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Phytochemical Investigation on the Root of *Erythrina Abbyssinica* (Korch)

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Abstract: *Erythrina abbyssinica* (korch) in the family of *Fabaceae* is a species native to Ethiopia. It has medicinal values and is important in the traditional medicine like the other species in the genus. In this work, the chemical constituents in methanol extract of the Ethiopian traditional medicinal plant *Erythrina abbyssinica (E.abbyssinica)* were investigated and the antibacterial and anti-fungal activity of them were examined. The chemical constituents were isolated and purified by various chromatographic methods and their structures were elucidated mainly by using ^{1}H NMR, ^{13}C NMR, UV & FTIR spectra. The compound isolated was identified as 9-propyltridecyl benzoate. Several un-characterized pigments were also isolated.

Keywords: *Erythrina abbyssinica,* NMR spectroscopy, Bioactivity, 9-Propyltridecyl benzoate

1. Introduction

Phytochemical studies of plants, especially of medicinal plants, are great importance in developing drugs [1, 2]. Considered in earliest times as a connection to the divine, the use of medicinal plant is as old as human civilization itself [3]. Whole nations subjugated its secrets, often associated with magical and religious rites, searching in nature's resources to improve life conditions, and increase chances of survival [4]. In 1978, the World Health Organization (WHO) recognized traditional medicine and its beneficial effects to health, during the *Alma Ata* conference, which published in 1985 that approximately 80% of the global population resorted to traditional medicine as their primary health treatment. Medicinal plants have been used as a means of curing or preventing diseases, now called phytotherapy [5], in all areas of the world, with regional variations due to the influence of cultural characteristics of the population, as well as its flora, soil and climate [6].

Since nineteenth century, humanity discovered the endless and diverse therapeutic arsenal present in medicinal plants, due to the discovery of active substances that in their natural state or after chemical transformation showed biological activity, and often already confirmed by popular use and proven scientifically[7]. A new herbal medicine can be introduced to the market in two forms: as a finished product – industrially produced, or as an official product – manufactured in pharmacies. Both forms should ensure quality, safety and efficacy of the herbal medicines supplied to the consumer. On the other hand, medicinal plant utilization as a way to diminish the costs of public health programs since the utilization of these plants may constitute a very useful therapeutic value due to their efficacy coupled with low operating costs and the relative ease of obtaining the plants [8]. However, we cannot rule out the cultural importance that popular knowledge inputs, being transmitted from generation to generation.

The genus *Erythrina* is one among several types from the Fabaceae family. The genus *Erythrina* species are used as healing agent in traditional medicine in Africa [9]. Of 31 African species, 11 (35%) have ethnomediacal uses in sub-Saharan Africa. These are *E.abbyssinica*, *E. addisonia Harms, E.excels Bak, E. fusca Lour, E.latissiniae E.Mey, E.mildbraedii Harms, E.poeppigiana (Walp.), E. variegataL. and E. Vogelii Hook f* [10]. Erythrina species are widely prescribed in sub-Saharan traditional medicine against frequent diseases from microbial and parasitic origin [11]. According to prelude Medical Plants Database, *E. senegalensis* in West Africa and *E. abbyssinica* in central South Africa are most used species. Thirty nine medicinal usages were found for *E. senegalensis* and 60 for *E. abyssinica. E. abbyssinica* are prescribed in ethno veterinary medicine practices against brucellosis, oedema, hygroma, dropsy, bacterial infections, skin diseases [12]

E. abbyssinica is a species of leguminous tree as seen in Figure 1. 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 [13]. It is distributed in, Ethiopia, Eritrea, Uganda, Kenya, Tanzania and Zimbabwe. In northern and western Ethiopia, it is found at elevations between 1600 and 2100 m. *E. abbyssinica* (Luganda name: Muyirigiti or Jjirikiti; Runyakole name: Ekiko), is a deciduous grassland legume. It grows in open woodland and grassland. It has characteristic red overflowing flowers. It can be propagated through seedlings, cuttings and truncheons. In the south western rangelands of Uganda, it is sometimes planted along fences of paddocks to support barbed wires. It has various traditional medicinal applications in livestock. It is also used in traditional human medicine for treatment of bacterial, fungal, parasitic and viral diseases, gastrointestinal disorders, liver disorders, nervous disorders, liver disorders, sexual asthenia, nervous disorder, sterility, eyes diseases and kidney pain etc [14].

