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Research Article

Antibacterial and Antioxidant Activity of Isoflavans from the Roots of *Rhynchosia ferruginea* and *In Silico* Study on DNA Gyrase and Human Peroxiredoxin

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Abstract: *Rhynchosia ferruginea* (Udusalim, Afan Oromo) is a medicinal plant traditionally used to treat skin infection, intestinal problems and amoebiasis. Silica gel chromatographic separation of dichloromethane/methanol (1:1) roots extract yielded isoflavan (**1**), isoflavene (**2**) and 1, 3-dilinoleoyl-2-stearoylglycerol (**3**), reported herein for the first time from the genus. Antibacterial activity was examined using disc diffusion method against *E. coli, S. aureus, P. aeruginosa* and *S. pyogenes.* AutoDoc vina 4.2 software was used for molecular docking analysis of compounds against human peroxiredoxin 5 and DNA gyrase B enzymes. Isoflavan (**1**) displayed zone of inhibition of 9.67 ± 0.58 mm and 10.67 ± 0.58 mm whereas isoflavene (**2**) showed 10.33 ± 1.15 mm and 10 ± 1.00 mm against *E. coli* and *S. aureus*, respectively, compared to ciprofloxacin (15.67±0.58 mm for both strains). DPPH radical scavenging assay of the dichloromethane/methanol (1:1) roots extract and isoflavan (**1**) exhibited better radical scavenging activity with IC_{50} value of 17.7 and 32, respectively. Molecular docking analysis revealed that **1** and **2** exhibited similar binding affinity of -7.4 kcal/mol compared to ciprofloxacin (-7.3 kcal/mol). *In silico* analysis against human peroxiredoxin 5 (PDB ID: 1HD2) revealed minimum binding affinity of - 3.7 and - 2.0 kcal/mol for compounds **1** and **2**, respectively, compared to ascorbic acid (-4.9 kcal/mol). The *in vitro* antibacterial and antioxidant activity of compounds **1** and **2** suggest the potential use of these compounds as drug lead candidates which corroborate with the traditional uses of the roots of *R. ferruginea*.

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1. INTRODUCTION

From the very beginning of human existence man has familiarized himself with plants and used them in a variety of ways throughout the ages. The relationship between plants and man led to the discovery of many plants used as medicines (Petrovska, 2012). Out of 255 drugs which are considered as basic and essential by the World Health Organization (WHO), 11% are obtained

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from plants and a number of synthetic drugs are also obtained from natural precursors (Joseph*et al*., 2013).A huge number of natural product derived compounds in various stages of clinical development highlighted the existing viability and significance of the use of natural products as sources of new drug candidates, chemical models or templates for the design, synthesis, and semi synthesis of novel substances for treating humankind's diseases.

The genus*Rhynchosia* consists of approximately 300 species circulated throughout the tropical and subtropical areas around the world (Rungsung*et al*., 2015). The most evident substances in *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, antiinflammatory, antifungal, antibacterial and antiproliferative (Muhammad*et al*., 2019). *Rhynchosia ferruginea* A. Rich (Figure 1) is known with common name Udusalim (Afan Oromo) by traditional healers in Barbare district Bale Zone Oromia region of Ethiopia used for treatment of different ailments including skin infection, wound, stomachache and amoebiasis (Jima andMegersa, 2018).

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

2. MATERIALS and METHODS

2.1 Plant Material Collection and Identification

The plant materials were collected from Bale Zone, Berbere district, which is located at about 526 km to southeast of Addis Ababa. Berbere district is situated between 06°33'N and 06°75'N and 039°40'E and 040°29'E. The plant material was identified with the help of a botanist (MelakuWondaferash) and voucher specimen (number KH-001) was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

2.2 Instruments Used

Column chromatography was carried out using silica gel 60-120 mesh ASTM. TLC was performed using precoated aluminum-backed supported silica gel 60 F254 (0.3 mm thickness). Melting point was measured in glass capillaries using Thiele tube. TLC visualization was done using UV lamp at 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 Tetramethylsilane (TMS) was used as an internal standard.

2.3. Extraction and Isolation

The collected roots of *R. ferruginia* were air dried at room temperature and grounded into powder using grinding mills. The grounded roots (500 g) of *R. ferruginia* were extracted with 2.5 L dichloromethane/methanol (1:1) for 72 h with occasional shaking at room temperature. The solution was filtrated and concentrated by a vacuum rotary evaporator at 50 ºC to afford 24.48 g (4.89%) dark red crude extract. The marc left was further extracted with methanol (2 L) for 72 h with occasional shaking at room temperature. The solution was filtered and concentrated by a vacuum rotary evaporator at a temperature of about 50 ºC to afford 17.55 g (3.51%) dark red crude extract. The dichloromethane/methanol (1:1) crude extract (14g) was adsorbed on 14 g of silica gel (mesh size 60-120) and subjected to silica gel column chromatography (180 g of silica gel, using *n*-hexane for packing) and eluted with increasing gradient of ethyl acetate in *n*-hexane followed by methanol in ethyl acetate. A total of 115 fractions each 100 mL were collected. The purity of each fraction was checked by using TLC. Fractions that showed similar R_f values and the same characteristic color on TLC (visualized in UV lamp at 254 and 356 nm) were combined. Fraction 28 showed single spot on TLC (EtOAc/*n*-hexane as eluent, 3:7, R_f= 0.44, 40 mg) to afford compound **3**. Fractions 35-38 showed single spot with similar R_f values to afford isoflavan (**1**) (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 isoflavene (**2**) (EtOAc/*n*-hexane1:1 as eluent, R_f=0.43, 50 mg).

2.4. 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, Ethiopia. *In vitro* antibacterial activity of crude extracts and isolated compounds was 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 compounds of *R. ferrugenia*were placed on the surface of the inoculated and dried plate. After 24 h of incubation at 37 °C, thediameter of the zones of growth inhibition around the disks was measured with a ruler and compared with ciprofloxacin. The experiments were done in triplicates and results were presented as mean value ± standard deviation.

