

Isolation, Characterization and Antibacterial Activities Evaluation of *Rumex abyssinicus* Rootbark Extracts

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Abstract

The bioactive phytochemical constituents present in the plant play a significant role in the development of medicines and drug discovery. *Rumex abyssinicus* is one of the plants that grow in Ethiopia and different parts of this plant are used as a traditional medicine. The aim of this research was isolation and characterization of bioactive compounds from *Rumex abyssinicus* rootbark. The plant material was collected from Oromia region around Ilu Ababor Zone, Metu College of teachers' education campus during February 2016. The dried and powdered plant material was subjected to sequential solvent extraction using maceration technique to prepare the crude extract which was directly used for antibacterial studies. The lists of solvents taken for the extraction were n-hexane, chloroform, acetone and methanol. Antibacterial evaluation of crude extracts of the plant rootbark were screened using *in vitro* method against four pathogenic bacteria species namely Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. These sequentially obtained solvent extracts were filtered and concentrated under reduced pressure. The results showed the acetone extract followed by methanol crude extracts showed inhibitory effects against all of the tested bacterial strains, but the isolated compounds showed inhibitory effects on *S. aureus* and *P. aeruginosa* bacterial strains. The acetone extract was subjected to column chromatographic separation that led to isolation of two compounds. The structures of these compounds were characterized with the help of spectroscopic methods (IR and NMR). The isolated compounds were characterized as diisobutyl phthalate (**RA-2**) (isolated for the first time from the plant) and Emodin (**RA-3**) based on spectroscopic data and in comparison with literature reports.

Keywords: Antibacterial Activity; Characterization; Extraction; Emodin; Diisobutyl Phthalate; Isolation; *Rumex abyssinicus*; Structural Elucidation.

INTRODUCTION

In Ethiopia medicinal plants play major supplementary roles to the limited modern health care available. The rich traditional knowledge of the people has over the centuries led to the application of plants for food, medicine and other uses. In the country traditional health care is culturally deep rooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective medicinal value for some ailments of human and domestic animals. Thus, medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country. The major reasons why medicinal plants are demanded in Ethiopia are due to culturally linked traditions, the trust the communities have in the medicinal values of traditional medicine and relatively low cost in using them. Medicinal plants obtained from wild habitats are found in different natural ecosystems of the forests, grasslands, woodlands, wetlands, in field margins and garden fences, as weeds and in many other microhabitats from where they are harvested when the need arises (Gidey, 2010).

Rumex abyssinicus is widely spread medicinal plant in the highlands of tropical Africa and is a common weed of cultivated lands or disturbed grounds ranging from North Africa to Ethiopia (Mekonnen *et al.*, 2010). It is one of medicinal

plants used by Ilu Ababor people (Southwest Oromia, Ethiopia) and its root as well as rootbark is used to lower blood pressure, heal wound and treat stomach ache. The rhizomes yield a yellow and red dye which is also used to impart a red color to the feet and hands of women of this area. The present study was aimed at the evaluation of antibacterial activity, and thereby carrying out the isolation and characterization of bioactive compounds from the rootbark of *Rumex abyssinicus*.

Materials and Methods

Chemicals

Chemicals that were used during this study include petroleum ether, hexane, chloroform, acetone, ethyl acetate and methanol. Silica gel (100-120 mm mesh size), TLC plates, vanillin, Dimethyl sulfoxide (DMSO), Mueller Hinton agar, nutrient broth and standard antibiotic gentamicine. All the chemicals and reagents used were of analytical grade.

Apparatus and Instruments

Apparatuses like rotary evaporator (RE 52-F), round bottom flask (50 mL, 100mL, and 250 mL), volumetric flask, measuring cylinder, pestle and mortar, filter papers, cotton , weighing balances, water bath, oven for drying purpose, reflux condenser and glass columns for column chromatography were used for the study. Spectral recording were done using

Bruker 400 MHz avance NMR spectrometer and Deuterated DMSO as solvent. Infrared (IR) spectra (KBr) were obtained from Perkin-Elmer BX infrared spectrometer (400-4000 cm^{-1}). All spectroscopic analysis were carried out at the Department of Chemistry, Addis Ababa University.

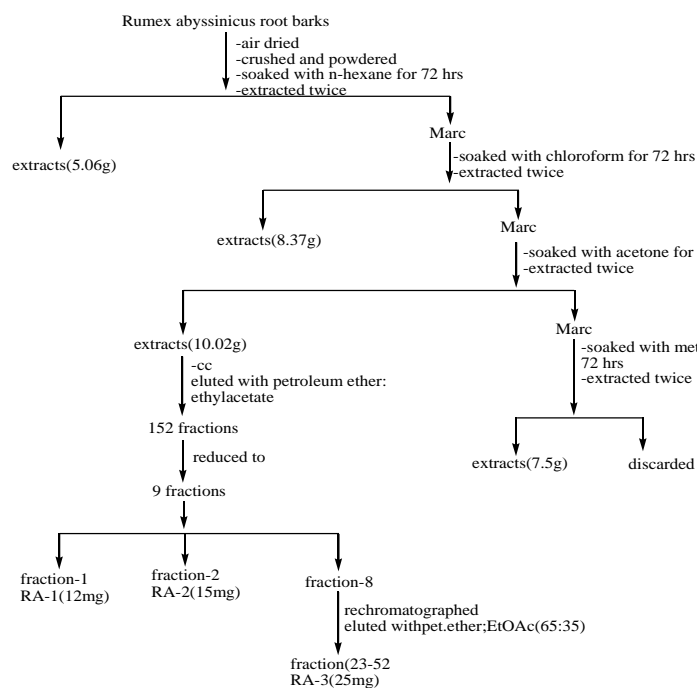
Collection and preparation of plant material

Fresh rootbark of *Rumex abyssinicus* were collected from Oromia region around Ilu Ababor Zone, Metu College of Teachers' Education campus during February 2016. The collected plant rootbark were washed with distilled water and shade dried in laboratory at room temperature. The dried part was grounded with manual grinder so as to enhance effective contact of solvent with plant material and make more homogenizing of the sample, increasing the surface area, and facilitating the penetration of solvents into cells of the plant powder. Botanical identification was made by Mr. Etana Tolesa (a botanist) and a specimen was deposited (voucher number Ze01) in the Herbarium of Department of Biology, Wollega University.

