ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Graduate Project (Chem.774)

Phytochemical Investigation On The Root of Rumex Abyssinicus (Makmako)

By
Bizuayehu Zinaye

Advisor: Dr. Ashebir Fiseha

In Partial Fulfillment of the Requirements for Master of Science Degree in Chemistry

---

July 2008

PHYTOCHEMICAL INVESTIGATION ON THE ROOT OF *RUMEX ABYSSINICUS* (MAKMAKO)

A GRADUATE PROJECT SUBMITTED TO THE OFFICE OF
RESEARCH AND GRADUATE PROGRAMME OF
ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

BIZUAYEHU ZINAYE

JULY, 2000

ADDIS ABABA UNIVERSTY
SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION ON THE ROOT OF *RUMEX ABYSSINICUS* (MAKMAKO)

BY
BIZUAYEHU ZINAYE
DEPARTMENT OF CHEMISTRY
SCIENCE FACULTY
JULY, 2008

APPROVED BY THE EXAMINING BOARD:

Dr. Ashebir Fiseha ______________________________
Advisor

Prof. Ermias Dagne ______________________________
Examiner

Prof. Wendimagegn Mammo ______________________________
Examiner

Dr. Gizachew Alemayehu ______________________________
Examiner

Acknowledgement

I express my sincere gratitude and appreciation for my project advisor Dr. Ashebir Fiseha for taking immense interest and permitting me to freely interact with him for his advice and suggestions related to this project work.

I would like to express my warm thanks to Prof. Wandimagegn Mammo who extends his tremendous support, encouragement and running NMR Spectroscopic data for this project work.

I also owe a deep sense of gratitude to my wife Nigiste Teshome who shouldered alone the burdens of family responsibility, which enable me to concentrate on my study, and this project always it remains with me live her and our children whole hearted cooperation support and best wishes through out my study

I would also like to express my deepest appreciation to my brother Tadesse Zinaye and his family for the encouragement and advice they gave me during my stay in the university.

I am also grateful to all academic staff and technical assistant of chemistry department of, Addis Ababa University.

Table of Contents

Content **Page**

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

ABSTRACT

PHYTOCHEMICAL INVESTIGATION ON THE ROOT OF *RUMEX ABYSSINICUS*

By: Bizuayehu Zinaye

Advisor.Dr. Ashebir Fiseha

The n-hexane: ethylacetate (7:3) extract of *Rumex abyssinicus* afforded two known anthraquinone, chrysophanol and Emodin. The root of *rumex abyssinicus* is traditionally used for the treatment of sexually transmitted disease such as gonorrhea, Lung-TB and leporsy. Structural determination was accomplished by means of IR, UV, 1D and 2D NMR.

1. INTRODUCTION
1.1. General

Phytochemical studies of plants, animals and microorganisms helps in order to have deeper understanding of the factor underlying for the growth, development and differentiation of their chemistry out comes[1]. Plant and animals produce a very large numbers of chemicals. These chemical products can be classified in to primary and secondary metabolites. Primary metabolites are those, which are common to all species and can be subdivided in to proteins, carbohydrates, lipids and nucleic acids. Primary metabolites are essentially ubiquitous and certainly essential for life and they function in cycle [2].

The secondary metabolite are often referred to as ‘’natural products’’ secondary metabolites can be subdivided in to trepenoids, alkaloids, steroids, shikimates and polyketides. The classification is based on the means buy which the materials were made. In principle, secondary metabolites are non- essential to life but they definitely contribute to the species fitness of survival. Different plant and animal species can employ different biosynthetic route to produce the same metabolites [3, 4].

Natural product as the name implies, are those chemical compounds derived from living organisms, plants, animals, insects and the study of natural product accounts in the investigation of their structure, formation, use and purpose in the organisms [5] .Natural products isolated from plants and arid microorganism have been providing noble, clinically active drugs. The key to the success of discovering naturally occurring therapeutic agents rests on bioassay guided fractionation and purification procedures [6].
Individual secondary metabolites may be common to a number of species or may be produced by only one organism. Relative species often have related patterns of secondary metabolite production, according to the secondary metabolite they produce species can be subdivided in to different parts. Such classification is known as chemotaxonomy. Tow plant may be found to have identical physical make up which botanists use for classification, but differ in the secondary metabolites thy produced. Such different

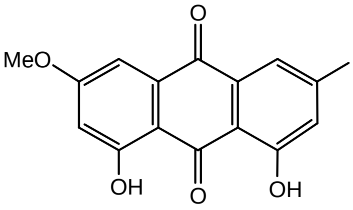
situation is known as chemo types [7]. Natural product chemistry covers the chemistry of naturally occurring organic compounds, their biosynthesis, function in their environment, metabolism and structure elucidation and synthesis [8]**.** Natural products are generally classified according to structure, physiological activity, taxonomy, and biochemical origin [9].

The World Health Organization (WHO) estimated about 80% of the people in the developing countries relies on traditional medicine for primary health care needs, of which a major proportion corresponds to plant extracts [10]. Herbal remedies have been used for centuries but more recently, the compounds that are active have been identified, extracted and purified. Synthetic organic chemists have then been able to produce the molecules in vitro and so produce them on large scales [11].

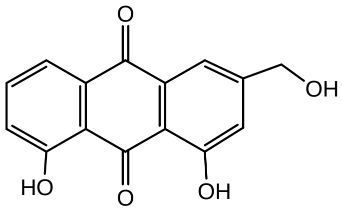
1.2. MEDICINAL USE OF RUMEX SPECIES

Natural product is a major source of drugs, with more than 25% of the pharmaceuticals in use today derived from natural product, therefore interest in natural product research remain strong [12].

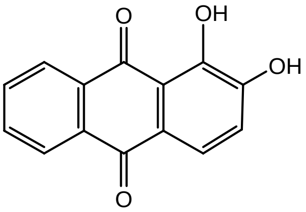
Several species of rumex have important medicinal properties and they have been the subject of several pharmalogical investigation. The most important medicinal compounds in this species are anthroaquinone .The monomeric anthraquinones extracted from rumex species such as physcion (1) , Aloe-emodin (2), Alizarin (3), Chrysophanol (4) from all species of Rumexs , Emodin (5), Fallacinol (7) from root of R.crispus, Xanthorin (8) from the seeds of R.vesicarius, Rubiadin (9) from the root bark of R.epalensis, Islandicin (10) from the seeds of R.nervosus posses anti bacterial activity against streptococcus pyogenes and staphylococcus aureus. They are also used for the treatment of gonorrhoea, mild form of diabetes, lung Tb, leporsy and fever [13].



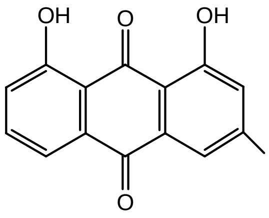
Physcion (1)



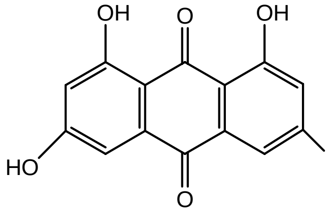
Aloe-emodin (2)



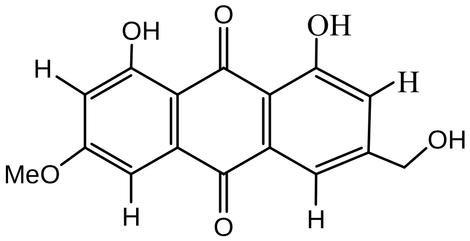
Alizarin (3)



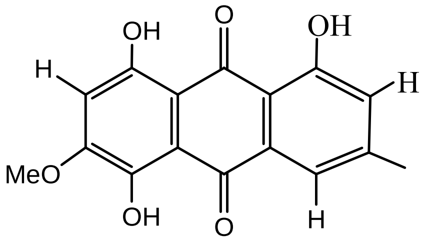
Chrysophanol (4)



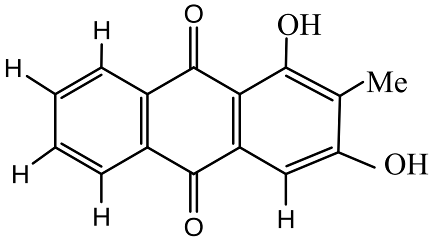
Emodin (5)



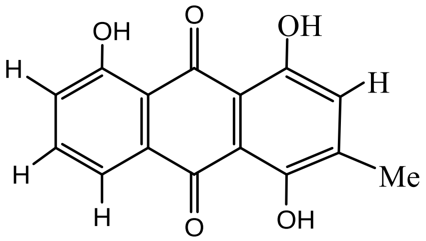
Fallacinol (7)



Xanthorin (8)



Rubiadin (9)



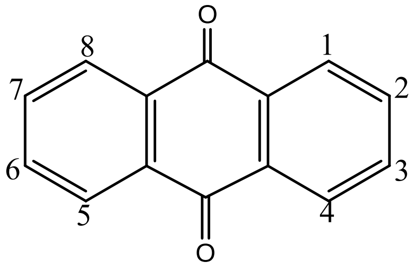
Islandicin (10)

2. ANTHRAQUINONES

2.1. Chemical investigation

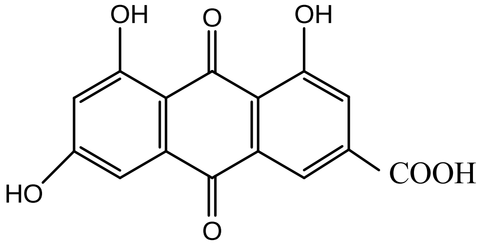
Anthraquinones are widely spreded in lower and higher plant and occur in the animal kingdom. They are present as qlycosides in young plant. Anthraquinones are the largest group of quinones. They have been used as mordant dyes, chemical indicator of acidity or alkalinity and as purgatives. They lost their importance, like so many other natural dyes, with the development of the synthetic dye industry [14, 15].

The quinones typically form strongly colored pigment covering the entire visible spectrum typically, however, they are found in the internal region of the plant and thus do not impart a color to the exterior of the plant. Anthraqinone are widely distributed in nature occurring both free and glycosidic forms, the latter being more common.There are a great many varieties of anthraquinone derivative, found in several plant families. They all show the same basic molecular configuration.



Anthraquinone skeleton

They tend to be found in the form of their qlycosides (the aglycone combined with one or more sugar molecules) which, because of the variety of possible sugars, increase the range even further. Direct anthraqunone derivatives include the following aglycones: Rhein(6) from Rhem, Rumex, cassia supp. Emodin (5) from rhamnus and rumex. Aloe-emodin (3) from Rheum and cassia spp. Chrysophonol (4) from Rheum and rumex species[14].



Rhein (6)

2.2. Identification of Anthraquinones

2.2.1. Color reaction

Crude extracts of certain plant show color formation when treated with certain chemical compounds. Plant extract containing anthraquinone, on addition of aqueous ammonia or sodium hydroxide results in the formation of pink, red, or violet color and these confirms the presence of hydroxyl anthraquinones [17]. Anthraquinones are generally yellow, orange or brown colored solid Anthraquinone can be distinguished from benzoquinones and naphthoquinones as they usually give red solutions on reduction in alkaline solution (in aqueous sodium hydroxide [18].

2.2.2. Ultraviolet – Visible Spectra

The UV-Vis spectra of anthraquinone pigment are dependent on the polyhydroxy or alkoxy derivative present in the molecules. The conjugation of ethylenic group with the carbonyl functional group of the anthraquinone causes for both the ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$) transition to longer wave length to the region 220-260 and 310-330 nm respectively. This is due to the lower energy difference created between the π and π^* orbital by the conjugation of the double bond with the π system of the carbonyl group.

Anthraquinones show intense benzenoid absorption at 240-260 nm, medium absorption at 320-330 nm a strong quinonoid electron transfer band at 270-290 nm accompanied by a weak quinonoid absorption band at 405 nm.

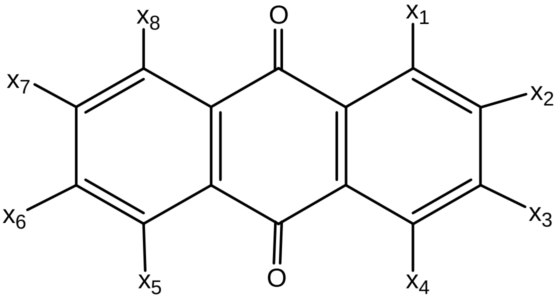
Hydroxyl groups in position 2, 6 or 7 give rise to stronger red shift bands than those with hydroxyl in 1, 4 or 5 position probably because in the former ahydroxyl group is para to one of the carbonyl group**.** The Uv-vis spectra of 1,8 di hydroxyanthraquinone show a peak at 430-450 nm and those of 1,4 di hydroxyanthraquinone show absorption at 470-500 nm [19,20].