2.5. 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 radicalswhich 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 antioxidative activity (Ansari*et al*., 2013). Four different concentrations of the samples and positive control (1 mL each), ascorbic acid (100, 50, 25 and 12 µg/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 37°C for 30 min and subjected to UV-Vis spectrophotometer to record absorbance at 517 nm.

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

$$\% \text{ of } I = [(A_{control} - A_{sample})/A_{control}] \times 100$$

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 min).

2.6. Molecular Docking Study of Compounds

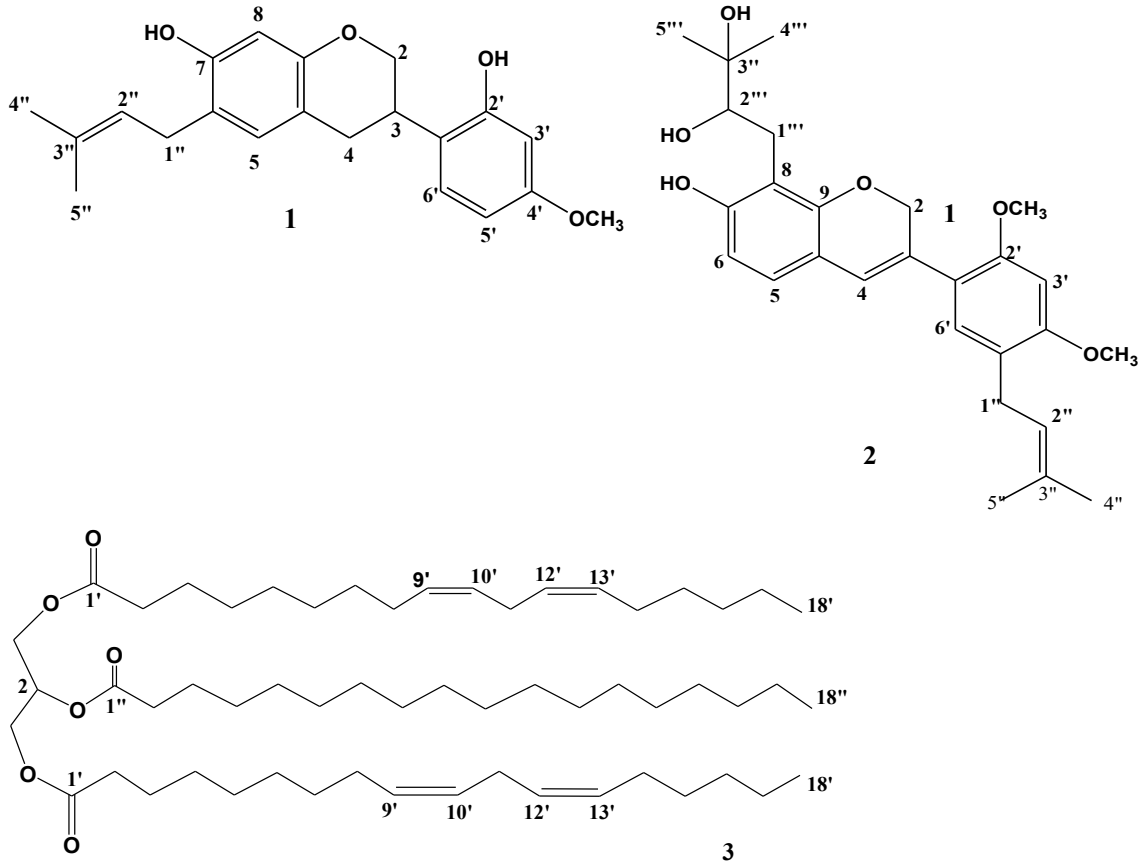
To have a better understanding about the inhibitory mechanism as well as the mode of interactions of the phytochemical compounds of the crude extract, molecular docking analysis was accomplished using the ADT version 1.5.2 and AutoDock vina version 4.2 docking program. The crystal structure of receptor molecule *E. coli* gyrase B (PDB ID: 6F86) and human peroxiredoxin (PDB ID 1HD2) were downloaded from protein data bank. The protein preparation was done using the reported standard protocolby removing the co-crystallized ligand, selected water molecules and cofactors, the target protein file was prepared by leaving the associated residue with protein by using auto preparation of target protein file AutoDock 4.2 (MGL tools1.5.7) (Eswaramoorthy *et al*., 2021). The graphical user interface program was used to set the grid box for docking simulations. The grid was set so that it surrounded the region of interest in the macromolecule. The docking algorithm provided with AutoDock Vina 4.2 was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of nine conformers were considered for each ligand. The conformations with the most favorable (least) free binding energy were selected for analyzing the interactions between the target receptor and ligands by discovery studio visualizer and PyMOL. The ligands are represented in different color and H-bonds and the interacting residues are represented in ball and stick model representation (Mansourian*et al*., 2015; Galma *et al*., 2021; Anza *et al*., 2021).

3. RESULTS and DISCUSSION

The root extracts of *R. ferruginea* after silica gel column chromatography furnished isoflavan (**1**), isoflavene (**2**) and 1, 3-dilinoleoyl-2-stearoylglycerol (**3**, Figure 2), are reported herein for the first time from the genus. The detailed characterization of these compounds ispresented in Figure 2.

