

## Original Research Article

# Chemical analysis and biological studies of leaf extracts of *Smallanthussonchifolius*(Poepp.) H. Rob. with *in-silico* assessment of GC-MS identified compounds

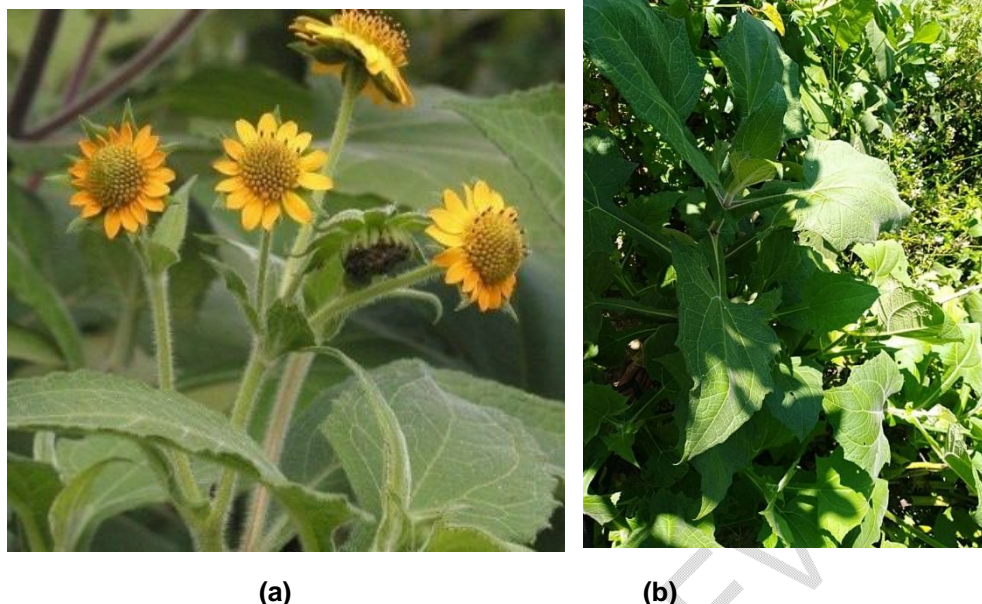
### ABSTRACT

*Smallanthussonchifolius*, from the Asteraceae family, is acknowledged for its therapeutic potential, especially in traditional medicine. This study focuses on the biological activities of its leaf extract, utilizing gas chromatography-mass spectrometry (GC-MS) to identify bioactive phytochemicals and explore antidiabetic properties through molecular docking and pharmacodynamic properties via ADMET predictions. The study involved ultrasonic extraction of shade-dried, powdered leaves using five solvents, followed by screening for phytochemical content and antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*. Antimicrobial testing showed hexane and methanol extracts were effective against *Candida albicans*, with inhibition zones comparable to kanamycin. The chloroform extract exhibited the highest phenolic content (21.4 mg GAE/g) and flavonoid content (350.87 mg QE/g). In contrast, the methanol extract showed superior antioxidant activity ( $IC_{50} = 242.74 \mu\text{g/mL}$ ) and  $\alpha$ -amylase inhibition ( $IC_{50} = 733.83 \mu\text{g/mL}$ ). The methanol extract exhibited moderate lethality in the brine shrimp assay with an  $LC_{50}$  value of 216.81  $\mu\text{g/mL}$ . GC-MS analysis identified 16 compounds in hexane extract and 4 in methanol extract. Molecular docking demonstrated strong binding affinities for diazprogesterone and pentadecafluorooctanoic acid, dodec-2-en-1-yl ester with  $\alpha$ -amylase, surpassing reference drug miglitol. *In-silico* drug-likeness and ADMET predictions indicated potential drug-like properties of the compound diazprogesterone but further experimental studies are suggested to explore their pharmacological and safety profiles.

**Keywords:** *Smallanthussonchifolius*, antioxidant, antidiabetic, GC-MS, molecular docking.

## 1. INTRODUCTION

*Smallanthussonchifolius* (Poepp.) H. Rob. (*S. sonchifolius*), often called "yacon," is a perennial herbaceous plant of the Asteraceae family with huge dark green leaves that thrive in a warm, temperate climate between 880 and 3500 m[1]. It is reported that *S. sonchifolius* has antioxidant, anti-inflammatory [2], antimicrobial [3], and antidiabetic effects[4] as it contains bioactive substances like fructooligosaccharides, inulin, and phenolic compounds with various phytochemicals [5]. It has been used to cure liver and kidney cancer [6], [7], diabetes, digestive problems, food, and tea production [8]. Additional research on the *S. sonchifolius* plant has revealed that its leaf contains high levels of carboxylic acids [9], polymatin B, allo-schkuhriolide, new phenylpropanoid smallanactone A, smallanthaditerpenic acids [10, 12], and sesquiterpene lactones enhydrin, dimer enhydrofolin[7] etc., where enhydrin and 8 $\beta$ -methacryoxymelampolid-14-oic acid ester were found to have antimicrobial properties and smallanthaditerpenic acids had anti-diabetic properties [6, 10]. The structures of some of the chemical components found on the plant's leaf are displayed in supplementary information (Figure S1). Because of this plant's diverse biological and pharmacological properties, bioactive chemicals have been isolated and employed to generate innovative medications in recent years.



**Figure 1:** Different parts of *S. sonchifolius* plant (a) flowers; and (b) leaves

Plants contain phytochemicals that can be identified and isolated using various analytical methods [13]. GC-MS combines chromatographic separation with spectrometric detection, allowing for the identification of bioactive substances in samples [14]. Due to their medicinal uses and minimal side effects [15], phytochemicals are increasingly popular for treating conditions like asthma, rheumatoid arthritis, and cancer, earning them the term "man-friendly medicines" [16].

Molecular docking helps to predict the interaction of small molecules with protein binding sites, to understand biochemical processes at the atomic level [17] and useful in drug discovery, including for diabetes, a major global health concern [18]. Inhibiting  $\alpha$ -amylase, a key enzyme in carbohydrate breakdown, can slow digestion and glucose absorption, making it a valuable target for docking studies with therapeutic phytochemicals in diabetes management [19]. Drug-likeness screening evaluates whether an organic compound has the properties to become an orally active drug [20]. Lipinski's "rule-of-five" is widely used to guide the selection of molecules with therapeutic potential [21]. Key pharmacokinetic factors for drug success or failure include absorption, distribution, metabolism, excretion, and toxicity (ADMET) [22, 23].

While the literature shows *S. sonchifolius* has various therapeutic properties, its phytochemicals have not been thoroughly explored, nor has their potential been assessed using *in silico* methods. This study aims to analyze the plant's extract, demonstrate its biological activities, and perform molecular docking of identified compounds against diabetes-related protein, followed by ADMET analysis to assess the drug-likeness of these compounds, laying the foundation for future drug development for diabetes.

## 2. MATERIAL AND METHODS

### 2.1 Chemicals and Reagents

All organic solvents used, including hexane, chloroform, ethyl acetate, and methanol (Fisher Scientific), were of analytical grade and sourced locally. Chemicals like ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), KOH, conc. H<sub>2</sub>SO<sub>4</sub>, conc. HCl, AlCl<sub>3</sub>, and phenol were lab-grade, while reagents like Mayer's, Dragendroff's, and Fehling's were prepared in the lab using laboratory-grade chemicals.

## 2.2 Collection of Plant and Extraction

*S. sonchifolius* leaves were collected from Kashikhanda municipality of Kavrepalanchwok district, Nepal, at 1219 m altitude and authenticated by the National Herbarium and Plant Laboratory. After air-drying in the shade, the leaves were powdered. For extraction, 400 g of powdered leaves were treated with hexane, ultrasonicated, and filtered. The filtrate was concentrated using a rotary evaporator (IKA, RV 10 D S96) and dried for storage. The process was repeated to produce three batches. Chloroform, ethyl acetate, methanol, and distilled water extracts were similarly prepared in increasing order of polarity.

## 2.3 Phytochemical Screening

Phytochemical screening of five leaf extracts of *S. sonchifolius* was done by using chemical methods based on the methodology given by Banu and Cathrine [16] to determine the presence of various phytochemicals.

## 2.4 Total Phenolic Content (TPC) Analysis

The total phenolic content in the methanol and chloroform extracts was measured at 760 nm using the Folin-Ciocalteu (FC) method, with gallic acid as the standard [23]. A calibration curve was made from various concentrations of gallic acid, with a 1000 µg/mL stock solution diluted in water. In a 96-well plate, 20 µL of standard solution was added in triplicate which was same for sample, followed by 100 µL of diluted FC reagent to initiate reaction and 80 µL of 7.5% Na<sub>2</sub>CO<sub>3</sub> to stabilize the color. After 2 hours in the dark, absorbance was measured at 760 nm using a UV-vis spectrophotometer (Labtronics LT-2802) and the results were expressed in mg GAE/g dry weight.

## 2.5 Total Flavonoid Content (TFC) Analysis

Quercetin was used as the reference in a colorimetric assay with aluminum chloride at 425 nm to measure total flavonoid content in the chloroform and methanol extracts [24]. A calibration curve was created using various quercetin concentrations, starting with a 1000 µg/mL stock solution. In a 96-well plate, 100 µL of the standard solution was loaded in triplicate which was the same for the sample, followed by 110 µL of distilled water and 100 µL of 2% AlCl<sub>3</sub> to initiate and stabilize the reaction. After 10 minutes in the dark, absorbance was measured at 425 nm using a UV-vis spectrophotometer (Labtronics LT-2802). Results were expressed as mg quercetin equivalent per gram of dry weight (mg QE/g).