2.2.3. Infrared spectroscopy

The presence of the main anthraquinone unit is confirmed by the carbonyl and haydroxy vibrational absorption frequencies. The Vibrational frequency absorption for a carbonyl between 1678 and 1653 cm^{-1} was mostly for anthraquinone with no-alpha hydroxyl group. A second carbonyl band at lower frequency can be observed if the anthraquinone contains a hydroxyl group in the α - position due to chelation and conjugation. If a hydroxyl group is present at the beta position, a sharp hydroxyl stretching band will be seen between 3600 and 3150 cm^{-1}. Alpha hdroxylanthraquinones show a broad and weak absorption band at approximately 3468 cm^{-1}, which corresponds to the streaching frequency of chelated hydroxyl group [21].

From the study of several anthraquinones, Briggs and Co-workers, found the correlation between the carbonyl frequency range and the number of alpha hydroxyl group as shown below.

Table 1: carbonyl absorption frequency of hydrox anthraqinone in cm^{-1} [22].



Anthraquinones	Carbonyl frequencies (cm^{-1})
No alpha OH	1678 – 1653
$x_1 = x_4 =$ OH or $x_1 = x_5 =$ OH	1645 – 1608 (chelated)
$x_1 = x_8 =$ OH	1678-1661(unchelated, C – 10)

	1626-1616 (chelated, C – 9)
$x_1 = x_4 = x_5 = OH$	1616 – 1592 (chelated)
$x_1 = R_4 = x_5$ and $x_8 = OH$	1675 – 1647 (unchelated) 1637 – 1621 (chelated)

2.2.4. Nuclear magnetic resonance spectroscopy

In the structural elucidation of anthraquinone, the NMR spectroscopy (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HMQC and HMBC) are an important techniques for the structural elucidation of anthraquinone. In ^{1}H NMR the α and β proton in 9, 10 – anthraquinone give multiplate centered at 8.07 and 7.67 ppm. respectively, and are modified by substitution. Chelated α - hydroxyl groups at position 1, 4, 5 and 8 are easily distinguished by their appearance at very low field resonance between 11 and 14 ppm. Information about orientation of substitutes around the aromatic ring system can be obtained from the chemical shift positions of an aromatic proton. Since many quinones are phenolic, calculation of theoretical chemical shift by the use of shielding parameters compiled for phenoli compounds can be used to predict the chemical shift of the aromatic protons and hence orientation of substituents [23] **.**

The orientation of substituents can be analyzed from the splitting patterns and the value of coupling constants. Coupling constant show wide variation depending on bond angles and bond hybridization. However, aromatic coupling constants are almost constant ortho coupling constant (J_o) is usually around 7-9 Hz; meta coupling (J_m) is 2 – 3 Hz and paracoupling constant (J_p) is 1 Hz. The coupling with a Para proton is very small it is only the ortho and meta coupling which are considered **[**23] .Any single aromatic proton may exhibit only one of seven possible first – order splitting patterns, as listed in the following table.

Table 2: Peak multiplicity for first – order splitting patterns [24].

Coupling	splitting patterns
Ortho	broad doublet
di-ortho	broad triplate
Meta	narrow doublet
di-meta	narrow triplate
Ortho – meta	double of doublate
Di ortho – meta	triplate of doublate
Ortho – dimeta	doublate of triplate

Anthraquinine of the chrysophanol (4) type show ortho-meta splitting pattern for the proton C-5 or C-7and di-ortho splitting patterns for the proton on C-6 of the benzene ring.. They also show the meta multiplicity for the proton at position 2 and 4, where the signal are broadened by the allelic coupling with the methyl protons at position 3. The Emodin and physion type anthraquinones show only the meta coupling pattern. The other splitting pattern mentioned in **table 2** is not common in anthraquinones.

2.3. Biosynthesis of Anthraquinones

The reaction path leading to a particular natural product is called the biosynthetic path, and the corresponding event is known as the biogenesis. Different plant and animal species can employ dramatically different biosynthetic pathways to produce the some metabolite. This feature can be employed in the classification of plant in terms of their chemotaxonomy [25].

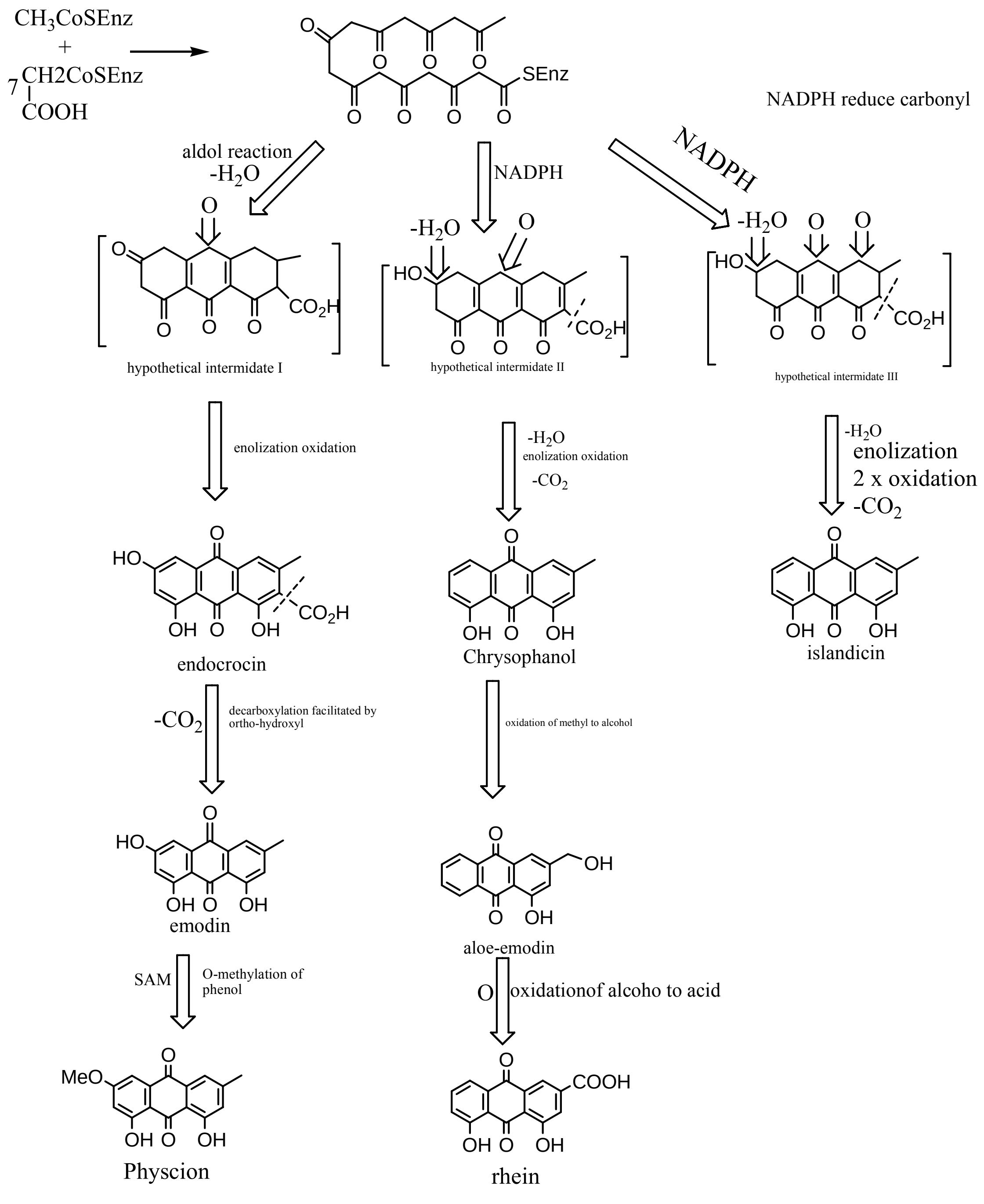
Two techniques have been used for the biosynthesis of anthraqunione from primary metabolites of the cells, these are

1. Polyketide pathway – it starts from acetyl and malonyl CoenzymeA
2. Shikimate pathway-it starts from carbohydrates.

Anthraquione with substitutent on both benzinoid ring follows the polyketide biogenetic route. Here acetic acid and its biosynthetic equivalent, acetyl CoA, occupy central position in the synthesis of anthraqunione. Repeated claisen condensation, reduction carboxylation, oxidation, dehydration, cyclization and aromatization are the reaction path way that leads to anthraquione.The biosynthesis of anthraquione is accomplished by multi enzyme complex.the carboxylation of acetyl CoA to malonyl CoA by a biotin-linked carboxylase is the first step in the pathway. This reaction is mediated by ATP and biotin act as carbondioxide carrier [26, 27].

As a general rule fungal anthraquinone and plant anthraquinone with hydroxyl groups on both ring are derived from polyketides ,whereas plant anthraquinone devoid of hydroxyl group in one ring, example, alizarin come from mixed path way [26,27] **.**

Emodin, chrysophanol, physcion, aloe-emodin and rhein are some of the anthraqunione formed from one acetyl CoA and seven malonyl CoA unit following the polyketide pathway as shown in **scheme 1** below.



Scheme 1: Biosynthesis of Anthraquinone Via the polyketide path way

3. *RUMEX ABYSSINICUS*

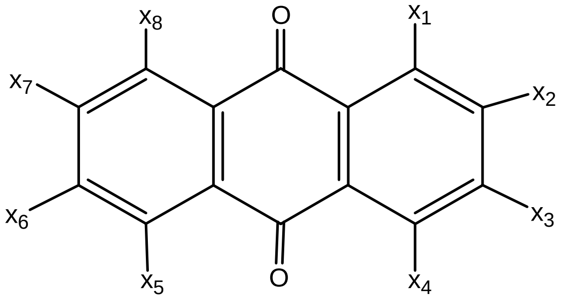
3.1. Botanical background

Rumex abysinicus, which belong to the family polygonaceae, is a perennial herb, up to 3m tall, the leaf of the plant is usually sagittate, and inflorescence and much branched. The rhizomes are used to refine butter and give it a rich yellow color. The family polygonaceae comprises some 5 genera and 200 species largely concentrated in temprate areas of the northern hemisphere and tropical-subtropical mountains. Some of the species which are found in Ethiopia are *Rumex epalensis, Rumex crispus, Rumex vesicarius, Rumex abyssinicus, Rumex nervosus, R.obtusifolius, R.palustaries, R.ecklonianus, R.hydrolapathum, R.scutatus, R.altissimus, R.stenophyllus, R.arifolius, R.patientia, R.confertus, R.sanguineus, R.brownii, R.pulcher,R.acetosa, R.conglumeratus R.acetosella, R.nepalensis, R.maritimus, R.alpinus, R.palustris and R.obtusifolius* [28].

3.2. Secondary metabolite from *Rumex abyssinicus*

Phytochemical study on the genus Rumex has led to the isolation and characterization of different classes of secondary metabolites. Anthraqinones constitute an important class of compounds with important biological properties. These compounds elaborated both by higher and lower plant, are also one of the most well known naturally occurring pigments. They range in color from yellow to orange and also to red. Anthraquinones and related compounds in higher plants are located in all parts of the plant, including root, bark leaves, seeds, and flowers often occur as glycosides. The monomeric anthraquinones extracted from the *Rumex abyssinicus* are summarized in **table 3.**

Table 3: Anthraquinones extracted from Rumex abyssinicus



Name	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	Source	Reference
Emodic acid	OH	H	COOH	H	H	OH	H	OH	root	31
Catenarin	OH	H	Me	H	H	OH	H	OH	root	30
Chrysophanol	OH	H	Me	H	H	H	H	OH	root	[29,30,31]
Emodin	OH	H	Me	H	H	OH	H	OH	*root*	*[29, 30]*
Damnacanthal	OMe	CHO	OH	H	H	H	H	H	*root*	*[30]*
Physcion	OH	H	Me	H	H	OMe	H	OH	root, seed	[29,31]
Rhein	OH	H	COOH	H	H	H	H	OH	seed	[30]

The following anthraquinones are also extracted from the powdered tubers of *Rumex abyssinicus* [31].