Extraction of Rumex abyssinicus Rootbark

Air dried powdered rootbark of *Rumex abyssinicus* was sequentially extracted with n-hexane, chloroform, acetone and methanol. The successive extraction with solvents of increasing polarity ensures that wide polarity

range of compounds were extracted. Six hundred seventy gram (670 g) of fine powdered material was soaked with hexane (3.35 L) for 72 hours (with manual shaking) and extracted with hexane. The combined extracts were filtered and concentrated by means of a rotary evaporator. The marc obtained from filtration of the hexane extract was then soaked with chloroform twice for 72 hours and extracted with the same solvent and the combined extracts were filtered and concentrated. The marc was then soaked with acetone twice for 72 hours and extracted and the combined extracts were filtered and concentrated. The methanol extract was also prepared in the same way as the chloroform and acetone extract (**Scheme 1**). The mixtures were filtered first by cotton plugged followed by Whatman No.1 filter paper and residual solvent in each gradient extract was removed using Rotary evaporator under reduced pressure. Fractions were then being placed in an oven at not more than 40 °C for about 48 hours to remove any residual solvent. The resulting semidried mass of each fraction were stored at 4 °C until used for experiments (Jones *et al.*, 2005).



Scheme 1: Extraction procedure followed to extract the plant material

I. Antimicrobial Assay of the Crude Rootbark Extracts

Under the antimicrobial assay, of course, evaluation of antibacterial activities were done for both crude rootbark extracts and isolated compounds in which both cases were explained in result and discussion sections.

Test strains

Microorganisms used for determination of antibacterial activities of the crude plant extracts were: gram positive (*Staphylococcus aureus*), gram negative (*Escherichia coli*), gram negative (*Pseudomonas aeruginosa*) and gram negative (*Salmonella thyphimurium*).

Preparation of test samples

The test solution were prepared by dissolving known weight of crude extract by serial dilution methods (400, 200, 100 and 50 mg) in 1 mL of Dimethyl Sulfoxide (DMSO) to achieve final stock concentration of 400, 200, 100 and 50 mg/mL respectively.

Preparation of fresh inoculums

Stock bacterial cultures were maintained at 4 °C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of bacterial cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) that was incubated without agitation for 24 hours at 37 °C. A cell suspension of each organism was freshly prepared by transferring isolated colonies selected from a 24 hours agar plate in to a broth and the suspension turbidity adjusted to a 0.5 McFarland turbidity standard (1x10⁸ CFU/mL) in sterile saline solution (Priya and Ganjewala, 2007).

Disc diffusion method

About 100 µL of bacterial suspensions obtained above was spread over the 90 mm Petri dishes containing Mueller-Hinton agar using a sterile cotton swab. Then six mm diameter sterile discs were bore red by cork borer on the surface of the inoculated Agar in Petri dishes, and 20 µL each test solutions were applied onto the discs. After addition of test solutions on the discs, the extract was allowed to diffuse for 5-10 minutes

and then the plates were kept in incubator at 37 °C until 24 hours. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler (Nascimento *et al.*, 2000). The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

II. Isolation and characterization of compounds

The crude acetone extract of *Rumex abyssinicus* rootbark was subjected to chromatographic separation using petroleum ether/ethyl acetate solvent combinations. Solvent system for elution of the column was determined after carrying out the TLC analyses in various combinations of petroleum ether, chloroform and ethyl acetate with different polarity. Among the solvent combinations tried petroleum ether/ ethyl acetate combination showed better separation of the visible spots. From the crude 10.02 g of acetone extract, 2.5g was adsorbed onto 10 g of silica gel. Then the dry sample was applied into the column that was packed with 100 g silica gel (100-120 mesh) slurry dissolved in petroleum ether. The column was eluted with petroleum ether and ethyl acetate mixture in different combination with increasing polarity (in the ratio 98:2, 95:5, 90:10, 85:15, 80:20, 75:25 up to 60:40 %) which is tabulated in Table 1 below.

Table 1: Column purification for acetone extract of *Rumex abyssinicus* rootbark

S. No	Fractions	Solvent ratio	Volume of solvent used (mL)
1	1-17	Petroleum ether 98:2 ethyl acetate	340
2	18-34	Petroleum ether 95:5 ethyl acetate	340
3	35-51	Petroleum ether 90:10 ethyl acetate	340
4	52-68	Petroleum ether 85:15 ethyl acetate	340
5	69-85	Petroleum ether 80:20 ethyl acetate	340
6	86-102	Petroleum ether 75:25 ethyl acetate	340
7	103-119	Petroleum ether 70:30 ethyl acetate	340
8	120-136	Petroleum ether 65:35 ethyl acetate	340
9	137-152	Petroleum ether 60:40 ethyl acetate	340

A total of 152 fractions each with 20 mL were collected in small beakers and TLC analysis was done. According to TLC profiles, these fractions were reduced to 9 fractions. This was done by comparison of observed spots on TLC and their R_f values, i.e., fractions with similar R_f values were mixed. The developed spots on TLC plates were visualized by spray of vanillin. Among reduced 9 fractions, fraction 1 eluted with petroleum ether: ethyl acetate (98:2) resulted single spot on TLC and showed an R_f value of 0.46 (98:2 petroleum ether: ethyl acetate). The product was yellow-orange crystalline solid and designated as Compound **RA-1** (12 mg). Fraction 2 eluted with petroleum ether: ethyl acetate (95:5) showed single spot on TLC and showed an R_f value of 0.35(98:2 petroleum ether: ethyl acetate). The product was yellowish crystalline solid and designated as compound **RA-2**(15mg). Fraction-8 was rechromatographed on silica gel eluting with petroleum ether: ethyl acetate (65:35) and a total of 52 fractions were collected each with 20 mL. Fractions 23-52 were combined and the product was yellowish- red crystal solid (25mg) and its R_f value was determined as 0.57 in petroleum ether: ethyl acetate (65:35). The compound was labeled as **RA-3**. The isolated compounds were then characterized by the spectral techniques namely, IR, ^1H NMR and ^{13}C NMR.

