PHYTOCHEMICAL ANALYSIS, BIOLOGICAL ACTIVITY AND MOLECULAR DOCKING STUDIES OF ROOT EXTRACTS OF *RHYNCHOSIA FERRUGINEA* A.RICH.

BY

KALID HUSSIEN

A Thesis Submitted to the Department of Applied Chemistry School of Applied Natural Science Presented in Partial Fulfillment of the Requirements for Master of Science Degree in Applied Chemistry (Medicinal Chemistry)

Office of Graduate Studies

Adama Science and Technology University

June, 2021

Adama, Ethiopia

Phytochemical analysis, Biological activity and molecular docking studies of the root extracts of *Rhynchosia ferruginea* A.Rich.

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DECLARATION

I hereby declare that this Master Thesis entitled "**Phytochemical Analysis, Biological Activity and Molecular Docking Studies of Root Extracts of *Rhynchosia ferruginea***" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

_____	_____	_____
Name of the student	Signature	Date

RECOMMENDATION

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Phytochemical Analysis, Biological Activity and Molecular Docking Studies of Root Extracts of *Rhynchosia ferruginea***" prepared under our guidance by kalid Hussien submitted in partial fulfillment of the requirements for the degree of Masters of Science in medicinal chemistry. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Name of major advisor	Signature	Date

_____	_____	_____
Name of co-advisor	Signature	Date

APPROVAL OF BOARD OF EXAMINERS

We, the advisors of the thesis entitled "**Phytochemical Analysis, Biological Activity and Molecular Docking Studies of Root Extracts of *Rhynchosia ferruginea A.Rich***" and developed by Kalid Hussien Shafi, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Kaild hussien shafi have read and evaluated the thesis entitled **Phytochemical Analysis, Biological Activity and Molecular Docking Studies of Root Extracts of *Rhynchosia ferruginea A.Rich***" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Medicinal Chemistry.

Chairperson	Signature	Date
Internal Examiner	Signature	Date
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

Department Head	Signature	Date
School Dean	Signature	Date
Office of Postgraduate Studies,	Dean Signature	Date

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LIST OF ACRONYMS AND ABBREVIATIONS

1D……..…One dimension

2D ……Two dimensions

$CDCl_3$… …….deuterated Chloroform

CD_3OD……..... deuterated methanol

DEPT……Distortionless Enhancement by Polarization Transfer

DMSO……..Dimethyl Sulfoxide

HPLC……High-performance Liquid Chromatography

IC_{50} …… Half maximal inhibitory concentration

NMR …….Nuclear magnetic resonance

TLC ….....Thin layer chromatography

ASTM.....American Society for Testing and Materials

ABSTRACT

Rhynchosia ferruginea (Udusalim, Afan Oromo) has been used by local community and traditional healers to treat various diseases like skin infection, wound, intestinal problems, and amoebiasis. Considering the absence of prior scientific report that supports ethno-medicinal uses of the roots of the plant, this study focused on isolation of chemical constituents, evaluation of the anti-bacterial and radical scavenging activities of the roots of *R. ferruginea*. The roots of *R. ferruginea* (500 g) were extracted by dichloromethane/methanol (1:1) and methanol to give 24.48 g (4.89%) and 17.55 g (3.51%) yield, respectively. Phytochemical screening of the roots extracts of *R. ferruginea* showed the presence of alkaloid, tannins, flavonoids, steroids, saponins, terpenoids, and phytosterol. The dichloromethane/methanol (1:1) extract was subjected to silica gel column chromatography to afford three compounds identified as isoflavan (**55**), isoflavene (**56**) and 1, 3-dilinoleoyl-2-Stearoylglycerol (**57**) reported here in for the first time from the genus. The structures of these compounds were characterized using spectroscopic methods (^{1}H NMR, ^{13}C NMR and DEPT-135).The extracts and isolated compounds were evaluated *in vitro* for their antibacterial activity using disc diffusion method against *E. coli*, *S. aureus. P. aeruginosa* and *S. pyogenes.* Compound **55** displayed 9.67±0.58 mm and 10.67±0.58 mm whereas compound **56** showed 10.33 ± 1.15 mm and 10 ± 1.00 mm zone of inhibition diameters against *E. coli* and *S. aureus*, respectively, compared to ciprofloxacin which showed inhibition zone of 15.67±0.58 for both *strains*. The extracts and isolated compounds were also assessed for their radical scavenging activity using DPPH assay. The dichloromethane/methanol (1:1) extract and compound **55** exhibited better radical scavenging activity with IC_{50} value of 17.7 and 32, respectively. Autodock vina software was used to perform molecular docking analysis of isolated compounds. Among the investigated ligands, compounds **55** and **56** exhibited similar binding affinity of -7.4 (kcal/mol) compared to ciprofloxacin -7.3 (kcal/mol) suggesting the compounds may act as potential inhibitors of DNA gyrase enzyme. Therefore, the *in vitro* antibacterial, radical scavenging activity and molecular docking analysis suggest the potential use of these compounds as potential drug lead candidates and gives hope for further investigation.

Keywords: Antibacterial activity, Phytochemical screening, *Rhynchosia ferruginea*, radical scavenging activity, molecular docking

1. INTRODUCTION

1.1. Background

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. During search of food, man has begun to distinguish those plants with definitive pharmacological action that suitable to relieve human suffering from disease. This relationship between plants and man leads to the discovery of many plants used to be as medicines (Kumar & Arvind, 2016). Out of 255 drugs which are considered as basic and essential by the World Health Organization (WHO), 11% are obtained from plants and a number of synthetic drugs are also obtained from natural precursors *(*Joseph *et al*., 2013).

Naturally occurring compound may be divided into two broad categories. The first class of this compounds are known as primary metabolite. These primary metabolites are found universally in all plants and play a central role in plant's normal growth, development and reproduction. Primary metabolite include nucleic acids, carbohydrates, proteins, fats and oils, and alcohols (Anulika, Ignatius, Raymond, Osasere, & Hilda, 2016). Secondary metabolites are organic compounds derived from primary metabolites that aid in the growth and development of plants but no fundamental role in the maintenance of life processes in the plants (Jain *et al*., 2019). Secondary metabolites generally, but not always, occur in relatively low quantities in specialized cells or tissue and their production may be widespread or restricted to particular families, genera or even species (Wink, 2015). The major class of secondary metabolites include tannins, glycosides, flavonoids, alkaloids, terpenoids, steroids, quinones, and saponins are among others.

The genus *Rhynchosia* consists of approximately 300 species distributed throughout the tropical and subtropical areas around the world (Rungsung, Ratha, Dutta, Dixit, & Hazra, 2015). The most evident substances in many *Rhynchosia* species are C-glycosides, O-glycosides, prenylated flavonoids and aglycones. Some of the isolated compounds of *Rhynchosia* genus and their plant extracts exhibit interesting biological activities, including antioxidant, anti-inflammatory, anti-fungal ,anti-bacterial and anti-proliferative (Muhammad *et al*., 2019). The roots of this plant has

been widely used traditionally in Bale Zone Barbare district by the local people for the treatment of different ailment including skin infection, wound, stomach ache and amoebiasis.

Motivated by wide spectrum of biological activities of *Rhynchosia* species, we hereby report antibacterial and radical scavenging activities and phytochemical constituent of roots of *Rhynchosia ferruginea A.Rich* which commonly known with common name Udusalim (Afan Oromo) among local community.

1.2. Statement of the Problem

The rapid development of multidrug resistant strain of bacteria increased the occurrence of bacterial infections that hampered treatment using conventional anti-microbial agents. Due to this, scientists are always searching for novel infection fighting strategies (Bello *et al.*, 2018). Enhancing the knowledge of screening of traditional medicinal plants for various biological activities as well as identification of bioactive chemical constituents may help in the use of natural products for drug discovery and development research.

Traditionally, people of Ethiopia have been using medicinal plants to treat different diseases. *R. ferruginea*is one of medicinal plants that have been commonly used by traditional healers in Barbare district Bale Zone Oromia region of Ethiopia. The roots of this plant has been widely used by the local people for the treatment of different ailment including skin infection, wound, stomach ache and amoebiasis. However, to the best of our knowledge there is no prior work on isolation and characterization of chemical constituents and evaluation of the biological activities of the roots extract of this plant in Ethiopia despite the wide traditional use of the plant. Therefore, the study was aimed at isolation and identification of compounds from the root of *R. ferruginea* and evaluation of biological activities of compounds and extracts to give scientific support for the traditional use of the plant.

1.3. Objectives of the Study

1.3.1. General Objective

The overall objective of this study was to isolate the chemical constituents present in the root extracts of *R. ferruginea*, evaluate their biological activities and molecular docking interaction with DNA gyrase B (PDB ID: 6F86).

1.3.2. Specific Objectives

The specific objectives of the study were as follows:

- To extract the roots of *R. ferruginea* using dichloromethane/methanol (1:1) and methanol.
- To isolate compounds from crude extracts by using silica gel column chromatographic techniques.
- To elucidate the structure of isolated compounds using NMR spectroscopic method and comparison with literature data.
- To test antibacterial activity of the crude extracts and isolated compounds using disk diffusion method against two Gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains.
- To test radical scavenging activity of crude extracts and isolated pure compounds from root extracts of *R .ferruginea* using DPPH assay method.
- To examine molecular docking study of isolated compounds which showed promising antibacterial activity using Autodoc vina version 4.2 with DNA gyrase B (PDB ID: 6F86).

1.4. Significance of the Study

This research is expected to:

- Provide information on the chemical constituents of the roots of the plant.
- Provide antibacterial activity information of the plant extracts and isolated compounds against selected microbial strains to provide scientific support to traditional use of the plant to treat infectious diseases.
- Provide information on molecular docking analysis so as to have better understanding of the molecular mechanisms of interaction between the compounds with promising antibacterial activity and selected protein domains.
- Provide baseline information for future related scientific studies on the plant.

2. LITERATURE REVIEW

2.1. Family Fabaceae

The Fabaceae or Leguminosae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. It includes trees, shrubs and herbaceous plants perennials or annuals, which are easily recognized by their fruits (legume) and their compound, stipulated leaves. The group is widely distributed and is the third-largest land plant family in terms of number of species (Rahman & Parvin, 2014). The family Fabaceae contain around 650 genera and approximately 18,000 species, representing one of the largest families of Angiosperms and one of the leading economically. Fabaceae family is known by its high content of flavonoids and related compounds. About 28% of total flavonoids and 95% of all known isoflavonoid aglycone structures in the plant kingdom are produced by this family (Lima *et al.,* 2017).

Fabaceae family are also reported to contain prenylated flavonoids as reported from some genus of this family such as *Derris, Flemingia, Glycyrrhiza, Lonchocarpus* and *Tephrosia.* Other classes of secondary metabolites have been identified in species of Fabaceae, such as anthraquinones, alkaloid, piperidine, pyridine and pyrrolidine derivatives, coumarins, cyanogenic glycosides, terpenes (including essential oils, diterpenes, phytosterol, triterpenes and saponins), hydrolysable and condensed tannins (Wink, 2013).

2.2. Botanical Description of *Rhynchosia ferruginea*

The *rhynchosia* is one of the genus in the family of Fabaceae or Leguminosae. Its name derives from a Greek word “rhynch” meaning “beak” in reference to the shape of the keel in *R. volubilis* one of species in this genus (Jaca & Moteetee, 2018). More than 230 species of genus *Rhynchosia* distributed predominantly in Africa. They are also found in other continents, particularly in climates characterized by warm temperatures, such as tropical Asia, northern Australia, and in tropical and subtropical America. Rhynchosia is closely related to the genus Eriosema (DC.), sharing mostly pinnately trifoliate leaves, which are prominently veined

underneath, yellow flowers, and generally 2-seeded fruits. The mode of funicular attachment of the seed in relation to the hilum is the only distinguishing character between these two genera (Moteetee *et.al*., 2012).

Rhynchosia ferruginea is one of the species from the genus *rhynchosia*. R. ferruginea is a flora of Tropical Africa with stems densely covered with pale ferruginous hair. It is has a flower in lax racemes, which exceed the leaves. Calyx 2.5 -3 mm long with densely ferruginous and the teeth linear-setaceous is reaching down nearly to the base. It has a corolla equal with the calyx, look standard yellow, veined with purple and not silky with the keel obtuse and Pod not seen. Its Petioles is around 2–3 cm long (Lock, 1990). It is perennial herb 1.5-2 m long from a woody rootstock. It has leaves with paler beneath, ovate with the laterals slightly oblique with 0.5-4 cm long and 0.3-3.5 cm wide round acute at the apex and round at the base. Rhynchosia ferruginea grow on upland associated with evergreen forest patches, on bushland, on cultivated ground and sometimes in swampy area. It is a plant native to Eritrea, Ethiopia, Kenya, Rwanda and Tanzania (Hedberg & Edwards.S 1991).

Figure 1. Picture of *R. ferrugenia* (Picture taken by Kalid Hussien, 17/8 2020)

2.3. Chemical Constituents of the Genus *Rhynchosia*

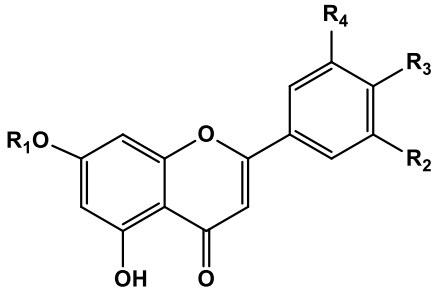
Numerous secondary metabolites like isoflavones, flavonols, flavan-3-ol, flavanonols, phenolic acid, xanthone and sterol with different biological activities have been isolated from Genus *Rhynchosia* (Rondo, 2017). Several flavones and flavone glycosides (**1-15**) were isolated from the genus *Rhynchosia.* This including apigenin (**1**) and luteolin (**2**) from the leaves of *R. heynei* (Bhakshu & Raju, 2009) and tricin (**3**), apigenin-7-*O*-β-d-glucopyranoside (**4**), luteolin-7-O-β-d-glucopyranoside (**5**) from roots of *R. volubilis* (Li & Xiang, 2011). 3′, 4′-Di-O-methylluteolin-7-*O*-glucuronide (**6**), vitexin (**7**), isovitexin (**8**) were reported from the leaves of *R. beddomei* (Adinarayana, Gunasekar, Seligmann, & Wagner, 1980) whereas vicenin-1 (**9**), vicenin-2 (**10**) and vicenin-3 (**11**) from the leaves *R.bracteatan* and *R.sublobata* (Ali, Sreeramulu, & Gunasekar, 1992). Schaftoside (**12**), orientin (**13**), isoorientin (**14**) were isolated from the leaves of *R. minima* (Jia et al., 2015) whereas lucenin-2- (**14**) reported from flowers of *R. suaveolen* (Rammohan *et al*, 2015).

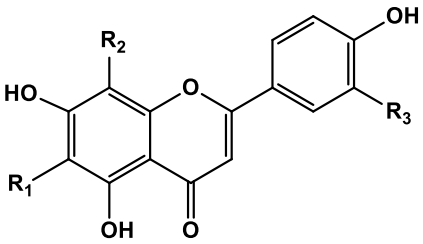
Flavonols and flavonol glycosides (**16-21**) were isolated from various *Rhynchosia* species. For example kaempferol (**16**) and quercetin (**17**) were isolated from the roots of *R. volubilis.* kaempferol-3-*O* methylether (**18**) from leaves of *R. rufescens* (Li & Xiang, 2011). Quercetin-7-*O*-methylether (**19**) and rutin (**20**) were reported from flowers of *R. beddomei* (Rammohan et al., 2019). Rhynchosin (**21**) from were isolated from the leaves of *R. beddomei*. Naringenin (**22**) and lupinifolin (**23**) were reported from the leaves of *R. beddomei* (Adinarayana *et al.* 1980) and roots of *R. precatoria* (Coronado-aceves *et al.* 2017), respectively. In a related study, 6-C-Prenyltoxifolin-7,3′-dimethylether (**24**) and lupinifolinol (**25**) were reported from the leaves of *R. densiflora* (Rao & Gunasekar, 1998) and roots of *R. precatoria* (Coronado-aceves *et al.* 2017), respectively.

Isoflavones (**26-40**) were reported from five species of genus *Rhynchosi.* From this diadzein (**26**), calycosin (**27**), biochaninA (**28**) and 4′-(1″-Methoxy)-propylgenistein (**37**) were reported from the root of *R. volubilis* (Li & Xiang, 2011). Genistein (**29**), licoisoflavone A (**30**), rhynchoviscin (**31**) and Sophoraisoflavone A (**38**) were reported from the roots of *R. viscosa* (Bohni et al., 2013). Rhynedulin A (**32**), rhynedulin B (**33**), rhynedulin C (**34**), cyclochandalone

(**35**) and ulexin B (**36**) were isolated from the bark of *R. edulis* (Guo et al., 2011). In a related study, precatorin C (**39**) and precatorin B (**40**) were reported from roots of *R. precatoria* (Coronado-aceves *et al.* 2017). Epicatechin (**41**) (Li & Xiang, 2011), 7-O-galloylcatechin (**42**) (Kinjo, Nagao, Tanaka, Nonaka, & Okabe, 2001) and proanthocyanidin (**43**) (Rangaswamy *et.al*., 1974) are the flavan-3-ols isolated from various *Rhynchosia* species. Two xanthone- C-glycosides , mangiferin (**44**) and isomangiferin (**45**) have been isolated from *R. suaveolens* (Rammohan *et al.*, 2015).

