (C+S)	% Repellents (C-S) x 100 / (C+S)
Olive Methanol trapped smoke	10	6.00	3.00	3.00	9.00	1.00	33.33 ± 0.82
	20	4.66	2	2.66	6.66	3.34	39.94 ± 0.47
	30	6.00	2.33	3.67	8.33	1.67	44.06 ± 0.47
	40	6.33	1.33	5.00	7.66	2.34	65.27 ± 0.47
	50	7.00	1.00	6.00	8.00	2.00	75.00 ± 0.00
	60	**8.00**	**0.33**	**7.67**	**8.33**	**1.67**	**92.10 ± 0.82**
Olive Hexane trapped smoke (OH)	10	2.66	2.00	0.66	4.66	5.34	14.16 ± 0.47
	20	3.00	1.66	1.34	4.66	5.34	28.75 ± 0.82
	30	4.66	1.33	2.33	5.99	4.01	38.90 ± 0.47
	40	5.66	1.66	4.00	7.32	2.68	54.64 ± 0.47
	50	5.00	0.33	4.33	5.33	4.67	66.67 ± 0.00
	60	**5.33**	**0.66**	**4.67**	**5.99**	**4.01**	**77.96 ± 0.47**
Neem Methanol trapped smoke (NM)	10	3.33	1.33	2.00	4.66	5.34	42.92 ± 0.47
	20	5.66	1.66	4.00	7.32	2.68	54.64 ± 0.47
	30	5.00	1.00	4.00	6.00	4.00	66.67 ± 0.82
	40	5.00	0.66	4.34	5.66	4.34	76.68 ± 0.47
	50	7.00	0.66	6.34	7.66	2.34	82.77 ± 0.81
	60	**8.00**	**0.00**	**8.00**	**8.00**	**2.00**	**100.00 ± 0.82**

Neem	10	3.00	2.00	1.00	5.00	5.00	20.00 ± 0.00
Hexane trapped smoke (NH)							
	20	3.33	1.66	1.67	4.99	5.01	33.47 ± 0.47
	30	3.66	1.33	2.33	4.99	5.01	46.69 ± 0.47
	40	4.00	1.00	3.00	5.00	5.00	60.00 ± 0.81
	50	5.33	1.00	4.33	6.33	3.67	68.40 ± 0.47
	60	**5.00**	**0.33**	**4.33**	**5.33**	**4.67**	**81.24 ± 0.47**

Note: Olive smoke trapped in methanol (OM), olive smoke trapped in hexane (OH), neem smoke trapped in methanol (NM), and neem smoke trapped in hexane (NH).

Table 16 shows that the trapped smokes have positive effect on the treated insects. Especially smoke tapped in methanol for both plants showed stronger repellency when compared to smoke trapped in hexane. This tells us that polar compounds trapped in methanol were responsible for the effect. Hence, the polar extract part of the plant can be used as insect repellent without further fractionation.

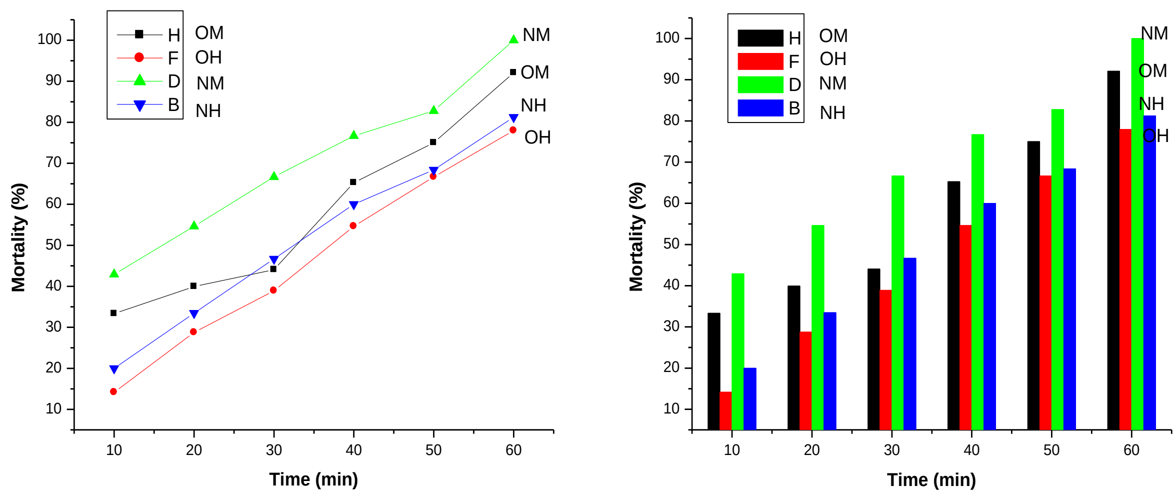
Between neem and olive, smoke from the neem showed higher repelling activity which is 100% at 60 min. While olive smoke exhibited 92% repellency at the end of one hour. From the data it can be inferred that the repelling nature of the extract increases with time. For the first 10 min all extracts showed lower repelling values with maximum repellency of 42.92% by the neem smoke trapped in methanol.

Table 17: Mean repelling values of the extracts.

Sample trapped smoke	Time interval and the average values						
	10min	20 min	30 min	40 min	50 min	60 min	Mean (%)
Olive Methanol	33.33 ± 0.82	39.94 ± 0.47	44.06 ± 0.47	65.27 ± 0.47	75.00 ± 0.00	92.10 ± 0.82	58.28 ± 0.51
Olive Hexane	14.16 ± 0.47	28.75 ± 0.82	38.9 ± 0.47	54.64 ± 0.47	66.67 ± 0.00	77.96 ± 0.47	46.85 ± 0.45
Neem Methanol	42.92 ± 0.47	54.64 ± 0.47	66.67 ± 0.82	76.68 ± 0.47	82.77 ± 0.81	100.00 ± 0.82	70.61 ± 0.64
Neem Hexane	20.00 ± 0.00	33.47 ± 0.47	46.69 ± 0.47	60.00 ± 0.81	68.4 ± 0.47	81.24 ± 0.47	51.63 ± 0.45

Table 17 shows that except the olive smoke trapped in hexane, all the rest showed average values above 50%. The highest recorded was for the neem smoke trapped in methanol.

The killing ability of the extracts go parallel with the repelling ability. This can not be a surprise as the two effects are interrelated. The killing nature of an insecticide is mostly preceded by repelling.



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Note: Olive smoke trapped in methanol (OM), olive smoke trapped in hexane (OH), neem smoke trapped in methanol (NM), and neem smoke trapped in hexane (NH).

Figure 15: Graphs showing rate of repellency

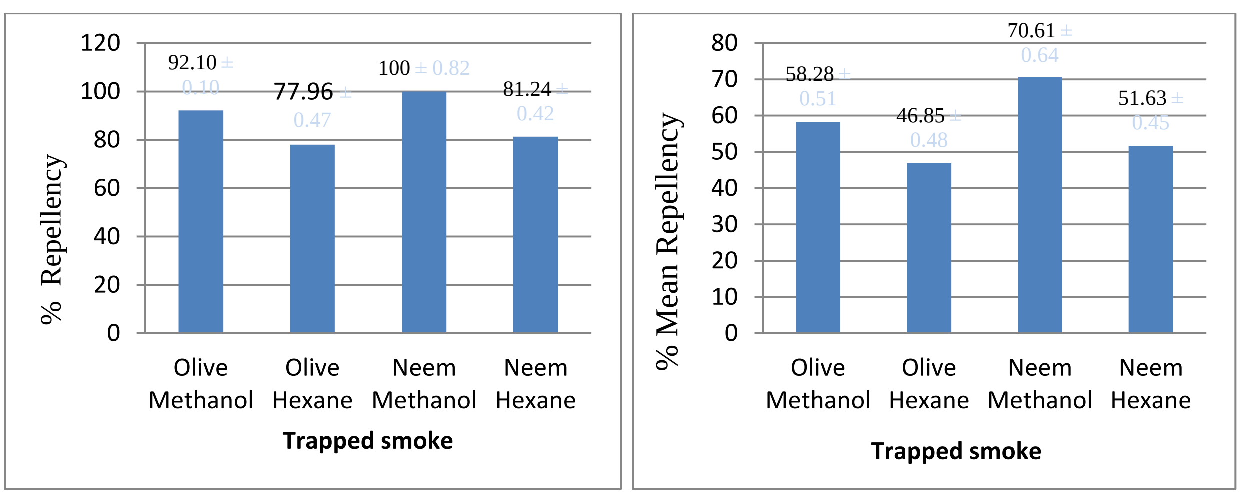

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Figure 16: Graphs showing repelling nature of the smokes components

Table 18: **Bioassay repellents of smokes obtained from neem and olive leaves.**

Extraction	Repellent time (min)	
Methods	RT_{50}	RT_{95}
Olive methanol trapped smoke	28.10	61.42 (HS)
Olive hexane trapped smoke	37.46	72.58 (HS)
Neem methanol trapped smoke	16	57.45
Neem Hexane trapped smoke	33.79	70.42 (HS)

Note: R = Repellent, T = Time, HS = out of the range (highly significant).

From table 18 the least time needed to repellent insects is the most efficient repellent extracts. The neem methanol trapped smoke at TR_{50} (16 min) the top repellent, followed by the olive methanol trapped smoke with the values RT_{50} (28.10 min) is the second level of repellent, neem hexane trapped smoke was also the third alternative repellent at RT_{50} (33.79 min) but, olive hexane trapped smoke is the least repellent at RT_{50} (37.46 min) from all extracts.

5.5. Mortality rate results of the solvent trapped smoke

5.5.1. Mortality rate of Cockroaches using the smoke trapped from neem and olive leaves

Insecticidal activity varied according to the plant part extracted and method of extraction. The mortality rates of the smokes were compared using cockroach as experimental insects. In each experiment that had been conducted, 5 insects were used and the experiments were conducted in triplicates.

Different concentrations were used over 36 hrs to see the effects. The data tabulated in table 19 shows that 100% mortality was recorded at 6 hrs for neem smoke trapped in methanol at the concentrations of 5 and 10%. Whereas, similar result was recorded for olive smoke trapped in methanol at 24 hrs by all the test concentrations (1.25%, 2.5%, 5% and 10%). For the neem smoke trapped in hexane, the maximum mortality (80%) was recorded at 36 hrs with the highest concentration (10%). At the same time by the same concentration, the olive smoke trapped in hexane showed 60% mortality.

It is clear from the data that the neem methanol trapped part of the smoke was effective in killing the insects. This is a good news for our society living in remote part of the country that do not have access to modern pesticides as they can easily use the plant part as fumigant to kill the pests.

In this test, the % mortality rate was calculated using Abbotts' formula equation. The smoke of neem methanol extracted of the leaf caused higher mortalities for cockroaches compare to the other extracted in reference of time. This indicating the presence of toxic compounds in the leaves, the smoke hexane extracted of the samples caused lower mortality compare to the smoke methanol extracted of leaves. In this study mortalities ranged from 6.66% to 100% in all extracted at lowest concentration (1.25%), after 3 hours the percent mortalities of cockroaches calculated and corrected with Abbotts' formula equation [61].

$$\text{Mortality (\%)} = \frac{(\text{death due to treatment of sample} - \text{death in control}) \times 100}{\text{Total number of cockroaches treated}} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Table 19: Mortality data of smoke of neem and olive leaves

Sample extract	Concentration (%)	Mean Mortality Rate of Cockroach In Time (h)					
		3 h	6 h	9 h	12 h	24 h	36 h
Neem Methanol trapped Smoke	1.25%	1.00	4.66	4.66	5.00	5.00	5.00
	2.5%	1.33	4.66	5.00	5.00	5.00	5.00
	5%	2.33	5.00	5.00	5.00	5.00	5.00
	10%	2.66	5.00	5.00	5.00	5.00	5.00
Neem Hexane trapped Smoke	1.25%	0.33	0.33	0.66	0.66	1.33	2.66
	2.5%	0.33	0.66	1.66	2.00	2.33	3.00
	5%	0.66	1.00	1.66	2.00	2.66	3.66
	10%	0.66	1.00	2.33	2.66	3.33	4.00
Olive Methanol trapped Smoke	1.25%	1.00	1.33	2.00	2.66	5.00	5.00
	2.5%	1.33	1.66	2.33	3.33	5.00	5.00
	5%	1.33	2.66	2.66	3.66	5.00	5.00
	10%	1.66	2.66	3.66	4.33	5.00	5.00
Olive Hexane Trapped Smoke	1.25	0.33	0.33	0.66	0.66	1.00	1.33
	2.5%	0.33	0.33	1.00	1.00	1.33	2.00
	5%%	0.33	0.33	1.00	1.33	1.66	2.66
	10%	1.00	1.33	1.33	1.66	2.33	3.00
Controls	DCM	0.00	0.00	0.00	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00	0.00	0.00	0.00

Table 20: Percent mortality of Cockroaches

Sample extract	Con. (%)	Mortality rate of Cockroaches at a given time (h)					
		3 h	6 h	9 h	12 h	24 h	36 h
Neem Methanol Trapped Smoke	1.25%	20.00 ± 0.00	93.32 ± 0.47	93.32 ± 0.47	100.00 ± 0.47	100.00 ± 0.47	100.00 ± 00
	2.5%	26.66 ± 0.47	93.32 ± 0.47	93.32 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
	5%	46.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
	10%	53.32 ± 0.47	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
Neem Hexane Trapped Smoke	1.25%	6.66 ± 0.47	6.66 ± 0.47	13.32 ± 0.47	13.32 ± 0.82	20.00 ± 0.47	53.32 ± 0.00
	2.5%	6.66 ± 0.47	13.32 ± 0.0	33.32 ± 0.47	40.00 ± 0.82	46.66 ± 0.47	60.00 ± 0.47
	5%	13.32 ± 0.47	20.00 ± 0.47	33.32 ± 0.47	40.00 ± 0.82	53.32 ± 0.47	73.32 ± 0.47
	10%	13.32 ± 0.47	20.00 ± 0.47	46.66 ± 0.82	53.32 ± 0.47	66.66 ± 0.47	80.00 ± 0.82
Olive Methanol Trapped Smoke	1.25%	20.00 ± 0.00	26.66 ± 0.47	40.00 ± 0.00	53.32 ± 0.41	100.00 ± 0.00	100.00 ± 0.00
	2.5%	26.66 ± 0.47	32.32 ± 0.47	46.66 ± 0.47	66.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
	5%	26.66 ± 0.47	53.32 ± 0.41	53.32 ± 0.47	73.32 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
	10%	33.32 ± 0.47	53.32 ± 0.47	73.32 ± 0.47	86.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
Olive Hexane Trapped smoke	1.25%	6.66 ± 0.47	6.66 ± 0.47	13.32 ± 0.47	13.33 ± 0.47	20.00 ± 0.47	33.32 ± 0.00
	2.5%	6.66 ± 0.47	6.66 ± 0.47	20.00 ± 0.00	20.00 ± 0.00	26.66 ± 0.47	40.00 ± 0.82
	5%%	6.66 ± 0.47	6.66 ± 0.47	20.00 ± 0.00	26.66 ± 0.47	33.32 ± 0.47	53.32 ± 0.82
	10%	20.00 ± 0.00	26.66 ± 0.47	26.66 ± 0.47	33.32 ± 0.47	46.66 ± 0.47	60.00 ± 0.00

5.5.2. Correlation coefficient (Pearson’s correlation coefficient):- is a statistical measure of the strength of a linear relationship between paired data. In a sample it is denoted by r and is by design constrained as:-, $-1 \leq r \geq +1$ Furthermore, positive values denote positive linear correlation, Negative values denote negative linear correlation and a value of ‘’0” denotes no linear correlation. The closer the value is to 1 or –1, the stronger the linear correlation. In the figures various samples and their corresponding sample correlation coefficient values are presented. The first three represent the “extreme” correlation values of -1, 0 and 1: Correlation is an effect size, and so we can verbally describe the strength of the correlation using the guide that Evans suggests for the absolute value of r values from 0.00-0.19 “very weak” r = from 0.20-0.39 “weak” r from 0.40 to 0 .59 is “moderate “and r = from 0 .60 to 0.79 “strong” and from 0 .80-1.0 “very strong “correlation [62].

Table 21: Correlation of mortality vs concentration and time

I) Correlation of mortality (%) with Concentration (%)

A) Neem smoke trapped in methanol and hexane

Neem Methanol	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.996	1		
5%	0.995	0.996	1	
10%	0.996	0.996	1	1

Neem Hexane	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.824	1		
5%	0.911	0.976	1	
10%	0.816	0.998	0.976	1

B) Olive smoke trapped in methanol and hexane

Olive Methanol	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.991	1		
5%	0.948	0.962	1	
10%	0.892	0.930	0.948	1

Olive Hexane	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.980	1		
5%	0.983	0.991	1	
10%	0.945	0.934	0.949	1

II) Correlation of mortality (%) with time (h)

C) Neem smoke trapped in methanol and hexane

Neem Methanol	3 h	6 h	9 h	12h
3 h	1			
6 h	0.970	1		
9 h	0.970	0.999	1	
12 h	-	-	-	1
24 h	-	-	-	-
36 h	-	-	-	-

Neem Hexane	3 h	6 h	9 h	12 h	24 h	36 h
3 h	1					
6 h	0.905	1				
9 h	0.700	0.886	1			
12 h	0.688	0.899	0.996	1		
24 h	0.784	0.946	0.990	0.990	1	
36 h	0.948	0.954	0.886	0.871	0.931	1

D) Olive smoke trapped in methanol and hexane

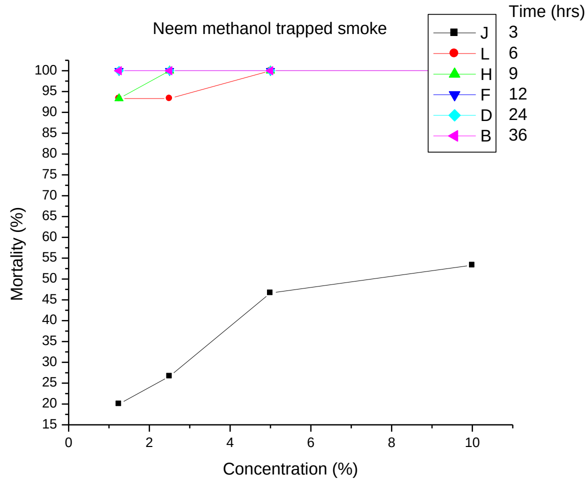
Olive Methanol	3 h	6 h	9 h	12h
3 h	1			
6 h	0.780	1		
9 h	0.945	0.822	1	
12 h	0.981	0.885	0.964	1
24 h	-	-	-	-
36 h	-	-	-	-

Olive Hexane	3 h	6 h	9 h	12 h	24 h	36 h
3 h	1					
6 h	0.999	1				
9 h	0.813	0.815	1			
12 h	0.774	0.774	0.948	1		
24 h	0.878	0.879	0.956	0.982	1	
36 h	0.732	0.731	0.894	0.990	0.963	1

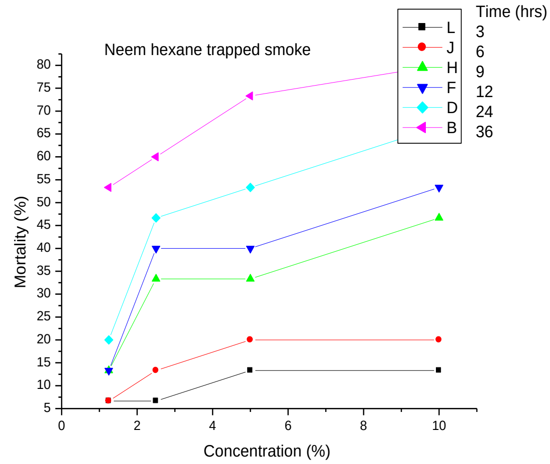
From table 21 the correlation of mortality of cockroaches with concentration (Table A and B) possess between 0.82 - 1.0 which is very strong positive correlated. The correlation of mortality of cockroaches with time average (Table C&D) possess between 0.688 - 0.79 “strong” and 0.80-1.0 “very strong, which is very strong positive correlated.

Statistical method percentage mortalities and probit analysis were used to analyze the number of mortality recorded and the percentage mortality to determine LC_{50} and LT_{50} [63].

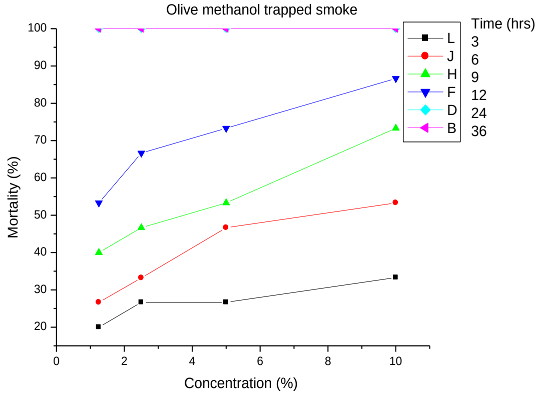
The results were subjected to one way Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version 23) to determine significant difference between various treatments and control, test was used to separate differences among means, differences were considered significant at ($P < 0.05$) [64].