Results and Discussions

Fractional Extraction of Rootbark Extracts of *Rumex abyssinicus* Plant species

The rootbark part of the plant species was successively extracted with n-hexane, chloroform, acetone and methanol by using maceration. These extracts after removal of the solvents were used for the both antibacterial assay and isolation. The yield from different solvent extracts is presented in Table 2 below.

Table 2: Percentage yields of *Rumex abyssinicus* rootbark extracts

Extraction solvents	Mass extracted (g)	% Yield
n-hexane	5.06	0.755
Chloroform	8.37	1.249
Acetone	10.02	1.5
Methanol	7.5	1.119

Evaluation of antibacterial activities of crude extracts from *Rumex abyssinicus* rootbark

The inhibitory activity of the four different extracts of *Rumex abyssinicus* against some human pathogenic bacteria was presented in Table 3. Among the four different extracts tested, acetone extract showed good antibacterial activity followed by methanol extract, whereas no significant antibacterial activity was observed in n-hexane and chloroform extracts (Appendix-11). While the standard test gentamicine showed the maximum antibacterial activity compared to the four

extracts, the negative control DMSO did not inhibit any of the bacteria tested. The acetone extract exhibited antibacterial activity with zone of inhibition ranging from 17mm to 21mm at 100mg/mL concentration depending upon bacterial species. The most susceptible organism in the present investigation was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Salmonella thyphimurium*, whereas *Escherichia coli* were found to be most resistant bacteria against all the extracts tested (Table 3). The effectiveness of the extracts varies with the kind of bacteria used in the study. These differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate their cell wall and cell membrane structures.

The results suggest that the acetone extract has broad spectrum against bacterial pathogens. Therefore, the crude acetone extract of *Rumex abyssinicus* rootbark was selected as the best candidate for chromatographic isolation of compounds.

Table 3: Antibacterial activity of different solvent extracts of *Rumex abyssinicus* rootbark against bacterial strains

Bacteria strain	Conc. mg/mL	Diameter of zone in mm				Ge (10 µg)
		HE	CE	AE	ME	
<i>Escherichia coli</i>	100	na	na	17	16	25
<i>Staphylococcus aureus</i>	100	na	na	21	20	25
<i>Salmonella thyphimurium</i>	100	na	na	19	13	21
<i>Pseudomonas aeruginosa</i>	100	na	na	19	19	21

Note; na= not active; HE-hexane extract; CE-chloroform extract; AE-acetone extract; ME-methanol extract; Ge-Gentamicin

Structural elucidation of the isolated compounds

The structures of compounds (RA-2 and RA-3) those isolated from acetone extract of *Rumex abyssinicus* rootbark were discussed below. But the structure of RA-1 was not analyzed due to small (insufficient) quantity of the sample for NMR spectroscopic techniques. The compounds were characterized using spectroscopic techniques (IR and NMR) and the structural elucidation was done by comparing the observed spectra with the reported data of these compounds in the literature.

The structural elucidation of RA-2

The IR spectrum of RA-2(Appendix-1) showed the absorption band at 2924cm⁻¹ corresponding to the stretching of C-H of alkane. The band at

1670 cm^{-1} most probably belongs to the carbonyl frequency and the band at 1250cm^{-1} showed the C-O stretching carbonyl frequency of ester. The broad band around 3400 cm^{-1} indicates the presence of hydroxide group. This may be due to the moisture absorbed by the sample or by KBr.

^1H NMR spectrum of compound **RA-2** (Appendix-2) showed a peak at δ 2.5 observed due to solvent(DMSO- d_6); the peak at δ 0.95(H-1) indicates protons of methyl groups. Signal at δ 4.00(H-3) indicates protons that were attached with oxygenated carbon, the peak at δ 7.74 (H-6) double doublets and δ 7.69 (H-7) triple triplets might be proton on aromatic ring, and a peak at δ 1.90 (H-2) septets indicates the signal of methine carbon flanked between methylene and methyl carbons. The ^1H NMR data of **RA-2** is shown in Table 4 below.

Table 4: ^1H -NMR data of **RA-2**

Position of H	Observed data of ^1H -NMR (δ in ppm)
H-1	0.95 d J = 6.8 Hz
H-2	1.95 m
H-3	4.00 d
H-6	7.74 dd
H-7	7.69 tt

The ^{13}C NMR (Appendix-3) spectrum showed one methyl carbon signals at δ 19.30, one carbonyl carbons at δ 167.41(C-4), one

oxygenated carbon at δ 71.60 (C-3) and one methyne carbon at δ 27.65(C-2).

The DEPT-135 spectrum (Appendix-4) of **RA-2** indicates the presence of one methyl proton at δ 19.30 and two aromatic C-H signals at δ 132.01(C-6) and δ 129.13(C-7). Additionally two quaternary carbon signals are shown at δ 167.41(C-4) and δ 132.14(C-5).

Table 5: The observed ^{13}C NMR and DEPT-135 spectroscopic data of (DMSO- d_6) **RA-2**

Position of carbon	^{13}C NMR data	DEPT-135 data	multiplicity
C-1	19.30	19.30	CH_3
C-2	27.65	27.65	CH
C-3	71.60	71.60	CH_2
C-4	167.41	-	Quaternary
C-5	132.14	-	Quaternary
C-6	129.13	129.13	CH
C-7	132.01	132.01	CH

The HSQC spectrum (Appendix-5) of **RA-2** correlates the chemical shift of proton with the directly bonded carbon atom. The HSQC spectrum showed connectivity between the following pairs: $\delta_{\text{H}}0.95$ and $\delta_{\text{C}} 19.30$ (C-1); $\delta_{\text{H}}1.90$ and $\delta_{\text{C}} 27.65$ (C-2); $\delta_{\text{H}}4.00$ and $\delta_{\text{C}}71.60$ (C-3); $\delta_{\text{H}}7.74$ and $\delta_{\text{C}}129.13$ (C-6); $\delta_{\text{H}}7.69$ and $\delta_{\text{C}} 132.01$ (C-7).

