JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

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M. Sc THESIS

ON

PHYTOCHEMICAL INVESTIGATION OF *DOVYSLIS ABYSSINICA* ROOTS AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITIES

BY

BELETE DELDIL

AUGUST, 2020

JIMMA, ETHIOPIA

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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.

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AUGUST, 2020

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Declaration

I declare that this thesis is my original work and has not been presented anywhere for award of any degree or diploma in any University.

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Abbreviations and Acronyms

^{13}C NMR	Carbon -13 Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
CC	Column Chromatography
$CHCl_3$	Chloroform
COSY	Correlated Spectroscopy
DMSO	Dimethyl-Sulfoxide
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
Me_2CO	Acetone
MeOH	Methanol
MPs	Medicinal Plants
NMR	Nuclear Magnetic Resonance Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
SNNP	South Nations, Nationalities and Peoples
TLC	Thin Layer Chromatography
WHO	World Health Organization

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Abstract

Compounds and extracts derived from the medicinal plants have profound uses in medicine. The products from medicinal plants are believed to possess different classes of chemicals that take part in the treatment of ailments. Several modern drugs have been developed from these chemicals isolated mainly from traditionally used medicinal plants. *Dovyalis abyssinica* is one of traditionally used medicinal plant for the treatment of various ailments such as amoeba, tapeworm, abdominal pain, headache, wound infection, typhoid, diarrhea, and eye infection. Even though, the chemical composition and anti-microbial activity of the root extracts of *Dovyalis abyssinica* has been the subject of previous studies, however; it is not exhaustively investigated as per its widely practiced by the traditional healers for the treatment of different infectious diseases. Therefore, this study was undertaken to identify bioactive secondary metabolites from the roots of *Dovyalis abyssinica* and evaluation of its anti-microbial activities. With this regard, the air dried roots of *Dovyalis abyssinica* was extracted (3x24 hr) with chloroform/acetone (1:1) by maceration. The extract was evaluated for its anti-bacterial and ant-fungal activities; and showed good activities against *P. aeruginsa* (bacterial strain) and *C. albicans* (fungal strain) with zone of inhibition 14.1±0.5, 14.4±0.7 mm, respectively. The crude extract was then subjected to column chromatography (CC) over silica gel, which was then eluted with petroleum ether containing increasing amounts of ethyl acetate, and resulted one compound (**41)**. The structure of the isolated compound was elucidated using 1D (^{1}H, ^{13}C) and 2D NMR spectroscopic techniques, and comparison with the literature. The isolated compound was also evaluated and showed good activities against *E. coli, S. aureus*, and *P. aeruginsa* with zone of inhibition 19.7±2.7, 21.2±0.7, and 21.3±2.5 mm, respectively and against the fungal strain (*C. albicans*) with the inhibition zone, 20.5±1.4 mm. The finding could be used for comprehensive evaluations of the phytochemicals for their microbial activities and also support the claim that the plant is used for the treatment of microbial disease.

Key words: *Dovyalis abyssinica*, 4-hydroxytremulacin, root extract, NMR, Traditional Medicinal Plants

1. Introduction

1.1 Background of the Study

Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, for medicines to alleviate or relieve and treat or control of different diseases and ailments. The history of the use of medicinal plants is probably dates back to the beginning of human civilization to deal with the treatment and management of diseases and ailments. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China, and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians. According to WHO medicinal plants defined as plants that possess healing properties or compounds that can be used for therapeutic purposes that synthesize metabolites to produce useful drugs [1]. Medicinal plants are also called medicinal herbs. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants that can be administered orally, inhaled or directly applied in the skin [2]. The medicinal value of these plants (TMPs) lies in bioactive phytochemical constituents that are produced by definite enzymatic or physiological action [3]. Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, carotenoids, glycosides and many more. Phytochemicals may be derived from different parts of plant including the bark, leaves, flowers, roots, grains or seeds, fruits, rhizomes, pulps and others. These natural compounds are of the bases for the production or the manufacture of modern drugs [4].

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their health care needs [5]. In the African continent long before colonization and arrival of Western values, plants were used for medicinal purposes. Extraction of medicine from herbs was indeed an integral part of the lifestyles of the people of Africa [6].

Traditional medicine has been practiced in Ethiopia since long time and the knowledge has been transferred orally from one generation to the next through healers or traditional herbalist, knowledgeable elders and/or ordinary people. It is estimated that about 80% of

the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants [7]. Different traditionally used medicinal plants were used for the treatment of different diseases were carried out in different parts of our country Ethiopia. For example: *Acanthus polystachyus Delile* (Root part), *Achyranthes aspera* L. (Root and Leave parts), *Acokanthera schimperi* (A.D) used for the treatment of worms, bleeding (skin-cut), bleeding after delivery, and wound, respectively. *Rumex nepalensis and Leucas deflexa* for the treatment of gastro-intestinal complaints [8]. The rhizomes of *Rumex abyssinicus,* possesses secondary metabolites such as tannins, saponins, flavonoids, steroids and anthraquinones. Flavonoids and tannins have been shown to be important for wound healing due to their antioxidant, anti-inflammatory and antibacterial activities [9]. *Croton macrostachyus* (leaf part) used for the treatment of skin fungus it has also showed larvicidal activity against larvae of *Anophele arabiensis*, a potent malaria anticonvulsant and sedative effects [10]. Among the most commonly used plants in Ethiopia were *Calpurnia aurea, Coffea arabica, Cordia africana, Rumex nepalensis, Zehneria scabra, Verbena officinalis, Verbascum sinaiticum, and Amaranthus* were used to treat diarrhea [10].

Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example tetrahydropalmatine(**1**) from *Corydalis yanhusuo* plant, is use for treating analgesic; tetramethyl-pyrazin(**2**) from *Ligusticum chuanxiong*, used for the treatment of Mmyocardial ischemia-reperfusioninjury ; puerarin (**3**) from *Pueraria,* to treat diabetes; gastrogin(**4**) from *Gastrodia (*BL), used as anti-convulsion and treat analgesic; aspirin (**5**) from *Filipendula ulmaria* plant, to treat analgesicand inflammation [11]; cocaine (**6**) from *Erythroxylm coca*, to treat analgesic and anti- *tussaive* [12]; salvianolic acid B (**7**) from *salvia miltiorrhiza* (Bunge), to cure and treat cardiovascular and cerebrovascular diseases, and many more (Figure 1).

Dovyalis abyssinica, traditionally pretty much of its part is used to treat or control, cure and prevent different diseases to humans and even live stokes [13]. Among the most human ailments, which are treated by *Dovyalis abyssinica* are gonorrhea, diarrhea, typhoid, cancer, HIV, malaria, skin diseases, headache, wound infection, eye infection, colic pain in infants, chest pain, amenorrhea, throat problem, colds, cough, syphilis and may more [14, 15, 16].

Chemical composition and anti microbial activity of the root extracts of *Dovyalis abysinica* was the subject of previous studies. However as per it's exploited by traditional practitioners because of its medicinal application, it was not exhausted very well. Therefore, this research project was aimed to carry out further extraction, biological activities with different concentrations, isolation and characterization of bioactive constituents of the plant root extract to identify active compound against a variety of anti-microbial activities.

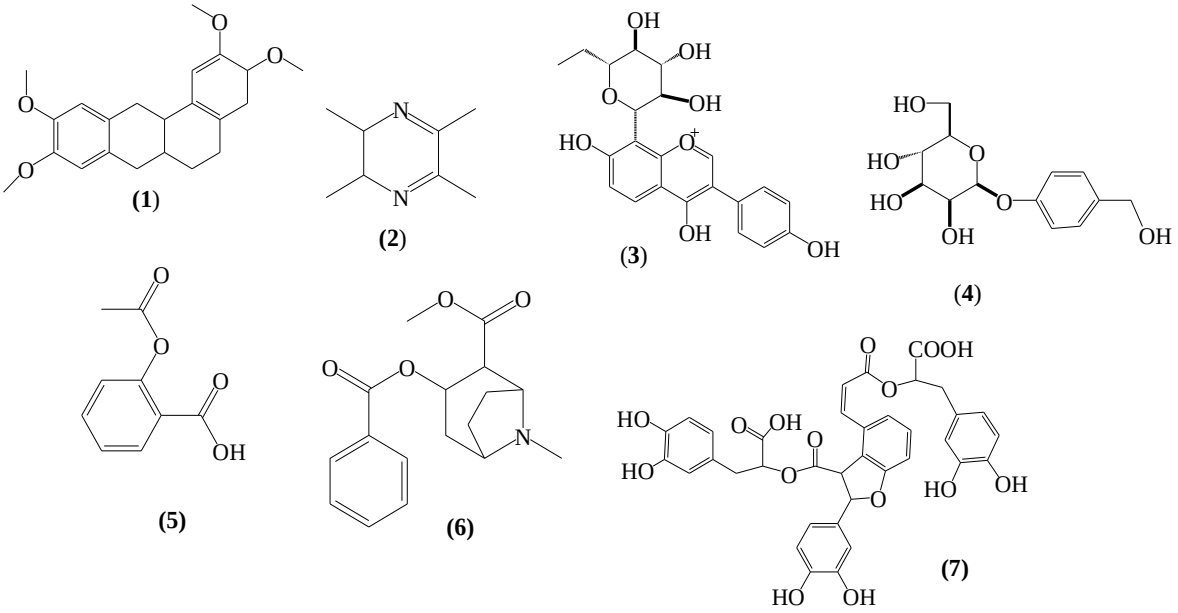


Figure 1 Some drugs isolated from medicinal plants.

1.2 Statement of the Problem

In the past years, the pharmacological industries have produced a number of anti-biotic and anti-fungal agents. Despite this effort however, the incidences of drug resistance by microorganisms has increasing. 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. Therefore, the urgent need discover alternative anti-microbial agents to address the current problem of drug resistance is required [17]. Medicinal plants are increasingly gaining attentions by researchers, due to the increasing inefficacy or potency of many modern drugs as well as increase in resistance by several bacteria to

various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health. Among the most common medicinal plants, *Dovyalis abyssinica* is one of the plant used in Ethiopia for the treatment of various diseases. Phytochemical analysis tests carried out in aqueous extract of *Dovyalis abyssinica* roots demonstrated that, it possessed bioactive compounds which include: alkaloids, falavonoids, tannins, saponins, carotenoids, betulinic acid, coumarin, polyphenols, and glycosides [18]. Previousreports have relied on phytochemical investigation from the stem bark, fruit, seeds and leave part, and very few reports on root part of *Dovyalis abyssinica* and evaluations of its anti-microbial activities.*Dovyalis abyssinica* is by far a rich source of bio-active compounds and it needs further investigation using different solvents. This study was focused on the phytochemical investigation of *Dovyalis abyssinica* roots and evaluation of its anti-microbial activities of the crude extracts and the pure isolated compound.

1.3 Objective of the Study

1.3.1 General Objective of the Study

- The main objective of this study was to investigate secondary metabolites from the roots of *Dovyalis abyssinica* and to evaluate their anti-microbial activities.

1.3.2 Specific Objectives of the Study

- To isolate secondary metabolites from the roots of *Dovyalis abyssinica* using chromatographic techniques;
- To elucidate the structure of the isolated compound using spectroscopic techniques, including 1D and 2D NMR and MS;
- To evaluate the antibacterial and antifungal activities of the crude extracts and isolated compound against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans* strains.

1.4 Significance of the Study

In Africa, the use of medicinal plants still plays an important role in microbial treatment. Taking into account that plants were potential sources of the existing first line antimicrobial drugs, there is still great potential of identifying antimicrobial drugs from plant-based

sources. Thus, screening and identifying the bioactive constituents of traditional medicinal plants significantly used to treat infectious diseases will have a great contribution in addressing this global problem. Phenolicglycosides (phenolics) could exhibit promising activity against different types of ailments and diseases. Members of the subfamily *Salicaceae* including the genus *Dovyalis* produce a broad variety of alkaloids and phenolicglycosides, with wide range of bioactivities. Thus, the study of *Dovyalis abyssinica* may yield promising anti-bacterial and anti-fungal agents. Furthermore, the findings of this research could be useful to provide information of the chemical profile of the plant and can be used as a database and guideline for further isolation and purification of the active principles.

2. Review of Related Literature

2.1 Botanical information and Ecology of *Dovyalis abyssinica*

The genus *Dovyalis* contains eighteen species. Of these fifteen species are native to Africa . The following are the most common *Dovyalis* species: *Dovyalis abssinica* (A.Rich.) Warb, *Dovyalis caffra* (Hook. And Hary.) Warb, *Dovyalis hebecarpa* (Gardner) Warb, *Dovyalismacrocalyx* (Oliy.) Warb, *Dovyalis mollis* (Oliy.) Warb, *Dovyalis rhamnoides* (Burch.Ex Dc) Burch.,ex Hary. And Sond, *Dovyalis rotundifolia* (Thunb.) Hary,*Dovyalis verrucosa* (Hochst.) Lign. And Bey, and *Dovyalis zeyheri* (Warb) [19].

The genus *Dovyalis* has traditionally been placed in the family *Flacourtiaceae*, which has long been recognized as a polyphyletic taxon, having a highly diverse and controversial circumscription. However, the cyanogenic tribes of *Flacourtiaceae* were recently included in the family *Achariaceae*, and the noncyanogenic tribes, including *Dovyalis*, were united with *Salicaceae*. *Dovyalis abssinica* is in the family of *Salicaceae*. *Dovyalis* is found primarily in subtropical and tropical regions of Africa, with one species *Dovyalis abyssinica*, which is widely distributed in tropical regions in Ethiopia. *Dovyalis abyssinica* occur naturally from Ethiopia, Eritrea and Somalia in the North through Kenya and Tanzania to Malawi in the South and grows in upland rainforest, dry evergreen forest, and sometimes in more open woodland. In Ethiopia, usually found in humid lower highland forest and mostly grew up in the Weyna Dega and Kolla climatic zones of Ethiopia [20]. It is known by the common names in Amharic: *Koshim*, in Afan Oromo, *Koshomo/Koshommii*, in Somaligna, *Ongolatz*, and in Tigrigna, *Aihada* [20].