Gallic acid (**46**), glucogallin (**47**), 1,6-Di-*O*-galloylglucose (**48**), trigalloylgallic acid (**49**) are simple polyphenolic compounds isolated from seeds of *R. volubilis* (Kinjo et al., 2001). A unique trioxygenated simple benzophenone, 2-Hydroxy-3,4-dimethoxybenzophenone (**50**) was reported from the flowers of *R. suaveolens* (Rammohan et al., 2015). In a related study, β-Sitosterol (**51**), stigmasterylgalactoside (**52**), lupeol (**53**) and 3-O-β-D-galactopyranosyl-stigmasta-5, 22-diene (**54**) are steroids isolated from the whole plant of *R. minima* (Ahmed *et al*. 1992).





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4 $R_1=b\text{-}Glc,R_2=R_4=H,R_3=OH$

5 $R_1=b\text{-}Glc,R_2=R_3=OH,R_4=H$

6 $R_1=b\text{-}Glur,R_2=R_3=OMe,R_4=H$

7 $R_1=R_3=H,R_2=Glc$

8 $R_1=Glc,R_2=R_3=H$

9 $R_1=Xyl,R_2=Glc,R_3=H$

10 $R_1=R_2=Glc,R_3=H$

11 $R_1=Glc,R_2=Xyl,R_3=H$

12 $R_1=Glc,R_2=Ara,R_3=H$

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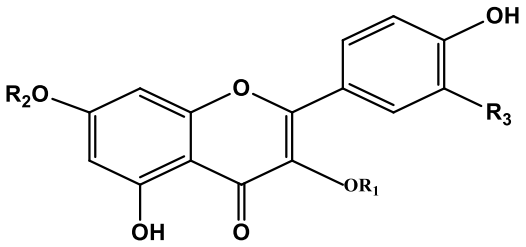

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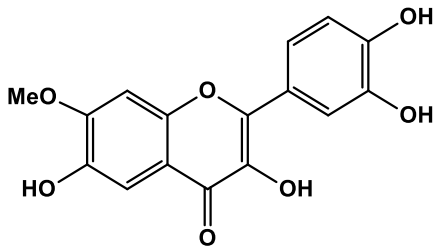

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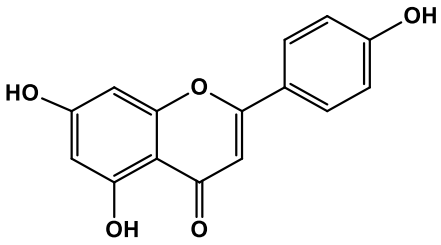

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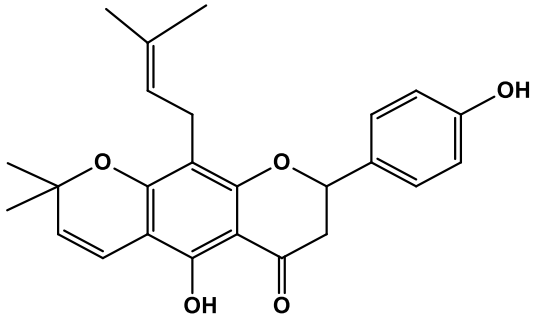

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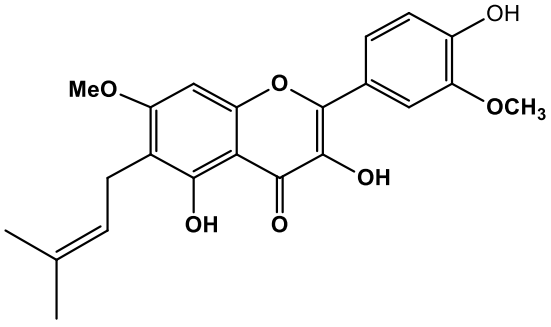

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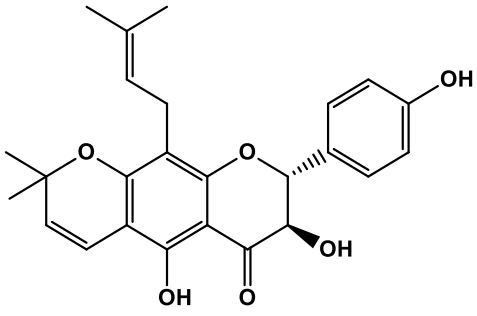

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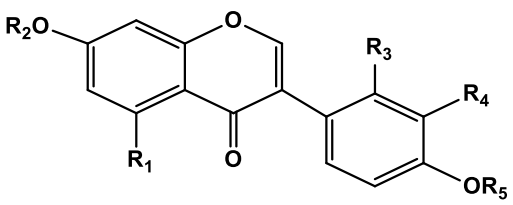

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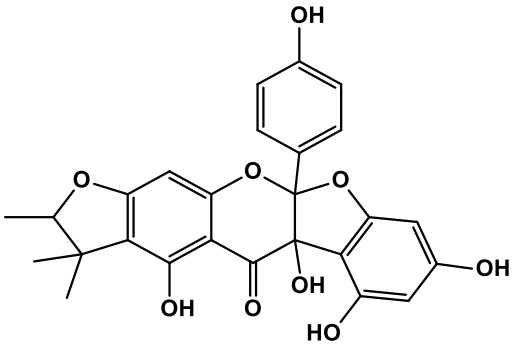

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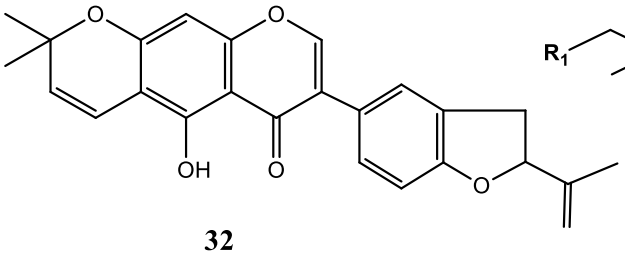

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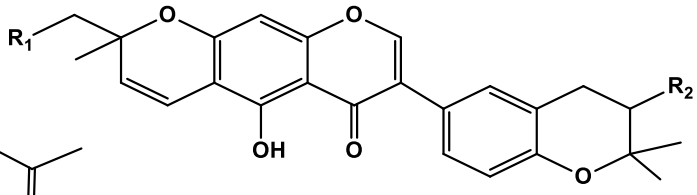

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34 $R_1=H$, $R_2=OH$

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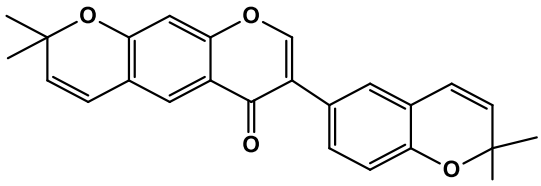

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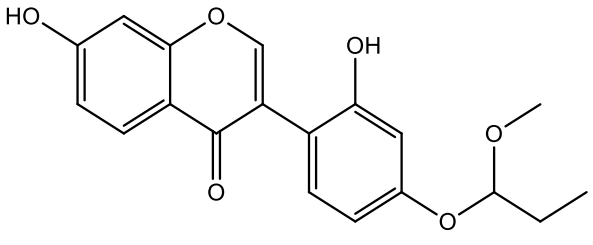

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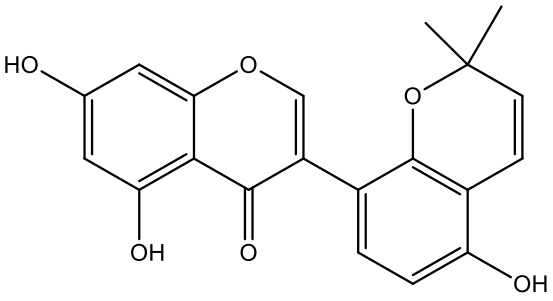

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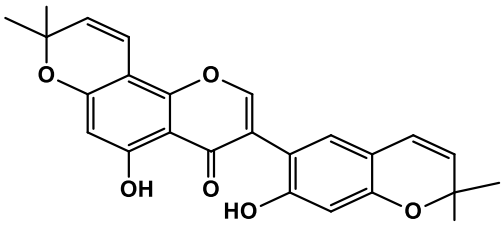

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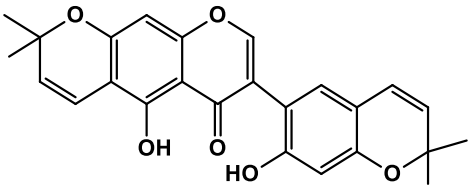

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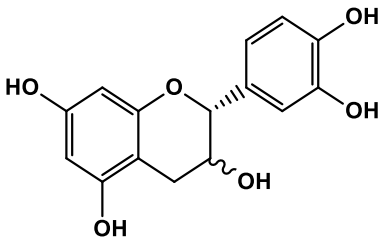

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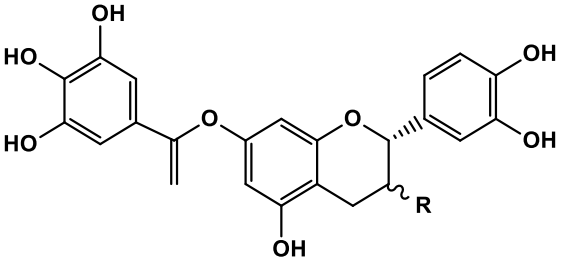

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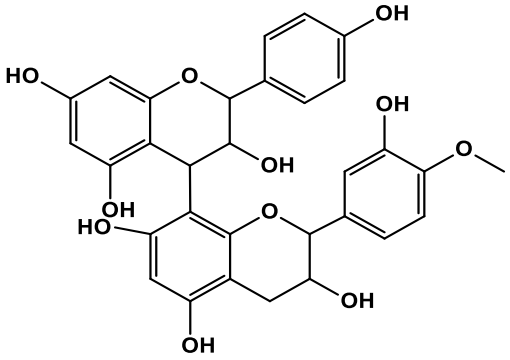

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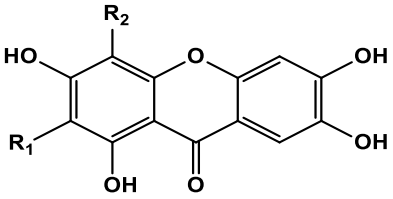
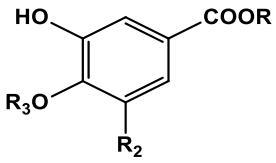


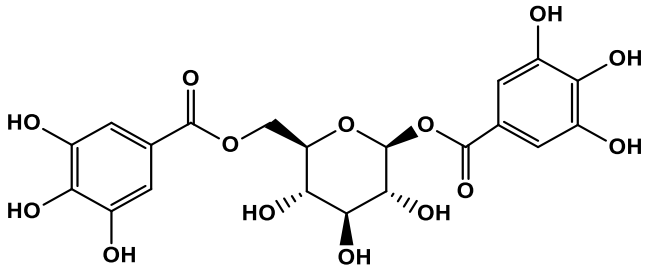
44 R_1=Glc, R_2=H

45 R_1=H, R_2=Glc



46 $R_1=R_3=H$, $R_2=OH$

47 $R_1=Glc$, $R_2=OH$, $R_3=H$



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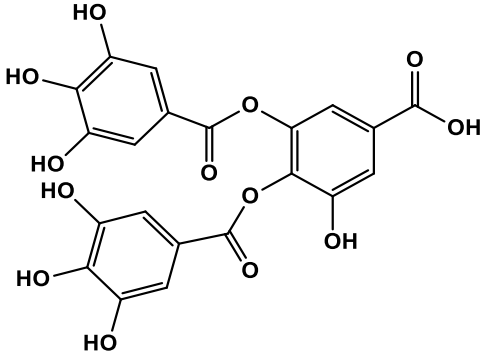

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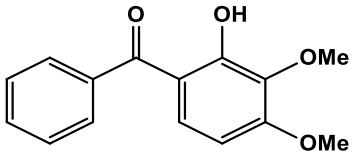

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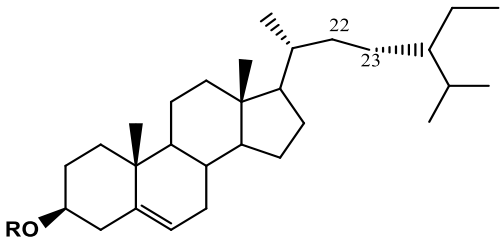
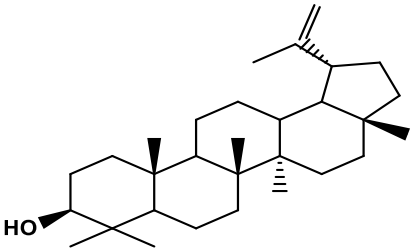


51 R=H

52 R=Glc,$\Delta^{22,23}$



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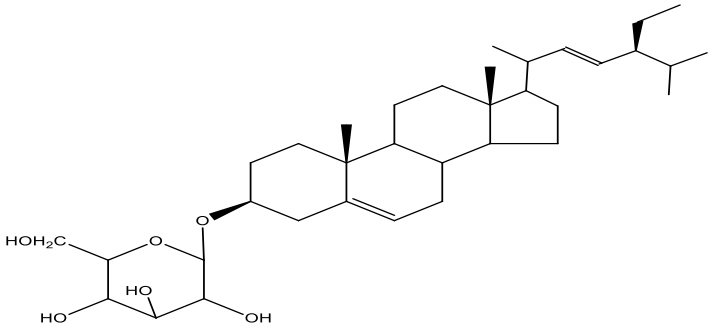

54

Figure 2.The chemical structures of isolated compounds (**1-54**) from genus *Rhynchosia*

2.4. Biological Activity of Genus *Rhynchosia*

2.4.1. Antibacterial Activity of Compound Isolated From the Genus *Rhynchosia*

An intensive literature search found that antibacterial activities were reported from studies of species of the genus, *Rhynchosia*. Four flavonoids namely, isovitexin (**8**), isoorientin (**14**), quercetin-7-*O*-methylether (**19**) and biochanin A (**28**) that were isolated from the flowers of *Rhynchosia beddomei* were tested for their antimicrobial activity against gram-positive and gram negative bacteria and fungi using disc diffusion method. Isoorientin (**14**) and quercetin-7-*O*-methylether (**19**) showed potent inhibitory concentrations against *Pseudomonas aeruginosa* and *Candida albicans* (Rammohan et al., 2019).

Flavonoids that were isolated from the roots of *Rhynchosia precatoria* like lupinifolin (**23**), lupinifolinol (**25**), precatorin C (**39**) and precatorin B (**40**) were screened for anti-mycobacterial activity. Among them the compound lupinifolin (**23**) exhibited highest activity against *Mycobacterium tuberculosis* (Coronado-aceves *et al.* 2017).

The ethyl acetate extracts of *R. scarabaeoides* demonstrated a broad spectrum of antibacterial activity ranging from gram positive to gram negative bacteria. Interestingly, ethyl acetate extracts of leaves were found to demonstrate significant activity against *Proteus vulgaris*, *Staphylococcus aureus*, *Klebseilla pneumonia* and *Escherichia coli* (Surekha et al., 2011). Petroleum ether and ethyl acetate extracts of *R. beddomei* leaves were found to be active against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus),* two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) (Bakshu & Raju, 2001). The essential oil of *R. minima* exhibited antibacterial activity against *Bacillus subtilis*, *Citro bacterfreundii*, *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhii, Staphylococcus aureus and Yersinia enterocolitica* (Gundidza et al., 2009).

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2.4.2. Antioxidant Activity of Genus *Rhynchosia*

The essential oil from *R. minima* showed anti-oxidant activity, with a mean zone of color retention of 6.8 mm compared to 10 mm for the positives control ascorbic acid suggesting that monoterpenes found in this essential oil may act as radical scavenging agents (Gundidza et al., 2009). The flavonoids isolated from *R. suaveolens* like isoorientin (**15**), mangiferin (**44**) and 2-hydroxy-3,4-dimethoxybenzophenone (**50**) were evaluated for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and showed significant radical scavenging activity comparable with that of ascorbic acid (Rammohan et al., 2015).

Rhynchosia nummularia has been used in the traditional medicine for the treatment of pain, inflammation and diabetes. *In-vitro* antioxidant activity was determined by DPPH to active compounds present in ethanol extract of aerial parts of *Rhynchosia nummularia* and showed potent antioxidant activity (Sekar *et al*., 2019). *In vitro* anti-oxidant activity of ethanol extract of the leaves of *Rhynchosia beddomei* was evaluated by total phenol content, flavonoid content, DPPH radical scavenging activity, nitric oxide scavenging activity, and hydrogen peroxide scavenging activity by using gallic acid and ascorbic acid as standard. The evaluation has confirmed the effectiveness in scavenging free radicals and potent antioxidant activity of *Rhynchosia beddomei* (Jaya and Deval, 2013).

