JMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

SCHOOL OF GRADUATED STUDIES

DEPARTMENT OF CHEMISTRY

M.SC THESIS

ON

PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY EVALUATION OF ROOTS OF *Rumex abyssinicus*

BY

MUAZ SHIFA

JULY 2020

JIMMA, ETHIOPIA

PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL ACTIVITY EVALUATION OF ROOTS OF *Rumex abyssinicus*

BY

MUAZ SHIFA

M.Sc THESIS SUBMITTED TO THE SCHOOL OF GRADUTE STUDIES JIMMA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

ADVISORS

TSEGAYE GIRMA (PhD)

DALE ABDISSA (M.Sc)

JULY 2020

JIMMA, ETHIOPIA

As research advisors and examiners of the M.Sc thesis open defense examination, we certify that we have read and evaluated the paper prepared by Muaz Shifa entitled phytochemical investigation and antimicrobial activity evaluation of roots of *Rumex abyssinicus*. The thesis is accepted for the fulfillment of the requirements for the degree of Master of Science in chemistry.

Name of advisor	Date	Signature
Dr. Tsegaye Girma Jimma University College of Natural Sciences Department of Chemistry	_____	_____

Name of co-advisor	Date	Signature
Mr. Dale Abdissa Jimma University College of Natural sciences Department of Chemistry	_____	_____

Name of examiners	Date	Signature
1. _____	_____	_____
2._____	_____	_____

Department head name	Date	Signature
Mr. Eferem Tilahun	_____	_____

Table of Contents

Contents Page

List of table page

Table figure Page

List of appendices Page

Abbreviation and Acronyms

^{13}CNMR	Carbon nuclear magnetic resonance
COSY	Correlated Spectroscopy
DMSO	Dimethyl Sulfoxide
HMBC	Heteronuclear multiple bond correlation
^{1}HNMR	Proton nuclear magnetic resonance
HSQC	Heteronuclear single Quantum Correlation
NMR	Nuclear Magnetic Resonance
SD	Standard Deviation
TLC	Thin Layer Chromatography
TMDs	Traditional medicinal plants
WHO	World Health Organization

Acknowledgment

I am extremely grateful to my research advisor Dr. Tsegaye Girma for his continuous support, supervision, encouragement and valuable guidance during the course of this study. I would like to express my deepest appreciation to Mr. Dale Abdissa who shared his laboratory experiences, for his encouragement and valuable comments he gave me during this project work. I am also grateful to my families and my friends for their encouragements and advice they gave me during this project work. I also wish to express my thanks to all organic stream staff of Jimma University who in some way contributed to this project. Finally, I would like to thanks Jimma University for financial supports and Chemistry Department for all laboratory materials support.

Abstract

The genus *Rumex* species belongs to the family *Polygonaceae* that contains a large number of chemically complex bioactive compounds such as anthraquinones, flavonoids, terpenes, steroids, leucoanthocyanidins and phenolic acids. The roots of *Rumex abyssinicus* is used traditionally for treating different diseases such as hepatitis, hemorrhoids, gonorrhea, typhus and wound. Therefore, this study was undertaken to identify bioactive secondary metabolites from the roots of *Rumex abyssinicus* and evaluation of its antimicrobial activities. With this regard, the air-dried, powdered root of *R. abyssinicus* was subjected to sequential extraction with petroleum ether, chloroform, acetone and methanol by maceration. Each of the extracts was filtered and concentrated using rotary evaporator, separately. Meanwhile, the crude extracts were kept in desiccator until dry for the next step. Then, the antimicrobial activities of crude extracts were tested against four bacterial strains (*S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae)* and two fungal strains (*C. albicans* and *S. Cervecia)*. Among the various root extracts *of R. abyssinicus*, the acetone crude extracts demonstrated the best zone of inhibition and subjected to column chromatography over silica gel eluted with petroleum ether containing increasing amount of the polarity of ethyl acetate, which resulted with the identification of chrysophanol and emodin. The structures of isolated compounds were elucidated using 1D and 2D NMR spectroscopic methods. The isolated compounds were also evaluated against microbial strains and indicate good inhibition zone against *S. aureus* (23.0±0.1) *K. pneumoniae* (22.5±0.4) and *C. albicans* (20.0±0.4 for compound-1 *and* 22.0±0.7 for compound-2*)*

Keywords: *Rumex abyssinicus,* root extract, NMR, emodin, chrysophanol

1. Introduction

1.1. Background of the study

Medicinal plants are plants that possess therapeutic properties, where one or more of its organs contain substances that can be used for therapeutic purposes or precursors for chemo-pharmaceutical synthesis. They contain medically active organic compounds that may provide definite physiological action on the human body to control diseases condition. Plant products used in the treatment of diseases could be obtained from barks, leaves, flowers, roots, fruits or seed part of the plants [1].

The therapeutic uses of the plants sources as herbal medicine in curing human disease worldwide disseminated since the old time to date. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian [2]. Over the years, they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as scientists use them in search for alternative sources of drugs. About 3.4 billion people in the developing world depend on plant-based traditional medicines [3]. This represents about 88% of the world's inhabitants, who rely mainly on traditional medicine for their primary health care [4]. Several people of developing country still use traditional medicine due to its low cost and availability. 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 [5].

Phytochemicals are clearly known that they have values in the protection of human health, where their dietary intake is significant [6]. These chemical products classified in to primary and secondary metabolites. Primary metabolites are those common to all species and can be classified as proteins, carbohydrates, lipids and nucleic acids. Primary metabolites are essentially ubiquitous and certainly essential for life and they function in life cycle. The secondary metabolite are often

referred to as ‘’natural products’’ or phytochemicals and mostly includes alkaloids terpenoids, flavonoids and polyphenols, steroids, and anthraquinones. They exhibit some antibiotic activity and serve as a source of antimicrobial agents against human pathogens [7].

The chemistry of the natural products include their biosynthesis, extraction, identification, quantification, structural elucidation, physical and chemical properties and reactions[8]. Phytochemicals play an important role in the ancient traditional medicine.

Several numbers of modern drugs have been derived from natural sources, many based on their use in traditional medicine. The use of biological resources for various therapies have been known in many different parts of the world, especially in remote regions where traditional medicines provide an alternative to "modern" health care system [9].

Currently, traditional medicinal plants are getting more attention of the scientific communities, particularly researchers in the field of natural products. It involves the isolation and identification of secondary metabolites produced by plants and use them as active principles in drug preparations. These compounds have potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus [10]. Although several plant species have been tasted for antimicrobial properties, the vast majority of them have not been exhaustively evaluated. Herbal remedies have been used for centuries but more recently, the compounds that are active have been identified and synthetic organic chemists have then been able to produce the molecules on large scale along with their synthetic analogues [5].

In Ethiopia, using traditional medicines is based on indigenous knowledge and has been practiced long ago to treat various illnesses. The people heavily relied on traditional medicine for centuries to treat various physical and mental disorders. About 80% of the population in the country still depends on traditional medicine as their major primary healthcare system [11] and more than 95% of traditional

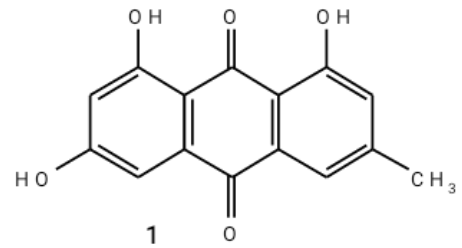
medicinal preparations made from plant origin. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people include the use of medicinal plants.

Ethno botanical studies are required to reveal locally important plant species for the development of crude drugs [12]. Plants and natural sources form the basis of today's modern medicine and contribute largely to the commercial drug preparations manufactured today, where about 25% of drugs prescribed worldwide are derived from plants [13].

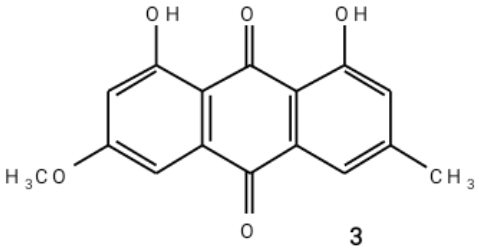
The genus *Rumex* belongs to the family *Polygonaceae* contains a large number of bioactive compounds such as anthraquinones, flavonoids, terpenes, naphthalenes stilbenoids and steroids, flavonoida glycosides, leucoanthocyanidins and phenolic acids. Traditionally, people use different parts of *Rumex* species to treat several health problems such as infections, diarrhoea, constipation, mild diabetes, oedema, jaundice, skin, liver and gallbladder disorders and inflammation, and as an antihypertensive, diuretic and analgesic preparation [14]. Due to its medicinal value and phytoconstituents, several researchers have been pursued several studies to extract and isolate valuable phytochemicals from *Rumex* species. The extracts of these plants and isolated compounds from them, have been demonstrated to possess various pharmacological activities including anti-inflammatory, antioxidant, antitumor, antibacterial, antiviral and antifungal properties *in vitro* and *in vivo*[15-16].

Rumex abyssinicus is widely spread medicinal plant in the highlands of tropical Africa and is a common weed of cultivated lands that disturbed grounds ranging from North Africa to Ethiopia at altitudes between 1200 and 3300 m [17]. It is a potential genetic resource for bio prospecting due to its active parts rhizome, roots and leaves. It is used in treating various diseases. The decoction of leaf or

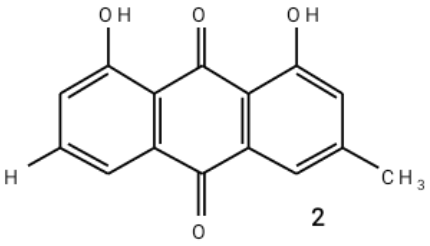
root powder is taken as vermifuge. It is also used to treat malaria, gonorrhea, poisoning, hepatitis, constipation, sciatic neuralgia, hypertension, migraine, rheumatism, breast cancer, stomach distention, earache, liver diseases, hemorrhoids, typhus, rabies and wound [18]. Its roots were used to control microbial activities. In addition, it is used for coloring wickerwork. The most important secondary metabolites reported from *R. abyssinicus* are given in Fig.1 including emodin (**1**), Chrysophanol (**2**) physcion (**3**), oxalic acid (**4**), Betulone (**5**), di isobutyl Phthalate (**6**) and Oleic acid (**7**) [32,81,83].



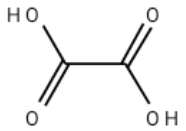
1



3



2



4

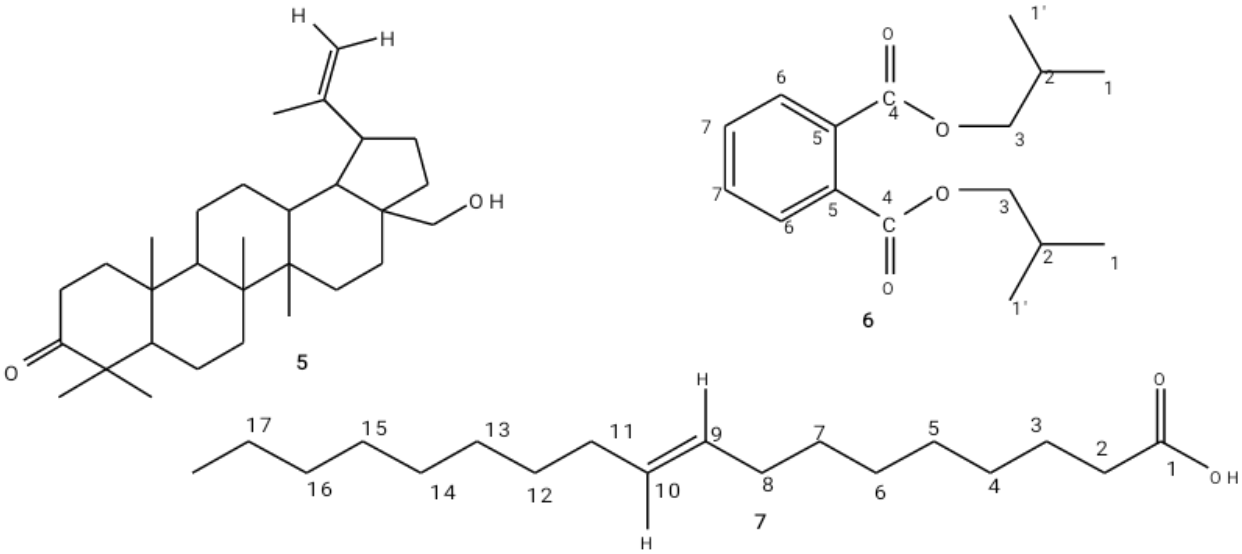


Figure 1.Some compound isolated from *R. abyssinicus*

1.2. Statement of the problem

Infection diseases are the main cause of death in developing countries, which accounts 43% of the total deaths. Since ancient time, traditional healers long have used plants to prevent infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity, and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. However, many infectious diseases are increasingly becoming difficult to treat because of anti-microbial resistance organisms. The problem of microbial resistance is growing and the outlook for the use of the current anti-microbial drugs in the future is still uncertain. So, it is worthwhile to study plants and plant products for antimicrobial activity. Traditionally people in Ethiopia have been used medicinal plants to treat different disease and also it has great contribution in primary health care systems, however, most of the existing medicinal plants are not well investigated to identify the chemical principles for their claims. Phytochemicals of plants also become more popular as wide natural sources of antimicrobial agents in modern medicine [19]. Previous reports have relied on

phytochemical investigation from the root bark and root *R. abyssinicus* and evaluations of its anti-bacterial activities. *R. abyssinicus* is by far a rich source of bioactive compounds and it needs further investigation using different solvents. Therefore, this study was intended to investigate the phytochemical constituents of *Rumex abyssinicus* roots and its antimicrobial activity.

1.3. Objective of the study

1.3.1. General objective

This study was aimed to understand the antimicrobial activity and the bioactive constituents of root extract of *Rumex abyssinicus*

1.3.2. Specific objective

The specific objectives of this study are:

- ✓ To extract and isolate bioactive compounds from crude extracts of roots of *R. abyssinicus;*
- ✓ To evaluate the antibacterial activity tests on the crude extracts and isolated compounds.
- ✓ To evaluate the antifungal activity of the crude extract and isolated compounds
- ✓ To elucidate the structure(s) of the isolated compound(s) by using different spectroscopic techniques.

1.4. Significance of the study

Natural products have been isolated from several plants species and play a key role in human health care in the century and certainly continued to be considered as one of the major sources of new drug in the future because only 5-10% of 250,000 species of higher plants that exist on the world have been investigated so far [20]. In addition, re-investigation of previously studied plants has continued to

exhaust their phytochemical constituents and produce new bioactive compounds for drug discovery. The therapeutic potential of plants has been explored over a long period of time that associated with antivirus, antitumor, antimalarial and analgesic [21]. Biologically active molecules of plants are either directly used as medicine or provide bioactive compounds used as raw material for the production of synthetic drug. The medicinal application of plants, for instance, *Rumex dentatus* have been used in Chinese herbal medicine for the treatments of many kinds of bacterial and fungal infection*, Rumex hastatus* for the healing of coughing, headache and fever and *Rumex nepalensis* used for the treatment of haemostasis and tinea [22]. Because of this, the search for antimicrobial agent is necessary and a continuous process in order to reduce or eliminate fatal infection of both microbial and non-microbial origins.

Therefore, the outcomes of this study will have the following contributions:

- ✓ give information about the phytochemical constitutes of *Rumex abyssinicus* and can help as a springboard for further study
- ✓ aware the community about the phytochemical constitutes and bioactivity of *Rumex abyssinicus*
- ✓ give valuable information for future pharmacological and bio prospecting studies

2. Review related literature

2.1. Botanical Information *Rumex genus*

The *Polygonaceae* family is distributes in a wide range of habitats from the arctic to tropic from mountain to lowland and from arid to aquatic situation [23]. The family is present worldwide but most diverse in North Temperate Zone that 35 of the 50 genera described below occurs in North America whereas few numbers are native to the southern hemispheres. The polygonaceae comprise about 1200 species in approximately 50 genera. The largest genera are Eriogonom, which comprises 240 species, Rumex 200 species, coccoloba 120 species, persicaria 100 species and colligonom 80 species [24].

Rumex is the second largest genus of family *Polygonaceae* with almost 200 species distributed in Europe, Asia, Africa and North America, mainly in the northern hemisphere. It is grown between 1200 m(4300 m. The name *Rumex* originated from the Latin word for dart, alluding to the shape of the leaves [25]. From 975 reported species of the *Rumex* genus, only 183 of them correspond to an accepted scientific name, and others are synonyms or unresolved names while those are used traditionally to treat diseases [26].

Rumex abyssinicus is perennial herb, which grows up to 3 m tall, with thick, fleshy rhizome. Its local name is *Momoko,* Afan Oromo´[27]. It is widely spread in the highlands of tropical Africa. It is widespread throughout Ethiopia at altitudes between 1200 and 3300 m. It is a common and tolerated weed fields and plantations. It also occurs along paths and water, in secondary scrub, grassland and margins of rain forest. This plant species will remain locally an important vegetable from the wild [28]. Table 1 gives the specific *Rumex* species and their traditional uses in Ethiopia.

Table 1. Some Rumex species available in Ethiopia and their traditional uses

Rumex species	Local area	Traditional use	Reference
R .abyssinicus	Oromia, Horroguduru zone	To treat typhus, rabies, fungal infection, diabetes, and lung TB and leprosy	29
R. nepalensis	Dejen district, N. Eth	Used as :Antifungal activity, cooked as food, stomach dieses	30
R. crispus	East Showa, Arsi, Bale (Oromia region),North Gondar (Amhara region)		31
R. steudelli (Tult)	South West Ethiopia	Root the plant traditionally used as contraceptive	32
R. nervous	Bale zone, Oromia Region; South Eastern Ethiopia.	Used for the eye disease, taeniacapitis, hemorrhoids, infected wounds, abscess	33

		and gynecological disorders	

2.2. Phytochemistry of *Rumex* Species

The genus Rumex is characterized by the accumulation of anthraquinones, naphthalenes, flavonoids and stilbenoids.

2.2.1 Anthraquinones

Anthraquinones are the largest class of aromatic compounds that derived from anthracene, which have two-ketone group at position 9, 10-dioxoanthracene core (Fig.2). Anthraquinones are occurring in plants to great extent than in animals. These compounds also occur as free state and glycosidic forms in nature [34]. They have used in manufacturing of dyes, as chemical indicators of alkalinity and acidity [35]. Figure 3 shows structures of some of the common anthraquinones.

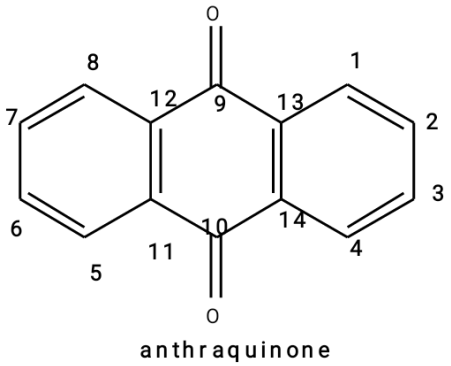


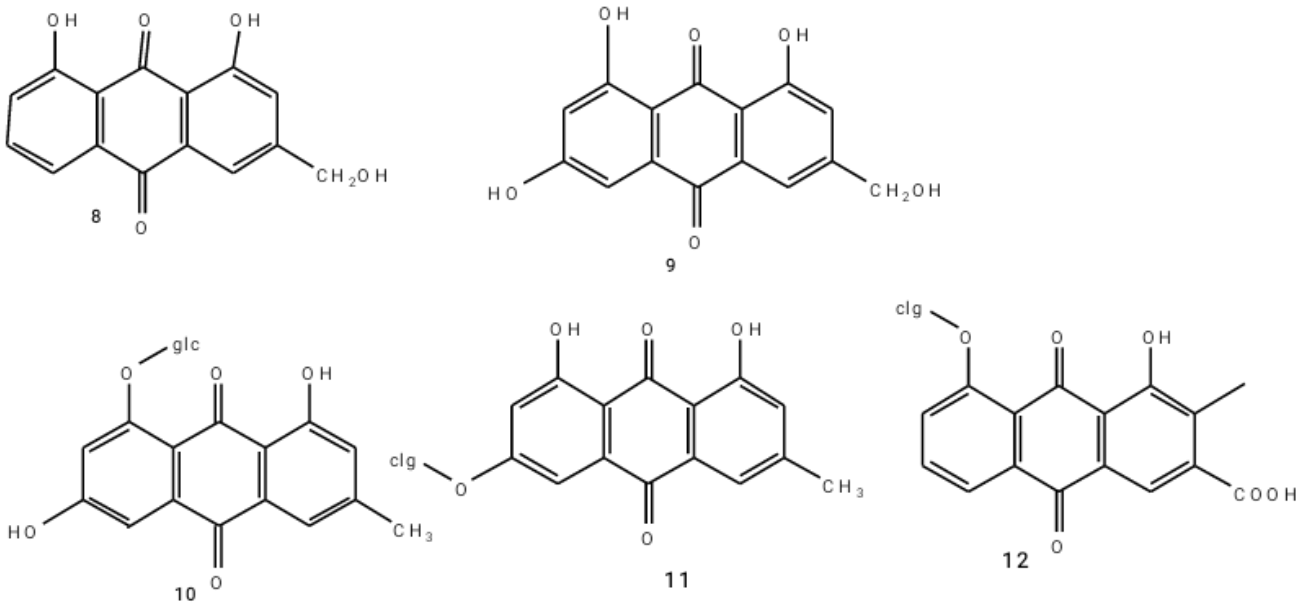
Figure 2. Chemical structure of anthraquinone

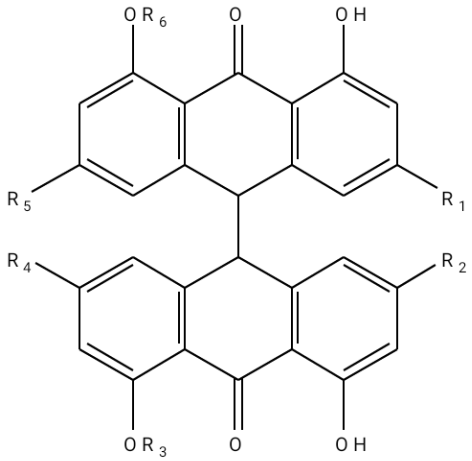
The most important secondary metabolites reported from *R. abyssinicus* are emodin (**1**), Chrysophanol (**2**) physcion (**3**), oxalic acid (**4**), Betulone (**5**), di isobutyl Phthalate (**6**) and Oleic acid (**7**) [36]. In addition, Emodin (**1**), chrysophanol (**2)** and physcion (**3**), aleo emodin (**9**) have investigated from the methanolic extract of the

root tubers of *R. dentatus* by reverse-phase (RP) HPLC [37]. A phytochemical investigation of the aerial parts of *R. aquaticus* resulted citreoresin (**8**) [48]. Emodin-6-O-β-D-glucopyranoside (**10**), emodin-8-O-β-D-glucopyranoside (**11**) and chrysophanol-8-O-β-D-glucopyranoside (**12**) were isolated from the roots of *R. patientia.* [39]. Methanolic extraction of *R. acetosa, R. acetosella, R. confertus* were contains Oxanthrone-C-glycos, endocrociides, patientosides A (**13**) and B (**14**), rumejaposides E (**20)** and I (**28**), and cassialoin (**29)** [40].