## 2.6 Antioxidant Activity

The DPPH method was used to evaluate the antioxidant activity of methanol and chloroform extracts as used before with slight changes [25]. A calibration curve was created using different concentrations of ascorbic acid (positive control) starting with a 1000 µg/mL stock solution and methanol was used as the negative control. In a 96-well plate, 50 µL each of positive control and negative control, and were added in triplicate which was the same for the sample, followed by the addition of 150 µL of DPPH reagent to initiate reaction. After 20

minutes of incubation in the dark, absorbance was measured at 520 nm using a UV-vis spectrophotometer (Labtronics LT-2802). The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{Radical scavenging (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\% \dots \dots \dots (1)$$

## 2.7 $\alpha$ -Amylase Inhibition Analysis

The  $\alpha$ -amylase inhibition of chloroform and methanol extracts was tested using the 3,5-dinitrosalicylic acid (DNSA) method as mentioned previously [26] with slight modification, with acarbose as the standard. The extract was diluted in 10% dimethyl sulphoxide (DMSO), dissolved in buffer with NaCl (pH 6.9), and mixed 200  $\mu$ L of it with 200  $\mu$ L of  $\alpha$ -amylase solution. After 10 minutes of incubation at 30°C, 200  $\mu$ L of 1% starch solution was added and incubated for 3 minutes. The reaction was stopped with 200  $\mu$ L of DNSA, boiled for 10 minutes in a water bath at 85-90 °C, cooled, and diluted with 5 mL distilled water. Acarbose was used as the standard. Inhibition activity was measured at 540 nm with a UV-vis spectrophotometer (Labtronics LT-2802). The percentage of  $\alpha$ -amylase inhibition activity was calculated using the following formula.

$$\% \alpha\text{-amylase inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\% \dots \dots \dots (2)$$

## 2.8 Antimicrobial Activity

The antimicrobial activity of five *S. sonchifolius* leaf extracts was tested using the agar disc diffusion method [27] against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Liquid broth (LB) media was made by dissolving 2 g of LB powder (Sisco Research Laboratories Pvt. Ltd, India) in 100 mL distilled water, autoclaving for 45 minutes at 15 psi and 121 °C. This media was used for bacterial and fungal cultures, incubated at 37 °C for 24 hours. Mueller-Hilton Agar (MHA) plates were prepared by dissolving 2.5 g of MH agar powder (Sisco research laboratories Pvt. Ltd, India) in 100 mL distilled water, autoclaved for 25 minutes at 15 psi and 121 °C, then cooled and poured into Petri dishes. Each plate received 150  $\mu$ L of microbial seed and 10  $\mu$ L of each leaf extract (15 mg in 100  $\mu$ L DMSO) on 7 mm paper discs. Kanamycin (5 mg/1000 mL) served as the positive control, and DMSO as the negative control. After 24 hours of incubation at 37 °C, the zone of inhibition was measured to assess antimicrobial activity.

## 2.9 Brine Shrimp Lethality Assay

Brine shrimp (*Artemia salina*) lethality tests are used to assess the cytotoxicity of bioactive compounds [28] by counting the number of dead nauplii. Artificial seawater was prepared with 3.2 g of rock salt in 100 mL of distilled water. Brine shrimp eggs (50 mg) were hatched in this water at 22-29 °C with continuous aeration. Nauplii were used for the assay after 48 hours. 2 mL of different concentrations of sample solutions of chloroform and methanol extracts were loaded, prepared by serial dilution of 1 mg/mL (2 mg in 2 mL DMSO), and tested in triplicate in a 96-well plate for 24 hours. Cytotoxicity was evaluated by counting motile nauplii, with extracts deemed active if mortality exceeded 50%. The following equation was used to get the fraction of mortality:

$$\% \text{Mortality} = \frac{\text{No. of dead shrimps}}{\text{Total No. of shrimps}} \times 100 \% \dots \dots \dots (3)$$

## 2.10 Gas Chromatography-Mass Spectroscopy

The GC-MS experiment of hexane and methanol extracts was performed using the GCMS-QP 2010 instrument under specific operating conditions. Carrier gas helium was flowed

through a Rtx-5MS column of dimensions 30m×0.25mm×0.25µm under the temperature from 80 °C to 300 °C, with hold times at 2.0 and 5.0 min, respectively. Consistently, the ion source and interface temperature were maintained at 200 °C and 250 °C. The compounds obtained were identified through MS comparison to those in the National Institute of Standards and Technology (NIST) library [29].

## 2.11 Computational Methodology

20 compounds identified by GC-MS experiment were used as ligands with their 3D structures retrieved from PubChem server [30]. It was optimized in the Avogadro (version 1.2.0) program [31] using the UFF force field, 5000 steps, conjugate gradients algorithm, and 10<sup>-8</sup> kcal/mol convergence. The ligands were converted to PDBQT format with Gasteiger charges via AutoDock Tools [32]. The 3D crystal structure of α-amylase (PDB ID 2QV4, X-ray diffraction, 1.97 Å resolution) was obtained from the RCSB database [33]. Using the PyMol program [34] water molecules, ions, and co-crystallized ligands were removed from the structure, which was then converted to PDBQT format with added polar hydrogens and Kollman charges using AutoDock Tools.

Molecular docking was performed using the AutoDock Vina (version 1.5.7) [33]. The grid box for docking was centered on the ligand binding site, with coordinates set as (x: 14.029, y: 49.559, z: 20.381) and a box size of (38, 40, 38) Å for α-amylase. Miglitol was used as a reference drug for comparison.

## 2.12 In-silico Drug-likeness and Toxicity Prediction

The drug-likeness was carried out by using the ADMETlab3.0 web server [22] and toxicity by using the ProTox3.0 web server [35].

## 3. RESULTS AND DISCUSSION

### 3.1 Extracts Yield and Phytochemical Analysis Screening Analysis

Table 1 shows the yield of *S. sonchifolius* extracts, with the aqueous extract yielding the most, followed by hexane and methanol extracts. Carbohydrates and phenolic compounds were present in all extracts, while flavonoids were only found in four extracts, excluding the aqueous one. The differences in phytochemical content across extracts likely result from the varying solvent polarities [18]. The phytochemical screening data is shown in Table 2.

**Table 1:** Table showing percentage yield of various extract

Plant parts	Extracts yield				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Leaf Yield (g)	12.71	6.54	0.43	10.0	25.59
Leaf Yield (%)	3.18	1.64	0.11	2.50	6.40

**Table 2:** Phytochemical analysis of leaf extract of *S. sonchifolius*

S.N.	Phytochemicals	Test Names	Results of Extracts				
			Hex	Chl	EtOAc	MeOH	Aqueous
1	Alkaloids	Mayer's Test	-	-	-	+	+
		Dragendroff's Test	-	-	-	+	+
2	Carbohydrates	Molisch's Test	+	+	+	+	+
3	Phenolic compounds	Ferric Chloride Test	+	+	+	+	+
4	Tannins	Ferric Chloride Test	-	-	-	+	+
5	Flavonoids	Shinoda's Test	+	+	+	+	-
6	Terpenoids	Chloroform Test	+	-	-	+	+
7	Quinones	NH <sub>4</sub> SCN, FeSO <sub>4</sub> and conc. H <sub>2</sub> SO <sub>4</sub>	-	+	+	+	+
8	Reducing sugar	Fehling's Solution Test	-	+	-	+	-
9	Saponins	Froth Test	-	-	-	+	+

**Note:** Hex: - hexane; Chl: - chloroform; EtOAc: - ethyl acetate; MeOH: - methanol.

### 3.2 Total Phenolic Content and Total Flavonoid Content Analysis

From the quantitative TPC and TFC analysis, chloroform was found to have the highest TPC (21.4 mg GAE/g) and TFC (350.87 mg QE/g) value with methanol extract having a TFC value of 82.05 mg QE/g. These results indicate high phenolic and flavonoid content in *S. sonchifolius* leaf extracts. These compounds are linked to antioxidant activity because these compounds operate as reducing agents, supplying hydrogen atoms and scavenging free radicals [20], which may contribute to the plant's antidiabetic properties, reflecting its traditional use in herbal medicine. TPC and TFC values are shown in Table 3.

**Table 3:** TPC and TFC in chloroform and methanol extracts of *S. sonchifolius*

Extract	Total phenolic content	Total flavonoid content
	(mg GAE /g extract)	(mg QE/g extract)
Chloroform (mg GAE/g)	21.4	350.87
Methanol (mg GAE /g)	2.10	82.049

### 3.3 Antioxidant Activity Screening Analysis

From the DPPH-radical scavenging assay, the IC<sub>50</sub> value was found to be 242.74 µg/mL for methanol extract and 1555.73 µg/mL for chloroform extract. In contrast, standard ascorbic acid exhibited an IC<sub>50</sub> value of 14.38 µg/mL. This suggests that methanol extract is effective as an antioxidant but less potent than ascorbic acid. The high scavenging activity of the methanol extract may be attributed to its higher flavonoid content [36], which helps mitigate oxidative stress by donating hydrogen and chelating metal ions [37] IC<sub>50</sub> values of the samples and ascorbic acid standard are listed in Table 4.

**Table 4:** IC<sub>50</sub> values of the tested samples and standard drug

Samples	IC <sub>50</sub> (µg/mL)
Chloroform extract	1555.73
Methanol extract	242.74
Ascorbic acid (Standard)	14.38

### 3.4 α-Amylase Inhibition Analysis

α-Amylase breaks down glycosidic bonds in starch and glycogen and its inhibition can help manage diabetes by reducing glucose absorption and preventing postprandial hyperglycemia [20]. From the DNSA method, the IC<sub>50</sub> values were found to be 1819.11 µg/mL for methanol extract and 733.83 µg/mL for chloroform extract which was listed in Table 5 along with that of standard acarbose. Methanol extract, with an IC<sub>50</sub> between 500-1000 µg/mL, may have anti-diabetic effects [38] but is less effective than acarbose (IC<sub>50</sub> = 52.02 µg/mL).

