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ON

EVALUATION OF FIXED OIL FROM SEEDS OF *Maesa Lanceolata (MYRSINACEAE)* FOR PHYTOCHEMICAL CONSTITUENTS, PHYSICOCHEMICAL CHARACTERISTICSAND BIOLOGICAL ACTIVITY

BY: WUBAYEHU SEWAGEGN

OCTOBER, 2017

JIMMA, ETHIOPIA

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THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE (MSc) IN CHEMISTRY.

BY

WUBAYEHU SEWAGEGN

ADVISORS

YINEBEB TARIKU (Asst. Prof.)

MELKAMU FEYERA (MSc.)

OCTBER, 2017

JIMMA, ETHIOPIA

Evaluation of fixed oil from seeds of *Maesa lanceolata* (Myrsinaceae) for phytochmical constituents, physicochemical characterization and biological activitiy

Declaration

I, the undersigned, declare that this thesis is my original work, not presented for any degree in any universities, and that all the sources used for it are duly acknowledged.

_____	_____	_____
Name	Signature	Date

Confirmation and Approval

This thesis has been submitted for examination with our approval as thesis advisors.

Principal Advisor:

_____	_____	_____
Name	Signature	Date

Co-Advisor:

_____	_____	_____
Name	Signature	Date

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ACRONYMS

AOAC Association of official analytical chemist

DMSO Dimethylsulfoxide

MLO *Maesa lanceolata oil*

NMR Nuclear magnetic resonance

OAc Acetoxy

OMe Methoxy

TLC Thin-layer chromatography

Abstract

The present study reports the yield, physicochemical properties, phytochemical constituents and biological activities (insecticidal & antimicrobial) of *M. lanceolata* seed oil and compounds isolated from it. The oil was extracted by soaking the seeds in petroleum ether for five days and the isolated oil was subjected to physicochemical and phytochemical analysis employing standard test protocols available including AOAC. Pure compound isolation and characterization was carried employing chromatographic separation of crude oil, physical and spectral analysis of isolated compounds. Serial concentration range (0.3125 % to 5 %) of the crude oil and isolated compounds were evaluated for biological activities on three insect pests (*S. zeamais*, *O. formosus* and *C. lectularius* employing no-choice assay) & culture of five pathogenic microorganisms (*B.cerus, S. aureus, E. coli*, *Aspargillus spp.* and *Fusarium spp in vitro* using *disc diffusion* test method). Positive and negative controls were included in each test. The acid-base indicator potential of crude oil and major fraction was also evaluated using simple titration method using phenolphthalein for comparison. The seed had 30.4% oil yield. Physicochemical tests performed gave acid value (0.8±0.15), peroxide value (0.375±0.1) and saponification value (106.59±0.57). Preliminary phytochemical tests performed on the oil indicate presence of terpenoids, quinines and alkaloids. Insecticidal activities carried using the crude oil and the major fraction isolated (MLO-4) confirm both to possess concentration dependent activities on all pests tested. 100% mortality was recorded for 5.00 % test samples (*MLO*/MLO-4) at 9/ 6; 3½/ 3½ and 3/- hrs against Maize weevil, worker termite and bed bug respectively. The antimicrobial tests carried confirmed that crude oil and its fractions to have effect on all tested microorganisms except *E. coli* and *Aspargillus spp.* and the crude oil had better activity than its fractions. The Inhibition zones recorded (in mm) for susceptible organisms for the crude oil and its major fraction (MLO-3, (MLO-4) respectively were 9 mm, 12 mm 14 mm and (for *B. cerus*), crude oil and MLO-2 14 mm & 10 mm, (for *S. aureus*) and 11, 9, 11 and 8 (MLO, MLO-2, MLO-3 and MLO-4) respectively. The crude oil and its major fraction (MLO-4) both have shown sharp end point color change (from yellow to purple) almost similar to endpoint for Phenolphthalein. Column chromatographic separation of the crude oil led to isolation of MLO-2 (Rf 0.66). Based on spectral data (^{1}H, ^{13}C NMR and DEPT) and melting point data of MLO-2 we propose this compound to be an isomeric mixture of monohydrated alkylbenzoquinone.

1.0. INTRODUCTION

1.1. Background of the study

Fixed oils are plant derived non-volatile liquids with thick, viscous consistency and mostly with yellow color and characteristic odor. Fixed oils may occur in various parts of the plant mainly in fruits, seeds and nuts. Seeds contain larger quantities of oils than other parts and they are usual sources of fixed oils [1]. Several species of plants are known to contain fixed oils. Some of these include Palm, Cotton, Sunflower, Niger, Canola, Jatropha, Soy, Pea nut and Sesame [2].

Chemically fixed oils are composed typically of triglyceride consisting of other lipophilic constituents such as phospholipids, sphingolipids, waxes, lipid vitamins and lipophilic phytochemicals. They are mostly extracted using organic solvents such as hexane/petroleum ether or mechanically by expression [3].

Fixed oils possess diverse application in food/feed, industry, medicine and agriculture [4]. Plant oils contain chemicals that play role in food flavor, provide energy and to supplement important nutrients for body and thus are common food staffs recommended. Industrially fixed oils are important raw materials during manufacturing soaps, paints, varnishes, pharmaceuticals (as stimulant, cathartic, lubricant, emollient in cosmetics, solvent in preparation of certain injections and laxative) [5]. Some fixed oils might have potential as bio fuels/ biodiesel and hydraulic fluid [6].

Many fixed oils and components isolated from such as free fatty acids and other phytochemicals were investigated for diverse biological activities (antioxidant, antibacterial, antifungal, insecticidal, antiparasitic etcactivities and most were confirmed to have medicinal potential [7-10] . Epidemiological and clinical studies carried on plant oil also show potential of fixed oils reduces the risk of Alzheimer's disease, stroke, inflammation and certain type of cancer [5].

1.2. Statement of the problem

Cereals are the dominant source of nutrition especially in developing and developed countries of sub-Sahara Africa and south East Asia where one-third of the world population exist [11]. Among the cereals; rice, wheat and maize constitute about 85%of total global production. Maize is an important cereal crop in Africa serving as source of food, feed and industrial raw material.

Pre/post harvest loss of crops constituents a great constraint to the realization of food security worldwide. Pests are the primary causes of pre/post harvest loss for maize grains in storage and field. Among several pests that attack maize in storage and field are insects (weevils and termites), soil nematodes and fungi (Aspergillus, Fusarium and Penincllium) are the most serious in Southwest Ethiopia. [7, 11]. Infectious diseases caused by pathogenic micro-organisms (such as bacteria)[12-14] and bedbug infestations [15, 16]are a serious threat for human health in Southwest Ethiopia. The condition is getting worse with hot and humid environment dominant in this areas.

To overcome problems pests and microorganisms pose, different control methods have been developed worldwide including use of commercially available synthetic pesticides and antibiotics. But many of these products have problems associated including high cost, development of target resistance, environmental pollution and toxic effect on non-targeted organisms and direct user [17,18].These problems all erg development of new products that are less coasty and with acceptable or no toxicity best from natural source such as plants.

Maesa lanceolata is a plant widely distributed in Ethiopia and is known for its fire woods, timber, oil bearing seeds and all parts used for traditional managing of wide range of human and cattle ailments [19,20]. Its seeds are used for greasing clay made pan while baking “Injera”. The plant was also investigated for diverse biological activities (antioxidant, antibacterial, antifungal, insecticidal, antiparasitic activities [21-24] and most confirmed its potential medicinal role.Phytochemicals analysis was carried on the plant also confirm presence of alkaloids, tannins, phenolics, saponins glycoside and terpenoids [10, 25-27].

But studies on carried fixed oil content, physicochemical properties of fixed oil, some biological activities (antibacterial, antifungal, insecticidal) and acid-base indicator potential of seed oil and fractions of *Maesa lanceolata* seed was not carried.

Therefore in this study attempt was made isolate fixed oil from dried seed of *Maesa lanceolata*, evaluate its physicochemical characteristics, phytochemical constituents, biological activities and acid–base indicator potential of the crude oil and compounds isolated employing standard test protocols**.**

1.3. Objectives of the study

1.3.1. General objective

- To investigate fixed oil composition, physicochemical/phytochemicals analysis and biological activity of *Maesa lanceolata* seed oil.

1.3.2. Specific Objective

- To determine the percentage oil content of *Maesa lanceolata* seeds
- To determine the physicochemical characteristics and acid-base indicator of seed oil of *Maesa lanceolata* using of the volumetric titration method and employing standard test procedures.
- To carry out preliminary phytochemicals analysis of the seed oil using standard test procedure.
- To isolate and characterize compounds from the seed oil of *Maesa lanceolata* using chromatography and spectroscopic techniques, respectively
- To evaluate the biological activities (anti-bacterial, anti-fungal and insecticidal) of crude oil and isolated compounds against standard reference test organisms *in vitro* employing standard test protocols.

1.3. Significant of the study

This work is aimed to investigate fixed oil composition physcochemical/phytochemical characteristics and biological activity of *Maesa lanceolata* seed oil.

Findings of this study would help to:

- Recommend potential application of seed oil of *Maesa lanceolata*
- Identify new compounds for possible drug development
- Confirm use of *Maesa lanceolata* as local medicinal plant

2.0. LITERATURE REVIEW

2.1. Botanical Information

2.1.1. The genus *Maesa*

The genus *Maesa* belongs to the family *Myrsinaceae* and consists of about 150 species with scramblers, shrubs and small trees distributed throughout the Old World tropics. Members of the genus are commonly found in secondary and disturbed habitats, but are also in the under storey of primary lowland and montane tropical forest [20]. *Maesa* was first recorded in the Philippines by de Candolle (1841) [28]. *Maesa lanceolata* is a very variable species as it can be a straggling shrub, 2 to3 m tall, or a small tree with a single stem up to 9 m tall, or a rounded bushy tree with branches almost at ground level. Leaves spirally arranged, lance shaped, tapering to pointed tips, sometimes with blunt or round tips, simple pale or dark green, with toothed margins. The midrib and lateral veins are conspicuously raised below. Sometimes the leaves are large and thick and resemble those of *Curtisia dentata*, the assegi, and are often mistake for this plant them, but those the leaves of C. dentata, opposite not alternate, as those of the false assegai [19].

The bark is usually smooth and brown, flowers are minute bisexual, sweetly scented white or yellow, in many – flowered sprays in the axils of leaves and at the ends of branches. Flowering in spring (peak in October), but it has been observed flower in on and off throughout the year. Matured fruit are usually found in March, usually crowded in dense sprays, are creamy – white, edible berries that have remnants of calyx lobes at top 3–6mm [19,20].The white, sweetly scented flowers are typical of those which are pollinated by night-flying insects such as moths and birds[19]. It grows well in moist and wet with The altitude ranging from 1350-3000m including Central and East Africa, especially Ethiopia and Kenya. In Ethiopia it occurs in gallery forest, dry evergreen forest margin, woodland and on mountain slopes with Acacia, Carissa and Euclea [19].

(A) (B) (C)

Figure : *Maesa lanceolata* Whole part (A), Inflorescence (B) and matured fruits(C) *(***Source:** *https,//www.prota4u.org/database/prota v8.asp?g=psk&p=Maesa+lanceolata+ For ssk. and photo by Wubayehu S., 2010 E.C)*

2.2. Phytochemical constituent of *Maesa lanceolata* /

Phytohemicas are mainly the secondary metabolites offering medicinal attribute to the plants. Plant constitutes have become an important source of active natural products which differ widely in terms of their structure and biological properties. Recent research demonstrates that many phytochemicals can also protect human against disease. The class of compounds isolated from *Maesa lanceolata* includes flavonoids, terpenoids, anthraquinones, benzoquinones with long aliphatic side chains and long chain aliphatic ketone, triterpenoid saponins, and benzophenons. [25, 26, 30-32]

2.2.1. Quinones

Quinones are large class of compounds endowed with rich and fascinating chemistry.1, 4-benzoquinone or p-benzoquinone is the basic structure of quinonoid compounds. They are widely distributed in the natural world being found in bacteria, plants and arthropods and hence quinones are ubiquitous to living system [29].