Investigation of an aqueous acetone extract of *R. japonicus*, resulted rumejaposides A–E (**15**–**19**) [41]. Three epimeric pairs of C-glucosyl anthrones [rumejaposides E (**15**), F (**20**), G (**21**), H (**22**), I (**23**) and cassialoin(**24**)] were also reported from the roots of *R. dentatus* by on-line HPLC-UV-CD analysis [42].

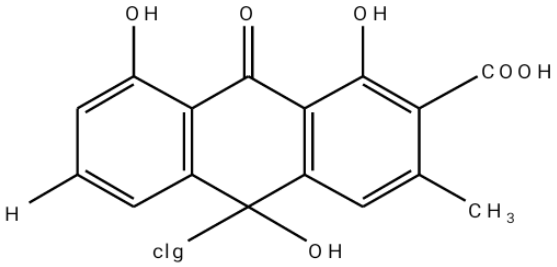
From the roots of *R. crispus*, few anthraquinones like 1,5-dihydroxy-3-methylanthraquinone (ziganein,**25**), 1,3,5-trihydroxy-6-hydroxymethylanthraquinone (**26**) and 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**27**), together with rumexone (**28**) were isolated [43].

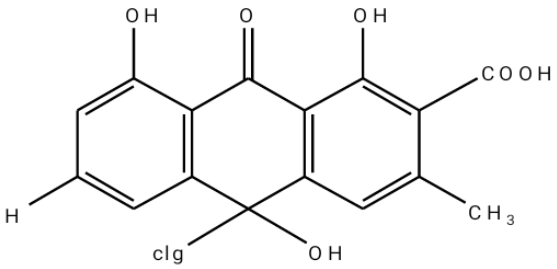




13.R_1=COOH,R_2=COOH,R_3=R_6=glcR_4=R_5=H

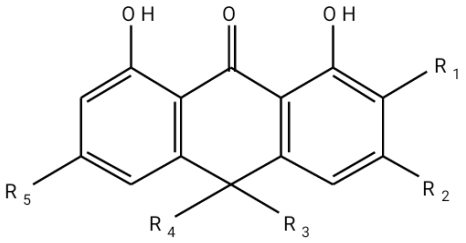
14.R_1=COOH,R_2=COOH,R_3=R_6=glc,R_4=R_5=H





15

16



17. R_1=COOH, R_2=CH_3,R_3=OH,R_4=glc, R_5=OH,

18. R_1=H, R_2=CH_2OH, R_3=OH,R_4=glc, R_5=OH

19. R_1=H, R_2=CH_3,R_3=OH, R_4=glc, R_5=OH,

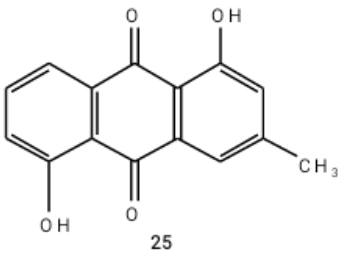
20. R_1=H, R_2=CH_3,R_3=OH, R_4=glc, R_5=OH,

21. R_1=H, R_2=CH_3,R_3=H, R_4=glc, R_5=OH,

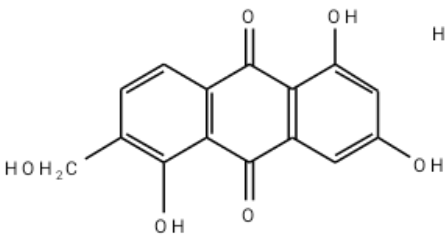
22. R_1=H, R_2=CH_3,R_3=H, R_4=glc, R_5=OH,

23. R_1=H, R_2=H, R_3=OH, R_4=glc, R_5=CH3,

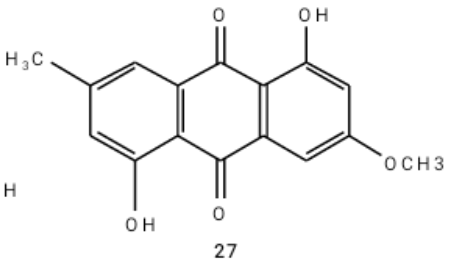
24. R_1=H, R_2=CH_3,R_3=OH, R4=glc, R_5=H



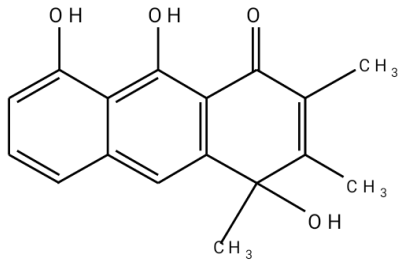
25



26



27



28

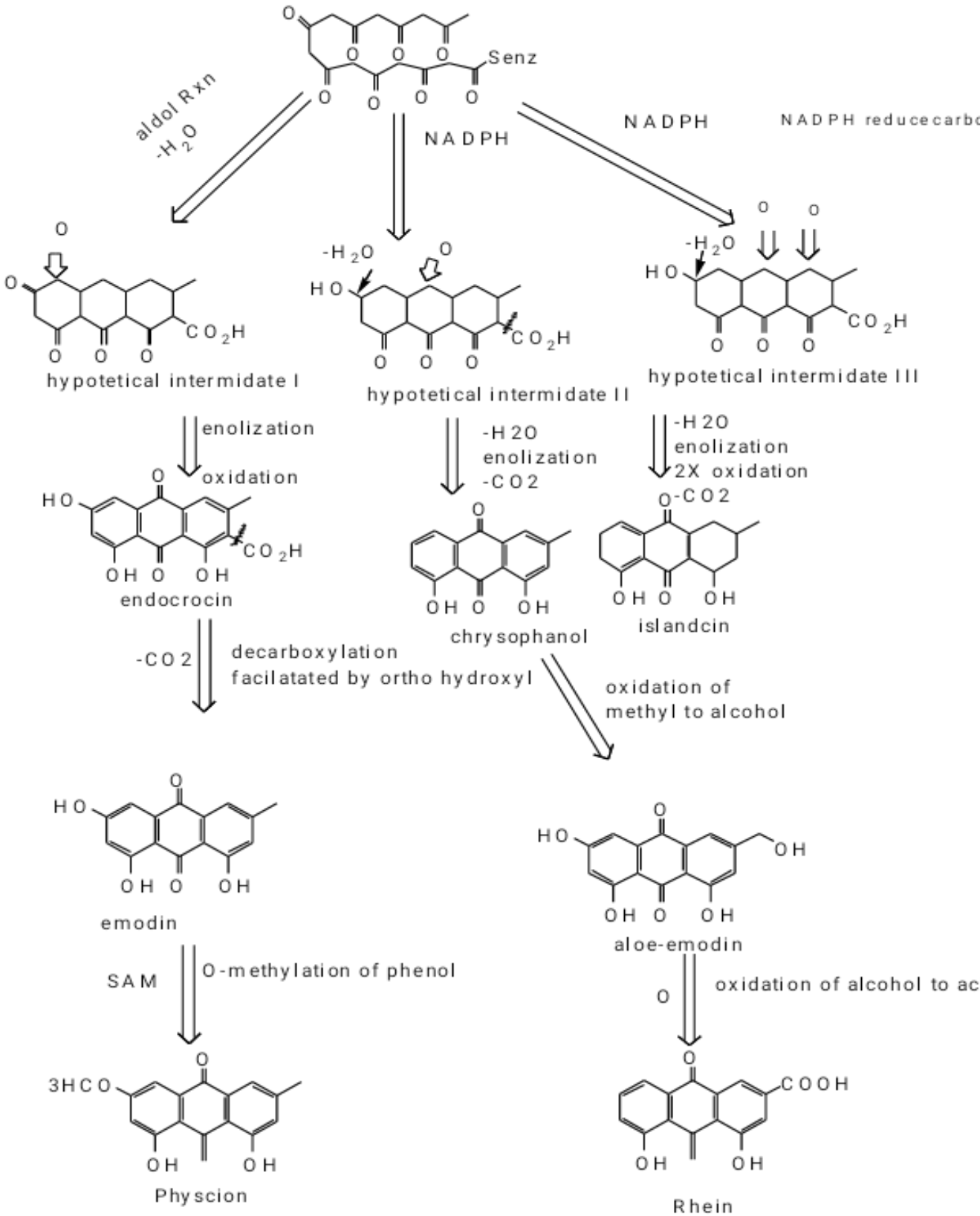
Figure 3. Chemical structure of some anthraquinone

2.2.2. 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. In higher plants, anthraquinones are biosynthesized either via acyl polymalonate (as in the plants of the families Polygonaceae and Rhamnaceae) or via shikimic acid pathways (as in the plants of the families Rubiaceae and Gesneriaceae) [37].

Anthraquinone with substituent on both benzenoid rings 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 pathway that leads to anthraqunione. The biosynthesis of anthraquinone is accomplished by multi enzyme complex [44].

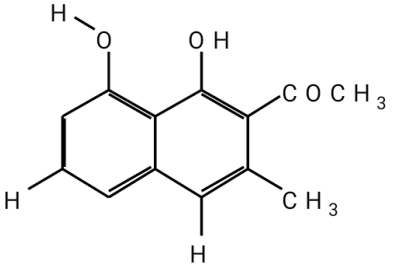
As a general rule fungal anthraquinone and plant anthraquinone with hydroxyl groups on both ring are derived from Polyketide, whereas plant anthraquinone devoid of hydroxyl group in one ring, example, alizarin come from mixed path way. Emodin, chrysophanol, physcion, aloe-emodin and rhein are some of the anthraqunione formed from one acetyl CoA and seven-malonyl CoA unit following the Polyketide pathway [45].



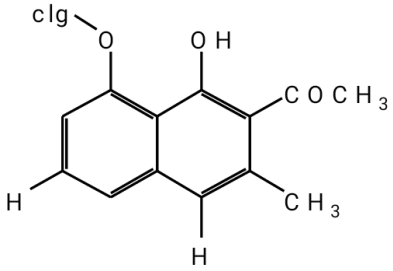
Scheme 1 Biosynthesis of Anthraquinone via the Polyketide path way

2.2.3. Naphthalenes

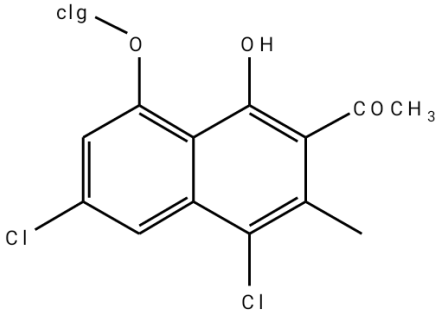
A phytochemical investigation of the roots of *R. Alpinus* resulted in the isolation of the naphthalene-1,8-diols nepodin (**29**), nepodin mono glucoside (**30**) and methoxynepodin (torachrysone,**31)** [46]. Rumexoside (**32**), orientaloside (**33**) and torachrysone (**34**) were isolated from the roots of *R. nepalensis* (Fig.4). The presence of nepodin-8-O-β-D-glucopyranoside (**35**), torachrysone (**36**) were reported from the EtOAc fraction of the roots [47].



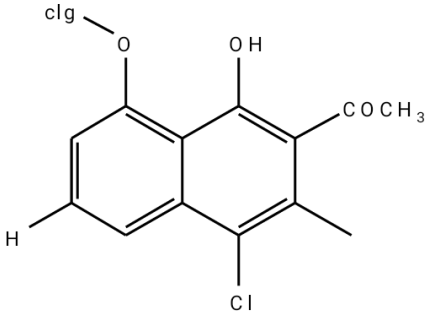
29



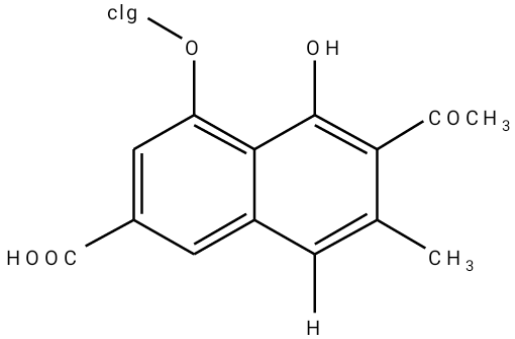
30



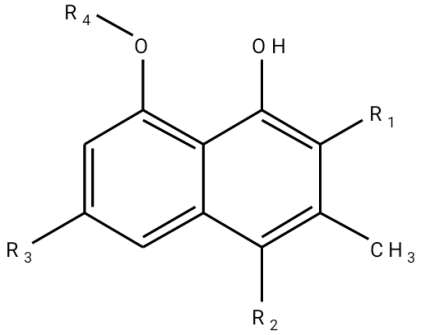
31



32



33



34. R_1=$COCH_3$,R_2=H, R_3=H,R_4=glc-glc,
35.R_1=$COCH_3$,R_2=H,R_3=OCH3,R_4=H,

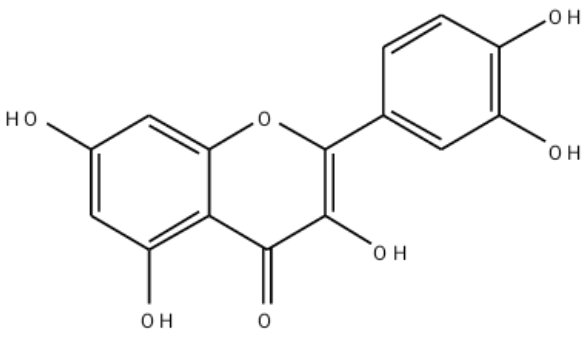
36. R_1=$COCH_3$,R_2=H, R_3=OCH_3,R_4=glc

Figure 4. Chemical structure of some naphthalene

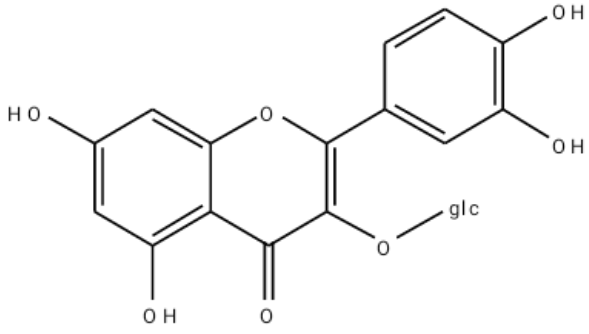
2.2.4. Flavonoids

Flavonoids are another important class of secondary metabolite that isolated from *Rumex* species (Fig. 5). Polyphenolic compounds occurring in plants were either as flavonols or their O/C-glycosides. Quercetin (**37**), kaempferol-3-O-β-D-glucosides (astragalin, **38**), quercitrin (**39**), isoquercitrin (**40**) and (þ)-catechin (**41**) were isolated from EtOAc extract of *R. japonicas* [48] and rutin (**42**) and epicatechin (**43**) were obtained from aqueous acetone extract of the root *R. japonicas* [43].

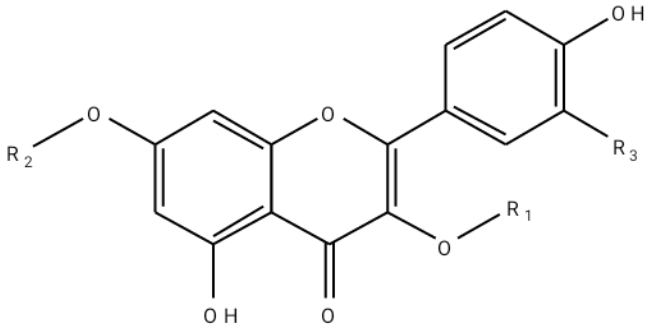
From the aerial parts of *R. aquaticus*, kaempferol-3-O-β-D-glucuropyranoside (**44**), quercitrin (**45**) and quercetin-3-O-β-D-glucuropyranoside (**46**) were isolated [49]. Later, quercetin-3-O-galactoside (hiperoside, **47)** and quercetin-3-O-arabinoside (guaijaverin,**48**) were yielded from the plant [53]. The phytochemical investigation of the EtOAc extract of the *R. Acetosa* herb were yielded numerous flavan derivatives catechin (**79**), epicatechin (**50**), epicatechin-3-O-gallate (**51**) [50].



37



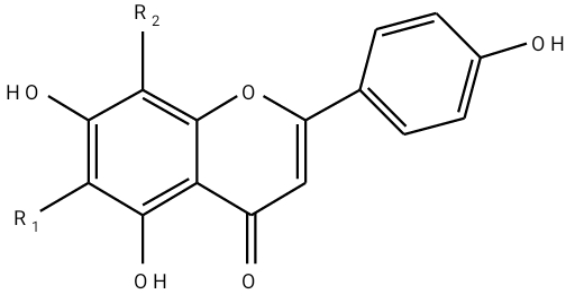
38



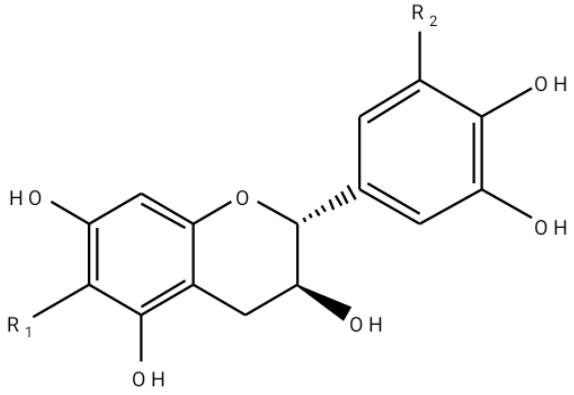
39. R_1=glu, R_2=H, R_3=H

40. R_1=gal, R_2=H, R_3=H,

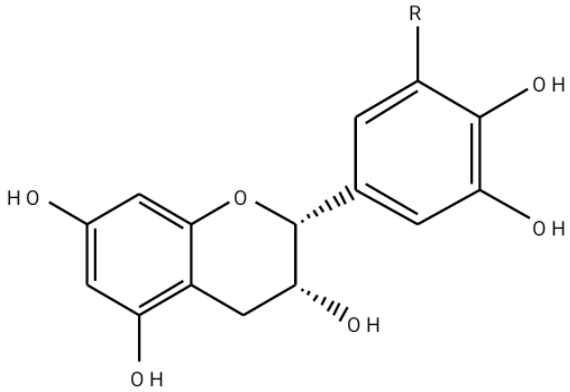
41. R_1=ara, R_2=H, R_3=H **42**. R_1=rha, R_2=H, R_3=OH, **42**. R_1=glc, R_2=H, R_3=OH,



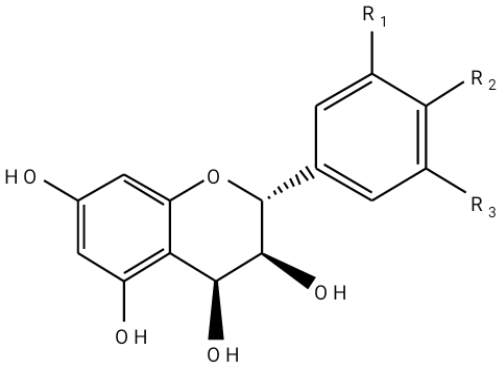
43. R_1=H, R_2=H **44**. R_1=H, R_2=glc **45**. R_1=glc, R_2=H



46. R_1=H, R_2=H **47**. R_1=H,R_2=OH, **48.** R_1=Cl,R_2=H



49.R=OH **50**. R=H



51. R_1=OH, R_2=OH, R_3=H **52**. R_1=OH, R_2=OH, R_3=OH,

Figure 5. Chemical structure of some flavonoid

2.2.5. Carotenoids

From steam-cooked *R. rugosus*, anhydroluteins I (**53**) and II (**54**) were isolated (Fig.6). These compounds could be formed by the acid-catalysed dehydration of lutein (**55**) [51]. In a comparative study, the lutein (**53**) and β-carotene (**56**) contents of frequently consumed uncultivated and cultivated leafy vegetables were investigated.

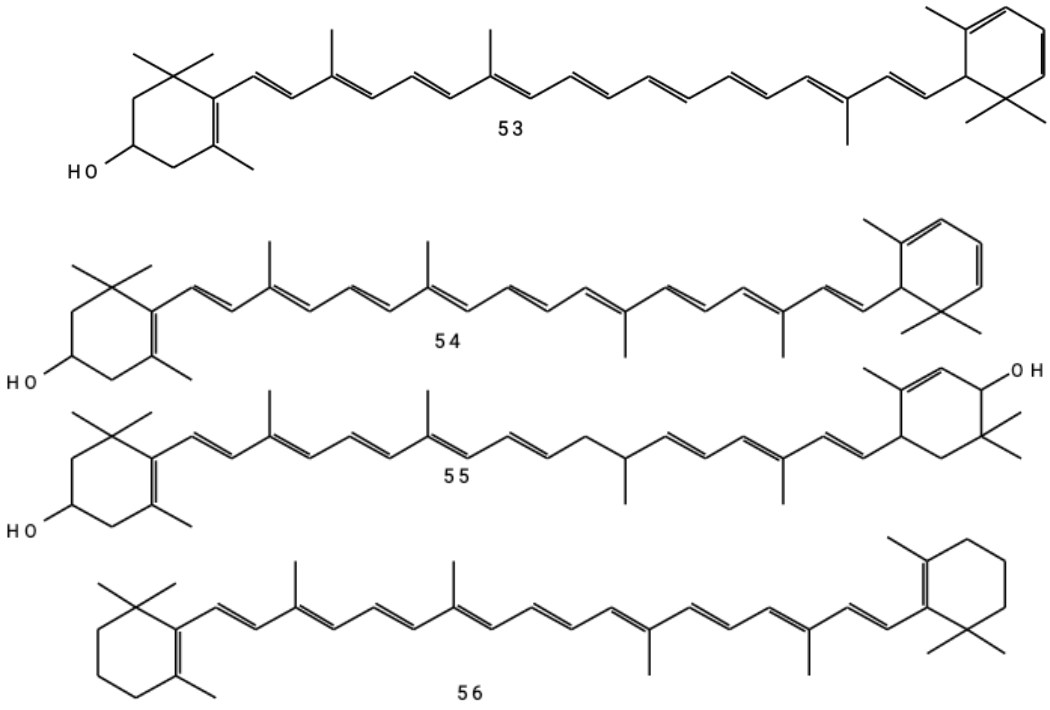
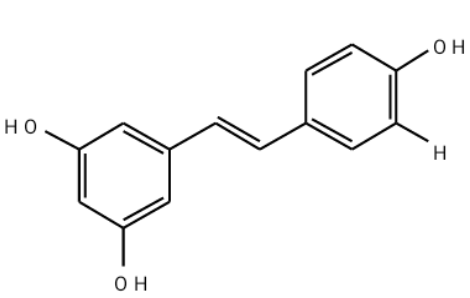


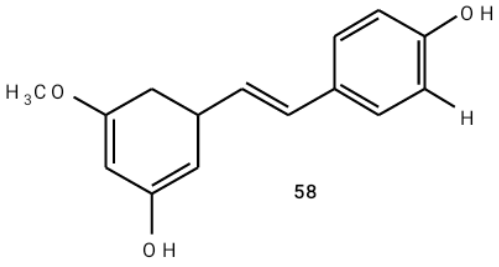
Figure 6. Chemical structure of carotenoids

2.2.6. Stilbenoids

Hydroxylated stilbenes are among the most interesting and therapeutically important groups of plant-derived polyphenols. Figure 7 gives strictures of some common stilbenoids. The most studied one are trans-resveratrol (**57**) and its glycoside, piceid (**58**). Resveratrol (**57**) has been reported to provide protection against cardiovascular diseases through its lipid-lowering activity and by inhibiting lipid peroxidation in humans [52]. Two mono methylated stilbene derivatives [5,4'-dihydroxy-3-methoxystilbene (**58**) and 3,5-dihydroxy-4'-methoxystilbene (**59**)], were reported from the EtOAc extract of the roots of *R. bucephalophorus* [53]. Other study was identified four stilbenoids resveratrol (**57**), 4-[(E)-2-(3,5-dihydroxyphenyl)ethenyl]-1,2-benzenediol (**60**), 4-[(E)-2-(3,5-dihydroxyphenyl) ethenyl] phenyl-hexopyranoside (**61**) and 4-[(E)-2-(3,5-dihydroxyphenyl)ethenyl]-2-hydroxyphenyl-hexopyranoside (**62**)] from the roots of *R. hymenosepalus* [54].



