Phytochemical, Antibacterial and Antioxidant Studies of the Roots Extracts of *Bersama abyssinica* Fresen

Adama Science and Technology University

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By

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Thesis Submitted to Office of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Chemistry (Organic Chemistry)

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Adama

SUPERVISORS'/ADVISORS' APPROVAL SHEET

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Subject: Thesis Submission

This is to certify that the thesis entitled "**Phytochemical, Antimicrobial and Antioxidant Study of the Roots Extracts of *Bersama abyssinica* Fresen sub species *abyssinica"*** is submitted to the Department of Applied Chemistry in partial fulfillment of the requirement for the degree of Master's in science in Applied Chemistry and it has been carried out by Emebet Getaneh , under our supervision. Therefore, we recommended that the student has fulfilled the requirements and hence she can submit the thesis to the Department for evaluation

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DECLARATION

I, the undersigned, hereby declare that this M.Sc Thesis is my original work and has not been presented for a degree in any other University, and all sources of material used for this thesis have been duly acknowledged.

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This is to certify that we have read and evaluated this Thesis prepared, under our guidance by Emebet Getaneh , entitled: **Phytochemical, Antibacterial and Antioxidant Studies of the Roots of *Bersama abyssinica* Fresen .** We recommend that the thesis fulfils the requirement for the Degree of Masters of Science in Organic Chemistry and complies with the regulations of the university and meets the accepted standards with respect to the originality and quality and signed by the examining committee.

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LIST OF ABBRIVIATIONS AND ACRONMYS

^{13}C-NMR	Carbon Thirteen Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
DPPH	2, 2'-DiPhenyl Picryl Hydrazyl
GC-MS	Gas Chromatography-Mass Spectrometry
IR	Infrared
NMR	Nuclear Magnetic Resonance
PTLC	Preparative Thin Layer Chromatography
TLC	Thin Layer Chromatography
CC	Column Chromatography
UV	Ultra Violet
WHO	World Health Organization
STD	Sexually Transmitted Diseases

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ABSTRACT

Medicinal plants contain a wide range of secondary metabolites that can be used to treat chronic illness as well as infectious diseases. Bersama abyssinica is selected for this study because of its uses in traditional medicine as an antimicrobial agent. The bark, root, and leaf decoctions are taken to treat a range of stomach disorders such as abdominal pain, colic, diarrhoea, intestinal worms and amoebiasis. A stem bark decoction is drunk to cure cancer and rheumatism. The overall objective of this study was to isolate and characterize antioxidant and antibacterial compounds from Bersama abyssinica. The roots were extracted successively with n-hexane, chloroform, ethyl acetate and methanol followed by separation using silica gel column chromatography which afforded two compounds ABS-EM and ABS 120. ABS-EM and ABS-120 were identified as isomer of betunilic acid and phthalate, respectively. The structures of the compounds were elucidated by using spectroscopic analysis (IR and 1D NMR). Furthermore, the extracts were assessed for its antibacterial activities using disk diffusion method (gram positive (S.aureus, B. subtilis) and gram negative (E. coli, P. mirabilis, P. pneumonia). Results revealed that the two compounds isolated in the present work showed pronounceable against S. aureus and B. subtilis for isomer of betunilic acid and S. aureus, E.coli for phtalate. B. abyssinica roots of methanol extract had high antioxidant activities comparable with ascorbic acid.

Key words: Bersama abyssinica , phytochemical, antibacterial, antioxidant, betunilic acid, phthalate

1. INTRODUCTION

1.1. Background

Humans have used them throughout history to either cure or lessen symptoms from an illness. It used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations uses traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie *et al.*, 1996), together with their side effects and lack of healthcare facilities (Griggs *et al*., 2001). Rural areas of many developing countries on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al.*, 1999; Idu *et al*., 2007; Mann *et al*., 2008; Ammara *et al.*, 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari *et al.*, 1986; Van Wyk *et al.*, 2000).

Medicinal plants have been used since prehistoric period for the cure of various diseases. Since these are in common use by the local people and are of great importance, many people are engaged in the trade of important medicinal herbs throughout the world. Besides, the villages are far away from cities and mostly lack proper health facilities (Elisabetsky, 1990). Medicinal plant products were proved useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health (Kaushik and

Dhiman, 2002). The increasing global interest in the medicinal potential of plants during the last few decades is therefore quite logical.

Medicinal ethnobotany is the sub-discipline of ethnobotany which refers to the study of traditional uses of plants and folk knowledge concerning plants and human health care, including prevention and curing of human illness using plants. Ethno medicinal/botanical information on medicinal plants and their uses by indigenous cultures is useful not only in the conservation of traditional cultures and biodiversity, but also for community health care and drug development. This information is utilized as a guide for drug development under the assumption that a plant that has been used by indigenous people over a long period of time may have an allopathic application (Farnsworth , 1993). One of traditionally used herbal medicine for different disease treatment is *Bersama abysinnica* .

B. abyssinica (Melianthaceae) belongs to the genus *Bersama. B. abyssinica* is a poisonous plant and have been implicated in killing human and live stocks. Therefore, for internal use, the dosage is critical. Extracts of growing shoots are used for external treatment of burns, ulcers and to clean wounds. *B. abyssinica* can be propagated by seed, cuttings, wildlings or root suckers (Djemgou *et al*. , 2010). Fruits are red woods capsules (Bosch *et al*., 2008) . *B. abyssinica* growing in Ethiopia is recognized as subspecies *abyssinica* where it is known locally as Azamer (Amharic) and Lolchissa (Oromiffa) (Jansen, 1981 and Verdcourt, 1989).

1.2. Statement of the problem

B. abyssinica is traditionally used to treat rheumatism (Simbo, 2010). Bark, leaf and root decoctions are widely taken as a purgative to treat a range of stomach disorders, such as abdominal pain, colic, diarrhea, cholera, intestinal worms, amoebiasis and dysentery. Rabies, syphilis, gonorrhea and malaria are also treated with these decoctions (Verdcourt, 1989). Search for newer drugs from plants has been important due to the emergence of new drug resistant pathogens diseases and alarming side effects of synthetic drugs. In view of this, and the fact that several medicinal plants including *B. abyssinica,* used in Ethiopia in traditional medicine have not been properly evaluated for their biological activities and chemical constituents, the plant was chosen for this study. Hence this study aims at isolation and characterization of secondary metabolites from the roots of *B. abyssinica*.

1.3. Significance of the study

B. abyssinica is used in Ethiopia for traditional therapy of microbial infection, but has not been fully investigated. There is no reported isolation and characterization of phytochemicals or any antibacterial substances reported so far on the basis of literature search. Therefore, this is research study was undertaken for isolation, characterization, phytochemical and biological investigations of the roots of *B.abyssinica*. The focus was given to the roots because the root is the most commonly exploited part in this species. The study would serves as a springboard for researchers who are interested to conduct further studies in related areas. The study on the extracts of the roots of *B.abyssinica* would provide information on new natural products.

1.4. Objectives of the Study

1.4.1. General Objective

- To carry out phytochemical investigations, antibacterial and Antioxidant studies of roots extracts of *B. abyssinica*.

1.4.2. Specific Objectives

- To extract the roots with n-hexane, chloroform, ethyl acetate and methanol successively by maceration
- To conduct Phytochemical screening test of the crude n-hexane, chloroform, ethyl acetate and methanol extracts.
- To isolate and characterize compounds from methanol extracts.
- To test the antibacterial activity of crude and fractions of n-hexane, chloroform, ethyl acetate and methanol extract against some selected bacterial strains, from Gram positive bacteria (*S. aureus*, *B. subtilis*) and Gram negative (*E.coli, P. mirabilis,* and *K. pneumonia)* by disc diffusion method.

2. LITERATURE REVIEW

2.1 The family Melianthaceae

The Melianthaceae family consists of two genera; *Melianthus* and *Bersama*. *Melianthus* consists of 8 species and is a genus of shrubs endemic to southern Africa. The genus Bersama comprises of four species namely; *B. swinnyi*, *B. yangambiensis, B. engleriana* and *B. abyssinica* . Trees and shrubs belonging to the genus *Bersama* are spread throughout tropical and sub-tropical Africa, and various parts of the plant such as the leaves, bark, roots and seeds have been used for medicinal purposes (Burkill, 1997).

2.1.1. *B. abyssinica* Fresen

B. abyssinica Fresen is a species of medium-sized evergreen tree in the Melianthaceae family. The leaves are pinnately divided with a strongly winged rachis (hence the common name Winged *Bersama* (Heywood, 1993). This species is distributed across sub-Saharan Africa (Mikkelsen and Serberg, 2001; Burkill, 1997).

(i) Aerial part of *B. abyssinica*

(ii) Roots of *B. abyssinica*

Fig.1. *Bersama abyssinica* Plant, Picture Taken at Asella, Arsi by Emebet Getaneh on January, 2016

2.2. Biological and pharmacological activities of *B. abyssinica*

All parts of *B. abyssinica* are poisonous when ingested and have been implicated in killing humans and livestock. For internal use, the dosage is therefore critical (Burkill, 1997). Bark, leaf and root decoctions are widely taken as purgative to treat a range of stomach disorders such as abdomen pains, colic, diarrhea, cholera, intestinal worms, amoebiasis and dysentery. Rabies, syphilis, gonorrhea and malaria are also treated by these decoctions. A stem bark decoction is drunk to cure cancer and rheumatism (Makonnen and Hagos, 1993).

As an aphrodisiac, powdered stem bark or leaves is added to beer. A stem bark poultice is applied on the back, a leaf decoction is drunk to cure lumbago, stem bark and leaves decoction is used to treat diabetes mellitus. Leaf decoctions are also taken to treat feverish pains, loss of appetite, debility, jaundice, diarrhea and leprosy. Extracts of growing shoots are used for external treatment of burns, ulcer and to clean wounds. To treat convulsions and snake bites, leaves are

pounded and mixed with water and the mixture is drunk and applied on the body (Edeoga *et al*., 2005). The root bark infusion is drunk, leaf sap is applied as eye drops or leaf powder is sniffed to treat migraine, headache and colds. A root decoction is used to treat hemorrhoids and epilepsy (Taniguchi and Kubo, 1993). Shoots and leaves are pounded and used to control stalk borers in maize. An aqueous stem bark crude extracts showed activity against *Candida albicans* (Makonnen and Hagos, 1993). *B. abyssinica*, species was reported to possess anthelmintic, antitumour (Burkill, 1997) purgative, pesticidal and cardiogenic activities.