Compound **1**was isolated as yellow solid (R_f=0.52 using 50 % EtOAc in *n*-hexane as eluent, melting point of 136-137 ^{0}C). Its ^{1}H NMR spectrum (Table 1) displayed a set of aliphatic proton signals at δ4.31 (dd, *J* = 15.7, 5.3 Hz, H-2), 3.99 (m, H-3), 2.94 (dd, *J* = 15.9, 5.2 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 δ2.94 (dd, *J* = 15.9, 5.2 Hz) and 2.86 (dd, *J* = 15.7, 5.2 Hz) as diastereotopic protons in agreement with the presence of chiral center C-3 position. This observation was also supported by the carbon resonances at δ70.0, 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. A set of ortho-coupled aromatic protons with AB spin pattern at δ6.90 (H-6', d, *J*= 8 Hz) and δ6.36 (H-5', d, *J*= 8 Hz) and three aromatic protons having ABX spin pattern were observed atδ6.36 (H-5', d, *J*=8Hz), 6.90 (H-6', d, *J*=8Hz), 6.38 (H-3', d, *J* = 2.2 Hz). Signals at δ1.78 (H-5", s), 1.73 (H-4", s), 3.26 (H-1", d, J= 7.5) and5.31 (H-2", t) suggest the presence of a prenyl group.

Figure 2. Compounds isolated from the roots of *R. ferruginea*.



The ^{13}C NMR and DEPT-135 spectrum (Table 1) revealed the presence of eight quaternary carbons, four of which are sp^2 oxygenated quaternary aromatic carbons at δ 154.8 (C-7), 152.8 (C-9), 155.2 (C-2') and 156.4 (C-4'). Aromatic methine peaks observed at δ99.2(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 δ55.5 suggest the presence of methoxy moiety. Thus, based on the above spectral data and comparison with literature, compound **1** was found to be identical with that of 7, 2'-dihydroxy-4'-methoxy-6-(3", 3"-dimethylallyl) isoflavan (**1**) (Figure 2, Table 1) previously reported from *Glycyrrhizauralensis* (Fan *et al*., 2020). This is the first report of the compound from the genus *Rhynchosia*.

Compound **2** was isolated as yellow solid (R_f=0.43using 50 % EtOAc in *n*-hexane as eluent, melting point of 124-125 ^{0}C). Its ^{1}H NMR spectrum (Table 2) revealed signals atδ 4.44 (s, H-2) and 6.78 (s, H-4) which are characteristic signals of flavan-3-ene ring C skeleton. On the basis of literature precedence, the signals at δ2.73 (H-1'"), δ 3.28 (H-2'"), 1.16 (H-4'") and 1.12 (H-5'") with corresponding carbon signals at δ70.5 (C-3'") and δ77.2 (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 δ1.62 (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).

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

Position	**1**			Fan *et al*., 2020	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
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*= 4.8,10.4 Hz); 3.86 (t, *J*=10.0 Hz)	69.1
3	3.50 (m)	31.7	-	3.30 (m)	31.2
4 ax 4 eq	2.94 (dd, *J* =15.9, 5.2 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, 1H)	129.7
6	-	122.5	-	-	119.8
7	-	154.8	-	-	153.6
8	6.40 (s, 1H)	99.2	99.2	6.23 (s, 1H)	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 (d, *J*= 8 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*=7.2 Hz)	27.4
2"	5.31 (t, 1H)	123.0	123.0	5.23 (t, 1H)	123.1
3"	-	132.0	-	-	130.4
4"	1.73 (s, 3H)	17.8		1.67 (s, 3H)	17.6
5"	1.78 (s, 3H)	25.9		1.73 (s, 3H)	25.5
OCH_3	3.77 (s, 3H)	55.5		3.66 (s, 3H)	54.8

The ^{13}C NMR and DEPT-135 spectra (Table 2) revealed the presence of eleven quaternary carbons, four of which are sp^2 oxygenated aromatic quaternary carbons at δ 155.4 (C-7), 152.7 (C-9), 156.0 (C-2'),155.9(C-4') and sp^3oxygenated quaternary carbon at δ 70.5 (C-3'"). The peak at δ77.23 suggests sp^3 oxygenated methine (C-2'"). Aromatic non-oxygenated quaternary carbon appeared atδ113.5 (C-8), 118.7 (C-10), 121.7 (C-1') and 122.6 (C-5'). Non aromatic sp^2quaternary carbon appeared at δ127.4 (C-3) and 131.4 (C-3"). Aromatic methines were observed at δ129.5 (C-5), 102.2 (C-6), 98.7 (C-3') and 129.8 (C-6'). Non aromatic sp^2methines were observed at δ127.2 (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 δ69.8 suggest oxygenated methylene, pointing down in DEPT-135 spectrum, and peaks atδ54.9 and 54.8 suggest methoxygroups supported by DEPT-135 spectrum pointing up. From the above spectral data and comparison with literature (Tanaka *et al*., 2002), compound **2** was identified and labeled as 7-hydroxy-2', 4'-di-methoxy-8-(2'",3'"-dihydroxy-3'"-methylbutyl)-5'-(3",3"-dimethylallyl) isoflav-3-ene (**2**) (Figure 2, Table 2).

Table 2. ^{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 (**2**) in $CDCl_3$.

Position	**2**			Tanaka *et al*., 2002	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
1	-	-	-	-	-
2	4.44 (s, 2H)	69.8	69.8	4.97 (s, 2H)	68.4
3	-	127.4	-	-	128.9
4	6.78 (s, 1H)	127.2	127.2	6.5 (s, 1H)	121.9
5	6.71 (d, *J* = 9.0 Hz)	129.5	129.5	6.82 (d, *J*= 8.1 Hz)	125.0
6	6.25 (d, *J* =8.9 Hz)	102.2	102.2	6.40 (d, *J*= 8.1 Hz)	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, 1H)	98.7	98.7	6.42 (s, 1H)	99.2
4'	-	155.9	-	-	156.6
5'	-	122.6	-	6.41 (dd, *J*=8.1,2.2 Hz)	107.3
6'	6.59 (s, 1H)	129.8	129.8	7.1 (d, *J*=8.1 Hz)	129.4
2 x OCH_3	3.67 (s, 3H) 3.68 (s, 3H)	54.8 54.9	54.8 54.9	3.75 (s, 3H) -	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, 1H)	122.7	122.7	5.27 (t, *J*=7.3)	121.8
3''	-	131.4	-	-	134.5
4''	1.60	17.0	17.0	1.74	17.9
5''	1.62	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, 3H)	24.3
5'''	1.12 (s, 3H)	28.0	28.0	1.23 (s, 3H)	23.6