Dovyalis abyssinica (Fig 2B) is a spiny evergreen shrub or tree, up to 6-10 height, with a rounded crown. The bark is ash grey, almost always supporting lichens. Branches armed with stout spines, up to 1½ cm long. The branch lets are covered with numerous dotted pores (lenticels). Leaves are oval to obovate, up to 5-7 cm long and 3 cm wide with a rounded tip, edges unevenly rounded. It is shiny, dark green, with reddish stalks and veins. Flowers are unisexual, yellow-green or greenish without petals, 5-7 mm long. Female flowers are single or in 2-3 flowered fascicles. Male flowers occur in clusters, with 40-60 stamens. It has powerful tap root system, a useful adaptation feature critical for drought tolerance. It is known that the capacity of plants to take up both water and mineral nutrients

from the soil depends on their ability to develop both extensive and intensive root systems. The wood is hard and suitable for tool handles, spoons, and bedsteads. In addition to medicinal values it is used for fencing, home gardens, and fodder for Goats and Sheep during drought conditions, fire wood [21].

Figure 2 Picture of *D. abyssinica* (taken by Belete Deldil)

2.1.1 The Genus *Dovyalis*

The genus name *Dovyalis* is a Greek word, meaning spear (in reference to the plant's long, strong, and sharp spines). *Dovyalis* is a genus of shrubs and small trees. The genus *Dovyalis* has traditionally been placed in the family *Flacourtiaceae*, which has long been recognized as a polyphyletic taxon, having a highly diverse and controversial circumscription. However, the cyanogenic tribes of *Flacourtiaceae* were recently included in the family *Achariaceae*, and the noncyanogenic tribes, including *Dovyalis*, were united with *Salicaceae* [22]. The distributions of the genus *Dovyalis* is limited to in Africa. Literature sources show that the genus *Dovyalis* is rich in saponins, alkaloids, flavonoids, glucosides, steroids, anthocyanins, phyosterols, phenolic compounds, novel spermidine alkaloids, terpens, etc [23, 24].

2.1.2 The *Salicaceae* Family

The *Salicaceae* are a family, the *willow* family of flowering plants. The family *Salicaceae* comprises 55 genera and 1, 200 species. The *Salicaceae* family traditionally comprises Salix (willow) and *Populus* (poplar, cotton), which are common in northern temperate regions. Botanically, the *Willow family* consists of bushes and trees with simple, alternate leaves and sometimes shadow. The flowers are unisexual with male and female flowers

appearing in catkins on separate plants (dioecious). *Salicacea family* and members of the *Salicaceae* are distributed mostly worldwide, from tropical to cold-temperate climates, tropical to north temperate and boreal regions [25]. Species on the family *Salicaceae* are known for producing a great number of bioactive substances. Some of its relevant compounds are lignins, terpenoids, coumarins, alkaloids, saponins and flavonoids and many more. Anti-inflammatory, anti-fungal, anti-ulcers, and antibacterial activities are reported for *Salicaceae* species. Numerous secondary metabolites were isolated from *Salicacea family* include flavonoids, salicin derivatives, phenolic acids, anthocyanins, salicinoids and polysaccharides [26]. The isolated compounds from *Salicaceaa family* such as flavonoids, salicin, phenolic alcohols, phenolic aldehyde and sterols are used for the treatment of fever, pain and inflammation. And also the members of this family contain varying amounts of the simple phenol glycosides populin, salicin, and pectin and methyl salicylate from which the common aspirin was originally derived; which are used to treat ulcers, and pain [26]. In general speaking *Salicaceae* family are known sources of secondary metabolites, which have anti-microbial effects or activities than other families [27].

2.1.3 Ethno-pharmacological use of *Dovyalis abyssinica*

The genus *Dovyalis* is widely used in the treatment, cure and prevent or control of human diseases and ailments including abdominal pain, colds, chest pains, stomachache, cough colic pain in infants, teeth problems, typhoid fever, headache, gonorrhea, brucellosis, diarrhea, skin diseases, wound infections, and infertility in women and many more [28].Reports in the litreture indicate that traditional healers throughout Africa have confined themselves almost exclusively to the use of species from the genus *Dovyalis* for the treatment of a wide range of infectious diseases [29]. All parts of *Dovyalis abyssinica* are utilized in the local and popular medicine, which serve as remedies against various diseases. In traditional medical practices of Ethiopia the aqueous and dichloromethane extracts obtained from the leaves and root bark of *D. abyssinica* have a longstanding reputation for the treatment of, tooth-ache, malaria, trypanosomosis, thetse, hemorrhoids, ulcers, and swelling of the throat, acariasis, bleeding gum, typhoid fever, diarrhea and also the fruits of *D. abyssinica* promote wound healing, a concoction made from the boiled the

root of the plant is used to treat eye infection. Moreover, it is used to treat acariasis and cancer when boiled seeds with water and eat fresh fruit [29, 30].

The medicinal values of *Dovyalis abyssinica* are largely experienced in different parts of the continent, Africa including Ethiopia. Reports and literatures showed that the plant *Dovyalis abyssinica* can treat various infectious diseases even including HIV [31]. In Kenya, *D. abyssinica*is used for the treatment of the ailment of cough, splenomegally, arthritis, infertility in women, rental disorder, cancer, colds, chest pains, stomach-ache, cough, malaria, amenorrhea, oral hygiene, hemorrhoids, arthritis, throat inflammation, eye disease [31]. In Tanzania, the root of *D. abyssinica* is used to treat syphilis, malaria, stomach problem, amoebiasis [32].

In our country Ethiopia, eating 6 – 10 fruits a day is used to treat abdominal pain and its fruit is eaten as food for the case of intestinal parasite before breakfast every morning; concocted, pounded & steam bath of its root used to treat syphilis and constipation (indigestion and fibroids), the leaves and roots decoction is used to treat malaria, boil seeds with water and drink is used to treat acariasis, eating fresh fruit is used to treat bleeding gum and cancer [33]. Decoction of the roots of *D.abyssinica* are used to treats gonorrhea and also serves as a tonic against harsh environmental conditions. Also the roots of *D.abyssinica* mixed with the roots of *Solanum aculeastrum* and then cooked in water and the resulting infusion is drunk twice daily to treat gonorrhea and malaria, until recovery [34]. Even more when some branches of *D. abysinica* placed on fire and fumigated during illness is used to treat evil eye in, Kenya [35].

2 .1.4 Phytochemicals Isolated from *Dovyalis* Species

A great diversity of chemical classes are found in the species of *Dovyalis*, mainly alkaloids, flavonoids, phenolics, tannins, terpenes, sterols, saponins, proteins, anthocyanins, carotenoids, glycosides, coumarin, etc [36, 37]. The major secondary metabolites so far reported are flavonoids, alkaloids, anthocyanins, phenolics, sperimidine type alkaloids, phenolic acids and sterols [38] which are summarized in Table 1.

Table 1: List of compounds isolated from *Dovyalis* species**.**

Compound Class	Compound Name	Plant Source	Ref.
Sterols	α-amyrin (**17**), β-amyrin (**18**), β-sistesterol (**19**), β-3-O-β-D-glucoside (**20**)	From leave part of *Dovyalis caffra*	[39]
Flavonoids	apigenin (**21**), luteolin (**22**), apigenin-7-O- β-glucoside (**23**),	,, ,, ,, ,, ,,, ,,, ,, ,, ,,,	[39]
Alkaloids	N'pentyl-10-(1,3-dimethylhaxahydro-2-pyrimidinyl)-1-decanamine, (Aberiamine) (**24**), N'-[{(E)-1-butenyl 17(dimethylcarboxamidoheptadecyl)amino}methyl]N'-methylacetamide,(Abriamide)(**25**)	,, ,,, ,,, ,,, ,,, ,,, ,,,,	[39]
Anthocyanins	Palargonidin **(28),** Cyanidin (**29),** Cyanidin (**30),** Peonidin (**31**), Petunidin(**32),** Malvidine (**33**).	From the root part of *D.hebecarpa*	[40]
Spermidine Alkaloids	Dovyalicin A (**8**), dovyalicin B (**9**), dovyalicin E (**10**), dovyalicin F (**11**), dovyalicin C (**12**)	From the leaves of *D. macrocalyx D. abyssinica* and *D. hebecarpa*	[40] [41]
Phenolics	methyl1-hydroxy-6-oxocyclohex-2-enecarboxylate (**13**), 4-hydroxytremulacin (**14**), tremulacin (**15**), 1,2-cyclohexanediol glucoside (**16),** Betulinic acid (**26**), Benzoic acid (**27**).	From the leaves of *D. zeher*i and *D.abyssinica*	[42]
Phenolic acids	hydro-p-coumaricacid **34**), Caffeic acid (**35),** m-hydroxybenzoic acid (**36),** p-hydroxyphenyl acetic acid (**37**), 3-methoxy-4- hydroxyphenylpropionic acid (**38**), p-coumaric acid (**39**), protocatechuic acid (**40)**	From the fruit of *Dovyalis caffra.*	[41]

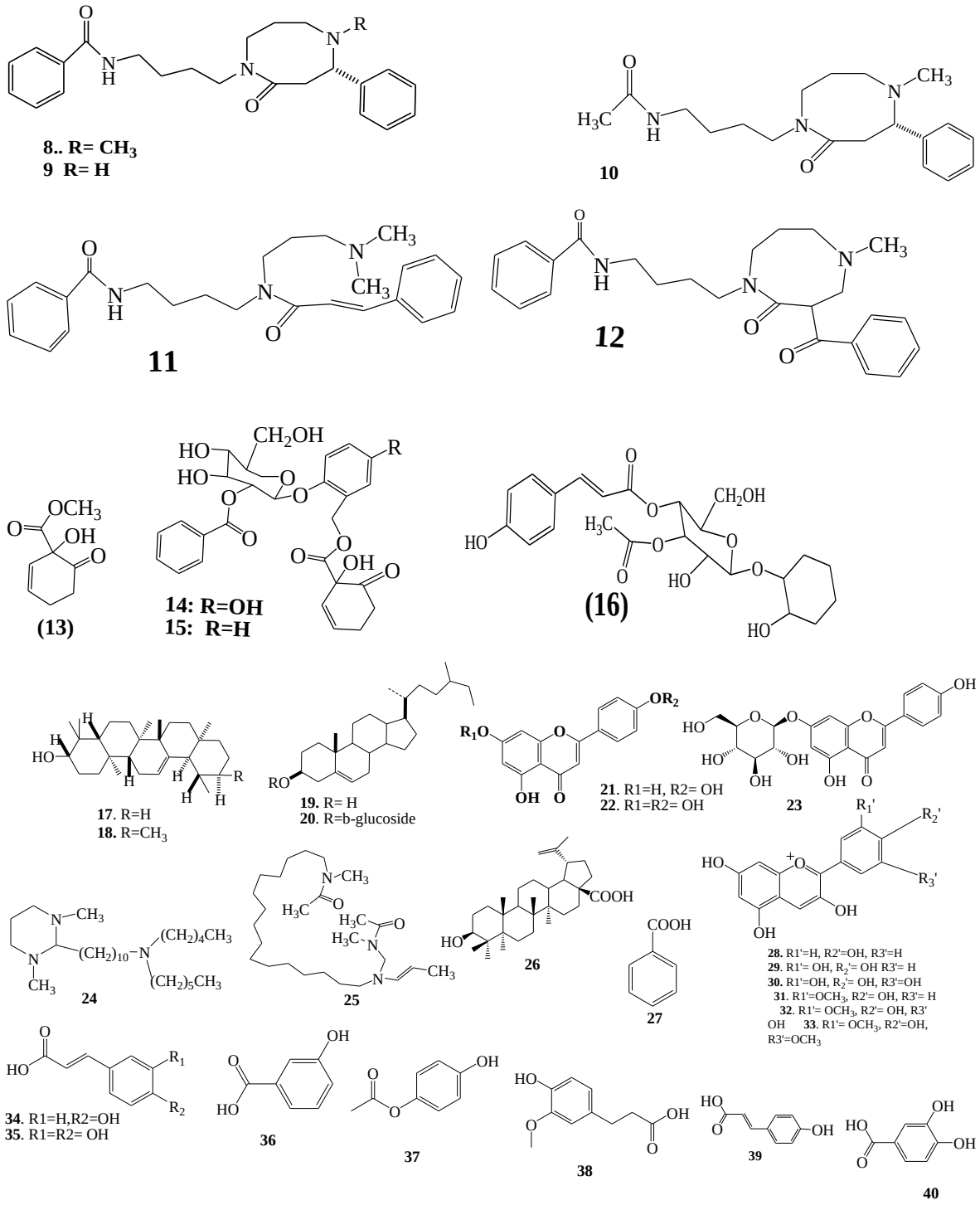


Figure 3 The structure of some of the isolated compounds from different *Dovyalis* species.

Very few researches have been done on the root, stem, and seed and fruit part of *D. abyssinica.* Petroleum ether, methanol: chloroform mixture and methanol crude extracts

were carried out for dried leaf sample of *D.abyssinica*.Flavonoids, tannis, steroids, saponins, phenolics, glycosides, coumarins and terpenoids were reported [43].

2.2 Biological activity tests carried out on *D. abyssinica*

It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in their anti-microbial activities; *D. abyssinica* is the one promising antimicrobial activities against different bacterial and fungal strains due to the presence of the listed secondary metabolite. So *D. abyssinica* is known for their healing properties and used for the treatment of various human diseases ranging from stomachache problem, headache, diarrhea, chest pain, skin diseases, colds, typhoid, diarrhea, and cough and wound infections among others. So far reports showed that methanol, water and acetone extracts of the leave and root parts of the plant *D. abyssinica* showed good antifungal and anti-bacterial activities [44]. The *in vitro* and *in vivo* anti-trypanosomal effects of crude dichloromethane and methanol leaf extracts of *D. abyssinica* on the most pathogenic East African animal trypanosome, T. congolense showed an excellent activity. Extracts of roots of *D. abyssinica* was tested for anti-diabatic properties and showed good and prominent ant- activities/healing power. The aqueous extracts of the roots of *D. abyssinica* are used by the Kipsigis Community, in Kenya, in folk medicine, as anticancer agent and in managing other ailments [45-48].