57



58

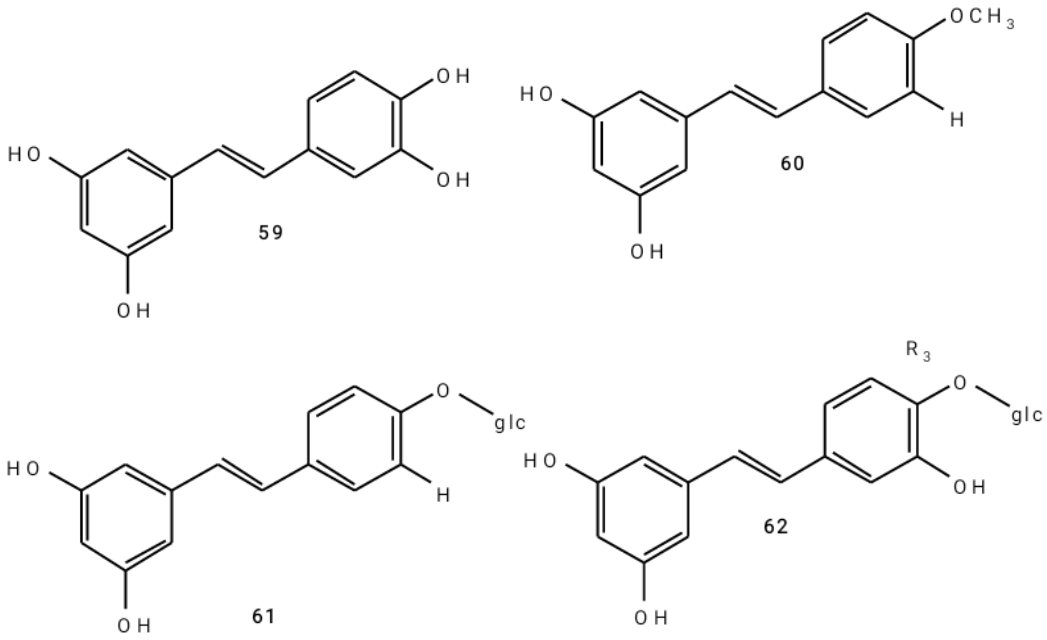


Figure 7. Chemical structure of few stilbenoids from Rumex species.

2.3. Traditional Use of Rumex Species

The genus *Rumex* plants have been used traditionally either as edible plants or for the treatment of several diseases in many parts of the World.

2.3.1. *Rumex* species as foods and coloring agents

The aboveground parts of numerous species (e.g. *R. acetosa, R. acetosella, R. crispus, R. patientia and R. pseudonatronatus*) gathered mainly during the spring and used as vegetables [55]. In most cases, the roots applied in therapy, but other plant parts, such as the leaves, fruits, and, the seeds are also used. On occasion, leaves are used for sauces and soups or dressed with olive oil and sometimes mixed with boiled potatoes to mitigate their acidity [57]. Some plants (e.g. *R. acetosa, R. acetosella, R. alpinus and R. nepalensis*) are consumed fried in olive oil or sautéed with butter or lard or are used as filling for pie [58]. The stems of *R. acetosa* and *R alpinus* are consumed as raw snacks, while the roots of *R. Hymenosepalus* used as

chewing gum in North America [59].

In some regions of India, almost all parts of *R. crispus* is used either as food or as a medicine. The very young leaves of the plant are added to salads, cooked as a potherb or added to soups; stems are peeled and the inner parts eaten, and finally seeds are grounded into a powder and used as flour for making pancakes [60]. The rhizomes of *Rumex abyssinicus* are used to refine butter and give it a yellow color [61]. Moreover, the tuberous roots of some plants (e.g. *R. abyssinicus, R. hymenosepalus*) have been used in Kenya and North America as a source of a yellow dye, which renders cellulose fibres red□brown when applied in the presence of sodium carbonate [20]. The leaves of *Rumex abyssinicus* and other Rumex species are utilized as foods, mainly in the forms of sour soups in milk, sauces and salads in some regions of the world [62].

2.3.2. *Rumex* species in traditional medicines

Several Rumex species are traditionally used as medicinal plants for treating different kind of dieses throughout the globe. Decoctions or infusions are prepared from the plant for medicinal application by utilizing other several processes.

In Africa, the aqueous root extracts of *R. abyssinicus, R. usambarensis* and *R. bequaertii* (*syn. Rumex nepalensis Spreng*) have been utilizes as remedies for various types of stomach disorder, while the extracts of *Rumex Abyssinicus* drunk to control mild diabetes, and as an antihypertensive, diuretic and analgesic. R. steudelii is one of the antifertility plants used in the folk medicine in Ethiopia. The roots of the plant, in combination with other medicinal plants, are additionally used in the

treatment of rectal prolapse, haemorrhoids, wounds, eczema, swelling, leprosy, abdominal colic and tinea nigra [63]. R. nepalensis is applied to treat stomach ache in Ethiopian regions. R. vesicarius is a wild edible Egyptian herb. In folk medicine, it is used as a tonic and analgesic and for the treatment of hepatic diseases, constipation, poor digestion, spleen disorders, flatulence, asthma, bronchitis, dyspepsia, vomiting and piles, among others [61-63]

In Europe, different parts of Rumex species mainly *R. acetosa, R. acetosella, R. alpinus, R. confertus, R. crispus* and *R. obtusifolius* are uses for the treatment of different diseases such as constipation, diarrhea, swellings, sores, rashes and wounds and as an astringent [64]. Leaves and roots of *R. alpinus* have used internally for the treatment of viral infections in Austria [65]. In Britain and Ireland *R. acetosa* is used for the treatment of scurvy, wounds, warts, bruises, jaundice and sore throat. Moreover, *R. hydrolapathum, R. conglomeratus* and *R. palustrisare* also applied in case of scurvy, as blood purifier, for bathing rashes and sunburn, and cancer cure [66].

In the traditional Chinese Medicine, several Rumex species (*R. dentatus, R. hastatus, R. nepalensis, R. japonicus* and *R. aquaticus*) have been used for the therapy of different diseases including bacterial and fungal infections, coughing, headache, fever, eczema, dysentery, diarrhoea, constipation, jaundice, haematemesis and uterine haemorrhage [67].

2.4. Antimicrobial Activity

Phytoconstituents employed by plants to protect them against

pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine [68]. Antimicrobial activity of some Rumex species were evaluated for the treatment of infectious diseases against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* [69].

Various antibacterial activities have been reported for extracts from different parts of Rumex species using different solvents [70]. Table 2 gives antimicrobial activities of some compounds that were isolated from Rumex species. The antimicrobial investigation of ether, ethanol, and hot water extracts of the leaves and seeds of *R. Crispus* indicated that the ether extracts of both plant parts and the ethanol extract of the leaves possessed activities against *S. aureus* (diameter of inhibition zone: 8 mm) and *B. subtilis* (diameter of inhibition zone:8 mm) [71]. *Rumex nervosus* and *Rumex abyssinicus* exhibited antibacterial activity against *S. Pyogenes, R. nervosus,* and *S. aureus*. Anti-inflammatory property of *R. abyssinicus* could justify for the treatment of several skin diseases [72].

The antifungal activities of *Rumex* species were tested against a number of fungal pathogens (*Aspergillus fumigatus, A. flavus, A. versicolor, A. Niger, Blastoschizomyces capitatus, Fusarium oxysporum, F. moniliforme, F. semitectum, Pythium sp., Rhizopu ssp., Sporotrichum sp.,* and *Thermomyce ssp.*)[73]. The antifungal effects of *R. dentatus* alcoholic extracts were evaluated against *A. versicolor*, *A. flavus, Acremonium spp., Penicilium dimorphosporum, Candida albicans, C. kruesieand C. parapsilosis.* The highest effect was observed against *C. albicans* (1472.0 mm at 500 µg/mL) [74].

R. abyssinicus roots were used for anti-microbial activities. The roots are

reported to possess antibacterial activity against *Streptococcuspyogenes.* The plant has also strong antiviral activity against *Coxsackie virus and influenza A virus* [75]. *In vitro,* it demonstrated proliferation of murine macrophage cells, suggesting that it may have a role in improving the immune system of the body. It also contains promising bioactive compounds that might be useful in the control of helminthes infections by interrupting the worms' life cycle and preventing their growth [76].

Table 2. Compound of Rumex species and their antibacterial activity

Rumex species	Plant part	Extract	Isolated compound	*Bacterial strain*	Ref.
R. abyssinicus	Root	80% MeOH MeOH	1.2,3,4,5,6,7	*S. pyogenes, S. aureus, Corynebacterium Diphtheria*	57
R .patientia	Root		1-3, 6,7,8		[43]
R .acetosa *R. acetosella* *R. confertus*		EtOAc	4,9,10,11,12,13,14,51,44, 46	*S. aureus, S. epidermidis, E. coli, Proteus mirabilis*	46,2,
R. dentatus	Root	MeOH Online HPLC	13,4,20,21,22,23,24	*Bacillus megaterium, B. subtilis, Enterobacter cloacea, P. aeruginosa*	[42]
R. aquatic	Arial part		5,33,47,48,49,50	*B. subtilis, B. cereus, E. coli*	42,45
R. vesicarius	Seeds	EtOH		*P. aeruginosa, E. coli, S. pneumoniae, S. aureus*	62
R. japonicus	Leaf, root, fruits,stem	EtOH, n- hexane, $CHCl_3$, EtOAc, H_2O	15,19,40,41,42,43,44,45,4 6	*B. subtilis, B. cereus, E. coli*	46,47
R. nepalensis	Root	n-Butanolic EtOAc	5,25,26,27,,33,36,37,38,3 9	*S. aureus, E. coli, B. subtilis, P. aeruginosa*	42,4345,
R. crispus	Root	ether, EtOH, H_2O	28,29,30,31	*P. aeruginosa, E. coli, S. pneumoniae,* *S. aureus*	43,45
R alpinus			32,33,38		45
R. rugorus	Steam	Acid canalized	52,53,54,55		53
R. bucephalophours	Root	EtOAc	56,57,58,59	*S. aureus, E. col*	54,55
R.	Root and		60,61,62	*P. aeruginosa, E. coli, S.*	56

hymenosepalus	tuber			*pneumoniae, S. aureus*	

3. Material and Methods

3.1. Chemicals

Chemicals that used for this study were petroleum ether, chloroform, acetone, ethyl acetate (analytical grade, Loba chemie put, India), for gradient extraction and column elution. Iodine was used for detection of spots on TLC. In addition, silica gel 60-120 mm mesh size (Loba Chemie, India) for isolation of compounds was used. Dimethyl sulfoxide (DMSO), Mueller Hinton agar, and nutrient broth were used for antimicrobial test. All chemicals and reagents used were analytical grade.

3.2. Apparatus and Instrument

Apparatus such as Rota vapor (Heidolph, Germany, laboratory 4000, No, 519-0000-00-2) plates, round bottom flask size 250,500, and 1000 mL, measuring cylinder different volumes, filter papers (cotton swab), weighing balance (model NWT100001X) oven (N5OC GENLAB WIDNES, England) for drying purpose. Analytical TLC was performed on pre- coated silica gel 60 F_{254} plates. Incubator (Gene lab incubator) and Hood (CLB-201-04, vertical laminar cabinet) were used for antimicrobial activity study. Glass column chromatography (500 mm, B-34/35) and UV chamber (254nm and 365nm) were used for isolation of compounds and detection of the spot on TLC, respectively. NMR spectra were recorded on an Avance 600 MHz spectrometer (Bruker, Billerica, MA, USA) using DMSO solvent.

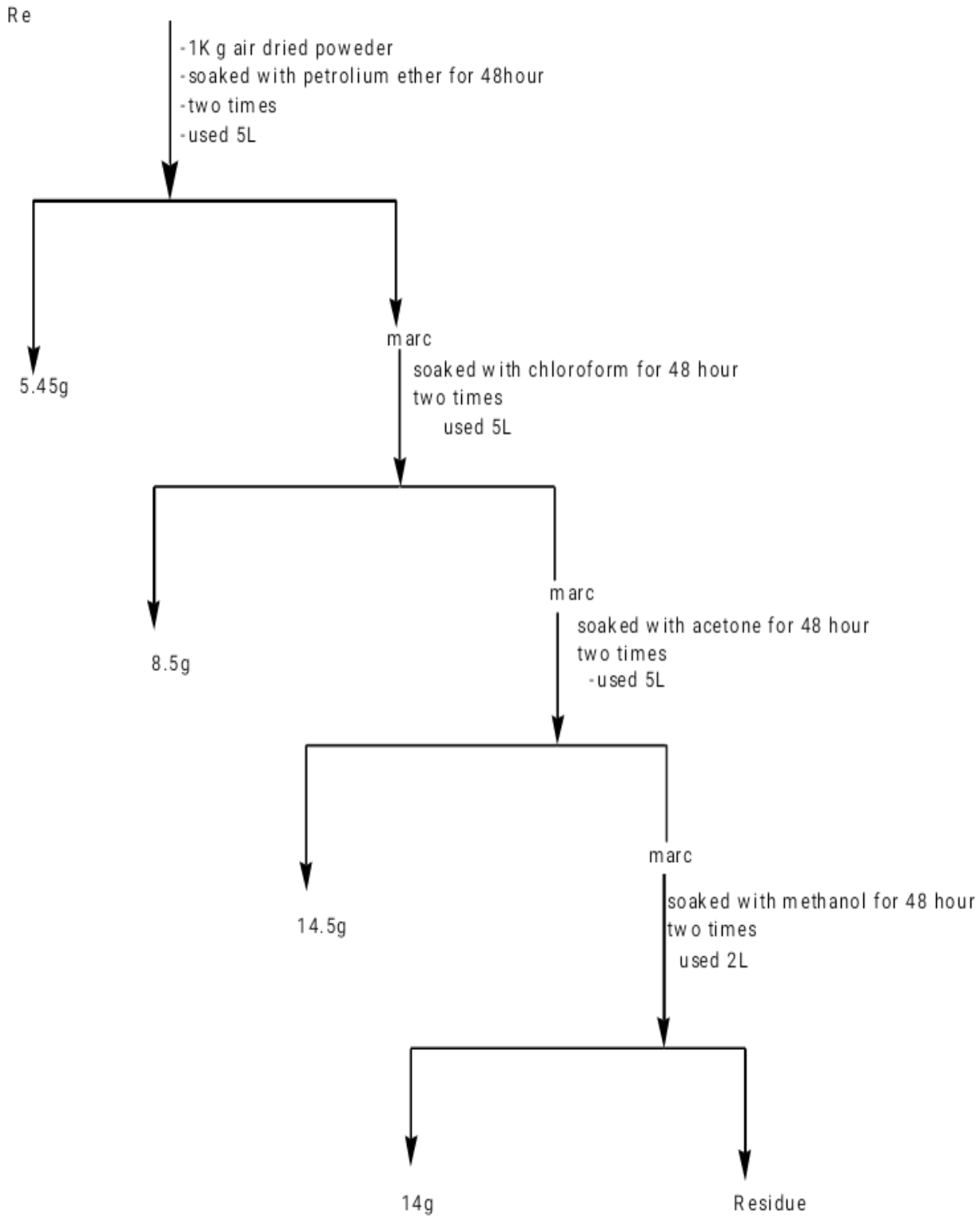
3.3. Sample Collection and Preparation

The plant material, roots of *Rumex abyssinicus* were collected from Oromia

regional state, Jimma zone, Gomma District around Limusapa keeled in July 2019, which is located at 420 Km away from Addis Ababa, Ethiopia. The collected root of the *R. abyssinicus* plant was washed, and then chopped in to smaller pieces and shade air-dried at room temperature in laboratory. The sample was more homogenized to suitable size to increasing rate dissolution of solvent in to the cells by using mechanical grinding.

3.4. Extraction

The powdered plant material (1kg) was sequentially extracted with petroleum ether, chloroform, acetone, and methanol using maceration technique (2 x 2.5 L) for 48 h for each case, in increasing order of polarity. That is, the finely powdered plant material (1kg) was socked with petroleum ether (2 x 2.5 L) for 48 h. The combined extracts were filtered and concentrated by means of a rotary evaporator. The solvent free marc was then socked with chloroform (2 x 2.5 L) for 48 h and extracted. The extracts were filtered and concentrated. The solvent free marc again socked with acetone (2x2.5 L) for 48 h and extracts were filtered and concentrated. At the last, the marc was socked with methanol (2x1 L) and extracts were filtered and concentrated. Filter paper and cottons were used during filtration. The residual solvent in each gradient extract was removed using rotary evaporator under reduced pressure at 45 (C. The resulting semidried mass of each fraction were stored at 4 °C until used for further experiments. Scheme 1 shows the general procedure of extraction the root of *R. abyssinicus*.



Scheme2. General procedure of extraction the root of *R. abyssinicus*

3.5. Isolation of compounds from *R. Abyssinicus*

In this phytochemical investigation, the acetone extract of root of *R. abyssinicus* showed three spots on TLC (Fig.8) and better antimicrobial activity than the other solvents extracts. As a result, it was subjected to column chromatographic separation using bare petroleum ether which followed by a binary solvent system (petroleum ether/ ethyl acetate) to isolate compounds (Fig. 9). Since petroleum ether/ ethyl acetate combination showed better separation of the compounds on TLC tests. The silica gel (60-120 mesh) was activated in hot air oven at 100 °C for 2 h and a glass column was packed with the activated 250 g silica gel slurry in petroleum ether. The crude acetone extract (14 g) was adsorbed on to 10 g silica gel and loaded onto the column packed with silica gel.

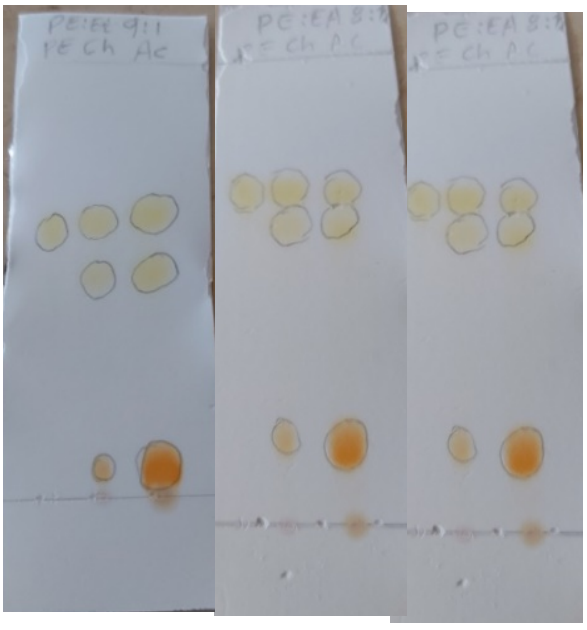


Figure 8.TLC profile of the crude extracts

The elution was started with petroleum ether and increasing polarity using 1 up to 100% ethyl acetate. Meanwhile, total of 148 fractions were collected each with 20 mL in small beakers. The first 30 fractions were mixtures after TLC analysis. Using TLC and Rf value results, fractions with similar Rf values were combined together. Fractions 52-65 were combined and washed repeatedly with n-hexane to afford compound-**1** (4 g). The elution was continued by increasing the polarity of solvent. The second pure compound (2) was obtained from fraction 66-81. It shows one spots and some impurities which was later purified by continues washing it with n-hexane. Then, crystalline orange colour product of 0.38 g was obtained. The isolated compounds were characterized by using ^{1}HNMR, ^{13}CNMR, 2D NMR spectroscopy techniques.

Figure 9.Column set up of the experiment

3.6. Antimicrobial activity test

3.6.1. Test strains and Preparation of test samples

Microorganisms used for evaluation of antibacterial activities of the crude plant extracts were Gram positive: *Staphylococcus aureus* (*ATCC25903*) and Gram negative: *klebsiella pneumoniae (NCTC13368*), *Escherichia coli* (*ATCC 25722) and Pseudomonas aeruginosa* (*DSMZ 1117*). *Candida albicans* and *S. cerevisiae* were used for determination of antifungal activities of crude extracts.

The test solutions were prepared by dissolving 50 mg of crude extract of the plant in 1 mL of Dimethyl Sulfoxide (DMSO) to achieve final stock concentration of 50 mg/mL solution for both antibacterial and antifungal activities evaluation. Gentamicin (10 mg) and Mancozeb (10 mg) were used as reference drug (positive control) to check antibacterial and antifungal activities, respectively.

3.6.2. Preparation of fresh inoculums

Agar disk diffusion method was used to evaluate the antibacterial activity of both crude extracts and isolated compounds on nutrient agar. The stock cultures were maintained on the nutrient agar slants which were stored at 4°C. The antimicrobial activity tests were done by disc diffusion method using standard procedures. Nutrient agar 15 mL poured into petri dishes and allowed to crystalline in lamina flow apparatus under sterile conditions [78]. The test carried out against four bacterial and two fungal strains by distributing 1 mL of each microbial suspension on agar petri dishes prepared separately. Then, 6 mm filter paper discs were soaked in each stock solution of crude extracts and fractions in DMSO at concentration of

50 mg/mL. Then, put onto petri dishes seeded with test organisms. The standard disc containing 10 mg/mL Gentamicin and 10 mg/mL Mancozeb were used for bacterial and fungal, respectively as positive control and DMSO as negative control. The dishes finally incubated for antimicrobial activity (antibacterial 37 °C and antifungal 27 °C) sensitivities confirmed after 24 h of incubation by measuring the diameter of the zone of inhibition (M±SD) (Appendix 1). All tests were performed in duplicates.

Active cultures for this experiment were prepared by transferring a loop full of bacterial cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) that incubated without agitation for 24 h at 37 °C. A cell suspension of each organism was freshly prepared by transferring isolated colonies selected from a 24 h agar plate in to a broth and the suspension turbidity adjusted to a 0.5 McFarland turbidity standard ($1x10^8$ CFU/mL) in sterile saline solution.

Potato dextrose agars (PDA) have maintained at 4 °C to prepare stock concentration of fungal cultures. Active cultures for the experiment were prepared by transferring a loop full of fungal cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB); which is the same methods with bacterial stock strains preparation except the condition it undergo here potato dextrose agar (PDA) as the test media. Mancozeb (10 mg/mL) as standard antifungal agent and longer incubation pared (24(48 h) was employed (Appendix 2).

4. Results and Discussion

4.1. Yield of extraction

The root part of the plant species (1 kg) was sequentially extracted with petroleum ether, chloroform, acetone and methanol by using maceration method. These extracts were weighted after removal of the solvents and obtained 5.5 g of petroleum ether extract, 8.5 g of chloroform extract, 14.50g of acetone extract, and 14.0 g of methanol extract. The highest percentage yield was obtained from acetone extract, whereas the lowest percentage yield was obtained from petroleum ether extract. The amounts of the crude extracts and their percent yields obtained from extraction of plant material are given below (Table 3).

