SUPPLEMENTARY MATERIAL

Exploring the anti-obesity bioactive compounds of *Thymelaea hirsuta and Ziziphus spina-christi* through integration of lipase inhibition screening and molecular docking analysis

ABSTRACT

Activity-guided fractionation of the ethanolic extracts of *Thymelaea hirsuta and Ziziphus spina-christi* furnished eight compounds having pancreatic lipase inhibitory activity. Six compounds were isolated from the chloroform fraction of *T. hirsuta*. It is worth mentioning that this is the first report for the isolation of 5,7,4′-trihydroxy-8-methoxycarbonyl flavanol (**2**), daphnodorin G-3″-methyl ether (**4**) and daphnodorin G (**5**) from genus *Thymelaea*. Moreover, daphnoretin (**1**), neochamaejasmin A (**3**) and daphnodorin B (**6**) were also isolated from the chloroform fraction of the same plant. On the other hand, quercetin 3-*O*-α-L-rhamnopyranosyl- (1 → 2)- [α-L-rhamnopyranosyl-(1 →6)]-*β*-D-galactopyranoside (**7**) and 3-*O*- [α -L-fucopyranosyl (1→2) -*β* -D-glucopyranosyl (1→3)-α -L-arabinopyranosyl] jujubogenin (christinin A) (**8**) were isolated from the *n*-butanol fraction of *Z. spina-christi*. Structure elucidation of the isolated compounds was carried out by detail analysis of 1D and 2D spectral data. These compounds showed percentage inhibition 72% (**1**), 52% (**2**) and 61.8% (**3**), 39% (**4**), 69.5% (**5**), 3.5% (**6**), 68% (**7**) and 75% (**8**) at the concentration of 250 μM and XP-G scores of lipase inhibition were 11.40 (**1**), 8.71 (**2**) and 6.13 (**3**), 8.23 (**4**), 6.22 (**5**), 9.76 (**6**), 14.66 (**7**) and 12.00 (**8**). This is the first report of the isolation of lipase inhibitors from both plants *T. hirsuta and Z. spina-christi*. In addition to that, this might corporate in presenting the biscoumarin; daphnoretin and the dammarane saponin; christinin A as potent lipase inhibitors.

Keywords: Lipase inhibitor, *Thymelaea hirsuta*, *Ziziphus spina-christi*, Christinin A, Daphnoretin.

Experimental

Lipase inhibition assay

1. Preparation of the plant extract stock solution:
   Both plant extracts (40 mg of each) were dissolved separately in 10 ml of tris base buffer solution containing 500 µl DMSO to reach a final concentration (4 mg/ml) and the resulting solution was then filtered.

2. Preparation of Tris base buffer solution:
    Tris base buffer (2.27 g) was dissolved in 250 ml distilled water to reach to a final concentration of 75 mM, and then few drops of concenterated hydrochloric acid was added to adjust the PH of the buffer solution at 8.5 using JENWAY PH meter.

3. Preparation of the substrate solution:
P-nitrophenylpalmitate (PNP) substrate (3.775 mg) was dissolved in acetonitrile (1 ml) to give a stock solution with a concentration of 10 mM. Ethanol was then added to a final concentration of 1:2 (v/v) acetonitrile: ethanol, resulting in 3.33 mM PNP and the solution was then stored at -20°C.

4. Preparation of the lipase enzyme solution:
Porcine pancreatic lipase (type II, crude, Sigma) (15 mg) was dissolved in 3 ml of freshly prepared distilled water to reach to a final concentration of 5 mg/ml and the solution was then stored at -20°C.

5. Microplate setup:
The method of Birari and Slanc et al. was employed [1,2]. Reaction mixtures containing 20 µl of the enzyme solution, 50 µl of the tested sample extract solution and 120 µl of Tris-base buffer solution, were incubated at 37°C for 25 min. Then 10 µl of PNP substrate was added to the reaction mixture, followed by incubation at 37°C for 30 min. Finally, the absorbance of released p-nitro phenol was measured at 405 nm using a microplate reader.

The activity assay was performed in triplicate for each treatment. Orlistat is used as a positive control.