Antispasmodic use of the plant is one of the claimed traditional applications (Asres *et al*., 2001). Cardiac glycosides and unsaturated sterols were identified in tests in Ethiopia on stem bark and root bark. Leaf extracts have cardiogenic, spasmolytic and hypoglycaemic activities. Crude stem bark extracts slow down growth of *Bacillus cereus*, *Staphylococcus aureus*, *Shigella flexineri* and *Shigella dysentriae*, a root bark extract slows down that of *Bacillus subtilis* (Zekeya *et al*., 2014). An aqueous stem bark extract showed antispasmodic effects on isolated guinea-pig ileum. A methanolic leaf extract had an inhibitory effect on HIV-1 replication (Makonnen and Hagos, 1993; Ngemenya *et al*., 2005). Much of the work on the species *B. abyssinica* have been stimulated by the reported three hellebrigenin acetates isolated from the bark of that species with anti-tumour activities . Since that time several other acetate and orthoacetate derivatives have been reported. *B. abyssinica* was reported to possess anthelmintic, antitumour, purgative (Ngemenya *et al*., 2005) and cardiogenic activities. In Kenya, boiled root and leaves of the plant is traditionally used in the treatment of general sexually transmitted diseases (STD) (Njoroge *et al*., 2009). In Tanzania, *B.abyssinica Fressen subsp. Paullinioides* is locally used for treatment of various infectious diseases including mycobacterial illness though the plant has never been investigated for antimycobacterial activity (Zekeya, *et al*, 2014). In Babungo (Cameroon), *B. abyssinica* is known as Fuaveti and Timber is used for construction purposes (Simbo, 2010).

Previous biological analysis of *B. abyssinica* revealed the presence of antimicrobial secondary metabolites (Tapondjou *et al*., 2006). In Ethiopian traditional medical practices, the leaves extracts of *B. abyssinica* are administered orally for treating dysentery, tumor (Abate *el*

at.,2000), stomach disorders such as abdominal pain, colic, diarrhoea, cholera, intestinal worms, amoebiasis, rabies, syphilis, gonorrhoea and malaria are also treated with these decoctions (Verdcourt, 1989). Root bark extract of *B. abyssinica* displayed antiviral activity at concentrations that were nontoxic to MT-4 cells. *B. abyssinica* displayed anti-HIV activity against both HIV-1 and HIV-2 strains (Asres *et al*., 2001).

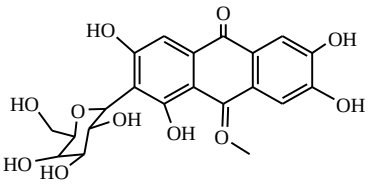
It is reported that methanolic extracts from the roots, stem bark, leaves and wood of *B.engleriana* showed antitumor, antioxidant and antimicrobial activities. The DPPH radical scavenging activity showed that the extract from the leaves was the most active. The results of antimicrobial activity showed that all tested extracts were active against all tested microbial species, including Gram-positive and negative bacteria, the two candida species and mycobacterium. Under the conditioning of the studied toxicity, all extracts were found to be non-toxic (Victor *et al*., 2008; Kupchan *et al*., 1971). Hellebrigenin **(7)** is the chemical constituent responsible for anticancer activity of the plant (Patel *et al*., 2010). Ethanolic extract, water extract and methanol extract of stem bark and root bark of *B. abyssinica* also show antibacterial activity against various bacterial strains at different concentration (Geyid *et al*., 2005).

2.3. Chemical Constituents of Genus *Bersama*

2.3.1. *B. engleriana* Gurke

B. engleriana Gurke is a tree that occurs in forests and forest margins of tropical and subtropical Africa. This species is used to treat a range of diseases by various communities in Africa. It is reported that phytochemical studies revealed the presence of five 3-O-glucuronide triterpene saponins isolated from the stem bark of *B.engleriana* along with two saponins, and one major C-

glycoside xanthones; mangiferin (**1**). These included 3-O-[ß-d-glucopyranosyl-(1→2)-ß-d-glucuronopyranosyl]-28-O-[ß-d-gluco-pyranosyl]-betulinacid (**2**); (3-O-[ß-d-glucopyranosyl-(1→2)-[ß-d-galactopyranos- yl-(1 →3)]-ß-d-glucuronopyranosyl]-oleanolic acid (**3)**; 3-O-[ß-d glucopyranosyl-(1→3)-ß-d-glucurono-pyranosyl]-28-O-[ß-d-xylopyranosyl-(1→6)-ß-d glucopyranosyl]-oleanolic acid (**4)**; 3-O-[ß-d-galactopyranosyl-(1→ 3)-ß-d-glucuronopyranosyl]-28-O-[ß-d-glucopyranosyl-(1→4)-ß-d-gluco -pyranosyl]-oleanolic acid (**5)** and 3-O-[ß-d-glucopyranosyl-(1→3)-ß-d-galactopyranosyl-(1→3)-ß-d-glucuronopyranosyl]-28-O-[ß-d-xylopyranosyl-(1 → 6)-ß-d-gluc-opyranosyl]-oleanolic acid (**6**) (Tapondjou *et al*., 2006). Hellebrigenin 3-acetate **(7)** was also isolated from *B.engleriana* plant.



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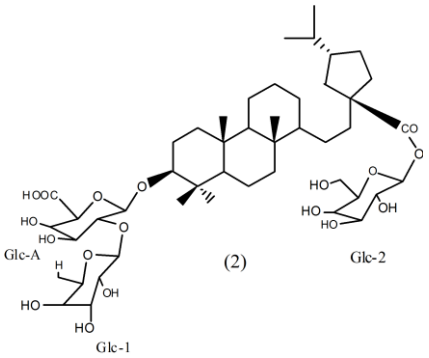

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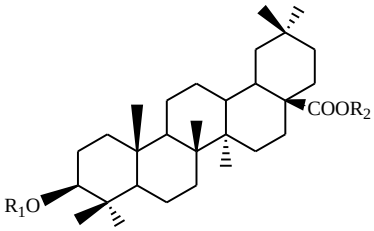
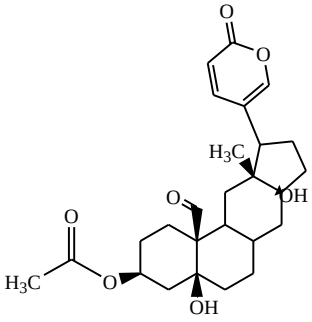




(7)

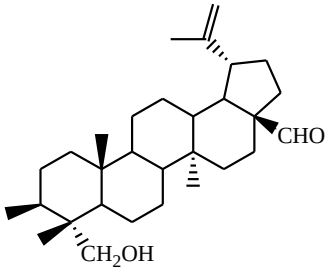
	R1	R2
(3)	Glc-(1→2)(Gal-(1→3))-GlcA-	H
(4)	Glc-(1→3)-GlcA-	xyl-(1→6)-Glc-
(5)	Gal-(1→3)-GlcA-	Glc-(1→4)-Glc
(6)	Glc-(1→3)-Gal-(1→3)GlcA-	xyl-(1→6)-Glc-

Fig.2. Reported Structures from *B. engleriana* Gurke

2.3.2. *B. swinnyi* Phil

B. swinnyi Phil is a tree that occurs in forests, on forest margins and sandstone outcrops of Eastern South Africa (Hutchings *et al*., 1996).

A triterpene,(3ß, 23-dihydroxylup-20(29)-en-28-al) (**8**), a triterpenoid, along with lupeol (**9**) were isolated from the chloroform extract of the bark of *B.swinnyi* (Thabo *et al*., 1998). Oleanolic acid (**10**) was isolated from the leaves (Monkhe *et al*., 1998) of the same plant



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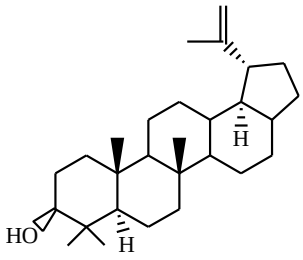

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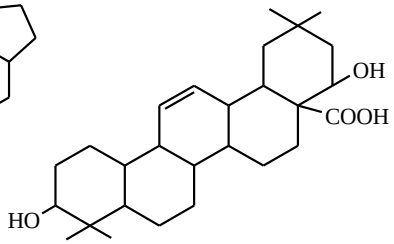


(10)

Fig.3. Reported Structures from various parts of B. *swinnyi*

3. MATERIALS AND METHODS

3.1. Apparatus and Chemicals

3.1.1. Apparatus and Instruments

The apparatus and instruments which were used in this study are oven, TLC plate, Whatman filter paper No.1, mechanical grinder, pipettes (different size), beakers (different size), UV light cabinet, electronic balance, flasks (different size), measuring cylinder, Rota vapor and TLC chamber. Analytical thin layer plates used were 0.2 mm thick layer of silica gel GF254 (Merck) coated on aluminium plate. Column chromatography was performed using silica gel (230-400 mesh) Merck. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Infrared (IR) spectra were obtained on Perkin-Elmer 65FT (IR KBr (4000-400) cm^{-1}) infrared spectrometer.

3.1.2. Chemicals

The chemicals and reagents used in this study were: solvents (n-hexane, chloroform, ethyl acetate and methanol), sulphuric acid, vanillin, silica gel and the microbial strains used were Gram positive (*S.aureus*, *B. subtilis*) and Gram negative (*E.coli, P.mirabilis, k.pneumonia).*

3.2. Collection of the plant material

The roots of *B.abyssinica* were collected from Asella, East Arsi 175 km far from Addis Ababa capital city of Ethiopia in the month of January, 2016. Botanical specimen of this plant was collected and submitted to Herbarium of Addis Ababa University for authentication voucher

number of EG/ 001/07/09/2016. After collection, the roots were washed repetitively and air dried in the shade to make it easily pulverized into fine particles.

3.3. Extraction of root of *B. abyssinica*

3.3.1. Solvent Extraction

The roots of the plant (2.0 kg) were grounded using mechanical grinder. The resulting powder was packed in polyethylene bag until the experiment was conducted.

Ground roots of *B. abyssinica* (500 g) were extracted successively with each 2000 mL of n-hexane, chloroform, ethyl acetate and methanol for 72 hrs at room temperature. Then each extract was filtered and concentrated using rotatory evaporator yielded the corresponding crude extracts. The yield of the crude extracts was determined and color of the extract in each solvent was indicated.

3.4 Preliminary phytochemical screening of the *Bersama abyssinica* Extracts

3.4.1. Phytochemical test

Detection of Alkaloids : The extract (15 mg) was dissolve in their respective solvents used for extraction and were stirred separately with 1% HCl (6 mL) in the beaker on a water bath holding by tong for 5 min and filtered into test tubes. Few drops of Wagner's reagent were added; a brown/reddish colored precipitate indicates the presence of alkaloids (Iqbal *et al*., 2015).

Detection of Anthraquinone glycosides: The extract solution (1 mL) in the first test tube, add 5% H_2SO_4 (1 mL). Boil the mixture in a water bath and then filter it into the second test tube. Shake the filtrate with equal volume of chloroform and let it to stand for 5 min. Then shake the lower layer of chloroform with half of its volume of dilute ammonia. The formation of rose pink to red color of the ammoniacal layer gives indication of anthraquinone glycosides (Iqbal *et al*., 2015; Shah& Seth, 2010).

Detection of Cardiac Glycosides: Shake extract (0.5 g) with distilled water (5 mL) in a test tube. To this, add glacial acetic acid (2 mL) containing a few drops of ferric chloride, followed by H_2SO_4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring (Iqbal et al., 2015) or a brown ring obtained at the interface indicates the presence of de-oxysugar characteristics of cardenolides (Patil & Paikrao, 2015; Shah& Seth, 2010).

Detection of Anthranol Glycosides: Hydrolyze the extract (10-20 mg) with dil. HCl in a test tube and treat it with 5% Ferric Chloride solution and immerse it in boiling water for about 5 minutes. Cool the mixture and extract it with equal volumes of benzene. Separate the benzene layer and treat it with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides (Shah& Seth, 2010).

