JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

SCHOOL OF GRADUATE STUDIES

DEPARTEMENT OF CHEMISTRY

M.Sc. THESIS ON:

EVALUATION OF STEM BARK OF *Erythrina abyssinica* FOR ANTIMICROBIAL AND TERMITICIDAL PRINCIPLES

BY

GIZATU MOSISA

OCTOBER, 2017

JIMMA, ETHIOPIA

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

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Abstract

Erythrina species are used as healing agent in traditional medicine in Africa and are prescribed against wide range of diseases including those with microbial and parasitic origin. *Erythrina abysinica* is a species widely distributed in Southwestern Ethiopia and is well known for its decay/decomposition tolerant bark from matured stem. The main objective of this study was to carry out phytochemical investigation of the stem bark of matured *Erythrina abysinica* and evaluation of its antimicrobial and termiticidal activities. The bark of *Erythrina abysinica* was collected from Jimma town, dried and powdered. The powdered sample was extracted with methanol using maceration technique. Phytochemical analysis of the crude extract was carried out using both chemical test and column chromatography. The isolated compounds were characterized using physical and spectral (1H NMR, ^{13}C NMR, and DEPT) data. The Crude extract and purified compounds were subjected to tests on selected strains of bacteria (*S. aureus, E. coli*, and *B. subtilus)* and fungi (*Aspargillus spp., Fusariun spp., penicillium spp & Trichophyton spp)* using disk diffusion method. Termiticidal activity was also carried out on *C. formosans* employing No-Choice assay method. The phytochemical screening tests carried on crude methanol extract (53 g, 4.82 %) confirmed the presence of alkaloids, saponins, terpenoids and flavonoids only. Column chromatographic analysis yielded two pure compounds E.A1 and E.A2 which were proposed to be lupinifolin and 9-ethyldodecyl-4-methoxybenzoate respectively by comparison of physical spectral data with those reported in literature. The crude extract was not active against all bacterial trains tested but E.A1 *and* E.A2 were active both with zone of inhibition 9.0 mm against *Bacillus subtilus and E. coli*. The crude extract and isolated compounds *(E.A1* and *E.A2)* were active against all fungal stains tested with zone of inhibition 10-18 mm except *Aspergillus spp.* Activities recorded were highest on *Fusariun spp.* and lowest on *Aspergillus spp. Aspergillus spp* was responsive only to E.A 2 and Mancozeb. Killing effects caused by the extracts on termites were both time and concentration dependent. The highest killing effect (100 %) was observed for 5% crude extract solution at 30 minutes which was also the same for 5% Malathion. Other test samples produced < 100 % killing effects even after 5 days. Most results attained in this study were in agreement with earlier reports. The termiticidal and antifungal activities observed here justify decay resistant nature of *Erythrina abyssinica* stem bark. Isolation of more phytoconstituent and detailed studies on organisms involved in decay process are recommended.

1. INTRODUCTION

1.1. Background Information

The process of decomposition, the breakdown of raw organic materials to finished compost is a gradual complex process which changes organic matter to into compost. The decomposition (stabilization) of organic matter by biological action has been taking place in nature since life first appeared on our planet. It can generally involve both chemical and biological processes. The breakdown of vegetation is highly dependent on organisms involved, oxygen and moisture levels and nutrients available.

A wide range of organisms takes part in the decomposition process, including beetles and their larvae, flies and maggots (the larvae of flies), woodlice, *fungi*, slime moulds, bacteria, slugs and snails, millipedes, springtails termites and earthworms. Most of them work out of sight, with their handiwork not immediately apparent, but they are the forest's unsung heroes of recycling. During decomposition, microorganisms require oxygen for their respiration. If anaerobic conditions dominate the decomposition environment, microbial activity will be slow and thus decomposition will be slow. Appropriate moisture levels are required for microorganisms to proliferate and to actively decompose organic matter. In arid environments, bacteria and fungi dry out and are unable to take part in decomposition. In wet environments, anaerobic conditions will develop and decomposition can also be considerably slowed down. Decomposing microorganisms also require the appropriate plant substrates in order to achieve good levels of decomposition. This usually translates to having appropriate carbon to nitrogen ratios (C: N).The ideal composting carbon-to-nitrogen ratio is thought to be approximately 30:1. As in any microbial process, the decomposition of plant litter by microorganisms will also be dependent on temperature. For example, leaves on the ground will not undergo decomposition during the winter months where snow cover occurs as temperatures are too low to sustain microbial activities.

Wide range of compounds in plants as flavonoids, isoflavonoids, lectins, saponins phytochemicals were identified to delay or inhibit/resist the decay process owing to their biological activity or toxicity they have on soil biota. Plant phenolics are often inhibitory to microbial activity by which sequester nitrogen in protein. High molecular-weight carbohydrates such as cellulose, hemicelluloses and lignin are often highly resistant to decomposition [1].

1.2. STATEMENT OF THE PROBLEM

Erythrina abyssinica Lam. is a medium-sized tree native to Eastern and Southern Africa, where is considered as a medicinal plant. It is member of the genus Erythrina (Fabaceae), which comprises over 100 species widely distributed in tropical and subtropical regions worldwide [2]. The bark of this plant is extensively used in traditional medicine of Eastern Africa for the treatment of various diseases such as bacterial, fungal, parasitic and viral diseases, gastrointestinal disorders, nervous disorders, liver disorders, sexual asthenia, sterility, eyes diseases and kidney pain etc [3]. The last two decades several phytochemical studies have been carried on the stem bark of E. abyssinica giving rise to the isolation of different secondary metabolites such as C-glycosyl flavones, prenylated flavanones, chalcones, pterocarpans and triterpenoid saponins [5, 7].], which have been found to possess a wide range of biological activities including anti-plasmodial, inhibition of the protein tyrosine phosphatase 1B (PTP1B), antioxidant, antiallergic and anti-inflammatory and cytotoxicity [4,6].

Erythrina abysinica is a species widely distributed in Southwestern Ethiopia and is well known for its decay/decomposition tolerant bark from matured stem. Therefore, the main objective of this study was to investigate the phytochemical constituents of stem bark of *Erythrina abyssinica* and evaluation of their activity against soil microbes (bacteria, fungi and termites) commonly thought to involve in decomposition/decay process in the soil.

The following basic research questions will be thought to be addressed in this research.

i) Do the chemical constituents of the plant stem bark of crude extracts of *E. abyssinica* possess potential to inhibit microbial, termiticidals and insecticidal growth?

ii) Which photochemicals in the plant can be associated with its decay inhibitory effect?

1.3. Objectives of the study

1.3.1. General objective

- To carry out phytochemical and biological investigation on stem bark of *E. abysinica*

1.3.2. Specific objectives

- To isolate compounds from the stem bark of *E. abyssinica* using chromatographic techniques.
- To elucidate the structures of the isolated compounds using physical data and spectroscopic techniques;(^{1}H NMR, ^{13}C NMR and, DEPT)
- To evaluate biological activity of crude extract and isolated compounds againsts different strains of fungi (*Trichophyton spps, Fusariun spp*., *Aspergillus spp.* and *Penicilliu .spp.)* and bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilus)* using disc diffusion methods
- To evaluate termiticidal activity of crude extract on *subterranean* termite employing no-choice test.

1.4. Significance of Study

The project focuses on the isolation, structure elucidation of chemical constituents of methanol extract of the stem bark of *E*. *abyssinica*. Ethiopian traditional medicinal plants *E*. *abyssinica* were investigated and the antibacterial and anti-fungal activities of them were examined by using disc diffusion method and phytochemical investigation on the stem bark of *E. abyssinica* (*Fabaceae*). The finding of this study would help:-

- To identify some compounds that could be used as leads to discover antimicrobial and termiticidal drugs.
- Give in providing baseline information about the constituent of the stem barks of *E. abyssinica* of responsible for antimicrobial activity and termiticidal principles and by side it can also help other researchers as secondary source of data.
- To scale up the commercial uses of this crude extracts of this plant.

2. REVIEW OF RELATED LITERATURE

2.1. Botanical description and distribution of *E. abyssinica*

2.1.1 The family Fabaceae

It is Also known as a sub-family of Leguminosae, the Fabaceae family is one of the largest botanical families and widely distributed around the world, spread out over temperate, tropical and cold regions. The family is composed of 32 tribes, whose genera are chemically represented by a variety of flavonoid skeletons, notably pterocarpans and isoflavones. There are about 650 genera comprising about 18,000 species [8]. Fabaceae family produces valuable medicinal drugs, ornamental species, fodders plants, oil producing plants, insecticides and species with various other functions [9].

2.1.2 The genus *Erythrina*

The genus *Erythrina* is one among several types from the *Fabaceae* family and sub family *Papilionideae* that comprises over 110 species of trees, shrubs and herbicaceous plants that are widely distributed through the tropical warm regions of the world. The origin of the name *Erythrina* comes from the Greek word "erythros" which means red, alluding to the bright red flowers of the trees of the genus [10]. Thirty one *Erythrina* species are known to grow in African. Seven of these species *E. caffra Thunb*, *E. decora Harms*, *E. humeana Sprengel*, E. *stoniana Baker*, *E. lystemon* Hutch, *E. abyssinica* and *E. latissima* are common to East and Southern Africa including Ethiopia, Eritrea, Uganda, Kenya, Tanzania and Zimbabwe [11].

2.1.3. Erythrina abyssinica

E. abyssinica is a deciduous legume that grows in open woodland and grassland. It has characteristic red overflowing flowers [12]. In Ethiopia it is found widely distributed in northern and western parts at elevations between 1600 and 2100 m.