Neem methanol trapped smoke
Time (hrs)
J 3
L 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
0
2
4
6
8
10

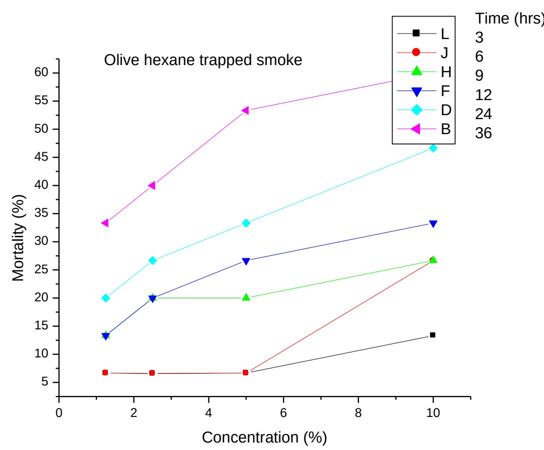
a


Neem hexane trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
2
4
6
8
10

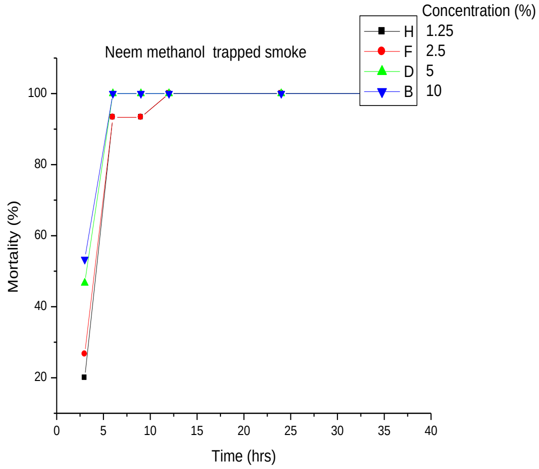
b


Olive methanol trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)
100
90
80
70
60
50
40
30
20
0
2
4
6
8
10

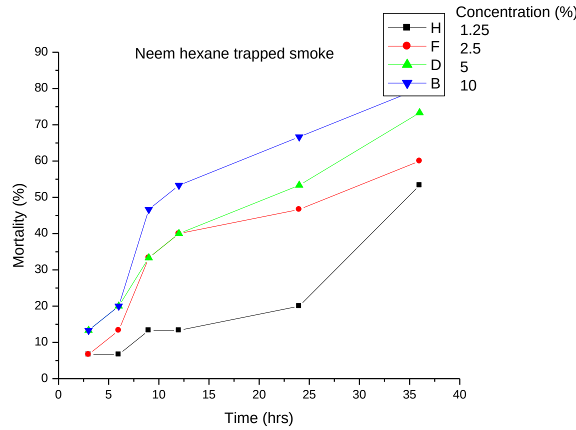
c


Olive hexane trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)
60
55
50
45
40
35
30
25
20
15
10
5
0
2
4
6
8
10

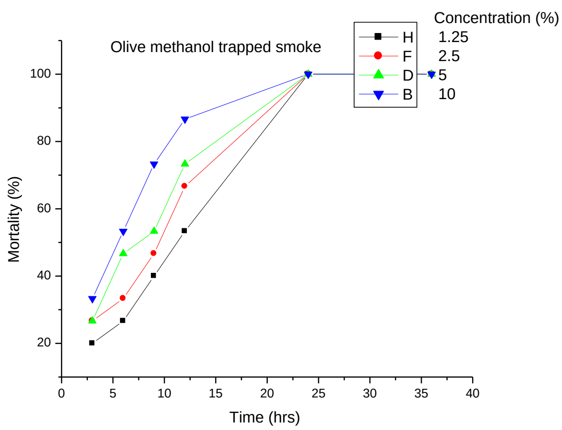
d



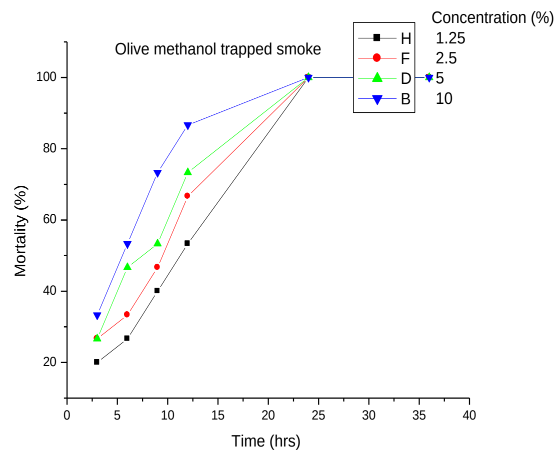
e



f



g



h

Figure 17: Percent mortality in relation to concentration (graph a, b, c, and d), and time (graphs e, f, g, and h) of the trapped smokes.

Figure 17: also shows the % mortality was positively correlated with times and concentrations of the samples.

5.5.3. Efficacy tests

The lethal time LT_{50} and LT_{95} data was calculated after 3, 6, 12, 24 and 36 hours for the lowest concentration (1.25%). Whereas, the lethal concentration LC_{50} and LC_{95} were evaluated at 1.25%, 2.5%, 5%, and 10% concentrations for the smallest time (3 h) after treatment.

Percent mortality versus concentration and time graphs for all treatments indicated in Figure 17 values for different samples are shown.

Table 22: Bioassay efficacy of smokes obtained from neem and olive leaves.

For lethal concentration LC_{50} and LC_{95} at the smallest time (3 hours), and for lethal time LT_{50}, and LT_{95} at the smallest concentration is (1.25%) after treatments.

Extraction	lethal concentration (%)		lethal time (hours)	
Methods	LC_{50}	LC_{95}	LT_{50}	LT_{95}
Neem methanol			4.6	10
Trapped smoke	6.4	HS		
Neem hexane			35	HS
Trapped smoke	HS	HS		
Olive methanol			10.20	20.40
Trapped smoke	HS	HS		
Olive hexane			HS	HS
Trapped smoke	HS	HS		

Note: C = concentration, T = Time, HS = out of the range (highly significant).

The data of the LC_{50}, LC_{95}, LT_{50} and LT_{95} values were obtained from probit analysis. It shows methanol trapped part of the smoke obtained from the neem leaf has a higher efficacy at the lowest concentration (1.25%). At this particular concentration, the extract was enough to kill more than 50% of Cockroaches. The olive hexane trapped smoke was the least active compared to others. Which has promoted 50% death of the Cockroaches at given concentration, our results show quantitative and qualitative differences in the chemical composition and insecticidal activities of the plants extracts. All the extracts were found to be toxic they were able to kill the insects.

From table 22 the least time and concentration needed to kill insect show the most efficient plants extracts. The neem methanol trapped smoke at LC_{50} (6.40%) and LT_{50} (4.60 h) is the top insecticidal, followed by the olive methanol trapped smoke extract with the values LC_{50} (HS) and LT_{50} (10.20 hrs).

5.5.4. Mean mortalities of Cockroaches

Table 23: Mean mortalities rate of all trapped smokes

Sample trapped smoke	mortality of Cockroaches (mean values)					Mortality
	1.25%	2.5%	5%	10%	Mean	(≥ 50%)
Olive Methanol	55.00 ± 0.19	62.77 ± 0.38	66.1 ± 0.38	69.44 ± 0.38	63.33 ± 0.33	High
Olive Hexane	15.55 ± 0.00	18.87 ± 0.28	23.33 ± 0.38	30.05 ± 0.38	21.95 ± 0.38	Very Low
Neem Methanol	84.44 ± 0.24	86.66 ± 0.18	91.11 ± 0.12	92.22 ± 0.12	88.61 ± 0.16	Very High
Neem Hexane	18.88 ± 0.47	33.33 ± 0.54	38.88 ± 0.45	46.66 ± 0.54	34.30 ± 0.50	Low

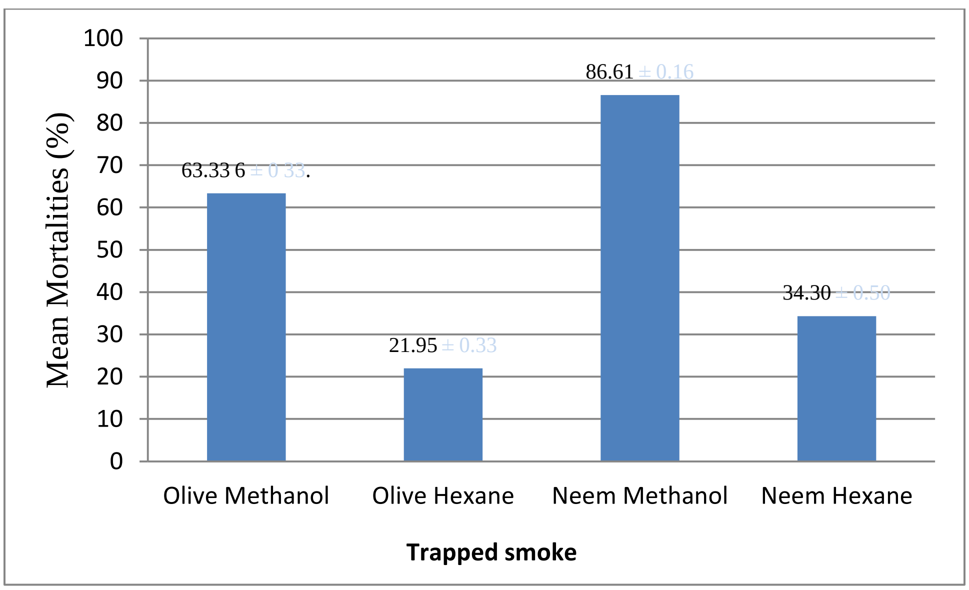


Figure 18: Mean mortalities of Cockroaches

Figure 18 and Table 23 mean mortalities shows insecticidal activities of the extracts with methanol extract of the neem leaf caused death to a greater extent compared to other extracts, and then olive methanol extract has shown better insecticidal activity when compared with others.

1.25% OM 2.5% OM 5% OM 10% OM

1.25% NM 2.5% NM 5% NM 10% NM

Control-1 Control-2 Triplicate (T) -1 T- 2 T-3

Figure 19: Cockroaches inside Petridishes

6. CONCLUSION

The mounts (% yield) of the essential oils of neem and olive leaves were 0.27% and 0.18% respectively. The % yields for neem smoke trapped in methanol and hexane were 1.83% and 1.07% respectively, while for olive smoke trapped in methanol and hexane were 2.333%, and 1.27% respectively. The smoke methanol extracts obtained was the highest yield (%) compares to the other extracts.

The major compounds detected in neem and olive essential oils were predominantly Phenolic with 40.42 %, and 36.80 % respectively. While, in the neem hexane trapped smoke was ester 98.19 %, and olive hexane trapped smoke was ester 81.00%. Neem and olive methanol trapped smoke showed phenolic compound with 34.74% and 43.10% respectively

The plants neem and olive leaves smoke trapped in methanol assayed by FRAP method was detected antioxidant activities. There is strong correlation between the concentration of sample with their absorbance and antioxidant. The measured average antioxidant concentrations for neem and olive smoke trapped in methanol was 19.65% and, 4.91 % respectively. This study suggests that the plant extraction of neem and olive have a source of natural antioxidant. The polar compounds trapped in methanol were responsible for the effect. Those plants can be used as a source of natural antioxidant to prevent disease associated with free radical and anti-oxidant activity. This value is consistent with the literature data that promote the medicinal values of the neem and olive plants.

The neem and olive trapped smokes indicated insecticidal (repellents, and mortalities) which, used as best an alternative to synthetic insecticidal. The neem smoke trapped in methanol was the most efficient repellent (100%). Olive methanol trapped smoke (92.10%), Neem hexane trapped smoke (81.24%) and Olive hexane trapped smoke (77.96%) at 60 min. The killing nature (mortalities) of an insecticide is mostly preceded by repelling. The mortalities of neem and olive smoke trapped in methanol at 10% concentration were 100% at 6 and 24 hrs respectively, which is efficient. While for neem and olive smoke trapped in hexane at 10% concentration were 80% and 60% respectively for 36 hrs.

7. RECOMMENDATIONS

Plant antioxidants are safer than synthetic antioxidant in reducing the risk of certain diseases such as cancer, heart diseases, DNA damages, and stroke. Large number of plants has been identified so far for their insecticidal property and can be used as an alternative pest management for synthetic pesticides. Smoke based remedies are available, safe, rapid delivery to the brain, more efficient absorption by the body, and lower cost of production. However, further Studies are required to know multipurpose application of plant derived smoke. The identification and isolation of bioactive compounds extracts of the neem and olive plants must be done as key issue for further study, especially with focus on pandemic antiviral.

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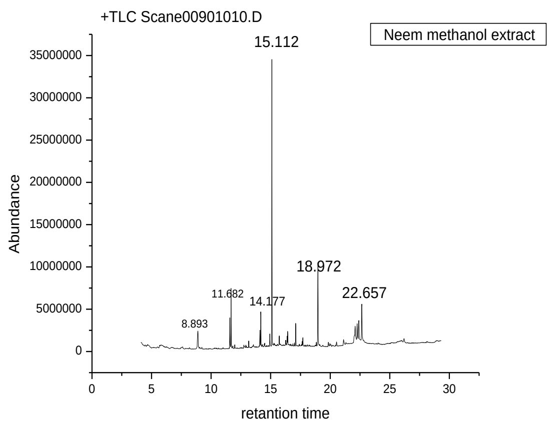
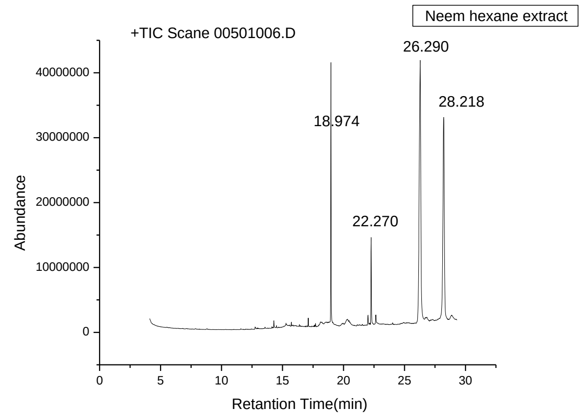
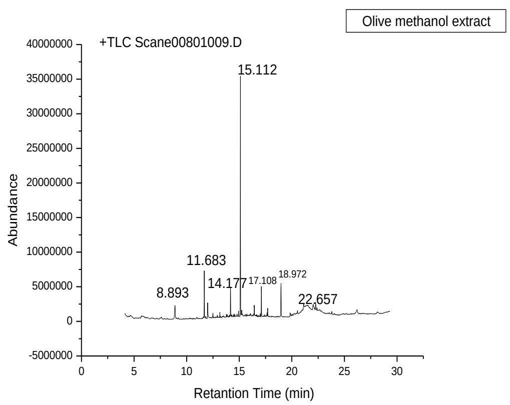
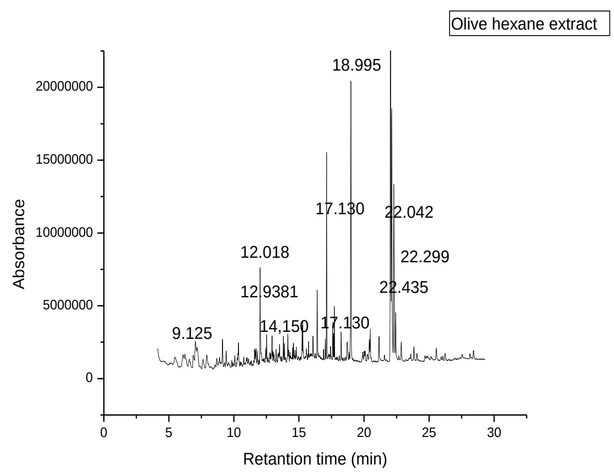
9. APPENDIXES

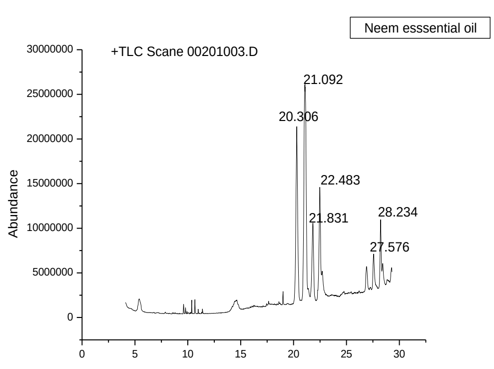
APPENDIX-1: GC-MS Analysis of neem and olive leaves essential oils and trapped smokes

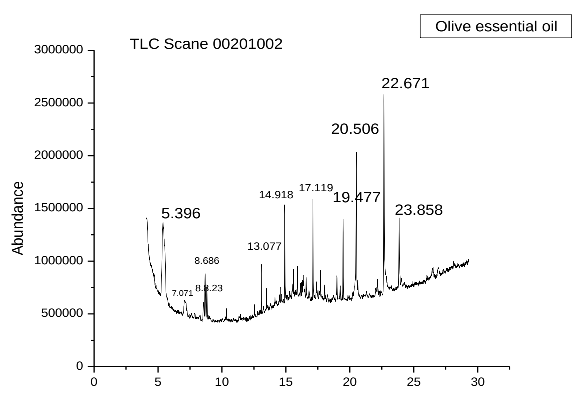






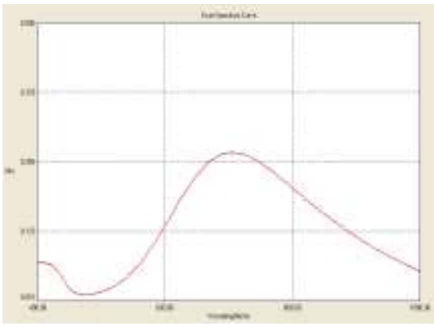




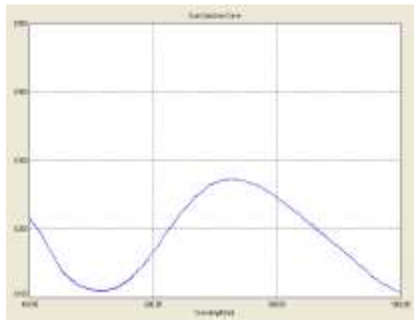


APPENDIX-2

UV-VISSIBLE Analysis of the olive smoke trapped in methanol at concentrations of 76.10 µ/mL, 152.20 µ /mL, 228.29 µ/mL in triplicate (T-1, T-2, and T-3)



76.10 µg/mL (T-1)



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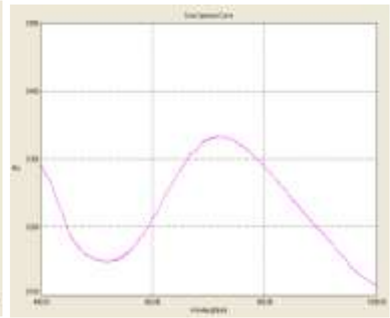
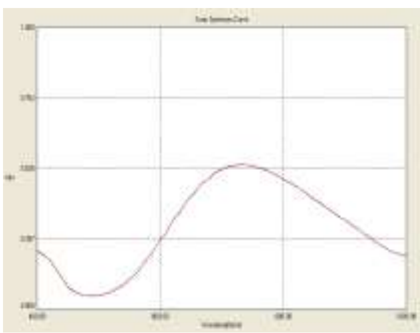
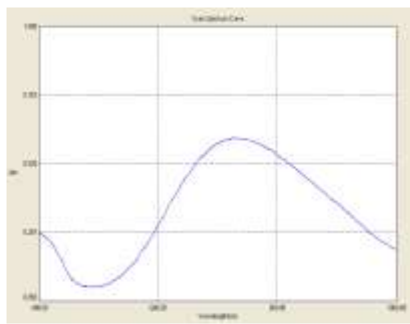


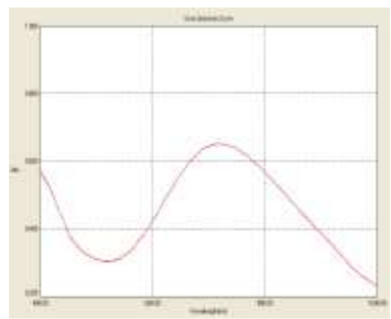
76.10 µg/mL (T-1)



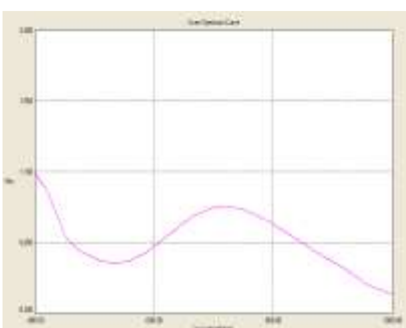
152.20 µg/mL (T-1)



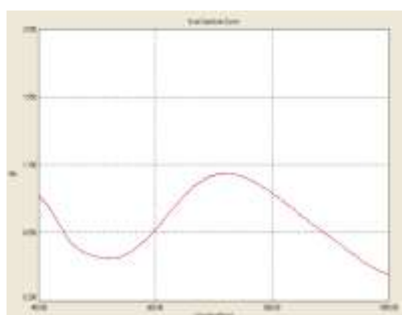
152.20 µg /mL (T-2)



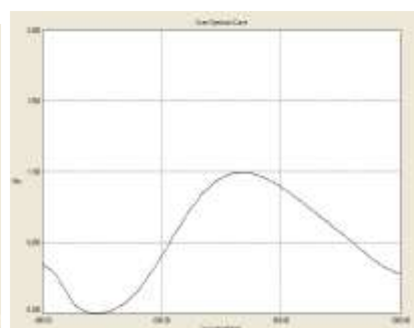
152.20 µg/mL (T-3)



228.29 µg/mL (T-1)



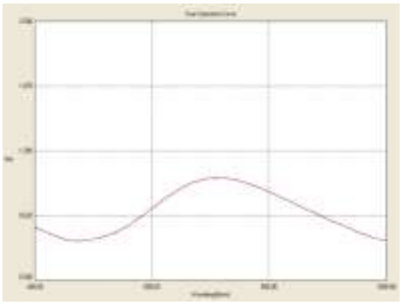
228.29 µg/mL (T-2)



228.29 µg/mL (T-3)

APPENDIX-3

UV-VISSIBLE Analysis of the neem smoke trapped in methanol at concentrations of 76.10 µ/mL, 152.20 µ/mL, and 228.29 µ/mL in triplicates (T-1, T-2, and T-3).