Detection of Saponins: To about 0.5 g of the plant extract in test tube add distilled water (10 mL) and if shaking results frothing, which persists on warming using water bath for 5 min, considered as preliminary evidence for the presence of Saponins (Iqbal et al., 2015; Patil & Paikrao, 2015; Shah& Seth, 2010).

Detection of Flavonoids: Alkaline reagent test: the extracts of *B. abyssinica* were treated with few drops of NaOH solution. The formation of intense yellow color, which becomes colorless on addition of diluted acid, indicates the presence of flavonoids (Siddiqui *el at*., 2009).

Detection of Terpenoids: The extracts (about 100 mg) were shaken with chloroform (2 mL) in the test tube followed by the addition of concentrated H_2SO_4 (2 mL) along the side of the test tube using dropper, a reddish brown coloration of the interface indicates the presence of terpenoid (Iqbal et al., 2015).

Detection of Tannin: 10 mL of freshly prepared 10% potassium hydroxide (KOH) in a beaker add 0.5 g of extract and shake to dissolve. Formation of a dirty precipitate indicates the presence of tannin (Patil & Paikrao, 2015).

Detection of Coumarins: 3 mL of 10% NaOH was added to 2 ml of extracts formation of yellow color indicates the presence of coumarins (Falodun et al., 2006).

3.5 Isolation of compounds

3.5.1 TLC Analysis

Analytical TLC was applied in the detection and monitoring of compounds through a separation process, and was used to optimize solvent systems for column chromatography. The band separation on the TLC describing the separation of compounds was detected under UV absorbance at 254 nm (absorption) and 366 nm (fluorescence), followed by dipping the TLC plates with vaniline-H_2SO_4 reagent and subsequent heating at 110°C. TLC was conducted prior

to further work to track the identity of each fraction and the qualitative purity of the isolated compounds. Different phytochemical gave different RF values in different solvent system. This variation in RF values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio was used for separation of pure compounds from plant extract. The selection of appropriate solvent system for a particular plant extracts was achieved by analyzing the RF values of compounds in different solvent system. The TLC analysis of the methanol extract indicated the best resolution using chloroform and methanol (7:3) solvent system compared with other crude extracts.

3.5.2. Column Chromatography

As described earlier, the crude extracts were analyzed by TLC to choose best mobile phase for column chromatography. Then the methanol crude extract was subjected to column chromatography over silica gel and eluted with solvents of different polarities (Table 1). Different fractions were collected and concentrated over rotary evaporator.

The composition of the fractions collected was monitored by TLC and UV. The spots were detected by their UV absorption and fluorescence under 254 and 366 nm respectively, as well as by spraying 1% vanillin in sulphuric acid for UV inactive constituents.

Most of experimental techniques such as extraction, separation and isolation were conducted at the laboratory of the Department of Chemistry, Adama Science and Technology University.

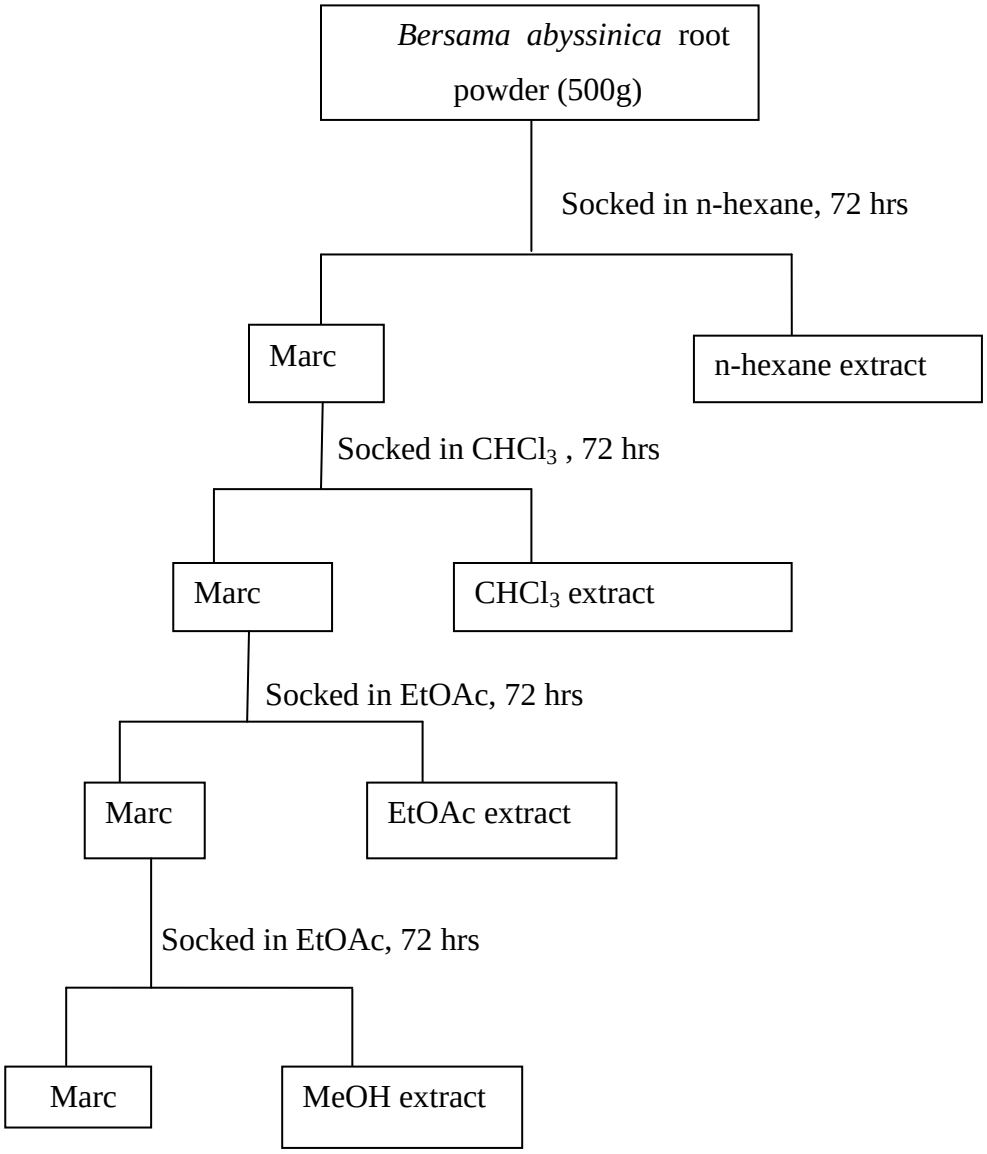
Whereas spectroscopic data such as IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135 of the isolated compounds were obtained from Addis Ababa university .

3.5.3. Fractionation of the extract

The methanol extract (6g) was fractionated on the silica gel column starting with 100% hexane and increasing the polarity by adding increasing amounts of ethyl acetate and then chloroform in methanol. The column was eluted with n-hexane:EtOAc, chloroform:MeOH of increasing polarities to furnish two compounds (Scheme 1).

Table 1:- chromatographic fractionations of the methanol crud extract.

Solvent system	Ratio of solvent used	Fractions collected	Volume of each fraction
n-hexane	100%	F1-F9	100 mL
n-hexane/EtOAc	90:10	F10-F12	100mL
n-hexane/EtOAc	80:20	F13-F21	100mL
n-hexane/EtOAc	70:30	F22-F26	20mL
n-hexane/EtOAc	60:40	F27-F29	100mL
n-hexane/EtOAc	50:50	F30-F36	20mL
n-hexane/EtOAc	40:60	F37-F42	100mL
n-hexane/EtOAc	30:70	F43-F46	100mL
n-hexane/EtOAc	20:80	F47-F50	100mL
n-hexane/EtOAc	10:90	F51-F57	20mL
EtOAc	100%	F58-F62	20mL
$CHCl_3$/MeOH	90:10	F63-F66	20mL
$CHCl_3$/MeOH	80:20	F67-F69	50mL
$CHCl_3$/MeOH	70:30	F70-F73	50mL
$CHCl_3$/MeOH	60:40	F74-F78	50mL
$CHCl_3$/MeOH	50:50	F79-F84	100mL
$CHCl_3$/MeOH	40:60	F85-F89	100mL
$CHCl_3$/MeOH	30:70	F90-F95	100mL
$CHCl_3$/MeOH	20:80	F96-F101	100mL
$CHCl_3$/MeOH	10:90	F102-F129	100mL
MeOH	100%	F130	100Ml



Scheme 1: Fractionation and isolation of compounds from *B. abyssinica* roots

3.6. Anti-bacterial activity test

The antibacterial activity of the crude extract and isolated compounds were tested against selected strains of bacteria in *vitro* by Disk-diffusion method.This was carried out *in vitro* using disc diffusion method. Small, sterile discs of filter papers (5 mm) were used as carriers of antibiotics and/ (or) crude extracts solutions to be tested. The test Organisms (Gram-positive and

Gram-negative) used in the study is listed in Table 4. These were obtained from Oromia public Health Research, Capacity building and Quality assurance laboratory center in Adama. The test bacteria were prepared by culturing the required bacterium in nutrient broth medium from stock cultures and later were transferred onto the nutrient agar in the Petri dishes (Clinical and Laboratory Standard Institute, 2013).

3.7. Antioxidant Activity test

The antioxidant activity of the methanol extract and its two constituents were evaluated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay by using procedures indicated in literature with little modification (Molyneux, 2004; Lewis, 2012; Garcia, 2012; Grujic *et al*., 2014). Thus, 4 mg DPPH was dissolved in 100 ml methanol to obtain 40µg/ml and stored in dark bottle at 4 ℃ by covering with aluminum foil. 5 mg of each of the methanol extract, ABS- EM and ABS-120 were dissolved in 5 ml methanol to obtain 1 mg/ml and were diluted to 500, 250, 125, 62.5 μg/ml concentrations to 4 ml final volume by using methanol. 4 ml DPPH in 4 ml methanol (control) and 4 ml DPPH with each prepared concentration (experimental part) were placed at 37 ℃ in oven for 30 min. The absorbance of both control and experimental part was taken by UV-Vis spectrometer at 517 nm using methanol as blank. The percent (%) inhibition of DPPH free radical by the sample was calculated as:

$\%\ \text{Inhibition} = \left(\frac{\text{Ac} - \text{As}}{\text{Ac}}\right) \text{x}\ 100\%$. Where A_c: absorbance of control and A_s: absorbance of sample. IC_{50} (the concentration of sample that inhibit 50 % of DPPH free radical) was calculated by using graph pad prism software from regression line (Khlifi *et al*., 2011). A lower value of IC_{50} indicates greater antioxidant capacity (Castro *et al*., 2014).