Table 3. Percentage yields of the main crude extracts of *R. abyssinicus*

Extraction solvents	Observed data of Extract root of *Rumex abyssinicus*	
	Mass (g)	Yield (%)
Petroleum ether extract	5.50	0.55
Chloroform extract	8.50	0.85
Acetone extract	14.50	1.45
Methanol extract	14.0	1.40

4.2. Evaluation of antibacterial activities of crude extracts

The antibacterial activity of the petroleum ether extract, chloroform extract, acetone extract and methanol extract of root of *Rumex abyssinicus* was

tested using agar disc diffusion method against Gram negative bacteria (*E. coli and P. aeruginosa*) and Gram positive bacteria (*S. aureus* and *Klebsiella pneumoniae*). The bioassay test was also conducted on the two fungal strains (*S. cerevisiae* and *C. albicans*) to determine antifungal activity of the extracts.

Among the four tested extracts, acetone extract showed the highest inhibition zone against Gram positive bacteria (*S. aureus* 21.0 ±1.4) and Gram negative bacteria (*K. pneumoniae* 22.5±0.7, *P. aeruginosa* 15.5±0.4 and *E. coli* 18.0±0.4) (Table 4). Whereas, petroleum ether extract has not shown any inhibition zone against *P. aeruginosa* and *K. pneumoniae* strains. Similar susceptibility towards the test organisms for the different extracts was observed [79].

Table 4. Antimicrobial inhibition zones of crude extracts and isolated compound of root of *R. abyssinicus*

Bacterial strain	Zone inhibition of Plant extract(M±SD)				Positive control	Negative control	Isolated compounds	
	PE	CE	AE	ME	G	DMSO	C-1	C-2
S. aureus	9.5±0.7	17.5±1.4	21.0±1.4	18.0±0.1	34.5±0.7	NI	23.0±0.1	20.0±0.2
P. aeruginosa	NI	12.5±0.7	15.5±0.4	13.0±0.1	30.5±0.7	NI	18.0±0.2	19.0±0.3
E. coli	10.5±0.7	14.0±0.2	18.0±0.4	13.5±0.7	32.0±0.1	NI	18.0±0.4	17.0±0.7
K. pneumoniae	NI	11.5±0.7	22.5±0.7	15.0±0.2	30.0±0.1	NI	22.5±0.4	20.0±0.7
G							26.0±0.3	26.0±0.2
DMSO							NI	NI
Fungal strain					Mc		C-1	C-2
C. albicans	9.0±1.4	12.0±0.1	17.5±0.7	13.0±3	20.5±.07	NI	20.0±0.4	22.0±0.7
S. cerevisiae	7.5±0.7	10.0±0.7	15.0±0.7	15.0±1.4	24.5±0.7	NI		
Positive control	-	-	-	-	-	-	22.0±0.1	22.0±0.2

(Mc)								
Negative control (DMSO)	-	-	-	-	-	-	NI	NI

PE= petroleum ether extract CE=chloroform extract AE=acetone extract C-1 Compound-1 C-2=Compound-2 ME=methanol extract G=Gentamycin Mc=Mancozeb NI=no inhibition

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 [79].

4.3. Evaluation of antifungal activities of crude extracts of Root of *R. abyssinicus*

The results of the study have also shown that acetone extract exhibited good antifungal activities against both *S. cerevisiae* (15.0±1.4) *and C. albicans(*17.5±0.7) than chloroform and petroleum ether extracts, while methanol extract showed comparable activity to acetone extract against *S. cerevisiae* (Table 4). However, all the crude extracts showed less activity than the observed activity for the reference drug (Mancozeb). Generally, the highest inhibition zone of antimicrobial activity was observed by acetone extract. So, based on its high bioactivity, good TLC profile, and high yield; the acetone extract was selected for further isolation of compounds using column chromatography.

5. Characterization of isolated compound

In this phytochemical investigation, two compounds were isolated from acetone extract of the root of *Rumex abyssinicus*. Based on spectroscopic data (^{1}HNMR and ^{13}CNMR) and literature reports, these two compounds were characterized. The details structural elucidations of the compounds are discussed in the following sections.

5.1. Characterization of Compound-1

Compound-1 was isolated as yellowish crystalline solid from the fractions (52-65). Its R_f value was 0.65 in a solvent system containing petroleum ether: ethyl acetate (70: 30, v/v) as eluent. The proton NMR spectrum (Appendix 3 & 4) analysis showed the presences of five aromatic protons at δ_H 7.22, δ_H 7.39, δ_H 7.55, δ_H 7.71 and δ_H 7.81. In addition, hydroxyl groups observed at δ_H 11.93.

^{1}H-NMR spectroscopic data of compound-1showed a proton signal at δ_H 2.44 ppm, integrated for three protons. This represents a methyl group that attached to C-3 of aromatic ring. The two-broad singlet peaks that observed at (δ_H7.55, 1H) and (δ_H7.22, 1H) represent the proton attached on C-2 and C-4 of aromatic ring, respectively. The aromatic protons that attached to C-5 and C-7 are doublet at (δ_H 7.72, d, J=5, 1H) and (δ_H 7.39, d, J=5), respectively. The triplet proton observed at (δ 7.82, t, J=5,10, (m,1H)). In addition, the presence of two chelated protons at peri-position of C-1 and C-8 of the aromatic ring, highly deshielded. The observed proton and carbon NMR spectral values are summarized in Table 5 below.

Table 5. Comparison of the ^{1}HNMR and ^{13}CNMR Spectroscopic Data (600MHz) of compound-1 δ in ppm with reported data (400 MHz) [83]

	Compound-1		Reported data chrysophanol [81]		Remark
Position	δ^{1}H	δ^{13}C	δ^{1}H	δ^{13}C	
1	11.93 s	161.8	12.06 s	162.45	-
2	7.22 s	124.6	7.13 s	124.57	CH
3		149.6		149.35	-
4	7.55 s	120.0	7.67 s	121.37	CH
5	7.72 (d J=6,)	119.8	7.85 dd (J=1.2, 8Hz)	119.94	CH
6	7.82 t (J=6 Hz)	137.8	7.70 t(J=6,1OHz)	136.96	CH
7	7.39 d (J=6Hz)	124.9	7.29 dd (J=1.2, 8Hz)	124.577	CH
8		162.0.		162.75	-
9		192.0		192.58	Quaternary
10		181.0		182.02	Quaternary
11		133.8		133.68	Quaternary
12		116.3		113.77	Quaternary
13		114.2		133.32	Quaternary
14		133.5		115.90	Quaternary

C-Me	2.44 s	22.1	2.5 s	22.26	CH_3

^{13}CNMR spectrum showed 15 signals (Appendix 5 & 6) that represented 15 carbons including one methyl group at δ 22.09, five CH aromatic groups at C-2 (124.56), C-4 (121.01), C-5 (δ119.78), C-6 (137.79), C-7 (124.88) as evidenced from HSQC. In addition, The spectra also showed two-hydroxyl carbons at δ 162.05 and 161.79 which represent C-1 and C-8 and two aromatic carbonyl group observed at δ 181.96 (C-9) and δ 192.09 (C-10).The proposed structure is supported with COSY(Appendix 7), HSQC (Appendix 8 & 9) and HMBC(Appendix 10 & 11) spectra data.

The COSY correlation data show the coupling between δ_H (δ7.82) with δ_H (7.39) and δ_H (7.72), the coupling of proton δ_H (7.22) with δ_H (7.55) and δ_H (7.39) The other important correlation observed in the COSY spectrum is correlation between protons of CH_3 and δ_H (δ7.22). The H-C coupling is observed from the HSQC spectrum as shown in Table 6. The correlation of H-2 (7.22) with C-2 (124.56), H-4(7.55) with C-4 (121.01), H-5(7.72) with C-5 (119.78), H-6(7.82) with C-6 (137.79), and H-7(7.39) linked with C-7(124.88).

HMBC spectrum shows the correlation of proton with carbon that is two to four bonds distance. HMBC spectra data (Table 6) showed the proton at δ 7.22 (H-2) correlated with C-13(114.24), C-4 (δ120.0), Me (22.1) and correlation with C-1 (161.79). The correlation at δ7.55 (H-4) showed a long-range correlation with C-10 (181.02), C-2 (124.56), CH_3 (22.4) and with C-13 (114.24). The partial structure of ring A of compound-1 can be constructed as shown in Fig.10. The observed HSQC and HMBC of the compound is given in (Table 6)

Table 6. HSQC and HMBC spectral data of compound-1

δC		δH	m	HSQC	HMBC spectral data	Remarks
1	161.8	OH(δ 11.93)	s			
2	124.6	H-2(δ 7.22)	s	C-2 ⟷ H-2	H-2 ⟶ C-1,C-4,C-13, CH_3	CH
3	149.6	H-Me(δ 2.44)	s		-	CH3
4	120.0	H-4 (δ 7.55)	s	C-4 ⟷ H-4	H-4 ⟶ C-2,C-13, C10,CH_3	CH
5	119.8	H-5 (δ 7.72)	d	C-5 ⟷ H-5	H-5 ⟶ C-12,C-7,C10	CH
6	137.8	H-6 (δ 7.82)	t	C-6 ⟷ H-6	H-6 ⟶ C11,C-8	CH
7	124.9	H-7 (δ 7.39)	d	C-7 ⟷ H-7	H-7 ⟶ C-5,C-8, C-12,C-8	CH
8	162.0	OH			-	
9	192.0	_				Quaternary
10	181.0	_				Quaternary
11		_				Quaternar

	133.8					y
12	116.3	_				Quaternary
13	114.2	_				Quaternary
14	133.5	_				Quaternary
Me	22.4	2.44			H-Me → C-2,C-4,C-3	CH_3

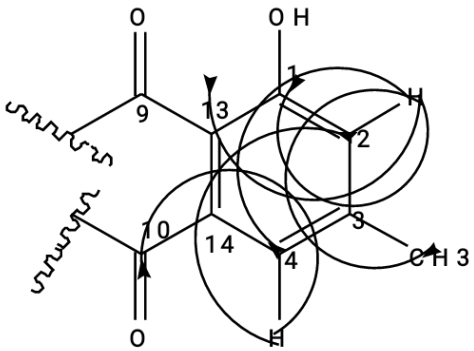


Figure10. Partial coupling structure of compound-1 (cycle. A)

The proton at δ_H7.72 (H-5) correlated with C-7 (124.87), C-10 (181.96) and coupled with C-12 (116.34). The correlation of H-7 with C-5 (119.78), C-12 (116.34) and with C-8 (124.87) was observed. Proton attached on C-6 at δ137.79 showed a long-range correlation with C-11 (δ133.78) and correlated with C-8 (δ162.05) which gives valuable information to construct partial structure of ring B of the compound-1 (Fig. 11).

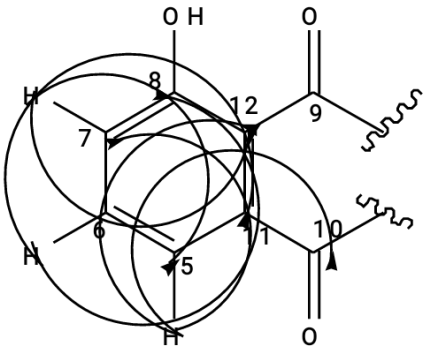


Figure 11. Partial structure of compound-1(cycle B)

All spectroscopic data were in good agreement with the suggested structure for compound-1 to be chrysophanol (Fig. 12).

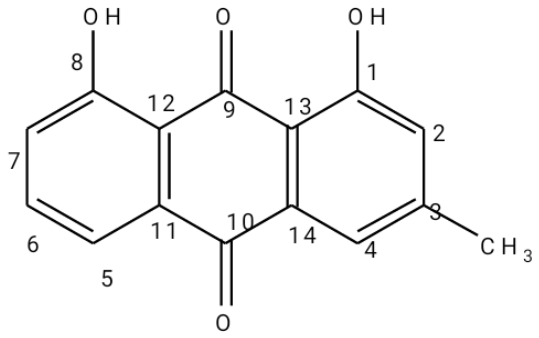


Figure 12. Complete Structure of compound-1 (chrysophanol)

5.2. Characterization compound-2

Compound-2 was isolated as an orange crystalline solid with R_f (0.58) value in petroleum ether-ethyl acetate (70:30). The ^{1}HNMR spectrum of the compound shows the presence of four aromatic proton at δ_H7.42 s, δ_H 7.11 br s, δ_H 7.06 d, J=1.2 and δ_H 6.55 d, J=1.2 (Appendix 12 & 13). It also revealed the presence of one set of methyl proton at δ_H 2.51 and two-hydroxyl proton at δ_H 12.23 s, and δ_H 12.32 s.

The ^{13}CNMR data of compound-2 is shown in Appendix 14 & 15, which

indicates the presence of 15 carbon atoms (Table 8). Among these carbons, there are four CH group of aromatic ring at δ_C (124.5), δ_C (120.9), δ_C (109.2) and δ_C (108.3), two-carbonyl carbons that attached to benzene ring at δ_C (181.7) and δc (190.1), three hydroxyl containing carbons of aromatic ring at C-1(δ161.9), C-6 (δ164.9), C-8 (δ166.0) and five quaternary carbons. The ^{13}C NMR spectrum also showed the presence of one methyl carbon at δ 22.0 and one methyl substituted carbon observed at δ 148.7(C-3).

Table 7. Comparison of the observed ^{13}CNMR spectroscopic data of Compound-2 with literature [79]

Position	Compound-2 [600MHz]		Emodin [400 MHz]	
	δ_H	δ_C	δ_H	δ_c
1	11.96 s	161.9	121	161.88
2	7.11b s	124.5	7.18 b s	124.63
3	-	149.7	-	148.77
4	7.42 s	121.0	7.50 b s	121.0
5	7.06 d J=1.2	109.2	7.20 d J=1.6	109.5
6	-	164.9		164.9
7	6.55 d J=1.2	108.3	6.49d.J=1.6	108.4
8	12.04 s	166.03	12.15 s	166.2
9	-	181.7		182.2
10	-	190.1		190.7
11		135.5		135.6
12	-	109.3		109.4
13	-	113.7		113.9
14	-	133.2		133.3
Me	H-Me δ 2.50 s	C-Me δ 21.97	H-Me 2.40 s	C-Me δ 21.98

The structural elucidation of compound-2 was also supported by data of 2D NMR spectra correlation (COSY) (Appendix 16), Heteronuclear Single Quantum Correlation (HSQC) (Appendix 17 & 18) and Heteronuclear Multiple Bonds Correlation (HMBC) (Appendix 19 & 20) as shown in (Table 9). The correlation of OH (δ 11.96) to C-1(δ 161 .9), H (δ 7.11) with C-2 (δ 121.0), H (δ 7.42) to C-4 (δ 124.5), H (δ 7.06) to C-5 (δ 108.3), H (δ 6.55) to C-7 (δ 109.2), OH (δ 12.04) to C-8 (δ 164.9) and H (δ 2.44) to CH_3 data obtained from HSQC data.

Table 8. Observed correlation in COSY, HSQC and HMBC spectroscopic data of Compound-2

Position	δ C	δH	HSQC data	HMBC
1	161 .9	11.96 s	OH-1 ⟷ C-1	OH-1 ⟶ C-1,C-2,C-13
2	121.0	7.11b s	H-2 ⟷ C-2	H-2 ⟶ C-1,C-4,C-13,CH_3
3	148.7	-	H-Me ⟷ C-Me	H-Me ⟶ C-13,C-2,C-4
4	124.5	7.42 s	H-4 ⟷ C-4	H-4 ⟶ C-1,C-2,C-13,C-9 CH_3
5	108.3	7.06 d J=1.2	H-5 ⟷ C-5	H-5 ⟶ C-6,C-9,C-11,C-12
6	166.0	-	-	-
7	109.2	6.55 d J=1.2	H-7 ⟷ C-7	H-7 ⟶ C-5,C-6,C-8
8	164.9	12.04 s	OH-8 ⟷ C-8	
9	181.7			
10	190.1			-
11	135.5			-
12	109.3			_
13	113.7			_

14	133.17			–
Me	21.97		H-Me ⟷ Me	H-Me ⟶ C-13,C-2,C-4

HMBC spectrum showed the correlations of protons H-2 (δ7.11) with C-1 (δ161.85),C-4 (δ124.54) C-13 (δ113.73) and Me (21.97) and H-4 (δ7.42) to C-1 (δ161.85),C-4 (δ124.54) C-13(δ113.73) C-9(δ 181.7) and Me (21.97) (Appendix 19 & 20). These correlations give us the information of partial aromatic structure of compound-2 (ring A of Fig. 13). In this cycle, the following correlations are important to note. The correlation from 2.50 ppm (H-Me) indicates that the methyl group is present in this cycle that has the meta aromatic protons.

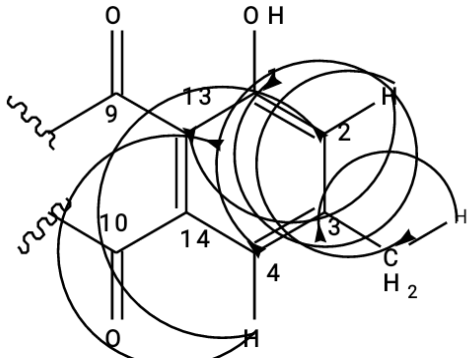


Figure 13. Figure shows the HMBC correlations on the aromatic cycle A.

Correlations of protons H-5 and H-7 give us the presence of another partial aromatic cycle structure (ring B) as shown in Fig. 14. In this cycle, it is important to remark the following correlations. The coupling correlation of H-5 (δ 7.06ppm) to C-6(δ 166.03),C-9(δ 181.70),C-12(δ 109.33), also the coupling of H-7 (δ 6.55) to C-5(δ 108.34),C-6(δ 166.03),C-8(δ 181.70). The correlation of H-7 with C-8 and C-6 indicates the presence of two alcohols in this meta aromatic cycle. The presences of anthraquinone also confirmed by the correlations of H-5 to the carbonyl signal C-9 and from H-2 (7.11

ppm) to carbonyl signal C-9. In addition, the correlation of H-5 to C-6 (an oxygenated quaternary carbon) indicates the presence of an alcohol group.

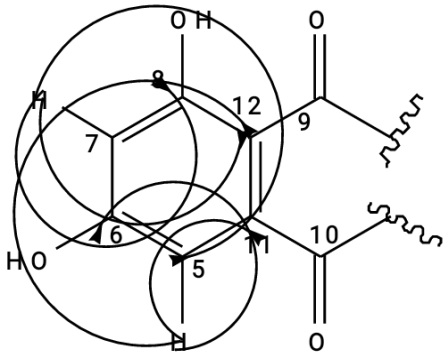


Figure 14. Figure that shows the HMBC correlations on the aromatic cycle B

From the observed and reported literature data of ^{1}H NMR and ^{13}-C NMR as well as spectral data from 2D NMR revealed that compound-2 is a hydroxyl anthraquinone known as Emodin (Fig. 15).

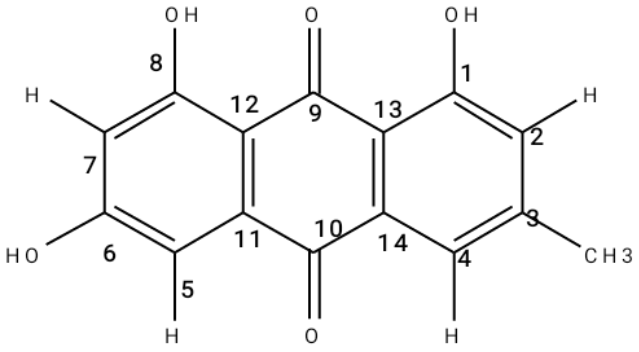


Figure 15. The Proposed Structure of Compound-2 (Emodin)

5.3. Evaluation of Antibacterial and Antifungal Activities of the Isolated Compounds

In the present study, the antimicrobial activity of the purified compounds were evaluated on top of that crude extracts. The antimicrobial and antifungal activities of isolated compound (compound-1 and compound-2) were carried out using four human pathogenic bacteria species; namely *P. aeruginosa*, *K. pneumoniae S. aureus* and *E. coli* and one fungal strains namely *C. albicans* by disc diffusion method (Appendix 21 & 22). The obtained results are summarized in (Table 10). Chrysophanol (compound-1) exhibited high zone of inhibition against *S. aureus* (23.0±0.1) and *K. pneumoniae* (22.5±0.4) than *P. aeruginosa* (18.0±0.2) and *E. coli* (18.0±0.4) as shown in Appendix 13. Compound-2 also showed good zone of inhibition (activity) against *K. pneumoniae* (20.0±0.7) *S. aureus* (20.0±0.2) than *p. aeruginosa* (19.0±0.3) and *E. coli* (17.0±0.7). The results indicated that compound-1 compound-2 showed better antibacterial activity against *K. pneumoniae* and *S. aureus* species and their activity was close to the standard Gentamicin that is used as a positive control.

As it has been indicated in the above (Table 4), the antifungal activity of both isolate compounds against *C. albicans* was very good. Particularly, the antifungal activity of compound-2 (22.0±0.7) is comparable with Mancozeb drug (22.0±0.2), the standard antifungal drug. This shows that compound-2 is more active compared to compound-1 against *C. albicans*. Generally, the antifungal activity of isolated compounds was better than the activity shown by all the crude extracts.

5.4. Conclusion

In the course of this present work, two compounds, compound-**1** and compound-**2** were identified from the roots of *R. abyssinicus.* Compound-1 was isolated from (54-65) fraction with R_f value 0.65 and compound 2 was isolated from 66-79 fractions with R_f 0.58 value in petroleum ether-ethyl acetate (70:30). The structures of these compounds were elucidated and antimicrobial activity of the root of *R. abyssinicus* crude extracts and pure compounds were tested based on the four bacterial and (one fungal for isolate and two for crude) strain.

Main crude which was designated as petroleum ether extract, chloroform extract, acetone extract and methanol extract showed considerable bacteria growth inhibition and fungal growth inhibition compared to the positive control Gentamicin for antibacterial and Mancozeb for antifungal. The isolated compounds showed comparable zone of growth inhibition compared to the positive control Gentamicin for antibacterial and Mancozeb for antifungal activity. The antibacterial activity of isolated compound-1 showed higher activity against to (*S. aureus* 22.5±0.4 *P. aeruginosa* 18.0±0.2, *E. coli* 18.0±0.4, *K. pneumoniae* 23.0±0.1) than compound-2; while antifungal activity compound-2 higher (*C. albicans* 22±0.7) than compound-1 *(C. albicans* 20±0.4).

5.5. Recommendation

The present study proved that the crude acetone extract as well as the isolated compounds support the traditional use of the plant against various infectious diseases. However, as evidenced from the activity of the crude

extract compared to individual isolated compounds; there are still more unidentified bio active compounds are present in the plant of *R. abyssinicus*. Therefore, further investigations are recommended on the plant for isolation of more compounds. Further investigation on the bioactivity of crude extracts and isolated compounds are also useful for detail understanding of the plant for discovery of new drugs.

6. References

1. Gidey, Y. Use of traditional medicinal plants by indigenous people. *J. Med. Plants Res*. **2010**, *4*:179 - 180

2. Ashenif, Tadel. Ethiopian herbal medicine research article profile part. *Traditional and Modern Medicine*. *Research Directorate Addis Ababa*, Ethiopia Compiled and Edited **2017**, 8, 2-4.