2.2.1 Antibacterial Activity Tests

Reports showed that *D. abyssinica* plant is by far the most prominent and promising plant species which is the ability to kill or suppress or inhibit the growth of bacterial strains even at low concentrations, 1000 µg/ml. For instance the MeOH leave extract of *D. abyssinica* at a concentration of 2000 µg/ml, 1000 µg/ml, 500 µg/ml, and 250 µg/ml showed significant antibacterial growth inhibition/killing their growth by its presence; the bacterial strains are: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Neisseria gonorrhoea and Escherichia coli*. The anti-bacterial test was conducted using the agar dilution method. These listed bacterial strains their growth inhibition was targeted by the presence of these most significant secondary metabolites tannins, terpenoids, alkaloids, polyphenols [49].

Methanolic fruit extracts of *D. abbyssinica* has been reported to be effective against *Staphylococcus* bacteria with a zone of inhibition of 7.02 mm. This bacterial growth inhibition was revealed to be the presence of phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins and in one case protein [50].

2.2.2 Anti-fungal Activity Tests

The methanolic leave extracts of *D. abyssinica* was tested against the following three fungal strains: *Aspergillus flavas, Aspergillus niger* and *Candida albicans (*clinical isolated strain) showed good inhibition activity. Methanolic fruit extracts of *D. abbyssinica* has been reported to be effective against *Trichophyton rubrum* fungi with a zone of inhibition of 8.60 mm [49, 50].

2.2.3 Anticancer Activity Tests

Plants have a long history of use in the treatment of cancer and it is significant that over 60% of currently used anti-cancer agents come from natural sources among that sources D. abyssinica plant is the one that is used to treat breast cancer in Ethiopia. According to World Health Organization, about 18.1 million new cancer cases and 9.6 million deaths occurred in 2018 [51]. The aqueous and methanolic extracts of the roots and stem bark of *D. abyssinica* are used by the Kenyan Community in folk medicine, as anticancer agent/activity [51]. MeOH and CH_2Cl_2 leave extracts of *D. abyssinica,* in Ethiopia traditionally used to treat cancer disease; it was showed promising healing activity [52].

2.3 Overview on Infectious Diseases

Infectious diseases mean illnesses caused by microbes (such as bacteria, viruses, and fungi) that enter the body, multiply, and can cause an infection. Infectious diseases are a leading cause of illness and death worldwide. Globally, infection cause over a fifth of all deaths and a quarter of all illnesses, disproportionately affecting poorer communities and resource-poor countries. It is estimated that worldwide each year around 5.5 million people die from malaria, tuberculosis (TB) and human immune deficiency virus (HIV) related infections, 1.8 million die from diarrheal disease and more than a million children die from other infectious diseases. The vast majority of these deaths are in the developing world, and similarly it has been suggested that in the case of an influenza pandemic up to 96% of deaths could occur in developing countries [53].

In recent years infections caused by bacteria resistant to multiple antibiotics have been a great problem around the world. The study of microbial diseases, availability of better diagnostic tools and discovery of improved therapeutic agents has stimulated a great deal of scientific activity in the area of Medical Plants. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Some commonly encountered pathogens have been associated with some of the human diseases. Methicillin resistant *Staphylococcus aureus* (MRSA) has been troubling hospital services all over the world. In order to prevent infectious diseases that are caused by

microorganism the researchers and scientists must be look and invest their time and economy towards drugs produced from medicinal plants like, *D. abyssinica* [54].

2.3.1 Bacterial Infections and Antibacterial Agent

Bacterial pathogens are derived from living organisms and affect the brain, spinal cord, or meninges. Bacterial infections, most often due to *Streptococcus, Haemophilus*, and *Neisseria* species, can cause significant meningitis. Bacteria are widely distributed and the most abundant group of unicellular organisms microscopic without nucleus on earth, capable of adapting in a diverse range of environments such as in soil, water and air. They are both useful and harmful to humans. Many parasitic bacteria do not harm their hosts. Some cause disease through different mechanisms including production of toxins and or hydrolytic enzymes. Bacteria have a prokaryotic cell type with a rigid wall which protects the cell against osmotic damage. The structure of the cell wall differs in Gram-positive and Gam-negative bacteria [55].

Gram-negative bacteria differ from Gram-positive by the presence of an outer membrane composed of lipopolysaccharides. It also contains specific proteins for transporting hydrophilic molecules. Other proteins are receptor sites for phages and bacteriocins. The outer membrane in Gram-negative bacteria covers the peptidoglycan layer, which is attached to the outer membrane by lipoproteins. The layer is separated by periplasm from the cytoplasmic membrane. Gram-positive bacteria have thicker peptidoglycan layers with no periplasm [56]. *Staphylococcus aureus* is a spherical Gram-positive parasitic bacterium that causes illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis and septicem other examples of spherical Gram-positive bacteria includes *streptococcus pheumoniae* and *streptococcus pyogenes* (it results in the disorder of pharyngitis or strep throat) [57].

The most Gram negative bacteria which have causative effect and they have curved or spherical shape includes: *Haemophilus influenza*, *Vibro cholera* and *Borrelia burgdorferi*. *Escherichia coli* is also a Gram-negative bacterium normally present in the intestinal tract of humans and other animals. *Escherichia coli* can sometimes be pathogenic, thus posing a threat to food safety, causing diarrhea, wound and urinary tract infections [58].

2.3.2 Fungal Infections and Antifungal Agent

Fungi are neither plants nor animals. Their size ranges from microscopic to easily seen with the naked eye. Pathogenic fungi have two forms: yeasts (unicellular) and molds (melti cellular). Basically fungal infections predominate in immune compromised hosts and are caused by yeasts, molds, and dimorphic fungi. *Cryptococcal meningitis* is the most common fungal infection, whereas *candidiasis* is the most commonnosocomial infection. *Mucormycosis* and *aspergillosis* are characterized by angioinvasiveness and are associated with high morbidity and mortality among immunocompromised patients. *Candida albicans and Trichophyton* are among the most causative fungal diseases that causes vaginal yeast and Athlete's foot infection respectively. Actually *C. albicans* infection is the most common nosocomial fungal infection; it is particularly common in patients receiving immunosuppressive therapy or with indwelling catheters [59].

The other problem associated with the emergence of various resistant organisms which limited therapeutic potency of many of the available drugs. Moreover, it has a serious global economic impact that affects the productivity of individuals, families and the society at large, since it causes energy loss, debilitation and loss of work capacity. Consequently, there is an urgent need for new, affordable and accessible antibacterial agents from nature with novel mechanism of action. The emergence and potential spread of strains of bacterial and fungal which are resistant to currently available drugs, has actually prompted the search for new drugs through the use of high-through put screening and combinatorial chemistry, genomics, and vaccine development. However, this effort has yet to deliver a single drug despite the enormous resources expended during the past many years. So, natural products will continue to serve as lead structures for the development of antimicrobial drugs. This is in fact a needle that new antimicrobial leads may emerge from plants, especially from those with accepted traditional uses like, *D. abyssinica.*

3. Materials and Methods

3.1 Chemicals

The chemicals used for this present work were petroleum ether (99.8, AR/ACS), chloroform (99.8, HPLC), acetone (99.8, HPLC) , methanol (99.8, AR/ACS) and ethyl acetate (99.8, AR/ACS) (analytical grades, Loba Chemie Pvt Ltd, India), silica gel 60-120 mm mesh size (Loba Chemie, India), Mueller Hinton agar and nutrient broth as culture media and dimethylsulfoxide (DMSO, 99.96) were used for antimicrobial activity test.

3.2 Apparatus and Equipment

Rotary evaporator (Heidolph, Germany, laboratory 4000, No, 519-0000-00-2) for solvent evaporation, Uv-Tech (254 and 365nm) chamber for detection of spots on TLC plate, glass column chromatography (500 mm, B-34/35) for separation and purification techniques, mortar and pestle and electrical grinder for grinding, round bottom flask of 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), Hood (CLB-201-04, vertical laminar cabinet) for antimicrobial activity were used in the study. NMR spectra were obtained on Bruker Advance 700 MHz spectrometer, using the residual solvent peaks as reference.

3.3 Plant materials collection and Preparation

The roots of *D. abyssinica* were collected from Amhara Region, South Wollo Zone (Dessie), Buanbua woha District, Buanbua Woha town, which is 401 Km away from Addis Ababa July, 2019. The plant material was air-dried under shade region and then powdered to suitable size by using mechanical and electrical grinder to improve the subsequent extraction by rendering the sample more homogenous, increasing the surface area, and facilitating the penetration of solvent into the cells.

3.4 Extraction of Roots of *Dovyalis abyssinica*

The air-dried and powdered roots of *D. abyssinica* 1 Kg was soaked in chloroform /acetone (1:1) 2.5 L and extracted three time for 24 hr each. The extract was concentrated using rotary evaporator at 50 °C under reduced pressure to yield 25 g (2.5%), which was stored at room temperature until used.

3.5 Isolation of Compound

Solvent selection was done for column chromatography by spotting dissolved sample solutions of the crude extract on TLC plate. Petroleum ether and ethyl acetate combinations of different polarities were selected as eluent solvents for column chromatography. A 20 g portion of the crude extract was adsorbed on 20 g silica gel (60-120 mesh) and subjected to column chromatography (500 mm diameter) on silica gel (240 g). The column was first eluted with petroleum with increasing polarity of ethyl acetate in petroleum ether from 0 to 100 % and; then eluted with EtOAc with increasing polarity of MeOH from 0 to 10% to provide 137 fractions. Fractions 107-115 (25 mg) were combined based on their TLC profile and washed repeatedly with n-hexane to afford compound-**41** (17 mg). Similarly fraction 118-123 (38 mg) were combined concentrated and washed repeatedly with petroleum ether, and chloroform; however, there was no pure compound isolated.

3.6 Biological activity tests

3.6.1 Anti-bacterial activity test

The test solutions were prepared by dissolving 50 mg of the extract in 1 mL of dimethyl sulfoxide (DMSO) to prepare the above different concentrations. The standard drug for antibacterial test Gentamicin and antifungal test Ketoconazole were used .The culture media was prepared by dissolving Muller Hinton Agar in distilled water and boiled to dissolve the media completely.

Agar disk diffusion method was used to evaluate the antibacterial activity of both crude extract and isolated compounds on nutrient agar. The stock cultures were maintained on the nutrient agar slants which were stored at 4℃. Agar cultures of the test microorganisms were prepared according to manufacture instruction. The test solutions were prepared by

dissolving 50 mg of the crude extract and 10 mg of isolated compound to achieve final stock concentrations of 0.1 mg/ml and 0.05 mg/ml in DMSO, respectively. Freshly prepared grown liquid culture of the test pathogens solutions were added over the Mueller-Hinton Agar medium with sterile swab. Filter paper pieces containing the test samples were put on Petri dish covered and incubated at 37 ℃ for 24 h. DMSO was used as a negative control and gentamicin as appositive control for each Petri dish. After the incubation clear zones were formed around each disk and measured in millimeter using ruler [60].

3.6.2 Ant-fungal activity test

A disc diffusion method was applied to test the extracts and isolates against the test fungi using standard antifungal agent ketoconazole as a positive control. The prepared culture media was autoclaved for 24 h at 121 °C temperature. Filter paper pieces containing the test samples were put on Petri dish and covered and incubated at 27 °C for 72 h. DMSO solvent was used as a negative control. Finally, the results were taken on the third day by measuring the diameter of zone of inhibition [61].

4. Result and Discussion

4.1 Structural elucidation of the Isolated Compound

About 1 kg of the air-dried root bark of *D. abyssinica* was extracted using chloroform/acetone (1:1) for 24 hr, three times and resulted 25 g (2.5 %). The chromatographic separation of the chloroform/acetone (1:1) extract of the roots of *D. abyssinica* has resulted one compound-41. Compound-41 was isolated as white amorphous solid. Its molecular formula was determined to be $C_{27}H_{28}O_{12}$ based on the ion peak observed at m/z 562.1926 for $[M + H_2O + H]^+$ in the ESI-MS.

The ^{1}H-NMR spectrum (Table 2) showed the presence of five mutually coupled multiple aromatic protons at δ_H 7.99 (2H), 7.66 (1H), and 7.53 (2H) confirmed by HMBC and COSY analyses indicated the presence of mono-substituted aromatic ring. It also revealed the presence ortho meta coupled three aromatic proton signals at δ_H 7.00 (d, J = 8.5 Hz), 6.64 (dd, J = 8.7, 3.2 Hz) and 6.55 (d, J = 3.0 Hz) for tri-substituted aromatic moiety. The up-field shifted chemical shift value of the later two aromatic protons could probably be due to the presence of ortho-oxygenation. In addition, cis-coupled olefinic protons at δ_H 5.65 (dt, J = 9.2, 1.5 Hz, 1H) and 5.02 (dt, J = 8.4, 3.6 HZ, 1H), and an ester glycoside, as evidenced from cluster of peaks from δ_H 3.80 - 5.34 ppm which are characteristic glycoside with the anomeric proton at δ_H 5.28 (d, J = 8.1 Hz) and an oxymetylene protons at δ_H 4.03 were also evident.

The ^{13}C-NMR spectrum showed the presence of 26 carbon atoms which are chemically distinct and attributed to seven quaternary carbons including three carbonyl carbons (206.2 (C-6'), 170.3 (C-7'), and 165.5 (C-1''')), two oxygenated aromatic carbons at δ_C 147.4 (C-1) and 153.2 (C-4); two aromatic quaternary carbons at δ_C 126.4 (C-2) and 130.2 (C-2'''), fifteen methine and four methylene carbons. Inconsistence with the ^{1}H NMR spectrum, the ^{13}C NMR spectrum also indicates the presence of *O*-linked carbons of the sugar moiety and aromatic carbons of the aglycone. The anomeric carbon resonated at δ_C 100.3 ppm while the aromatic carbons were observed in the region δ = 114.9 –165.5 ppm.