In this paper, we report on the isolation, structure elucidation of chemical constituents in methanol extract of the roots and stem bark of *E. abbyssinica*. Ethiopian traditional medicinal plant *E.abbyssinica* were investigated and the antibacterial and anti-fungal activity of them were examined by using disc diffusion method.

2. Experimental

General

Electric grinder (KIkA®_WERKE), digital measuring balance, 600mL round bottom flask, Whatman filter paper (hardened ash less circles 110mm),

Fig. 1. Erythrina abyssinica (A) tree (B) and its bark

Erlenmeyer flask, Conical flask, Rotary evaporator, TLC plate (pre-coated aluminum sheet, 20 x 20 cm), (silica gel 60 F_{254}),254 nm UV lamp, Column, Silica gel (230- 400 mesh size), UV –Vis spectrophotometer, NMR machine (Bruker advance 400 MHz spectrometer), IR spectrometer (400-4000cm^{-1}), Muller Hinton Agar Media (MHA), *Staphylococcus aurous* (gram positive) and *Shigela boydi* (gram negative) bacterial pathogen, *Candida albicans* (fungus) fungus pathogen, Autoclave Sterilizer, Vortex, Incubator, nutrient broth (NB), cotton swab, Petri dish plates, different organic solvents and chemicals, etc

Plant material

The stem, root and bark of *E. abbyssinica* were harvested and the collected specimens were identified at the department of Biology, University of Gondar, Gondar town, Ethiopia, in December 2014. The voucher specimen is deposited.

Drying and pulverizing plant material

The root of *E. abbyssinica* plant was chopped into small pieces and air dried at room temperature under shade to avoid direct sunshine that could degrade some of the compounds in the plant root. It was also spread out and regularly turns over to avoid fermenting and rotting. The dried root sample was milled (pulverized) using an electric grinder (KIkA®_WERKE) in to fine powder.

Extraction and Isolation

Air-dried milled root (450g) of the *E. abbyssinica* plant powder was soaked in methanol (2.5L) for seven days with frequent shaking at 25ºC. Thereafter the extract was filtered first with cotton wool followed by Whatman filter paper® to obtain the crude extract solution. The brown crude extracted solution was concentrated to a minimum volume by a rotary evaporator (STONE STAFFORDSHIRE ENGLAND ST15 OSA) at 45°C under reduced pressure. Then concentrated crude extract was allowed to evaporate to constant weight (45g) at room temperature. About 100ml of petroleum ether was added in to 45g of organic extract and then transferred in to separator funnel which forms the two layers (above part green solution and lower part red precipitate).

First: The above green solution was filtered and then concentrated (21g) with a rotary evaporator at 45°C under reduced pressure. This concentrated (21g) extract was adsorbed on 29g of silica gel and charged on to column packed with 70g silica gel of mesh size 230-400 by using petroleum ether. Then the column was eluted by using acetone: petroleum ether (1: 3) as eluting solvent continuously. A total of 6 fractions were collected. The volume collected and the ratio of solvent used is given in Table.1

Second: The lower red precipitate was dissolved with acetone: diethyl ether (2:3) and then filtered. This solution was concentrated (23g) with a rotary evaporator at 45°C under reduced pressure. Concentrated (23g) was adsorbed on 32g of silica gel and charged on to column packed with 72g silica gel of mesh size 230-400 by using petroleum ether.

Table 1: Ratio and volume of solvent used for column chromatography

Fraction	Solvent	Ratio	Volume
EA7	Acetone: petroleum ether	1:3	25 ml
EA8	Acetone: petroleum ether	1:3	28 ml
EA9	Acetone: petroleum ether	1:3	30 ml
EA10	Acetone: petroleum ether	1:3	20 ml
EA11	Acetone: petroleum ether	1:3	34 ml

Table 2: Ratio and volume of solvent systems used for column chromatography

Fraction	Solvent system	Ratio	Volume
EA1	Acetone: petroleum ether	2:3	20 ml
EA2	Acetone: petroleum ether	2:3	25 ml
EA3	Acetone: petroleum ether	2:3	18 ml
EA4	Acetone: petroleum ether	2:3	22 ml
EA5	Acetone: petroleum ether	2:3	21 ml
EA6	Acetone: petroleum ether	2:3	23 ml

Then the column was eluted by using acetone: petroleum ether (2:3) as eluting solvent continuously. A total of 5 fractions were collected. The volume collected and the ratio of solvent used is given in Table 2.