2.4.3. Anti- Inflammatory and Anti-Angiogenic Activities

Isolated compounds from *Rhynchosia viscosa* genistein (**29**), 3′-*O*-methylorobol (**28**), licoisoflavone A (**30**) and rhynchoviscin (**31**) were tested for anti-inflammatory activity using lipopolysaccharide (LPS)-enhanced leukocyte migration assay. Genistein (**29**) showed significant inhibition of leukocyte migration with IC_{50} 12.5. The other two compounds **28** and **30**, did not show any significant inhibition. Furthermore, the angiogenic activity of extracts and compounds was also carried out using Zebrafish-based vascular outgrowth assay. The compounds Genistein (**29**) and licoisoflavone A (**30**) showed good potency of anti-angiogenic activity with IC_{50} 24.2 and 16.7 μM, respectively (Bohni et al., 2013).

2.4.4. Anti-Proliferative Activity

The anti-proliferative properties of seeds of *Rhynchosia volubilis* were tested against human gastric adenocarcinoma cells (MK-1), human uterus carcinoma (HeLa) and murine melanoma (B16F10) using MTT assay (Kinjo et al. 2001). The MeOH extract showed potent growth inhibition of MK-1 (GI_{50}: 25 µg/ml), HeLa (GI_{50}: 30 µg/ml) and B16F10 cells (GI_{50}: 8 µg/ml). Furthermore, isolated polyphenol compounds of MeOH extract 7-O-galloylcatechin (**42**), gallic acid (**46**), glucogallin (**47**), 1, 6-di-O-galloylglucose (**48**) and trigalloylgallic acid (**49**) showed good inhibition against B16F10 than HeLa and MK-1 cells. Among all the isolates, trigalloylgallic acid (**49**) showed the highest activity with B16F10, HeLa and MK-1 cells (GI_{50}: 2.9, 9.3 and 10 µg/ml respectively) followed by the gallic acid (**46**), (GI_{50}: 7.1, 22 and 19 respectively µg/ml) (Kinjo et al., 2001).

3. MATERIALS AND METHODS

3.1. Plant Material Collection and Identification

The plant materials were collected from Bale Zone, Berbere district which located at about 526 km to south east Addis Ababa. Berbere district is situated between 06º33’ N and 06º75’N and 039º40’E and 040º29’E. The plant material was identified with the help of Botanist (Melaku Wondaferash) and voucher specimen (number KH-001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

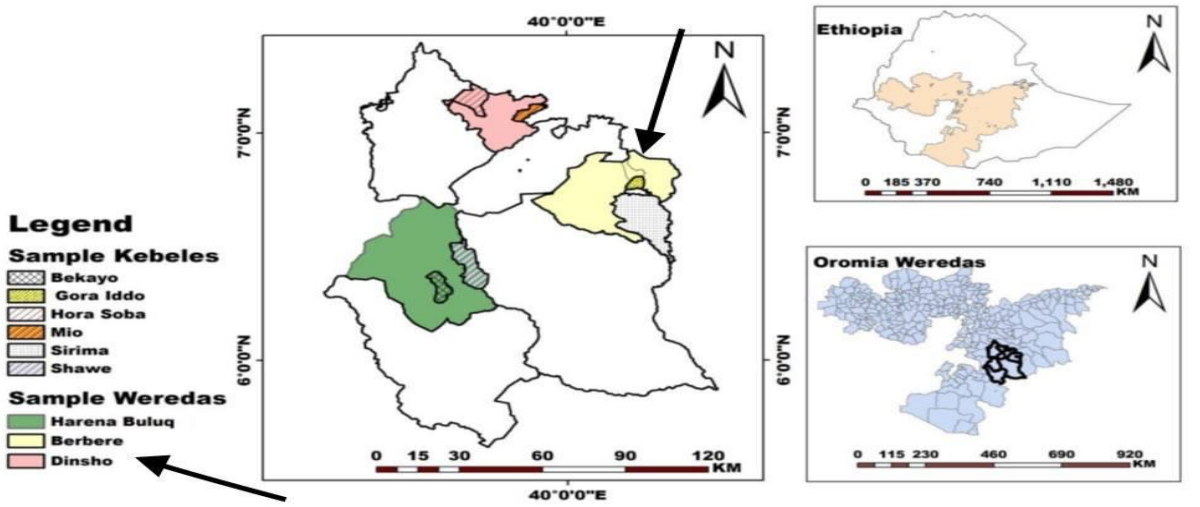


Figure 3. Map showing location of sample collection site (Kifle, 2016).

3.2. Chemicals and Reagents

The chemicals and reagents used in this investigation were silica gel mesh size 60-120, sulfuric acid, hydrochloric acid, ferric chloride, mayer’s reagent, Wagner’s reagent, glacial acetic acid and ammonia solution. Solvents used in this study were of methanol (99.8%) (HPLC grade), dichloromethane (99.6%) (HPLC grade), hexane 65-70deg C (85%), and ethyl acetate (99.5%) that are products of LOBA CHEMIE PVT. LTD (India). All the chemicals and reagents used in this study were analytical grade and are of pure Aldrich grade.

3.3. Instruments Used

Column chromatography was carried out using silica gel 60-120 mesh at ASTM. TLC was performed using Precoated aluminum-backed supported silica gel 60 F254 (0.3 mm thickness). Melting points were measured in glass capillaries using Thiele tube. TLC visualization was done using UV cabinet at λ 254 and 365 nm. The ultraviolet and visible (UV-Vis) spectrum was run using Spectroscopic Genesys™ 2PC UV-Vis scanning spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker avance 400 MHz spectrometer using $CDCl_3$ solvent and Tetra methyl silane (TMS) was used as an internal standard.

3.4. Extraction and Phytochemical Screening

3.4.1. Extraction

The collected roots of *R. ferruginia* were air dried at room temperature and grounded in to powder using grinding mills. The grinded roots (500 g) of *R. ferruginia* were extracted with 2.5 L dichloromethane/methanol (1:1) for 72 hr with occasional shaking at room temperature. The solution was filtrated and concentrated by a vacuum rotary evaporator at 50 ^{o}C to afford 24.48 g (4.89%) dark red crude extract. The marc left was further extracted with methanol (2 L) for 72 hr with occasional shaking at room temperature. The solution was filtered and concentrated by a vacuum rotary evaporator at a temperature of about 50 ^{o}C to afford 17.55g (3.51%) dark red crude extract. The TLC profile of dried crude extracts were checked and finally the extracts were collected in labeled sterile containers and put in deep freezer until used for further experiment.

3.4.2. Phytochemical Screening Tests

1. **Test for Alkaloid**

Crude extracts (DCM: MeOH (1:1) and MeOH, each 0.5 g, were separately stirred with 1 % HCl (0.5 mL) on a water bath for 5 min and filtered. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate was expected for the presence of alkaloids. These filtrates were divided into three equal parts. To one portion of

filtrate, Wagner's reagent (Iodine in Potassium Iodide) (1 mL) was added. Formation reddish precipitate gives indication of the presence of alkaloids. To other portion of filtrate Mayer's reagent (Potassium mercuric iodide solution) (1 mL) was added. Formation of yellow colored precipitate confirm an indication of the presence of alkaloids (Sheel *et al*., 2014).

2. Test for flavonoids

Crude extract (DCM: MeOH (1:1) and MeOH extracts, each 0.5 g, were dissolved with 10 mL of ethyl acetate and heated for 3 min using steam bath. The mixture was filtered and the filtrate was mixed with 1mL of dilute ammonia solution. Formation of intense yellow color indicate the presence of flavonoids (Karande *et al.,* 2016).

3. Test for saponnins

Crude extracts DCM: MeOH (1:1) and MeOH , each 0.5 g, were dissolved in 5 mL of distilled water and shaken while heating to boil. Frothing showed the presence of saponnins (Jaradat *et al.,* 2015).

4. Test for phenols

To crude extracts DCM: MeOH (1:1) and MeOH each 0.5 g, few drops of 2 % of $FeCl_3$ was added and the formation of bluish green to black color indicates the presence of phenols (Bargah and Rohit, 2015).

5. Test for tannins

Crude extract DCM: MeOH (1:1) and MeOH (0.5 g) were boiled in 5 mL of water in a test tube and filtered. To the filtrate, few drops of 0.1 % $FeCl_3$ was added to give blue-green precipitate which confirms the presence of tannins (Amin *et al.,* 2016).

6. **Test for terpenoids** (Salkowski test)

Crude DCM: MeOH (1:1) and MeOH extracts were mixed with 2 mL of chloroform and 3 mL concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids (Santhi and Sengottuvel, 2016).

7. **Test for steroids**

Crude extracts (5 mg) were dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer was turned red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids (Nwodo and Nkwocha, 2015).

8. **Test for glycosides** (Keller-Killani's Test)

Crude extracts DCM: MeOH (1:1) and MeOH (0.5 g) were treated with 2mL of glacial acetic acid solution which contain 1 drop of ferric chloride solution and 1mL of concentrated H_2SO_4. A reddish brown colour formed at the junction of the two layers was taken as an indication of the presence of glycoside (Kamal *et al.*, 2012).

3.5. Isolation of Compounds

3.5.1. Silica gel Column Chromatography

The dichloromethane/methanol (1:1) crude extract showed better TLC profile than methanol extract and hence selected for silica gel column chromatography separation. The crude extract (14 g) was adsorbed on 14 g of silica gel (mesh size 60-120), subjected to silica gel chromatography (180 g of silica gel) using *n*-hexane for packing and elution was carried out with increasing gradient of ethyl acetate in *n*-hexane followed by methanol in ethyl acetate as eluent. A total of 115 fractions each 100 mL (Table 1) were collected. The purity of each fractions was

checked by using TLC. Fractions that showed similar R_f values and the same characteristic color on TLC (visualizing in UV cabinet) were combined. Fraction 28 showed single spot on TLC (EtOAc/*n*-hexane, 3:7, R_f 0.44, 40 mg) to afford compound **57**. Fractions 35-38 showed single spot with similar R_f values to afford compound **55** (EtOAc/*n*-hexane as eluent, 1:1, R_f 0.52,120 mg). Fractions 43-45 showed single spot with similar R_f values to afford compound **56** (EtOAc/*n*-hexane, 1:1 as eluent, R_f 0.43, 50 mg).

Table 1. Summary of fraction of dichloromethane/methanol (1:1) extract.

Eluents	Ratio	Fractions	Eluents	Ratio	Fractions
n-hexane:EtOAc	100:0	F_1-F_4	EtOAc;MeOH	95:5	F_{65}-F_{70}
	99:1	F_5-F_6		90:10	F_{71}-F_{80}
	98:2	F_7-F_8		80:20	F_{81}-F_{85}
	97:3	F_9-F_{10}		70:30	F_{86}-F_{92}
	96:4	F_{11}-F_{12}		60:40	F_{93}-F_{97}
	94:6	F_{13}-F_{14}		50:50	F_{98}-F_{104}
	93:7	F_{15}-F_{16}		30:70	F_{105}-F_{110}
	92:8	F_{17}-F_{18}	MeOH	100	F_{111}-F_{115}
	90:10	F_{19}-F_{20}			
	85:15	F_{21}-F_{23}			
	80:20	F_{24}-F_{25}			
	70:30	F_{26}-F_{30}			
	60:40	F_{31}-F_{39}			
	50:50	F_{40}-F_{45}			
	25:75	F_{46}-F_{55}			
EtOAc	100	F_{56}-F_{64}			

3.5.2. Spectral data of isolated compounds

The spectroscopic techniques (NMR) were employed to elucidate the structure of isolated compounds. The ^{1}H NMR and ^{13}C NMR were obtained in $CDCl_3$ on Bruker Avance 400 MHz spectrometer.

3.6. Biological Evaluation

3.6.1. Antibacterial Activity

The antibacterial activities of crude extracts and isolated compounds were done in collaboration with the Department of Applied Biology, Adama Science and Technology University. *In vitro* antibacterial activity of crude extracts and isolated compounds were investigated by using Mueller-Hinton Agar (MHA) disc diffusion method against four bacterial strains *E. coli, S. aureus, P. aeruginosa* and *S. pyogenes.* The medium was prepared by dissolving 38 g of Mueller Hinton agar medium in 1000 mL of distilled water and the autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile plates (20-25 mL/plate) and the plates were allowed to solidify under sterile condition at room temperature. After solidification, the plates were seeded with colonies of pure bacterial culture approximately 1.5 X 10^8 CFU/mL with a sterile cotton swab on to the surface of the medium until an even distribution of the inoculum was achieved.

Using sterile forceps, antibiotic disk of standard drugs (Ciprofloxacin), and disk soaked with crude extract and pure compound of *R. ferrugenia* was placed on the surface of the inoculated and dried plate. After 24 hr of incubation at 37 °C, the diameter of the zones of growth inhibition around the disks were measured with a ruler and compared with ciprofloxacin. The experiments were done in triplicates and results were presented as mean value ± standard deviation.

3.6.2. Antioxidant Activity

DPPH is widely used to test the ability of compounds to act as free radical scavengers and to evaluate antioxidant activity of compounds. It is a stable free radical, which is due to the delocalized electron. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 5l7 nm (Hangun-Balkir and McKenney, 2012). The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It

is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the anti-oxidative activity (Qaiyum Ansari *et al*., 2013).

One ml from each four different concentrations of the samples and positive control, ascorbic acid (100, 50, 25 and 12 µ g/ml) were taken and mixed with 4 ml of DPPH solution that was prepared by dissolving 4 mg of DPPH in 100 mL of MeOH. The resulting solution was placed in an oven at 37ºC for 30 min and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm.

The percentage DPPH inhibition calculated according to the following formula (Proestos *et al.*, 2013).

$$I\% = \frac{Ao - A}{Ao} \times 100$$

where I = DPPH inhibition (%), A_O = absorbance of control sample (t = 0 h) and A = absorbance of a tested sample at the end of the reaction (t = 30 minite).

3.7. Molecular Docking Study of Isolated Compounds against DNA Gyraseb (PDB ID 6F86).

To have a better understanding about the inhibitory mechanism as well as the mode of interactions of the phytochemical compounds of the crude extract, molecular docking analysis was accomplished using the AutoDock version 4.2 docking program.

Crystal structure of the enzyme (PDB ID: 6F86) with resolution 2.3 Å was chosen as the protein model. The structures of ligands were optimized using the Hyper Chem 7.0 software. Auto Dock version 4.2 was used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms were added while nonpolar hydrogen atoms were merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, were allowed to rotate during the automated docking process and then prepared protein and ligand structures were saved in the PDBQT format suitable for calculating energy grid maps. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations were clustered and ranked by energy of

which the conformation with the best scored pose and the lowest binding energy was selected for the ligands (Mansourian *et al.*, 2015; Morris *et al.*, 1998)

4. RESULTS AND DISCUSSION

4.1. Phytochemical Screening

Qualitative phytochemical screening was carried out using several tests and results revealed that the roots of crude extracts of *R. ferrugenia* contains alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids, phytosterols and glycosides. The results were represented in Table 2. The results obtained in this study suggest the identified phytochemical compounds may be the bioactive constituents of this plant suggesting the plant as a valuable reservoir of bioactive compounds of substantial medicinal importance.

Table 2. Phytochemical screening results on root extracts of *R. ferrugenia*.

NO	Phytochemical screening	DCM: MeOH (1:1)	MeOH 100%
1	Saponnins	+	+
2	Flavonoids	+	+
3	Phenols	+	+
4	Tannins	+	+
5	Terpenoids	+	+
6	Steroids	+	+
7	Glycosides	+	+
8	Alkaloids	+	+

+ indicates presence,

4.2. Characterization of Compounds

Compound **55** was isolated as yellow solid with melting point of 136-137 ^{0}C. Its R_f value was 0.52 (50 % EtOAc in *n*-hexane as eluent). Its ^{1}H NMR spectrum (Appendix 1) displayed a set of aliphatic proton signals at δ 4.31 (dd, J = 15.7, 5.3 H-2), 3.99 (t, H-2), 2.94 (dd, J = 15.9, 10.2 Hz , H-4) 2.86 (dd, *J* = 15.7, 5.9 Hz, H-4) attributed to isoflavan nucleus (ring-C) in agreement with previous literature (Bedane *et al*., 2016). In agreement with the skeketon, methylene protons at C-4 appeared at δ 2.94 dd (J = 10.5, 5.3 Hz) and 2.86 dd (J = 15.7, 5.9 Hz) as diastereotopic

protons indicated the presence of chiral center C-3 position. This observation was also supported by the carbon resonances at δ C 70.0, 31.7 and 30.0 attributed to C-2, C-3 and C-4, respectively, of which the former is pointing down in DEPT-135 spectrum in agreement with methylene moiety. Three aromatic protons having ABX spin pattern were observed at δ H 6.90 (H-6', d, J=8), 6.36 (H-5', d, J= 8) and 6.38 (H-3', d, J = 2.2 Hz). Signals at δ 1.78 (H-5", s), 1.73 (H-4", s), 3.26 (H-1", d, J= 7.5), 5.31 (H-2", t) suggest the presence of a prenyl group.