It plays pivotal role in biological functions including oxidative phosphorylation and electron transfer. Their role as electron transfer agents in primary metabolic process like photosynthesis and respiration is vital to human life. Maesanin, 2,5 - dinydroxy - 3 - (nonadec-14 - enyl) - benzoquinon, lanciaquinon and alkylated hydroxy benzoquinones are obtained from the fruits of *Maesa lanceolata*[33].Maesanin, 2-hydroxyl-5-Methoxy-3-(10'-pentadecenyl)-1,4-berzoquinone

is a natural p -benzoquinonpossess pronounced biological activities including non-specific immunostimulation, lipoxygnase inhabitation [33].**(1-7)**Alkylatedbenzoquinones [33,34] **(8)** 2,5dihydroxy3(nonadec-14-eny) benzoquinone [33]. **(9)** Maesanin and **(10)** Maesanol [34] are from fruits and Lanciaquinon **(11)** and Ardisiaquinone **(12)** from leaves of *Maesa lanceolata* [34].

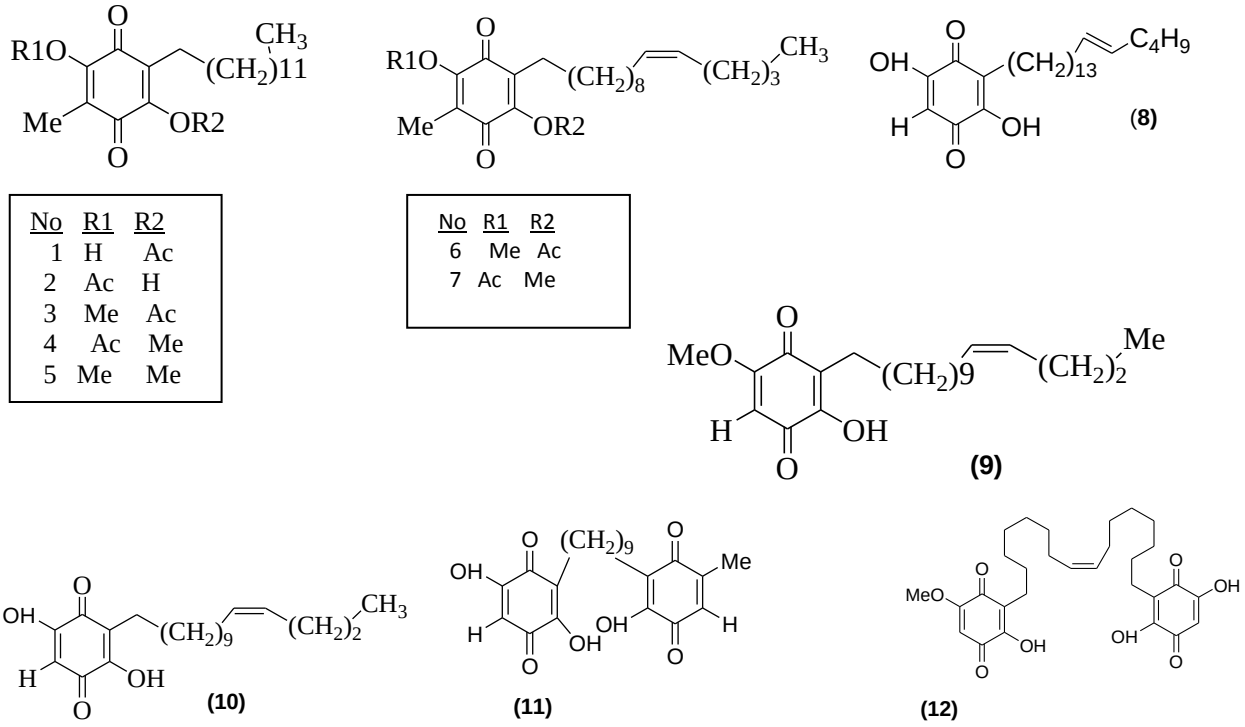
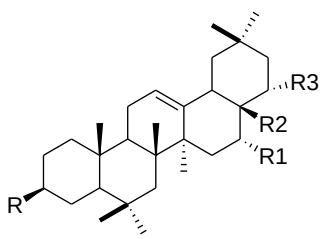
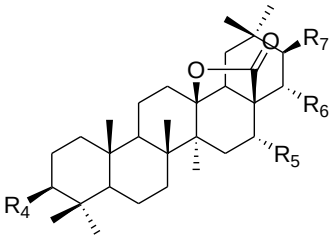


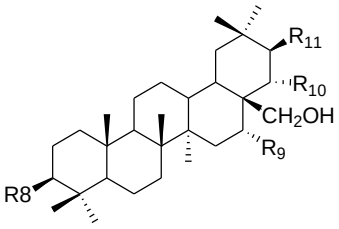
Figure : Structure of quinines obtained from *Maesa lanceolata* fruit and leaves

2.2.2. Triterpenoid saponins

Triterpenoid saponins are a large class of structurally diverse and biologically active specialized metabolites produced by numerous plant species. These amphipathic glycosides consist of a hydrophobic backbone or sapogenin with one or more hydrophilic sugar chains attached to it. Triterpenoid saponins display a wide range of commercial applications in the agricultural, food, cosmetic, and pharmaceutical sectors as pesticides, preservatives, surfactants, adjuvants, antimicrobial, anti-inflammatory, and anticancer agents [31]. Various triterpene saponins **(13-20)** were obtained from fruit *Maesa lanceolata* [31]







No	R	R1	R2	R3
13	O-glc	O	CH_2OH	H
14	O-glc	OH	CH_2OH	H
15	O-glc	OH	CH_2OH	OH
16	O-ara.	OH	CHO	OAc

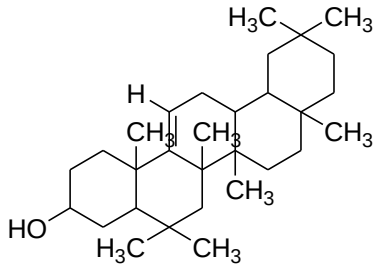
No	R4	R5	R6	R7
17	O-glc	OH	OAc	OH
18	O-glc	OAc	OAc	O-CO-C (CH_3) =$CHCH_3$

No	R8	R9	R10	R11
19	O-xyl	OH	OH	O-CO C (CH3)=CHCH3
20	O-ara	OH	OAc	O-CO-C (CH3)=CHCH3

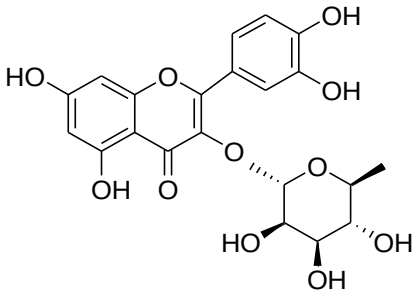
Figure : Structure of triterpene saponins isolated from *Maesa lanceolata*

2.2.3. Miscellaneous compounds

A triterpenoid, myrisene **(21)** and flavonoid, quercitirin **(22)** were also isolated from theleaves [32] and seeds [31] respectively of *Maesa lanceolata.*



(21)



(22)

Figure : Structure of myrisene and quercitirin isolated from of of *Maesa lanceolata*

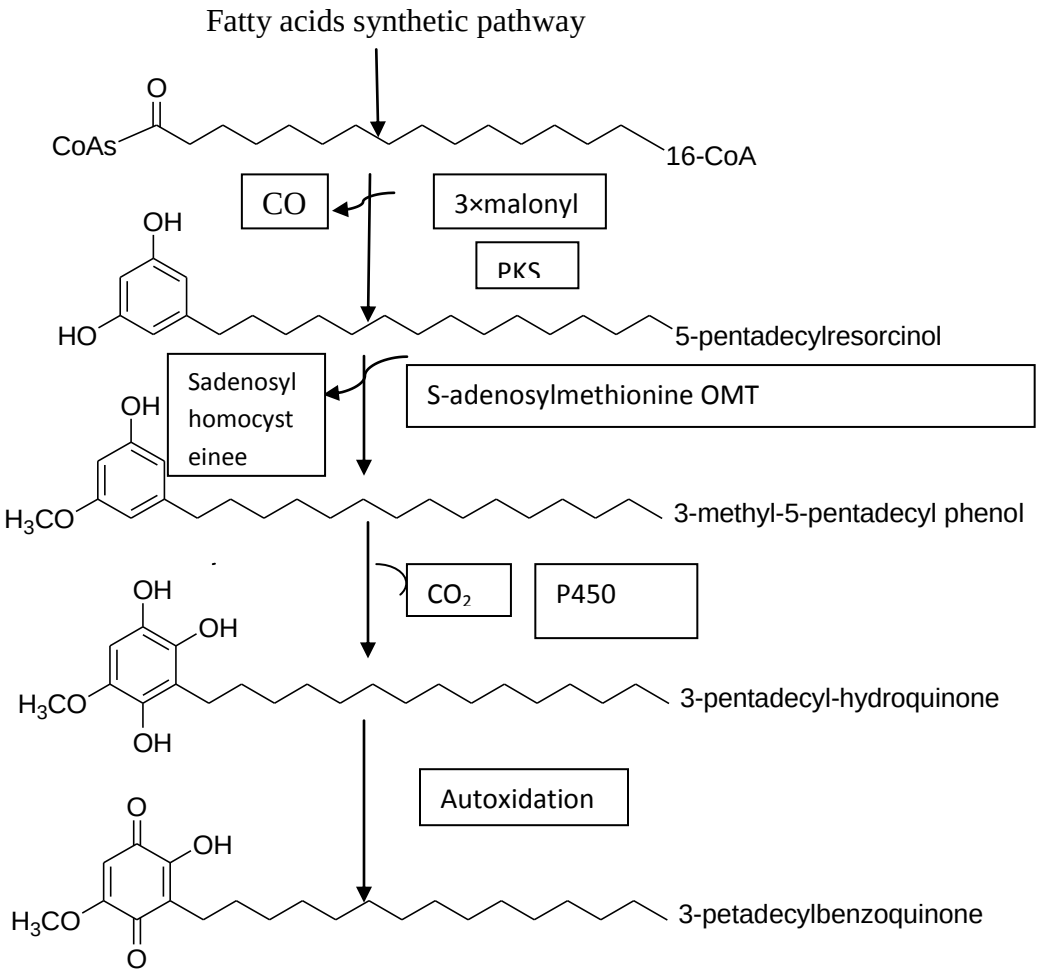
2.3. Traditional Use of *Maesa lanceolata*

Maesa species are used traditional medicine in many countries. *Maesa* plants could, however, also play a role in modern medicine, for example in cure of cancer or Leishmanisis [35]. *The seed* and fruits of *Maesa lanceolata* have been used traditionally to treat arthritis and antihelmenthic in human. The common method of seed and fruit preparation for medicinal use is seeds and fruits are ground in to a fine powder and boil in water or milk then sieve medical purposes for malaria [34]. Leaves, roots and fruits of *Maesa lanceolata* have been used to treat and manage most of the aliments and conditions, including Flu, antihalmenthic, appetizer, stomach ache, sexually transmitted disease (e.g. syphilis and gonorrhea), malaria, arthritis,

against bacterial infection in small intestine and viral infections in the liver [32, 34, 36] and it is also well known for fishing by Shinasha and Gumuze tribes in Benshangul gumuze region.

2.4. Biosynthesis of alkylatedbenzoquinones

Alkylated benzoquinones are mostly synthesized involving the mevalonate pathways for synthesis of long hydrocarbon chain and the polyketide pathways used to construct the 1, 4-benzoquinone structure. Route for biosynthesis of alkylated benzoquinones sorgoleone **(23)** in sorghum [37].



(23) Figure : Bio -synthesis of Sorgoleone from root hairs of Sorghum.

2.5. Plant products as natural acid-base indicators

Indicator is a substance that has a different color on acid and alkaline. They are usually weak acids or bases when dissolved in water dissociate slightly and form ions. Common indicators used in laboratories are phenolphthalein, methyl orange, methyl red, methyl blue and litmus. There are many natural acid-base indicators that can be obtained from flowers, fruits and vegetables. The plant pigments known as anthacyanins are responsible for many of the red, blue and violet colors seen in plants [38]. It exhibit different colors in acidic and basic medium and this substance give sharp distinct and stable color change on change of acid to alkaline .thus it may be used as acid base indicators in volumetric analysis [39].