4. RESULTS AND DISCUSSION

4.1. Percentage yield of *B. abyssinica* extracts

Extracts of roots of *B. abyssinica* were obtained successfully by using different solvents. After concentration, each of the dried crude extracts was weighed and the color of extracts recorded (Table 2)

Table 2: Extraction yields of the roots of *B. abyssinica*

S.No	Solvent	Color of extract	Percentage yield(%w/w)
1	n-hexane	Yellow	0.42
2	Chloroform	Yellow	0.64
3	Ethyl acetate	Yellow	0.82
4	Methanol	Brownish	8.04

Hexane extract had the lowest percentage and was oily in nature. The methanol extract had the highest percentage yield (8.04%). This indicates that the secondary metabolites present in the roots of *B. abyssinica* are polar. The extracts were subjected to phytochemical and antibacterial tests before fractionation commenced.

4.2. Phytochemical Screening of the Extracts

The root extracts of *B. abyssinia* were used to investigate preliminarily phytochemical studies. The selected part of plant was analyzed for phytochemical screening for the extracts obtained from cold extraction successfully using gradient solvent system, n-hexane, chloroform, ethyl acetate and methanol respectively.

Table 3:- Phytochemical screening of *B. abyssinica* roots

No	Phytoconstituents	Reagents	Extracts			
			n-hexane	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	Wagner's	-	-	+	+
2	Tannins	KOH	+	+	+	+
3	Flavonoids	Shinoda test	_	_	+	+
4	Terpenoids	Salkowiski	+	+	+	+
5	Anthraquinone glycosides	Born trager's	_	+	_	_
6	Cardiac glycosides	Keller killiani's	_	_	+	+
7	Anthranol glycosides	Modified born trager test	+	+	+	+
8	Saponins	Foam test	_	_	_	+
9	Coumarins	NaOH test	+	+	+	-

(+) indicates Present (-) indicates absent

The extracts were subjected to various qualitative tests for phyto-constituents such as alkaloids, flavonoids, triterpenoids, tannis, coumerins, saponins and anthraquinones.

The result of phytochemical screening test of the extracts of roots of *B. abyssinica* reveals the presence of tannins,terpenoids, anthranol glycosides in all four extracts (n-hexane, chloroform, ethyl acetate and methanol) while alkaloids, flavonoids, and cardiac glycosides were found only in ethyl acetate and methanol extracts. In comparison, phyto-constituents of the leaves of *B.abyssinica* from the literature presence of alkaloides, flavonoides and glycosides in all extracts(n-hexane, chloroform, Ethyl acetate and methanol) while coumerins were found only in ethyl acetate and methanol extract whereas triterpenes present only in methanol (Anza *et al.*, 2015).

The traditional use of the plant may be attributed to its high contents of flavonoids, tannins, coumarins, phenols, saponnins and anthraquinones constituents.

4.3. Characterizations of isolated compounds

The methanol extract of the roots was subjected to column chromatography with *n*-hexane, chloroform, ethyl acetate and methanol. The chloroform: methanol fraction (fraction-68 and fraction-120) was purified and two compounds (ABS-EM and ABS-120) were obtained (Fig. 4 & 5). By means of spectroscopic analysis, they were identified as isomer of betulinic acid (ABS-EM) (**1**), phthalic acid, ABS-120, (**2**).

4.3.1. Characterization of the compound ABS-EM

The compound ABS-EM was obtained as colorless amorphous solid isolated from the roots of *B. abyssinica*.TLC showed one spot at R_f 0.65 with chloroform: methanol (8:2) solvent as a mobile phase. TLC spot viewed under UV lamp showed a blue color; this was further analyzed by spraying the spot with 1% Vanillin in sulphuric acid and heating for few minutes.

In the IR spectrum of the compound ABS-EM (Appendix 1), the absorption band at 3435 cm^{-1} showed the OH stretching that indicating the presence of a hydroxyl group. The strong absorption band at 2928 cm^{-1} showed the presence of the CH stretching, due to the stretching vibrations of aliphatic CH group. The absorption band at 1710 cm^{-1} showed the presence of the C=O stretching. The absorption band at 1630 cm^{-1} showed the presence of the C=C stretching. The absorption band at 1469 cm^{-1} showed the presence of C-C stretching.

The ^{1}H-NMR spectrum (Appendix 2) of ABS-EM showed signals at δ4.34 (1H) and 5.12 (1H) which are accounted to the presence of olefinic protons. A singlet peak at δ1.23 integrated for three protons in the spectrum is indicative of a quaternary methyl proton. The ^{1}H-NMR spectra also indicated signals due to six quaternary methyl protons (all singlets), with their peaks at δ

(0.67, 0.79, 0.90, 1.03, 1.23 and 1.23), each integrating for three protons. There are also different peaks integrating for 29 hydrogens observed in the region between δ 1.44 - 4.34

The ^{13}C-NMR spectrum (Appendix 3) in $CDCl_3$ of compound **ABS-EM** , analyzed with DEPT-135 (Appendix 5) showed a well resolved resonance of 30 carbon atoms, indicated the presence of seven quaternary carbons (C), which were attributable to five aliphatic carbons, one olefinic carbon and one carbonyl (carboxylic acid). From the spectral data six methine (CH), which were attributable to one oxygenated carbon and five aliphatic carbons were observed. In addition, the spectral data indicated the presence of eleven methylene (CH_2), which were attributable to ten aliphatic carbons and one olefinic carbon and also six methyl (CH_3) all of them were attributable to aliphatic carbon. This structural type was further supported by the ^{13}C-NMR spectrum, in which the appearance of signals at δ 125.02 and at δ 138.64 (Table 6) indicated the presence of a double bond and the most downfield signal resonated at δ 178.80 is attributed to the carboxylic acid. However, some of the signals appeared in ^{13}C-NMR spectrum were not appeared in DEPT-135 below 30 ppm region of chemical shifts made the characterization difficult especially due to the absence of methylene (CH_2) signals in DEPT-135 . The complete ^{13}C-NMR data is given in table and Appendix. Besides to the observed relationships between ^{13}C-NMR and DEPT-135, ^{13}C-NMR spectral data of the proposed structure of compound **ABS-EM** was found isomer of betulinic acid (Hong-peng *et al*..,2014) (Table 6). Based on the NMR data, the structure of the compound **ABS-EM** is given (figure 4.). The betulinic acid is a known triterpenoid isolated from various organs and species of plants. This metabolite shows inhibitory activity on growth of human melanoma cells, and replicate of AIDS virus. The betulinic acid also has antibacterial property and inhinbits the growth of colonies of *Escherichia coli* and *Staphylococcus aureus* (Alaide *et al*..,2010). Triterpenoids possesses anti-microbial activities may be confirmed that a isomer of betulinic acid isolated from this plant would exhibit anti-microbial activity and supports the finding in this present study that extracts of the plant and the isolated compound may be useful in wound healing, gonorrhea, stomachache, headache and it may responsible for curing various ailments.

Table 4: ^{1}H-NMR and ^{13}C-NMR spectral data of compound ABS-EM

Position	^{1}H-NMR (in ppm)	^{13}C-NMR (in ppm)	DEPT-135 (in ppm)
1	1.44 (t,2H) ; CH_2	38.9	-
2	1.54 (m,2H) ; CH_2	24.3	-
3	4.34 (m,1H) ; CH	77.3	77.3
4	C	38.9	-
5	1.78 (m,1H) ; CH	52.8	52.8
6	CH_2,(m,2H) 1.41	18.4	-
7	CH_2,(m, 2H) 1.03	36.8	-
8	C, -	39.2	-
9	1.79 (m, 1H); CH	47.5	47.5
10	C, -	36.9	-
11	CH_2,(m, 2H) 1.30	23.3	-
12	CH_2, (m, 2H)1.413	23.7	-
13	C,	40.5	-
14	1.83 (m,1H) ; CH	38.8	38.9
15	CH_2,(m,2H) 1.22	28.7	-
16	CH_2,(m,2H) 1.45	33.2	-
17	C, -	55.2	-
18	1.89 (s,1H) ; CH	47.3	-
19	1.92 (m,1H) ; CH	42.1	38.9
20	C, -	138.6	-
21	CH_2, (m,2H) ; 1.48	38.7	-
22	CH_2, (m,2H) ; 1.47	27.9	-
23	0.67, (s, 3H) ; CH_3	27.4	-
24	0.79, (s,3H) ; CH_3	16.5	-
25	0.91,(s, 3H); CH_3	17.4	-
26	1.03,(s, 3H) ; CH_3	17.5	-
27	1.23, (s, 3H) ; CH_3	15.7	-
28	C, -	178.8	-
29	4.35, (m, 2H); CH_2 5.12 (m, 1H)	125.0	125.0
30	1.23, (s, 3H); CH_3	21.5	-

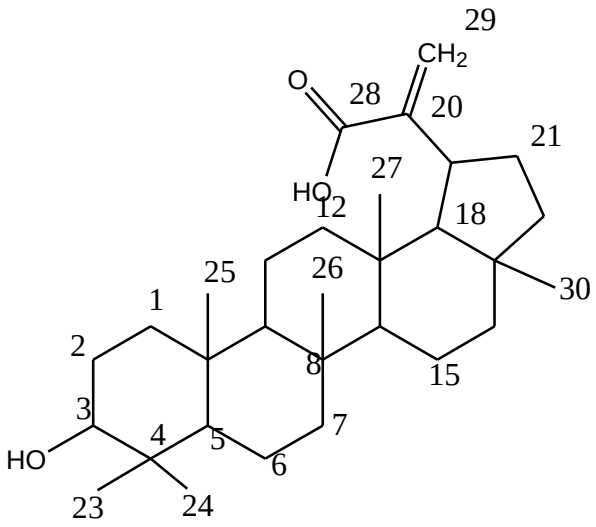


Figure 4: Proposed structure of compound **ABS-EM**

4.3.2 Characterization of compound ABS-120

The compound ABS-120 was obtained as a yellow amorphous solid isolated from the roots of *B. abyssinica*. It exhibited a blue colored spot on silica gel TLC when dipped in 1% vanillin in H_2SO_4 followed by heating for few minutes. The spot fluoresces under UV light prior to spraying and heating. It was soluble in organic solvents such as chloroform and methanol.

The IR spectral (Appendix 5) analysis showed stretching frequency at 2961 cm^{-1} suggests the presence of C-H stretching. Also observed was a band at 1730 cm^{-1} showed the presence of the C=O of esters stretching. The absorption band at 1278cm^{-1} and 1073cm^{-1} showed the presence of C-O stretching.

In the proton NMR of ABS-120(Appendix 5), at δ 7.65, and 7.75 two broad doublets each integrated for two protons were indicative of an aromatic ring system which was ortho-disubstituted most likely with similar substituents. A quartet at δ 4.21 integrated for four protons was indicative of two equivalent methylene groups that were oxygenated and make part of an ester functional group.