The ^{13}C NMR and DEPT-135 spectrum (Appendix 1.2 and 1.3) revealed the presence eight quaternary carbons of which four of them are sp^2 oxygenated quaternary aromatic carbons at δ 154.8 (C-7), 152.8 (C-9), 155.2 (C-2'),156.4 (C-4'). Aromatic methine peaks observed at δ 99.2(C-8), 130.0 (C-5), 103.2 (C-3'), 107.7(C-5') and 128.3 (C-6') of which the upfeild chemical shift value of C-8 and C-3' are in good agreement with 1,3-dioxygenated substitution pattern that contributed to shielding effect possibly due to delocalization of lone pair electrons of oxygen atoms attached to adjacent carbons (oxygenation at C-7 & C-9 affecting chemical shift of C-8 and oxygenation at C-2' & C-4' affecting chemical shift of C-3'). The peak at δ 55.5 suggest the presence of methoxy moiety. The NMR spectral data of compound **55** was compared with the literature reported for 7, 2'-dihydroxy-4'-methoxy-6-(3", 3"-dimethylallyl) isoflavan with the data depicted in Table 3.

Table 3.^{1}H, ^{13}C and DEPT-135 NMR spectral data of 7, 2'-dihydroxy-4'-methoxy-6-(3", 3"-dimethylallyl) isoflavan (**55**) in $CDCl_3$.

Position	Compound **55**			Fan *et al*., 2019	
	δ^{1}H (multiplicity, J value)	δ^{13}C	DEP	δ^{1}H (multiplicity, J value)	δ^{13}C
1	--	--	--	---	---
2 ax 2 eq	4.31 (dd, *J* = 15.7, 5.3 Hz,) 3.99 (t)	70.0	70.0	4.11, (dd, *J*= 4.8,10.4Hz); 3.86, (t, J=10.0Hz)	69.1
3	3.50 (m)	31.7	__	3.30, m	31.2
4 ax 4 eq	2.94 (dd, *J* =15.9, 10.2 Hz Hz) 2.86 (dd, *J* = 15.7, 5.9 Hz)	30.0	30.0	2.85, m; 2.68, (dd, *J*= 4.0, 10.4 Hz)	29.8
5	6.84 (s, 1H)	130.0	130.0	6.69 s	129.7
6	__	122.5	__	__	119.8
7	__	154.8	__	__	153.6
8	6.40 (s, 1H)	99.2	99.2	6.23 s	102.3
9	__	152.8	__	__	152.4
10	__	113.6	__	__	112.4
1'	__	120.0	__	__	119.8
2'	__	155.2	__	__	155.8
3'	6.38 (d, *J* = 2.2 Hz)	103.2	103.2	6.42 (d, J=2.4 Hz)	101.3
4'	___	156.4	___	___	158.7
5'	6.36 (dd, *J*= 8 Hz, 2.2 Hz)	107.7	107.7	6.34 (dd, J=8.8,2.4 Hz)	104.3
6'	6.90 (d, *J*= 8 Hz)	128.3	128.3	6.97 (d, J= 8.8 Hz)	127.6
1"	3.26 (d, *J*= 7.5 Hz)	27.8	27.8	3.11 (d, J=7.2Hz)	27.4
2"	5.31 (t, 1H)	123.0	123.0	5.23, t	123.1
3"	__	132.1	__	__	130.4
4"	1.73 (s, 3H)	17.8		1.67(s)	17.6
5"	1.78 (s, 3H)	25.9		1.65 (s)	25.5
OMe	3.77 (s, 3H)	55.5		3.66 (s)	54.8

Thus, based on the above spectral data and comparison with literature, the structure of compound **55** was proposed to be identical with that of 7, 2'-dihydroxy-4'-methoxy-6-(3", 3"-dimethylallyl) isoflavan (Figure 4) previously reported from *Glycyrrhiza uralensis* that belongs to genus *Glycyrrhiza* found in the family of Fabaceae (Fan *et al*., 2019). This is the first report of the compound from *Rhynchosia ferruginea* and the genus *Rhynchosia*.

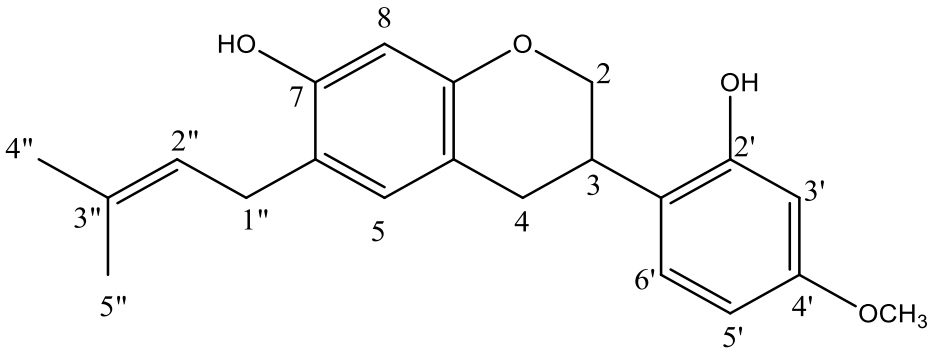


Figure 4. 7, 2'-dihydroxy-4'-methoxy-6-(3", 3"-dimethylallyl) isoflavan (**55**).

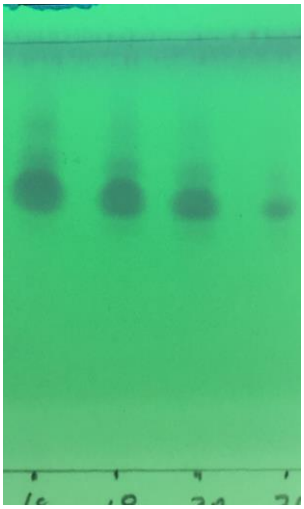


Figure 5. TLC profile of compound **55** (EtOAc/*n*-hexane as eluent, 1:1)

Compound **56** was isolated as yellow solid with melting point of 124-125 ^{0}C. Its R_f value was 0.43 (50 % EtOAc in *n*-hexane as eluent). Its ^{1}H NMR spectrum (Appendix 2.1) revealed signals at δ 4.32 (s, H-2) and 6.78 (s, H-4) which are characteristic signals of flavan-3-ene on ring C skeleton. On the basis of literature precedence, the signals at δ 2.73 (H-1'''), δ 3.28 (H-2'''), 1.16 (H-4''') and 1.12 (H-5''') with corresponding carbon signals at δ 70.5 (C-3''') and δ 77.2 (C-2''')

suggest the presence of 2,3-dihydroxy-3-methylbutyl moiety (Kamdem *et al.*, 2005; Nyandoro *et al.*, 2017). The presence of a prenyl group was evident from the signals at δ 1.62 (H-5", s), 1.60 (H-4", s), 3.12 (H-1", d, J= 8.1), 5.17 (H-2", t) with corresponding carbon peaks at δ 24.1 (C-1"), 122.7 (C-2"), 131.4 (C-3"), 17.0 (C-4") and 25.2 (C-5"). Two methoxy groups are observed at δ 3.67 (s, 3H) and 3.68 (s, 3H).

The ^{13}C NMR and DEPT-135 spectra (Appendix 2.2 and 2.3) revealed the presence of eleven quaternary carbons of which four of them are sp^2 oxygenated aromatic quaternary carbons at δ C 155.4 (C-7), 152.7 (C-9), 156.0 (C-2'), 155.9(C-4') and sp^3 oxygenated quaternary carbon at δ 70.5 (C-3'"). The peak at δ C 77.23 suggest sp^3 oxygenated methine (C-2'"). Aromatic non-oxygenated quaternary carbon appeared at δ C 113.5 (C-8), 118.7 (C-10), 121.7 (C-1') and 122.6 (C-5'). Non aromatic sp^2 quaternary carbon appeared at δ 127.4 (C-3) and 131.4 (C-3"). Aromatic methines were observed at δ C 129.5 (C-5), 102.2 (C-6), 98.7 (C-3') and 129.8 (C-6'). Non aromatic sp^2 methines were observed at δ 127.2 (C-4) and 122.7 (C-2"). The upfeild chemical shift values for C-3' are in good agreement with 1, 3-dioxygenated substitution pattern at C-2', 4' positions as in the proposed structure. The peak at δ C 69.8 suggest oxygenated methylene, pointing down in DEPT-135 spectrum, and peaks at δ C 54.9 and 54.8 suggest methoxy groups supported by DEPT-135 spectrum pointing up. The ^{1}H and ^{13}C-NMR spectral data of compound **56** is summarized in Table 4.

Table 4. ^{1}H, ^{13}C and DEPT-135 NMR spectral data of 7-hydroxy-2', 4' di-methoxy-8-(2''', 3'''-dihydroxy-3'''-methylbutyl)-5'- (3'', 3''-dimethylallyl) isoflav-3-ene (**56**) in $CDCl_3$.

Position	Compound **56**			Tanaka *et al.*, 2002	
	$\delta^{1}H$ (Multiplicity, J-value)	$\delta^{13}C$	DEPT-135 (δ C)	$\delta^{1}H$ (Multiplicity, J-value)	$\delta^{13}C$
1	--	--	--	---	---
2	4.32 (s)	69.8	69.8	4.97s	68.4
3	__	127.4	__	_	128.9
4	6.78 (s)	127.2	127.2	6.5 s	121.9
5	6.71 (d, *J* = 9.0 Hz)	129.5	129.5	6.82 (d, *J*= 8.1)	125.0
6	6.25 (d, *J* =8.9 Hz)	102.2	102.2	6.40 (d, *J*= 8.1)	108.7
7	__	155.4	__	__	155.2
8	__	113.5	__	__	114.4
9	__	152.7	__	__	151.9
10	__	118.7	__	__	117.1
1'	__	121.7	__	__	120.8
2'	__	156.0	__	__	158.4
3'	6.21(s)	98.7	98.7	6.42 (s)	99.2
4'	__	155.9	__	__	156.6
5'	__	122.6	__	6.41 (dd, J=8.1,2.2)	107.3
6'	6.59 (s)	129.8	129.8	7.1 (d, J=8.1)	129.4
OMe, OMe	3.67 3.68	54.8 54.9	54.8 54.9	3.75 s __	55.4 __
1''	3.12 (d, *J*= 8.1 Hz),	24.1	24.1	3.42(d, J= 7.3)	22.4
2''	5.17 (t, J=8.1 Hz)	122.7	122.7	5.27 (t, J=7.3)	121.8
3''	__	131.4	__	__	134.5
4''	1.60 (s)	17.0	17.0	1.74	17.9

5"	1.62 (s)	25.2	25.2	1.82	25.8
				(Nyandoro *et al.*, 2017)	
1"'	2.73 (d. *J* =15 Hz)	29.2	29.2	2.73 m	26.0
2"'	3.28 (d. *J* =15 Hz)	77.2	77.2	3.61m	77.7
3"'	__	70.5	__		72.0
4"'	1.16 (s, 3H)	31.3	31.3	1.19 s	24.3
5"'	1.12 (s, 3H)	28.0	28.0	1.23 s	23.6

From the above spectral data and comparison with literature, new compound **56** was identified and labeled as 7-hydroxy-2', 4'-di-methoxy-8-(2"', 3"'-dihydroxy-3"'-methylbutyl)-5'- (3", 3"-dimethylallyl) isoflav-3-ene (**56**, Figure 6).

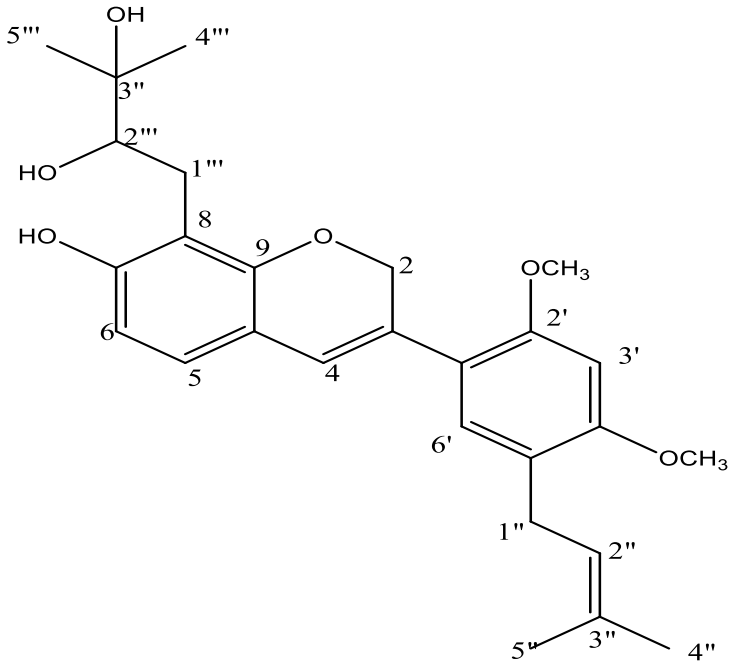


Figure 6. 7-hydroxy-2', 4'-di-methoxy-8-(2"', 3"'-dihydroxy-3"'-methylbutyl)-5'- (3", 3"-dimethylallyl) isoflav-3-ene (**56**).

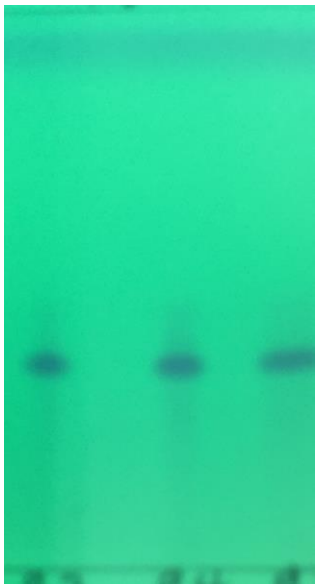


Figure **7**. TLC profile of compound **56** (50 % EtOAc in *n*-hexane as eluent)

Compound **57** was obtained as white semi solid with melting point of 43-45 ^{0}C. Its R_f value was 0.44 (EtOAc/*n*-hexane, 3:7 as eluent). Its ^{1}H NMR spectrum (Appendix 3.1) displayed peaks at δ H 0.90 and 0.89 suggesting the presence of two terminal methyl protons. The peaks at δ 1.29 and δ 1.64 indicate protons of aliphatic methylene (–CH_2) group, a peak at δ 2.06 indicates the presence of protons of a methylene group that is bonded to C=C and the triplet peak at δ 2.36 indicates the presence of methylene protons α to a carbonyl group. The glycerol moiety exhibited doublet of doublets at δ 4.17 for oxygenated methylene protons H-1, 3 and a multiplet at δ 5.10 attributed to oxygenated methine proton H-2 whereas olefinic protons were observed at δ 5.37 as multiplet.

The ^{13}C NMR and DEPT-135 spectra (Appendix 3.2 and 3.3) revealed the acyl function attributed to esters carbonyls at δ 174.0 and 173.9, oxygenated methylene at δ 65 pointing down in DEPT-135 spectrum, and oxygenated methine at δ 68, pointing up in DEPT-135 spectrum. Four sp^2 methines are clearly evident at δ 130.2, 130.0, 128.1 and 127.9. Overlapped methylene peaks are observed at δ 29 and two terminal methyl carbons appeared at δ 14.1 and 14.2.

Table 5. ^{1}H, ^{13}C and DEPT-135 NMR spectral data of 1, 3-dilinoleoyl-2-Stearoyl glycerol (**57**)

Position	Compound **57**			Bekele *et al*., 2013	
	$\delta^{1}H$ (Multiplicity, J-value)	$\delta^{13}C$	DEPT-135 (δ C)	$\delta^{1}H$ (Multiplicity, J-value)	$\delta^{13}C$
1	4.17(dd,J=10.6, 5.0 Hz)	65	65	4.20	62.1
2	5.10 m	68	68	5.25	68.9
3	4.17(dd,J=10.6, 5.0 Hz)	65	65	4.20	62.1
1',1"	___	174.0, 173.9	___	___	173.3, 172.8
2',2"	2.36 t	34.1	34.1	2.33	34.1, 34.2
3',3"	1.64	31.9, 31.5	31.9, 31.5	1.60	31.9, 31.5
4',4"	1.29	22.7	22.7	1.25	22.7
5',5"	1.29	22.6	22.6	1.25	22.7
6',6"	1.29	24.9	24.9	1.25	24.9
7',7"	1.29	24.9	24.9	1.25	24.9
8',8"	2.05	27.2	27.2	2.03	27.2
9',9"	5.37m	130.9, 29.7	130.9, 29.7	5.40	129.7, 130.2
10',10"	5.37m	128.1,29.7	128.1,29.7	5.40	127.9, 129.7
11',11"	2.05	25.6,29.7	25.6,29.4	2.80, 2.03	25.6, 27.2
12',12"	5.37m	130.0, 29.4	130.0, 29.4	5.40, 1.25	127.9, 128.1
13',13"	5.37m	127.9, 29.4	127.9, 29.3	5.40, 1.25	131.9, 130.2
14',14"	1.29	29.3	29.2	2.03, 1.25	27.2
15',15"	1.29	29.2	29.2	1.25	29.2–29.7
16',16"	1.29	29.1	29.1	1.25	29.2–29.7
17',17"	1.29	29.1	29.1	1.25	29.2–29.7
18,'18"	0.90	14.2 14.1	14.2 14.1	0.90	14.2, 14.1

By relating the above spectral data with previously reported compound from plant *Moringa stenopetala* in the family of Moringaceae (Bekele et al., 2013), the compound was found to be triglyceride and named as 1, 3-dilinoleoyl-2-Stearoylglycerol (**57,** Figure 8).