The term volumetric analysis refers to quantitative chemical analysis carried out by determining the volume of solution of accurately known concentration which is required to react qualitatively with a measured volume of a solution of the substance to be determined. The objective of volumetric analysis is to determine the equivalent quantity of the other substance required for neutralization. The point at which complete neutralization is achieved is called the end point or the equivalent point. Commercial indicators are expensive; some of them have toxic effects on users and causes environmental pollution. For this reason there has been increasing interest in searching for alternative source of indicators from natural source [40].

2.6. Harmful Organisms, Effects caused by them and modalities available for their management

2.6.1. Insect pest

Insect pests are major constrain on crop production, especially in developing countries. Pest insects can have adverse and damaging impacts on agricultural production and market access, the natural environment, and our lifestyle. Pest insects may cause problems by damaging crops and food production, parasitizing livestock, or being a nuisance and health hazard to humans [17].

2.6.1.1. Maize Weevil (*Sitophilus zeamais*)

The maize weevil (*Sitophilus zeamais*) is a species of beetle in the family Curculionidae. It can be found in numerous tropical areas around the world, especially in locations where maize is grown [41]. Maize weevil, *Sitophilus zeamais* , is a major pest of stored maize and of cob maize

prior to harvest [41]. Infestations initiated in the standing crop may further develop in storage as the grain dries whether stored as cobs or bulk grain. It may also infest other cereals if the moisture content is moderate or high. This species attacks both standing crops and stored cereal products, including wheat, rice, sorghum and cottonseed [42]. The maize weevil also infests other types of stored, processed cereal products such as pasta, cassava and various coarse, milled grains. It has even been known to attack fruit while in storage, such as apples [43].

Apart from the indirect effects, arising from the production of heat by the insects, the major effect of infestation by the maize weevil *Sitophilus spp*. is the damage to grain by feeding activities of the adults and the development of immature stages within the grain. This not only reduces the grain quality but also produces a considerable amount of grain dust mixed with frass [41]. Early detection of infestation is difficult. As *Sitophilus zeamais* larvae feed on the interior of individual grains, often leaving only the hulls, a flour-like grain dust, mixed with frass is evident. Infested grains contain holes through which adults have emerged.

A possible indication of infestation is grain, when placed in water, floating to the surface [41]. In large stores of grain, an increase in temperature may be detected. The most obvious sign of infestation is the emergence of adults. One study recorded, 5 weeks after infestation the emergence of 100 adults per kg per day [42] . Complete development time for the life cycle of *S. zeamais* averaged 36 days (range 33-45) at 27 ± 1^0C and 69 ± 3% RH. Maximum daily rate of fecundity (6.7 eggs per female in 24 hours), duration of development, and number of progeny produced were optimal at 30^{o}C and the lower limit for development from egg to adult weevils was 15.6^{o}C and the upper limit was 32.5^{o}Cat75%RH [42].

2.6.1.2. Termites *(Odontotermes formosus)*

Termites are social insects comprise the order isopteran. The individuals are differentiated into various morphological forms or castes which exhibit division of labor performing different biological functions and which live in highly organized and integrated units, societies or colonies. They differ from hymenoptera social insects(ants ,bee ,warps)in that they are hem boles, their castes are usually bisexual and they have no sub social groups [44].Termites are devasting insects pests which lead to sever soil degradation by reducing vegetation and leaving the soil surfaces and barren and exposed to the elements of erosion [45].The consequence of termites attack reduces farm productivity and increases land degradation [46].In general

agricultural production is very difficult in termite infested areas. The crops are attacked while they are standing in field and the soil are compacted and difficult to plough ,this in turn resulting in lower production ,low income and famine in the rural society of the area. As result farmers are forced to leave their farm lands and exposed to sever poverty.

2.6.1.3. Bedbugs *(Cimex lectularius)*

Bed bugs are parasitic insects of the cimicidal family that feed exclusively on blood [47]. *Cimex lectularius,* the common bed bug, is the best known as it prefers to feed on human blood and the species best adapted to human environments [48].

It is found in temperate climates throughout the world [48]. They obtain all the additional moisture they need from water vapor in the surrounding air [49]. Bed bugs are attracted to their hosts primarily by carbon dioxide, secondarily by warmth, and also by certain chemicals [50]. Bedbugs prefer exposed skin, preferably the face, neck, and arms of a sleeping person. Although under certain cool conditions adult bed bugs can live for over a year without feeding, under typically warm conditions they try to feed at five- to ten-day intervals, and adults can survive for about five months without food [51].

Bed bugs can survive a wide range of temperatures and atmospheric compositions [47] .Below 16.1 °C, adults enter semi hibernation and can survive longer; they can survive for at least five days at −10 °C, but die after 15 minutes of exposure to −32 °C. They show high desiccation tolerance, surviving low humidity and a 35–40 °C range even with loss of one-third of body weight**.** The thermal death point for *C. lectularis* is 45 °C; all stages of life are killed by 7 minutes of exposure to 46 °C [47].

Bedbugs, while significant social problem, do not transmit disease to humans .However, bed bug bites can cause red ,raised itchy lesion on the skin that may take up to 14 days to develop and allergic reactions to the bites have been reported [15].Scratching bug bites can also lead to secondary skin infections. Some individuals report significant psychological distress, disruption of sleep, nervousness agitation when dealing with bed bug infestation [16].

2.6.2 Infectious Agents

The agents that cause disease fall into five groups: viruses, bacteria, fungi, protozoa and helminthes (worm).Infectious disease is one of the leading causes of death worldwide. Many diseases become difficult to control if the infectious agents evolve resistance to commonly used drugs. Some infectious bacteria give off toxins which can make some disease and spread in many ways including Spread by surface and skin contact. For instance Staphylococcus *aureus* can diffuse through coughing and sneezing [37].

Fungi are the major cause of plant diseases and are responsible for large scale harvest failures in crops like maize and other cereals all over the world [8, 52]. The fungi genera typically found in stored grains are Aspergillus, Penicilliom, fusarium and some xerophytic species, several of them with capabilities of producing toxins [9]. The impact that fungi have with regards to plant health, food loss, and human nutrition is staggering. Some of the world's great famines and human suffering can be blamed on plant disease-causing fungi and Wheat crops of the Middle Ages were commonly destroyed when the grains became infected with a dark, dusty powder now known to be the spores of the fungus called bunt or stinking smut [53].

3.0. MATERIALS AND METHODS

3.1. Apparatus and chemicals

3.1.1. Apparatus

The apparatus which was used in the experiments were NMR, Rotary evaporator (Heidolph, UKLABOROTA 4000), Magnetic stirrer, filter paper, round bottom flask, electrical beam balance, hot plate, UV-254-UV-365(Uvitec) chamber, volumetric flask, test tube, burette, oven, beaker, TLC plates,. TLC tank and lid, pipette,500 mL glass jar, reflux condenser, measuring cylinder, peteridishes, plastic box ,100 mL volumetric flask,50ml volumetric flask, cotton cloth, digital balance, incubator, Bunsen burner and autoclave.

3.1.2. Chemicals

Analytical grade chemicals and reagents are used 1%starch indicator ,glacial acetic acid, acetone (LOBAChemie)agar,Chloroform(LOBAChemieIndia),diethylether,ethanolabsolute99.8%(FDR), ethylacetate(LOBAChemieIndia),hydrochloricacid,methanol(LOBAChemieIndia),petroleum ether(LOBAChemieIndia),phenolphthalein,potassiumhydroxide,potassiumiodide,sodiumhydroxideandsodiumthiosulphate,boricacid,malathion,DMSO,mancozeb80%wp(CoromandelInternationalLtd.) and gentamicin (Pharmaceuticals pvt. Ltd.)

3.2. Sample collection and preservation

The seed of *Maesa lanceolata* used for this study was obtained from the fruit of the plant. Mature and healthy seeds of *Maesa lanceolata* plant was selected and collected from Benishangul gumuze region Metekel zone Wonbera district with altitude 2769 m. The seed was cleaned (undesired materials) removed and shade dried at room temperature (25^{o}C) for 30 days. The voucher specimens were brought to Jimma university for identification using taxonomic keys given The Flora of Ethiopia and Eritrea book 8(4,1).

3.3. Extraction of oil

One kg of dried seed was ground into fine powder and soacked with 2 liters of petroleum ether in 5000 mL of round bottom flask. The maceration was carried out for 24 hrs with intermittent

agitation .The extracted portion was filtered with the aid of cotton cloth and the marc (the residual material) was macerated successively five times with petroleum ether and extracts obtained in each step were pooled together and finally concentrated using rotary evaporator at 60 ^{o}C. The volume of extracted oil was recorded and the percentage of oil obtained was determined. The same procedure was applied for the second batch. The resulting extract was stored at 4^{o}C until required use.

3.4. Isolation of compounds

Isolation of crude sample was carried out using column chromatography employing gradient elution method using the solvent chosen. First TLC analysis and preparation of sample for column separation was performed. TLC analysis was carried using different solvent systems available and among which binary solvent system Petroleum ether: ethyl acetate was found most effective. The sample was separation for column separation by mixing 50 g of sample with 50 g Silica gel that was deactivated for 2 hrs at 105^{0}C in an oven. Chromatographic column was packed by continuously passing slurry of 269 g of deactivated silica gel, 8 g of Oxalic acid ($C_2H_2O_4.2H_2O$) and sufficient amount of petroleum ether.

Column elution was carried out by continuously passing the solvent system chosen petroleum ether: ethyl acetate (100:00 to 78:22) each with 200 mL amount and fractions (each with 25-30 ml) were collected. Each of the fractions collected were checked with TLC and those with similar TLC profile were pooled together.

For the second, column chromatography separation of fraction pooled on the bases of TLC profile (100:0, 99:1 and 98:2; 30g in mass) was carried on 150g of silica gel using petroleum ether: acetone (100:00 to 89:11 each with 100 mL) collecting fractions with 20-25 mL each.

3.5. Physicochemical analysis

The AOAC method of analysis was employed in the determination of physicochemical properties of the oil. The chemical properties of the oil determined include acid, peroxide and saponification value. [1] And the percentage of oil content can be calculated as below.

$$\% \text{ of oil} = \frac{\text{Wt of oil obtained in gram}}{\text{Wt. of seed taken in gram}} \text{X } 100$$

3.5.1. Determination of acid value

Diethyl ether 25mL and 25 mL of ethanol was mixed in 250 mL beaker. The resulting mixture was added to 10 g of oil in a 250 mL conical flask and a few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1 M N KOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1 M KOH (Vo) was noted. Acid value (Av) was then calculated as:

$$Av = \frac{VO}{Wo}$$; *Where* VO= ml of 0.1M KOH and WO= sample weight

3.5.2. Determination of saponification value

Two g of oil extract was weighed in to a conical flask and 250 mL of 0.1 Nethanolic potassium hydroxide was added. The content which was constantly stirred and allowed to boil gently for 60 min. attached with a reflux condenser. After boiling few drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5 M HCl to the end point until the pink color of indicator just disappeared. The volume of HCl and blank solution was recorded. 0.1 N ethanolic potassium hydroxide solution was taken as blank solution.

The expression for saponification value (SV) is given by

$$SV=56.1N\ (S-B/M)$$

Where Sample titer volume, B= Blank titer volume, N=Actual normality of HCl used, M=Mass of the sample

3.5.3. Determination of peroxide value

To one g of the oil sample one g of potassium iodide and 20 mL of solvent mixture (glacial acetic acid/ chloroform 2:1 by volume) was added and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20 mL of 5 % potassium iodide. A few drops of starch solution were added to the mixture and the latter was titrated with 0.25 N sodium thiosulphate. The peroxide value was determined as follows;

$$Pv = \frac{SN\ 10^3}{W}$$

Where, S = mL of $Na_2S_2O_3$; N=Normality of $Na_2S_2O_3$; W=Weight of oil sample (g)

3.6. Phytochemical Analysis

The qualitative screening of bioactive component in petroleum ether extracted oil was carried out employing standard method [10, 27]. Qualitative test methods were employed to evaluate for the presence of saponins, alkaloids, tannins, flavonoids, terpenoids, glycosides and quinones. All tests were done in replicates.