**Table 5:** IC<sub>50</sub> values of the tested samples and standard drug

Samples	IC <sub>50</sub> (µg/mL)
Chloroform extract	1819.11
Methanol extract	733.83
Acarbose (Standard)	52.02

### 3.5 Antimicrobial Screening Analysis

Antimicrobial activity was evaluated by measuring inhibition zones (ZOI) [39] against three microbial strains *Candida albicans*, *Bacillus subtilis*, and *Escherichia coli*. At a concentration of 1.5 mg/mL, all five leaf extracts showed some zone of inhibition against the tested microbial strains. Hexane and chloroform extracts demonstrated good ZOI of 11.0 mm against the fungus *Candida albicans*, comparable to the positive control, kanamycin (5 µg/mL), indicating potential antimicrobial activity. The ZOI of the tested extracts is shown below in Table 6.

**Table 6:** Antimicrobial activity shown by the different leaf extracts

Micro-organisms	Reference culture	Positive control	Zone of Inhibition (ZOI) of extracts in mm (7 mm diameter)					
		Kanamycin ZOI (mm)	Negative control (DMSO)	Hex	Chl	EtOAc	MeOH	Aqueous extract
<i>Bacillus subtilis</i>	(ATCC 6051)	12.0	0.0	4.0	3.0	3.0	2.0	2.0

<i>Escherichia coli</i>	(ATCC 8739)	12.0	0.0	4.0	3.0	3.0	2.0	0.0
<i>Candida albicans</i>	(ATCC 2091)	11.0	0.0	11.0	11.0	4.0	4.0	4.0

**Note:** ATCC: - American type culture collection; Hex: - hexane; Chl: - chloroform; EtOAc: - ethyl acetate; MeOH: - methanol.

The findings demonstrate the potential antibacterial activity of the *S. sonchifolius* plant and imply that these extracts may prove to be effective antimicrobial agents providing a basis for future antimicrobial drug discovery.

### 3.6 Brine Shrimp Lethality Analysis

The % mortality of the chloroform and methanol extract is different in different concentrations which is shown in Table 7.

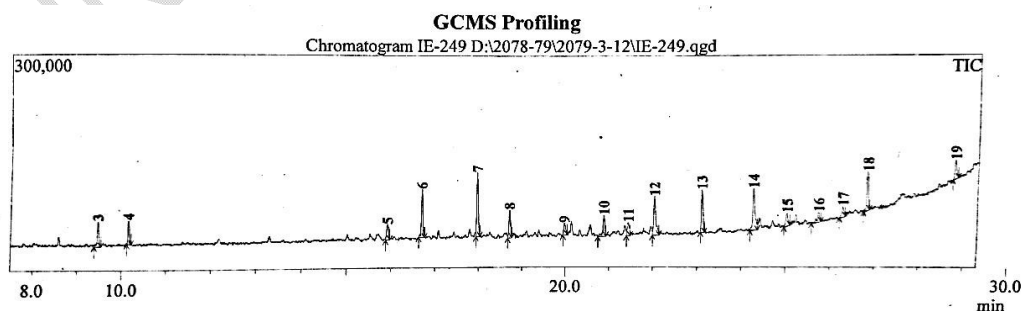
**Table 7:** LC<sub>50</sub> values of the tested samples

Samples	LC <sub>50</sub> (µg/mL)
Chloroform extract	92.76
Methanol extract	216.81

Chloroform and methanol leaf extract of *S. sonchifolius* were reported to have lethality concentration (LC<sub>50</sub>) of 92.76 µg/mL and 216.81 µg/mL, respectively. The outcomes imply that these extracts are effective against the lethality test for brine shrimp. The LC<sub>50</sub> value of the two extracts showed that the chloroform extract was found to be toxic, while the methanol extract was determined to be moderately toxic[40]. According to the test, the plant may contain probable anticancer components and high cytotoxic substances as LC<sub>50</sub> values are below 1000 µg/mL [35]. This offers proof of the plant's historical usage in treating kidney and liver cancer. It also lays the groundwork for the isolation of an anticancer agent for use in further medication research and discovery.

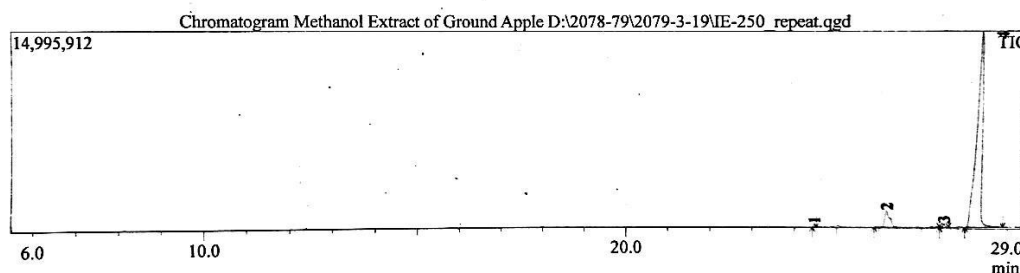
### 3.7 GC-MS Spectra Analysis

The GC-MS analysis was done by using GCMS-QP 2010 which revealed the presence of 19 peaks in hexane leaf extract and 4 peaks in methanol leaf extract as shown in Figures 2 and 3.



**Figure 2:** Chromatogram of hexane leaf extract

### GCMS Profiling



**Figure 3:** Chromatogram of methanol leaf extract

A total of 20 different compounds were obtained which was recorded in Table 8 with their PubChem CID, retention time (RT), molecular formula, and peak area. In hexane leaf extract, 3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl- (27.02%) most abundant whereas in methanol, 4-Bromobutyric acid, 3-methylbut-2-yl ester (91.70%) was most prevalent one. The mass spectra of each phytochemical identified by GC-MS are presented in the supplementary information (Figures S14 and S15).

**Table 8:** Components based on GC-MS analysis

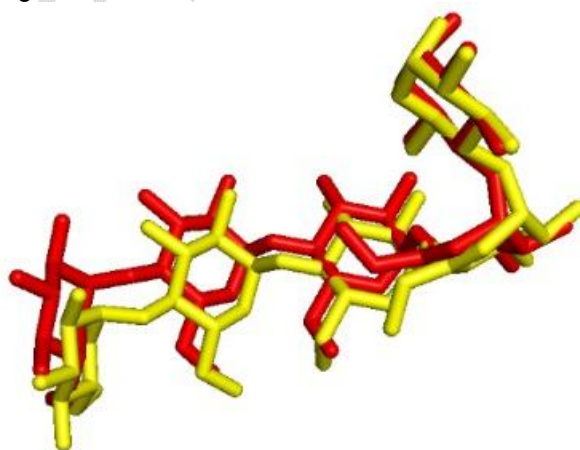
Extracts	Name of compounds	Code	PubChem CID	RT	Molecular formula	Peak Area (%)
	Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl-	1	79022	6.300	C <sub>10</sub> H <sub>16</sub>	2.17
	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl-	2	134900801	7.067	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub>	27.02
	9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane	3	556763	15.925	C <sub>12</sub> H <sub>16</sub>	2.37
	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-(1.α.,4.α.,4a.α.,10a.α.)-	4	5369975	17.983	C <sub>13</sub> H <sub>20</sub> N <sub>2</sub>	8.22
Hexane	3,3,6,6,9,9-hexamethyltetracyclo [6.1.0.0 <sup>2,4</sup> .0 <sup>5,7</sup> ] nonane	5	142890	18.726	C <sub>15</sub> H <sub>24</sub>	3.93
	Diazoprogestrone	6	543575	20.583	C <sub>21</sub> H <sub>30</sub> N <sub>4</sub>	7.19
	1.8-Cyclotetradecadiyne	7	137070	20.900	C <sub>14</sub> H <sub>20</sub>	3.67
	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	8	8748	23.125	C <sub>10</sub> H <sub>18</sub> O	5.60
	2-isopropyl-3-methylcyclohexanol	9	228304	24.292	C <sub>10</sub> H <sub>20</sub> O	7.91
	Pentadecafluorooctanoic acid, dodecyl ester	10	15918850	24.425	C <sub>20</sub> H <sub>25</sub> F <sub>15</sub> O <sub>2</sub>	

	Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester	11	91694721	25.042	$C_{20}H_{23}F_{15}O_2$	2.56
	8-Methylnonanoic acid, methyl ester	12	20619411	25.750	$C_{11}H_{22}O_2$	2.39
	Decanoic acid, silver (1+) salt	13	21226017	26.292	$C_{10}H_{19}AgO_2$	2.16
	Octanoic acid, 4-methyl-, ethyl ester, (+/-)-	14	92058	26.842	$C_{11}H_{22}O_2$	5.08
	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	15	84846	27.642	$C_{16}H_{32}O_3S$	
	3,7-Dimethyl-6-nonen-1-ol	16	5364807	28.800	$C_{11}H_{22}O$	3.00
	Nonanoic acid, methyl ester	17	15606	24.473	$C_{10}H_{20}O_2$	0.38
Methanol	Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester	18	590779	26.167	$C_{16}H_{31}O_2P$	7.21
	2-Nonen-1-ol, 2-methyl-	19	5366241	27.514	$C_{10}H_{20}O$	0.71
	4-Bromobutyric acid, 3-methylbut-2-yl ester	20	91720329	28.308	$C_9H_{17}BrO_2$	91.70

### 3.8 Analysis of Computational Outputs

#### 3.8.1 Molecular Docking Scores

The effectiveness of natural inhibitors is related to their binding affinity with target proteins[42]. While binding affinity does not always directly correlate with inhibitory potential, understanding these interactions is crucial for developing effective therapies [41]. A good RMSD value of 1.3 Å and a binding affinity of -10.4 kcal/mol for  $\alpha$ -amylase confirmed the docking protocol's accuracy. A superimposed image of native and docked ligands for  $\alpha$ -amylase is shown in Figure 2.