Table 2: The ^{1}H and ^{13}C NMR spectral data of compound-**41** (in DMSO-d_6) and 4-hydroxy tremulacin (in CD_3OD)[62].

Position	**Compound-41**		**4-hydroxy tremulacin**		Remark
	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)	δ_C	
1		147.4		149.6	Quaternary
2		126.4		127.6	Quaternary
3	6.55 (d, J = 3.0)	114.9	6.61(d, J = 2.9)	116.5	CH
4		153.2		154.2	Quaternary
5	6.64 (dd, J = 8.7, 3.2)	115.8	6.67(dd, J = 8.9, 3.1)	116.9	CH
6	7.00 (d, J = 8. 5)	118.2	7.07(d, J = 8.5)	119.5	CH
7	4.89 (d, J = 12.2), 4.76 (d, J = 12.2)	61.9	2d(4.85, 4.96, J = 12.5)	63.9	CH_2
1’		77.7		79.2	CH
2’	5.65 (dt, J = 9.2, 1.5)	129.8	5.68 (dt, J = 9.8, 1.8)	129.3	CH
3’	5.02 (dt, J = 3. 6, 8.4)	132.0	6.13 (dt, J = 9.8, 3.7)	133.4	CH
4’	2.50 (m), 2.56 (m)	26.3	2.54 (m), 2.56 (m)	27.3	CH_2
5’	2.50 (m),	36.0	2.49 (m), 2.85 (m)	36.9	CH_2
6’		206.2		207.30	Quaternary
7’		170.6		171.20	Quaternary
1’’	5.28 (d, J = 8.1)	100.3	5.06 (d, J = 8.0)	102.3	CH
2’’	6.07 (m)	74.4	5.22 (t, 8.0)	75.7	CH
3’’	3.74-3.75 (m)	74.8	3.78 (dd, 9.6, 8.7)	76.1	CH
4’’	3.53 (m)	70.4	3.54 (dd, J = 9.7, 8.7)	71.7	CH
5’’	3.52-3.54 (m)	77.7	3.49 (ddd, J = 9.7, 5.5, 2.3)	78.4	CH
6’’	4.03(q, J = 2.4)	61.1	3.76 (dd, J = 12.0, 2.3), 3.94 (dd, J = 12.0,5.5)	62.6	CH_2
1’’’		165.5		167.3	Quaternary

2’’’		130.2		131.3	Quaternary
3’’’/7’’’	7.99 (m)	129.1	8.08 (m)	130.9	CH
4’’’/6’’’	7.66 (m)	129.2	7.48 (m)	129.7	CH
5’’’	7.53 (m)	133.8	7.60 (m)	134.5	CH
1’-OH	9.20 (s)				

From the above the above spectral data, the partial structure of compound was shown below.

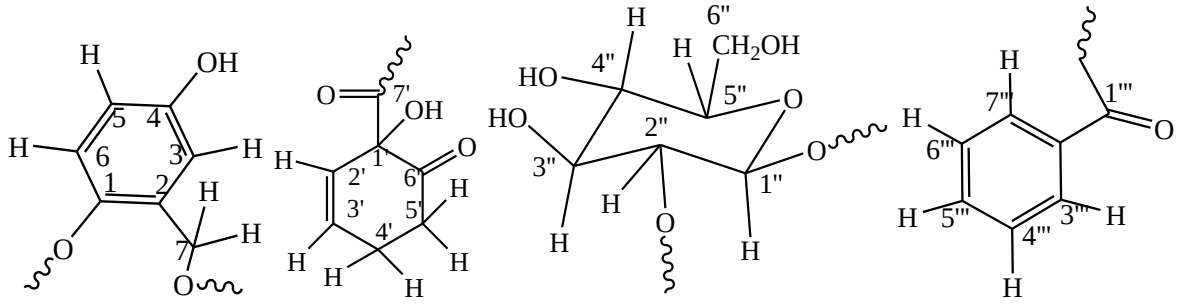


Figure 4 Partial structure of compound-**41** based on ^{1}H and ^{13}C NMR spectral data

The ^{1}H-^{1}H COSY spectrum showed the correlation between three aromatic protons each symmetrically placed on ortho and meta substituted on benzene ring at δ_H 6.64, δ_H 6.55 and δ_H 7.00. The two protons are found coupled to each other symmetrically. The ^{1}H-^{1}H COSY also showed the correlations between two cis protons appeared at the δ_H 5.65 and 5.02. The ^{1}H-^{1}H correlations are observed from its COSY spectrum (Appendix 7) as illustrated in Figure 5.

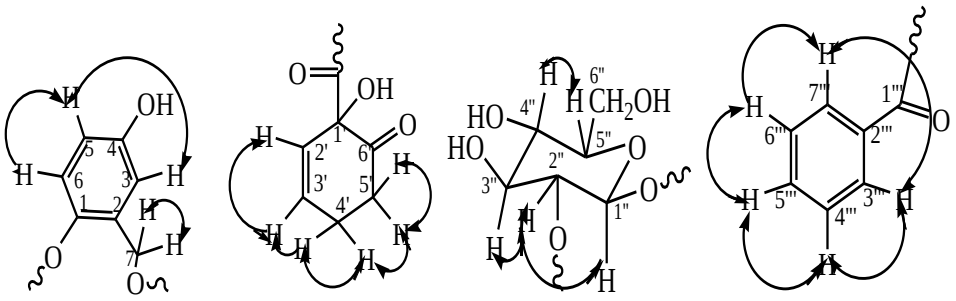


Figure 5 Structure of compound-**41** based on COSY spectrum.

Table 3: HSQC data for compound-**41**.

Carbon (^{13}CNMR)		**Proton(^{1}HNMR)**		**Multiplicity**	**Remark**
No	δ_C (ppm)	No	δ_H (ppm)		
3	114.9	3	6.65	d	CH
5	115.8	5	6.63	dd	CH
6	118.2	6	7.00	d	CH
7	61.9	7	4.90, 4.88	2d	CH2
2’	129.8	2’	5.65	m	CH
3’	132.0	3’	5.02	m	CH
4’	26.3	4’	2.61	m	CH_2
5’	36.0	5’	2.51	m	CH_2
1’’	100.3	1’’	6.07	d	CH_2
2’’	74.4	2’’	4.66	dd	CH
3’’	74.8	3’’	3.74	m	CH
4’’	70.4	4’’	3.55	m	CH
5’’	77.7	5’’	3.52	m	CH
6’’	61.1	6’’	4.01	m	CH_2
3’’’/7’’’	130.2	3’’’/7’’’	7.99	m	CH
4’’’/6’’’	129.15	4’’’/6’’’	7.65	m	CH
5’’’	133.78	5’’’	7.52	m	CH

The HSQC spectrum showed correlation such that protons doublet at δ 6.65, 7.00 and 4.03 correlate with the carbons at δ_C 114.9, 118.2 and 100.3, respectively and other correlations observed were between aromatic protons at δ 7.99 (2H, m), 7.65(2H, m) and 7.52(1H, m) with aromatic carbon signals at δ_C 129.1 (C-3’’’ & C-7’’’), 129.2 (C-4’’’ & C-6’’’) 133.8 (C-5’’’), respectively as shown in table 3 (Appendix 8)

Table 4: Observed correlations in HMBC data of compound-**41**.

Carbon		HMBC (^{1}H-^{13}C)
No	δ_C	
C-3	114.9	H-3↔C-1, H-3↔C-7, H-3↔C-5,
C-5	115.8	H-5↔C-1, H-5↔C-6,
C-6	118.2	H-6↔C-4, H-6↔C-2,
C-7	61.2	H-7↔C-7’
OH on C-4		H(4-OH) ↔C-4, H(4-OH) ↔C-5, H(4-OH) ↔C-6, H(4-OH) ↔C-3
C-2’	129.8	H-2’↔C-3’, H-2’↔C-4’, H-2’↔C-7’,
C-3’	132.0	H-3’↔C-4’, H-3’↔C-5, H-3’↔C-1’
C-4’	26.3	H-4’↔C-5’, H-4’↔C-6’
C-5’	36.0	H-5’↔C-6’, H-5’↔C-1’
OH on c-1’		H(1’-OH) ↔C-1’,
C-3’’’	130.2	H-3’’’↔C-4’’’, H-3’’’↔C-5’’’, H-3’’’↔C-7’’’,
C-4’’’	129.2	H-4’’’↔C-5’’’, H-4’’’↔C-6’’’, H-4’’’↔C-2’’’,
C-5’’’	133.8	H-5’’’ ↔ C-6’’, H-5’’’↔C-7’’’,
C-7’’’	130.2	H-7’’’↔C-1’’’
C-1’’	100.3	H-1’’↔C-2’’, H-1’’↔C-3’’, H-1’’↔C-1
C-2’’	74.4	H-2’’↔C-3’’, H-2’’↔C-4’’, H-2’’↔C-1’’’
C-3’’	74.8	H-3’’↔C-4’’, H-3↔’’C-5’’
C-4’’	70.4	H-4’’↔C-5’’
OH on C-3’’		H(3’’-OH) ↔C-3’’
OH on C-4’’		H(4’’-OH) ↔C-4’’
OH on C-6’’		H(6’’-OH) ↔C-6’’

The HMBC spectral analysis revealed that the proton signal at δ_H 5.65 (H-2’’) showed long range coupling with the carbon signals at δ_C 165.49 (C-1’’’), and 5.28(H-1’’),) and 4.76(H-7) coupled with the carbons signals at 147.44(C-1) 170.25(C-7’) confirming the positions of benzoyl group, glucosyl moiety and oxocyclohexenecarboxylic acid moiety and alsot he

most downfield shifted signal in the ^{1}H NMR at δ_H 9.20 (4-OH) showed HMBC cross coupling with its neighboring carbons, C-4 (δ 153.16), C-3 (δ 114.92) and C-5 (δ 115.77) confirming the position of this hydroxyl group at C-4 as showed in Figure 6 and (Appendix 9).

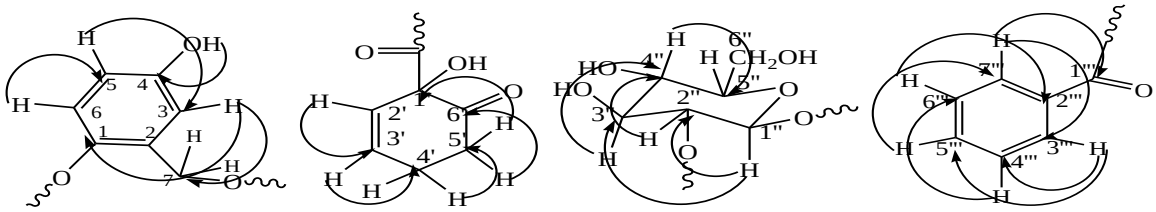


Figure 6 Structure of compound-**41** based on HMBC spectrum.

In the ^{1}H-^{1}H NOESY spectrum of compound-**1** showed correlations between δ_H 4.76 (2H-7) with 6.55 (H-3), 6.64 (H-5) with 7.00 (H-6), 6.07 (H-2') with 2.50 (H-4''). Furthermore the ^{1}H-^{1}H NOESY spectrum revealed that the long- rage correlation between hydrogen δ_H 5.28 (H-1'') to 3.74(H-3''), 3.52 (H-5'') this is observed from its NOESY spectrum (Appendix 10) as illustrated in figure 7.

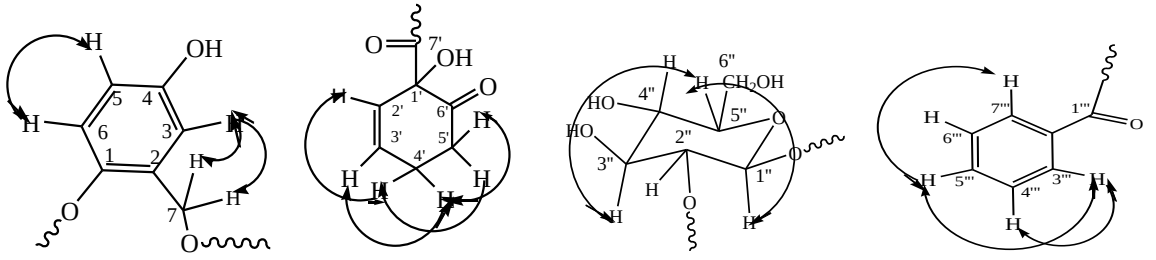
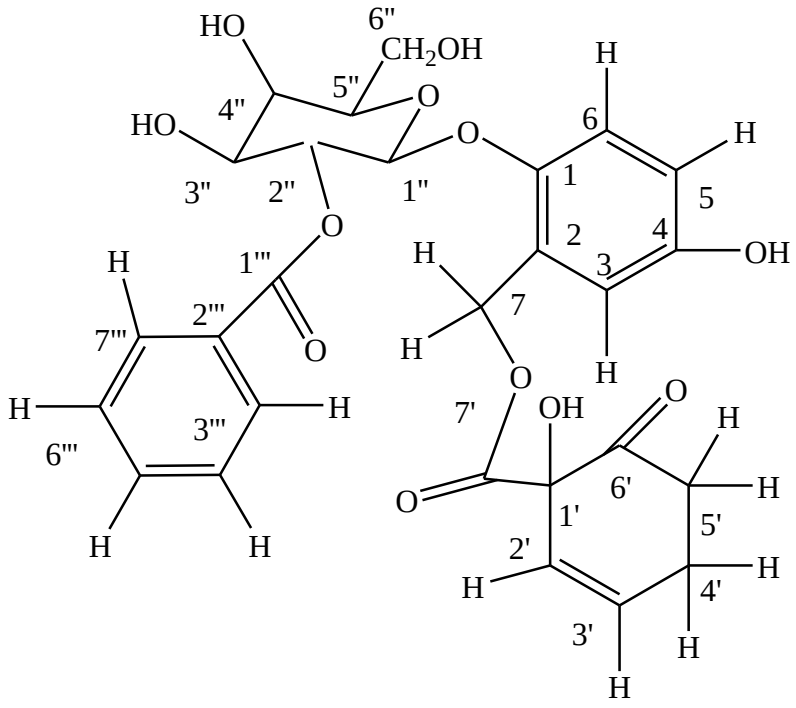


Figure 7 Structure of compound-**41** based on NOESY Spectrum.