2.2. Ethnobotanical uses of *Erythrina spp*.

Erythrina species are used as healing agent in traditional medicine in Africa [13]. Of thirty one African species, eleven (35%) have ethnomedicinal uses in sub-Saharan Africa traditional medicine against frequent diseases of microbial and parasitic origin [14]. Thirty nine medicinal usages were found for *E. senegalensis* and 60 for *E. abyssinica*. *E. abyssinica* is well known as a medicinal plant for treatment of bacterial, fungal, parasitic and viral diseases, gastrointestinal

disorders, liver disorders, nervous disorders, sexual asthenia, nervous disorder, sterility, eyes diseases and kidney pain etc [15].

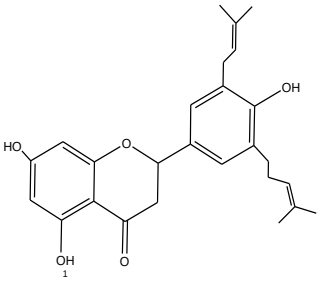
Figure1 Picture of *Erythrina abyssinica* marure tree (A) and inflorescence (B) (by Gizatu Mosisa, take from Jimma town "Bosa Kito Kebele").

The bark is commonly used in traditional medicine, to treat snakebites, malaria, sexually transmittable diseases such as syphilis and gonorrhea, amoebiasis, cough, liver inflammation, stomach-ache, colic and measles. Roasted and powdered bark is used to burns, ulcers and swellings. The liquid from crushed bark of green stems is used to cure conjunctivitis caused by Chlamydia trachoma is (trachoma), whereas bark sap is also drunk as an antihelmintic. The bark is also applied against vomiting. Pounded flowers serve to treat dysentery. A maceration of the flower is drunk as an abortifacient, and applied externally to treat earache. Roots are taken to treat peptic ulcers, epilepsy, malaria, and schistosomiasis. Leaves are taken to treat peptic ulcers; they are also used for treatment of diarrhea. A leaf decoction serves as an emetic. Fruit extracts are taken to treat asthma and meningitis. The plant is also known in ethnoveterinary medicine practices against brucellosis, oedema, hygroma, dropsy, bacterial infections, and skin diseases [16]. Leaves are applied externally to wounds and painful joints; they are also applied to treat skin diseases in cattle. The bark *E. abyssinica* is sometimes used as a source of brown dye for textiles and the sap gives a red color. Cork from the bark is used as floats for fishing nets. The leaves are eaten by sheep and goats. The seeds are locally popular for making curios and necklaces. The flowers provide nectar and pollen for bees at the end of the dry season, strengthening bee colonies in this difficult period [17].In Ethiopian traditional medicinal plant *E. abyssinica* will be investigated and the antibacterial and anti-fungal activity of them will be examined by using disc diffusion method [18].

2.3 Phytochemical constituents of *Erythrina spp.*

Phytochemical analysis carried out on various *Erythrina spp*. demonstrated the presence of terpenes, alkaloids [19], and flavonoids, especially, pterocarpanes, flavanones and isoflavanones [20]. Some of these flavonoids have demonstrated a wide variety of biological activities [21]. The following flavonoids have been isolated from the roots of E. abyssinica; abyssinone (**1**), cristacarpin (**2**), erythrabyssin II (**3**), phaseollidin (**4**) [20]. From the seeds the alkaloids produced are of the *erythrina* type, some of which has been shown to have curare-like activity on the central nervous system. These were erythraline **(5)**, 11-hydroxysodine **(6)**, erysopine **(7)** and erythracine **(8)** [20].

Flavonoids isolated from the roots of *E. variegate* Linn. are; erycristagallin **(9)**, erythrabyssin II, eryvariestyrene, isobavachin **(10)**, phaseollidin, phaseollin and scandenone **(11)**, from the bark alphinumisoflavone **(12)**, erythrinin A, erythrinin C **(13)**, osajin **(14)** and wighteone **(15)** **[**22].From flowers pelargonidin **(16)** and quercetin-rutinoside **(17)** were identified [42]. The alkaloids found in leaves were choline (**18),** coreximine **(19**), erybidine **(20),** were reported [23]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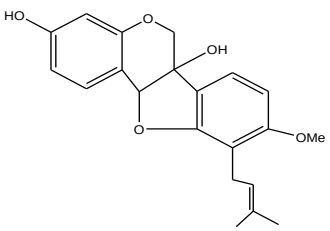
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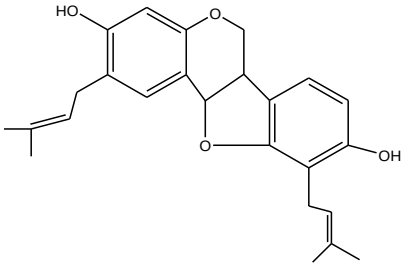
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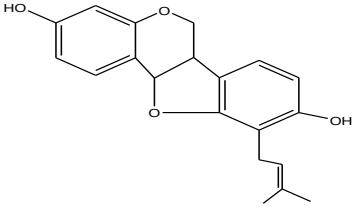
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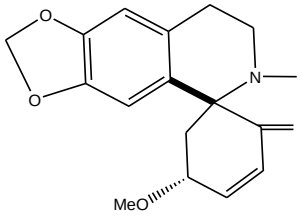
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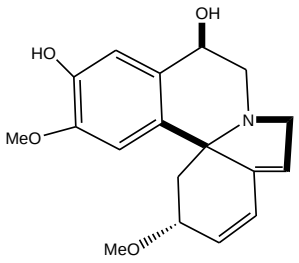
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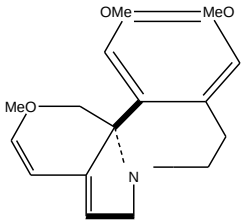
HO
MeO
N
MeO

6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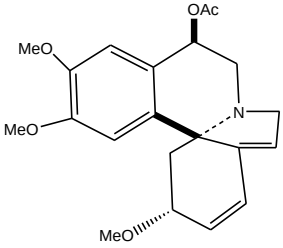
7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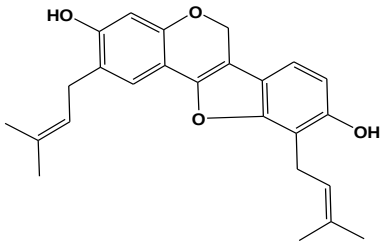

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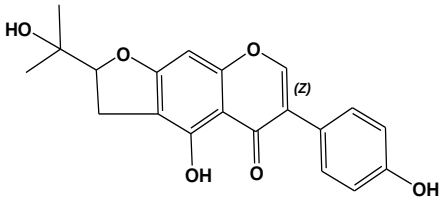

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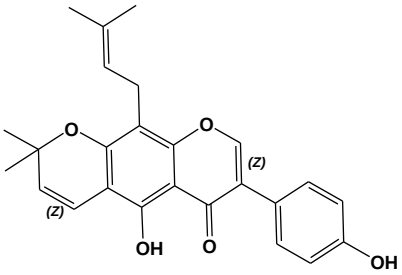

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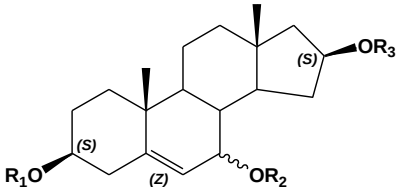

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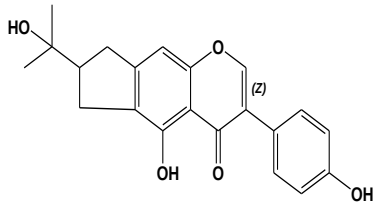

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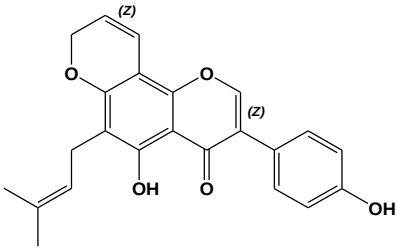

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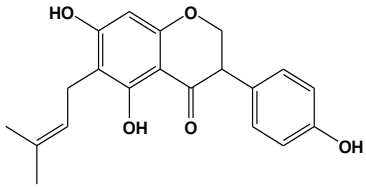

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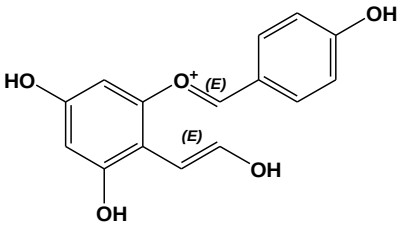

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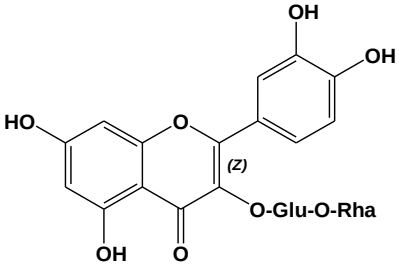

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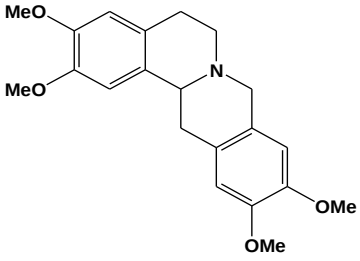

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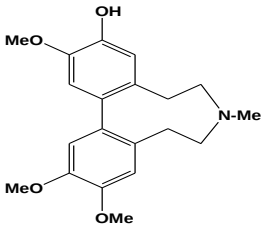


20

$HOCH_2CH_2N^+(CH_3)_3$ (18)

Figure 2 Photochemicals from different parts of *Erythrina* species.