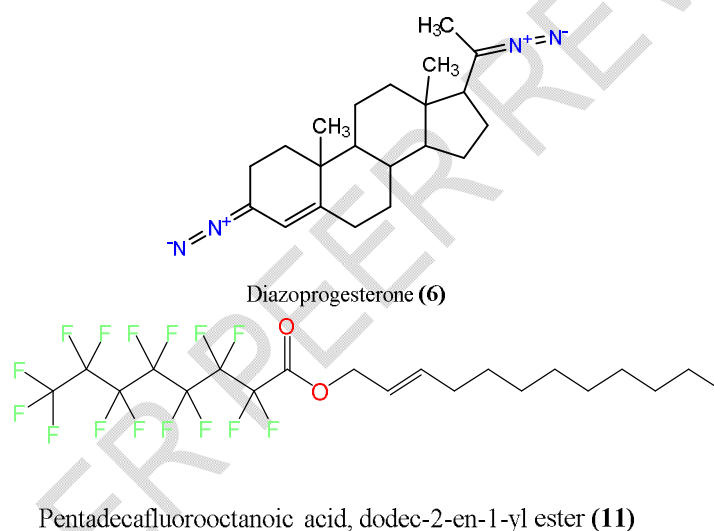
**Figure 4:** Superimposition of native ligand (yellow) with docked ligand (red) in  $\alpha$ -amylase protein (RMSD of heavy ligand atom = 1.3 Å)

The docking score of the studied compounds demonstrated that compounds 6 and 11 have the highest affinity exceeding reference drug, as shown in Table 9. The GC-MS compound

data are present in the supplementary information (Table S13). The molecular structures of the top two compounds are shown in Figure 3, with all 20 compounds in supplementary information (Figure S16).

**Table 9:** Binding affinities of top two GC-MS docked compounds, native ligand, and reference drugs with  $\alpha$ -amylase protein

Extracts	Ligands	Binding Affinities (kcal/mol) with $\alpha$ -amylase protein	
		(PDB ID 2QV4)	
Hexane	6	-9.1	
	11	-8	
Reference Drugs	Miglitol	-5.8	
Native ligand	QV4	-10.4	



**Figure 5:** Molecular structures of top two GC-MS compounds with  $\alpha$ -amylase

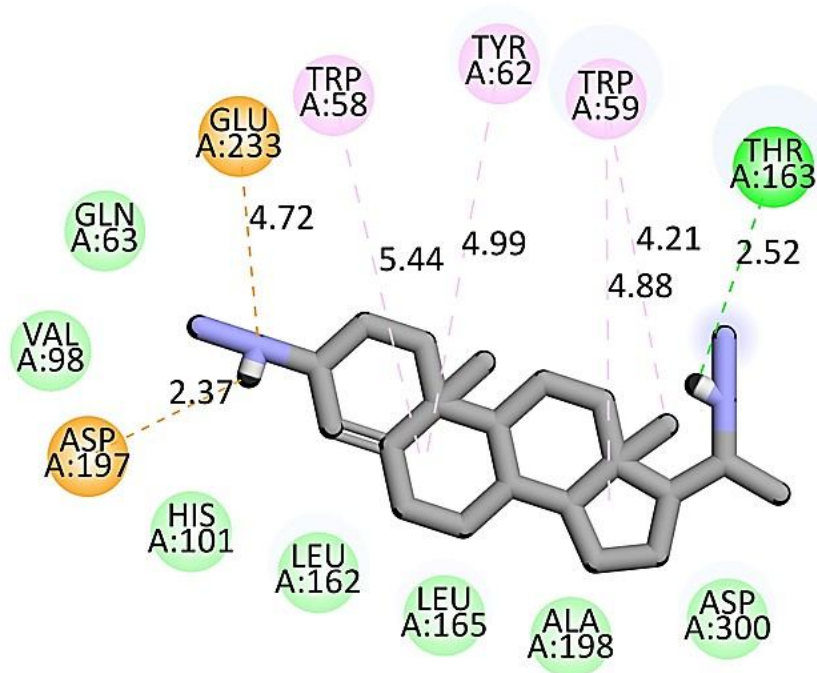
### **3.8.2 Protein-ligand interaction**

The 2D interaction analysis revealed conventional hydrogen bonds in both the top compounds, with a distance range of 2.20 to 2.73 Å, similar to that of the native ligand, indicating strong binding to the protein's active site. Both of the top two compounds exhibited pi-alkyl interactions with the protein's amino acids, with additional alkyl interactions observed in the protein-11 complex and unique halogen bond. Salt bridges and attractive charges were specific to protein-6, while a pi-donor hydrogen bond was unique to protein-11 complex. The native ligand also showed a pi-donor hydrogen bond with the protein. The top two compounds interacted with the key amino acid residues TRP58, TRP59, TYR62, LEU162, THR163, LEU165, ASP197, ALA198, GLU233, and ASP300, similar to that observed in case of the native ligand. The overall interactions of the top two docked compounds and native ligands with  $\alpha$ -amylase are demonstrated in Table 10 and Figures 4. The comparative interaction study between the top two docked compounds and the native

ligand with amino acid residue of protein showed that the compounds found to bind with protein in a binding pocket strongly comparable to that of the native ligand with the protein to form a complex.

**Table 10:** Different types of interactions between the top three ligands and key amino acid residues in the protein-ligand complexes along with the distances for  $\alpha$ -amylase protein

Complex	Type of interactions	Amino acid residues with distance (Å)
<b>2QV4-6</b>	Conventional hydrogen bonds	THR163 (2.52)
	Pi-alkyl	TRP58 (5.44), TRP59 (4.21, 4.88), TYR62 (4.99)
	Salt bridge	ASP197 (2.37)
	Attractive charge	ASP197 (2.37), GLU233 (4.72)
	van der Waals	GLN63, VAL98, HIS101, LEU162, LEU165, ALA198, ASP300
<b>2QV4-11</b>	Conventional hydrogen bond	GLN63 (2.69), THR163 (2.73)
	Halogen bond	TRP59 (3.26), ASP197 (3.68), ASP300 (3.03)
	Pi-alkyl	TYR151 (5.26), HIS201 (4.34, 5.08, 5.37)
	Alkyl	LEU162 (5.11), LEU165 (4.44), ALA198 (4.23), LYS200 (3.97), ILE235 (4.06, 4.74, 5.11)
	van der Waal	TRP58, TYR62, ARG195, GLU233, VAL234, HIS299, HIS305
<b>Native-complex</b>	Conventional hydrogen bond	GLU63 (2.92, 3.38), ASN105 (2.98), ALA106 (3.16), THR163 (3.25), ARG195 (3.17), GLU233 (3.21), HIS299 (3.30), ASP300 (2.91), HIS305 (3.17)
	Carbon hydrogen bond	GLY164 (3.51), GLU233 (3.55), THR163 (3.49)
	Pi-donor hydrogen bond	TYR62 (3.52)
	van der Waal	ILE51, TRP58, TRP59, HIS101, GLY104, VAL107, TYR151, LEU162, LEU165, ASP197, ALA198, LYS200, HIS201, ILE235

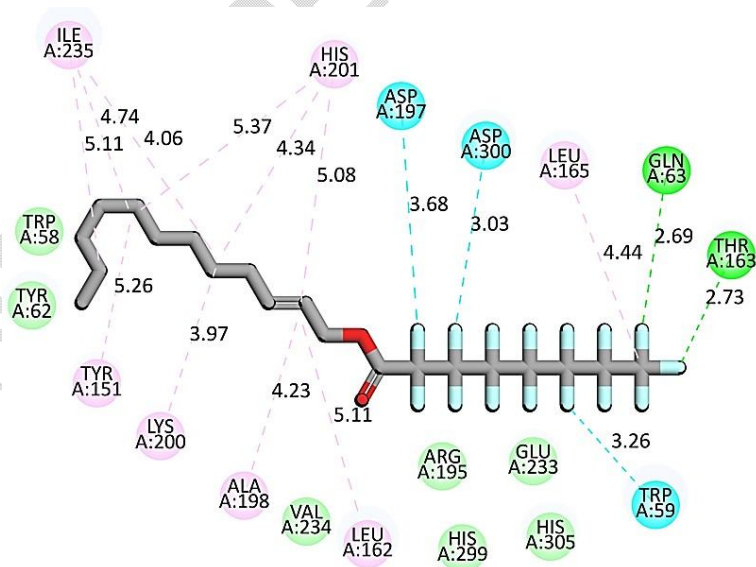


**Interactions**

- van der Waals
- Salt Bridge
- Attractive Charge

- Conventional Hydrogen Bond
- Pi-Alkyl

**2QV4-Diazoprogesterone complex**

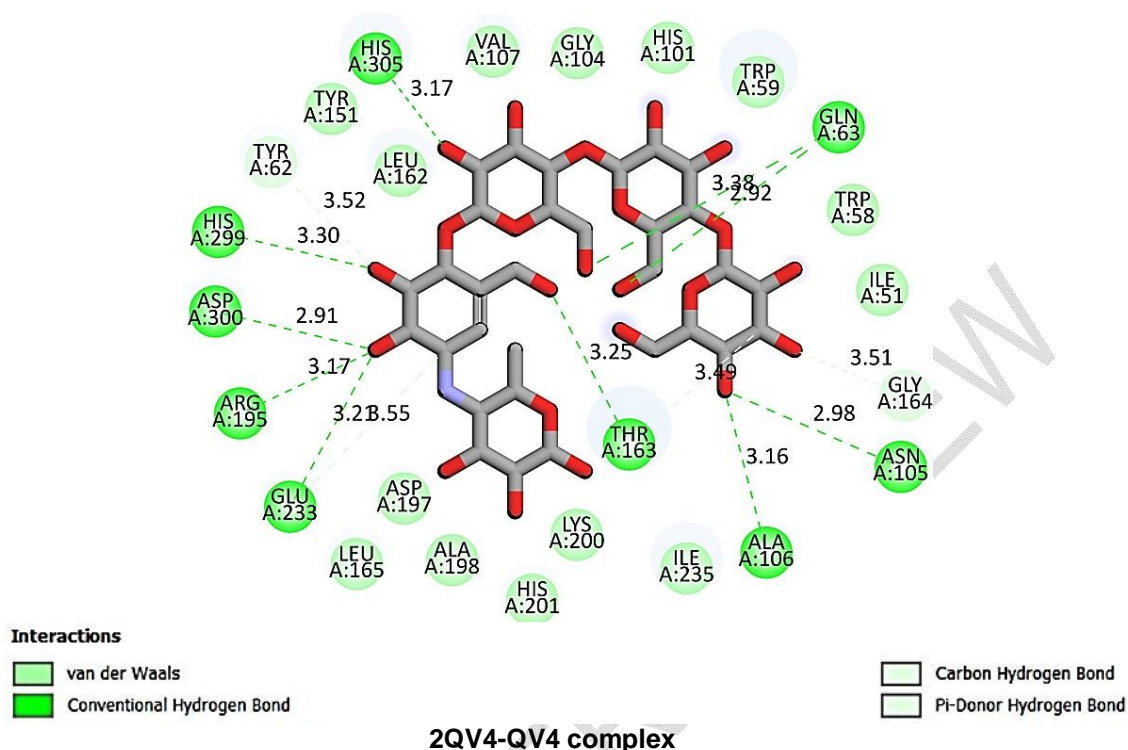


**Interactions**

- van der Waals
- Conventional Hydrogen Bond
- Halogen (Fluorine)