The percent PPL inhibition was calculated according to the following equation:

$$= \frac{(Abs_{\text{Negative control}} - Abs_{\text{Negative control blank}}) - (Abs_{\text{Test inhibitor}} - Abs_{\text{Test inhibitor blank}})}{(Abs_{\text{Negative control}} - Abs_{\text{Negative control blank}})} * 100$$

Abs: absorbance values.
Negative control: it contained all the above stated reaction components, except the sample or the test inhibitor.
Blank: blanks are prepared for all the tested samples and for the negative control, where it contained all the above stated reaction components, except the enzyme.

6. Preparation of test substance solution:
   A 1 mM stock solution of each isolate was first prepared by dissolving an accurately weighted mass in 4 ml of Tris buffer solution containing 200 µl DMSO. This final solution was employed for preparing the test substance.

7. Plate setup:

Eight rows were consumed for testing the eight isolates along with the reference orlistat. The method of [1,2] was employed with some modifications. Reaction mixtures were incubated at 37°C for 25 min. Then PNP substrate was added to the reaction mixture, followed by incubation at 37°C for 30 min. Finally, the absorbance of released *p*-nitro phenol was measured at 405 nm using a microplate reader. The composition of the reaction mixture in each well was: 10 μl of 3.33 mM PNP, 120 μl of 75 mM Tris base buffer (pH = 8.5), 50 μl of the extract and 20 μl of the enzyme solution. The activity assay was performed in triplicate for each treatment. Orlistat is used as a positive standard.

References

[1] Birari, R., Roy, S. K., Singh, A., & Bhutani, K. K. (2009). Pancreatic lipase inhibitory alkaloids of Murraya koenigii leaves. *Natural Product Communications*, *4*(8), 1089–1092. https://doi.org/10.1177/1934578x0900400814

[2] Slanc, P., Doljak, B., Kreft, S., Lunder, M., Janeš, D., & Štrukelj, B. (2009). Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytotherapy Research*, *23*(6), 874–877. https://doi.org/10.1002/ptr.2718
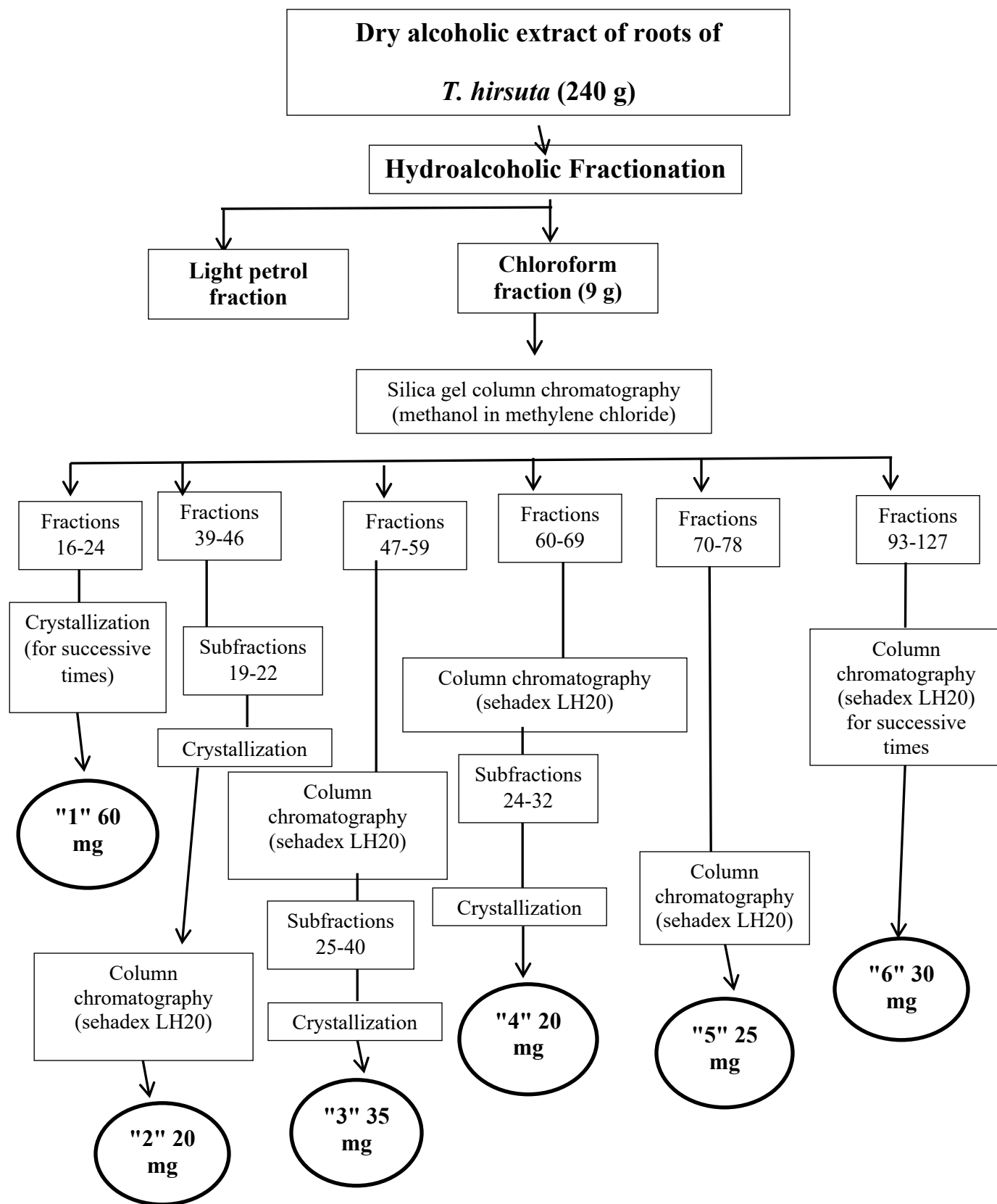
Dry alcoholic extract of roots of