3. Sarker, SD.; Nahar, L. Chemistry for pharmacy students general organic and natural product chemistry. *England,John Wiley and Sons.* 2007, 283-359

4. Krishnananda, P.; Ingle, Amit G.; Deshmukh, Dipika A.; Padole, Mahendra S.; Dudhare, Mangesh.; P, Moharil and Vaibhav C. Khelurkar. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *J. Pharmacognosy and Phytochemistry*. **2017**, 6,32-36

5. Addis, G.; Abebe, D.; Urga, K.; A survey of traditional medicine in Shirka District, Arsi Zone, Ethiopia. *Ethiop pharm*. **2001**, 19, 30-47

6. 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**, 2, 289-293

7. Leland, J.C., Ara, K., Peter, P. K., Sara, L. W., James, A. D., and Harry, L. B. *Natural products from plants, Phytochemicals:* The Chemical Components of Plant. 2nd ED Taylor and Francies 2006, 2-4

8. Suffredini, I.B.; Sader, H.S.; Gonçalves, A.G.; Reis, A.O .; Gales, A.C.; Varella, A.D.; Younes, R.N. Screening of antibacterial extracts from plants native to the Brazilian amazon rain forest and Atlantic forest*. Braz. J. Med.*

Biol. Res. **2004**, 37, 379384

9. Satyajit, D S., Zahid, L.; and Alexander, I.; G. Natural products isolation, Natural Products: Present and Future.2nd ED. *Humana press, Totowa New Jersey*, 2006, 4-6.

10. Nair. R.; Kalariya. T.; Sumitra. C. Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol*. **2005**, *29*: 41-47.

11. Negero, G.; Ashenif, T.; Biruktawit, G.; and Frehiwot, T. Proceeding of the Workshop on Ethiopian Traditional Medicine: Past, Current and Future. Adama Ethiopia. **2015,**7, 4-8

12. Fatima, N.; Zia, M.; Rizvi, Z. F.; Ahmad, S.; Mirza, B.; Chaudhary, M. F. Biological Activities of Rumex Dentatus L : Evaluation of Methanol and Hexane Extracts. **2009**, *8*, 6945–6951.

13. Hasan, A.; Ahmed, I.; Jay, M.; Voirinn, B. Flavonoid glycosides and an Anthraquinone from Rumex chalepensis. *Phytochemistry*. **1995**; 39: 1211-1213.

14. Rouf, A.; S, I.; M. S.; and T, R. M. S. Evaluation of Antidiarrheal Activity of Rumex maritimus root. *Ethnopharmacol*. **2002**, 84: 307-310

15. Cos P.; Hermans, N.; Bruyene, T. Antiviral activity of Rwandan medicinal plants against human immunodeficiency virus type-1 (hiv-1). *Phytomedicine*, **2002**, 96: 2-68.

16. Lee, N J.; Choi H, J.; Koo B, S.; Y, R. S. Antimutagenecity and Cytotoxicity of The Constituents from the Arial parts Rumex. *Biol, pharm*. **2005,** Bull, 28: 2158-2161.

17. Teshale, Mekonnen,; Kelbesa, Urga and Ephrem Engidawork. Evaluation of the diuretic and analgesic activities of the rhizomes of *Rumex*

abyssinicus. *Jinmice. J. Ethnopharmacol.* **2010,** 127, 433–439.

18. Tesso, H.; Nisha, A. K.; Kumsa, K. Antibacterial Activity and Phytochemical Screening of Some Important Medicinal Plants against Human Diarrheal Pathogens in Adama City, Ethiopia. *Int. J. Microbiology and Immunology Research,* **2015**, 3, 29–35.

19. Tilahun, Teklehymanot.; Mirutse, Giday,; Girmay. Medhin.; Yalemtshay, Mekonnen .Knowledge and use of medicinal plants by people around Debre Libanos monastery in Ethiopia*. J. Ethnopharmacol.* **2007** ,111, 271–283.

20. Teklehymanot, T. Ethno botanical study of knowledge and medicinal plant use by the people in Dek Island in Ethiopia. *J. Ethno pharm.* **2009** *124*, 69-78.

21. Himani, R,; Garima, S,; Nupur, J,; Neelam, S,; Navneeta, B. phytochemical importance of medicinal plants as potential source of anticancer agents. *Turk. J. Bot*. **2014,** 38, 1027-1035

22. Javid,A,;Parray,Rehana,H,;Azra,N,;Kamili, Now sheen, S,; Sumira, J,. Biological Efficacy and radical scavenging potential of shikonin in Arabia benthami (Wall ex. G Don). Johnstan. *Ind. crop. Prod.(* **2015),** 15, 434-439

23. The Plant List. Published on the Internet. http://www.theplantlist.org/ 2013. Version 1. (accessed 20th March 2015)

24. Schunko, C,; Grasser, S,; Vogl, C.R. Explaining the resurgent popularity of the wild: motivations for wild plant gathering in the Biosphere Reserve Grosses Walsertal. *Austria. J. Ethnobiol. Ethno med*. **2015,** 55, 1–14

25. Wegiera, M.; Kosikowska, U.; Malm, A.; Smolarz, H. D. Antimicrobial Activity of the Extracts from Fruits of *Rumex L*. Species. **2011**, 6. https://doi.org/10.2478/s11535-011-0066-0

26. Saleh, N.A.M.; M.N, El-Hadidi. R.F.M, Arafa Flavonoids and anthraquinones of some Egyptian Rumex species (Polygonaceae). *Biochem. System. Ecol*. **1993**, *21*, 301-303

27. The Plant List.2013.Version 1.Published on the Internet. http://www.theplantlist.org/ accessed 20th March, **2015.**

28. Ermias Dagne. Natural Database for Africa (NDA). Addis Ababa, Ethiopia. **2009**, 6, 6-15.

29. Edwards, S,; Mesfin, Tadesse,; Sebsebe, Demissew and Hedberg, I. Flora of Ethiopia and Eritrea. Addis Ababa University and Uppsala University, Uppsala.**2000,** 1, 2-9.

30. Fufa, M. F. Phytochemical Investigation and In Vitro Antibacterial Evaluation on Root Natural Products Chemistry & Research Phytochemical Investigation and In Vitro Antibacterial Evaluation on Root Extracts of *Rumex Abyssinicus*. **2018**, 8,5-12

31. Nusrat, Sh.; Muhammad, S.; Sumaira, K.; Madiha, S.; Syed, M..; Hussain, F. J. Investigation of genus Rumex for their biologically active constituents. *Life Science Informatics Publications.* **2017**, 24, 54-63.

32. Jibat, M.; Getachew, W.; Getu, A.; Kifelew, H. Survey and Identification of Major Weeds of Seeds Spice in Ethiopia Plant Pathology & Microbiology. 1–4. https://doi.org/10.35248/2157-7471.10.477

33. Alemu Tadesse Feroche. Evaluation of abortifacient efficacy of Rumex steudelli (Tult) root traditionally used medicinal plant in South West Ethiopia. *J. Phytopharmacology.* **2015**, 4,221-223

34. Habtamu, Tedila.; Addisu, Assefa. In vitro antibacterial activity of Rumex nervosus and Clematis simensis plants against some bacterial human pathogens. *Afr. J. Microbiol. Res*. **2019**, 13, 14-22, 7

35. Nigussie, G. Isolation, Characterization and Structural Elucidation of the Roots of *Rumex* Organic Chemistry , Current Research. 1–7. https://doi.org/10.35248/2161-0401.20.9.200.

36. Khan, M. E.; Williams, E. T.; Abel, A. Emodin Isolation from the Leaves of PteridiumAcquilinum. **2019**, 6, 1–6. https://doi.org/10.9734/CJAST/2019/v33i630106

37. Satyajit, D. Sarker.; Lutfun, Nahar. Chemistry for Pharmacy Students. General, Organic and Natural Product Chemistry. University of Ulster, Colerain, Northern Ireland, UK. , 2nd ed. **2007**, 169, 324-339

38. Tadesse, G.; Reneela, P.; Dekebo, A. Isolation and Characterization of Natural Products from *Helinus Mystachnus* (*Rhamnaceae*). *J. Chem. Pharm. Res.* **2012**, *4* (3), 1756–1762.

39. Liu, SY,; Sporer. F.; Wink, M.; Jourdane, J,; Henning, R,; Li ,YL,; Ruppel, A. anthraquinone in rheum palmatum and Rumex dentatus and phorbol esterin jatrophacurcas with molusiccidal activity against the schistosome vector snaile on comelania biomphalaria and bulinus. *Trop. Med. Int. Health*. **1997**, 2: 179–188

40. Mei, R.; Liang, H.; Wang, J.; Zeng, L.; Lu, Q.; Cheng, Y. new seco-anthraquinone glucoside activity from root Rumex nepalensis. *Planta Med.* **2009**, 75: 1162–1164.

41. Demirezer, LÖ.; Kuruüzüm, A.; Bergere, I.; Schiewe, HJ.; Zeeck, A. Five naphthalene glycosides from the roots of *Rumex patientia* Phytochemistry. *Phytochemistry*.**2001**; 56: 399–402

42. Yang, Y.; Yan, Y.; Wei, W.; Luo, J.; Zhang, L.; Zhou, X. ChemInform Abstract : Anthraquinone Derivatives from Rumex Plants and Endophytic Aspergillus Fumigatus and Their Effects on Diabetic Nephropathy .

Bioorganic & Medicinal Chemistry Letters Anthraquinone Derivatives from Rumex Plants and Endophytic Aspergillus Fumigatus and Their Effects on Diabetic Nephropathy Q. Bioorg. Med. Chem. Lett. 2013, 23 (13), 3905–3909. https://doi.org/10.1016/j.bmcl.2013.04.059.

43. Dewick, P. M. Medicinal Natural Products. A Biosynthetic Approach, 2nd. ed. England. *John Wiley & Sons Ltd*. **2004**, 8, 145-164.

44. Manitto, P. Biosynthesis of Natural Products, England. *Ellis Horwood Ltd.* **1981**, 10, 177-195.

45. Jiang, L.; Zhang, S.; Xuan, L. Oxanthrone C-glycosides and epoxynaphthoquinol from roots of *Rumex japonicus*. *Phytochem*. **2007**, 68: 2444–2449

46. Zhu J.J., Zhang, C.-F., Zhang, M. and Wang Z.-T. Anthraquinones and chromones from *Rumex dentatus*. *Biochemical Systematics and Ecology*, **2006**, 34: 753-756.

47. Baskan, S.; Daut-Ozdemir, A.; Gunaydin, K.; Erim, F.B. Analysis of anthraquinones in *Rumex crispus* by micellarelectrokinetic chromatography. *Talanta*. **2007,** 71: 747 45

48. Van den Berg, A. J. J.; Labadie, R. P. The Production of Acetate Derived Hydroxyanthraquinones, -Dianthrones, -Naphthalenes and -Benzenes in Tissue Cultures from Rumex Alpinus. *Planta Med.* **1981**, *41* (2), 169–173. https://doi.org/10.1055/s-2007-971695.

49. Liang, HX,; Dai, HQ.; Fu, HA.; Dong, XP.; Adebayo, AH.; Zhang, LX.; Cheng, YX. Bioactive compounds from *Rumex* plants. *Phytochem. Lett*. **2010**; *3*: 181–184.

50. Tavares, L.; Carrilho, D.' Tyagi, M.; Barata, D.; Serra, AT.; Duarte, CMM.;

Duarte, RO.; Feliciano RP.; Bronze, MR.; Chicau, P.; Espirito-Santo, MD.; Ferreira, RB.; dos Santos, CN. *Molecules .* **2010**, 15, 2576–2592.

51. Orbán- Gyapai. flavonoid isolated from Rumex aquaticus exhibits neuroprotective and neuroprospative properties by enhancing neurite outgrowth and synaptophysin. *CNS and neurol.disord drug targets*. **2014,** 13, 1458-1564.

52. Bélanger, J.; Balakrishna, M.; Latha, P.; Katumalla, S.; Johns, T. Contribution of selected wild and cultivated leafy vegetables from South India to lutein and β-carotene intake. *Asia Pac. J. Clin. Nutr* .**2010.** 19, 417–424

53. Molnár, P.; Ősz, E.; Zsila ,F.; Deli. Isolation and structure elucidation of anhydroluteins from cocked sorrel (*Rumex rugorus* campd). *J. Chem. Chem Biodivers*. **2005**; 2: 928–935

54. Fremont, L.; Bulguendouz, L., Delpal, S. Antioxidant activity of resveratrol and alcohol free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *life sci*. **1999**, 64,2511-2521.

55. Kerem, Z.; Bilkis, I.; Flaishman, M. A and Sivan, L. Antioxidant Activity and Inhibition of r-glucosidase by trans-Resveratrol, Piceid, and a Novel trans-Stilbene from the Roots of Israeli Rumex bucephalophorus L. *J. Agriculture and Food Chemistry*. **2006**, 54: 1243-1247.

56. Rivero-Cruz, I.; Acevedo, L.; Gurerrero, JA.; Martinez, S,.; Bye, R.; Pereda-Miranda, R.; Franzblau, S.; Timmermann, BN.; Mata, R. *J. Pharm. Pharmacol.* **2005**, 57: 1117–1126.

57. Getie, M.; Mariam, T.; G.-, Reitz R, Höhne, C.; Huschka, C.; Schmidtke, M.; Abate A and Neubert RHH Evaluation of the Anti-Microbial and Anti-Inflammatory Activities of the Medicinal Plants Dodonaeaviscosa,

Rumex nervosus and Rumex abyssinicus. *Fitoterapia*. (**2003**), 74:139-143.

58. Pardo-de-Santayana, M, Tardío, J, Morales; R, the gathering and conception of wild edible plants in the Campoo (Cantabria, Spain), *int. food. J. food Sci*. **2005**

59. L, Łuczaj,; W.M, Szymański. Wild vascular plants gathered for consumption in the Polish countryside: *a review J. Ethnobiol. Ethno med*. **2007**, *3*: 1-22

60. Abbet, C. Maor, R. Roguet; D, Spichger; R. Hamburger; M, Potterats, O. ethinobotanical survey on wild alpine food plants in lower and central valaies (Switzerland),*J.ethinopharmacol*.**2014**,151,624-634

61. Elvin-Lewis, M.J. Should we be concerned about herbal remedies? *Journal of Ethno pharmacology*. **2001,** 75 (2-3): 141-164

62. Kumar, R.S., Gupta, M., Mazumdar, U.K., Rajeshwar, Y., Kumar, T.S., Gomath, P. & Roy, R. Effects of methanol extracts of Caesalpinina bonducella and Bauhinia racemosa on hematology and hepatorenal function in mice. *J. Toxicological Sciences*. **2011**, 30 (4): 265-.; 3: 240–242.

63. Munavu, R,; M, Mudamba, L.; O, Ogur. A. Isolation and characterization of the major anthraquinones pigments from *Rumex abyssinicus*. *J. Plant Med*. **1984,** *50,* 111

64. Solomon, T.; Largesse, Z,; Mekbeb, A,; Eyasu, M,; Asfaw, D. Effect of Rumex steudelii methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats. *Afr. Health Sci*. **2010**, *10*: 353–361

65. El-Hawary SA, Sokkar NM, Ali ZY, Yehia MM. A profile of bioactive compound of *R. vesicarius L*. *J. Food Sci.* **2011**; 76: 1195–1202

66. Dénes, A.; Papp, N.; Babai, D.; Czúcz, B.; Molnár, Z.; Dunántúli Dolgozatok

(A) Természettudományi Sorozat **2013**; 13: 35–76

67. Bogl, S,; Picker. P,; Mihaly-Bison, J,; Fakhrudin N, Atanasov AG, Heiss EH, Wawrosch C, Reznicek, G,; Dirsch, VM,; Saukel, J,; Kopp, B. *J. Ethnopharmacol.***2013**, *149*: 750–771

68. Allen, D.E., Hatfield, G. Medicinal Plants in Folk Tradition: An Ethno botany of Britain and Ireland. Timber Press, Portland, Cambridge **2004**

69. Zhu JJ, Zhang CF, Zhang M, Bligh SWA, Yang, L, Wang ZM, Wang ZT. Separation and identification of three epimeric pairs of new C-Glycosyl anthrones from R. dentatus by online HPLC circular dichroism analysis. *J. Chromatogr*. **2010**; 1217: 5384–5388

70. Nascimento,; G.G.F.; Locatelli, J.; Freitas, P.C. & Silva1, G.L. Antibacterial activity of plant extracts and phytochemicals on antibioticresistant bacteria. *Brazilian J. Microbiology*. **2003**,1:247-256

71. Neuwinger, H.D,; Chapman and Hall. *African Ethno botany*. Poisons and Drugs London. **1996**, pp,12-36

72. Mkhize, S. S.; Zharare, G. E.; Basson, A. K.; Mthembu, M. S.; Cloete, J. Performance of Pleurotus Pulmonarius Mushroom Grown on Maize Stalk Residues Supplemented with Various Levels of Maize Flour and Wheat Bran. *Food Sci. Technol.* **2015**, *37* (4), 570–577. https://doi.org/10.1590/1678-457x.27216.

73. Yildirim, A,; Mavi, A,; Kara, AA. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts*. J. Agric. Food Chem*. **2001**,*49,* 4083–4089

74. Getie, M ,; Gebre-Mariam, T,; Rietz, R;, Höhne, C., Huschka, C,; Schmidtke, M,; Abate, A,; Neubert, R.H.H. Evaluation of the anti-microbial and

anti-inflammatory activities of the medicinal plants. *Dodonaeaviscosa, Rumex nervosus and Rumex abyssinicus*. *Fitoterapia*. **2003**, *74*, 139–143

75. Humeera N,; Kamili AN,; Bandh SA,; Amin S, Lone BA,; Gousia N. Microbiol. Path. **2013**; 57: 17–20

76. Sharma, R.S.; Mishra, V.; Singh, R..; Seth, N.; Babu, C. R. Anti-fungal Activity of some Himalayan Plants and Cultivated ornamental species. Fitoterapia. **2008**., 79: 589–591

77. Diallo, D., Hveem, B, Mahmoud, M.A., Betge, G., Paulsen, B.S. and Maiga, A. An ethno botanical survey of herbal drugs of Gourma district, Mali. *Pharm. Biol*. **1990**, 37:80-91.

78. Basha, S.N,; Rekha, R,; Sirak, Saleh and Samuel Yamane. Evaluation of in vitro anthelmintic activities of Brassica nigra, Ocimum basilicum and Rumex abyssinicus. *Pharmacognosy Journal*. **2011**, *3(2).*88-92.

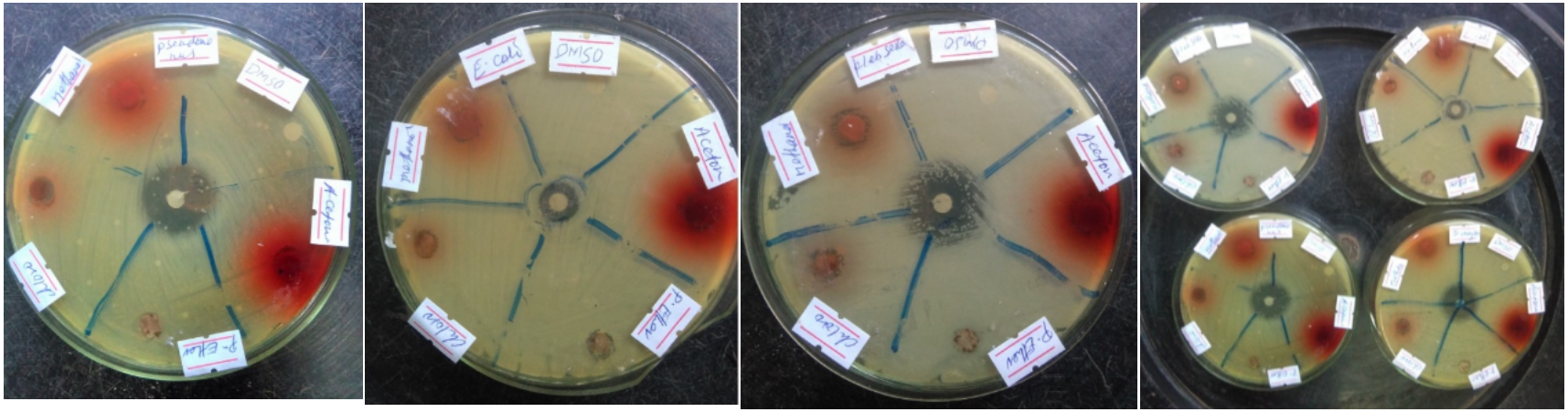
79. Z.; De, D. Natural Products Chemistry & Isolation , Characterization and Antibacterial Activities Evaluation of Rumex Abyssinicus Root bark Extracts. **2019**, *7* (1), 1–8. https://doi.org/10.4172/2329-6836.1000353.

80. Nighat Fatima Muhammad ZiaRiaz-ur-Rehman Zarrin Fatima Rizvi Safia Ahmad Bushra Mirzaand M. Fayyaz Chaudhary. Biological activities of Rumex dentatus L: Evaluation of methanol and hexane extracts. *African Journal of Biotechnology.* **2009,** Vol. 8 (24), pp. 6945-6951.

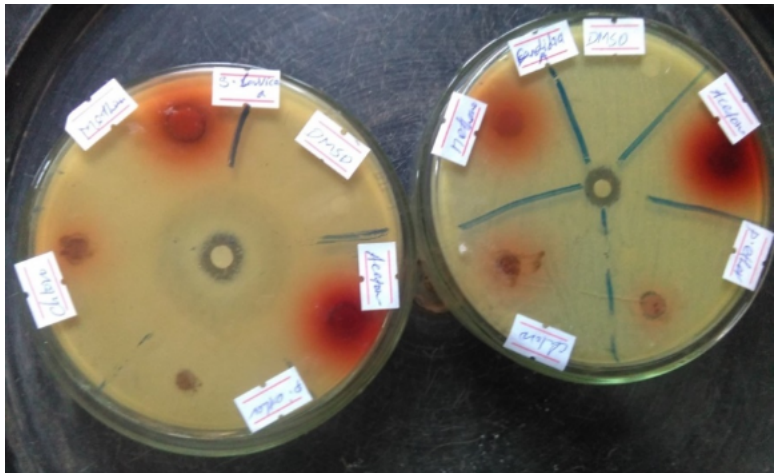
81. Zinaye, B. and Fiseha, A. Phytochemical investigation on the root of *Rumex abyssinicus* (MAKMAKO), (2008); AAU electronic library.