- Alkyl
- Pi-Alkyl

## 2QV4-Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester complex



**Figure 6:** 2D representations of the binding modes to  $\alpha$ -amylase protein (PDB ID: 2QV4) with compounds diazoprogerone and pentadecafluorooctanoic acid, dodec-2-en-1-yl ester and native QV4

## 3.9 Drug-likeness and ADMET Profiling

### 3.9.1 Drug-likeness

Drug-likeness predictions for the top two compounds are detailed in Table 11. Among the two, only compound 6 meets Lipinski's criteria, suggesting it is likely orally active, similar to reference drugs. Compound 6 has a TPSA of  $72.78 \text{ \AA}^2$ , while compound 11 has a TPSA of  $26.30 \text{ \AA}^2$ , indicating good bioavailability. Compound 6 also has rotatable bonds below 10, unlike compound 11. Log P values are 4.59 for compound 6 and 7.04 for compound 11, suggesting that compound 11 is more lipophilic and may penetrate biological membranes more effectively than compound 6 and the reference drugs [42].

**Table 11:** Drug-likeness predictions of the top two docked compounds, computed by ADMETlab

Drug-likeness parameters	Compounds	Mol. Wt. (g/mol)	NRB	NHA	NHD	TPSA ( $\text{\AA}^2$ )	LogP	Lipinski Rule (RO5)
Ligands	6	338.25	1	4	0	72.78	4.59	Accepted
	11	580.15	18	2	0	26.30	7.04	Rejected
Reference	Miglitol	207.11	3	6	5	104.39	-2.27	Accepted

## drugs

**Note:** NRB-Number of rotatable bonds, NHA-Number of hydrogen acceptor, NHD-Number of hydrogen donor, TPSA-Total polar surface area.

### 3.9.2 ADMET profiling

ADMET studies for the top two GC-MS compounds are detailed in Table 12. Compounds 6 and 11 showed high Caco-2 permeability (-4.49 and -4.99 log cm/s), better than the reference drug miglitol (-6.25 log cm/s), indicating strong absorption[43]. Both compounds also demonstrated high intestinal absorption, better than the reference drug miglitol. They are not P-glycoprotein (P-gp) substrates, enhancing absorption as they are less likely to be expelled from cells[44]. Compounds 6 and 11 both showed high binding with plasma protein binding (PPB) with low blood-brain barrier (BBB) penetration (comparable to reference drug miglitol), indicating no effects on the central nervous system (CNS). Compound 6 did not inhibit any CYP P450 enzymes studied, suggesting efficient metabolism, while compound 11 inhibited CYP2C19, CYP2C9, and CYP1A2, indicating slower metabolism [45]. Plasma clearance rates were moderate for both compounds (5.64 and 11.33 mL/min/kg, respectively), similar to the reference drug. Toxicity predictions from ProTox3.0 classified both compounds under toxicity class 5 (non-toxic), with LD<sub>50</sub> values of 3700 mg/kg for compound 6 and 5000 mg/kg for compound 11. Compound 11 was non-hepatotoxic, non-neurotoxic, non-immunotoxic, non-mutagenic, and non-cytotoxic, whereas compound 6 was identified as neurotoxic and immunotoxic. Overall, compound 6 showed the best pharmacokinetic properties among the two, comparable to those of established drugs. However, further *in vitro* and *in vivo* studies are needed to confirm the compounds' safety and drug-like characteristics.

**Table 12:** ADMET predictions of the top two docked compounds, computed by ADMETlab and ProTox

ADMET parameters	Ligands		Reference drug	
	6	11	Miglitol	
A				
	Caco-2 permeability (Log cm/s)	-4.49	-4.99	-6.25
	HIA	---	--	+
	P-gp substrate	---	---	++
D				
	PPB	98.3 %	99.8 %	1.9 %
	BBB	---	---	-
M				
	CYP1A2 inhibitor	---	+++	---
	CYP2C19 inhibitor	---	+++	---
	CYP2C9 inhibitor	---	+++	---
	CYP2D6 inhibitor	---	---	---
	CYP3A4 inhibitor	---	---	---
E				
	CL <sub>plasma</sub> (ml/min/kg)	11.33	5.64	5.36
T				
	Toxicity class	5	5	4

<b>LD<sub>50</sub> (mg/kg)</b>	3700	5000	1200
<b>Hepatotoxicity</b>	Inactive	Inactive	Inactive
<b>Neurotoxicity</b>	Active	Inactive	Inactive
<b>Immunotoxicity</b>	Active	Inactive	Inactive
<b>Mutagenicity</b>	Inactive	Inactive	Inactive
<b>Cytotoxicity</b>	Inactive	Inactive	Inactive

where, “---” = (0-0.1), “--” = (0.1-0.3), “-” = (0.3-0.5), “+” = (0.5-0.7), “++” = (0.7-0.9), “+++” = (0.9-1.0) probability values

**Note:** Caco-2: - human colon adenocarcinoma cell line; HIA: - Human-intestinal absorption; PPB: - Plasma protein binding; BBB: - Blood-brain barrier; P-gp: - P-glycoprotein; CYP: - Cytochrome-P; CL<sub>plasma</sub>: - Plasma clearance.

#### 4. CONCLUSION

The antimicrobial evaluation of five leaf extracts from *S. sonchifolius* revealed notable antifungal activity, highlighting its potential in addressing microbial infections. A detailed quantitative analysis of the chloroform and methanol extracts identified a high concentration of phenolic and flavonoid compounds, which are believed to be responsible for the plant's biological effectiveness. Furthermore, the DPPH free radical scavenging assay confirmed strong antioxidant activity, while the  $\alpha$ -amylase inhibition assay demonstrated moderate antidiabetic potential. The brine shrimp lethality assay showed toxic to moderately toxic effects on larvae, possibly due to carcinogenic phytochemicals in the plant. Molecular docking studies of GC-MS-identified compounds further supported these results, showing that diazoprogestosterone and pentadecafluorooctanoic acid, dodec-2-en-1-yl ester exhibited strong binding affinities to the diabetic  $\alpha$ -amylase protein, suggesting their potential in diabetes management. *In-silico* drug-likeness and ADMET predictions revealed that the compound diazoprogestosterone shows drug-likeness property and belong to toxicity class 5, presenting promise in drug development. However, further research is needed to comprehensively evaluate their pharmacological properties and safety. Overall, this study supports the traditional use of *S. sonchifolius* in managing diabetes and severe infections, providing valuable insights into its bioactive components and mechanisms of action for future investigation.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors affirm that no generative AI technologies, including Large Language Models (e.g., ChatGPT, COPILOT) or text-to-image generators, were utilized in the writing or editing of the manuscripts.

## CONSENT (WHERE EVER APPLICABLE)

Not applicable

## ETHICAL APPROVAL (WHERE EVER APPLICABLE)

The authors confirm that this article does not include any research involving human or animal subjects.