T. hirsuta (240 g)

↓

Hydroalcoholic Fractionation

Light petrol fraction

Chloroform fraction (9 g)

↓

Silica gel column chromatography
(methanol in methylene chloride)

Fractions 16-24

Fractions 39-46

Fractions 47-59

Fractions 60-69

Fractions 70-78

Fractions 93-127

Crystallization (for successive times)

Subfractions 19-22

Crystallization

Column chromatography (sehadex LH20)

Column chromatography (sehadex LH20) for successive times

"1" 60 mg

Column chromatography (sehadex LH20)

Subfractions 24-32

Subfractions 25-40

Crystallization

Column chromatography (sehadex LH20)

Column chromatography (sehadex LH20)

Crystallization

Crystallization

"4" 20 mg

"5" 25 mg

"6" 30 mg

"2" 20 mg

"3" 35 mg

Figure S1: Fractionation of _T. hirsuta_ extract showing the isolation of compounds (**1-6**)

Dry alcoholic extract of *Z. spina christi* aerial parts (220 g)

↓

Hydroalcoholic Fractionation

Light petrol fraction

Chloroform fraction

Ethyl acetate fraction

Butanol fraction (11 g)

↓

Silica gel column chromatography (methanol in methylene chloride)

↓

Fractions 6-15

Fractions 27-52

Reversed phase column chromatography

Crystallization

Subfractions 30-40

Crystallization

"8" 47 mg

"7" 18 mg

Figure S2: Fractionation of *Z. spina christi* extract showing the isolation of compounds (**7-8**)