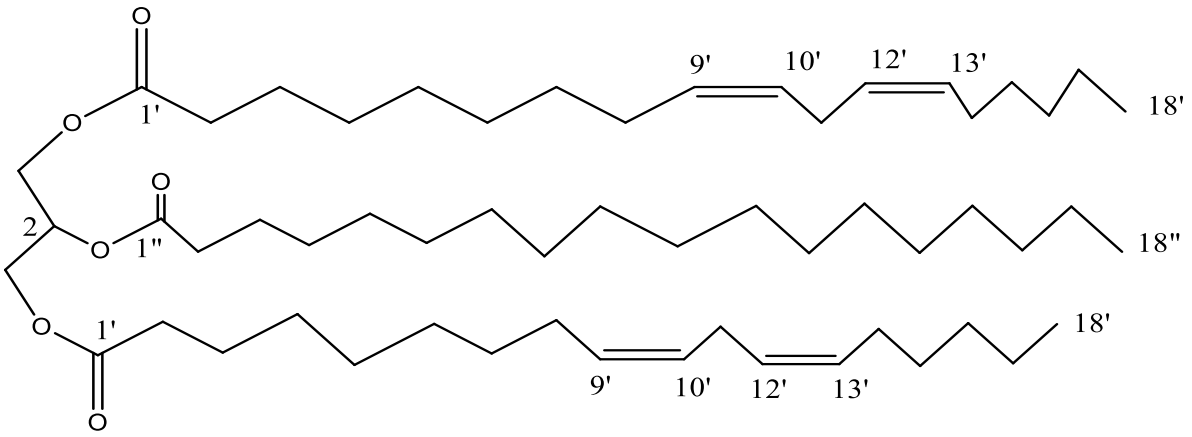


Figure 8. Structure 1, 3-dilinoleoyl-2-Stearoylglycerol (**57**).

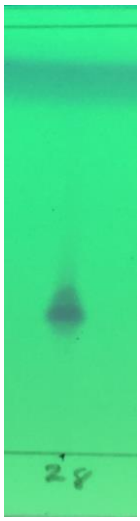


Figure 9. TLC profile of compound **57** (EtOAc/*n*-hexane, 3:7 as eluent).

4.3. Biological Activity of Crude Extract and Isolated Compounds of *R. Ferrugenia*

4.3.1. Antibacterial Activity

In vitro antibacterial activity of dichloromethane/methanol (1:1) crude extract and isolated compounds were tested for their antibacterial activity against the bacterial strain *E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes* at two different concentration (1 mg/mL and 1.5 mg/mL) (Table 6). Ciprofloxacin was used as positive control. The antibacterial activity of crude extracts and compounds showed dose-dependent increment for both crude extracts and compounds.

Table 6. Zone of bacterial growth inhibition diameter (mm).

Sample	Concentration in mg/mL	Inhibition diameter (mm) ±SD			
		E. coli	***S. aureus***	***P. aeruginosa***	***S. pyogenes***
Methanol/dichloromethane (1:1) extract	1	6.67±0.58	6.67±0.58	6.00±0.00	6.00±0.00
	1.5	7.33±0.58	7.33±0.58	6.33±0.58	6.00±0.00
Compound **55**	1	8.67±0.58	10.33±0.58	8.33±0.58	6.33±0.58
	1.5	9.67±0.58	10.67±0.58	8.67±0.58	7.33±0.58
Compound **56**	1	9.67±1.15	9.67±0.58	6.67±0.58	6.00±0.00
	1.5	10.33±1.15	10±1.00	7.33±0.58	6.33±0.58
Compound **57**	1	6.33.58	6.67±0.58	6.33±0.58	6.00±0.00
	1.5	6.67±0.58	7.67±0.58	6.67±0.58	6.00±0.00
Ciprofloxacin	1	15.33±0.58	15.33±0.58	15.67±0.58	16.33±0.58
	1.5	15.67±0.58	15.67±0.58	17.33±1.53	17.33±1.00

Compound **55** (1.5 mg) displayed 9.67±0.58 mm and 10.67±0.58 mm zone of inhibition whereas compound **56** (1.5 mg) showed 10.33±1.15 mm and 10±1.00 mm zone of inhibition against *E. coli* and *S. aureus*, respectively, compared to ciprofloxacin (15.67±0.58 mm zone of inhibition for both strains). The values displayed by these compounds (**55** and **56**) against these two strains can be considered as promising antibacterial results. All compounds and crude extract showed weak zone of inhibition against *P. aeruginosa* and *S. pyogenes*. The antibacterial activity of dichloromethane/methanol (1:1) extract and compound **57** were found to be less than that of compound **55** and **56** with weak antibacterial activity against *E. coli* and *S. aureus*. Likewise, from the genus *Rhynchosia* the antibacterial activities of essential oil of *Rhynchosia Minima* also exhibited significant activity against *E. coli* and *S. aureus* more than other strains, with inhibition

zone diameter of 18.2 and 12.5mm respectively at concentration of 100µg/ml (Gundidza *et al.*, 2009).

4.3.2. Antioxidant Activity

DPPH is widely used to test the ability of compounds to act as free radical scavengers and to evaluate antioxidant activity of compounds. The radical scavenging activity of the extracts and isolated compounds were assessed by measuring the reduction of DPPH absorbance at 517 nm. Crude extracts and pure compounds of *R. ferrugenia* reduced the DPPH in dose-dependent manner (Table 7). DCM/methanol (1:1) extract, compound **55**, compound **56** and compound **57** displayed 73%, 70%, 59% and 50% of inhibition, respectively, at concentration of 100 µg/mL. The positive control, ascorbic acid (Figure 10) showed maximum scavenging effect from very low to high concentration (85, 93, 97 and 99 % at 12, 25, 50 and 100 µg/mL respectively). Over all, the dichloromethane/methanol (1:1) extract and compound **55** displayed promising radical scavenging activity with IC_{50} of 17.7 and 32, respectively.

Table 7. Scavenging effect of the extracts and isolated compounds of the root of *R. ferrugenia.*

Conc (µg/ml)	DCM/MeOH(1:1) extract		Compound **55**		Compound **56**		Compound **57**		Ascorbic acid	
	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity	A	% scavenging activity
12.5	0.6	45	0.65	40	0.8	27	0.82	25	0.16	85
25	0.5	54	0.55	50	0.64	41	0.75	31	0.08	93
50	0.4	63	0.45	59	0.55	50	0.60	45	0.03	97
100	0.3	73	0.33	70	0.45	59	0.55	50	0.01	99
IC_{-50}	17.7		32		64.5		90.5			
Absorbance of DPPH =1.1										

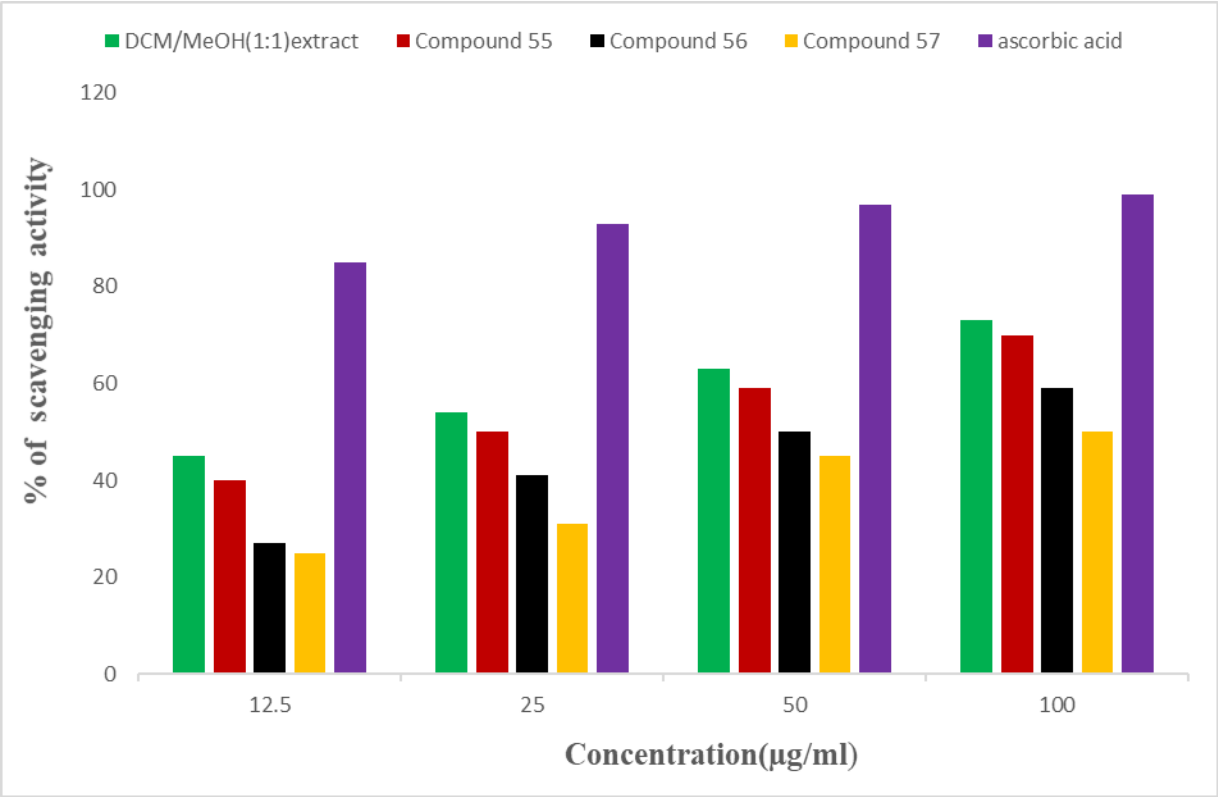


Figure 10. DPPH scavenging activity (%) of *R. ferrugenia* DCM:MeOH(1:1) extract, isolated compounds and the positive control (Ascorbic acid).

4.4. Molecular Docking Study of Isolated Compounds against DNA Gyraseb (PDB ID 6F86)

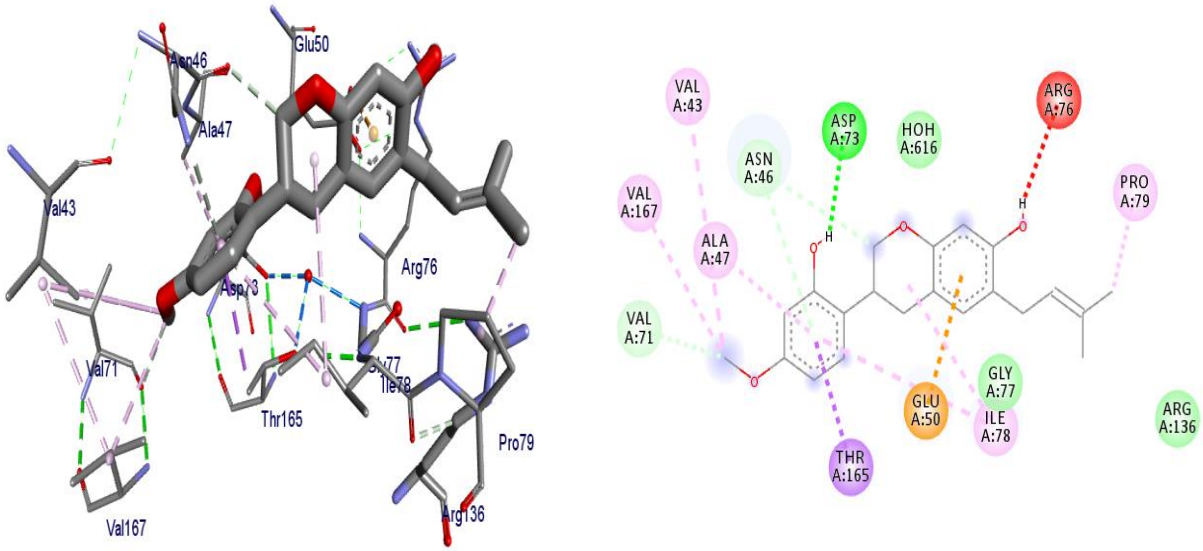
Molecular docking analysis of isolated compounds was done in comparison with ciprofloxacin to predict the orientation and binding affinity of ligand molecules at the active site of the receptor. Compound **55** and **56** (Table 8) displayed similar binding affinities of -7.4 kcal/mol and exhibit hydrogen bond with three active site amino acid residue compared to ciprofloxacin -7.3 kcal/mol. Compound **55** binding conformation within the active pocket of PDB ID 6F86 exhibits four hydrogen bonding interaction. From the Figure 11 the green dash lines indicates a possible hydrogen bond formed between the connections residues and pink lines indicated hydrophobic bond interaction. For instance, 2'-OH, H-2, H-5' and OCH_3 made hydrogen bond with Asp-73, Asn-46, Val-71, respectively.

Table 8. Molecular docking results of compounds isolated from the root of *R. ferrugenia* and ciprofloxacin against E.coli DNA gyraseB (PDB ID 6F86)

Ligands	Affinity (kcal/mol)	Amino acid residue form H-bond with ligands	Residual Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions
Compound **55**	-7.4	Asp-73,Asn-46, Arg-76	Ile-78, Glu-50, Thr-165, Pro-79, Ala-47, Val-43, Val-167, Gly-77, Arg-136
Compound **56**	-7.4	Asp-73,Asn-46, Thr-165	Ile-78, Val-43, Ala-47, Val-167, Val-120, Asp-49, Gly-77, Gly-119, Ser-121
Compound **57**	-5.2	Asn-46, Val-120	Arg-76, Ile-78, Pro-79, Ile-94, Val-93, Val-90, Val-93, Val-43, Val-47, Val-71, Val-167
Ciprofloxacin	-7.3	Asp-73,Asn-46, Arg-76	Ala-47, Glu-50, Gly-77, Ile-78, Pro-79, Ile-94

Compound **56** showed hydrogen bond interaction with three active site amino acid residue Asp-73, Asn-46, Thr-165 (Table 8). The hydrogen bond formed (Figure 8) between O atom of C ring with Asn-46, O atom of methoxy on C-4' with Thr-165 and H atom of methoxy on C-4' with Asp-73.The two methyl of prenyl moiety on C-5' displayed hydrophobic interaction with Val-43, Ala-47, Val-167 and Val-120.Compound **57** showed binding affinity of -5.2 kcal/mol (Table 8).The oxygen atoms on esters functional group of compound **57** form hydrogen bonding with two amino acid Asn-46 and Val-120 (Figure 11). In this study, compounds **55** and **56** exhibited better docking efficiency with DNA gyrase and may act as potential inhibitors of DNA gyrase enzyme.