Table 6: ^{13}C NMR (DEPT-135) and HSQC spectroscopic data of **RA-2**

Position of carbon	¹³ C NMR data of RA-2(δ in ppm)	HSQC spectra data of RA-2
C-1	19.30	C-1↔H-1
C-2	27.65	C-2↔H-2
C-3	71.60	C-3↔H-3
C-6	129.13	C-6↔H-6
C-7	132.01	C-7↔H-7

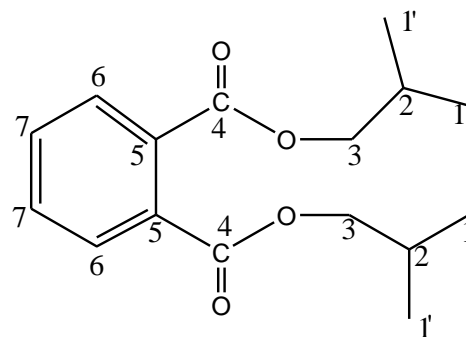


Figure 1: Proposed structure of **RA-2**

The HMBC (Appendix-6) was also used to propose the structure of a compound by correlation of proton with carbon that is two or three bond away.

Table 7: Observed correlation in HMBC spectroscopic data of **RA-2**

Position of carbon	¹³ C NMR data of RA-2(δ in ppm)	HMBC spectra data of RA-2
C-1	19.30	H-1→C-1,C-2 and C-3
C-2	27.65	H-2→C-1 and C-3
C-3	71.60	H-3→C-1,C-2 and C-4
C-4	167.41	-
C-5	132.14	-
C-6	129.13	H-6→C-7 and C-4
C-7	132.01	H-7→ C-6

As can be deduced from all spectroscopic data above, the possible structure of RA-2 is given in Figure 1 and is named as diisobutyl phthalate.

The structural elucidation of RA-3

The IR spectrum of **RA-3** (Appendix-7) showed that the absorption band at 3424cm⁻¹ corresponding to the stretching vibration peak of the hydroxyl (OH) group. The band at 2936cm⁻¹ indicates the C-H stretching and the bands at 1680 cm⁻¹and 1650cm⁻¹ correspond to the un-chelated and chelated carbonyl carbons absorption, respectively.

The ¹H-NMR of this compound (DMSO-d₆, Appedix-8) exhibited two hydroxyl protons at δ11.87 (OH-1) and δ 11.95(OH-8), two meta coupled doublet at δ 6.49(H-7) and δ 6.98(H-5), two broad singlet signals at δ 7.01(H-2) and δ7.31 (H-4) and one methyl group at δ 2.33. The result is also comparable with the ¹H-NMR spectra data of Emodin from literature as shown in Table 8 below.

Table 8: ¹H NMR data of **RA-3** with reported data of Emodin

H	¹ H-NMR data of RA-3(δ,ppm)	Reported data of Emodin* (δ,ppm)
OH-1	11.87 s	12.01 s
H-2	7.01 br.s	7.18br.s
CH ₃ -3a	2.33 s	2.40 s
H-4	7.31 br.s	7.50 br.s
H-5	6.98 d J=2.4 Hz	7.20 d,J=1.6Hz
H-6	-	-
H-7	6.49 d J=2.4 Hz	6.60 d,J=1.6Hz
OH-8	11.95 s	12.15 s

*Data from Zinaye and Fiseha, 2008 AAU electronic library

The ¹³C NMR (Appendix-9) spectrum showed one methyl carbon signal at δ 21.92 and three oxygenated carbons at δ 161.78(C-1), δ 165.95 (C-6) and δ 164.83 (C-8) .

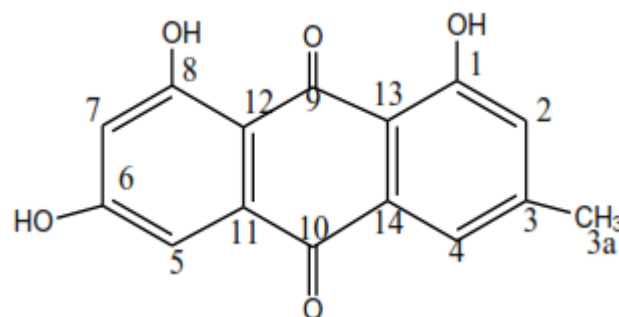
The ¹³C NMR also shows two carbonyl carbon at δ 189.91(C-9) and δ 181.50(C-10).One methyl substituted carbon at δ 148.55(C-3). According to the DEPT 135(Appendix-10) spectra the peaks at δ 135.26(C-11), δ 109.16(C-12), δ 113.53(C-13) and δ 132.96(C-14) belong to the quaternary carbons and the peaks at δ124.41(C-2), δ120.78(C-4), δ109.18(C-5) and δ108.24(C-7) characteristics of one proton carbons. The chemical shift of carbon at δ 165.95(C-6) indicate the presence of hydroxyl group on the benzene ring at this position.

Table 9: Comparison of the observed ¹³C NMR and DEPT-135 spectroscopic data (DMSO-d₆) of RA-3 and Emodin

Position of carbon	Observed data (δ in ppm)	Observed DEPT-135 data (δ in ppm)	Reported data of Emodin* ¹³ C NMR	Reported data of Emodin *DEPT-135	Nature of carbon
C-1	161.8	-	161.9	-	Quaternary
C-2	124.4	124.4	124.6	124.6	CH
C-3	148.5	-	148.8	-	Quaternary
C-4	120.8	120.8	120.9	120.9	CH
C-5	109.2	109.2	109.3	109.3	CH
C-6	165.9	-	166.1	-	Quaternary
C-7	108.2	108.2	108.4	108.4	CH
C-8	164.8	-	164.9	-	Quaternary
C-9	189.9	-	190.1	-	Quaternary
C-10	181.5	-	181.9	-	Quaternary
C-11	135.3	-	135.6	-	Quaternary
C-12	109.3	-	109.4	-	Quaternary
C-13	113.5	-	113.8	-	Quaternary
C-14	132.9	-	133.3	-	Quaternary
3a-CH ₃	21.9	21.9	21.9	21.9	CH ₃

*Data from Zinaye and Fiseha, 2008 AAU electronic library

From the observed and reported literature data of ¹H NMR, ¹³C NMR and DEPT-135 RA-3 is a hydroxyl anthraquinone known as Emodin.