Antibacterial and Antifungal Property of Samples

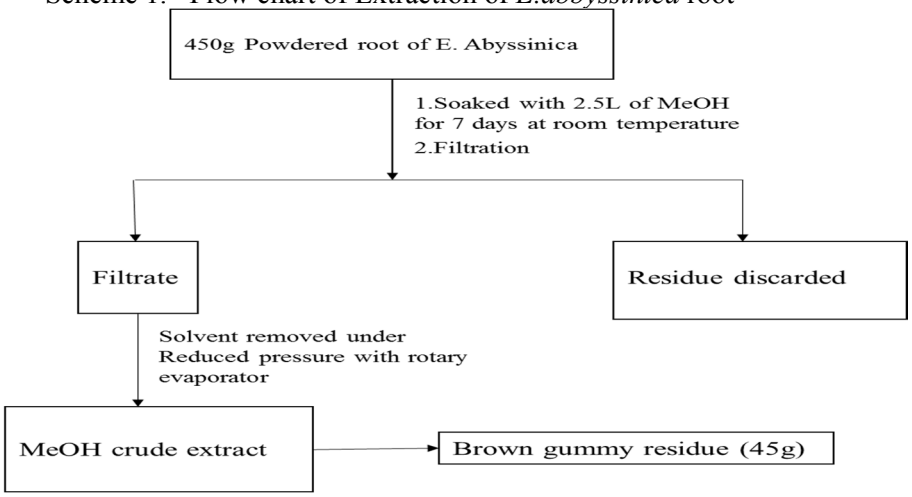
Muller Hinton (MH) agar medium was prepared by adding yeast extract (0.5 g), tryptophan (1 g), sodium chloride (1 g) and bacterial grade agar (2.5 g) in distilled water (100 ml). Then the agar medium was sterilized by autoclaving at a pressure of 15 psi and 120^{o}C temperature for 30 min. This medium was transferred into sterilized Petri dishes in a laminar air flow. After solidification of media, overnight culture of *S. aurous* (gram positive), *S. boydi* (gram negative) and *C. albicans* (fungus).was spread separately on the solid surface of the media. Sterile discs were kept on these inoculated plates with the help of sterile forceps. Sample of EA1 (2.17g), EA2 (1.2g), EA3 (4.9g), and EA4 (3.7g) *isolated* compounds were dissolved by acetone, and chloroform solvents respectively. (60 µl) solutions were placed on these discs and were incubated at 37 ^{o}C for 24 h for bacteria and 48hrs at 28^{o}C for fungi in a bacterial incubator. The zone of inhibition (ZOI) that appeared around the disc was measured and recorded as the antibacterial effect of the isolated compounds [15] (EA1, EA2, EA3, and EA4).

3. Result and Discussions

The plant material (root of *E. abbyssinica*) was collected by Ato Tadese Belay. The air dried root was first crashed to powder before extraction.

Extraction of the plant material

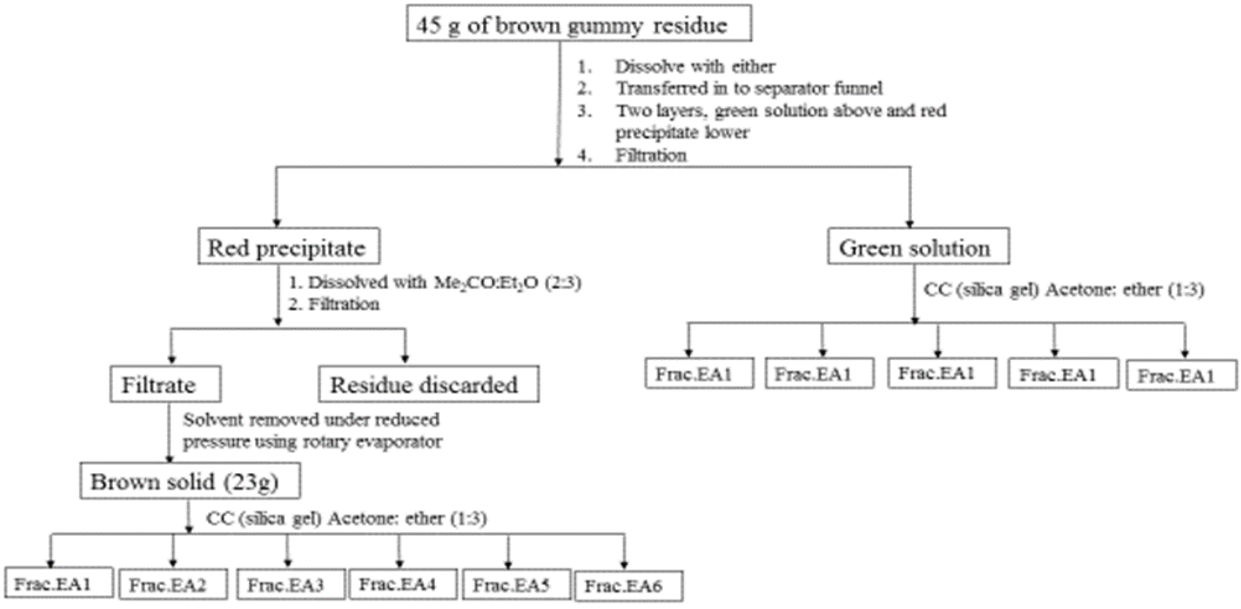
Scheme 1. Flow chart of Extraction of *E.abbyssinica* root