A)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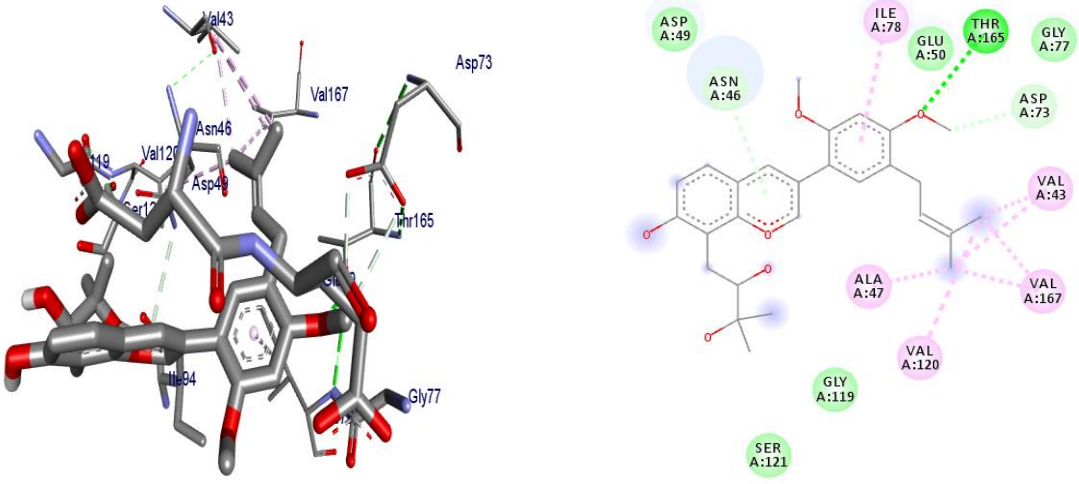
B)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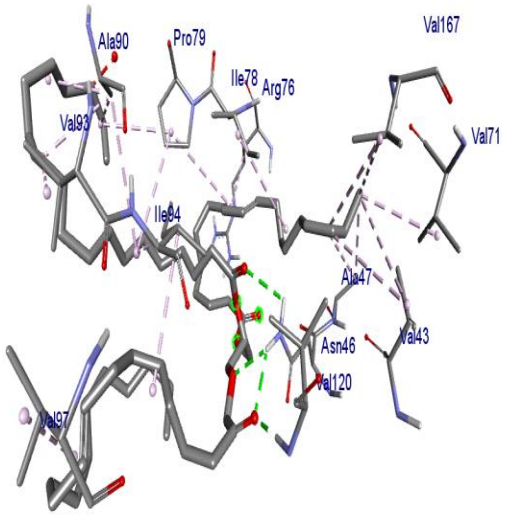
SER A:121

C)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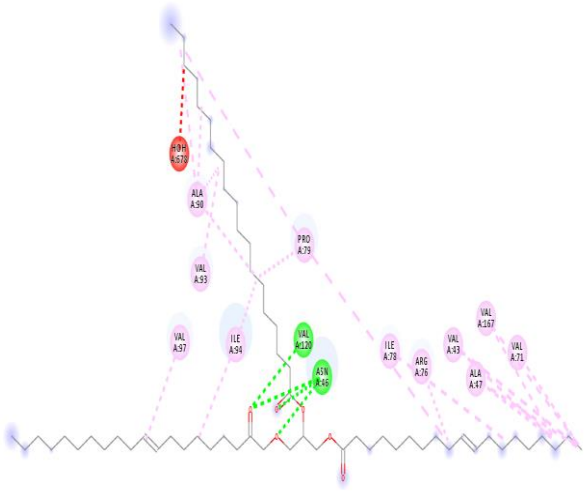

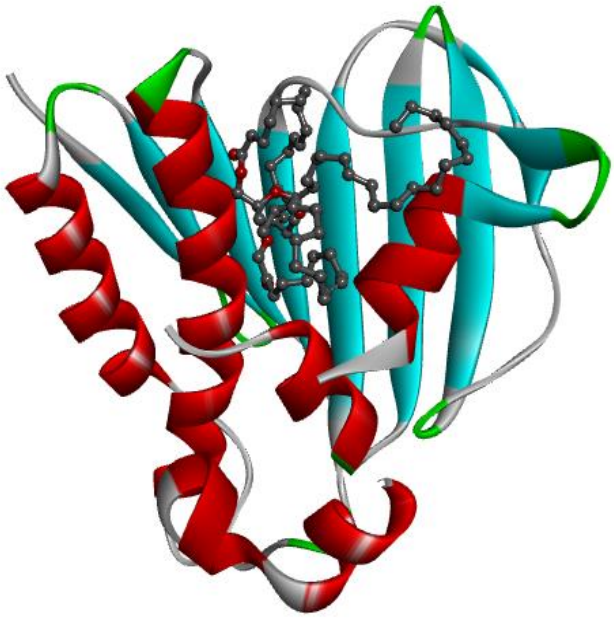

D)

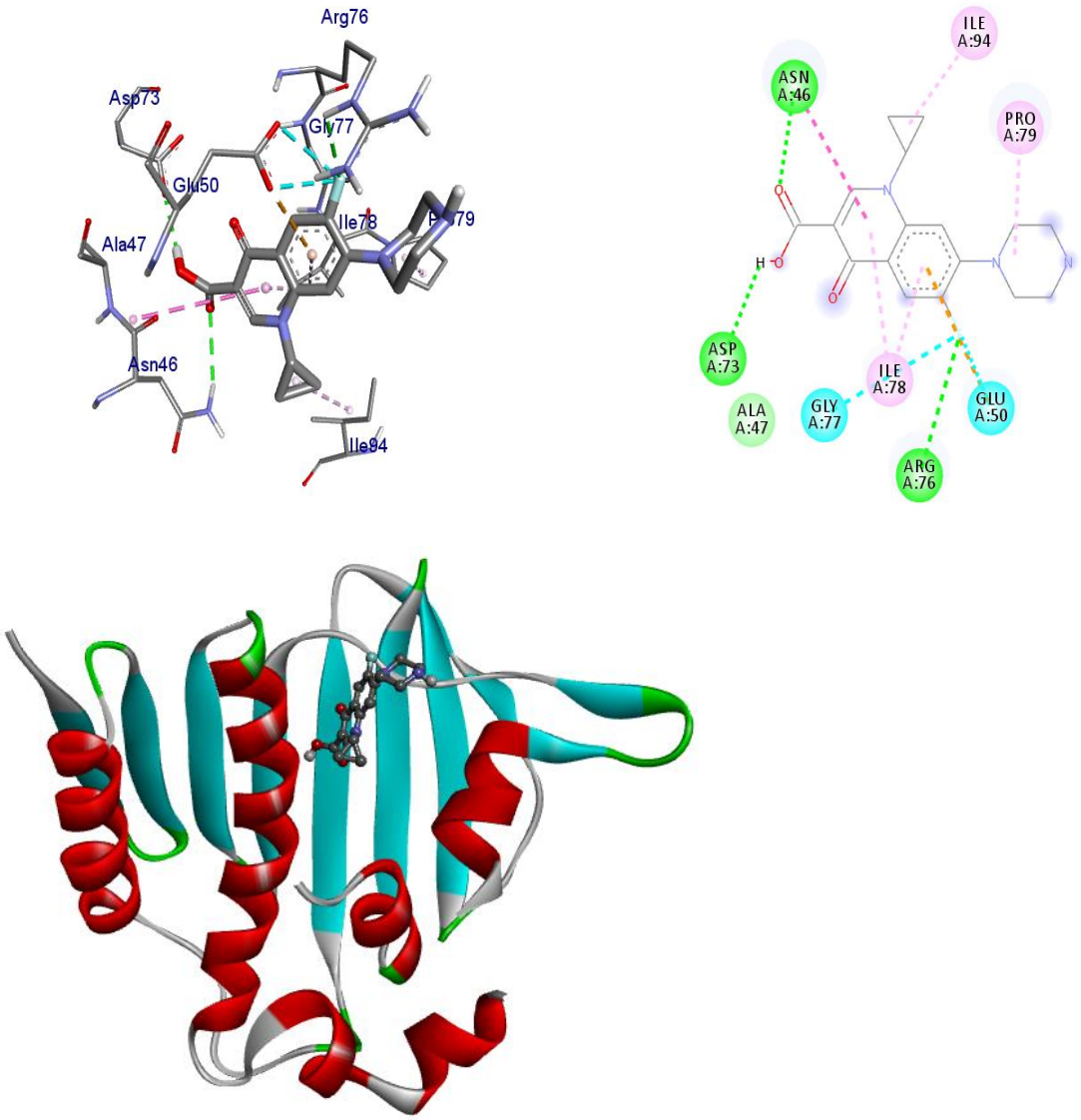

Figure 11.The 2D and 3D binding interactions of ligands (A, B, C, D) against DNA gyrase B (PDB ID: 6F86). A-compound **55**, B-Compound **56**, C-Compound **57**, D-Ciprofloxacin

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In this study, the phytochemical screening tests showed that crude extracts of roots of *R. ferrugenia* contain alkaloids, flavonoids, saponins, phenols, tannins, terpenoids and steroids. Silica gel column chromatographic analysis of the DCM:methanol (1:1) extract furnished three compounds and named as 7,2'-dihydroxy-4'-methoxy-6-(3",3"-dimethylallyl) isoflavan (**55**), 7-hydroxy-2',4'-dimethoxy-8-(2"',3"'-dihydroxy-3"'-methylbutyl)-5'-(3",3"dimethylallyl) isoflav-3-ene (**56**) and 1,3-dilinoleoyl-2-stearoyl glycerol (**57**). These compounds were reported for the first time from the plant. Compound **55** and **56** showed promising antibacterial activity against *E. coli* and *S. aureus*. Dichloromethane: methanol (1:1) extract and compound **55** showed promising DPPH radical scavenging activity with IC_{50} of 17.7 and 32, respectively, compared to ascorbic acid. Molecular docking that was done against DNA gyrase B (PDB ID: 6F86) for isolated compounds showed best scoring pose (lowest energy) with value of -7.4 Kcal/mole for both compound **55** and compound **56**. Therefore, the in vitro antibacterial, radical scavenging activity and molecular docking analysis suggest that the plant has valuable compounds with interesting biological activity and gives hope for further investigation

5.2. Recommendation

In this study only 1D NMR spectrum and literature data was used for structures elucidation of isolated compounds. Further 2D NMR and mass spectroscopic analysis is recommended for compound **56**. As traditional healer uses the plant for many type of disease this work recommend further activity screening such as anti-fungal, anti-protozoal, anti-viral and hormonal disorder diseases like diabetes mellitus (hypoglycemic activity). It is recommended if *in vivo* and toxicological studies are carried out for compounds **55**, **56** and crude extract DCM: MeOH (1:1) extract. It is also recommended if further research work is carried out for the isolated compound about their structure-activity relationships studies to have a better understanding of structural and chemical parameters required to develop potent compounds. Additional research efforts are also recommended to determine the concentration and target of the compounds and application of computational analysis like docking to have a deeper understanding of the molecular interaction.

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6. REFERENCE

Adinarayana, D., Gunasekar, D., Seligmann, O., & Wagner, H. (1980). Rhynchosin, a new 5 deoxyflavonol from Rhynchosia beddomei. *Phytochemistry*, *19*, 483–484.

Ahmed, W., Jemal, Z., & Mali, A. (1992). Stigmasteryl galactoside from Rhynchosia minima. *Phytochemistry*, *31*, 635–649.

Ali, M., Sreeramulu, K., & Gunasekar, D. (1992). Constituents of Rhynchosia bracteata and Rhynchosia sublobata leaves. *Fitoterapia*, *46*(2), 141–146.

Amin, M., Parihar, K., Tabasum, U., & Kumari, E. (2016). Estimation of alkaloid , saponin and flavonoid , content in various extracts of Crocus sativa. *Journal of Medicinal Plant Studies*, *4*(5), 171–174.

Anulika, N., Ignatius, O., Raymond, E., Osasere, O., & Hilda, A. (2016). The Chemistry Of Natural Product : Plant Secondary Metabolites. *International Journal of Technology Enhancements and Emerging Engineering Research*, *4*(8), 1–8.

Bakshu, L., & Raju, R. R. V. (2001). Antimicrobial activity of Rhynchosia beddomei. *Fitoterapia*, *72*(9), 579–582.

Bargah, & Rohit. (2015). Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. *Journal of Pharmacognosy and Phytochemistry*, *4*(1), 7–9.

Bedane, K. G., Masesane, I., B., S., Majinda, & R.T., R. (2016). New isoflavans from the root bark of Erythrina livingstoniana. *Phytochemistry Letters*, *17*, 55–58.

Bekele, B., Adane, L., Tariku, Y., & Hailu, A. (2013). Evaluation of antileishmanial activities of triglycerides isolated from roots of Moringa stenopetala. *Medicinal Chemistry Research*, *22*(10), 4592–4599.

Bello, O., Ayanda, O., Aworunse, K., & Olukanmi, B. (2018). Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution? *Pharmacognosy Reviews*, *1*(2), 8–15.

Bhakshu, L., & Raju, R. (2009). Chemical composition and in vitro antimicrobial activity of essential oil of Rhynchosia heynei, an endemic medicinal plant from Eastern Ghats of India. *Pharmaceutical Biology*, *47*(11), 1067–1070.

Bohni, N., Cordero-Maldonado, M., Maes, J., Siverio-Mota, D., L, M., Munck, S.,Witte PA, C. A. (2013). Integration of microfractionation, qNMR and zebrafish screening for the in vivo bioassay-guided isolation and quantitative bioactivity analysis of natural products. *PLoS One*, *8*, e64006.

Coronado-aceves, Wenceslao, E., Gigliarelli, G., Garibay-escobar, A., Robles, E., Curini, M., Carla, M. (2017). New Isoflavonoids from the extract of Rhynchosia precatoria (Humb. & Bonpl. ex Willd.) DC. and their antimycobacterial activity. *Journal of Ethnopharmacology*, *206*, 92–100.

Fan, J., Kuang, Y., Dong, Z., Yi, Y., Zhou, Y., Li, B., Ye, M. (2019). Prenylated Phenolic Compounds from the Aerial Parts of Glycyrrhiza uralensis as PTP1B and α - Glucosidase Inhibitors. *Natural Products*, *2*(3), A-K.

Gundidza, Gweru, N., Magwa, M. L., Ramalivhana, N. J., Humphrey, G., Samie, A., & Mmbengwa, V. (2009). Phytochemical composition and biological activities of essential oil of Rhynchosia minima (L) (DC) (Fabaceae). *African Journal of Biotechnology*, *8*(5), 721–724.

Guo, Y., Yin, W., Liu, P., Hu, Y., Yan, M., & Sun, X. (2011). Extraction and separation of isoflavonoids from Rhynchosia volubilis lour using ionic liquid. *Chinese Journal of Applied Chemistry*, *28*, 537–541.

Hangun-Balkir, Y., & McKenney, M. (2012). Determination of antioxidant activities of berries and resveratrol. *Green Chemistry Letters and Reviews*, *5*(2), 147–153.

Hedberg.I, & Edwards.S. (1991). Flora of Ethiopia and Eritrea. In *The National Herbarium. Addis abeba universty, Ethiopia.*

Jaca, T., & Moteetee, A. (2018). Taxonomic notes on the Rhynchosia densiflora group (Phaseoleae , Fabaceae) in South Africa and its segregation from Rhynchosia section Arcyphyllum. *Bothalia - African Biodiversity & Conservation*, *48*(1), 1–10.

Jain, C., Khatana, S., & Vijayvergia, R. (2019). Bioactivity of secondary metabolites of various plants: A review. *International Journal of Pharmaceutical Sciences and Research*, *10*(2), 494–504.

Jaradat, N., Hussen, F., Ali, A., Alniss, H., & Dweikat, M. (2015). Phytoconstituents , Free Radical Scavenging Potential , Total Phenols and Total Flavonoids Assessments for Violet Horned Poppy from Jerusalem Mountains. *Journal of Material and Environmental Science*, *6*(10), 2958–2966.

Jaya, S., & Deval Rao, G. (2013). Evaluation of in vitro antioxidant activity of ethanolic extract of leaves of Rhynchosia beddomei Baker. *International Journal of Research in Pharmaceutical Sciences*, *4*(4), 569–579.

Jia, X., Zhang, C., Qiu, J., Wang, L., Bao, J., Wang, K., … He, C. (2015). Purification, structural characterization and anticancer activity of the novel polysaccharides from Rhynchosia minima root. *Carbohydrate Polymers*, *132*(7), 67–71.

Joseph, B., Kumbhare, P., & Kale, M. (2013). Preliminary phytochemical screening of selected Medicinal Plants. *Inernational Research Journal of Science and Enjineering*, *29*(2), 12–16.

Kamal, T., Muzammil, A., Akintunde, R., Rahma, M., & Omar, M. (2012). Preliminary phytochemical screening test of Garcinia griffithii Plant. *Innova Cienta*, *4*(4l).

Kamdem, W., Coombes, P., Mulholland, A., Nkengfack, E., & Fomum, T. (2005). Flavones and isoflavones from the west African Fabaceae Erythrina vogelii. *Phytochemistry*, *67*(5), 459–463.

Karande, A., Kamble, H., Kumbhar, V., Kane, S., Magdum, C., & Kane, S. (2016). Preliminary phytochemical screening of Glochidion ellipticum. *Europian Journal of Expermental Biology*, *6*(4), 41–45.

Kifle, T. (2016). Land Management Practices and Their Contribution to Livelihoods. *MSc THESIS*.

Kinjo, J., Nagao, T., Tanaka, T., Nonaka, G., & Okabe, H. (2001). Antiproliferative constituents in the plant 8. Seeds of Rhynchosia volubilis. *Biological and Pharmaceutical Bulletin*, *24*(12), 1443–1445.

Kumar, A., & Arvind, S. (2016). Medicinal plants: Future source of new drugs. *Nternational Journal of Herbal Medicine*, *4*(4), 59–64.

Li, S., & Xiang, Q. (2011). Chemical constituents in Rhynchosia volubilis. *Chin Trad Herbal Drugs*, *42*(2), 1276–1278.

Lima, N., Santos, V., Carli, A., & Soares, C. (2017). Genus Deguelia : Chemistry , Chemotaxonomy , Ethnopharmacology and Pharmacological Characteristics – A Review. *The Pharmaceutical and Chemical Journal*, *4*(5), 13–26.

Lock, J. (1990). *legumes of africa check list*.

Mansourian, M., Fassihi, A., Saghaie, L., Madadkar-S, Mahnam, K., & Abbasi, M. (2015). QSAR and docking analysis of A2B adenosine receptor antagonists based on non-xanthine scaffold. *Medicinal Chemistry Research*, *24*(1), 394–407.

Morris, D, G., R, H., R, H., W, H., & Belew, K. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, *19*(14), 1639–1662.

Moteetee, A. N., Boatwright, J. S., & Jaca, T. P. (2012). Short communication A review of Rhynchosia section Cyanospermum (Phaseoleae , Fabaceae) in South Africa. *South African Journal of Botany*, *81*, 124–127.