1. Plamidin C (emodin-chrysophanol bianthrone)
2. Chrysophanol-8-β-D-glucoside and
3. Emodin-8- β-D- glucoside.

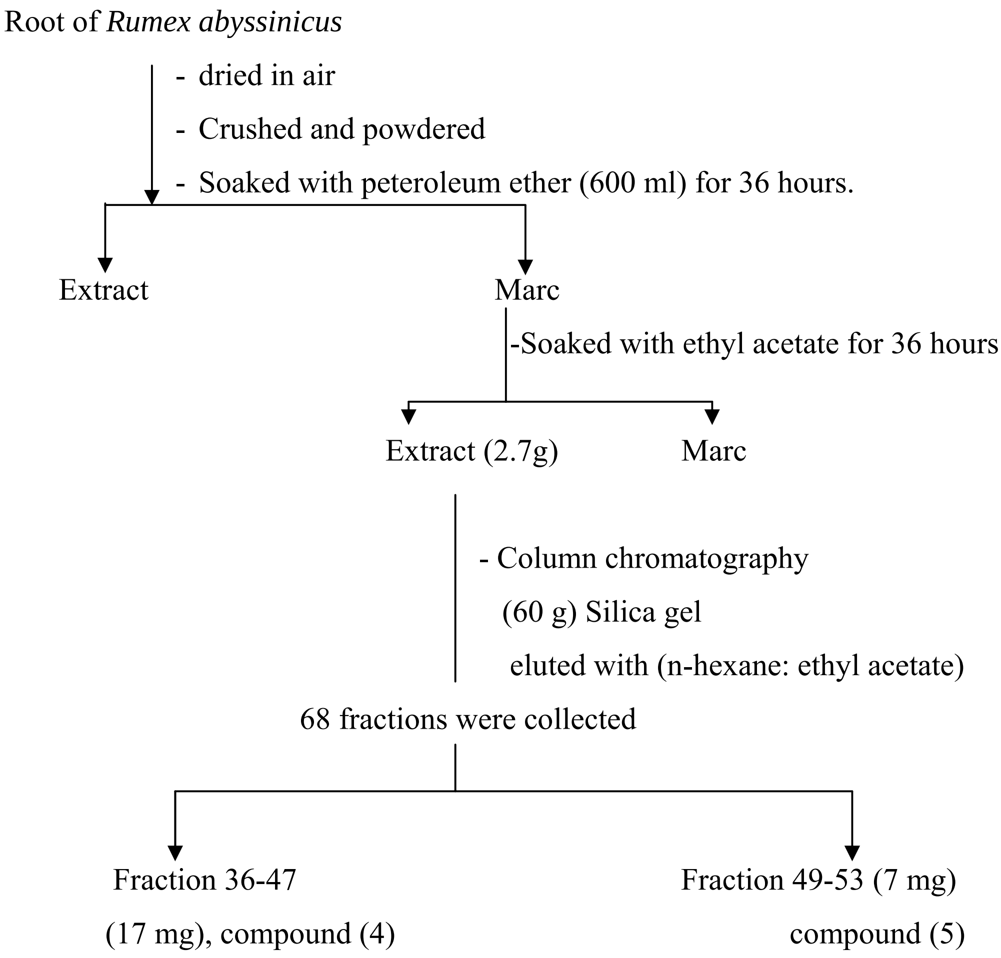
4. OBJECTIVES OF THE STUDY

The main objective of this study was isolation and structural elucidation of constituents of the root of *Rumex abyssinicus*. The plant was selected for this study because it was used in Ethiopia as traditional medicines for the treatment of sexually transmitted disease, diabetes, lung TB and leporsy.

5. Result and Discussion

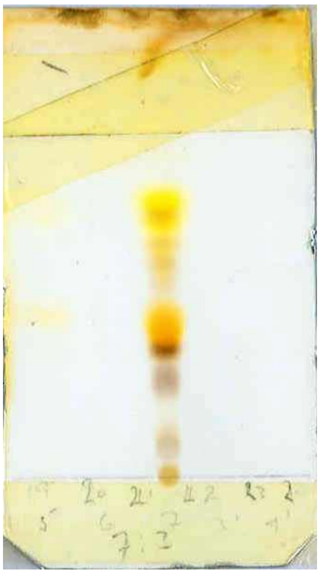
The air dried and powdered root of *rumex abyssinicus* was extracted with ethyl acetate. The brown- gummy extract when developed on TLC (n-hexane: ethylacetate) show more than four spot with visible color. The brown-gummy organic extract was subjected to column chromatography and 68 fractions were collected. This study has resulted in the isolation and characterization of two known anthraquinone,Chrysophanol (4) and Emodin

(5) from the root of the plant material. These Compounds were identified on the basis of both 1 D and 2 D NMR spectroscopic data and comparig the result with reported value from literature. The scheme of the extraction was shown below.



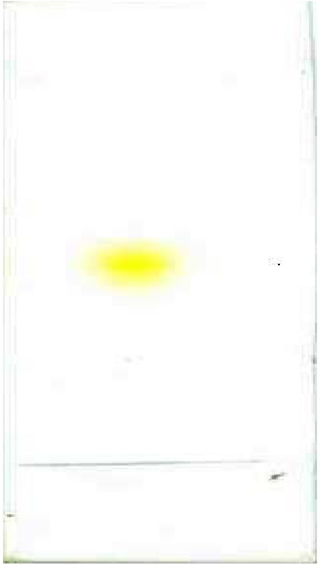
Scheme 2: Method used to extract plant material

The TLC of the crude extract, compound (4) and compound (5) was shown in the following diagrams.



TLC for the crude extract

TLC for compound (4)



TLC for compound (5)

5.1. Characterization of Compound (4)

Compound (4) was obtained as a bright- yellow solid. This compound has RF Valve 0.34 using n-hexane: ethylacetate (7:3) as a solvent. It shows a color change to violet when the TLC was sprayed with 5% methanolic KOH

From UV-Vis spectrum (Appendix 1.1), the absorption band at λ_{max}259, 289 and 433 nm indicate characteristics absorption chromophore center for anthraquinone. The absorption band at 433 nm especially shows the presence of conjugation in the molecules.

In the IR spectrum of the compound (Appendix 1.2), the absorption band at 3468 cm^{-1} was due to hydroxyl groups. The absorption bands at 1672 cm^{-1} and 1629 cm^{-1}indicate the absorption of the un-chelated and chelated carbonyl carbon respectively.

The ^{1}H NMR spectrum (Appendix 1.3) of Compound (4) , Showed a single two intense singlet signal at δ 12.06 and 12.15 PPm indicate the chelated hydroxyl proton resonance attached on C-1 and C-8 of the aromatic ring respectively. The two doublet of doublet signal at δ 7.85 and 7.29 ppm correspond to the aromatic proton attached on C-5 and C-7 respectively. The triplet signal which was integrated for three protons at δ 7.70 ppm indicated for aromatic proton attached on C-6. A broad singlet signal at δ 7.13 ppm is for the aromatic proton attached on C-2 and abroad singlet signal at δ 7.67 ppm is for aromatic proton attached on C-4. A strong intense signal around 2.5 ppm integrated for three protons are for methyl proton attached on C-3 of the aromatic ring. The result obtained here is comparable with ^{1}H NMR spectral data of chrysophanol from literature (**table 4** below) and the proposed structure of compound (4) was shown below.

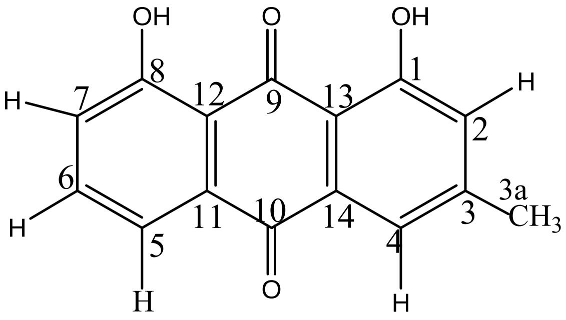


Figure 1: Structure of Compound (4)

Table 4: Comparison of the observed ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data of Compound (4) with the reported value of Chrysophanol [32].

H	Observed data (δ in ppm)	Reported datd (δ in ppm)
1-OH	12.06 s	12.00 s
2-H	7.13 br s	7.09 br s
3a-Me	2.5 s	2.48 s
4-H	7.67 br s	7.64br s
5-H	7.85 dd, J=1.2, 8Hz	7.81 dd, J=1.1, 7.5 Hz
6-H	7.70 t, J=6,1OHz	7.67 t, J=8.3, 7.9 Hz
7-H	7.29 dd, J=1.2, 8Hz	7.29dd,J=1.2, 8.3Hz
8-OH	12.15 s	12.03 s

Furthermore, the melting point of the compound (4) is 196-198^{o}C, which is in agreement with the reported value of chrysophanol (196^{o}C)

The ^{13}C NMR spectrum (Appendix 1.4) showed one methyl carbon at δ 22.26, two oxygenated carbons at δ 162.45(C-1) and 162.75(C-8), two carbonyl carbons at δ192.58(C-9) and 182.02(C-10), one methyl substituted carbon at δ 149.35(C-3), five unsbstituted carbons at (δ 124.57, C-2, 119.94, C-4, 136.96, C-5, 124.37, C-6 and 121.37, C-7)

The DEPT spectrum (Appendix 1.5) of compound (4) indicate the presence of one methyl proton at δ 22.3, and five C-H signal at(δ124.57,C-2, 119.94, C-4, 136.96, C-5, 124.37, C-6, and 121.37, C-7) on the aromatic rings. Since the ^{13}C NMR showed well resolved 15 carbon atom signals and the DEPT spectrum shows five aromatic C-H signal and one methyl carbon signal, the presence of nine quaternary carbon atoms and absence of methylen group can be known from the given spectral data of the compound(4). From this data compound (4) is a monomer and the proposed structure is in agreement with **figure 1** above.

Table 5: Proton decupled ^{13}C NMR and DEPT spectroscopic data of Compound (4)

Position of carbons	^{13}C data (δ in ppm)	DEPT 135 data (δ in ppm)	Remarks
C-1	162.45	-	quaternary carbon
C-2	124.57	124.57	C-H
C-3	149.35	-	quaternary carbon
C-4	119.94	119.94	C-H
C-5	136.96	136.96	C-H

C-6	124.37	124.37	C-H
C-7	121.37	121.37	C-H
C-8	162.75	-	quaternary carbon
C-9	192.58	-	quaternary carbon
C-10	182.02	-	quaternary carbon
C-11	133.68	-	quaternary carbon
C-12	115.90	-	quarternary carbon
C-13	113.77	-	quaternary carbon
C-14	133.32	-	quaternary carbon
3a-CH_3	22.26	22.26	CH_3

The proposed structure was supported with COSY, HSQC and HMBC. The ^{1}H – ^{1}H correlation spectroscopy (COSY) spectrum (Appendix 1.6) shows the correlation of one proton with other proton in the compound.

The COSY correlation indicates the presence of ortho coupling between (H-6 and H-7; H-5 and H-6) and meta coupling between (H-5 and H-7; H-2 and H-4). The other important correlation observed in the COSY spectrum is correlation between CH_3 and H-2 and CH_3 and H-4

The HSQC spectrum (Appendix 1.7) correlates the chemical shift of proton with the directly bonded carbon atom. The HSQC spectrum showed connectivity between the following pairs: δ_H 7.13 and δ_C 124.37(C-2); δ_H 2.5 and δ_C 22.26 (CH_3); δ_H 7.67 and δ_C 121.37 (C-4); δ_H 7.85 and δ_C 119.94 (C-5); δ_H 7.70 and δ_C 115.90 (C-6)

Table 6: ^{13}C NMR (DEPT) and HSQC spectroscopic data of compound (4)

Position of Carbon atom.	^{13}C NMR data of compound (4) (δ in ppm)	HSQC spectral data of compound (4)
C-2	124.37	C-2 ↔ H-2
C-3	22.26	C-3 ↔ Me
C-4	121.37	C-4↔ H-4
C-5	119.94	C-5 ↔ H-5
C-6	115.90	C-6 ↔ H-6
C-7	113.77	C-7 ↔ H-7

Another spectroscopic technique, which was used to propose the structure of compound in addition to the above experiment, is HMBC (Appendix 1.8). In the HMBC spectrum, correlation of proton with carbon that is two or three bond away was seen.