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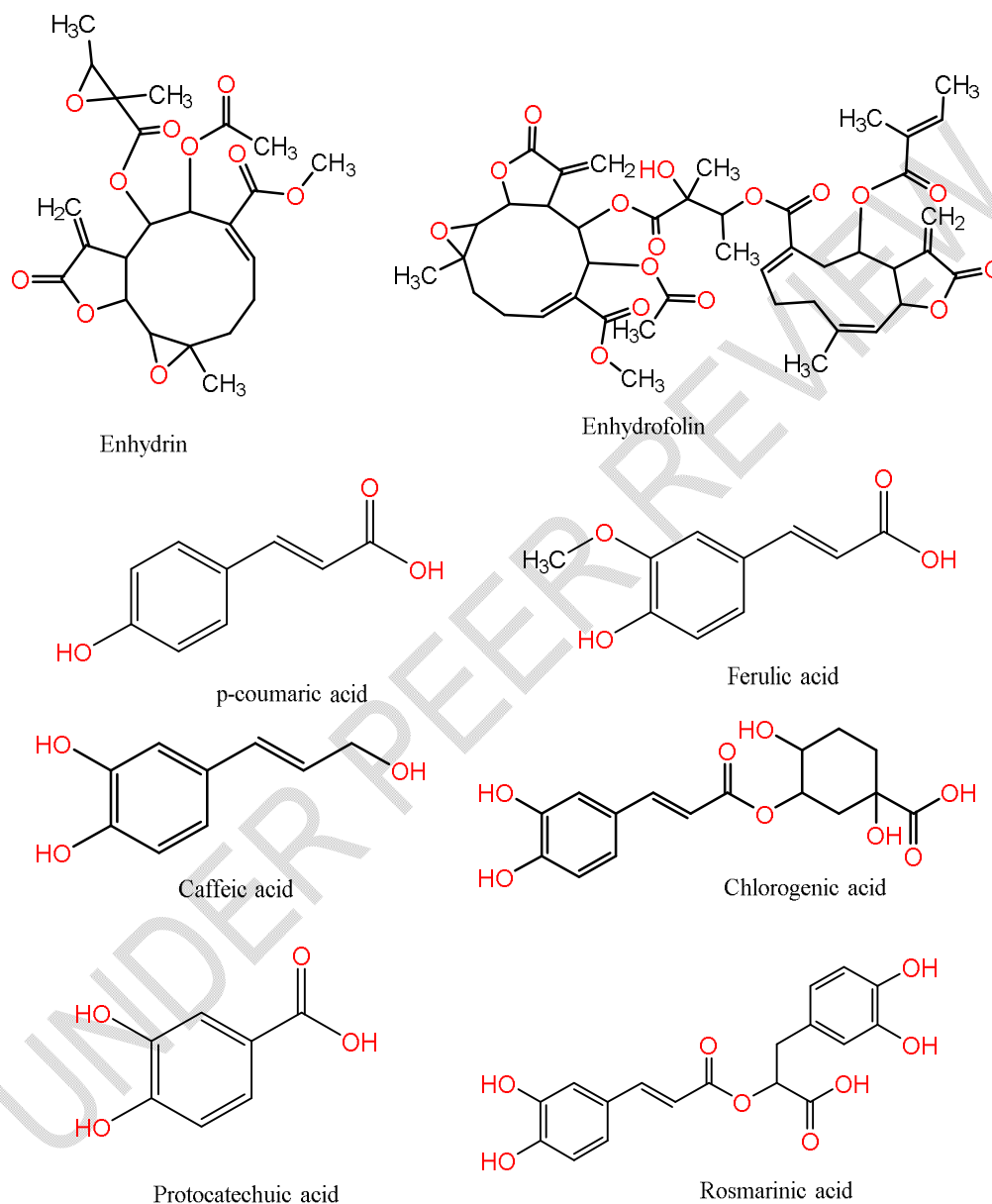
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### SUPPLEMENTARY INFORMATION

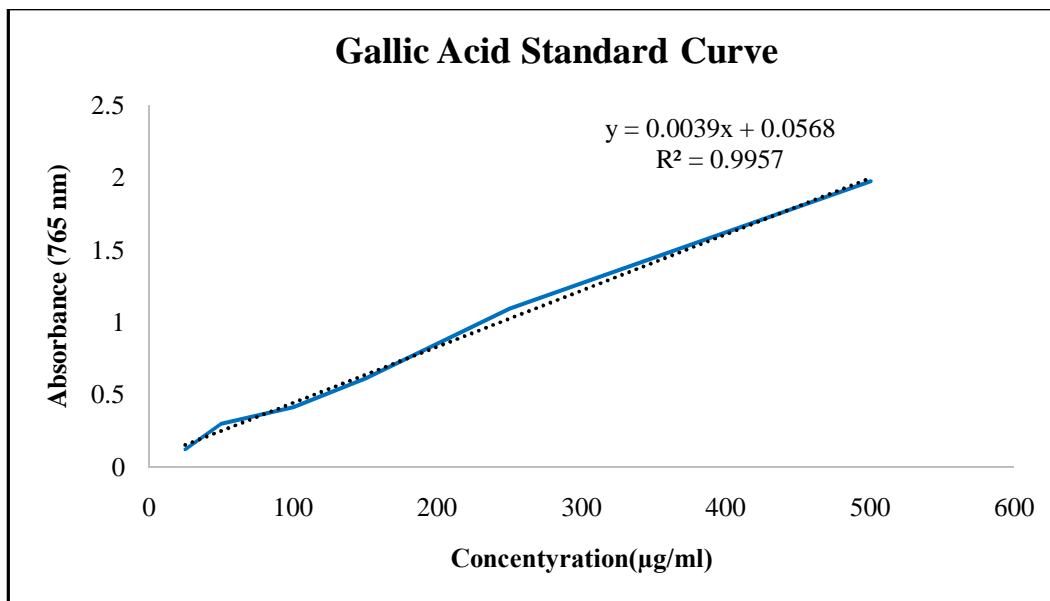


**Figure S1:** Structures of some chemical compounds found in the leaf of *S. sonchifolius*

**Table S1:** Gallic acid standard

Conc. ( $\mu\text{g/mL}$ )	Triplicates Absorbance data			Average Absorbance	Absorbance
500	2.332	2.121	2.208	2.22	1.976
250	1.3	1.344	1.369	1.338	1.094

150	0.891	0.854	0.821	0.855	0.611
100	0.675	0.635	0.664	0.658	0.414
50	0.544	0.554	0.53	0.543	0.299
25	0.374	0.366	0.353	0.364	0.12
<b>Control</b>	<b>0.235</b>	<b>0.231</b>	<b>0.266</b>	<b>0.244</b>	



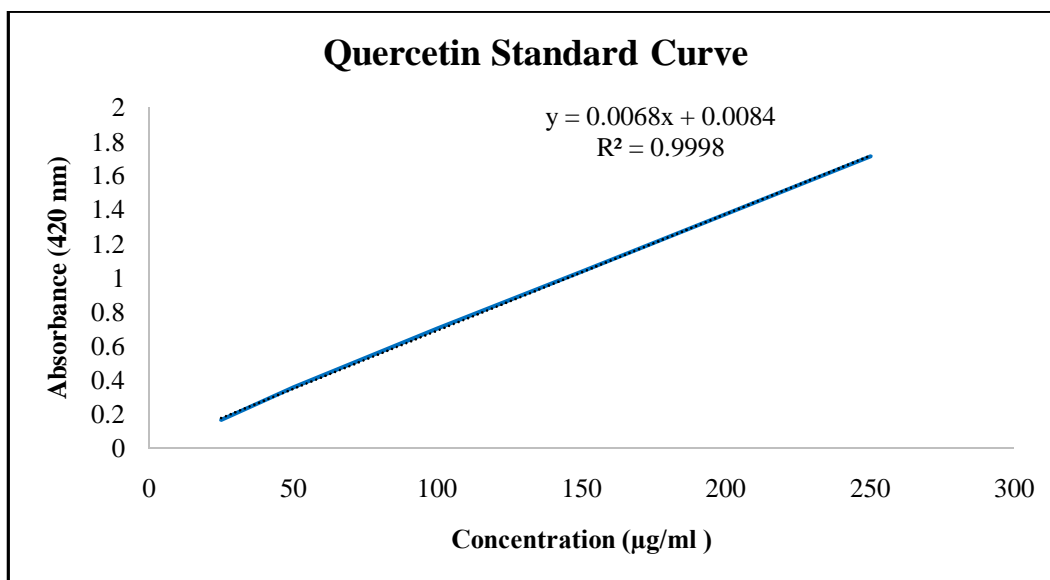
**Figure S2:** Calibration curve for standard gallic acid

**Table S2:** Total phenolic content in chloroform and methanol leaf extract

Extracts	Concentration (µg/mL)	Absorbance <sub>Sample</sub>	Absorbance <sub>control</sub>	Absorbance value
Chloroform	1000	0.213	0.073	0.14
Methanol	1000	0.124	0.059	0.065

**Table S3:** Quercetin standard

Conc. (µg/mL)	Triplicates Absorbance data			Average Absorbance	Absorbance (Av-blank)
250	1.731	1.76	1.829	1.773	1.712
150	1.117	1.089	1.093	1.100	1.038
100	0.762	0.771	0.76	0.764	0.702
50	0.416	0.416	0.422	0.418	0.356
25	0.225	0.228	0.23	0.228	0.166
<b>Control</b>	<b>0.054</b>	<b>0.066</b>	<b>0.065</b>	<b>0.062</b>	



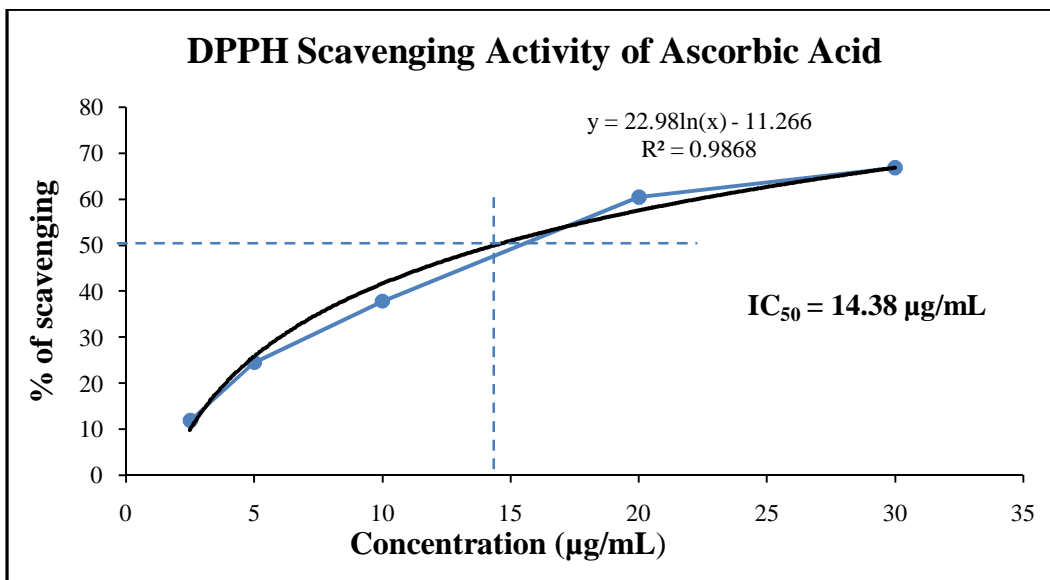
**Figure S3:** Calibration curve for standard quercetin