Muhammad, N., Uddin, N., & Ali, N. (2019). Ethnomedicinal Uses and Inter Specific Diversity Encourage Conservation of Rhynchosia species Growing in Hilly Areas of Swat. *Agricultural Research & Technology: Open Access Journal*, *20*(2), 0096–00107.

Nwodo, O., & Nkwocha, P. (2015). Qualitative and Quantitative Phytochemical Screening of the Aqueous Leaf Extract of Senna mimosoides : Its Effect in in vivo Leukocyte mobilization induced by inflammatory stimulus. *International Journal of Current Microbiology and Appliied Science*, *4*(5), 1176–1188.

Nyandoro, S., Munissi, J., Kombo, M., Mgina, C., Pan, F., Gruhonjic, A., Erdélyi, M. (2017). Flavonoids from Erythrina schliebenii. *Journal of Natural Products*, *80*(2), 377–383.

Proestos, C., Lytoudi, K., Mavromelanidou, O., Zoumpoulakis, P., & Sinanoglou, V. (2013).

Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants*, *2*(6), 11–22.

Qaiyum Ansari, A., Abrar Ahmed, S., Waheed, M., & Juned, S. (2013). Extraction and determination of antioxidant activity of Withania somnifera Dunal. *Pelagia Research Library European Journal of Experimental Biology*, *3*(5), 502–507

Rahman, A., & Parvin, M. (2014). Study of Medicinal Uses on Fabaceae Family at Rajshahi, Bangladesh. *Research in Plant Sciences*, *2*(1), 6–8.

Rammohan, A., Bhaskar, B., Venkateswarlu, N., Rao, V., Gunasekar, D., & Zyryanov, G. (2019). Isolation of flavonoids from the flowers of Rhynchosia beddomei Baker as prominent antimicrobial agents and molecular docking. *Microb Pathog*, *136:*, 103667.

Rammohan, A., Gunasekara, D., Reddyb, N., Vijayab, T., Devillec, A., & Bodo, B. (2015). Structure Elucidation and Antioxidant Activity of the Phenolic Compounds from Rhynchosia suaveolens. *Natural Product Communications Structure*, *10*(4), 609–611.

Rangaswamy, N., Krishnamurthy, H., & Rawat, M. (1974). Proanthocyanidins in seeds of the leguminous weed, Rhynchosia minima. *Current Science*, *5*(4), 1827–1837.

Rao, K., & Gunasekar, D. (1998). C-prenylated dihydroflavonol from Rhynchosia densiflora. *Phytochemistry*, *48*, 1453–1455.

Rondo, M. (2017). Phytochemical and biological studies on some south african plants used in traditional medicine for skin hyperpigmentation. (Vol. 3).

Rungsung, W., Ratha, K., Dutta, S., Dixit, A., & Hazra, J. (2015). Secondary Metabolites of Plants In Drugs Discovery. *World Journal of Pharmaceutical Research*, *4*(7), 604–613.

Santhi, K., & Sengottuvel, R. (2016). Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*, *5*(1), 633–640.

Sekar V, Gopal, V., & Devanna, N. (2019). Gc-Ms Analysis, In-Vitro Anti-Diabetic and Anti-Oxidant Activities of Rhynchosia nummularia (L.) DC. *Inernational Research Journal of Pharmaceutical*, *10*(3), 237–241.

Sheel, R., Nisha, K., & Kumar, P. J. (2014). Preliminary Phytochemical Screening of Methanolic Extract Of Clerodendron infortunatum. *IOSR Journal of Applied Chemistry*, *7*(1), 10–13.

Surekha, J., Rajam, K., Kasapu, V., Vishnu, S., Tanneeru, S., Visakhapatnam, … Kantamreddi, H. (2011). Antibacterial properties of an Indian traditional medicinal plant, Rhynchosia scarabaeoides (L.) DC (Fabaceae. *Alternative Medicine Studies*, *1*(17).

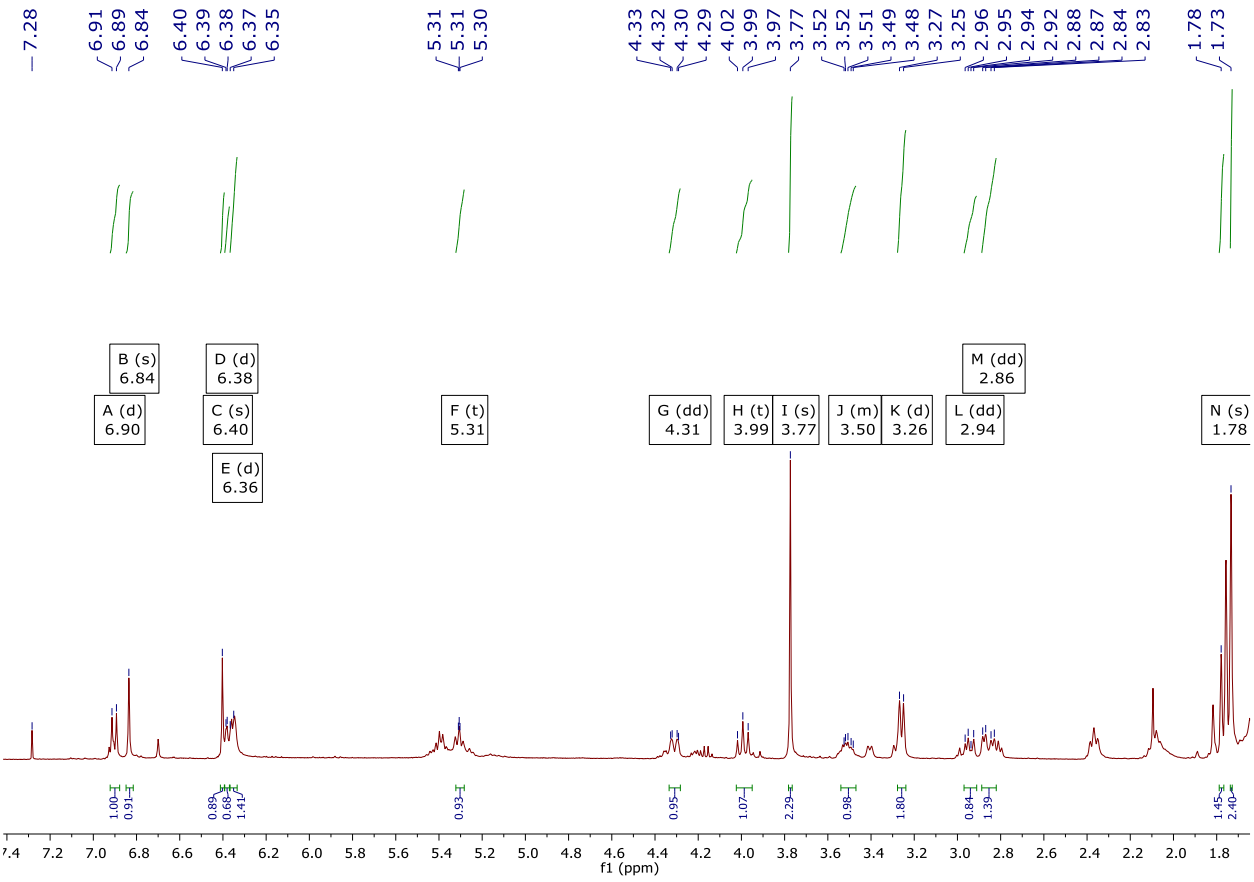
Tanaka, H., Oh-Uchi, T., Etoh, H., Shimizu, H., & Tateishi, Y. (2002). Isoflavonoids from the roots of Erythrina poeppigiana. *Phytochemistry*, *60*(8), 789–794.

Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). *South African Journal of Botany*, *89*(7), 164–175.

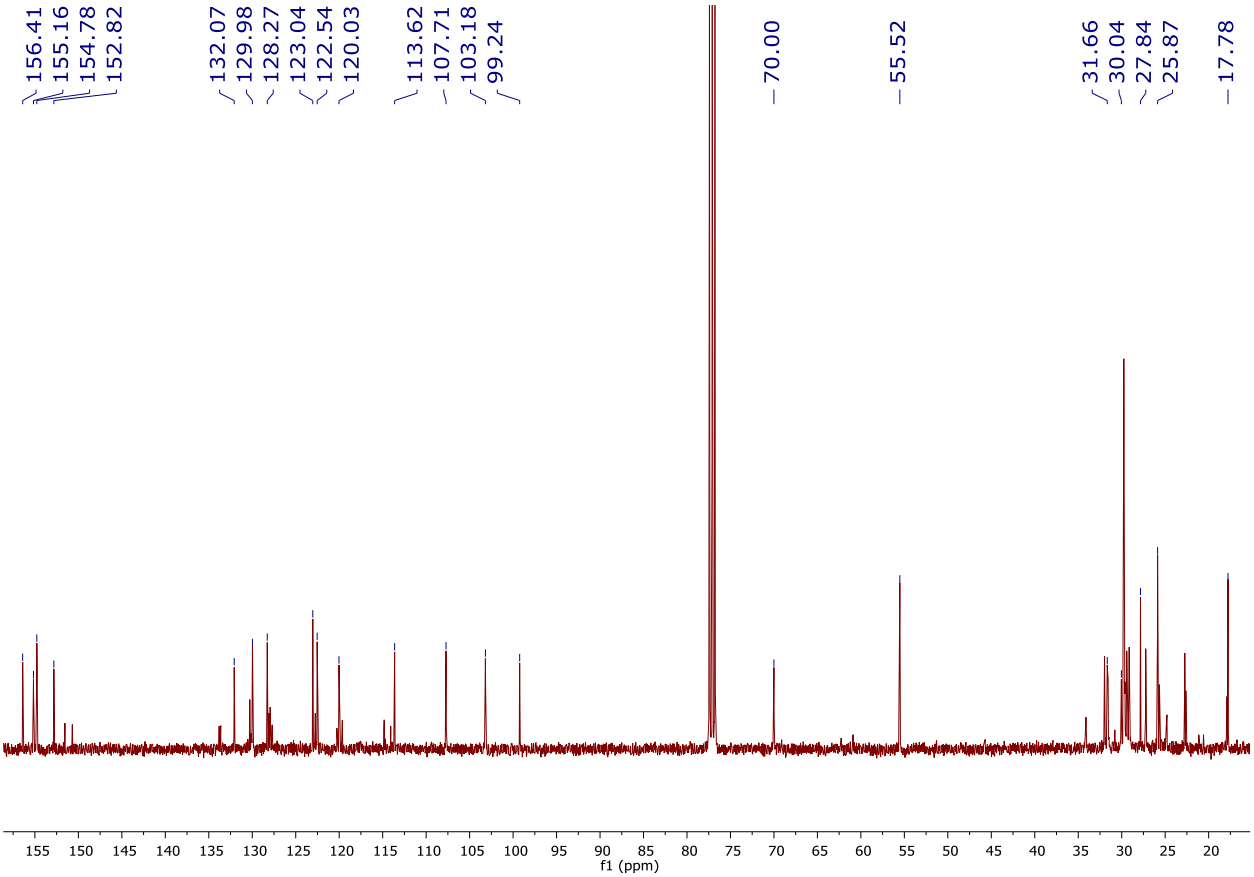
Wink, Michael. (2015). Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines*, *2*(3), 251–286.

APPENDIXES

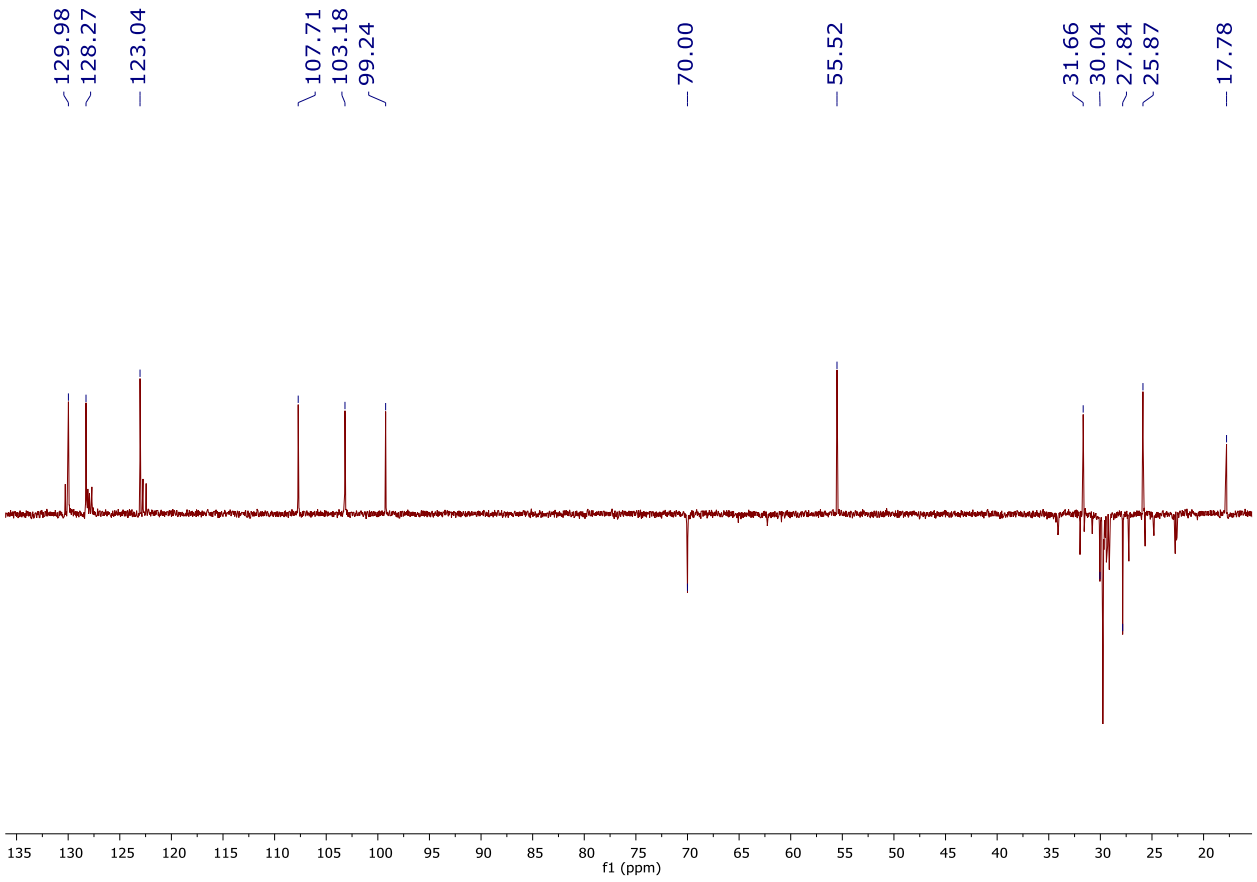
Appendix 1:1 The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **55**



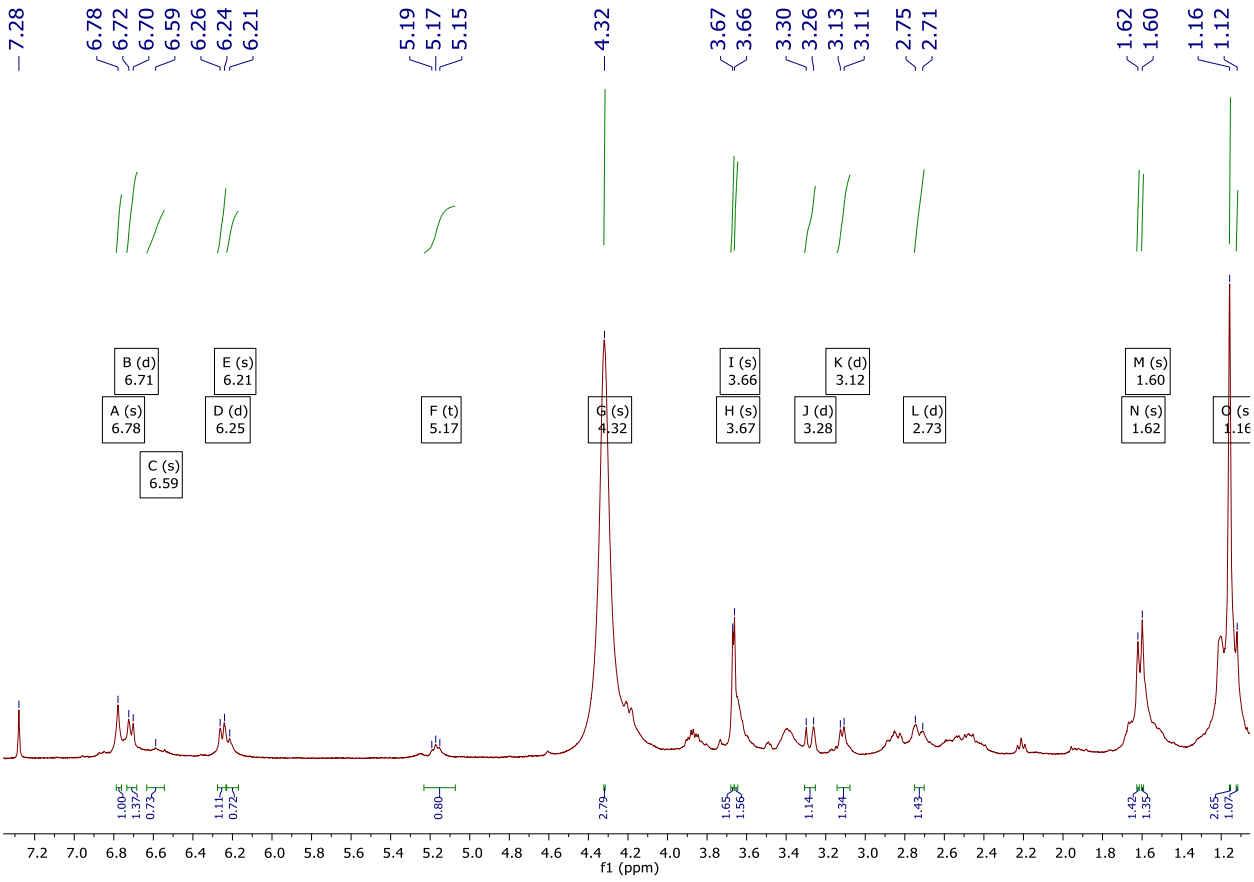
Appendix1.2. The ^{13}C-NMR spectrum (101 MHz, $CDCl_3$) of compound **55**