Finally from the assignments made on the basis of ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC NOESY and MS correlations and the literature values, the structure of the isolated compound was deduced to be 4-hydroxy tremulacin, as shown in Figure 8, which was previously isolated from the leaves of *Dovyalis caffra* and *Dovyalis zeyheri* [63].



Compound-41

Figure 8 Partial Structure of Isolated Compound.

4.2 Antimicrobial activity Evaluations of crude extract and Isolated Compound

The crude extract, and the isolated compounds; were evaluated for antibacterial activity against four bacterial strains; *P. aeruginos, B.subtilis, S.aureus,* and *E. coli.* However, the crude extract was evaluated only against one bacterial strain; *P. aerugiosa*, and the remaining three bacterial strains were rejected due unexpected contamination.

The disk diffusion method was used and the zone of growth inhibition of crude extract and the isolated compound were measured in millimeter (mm) compared to the standard positive control, gentamicin and negative control, DMSO. The zone of inhibition measured was presented in Table 5.

Table 5: *In vitro* antimicrobial activities of the crude extract and isolated compound

Test Strain	Crude extract	Compound-41	Gentamicin	Ketoconazol	DMSO
Conc. (mg/mL)	50	10	25	25	-
P. aeruginosa	14.1±0.5	21.3±2.5	22.1±1.4	-	NI
E. coli	-	19.±2.7	23.5±2.6	-	NI
B. subtilis	-	12.5±2.5	20.4±3.5	-	NI
S. aureus	-	21.2±0.7	22.8±2.5	-	NI
C. abicans	14.4±0.7	20.5±1.4	-	24.0±0.7	NI

Key: "-": Not tested, NI: No Inhibition

The zone of growth inhibitions (Table 5) of the crude extract and isolated compound are different. The crude extract exhibited higher zone of growth inhibition (14.1±0.5 mm) against *P. aeruginosa* bacterial strain. Whereas the isolated compound showed good activities against the tested bacterial strain with the highest activity was observed against the two strains; *P. aeruginosa* and *S. aureus* with the inhibition zone of 21.3±2.5 mm and 21.2±0.7 mm, respectively, which is comparable to that of the reference drug; gentamicin (22.8±1.4 mm). Whereas, both the crude extract and the isolated compound showed marginal activity against the fungal strain; *C. abicans*. The isolated compound showed batter activity that the crude extract, which could probably due to the antagonistic effect of the several compounds present in the crude extract.

5. Conclusion and Recommendation

5.1 Conclusion

The chromatographic separation of the chloroform/acetone (1:1) extract from the root of *D. abyssinica* has resulted one compound, which was characterized and identified as 4-hydroxytremulacin. This is the first report of its kind from *D. abyssinica* having previously been reported from leaves of *Dovyalis caffra* and *Dovyalis zeyheri*.

The crude extract and the isolated compounds were assayed against four bacterial and one fungal strain. Compound-**41** showed superior activity against some bacterial (*P. aeruginosa*) and fungal (*C. abicans*) strains with mean zone inhibition of 21.3±2.5 and 20.5±1.4 mm, respectively. The little activity was observed against *B. subtilis* with mean zone of inhibition of 12.5±2.5 mm. Generally, the superior activity of the pure compound than the crude extract was exhibited and this may be due to negative synergetic effects of several compounds present in the crude extract. The microbial activity observed for the crude extract and isolated compound is in line with the traditional uses of the plant and the compound could be of pharmaceutical interest for therapeutical application as complementary antibacterial agents for infectious disease.

5.2 Recommendation

- In this work isolation was done chloroform/acetone (1:1) crude extract, thus isolation should be done from other fraction in order to confirm the presence of other phytochemicals from root bark of this plant.
- Further phytochemical investigation should be take place in order to confirm the diverse structures of compounds of phenolic glycosides.
- Further studies should be conducted on seed, leave, twigs and on stem part of *D.abyssinica* to isolate, purify and identify bioactive principles/phytochemicals responsible for antibacterial and antifungal activities of the plant.
- Alternative method of extraction and isolation should be done well as insecticide and termiticidal activities of this plant are recommended for further researchers.

- Further work should be done to isolate, characterize and elucidate the chemical structures of the bioactive compounds from this medicinal plant. Considering the importance of this plant, it should be cultivated for more extensive scientific investigations that could reveal new potent antimicrobial agents.

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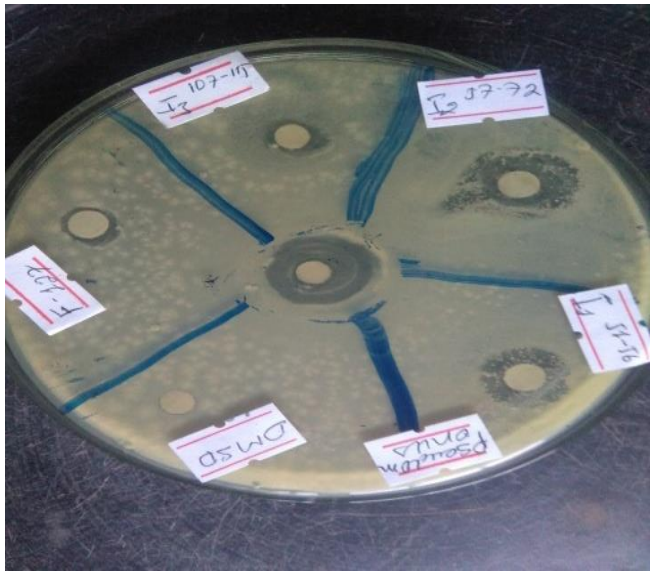
Appendices

Appendix-1 Biological activity tests of crude & isolated compounds, zone of growth inhibition (mm) for selected anti-bacterial strain.

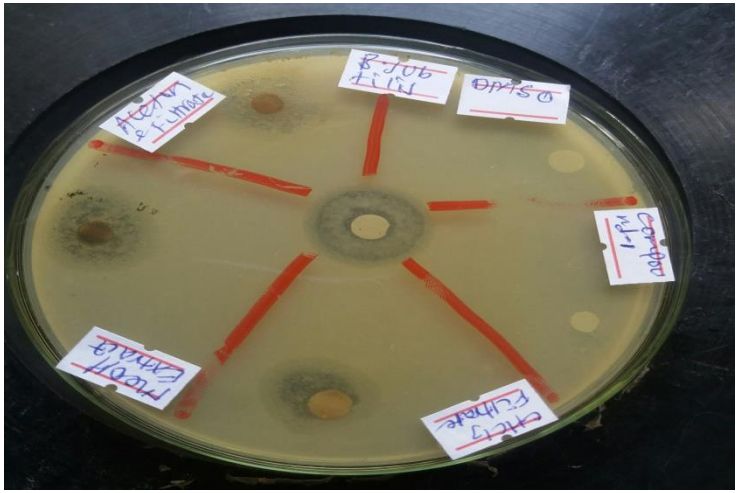


Pseudomonas aeruginosa

Appendix-2 Biological activity tests of crude and isolated compounds, zone of growth inhibition (mm) for selected anti-fungal strain.

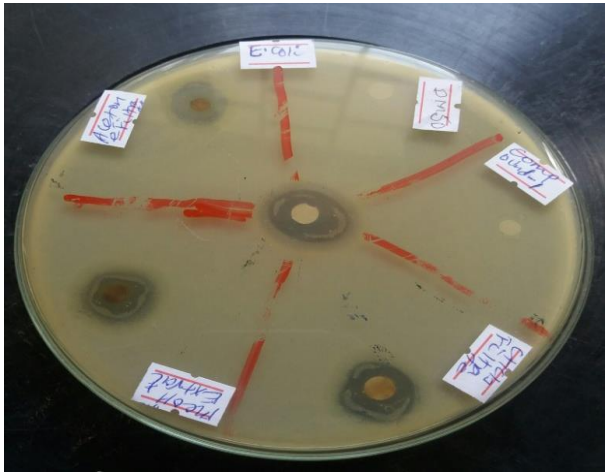
Candida albicans

Appendix3 Biological activity tests of fractionated extract and pure compound, zone of growth inhibition (mm) for selected anti- bacterial strains.