Saponins

In test tub 0.5 mg of extract oil was added and diluted with 20 mL distill water and agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin.

Alkaloids

One mL of oil extract was added in test tub and 3 drops of Wagner's reagent added the presence of alkaloids indicated by the formation of reddish brown precipitate.

One mL of oil extract was added in test tub and3 drops of Mayer's reagent was added the presence of alkaloids indicated by the formation of creamy yellow precipitate

Tannins

In test tub one mL 5% ferric chloride ($Fecl_3$) was added to solvent free 0.5mg extract. The presence of tannin is indicated by the formation bluish black or greenish precipitate.

One % lead acetate was added to the test solution. The presence of tannins is indicated by the formation of yellow precipitate.

Flavonoids

In test tube 1mL of test solution, a few drop of dilute sodium hydroxide (NaOH) was added, an intense yellow color was produced in the plant extract which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoid.

A few drops of NaOH and HCl added to test solution. The presence of flavonoid indicated by the formation of yellow/orange color

Terpenoids

In a test tube 5 mL of test solution 2mL of chloroform and 3ml of concentrated sulphuric acid (H_2SO_4) was added to form a layer. The yellow color in lower layer indicates the presence of terpenoids.

Glycosides

In test tube one g of oil was dissolved in water and then aqueous 0.5 mL NaOH solution was added formation of yellow color indicates the presence of glycoside

Quinones

In test tube a small amount of seed oil was treated with con. HCl. Formation of yellow precipitate (or coloration**)** confirms presence of quinines**.**

3.7. Test for natural acid-base indicator potential

The indicators were prepared by dissolving 1 mg of phenolphthalein or extracted oil/MLO-4 in 10 mL of acetone and ethanol respectively. Then titration of 10 mL of 0.1M HCl solution was carried using 0.1M of NaOH as standard and 3 drops of phenolphthalein or crude oil/MLO-4 as indicators. End point color changes and volume of NaOH were recorded. Each experiment was performed in replicates.

3.8. Organism culture preparation and Bioassay procedures

3.8.1. Insecticidal activities

3.8.1.1. Evaluation of *Maesa lanceolata* seed oil in the control of Maize weevil, *Sitophlus zeamais (Matschulsky)*

The initial generation of *S. zeamais* was obtained from maize store culture of Jimma Markato superMarket stores with maize grains. The established S.zeamais was reared on 5 Kg maize grains in bucket covered with cotton cloth in home at 27^{o}c and 50 –70 RH in Jimma for 45 days.

ii. Bio-assay procedure

The oil extract and its fraction (MLO-4) were weighed and incorporated in acetone in 100 ML volumetric flask to prepare serial concentration of 0.3125, 0.625, 1.25, 2.500 and 5.00 % by dilution. The prepared concentrations and 25 g healthy disinfected maize grain seeds were put in 500 mL glass jars and mixed with each concentration. The jar contents (treatment maize grain) was shaken thoroughly for about five minutes to ensure uniform distribution of the oil and allowed the solvent to evaporate for 12 h. Then, 20 early emerged adults of almost same aged male and female *S. zeamais* was collected from the previously reared culture of insects and induced into jar. Insect mortality was followed and assessed on 3, 6, 9, 12, and 24 hrs. 5 % malathion and acetone were used as controls. Insects were considered dead when gently probed with sharp objects and there were no responses. Percent adult mortality was determined as using the following formula

$$(\%)\text{Mortality} = \frac{\text{No of dead insects}}{\text{Total number of insects}} X\ 100$$

3.8.1.2. Insecticidal activities of *Maesa lanceolata* seed oil against termites

Population of *Odontotermes* formosus were collected from Jimma University main campus. Termite mounds were dug up from tree and soil containing termites were put on plastic sheets. Termites populations survived were then transferred to Petri dishes with filter papers and left in cool and dark area (inside a wooden cap board) for one day just to make them adapt the laboratory environment.

Two milliliters of the crude oil and MLO-4 samples prepared with concentration 0.3125, 0.625, 1.25, 2.50 and 5.00% were each transferred into filter paper (Whatman No 1) of 9 cm diameter and solvent from moistened paper were allowed to evaporate for 12 hr. Then the treated filter papers were placed inside petri dishes and covered after introduction of twenty termites (15 workers and 5 solder) and wad of water moistened cotton. In all experiments 2 mL of 5% boric acid and acetone were used as positive and negative controls respectively. Finally all the treated Petri dishes were placed in the cap board where conditions (25 -30^{o}c and 60 – 70% Rh) can be achieved and to simulate the dark galleries of termites. The mortality of termite was be recorded on 30 min,1,1:30,2,2:30,3,3:30,4,4:30,5,5:30,and 6 hrs after treatment application.

Live and dead termites (both worker and solder) were counted and percent mortality was calculated according to the following equation.

$$\text{percent Mortality} = \frac{\text{No of dead termite}}{\text{Total Numberof termte}} \text{ X } 100\%$$

3.8.1. 3.Insecticidal activities of *Maesa lanceolata* seed oil against Bedbug

Population of bedbug was collected from Jimma University dormitories. Bedbug populations were collected using cotton cloth and placed in plastic bottle (polyethylene plastic bottle).

Bioassay procedures were conducted almost the same way as that of termites except insect mortality was recorded on 1, 2 and 3 days after treatment application.

3.8.2. Antibacterial and Antifungal assay (disc diffusion assay)

Microorganisms selected for this study were two gram positive bacteria *Bacillus cereus* and *Staphylococcus aureus* and a gram negative bacteria Escherichia Coli, two fungi , *Aspergillus spp. and Fusarium* spp. All the identified bacterial and fungal species were obtained from Microbiology Research laboratory, College of Natural Sciences, Jimma University,

Culture medium for bacterial strains (Muller hinton agar) was made by dissolving 4.56 gm agar in 120 ml distilled H_2O in 250 ml flask with continuous mixing. Then the dissolved matter was autoclaved at120 ^oC and 15 mL amount of solution cooled to 50^0C was dispersed (poured) in to sterilized Petri dishes inside the laminar flow bench. The solidified agar was then test cheeked for sterility for 24 hrs. Culture medium for fungal strains (Potato dextrose agar) was prepared almost in similar way.

Then 0.1 ml of 24 hrs cultured three bacteria and two fungi were aseptically transferred on solidified medium and spread by use of swab and spreading glass rod. After spreading on each medium sterile disc pre-immersed in test samples were put on them and dishes were incubated at 37 ^oC for 24 hrs for bacteria 72 hrs for antifungal strains with daily assessment. Finally zone of inhibition of each sample was determined by measuring diameter of clean zone around discs using ruler. Concentration of crude oil (MLO) and its fractions (MLO-2, MLO-3and MLO-4) were all 100 mg/mL in DMSO. Gentamicin and Mancozeb (100 mg/mL) were used as positive controls for bacteria and fungi respectively while DMSO used as negative control (NC).

The inhibitory effects of each test samples were calculated and compared by measuring the activity index.

$$\text{Activity Index (AI)} = \frac{\text{zone inhibition of extract}}{\text{zone of inhibition of antibiotic}}$$

4.0. RESULTS AND DISCUSSION

4.1. Oil yield and Physicochemical test data

The percentage oil yield of *M. lanceolata* seed was 30.4. The yield is less than the value reported in *DelonixRegia* seed oil 30.8% ,*Jatropha curcas* seed oil was to be 42.19% [54] and higher than Cotton seed with 14% ,*Cissus avolioides* seed 23% [5]. This indicates that *M. lanceolata* oil has moderately higher oil yield. The oil obtained had deep red color and viscous appearance and noxious odor. Relative density of oil was 0.75. The higher mass of oil would higher energy available for work out put per unit volume and less than water [5]. Physicochemical characteristics of oil were also determined and results are indicated in table 1.

Table : Physical properties of the oil

Characteristics observed/measure	**Result**
Seed oil yield (%)	30.4
Relative density	0.75
Color	Deep red
Physical state at room temperature	Viscous liquid
Odor	Noxious
Acid value(mg KOH/g)	0.8±0.15
Peroxide value(meq/kg)	0.375±0.1
Saponification value($mgKOHg^{-1}$)	106.59±0.57

Acid and peroxide indexes are parameters that demonstrated the quality of the oil [5] From table 1 above the acid value 0.8 mg KOH/g is lower than limited value (4mg KOH/g) Codex STAN 19(19983a) Acid value is direct measure of free fatty acids in a given amount of oil. It is a measure of extent to which the triglycerides in the oil have been decomposed by lipase action in to free acids [54]. Acid value depend on the degree of rain acidity this is the indicator of that oil cannot easily go rancid. Peroxide value is an index of primary oxidation status of oils that information about the concentration of hydro peroxide (primary oxidation products), which are unstable and easily can decompose secondary oxidation products such as ketones and aldehydes. These low per oxide value increase stability of the oil for long time storage due to a low level of oxidative and lipolytic activities. The peroxide value for *M.lanceolata* seed was found to be

0.375meq/kg. This value is relatively low compared with the value of limited (10meq /kg) Codex STAN 19,(19983a) and other oil of wild plants. High peroxide value is associated with high rancidity rate. Thus, with this fact, the low per oxide value obtained from the oil is simply an indication of the oil less liable to rancidity at room temperature. [55]

The saponification value is a measure of the alkali reactive groups in fats and oils and predicating the type of glycerides in an oil sample, which gives an idea of approximate chain length of the oil and was found to be 106±0.55 KOHg-1 .The oil with a saponification value of 200 mgKOHg-1 and above is regarded as high molecular weight fatty acid oil and used in making of soap [54]. Saponification value is measure of the equivalent weight of acid present and therefore it is an indicator of purity. This type of oil with saponification value of 106mg $KOHg^{-1}$, is of a very candidate in soap making industries. However, the oil can subjected to refining process in order to find place in soap making industries and to be as emulsifiers [55].

4.2. Phytochemical Analysis

Phytochemical analysis of petroleum ether oil extract of *M .lanceolata* seed revealed that the oil extract has a potential source of bioactive components, such as quinones, alkaloids and terpinoids the presence of these substances in the investigated plant accounts for its use fullness as medicinal plant and the absence of saponin, tannin and glycosides is due to their polarity .The plant can be useful for natural indicator, antimicrobial and anti insecticidal. Information obtained is used to facilitate quantitative estimation and qualitative separation of constituents from seed.

Table : Phytochemical screening test performed on oil extract of *M. lanceolata* seed.

Alkaloid	Saponin	Tannin	Qunones	Terpinoids	Glycosides
+	–	–	+	+	–

+=Presence of bioactive compounds

4.3. Compounds isolated from *M. lanceolata* seed oil

The column was eluted first with petroleum ether then with petroleum ether: ethyl acetate ratio. Each beaker contains 25-30 ml and all fractions were optimized using TLC and collected in one beaker those have the same polarity.100 % (1-7), 99:1(1-7) and98:2(1-7) have the same polarity and separated component are observed and chosen for the second column. 97:3(1-7), 96:4(1-7), 95:5(1-7) and 94:6(1-7), have the same polarity and observed more than three components .93:7(1-7), 92:8(1-7)), 91:9(1-7)and 90:10(1-7) have the same polarity 89:11(1-7),88:12(1-7),87:13(1-7)and 86:14(1-7)have the same polarity, it is gum substance and more than four components are observed .85:15(1-7) ,84:16(1-7)and 83:17(1-7)have the same polarity, more than two components and small amount.82:18(1-7),81:19(1-7)and80:20(1-7) have the same polarity and yellow crystalline solid compound(MLO-2) is eluted.79:21(1-7) and 78:23(1-7)have not observed any spot on TLC(fractionated component).