Compound **3** was obtained as white semi solid with R_f value of 0.44 (EtOAc/*n*-hexane, 3:7 as eluent) and melting point of 43-45 ^{0}C. Its ^{1}H NMR spectrum (Table 3) displayed peaks at δ0.90 and 0.89 suggesting the presence of two terminal methyl protons. The peaks at δ1.27 and δ 1.62 indicate protons of aliphatic methylene ($-CH_2$) group, a peak at δ2.06 indicates the presence of protons of a methylene group that is bonded to C=C bond and the triplet peak

atδ2.36 indicates the presence of methylene protons α to a carbonyl group. The glycerol moiety exhibited doublet of doublets at δ4.17 for oxygenated methylene protons H-1, 3 and a multiplet at δ5.10 attributed to oxygenated methine proton H-2 whereas olefinic protons were observed at δ5.37 as multiplet.

The ^{13}C NMR and DEPT-135 spectra (Table 3) revealed the acyl function attributed to esters carbonyls at δ174.0 and 173.9, oxygenated methylene at δ65 pointing down in DEPT-135 spectrum, and oxygenated methine at δ68, pointing up in DEPT-135 spectrum. Four sp^2 methines are clearly evident at δ130.2, 130.0, 128.1 and 127.9. Overlapped methylene peaks are observed at δ28-29 and two terminal methyl carbons appeared at δ14.1 and 14.2. From the above spectral data the compound was found to be triglyceride compound named as 1, 3-dilinoleoyl-2-stearoylglycerol (**3,** Figure 2) by relating with a compound previously reported from plant *Moringastenopetala* (Bekele *et al*., 2013).

Table 3. ^{1}H, ^{13}C and DEPT-135 NMR spectral data of 1, 3-dilinoleoyl-2-Stearoyl glycerol (**3**) in $CDCl_3$.

Position	**3**			Bekele *et al*., 2013	
	^{1}H NMR	^{13}C NMR	DEPT-135	^{1}H NMR	^{13}C NMR
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"	1.30	22.7	22.7	1.25	22.7
5', 5"	1.30	22.6	22.6	1.25	22.7
6', 6"	1.30	24.9	24.9	1.25	24.9
7', 7"	1.30	24.9	24.9	1.25	24.9
8', 8"	2.05	27.2	27.2	2.03	27.2
9', 9"	5.37 (m)	130.9, 29.7	130.9, 29.7	5.40	129.7, 130.2
10', 10"	5.37 (m)	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"	5.37 (m)	130.0, 29.4	130.0, 29.4	5.40, 1.25	127.9, 128.1
13',13"	5.37 (m)	127.9, 29.4	127.9, 29.3	5.40, 1.25	131.9, 130.2
14',14"	1.30	29.3	29.2	2.03, 1.25	27.2
15',15"	1.30	29.2	29.2	1.25	29.2–29.7
16',16"	1.30	29.1	29.1	1.25	29.2–29.7
17',17"	1.30	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

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 species *E. coli, S. aureus*, *P. aeruginosa* and *S. pyogenes*at two different concentration (1 mg/mL and 1.5 mg/mL) (Table 4). Compound **1** (1.5 mg/mL) displayed 9.67 ± 0.58 mm and 10.67 ± 0.58 mm zone of inhibition whereas compound **2** (1.5 mg/mL) 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 compounds **1** and **2** 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 **3** were found to be less than that of compound **1** and **2** with weak antibacterial activity against *E. coli* and *S. aureus*. The result obtained is comparable with previous report on essential oil of *Rhynchosia minima* displaying inhibition zone diameter of 18.2 and 12.5mm against *E. coli* and *S. aureus*, respectively, at concentration of 100 µg/mL (Gundidza*et al*., 2009).

Table 4. 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
1	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
2	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
3	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

SD = standard deviation

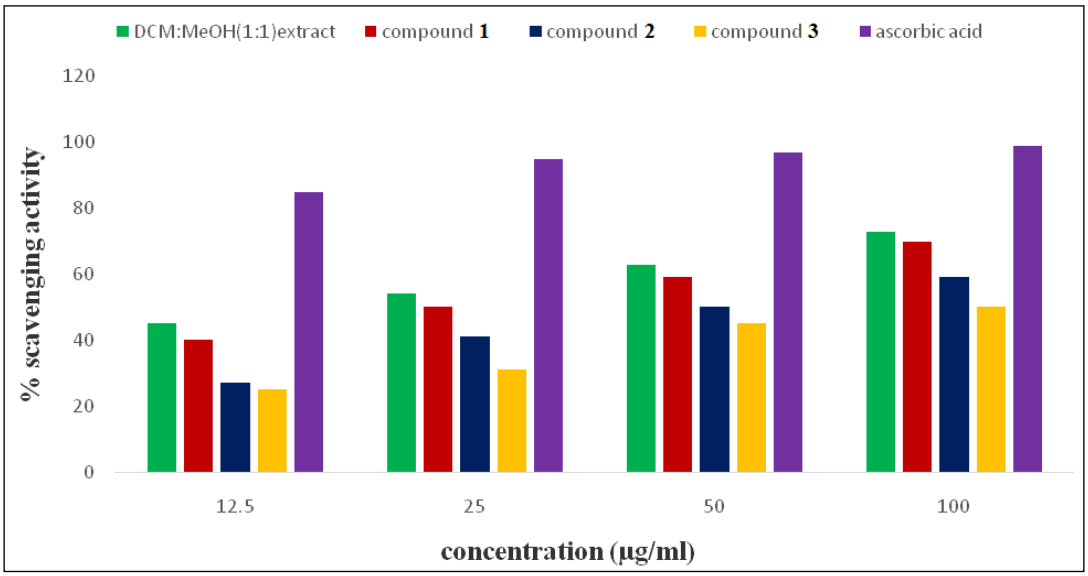
3.2. Antioxidant Activity

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 5, Figure 3). Compound **1**, DCM/methanol (1:1) extract, compound **2** and compound **3** displayed 70%, 73%, 59% and 50 % inhibition, respectively, at concentration of 100 µg/mL. The positive control, ascorbic acid (Figure 3) showed maximum scavenging effect from very low to high concentration (85, 93, 97 and 99 % at 12, 25, 50 and 100 µg/mL, respectively). Overall, the dichloromethane/methanol (1:1) extract and compound **1** displayed promising radical scavenging activity with IC_{50} of 17.7 and 32, respectively.