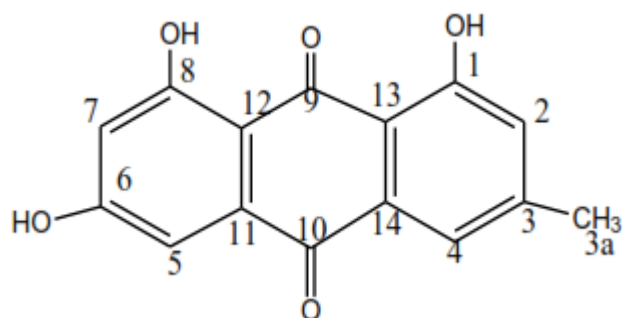


Figure 2: The Structure of Emodin

Emodin forms the basis of a purgative anthraquinone derivative and from ancient times has been widely used as a laxative compound (Evans, 1996; Bruneton, 1999). It is believed that the presence of hydroxyl groups in position 1 and 8 of the aromatic ring system is essential for the purgative action of this compound (Paneitz and Westendorf, 1999).

Evaluation of antibacterial activities of the isolated compounds

The antimicrobial activities of two isolated compounds (**RA-2** and **RA-3**) were carried using four human pathogenic bacteria species namely *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli* by disc diffusion method. The growth inhibition zones (in mm) of the compounds were given in Table 10. The result indicated that **RA-2** and **RA-3** showed a better

antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial species. On comparison basis, the antibacterial activity of **RA-3** showed more than **RA-2** against the two of the bacterial strains (Table 10). Thus these results prove the antimicrobial potential of Emodin isolated from the *Rumex abyssinicus* root bark and provide additional support for the use of this plant as traditional medicine. Both **RA-2** and **RA-3** showed no activity against *Escherichia coli* and *Salmonella typhimurium* bacterial strains. Similar to the crude extracts, the antibacterial activities of the isolated compounds were lower than that of the reference drug (gentamicine) against all bacterial species used in the experiment. However, as evidenced from the activity of the acetone crude extract compared to individual isolated compounds there are still more active and unidentified secondary metabolites. The most susceptible organism was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* whereas *Escherichia coli* and *Salmonella typhimurium* were found to be most resistant bacteria against all the isolated compounds (Appendix-12).

Table 10: Antibacterial activity of isolated compounds by disc diffusion methods.

Bacteria strain	Conc. mg/mL	Diameter of zone in mm			
		RA-2	RA-3	Ge(10 µg)	DMSO
<i>Escherichia coli</i>	100	na	na	24	na
<i>Staphylococcus aureus</i>	100	11	14	25	na
<i>Salmonella thyphimurium</i>	100	na	na	21	na
<i>Pseudomonas aeruginosa</i>	100	10	12	21	na

Note; na = not active; Ge-Gentamicin

Conclusion

From the results of this study two compounds (**RA-2** and **RA-3**) were isolated from the crude acetone extract rootbark of *Rumex abyssinicus*. The compounds were identified as diisobutyl phthalate and Emodin based on spectroscopic (IR and NMR) data as well as literature reports. The isolation of **RA-2** (diisobutyl phthalate) is reported for the first time from *Rumex abyssinicus* plant. The observed antibacterial activities of the crude extract and isolated compound **RA-3** could justify the traditional use of the plant for the treatment of bacterial infection.

Recommendation

As evidenced from the activity of the crude extract compared to individual isolated

compounds there are still more active and unidentified secondary metabolites. Further work is needed to isolate more bioactive compounds from the rootbark of *Rumex abyssinicus*. In addition, further test is recommended on large number of bacterial strains to conclude as the potential candidate in the development of antibacterial drugs.

References

- Bruneton, J. (1999). Pharmacognosy, phytochemistry, medicinal plants, 2nd ed. Paris, France: Intercept Ltd and Lavoisier Publishing.
- Evans, W.C. (1996). Treas and evans pharmacognosy, 4th ed. London, UK: W.B. Saunders Company.
- Gidey, Y. (2010). Use of traditional medicinal plants by indigenous people. *J. Med. Plants Research*, 4 (17), 1799-1804.
- Jones, W. P.; Kinghorn, A.D. (2005). Natural product isolation: Extraction of Plant Secondary Metabolites. *Methods in Biotechnology*, 20, 323-351.
- Mekonnen, T.; Urga, K.; Engidawork, E. (2003). Evaluation of the diuretic and analgesic Activities of the rhizomes of *Rumex abyssinicus* J. in mice. *Journal of Ethnopharmacology*, 127, 433-439.
- Nascimento, G. F.; Locatelli, J.; Freitas, P.C.; Silva, G.L. (2000). Antibacterial activity

of plant Extract and phytochemicals on antibiotic resistant bacteria. *Braz. Journal of Microbiol.*, 31, 247-256.

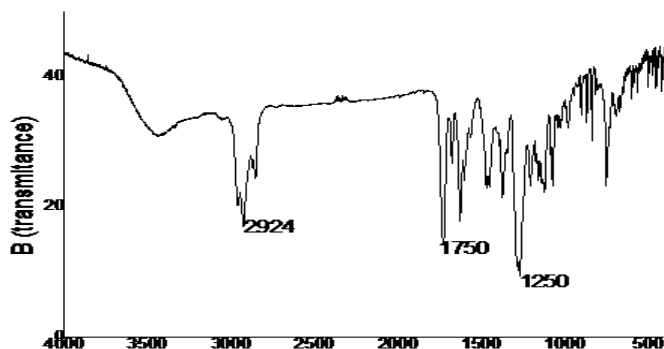
Paneitz, A. and Westendorf, J. (1999). Anthranoid contents of rhubarb (*Rheum undulatum* L.) and other *Rheum* species and their toxicological relevance. *Europ. Food Res. Technol.*, **210**:97-10.