Isolation of Compounds from Extracts of Root of *E. abbyssinica*

The following flow chart shows that the separation scheme.

Scheme 2. Flow chart of isolation of *E. Abbyssinica* components.



Thin layer chromatography (TLC) was performed on alumina plates coated with silica gel (Merck silica gel 60 F 254, layer thickness 0.2 mm). The TLC method was used to monitor the purity of the isolate. Visualization was accomplished by UV-light (wavelength 254 nm). A total of 11 fractions with TLC spots and retention factor (R_f) values are given in Table 3.

4. Characterization

Isolated compound characterized by using following spectroscopic techniques namely UV-Vis, FTIR, ^{1}H NMR

^{13}C NMR and DEPT.

UV-Visible Spectra

UV-Vis absorption spectrum (Figure-2) of EA2 was showed a band around 287nm which may be due to the presence of conjugated carbonyl chromo-phore with phenyl group.

Table 3. R_f value and color of fractions of *E. abbyssinica* extractions.

Fractions	Solvent system	Ratio	TLC	R_f value
EA1	Acetone: petroleum ether	2:3	one spot	0.95
EA2	Acetone: petroleum ether	2:3	one spot	0.56
EA3	Acetone: petroleum ether	2:3	one spot	0.92
EA4	Acetone: petroleum ether	2:3	2 spot	-----
EA5	Acetone: petroleum ether	2:3	5 spot	-----
EA6	Acetone: petroleum ether	1:3	3 spot	------
EA7	Acetone: petroleum ether	1:3	4 spot	------
EA8	Acetone: petroleum ether	1:3	4 spot	------
EA9	Acetone: petroleum ether	1:3	5 spot	------
EA10	Acetone: petroleum ether	1:3	3 spot	------
EA11	Acetone: petroleum ether	1:3	5 spot	------