The second column chromatography was packed to purify the chosen fraction (100 %, 99:1and 98:2) .It has 30g mass, viscous solid and orange color. The sample was absorbed with 30g and packed with 150g of silica gel. The column was purified first with petroleum ether then with petroleum ether: acetone ratio by using gradient elution method.

Each beaker contains 20-25 ml and all fractions were optimized using TLC and collected in one beaker those have the same polarity. 100 % (1-3) elute has no component observed100% (5-6) and 99:1 (1-2) have the same polarity and white crystalline solid (MLO-1) component is eluted, Components 99:1 (3-4)and 98:2 (1-4)have the same polarity, viscose solid and more than two components observed,97:3 (1-4) have the same polarity, orange viscose solid (MLO-3) substance is obtained,96:4 (1-4),95:5 (1-4) and 94:6 (1-4) have the same polarity, orange viscose solid (MLO-4) obtained,93:3(1-4) and 92:8 (1-4) have the same polarity ,small amount and more than two components observed and 91:9 (1-4),90:10 (1-4) and 89:11(1-4)have the same polarity, small amount and has more than one component.

4.4.Characterization of compounds isolated

Among three relatively pure compounds isolated MLO-1,MLO-2 and MLO-3 only MLO-2 was successfully characterized using physical data (M.pt) and NMR. MLO-2 was harvested as yellow crystalline solid (melting point 101-102^{0}C, Rf = 0.66 in petroleum ether: ethyl acetate 8:2 .The ^{1}H and ^{13}C NMR data of this compound is given below.

In the 1H-NMR spectrum of compound MLO-2 displayed eight peaks. the triplet peak at δ 0.90 indicated the presence of terminal methyl group adjacent to methylene carbon, the peak at δ 1.14-1.36 indicates proton of aliphatic methylene (-CH_2) group, peak at δ 2.42 indicate the presence of methyl group bonded to ester carbonyl carbon, the peak δ 2.33 indicates the presence of protons of a methylene group bonded to aromatic carbon and the peak δ 3.45 indicates the presence of proton attached to oxygen.

The ^{13}C NMR spectrum showed presence of 44 Carbons (table 4) of which fourteen are quaternary carbon 2 x(C-1, C-2, C-3,C-4,C-5,C-6 and C-OAc),twenty four methylene 2 x (C-1'-C-12') and six methyl 2x(C-13',C-14 and C-15). In the ^{13}C-NMR spectrum of compound MLO-2,The peaks in the range of chemical shift values at183.32-180.18ppm indicate C=O bonds; the peaks at 167.78 and 167.50 ppm indicate quaternary carbon atoms of ester carbonyl group, the peaks at151.71-115.06 quaternary carbon substituted in aromatic (benzene ring).On the other hand chemical shift values in range of 6.88-19.43ppm indicated the presence of methyl(-CH_3)carbon and 22.7-33.31ppm indicated the presence of aliphatic saturated long hydrocarbon chain.

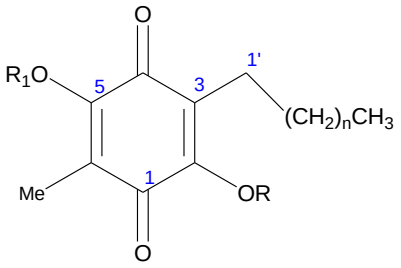
In DEPT spectrum, the data are collected in such way that the resulting signal is either positive (CH_3) or negative (CH_2) depending on the number of protons attached. Accordingly, twenty four signals are pointed down (negative) indicating that there are twenty four methylene groups in MLO-2 and six signals pointing upward (positive) indicating six carbons attached with three hydrogen atoms. The DEPT-135 spectrum show peaks of presence of methyl (-CH_3) carbon atoms at 6.61-19.42 ppm, methylene carbons at 22.01-33.30 ppm and the absence of peaks at183.32-115.06ppm in DPT-135 spectrum indicated quaternary carbon atoms of (ketone and

ester carbonyl group and aromatic substituted carbon atoms).The peaks were observed in ^{13}C NMR spectrum but not in DPT-135.

Based on information from ^{1}H,^{13}C and DEPT-135 spectra for the sample analyzed we proposed presence of an isomeric mixture of tetra-substituted 1,4–benzoquinone nucleus which is common to the family *Myrsinaceae*. Our search on literatures for physical and spectral data of plant derived compounds we found isomeric mixture of 1,4-benzoqinone; 5-Acetoxy-2-hydroxy-6-methyl-3-tridecyl-1,4-benzoquinone **[1]** and 2-Acetoxy-5-hydroxy-3-methyl-6-tridecyl-1,4-benzoquinone **[2]** both derived from *M .lanceolata* fruits to have yellow color, Rf (0.60 hexane: ethyl acetate1:4), melting point (101-102^{o}C), and NMR spectral data comparable with ours.

Table : ^{1}H & ^{13} C NMR data of compound MLO-2 in comparison with reported data [56].

Carbon number	**^{1}H-NMR MLO-2**	**^{1}H-NMR Reported compound**	**Multiplicity(DPT)**	**^{13} C-NMR *MLO-2***	**^{13}C-NMR Reported compound**	***Remark***
1	-	-	C	180.35,183.32	180.5,183.3	Quaternary carbon
2	-	-	C	149.63,152.71	150.3,151.0	Quaternary carbon
3	-	-	C	132.57,128.92	132.7,128.7	Quaternary carbon
4	-	-	C	180.18,182.94		Quaternary carbon
5		-	C	149.83,152.84	151.1,150.3	Quaternary carbon
6	-	-	C	115.06,119.43	115.8,120.1	Quaternary carbon
1’	2.33	-	CH_2	22.26,22.95	22.7,23.3	Methylene
2’	1.41	-	CH2	28.02, 28.41	28.1,28.2	Methylene
12’	1.39	-	CH2	19.41,19.36	22.6,22.6	Methylene
13’	0.92	-	CH_3	13.56,	14.0,14.0	Methyl
6-Me	1.91	-	CH_3	7.88,6.98	7.8,8.7	Methyl
OAc	2.42	-	C	167.78,167.50	167.8,168	Quaternary carbon
		-	CH_3	19.43,19.36	20,9,20.9	Methyl
Other C	1.14-1.36	-	2(9CH_2)	28.60-33.31	29.2-31.9	Methylene



No	R	R1
1	Ac	H
2	H	Ac

Figure : Isomeric mixture of monohydroxy alkaylatebenzoquinone (proposed compound).

4.5. Test results for potential acid- base indicator

The result obtained in titrations was due to the presence of bioactive substance, sharp color change occurred at end points of the titration. Neutralization point obtained by *M.lanceolata* seed oil and *MLO-4* were much closed with equivalence point obtained by standard indicator (phenolphthalein).This represent usefulness of *M. lanceolata* oil and MLO-4 as indicator in acid base titration. Titrate and titrant with indicators show sharp and intense color change at the equivalence point that is at neutralization.

Table : Comparison of Phenolphthalein, *Maesa lanceolata* oil (MLO) and isolated compound (MLO4)

Titrant	Titrate	Indicators	Color Change	Volume of titrate consumed
0.1MHCl	0.1MNaOH	Phenolphthalein	Colorless- Pink	10.3±0.2
0.1MHCl	0.1MNaOH	MLO	Yellow- Pink	10.2±0.2
0.1MHCl	0.1MNaOH	MLO-4	Yellow- Pink	10.1±0.2

4.6. Insecticidal activity test results

Insecticidal activity test of the oil extract and its fraction (MLO-4) on insects (*S.zeamais*, termite and bed bug) is presented in table below. Adult mortality significantly increased with increase in concentration and hour of exposure on three different insect species.

The highest value of 100% mortality of Maize weevil was observed in the treatment of oil extract 5.00% on 9 hours, This followed by 2.50%, 1.25%, 0.625% and 0.3125%.However on MLO-4 treatment the highest value of 100%mortality observed on 6 hours The oil extract and its fraction on application, covered the outer layer of the grains (there by serving as food poison to the adult insects). The mortality of Maize weevil has been graphically represented in figure.

Table : Effect of seed oil on adult Maize Weevils (*Sitophilus zeamais*)

Plant extract	Concentration(%,v/w)	Mean mortality(%) at 3-12hrs post treatment			
		3	6	9	12
Maesalanceolata crude Oil	0.3125	7.50	22.50	40.50	55.50
	0.625	10.00	35.00	60.00	75.00
	1.25	17.50	42.50	50.00	82.50
	2.50	20.00	50.00	80.00	90.00
	5.00	27.50	82.50	100.00	
	Standard (Malathion 5%)	52.00	92.50	100.00	
	Control(solvent-treated)	2.50	00.00	00.00	00.00

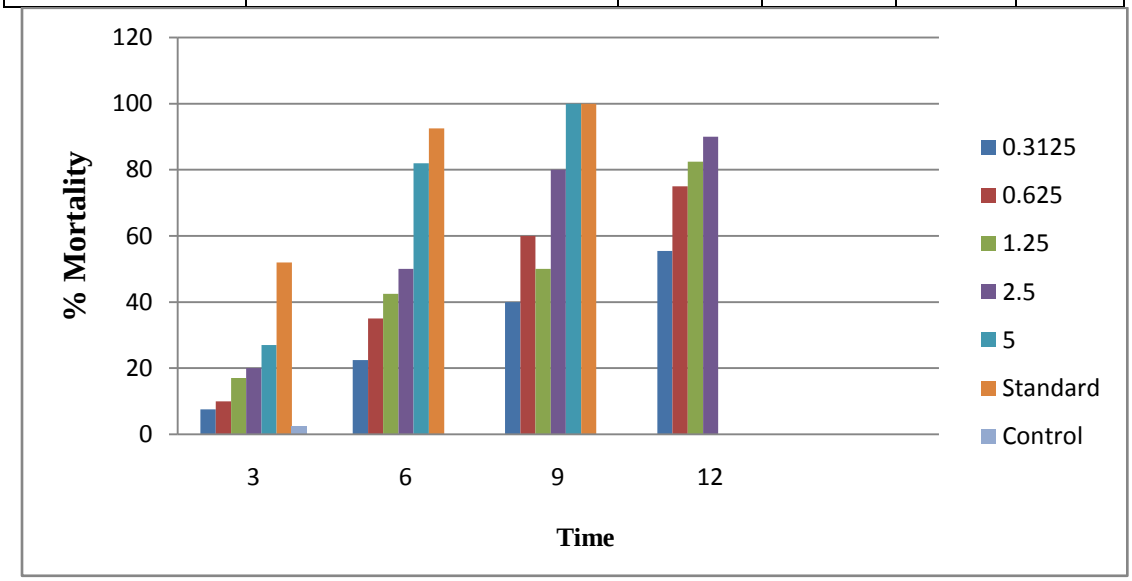


Figure : Plot of percent mortality of maize weevil time with different concentration of *M.lanceolata* seed oil

Table : Effect of *MLO-4* on Maize Weevils (Sitophilus zeamais)

Plant extract	Concentration(%,v/w)	Mean mortality(%) at3-9 hrs post treatment		
		3	6	9
MLO-4	0.3125	20.00	60.00	80.00
	0.625	20.00	60.00	90.00
	1.25	40.00	80.00	100.00
	2.50	65.00	85.00	100
	5.00	85.00	100.00	-
	Standard (Malathion5%)	90.00	100.00	-
	Control(solvent)treated	00.00	00.00	00.00

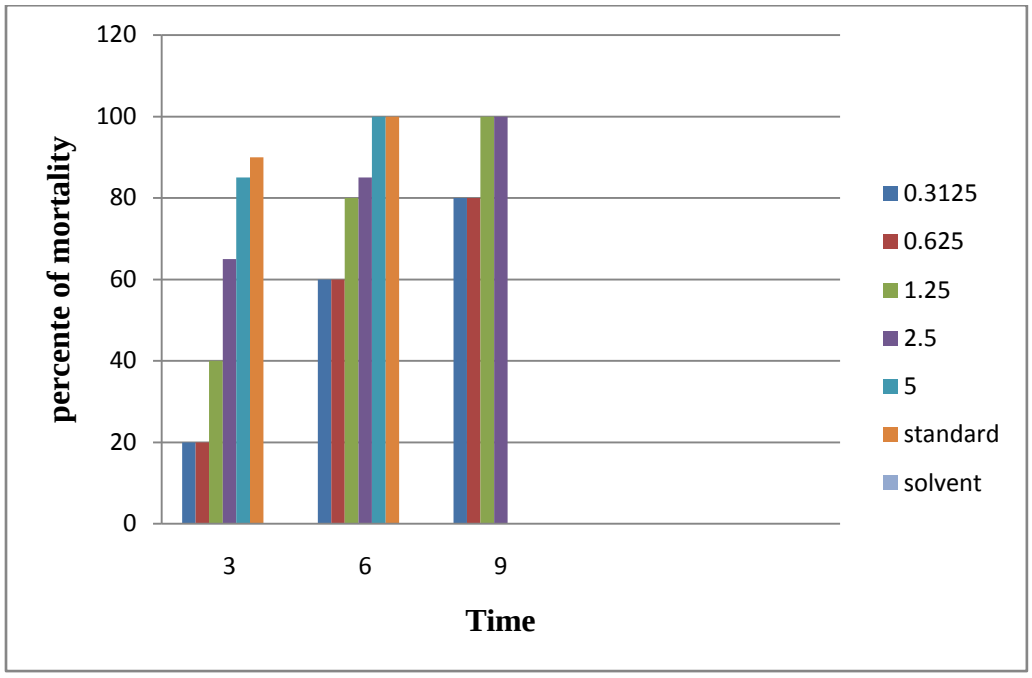


Figure : Plot of percent mortality of maize weevil time with different concentration of MLO-4.