7. Appendices

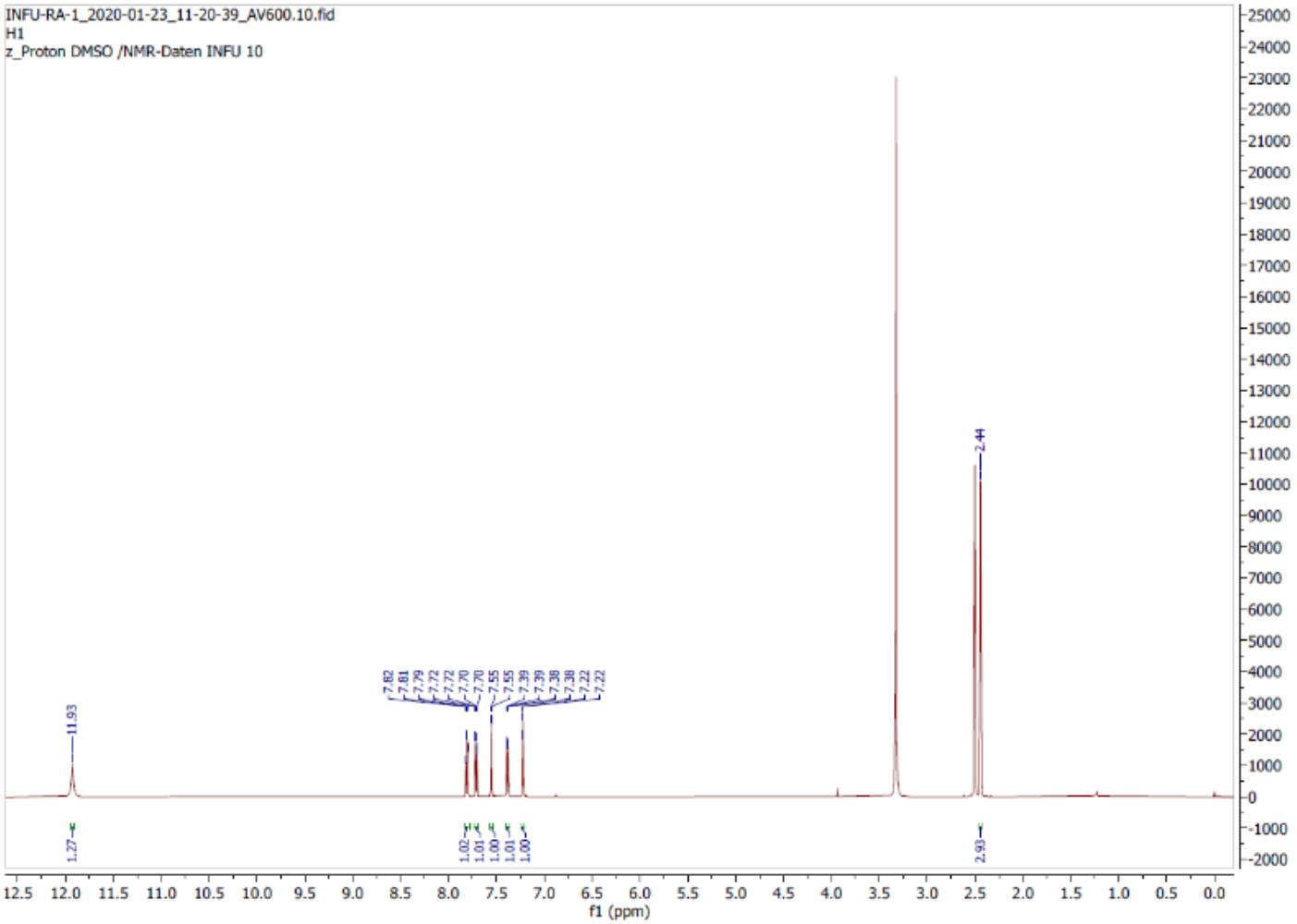
Appendix 1. Antibacterial activity of crude extract of root of *R. abyssinicus*



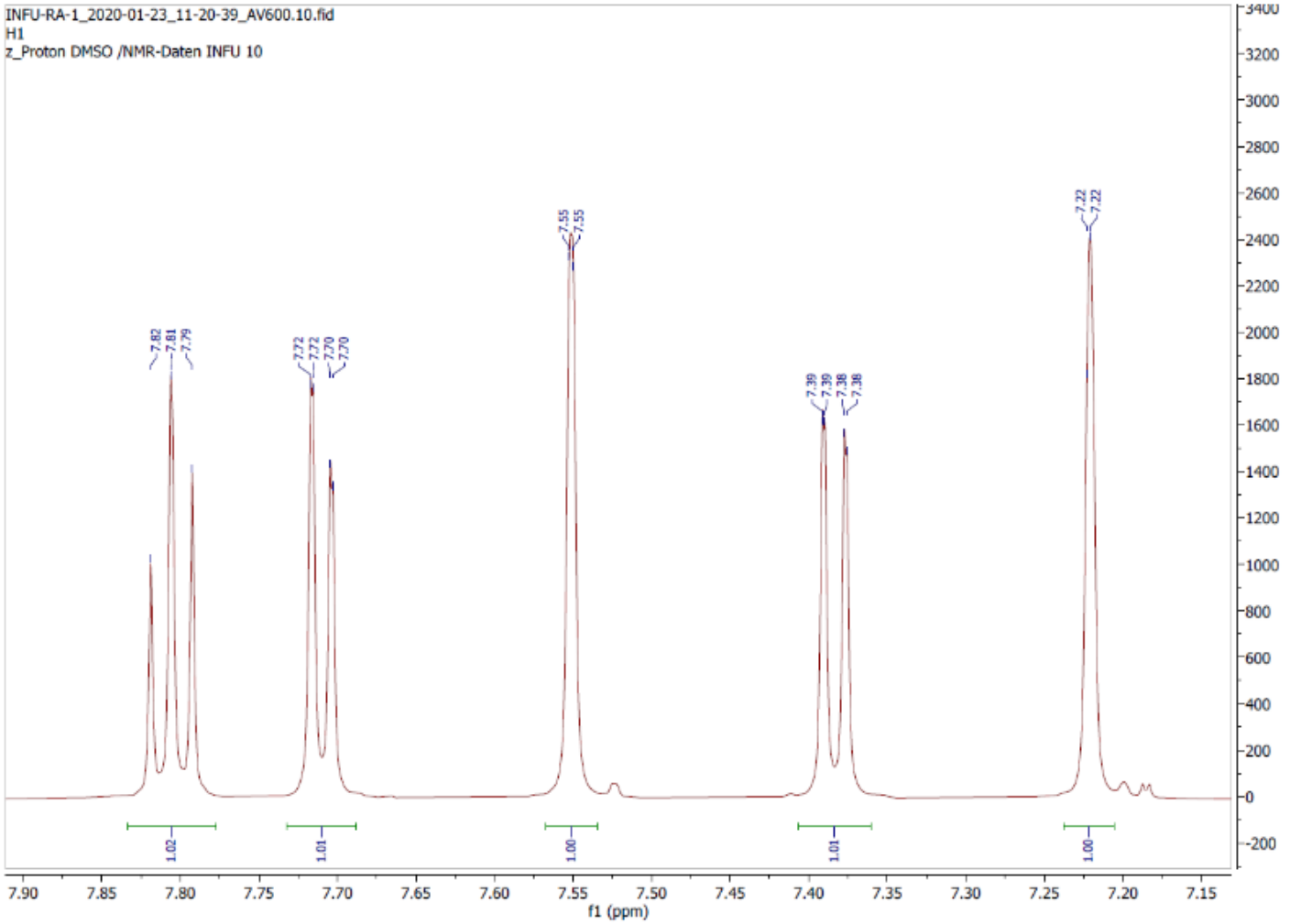
Appendix 2. Antifungal activity crude extract of root of *R. abyssinicus*



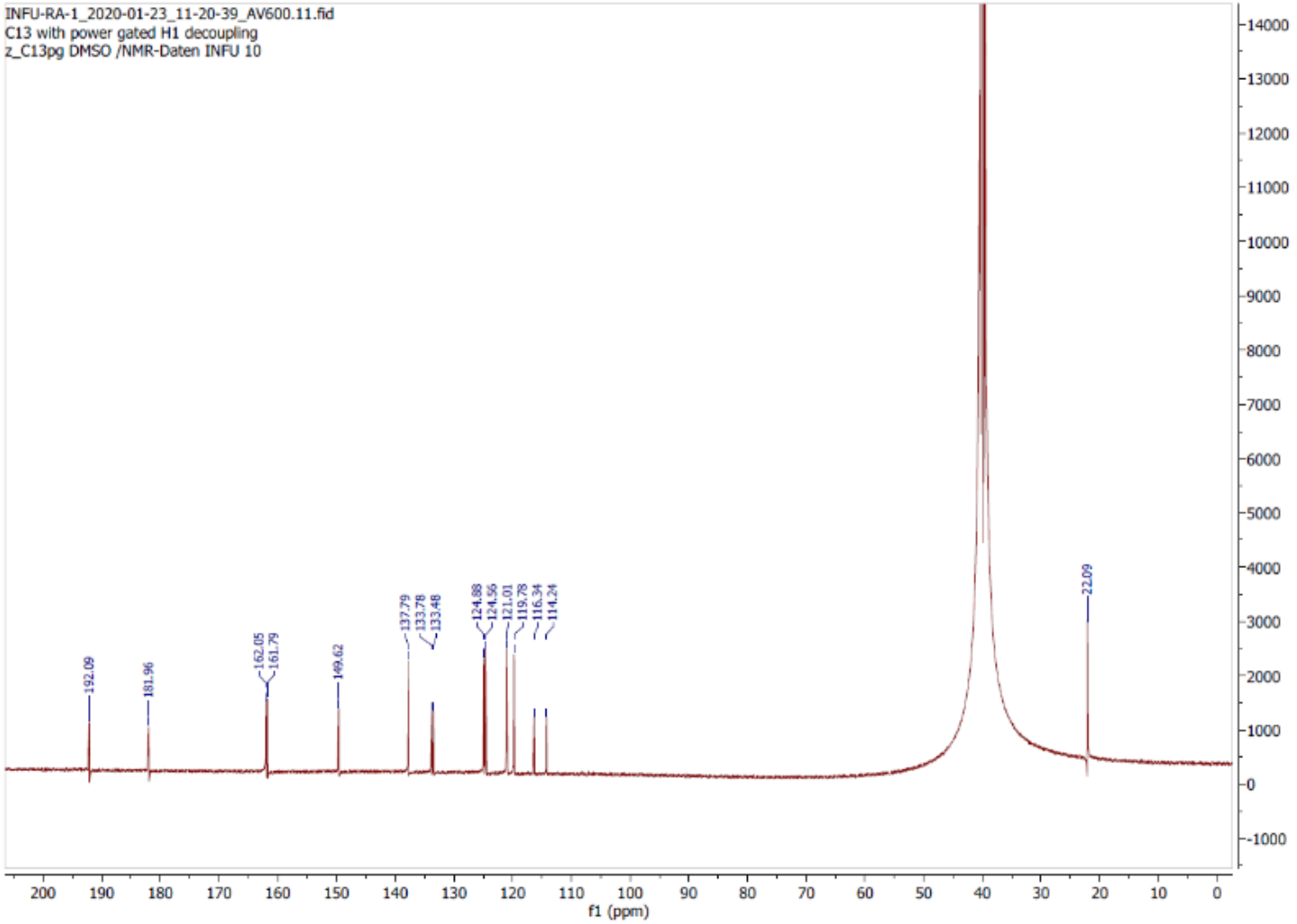
Appendix 3. [1]HNMR spectral data of compound-1



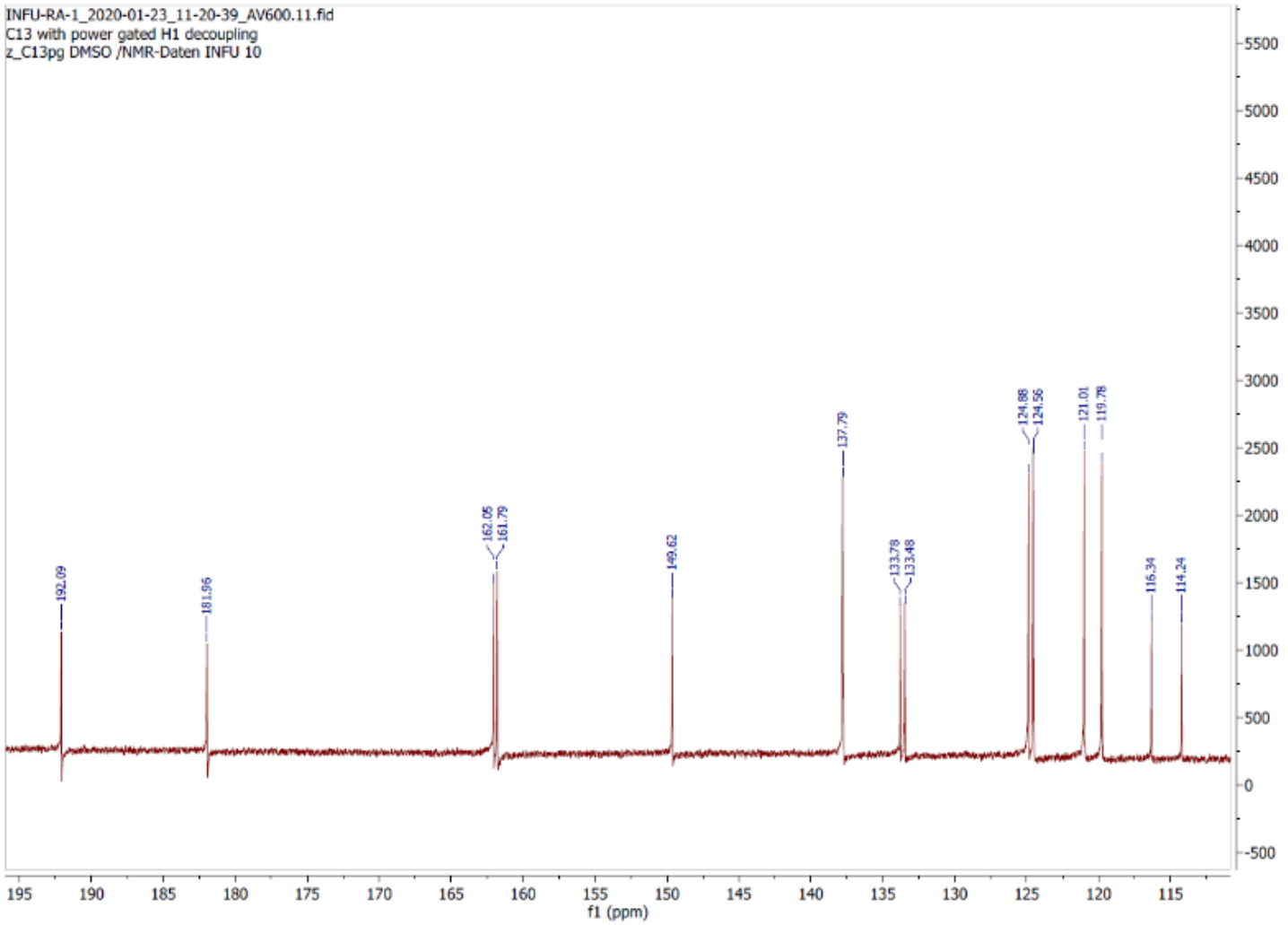
Appendix 4. Expanded [1]HNMR spectral data of compound-1from 7.15-7.90 ppm



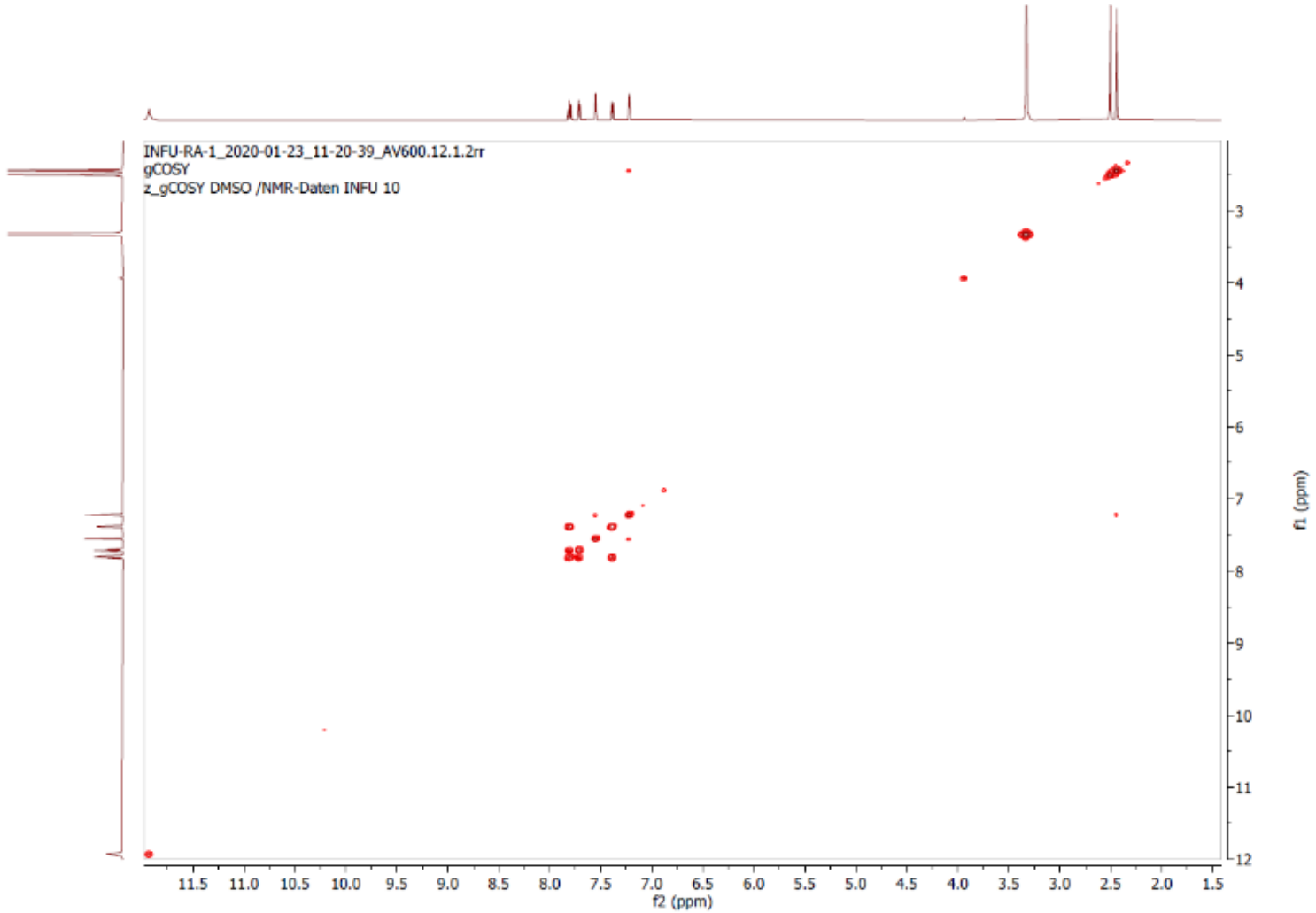
Appendix 5. ^{13}CNMR spectral data of compound-1



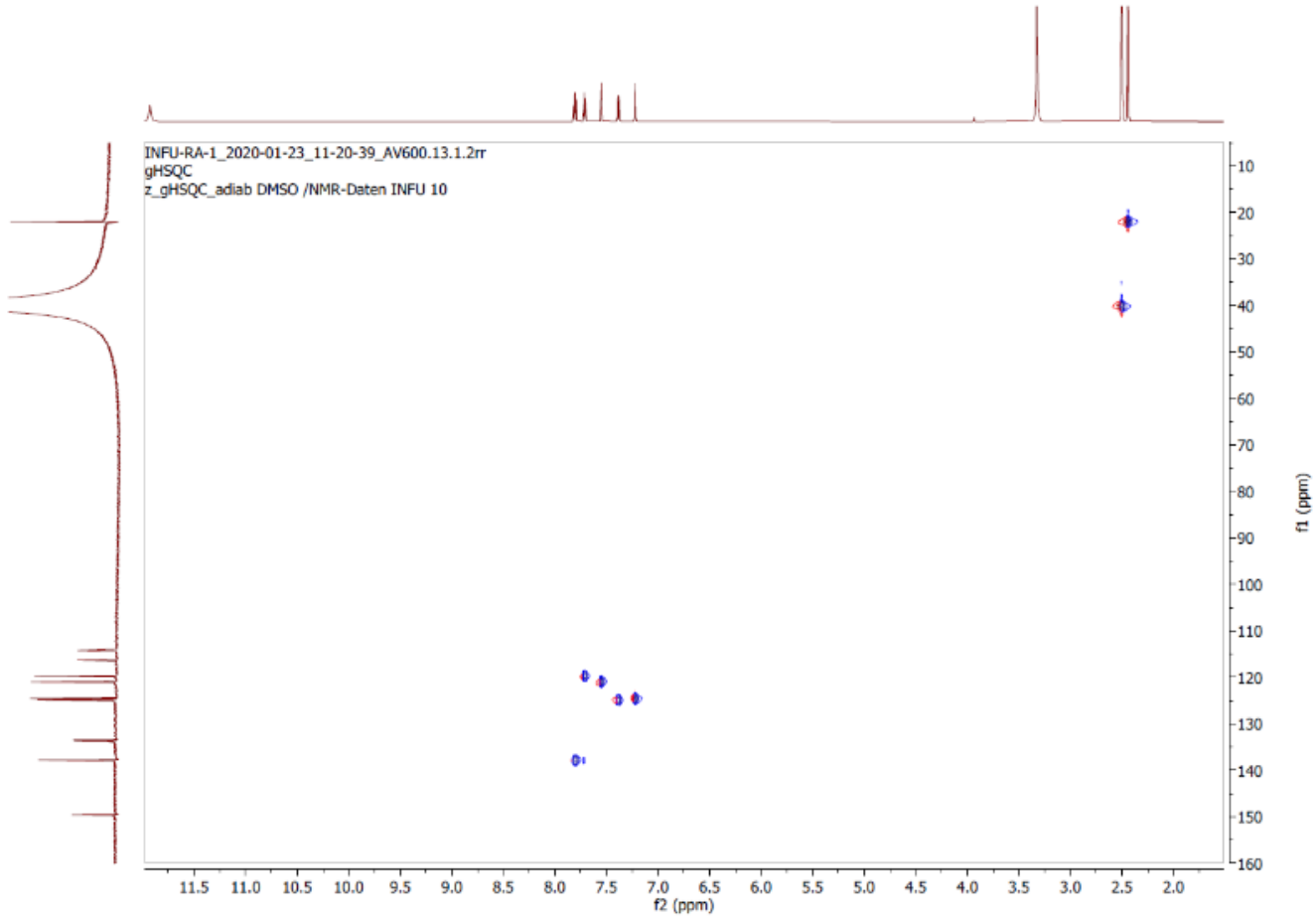
Appendix 6. Expanded ^{13}CNMR spectral data of compound-1from 115-195 ppm