Table 5: ^{1}HNMR^{13}C NMR and DEPT-135 spectroscopic data of ABS-120

Position	^{1}H-NMR (in ppm)	^{13}C-NMR (in ppm)	DEPT-135 (in ppm)
1&34	1.01 (m,3H) ; CH_3	22.5	23.5
2&33	1.30 (m,1H) ; CH	22.8	23.7
3&32	1.35 (m,2H) ; CH_2	25.9	25.4
4&31	1.42(m,2H);CH_2	29.0 intense peak	29.4
5&30	1.44 (m,2H) ; CH_2		
6&29	1.44,(m,2H) ; CH_2		
7&28	1.47(m, 2H) ; CH_2		
8&27	1.49(m,2H); CH_2		
9&26	1.60(m,2H); CH_2		
10&25	1.69(m,2H); CH_2	30.3	30.3
11&24	1.71(m, 2H) ; CH_2	31.8	31.8
12&23	1.96(m, 2H); CH_2	38.7	38.8
13&22	4.26(t,2H); CH_2	67.5	67.5
14&21	C,	167.1	-
15&20	C,	131.1	-
16&19	7.65(m,1H)	128.8	128.8
17&18	7.66(m,1H)	132.6	132.6
35&36	1.01(m,3H)	22.5	23.5

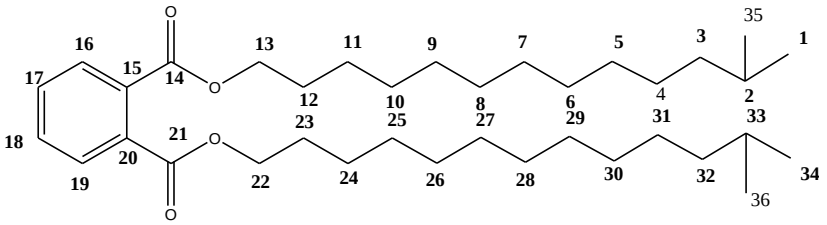


Figure .5 Proposed structure of ABS-120

4.4. Anti-bacterial activities of methanol extract and its fractions

The extracts and the isolated compounds were evaluated for their antibacterial activities against Gram positive (*S.aureus*, *B.subtilis*) and Gram negative (*E.coli*, *P.mirabilis*, *K.pneumonia*) with the results presented in Table 6.

Table 6: Antibacterial activities of ABS-EM and ABS-120, extracts and the standard drugs (Inhibition zones in mm)

Samples	Conc.in µg/mL	Types of bacteria with mean inhibition zone (mm)				
		S.aureus(+)	*B.subtilis*(+)	*E.coli* (-)	*P.mirabilis(-)*	*K.pneumonia(-)*
n-Hexane ext	20	6(R)	6(R)	6(R)	6(R)	6(R)
$CHCl_3$ ext	20	15(S)	16(S)	5(R)	18(S)	13(S)
EtOAc ext	20	16(S)	15(S)	13(S)	10(R)	12(S)
MeOH ext	20	16(S)	15(S)	6(R)	7 (R)	7(R)
ABS-EM	20	18(S)	16(S)	7(R)	7(R)	6(R)
ABS-120	20	15(S)	6(R)	12(S)	10(R)	9(R)
Cipro.	0.05	20	18	19	33	18

Cipro: Ciprofloxacin ; S: Sensitive; R: Resistive; ext: extract

The antibacterial test was performed using the agar disc diffusion method. The antibacterial activities in terms of zone of inhibition (in mm diameter) of the extracts and two isolated compounds were tested against selected gram positive (*S.aureus,B. subtilis*) and gram negative (*E.coli,P.mirabilis, k.pneumonia)* bacterial strains. The crude extracts and isolated compounds showed significant sensitivity to the different strains of bacteria used. The chloroform extract of *B. abyssinica* had inhibitory activity against two gram positive (*S. aureus, B. subtilis*) and in gram negative (*P. mirabilis, K. pneumonia*). Ethyl acetate extract had inhibitory activity against in all bacteria except one gram negative (*P.mirabilis).* Methanol extracts activity against two gram positive bacteria (*S.aureus, B.subtilis*). This was done in comparison with the inhibition zones of the standard reference as shown in Table 4. The values that are close to the values of the standard used show high activity and those that are half show moderate activity while those that are far below shows mild activity. Hexane did not have any activity since all the inhibition zones had 6mm which is the same value that was the diameter of the disc. *S. aureus* is an organism causing wound infection. The antibacterial activity displayed by the extracts and isolated compounds supports the traditional uses of this plant against bacteria.

4.5. Antioxidant activity of methanol extract and its fractions

The result of the antioxidant activity of the crude extract and the purified compounds evaluated by DPPH was shown as DPPH % inhibition as indicated on Fig. 6. This was calculated from absorbance of each concentration for methanol extract and purified compounds in terms of the absorbance of the control solution at 517 nm as shown on Table 7.

Table 7: Antioxidant Activities of the MeOH Extract, its Fractions and DPPH % inhibition

Sample concentration (µg/ml)	Absorbance			DPPH % inhibition		
	Methanol extract	ABS-120	ABS-EM	MeOH extract	ABS-EM	ABS-120
62.5	0.089	0.227	0.154	82.0	68.9	54.2
125	0.063	0.206	0.127	87.3	74.4	58.5
250	0.047	0.183	0.094	90.5	81.0	63.1
500	0.042	0.130	0.078	91.5	84.3	73.8
Control	0.496					

At 500 and 250 µg/ml % inhibition of *B. abyssinica* roots was around 65 % and 25 %, respectively. In other way Amarowic &Troszyñska (2003) compared the scavenging effect of *B. abyssinica* crude extract and fractionated from this crude on DPPH free radical. IC_{50} was calculated from regression line as shown on Fig. 6 (**B**). Therefore, IC_{50} value for ABS-EM was 10.07µg/ml and for ABS-120 was 46.90µg/ml. The result indicated ABS-EM has more antioxidant activities (Castro *et al*., 2014).

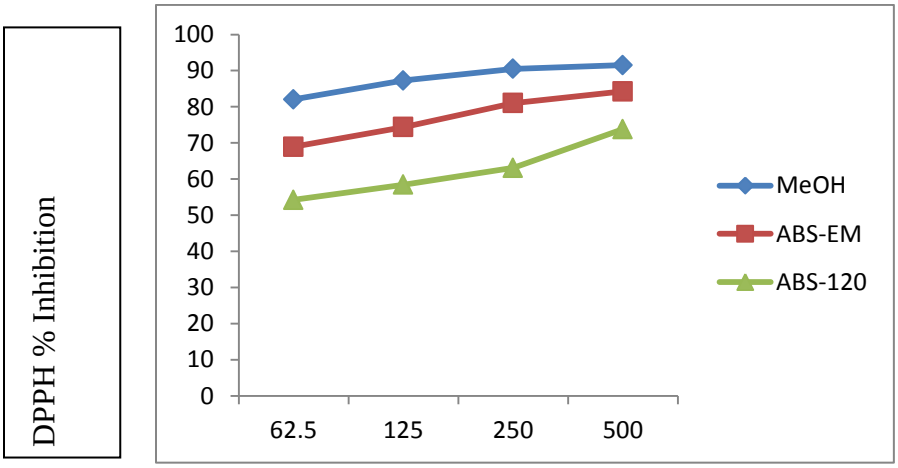


Figure 6. Antioxidant activity assay results

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The result showed that the percentage yields of the crude extracts increased from n-hexane to $CHCl_3$ to EtOAc to MeOH. These result suggested that the roots of *B. abyssinica* is very rich in polar metabolites. The band separation on the TLC describing the separation of compounds was detected under UV absorbance at 254 nm (absorption) and 366 nm (fluorescence), followed by dipping the TLC plates with vaniline-H_2SO_4 reagent and subsequent heating at 110^oc. phytochemical screening of *B.abyssinica* roots extract contain tannin, terpenoids and glycosides. The methanol extract was subjected to column chromatography and yielded isomer of betunilic acid and phthalate. The antibacterial activity evaluation of methanol extract and fractions showed was moderately sensitive against S.*aureus* and *B.subtilis*, which support its traditional medicine in the treatment of wounds. Antioxidant test of *B.abyssinica* roots methanol extract was good (pronouncable) result. Isomer of betunilic acid was isolated from roots of *B. abyssinica.* This structure of the triterpene compound was identified on the basis of spectroscopic and by comparing physical property and spectral data of betunilic acid reported in the literature.

The chemical structure of compound from ABS-120 was not proposed because it was masked by a pollutant which interfered during the analytical process. This pollutant may absorb di-n-octylphthalate from soil. According to Kirchmann et al (1991), phthalates released to environment can be deposited on or taken up by crops.

5.2. Recommendation

(i) The isolated compound had varied bioactivities on the test bacteria hence the plant can be used in treatment of the diseases caused by the respective pathogens. It recommends further works.

(ii) The crude extract for methanol possesses significant activity as seen in its ability to inhibit the growth of most of the test bacteria. Since methanol is a polar solvent it should be most preferred solvent in extraction.

(iii) The isolated compound is not the only compound in the plant show from TLC analysis, so for the future work should aim at isolating other compounds.

6. REFERENCES

Abate D., Debella, A., Urga, K., (2000). Medicinal Plants and other Useful Plants of Ethiopia, Camerapix Publishers Int., and Nairobi, Kenya, p.19.

Alaide, S., Barreto, Glaucio, D., Feliciano, Claudia Cristina Hastenreiter da Costa Nascimento, Carolina, S., Luna, Bruno da Motta Lessa, Carine, F.,da Silveira, Leandro da, S.,Barbosa, Ana, C.F., Amaral and Antonio, C., Siani, (2010). Analysis of the presence of the betunilic acid in the leaves of Eugenia floridaby using the technique GC/MS, GC/FID and HPLC/DAD: A Seasonal and Quantitative study.*J.Nat. product*.66 (4), 184-193

Ammara, H., Salma, R., Farah, D. & Shahid, M. (2009). Antimicrobial activity of some plant extracts having hepatoprotective effects. *Journal of Medicinal Plant Research* **3**(1):20-23.

Asres, K., Bucar F., Kartining T., Witvrouw M., Pannecoupue C., De clercq E. (2001).Antiviral activity against human immunodeficiency virus type 1(HIV-1) and type 2(HIV-2) of ethno botanically selected Ethiopia medicinal plants. *Phytotherapy Research*; 15:62

Ayoola, GA., Coker., HAB, Adesegun, SA., Adepoju-Bello, AA., ObaweyaK, Ezennia, EC., (2008). Atangbayila TO phytochemical screening and antioxidant activities of some selected medicinal lants used for malaria therapy in south western Nigeria. Trop J pharm. Res;7: 1019-1024. *Bioactive Natural Products*: *an update* in: *Studies in Natural Products Chemistry*, Atta-ur-Rahman (Ed.), Elsevier: Amsterdam; 99, p 383.

Bosch, CH., *Bersama abyssinica* Fresen. In: Schmelzer, GH., Gurib-Fakim, A.,(Editors) (2008). Plant Resources of Tropical Africa 11(1). Medicinal plants 1.PROTA Foundation. Wageningen, Netherlands :Backhuys publishers, p.116.

Burkill, H.M., (1997). The useful plants of West Tropical Africa. 2nd Edition.Volume 4, Families compounds Table of spectral data" springer-verlag Berlin Heidelberg pp 71-150.

Dahanukar, S.A., Kulkarni, R.A., and Rege, N.N., (2000). Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology* **32**: 81-118.

Djemgou, PC., Hussien, TA., Hegazy, MEF., Ngandeu, F., NeguimG,Tane , C., (2010).Glucosidexanthane from the stem bark extracts of Bersamaengleriana, phcog Res, 2:229-232.

Edeoga, H.O., Okwu, D.E and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**: 685-688.

Elisabetsky (1990). Plants used as analgesics by Amazonian cabocols. *International Journal of Crude Drug Research*. 28: 309-320.