Compound MLO-4 was relatively more active than seed oil (MLO), comparison of these data

with the observed 100% mortality of maize weevil on 6hrs in reference compound (Malathion5%) suggested that comparable activities. The highest of 100% mortality of worker termite was observed in the treatment of oil extract 5%on 1:30 hrs and 0.3125% on4:00 hr, but80% mortality of solder termites was on 4:00 hr in the treatment of 5% and 20% in the 0.3125% treatment.100%mortality of worker and Solder termites highest on 1:30 hrs in the treatment MLO-4 of 5.00% followed by 2.50, 1.25, 0.625 and 0.3125%..Compound MLO-4 was relatively more active than seed oil (MLO).Comparison of these data with the observed 80% mortality of worker and solder termite on 4 hrs in reference compound (Boric acid) suggested that MLO and MLO-4 were higher activities than standard.

Table : Effect of MLO on Termite

Time (hrs) post treatment		Mean mortality (%) with concentration (v/w %)						
		0.3125	0.625	1.25	2.50	5.00	Boric acid %)	Acetone
0.30 min	W	13.33	13.33	33.33	40.00	46.66	13.33	00.00
	S	-	-	-	-	-	-	00.00
1:00	W	33.33	33.33	53.33	66.66	73.33	33.33	00.00
	S	-	-		-	40.00	20.00	00.00
1:30	W	46.66	46.66	66.66	73.33	100.00	53.33	00.00
	S	-	-	20.00	20.00	40.00	40.00	00.00
2:00	W	60.00	60.00	73.33	86.66	-	66.66	00.00
	S	-	-	20.00	40.00	60.00	0.00	00.00
2:30	W	73.33	73.00	86.66	100.00	-	73.33	00.00
	S	-	20.00	40.00	40.00	60.00	40.00	00.00
3:00	W	86.66	93.33	100.00	-	-	73.33	00.00
	S	-	20.00	40.00	40.00	60.00	60.00	00.00
3:30	W	93.33	100.00	-	-	-	80.00	00.00
	S	20.00	20.00	40.00	60.00	60.00	80.00	00.00
4:00	W	100.00	-	-	-	-	80.00	00.00
	S	20.00	20.00	40.00	60.00	80.00	80.00	00.00

W worker termite

S solider termite

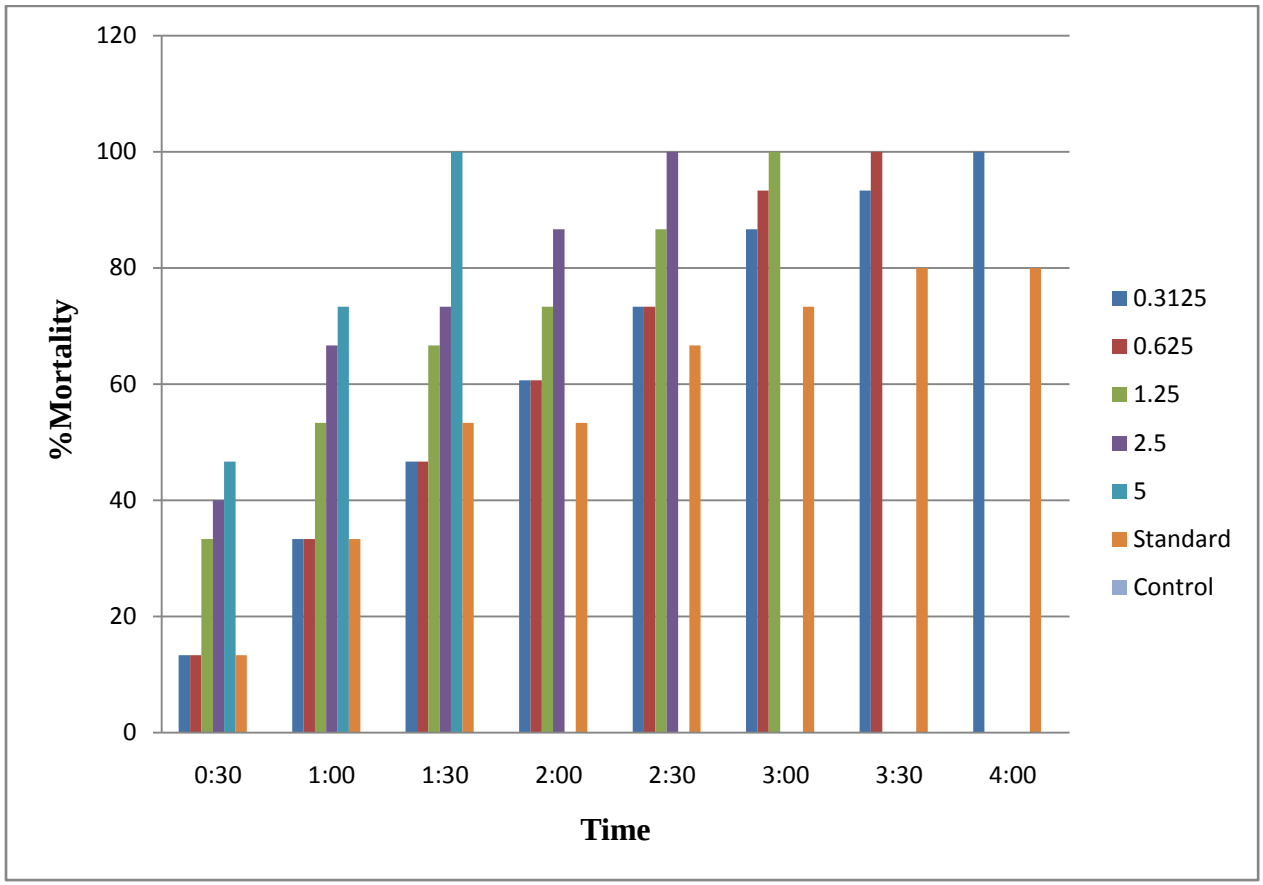


Figure : Percent mortality of worker termite time with different concentration of seed oil

Table : The effect of MLO-4 on termite

Time (hrs) post treatment		Mean mortality (%) with concentration (v/w %)						
		0.3125	0.6250	1.2500	2.500	5.0000	Standard (5% Malathion)	Control(Solvent treated)
30min.	W	20.00	33.33	36.66	56.66	66.66	86.66	00.00
	S	0.00	0.00	20.00	20.00	40.00	60.00	00.00
1:00	W	40.00	53.33	56.66	63.33	93.33	100.00	00.00
	S	0.00	20.00	20.00	40.00	80.00	80.00	00.00
1:30	W	53.33	66.66	73.33	86.66	100.00	-	00.00
	S	20.00	40.00	40.00	60.00	100.00	100.00	00.00
2:00	W	60.00	73.33	86.66	93.33	-	-	00.00
	S	40.00	40.00	60.00	80.00	-	-	00.00
2:30	W	73.33	80.00	93.33	100.00	-	-	00.00
	S	60.00	60.00	60.00	100.00	-	-	00.00
3:00	W	86.66	93.33	100.00	-	-	-	00.00
	S	60.00	60.00	80.00	-	-	-	00.00

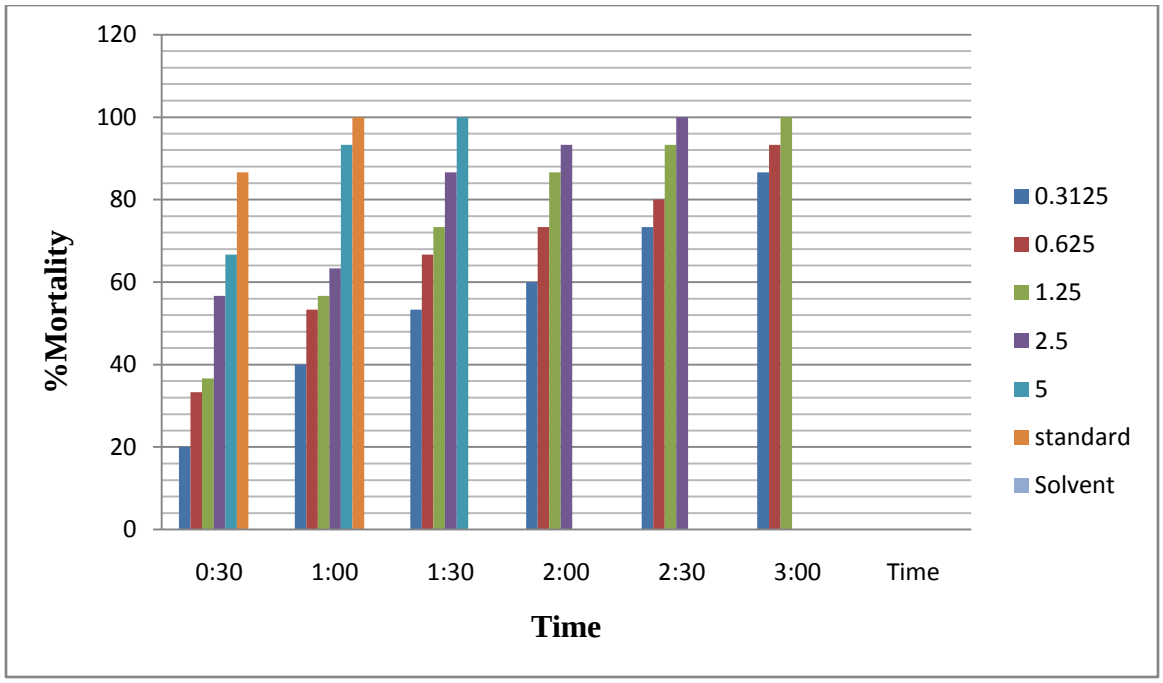


Figure : Plot of percent mortality of worker termite time with different concentration of MLO-4

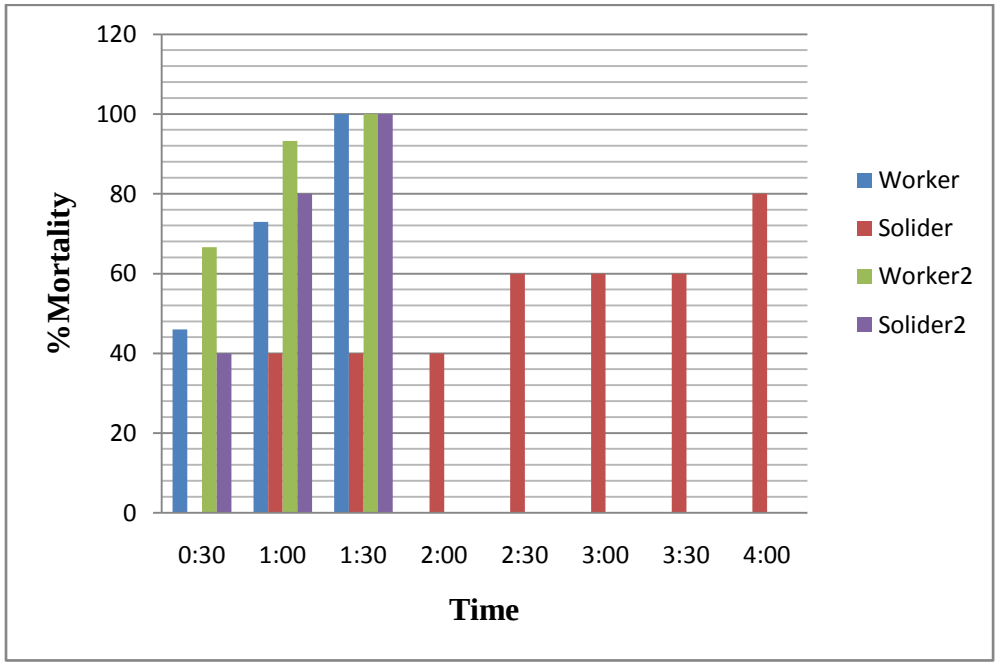


Figure : Plot of percent mortality comparison of worker and solder of termite time with 5.00% concentration of seed oil and MLO-4

The highest 100% mortality of bed bug observed on 3 days in the treatment of 5%and 75%on 4 days in the small treatment(concentration).