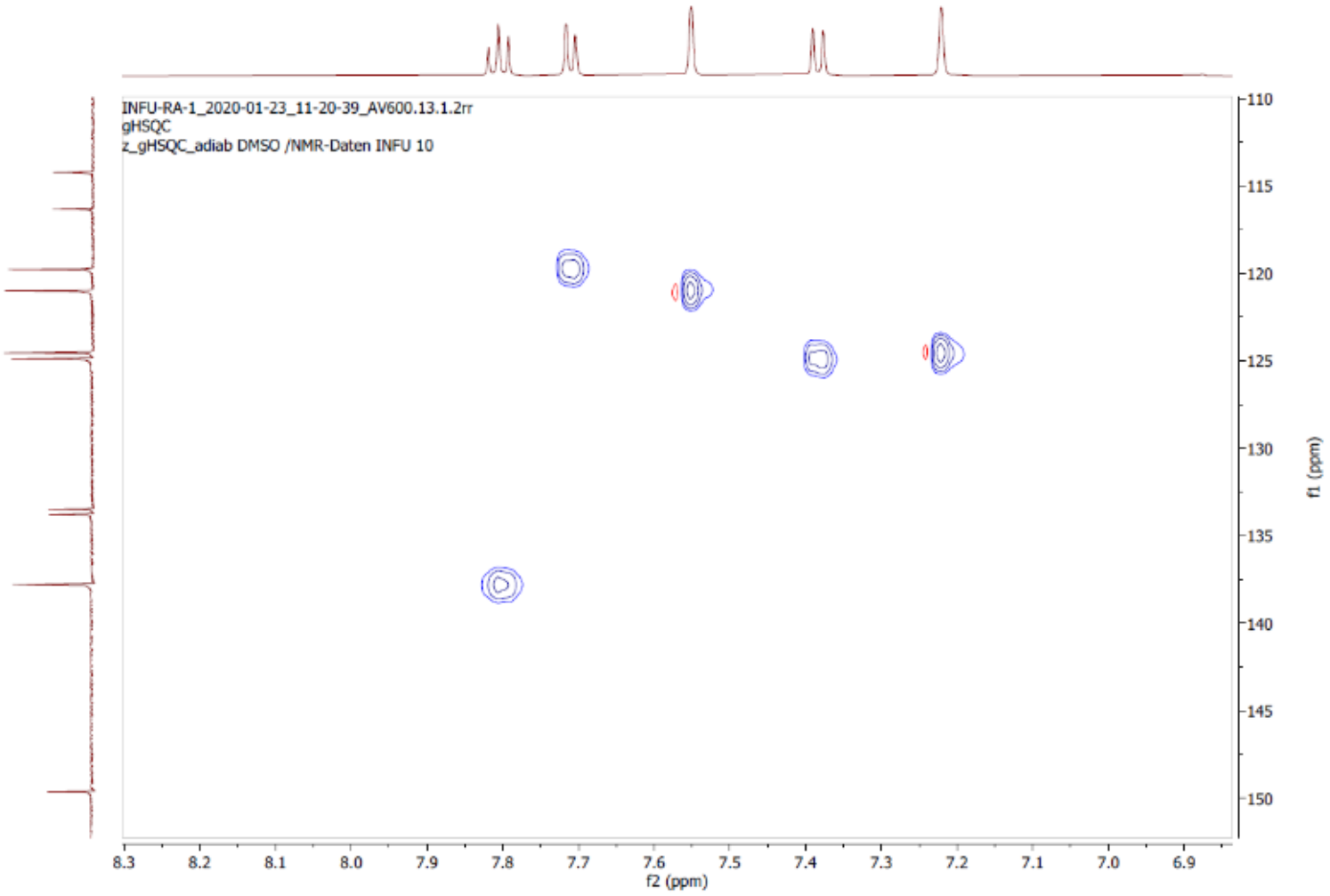
Appendix 7. COSY spectral data of compound-1



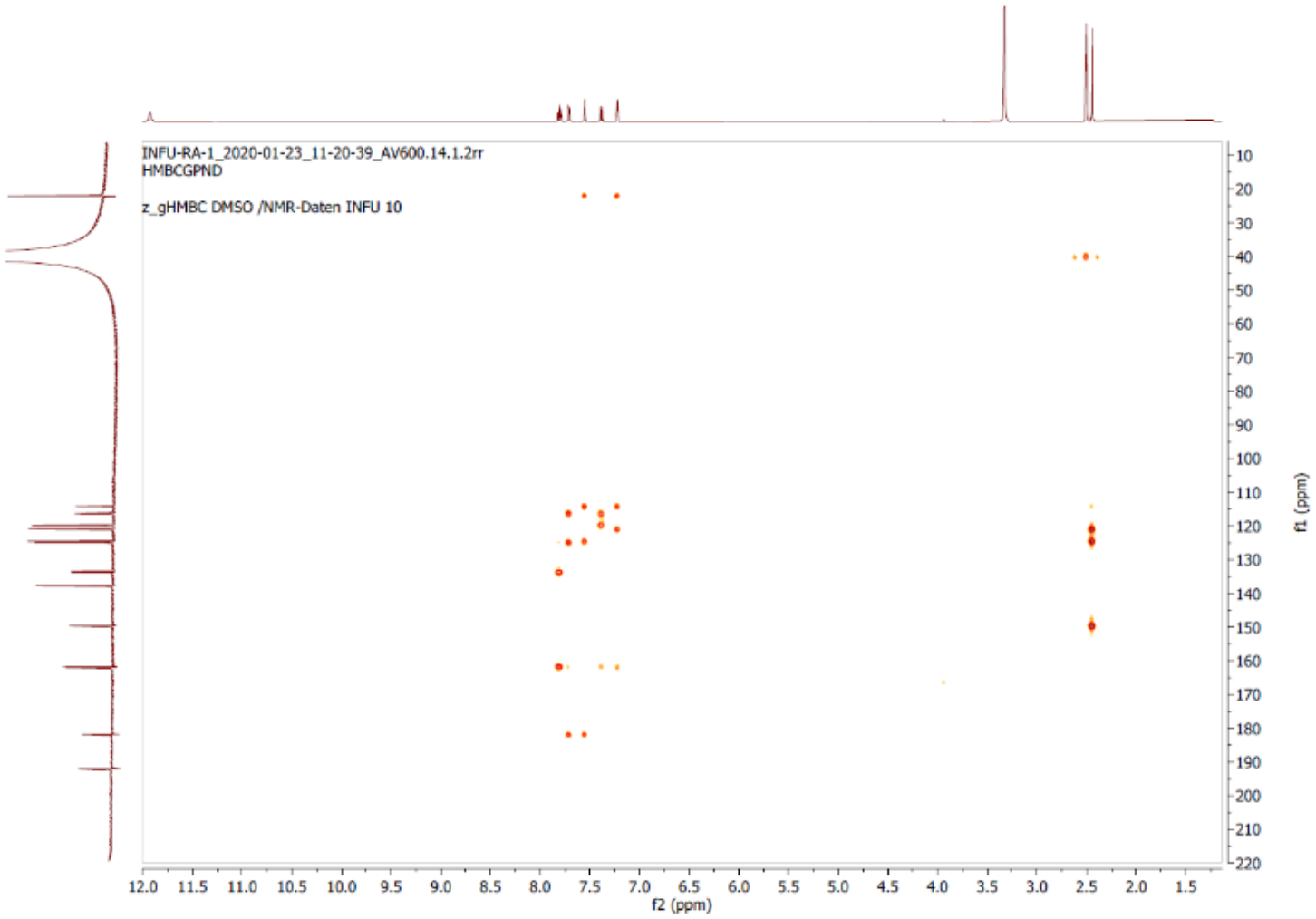
Appendix 8. HSQC spectral data of compound-1


INFU-RA-1_2020-01-23_11-20-39_AV600.13.1.2rr
gHSQC
z_gHSQC_adiab DMSO /NMR-Daten INFU 10
f2 (ppm)
f1 (ppm)

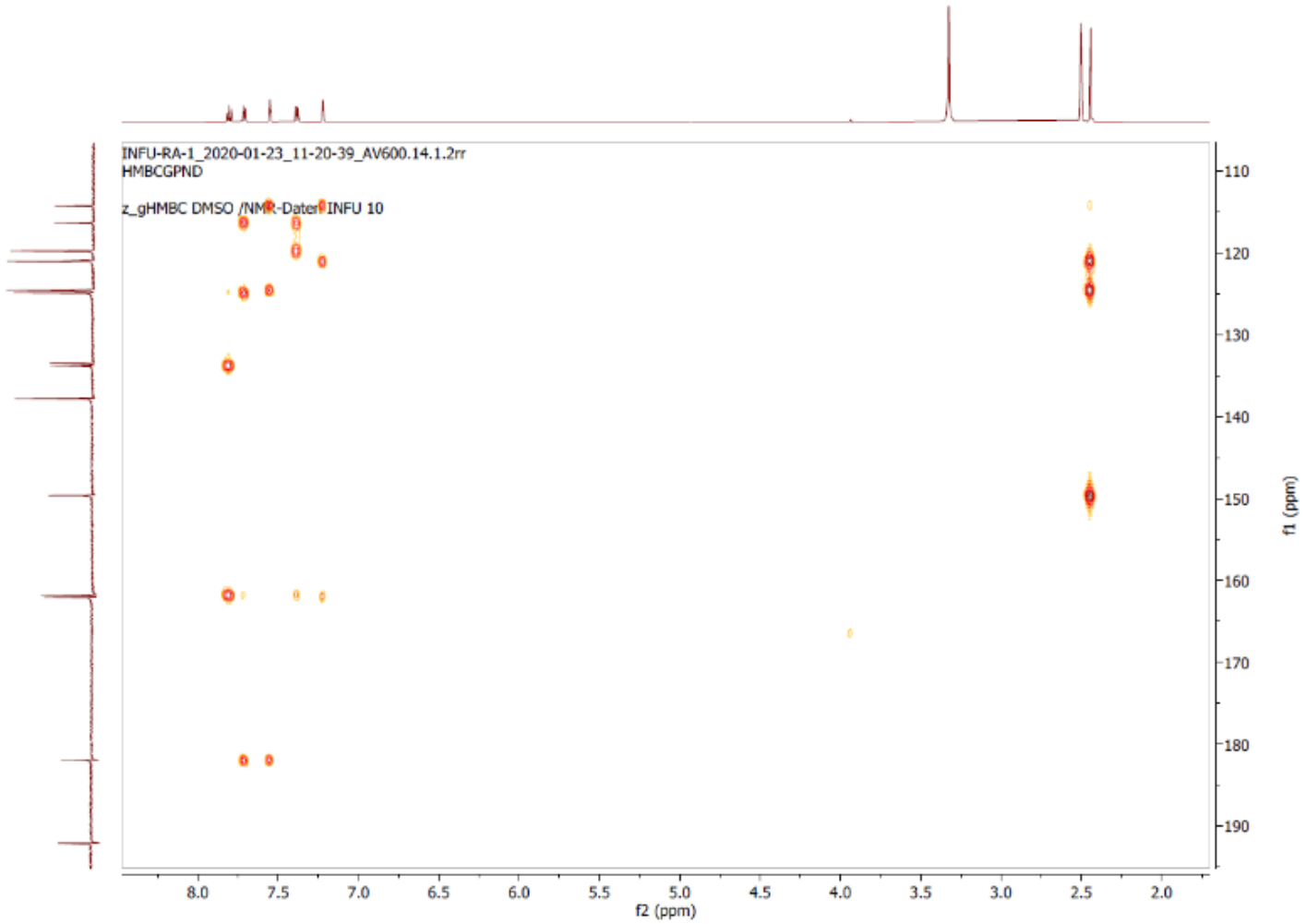
Appendix 9. Expanded HSQC spectral data of compound-1



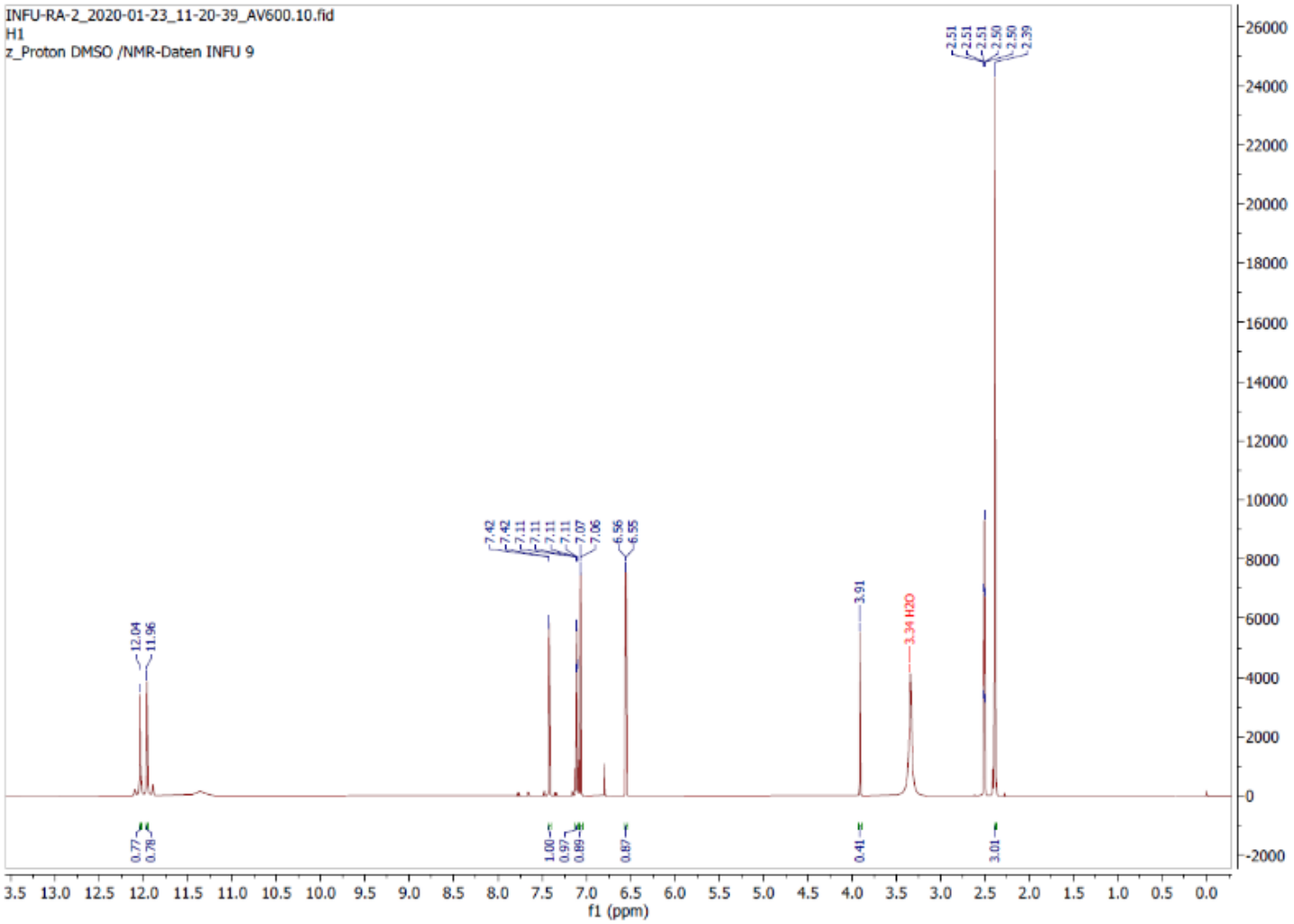
Appendix 10. HMBC spectral data of compound-1



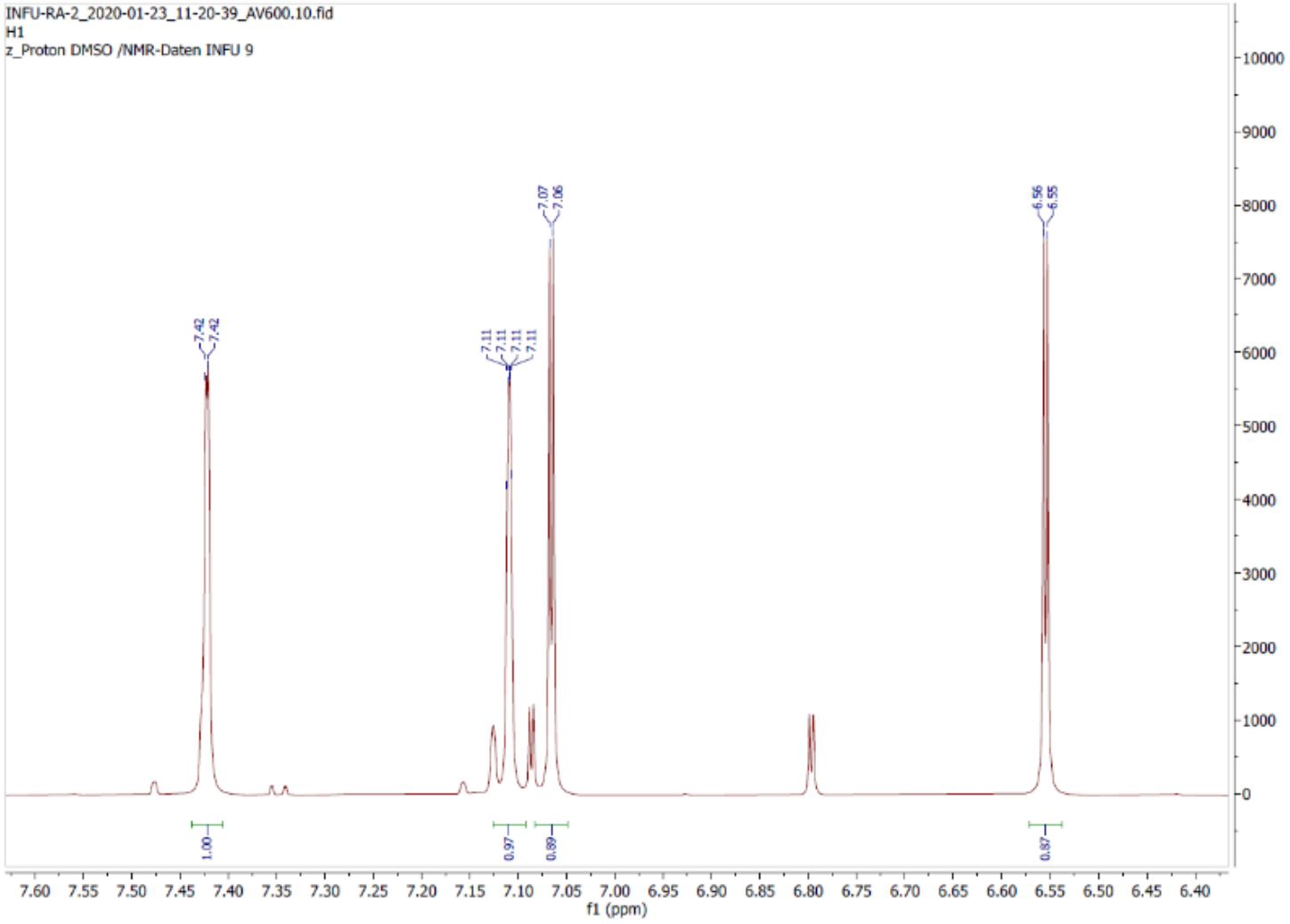
Appendix 11. Expanded HMBC spectral data of compound-1


INFU-RA-1_2020-01-23_11-20-39_AV600.14.1.2rr
HMBCGPND
z_gHMBC DMSO /NMR-Daten/INFU 10
110
120
130
140
150
160
170
180
190
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
f2 (ppm)

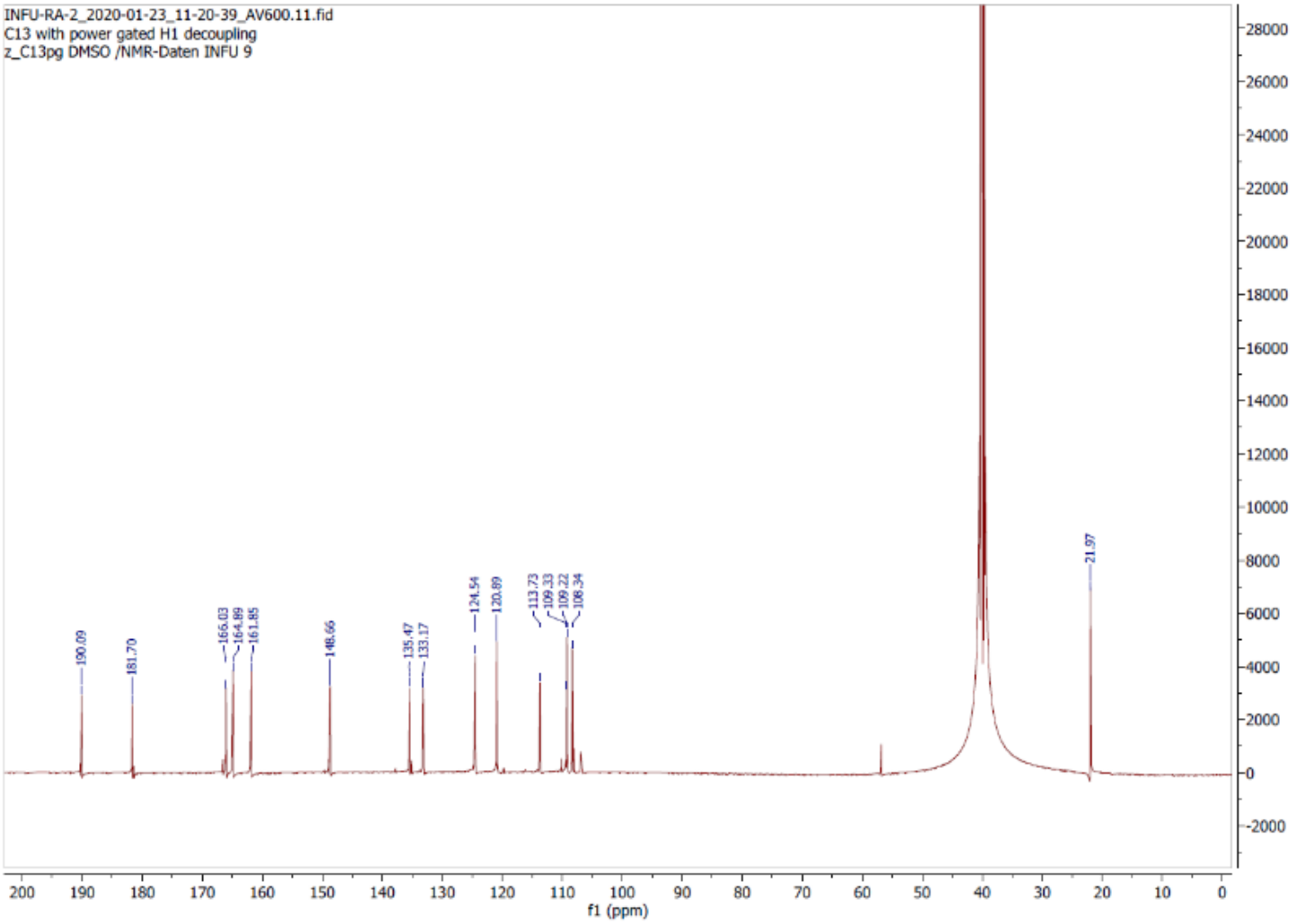
Appendix 12. [1]HNMR spectral data of compound-2