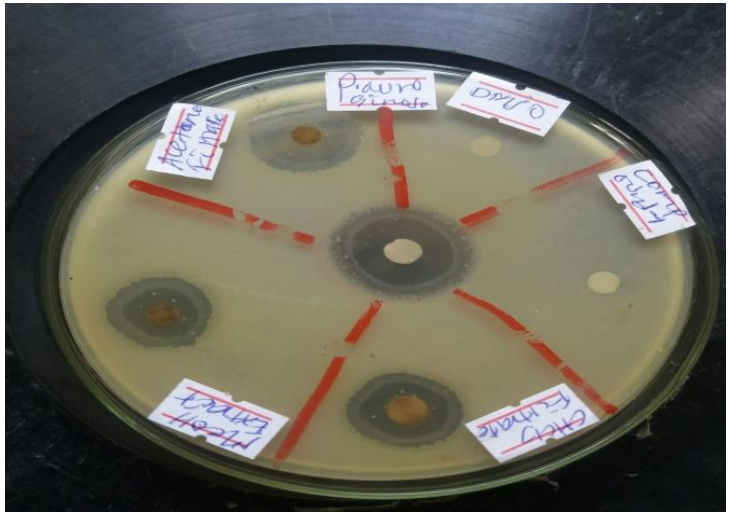


Bacillus Subtilis

Staphylococcus aureus

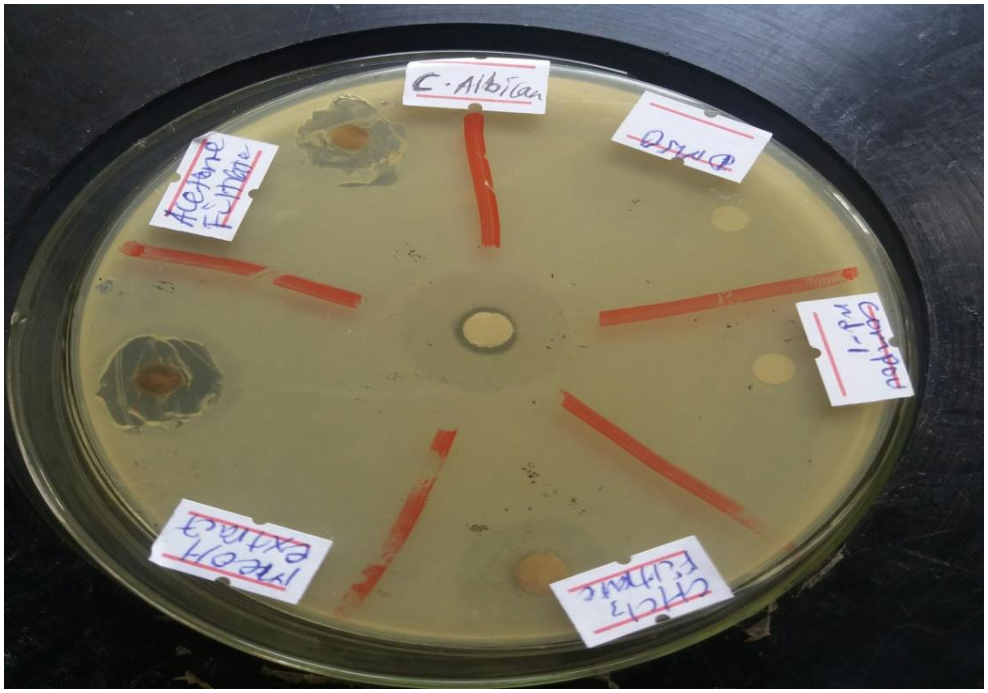


Escherichia coli



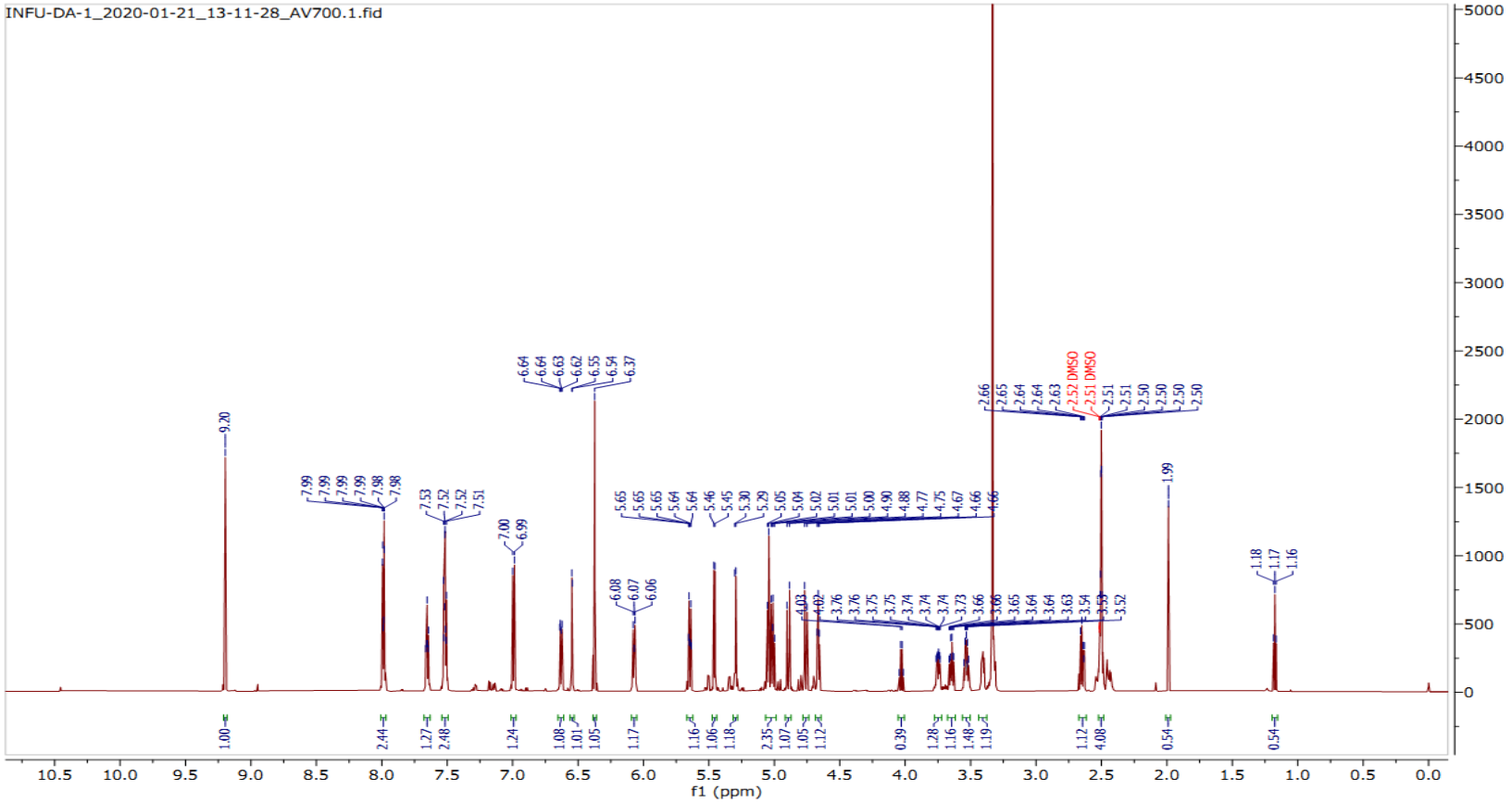
Pseudomonas aeruginosa

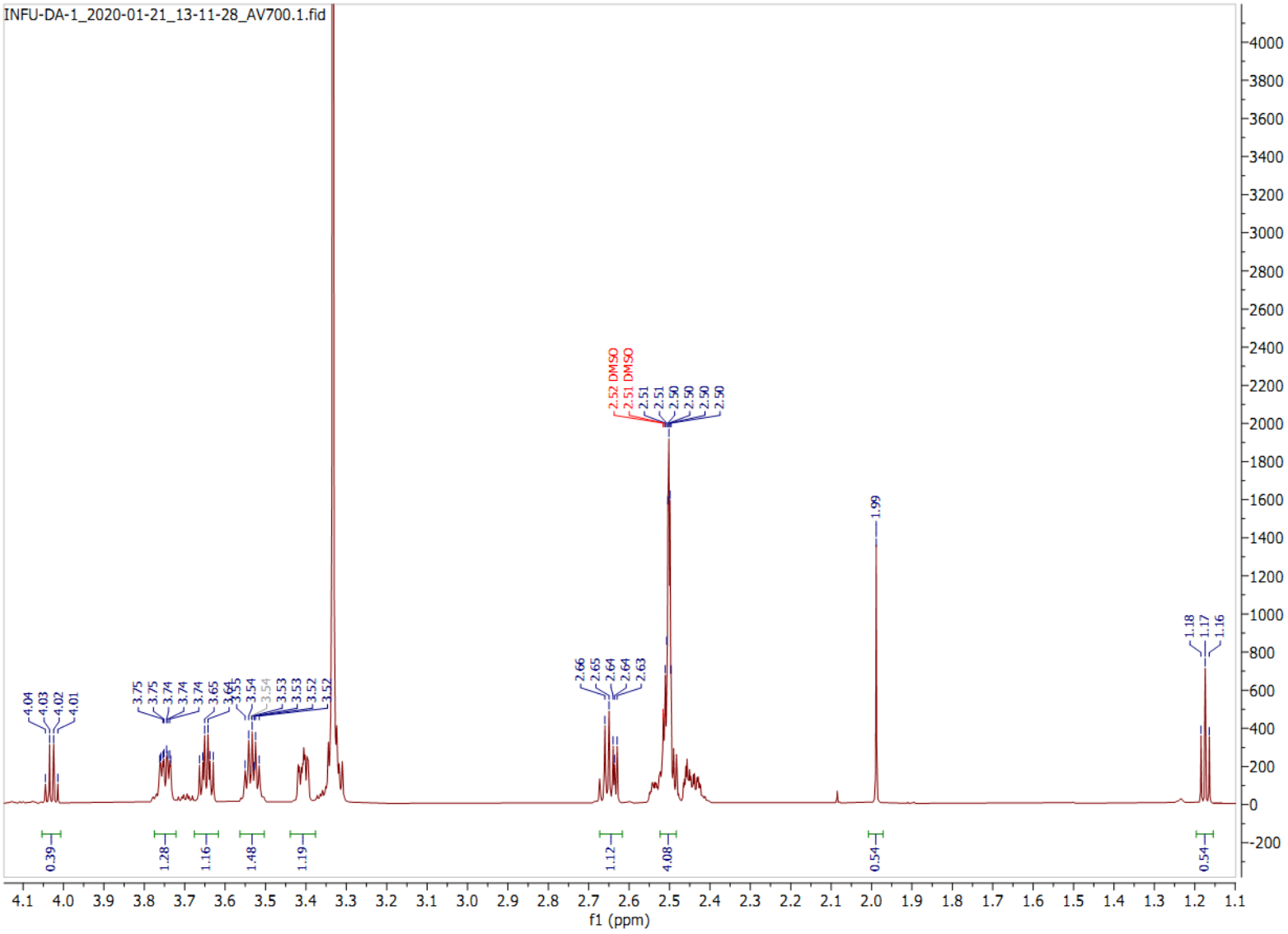
Appendix-4 Biological Activity Tests of fractionated extracts and pure compound, zone of growth inhibition (mm) for selected antifungal strain.

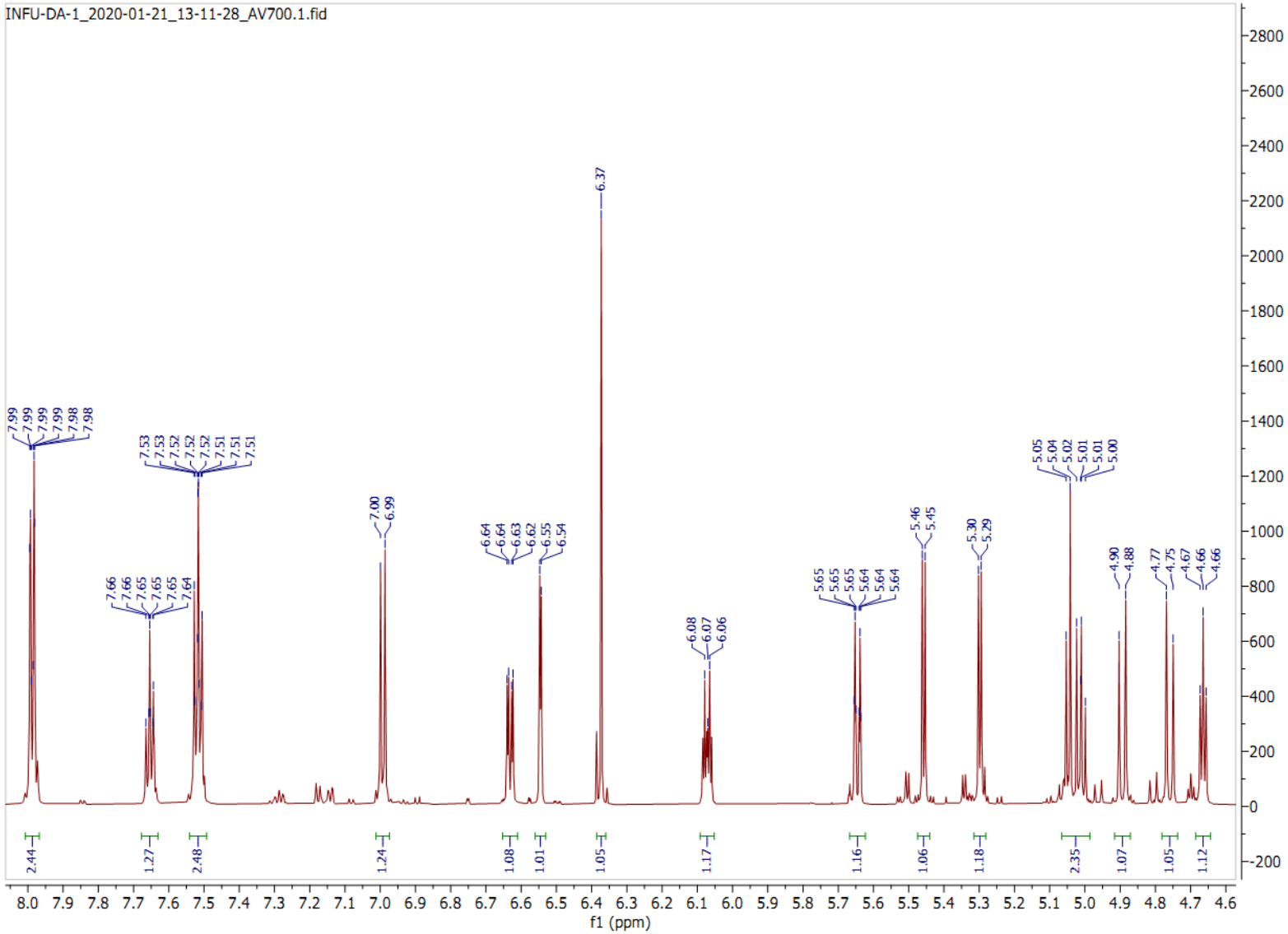


Candida albicans

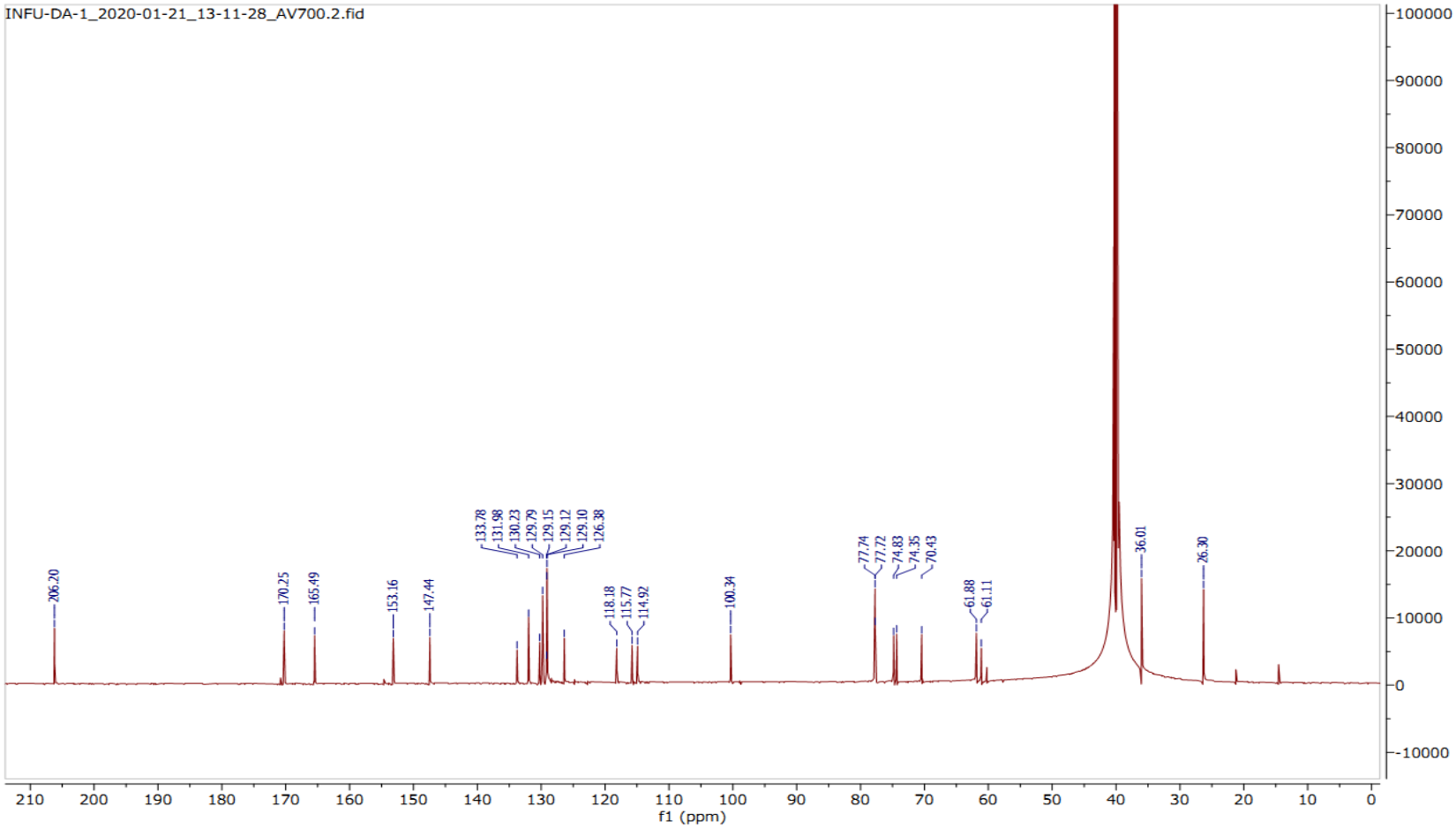
Appendix-5 ^{1}H-NMR Spectrum of Compound-**41** in DMSO-d_6