Table : Effect of *Maesa lanceolata* seed oil on Bedbug

Oil Concentration(%,v/w)	Mean mortality(%) at 1-4 days post treatment			
	1	2	3	4
0.3125	35.00	45.00	60.00	75.00
0.625	40.00	50.00	65.00	85.00
1.25	55.00	70.00	85.00	90.00
2.50	60.00	75.00	85.00	95.00
5.00	80.00	90.00	100.00	
Standard (Boric acid 5%)	10.00	15.00	30.00	35.00
Control(solvent-treated)	00.00	00.00	00.00	00.00

Each value is the mean of three replicate.

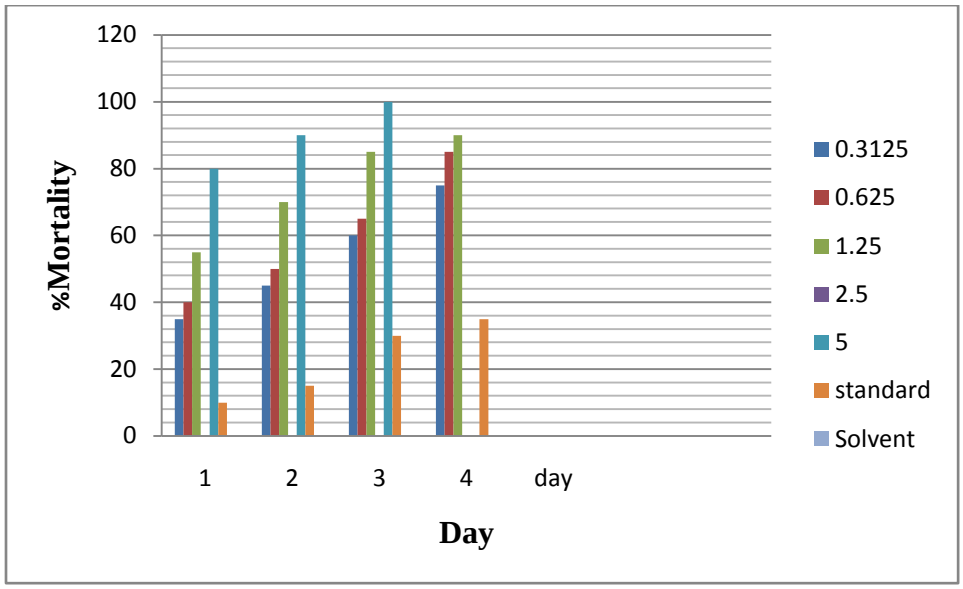


Figure : Plot of percent mortality of bed bug day with different concentration of seed oil

4.7. Antibacterial activity test results

Antibacterial activity test of seed oil and its three fractions were performed using disc diffusion method .The zone of inhibition were measured in millimeters (mm) and compared the zone of inhibition of antibiotic (gentamicin) which was used as positive control. Antibacterial activity of seed oil and its fractions with positive control has been shown in the table 11 and the comparison of zone of inhibition graphically represent in figure.

Table 11shows the comparative results between the seed oil, its different fractions and the positive control against Bacillus cereus, Staphylococcus aureums and Escherichia coli

Table : Anti bacterial activity (and activity index value) of the oil extract and its fractions

Organisms	zone of inhibition in mm(Activity index)					
	MLO	MLO-2	MLO-3	MLO-4	Gentamicin	DMSO
B. cereus	9 (0.5)	NI	12 (0.66)	8 (0.44)	18	NI
S. aureus	14 (0.77)	10 (0.55)	NI	NI	18	NI
E. coli	NI	NI	NI	NI	18	NI

NI-not inhibitory

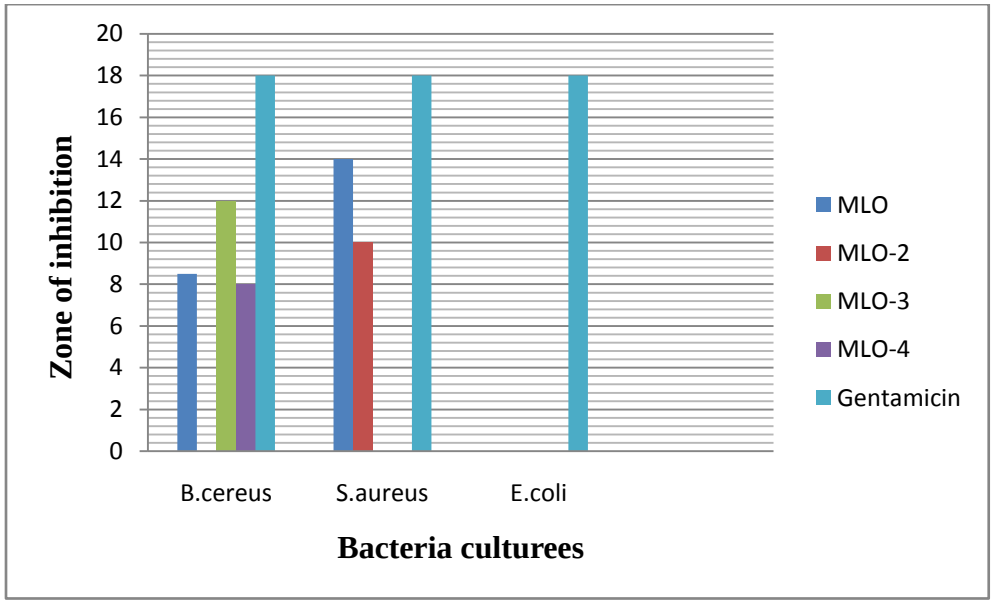


Figure : Zone of inhibition of seed oil, its fractions and antibiotics

4.8. Antifungal activity test result

Antifungal activity test of seed oil and its three fractions were performed using disc diffusion method .The zone of inhibition were measured in millimeters (mm) and compared the zone of inhibition of antibiotic (mancozeb) which was used as positive control. Antifungal activity of seed oil and its fractions with positive control has been shown in the table 11 and the comparison of zone of inhibition graphically represent in figure.

Table : Zone of inhibition and activity index of seed oil and its fractions against Fusarium ssp.

Test samples	Inhibition zone(inmm) and activity index	
	Inhibition zone	Activity index
MLO	11	0.92
MLO-2	9	0.75
MLO-3	11	0.92
MLO-4	8	0,66
Mancozeb	12	-
DMSO	NI	-

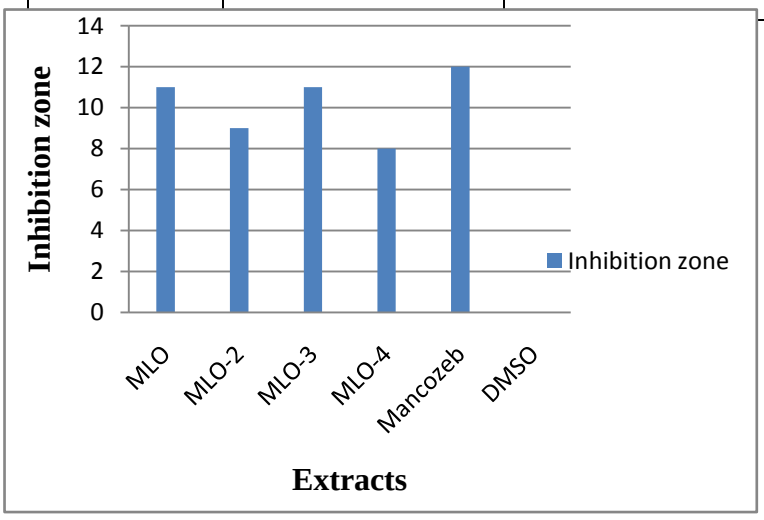


Figure : Zone inhibition inhibition of seed oil and its fractions against fusarum spp.

5.0. CONCLUSION AND RECOMMENDATIONS

5.0. Conclusion

Fixed oils possess divers' application in food/feed, medicine, industry, agricultur and fuel. The extraction and characterization of *M. lanceolata* seed oil results obtained from the various tests and analysis carried out on *M. lanceolata* seed oil, it showed that oil can be extracted from the *M.lanceolata seeds*. The percentage oil content is high and suggests that its extraction on commercial scale is possible and economical. The physico chemical parameter of *M.lanceolata* seed oil indicates this oil can be used in liquid soap production, detergents and shampoo industries. Because oil exhibit low saponification value and low free fatty acid indicates that oil have low deteriorating rate and high ediblity. This makes the oil good in quality and higher in molecular mass. Isolated mixture of monohydroxy alkylbenzoquinone(MLO-2) was isolated from petroleum ether:ethylacetate crude extractoil of *M.lanceolata,*Itsidentity was determined to be 2-acetoxy-5-hydroxy-3- tridecyl-1,4-benzoquinone.Based on similarties of observed ^{13}C-NMR and mp.with that of reported for isomeric mixture of monohydroxy alkylbenzoquinone.

The oil extracted has potential source of bioactive components, such as quinones, alkaloids, terpinoids and flavonoids. The plant may be used for insecticidal, antibacterial, antifungal and an indicator. The result obtained in acid base titrations lead to as conclude that in strong acid-strong base titration was found to be more significant over standard indicator as it gives sharp color change at equivalence point due to the presence of quinines sharp color changes at end point of the titration and showed that phenolphthalein indicator replaced successfully by *M.lanceolata* seed oil, it is simple, accurate, economical, precise and prepared easily. The proposed oil indicator can be used as substitute to synthetic (phenolphthalein) indicator. Based on Bronsted-Lowery acid base theory the proton from the *M. lanceolata* seed oil received by $^{-}$OH ions thus causing color change.The seed oil produces different chemical as secondary metabolites and used as controlling against insects, bacteria, and fungi. In the present study the petroleum extract of *M. lanceolata* oil and its fractions show significant insecticidal activity against maize weevil, termites and bed bug, anti bacterial and anti fungal activity against bacillus cereus and *stphylcocous aureaus* and a fusarium respectively.It was previously *M.lanceolata* reported that does have anti bacterial and anti fungal activity of different parts of plants.

5.2 Recommendation

Based on the present work the following are suggestions for further work forwarded

- Iodine value and transesterification of *M.lanceolata* seed oil is strongly recommended.
- The use in strong acid -strong base titration was found to be more significant the further investigation on strong acid-weak base, strong base-weak acid and weak acid-weak base.
- Further studies would still required for better understanding of especially the chemistry of *M lanceolata* seed oil NMR (2D), GM-MS, MS, LC-MS and elementary analyzer.
- The researchers believe that field level would need to further validate and reproduce insecticidal potential *of M.lanceolata* seed.