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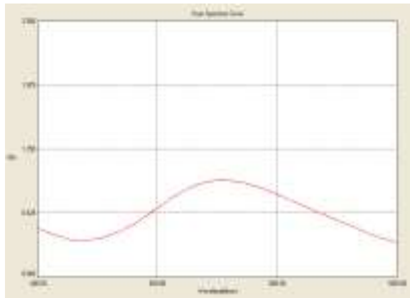

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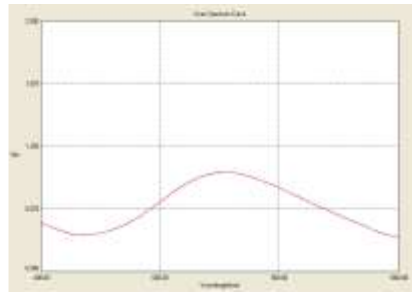
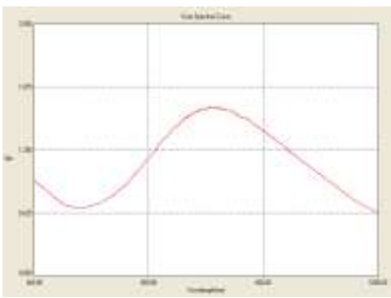
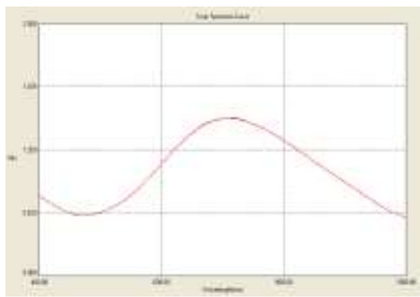


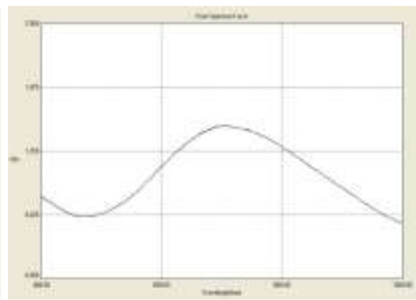
76.10 µg/mL (T-1)



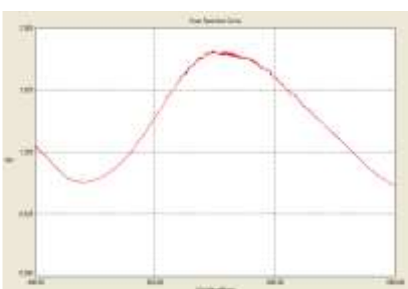
152.20 µg/mL (T -1)



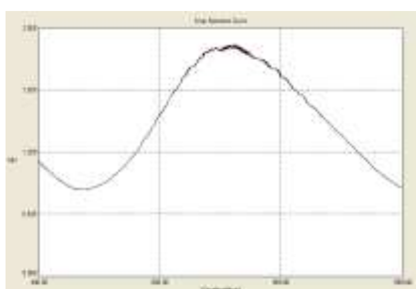
152.20 µg /mL (T-2)



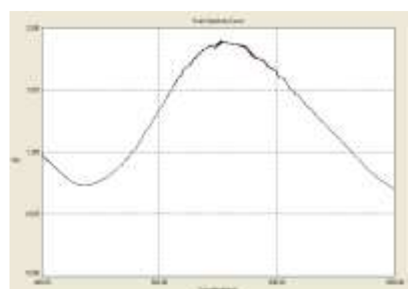
152.20/ µg/mL (T-3)



228.29 µg/mL (T-1)



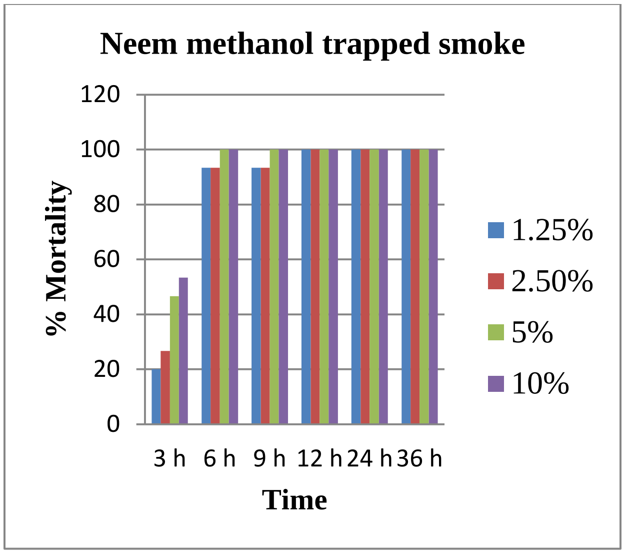
228.29 µg/mL (T-2)

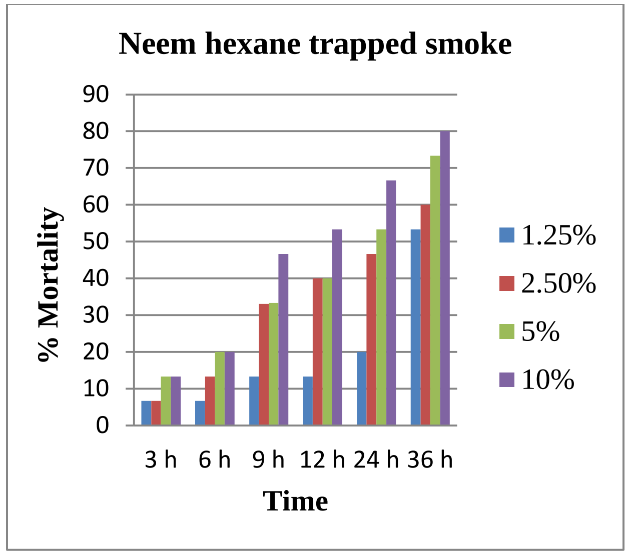


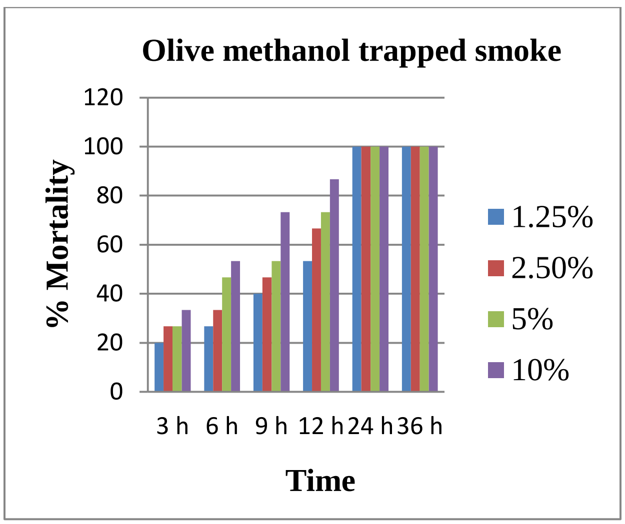
228.29 µg/mL (T-3)

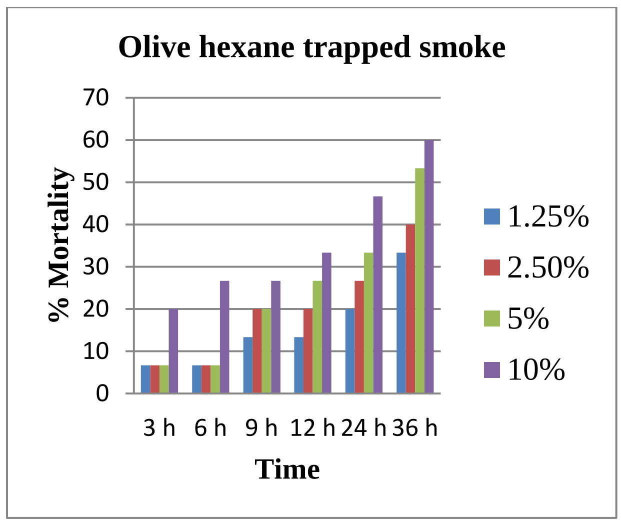
APPENDIX-4

Percent mortalities in relation to concentration and time of the neem and olive leave smokes at concentrations of 1.25, 2.5, 5, 10% and time intervals of 3,6,9,12,24, and 36 hours.









ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVIITIES OF SMOKE COLLECTED FROM *AZADIRACHTA INDICA (NEEM) AND OLEA EUROPAEA* (OLIVE) LEAVES

BY: Kibrom Teshome

Advisor: Dr. Estifanose Ele

July 2020

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

BY: Kibrom Teshome

July, 2020

Approved by:	Signature
Dr. Estifanose Ele (Advisor)	_______________
Dr. Mekonnen Abebayehu (Examiner)	________________
Dr. Merid Tessema (Examiner)	_________________

DECLARATION

I, the undersigned, declare that this Thesis entitled "CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVIITIES OF SMOKE COLLECTED FROM *AZADIRACHTA INDICA* (NEEM) AND *OLEA EUROPAEA* (OLIVE) LEAVES "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: Kibrom Teshome

Signature: ________________________________

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

Name: Estifanose Ele (PhD)

Signature: _________________________________

Place and date of submission: Addis Ababa University, Department of Chemistry

July, 2020

Addis Ababa, Ethiopia

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LIST OF ABBREVATIONS

DCM	Dichloride methane
TCA	Trichloro acetic acid
FRAP	Ferric Reducing Antioxidant Power
WHO	World Health Organization
NIST	National Institute of and Standard and Technology
GC-MS	Gas Chromatography Mass Spectroscopy
UV	Ultra-Violent
BHA	Butylated Hydroxy anisole
BHT	Butylated Hydroxy toluene
ppm	Part Per Million

ABSTRACT

This study aimed at the analysis of essential oil and smoke from neem and olive leaves for their biological (antioxidants and insecticidal) activities. Essential oil was extracted using hydrodistillation technique, and the smoke was collected using lab designed set of apparatus. The chemical compositions of the oils and smoke were analyzed by Gas Chromatography Mass Spectrometry (GC-MS). The test for antioxidant activities was performed using UV-Visible spectroscopy. The antioxidant concentration of the sample was determined by using a calibration curve constructed from standard ascorbic acid, and the insecticidal activity test (both repellent and mortality) was conducted using cockroaches.

The measured average antioxidant concentrations for neem smoke trapped in methanol was 19.65% for 152.20 µg/mL, and for olive smoke trapped in methanol was 4.91% for 152.20 µg/mL. While, the antioxidant concentration test in the neem and olive smoke trapped in hexane was found to be negligible. The time course insecticidal (insect repellent) activities were performed at 10, 20, 30, 40, 50, and 60 minutes using 15 mg of the smoke trapped in hexane and methanol against cockroaches.

For the neem hexane trapped smoke sample the minimum % repellent was 20 % at 10 minutes, and the maximum was 81.24 % at 60 min, and for olive hexane trapped smoke was 14.16 % at 10 min and 77.96 % at 60 minutes. The neem methanol trapped smoke showed 42.92 % at 10 min, and 100 % at 60 min. The olive methanol trapped smoke showed 33.33 % at 10 min, and 92.10 % at 60 minutes. The time course insecticidal (insect mortality) activities were performed at 3, 6, 9, 12, 24, and 36 hours using 1.25%, 2.5%, 5%, 10% samples concentration.

The minimum and maximum % mortality of neem hexane trapped smoke was 6.66 % at 1.25% concentration in 3 h, and 80 % at 10 % concentration in 36 h, For olive hexane trapped smoke the minimum rate was 6.66 % at 1.25% in 3 h, while the maximum was 60 % at 10 % over 36 h. For neem methanol trapped smoke the minimum was 20% at 1.25% concentration in 3 h and the maximum was 100 % at 5 and 10% concentrations in 6 h, for the olive methanol trapped smoke the minimum was 20 % at 1.25 % concentration at 3 h while, the maximum rate was 100% at 10 % concentration in 24 hours.

The major compounds detected in the neem hexane trapped smoke were Methyl Hexadecanoate (12.11%), Methyl stearate (4.88%). The major compounds detected in olive hexane trapped smoke were Methyl octadec-9-enoate (E)- (29.66%), Methyl (9Z,12Z) – octadeca-9,12-dienoate (15.35%), Methyl Hexadecanoate (13.54%), The methanol trapped smoke of the neem showed 3,5-ditert butyl phenol (29.35%), Methyl Hexadecanoate (12.93%), 1,2-15,16-Diepoxy hexa-decane (7.92%) as major compounds, and the olive methanol trapped smoke showed 2,6-ditert-butyl phenol (38.27%), Methyl Hexadecanoate (8.70%), (E) -Tetradec-2-ene (8.45%), as major compounds. From the leaves the essential oil of neem extract showed Methyl tetracosanoate (3.72%), 2,4-Bis (dimethyl benzyl)-6-t-butyl phenol (3.39%), Glabrol (2.79 %), as major compounds, and the olive essential oil extract were Benzene acetaldehyde (3.24%), N-desmethyltapentadol (1.90%), 1-(2,6,6-trimethyl cyclohexa-1,3-dien-2-en-1-one (1.88%) as major compounds.

Key word: Biological activities, antioxidant, insecticidal, bioassay, neem, and olive leaves.

1. INTRODUCTION

Plants are in use as sources of medicinal health care since the ancient time. The World Health Organization (WHO) estimated that 80% of the world uses traditional medicines which are produced mainly from natural products (animals and plants) for daily health care requirements. There are about, 35,000-70,000 species of plants that are used for medicinal purposes globally according to WHO. The medicinal plants are the major constitute of traditional system of medicine hence, many countries should give emphasis to develop and utilize local medications. Ethiopia is sixth center of biodiversity in the world that contains diversified flora and fauna. There are more than 6,500 species of plants of which 12% are endemic and about 887 species are used as medicinal plants. Almost 80% of the Ethiopian people depend on traditional medicine of which 95% of them are plant origin [1].

Plants and animals produce primary and secondary metabolites. The primary metabolites are like (proteins, carbohydrates, lipids, nucleic acid), and secondary metabolites are terpenoids, alkaloids, steroids, phenols, etc. which, are potential sources of bioactive compounds [2].

Bioactive compounds are highly beneficial for the treatment and preventions of diseases like cardiovascular and cancer. The protective abilities of bioactive compounds are associated to their antioxidant, insecticides, antimicrobial, antiviral or anti-carcinogenic effects. Plant extracts (polyphenols) are also protective ingredients in food, pharmaceutical and cosmetics industries as food additives, preservatives and dietary supplements instead of synthetic chemicals [3].

The neem tree commonly referred to *Azadirachta Indica* is from the meliaceae family. The neem tree can reach a height of 30 meters with a trunk girth of 2.5 meters. Its deep root system is well adapted to retrieving water and nutrients from the soil. The neem tree survives in hot, dry climates, even the shade temperatures often reach 50 degrees Celsius and annual rainfall ranges from 400 to 1,200 millimeters [4].

Olive tree is an evergreen tree, and the fruitiest tree in the world. It is believed to be originated from Mediterranean region. The plant is associated with human religious, social, culture, medicinal, and nutritional needs. The olive plant extract contains phenols, phenolic acids, catechol, tyrosol, and inhibited oxidative deterioration of refined vegetable oils. The olive leaves also, contain phenolic compounds which are used as antioxidant compounds [5].

Both neem and olive plants uses for antioxidant and insecticidal activities. Antioxidants are compounds that reduce free radical activity, and protect the components of cells, and biomolecules from oxidation by scavenging an electron from reactive oxygen species (ROS) such as superoxide, peroxy, and hydroxyl radicals. Many recent studies reported that people can reduce DNA damages, which cause risk for cancer and cardiovascular diseases, significantly by eating more fruits, vegetables, and other foods from plants that contain phytochemicals [6].

Insecticides are compounds that protect insects from attacking animals and plants, and it can be natural or synthetic. The compounds fall into several classifications including repellents, feeding deterrents, toxins, and growth regulators [7].

The objective of this work was to investigate biological activities (antioxidant & insecticidal) of the plants *Azadirachta indica* (neem) and *Olea eurpoaea* (olive) leaves. Investigation was made on the essential oils and smokes collected by burning the plant parts.

2. LITERATURE REVIEW

2.1. Medicinal Plants

Medicinal plants are important traditional medicinal systems in the world. Ethno pharmacological surveys are rational for the selection and scientific investigation of medicinal plants, since a huge number of people use an indigenous remedy effectively over extended periods of time. People uses many wild species of plants as food, medicine, cosmetics, clothing, perfume, shelter, fiber, fuel, religious, and other applications.

Developing countries use traditional medicinal plants as primary sources of healthcare. Ethiopia is a huge potential of medicinal plants, and their uses that provide a wide contribution to the treatment of human and livestock aliments. About 80% of the human population and 90% of livestock depend on traditional medicine for primary health care services, and most of these come from plants. As a result there are high expectation of enormous traditional knowledge, and uses of medicinal plant species [8, 9].

2.2. Phytochemicals of medicinal plants

Phytochemicals are chemical compounds of the plant origin; they are essential and have protective or disease preventive properties [10]. They act as natural defense system for host plant and provide color, aroma and flavor. The phytochemicals play a substantial role in the prevention from microbial, insecticidal or herbivorous. They contain secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, phenols, and glycosides which are antimicrobial, anthelmintic, antidiarrheal activities while, saponins and polypeptides are considered to have anticancer and antiviral activities respectively [11,12].

Table 1: Some medicinal plants used for different purposes in Ethiopia [13, 14.18.15]

#	Scientific name	Family name	Local name	Part	Mode of smoke	Treatments
1	*Allium staivvm.L*	Alliaceae	Nech shunkurt	. Root . Seed	. Inhalation . Oral	. Evil eye . Antibiotic
2	*Carissa spinarum L.*	Apocynaceae	Agam	Root	. Inhalation. . Fumigation . Fumigation	. Evil aye . Bleeding after delivery . Insect repellent
3	*Clausena ansata*	Ruteceae	Limch	. All part . Leaf	. Topical . Skin	. Exorcism . Skin rash
4	*Cordial aficana lam*	Boraginacea	Wanza	. Leaf / . Root	. Topical . Oral	. Nightmare, Liver disease, Amoebiasis
5	*Enchinops kebericho*	Asteraceae	Kebercho (O&A)	. Root . Shrub/root . Root . Root	. Inhalation, Smoke, /Nasally/Orally . Oral . Nasally, oral	. Evil aye . Febrile illness . Snake repellent . Internal Parasite . Febrile
6	*Ficus vasta forssk*	Moraceae	Shoal	Bark	. Inhalation . Anal	. Epilepsy . Hemorrhoid
7	*Ruta chalepensil L*	Rutaceae	Tenadam	Leaf	. Inhalation . Orally	. Evil aye . Deficient disorder . Common cold
8	*Crton macrostachyus*	Cuphorbiaceae	Bisana	. Root, Leaf . Leaf . Leaf, bark . Leaf	. Topical, and inhalation . Smoke, Skin . Nasal . Dermal	. Psychosis, . For good smell materials . Antifungal, Malaria. . Headache & Ring Worm
9	*Phytolacca*	Phytolacceae	Endod	. Root, Leaf . Root	. Skin . Inhalation, . Skin/ Orally	. Itching skin . Gonorrhea . Rabies
10	*Vernonia odoensis*	Asteraceae	Este mussie	Root Leaf	Oral	. Psychosis, Malaria . Tooth infection
11	*Vernanine amygdalina*	Asteraceae	Girawa	Root	Inhalation	Evil eye
12	*Otostegia integrifolia*	Lamiaceae	Tunjut (T)	. Leaf/bark . Root, Leaf, Herb	Inhalation, oral	. Psychosis Mallari Abagorba
13	*Aloe megalacantha*	Aloaceae	Ere (T)	. Bark, leaf juice, Herb	. Inhalational, oral . Skin,	. Evil eye . wound . Insect repellent
14	*Gymnosporia senegalensis*	Celastraceae	Kombolcha (O)	All parts	Oral	Evil eye
15	*Brachiaria brizontha*	Asteraceae	Maas'uwa (D)	All parts	Topical	Evil eye
16	*Silene macrosolen*	Caryophyllaceae	Wegert	Root	Inhalation	Evil eye
17	*Sida tenuicarpa Vollesen*	Malvaceae	Chifrig	Root, and Leaf	Topical, oral Skin	. Evil spirit . Foot wound

18	*Osyris quadripartita Decn.*	Santalaceae	Waatoo (Af)	Root	Inhalation	Evil sprite
19	*Plectranthus edulis Vatke*	Lamiaceae	Dinicha Oromo (O)	Leaf, root	Oral	Epilepsy
20	*Nigella sativa L*	Ranunculaceae	Tiqur-azmud	Seed	Skin	. Skin fungus . Antioxidant
21	*Ocimum lamiifolium*	Lamiaceae	Yemich-medhani	. Leaf . Leaf	. Skin . Nasal	. Mich . Headache
22	*Stereospermum kunthianum .C*	Bignoniaceae	Washinte	Stem bark	Oral	Snake and scorpion bites
23	*Azadirachta indica A. Juss.*	Meliaceae	Neem/kinin	Leaf, tree	Fumigation	Antioxidant, anti-malaria,
24	*Olea europaea subsp.*	Oleaceae	Weyra	. Leaf . Oil . Tree	. Inhalation . Smoking, and Fumigant	. Psychiatric disease . Body bath . Insect repellent

Local name of plants in Amharic (A), Oromigna (O), Tigrigna (T), Afar (Af), Debub H (D)

2.3. Plant derived smokes

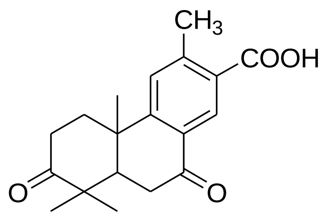
The use of smokes of medicinal plants has started from Mesopotamia, dated about 2600 BC; are still in use today for the treatment ailments ranging from coughs and colds to parasitic infections and inflammations [16]. In addition to its medicinal values, plant derived smoke has multiple uses, including air purification, ceremonial, flavoring [17].

Fumigation is commonly used mode processing plants parts for their use as medicine and insect repellents in Ethiopia. Mostly women use smoke as cosmetics to keep their skin clean and healthy [18]. There are different plant materials that are in use in the form of smoke to disinfect, heal and fragrance. This thesis focused on investigation of chemical compositions of smoke trapped from neem and olive leaves.

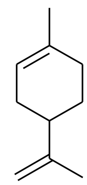
2.4. Chemistry of the Neem plant

Neem commonly known as *Azadirachta indica* is recently grow in about 72 worldwide countries, mostly in the tropical and subtropical countries. Neem has more than 135 unique bioactive compounds, which have been isolated from different parts of the plant. These compounds are categorized into isoprenoids including diterpenoids and triterpenoids containing liminoids, azadirone and its derivatives, genudin and its derivatives and csecomeliacins such as nimbin, salannin and azadirachtin. And non-isoprenoids including proteins (amino acids), carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, tannins, aliphatic compounds and phenolic acids [19,20].