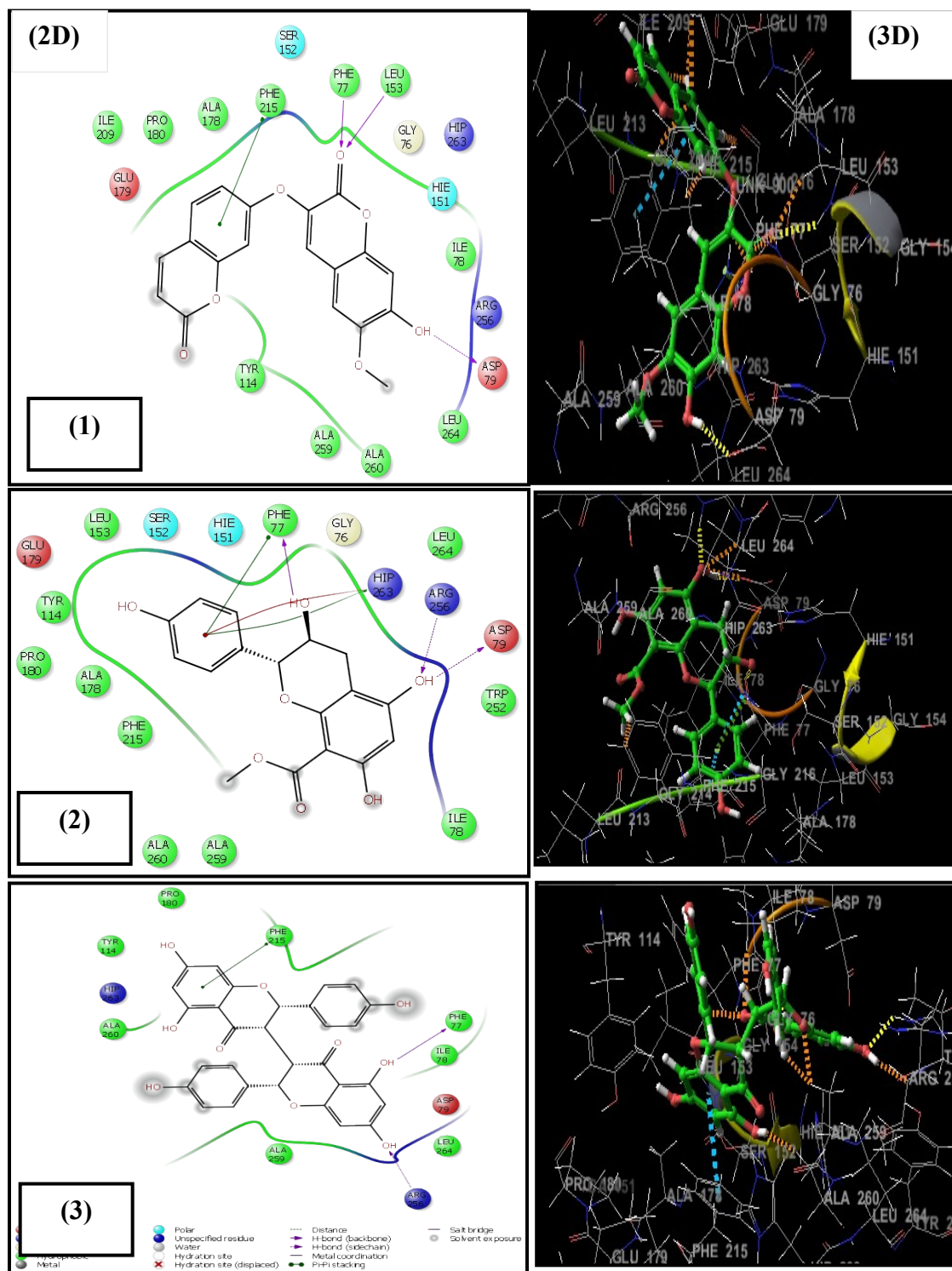**In-silico docking study of the lipase inhibitory activity of isolated compounds (1-8)**