**Table S4:** Total flavonoid content in chloroform and methanol leaf extract

Extracts	Concentration (µg/mL)	Absorbance <sub>Sample</sub>	Absorbance <sub>control</sub>	Absorbance value
Chloroform	1000	2.464	0.0697	2.394
Methanol	1000	0.636	0.0697	0.566

**Table S5:** Antioxidant activity of ascorbic acid

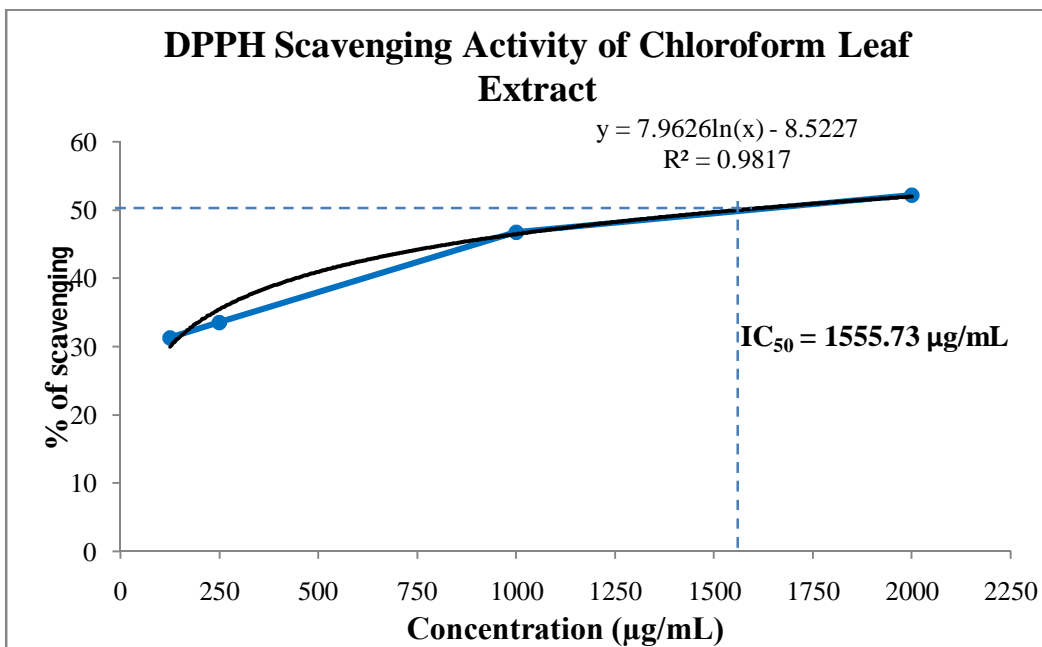
Concentration (µg/mL)	Absorbance(nm)			Average absorbance(nm)	% of scavenging
2.5	0.433	0.433	0.433	0.433	11.872
5	0.385	0.362	0.366	0.371	24.491
10	0.303	0.304	0.309	0.305	37.856
20	0.21	0.195	0.177	0.194	60.516
30	0.16	0.166	0.162	0.163	66.892
<b>Control</b>	0.48	0.524	0.47	0.491	18.33
<b>IC<sub>50</sub> = 14.38 µg/mL</b>					



**Figure S4:** Antioxidant activity of ascorbic acid

**Table S6:** Antioxidant activity of chloroform leaf extract

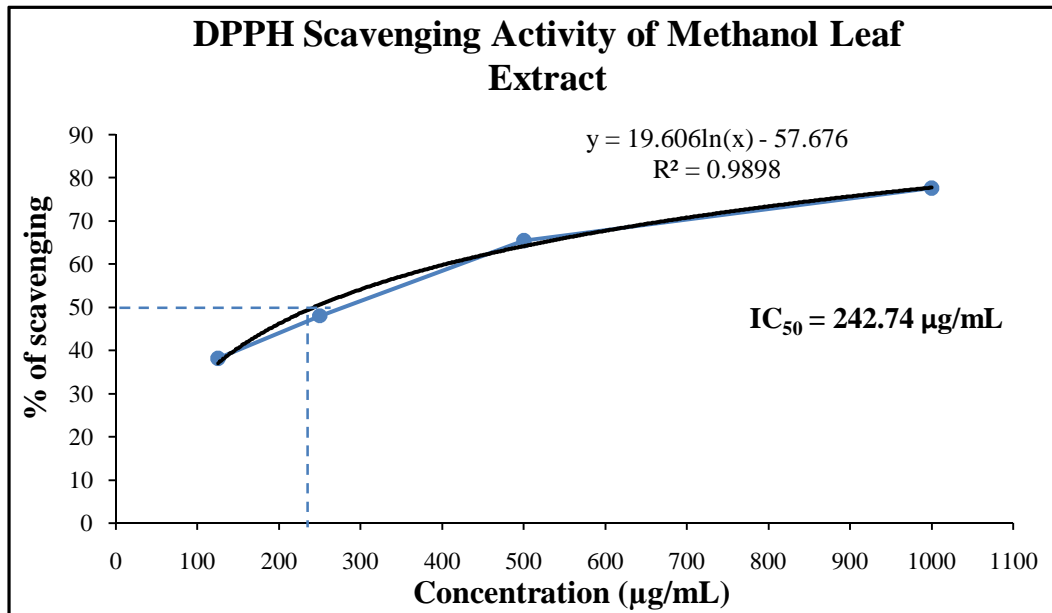
S.N.	Concentration (µg/mL)	% of scavenging
1	2000	52.244
2	1000	46.795
3	250	33.526
4	125	31.282
$IC_{50} = 1555.73 \mu\text{g/mL}$		



**Figure S5:** Antioxidant activity of chloroform leaf extract

**Table S7:** Antioxidant activity of methanol leaf extract

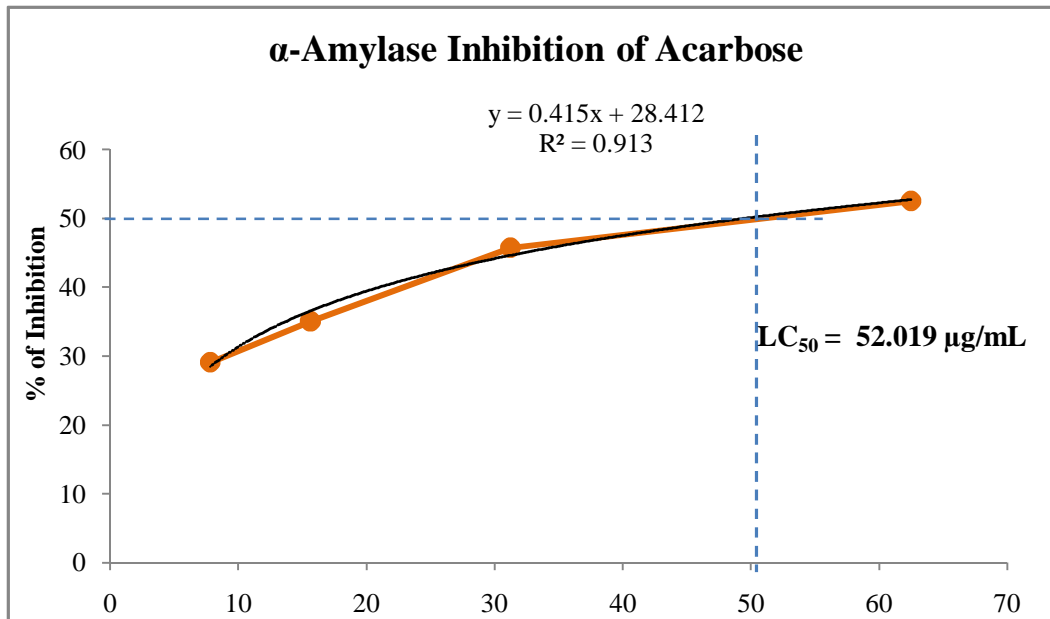
S.N.	Concentration (µg/ml)	% of scavenging
1	1000	77.692
2	500	65.512
3	250	48.077
4	125	38.205
$IC_{50} = 242.74 \mu\text{g/mL}$		



**Figure S6:** Antioxidant activity of methanol leaf extract

**Table S8:**  $\alpha$ -amylase inhibition of acarbose

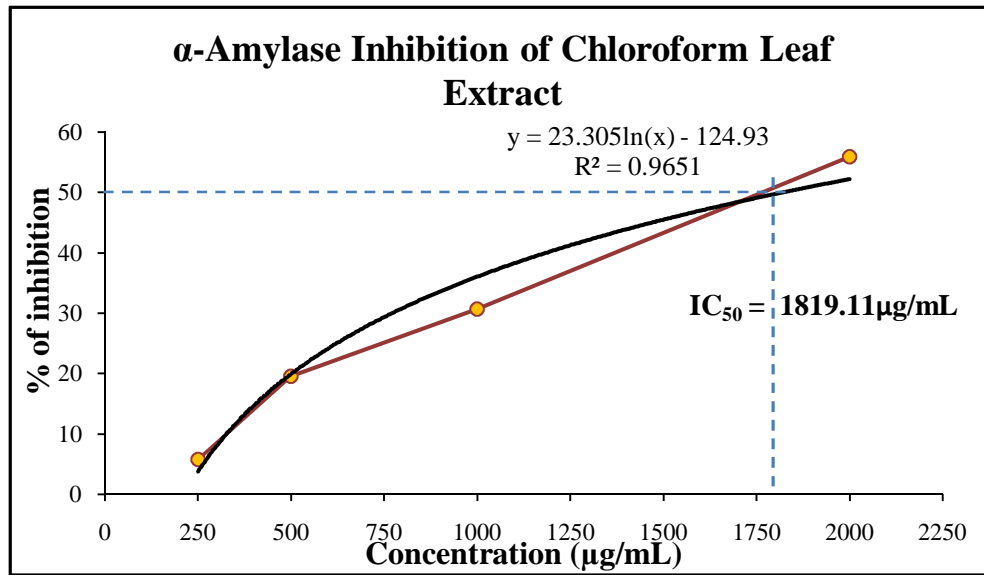
Concentration ( $\mu\text{g/mL}$ )	% of Inhibition
62.5	52.48
31.25	45.7
15.625	35.02
7.8125	29.08
$IC_{50} = 52.019 \mu\text{g/mL}$	