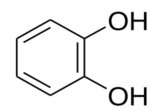
Terpenes are a unique group of hydrocarbon based natural products whose, structure is derived from isoprene. Terpenes are thus classified by the number of 5 carbon units they contain: Hemiterpenes C5, Monoterpene C10, sesquiterpene C15, Diterpene C20, Sesterterpene C25, Triterpene C30, and, tetratrepene C40.



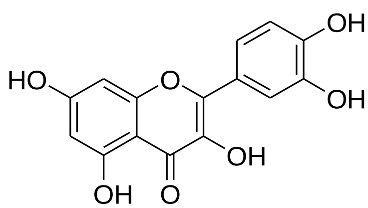
Margolonone (**1**)



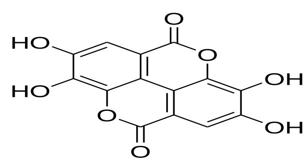
Limonene (**2**)



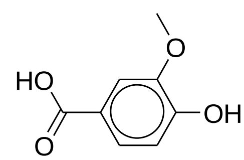
Phenol (catechol) (**3**)



Flavonoid (quercetin) (**4**)



Tannis (Ellagic acid) (**5**)

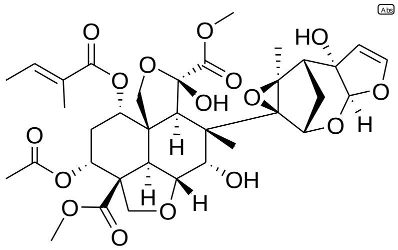


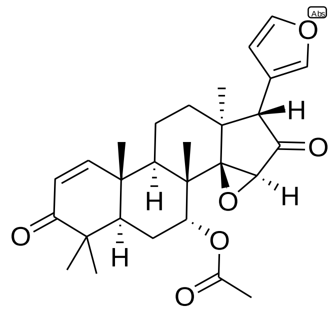
Phenolic acid (Vanillic acid) (**6**)

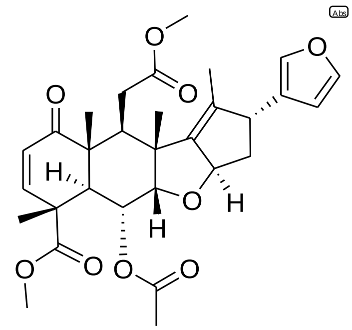
Figure 1 **:** Some bioactive compounds that are isolated from the neem plant [20, 21]

Table 2: Compounds that are isolated from neem plant, their sources, and biological activities and source [20, 21].

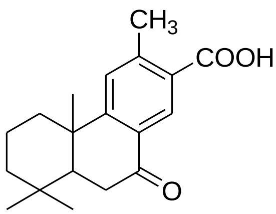
Compounds	**Source**	**Biological activity**	**Compounds**
Azadirachtin	Seed oil	Antimalarial	**7**
Nimbidin	Seed oil	Antigastric, antibacterial	**8**
Nimbin	Seed oil	Spermicidal	**9**
Margolone	Bark	Antibacterial	**10**
Epicatechin	Bark	Immunomodulatory	**11**
Catechin	Bark	Immunomodulatory	**12**
Cyclic trisulphide	Leaf	Antifungal	**13**
Gallic acid	Bark	Anti-inflammatory	**14**

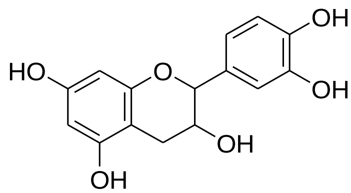


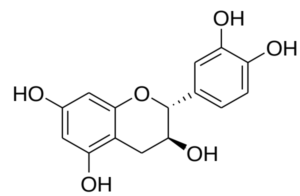




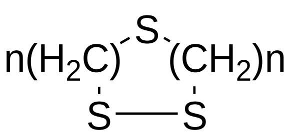
7 **8** **9**

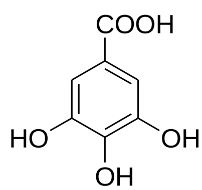






10 **11** **12**





13 **14**

Figure 2 : The bioactive compounds isolated from neem [21].

The most active compounds like alkaloids, ketones, phenolic compounds, carotenoids, flavonoids, steroids are effective against microorganism and ectoparacites including, bacteria, fungi, viruses, ticks, antimalarial, anticancer, antioxidant, antidiabetic, antifeedant, antitumor, antiseptic, antiviral, contraceptive, cosmetics, as oral deodorant, toothache reliever and tooth cleaners, insecticides, wound healing effects. Neem tree also used in agriculture as pesticides and fertilizer, medicine for diabetes, aids, cancer, heart disease, and, allergies. Generally, neem tree is one of the medicinal plants widely used in Ethiopia [22].

The compound Azadirachtin (**7**) is one a powerful insect repellent at low concentrations. Neem repels a wide variety of pests including, beet armyworm, aphids, the cabbage worm, white flies, mites, beetles, moth larvae, mushroom flies, leaf miners, caterpillars [23].

Neem plant used in the activation of antioxidative enzyme, that play a role in the control of problem caused by free radicals/reactive oxygen species. Most plant fruits, seeds, oil, leave, bark, and roots show an important role diseases prevention due to the rich source of antioxidant [24].

2.5. Chemistry of the olive plant

Olive tree, known as *Olea europaea* which belongs to the Oleaceae family have higher contents of bioactive compounds; including phenolic compounds, with diverse biological properties, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, in addition to regulating blood pressure, and cholesterol levels in animals. Thus, a previous study verified the effective antioxidant activity of olive leaf extract as a potential natural functional ingredient in food products [25].

Olive leaf is one of the potent sources of plant polyphenols having antioxidant, antimicrobial, antiviral properties due to its rich phenolic content. The most abundant phenolic component gives the bitter taste to olive and olive oil. Olive is rich in antioxidant compounds, especially tocopherols hydroxytyrosol, caffeic, protocatechuic, ferulic, syringic, and vanillic acids [26].

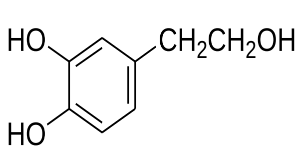
2.5.1 Phenolic compounds in the olive leaf and their antioxidants capacity

There are five groups of phenolic compounds present in olive leaves, namely oleuropeosides (oleuropein and verbasscoside), flavones (luteolin-7glucoside, apigenin-7-glucoside, diosmetin-7-, luteolin, and diosmetin), flavonols (rutin), flavan-3-ols (catechin), and substituted phenols (tyrosol, hydroxrosol, vanillin, vanillic acid, and caffeic acid). The antioxidant activity of flavonoids present in olive leaves are influenced by the present of functional group in their structure, mainly the B-ring catechol, the 3-hydroxyl group and the 2, 3-double bond conjugated [27].

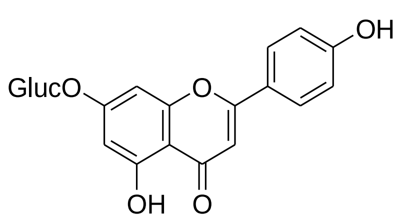
Table 3: The amounts of phenolic, and antioxidants extracted from olive leaves [28, 29].

Name of compounds	Amount in %	Antioxidant (TEAC) (mmol/L)
Olueropein	24.54	0.88 ± 0.09
Hydroxytyrosol	1.46	1.57 ± 0.12
Apigenin-7-glucoside	1.37	0.42 ± 0.03
Luteolin-7-glucoside	1.38	0.71 ± 0.04
Verbascoside	-	1.02 ± 0.07

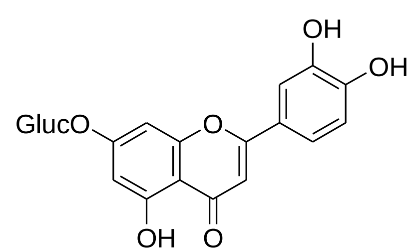
Antioxidant capacity of each individual phenolic compound based on their relative radical scavenging ability was found to be "Rutin > luteolin > olive leaf extract = hydroxytyrosol > caffeic acid > verbascoside > oleuropein > luteolin-7-glucoside-vannillic acid > apignin-7glucoside > tyrosol". Hydroxytyrosol a phenolic alcohol and one of the derivatives of oleuropein have demonstrated a good antioxidant property [29].



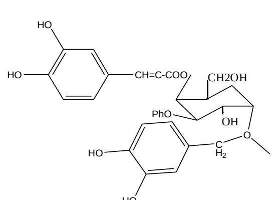
Hydroxtyrosol (**15**)



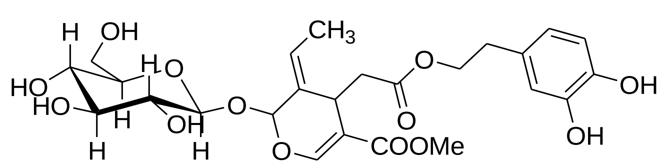
Apigenin-7-glucoside (**16**)



Luteolin-7-glucoside (**17**)



Verbasecoside (**18**)



Oleuropein (**19**)

Figure 3: Some of polyphenolic (antioxidant) compounds extracted from olive plant [30].

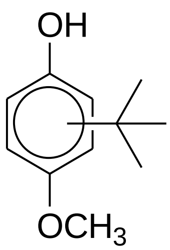
2.5.2 Oleuropein (19) and Its Properties

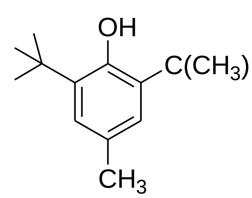
Oleuropein is the major phenolic compound in olive leaves. The Oleuropein is an ester of 2-(3, 4-dihydroxyphenyl) ethanol (hydroxytyrosol) with elenolic acid glucoside. Oleuropein has many benefits for health, including antioxidant, antimicrobial, anticancer, anti-inflammatory. Oleuropein uses as antioxidant at both prevention and intervention levels [31].

2.6. Antioxidant

2.6.1. Antioxidant Compounds

Antioxidants are compounds which are present at low concentrations compared to the oxidizable substrates that significantly delay or inhibit oxidization of those substrates. Antioxidants are mainly classified as; primary (chain breaking) and secondary (preventing). Antioxidants deactivate radicals by two major mechanisms, Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). Antioxidants that work with HAT mechanism quench free radicals simply by donating hydrogen atom while those of which work with SET mechanism transfer one electron to reduce any compound, such as metal ions, butylated hydroxyl toluene (BHT), and butylated hydroxyl anisole (BHA), most of them used in many foods additives, oils and fats [32].





Butylated hydroxyl anisole (BHA) (**20**)   Butylated hydroxyl tolune (BHT) (**21**)

Figure 4: The structures of some synthetic antioxidants

2.6.2. Free Radicals: - Free radicals are unstable and high energy atoms with an extra unpaired electron, produced unwanted metabolic by-products [33].

Free radicals in the body attack the nearby tissues by oxidizing membrane lipids, cellular proteins, DNA and causes complete shutdown of cellular activities such as respiration. Furthermore, the interaction of oxygen free radicals with members of lipidic portion of body leads us to formation of new radicals such as hydro peroxides, superoxide, lipoid oxides and hydroxyl radical whose type may interact with biological systems in a citotoxic manner [34].

DNA damage has been implicated as a cause of cancer prevention of oxidative stress caused by ROS and RNS has important implications for the prevention and treatment of diseases [35].

They interfere with the oxidation of lipids and other molecules by rapid donation to radicals (R):

$$R^{\cdot} + POH \rightarrow RH + PO^{\cdot} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

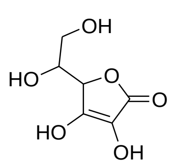
The phenoxy radical intermediates ($PO^{\cdot}$) are relatively stable due to resonance and therefore a new chain reaction is not easily initiated. Moreover, the phenoxy radical intermediates also act as terminators of propagation by reacting with other free radicals:

$$PO^{\cdot} + R^{\cdot} \rightarrow POR \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

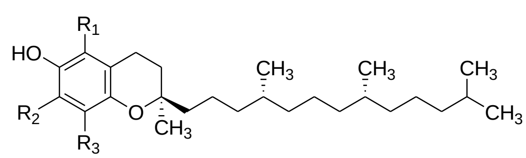
Phenolic compounds possess ideal structure for free radical scavenging activities because they have: (1) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; (2) extended conjugated aromatic system to delocalize an unpaired electron [36].

2.6.3 Compositions of Antioxidant compounds

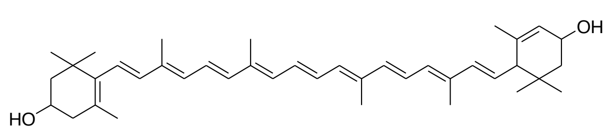
The most common antioxidants extracts or exists in plants are vitamin (C, A and E), Carotenoids, and phenolic compounds [37].



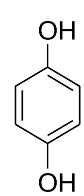
Vitamin C (ascorbic acid) (**22**)



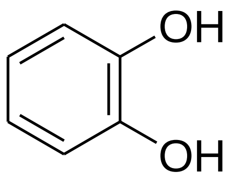
Vitamin E (Tocopherols) (**23**)



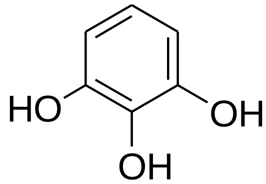
Carotenoid (Lutein) (24)



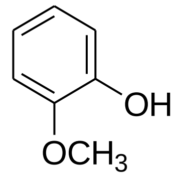
Hydroxy phenol (**25**)



Catechol (**26**)



Pyrogallol (**27**)



hydroxy anisole (**28**)

Figure 5: Representative of antioxidant compounds [37, 38].

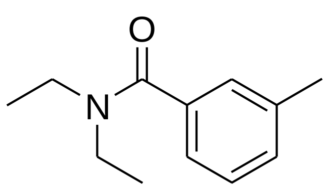
Table 4: Some plants commonly uses as the potential source of antioxidant [39].

Number	Name of plant	Common English name	Family	Local name	Plant part used
1	*Allium cepa*	Onion	Amaryllidacea	Key shunkurt	Bulp
2	*Aloe vera*	Aloe vera	Xanthorovhoeaceae	Ret	Leaf
3	*Azadirachta inidica*	Neem	Maliacea	Neem/kinin	Leaf
4	*Beta vuulgaris*	Beet root	Amaranthaceae	Key sir	Root
5	*Camellia sinersis*	Green tea	Theaceal	shay kittel	Green tea
6	*Cinnamomum tamala*	Tejpat	Lauranceae	Kerfa	Tejpat
7	*Curcum longa*	Turmeric	Lingiberaceae	Erd	Turmeric
8	*Daucuscarota*	Carrot	Apiaceae	Carrot	Root
9	*Emblica officinalis*	Amla	Euphorbiaceae	Machchew	Fruit
10	*Ocimum sanctum*	Tulsi	Lamiaceae	Damakesse	Leaf
11	*Zingiber officinale*	Ginger	Zigiberaceae	Zinjibele	Rhizome
12	*Solanum nigrum*	Blacknightshade	Soanaceae	Tikur awitt	Leaf
13	*Solanum tuberosum*	Potato	Soanacea	Dinich	Tuber
14	*Terminalia bellarica*	Behda	Combretaceae	Weyefba	Fruit

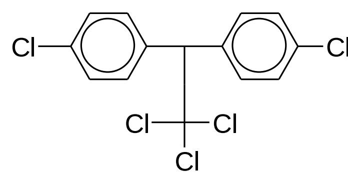
The medicinal plants are an important source of antioxidants which increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The secondary metabolites like phenolic and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots, and barks [39].

2.7. Insecticidal

Insecticides contain essential compounds preventing attack from phytophagous insects. The Insecticides are categorized as repellents, feeding deterrents, toxins, and growth regulators. Most can be grouped into five major chemical categories: (1) nitrogen containing compounds (primarily alkaloids), (2) terpenoids, (3) phenolic, (4) proteinase inhibitors, and (5) growth regulators. The gold-standard synthetic repellents DDT (Dichloro diphenyl trichloro ethane) and DEET (N, N-diethyl-3-methylbenzamide), are highly conserved across insect species.



N-N-Diethyl- m-toluamide (DEET) (**29**)



Dichloro diphenyl trichloro ethane (DDT) (**30**)

Figure 6: The structures of some synthetic insecticidal

Plant-based repellents are still extensively used in traditional way throughout rural communities as it is the only means of protection from mosquito in developing countries [40]. Plant-based repellents are safer than DEET since they are natural and less side effects, better for the environment than synthetic molecules, and simply have been used for a long time with tested safety record [41].

In Ethiopia, wogert, (Silene macroserene), kebericho (Echinops kebericho), tinjut (Ostostegia-integrifolia), and woira (Oleaeuropaea) and, neem have been shown to have repellent effects against insects is under laboratory conditions [42].

Table 5: Neem extract tested for Insect repellence.

The percentage repellency and EPI value of treated (cockroaches) with different concentrations of the power of *Azadirachta indica* for 1, 6, & 24 hours [43].

PLANT NAME	TREATMENT	TIME					
		1 Hours		6 Hour		24 Hour	
		EPI	PC	EPI	PC	EPI	PC
Neem	0d.5 gm (25%)	-0.38	70%	-0.52%	77%	-0.46	74%
	1.0 gm (50%)	-0.57	79%	-0.60	81%	-0.71	86%
	1.5 gm (75%)	-0.71	86%	-0.73	87%	-0.80	90%
	2.0 gm (100%)	-0.77	98%	-0.80	90%	-0.85	93%
	Control (biscuit)	0	0%	0	0%	0	0%

*PC = percentage of repellency *EPI = excess proportion index (EPI) < 1 Repellency, EPI > 1. Attractant, EPI = 1, Neutral

Table 5: Shows the repellency of Periplanata Americana (cockroaches), treated with different concentrations of the powder of *Azadirachta indica*. The highest repellency was recorded with the highest repellency dose of (2.0 g) neem powder.

Table 6: Mean percentage mortality of P. Americana (Cockroaches) treated with different concentrations of the powder of *Azadirachata indica* for 3 days [44].

Treatment	Mean Percentage Mortality For 3 Days		
	Day 1	Day 2	Day 3
0.5g (25%)	0.00	6.67	6.67
1.0g (50%)	8.33	33.34	33.34
1.5g (75%)	0.00	0.00a	33.34
2.0g (100%)	66.67	83.34	83.34
Control (biscuit)	0.00	0.00	0.00

The maximum mortality of cockroaches recorded at a maximum concentration was 83.34%.

2.8. Extraction of Essential oil

Essential oils contain volatile substances that are isolated by a physical method or process from plants. The essential oils are so termed perceived to represent the very essence of odor and flavor. The essential oils are of a great benefit to help protect our bodies and homes from pathogens. The immune system needs support and required endorsement of essential oils. Essential oil can be extracted using a variety of methods, depending on the plant material to be distilled, and the desired product. The essential oils can be extracted by steam distillation and hydrodistillation, microwave extraction, soxhlet extraction, supercritical fluid extraction method, solvent extraction, and cold pressed expression methods [45].

2.8.1. Steam distillation and hydrodistillation

Steam distillation method is used for the extraction of essential oil from the plant materials. It is a special type of distillation process for temperature sensitive materials like oils, resins, hydrocarbons, in which they are insoluble in water and may decompose at their boiling point [46]. Hydro distillation process involves that the plant materials to be extracted are completely immersed in water, and boiled by applying heat. The boiling water would carry the less volatile materials with it and condensed in a condenser attached to the round bottomed flask. It's recommended that the process is performed under atmospheric pressure at the boiling temperature about 100 °C [47].

2.8.2. Microwave assisted extraction (MAE)

Microwaves generate heat by interacting with polar compounds such as water and some organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms that allow the extraction of essential oils. The transfers of heat and mass are in the same direction in a MAE, which generates a synergistic effect to accelerate extraction and improve extraction yield. The application of MAE provides many advantages, such as increasing the extract yield in addition to avoid decomposition of the components [48, 49].

2.8.3. Soxhlet extraction

This method integrates the advantages of the reflux extraction and percolation, which utilizes the principle of reflux and siphoning to continuously extract the herb with fresh solvent. This extraction is an automatic continuous extraction method with high extraction efficiency, requires less time, and solvent consumption than the other. The high temperature and long extraction time in the Soxhlet extraction will increase the possibilities of thermal degradation [50].

2.8.4. Supercritical fluid extraction (SFE)

It has similar solubility to liquid and similar diffusivity to gas, and can dissolve a wide variety of natural products. Their solvating properties dramatically changed near their critical points due to small pressure and temperature changes. Supercritical carbon dioxide was widely used in SFE because of its attractive merits such as low critical temperature (31 °C), selectivity, inertness, low cost, non-toxicity, and capability to extract thermally labile compounds. The low polarity makes it ideal for the extraction of non-polar natural products such as lipid and volatile oil [51].

2.8.5. Solvent extraction

The hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing resin or a combination of wax, and essential oils remains. The principle of solid liquid extraction is when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent. The extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent and this takes place in a concentration gradient. It is one of the methods used in the preparation of oils from flowers, in which flower petals are mixed with volatile solvents such as a petroleum ether or benzene until all the volatile oils dissolve in the solvent, and the solvent is evaporated at a low pressure [52].

2.8.6. Cold pressing

This technique is an extraction without heating. This method is used to extract acid oils such as orange and lemon, grapefruit and mandarin. The principle of this mechanical process is based on machine squeezing the citrus at room temperature for the release of essential oils. One way is to puncture the oil glands and then the fruit to remove oil from the glands and wash with a little spray of water. It is important to note that oils extracted using this method have a relatively short shelf life [53].

3. OBJECTIVE OF THE WORK

3.1. General objective

The main objective of this study is:-
To extract, analyze and conduct biological activities of essential oils and smokes collected from plant materials that are used locally as fragrances and for disease control.