Farnsworth, N.R., (1993). Ethno pharmacology and future drug development the North American.*Journal of Pharmaceutical.* 38: 145-152.

Farnsworth,N.R., Akerele O., Bingel A.S., and Soejarto D.D., *(*1985). Medicinal plants in therapy effects.*World Health Organization*. 63: 83–97.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka ,F., et al (2005). Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *Journal of Ethnopharmacology*; 97:421-427.

Gibbs, RD., Chemotaxonomy of Flowering plants. Vol.1, McGill Queen's University press, Montreal and London 1974.

Griggs,J.K.,Manandhar,N.P.,Towers, G.H.N.and Taylor, R.S.L. (2001). The effects of storage on the biological activity of medicinal plants from Nepal. *Journal of Ethnopharmacology* 77:247-252.

Heywood, V.H., (1993). Flowering Plants of the World (2nd ed.). New York: Oxford University Press. pp 191–192.

Hong-peng, Yang,Sheng Que, Yan-Ping, Shi and Li-Tong Ban.(2014).triterpenoids from *Gentiana veitchiorum.J.Chemical and pharmaceutical Research*.6(7),1986-1990

Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A. (1996). Zulu medicinal plants; An Inventory. Pietermaritzburg, University of Natal Press. pp 190-191.

Idu, M., Omonigho, S. E., and Igeleke, C., L.(2007). Preliminary investigations on the phytochemical and antimicrobial activity

Iqbal, E., Abu Salim, K., & Linda, B.L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. Journal of King Saud University - Science, 27 (2), 224-232.

Iwu, MM., handbook of African medicinal plants.CRC press 1993.

Iwu, M. W., Duncan, D., R. & Okunji, C., O. (1999). New antimicrobials of plant origin. In: Janick, J. (Ed.) *Perspective on New Crops and New Uses*. ASHS Press. Alexandria, VA. 107-108.

Jansen P, C., Spices, M., (1981). Condiments and Medicinal Plants in Ethiopia: Their taxonomy and Agricultural Significance, Centre for Agricultural Pub. And Docu, Wageningen.The Netherlands.

Kaushik, P., and Dhiman, A.K., (2002). Medicinal Plants and Raw Drugs of India. http://www.vedicbooks.net/medicinal-plants-andrawdrugs of-indiap13886.html

Kirchmann, H., Astrum, G., and Jonsali, G., (1991). Organic pollutants in organic sewage slug1. Effect of toluene, naphthalene, 2-methylnaphthalene, 4nonylphenol, and di-2-ethylhexylphthalate on soil biological processes and their decomposition in soil. Swedish J. Agric. Res 21, 107-113.

Kupchan, M., Hemingway, RJ. Hemingway, JC., Tetrahedron Letters 149(1968); 1.org.Chem. 1969; 34:3894.

Kupchan, SM,, Moniot, JL., Sigel, CW., Hemingway, RJ., Tumor inhibitors(1971). LXV,Bersenogenin, berscillogenin, and 3-epiberscillogenin, three new cytotoxic bufadienolides from Bersamaabyssinica. J.Org.Chem.; 36(18):2611-2616.

Lather, A., Gupta, V., Tyagi, V., Kuma,r V., Garg, C.,(2010). Phytochemistry and Pharmacological Activity of *Bersamaabyssinica*Guerke-An overview. International Research Journal of Pharmacy; 1(1):89-94

Maheshwari, JK., Singh, KK., and Saha, S., (**1986).** Ethnobotany of tribals of Mirzapur District, Uttar Pradesh, Economic Botany information Service. *NBRI*, Lacknow,

Makonnen, E., and Hagos, E., (1993). Antispasmodic effect of *Bersama abyssinica* aqueous extract on guinea-pig ileum. *Phytotherapy Research* **7 (2):** 211-212.

Mann, M.E., Zhang, Z., Hughes, M.K., Bradley, R.S., Miller, S.K., Rutherford, S.,*(2008)* Proxy-Based Reconstructions of Hemispheric and Global Surface Temperature Variations over the Past Two Millennia, *Proc. Natl. Acad. Sci.*, 105, 13252-13257.

McLaughlin, J.R., Chang, C-J., Smith, D.L. (**1991**) "*Bench-Top"Bioassays for Discovery of*

Mikkelsen, K.S., and Seberg, O., (2001). "Morphometric analysis of the *Bersama abyssinica* Fresen. Complex (Melianthaceae) in East Africa". *Plant Systematics and Evolution* **227** (**4**): 157182

Mokaddas, E., Rotimi, V.O., and Sanyal, S.C., (1998). *In vitro* activity of Piperacillin/tazobactam versus other broad antibiotics against nosocomial Gram-negative pathogens isolated from burn patients. *Journal of Chemotherapy* **19**: 208-14.

Monkhe, T., Mulholland, D., and Nicholls, G., (1998). Triterpenoids from *Bersama swinnyi*. *Phytochemistry* **49:** 1819-1820.

Moshi, MJ.(2005) Current and Future prospects of integrating traditional and alternative medicine in the management of diseases in Tanzania. Tanzania Health Research Bulletin; 7:159-167.

Ngemenya, M., Titanji, V., Akam, T., Yong, J., Tane, P. and Fanso-Free, S. (2005).Nigerian medicinal plants. *African Journal of Biotechnology* **4**: 685-688.

Ohinishi, M.; Morishita, H.; Iwahashi, H.; Toda, S.; Shirataki, Y.; Kimura, M.; Kido, R.(**1994**)

Patel, B., Das, S., Prakash, R., Yasir, M., (2010). Natural Bioactive compound with Anticancer potential.International *Journal of Advances in pharmaceutical sciences*; 1:32-41.

Patil, A.S., & Paikrao, H.M. (2015). Phytochemical screening methods.

Perumal, S.R., and Ignacimuthu, S., (2000). Antibacterial activity of some folklore medicinal plants used by tribal in Western Ghats of India. *Journal of Ethno Pharmacology* **69**: 63-71.*Phytochemistry*, 36, 579.

Pretsch, E., Buhlmann, M., and Affolter, A., (2000). "Structure Determination of organic saponins from *Bersama engleriana*. *Phytochemistry* **67** (**19**): 2126-2132.

Retrieved from http://gobioplanet.com/phytochemical-screenin-methods/ on October 2/2015.

Salie, F., Eagles, P.F.K., Leng, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27–33

Siddiqui, S., Verma, A., Rather, AA,, Japeen, F., (2009). Meghvansi MK. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. *Advances in biological research*; 3(5-6): 188-195.

Simbo, DJ, (2010) An ethnobotanical survey of medicinal plants in Babungo, Northwest Region, cameroonsimbo Journal of Ethnobiology and Ethnomedicine; 6(8): 1-7.

Sofowora, A., (1982). Medicinal plants and traditional Medicine in Africa. John Wiley & Sons Limited, New York.

Taniguchi, M. and Kubo, I. (1993). Ethnobotanical drug discovery based on medicine men's Tanzanian *Bersama abyssinica. International Journal of Science and Research* **3**(**7**): 1150- 1154.

Tapondjou, A.L., Magimoto, T. and Lacailla-Dubois, M.A. (2006). Glucuronide triterpene trials in the African Savana: screening of East African plant for antimicrobial activity II. *Journal of Natural Products* **56**: 1539–1546.

Van Wyk, B.-E., Gericke, N., 2000. People's Plants. A Guide to Useful Plants of Southern Africa. Briza Publications, Pretoria

Verdcourt B., (Melianthaceae) The National Herbarium,Addis Ababa University, Ethiopia and the Department of Systematic Botany, Uppsala University, Sweden, In: Hedberg I, and Edwards Seds, Flora of Ethiopia,3(1989)511-512.

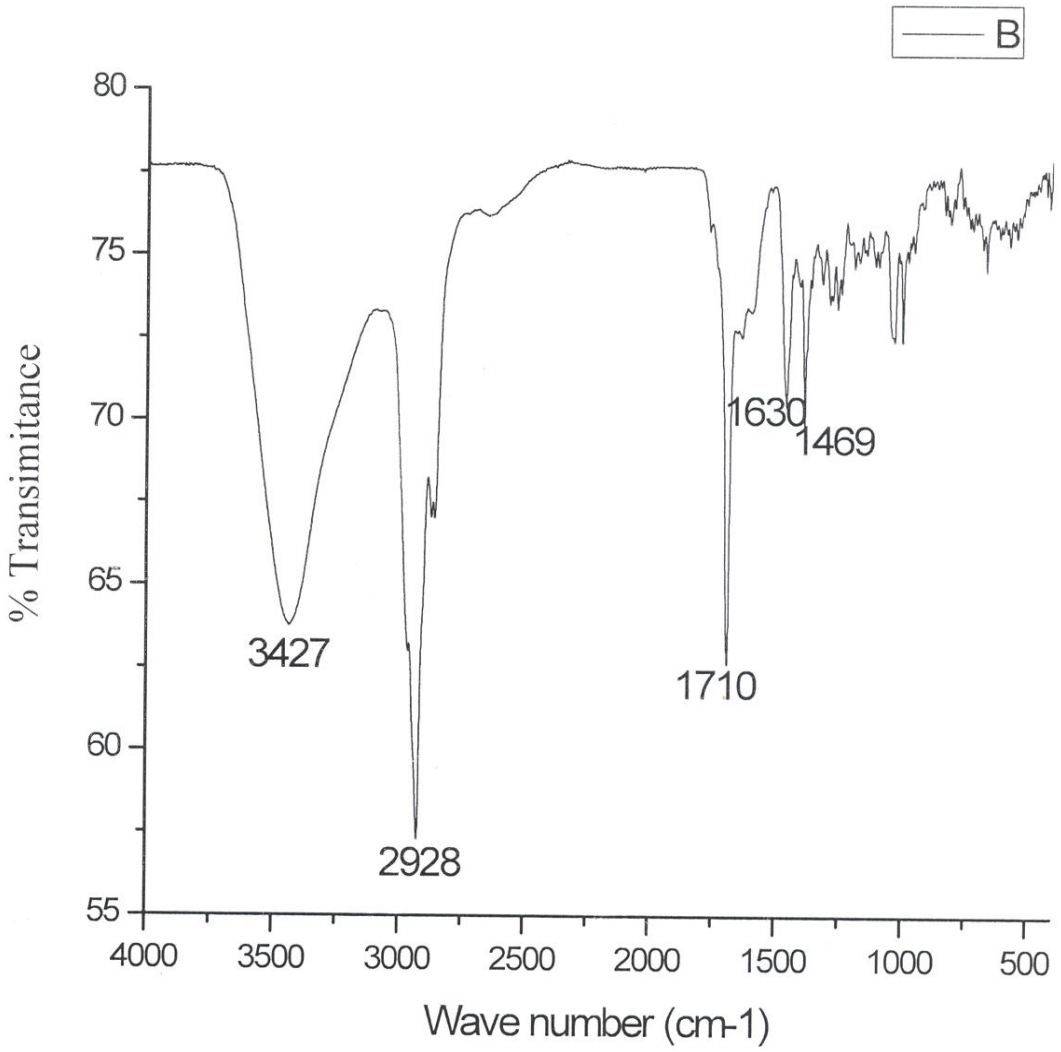
Victor K, Armelle TM, Maurice T, Veronique PB, Fancois- Xavier ETOA, Augustin EN Antitumor, Antioxidant and antimicrobial activities of *Bersama engleriana* (Melianthaceae). *Journal of Ethnopharmacology 2008; 115(3): 494-501.*

Yineger, H., yewhalaw, D,, Teketay, D. (2008). Ethnomedicinal plant knowledge and practice of the oromoethinic group in southwestern Ethiopia. Journal of Ethno biology and Ethno medicine; 4(11): 1-10.