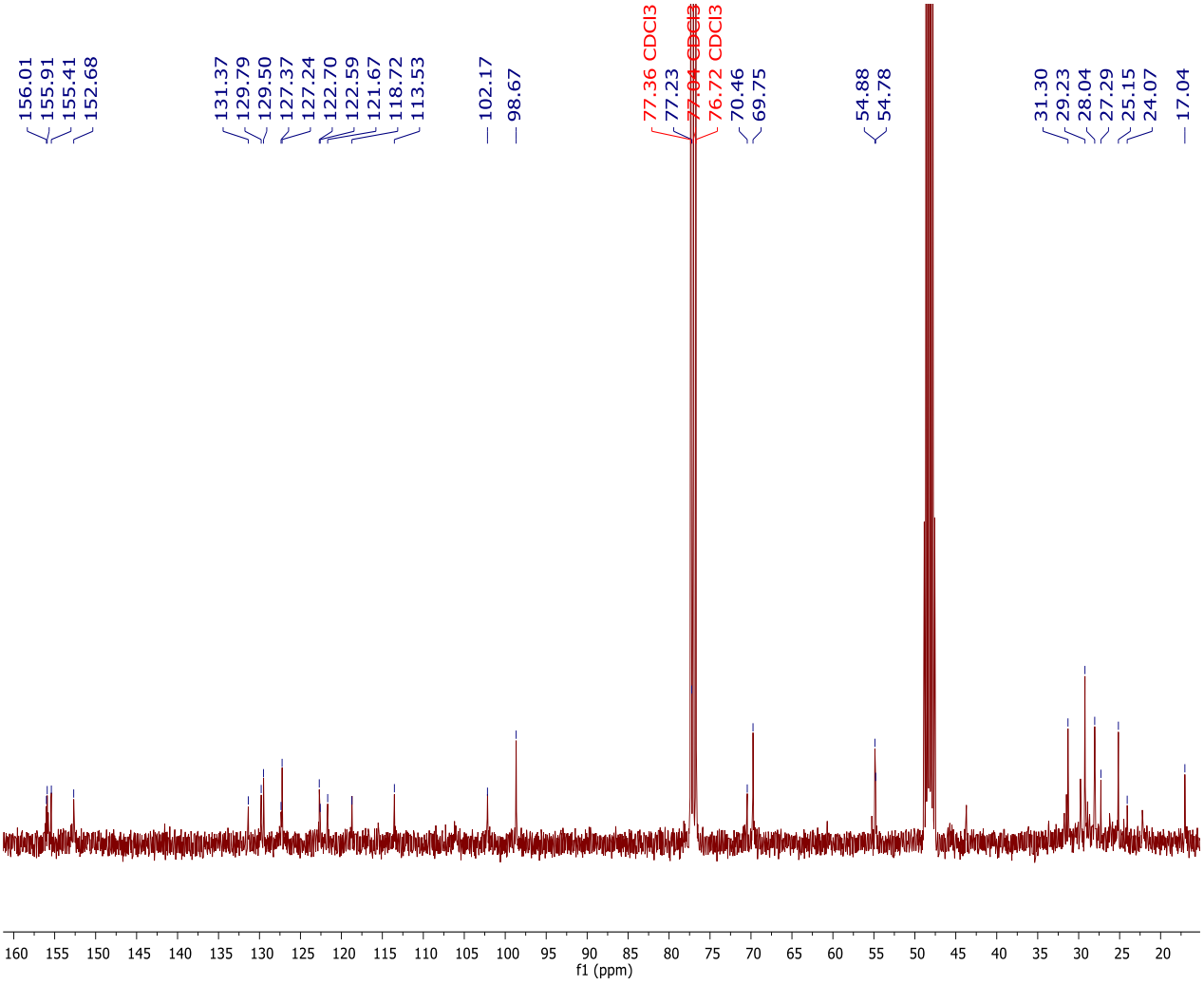
Appendix 1.3. The DEPT-135 spectrum (101 MHz, $CDCl_3$) of compound **55**



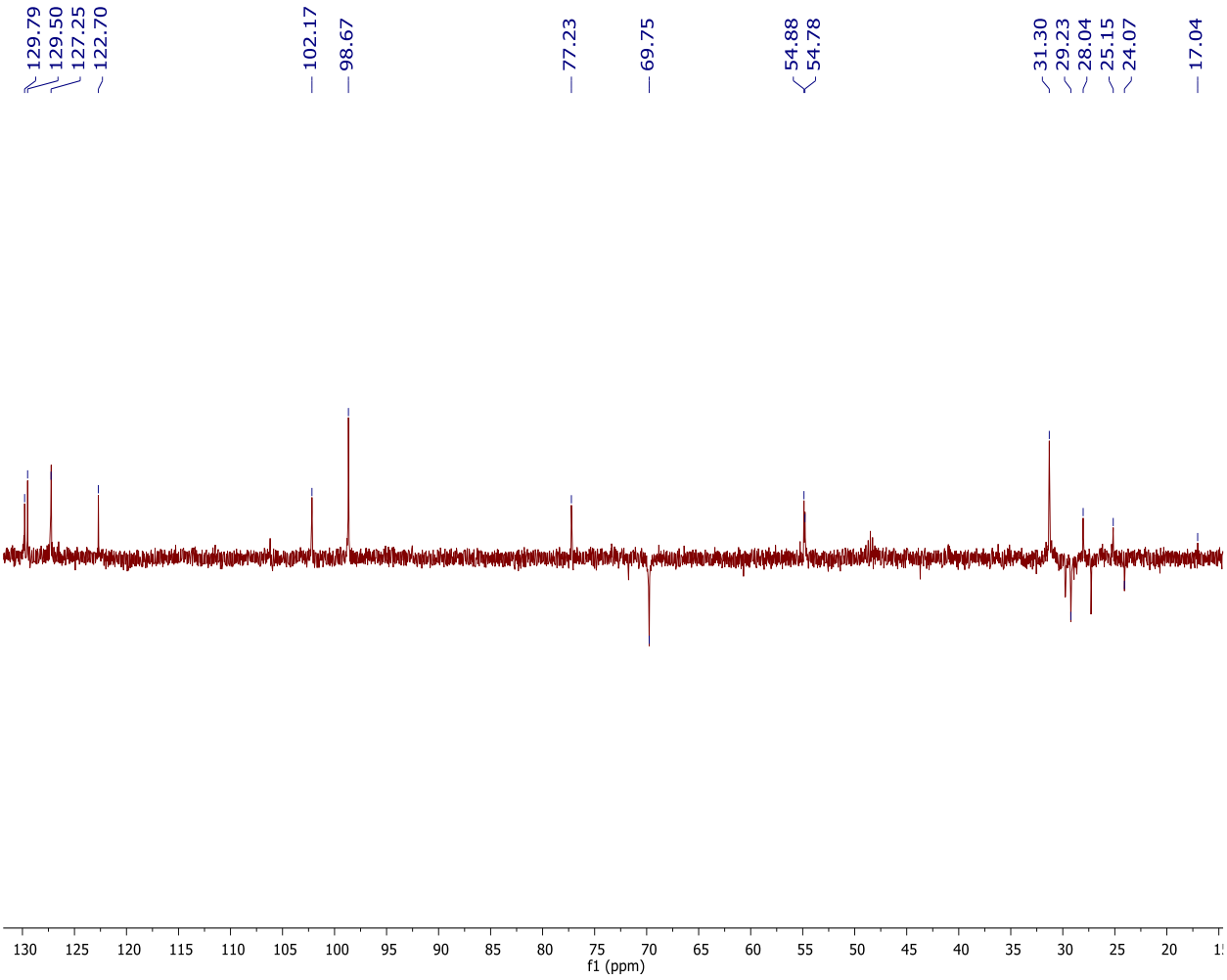
Appendix 2.1. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **56**



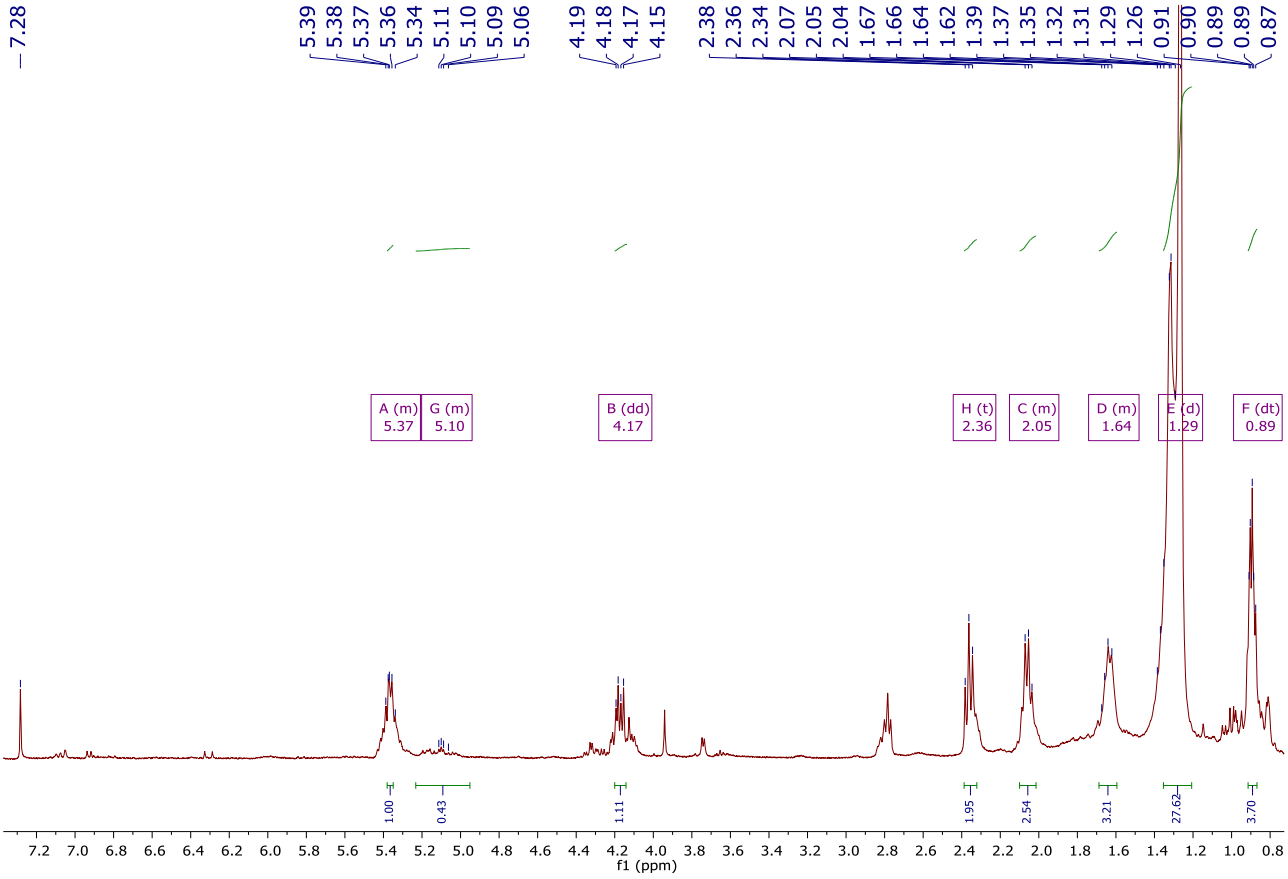
Appendix 2.2. The ^{13}C-NMR spectrum (101 MHz, $CDCl_3$ and CD_3OD) of compound **56**



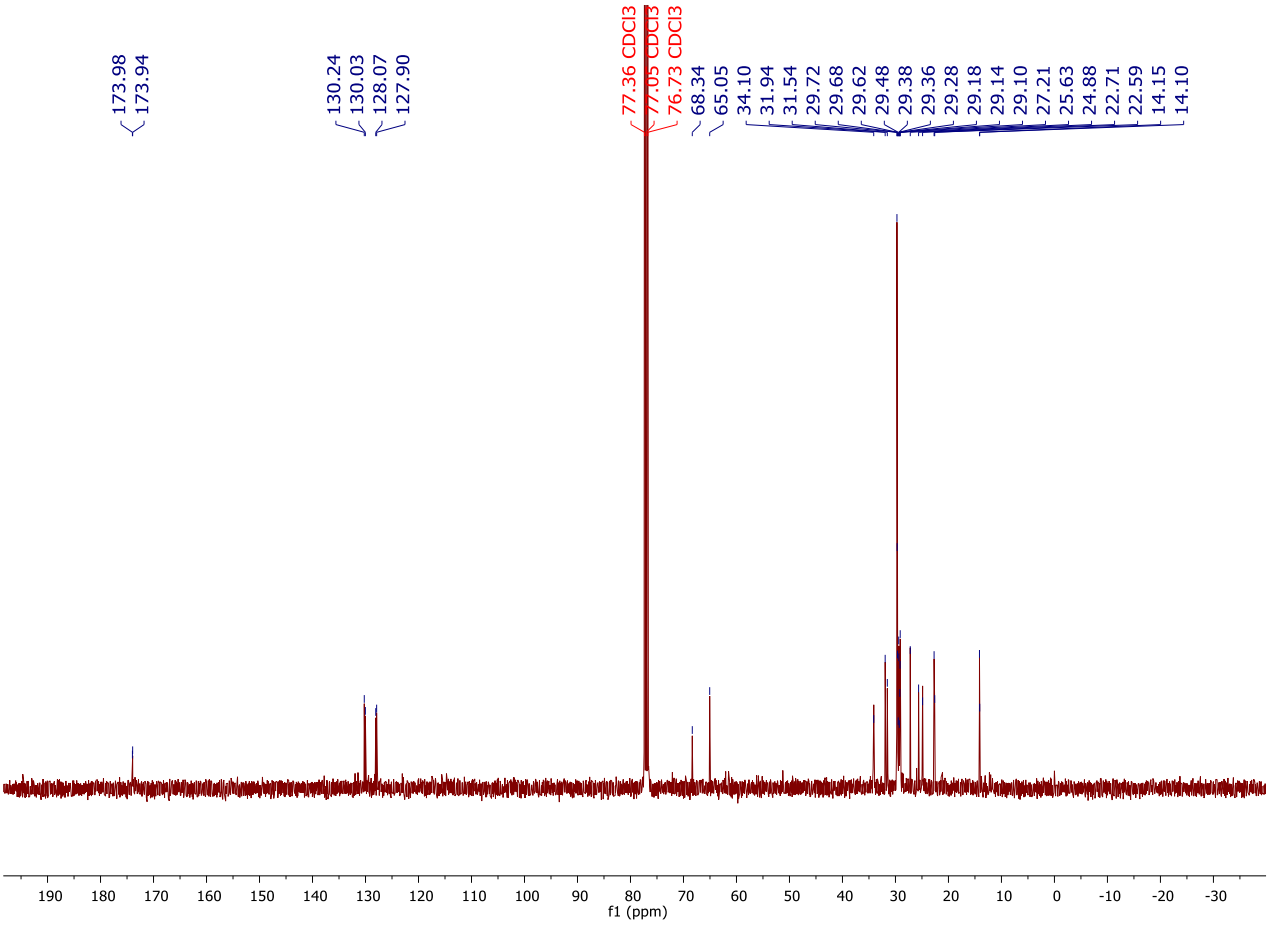
Appendix 2.3. The DEPT-135 spectrum (101 MHz, $CDCl_3$ and CD_3OD) of compound **56**



Appendix 3.1. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of compound **57**



Appendix 3.2. The ^{13}C-NMR spectrum (101 MHz, $CDCl_3$) of compound **57**



Appendix 3.3. The DEPT-135 spectrum (101 MHz, $CDCl_3$) of compound **57**