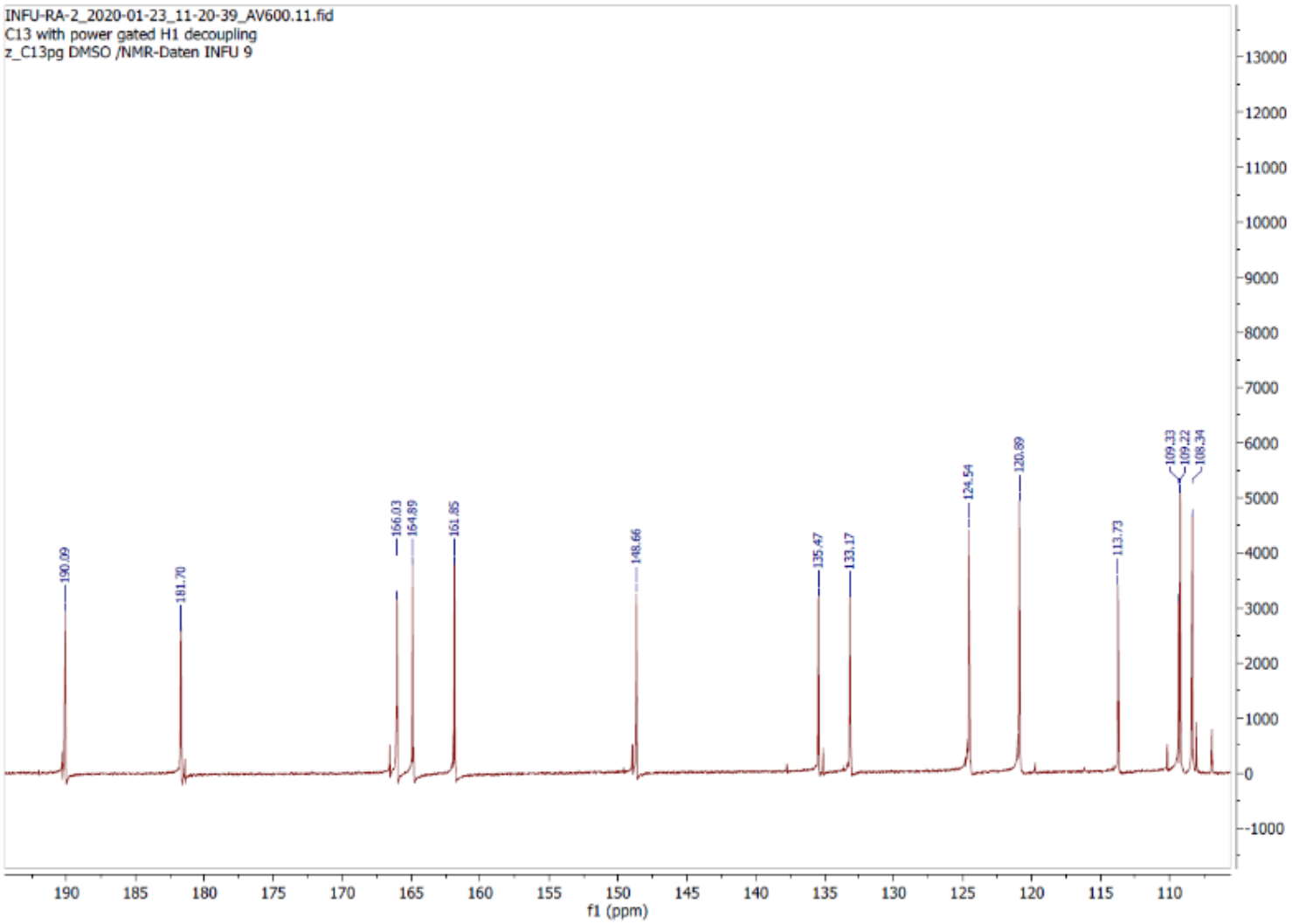
Appendix 13. Expanded ^{1}HNMR spectral data of compound-2 from 6.40-7.60 ppm



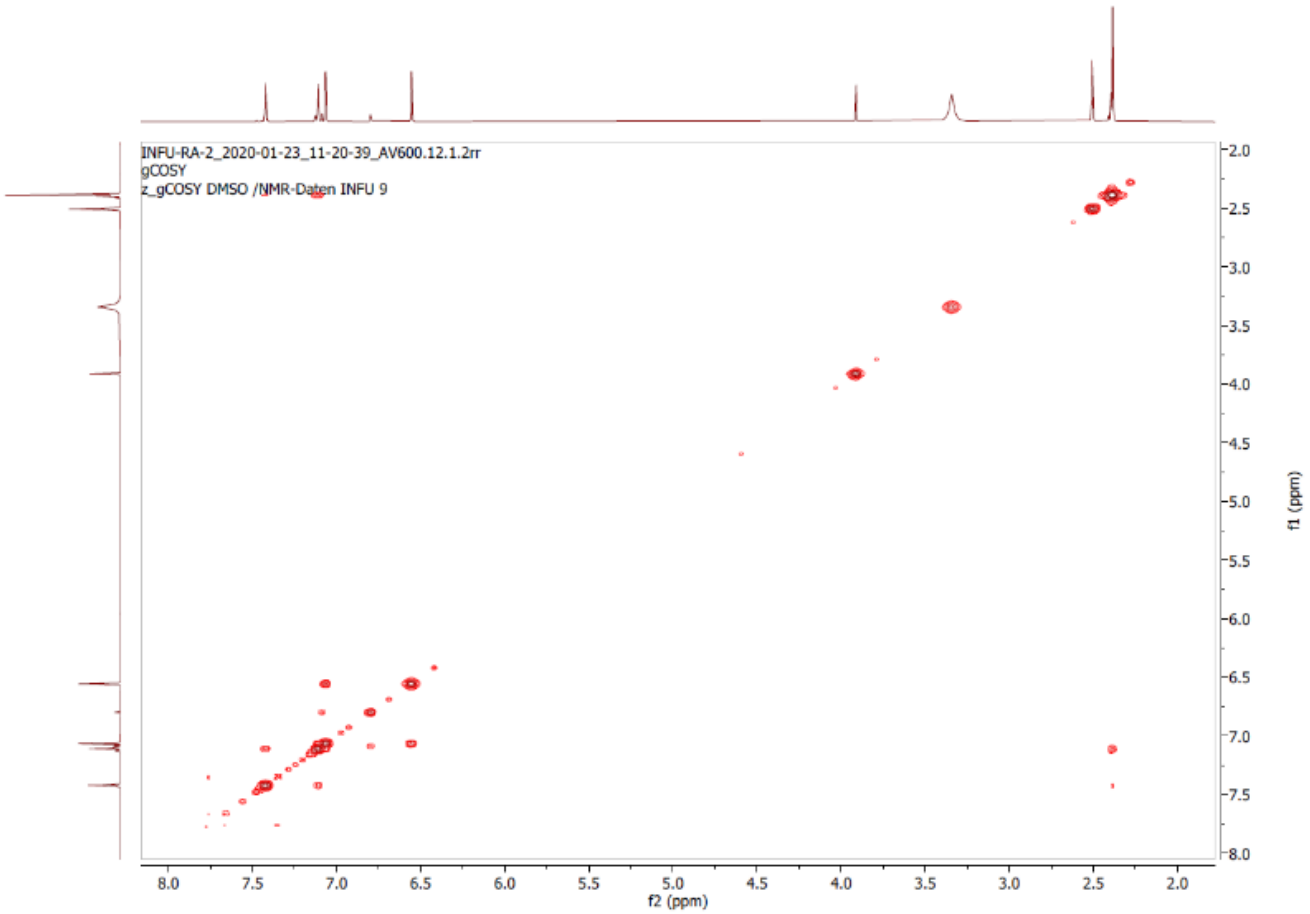
Appendix 14. ^{13}CNMR spectral data of compound-2



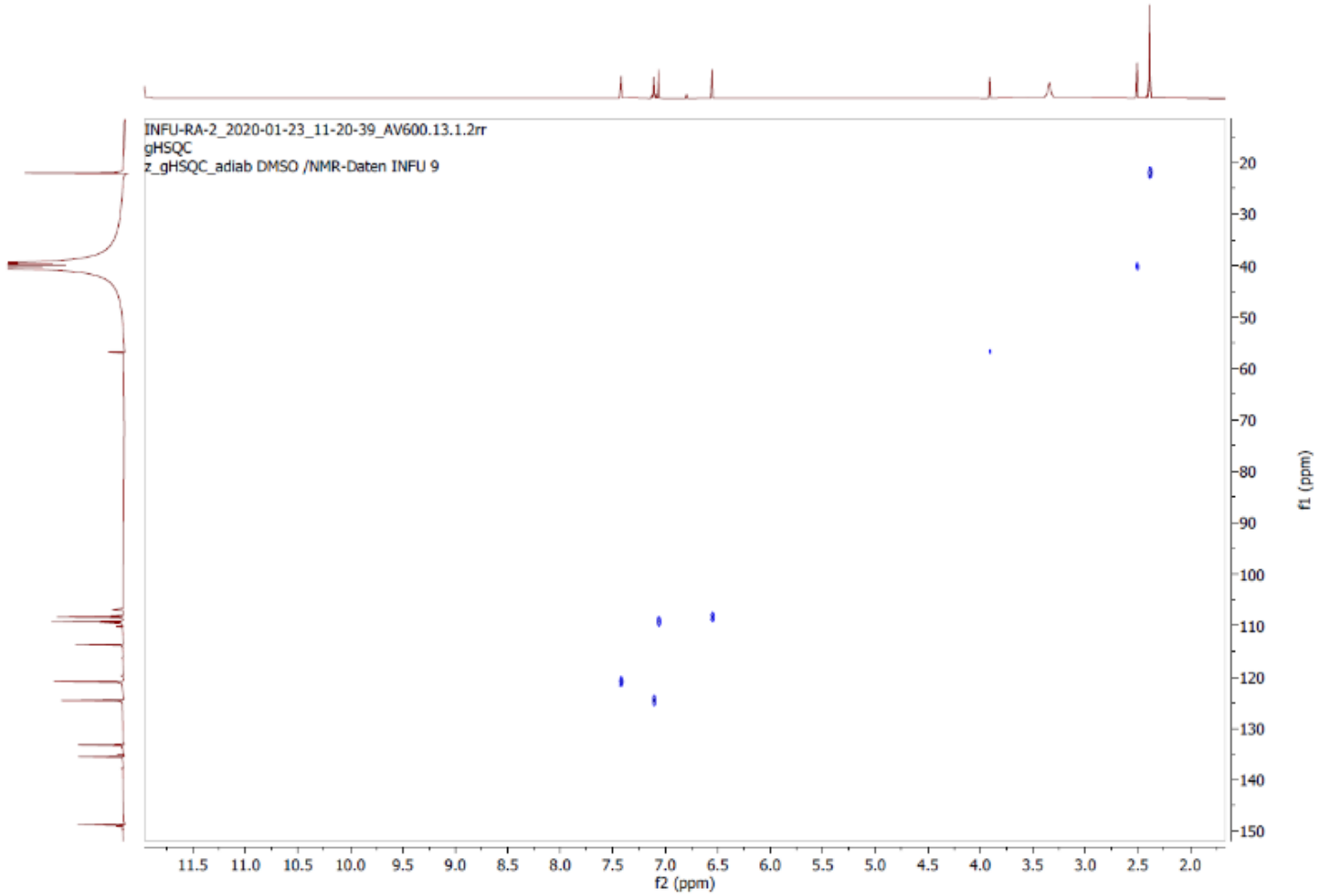
Appendix 15. Expanded ^{13}CNMR spectral data of compound-2 from 110-190 ppm



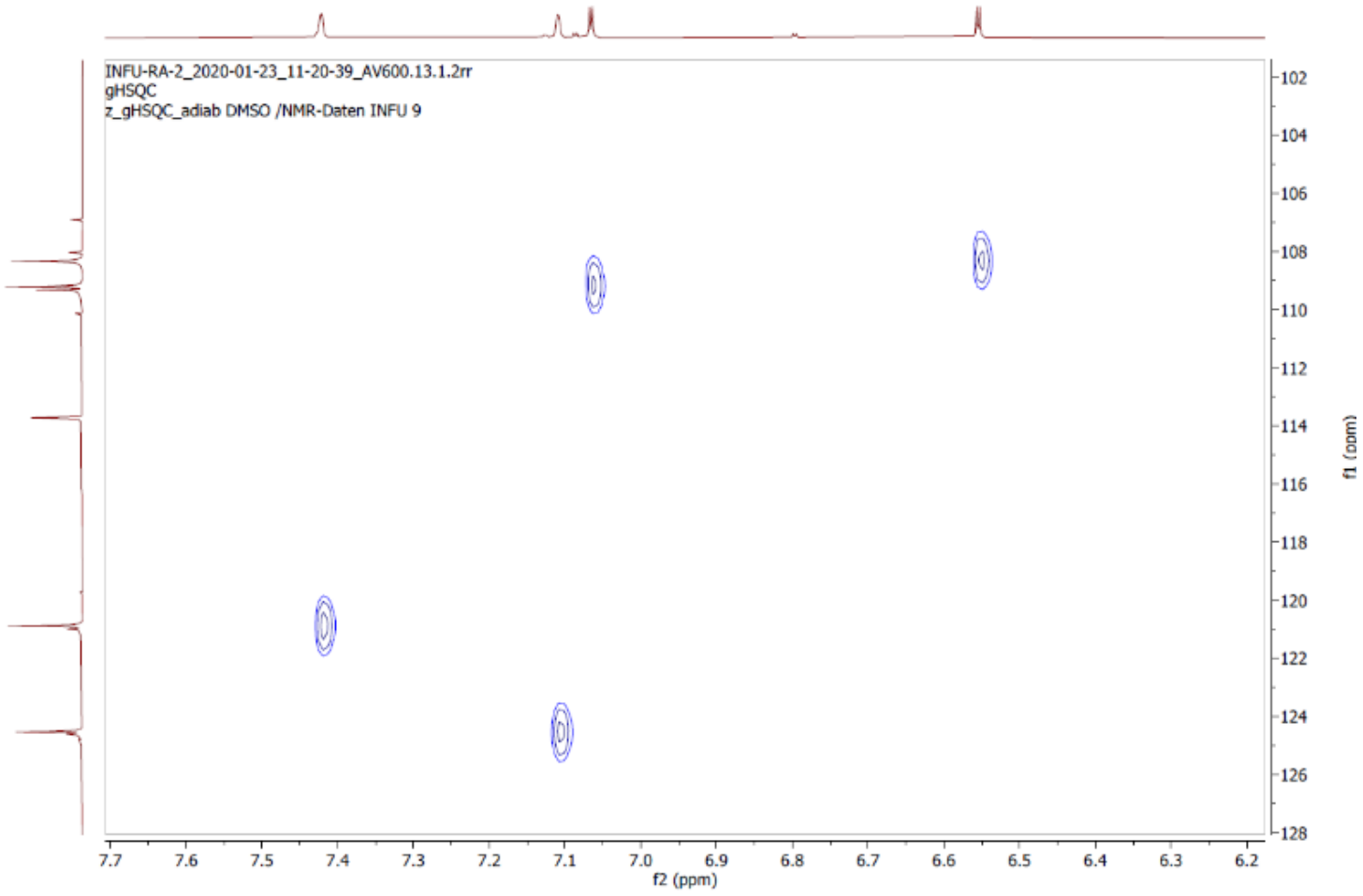
Appendix 16. COSY spectral data of compound-2


INFU-RA-2_2020-01-23_11-20-39_AV600.12.1.2rr
gCOSY
z_gCOSY DMSO /NMR-Daten INFU 9
f2 (ppm)
f1 (ppm)

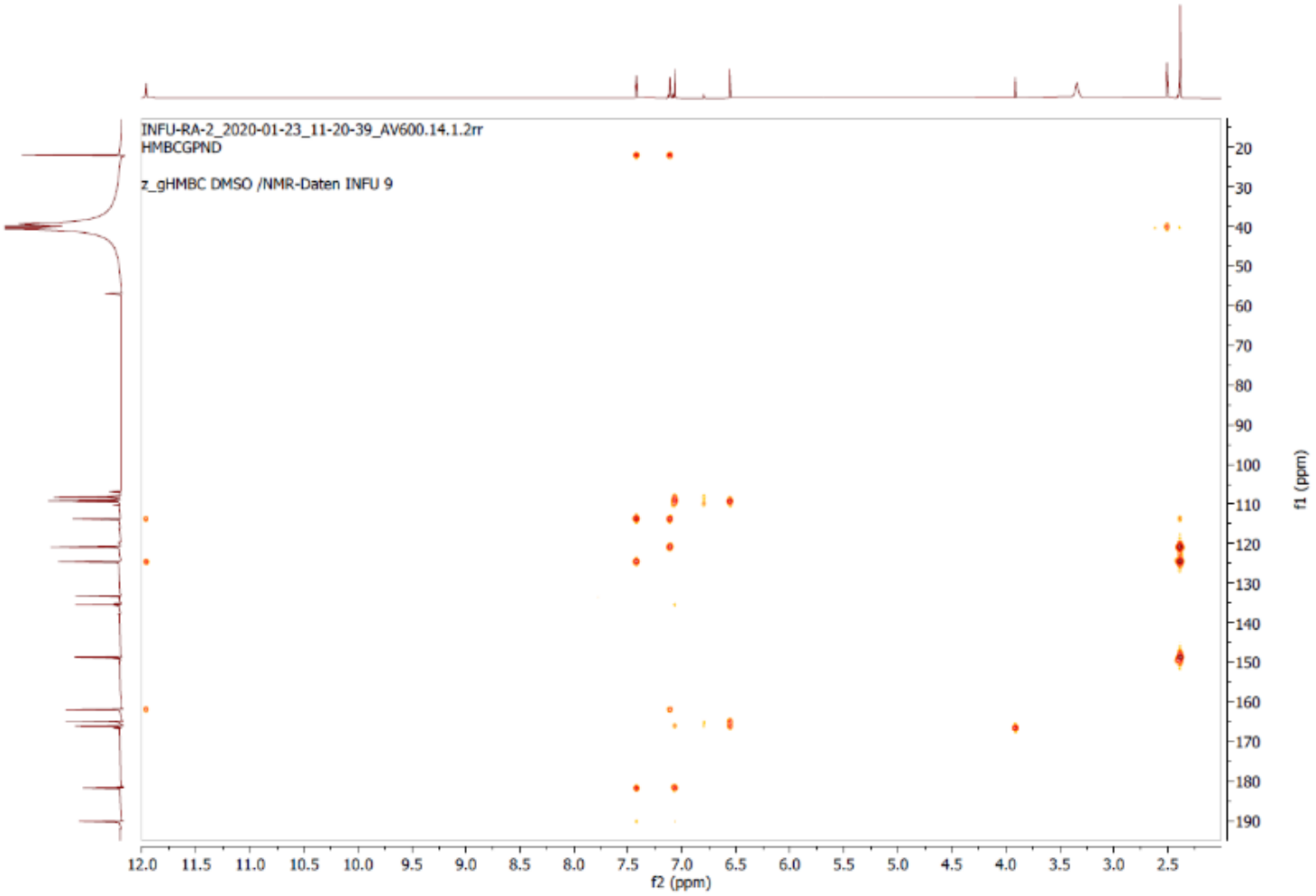
Appendix 17. HSQC spectral data of compound-2



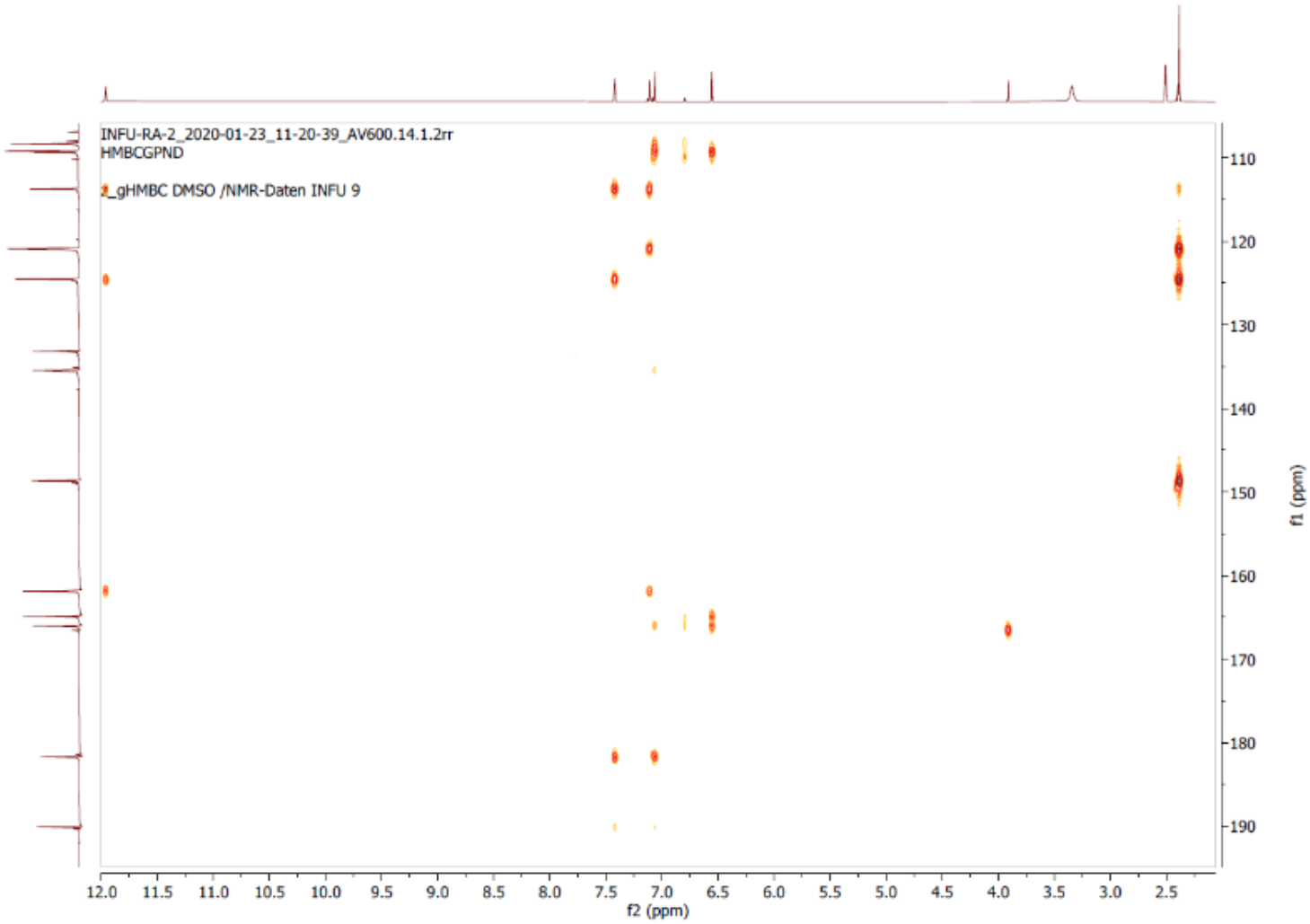
Appendix 18. Expanded HSQC spectral data of compound-2



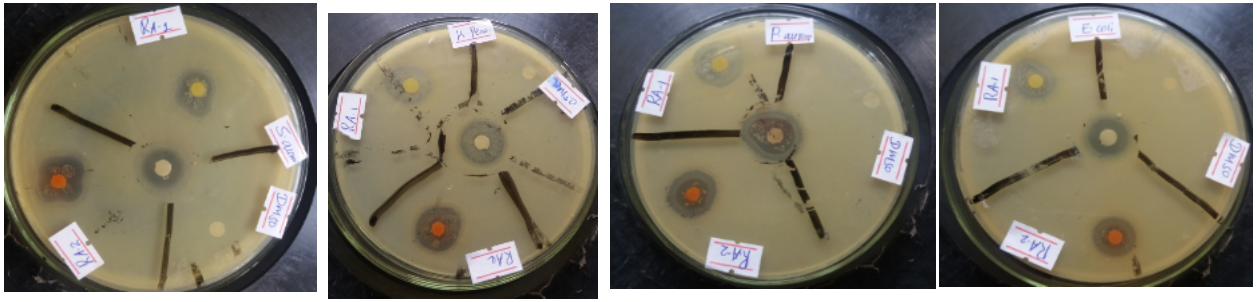
Appendix 19. HMBC spectral data of compound-2



Appendix 20. Expanded HMBC spectral data of compound-2



Appendix 21. Antibacterial activity of isolated compounds



Appendix 22. Antifungal activity of isolated compounds