Priya, K. and Ganjewala, D. (2007). Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of *Nyctanthes arbor-tristis* L. *Research Journal of Phytochemistry* 1, 61-67.

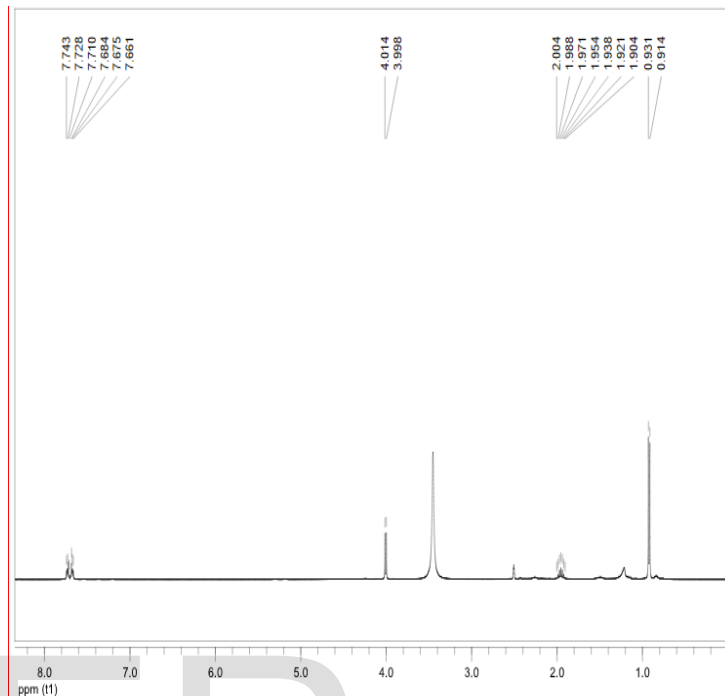
Zinaye, B. and Fiseha, A. (2008). Phytochemical investigation on the root of *Rumex abyssinicus* (MAKMAKO), AAU electronic library.

APPENDIX

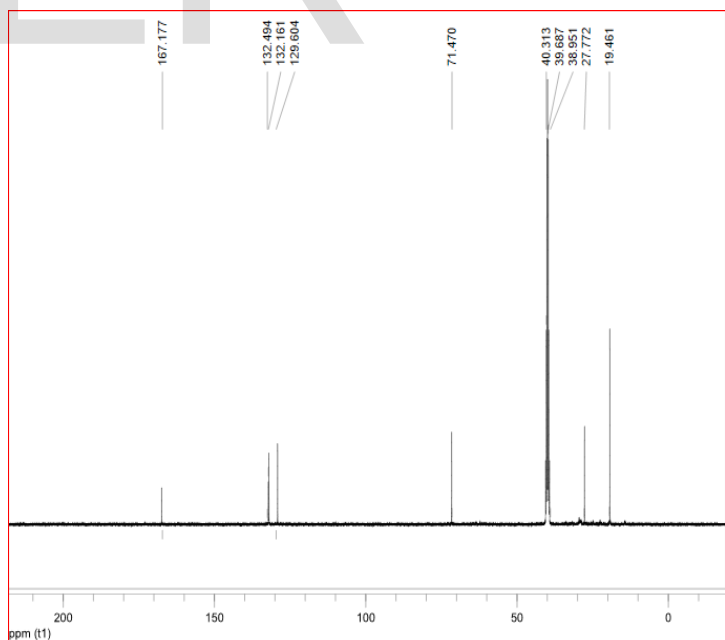
Appendix 1: IR spectrum for RA-2



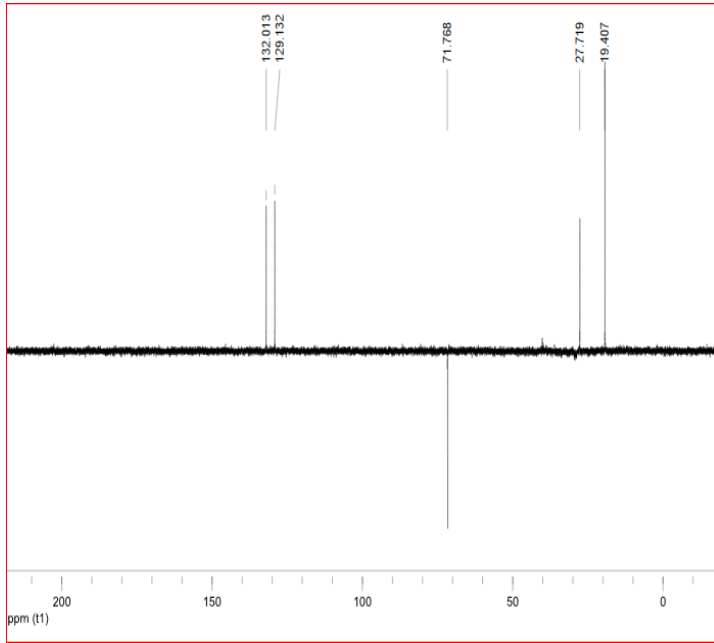
Appendix 2: ¹H NMR (DMSO-d₆) spectrum of RA-2



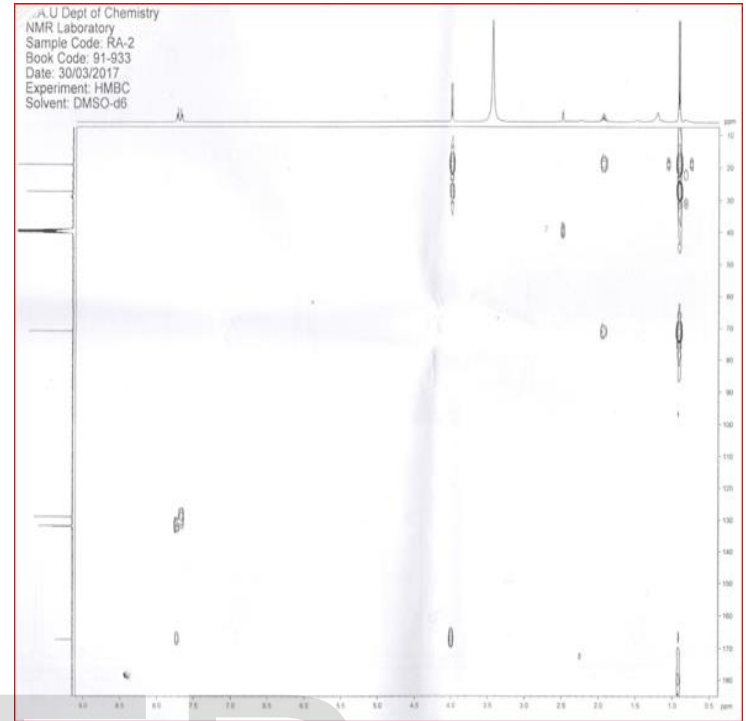
Appendix 3: ¹³C NMR (DMSO-d₆) spectrum of RA-2