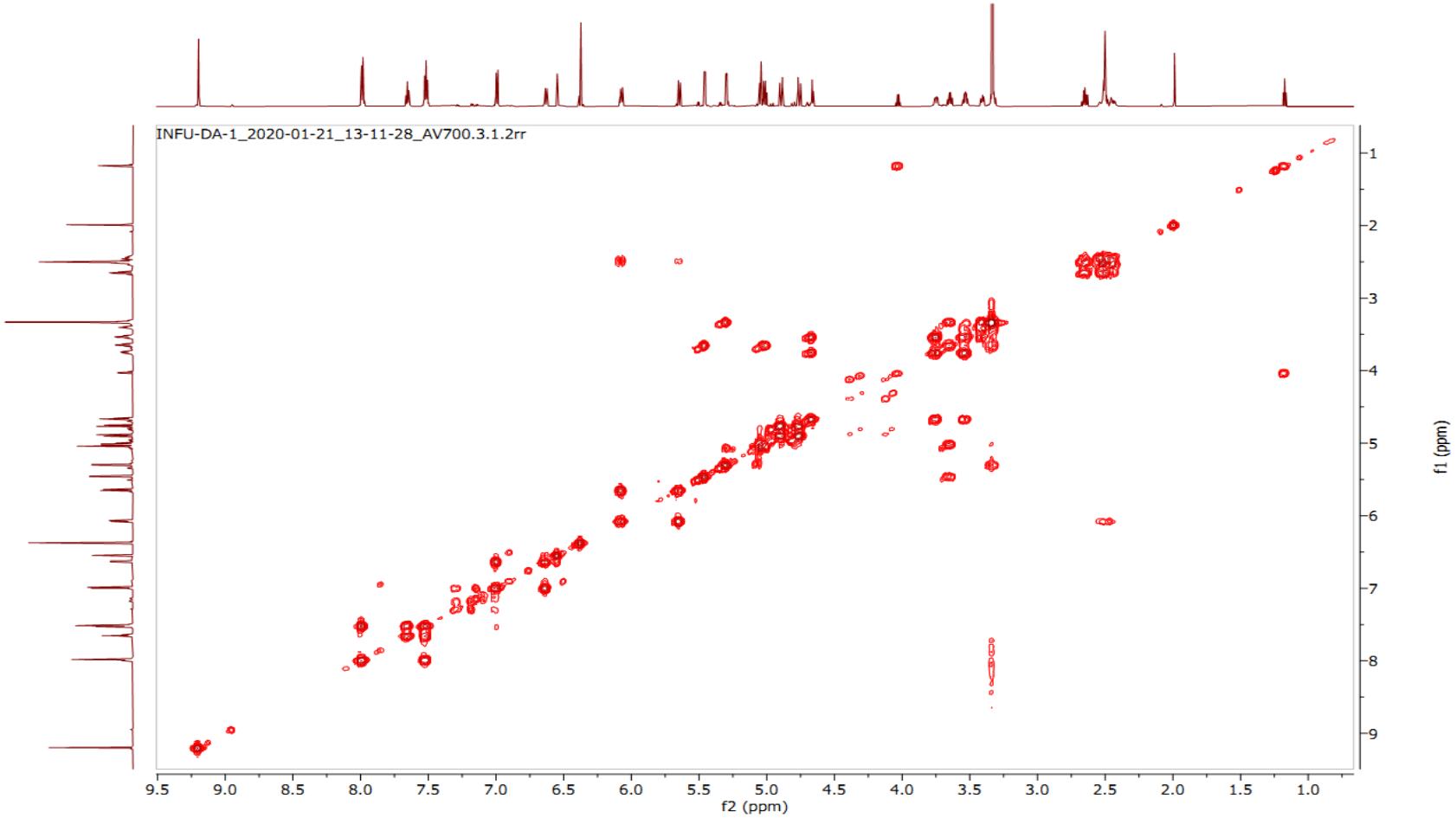

INFU-DA-1_2020-01-21_13-11-28_AV700.1.fid
4.04
4.03
4.02
4.01
3.75
3.75
3.74
3.74
3.74
3.65
3.64
3.55
3.54
3.54
3.53
3.53
3.52
3.52
2.66
2.65
2.64
2.64
2.63
2.52 DMSO
2.51 DMSO
2.51
2.51
2.50
2.50
2.50
2.50
1.99
1.18
1.17
1.16
0.39
1.28
1.16
1.48
1.19
1.12
4.08
0.54
0.54
f1 (ppm)


INFU-DA-1_2020-01-21_13-11-28_AV700.1.fid
f1 (ppm)

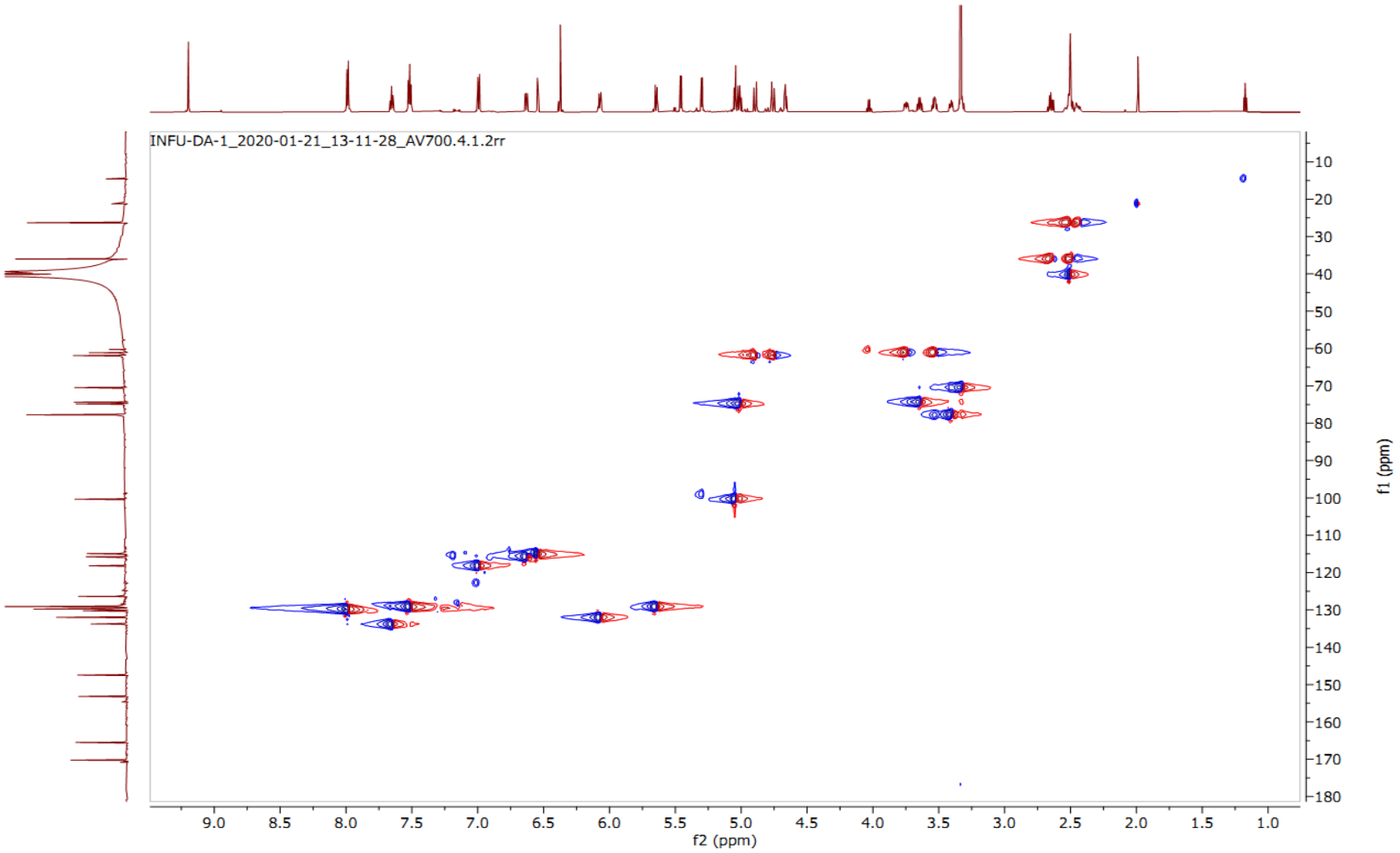
Appendix-6 ^{13}C-Spectrum of Compound-**41** in DMSO-d_6



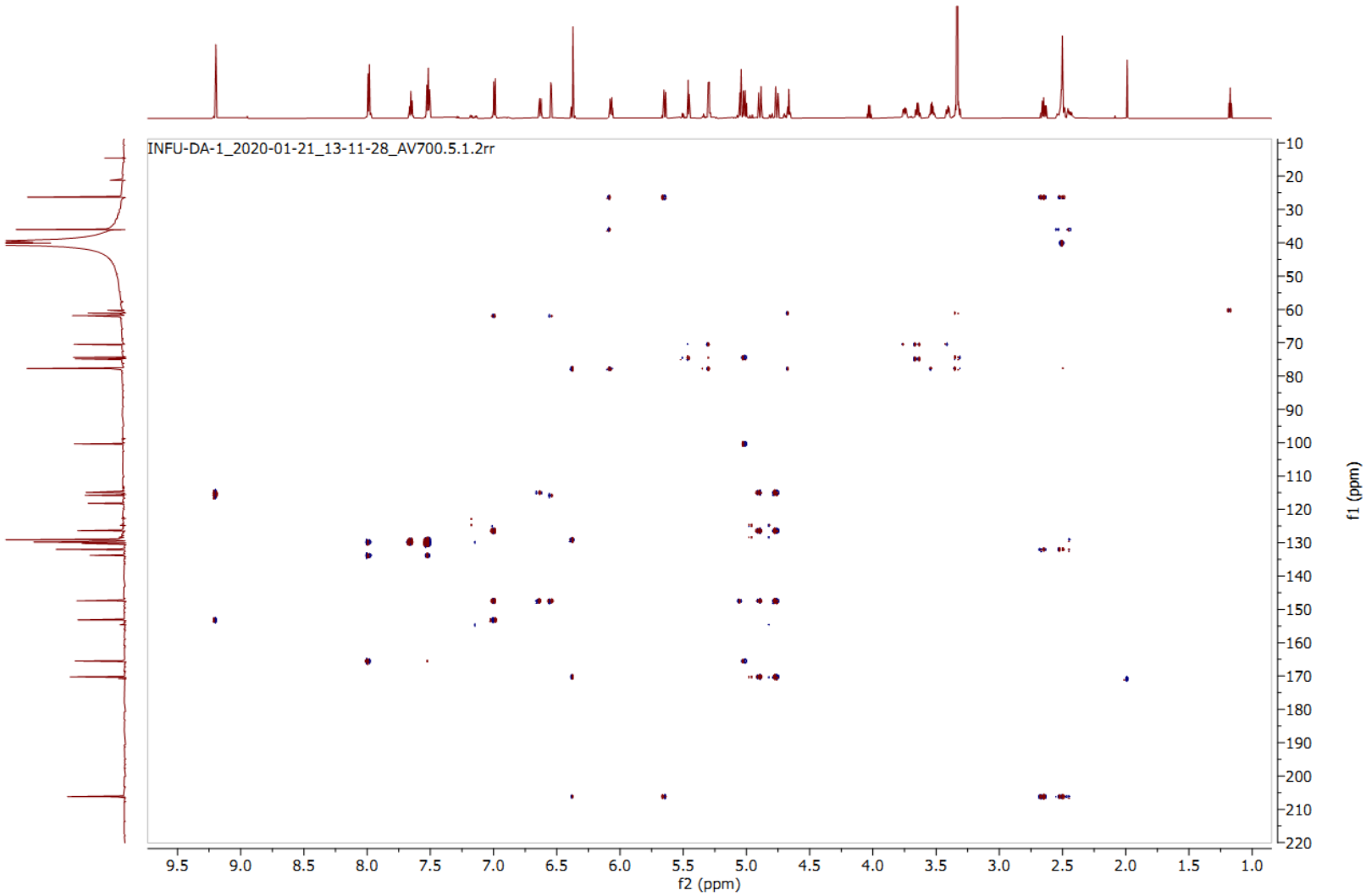
Appendix-7 COSY Spectrum of Compound-**41** in DMSO-d_6