Table 7: Observed correlation in HMBC spectroscopic data of Compound (4)

Carbon No.	^{13}C NMR(δ in ppm)	HMBC
1	162.45	OH-1→C-1, C-2 and C-13
2	124.57	H-2→C-4, 3a-CH_3, C-1 and C-13
3	149.35	-
4	119.94	H-4→C-10, 3a-CH_3, C-13, C-13
5	136.96	H-5→C-6, C-8, C-7,C-10 and C- 11
6	124.37	H-6→C-8

7	121.37	H-7→C-6
8	162.75	OH-1→C-6, C-8, and C-12
9	192.58	-
10	182.02	-
11	133.68	-
12	115.90	-
13	113.77	-
14	133.32	-
3a-CH_3	22.26	3a-CH_3→C-2, C-3 and C-12

From HMBC the proton at δ 7.13(H-2) showed a long- range correlation (^3J) with C-13 (δ 113.77), C-4 (δ 119.94) and ^2J correlation with C-1 (δ 162.45). The proton at δ 7.67(H-4) showed a long- range correlation (^3J) with C-10 (δ 182.02), 3a-CH_3 (δ 22.26), C-13 (δ 113.77) and ^2J correlation with C-14 (δ 133.32). The ^3Jcorrelation the OH proton attached on C-1 at δ 162.45 showed with C-13 (δ 113.77) and C-2 (δ 124.57) helped to construct partial structure of ring A of the Compound.

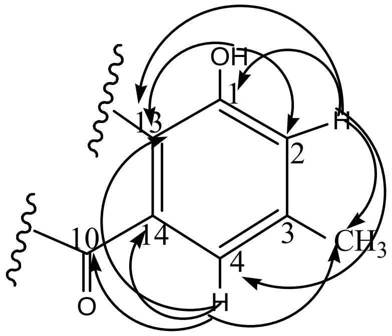


Figure 2: Partial structure of ring A of Compound (4)

The other possible correlation observed in the HMBC spectrum is used to construct the partial structure of the other ring of the compound. The proton at δ 7.85 (H-5) showed along range correlation (^{3}J) with C-7 (121.37) and ^{2}J correlation with C-11 (133.68). The correlation the OH proton attached on C-8 at δ 162.75 showed a long-range correlation (^{3}J) with C-7 (δ 121.37) and ^{2}J correlation with C-12 (δ 115.90) and H-5 with C-12 helped to construct partial structure of ring C of the Compound.

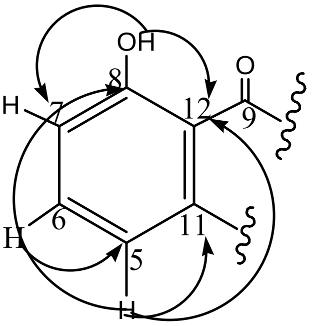


Figure 3: Partial structure of ring C of Compound (4)

To connect the two partial structures the correlation the OH proton attached on C-8 at (δ 162.75) and the OH proton attached on C-1 at (δ 162.45) makes a ^{2}J correlation with C-12 (δ115.90) and C-13 (δ 113.77). The ^{2}J, correlation the proton at δ 7.85 (H-5) showed with C-11 (δ133.68) and the ^{3}J correlation with C-12 (δ 115.90) and C-10 (182.02). The ^{2}J correlation that the proton at δ 7.67 (H-4) showed with C-14 (δ 133.32) and ^{3}J correlation with C-10 (δ 182.02) and C-13 (δ 113.77) are important to construct the two rings and the structure of the compound.

All spectroscopic findings were in good agreement with the suggested structure for compound (4) to be chrysophanol.

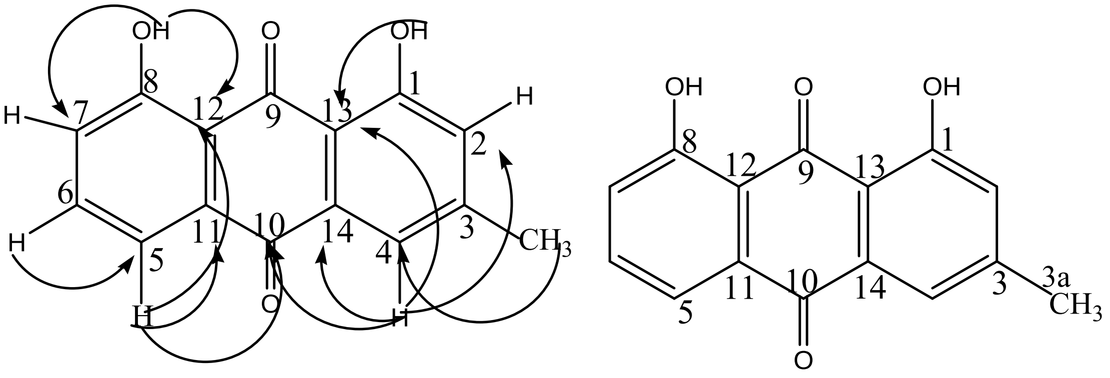


Figure 4: Structure of compound (4)

With selected HMBC

Structure of chrysophanol

5.2. Characterization of Compound (5)

Compound (5) was obtained as orange-red solid. This compound displayed UV-absorption (Appendix 2.1) at λ_{max} 265, 289 and 442 nm, characteristics of anthraquinone.

The IR spectrum (Appendix 2.2) shows band at 1670 and 1625 cm^{-1} correspond to the un-chelated and chelated carbonyl carbons absorption respectively. The ^{1}H NMR (DMSO-d_6, Appendix 2.3) shows two chelated hydroxyl proton resonating at δ 12.01(1-OH) and δ 12.15 (8-OH), two meta coupled doublet at δ 6.60 (7-H) and δ 7.20 (5-H), two broad singlet signals at δ 7.18 (2-H) and 7.50 (4-H) and one methyl group at δ 2.40 ppm. The ^{1}H NMR spectral data indicate the proton on Carbon five (2-H) and Carbon seven (7-H) only shows the meta splitting patterns and ortho-meta splitting is not observed. The ^{1}H NMR spectroscopic data analyses indicate the structure of Compound (5) to be the following.

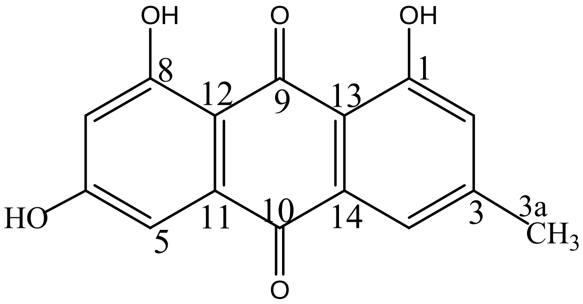


Figure 5: Structure of Compound (5)

The result obtained is also comparable with the ^{1}H NMR spectral data of Emodin from literature as shown in **table 9** below

Table 8: Comparison of the observed ^{1}H NMR spectroscopic data of Compound (5) with the reported value of Emodin [33].

H	Observed data of Compound (5) (δ in ppm)	Reported data of Emodin (5), (δ in ppm)
1-OH	12.01 s	12.09 s
2-H	7.18 br s	7.16 br s
3a-CH3	2.40 s	2.48 s
4-H	7.50 br s	7.59 br s
5-H	7.20 d, J=1.6 Hz	7.27 d, J=2.4 Hz
6-H	-	-
7-H	6.60 d, J= 1.6 HZ	6.68 , J=2.4 Hz
8-OH	12.15 s	12.21 s

The ^{13}C NMR (Appendix 2.4) spectrum showed one methyl carbon at δ 21.98 and three oxygenated carbon at δ 161.88 (C-1), 166.10(C-6) and 164.92(C-8). The ^{13}C

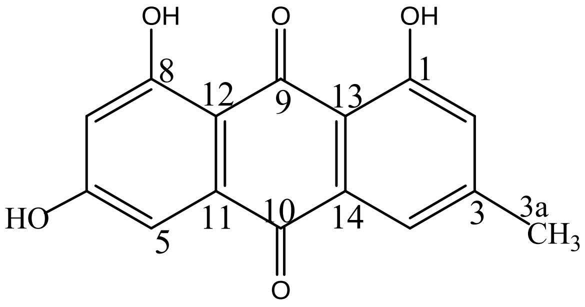
NMR also shows two carbonyl carbon at δ 190.14 (C-9) and 181.97 (C-10). One methyl substituted carbon at δ 148.77(C-3), four unsubstantiated carbons at (δ 124.63 (C-2), 120.97 (C-4), 109.30 (C-5), 108.44 (C-7) and four quaternary carbons at δ 135.61 (C-11), 109.4 (C-12), 113.87 (C-13) and 133.31 (C-14). The chemical shift of the carbon at δ 166.10 (C-6) indicate the presence of hydroxyl group on the benzene ring at this position.

Table 9: Comparison of the observed ^{13}C NMR spectroscopic data (400MHZ, DMSO-d_6) Compound (5) and Emodin [33].

Position of carbon	Observed 13C NMR data (δ in ppm)	DEPT135 data (δ in ppm)	Lit.data (CDCl$_3$ +MeOH-d_4. (δ in ppm)	Remarks
C-1	161.88	-	161.6	Quaternary
C-2	124.63	124.63	123.6	C-H
C-3	148.77	-	147.5	Quaternary
C-4	120.97	120.97	120.3	C-H
C-5	109.30	109.30	108.7	C-H
C-6	166.10	-	165.6	Quaternary
C-7	108.44	108.44	107.6	C-H
C-8	164.92		164.7	Quaternary
C-9	190.14	-	189.8	Quaternary
C-10	181.97	-	181.9	Quaternary
C-11	135.61	-	134.8	Quaternary
C-12	109.41	-	109.0	Quaternary

C-13	113.87	-	131.1	Quaternary
C-14	133.31	-	132.7	Quaternary
3a-CH_3	21.98	-	20.9	CH_3

From the observed and reported literature data of ^{1}H NMR and ^{13}C NMR Compound (5) is a hydroxyl anthraquinone known as Emodin.



Emodin

6. Experimental

6.1. General

^{1}H, ^{13}C and 2D NMR Spectra were recorded on a Bruker Advance 400 MHZ spectrometer in $CDCl_3$ with TMS as internal standard. The ultra- violet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer (200-800 nm). Infrared (IR) spectra were obtained on perkin Elmer BX infrared spectrometer (400-4000 cm^{-1}) using KBr. Melting points were recorded using Thomas HOOVER capillary melting point apparatus. Analytical thin layer chromatograms were run on a ready made 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on Aluminium foil. Compound on TLC were detected using eye protected UV- instrument.

6.2. Plant Material

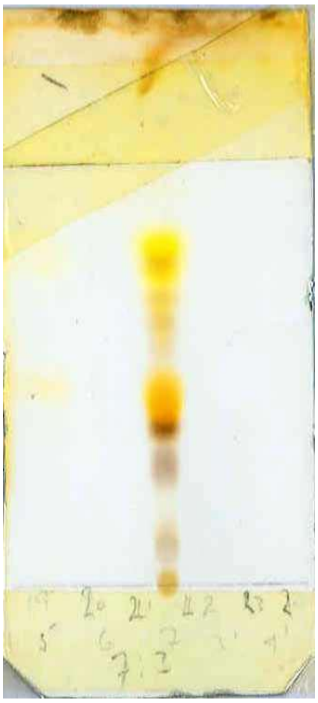
The root of *Rumex abyssinicus* was colleted from oromia regional state, welaga administrative zone which is 480 km from Addis Ababa on February 26/ 2008.

6.3. Extraction and Isolation

The air-dried and powdered plant material (500 g) was first soaked with petroleum ether for 36 hours and the extract was collected and discarded. The solvent free marc was then soaked with 400 ml ethylacetate for 36 hours and the extract was collected. This filtrate was evaporated under the reduced pressure using the Rotavapor and afforded 3.5 g a brown- gummy extract. When the TLC of this crude sample was checked using solvent system n-hexane:ethylacetate(7:3) four spot have been seen.

This 2g crude extract was dissolved in ethylacetate and applied to silica gel (60g) column chromatography, which was packed with n-hexane (100%). The column was eluted using the following solvent system. n-hexane (100%) fraction 1-7, n-hexane-ethylacetate (9.5:0.5) fraction 8-19, n-hexane-ethylacetate (9:1) fraction 20-27, n-hexane-ethylacetate (8.5:1.5) fraction 28-34, n-hexane-ethylacetate (8:2) fraction 35-40, n-hexane-ethylacetate (7:3) fraction 41-52, n-hexane-ethylacetate (6:4) fraction 53-58, n-hexane-ethylacetate (1:1) fraction 59-67, and ethylacetate (100%) fraction 68. Totally 68 fraction were collected each 45 ml.