Table 5. Radical scavenging activity of DCM/MeOH (1:1) extract and compounds (**1**-**3**).

Concentration	DCM/MeOH(1:1) extract		**1**		**2**		**3**	
	% scavenging activity	IC_{50}	% scavenging activity	IC_{50}	% scavenging activity	IC_{50}	% scavenging activity	IC_{50}
12.5	45	17.7	40	32	27	64.5	25	90.6
25	54		50		41		31	
50	63		59		50		45	
100	73		70		59		50	

Figure 3. DPPH scavenging activity (%) of DCM/MeOH (1:1) extract and compounds (**1-3**).



3.3. Molecular Docking Study Against DNA Gyrase B (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. Compounds**1**and **2** (Table 6) displayed similar binding affinity of -7.4 kcal/mol and exhibit hydrogen bond with three active site amino acid residue compared to ciprofloxacin -7.3 kcal/mol (Table 6, Figure 7). Compound **1**binding conformation within the active pocket of PDB ID 6F86 exhibits four hydrogen bonding interaction. The green dash lines indicates a possible hydrogen bond formed between the connections residues and pink lines indicated hydrophobic bond interaction (Figure 4).

Table 6. Molecular docking results of compounds (**1-3**) against *E.coli* DNA gyrase B (PDB ID 6F86).

Ligands	Affinity (kcal/mol)	Amino acid residue form H-bond with ligands	Residual Pi-Alkyl Hydrophobic/Pi-Cation/Pi-Anion/ interactions
1	-7.4	Asp-73, Asn-46, Val-71	Ile-78, Glu-50, Thr-165, Pro-79, Ala-47, Val-43, Val-167, Gly-77, Arg-136
2	-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
3	-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 **2** showed hydrogen bond interaction with three active site amino acid residue Asp-73, Asn-46, and Thr-165 (Table 7). The hydrogen bond formed (Figure 5) 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 methyls of prenyl moiety attached to C-5' displayed hydrophobic interaction with Val-43, Ala-47, Val-167 and Val-120. Compound **3**showed binding affinity of -5.2 kcal/mol (Table 7). The oxygen atoms on esters functional group of compound **3** form hydrogen bonding with two amino acids Asn-46 and Val-120 (Figure 6). In this study, compounds **1** and **2** exhibited better docking efficiency with DNA gyrase and may act as potential inhibitors of DNA gyrase enzyme.

Figure 4. The 2D and 3D binding interactions of compound **1** against DNA gyrase B (PDB ID: 6F86).

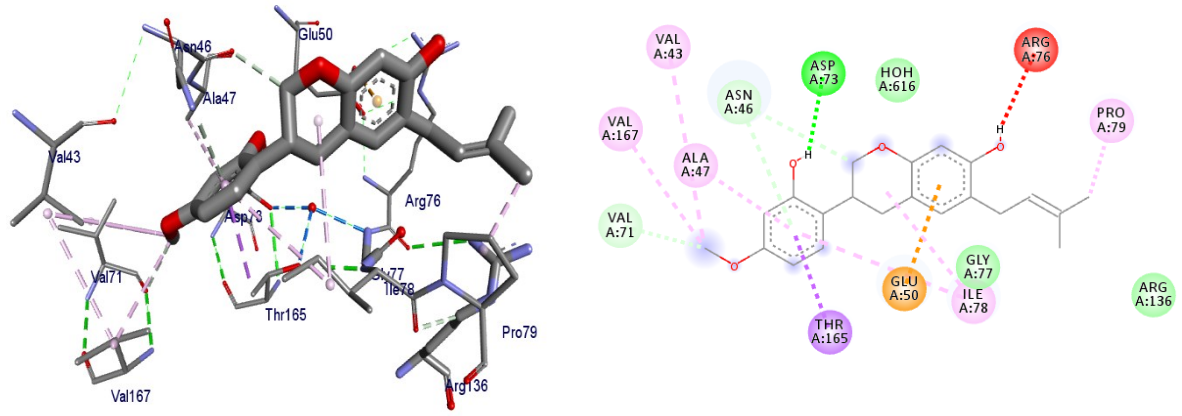


Figure 5. The 2D and 3D binding interactions of compound **2** against DNA gyrase B (PDB ID: 6F86).