Figure S3: 2D and 3D ligand interaction diagrams of daphnoretin (1), 5,7,4′-trihydroxy-8-methoxycarbonyl flavanol (2) and neochamaejasmin A (3) in the active site of lipase crystalline structure (1LPA)
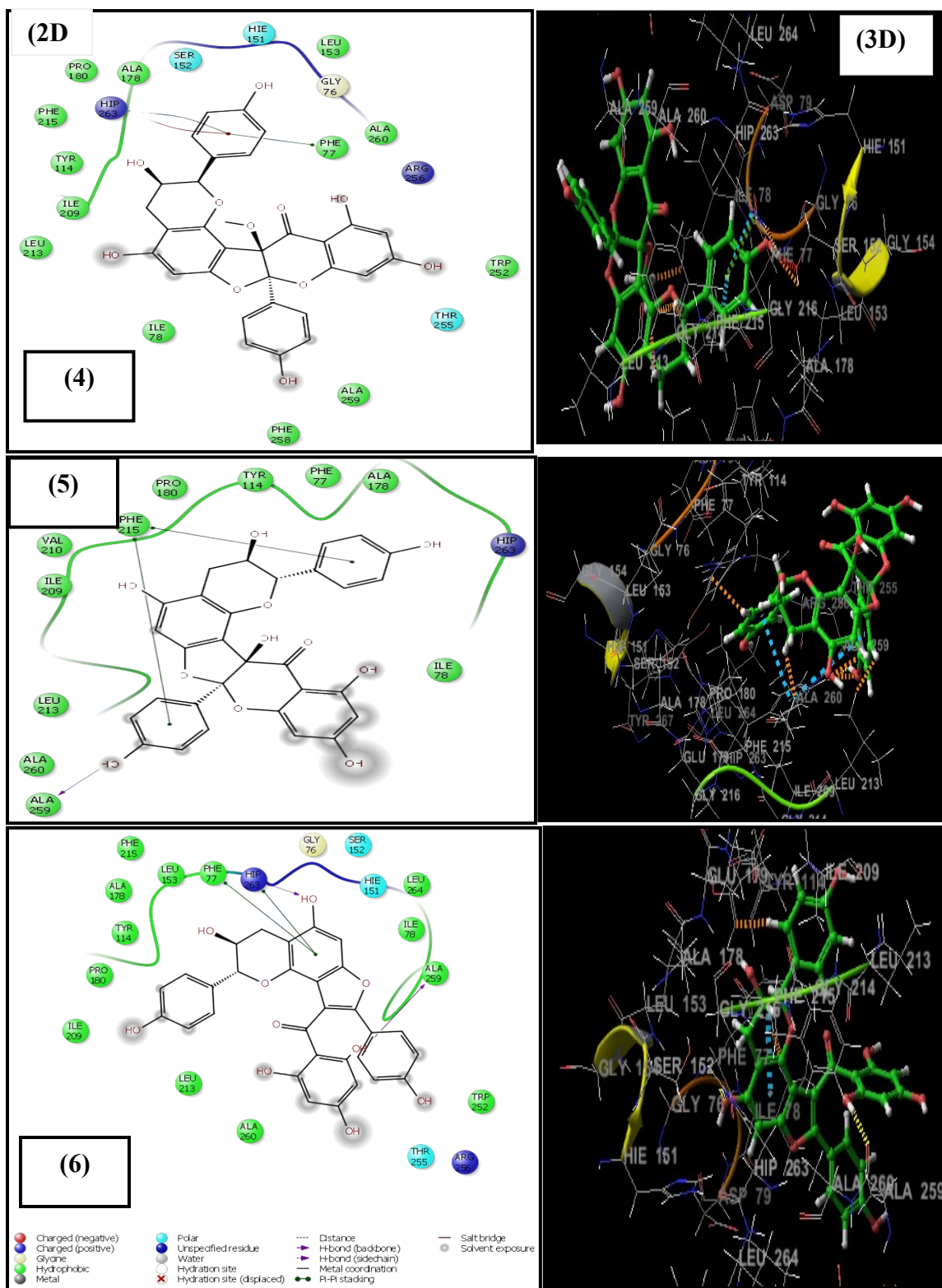
Figure S4: 2D and 3D ligand interaction diagrams of daphnodorin G-3"-methyl ether (4), daphnodorin G (5) and daphnodorin B (6) in the active site of lipase crystalline structure (1LPA)
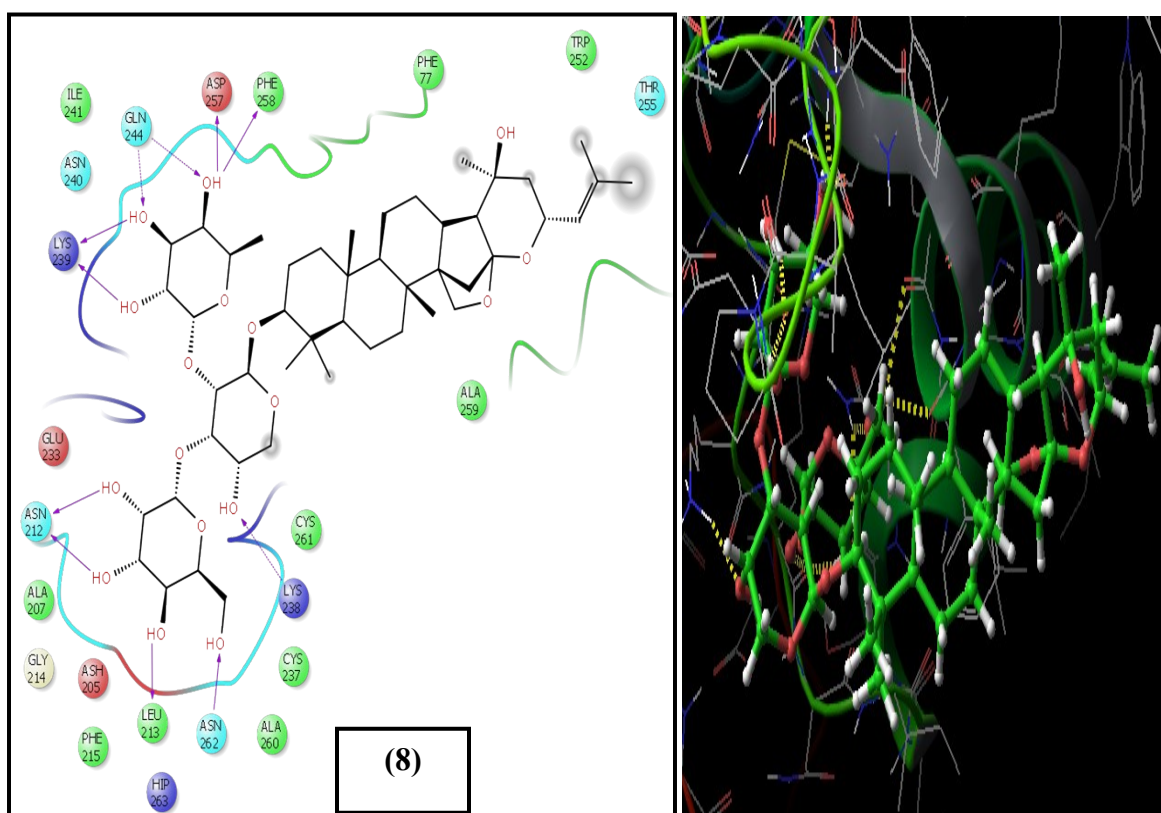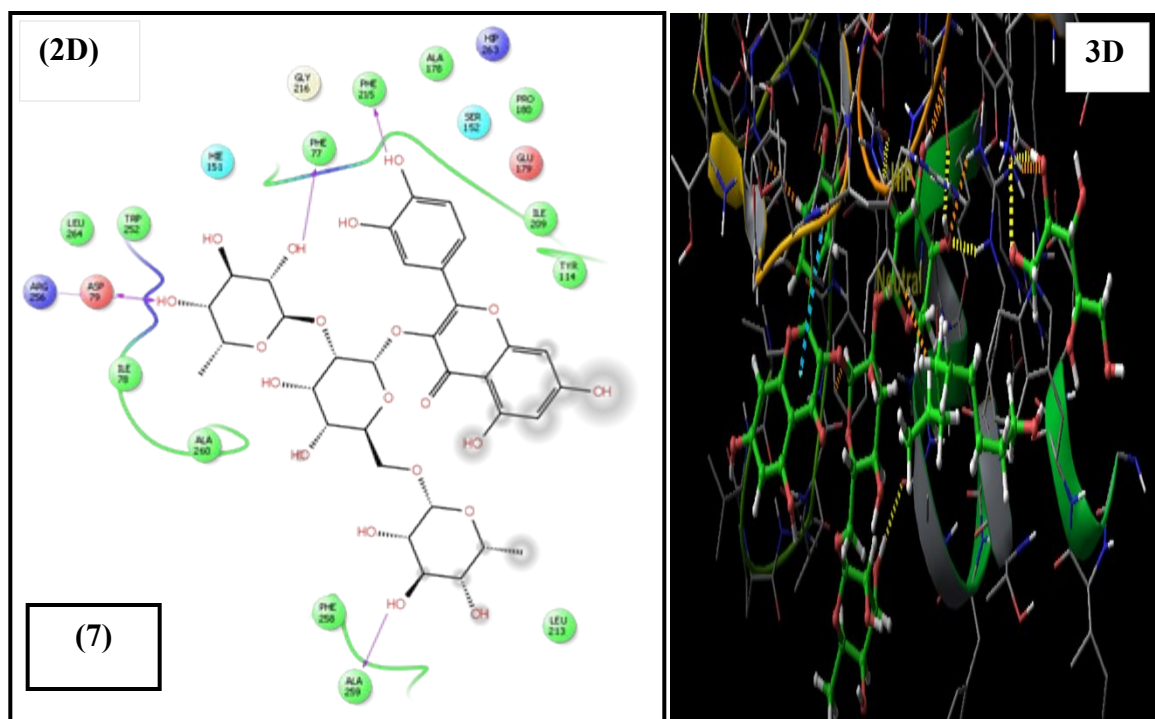
Figure S5: 2D and 3D ligand interaction diagrams of quercetin 3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-*β*-galactopyranoside) (7) and christinin A (8) in the active site of lipase crystalline structure (1LPA)