3.2. Specific objectives

- extract essential oils from the neem and olive leaves by hydro distillation.
- collect smoke from the neem and olive leaves by burning the plant materials and trapping the smokes using hexane and methanol solvents.
- analyze the extracts by GC-MS.
- test the antioxidant activities of the extracts using UV-visible spectroscopy.
- test the insecticidal activities (repellent and mortality) rates of the smokes on a selected insect.

3.3. Significance of the study

This study provide scientific information concerning smoke as traditional medicine, and it also led to the discovery of potential bioactive compounds from smoked medicinal plants which can help as antioxidant, and insecticidal activities.

4. EXPERIMENTAL PART

4.1. Materials and Methods

4.1.1. Plant materials

The neem leaf (mogersa, kinin) and olive leaf (weyra local name) used in this study were collected from Addis Ababa area. The plant materials were allowed to dry under shade and burned to collect smoke. Essential oils from both plants were extracted using Clevenger apparatus.

Figure 7: Photo of a) neem leaf b) olive leaf

4.1.2. Equipment's /Apparatus/ and Chemicals used

The equipment used to conduct this study was hydrodistillation set up, locally manufactured electrical stove, Petridish, insect repellent device set up, Suction flasks (250 mL), Rubber tube, funnel, different size Erlenmeyer flask, rotary evaporator, Spatula, Watch glass, oven, What-man number 1 filter paper, digital balance, UV-Visible spectra meter (model), thermometer, Gas chromatography-Mass spectrometer (GC-MS) (Model: GC-7820A, Agilent Technologies; Detector-5977EMSD, USA) Column: DB-1701, (30 m × 0.250 mm), and 0.25 micrometer particle size), tong, measuring cylinder, heating gun.

All chemicals, reagents, and solvents used in the experiment are hexane, methanol, Dichloro methane (DCM), ethyl acetate, ascorbic acid (standard)**,** buffer solution of (NaH_2PO_4, and Na_2HPO_4), 1% of potassium ferric cyanide, 10% of trichloro acetic acid (TCA), and 0.1% of ferric chloride ($FeCl_3$).

4.1.3. Essential oils extraction

Essential oil of the neem was obtained by hydro distillation. The fresh leaves (1 kg) of neem were inserted in to a distillation flask containing distilled water (2 L). The flask was attached to a Clevenger apparatus, which was attached to a condenser. Hydrodistillation continued at for 4 hrs after initial boiling. The essential oil was separated from the aqueous phase, and the essential oils were stored at 4 °C in refrigerator until it was analyzed by GC-MS. Following the same procedure, 1 kg fresh leaves of olive was used for extraction of the essential oil. The yields (v/w, mL/kg) were calculated using the amount of essential oil (mL) or mass (gram) obtained relative to the fresh weight (kg) of the sample used.

Figure 8: Hydrodistillation set up.

4.1.4. Smoke collection

The neem and olive leaves were chipped off separately into small pieces and air dried at room temperature. The dried sample plant materials (300 g) was burned by an electrical stove smoker with a small opening underneath which is designed for the inlet of air to keep the plant material burning and increase the amount of smoke entering in to methanol and hexane solvents. The smoke produced from the burning plant material was collected using the inverted funnel fitted with a heat resistance rubber tube. Then, smoke flows from the smoker through the heat resistance rubber tube, and, the other end of heat resistance rubber tube was fitted with a glass tube which passed through the hole of stopper (cork) and placed below the surface of 100 mL methanol in 250 mL suction flask. The stopper was chosen so that the outer diameter of the stopper fits the inner diameter of the mouth of the flask. The arm of the flask was connected to the second suction flask by rubber tube which was fitted into glass tube on the other end. Glass tube was passed through the opening of stopper on the mouth of the flask and placed below the surface of 100 mL hexane in 250 mL suction flask. The advantage of the second suction flask (100 mL hexane in it) was to condensate smoke which was not trapped by methanol in the first flask. A water aspirator is attached to the side arm of the second flask [54]. The vacuum generated by the aspirator was used to draw the smoke from the smoker into solvents (Methanol & Hexane), as shown in Figure 9. The smoke extracted was concentrated using a rotary evaporator and analyzed.

Figure 9: Smoke trapping and extraction system.

4.2. Gas chromatography mass spectroscopy (GC-MS)

The essential oils and smoke were analyzed by gas chromatography (Agilent technologies 7820A GC system coupled with Agilent technologies 5977E MSD, USA). The chromatographic capillary column (DB-1701) 30 m long and 0.25 mm in internal diameter was used. Ultra-high pure (99.999%) helium gas, as the carrier gas, was used at a constant flow mode. An Agilent 7820A auto sampler was used to inject 1 μL of the sample with a splitless injection mode into the inlet heated to 275 ºC. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min, and then, the temperature was increased at a rate of 10 ºC/min until the column temperature was reached 200 ºC, and then heated at the rate of 3 ºC/min till the temperature reached 240 ºC. No mass spectra were collected during the first 4 minute of the solvent delay. The transfer line, the ion source and ion filter temperature were 280 ºC, 230 ºC and 150 ºC respectively. The parameters, such as the quality, and probability values of peaks identifications was made through a library search using NIST 2014.

4.3. GC-MS sample preparation

4.3.1. Smoke sample preparation

A 0.2 mg of sample of hexane extracted smoke was dissolved in DCM solvent to form 10 mL solution with a concentration of 0.02 mg/ml (20 ppm). The smoke trapped in methanol was also prepared in similar way.

4.3.2. Essential oils sample preparation

5 µl of essential oil was added in to DCM solvent to form 25 mL solution (200 ppm). 0.1 mL solution of 200 ppm was added to 0.9 mL DCM solvent to form 1 mL (20 ppm) solution. One µL solution was analyzed by GC-MS.

4.4. Antioxidant activity determination

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. The presences of antioxidant in the extracts result into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} in to Fe^{2+}, there by changing the solution into various shades from green to blue, depending on the reducing power of the compounds. Strong reducing agents, however, formed Perl's Prussian blue color and absorbed at 700 nm. The higher the absorbance of the reaction mixture, the higher would be the reducing power. It has been reported that the reducing power of substances is probably because of their hydrogen-donating ability. The donors react with free radicals to convert them into more stable products, and then terminate the free radical chain reactions [55].

The reducing ability assay is based on the principle that substances, which have reduction potential, react with potassium ferric cyanide ($K_3Fe^{3+}(CN)_6$) to form potassium Ferro cyanide ($K_4Fe^{2+}(CN)_6$), which then reacts with ferric chloride to form a ferric-ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferric cyanide + ferric chloride} \xrightarrow{\text{antioxidant}} \text{potassium ferric cyanide + ferrous chloride}$$

$K[Fe(III)(CN_6)]^{3+}$ + Ph-OH → $Kx(Fe_n^{3+}[Fe^{+2}(CN)_6])$ + Ph-O• + H^+ .... (1)

(Yellowish color) (Color of essential oil) (Greenish-blue color)

$K_3Fe^{3+}(CN)_6$ (aq)+Iron chelator [red] (aq)→$K_4Fe^{2+}(CN)_6$ (aq)+ Iron chelator [ox] (aq)…..(2)

$K_4Fe^{2+}(CN)_6 (aq) + FeCl_3(aq) \rightarrow KFe^{3} + Fe^{2+}(CN)_6(aq) + 3KCl (aq)$ …………………(3) [56].

The antioxidant activities of the smoke extract was performed by UV-Visible analysis using the standard ascorbic acid solution and prepared solutions.

4.4.1. Preparation of reagents

A 200 mM sodium phosphate buffer solution (NaH_2PO_4, and Na_2HPO_4) prepared as, 34.836 g Na_2HPO_4 was dissolved in 100 mL distilled water to gives 2 M solution, and 27.218 g of NaH_2PO_4 was also dissolved in 100 mL of distilled water gives 2 M solution. The two solutions were mixed up in the ratio of 38.1 mL of Na_2HPO_4 and 61.9 mL of NaH_2PO_4. The PH of prepared solution has been checked using pH meter at room temperature (adjust if it is not pH = 6.6), the combined stock solution (2 M) was diluted to 1 litter with distilled water to obtain a 0.2 M solution. 1% potassium ferric cyanide was prepared by dissolving 1gram of potassium ferric cyanide in100 mL of distilled water. 10% TCA was prepared by dissolving 10 grams of TCA in 100 mL, and 0.1% $FeCl_3$ was prepared by dissolving 0.05 g of $FeCl_3$ in 50 mL.

4.4.2. Preparation of stock solution

The stock solution was prepared by dissolving 0.0088 grams of ascorbic acid in 500 mL to get a concentration 17.6 µg/mL. The remaining concentrations (0.176 µg/mL, 0.88 µg/mL, 1.76 µg/mL, 3.52 µg/mL, 7.04, 10.56 µg/mL) were prepared from the stock solution by a serial dilution method. The calibration curve was drawn from these concentrations, and the reducing power of the extract and smoke of a different concentration would be determined according to [57].

4.4.3. Samples preparation and UV analysis

The samples were prepared by dissolving 0.3 gram of smoke trapped in methanol in 2 mL of methanol solvent, then from the prepared sample solution 0.5 mL was dissolved in to 48 mL of methanol, and mixed with 2.5 mL of 200 mM or (0.2 M) sodium phosphate buffer of pH = 6.6 and 2.5 mL of 1% of potassium ferric cyanide [$K_3Fe(CN)_6$]. Shaked well using vortex shaker and the mixture were incubated in water bath at 50 ^{0}C for 20 minutes. Then 2.5 mL 10%TCA added to the mixture and centrifuged at 300 rpm for 10 minutes. Then 2.5 mL upper layer was taken and mixed with 2.5 mL of distilled and 0.5 mL of 1%$FeCl_3$ mixed well and let to stand for

10 minutes at room temperature. Then samples of 500 µL, 1000 µL, 1500 µL were diluted with a solvent (distilled water) 3.5 mL, 3 mL, & 2.5 mL respectively to form 4 mL volume solution. The prepared solution was added to cuvettes for UV measurement. The antioxidant activity of the samples was observed at 700 nm against the blank solution as control. The concentration of the sample complex was calculated by stating equivalent to regression equation of ascorbic acid calibration curve.

4.5. Insecticidal (repellents) tests

The insecticidal (repellent) activity was conducted using Hexane and Methanol trapped smoke. In each case 15 mg of the sample was placed on one side of the set up and control (water or solvent) 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.

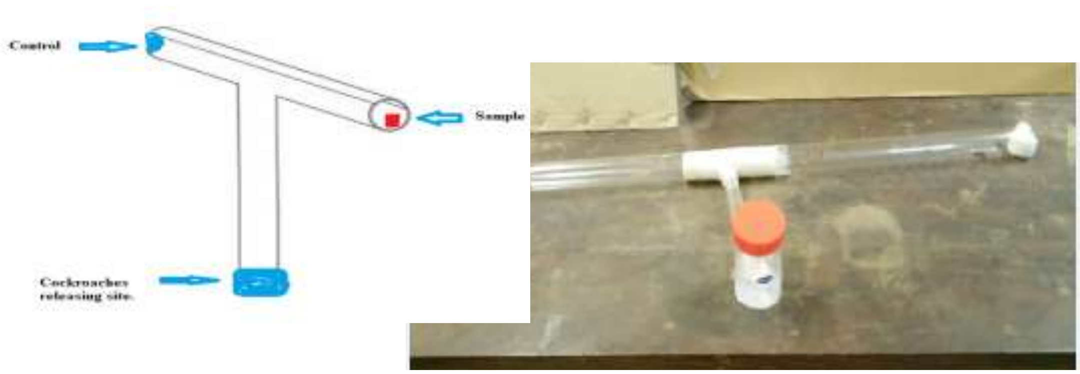


Figure 10: A set up to test repellent activity of the smoke samples

According to Abbot's formula's, and the device set up, the % insect repellent calculated as:

$$\text{Repellent (\%)} = \frac{(\text{\# of cockroach in the control side (CC)} - (\text{\# cockroach sample side (CS)})}{\text{Total \# of cockroaches in the control and sample sides}} \times 100$$

$$\text{Repellent (\%)} = \underline{(\text{\# CC - \# CS}) \times 100} \,/\, (\text{\# CS + \# CC})$$

4.6. Insecticidal (mortality) tests

4.6.1. Bioassays of extracts of neem and olive leave smokes against cockroaches

Bioassay methods that are commonly employed for insecticide toxicity evaluation are topical application, potter's tower method, and injection method, dipping method, contact or residual method, and film method. Film method is the technique used for this experiment.

Film method is the technique involves insecticide solution is usually deposited on glass surface such as Petri dish, flask, vial, wide mouth jar etc. In this approach Petri dishes (90 mm diameter) were lined with a filter paper and sample solutions (1 mL) was applied and allowed to dry. Samples were dissolved in appropriate solvents, methanol for the methanol extract, and DCM for the hexane extract. Using a 1.5 g solid sample, 20% stock solutions were prepared and diluted to get 10%, 5%, 2.5%, and 1.25% solutions and controls were prepared using blank solvents. After leaving for overnight for solvents to vaporize, 5 cockroaches were placed in a Petri dish. Experiments were conducted in a time course fashion to follow the mortality rate. Data was collected at 3, 6, 9, 12, 24 and 36 hours. All the measurements were done in triplicate and results were recorded as mean, and standard deviation. [58].

Figure 11: Mortality rate tests

5. RESULTS AND DISCUSSION

5.1. The percentage yield of the essential oil, and smoke extracts

The percentage yield of the extract was calculated from the relationship between the mass/volume of the extract and the mass or volume of the raw material used for the extraction. The % yield = (mass of the extract ×100) / mass of the sample, which is reported in the table below.

Table 7: Amount of essential oils, and smoke obtained from the extracts

No.	Extract Species	Volume obtained (mL)	Mass obtained (g)	Yield (%)
1	Neem essential oil	3	2.7	0.27
2	Olive essential oil	2	1.8	0.18
3	Neem methanol trapped smoke	-	5.5	1.83
4	Neem hexane trapped smoke	-	3.2	1.07
5	Olive methanol trapped smoke	-	6.1	2.33
6	Olive hexane trapped smoke	-	3.8	1.27

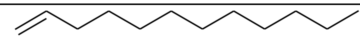
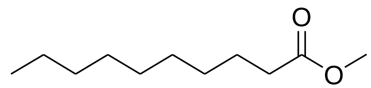
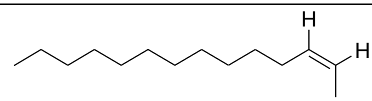
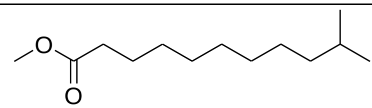
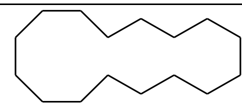
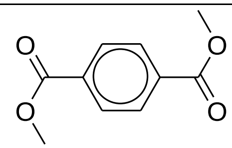
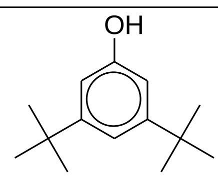
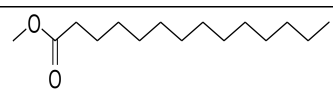
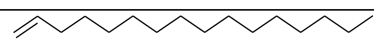
The amount of sample used for the essential oil extraction was 1 kg, and for the smoke extraction was 300 g. As it can be seen from the table above, the amount of smoke extracted in methanol is high in yield compared to the extract in hexane. The olive methanol smoke gave 2.33 % while that of neem was 1.83%. Relatively the amount of smoke in both solvents is higher than the essential oil extracted from the two plants. The extracts yield between 0.01 to 10% categorized as standard essential oils extract.

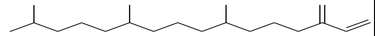
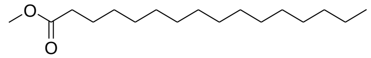
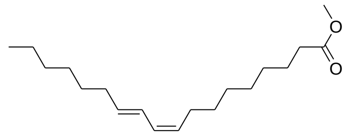
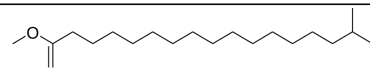
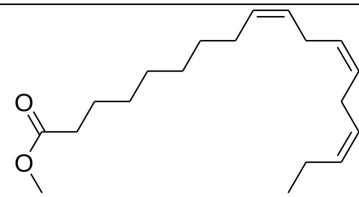
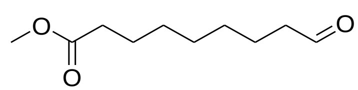
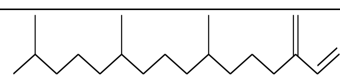
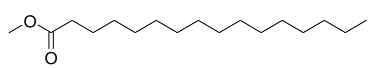
5.2.GC-MS analysis results

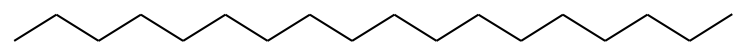
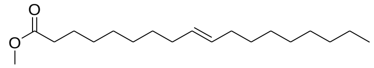
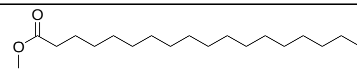
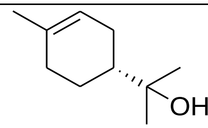
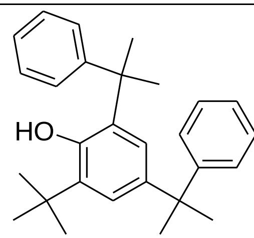
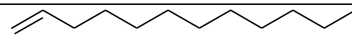
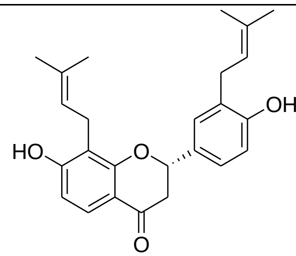
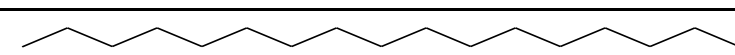
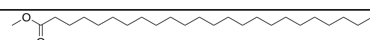
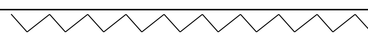
5.2.1. Characterization of essential oils and smokes

From GC-MS Analysis the result identified in terms of the total number of compounds and compounds with qualities ≥ 70% subjected to analysis with the help of NIST library 2014 mass spectral library. The following table shows GC-MS analysis results for the essential oils and smokes collected from neem and olive leaves.

Table 8: GC-MS analysis results of neem leaves essential oil and smokes (trapped by hexane and methanol).

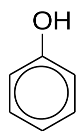
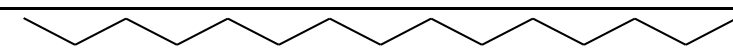
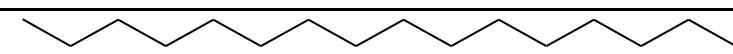
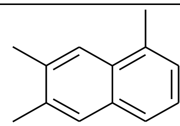
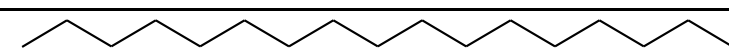
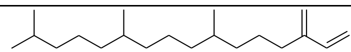
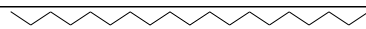
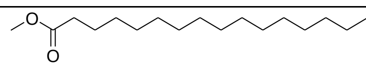
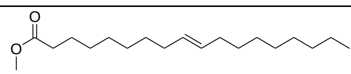
Sample name	Chromatogram Peak #	Name	Structure	Molecular Formula	MW	Retention time (t_R)	Area%	Retention index (RI)	Quality (%)
Neem leaves smoke trapped by methanol (NM)	1	1-Dodecene		$C_{12}H_{24}$	168.32	8.89	5.64	1092.22	96
	2	Methyl decanoate		$C_{11}H_{22}O_2$	186.29	11.57	3.96	1286.38	97
	3	(E)-2-Tetradecene		$C_{14}H_{28}$	196.37	11.68	6.51	1496.12	98
	4	Methyl, 10-methylundecanoate		$C_{13}H_{26}O_2$	214.34	14.10	2.28	1484.53	96
	5	Cyclohexadecane		$C_{16}H_{32}$	224.42	14.18	4.59	1649.10	94
	6	Dimethyl benzene-1,4-dicarboxylate		$C_{10}H_{10}O_4$	194.18	14.94	1.75	1081.56	97
	7	**3**,5-Di-t-butylphenol		$C_{14}H_{22}O$	206.32	15.11	29.35	14688.91	87
	8	Methyl tetradecanoate		$C_{15}H_{30}O_2$	242.40	16.39	1.0934	1686.95	97
	9	Cetene		$C_{16}H_{32}$	224.42	16.44	1.61	1745.30	99

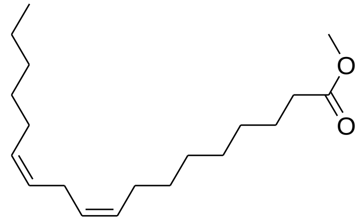
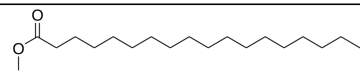
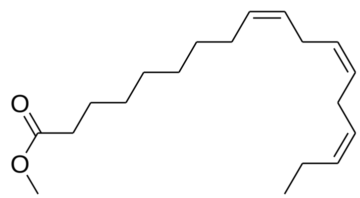
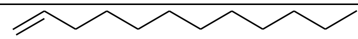
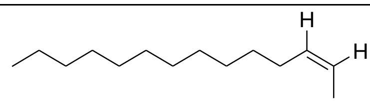
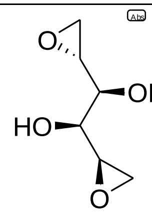
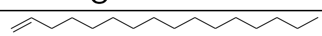
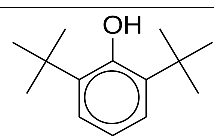
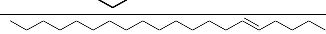
	10	Neophytadiene		$C20H_{38}$	278.52	17.11	2.86	1980.06	99
	11	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	12.93	1912.21	99
	12	Methyl (9*E*,11Z)-octadeca-9,11-dienoate		$C_{19}H_{34}O$	294.47	22.10	7.14	2119.73	98
	13	Heptadecanoic acid, 16-methyl-, methyl		$C_{19}H_{38}O_2$	298.50	22.27	4.57	2126.03	98
	14	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate		$C_{19}H_{32}O_2$	298.50	22.40	5.59	2131.57	99
	15	1,2-15,16-Diepoxyhexadecane		$C_{16}H_{30}O_2$	254.41	22.66	7.92	1680.09	86
Neem leaves smoke trapped by Hexane (NH)	1	Methyl 9-oxononanoate		$C_{10}H_{18}O_3$	186.25	14.30	0.33	-	70
	2	Neophytadiene		$C_{20}H_{38}$	278.52	17.11	0.3237	1882.03	99
	3	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	12.13	1912.26	99

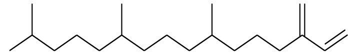
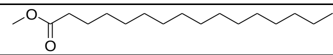
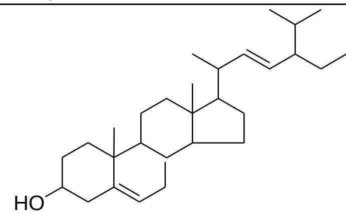
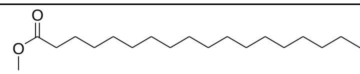
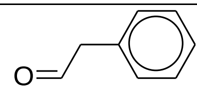
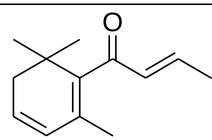
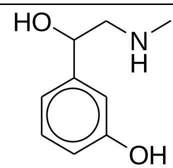
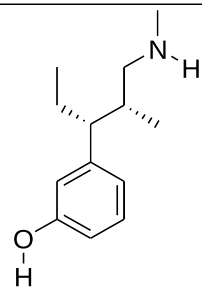
	4	Octadecane		$C_{18}H_{38}$	254.49	20.33	0.62	2014.73	89
	5	Methyl octade-9-enoate		$C_{19}H_{36}O_2$	296.49	22.01	0.59	2116.32	99
	6	Methyl stearate		$C_{19}H_{38}0_2$	298.50	22.27	4.88	2059.79	99
Neem Essential oil	1	Alpha.-Terpineol (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol)		$C_{10}H_{18}O$	154.25	10.37	0.29	-	93
	2	2,4-Bis(dimethylbenzyl)-6-t-butylphenol		$C_{28}H_{34}O$	386.65	14.63	3.40	-	99
	3	1-Docosene		$C_{12}H_{24}$	168.32	19.01	0.47	1725.22	99
	4	Glabrol		$C_{25}H_{28}0_4$	392.50	22.71	2.78	-	95
	5	Heptadecane		$C_{17}H_{36}$	240.47	26.90	2.60	2266.36	95
	6	Tetracosanoic acid, methyl ester		$C_{25}H_{50}O_2$	382.66	27.58	3.72	-	96
	7	Eicosane		$C_{20}H_{42}$	282.54	28.43	2.23	2026.16	92

Similarly the following table contains GC-MS data of the olive leaves sample

Table 9 GC-MS analysis results of olive leaves essential oils and smokes (trapped by hexane and methanol).