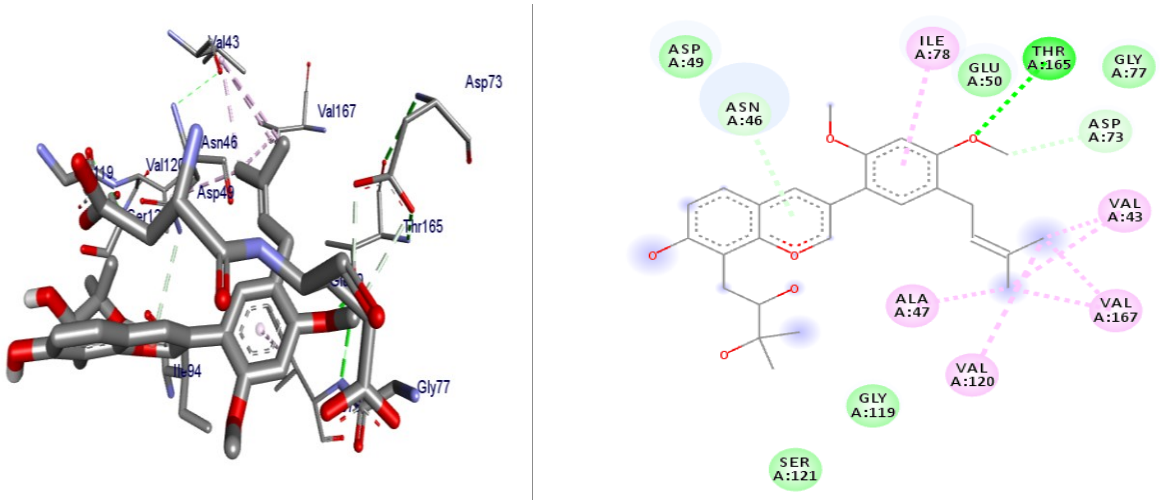
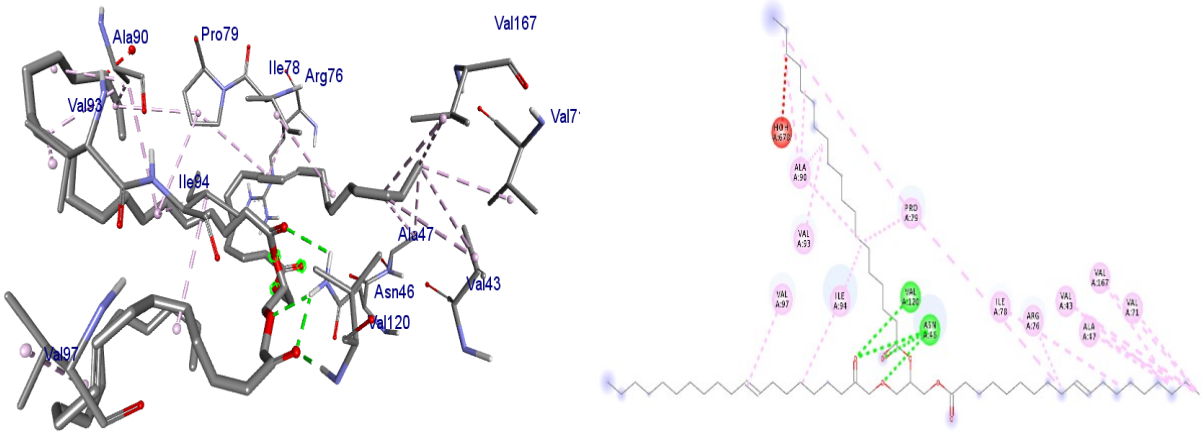


Figure 6. The 2D and 3D binding interactions of compound **3** against DNA gyrase B (PDB ID: 6F86).



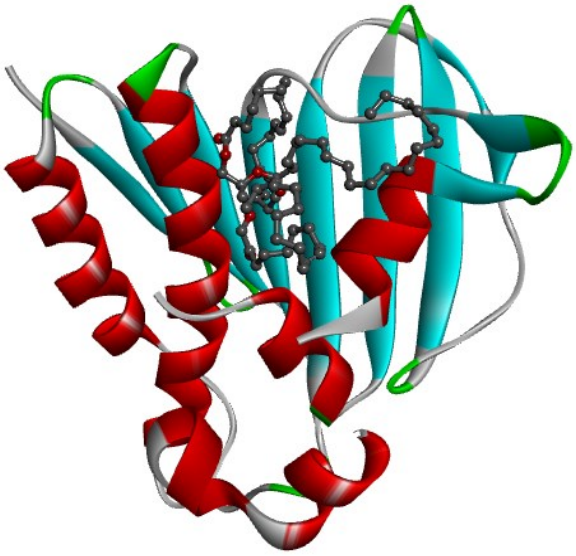


Figure 7. The 2D and 3D binding interactions of ciprofloxacin against DNA gyrase B (PDB ID:6F86).