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Annex I Pictures showing some of the major activities in this work

Sample collection

Harvested seeds

Ground seeds

Concentration of extracts by rotary evaporator

Sample extraction by socking with petroleum ether and filtrate

Solvent free extracted

Optimization of solvent system for column packing

Sample absorbed in silica gel



Process of isolation of compounds using column chromatography

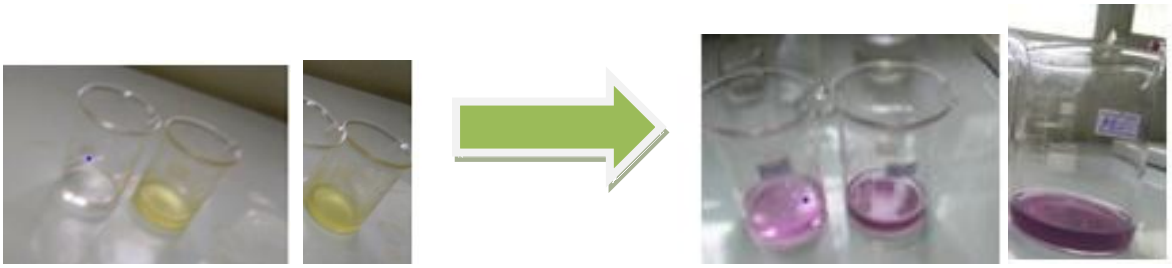




Purification process

Annex II. Pictures showing major test results obtained.

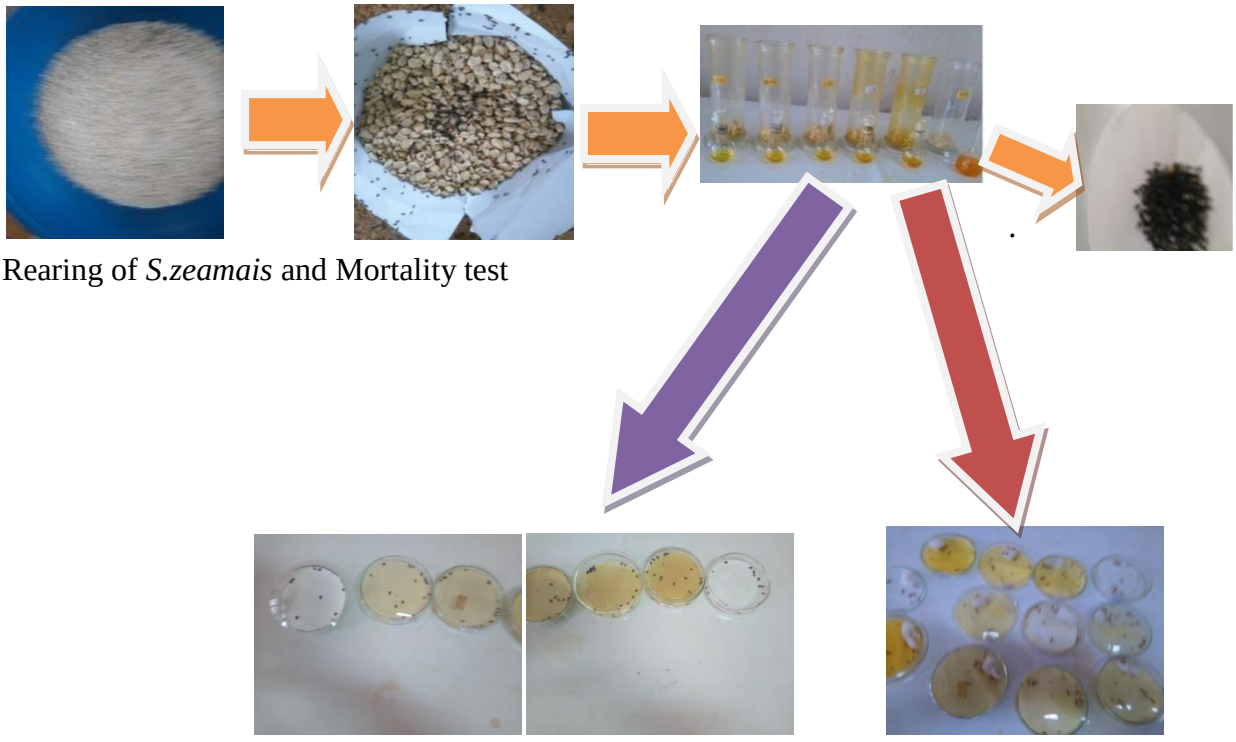
i) Color change of phenolphthalein (A), MLO-4(B) and crude oil (C) in the presence of acid before and at end point of titration



A B C A B C

Before end point of titration at end point of titration

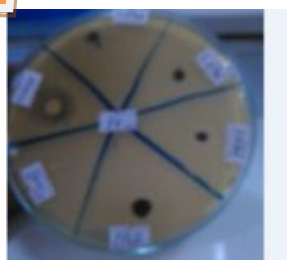
ii) Insecticidal assays



Rearing of *S.zeamais* and Mortality test

iii) Antimicrobial assays

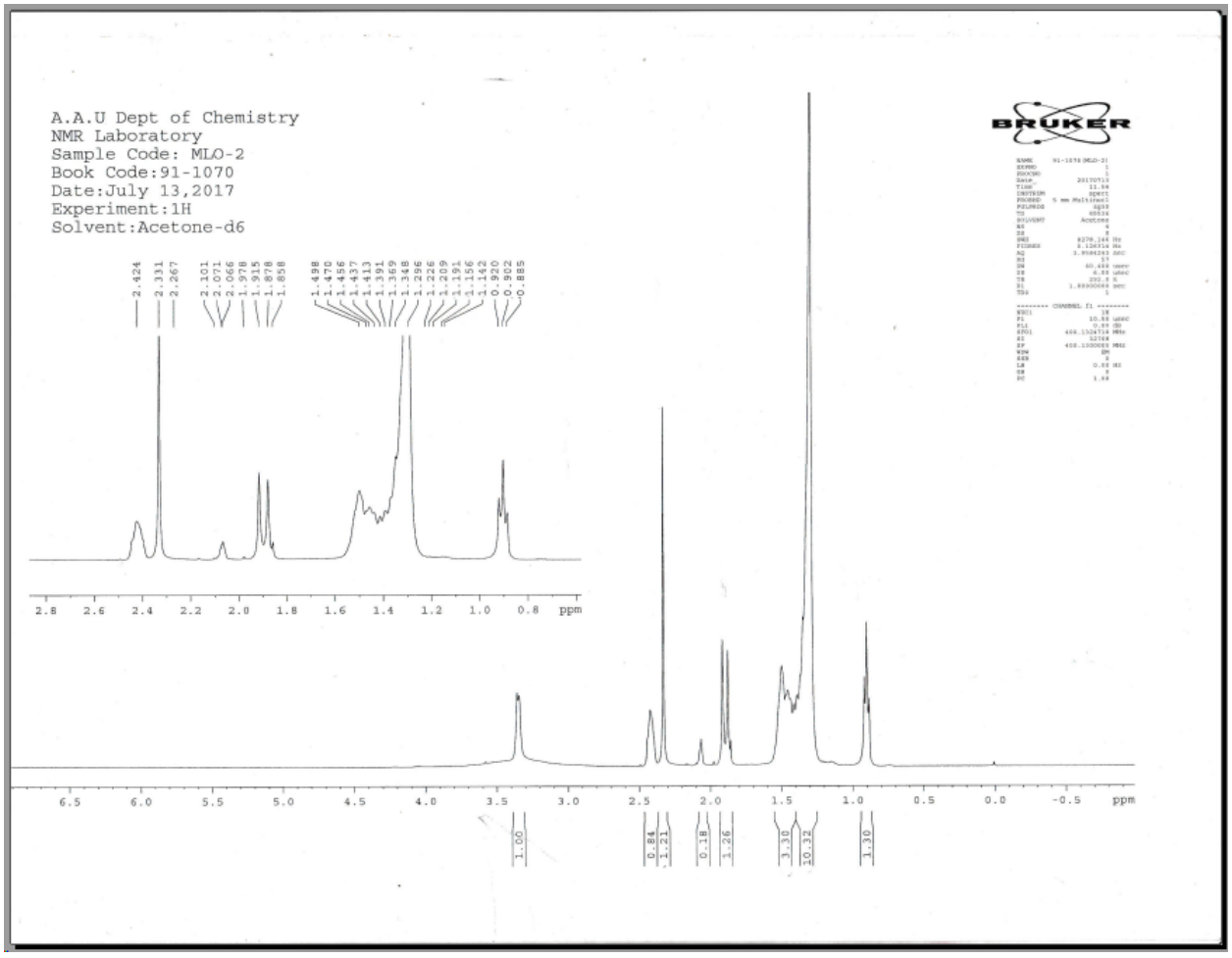
Anti bacterial activity of the oil extract and its fractions



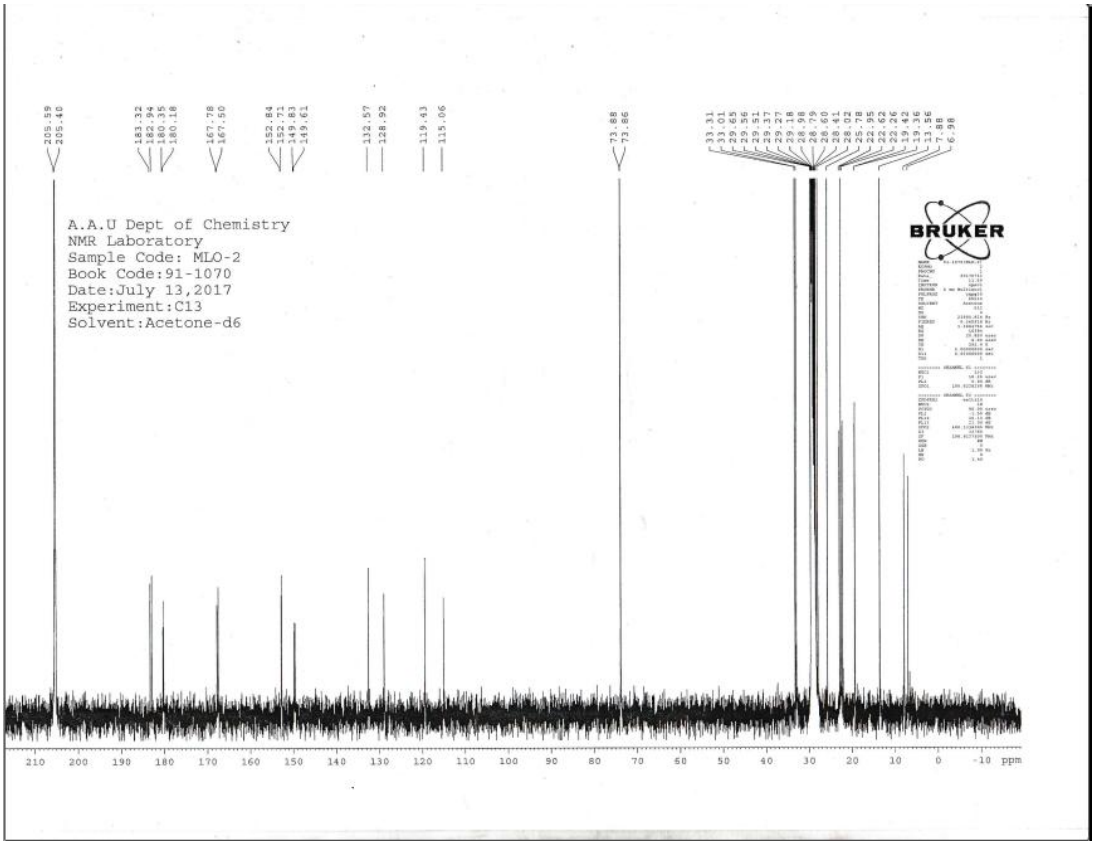
Anti fungal activity of the oil extract and its fractions

Annex III. NMR data

^{1}H Chemical shift of MLO-2



^{13}C Chemical shift of MLO-2



DPT-135 of MLO-2