2.4. Scientific studies carried on medicinal uses of *Erythrina spp.*

Based on the common use in folk medicine several phytochemical studies on the stem bark of *E. abyssinica* have been carried out in the last two decades, giving rise to the isolation of different secondary metabolites such as prenylated flavanones, chalcones and pterocarpans, which have been found to possess a wide range of biological activities including anti-plasmodial, inhibition of the protein tyrosine phosphatase 1B [24]. For various reasons, these preceding researches have been focused toward the medium polar constituents of the *E. abyssinica* stem bark, while the knowledge concerning to the most polar secondary metabolites remains limited up to now. In contrast, the stem bark from other *Erythrina* species have been previously studied for their polar constituents, resulting in the isolation of other classes of secondary metabolites such as triterpenoid saponins [25], also found to exert a wide range of pharmacological properties including cytotoxicity [26], and most recently C-glycosyl flavones [27]. Though flavonoids most often are distinguished by their antioxidant, antiallergic and anti-inflammatory activities, some representative cases such as quercetin, catechin, gallocatechin and apigenin have been investigated as potential chemo preventive or chemotherapeutic agents against cancer [28].

3. MATERIAL AND METHODS

3.1.1. Chemicals

Petroleum ether (batch No. 1268), Chloroform (batch No. 455180915119.38), ethyl acetate (batch No.1173), acetone (batch No. 13081), and methanol (batch No. B214771612) and ethanol (batch No. 99026009) were from LOBAL chemie laboratory reagent (Wodebhouse Road, jehangir villa Mumbai-400005, India). Silica gel (60- 120 mesh size, batch No. 42065LR), Muller Hinton Agar Media (MHA), (HiMedia laboratories pvt.Ltd, India), DMSO (Blulux laboratories (p) Ltd-121001, batch No.41784LR), Mancozeb, Gentamicin injection (80 mg/2mL, CSPS, ouyi pharmatucicals Co. Ltd. China, batch No.150711) and nutrient broth (NB) were used.

3.1.2. Equipments and instruments

Mortar and pestle, digital balance, Whatman filter paper (hardened ash less circles 110 mm), Rotary evaporator (LABOROTA4000, Heidolph), melting points (Gallen kamp, Sanyo, West Sussex, UK), TLC plate (pre-coated aluminum sheet, 20 x 20 cm, silica gel 60 F254), UV illuminator, Glass column, NMR spectrometer (Bruker advance 400 MHz), Autoclave, Vortex, Incubator, cotton swab, Petri dish plates, beaker, micro pipette, glove and desiccators.

3.2 Methods

3.2.1. Collection and preparation of plant sample

The stem bark of *E. abyssinica* was collected from Jimma town, Bosa Kito ‘‘kebele’’ during May 2017 E.C and the voucher specimens of the plant was deposited at Jimma University Herbarium, Biology department for future reference in August 2017. The sample was chopped into small pieces and air dried at room temperature under shade. The dried stem barks was milled (pulverized) by use of a manual mortar and pestle in the laboratory.

3.2.2 Extraction plant sample

The air-dried and powdered stem bark (1.1 kg) of *E. abyssinica* was extracted with methanol (2.5 Lx5) for seven days with frequent shaking at room temperature. Thereafter the extract was filtered first with cotton plugged followed by Whatman filter paper to obtain the crude extract solution. The crude extract was concentrated by a rotary evaporator at 65°C. Then concentrated crude extract was allowed to evaporate to constant weight (53 g) at room temperature.

3.2.3 Isolation of compounds

Column chromatography was used to fractionate the extracts. The silica gel was deactivated at 105 ^{o}C for 1 hrs. The column was packed with 300 g silica gel (60-120 mesh size) impregnated with oxalic acid (3%) using petroleum ether. Then 40 g of methanol extract pre adsorbed in 40 g of silica gel was applied to the column and elution was carried using petroleum ether: ethyl acetates 200:0,198:2,196:4,194:6,192;8,190:10,188:12,186:14,184:16,182:18,180:20,178:22,176:24,174:26,172:28, 170:30--- up to 100:100. In each case 200 ml of solvent system was used.

3.2.5. Antimicrobial activity test

3.2.5.1. Antibacterial activity test

The disc diffusion method was used to test the extracts and isolated compounds against the bacteria. Antibacterial sensitivity was confirmed by using standard discs containing 10 mg/mL Gentamicin as positive control and DMSO as anegative control. Nutrient agar (14 g) was dissolved in 0.5 liter of distilled water. This was sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. Nutrient agar (15 mL) was poured into Petri dishes in a lamina flow apparatus under sterile conditions. The test was carried out against four bacterial strains (Staphylococcus *aureus*, *Escherichia coli*, and *Bacillus subtilus).* Then, 0.1 mL of bacterial suspension was added to it. Stock solutions of crude extract and fractions in DMSO at concentration of 50 mg/mL & 20 mg/mL respectively were prepared. Then 6 mm filter paper discs were soaked in each stock solution and put onto Petri dishes seeded with test organisms. Dishes were finally incubated at 37ºC. The results were obtained after 24 hrs of incubation by measuring the diameter of the zone of inhibition [29].

3.2.5.2. Antifungal activity test

Four fungal strains i.e. *Trichophyton spp, Fusariun spp. Aspergillus spp.* and *Penicillium spp*. were used for antifungal activities. Standard antifungal agent Mancozeb (10 mg/mL) was used as positive control [30]. A disc diffusion method was used to test the plant extracts. Potato dextrose agar (PDA) autoclaved for 15 min at 121ºC temperature and 15 psi pressure poured into the Petri dishes. The antifungal solutions were poured into the: containing the PDA. 0.1 mL of the plant extracts solution; of dimethyl sulphoxide (DMSO) reference antifungal drugs and distilled water were added into the 6 mm discs and put on the top of PDA. DMSO solvent was used as a negative control for each Petri dish. The Petri dishes were covered and incubated at 37 ºC for 72 hrs. The

results were taken on the third and fourth day by measuring the diameter of zone of inhibition [31].

3.2.5.3. Termiticidal activity test

Termite *C. formosanus* were collected in Jimma University, Jimma Campus, in 2009 [32].

These termites were brought to the laboratory and kept in covered, 140-L Glass Petri dishes with filter paper until they were extracted for experiments.

The crude extracts of 5 % (0.050 g/mL), 2.5 % (0.025 g/mL), 1.25 % (0.0125 g/mL 0.625% (0.0625 g/mL) and 0.3125 % (0.03125 g/mL) were prepared by dissolving 5 mg samples in 1 mL of acetone serial dilution. Acetone treated filter paper was used as a negative control and 5% malathoin treated filter paper was served as positive control. Then 2 mL of each concentration of the solutions prepared were 2 mL applied to each filter paper with a 9.0 cm in diameter of filter paper samples (1.5 mm thickness) and dried in dry air for 1h. Then each of the tests contained 2 replicates. Then the 20 active termites (15 workers and 5 soldiers) were introduced onto each Petri dish (9 cm diameter and 1.6 cm height). Distilled water was added sterile cotton put on bottom of each Petri dish. All the Petri dishes with covers were placed the cap board and lastly the mortality of the termites was counted and recorded every 30 min for 72 hrs. The dishes in which all the termites died were evaluated every 30 minutes. The termites were tested for 24 hrs. Finally, we calculated the termite mortality (percent calculated). The termiticidal effect of each extractive was compared. The following equation was employed to calculate the termite mortality.

$$\text{Termite Mortality (\%)} = \frac{\text{Number of peti dishes termites all Died}}{\text{Total Number of petridishes}} \text{x}100\%$$

3.2.6. Phytochemical tests

The phytochemical screening tests were carried out on crude methanol extracts employing standard procedures described in [33-36].

I) Test for Alkaloids (Wagner's reagent)

0.2 g of the alcoholic extract was heated on a boiling water bath with 2N HCl (5 mL).After cooling, the mixture was filtered and the filtrate was divided in to two equal portions. One portion was treated with few drops of Mayer's reagent and the other with equal amounts of Dragende off's .Turbidity of the resulting precipitate in the both reagents was taken as evidence for the presence of alkaloids [33].

II) Flavonoids (Alkaline reagent test)

A crude extracts of 0.2 g was taken and dissolved in diluted NaOH and 1M of HCl was (5 mL each) added. A yellow solution that turns to colorless was taken as the indication for the presence of flavonoids [33].

III) Saponins (Foam test)

About 0.2 g of extracts was shaken in test tubes with 5ml of distilled and heated on water bath to boiled. Formation of strong and stable foam (1.7cm height) was taken as indication for the presence of Saponins [34].

III) Tannins (Braymer's test)

A 0.2 g of crude extract were mixed with10 mL of distilled water and heated on water bath. The mixtures were filtered and to each filtrate 5 % (w /v) solution of ferric chloride was added and the formation of dark green solution was taken as an indication for the presence of tannins [35].

V) Test for Quinones

A 0.2 g of crude extract was mixed with concentrated HCl and observed for the formation of yellow precipitate (or coloration) [36].