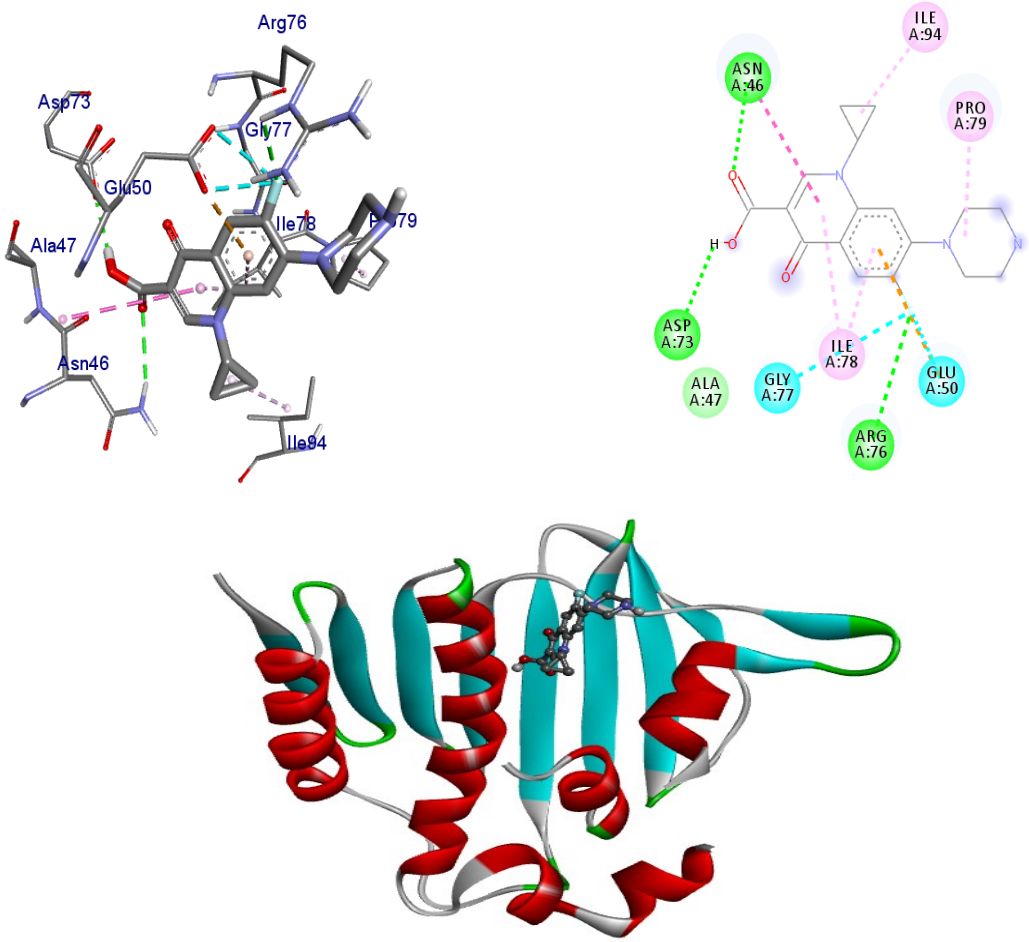
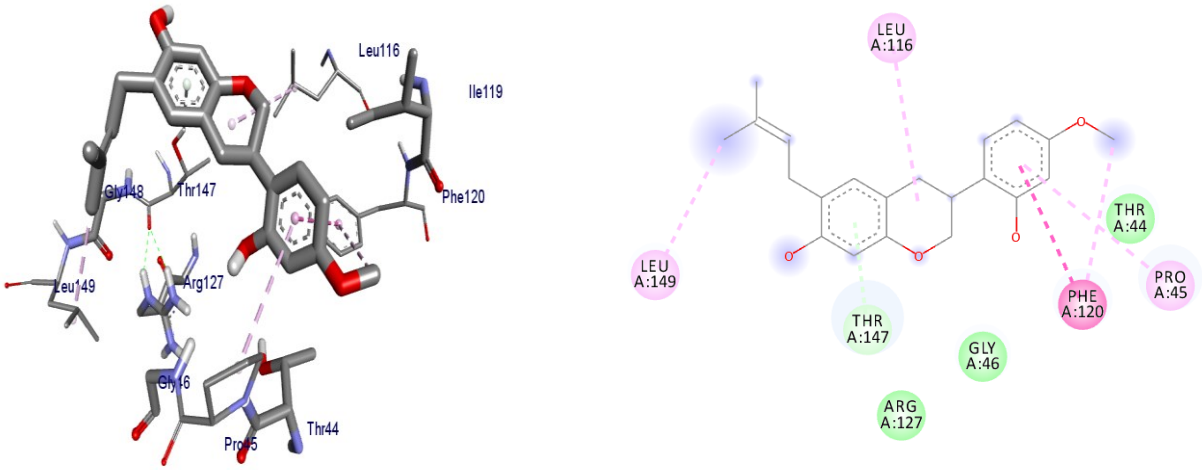


Figure 8. The 2D and 3D binding interactions of compound **1** against human peroxiredoxin 5 (PDB ID: 1HD2). Hydrogen bond between compounds and amino acids are shown as green dash lines, hydrophobic interaction are shown as pink lines.



3.4. Molecular Docking Binding Analysis Against Human Peroxiredoxin 5 (PDB ID:1HD2)

The molecular docking analysis of the isolated compounds was carried out to investigate their binding pattern with human peroxiredoxin 5 (PDB ID: 1HD2) and compared with the natural antioxidant ascorbic acid. Isoflavan (**1**) and Isoflavene (**2**) were found to have minimum binding energy of -3.7 and - 2.0 kcal/mol, respectively (Table 7). The molecular docking analysis also support the better docking affinity (-3.7 kcal/mol) for compound **1** within the binding pocket of human peroxiredoxin 5. The key amino acid residues within the active sites of peroxiredoxin 5 are Cys 47, Thr-44, Gly-46 and Thr-147. Compound **1** formed hydrogen bond interaction with Thr-147 (Figure 8) whereas compound **2** formed hydrogen bond interaction with Asp-145 and residual van darwaals interaction with residual amino acids Thr-147, Thr-44, Pro-45, Arg-127, Ile-119, Phe-120 (Figure 9, 10, Table 7).

Table 7. Molecular docking value of isolated compounds (**1** and **2**) and ascorbic acid against human peroxiredoxin (PDB ID: 1HD2).

Compounds	Affinity (kcal/mol)	H-bond	Residual Amino acid Interactions	
			Hydrophobic/Pi-Cation/Pi-Anion/ Pi-Alkyl interactions	Van-der Walls interactions
1	-3.7	Thr-147	Pro-45, Phe-120, Leu-116, Leu-149	Thr-44, Pro-40, Gly-46, Arg-127
2	-2.0	Asp-145	Cys-47, Pro-40, Leu-149	Thr-147, Thr-44, Pro-45, Arg-127, Ile-119, Phe-120
Ascorbic Acid	-4.9	Cys-47, Thr-44, Gly-46, Thr-147	Pro-40, Pro-45, Phe-120, Arg-127, Leu-149	--

Figure 9. The 2D and 3D binding interactions of compound **2** against Human peroxiredoxin 5 (PDB ID: 1HD2). Hydrogen bond between compounds and amino acids are shown as green dash lines, hydrophobic interaction are shown as pink lines.