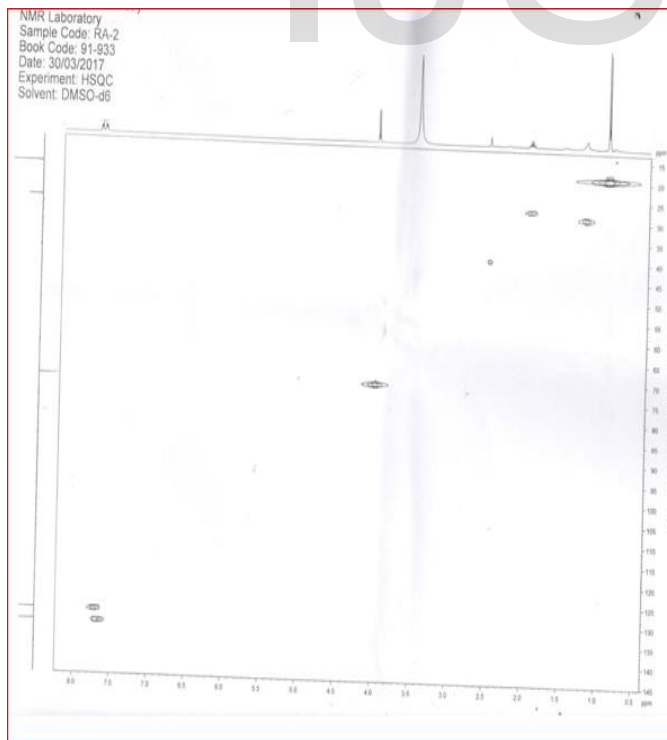
Appendix 4: DEPT-135(DMSO-d₆) spectrum of RA-2



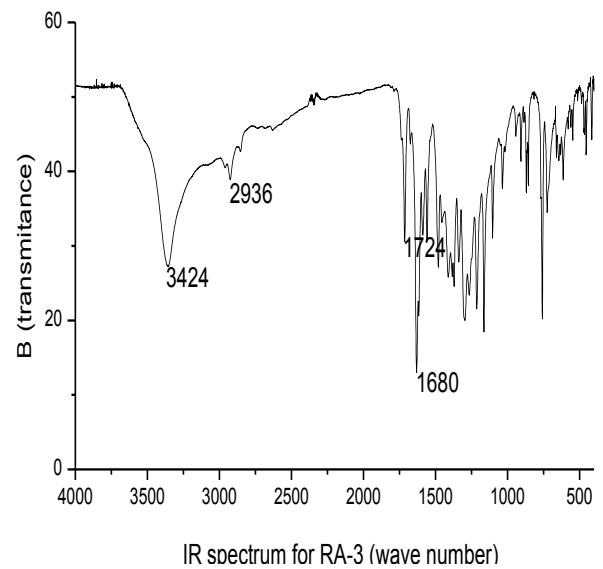
Appendix 6: HMBC (DMSO-d₆) spectrum of RA-2



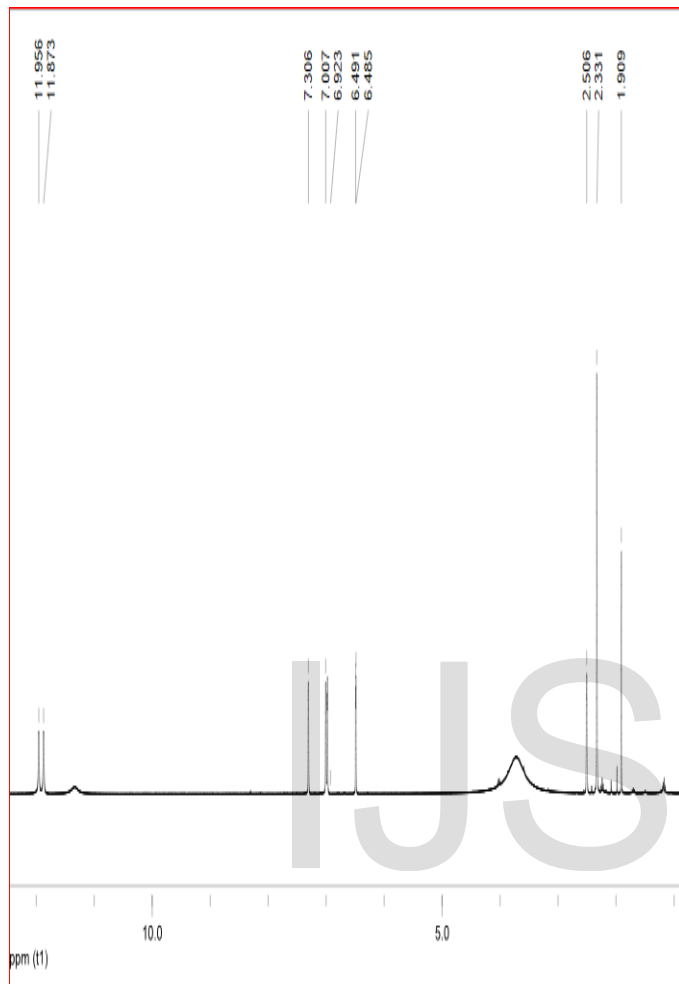
Appendix 5: HSQC (DMSO-d₆) spectrum of RA-2