VI) Test for terpenoids

0.2 g of methanol extract was mixed separately with 2 mL of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 mL) and then added carefully, to form a layer. A formation of reddish brown coloration of the solution at inert face was taken as the presence of terpenes [33]**.**

4.0 RESULT AND DISCUSSION

4.1 Extraction and phytochemical screening test results

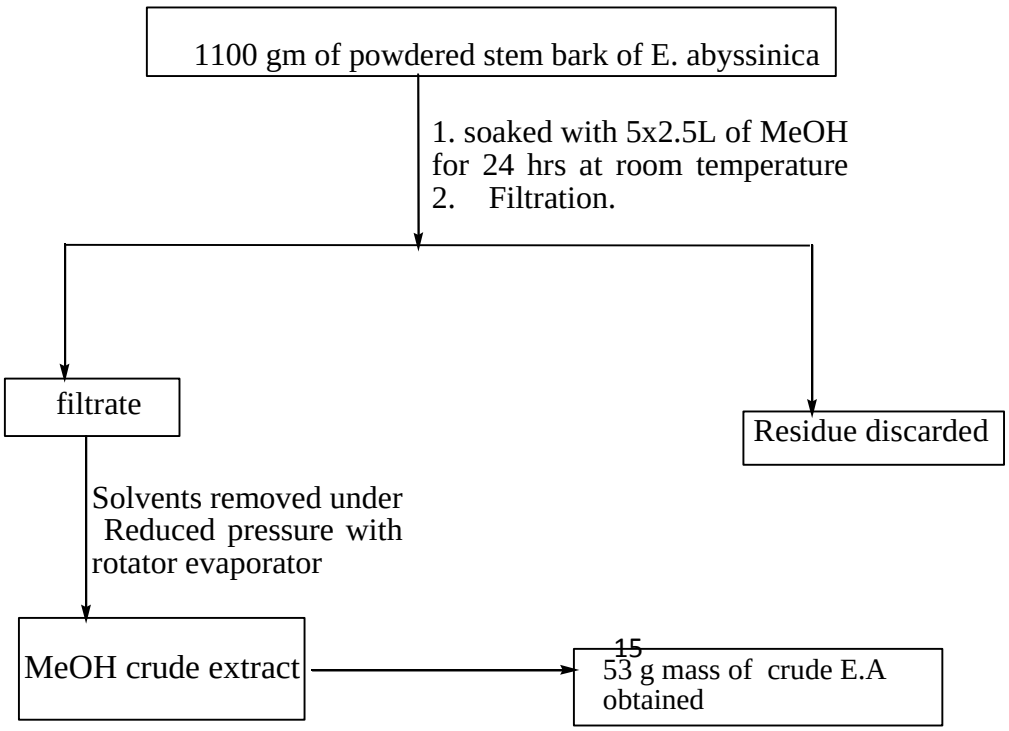
Extraction of 1.1 kg of powdered bark of *Erythrina abysisnica* gave 53 g (4.82 %) of dirty yellow powder up on complete removal of solvent. The phytochemical screening tests carried on crude methanol extract confirmed presence of alkaloids, saponins, terpenoids, flavonoids and absence of tannins and quinones (Table 1).

Table 1**: Phytochemical test data on crude methanol extract of *E. abyssinica* stem bark**

Phytochemical classes tested	Test results	Observed colors
Alkaloids	+	Reddish brown color
Flavonoids	+	Yellow color
Saponin	+	Persistent foam
Quinones	-	-
Terpenoids	+	Reddish brown color
Tannin	-	-

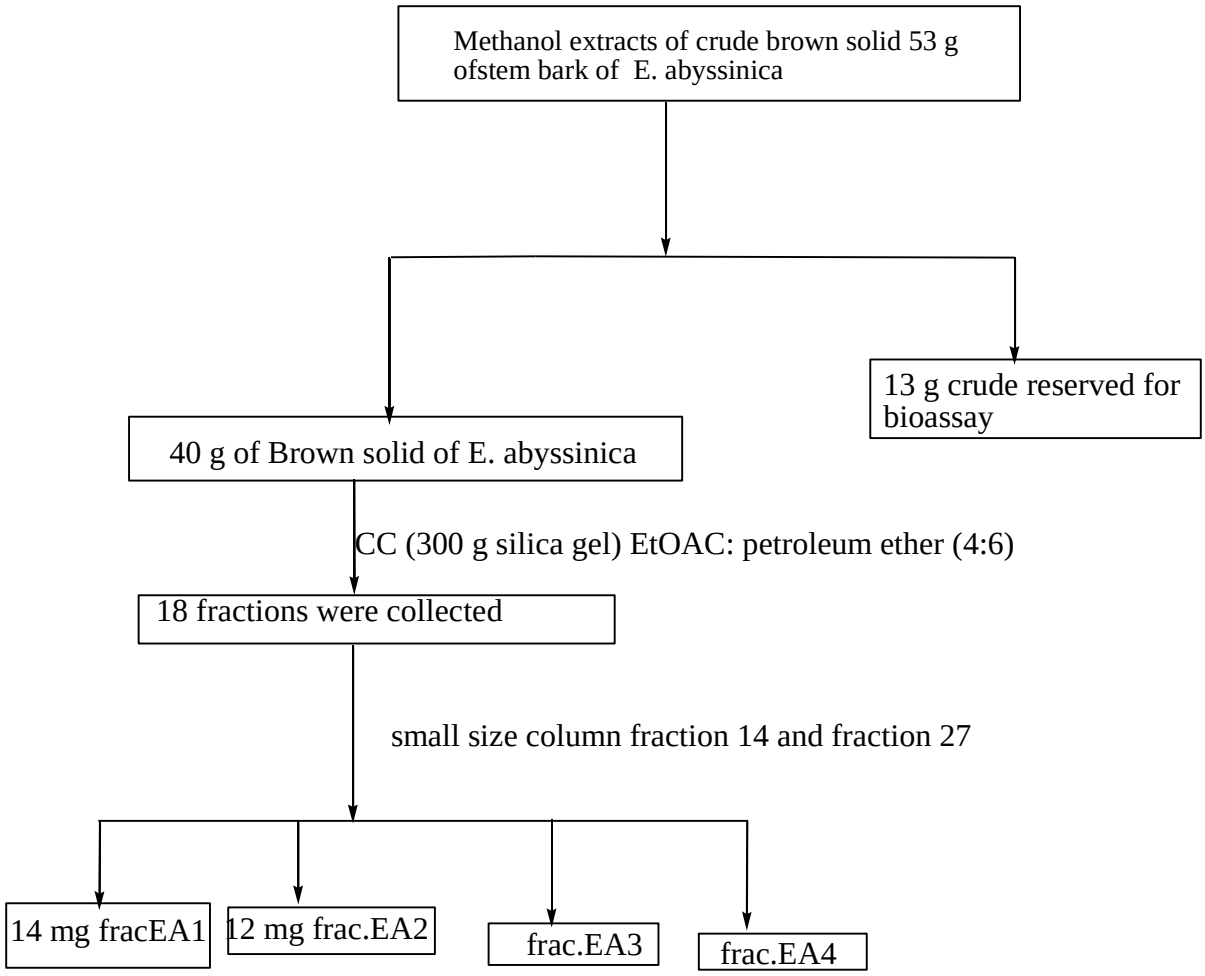
Keys + = presents - = absents

Figure 3.Flow chart of extraction of stem barks *E. abyssinica*.



4.2 Isolation of pure compound

Figure 4. Flow chart of isolation of *E. abyssinica* components



4.3. Characterization of the isolated compounds

Structural elucidation of isolated compounds was performed by comparing physical (melting point) and the spectroscopic (^{1}HNMR, ^{13}CNMR, and DEPT-135) data obtained in this study with those reported in literature.

4.3.1 Characterization Compound E.A1

Compound E.A1 was obtained as yellow crystals; melting point 116-119°C. It was analyzed by 1H NMR spectrum (400 MHz) using acetone-d6 as solvent and gave ten signals. The signal was identified as a hydroxyl proton singlet stabilized with the carbonyl group and resonating at a chemical shift δ 12.20 (1H, s, H-1). The signal integrating into two protons was identified as an aromatic proton doublet at a chemical shift δ 7.70 (2H, H-2′, H-6′) which showed significant correlation from spectrum with (H-3′, H-5′). The chemical shift of δ 6.98 (2H, d, H-3′, H-5′), the signal integrating into two protons was aromatic proton doublet. The chemical shift at δ 6.66 (1H, d, H4′′) was a vinylic proton doublet resonating. The chemical shift at5.47 (1H, H-3′′) was a proton doublet. The chemical shift at δ5.36 (1H, dd, H-2) was a proton double doublet. The chemical shift at δ 5.24 (1H, t, H-2′′′) was a proton triplet. The chemical shift at δ 3.70 (2H, H-1′′′), was doublet .The chemical shift at3.05 (1H, dd, H-3ax) was a double of doublet. The chemical shift at δ 2.82 (1H, dd, H-3eq), doublet of doublet. The chemical shift at δ 1.65 (6H, s, H-4′′′/ H-5′′′) was identified as a proton singlet. The chemical shift at δ 1.45 (6H, s, 2xCH_3) singlet,[37].

The ^{13}C NMR spectrum showed 21 carbon signals. The hydroxylated methine carbon was identified as δ 78.5 (C-2) and a methylene carbon resonating at δ 43.3 (C-3). The highly deshielded quarternary carbon was identified as a carbonyl carbon at δ 196.4 (C-4). The quartenary aromatic carbons were identified as, δ 102.6 (C-4a), δ 156.5 (C-5), δ 102.8 (C-6), δ 160.0 (C-7), δ 108.5 (C-8) and δ 159.5 (C-8a). The other aromatic carbon atoms were identified as δ 131.1 (C-1′), δ 127.7 (C-2′/6′) and δ 115.6 (C-3′/5′). The prenyl ring showed shifts at δ 80.0 (C-2′′), δ 126.0 (C-3′′), δ 115.6 (C-4′′). The hydroxylated olefinic group attached to benzene showed chemical shifts at δ 21.5 (C-1′′′), δ 122.5 (C-2′′′), δ 25.8 (C-4′′′) and δ 17.8 (C-5′′′). Most chemical shifts were in close agreement with those of lupinifolin **[37]** (Table 2).