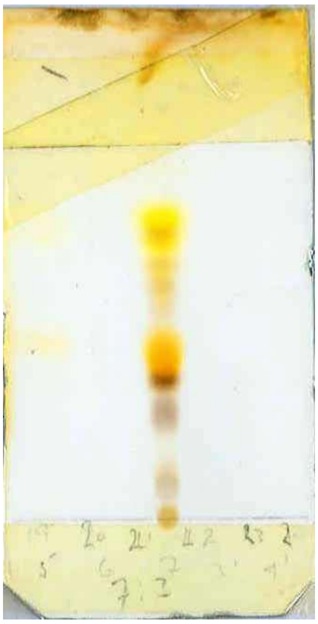
Out of 68 fraction colleted by increasing Solvent polarity using n-hexane:ethylacetate (7:3) solvent system, fraction from 36 to 44 show the same profile on TLC when mixed and the solvent is removed 17 mg of pure compound was obtained. Bright orange spot was formed on the TLC for this pure sample.



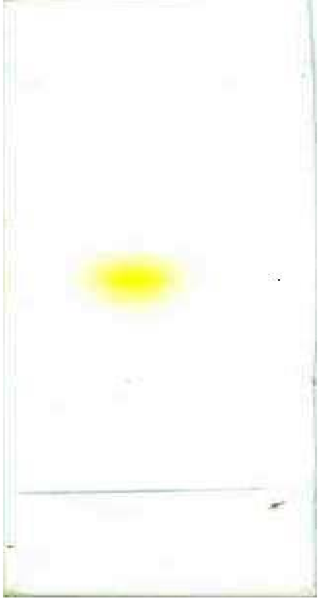
TLC for the crude extracts

TLC for compound (4)

Again when the polarity of solvent system (n-hexane: ethylacetate) increase to (8:2) fraction 49-53 show the same profile on TLC with a bright-orang red color , this portion afford only 7 mg of the second pure compound.



TLC for the crude extracts



TLC for compound (5)

6.4. Spectral data

Compound (4) is an orange solid m.p., 196 – 196 ^{o}c, Rf 0.340 in n – hexane-EtoAC (7:3), UV - Vis λ_{max} (CHCl$_3$) nm. 259, 289 and 440, IRυ_{max} (KBr) cm^{-1}: 3467, 2361, 1671, 1628, 1559, 1475, 1458, 1384, 1272, 1208 1156,, 1088, 1026, 901, 752, 'H-NMR (CDCl$_3$) at δ 2.47 (3H, s, CH$_3$), 7.11 (1H, brs, H-2) 7.30 (1H, dd, J=1.2 and 8.0 Hz, H-7), 7.67(1H, br s, H-4), 7.70 (1H, t, H-6), 7.85 (1H, dd ,J=1.2 and 8.0Hz , H-5),12.06 (1H, s, -OH), and 12.15 (1H, s, -OH).

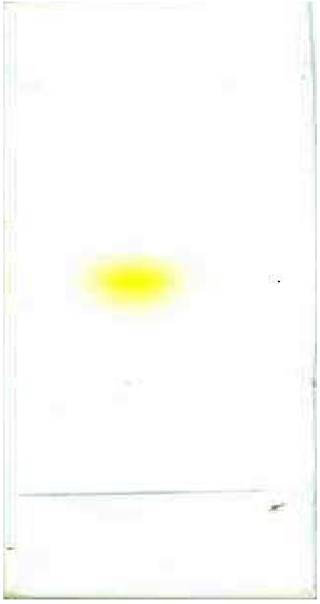
Compound (5) is an orange-red solid. M.p, 263 – 265^{o}C, Rf 0.280 in n-hexane: EtoAC (8:2), UV – Vis λ_{max} (acetone) nm. 265, 330, and 445. IRυ_{max} (KBr) cm^{-1}: 3391, 2341, 1630, 1560, 1480, 1458, 1385, 1338, 1297, 1267, 1213,1036, 909,870,760,727cm^{-1}. 'HNMR (DMSO-d$_6$) at δ 2.40 (3H, S, CH$_3$), 6.60 (1H, d, J = 1.54Hz, H – 7) 7.20 (1H, brs, H-2), 7.18 (1H, d, J = 1.50HZ, H-5), 7.50 (1H, S),12.01(1H, s, -OH) and 12.15 (1H, s,-OH)

7. CONCLUSION

Two anthraquinone were isolated and characterized from the root of *Rumex abyssincus*.

The n-hexane-ethyl acetate (7:3) affords 17 mg of compound (4) a known antraquinone chrysophanol. The n-hexane-ethyl acetate (8:2) gives 7mg of the second anthraquinone, compound (5) which is called Emodin. The amount obtained was very small for this compound. These two Compounds are identified by comparing their ^{1}H NMR and ^{13}C NMR spectroscopic data for similar compound from literature. Since the amount of compound (4) is large the 2D NMR spectroscopic data analysis is also used for its structural elucidation. The two compounds have different Rf value indicating that compound (4) is more polar than compound (5).

TLC for compound (4)



TLC for compound (5)

8. REFERENCES

[1] Harborne, J.B.; Lewis, N.G.; Phytochemistry, **1999**, Vol.50 No2, PP.361

[2] Mann, J.; Davidson, R.S.; R.S.; Hobbs, H.B.; Banthrope,O.V.; Harborne, J.B.; *Natural products. Their chemistry and Biological significance*, Addison Wesley long man Ltd, England, **1994**, PP.2, 289, 293

[3] Torssell, K .B. G. *Natural product chemistry, Amechanistic, biosyntheric and ecological approach*, second edition,Apotekarsocieten, Stockholm, Sweden, **1997**, PP. 16-17

[4] Koskinen, A.; Asymmetric synthesis of natural products, 1993, John wiley and Sons LTD, university of Oulu, Oulu, Finland.

[5] http://www. People. vcu. edu

[6] http://www. arbec. Com. my/top

[7] http://www.rsc.org/ebooks/archive/free/Bk9780854046812/Bk978085404681200001. Pdf

[8] Sarker, S.D.; latif, Z.; Gray, A.I. *Natural Products Isolation*, second edition, Humana press Inc., Totowa, New Jersey, **2006**, PP.1-2

[9] Peter, K.; Vollhardt, C.; Organic Chemistry, 2nd ed., W.H.Freeman and Company, Newyork, PP.124

[10] Salatino, A.; Faria Salatino, M.L.; Negri, G. *Journal of Brazilian chemical society*, **2007**, Vol.18, PP.11-33

[11] http://members.aol.com/MrDJReed/Private/plant2met.htm.

[12] Williams, A.D.; Lemake, L.T.; Fayes, principle *of medicinal chemistry, 5th*

ed., Philadelphia, Lippincott, Williams and Wilkins, **2002**, PP. 24-27

[13] http.//www.ibiblio.org/pfaf/ cgi-bin/arr-htm/? Rumex +abyssincus

[14] Kurt, B.; Torssell, G. *Natural product chemistry, 2nd ed.* Sweden, Stockholm, **1997**, PP. 213-214

[15] Sarker, S.D.; Latif, Z.; Gray, A.I. *Natural product isolation, 2nd* ed. Humana press Inc., Totowa, New Jersey, **2006**, PP. 169-171

[16] Kaufman, B….[etal]. *Natural product from plant*, NewYork, by CRC Press. LCC, **1999**, PP. 1-23

[17] Trease, G.E.; Evans, W.C.; pharmacognosy, 11th ed. Bailleric Tindal, London**,** **1978**, PP. 373-377

[18] Shibata, s.; Fakido, M.; tanaka, O.; *Jon. Am. Chem. .society*, 1950, vol.72, PP. 2789

[19] Thomson, R.H.; *Naturally occurring quinines, 2nd ed.* Champman and Hall LTD, London, **1971, PP**. 40-64

[20] Briggs, L.H.; Nicholells, G.A.; Paterson. R.M.L, Jon. chem. Soc. PP. 1716-1718

[21] Bloom, H.; Briggs, L.H.; Cleverley, B.; Jon.A.chem.soc. PP. 178-179

[22] Gatenbeck, s, Biogenesis Antibiot, 255; **1966**, Chem. Abstr., 64, 245d.

[23] Ballantine, J.A.; Pillinger, C.T.; Tetrahedron, **1967**, vol. 23, PP.1691

[24] Zanger, M.; Org. Mag. Res-4, **1972**, PP. 9-11

[25] Sarker, S.D.; Latif, Z.; Gray, A.I. *Natural product isolation, 2nd* ed. Humana press Inc., Totowa, NewJersey, **2006**, PP.169-171

[26] Paul, M., Dewick Medicinal Natural Product Abiosynthtic approach, 2nd ed, John Wiley and Sons, LTD, PP.63-65

[27] Mann, J.; Davidson, R.S.; R.S; Hobbs, H.B.; Banthrope, O.V.; Harborne, J.B.; *Natural products. Their chemistry and Biological significance*, Addison Wesley long man Ltd, England, **1994**, PP.2, 289 and 293

[28] Andrews, Flowering plant of *Sudan*, **1950**, Vol. 1, PP. 100-105

[29] Midiwo Ogweno, J and Rukunga Muriki, G, Distribution of anthraquinone in Rumex Species, Phytochemistry, vol. 24, No. 6, 1985, PP.1390-1392

[30] Fairbairn, W.J and EL-Muhtaid, J. F, Chemotaxonomy of anthraquinone in Rumex, Phytochemistry, vol. 11, **1972**, PP. 263-268

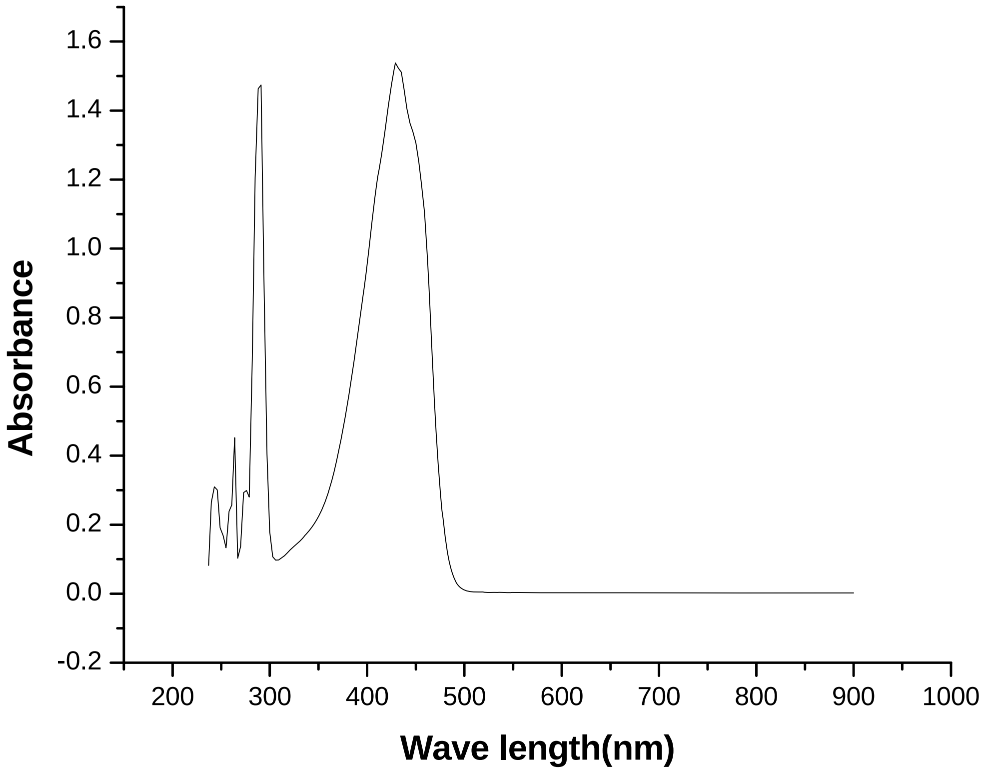
[31] Fassil. Y, Bezabeh. A and Abegaz. B, Anthracene derivative of Rumex abyssinicus, Journal of Natural products, Vol.48, No. 1, PP.148

[32] Muhanji, C., M.Sc. Thesis, Univesity of Botswana, 2001, Department of Chemistry, Botswana.