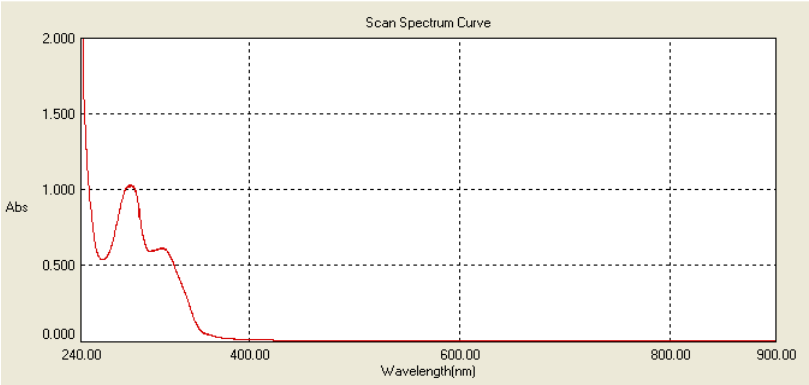


Figure 2. UV-Vis Spectrum of EA-2

FT-IR Spectra

The FTIR spectrum (Figure-3) revealed a stretching band at 1720 cm^{-1} indicating that the presence of carbonyl group of conjugated ester. The carbon-hydrogen stretching vibration which occurs at 2926 and 2851 cm^{-1} is due to the stretching vibration of the aliphatic C-H. It also showed stretching vibration band at 1605 and 1463 cm^{-1} indicating the presence of benzene carbon double bond, carbon (C=C) stretching. The stretching vibration band that occurs at 1276 cm^{-1} may be due to the presence of C-O stretching and Vibration band that occur at 1379 cm^{-1} is due to the presence of C-H bonding. The stretching vibration band at 735 and 650 cm^{-1} indicates that benzene C-H oops (out of plane bending). The spectrum around 900 cm^{-1} is ascribed to the skeletal and CH_2 rocking nodes.

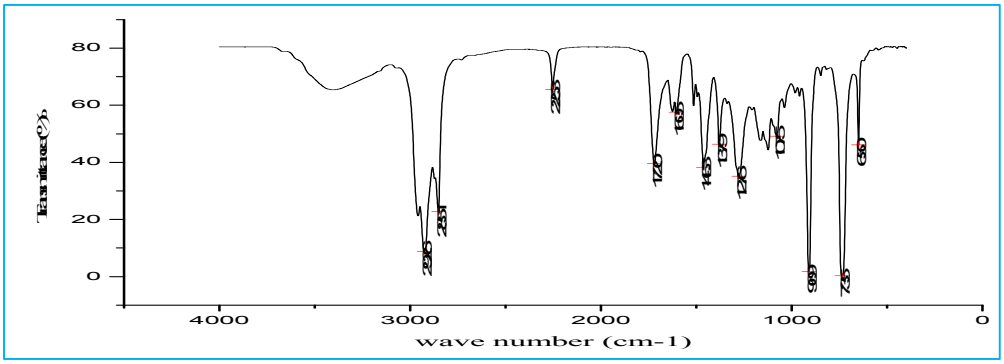


Figure 3. IR Spectrum of EA-2

Table 4.^{1}H NMR spectral analysis and multiplicity of EA-2

S. no.	δ ^{1}H	Remark
1	7.75-7.7(4H,dd)	Benzene carbon ortho and para protons
2	7.05-7.3 (1H, m)	Benzene carbon meta proton
3	6.4 (2H, t)	O-CH_2
4	1.5-4.0 (21H, m)	C_2-C_{11}
5	0.8 -1.4 (6H, t)	CH_3

[1]HNMR spectra

The [1]HNMR (Figure-4) the signals at δppm 7.70 – 7.75 (1H, dd), δ ppm 7.57 (1H, dd) and δppm 7.2 – 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 0.9 to 1.2 are stands for methyl protons. The δ ppm values and their corresponding multiplicity of the compound were given in Table 4.

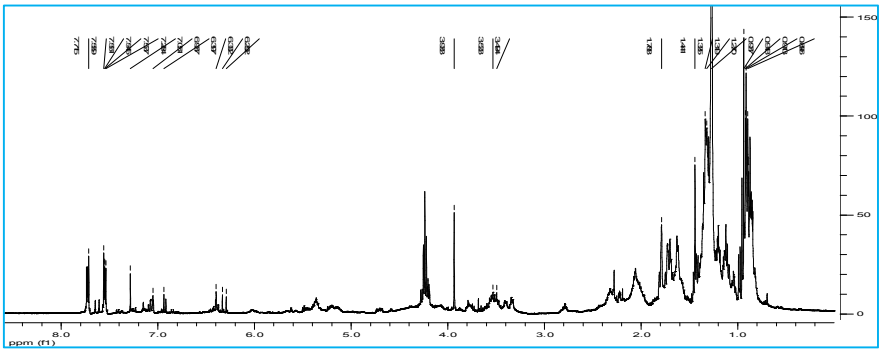


Figure 4. [1]H NMR Spectrum of EA-2

[3] C NMR and DEPT- 135 Spectrum

Proton decoupled ^{13}C spectrum (Figure-5) showed well resolved resonances of 23 carbon atoms with certain impurities. The multiplicity of each carbon atom was determined by using DEPT-135 Spectrum which showed the presence of two methyl, thirteen methylene, six methine, and two quaternary Carbons clearly. This indicates the presence of 38 hydrogen atoms in the molecule. The signals at δppm 167.8 of ^{13}C NMR 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}C NMR spectrum; because of an equivalent ^{13}C atoms appear at the same chemical shift value (the ortho and Meta carbons are doubled by symmetry). The signals at δppm 133.2, 130.9, 129.9 and 128.8 indicates the presence of mono substituted benzene. The ^{13}C NMR spectrum revealed that the signal at δppm 167.8 and 130.9 are due to quaternary carbons which do not appear in the DEPT-135 spectrum since it has no attached hydrogen.