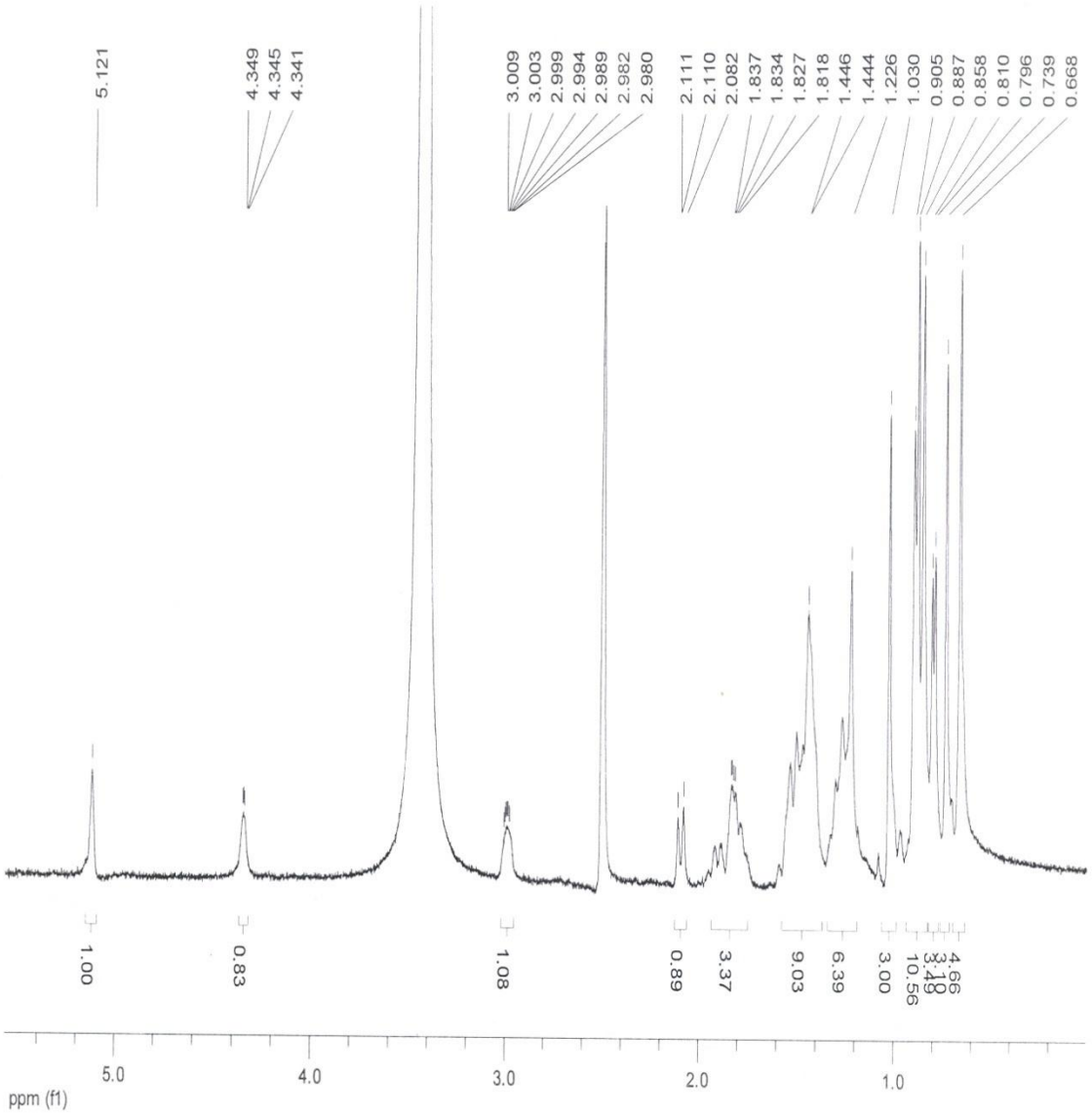
Zekeya, N.,Chacha, M. and Shahada, F. (2014). Antibacterial and antifungal activity of Tanzanian *Bersama abyssinica. International Journal of Science and Research* **3**(**7**):1150-115

7. APPENDIXES

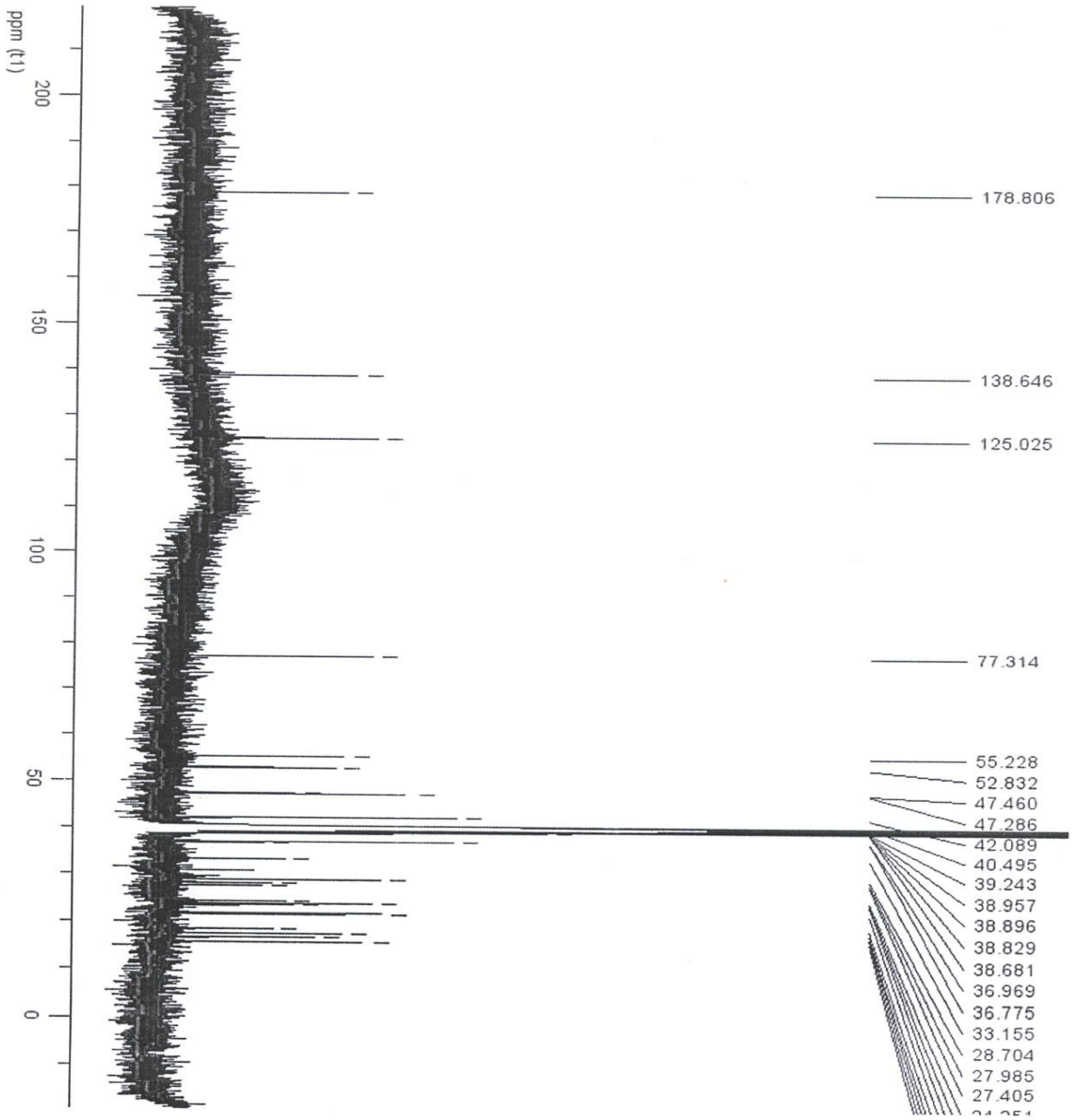
Appendix 1: IR Spectrum of ABS-EM



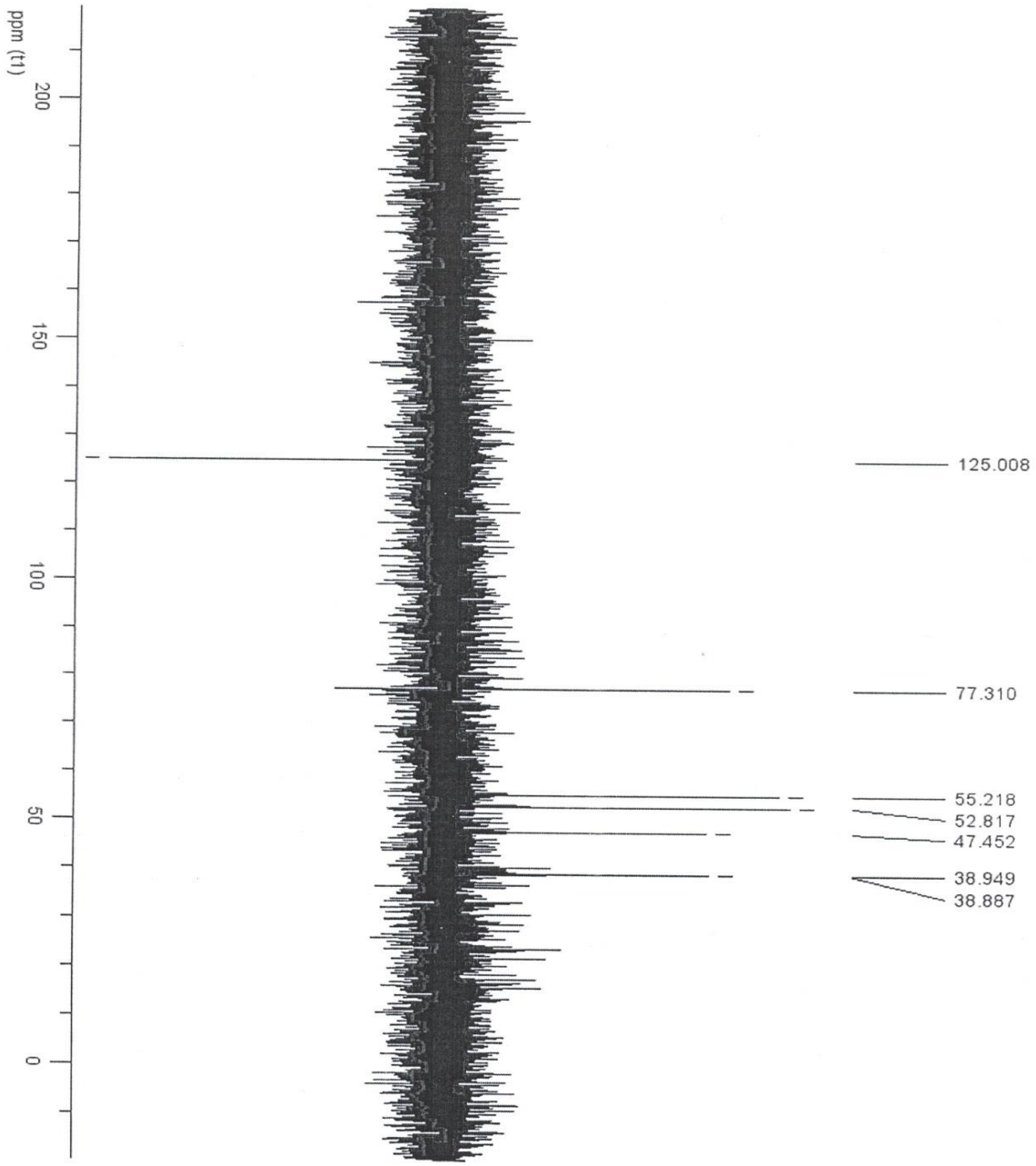
Appendix 2: ^{1}H-NMR Spectrum of ABS-EM