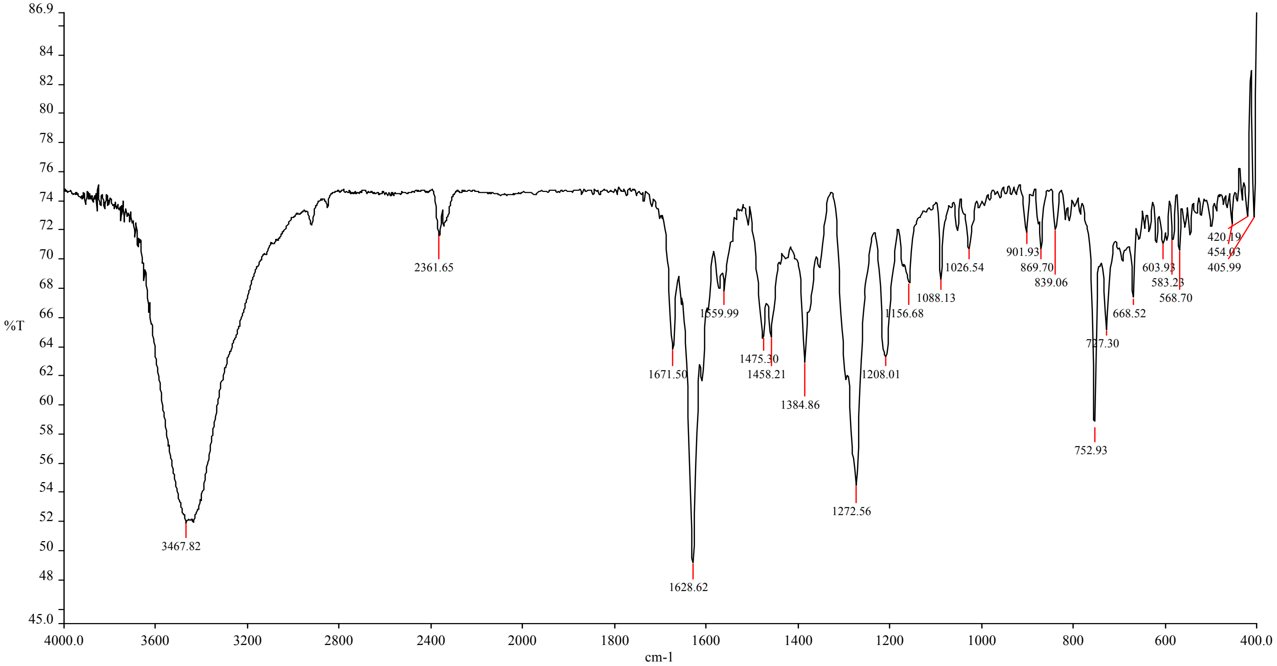
[33] Meselhy, R.M., Moleculs 2003, vol.8, 614-621.

9. Appendix

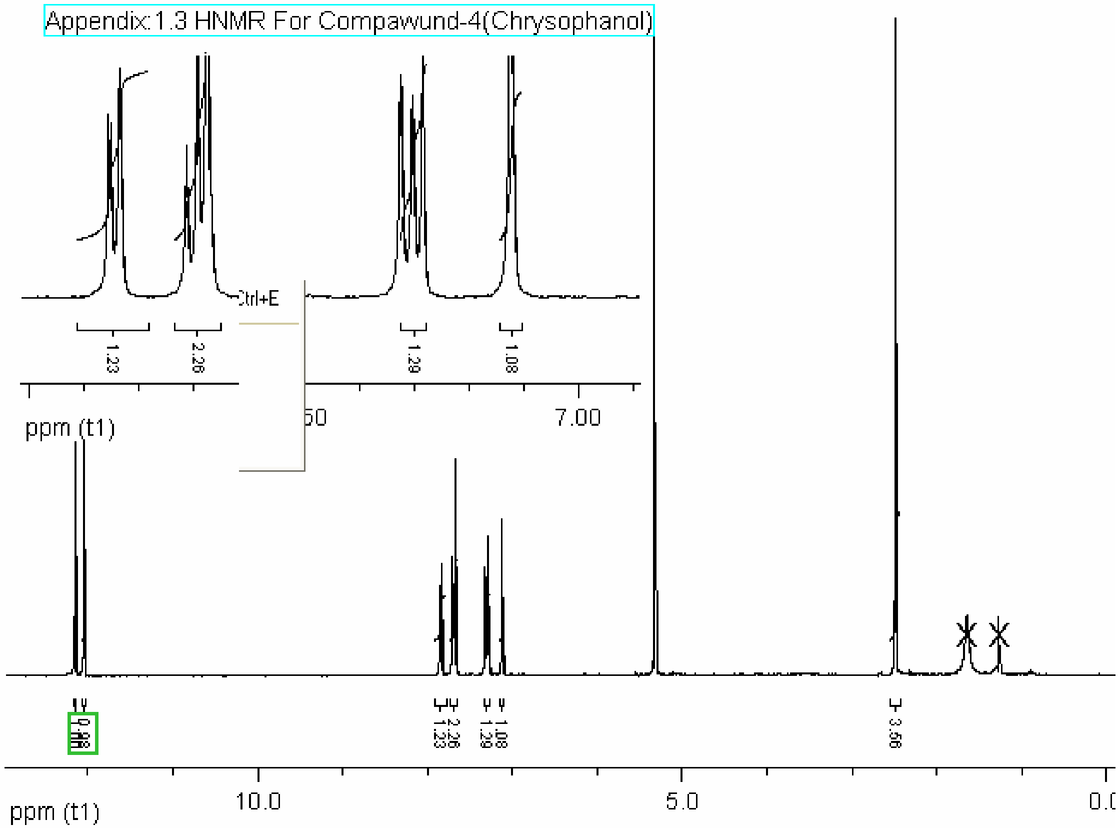
Appendix1.1. Uv-Vis spectrum for compound (4)

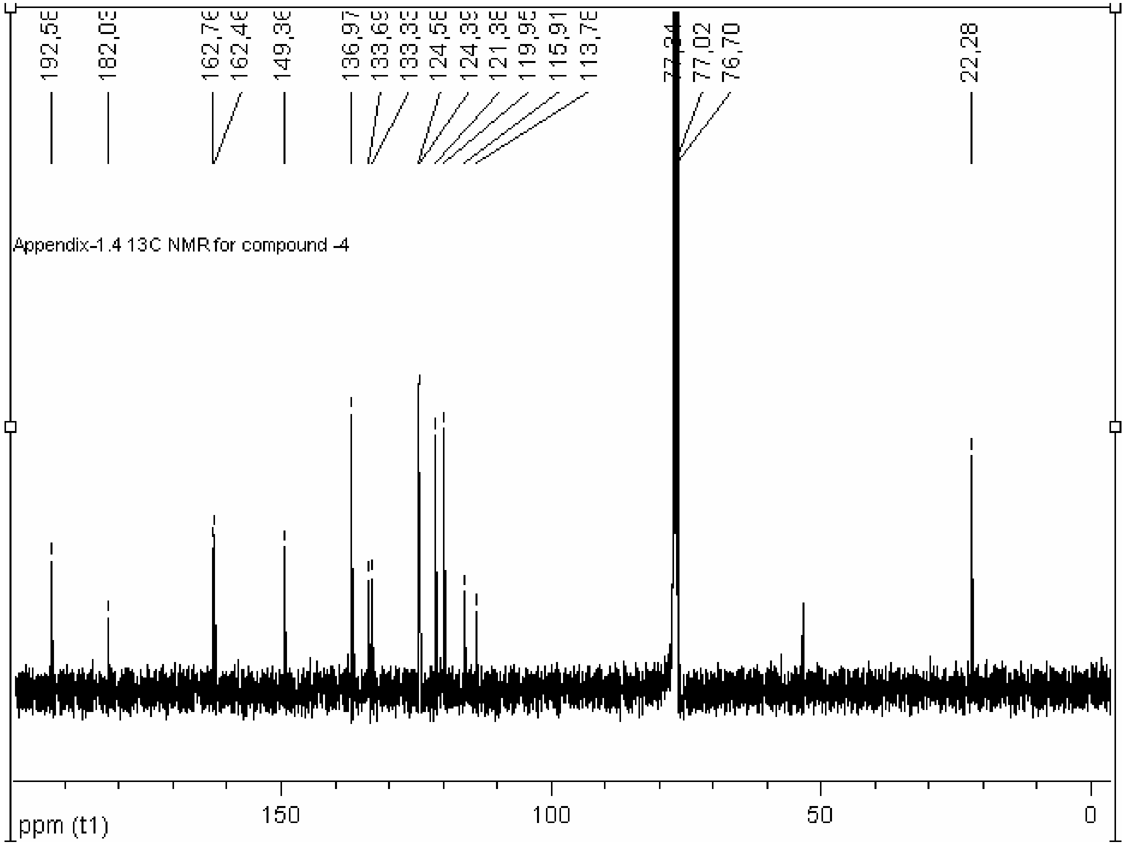


Appendix1.2. IR Spectrum of compound (4)

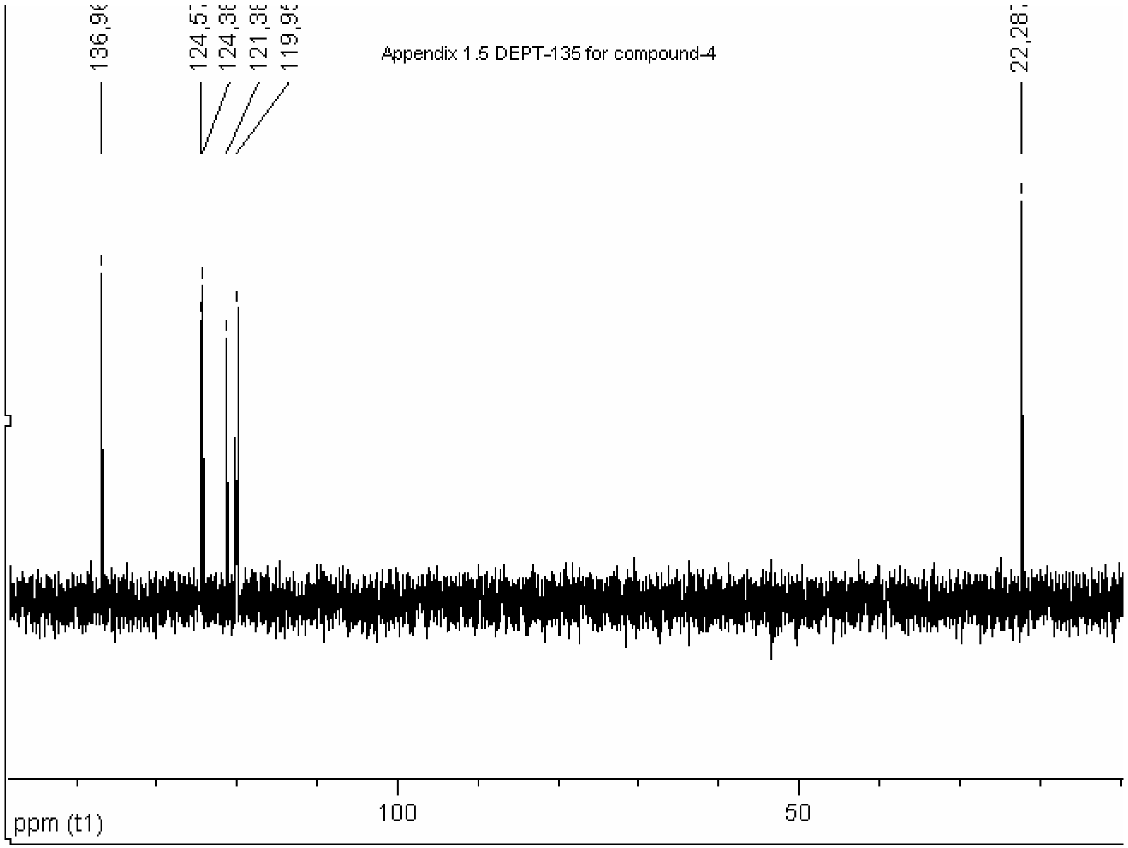


Appendix:1.3 HNMR For Compawund-4(Chrysophanol)