Table 5. The ^{13}C NMR and DEPT-135 spectral analysis of EA-2

δ ^{13}C	DEPT-135	Remark
167.84 (carbon e)	-	Carbonyl carbon
130.94 (carbon a)	-	Quaternary carbon
129.90 (carbon b)	129.90	benzene carbon
128.83 (carbon c)	128.83	benzene carbon
132.40 (carbon d)	132.40	Benzene carbon
68.19 (carbon 1)	68.19	Methylene carbon
38.73 (carbon 1')	38.73	Methylene carbon
36.00 (carbon 9)	36.00	Methine carbon
34.69 (carbon 8)	34.69	Methylene carbon
34.57 (carbon 10)	34.57	Methylene carbon
30.37 (carbon 6)	30.37	Methylene carbon
29.74 (carbon 11)	29.74	Methylene carbon
29.65 (carbon 5)	29.65	Methylene carbon
29.41 (carbon 4)	29.41	Methylene carbon
28.94 (carbon 2)	28.94	Methylene carbon
26.97 (carbon 7)	26.97	Methylene carbon
26.06 (carbon 3)	26.06	Methylene carbon
23.02 (carbon 12)	23.02	Methylene carbon
19.48 (carbon 2')	19.48	Methylene carbon
14.2 (carbon 3')	14.2	Methyl carbon
14.1 (carbon 13)	14.1	Methyl carbon