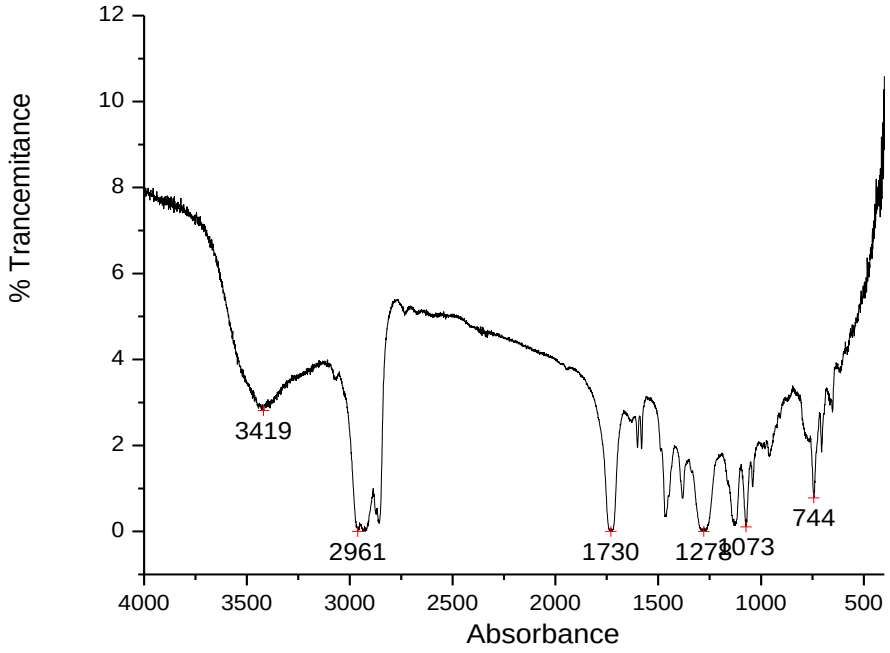
Appendix 3: ^{13}C-NMR Spectrum of ABS-EM



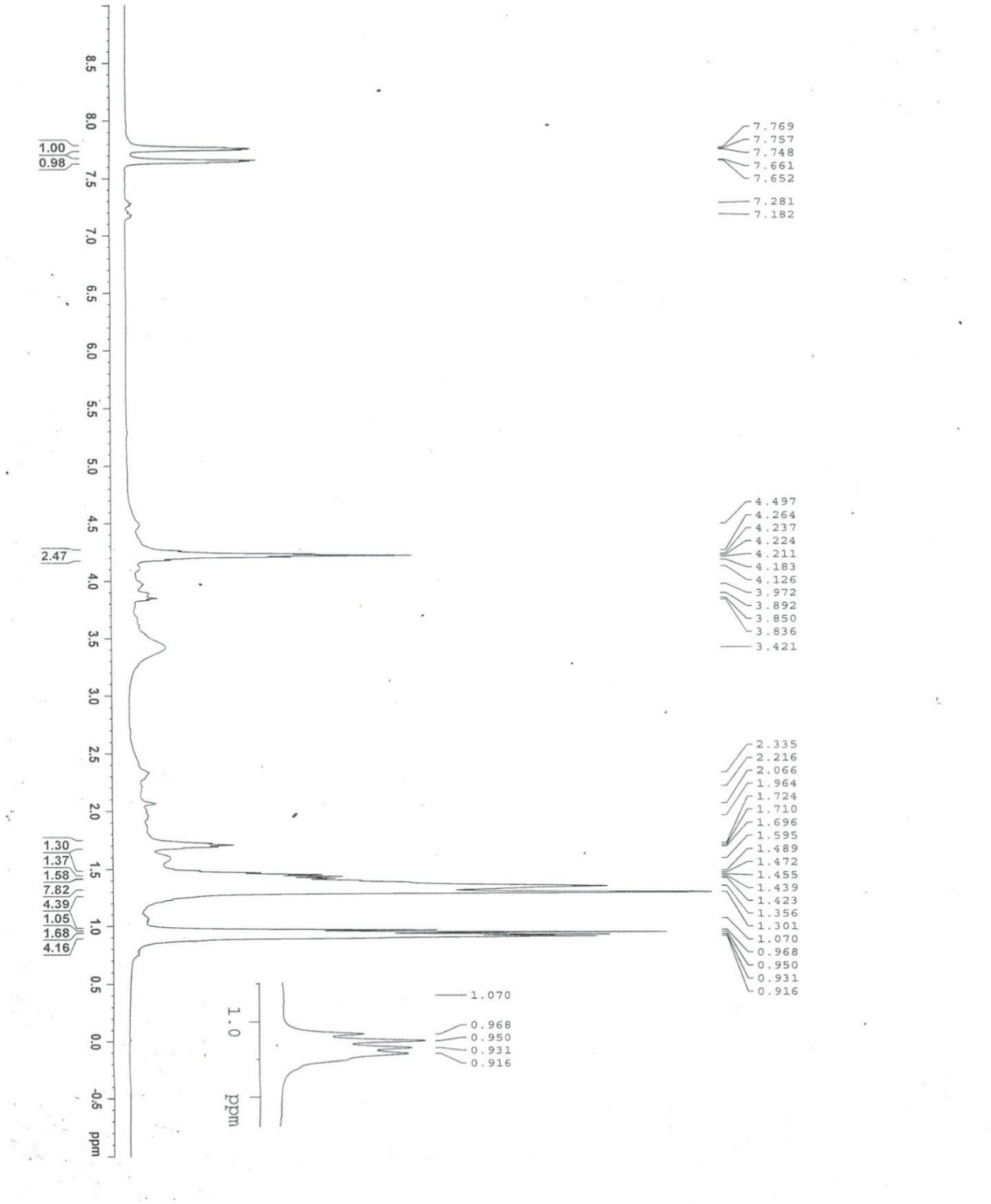
Appendix 4: DEPT-135 Spectrum of ABS-EM



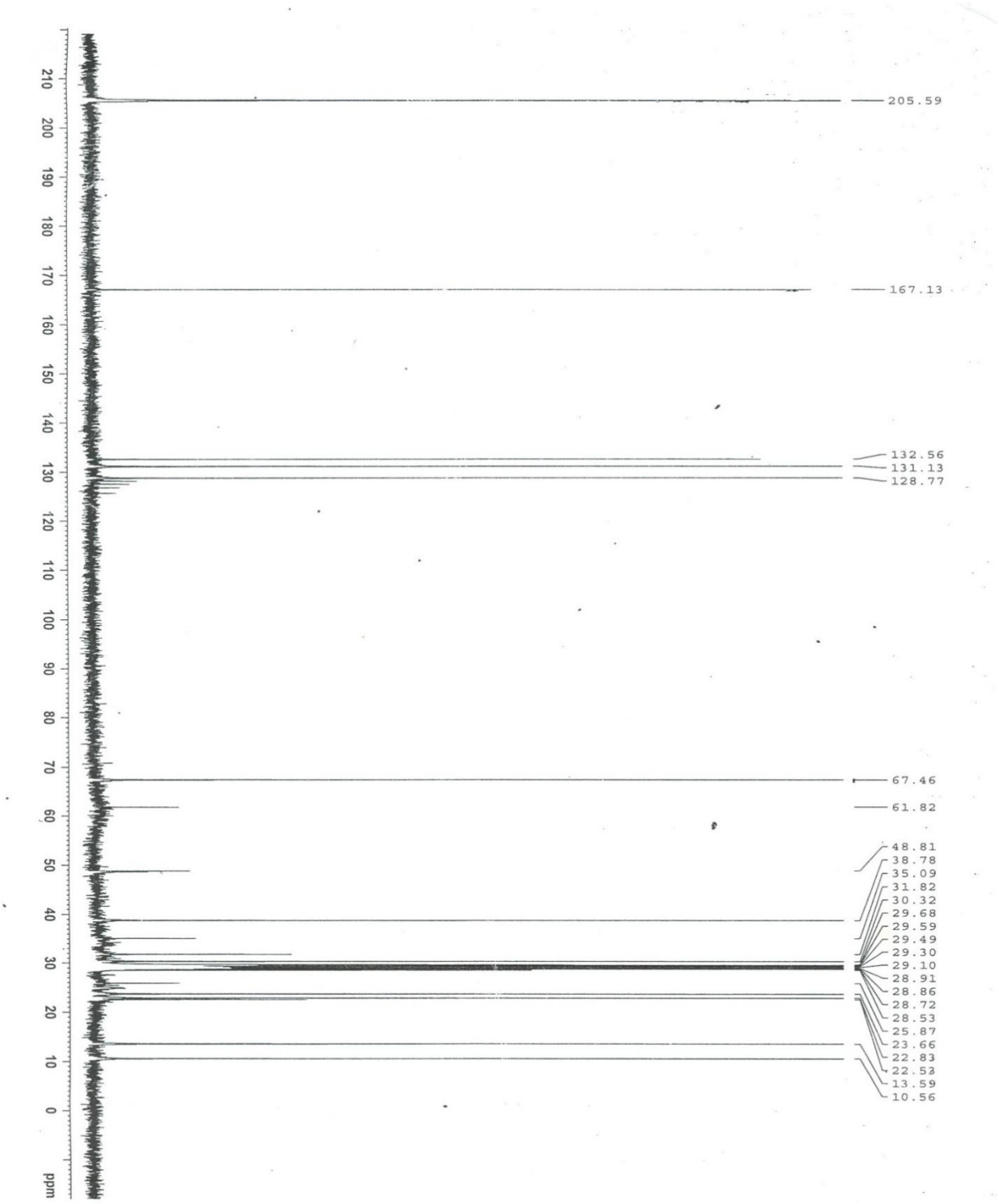
Appendix 5: IR Spectrum of ABS-120



Appendix 6: ^{1}H-NMR Spectrum of ABS-120



Appendix 7: ^{13}C-NMR Spectrum of ABS-120



Appendix 8: DEPT-135 Spectrum of ABS-120