Table 2: ^{1}H NMR and ^{13}C NMR chemical shifts of compound 1 and Reported data [37]

C	Position	Compound EA1 Spectral data		Reported value	
		δ13C NMR	δ H value	δ13C NMR	δH value
1	2	196.2		196.5 s	
2	3	164.0		160.0 s	
4	4	159.0		159.3 s	
5	4a	156.1		156.5 s	
6	5	155.0		155.9 s	
7	6	131.8		131.1 s	
8	7	130.0		130.9 s	
9	8	127.7	7.7 d	127.7 d	7.3 d
10	8a	125.8	5.4 d	125.9 d	5.4 d
11	1’	122.4	5.2	122.4 d	5.1 t
12	2’/6’	115.2	6.6 s	115.6 d	6.6 d
13	3’/5’	109.8		108.6 s	
14	4’	102.2		102.6 s	
15	1’’	79.4	5.4 dd	78.5 d	5.3 dd
16	2’’	78.1		78.1 s	
18	3α	43.3	3.1 dd	43.1 t	3.0 dd
19	3β	43.3	2.7 dd	43.1 t	2.8 dd
20	5’’	28.8	1.5 s	28.4 q	1.4 s
21’	6’’	28.1	1.4 s	28.3 q	1.4 s
22	4’’’	25.0	12.2 s	25.8 q	12.2 s
23	1’’’	22.0	3.8 (2H,d)	21.4 t	3.2 (2H,d)
24	5’’’	17.0	1.7 s	17.8 s	1.6 s
	5-OH			12.2 s	12.2 s

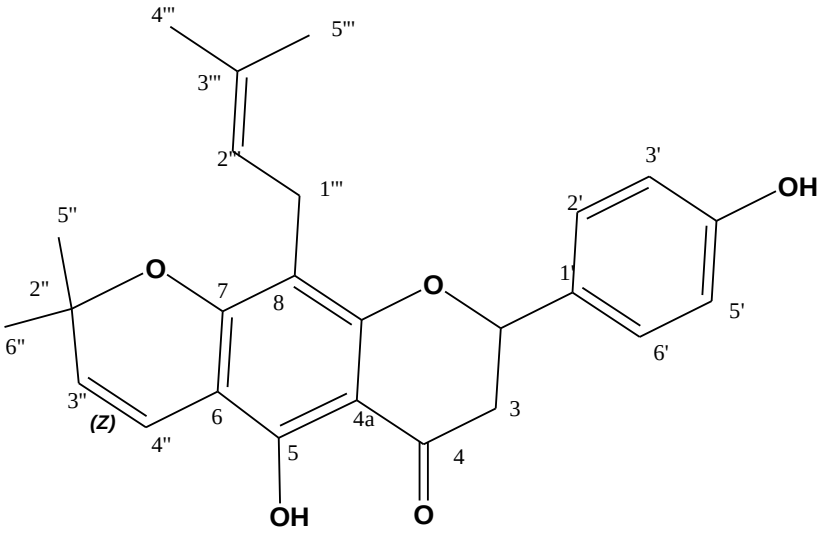


Figure 5. Proposed structure of compound 1 (**Lupinifolin,** $C_{25}H_{26}O_5$)

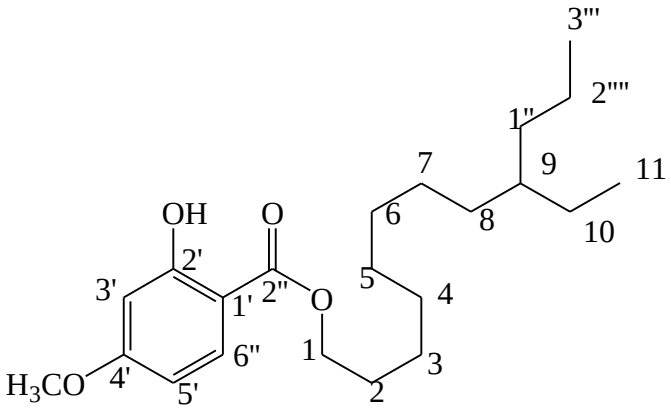
4.3.2 Characterization **Compound 2**

The compound 2 was identified to be deep yellow crystalline compounds, mp 80-83 °C, ^{1}HNMR the signals at δ ppm 7.70 – 7.75 (1H, *dd*), δ ppm 7.57 (1H, dd) and δppm 7.2 to 7.3 (1H, m) are peaks in the aromatic region which is due to aromatic protons. The signal at δ ppm 3.9 (2H, t) for CH_2 proton which one of the substituent on the carbon is oxygen. The signals 1.3 to 3.5 may be due to the presence of CH_2 protons and the signals in the range from 0.8 to 1.2 are stands for methyl protons. Proton decoupled ^{13}C spectrum showed well resolved resonances of 22 carbon atoms with certain impurities. The multiplicity of each carbon atom was determined by using DEPT spectrum which showed the presence of three methyl, eleven methylene, five methine, and four quaternary carbons atoms clearly. This indicates the presence of 36 hydrogen atoms in the molecule. The signals at δppm 166.0 of ^{13}CNMR spectra are characteristics of carbonyl carbon of ester. Compounds containing aromatic rings give rise to chemical shifts in the range 100 ppm to 175ppm. A mono substituted benzene ring shows four peaks in the aromatic carbon area of ^{13}CNMR spectrum; because of an equivalent 13C atoms appear at the same chemical shift value (the ortho and Meta carbons are doubled by symmetry). The signal at δ ppm 132, 101.9 and 106.9 indicates the presence of mono substituted benzene.

The ^{13}CNMR spectrum revealed that the signal at δ ppm 166.4, 162.8 and 107.6 are due to quaternary carbons which do not appear in the DEPT spectrum since it has no attached hydrogen (9-ethyldodecyl-4-methoxybenzoate**) [38].**

Table 3.The 1HNMR and ^{13}CNMR spectral compound EA2 with reported value [38].

No.	1HNMR OF EA2	1HNMRδ-Shift of Reported value	Position of C.	δ of EA2spectra	δ of reported value	Node	Comment (ppm rel. to TMS)
1	6.4	6.4	C-6’	164.4	167.0	CH	1-benzene
2	6.5	6.4	C-5	128.6	129.9	CH	1-benzene
3	7.7	7.7	C-1’	110.8	111.0	CH	1-benzene
4	4.2	4.2	C-3’	102.7	102.8	CH2	Methylene
5	6.4	6.5	C-2’’’	94.97	95.4	CH2	Methylene
6	5.3	5.2	C-1	59.7	58.2	CH2	Methylene
7	3.3	3.4	C-9	54.5	55.2	CH2	Methylene
8	3.1	3.2	C-1’’’	43.5	43.0	CH2	Methylene
9	2.6	2.5	C-1’’	31.8	32.0	CH2	Methylene
10	2.1	2.2	C-8	29.5	28.5	CH2	Methylene
11	1.9	1.9	C-6	29.5	29.3	CH2	Methylene
12	1.7	1.7	C-5	29.4	29.4	CH	Methine
13	1.6	1.5	C-4	29.2	29.5	CH2	Methylene
14	1.2	1.2	C-2	28.9	29.4	CH2	Methylene
15	1.2	1.3	C-10	28.7	28.4	CH2	Methylene
16	0.9	0.9	C-7	28.4	28.7	CH3	Methyl
17	0.8	0.9	C-3	25.0	25.0	CH3	Methyl
18	5.0	5.0	C-2’’’’	22.5	22.8	OH	aromaticCOH
19	3.7	3.7	C-3’’’	17.0	18.4	CH3	OCH_3
			C-11	13..6	13.2	CH3	Methyl
			OMe-4’	55.9	-	OCH3	Methoxy



9-ethyldodecyl 2-hydroxy-4-methoxybenzoate

Figure 6 Proposed structure of compound 2

4.4. Bioassay data

4.4.1 Antibacterial test

The crude extract of *E. abyssinica* and two of the isolated compounds E.A1 and E.A2 were tested against three bacterial strains; *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilus.* The crude extract was not shown any activity against all test strains but E.A1 *and* EA2 were active each with zone of inhibition 9.0 mm only against *Bacillus subtilus & E. coli* respectively (Table 4).

Table 4: Antibacterial activities of extracts and compounds isolated from *Erythrina abyssinica* stem bark.

Test sample type	Conc. mg/ml	Inhibition zone diameters measured in millimeters		
		S. aureus	*E. coli*	*B. subtilus*
EA1	20.0	NA	NA	9.0
EA2	20.0	NA	9.0	NA
Crude EA	50.0	NA	NA	NA
DMSO		NA	NA	NA
Gentamicin	10.0	19.0	19.0	18.0

Key: EA1= isolated compound 1 *E. abyssinica*, EA2= isolated compound 2 *E. abyssinica* NA= Not active

4.4.2. Antifungal test

The methanol crude methanol extracts of *E. abyssinica* and two compounds (E.A 1 & E.A 2) isolated from it were subjected to antifungal activities against four fungal strains; *Penicillium spp, Fusariun spp. Trichophyton spp* & *Aspergillus spp* using Mancozeb as positive control. Results are summarized on table 5 below.

Table 5**:** Antifungal activitys of crude extracts and compounds isolated from *Erythrina abyssinica* stem bark.

Test sample type	Conc.mg/ ml	Inhibition zone diameters measured in millimeter			
		Aspergillus spp.	Fusarium spp.	Trichophyton spp.	Penicillium spp.
*E.A*1	20.0	NA	16.0	12.0	10.0
*E.A*2	20.0	10.0	18	10.0	11.0
E.A-crude	50.0	NA	12	11	9
DMSO		NA	NA	NA	NA
Mancozeb	10.0	12.0	25.0	20.0	20.0

Key: EA1= isolated compound 1 *E. abyssinica*, EA2= isolated compound 2 *E. abyssinica* NA= Not active

Results display that both the extract and isolated compounds *(E.A1* and *E.A2)* were active (with zone of inhibition 10-18 mm) against all the tested stains except *Aspergillus spp.* Activities recorded were highest on *Fusariun spp.* and lowest on *Aspergillus spp* which was responsive only to EA 2 & Mancozeb. Our results also supplement earlier reports on resistance of *Aspergillus spp*

4.4.3. Termiticidal test

Termiticidal activity of methanol extracts of *E. abyssinica* was assessed against Formosan subterranean termites (*C. formosans*) utilizing Maltathion (5%) as appositive control. Results are summarized on table 6 below.