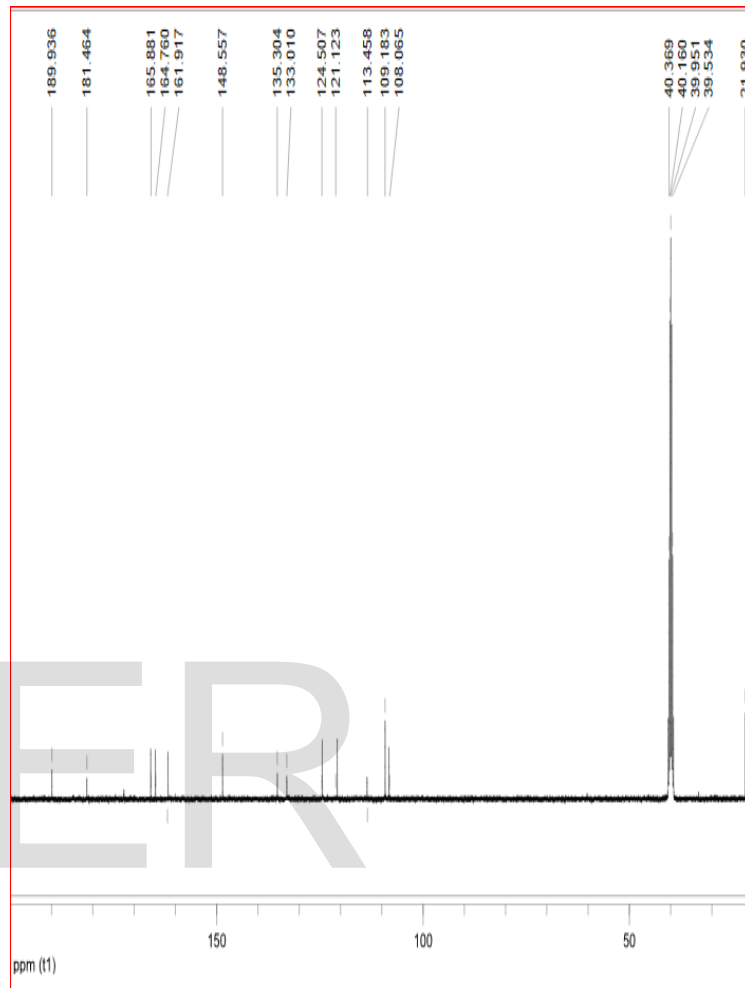
Appendix 7: IR spectrum for RA-3



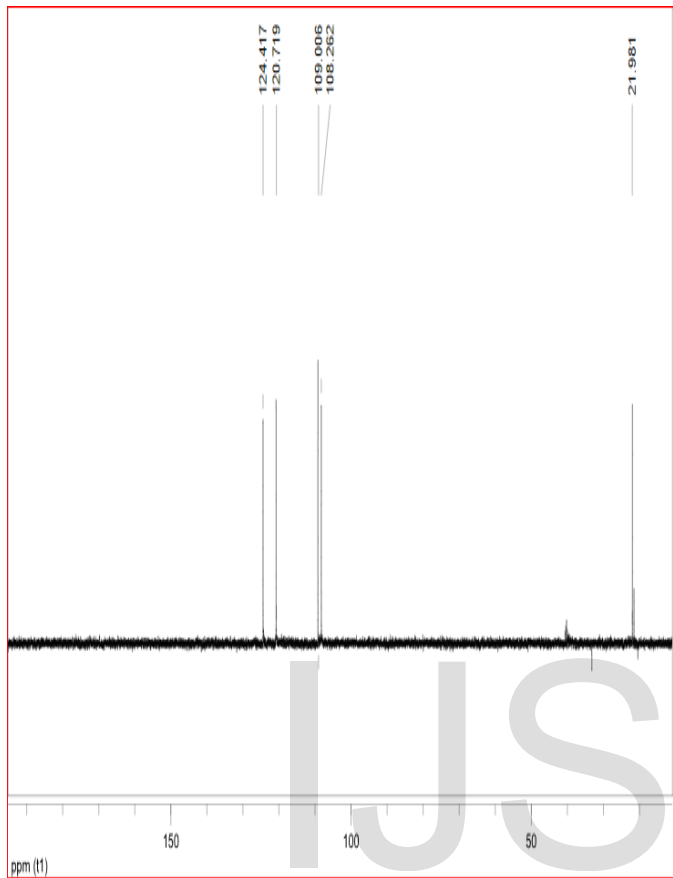
Appendix 8: ^1H NMR (DMSO- d_6) spectrum of RA-3



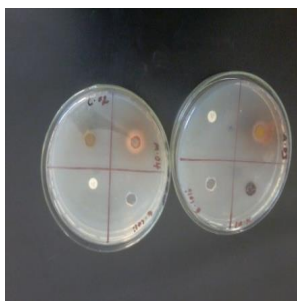
Appendix 9: ^{13}C NMR (DMSO- d_6) spectrum of RA-3



Appendix 10: DEPT-135(DMSO-d₆) spectrum of RA-3



Appendix-11 Bioactivity pictures of the crude extracts



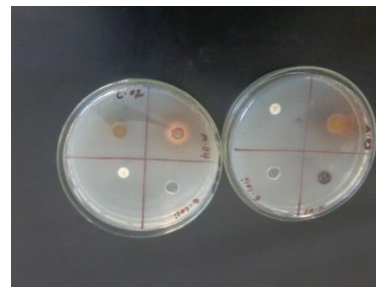
Salmonella typhimurium



Staphylococcus aureus



Pseudomonas aeruginosa



Escherichia coli

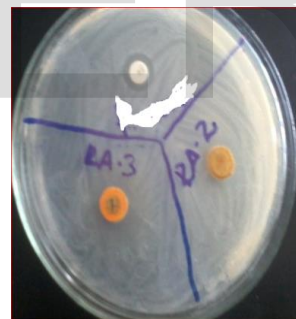
Appendix-12 Bioactivity pictures of the isolated compounds by disc diffusion



Staphylococcus aureus



Salmonella Typhimurium



Escherichia coli



Pseudomonas aeruginosa