Sample name	Chromatogram Peak#	Name	Structure	Molecular Formula	MW	Retention time (t_R)	Area %	Retention index (RI)	Quality (%)
Olive leaves smoke trapped by Hexane (OH)	1	Phenol		C_6H_6O	94.11	9.12	1.82	-	90
	2	Pentadecane		$C_{15}H_{32}$	212.41	12.94	1.27	1548.36	96
	3	Hexadecane		$C_{16}H_{34}$	226.44	14.15	1.71	1647.96	96
	4	Naphthalene, 1,6,7-trimethyl-		$C_{13}H_{14}$	170.25	15.25	1.42	1525.54	98
	5	Heptadecane		$C_{17}H_{36}$	240.47	15.30	1.43	2954.43	97
	6	**Neophytadiene** (7,11,15-trimethyl-3-methyl idenehexadec-1-ene)		$C_{20}H_{38}$	278.52	17.13	6.41	1980.77	94
	7	Nonadecane		C_{19}H40	268.52	17.60	1.31	1944.42	97
	8	Methyl **hexadecanoate**		$C_{17}H_{34}O_2$	270.45	19.00	13.54	1913.24	99
	9	Heneicosane		$C_{21}H_{44}$	296.57	20.48	1.30		98
	10	Methyl octadec-9-enoate		$C_{19}H_{36}O_2$	296.49	22.04	29.66	21175.51	99

	11	Methyl (9Z,12Z)-octadeca-9,12-dienoate		$C_{19}H_{34}O_2$	294.47	22.13	15.35	2120.99	99
	12	Methyl stearate		$C_{19}H_{38}O_2$	298.50	22.30	9.52	2127.51	99
	13	Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate		$C_{19}H_{32}O_2$	298.50	22.43	2.98	2132.80	99
Olive leaves smoke trapped by Methanol (OM)	1	1-Dodecene		$C_{12}H_{24}$	168.32	8.89	6.79	1250.02	96
	2	(E)-2-Tetradecene		$C_{14}H_{28}$	196.37	11.68	8.45	1449.52	98
	3	1,2:5,6-Dianhydromannitol		$C_6H_{10}O_4$	146.14	12.00	4.60	-	90
	4	Cetene		$C_{16}H_{32}$	224.42	14.18	5.72	1649.73	95
	5	Phenol, 2,6-bis(1,1-dimethylethyl)-		$C_{14}H_{22}O$	206.32	15.11	38.27	1578.59	87
	6	(E) - 5-Eicosene		$C_{20}H_{40}$	280.53	16.44	1.89	1956.60	99

	7	Neophytadiene		$C_{20}H_{38}$	278.52	17.11	5.85	1980.02	98
	8	Methyl hexadecanoate		$C_{17}H_{34}O_2$	270.45	18.97	8.7054	1912.19	98
	9	Stigma sterol		$C_{29}H_{48}O$	412.69	21.13	5.84	-	96
	10	Methyl stearate		$C_{19}H_{38}O_2$	298.50	22.27	2.68	2126.30	96
Olive Essential Oil	1	Benzene acetaldehyde		C_8H_8O	120.15	8.69	3.24		80
	2	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-		$C_{13}H_{18}O$	190.28	13.08	1.81	1447.92	80
	3	Dl-Phenylephrine		$C_9H_{13}NO_2$	167.20	16.30	1.04	-	72
	4	N-Desmethyltapentadol		$C_{13}H_{21}NO$	207.31	16.59	1.90	1573.19	72

From the above tables, phenolic compounds appeared in neem and olive methanol trapped smoke. The leaves hexane trapped smoke mainly contain esters and alkanes as major compounds which are consistent with the absence of antioxidant activities of the extracts.

Table 10 below shows the classes of compounds that were identifies by GC-MS in the extracts.

Table 10: Classification of the compounds

Sample extracted	Total number of Compounds	Number of Compounds ≥70% quality	Major compound	Number of compounds In (%)	Area (%)
Neem essential oil	15	7	Phenolic Alkane Ester Alkene Terpene	28.57 14.28 14.28 14.28 14.28	40.42 30.28 24.33 3.07 1.90
Olive essential oil	34	4	Phenolic Terpene / ketone Aromatic	50 25 25	36.80 22.65 40.55
Neem methanol Trapped smoke	17	15	Phenolic Ester Alkene Terpene	6.67 53.33 20 7.7	34.74 30.74 16.29 3.96
Neem hexane Trapped	9	6	Ester Alkane Terpene	66.67 16.67 16.67	98.19 3.39 1.75
Olive methanol Trapped Smoke	12	10	Phenolic Alkene Ester Terpene Alcohol	10 40 20 10 10	43.10 25.73 12.62 6.60 6.58
Olive hexane Trapped smoke	18	13	Ester Alkane Terpene Phenol	38.46 38.46 7.69 7.69	81.0 8.00 7.31 2.08

Area % of the compounds ≥ 70 % quality was calculated as:

$$\text{Area \%} = \frac{\text{Total area \% of the same functional group compounds in the extract}}{\text{Total area \% of the compounds in the extract}} \times 100 \quad \text{……... (1)}$$

The area % of the major compounds (≥ 70 % quality) of the neem and olive leaves essential oils were dominantly phenolic with 40.42 % and 36.80 % respectively. While the neem and olive smoke trapped in hexane contain ester 98.19 % and 81.00 % respectively. When it comes to the smokes trapped in methanol, the major compounds were phenolic with 34.74 % in neem and 43.10 % in olive extract.

5.2.3. The major compounds identified from GC-MS result

The top major compounds of both neem and olive essential oils, and smoke trapped in methanol were mainly phenolic compounds. While the neem and olive smoke trapped in hexane were fatty acid esters.

Table 11: The major compounds of olive and neem leaves essential oil

Extract	Name of the compounds	Area (%)	Compound type	Compound
Neem essential oil	Methyl tetracosanoate	3.72	Fatty acid ester	31
	2-Tert-Butyl-4,6-bis (1-methyl-1-phenyl ethyl) phenol	3.39	Phenolic	32
	Glabrol (2*S*)-7-hydroxy-2-[4-hydroxy-3-(3-methylbut-2-enyl) phenyl]-8-(3-methylbut-2-enyl)-2,3-dihydro chromen-4-one	2.79	Phenolic	33
Olive essential oil	Benzene acetaldehyde	3.24	Aromatic aldehyde	34
	N-Desmethyltapentadol (3-[(2*R*,3*R*)-2-methyl-1-(methyl amino)pentan-3-yl] phenol)	1.90	Phenolic	35
	1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one	1.88	Ketone	36

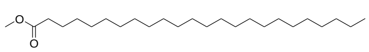
The top major compounds of both neem and olive essential oils were mainly phenolic compounds which were easily decomposed compared to fatty acid ester of smoke. The essential oils extracted contain compounds uses as insecticidal and antioxidant activities (example: 2-Tert-Butyl-4,6-bis (1-methyl-1-phenylethyl) phenol [59].

Table 12: The major compounds of the neem leaves smoke

Extracts	Name of compounds	Area (%)	Compound type	Cpd.
Neem hexane trapped smoke	Methyl hexadecanoate	12.11	Fatty acid ester	37
	Methyl stearate	4.88	Ester (Stearic acid)	38
Neem methanol trapped smoke	3,5-di*tert*-butylphenol	29.35	Phenolic	39
	Methyl hexadecanoate	12.93	Fatty acid ester	37
	1,2-15,16-Diepoxyhexadecane	7.92	Alkane (epoxy)	40

Table 13: The major compounds of olive leaves smoke

Extracted	Name of compounds	Area (%)	Compound type	Cpd.
Olive hexane trapped smoke	Methyl octadec-9-enoate	29.66	Fatty acid ester	41
	Methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate	15.35	Fatty acid ester	42
	methyl hexadecanoate	13.54	Fatty acid ester	37
Olive Methanol trapped smoke	2,6-Di-*tert*-butylphenol	38.27	Phenolic	43
	Methyl hexadecanoate	8.70	Fatty acid ester	37
	(E)- tetradec-2-ene	8.45	Alkene	44



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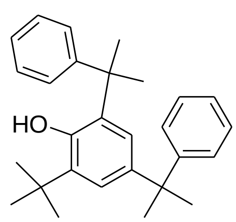

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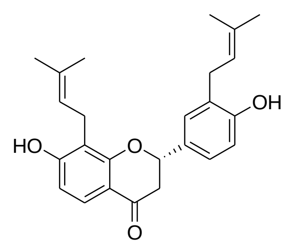

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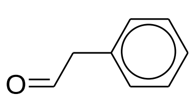

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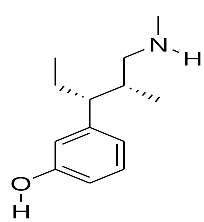

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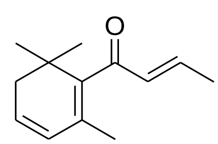

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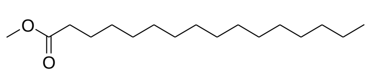

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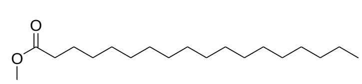

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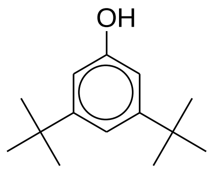

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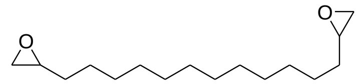

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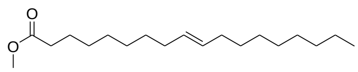

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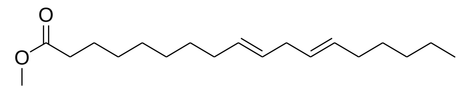

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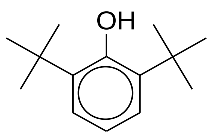

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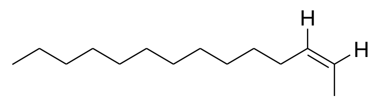

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Figure 12: The structures of the major compound of the extracts

The phenolic compounds 2-Tert-butyl-4,6-bis (1-methyl-1-phenylethyl)phenol and it analog's are good contributor for biological activities as antioxidant, and insecticidal [59]. While, the fatty acid esters reported in table 11, 12, and 13 are used as pesticides [60].

5.3. Antioxidant tests

5.3.1. Concentration and regression curve of ascorbic acid

Table 14: UV-Visible data of ascorbic acid

Sample Standard	Concentration (μ g/mL)	Absorbance
Ascorbic acid	0.176	0.2387 ± 0.0057
	0.88	0.2620 ± 0.0104
	1.76	0.2903 ± 0.0087
	3.52	0.3570 ± 0.0128
	7.04	0.5540 ± 0.0128
	10.56	0.7213 ± 0.0173

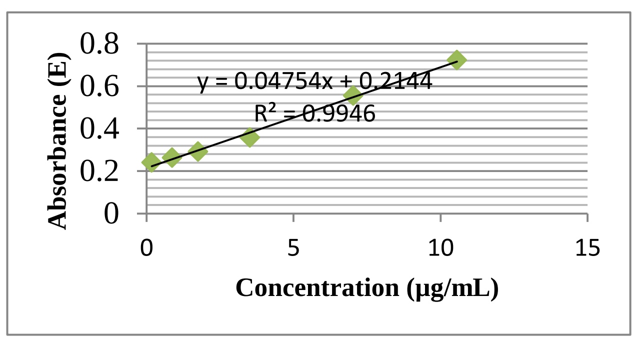


Figure 13: Linear regression curve of ascorbic acid

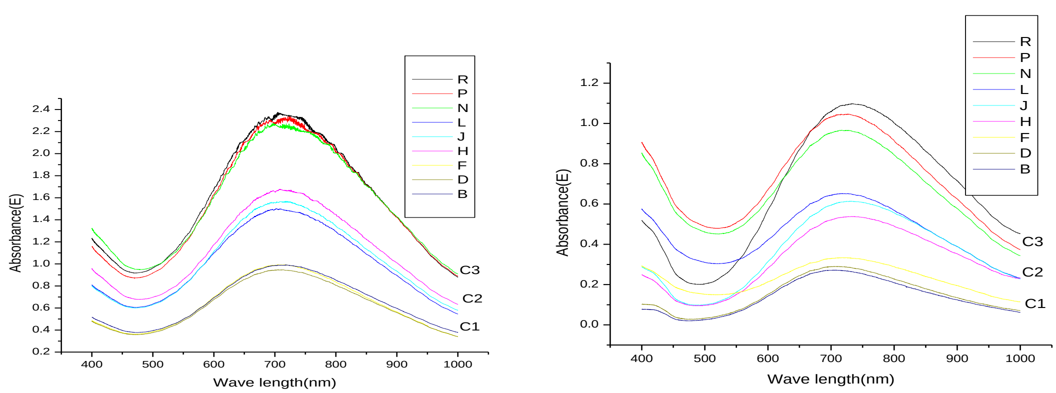
The relationship between concentration (μg/mL) of ascorbic acid and its average absorbance determined at 220 nm was used as positive control or standard calibration curve. The regression equation obtained for ascorbic acid was y = 0.04754x + 0.2144, (R^2 = 0.9946), which is used to calculate the concentration of antioxidant of the samples.

5.3.2. Concentration of antioxidants in the test samples

Table 15: Average absorbance of neem and olive leaves extracts

Sample of Methanol trapped Smoke	Concentration of Samples (µg/mL)	Absorbance Of Sample at 700 nm	Con. of Antioxidant Calculated from standard Calibration Curve (µg/mL)	% of antioxidant extract from the samples (%)	Mean % of antioxidant extract from the samples
Neem Leaf Trapped smoke	76.10	0.973 ± 0.0251	15.97	20.99	19.65 ± 0.84
	152.20	1.567 ± 0.05003	28.48	18.71	
	228.29	2.301 ± 0.0456	43.92	19.24	
Olive Methanol Trapped smoke	76.10	0.296 ± 0.0196	1.718	2.26	4.91 ± 1.81
	152.20	0.583 ± 0.0655	7.76	5.10	
	228.29	1.015 ± 0.0377	16.86	7.38	

The reducing power was determined according to Oyaizu (1984). The absorbance at 700 nm was measured. Increased absorbance of the reaction mixture indicated increased reducing power. or the concentrations of antioxidant compounds. A significant relationship was found between antioxidant potential and total phenolic content, suggesting that phenolic compounds are the major contributors to the antioxidant activity of neem and olive leaves. The antioxidants concentrations obtained were 19.65 ± 0.84 for the neem smoke trapped in methanol 4.91 ± 1.81 for olive smoke trapped in methanol. The results show that the antioxidant compounds in neem sample is in a higher concentration compared to that of olive. This value is consistent with the literature data that promote the medicinal values of the neem plant



a) b)

Figure 14 : Absorbance of a) Neem methanol smoke b) Olive methanol smoke

The antioxidant activity has direct relation with the concentration of the samples (Figure 14).

5.4. Insect repellent test result of solvent trapped smoke

The percentage repellent was calculated as:

$$\% \text{ repellent} = \frac{(\#\text{ of cockroaches in the control side} - \#\text{ cockroaches in the sample side}) \times 100}{\text{Total }\#\text{ of cockroaches in the control and sample}}$$

% repellent = (# CC - # CS) x 100 / (# CC + # CS)

For example: repellent (%) of olive methanol trapped smoke at 20 minutes calculated as;

Repellent (%) = (4.66 – 2.00) x 100 / 6.66 = 3 9.94%

Based on this, % repellent was calculated and shown in the table below.

Table 16: Insect (cockroaches) repellent test using neem and olive leaves smokes

Sample extracted	Time (min)	# Cockroaches move to control (C)	# cockroaches move to sample (S)	# cockroaches repellent (C-S)	# cockroaches treated (C+S)	#cockroaches un- treated 10 - (C+S)	% Repellents (C-S) x 100 / (C+S)
Olive Methanol trapped smoke	10	6.00	3.00	3.00	9.00	1.00	33.33 ± 0.82
	20	4.66	2	2.66	6.66	3.34	39.94 ± 0.47
	30	6.00	2.33	3.67	8.33	1.67	44.06 ± 0.47
	40	6.33	1.33	5.00	7.66	2.34	65.27 ± 0.47
	50	7.00	1.00	6.00	8.00	2.00	75.00 ± 0.00
	60	**8.00**	**0.33**	**7.67**	**8.33**	**1.67**	**92.10 ± 0.82**
Olive Hexane trapped smoke (OH)	10	2.66	2.00	0.66	4.66	5.34	14.16 ± 0.47
	20	3.00	1.66	1.34	4.66	5.34	28.75 ± 0.82
	30	4.66	1.33	2.33	5.99	4.01	38.90 ± 0.47
	40	5.66	1.66	4.00	7.32	2.68	54.64 ± 0.47
	50	5.00	0.33	4.33	5.33	4.67	66.67 ± 0.00
	60	**5.33**	**0.66**	**4.67**	**5.99**	**4.01**	**77.96 ± 0.47**
Neem Methanol trapped smoke (NM)	10	3.33	1.33	2.00	4.66	5.34	42.92 ± 0.47
	20	5.66	1.66	4.00	7.32	2.68	54.64 ± 0.47
	30	5.00	1.00	4.00	6.00	4.00	66.67 ± 0.82
	40	5.00	0.66	4.34	5.66	4.34	76.68 ± 0.47
	50	7.00	0.66	6.34	7.66	2.34	82.77 ± 0.81
	60	**8.00**	**0.00**	**8.00**	**8.00**	**2.00**	**100.00 ± 0.82**

Neem	10	3.00	2.00	1.00	5.00	5.00	20.00 ± 0.00
Hexane trapped smoke (NH)							
	20	3.33	1.66	1.67	4.99	5.01	33.47 ± 0.47
	30	3.66	1.33	2.33	4.99	5.01	46.69 ± 0.47
	40	4.00	1.00	3.00	5.00	5.00	60.00 ± 0.81
	50	5.33	1.00	4.33	6.33	3.67	68.40 ± 0.47
	60	**5.00**	**0.33**	**4.33**	**5.33**	**4.67**	**81.24 ± 0.47**

Note: Olive smoke trapped in methanol (OM), olive smoke trapped in hexane (OH), neem smoke trapped in methanol (NM), and neem smoke trapped in hexane (NH).

Table 16 shows that the trapped smokes have positive effect on the treated insects. Especially smoke tapped in methanol for both plants showed stronger repellency when compared to smoke trapped in hexane. This tells us that polar compounds trapped in methanol were responsible for the effect. Hence, the polar extract part of the plant can be used as insect repellent without further fractionation.