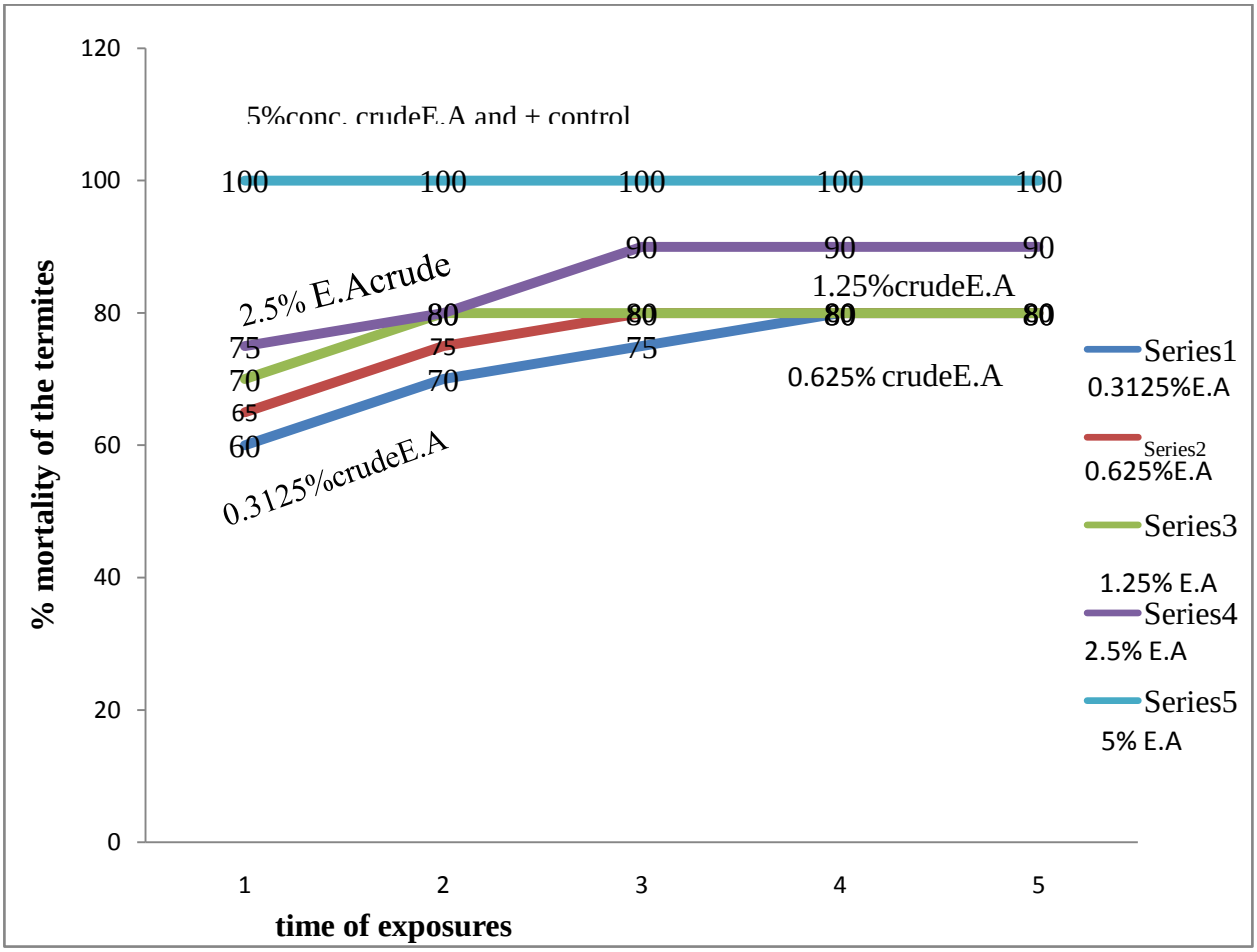
Table 6: Effect of the stem bark methanol extract of *E. abyssinica* on termites (*C. formosans*) assessed at different time using no-choice assay.

Test sample type	**Termite mortality recorded at time**											
	30'		**1 hr**		**2 hr**		**3hr**		**4r**		**5hr**	
	W	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**	**W**	**S**
E.A-crude (0.3125 %)	10	2	11	3	12	3	13	3	13	3	13	3
E.A- crude (0.625 %)	11	2	12	3	13	3	13	3	13	3	13	3
E.A- crude (1.25 %)	12	2	13	3	13	3	13	3	13	3	14	4
E.A- crude (2.50 %)	13	2	13	3	14	4	14	4	14	4	15	4
E. A-crude (5%)	15	5	15	5	15	5	15	5	15	5	15	5
Malathion (5%)	15	5	15	4	15	5	15	5	15	5	15	5

Key w = worker termites and S = solder termites.

Results display that killing effects caused by the extracts are both time and concentration dependent. The highest killing effect (100 %) was observed for 5% solution of the extract at 30 minutes which was also the same for 5% Malathion. Other test samples produced < 100 % killing effects even after 120 hrs.

Figure: 7 Plot of % mortality of termites Vs time with d/t concentration of crude extracted stem bark of *E. Abyssinica*.



5. Conclusion and recommendation

5.1. Conclusion

The bark of *Erythrina abysinica* was collected from Jimma town and dried powdered sample was extracted with methanol using maceration technique. Phytochemical analysis crude extract was carried using both chemical test and column chromatography. Purified compounds isolated were characterized using physical (M. pt) and spectral (1H NMR, ^{13}C NMR, and DEPT) data. The Crude extract and purified compounds were subjected to tests on selected strains of bacteria (*S. aureus*, *E. coli*, and *B. subtilus)* and fungi (*Aspargillus spp.*, *Fusariun spp.* , *penicillium spp & Trichophyton spp)* using disk diffusion method. Termiticidal activity was also carried on *C. formosans* employing No-Choice assay method. The phytochemical screening tests carried on crude methanol extract obtained (53 g, 4.82 %) confirmed presence of alkaloids, saponins, terpenoids and flavonoids only. Column chromatographic analysis yielded two pure compounds E.A 1 & E.A 2 which was proposed to be lupinifolin and 9-ethyldodecyl-4-methoxybenzoate respectively by comparison of physical spectral data gained in this study with those reported in literatures. The crude extract was not active against all bacterial trains tested but E.A1 *and* E.A2 were active each with zone of inhibition 9.0 mm against *Bacillus subtilus & E. coli*. The crude extract and isolated compounds *(E.A1* and *E.A2)* were active against all fungal stains tested with zone of inhibition 10-18 mm except *Aspergillus spp.* Activities recorded were highest on *Fusariun spp.* and lowest on *Aspergillus spp. Aspergillus spp* was responsive only to EA 2 & Mancozeb. Killing effects caused by the extracts on termites were both time and concentration dependent. The highest killing effect (100 %) was observed for 5% crude extract solution at 30 minutes which was also the same for 5% Malathion. Other test samples produced < 100 % killing effects even after 5 days. Most results attained in this study were in agreement with earlier reports.

5.2. Recommendations

The active constituents of the plants should be isolated, identified and characterized. These should then be tested on the decomposers of microorganisms. The termiticidal and antifungal activities observed here justify decay resistant nature of the *Erythrina abyssinica* stem bark. Isolation of more phytoconstituent and detailed studies on organisms involved in decay process are recommended.