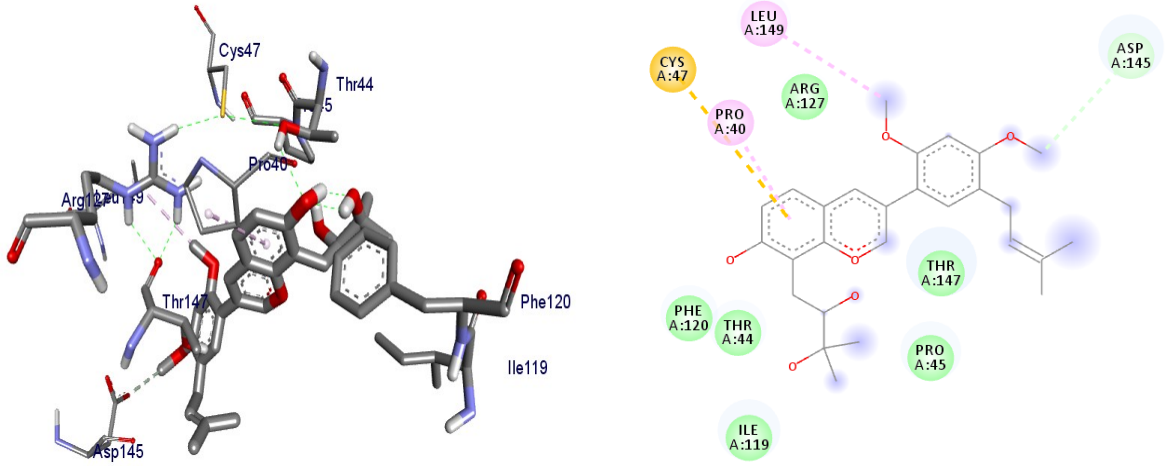
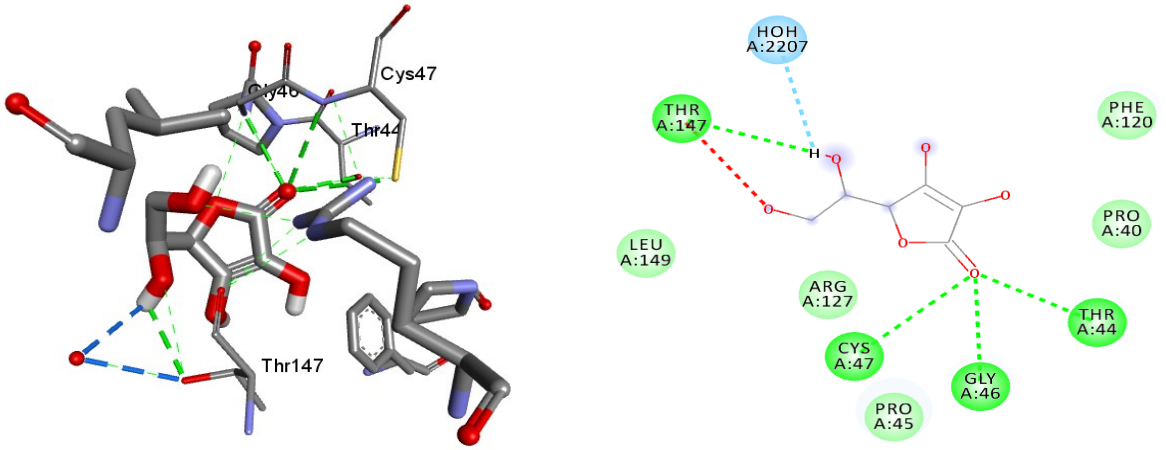


Figure 10. The 2D and 3D binding interactions of compound Ascorbic Acid against Human peroxiredoxin 5 (PDB ID: 1HD2). Hydrogen bond between compounds and amino acids are shown as green dash lines; hydrophobic interaction are shown as pink lines.



4. CONCLUSION

*R. ferrugenia*is one of traditional medicinal plants used by local traditional healers for treatment of skin infection, wound, stomachic and amoebiasis. Silica gel column chromatographic analysis of dichloromethan/methanol (1:1) extract furnished 7,2'-dihydroxy-4'-methoxy-6-(3",3"-dimethylallyl) isoflavan (**1**), 7-hydroxy-2',4'-dimethoxy-8-(2"',3"'-dihydroxy-3"'-methylbutyl)-5'-(3",3"-dimethylallyl) isoflav-3-ene (**2**) and 1,3-dilinoleoyl-2-stearoyl glycerol (**3**), reported herein for the first time from the genus. *In vitro* antibacterial activity of dichloromethane: methanol (1:1) and isolated compounds were tested for their antibacterial activity against the bacterial species *E. coli, S. aureus*, *P. aeruginosa* and *S. pyogenes.* Compound **1** displayed 9.67 ± 0.58 mm and 10.67 ± 0.58 mm zone of inhibition whereas compound **2** 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). Dichloromethane/methanol (1:1) extract and isoflavan (**1**) showed promising DPPH

radical scavenging activity with IC_{50} of 17.7 and 32, respectively, compared to ascorbic acid. Molecular docking analysis against DNA gyrase B (PDB ID: 6F86) revealed minimum binding affinity value of -7.4 kcal/mol for both compounds**1** and **2**compared to ciprofloxacin -7.3 kcal/molsuggesting the compounds may act as potential inhibitors of DNA gyrase enzyme. *In silico* analysis against human peroxiredoxin 5 (PDB ID: 1HD2) revealed minimum binding energy of - 3.7 and - 2.0 kcal/mol for compounds **1** and **2**, respectively, compared to ascorbic acid (-4.9 kcal/mol). The *in vitro* antibacterial activity and antioxidant activity of compounds **1** and **2**suggest the potential use of these compounds as potential drug lead candidates which corroborate with the traditional uses of the roots of *R. ferrugenia*.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Kalid Hussein: conducted experimental work and drafted the manuscript. **Rajalakshmanan Eswaramoorthy:** conducted the computational study. **Yadessa Melaku** and **Milkyas Endale:** supervised the experimental work and edited the manuscript.

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