In-silico prediction of QikProp pharmacokinetic ADMET properties

In the development stage, many drugs are rejected due to their poor pharmacokinetic (Absorption, Distribution, Metabolism, Excretion and Toxicity: ADMET) profiles. [34]. Qikprop is an easy, fast and accurate software that can be used to generate ADMET values. Qikprop module embedded in Schrodinger® Suite software package was used to investigate the relevant pharmaceutical properties (human oral absorption, central nervous system activity, octanol/water log P (QPlog Po/w), molecular weight, hydrogen bond donor, hydrogen bond acceptor, and Lipinski's rule of 5 [5]) of the eight isolated compounds from *T. hirsuta* and *Z. spina-christi* through the calculation of the ligands' ADMET properties [6] (Table 8.2). Lipinski's Rule of Five was used as a standard. This rule explains the different properties of a drug molecule as well as its interactions metabolism within the human body as well as its excretion.

Table (S1): Predicted ADME properties of the isolated eight molecules

Compound name	CNS[a]	Molecular weight[b]	Percentage Human Oral Absorption[c]	Donor HB[d]	Accpt HB[e]	QPlog Po/w[f]	Rule Of Five
Daphnoretin (**1**)	-2	352.3	79.19	1	7	1.835	0
5,7,4′-Trihydroxy-8-methoxycarbonyl flavanol (**2**)	-2	332.309	69.72	3	5.7	1.659	0
Neochamaejasmin A (**3**)	-2	542.498	35.33	4	8	2.629	2
Daphnodorin G-3″-methyl ether (**4**)	-2	572.524	25.50	5	9.45	2.644	2
Daphnodorin G (**5**)	-2	558.497	14.04	6	9.45	1.874	2
Daphnodorin B (**6**)	-2	542.498	35.18	5	7.45	2.83	2
Quercetin 3-*O*-(2,6-di-*O*-α-rhamnopyranosyl-*β*-galactopyranoside) (**7**)	-2	756.667	14.36	11	27.35	-3.501	3
Christinin A (**8**)	-2	913.107	17.28	9	26.05	0.886	3

Acceptable ranges:
a) Predicted central nervous system activity on a –2 (inactive) to +2 (active) scale
b) Molecular weight between 130.0 to 725.0
c) Predicted human oral absorption on 0 to 100% scale (acceptable range from 25%-80%)
d) Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution from 0.0 to 6.0

e) Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution from 2.0 to 20.0

f) Predicted octanol/water partition coefficient scale from –2.0 to 6.5 (the excretion process depends on the molecular weight and the partition coefficient of the compound, so small hydrophilic compounds are more likely cleared through the kidney and more safe than large hydrophobic moecules).