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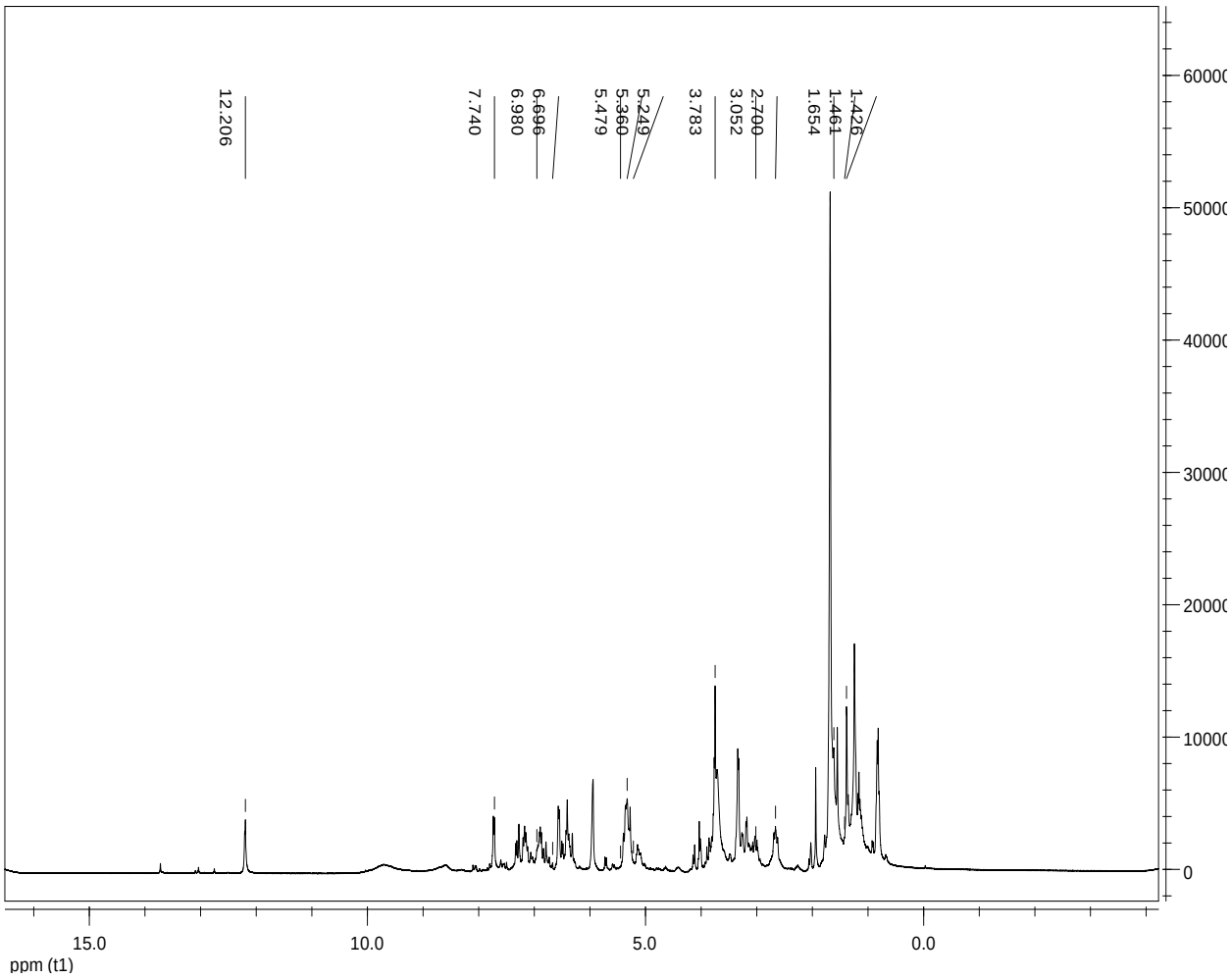
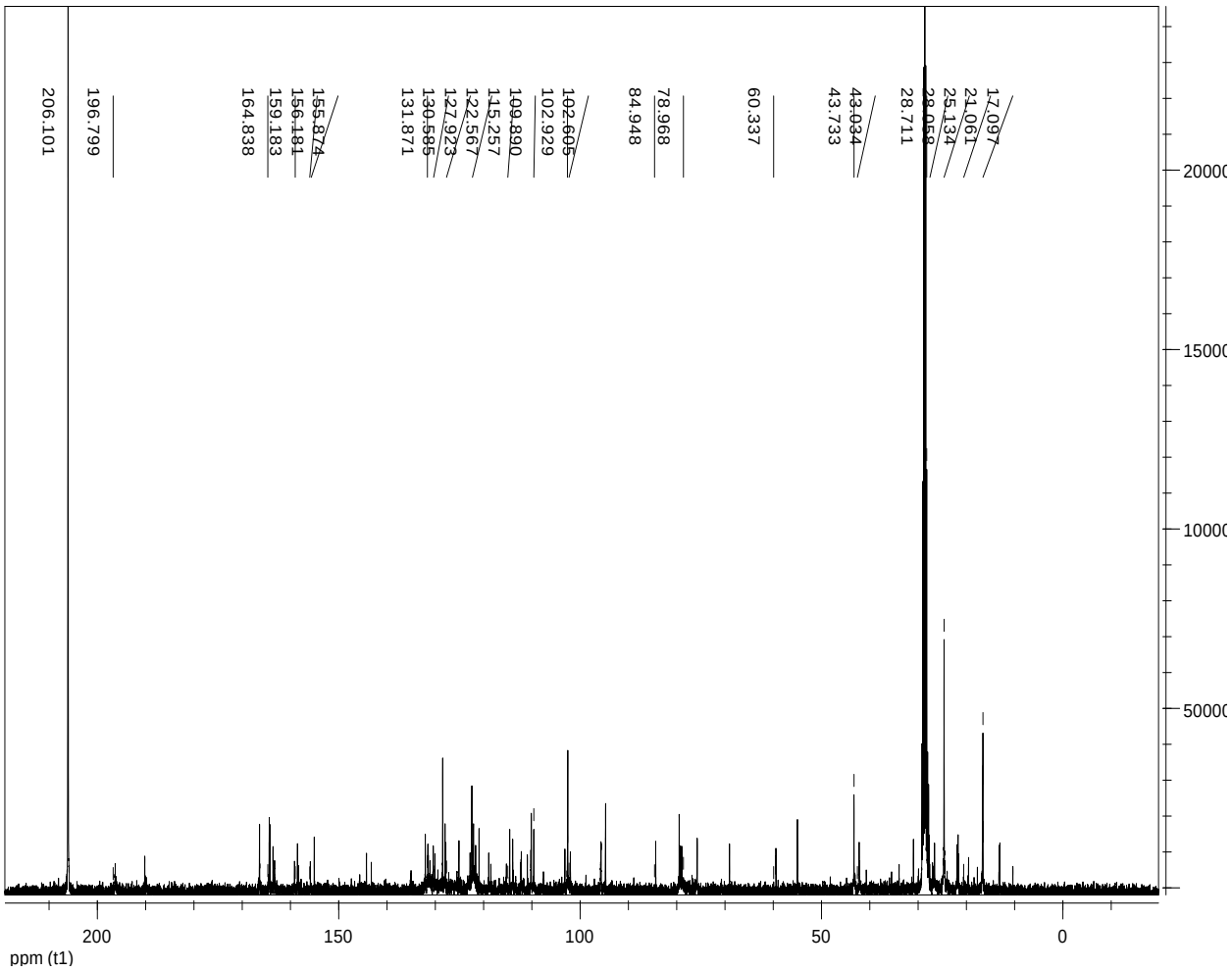
1. Appendices

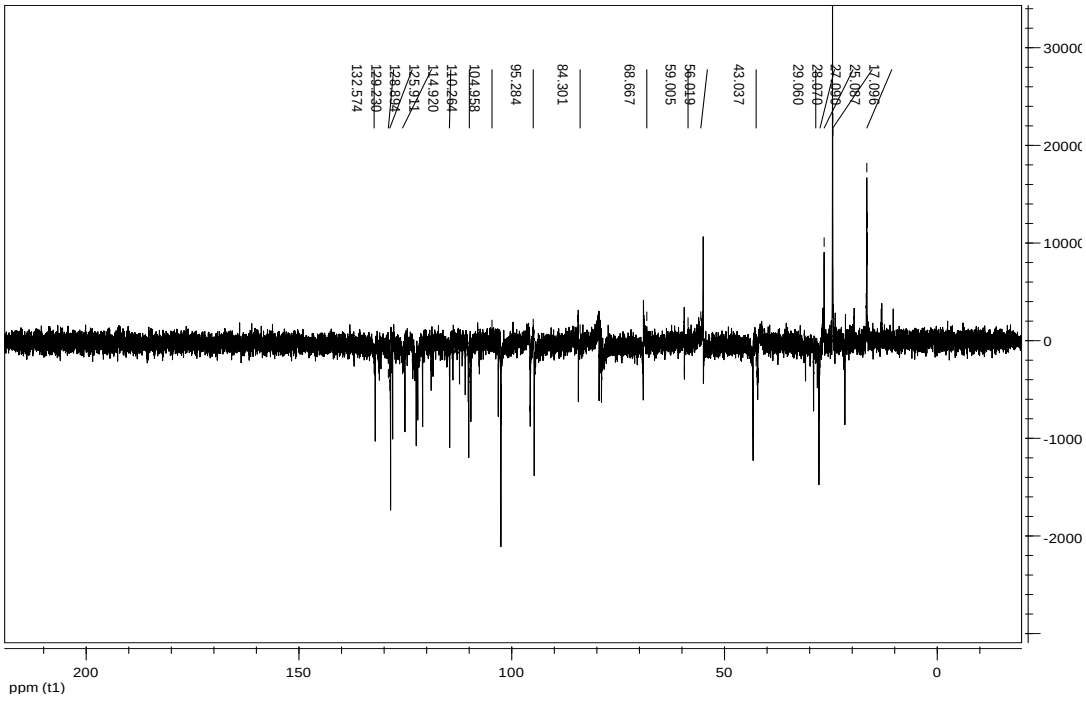
1.1.Appendix: 1^{1}HNMR Spectra of compound1



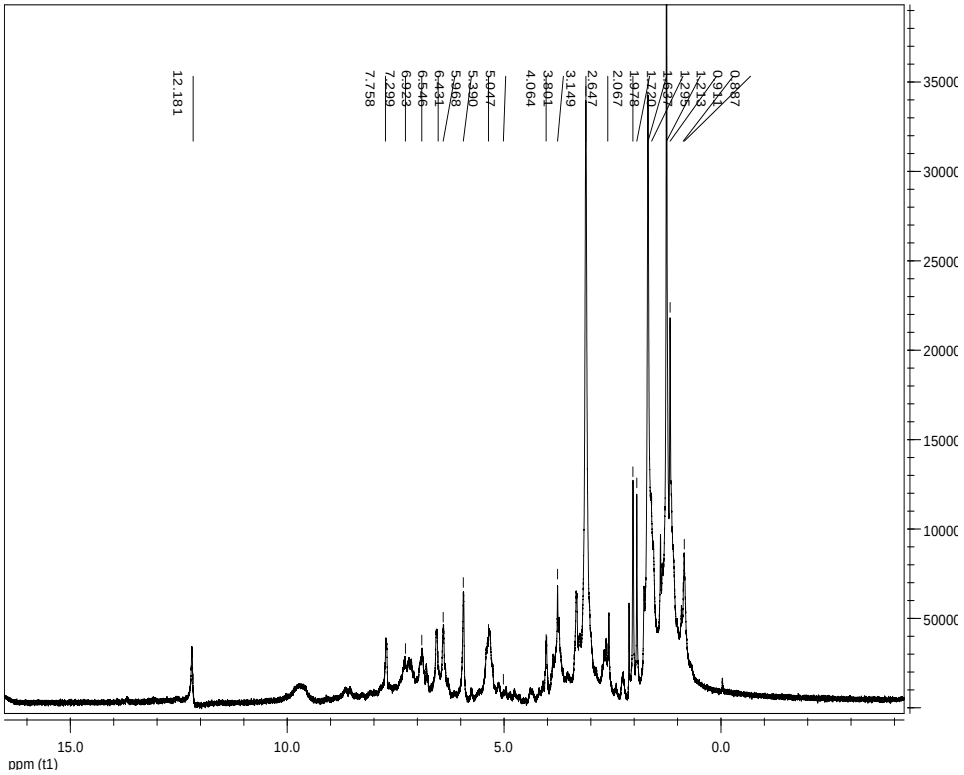
1.2.Appendix: 2 ^{13}CNMR spectra of compound EA1