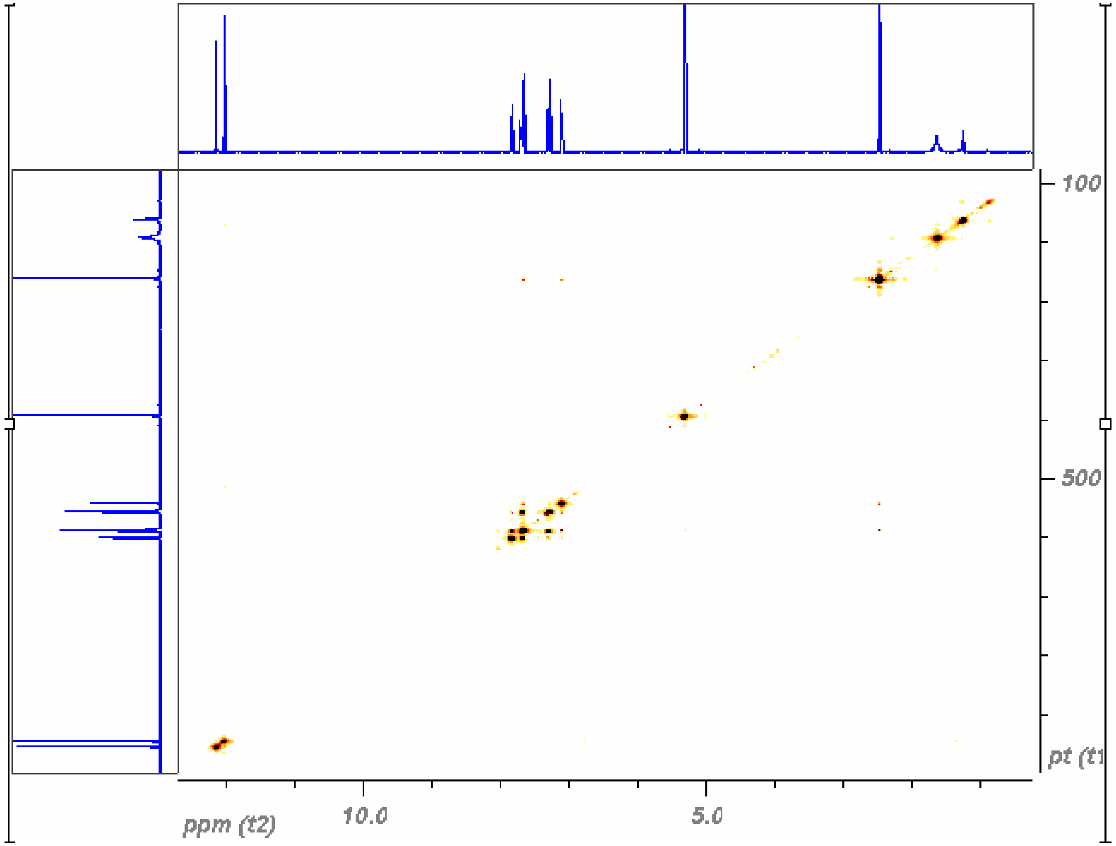




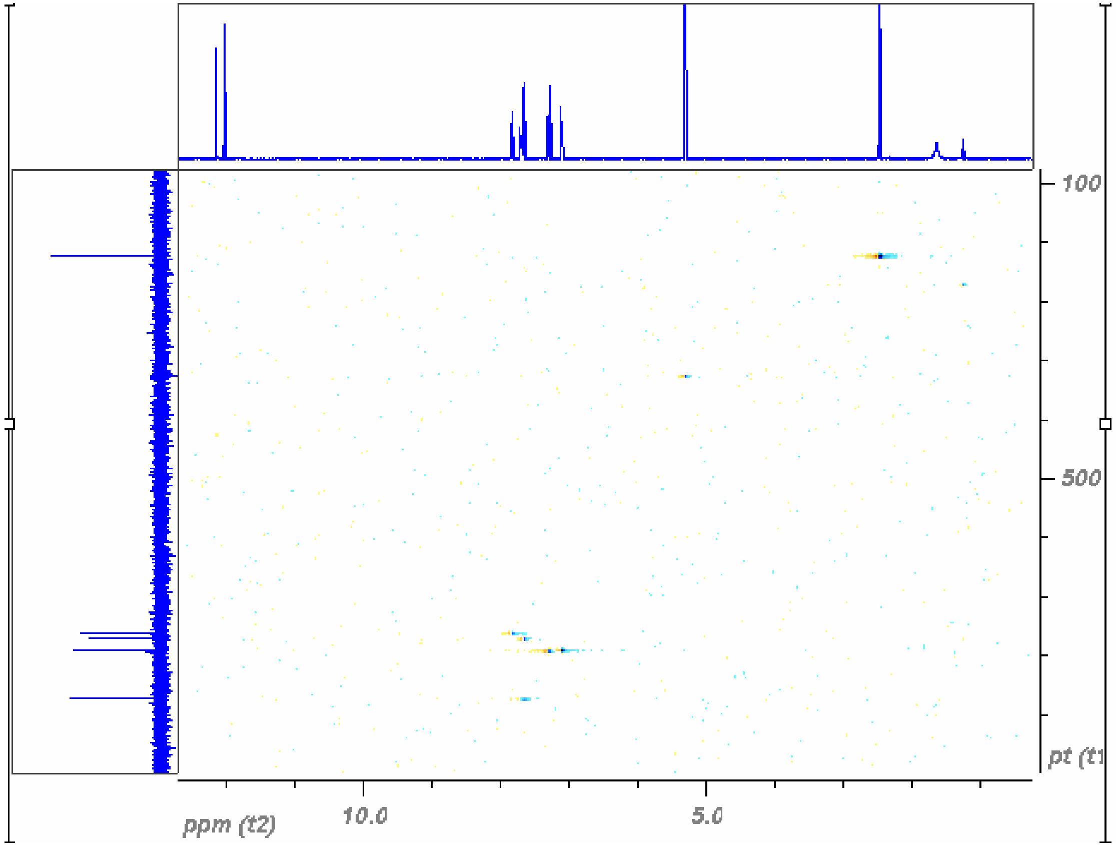
Appendix-1.4 13C NMR for compound -4


Appendix 1.5 DEPT-135 for compound-4

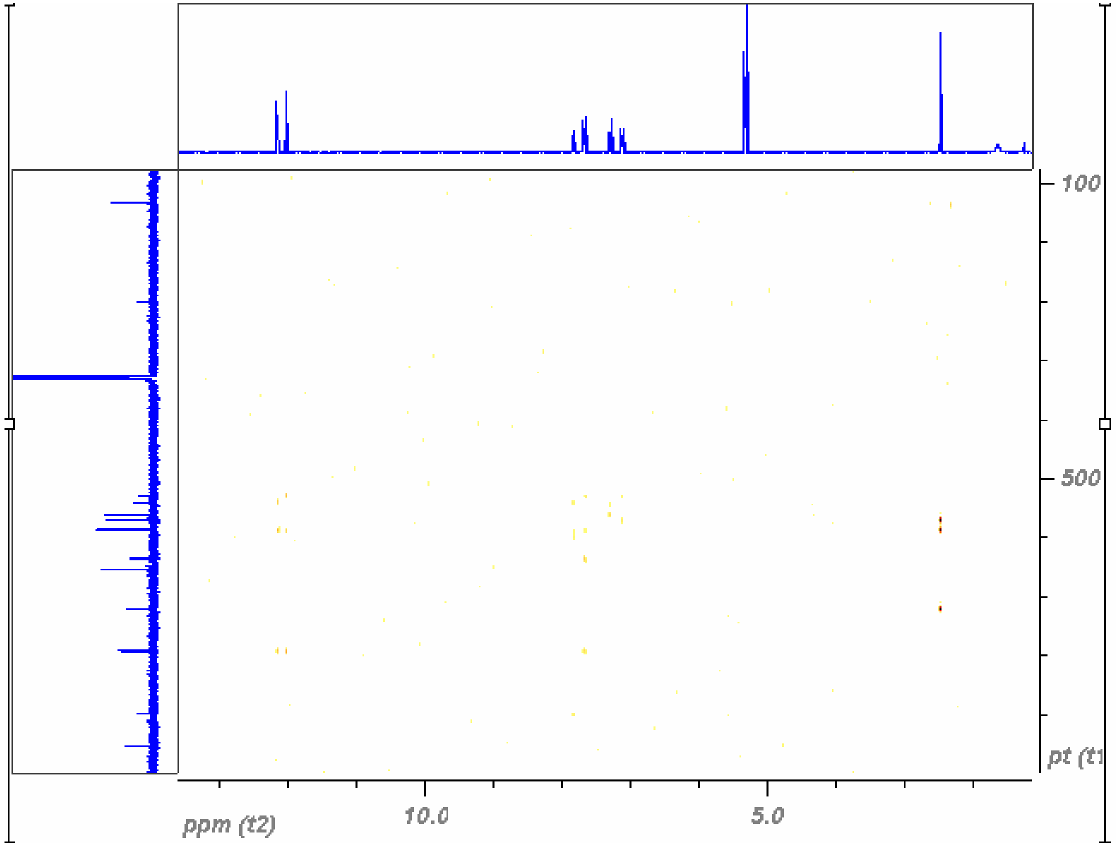
Appendix1.6. ^{1}H-^{1}H COSY Spectrum for compound (4)



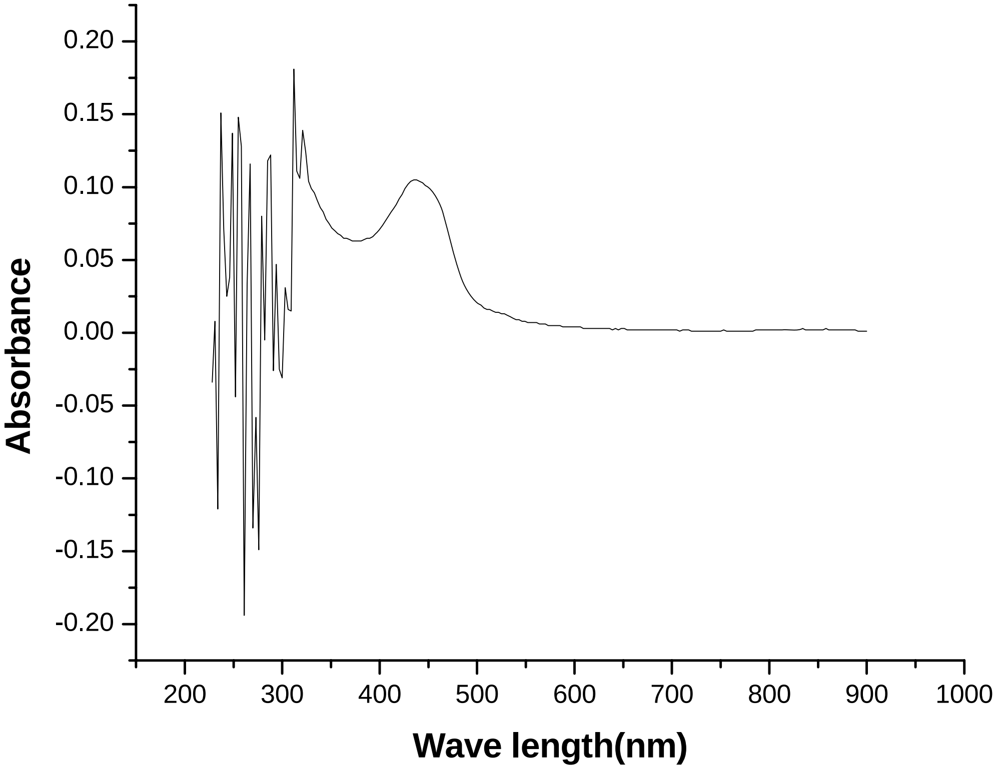
Appendix 1.7 .HSQC spectrum for compound (4)



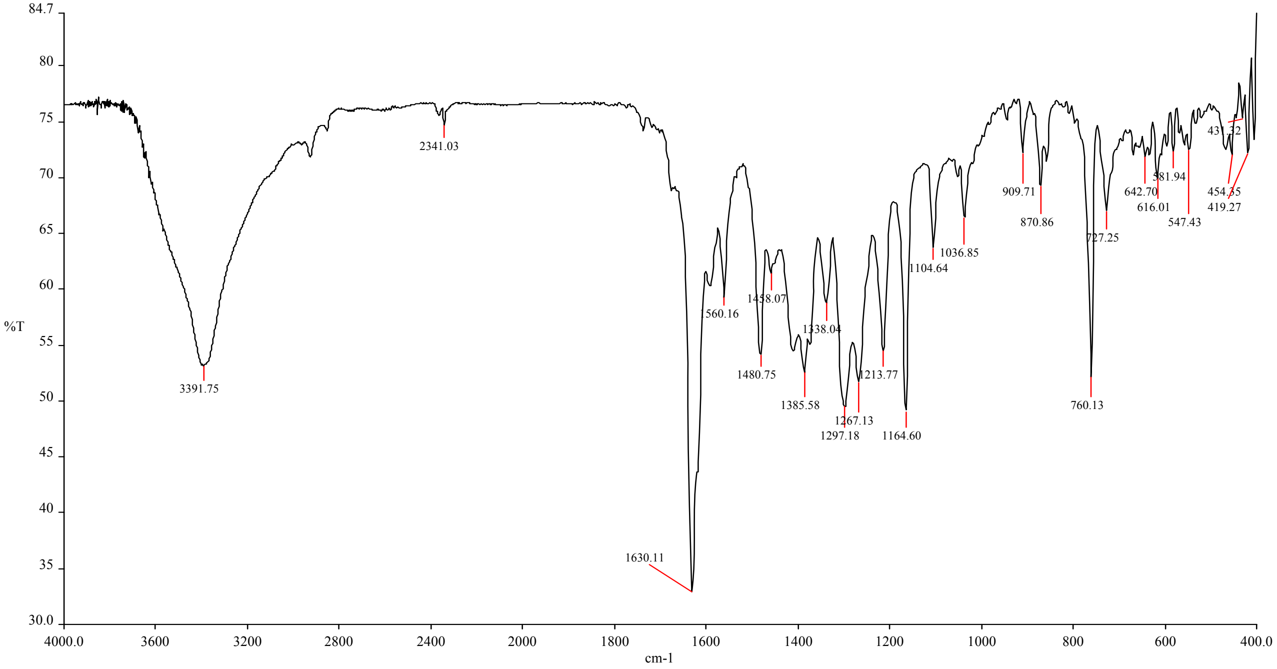
Appendix1.8. HMBC spectrum for compound (4)