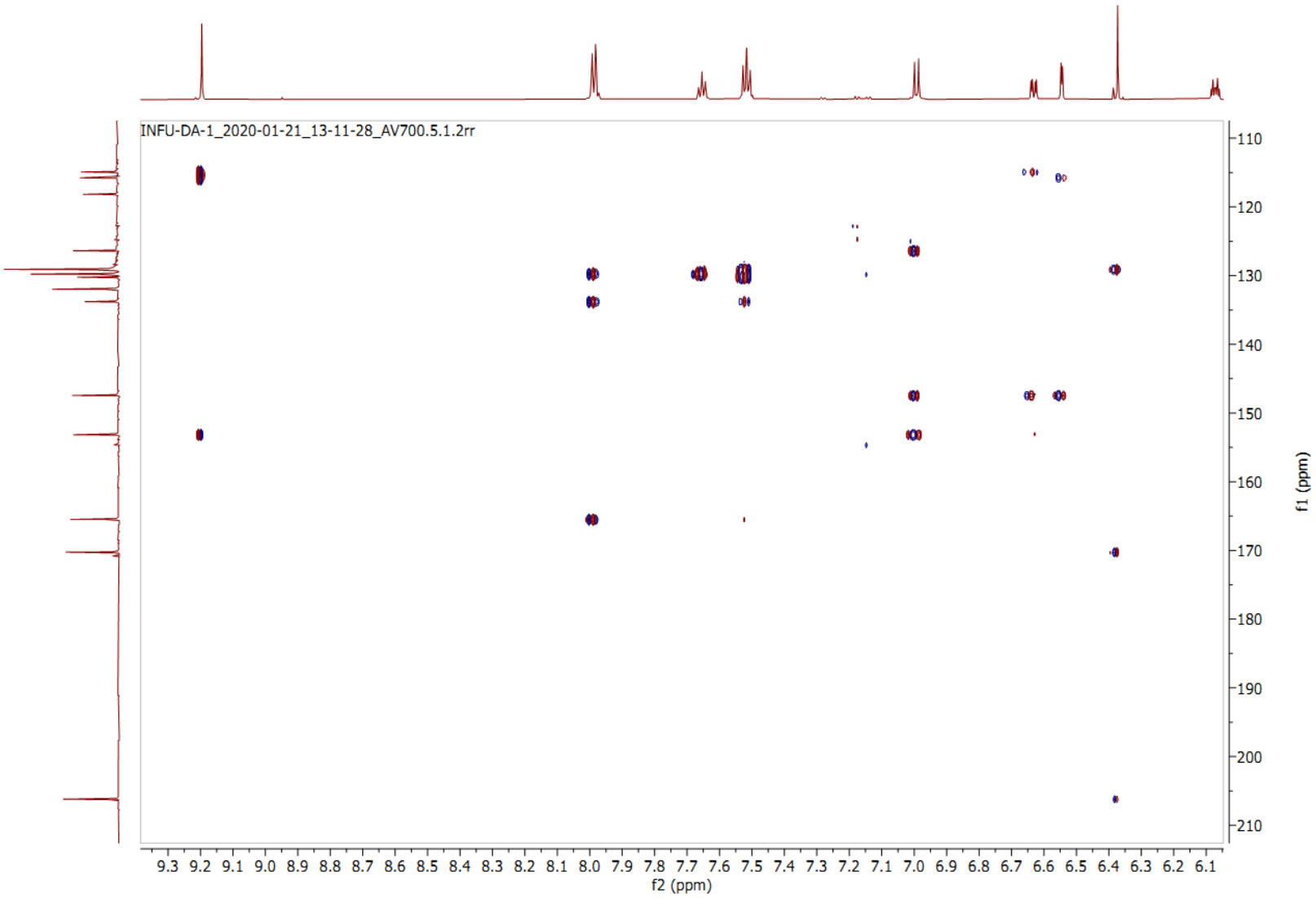


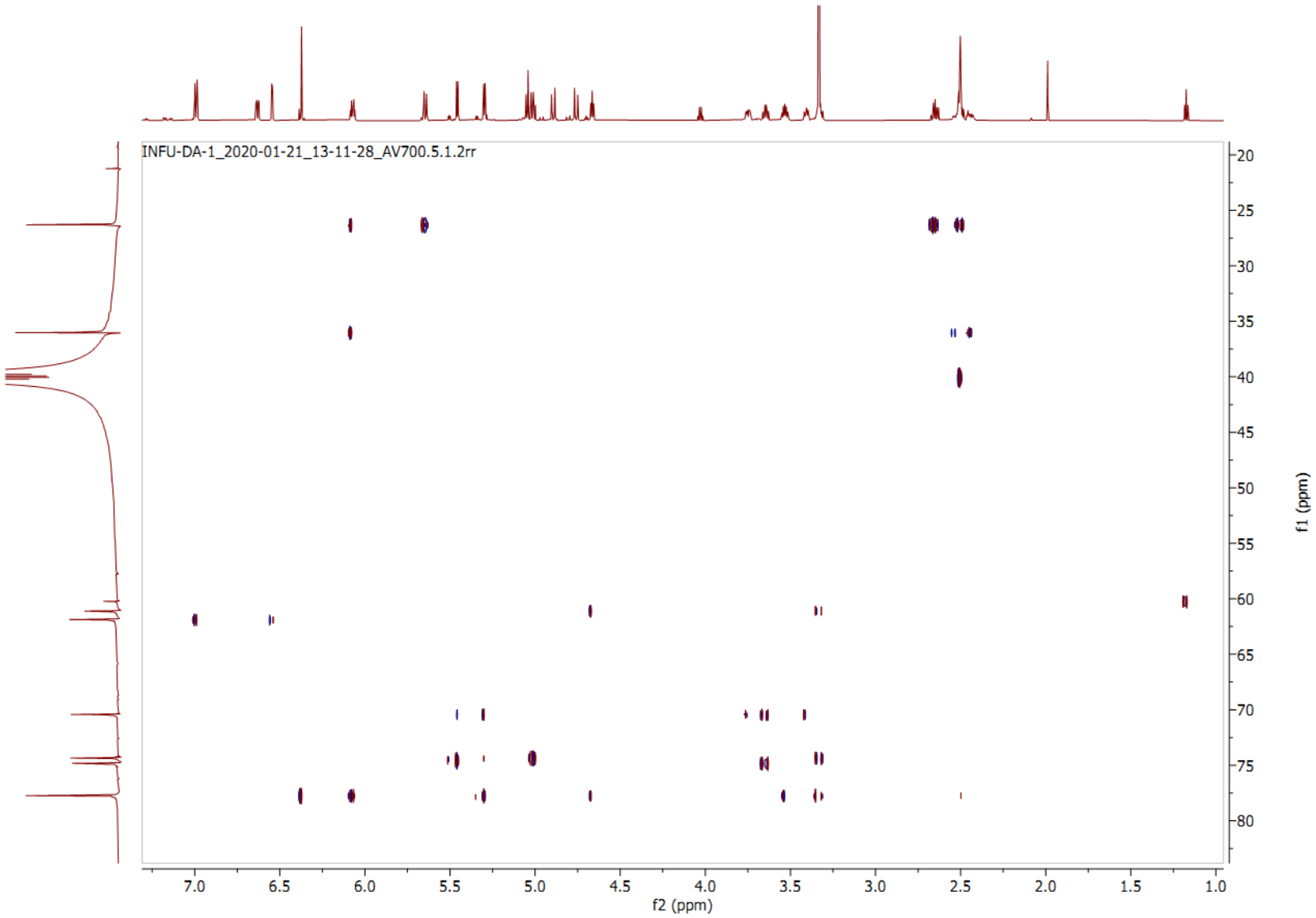
Appendix-8 HSQC Spectrum of Complound-**41** in DMSO-d_6

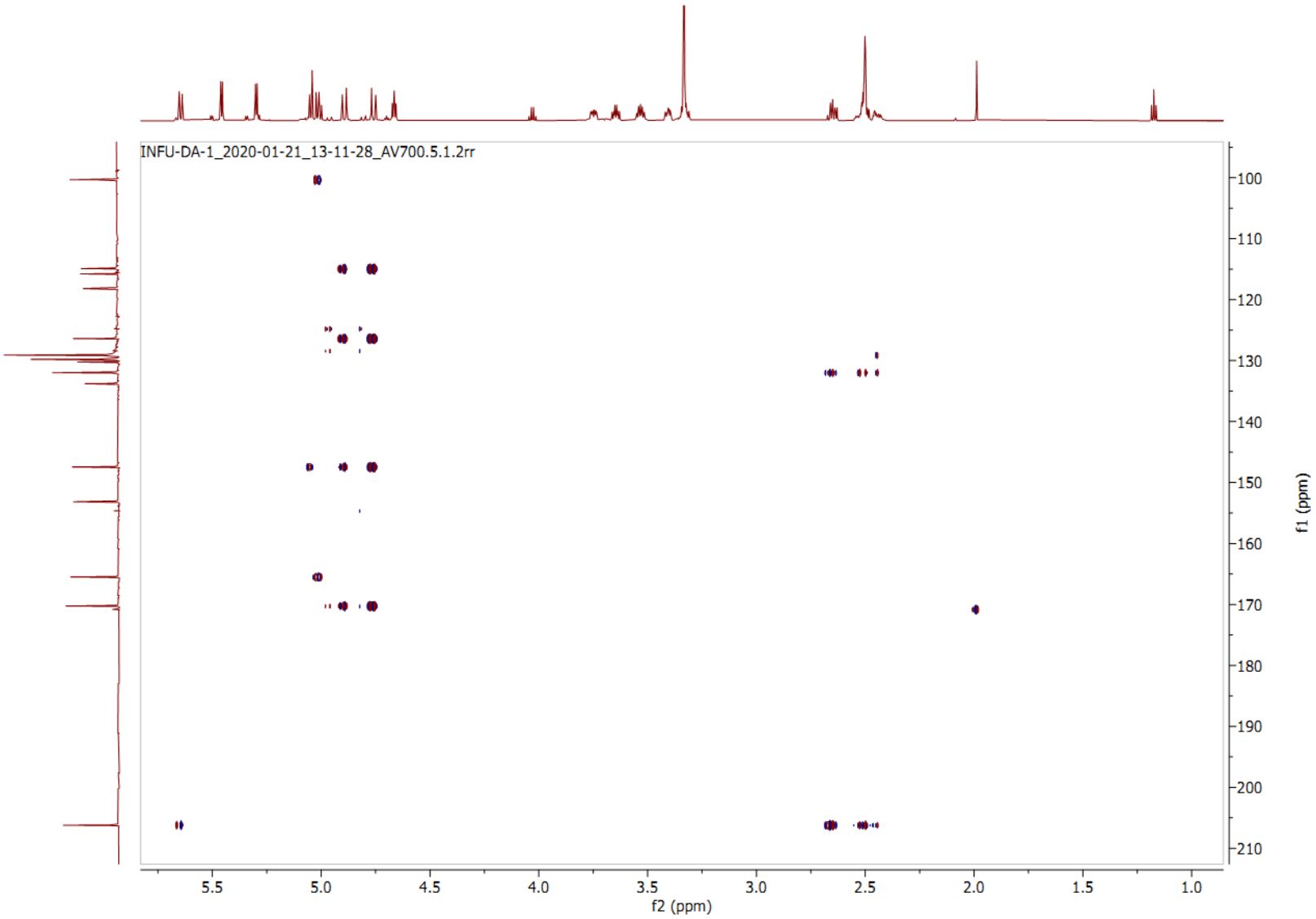


Appendix-9 HMBC Spectrum of Compound-**41** in DMSO-d_6

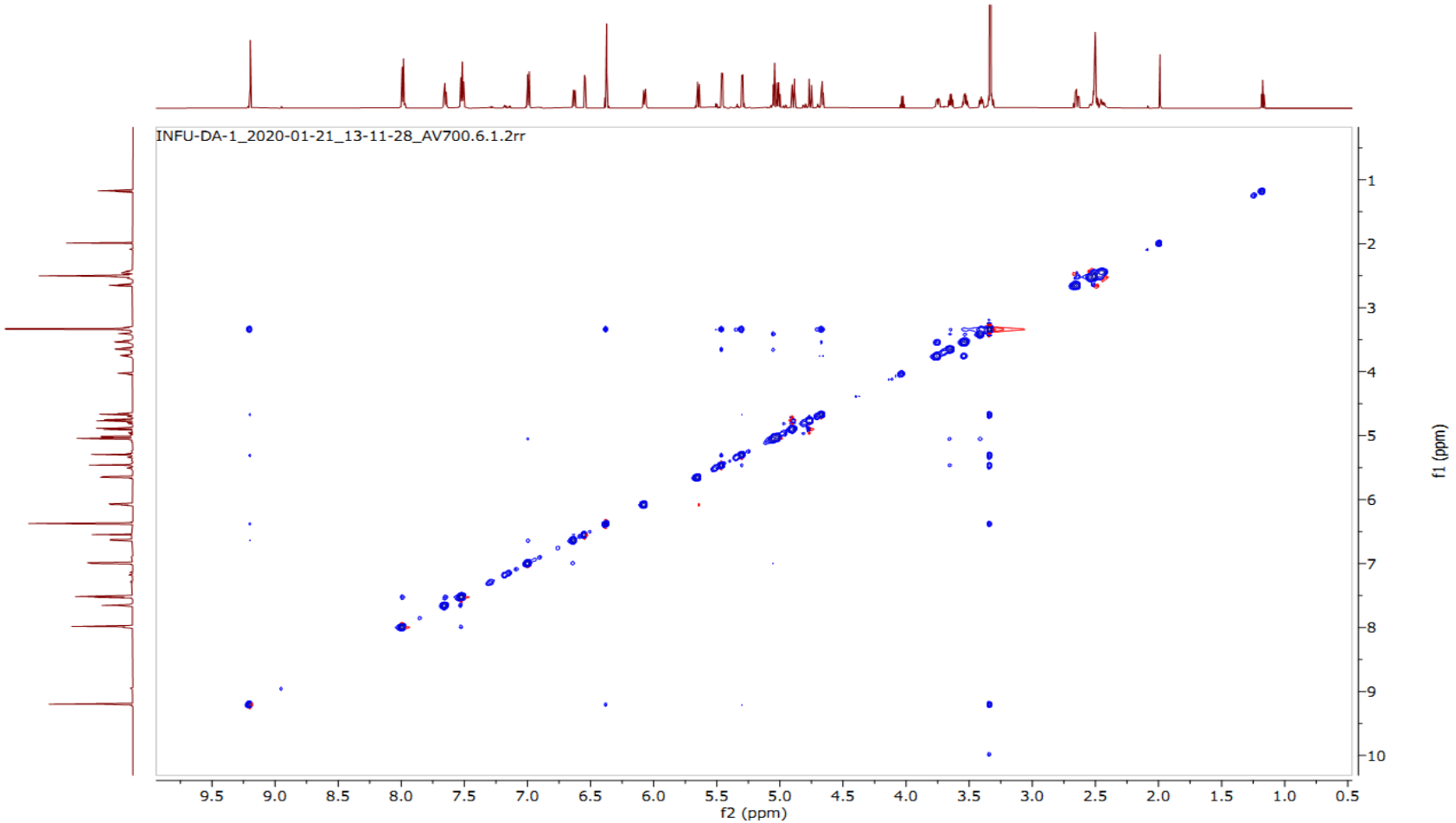



INFU-DA-1_2020-01-21_13-11-28_AV700.5.1.2rr
9.3 9.2 9.1 9.0 8.9 8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1
f2 (ppm)
110 120 130 140 150 160 170 180 190 200 210
f1 (ppm)


INFU-DA-1_2020-01-21_13-11-28_AV700.5.1.2rr
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)
20
25
30
35
40
45
50
55
60
65
70
75
80
f1 (ppm)


INFU-DA-1_2020-01-21_13-11-28_AV700.5.1.2rr
100
110
120
130
140
150
160
170
180
190
200
210
f1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)

Appendix-10 NOESY Spectrum of Compound-**41** in DMSO-d_6



Appendix-11 Mass Spectra of Compound-**41**