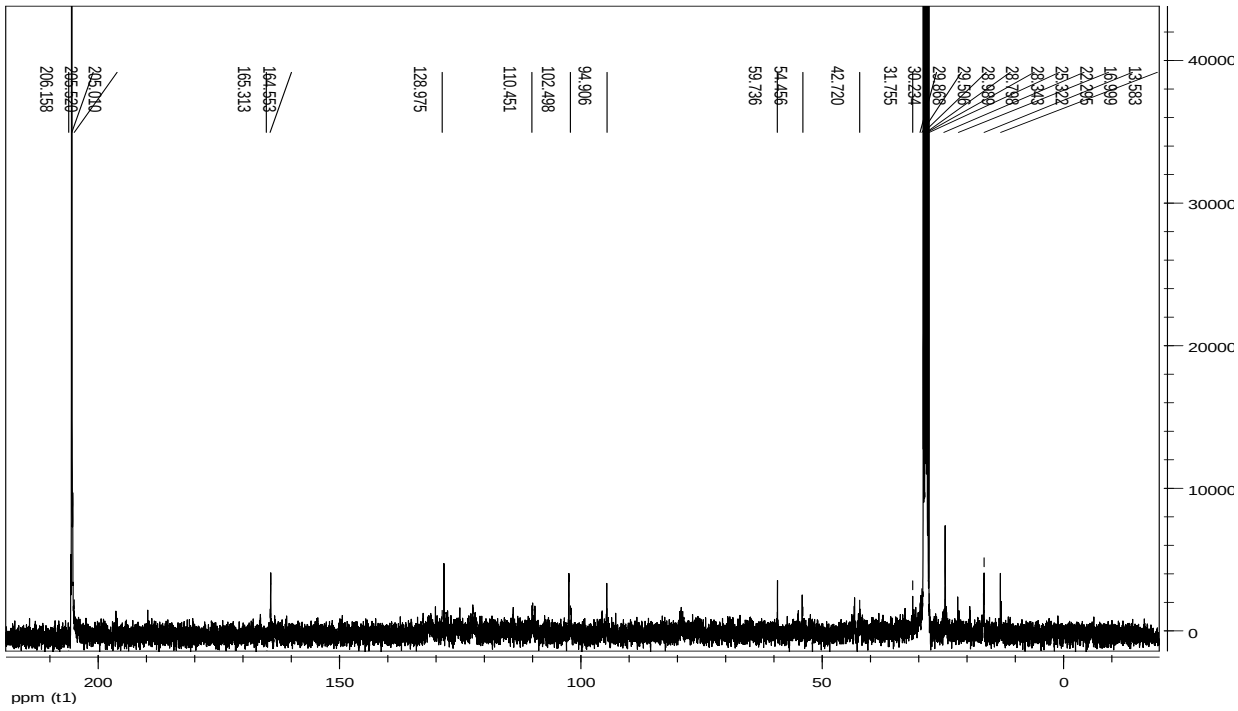
1.3.Appendix: 3**.**DEPT-135 spectra data of compound EA1



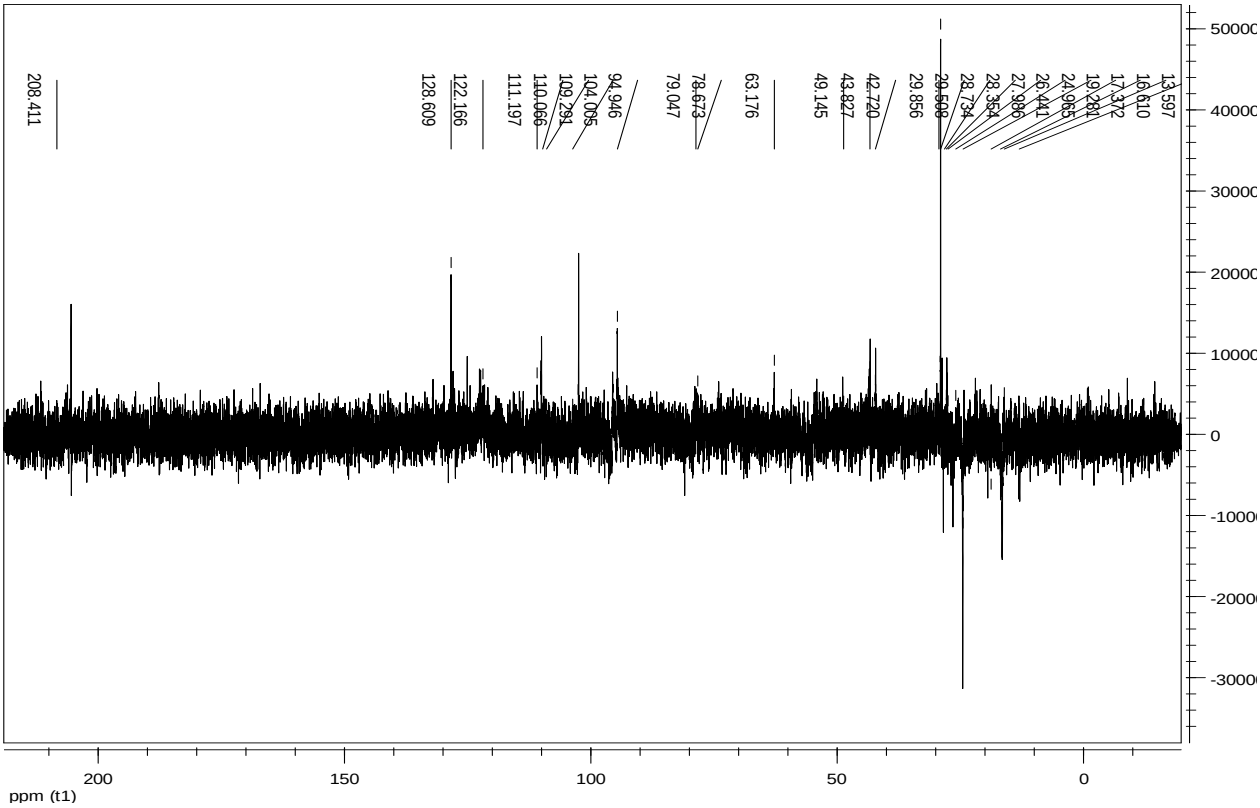
1.4. Appendix: 4. ^{1}HNMR spectra data of compound EA2



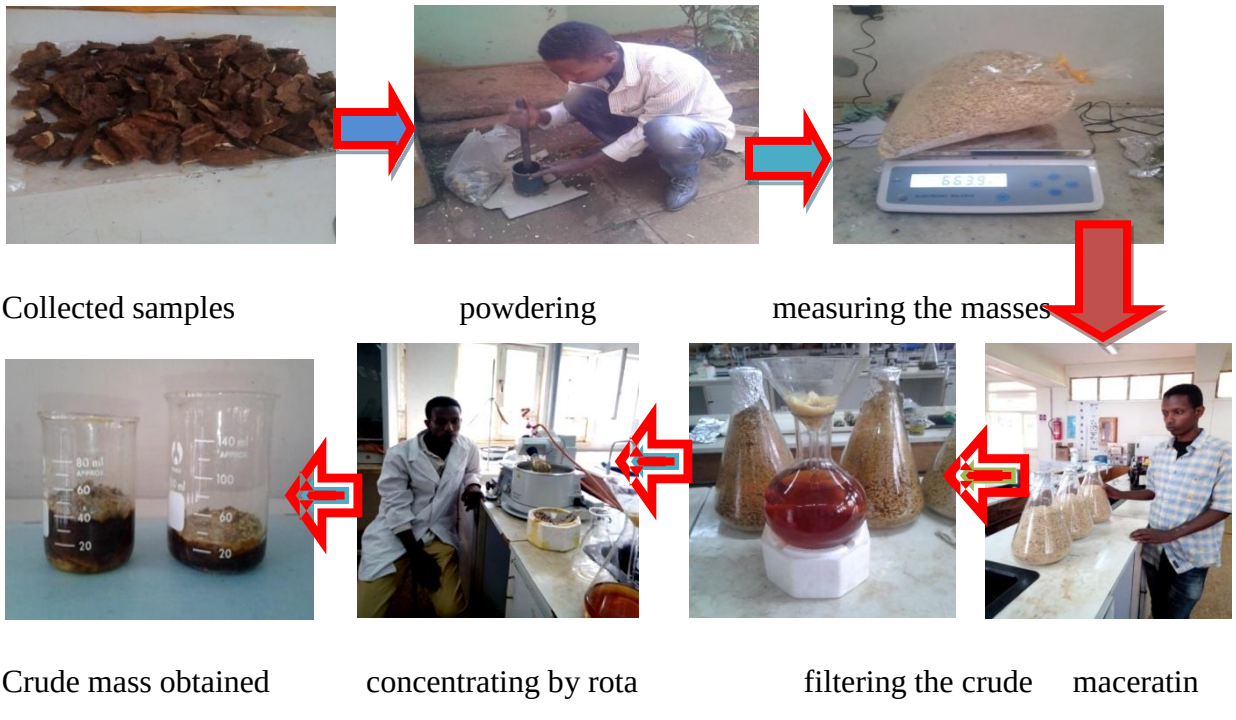
1.5. Appendix:5**.** ^{13}CNMR of compound EA2



1.6. Appendix: 6**.** DEPT-135 spectral data of compound E.A2



1.7. Appendix: 7 “Pictures taken during major steps in this study”.



1.8.Appendix 8 Pictures collections for bioassay test results obtained in this study”