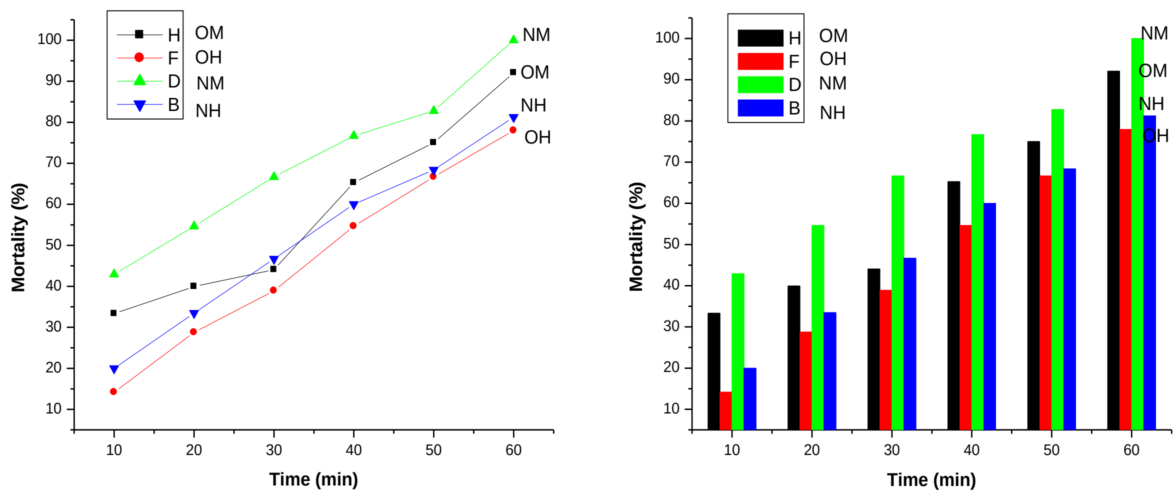
Between neem and olive, smoke from the neem showed higher repelling activity which is 100% at 60 min. While olive smoke exhibited 92% repellency at the end of one hour. From the data it can be inferred that the repelling nature of the extract increases with time. For the first 10 min all extracts showed lower repelling values with maximum repellency of 42.92% by the neem smoke trapped in methanol.

Table 17: Mean repelling values of the extracts.

Sample trapped smoke	Time interval and the average values						
	10min	20 min	30 min	40 min	50 min	60 min	Mean (%)
Olive Methanol	33.33 ± 0.82	39.94 ± 0.47	44.06 ± 0.47	65.27 ± 0.47	75.00 ± 0.00	92.10 ± 0.82	58.28 ± 0.51
Olive Hexane	14.16 ± 0.47	28.75 ± 0.82	38.9 ± 0.47	54.64 ± 0.47	66.67 ± 0.00	77.96 ± 0.47	46.85 ± 0.45
Neem Methanol	42.92 ± 0.47	54.64 ± 0.47	66.67 ± 0.82	76.68 ± 0.47	82.77 ± 0.81	100.00 ± 0.82	70.61 ± 0.64
Neem Hexane	20.00 ± 0.00	33.47 ± 0.47	46.69 ± 0.47	60.00 ± 0.81	68.4 ± 0.47	81.24 ± 0.47	51.63 ± 0.45

Table 17 shows that except the olive smoke trapped in hexane, all the rest showed average values above 50%. The highest recorded was for the neem smoke trapped in methanol.

The killing ability of the extracts go parallel with the repelling ability. This can not be a surprise as the two effects are interrelated. The killing nature of an insecticide is mostly preceded by repelling.



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Note: Olive smoke trapped in methanol (OM), olive smoke trapped in hexane (OH), neem smoke trapped in methanol (NM), and neem smoke trapped in hexane (NH).

Figure 15: Graphs showing rate of repellency

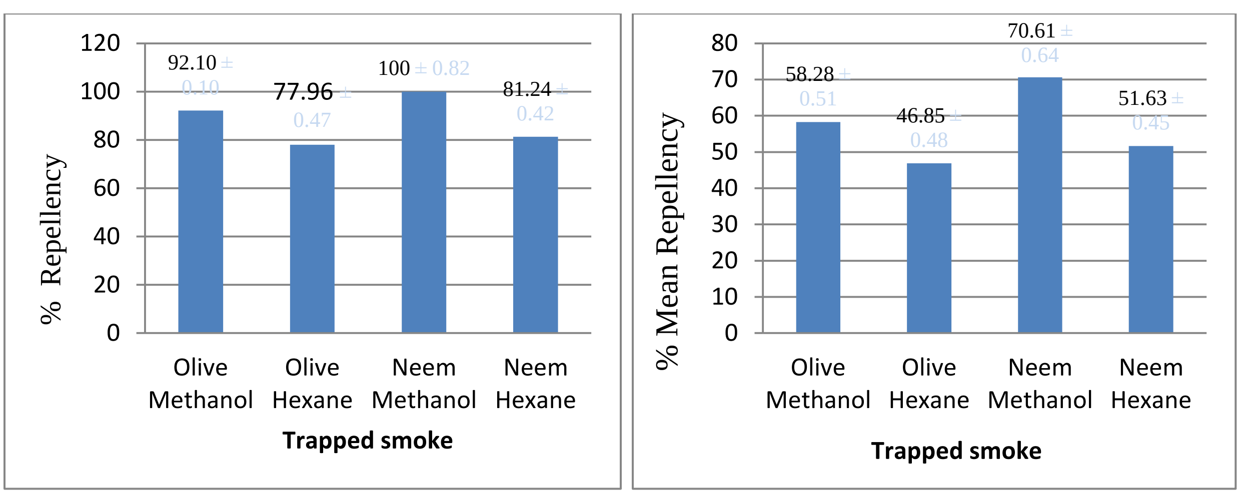

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Figure 16: Graphs showing repelling nature of the smokes components

Table 18: **Bioassay repellents of smokes obtained from neem and olive leaves.**

Extraction	Repellent time (min)	
Methods	RT_{50}	RT_{95}
Olive methanol trapped smoke	28.10	61.42 (HS)
Olive hexane trapped smoke	37.46	72.58 (HS)
Neem methanol trapped smoke	16	57.45
Neem Hexane trapped smoke	33.79	70.42 (HS)

Note: R = Repellent, T = Time, HS = out of the range (highly significant).

From table 18 the least time needed to repellent insects is the most efficient repellent extracts. The neem methanol trapped smoke at TR_{50} (16 min) the top repellent, followed by the olive methanol trapped smoke with the values RT_{50} (28.10 min) is the second level of repellent, neem hexane trapped smoke was also the third alternative repellent at RT_{50} (33.79 min) but, olive hexane trapped smoke is the least repellent at RT_{50} (37.46 min) from all extracts.

5.5. Mortality rate results of the solvent trapped smoke

5.5.1. Mortality rate of Cockroaches using the smoke trapped from neem and olive leaves

Insecticidal activity varied according to the plant part extracted and method of extraction. The mortality rates of the smokes were compared using cockroach as experimental insects. In each experiment that had been conducted, 5 insects were used and the experiments were conducted in triplicates.

Different concentrations were used over 36 hrs to see the effects. The data tabulated in table 19 shows that 100% mortality was recorded at 6 hrs for neem smoke trapped in methanol at the concentrations of 5 and 10%. Whereas, similar result was recorded for olive smoke trapped in methanol at 24 hrs by all the test concentrations (1.25%, 2.5%, 5% and 10%). For the neem smoke trapped in hexane, the maximum mortality (80%) was recorded at 36 hrs with the highest concentration (10%). At the same time by the same concentration, the olive smoke trapped in hexane showed 60% mortality.

It is clear from the data that the neem methanol trapped part of the smoke was effective in killing the insects. This is a good news for our society living in remote part of the country that do not have access to modern pesticides as they can easily use the plant part as fumigant to kill the pests.

In this test, the % mortality rate was calculated using Abbotts' formula equation. The smoke of neem methanol extracted of the leaf caused higher mortalities for cockroaches compare to the other extracted in reference of time. This indicating the presence of toxic compounds in the leaves, the smoke hexane extracted of the samples caused lower mortality compare to the smoke methanol extracted of leaves. In this study mortalities ranged from 6.66% to 100% in all extracted at lowest concentration (1.25%), after 3 hours the percent mortalities of cockroaches calculated and corrected with Abbotts' formula equation [61].

$$\text{Mortality (\%)} = \frac{\text{(death due to treatment of sample – death in control) x 100}}{\text{Total number of cockroaches treated}} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Table 19: Mortality data of smoke of neem and olive leaves

Sample extract	Concentration (%)	Mean Mortality Rate of Cockroach In Time (h)					
		3 h	6 h	9 h	12 h	24 h	36 h
Neem Methanol trapped Smoke	1.25%	1.00	4.66	4.66	5.00	5.00	5.00
	2.5%	1.33	4.66	5.00	5.00	5.00	5.00
	5%	2.33	5.00	5.00	5.00	5.00	5.00
	10%	2.66	5.00	5.00	5.00	5.00	5.00
Neem Hexane trapped Smoke	1.25%	0.33	0.33	0.66	0.66	1.33	2.66
	2.5%	0.33	0.66	1.66	2.00	2.33	3.00
	5%	0.66	1.00	1.66	2.00	2.66	3.66
	10%	0.66	1.00	2.33	2.66	3.33	4.00
Olive Methanol trapped Smoke	1.25%	1.00	1.33	2.00	2.66	5.00	5.00
	2.5%	1.33	1.66	2.33	3.33	5.00	5.00
	5%	1.33	2.66	2.66	3.66	5.00	5.00
	10%	1.66	2.66	3.66	4.33	5.00	5.00
Olive Hexane Trapped Smoke	1.25	0.33	0.33	0.66	0.66	1.00	1.33
	2.5%	0.33	0.33	1.00	1.00	1.33	2.00
	5%%	0.33	0.33	1.00	1.33	1.66	2.66
	10%	1.00	1.33	1.33	1.66	2.33	3.00
Controls	DCM	0.00	0.00	0.00	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00	0.00	0.00	0.00

Table 20: Percent mortality of Cockroaches

Sample extract	Con. (%)	Mortality rate of Cockroaches at a given time (h)					
		3 h	6 h	9 h	12 h	24 h	36 h
Neem Methanol Trapped Smoke	1.25%	20.00 ± 0.00	93.32 ± 0.47	93.32 ± 0.47	100.00 ± 0.47	100.00 ± 0.47	100.00 ± 00
	2.5%	26.66 ± 0.47	93.32 ± 0.47	93.32 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
	5%	46.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
	10%	53.32 ± 0.47	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00
Neem Hexane Trapped Smoke	1.25%	6.66 ± 0.47	6.66 ± 0.47	13.32 ± 0.47	13.32 ± 0.82	20.00 ± 0.47	53.32 ± 0.00
	2.5%	6.66 ± 0.47	13.32 ± 0.0	33.32 ± 0.47	40.00 ± 0.82	46.66 ± 0.47	60.00 ± 0.47
	5%	13.32 ± 0.47	20.00 ± 0.47	33.32 ± 0.47	40.00 ± 0.82	53.32 ± 0.47	73.32 ± 0.47
	10%	13.32 ± 0.47	20.00 ± 0.47	46.66 ± 0.82	53.32 ± 0.47	66.66 ± 0.47	80.00 ± 0.82
Olive Methanol Trapped Smoke	1.25%	20.00 ± 0.00	26.66 ± 0.47	40.00 ± 0.00	53.32 ± 0.41	100.00 ± 0.00	100.00 ± 0.00
	2.5%	26.66 ± 0.47	32.32 ± 0.47	46.66 ± 0.47	66.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
	5%	26.66 ± 0.47	53.32 ± 0.41	53.32 ± 0.47	73.32 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
	10%	33.32 ± 0.47	53.32 ± 0.47	73.32 ± 0.47	86.66 ± 0.47	100.00 ± 0.00	100.00 ± 0.00
Olive Hexane Trapped smoke	1.25%	6.66 ± 0.47	6.66 ± 0.47	13.32 ± 0.47	13.33 ± 0.47	20.00 ± 0.47	33.32 ± 0.00
	2.5%	6.66 ± 0.47	6.66 ± 0.47	20.00 ± 0.00	20.00 ± 0.00	26.66 ± 0.47	40.00 ± 0.82
	5%%	6.66 ± 0.47	6.66 ± 0.47	20.00 ± 0.00	26.66 ± 0.47	33.32 ± 0.47	53.32 ± 0.82
	10%	20.00 ± 0.00	26.66 ± 0.47	26.66 ± 0.47	33.32 ± 0.47	46.66 ± 0.47	60.00 ± 0.00

5.5.2. Correlation coefficient (Pearson's correlation coefficient):- is a statistical measure of the strength of a linear relationship between paired data. In a sample it is denoted by r and is by design constrained as:-, $-1 \leq r \geq +1$ Furthermore, positive values denote positive linear correlation, Negative values denote negative linear correlation and a value of ‘’0” denotes no linear correlation. The closer the value is to 1 or –1, the stronger the linear correlation. In the figures various samples and their corresponding sample correlation coefficient values are presented. The first three represent the “extreme” correlation values of -1, 0 and 1: Correlation is an effect size, and so we can verbally describe the strength of the correlation using the guide that Evans suggests for the absolute value of r values from 0.00-0.19 “very weak” r = from 0.20-0.39 “weak” r from 0.40 to 0 .59 is “moderate “and r = from 0 .60 to 0.79 “strong” and from 0 .80-1.0 “very strong “correlation [62].

Table 21: Correlation of mortality vs concentration and time

I) Correlation of mortality (%) with Concentration (%)

A) Neem smoke trapped in methanol and hexane

Neem Methanol	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.996	1		
5%	0.995	0.996	1	
10%	0.996	0.996	1	1

Neem Hexane	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.824	1		
5%	0.911	0.976	1	
10%	0.816	0.998	0.976	1

B) Olive smoke trapped in methanol and hexane

Olive Methanol	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.991	1		
5%	0.948	0.962	1	
10%	0.892	0.930	0.948	1

Olive Hexane	1.25%	2.5%	5%	10%
1.25%	1			
2.5%	0.980	1		
5%	0.983	0.991	1	
10%	0.945	0.934	0.949	1

II) Correlation of mortality (%) with time (h)

C) Neem smoke trapped in methanol and hexane

Neem Methanol	3 h	6 h	9 h	12h
3 h	1			
6 h	0.970	1		
9 h	0.970	0.999	1	
12 h	-	-	-	1
24 h	-	-	-	-
36 h	-	-	-	-

Neem Hexane	3 h	6 h	9 h	12 h	24 h	36 h
3 h	1					
6 h	0.905	1				
9 h	0.700	0.886	1			
12 h	0.688	0.899	0.996	1		
24 h	0.784	0.946	0.990	0.990	1	
36 h	0.948	0.954	0.886	0.871	0.931	1

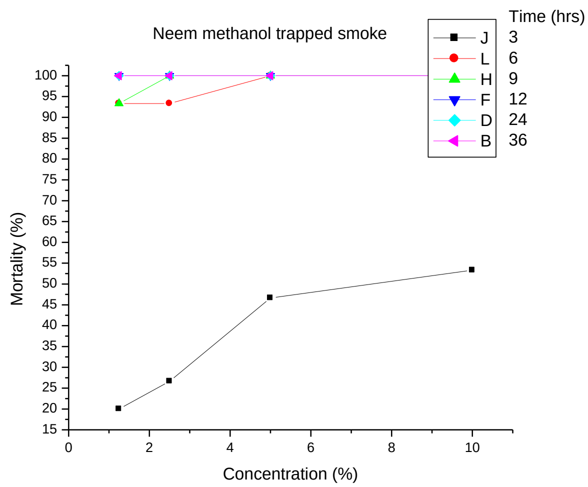
D) Olive smoke trapped in methanol and hexane

Olive Methanol	3 h	6 h	9 h	12h	Olive Hexane	3 h	6 h	9 h	12 h	24 h	36 h
3 h	1				3 h	1					
6 h	0.780	1			6 h	0.999	1				
9 h	0.945	0.822	1		9 h	0.813	0.815	1			
12 h	0.981	0.885	0.964	1	12 h	0.774	0.774	0.948	1		
24 h	-	-	-	-	24 h	0.878	0.879	0.956	0.982	1	
36 h	-	-	-	-	36 h	0.732	0.731	0.894	0.990	0.963	1

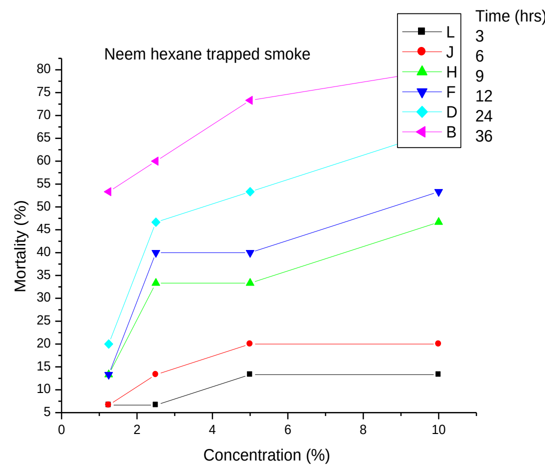
From table 21 the correlation of mortality of cockroaches with concentration (Table A and B) possess between 0.82 - 1.0 which is very strong positive correlated. The correlation of mortality of cockroaches with time average (Table C&D) possess between 0.688 - 0.79 “strong” and 0.80-1.0 “very strong, which is very strong positive correlated.

Statistical method percentage mortalities and probit analysis were used to analyze the number of mortality recorded and the percentage mortality to determine LC_{50} and LT_{50} [63].

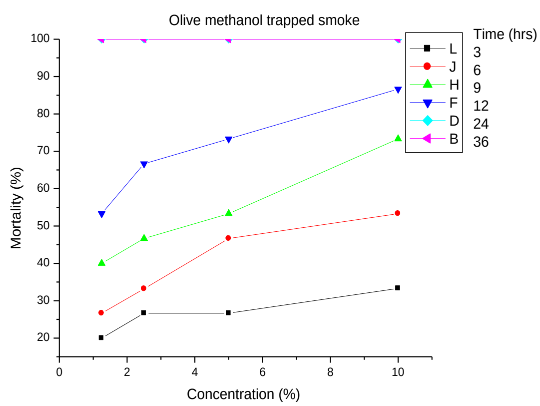
The results were subjected to one way Analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS Version 23) to determine significant difference between various treatments and control, test was used to separate differences among means, differences were considered significant at ($P < 0.05$) [64].


Neem methanol trapped smoke
Time (hrs)
J 3
L 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)

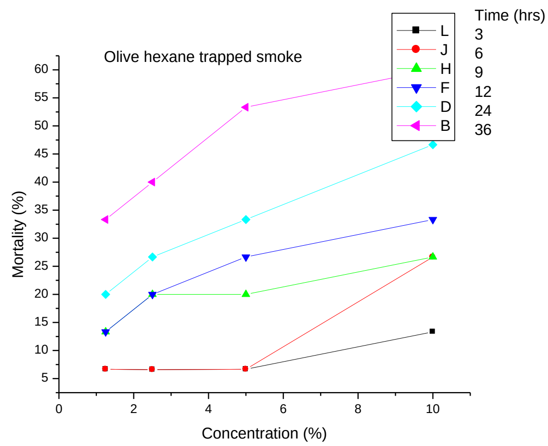
a


Neem hexane trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)

b


Olive methanol trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)

c


Olive hexane trapped smoke
Time (hrs)
L 3
J 6
H 9
F 12
D 24
B 36
Mortality (%)
Concentration (%)

d

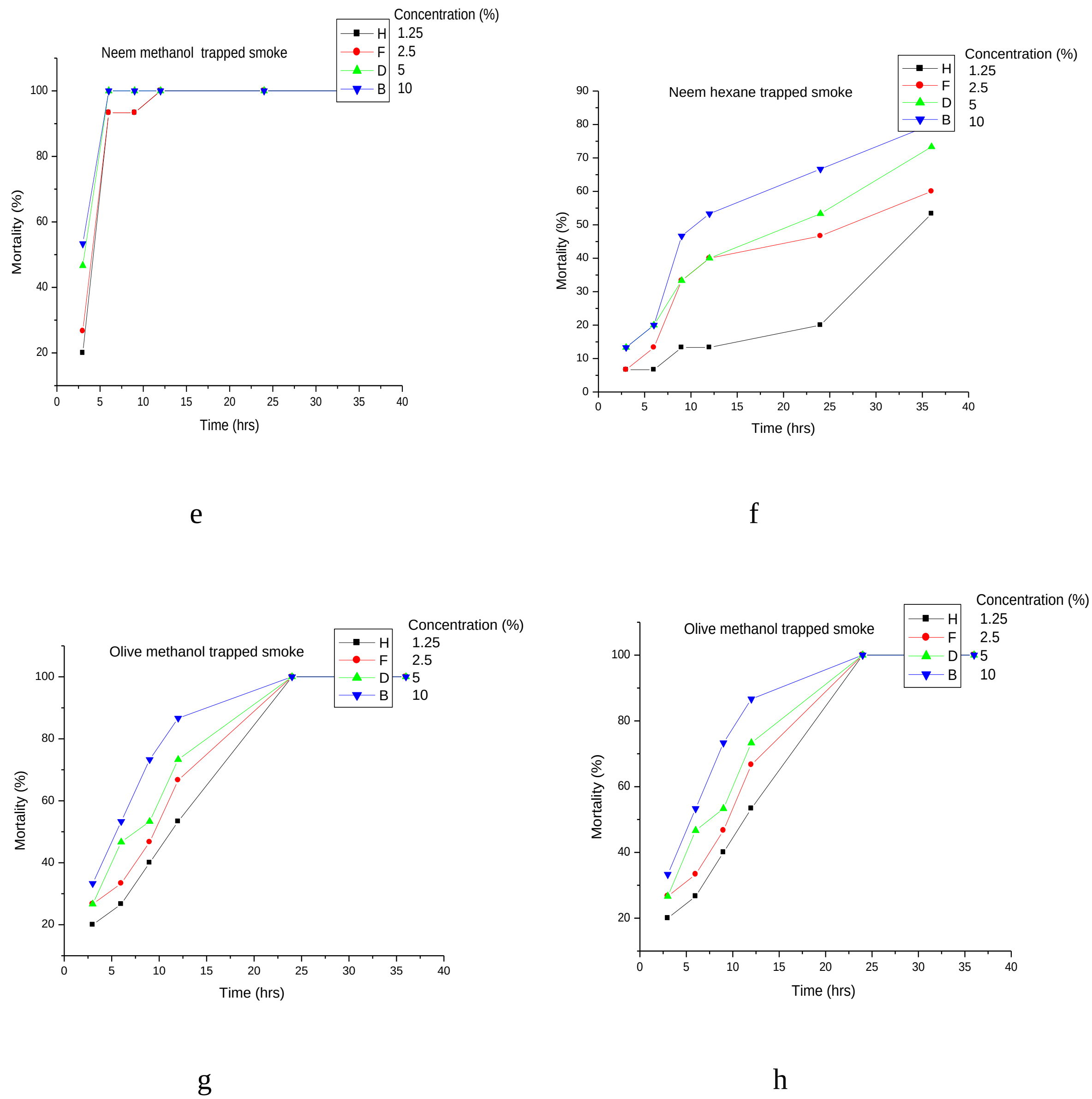


Figure 17: Percent mortality in relation to concentration (graph a, b, c, and d), and time (graphs e, f, g, and h) of the trapped smokes.