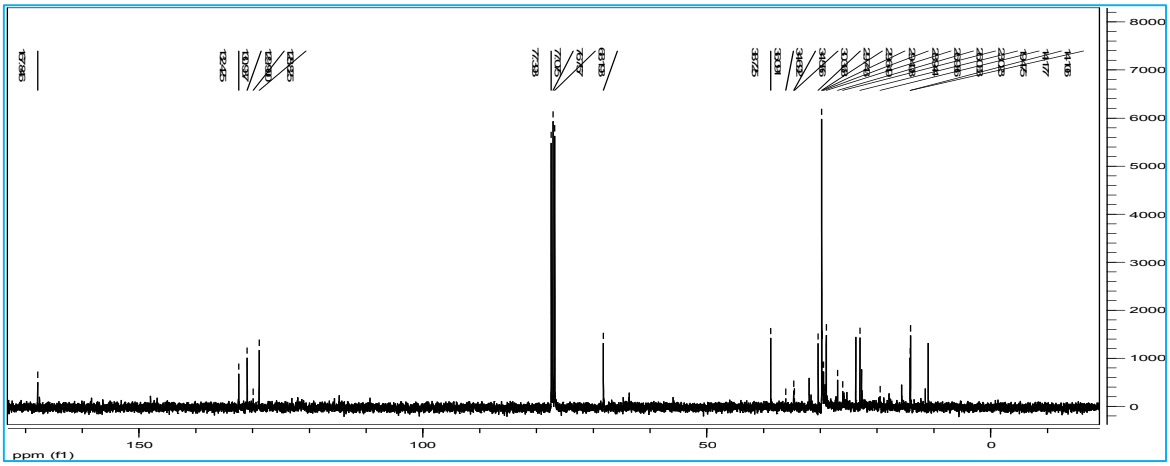


Figure 5. ^{13}C NMR Spectrum of EA-2

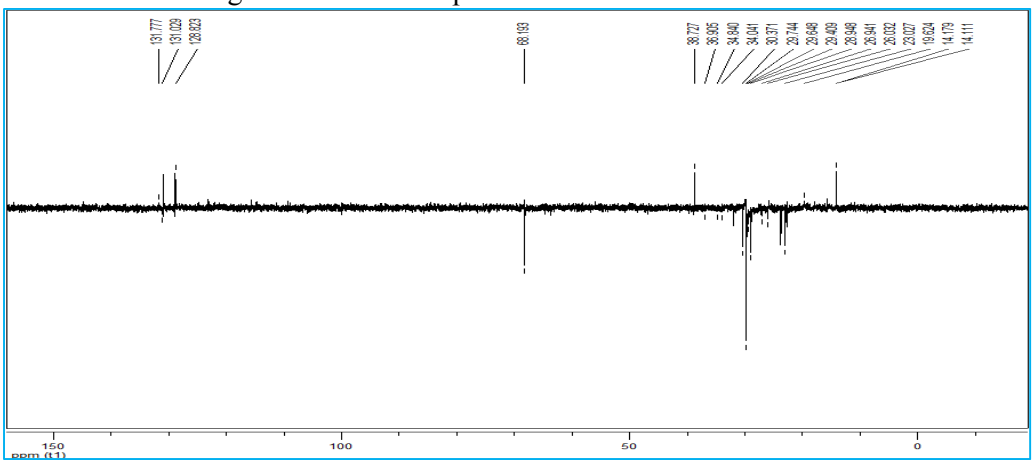
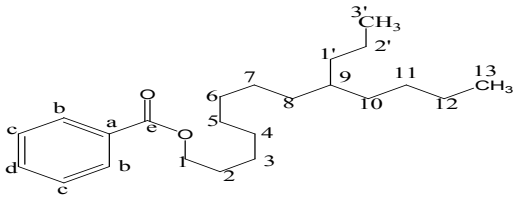


Figure 6. DEPT-135 Spectrum of EA-2

The proposed compound EA2 which isolated from root of *E. abbyssinica* by methanol extraction was 9-propyltridecyl benzoate.



Microbial and Fungal Activities

Agar well diffusion is a method in which the bacterial suspension is spread on the surface of agar in the plate by mixing together with the agar media using vortex. Then wells were made by using sterile cork borer. Finally each well was filled with 60µL doses of the test materials (extracts) and the test material diffuses out of the well onto the agar. Using this technique extracts of *E. abbyssinica* using different solvents were checked for their antibacterial activity against *S.boydi* (G-) and *S.aurous* (G+**)** bacterial pathogen. EA1–EA4 extracts were subjected to antibacterial test against *S.boydi* and *S.aurous* and all of them were found to be active with different inhibition zone as shown in table 6. EA2 and EA4 were found to be highly active than EA1 and EA3 to the both *S.boydi* (G-) and *S.aurous* (G+**)** bacteria pathogen with inhibition zone of 22mm and 23mm for *S.boydi and* 23mm and 24mm for *S.aurous* respectively on agar well diffusion method which is greater inhibition zone than that of the Tetracycline (which is used as a positive control) having inhibition zone of *S.boydi* 21mm and *S.aurous* 21.5mm. The inhibition zone of EA1–EA4 extracts are shown in the following table 6.

Table 6. Antimicrobial and antifungal efficiency of extracts EA1-EA4 against test organism (pathogen).

Methanol extracts	Inhibition zone(mm)	
	S.aurous (G-)	*S.boydi* (G+)
EA1	15	16
EA2	22	23
EA3	18	20
EA4	23	24
Tetracycline(standard)	21	21.5

Figure 7. Antibacterial test results of *E. abbyssinica* with *S. aureus* and *E.coli* after 24 h of incubation. As the sample of *E. abbyssinica* 1-4

Agar well diffusion is a method in which the bacterial suspension is spread on the surface of agar in the plate either by swabbing or by mixing together with the agar media. Then 60 µL doses of the test materials are applied on paper discs, placing them directly onto the agar surface. Finally the test material diffuses out of the disc onto the agar. Using this technique, extracts of the *E. abbyssinica* using different solvents were checked. Methanol extracts of the root of *E. abbyssinica* were subjected to antifungal test against *C. albican*. But all of the extracts of *E. abbyssinica* root were inactive to *C. albican,* which may be due to the reason that *E. abbyssinica* root is inactive to this fungal pathogen or may be the method of extraction is not suitable for extracting the active constituent of the *E. abbyssinica* root against *C. albican* fungi.

5. Conclusion

In conclusion, methonal extract of *E. abbyssinica* (9-propyltridecyl benzoate) compound is identified by using UV-Visible, FTIR ^{1}H NMR and ^{13}C NMR spectroscopy. The extracted *E.abbyssinica* compound showed a very good antibacterial properties against the bacterial pathogen *S. aurous* (gram positive), *S.boydi* (gram negative). The antifungal activity of all the extracts was found to be inactive against the *C. albicans* pathogen. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

6. References

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