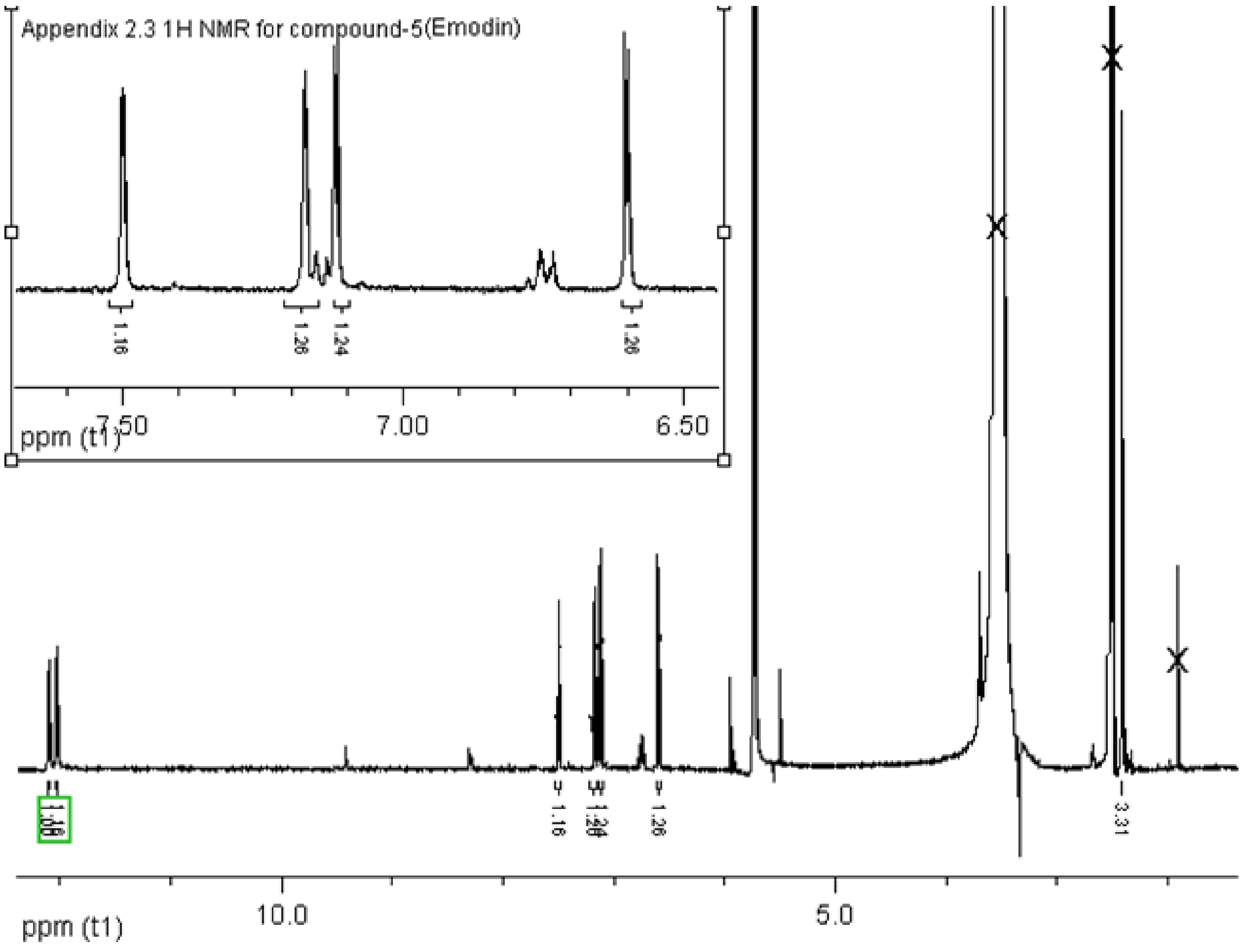


Appendix2.1. Uv-vis spectrum for compound (5)

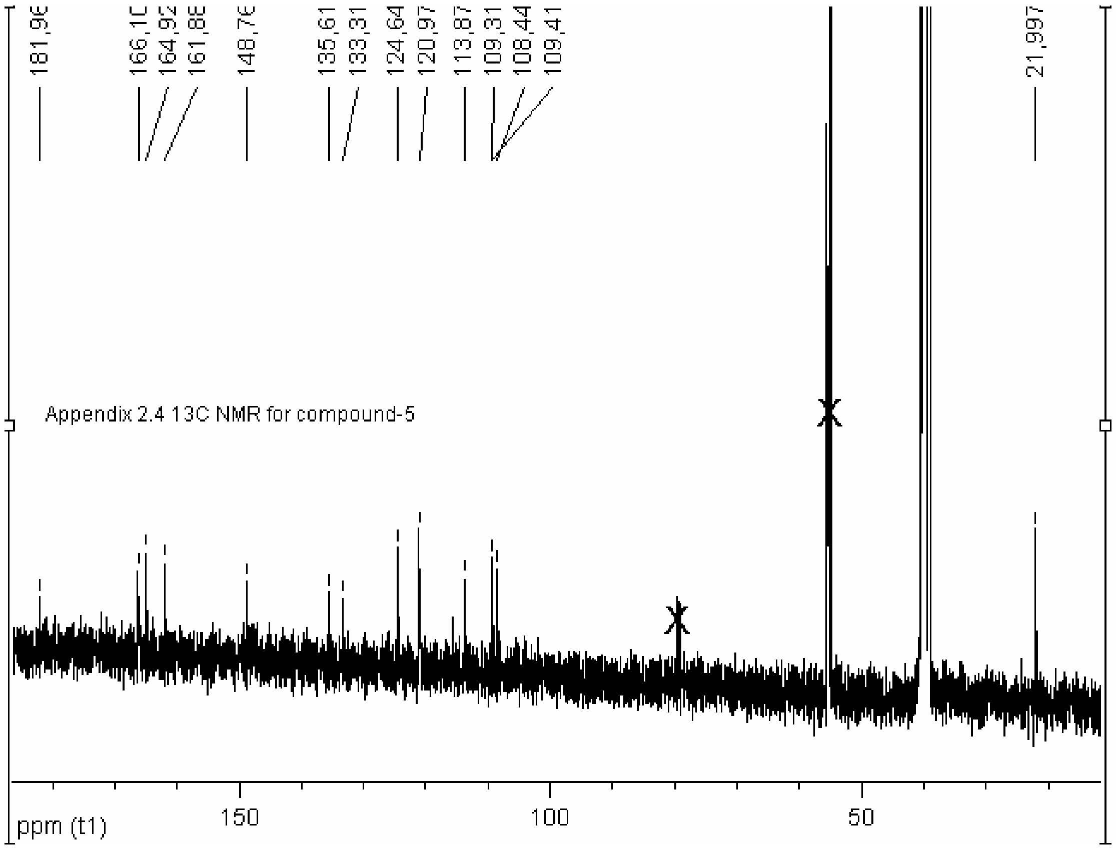


Appendix2.2. IR spectrum for compawund (5)

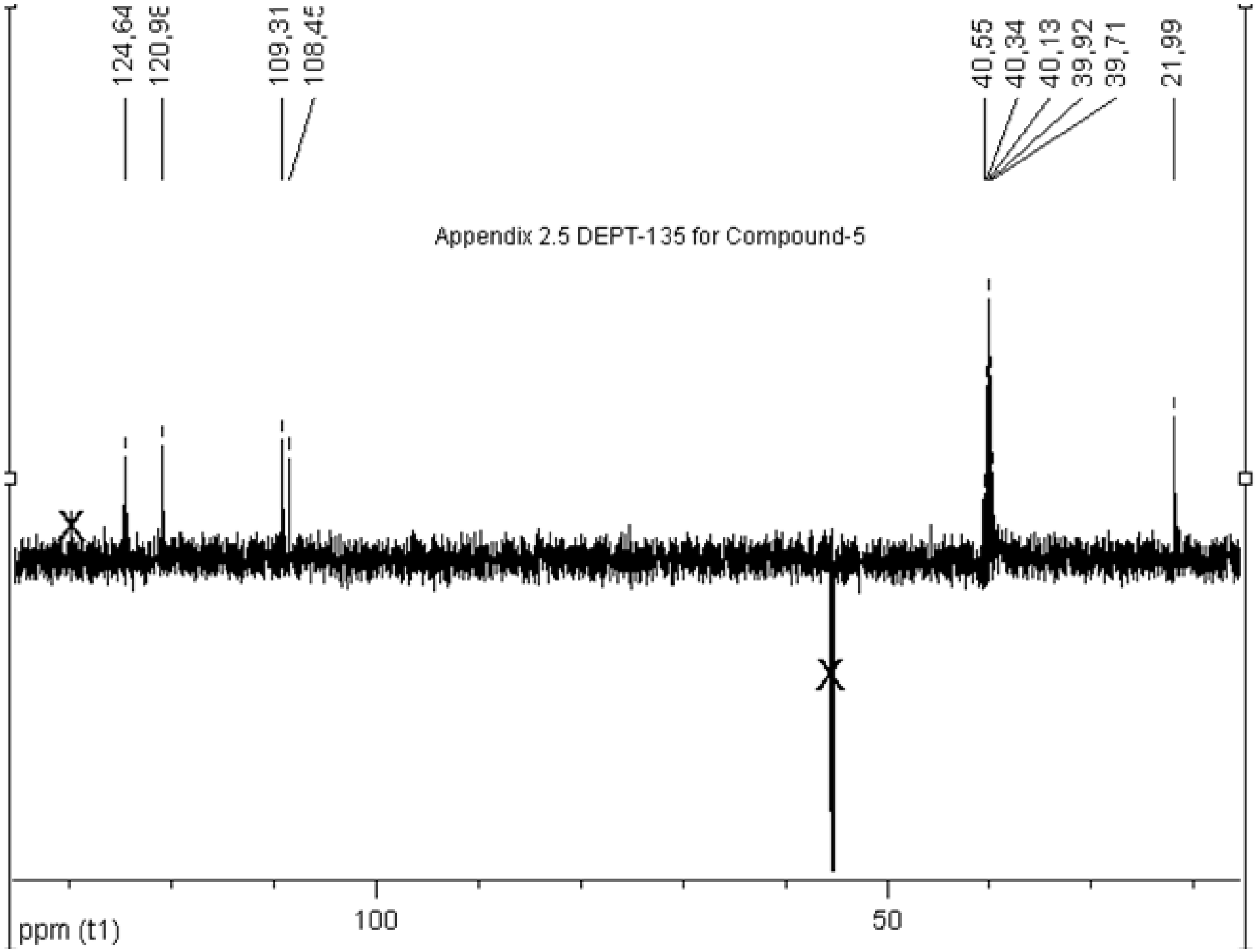




Appendix 2.3 1H NMR for compound-5(Emodin)



Appendix 2.4 13C NMR for compound-5



Appendix 2.5 DEPT-135 for Compound-5

DECLARATION

I the undersigned, declare that this project is my original work and has not been presented for a degree in any other University and that all sources of materials used for the project have been duly acknowledged.

Name: Bizuayehu Zinaye
Signature: ________________

This project has been submitted for examination with approval as University advisor

Advisor	Signature
Dr. Ashebir Fiseha	______________

Place and date of submission: School of Graduate Studies
Addis Ababa University
July, 2008