Figure 17: also shows the % mortality was positively correlated with times and concentrations of the samples.

5.5.3. Efficacy tests

The lethal time LT_{50} and LT_{95} data was calculated after 3, 6, 12, 24 and 36 hours for the lowest concentration (1.25%). Whereas, the lethal concentration LC_{50} and LC_{95} were evaluated at 1.25%, 2.5%, 5%, and 10% concentrations for the smallest time (3 h) after treatment.

Percent mortality versus concentration and time graphs for all treatments indicated in Figure 17 values for different samples are shown.

Table 22: Bioassay efficacy of smokes obtained from neem and olive leaves.

For lethal concentration LC_{50} and LC_{95} at the smallest time (3 hours), and for lethal time LT_{50}, and LT_{95} at the smallest concentration is (1.25%) after treatments.

Extraction	lethal concentration (%)		lethal time (hours)	
Methods	LC_{50}	LC_{95}	LT_{50}	LT_{95}
Neem methanol			4.6	10
Trapped smoke	6.4	HS		
Neem hexane			35	HS
Trapped smoke	HS	HS		
Olive methanol			10.20	20.40
Trapped smoke	HS	HS		
Olive hexane			HS	HS
Trapped smoke	HS	HS		

Note: C = concentration, T = Time, HS = out of the range (highly significant).

The data of the LC_{50}, LC_{95}, LT_{50} and LT_{95} values were obtained from probit analysis. It shows methanol trapped part of the smoke obtained from the neem leaf has a higher efficacy at the lowest concentration (1.25%). At this particular concentration, the extract was enough to kill more than 50% of Cockroaches. The olive hexane trapped smoke was the least active compared to others. Which has promoted 50% death of the Cockroaches at given concentration, our results show quantitative and qualitative differences in the chemical composition and insecticidal activities of the plants extracts. All the extracts were found to be toxic they were able to kill the insects.

From table 22 the least time and concentration needed to kill insect show the most efficient plants extracts. The neem methanol trapped smoke at LC_{50} (6.40%) and LT_{50} (4.60 h) is the top insecticidal, followed by the olive methanol trapped smoke extract with the values LC_{50} (HS) and LT_{50} (10.20 hrs).

5.5.4. Mean mortalities of Cockroaches

Table 23: Mean mortalities rate of all trapped smokes

Sample trapped smoke	mortality of Cockroaches (mean values)					Mortality
	1.25%	2.5%	5%	10%	Mean	(≥ 50%)
Olive Methanol	55.00 ± 0.19	62.77 ± 0.38	66.1 ± 0.38	69.44 ± 0.38	63.33 ± 0.33	High
Olive Hexane	15.55 ± 0.00	18.87 ± 0.28	23.33 ± 0.38	30.05 ± 0.38	21.95 ± 0.38	Very Low
Neem Methanol	84.44 ± 0.24	86.66 ± 0.18	91.11 ± 0.12	92.22 ± 0.12	88.61 ± 0.16	Very High
Neem Hexane	18.88 ± 0.47	33.33 ± 0.54	38.88 ± 0.45	46.66 ± 0.54	34.30 ± 0.50	Low

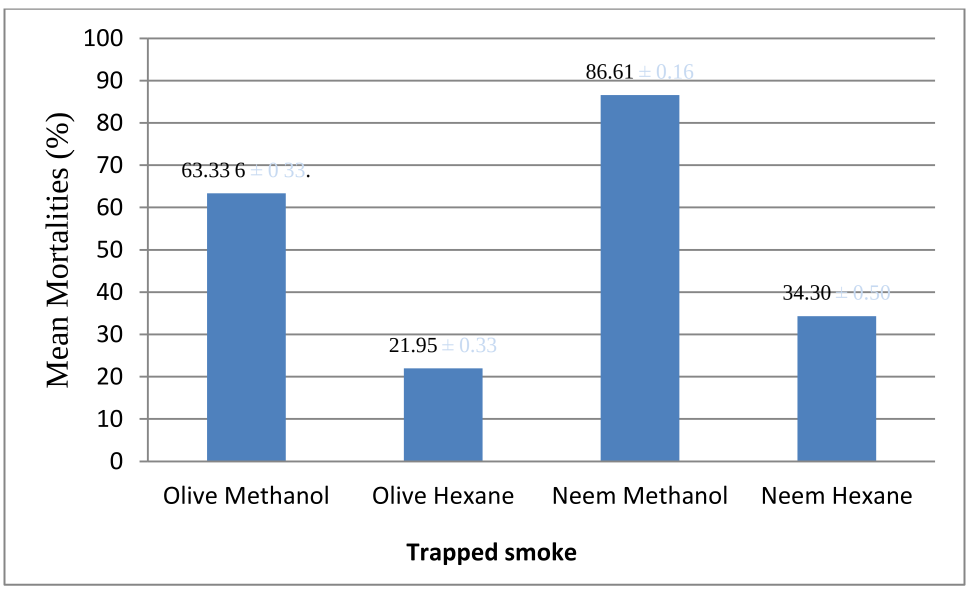


Figure 18: Mean mortalities of Cockroaches

Figure 18 and Table 23 mean mortalities shows insecticidal activities of the extracts with methanol extract of the neem leaf caused death to a greater extent compared to other extracts, and then olive methanol extract has shown better insecticidal activity when compared with others.

1.25% OM 2.5% OM 5% OM 10% OM

1.25% NM 2.5% NM 5% NM 10% NM

Control-1 Control-2 Triplicate (T) -1 T- 2 T-3

Figure 19: Cockroaches inside Petridishes

6. CONCLUSION

The mounts (% yield) of the essential oils of neem and olive leaves were 0.27% and 0.18% respectively. The % yields for neem smoke trapped in methanol and hexane were 1.83% and 1.07% respectively, while for olive smoke trapped in methanol and hexane were 2.333%, and 1.27% respectively. The smoke methanol extracts obtained was the highest yield (%) compares to the other extracts.

The major compounds detected in neem and olive essential oils were predominantly Phenolic with 40.42 %, and 36.80 % respectively. While, in the neem hexane trapped smoke was ester 98.19 %, and olive hexane trapped smoke was ester 81.00%. Neem and olive methanol trapped smoke showed phenolic compound with 34.74% and 43.10% respectively

The plants neem and olive leaves smoke trapped in methanol assayed by FRAP method was detected antioxidant activities. There is strong correlation between the concentration of sample with their absorbance and antioxidant. The measured average antioxidant concentrations for neem and olive smoke trapped in methanol was 19.65% and, 4.91 % respectively. This study suggests that the plant extraction of neem and olive have a source of natural antioxidant. The polar compounds trapped in methanol were responsible for the effect. Those plants can be used as a source of natural antioxidant to prevent disease associated with free radical and anti-oxidant activity. This value is consistent with the literature data that promote the medicinal values of the neem and olive plants.

The neem and olive trapped smokes indicated insecticidal (repellents, and mortalities) which, used as best an alternative to synthetic insecticidal. The neem smoke trapped in methanol was the most efficient repellent (100%). Olive methanol trapped smoke (92.10%), Neem hexane trapped smoke (81.24%) and Olive hexane trapped smoke (77.96%) at 60 min. The killing nature (mortalities) of an insecticide is mostly preceded by repelling. The mortalities of neem and olive smoke trapped in methanol at 10% concentration were 100% at 6 and 24 hrs respectively, which is efficient. While for neem and olive smoke trapped in hexane at 10% concentration were 80% and 60% respectively for 36 hrs.

7. RECOMMENDATIONS

Plant antioxidants are safer than synthetic antioxidant in reducing the risk of certain diseases such as cancer, heart diseases, DNA damages, and stroke. Large number of plants has been identified so far for their insecticidal property and can be used as an alternative pest management for synthetic pesticides. Smoke based remedies are available, safe, rapid delivery to the brain, more efficient absorption by the body, and lower cost of production. However, further Studies are required to know multipurpose application of plant derived smoke. The identification and isolation of bioactive compounds extracts of the neem and olive plants must be done as key issue for further study, especially with focus on pandemic antiviral.

8. REFFERENCES

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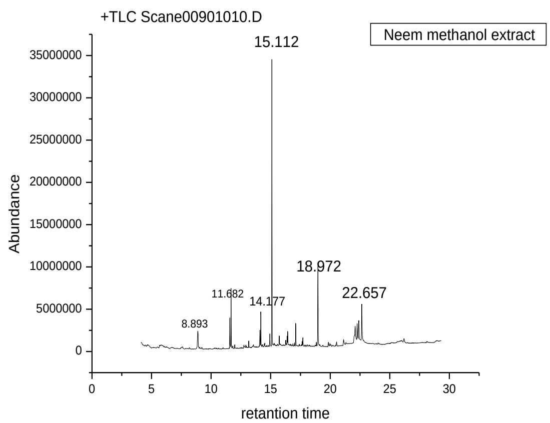
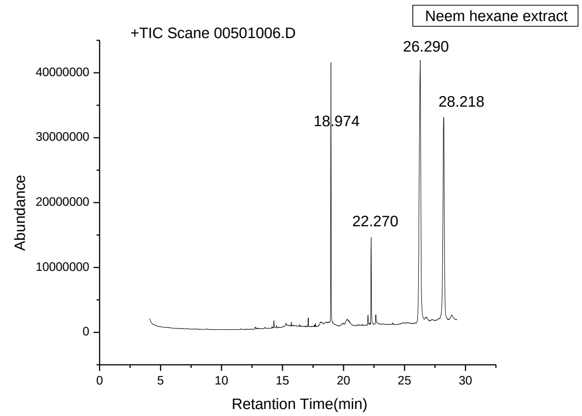
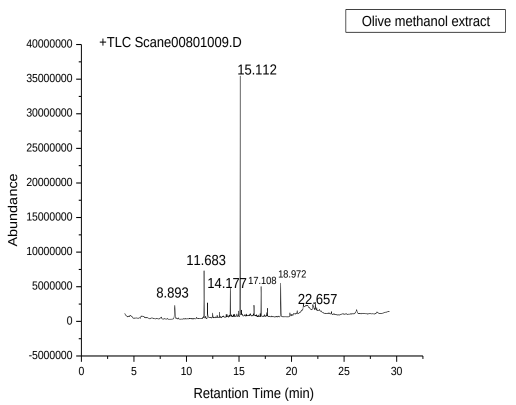
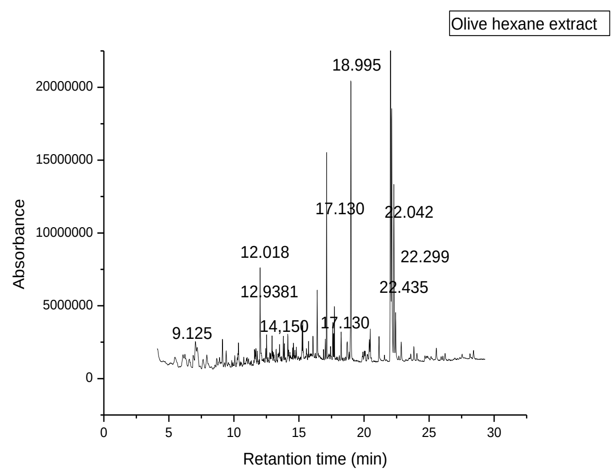
9. APPENDIXES

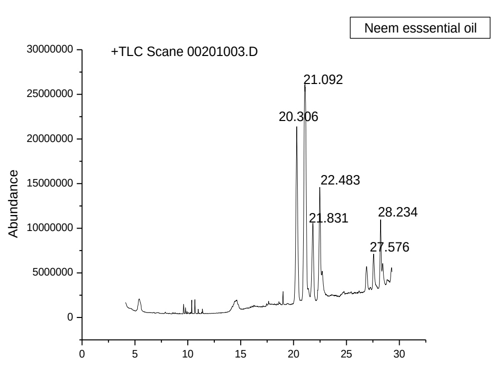
APPENDIX-1: GC-MS Analysis of neem and olive leaves essential oils and trapped smokes

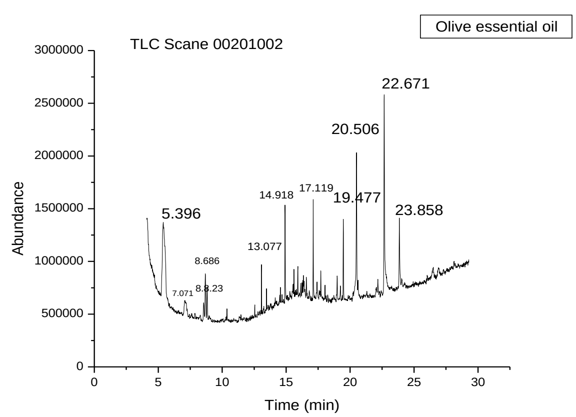






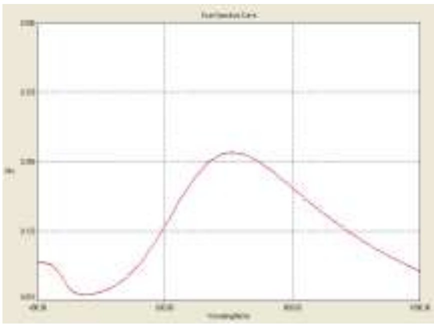




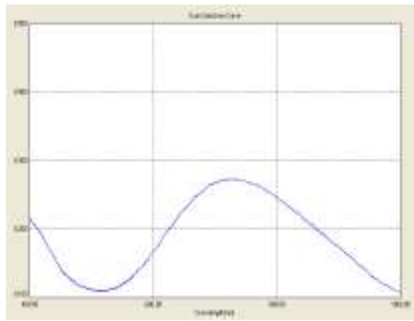


APPENDIX-2

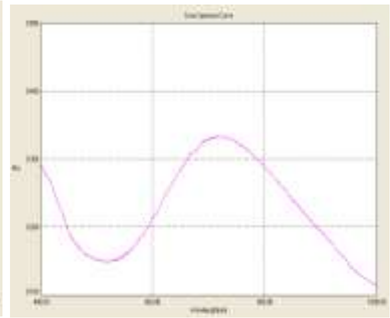
UV-VISSIBLE Analysis of the olive smoke trapped in methanol at concentrations of 76.10 μ/mL, 152.20 μ /mL, 228.29 μ/mL in triplicate (T-1, T-2, and T-3)



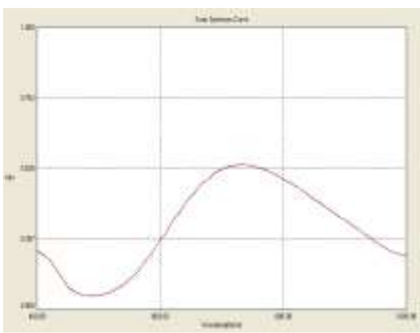
76.10 μg/mL (T-1)



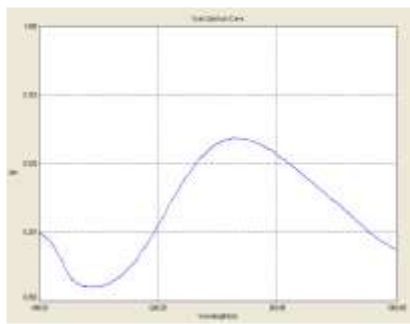
76.10 μg/mL (T-1)



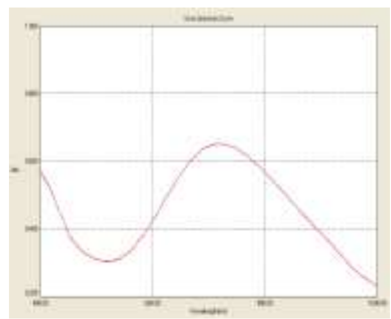
76.10 μg/mL (T-1)



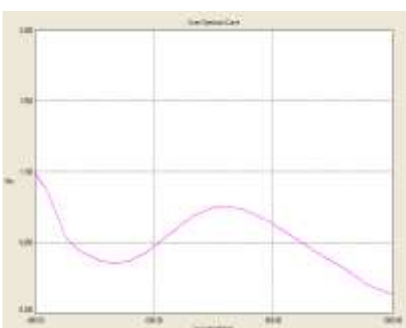
152.20 μg/mL (T-1)



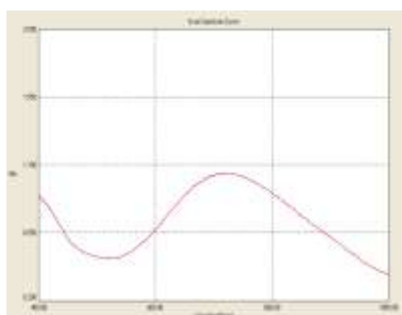
152.20 μg /mL (T-2)



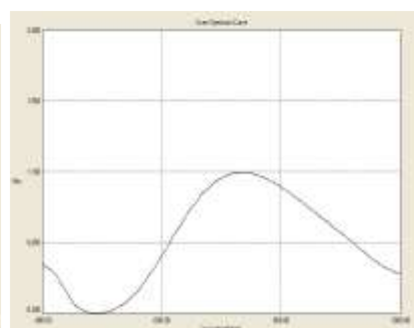
152.20 μg/mL (T-3)



228.29 μg/mL (T-1)



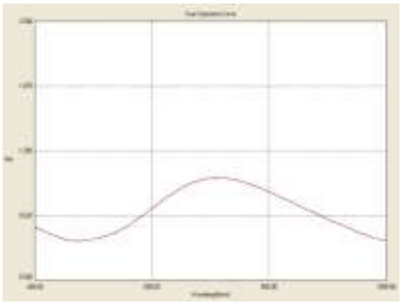
228.29 μg/mL (T-2)



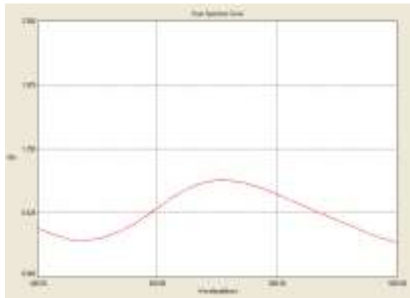
228.29 μg/mL (T-3)

APPENDIX-3

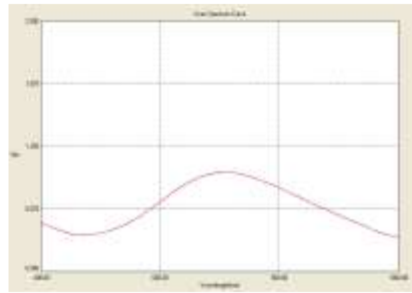
UV-VISSIBLE Analysis of the neem smoke trapped in methanol at concentrations of 76.10 µ/mL, 152.20 µ/mL, and 228.29 µ/mL in triplicates (T-1, T-2, and T-3).



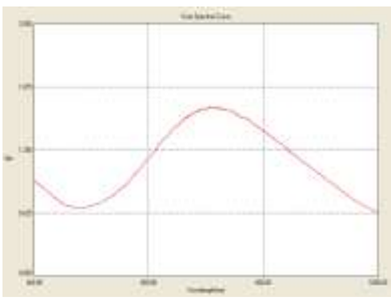
76.10 µg/mL (T-1)



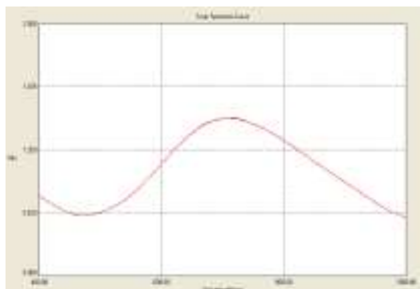
76.10 µg/mL (T-1)



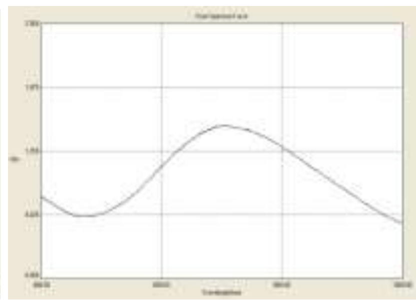
76.10 µg/mL (T-1)



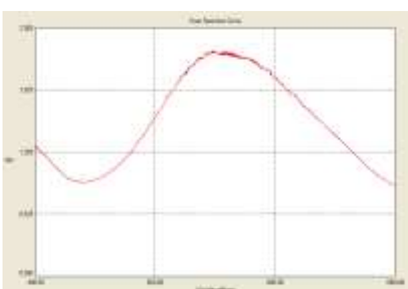
152.20 µg/mL (T -1)



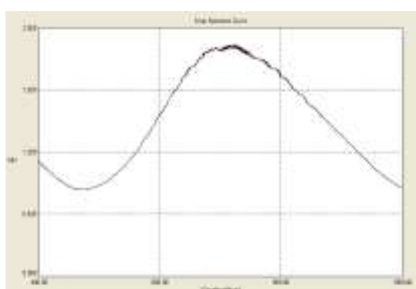
152.20 µg /mL (T-2)



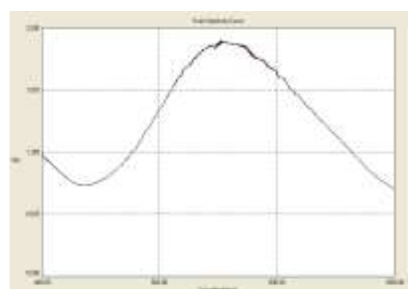
152.20/ µg/mL (T-3)



228.29 µg/mL (T-1)



228.29 µg/mL (T-2)



228.29 µg/mL (T-3)

APPENDIX-4

Percent mortalities in relation to concentration and time of the neem and olive leave smokes at concentrations of 1.25, 2.5, 5, 10% and time intervals of 3,6,9,12,24, and 36 hours.