**Figure S7:** α-amylase inhibition of standard acarbose

**Table S9:** α-amylase inhibition of chloroform leaf extract

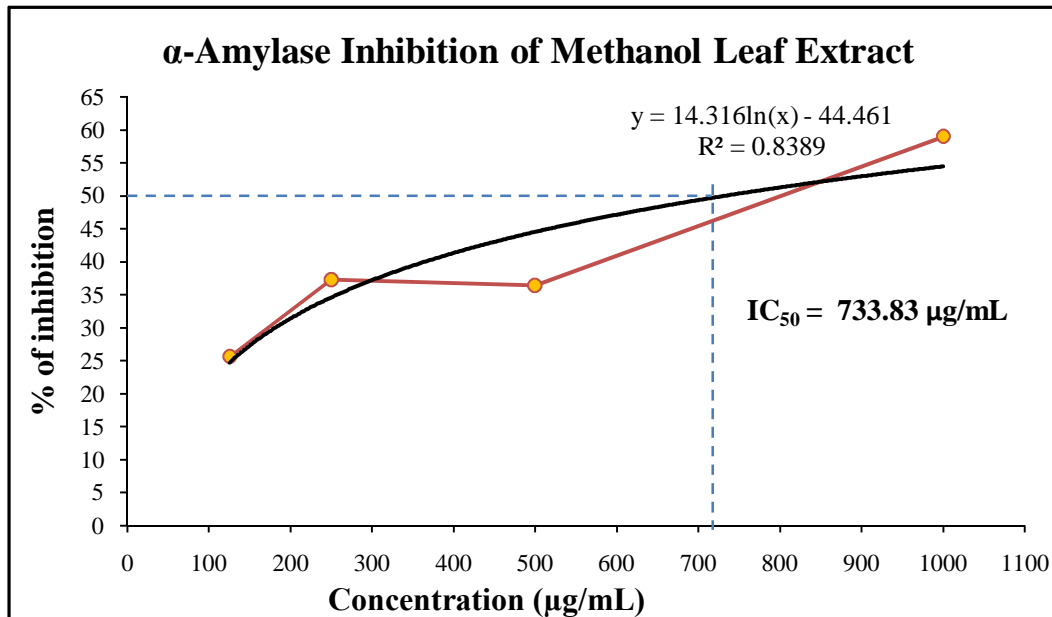
Concentration ( $\mu\text{g/mL}$ )	% of Inhibition
2000	55.918
1000	30.642
500	19.582
250	5.760
$IC_{50} = 1819.11 \mu\text{g/mL}$	



**Figure S8:**  $\alpha$ -amylase inhibition of chloroform leaf extract

**Table S10:**  $\alpha$ -amylase inhibition of methanol leaf extract

Concentration ( $\mu\text{g/mL}$ )	% of Inhibition
1000	58.959
500	36.383
250	37.260
125	25.589
$IC_{50} = 733.83 \mu\text{g/mL}$	



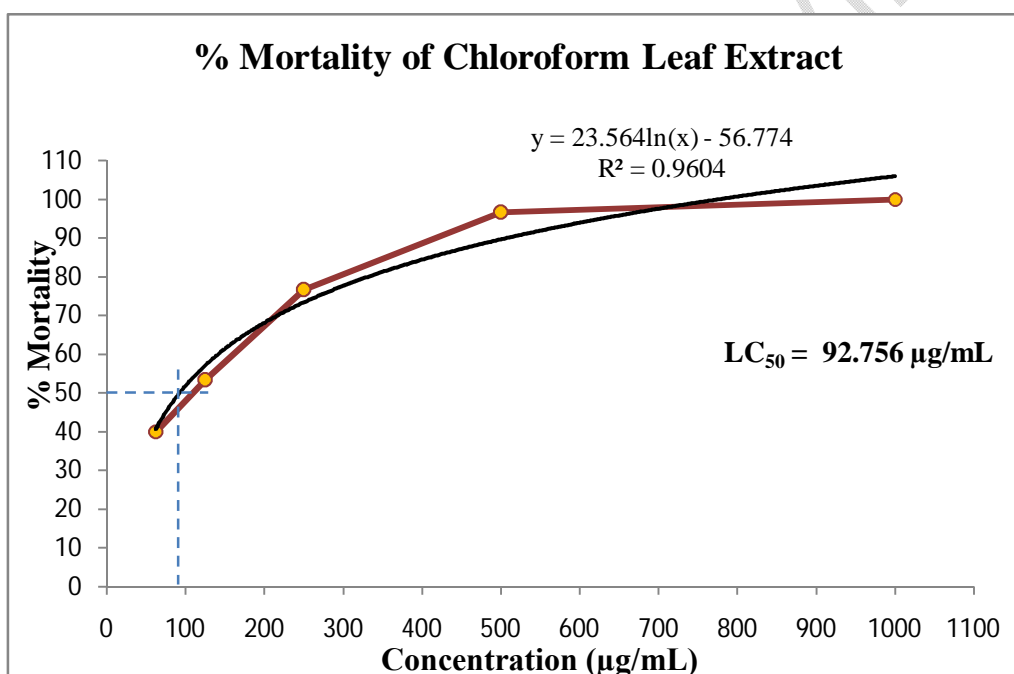
**Figure S9:** α-amylase inhibition of methanol leaf extract



**Figure S10:** ZOI of leaf extracts of *S. sonchifolius*

**Table S11:** Calculation of mortality % of chloroform leaf extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	10	10	10	100
500	9	10	10	96.667
250	8	7	8	76.667
125	5	5	6	53.333
62.5	4	4	4	40
<b>LC<sub>50</sub> = 92.76 µg/mL</b>				

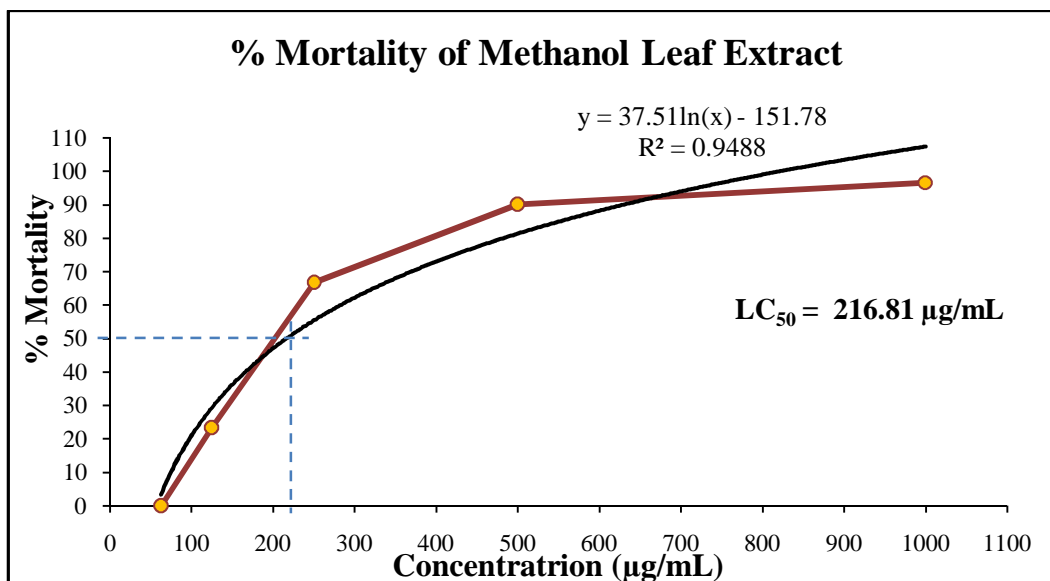


**Figure S11:** Cytotoxicity activity of chloroform leaf extract

**Table S12:** Calculation of mortality % of methanol leaf extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	9	10	10	96.667
500	8	9	10	90.0
250	6	7	7	66.667

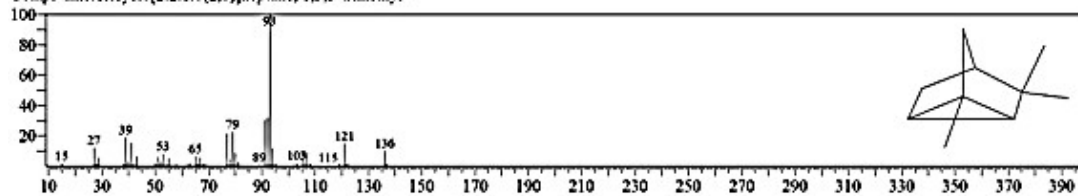
125	3	2	2	23.333
62.5	0	0	0	0
<b>LC<sub>50</sub> = 216.81 µg/mL</b>				



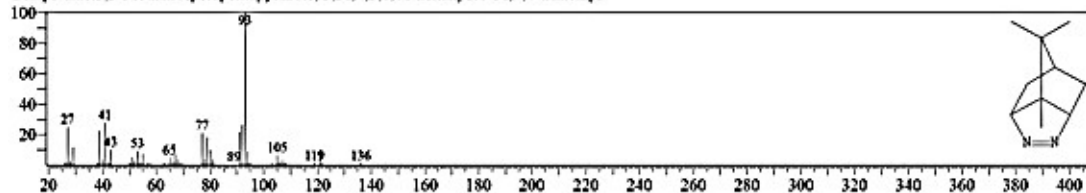
**Figure 7:** Cytotoxicity activity of methanol leaf extract

### Mass Spectral Data of Constituents Identified by GC-MS in Hexane Extract

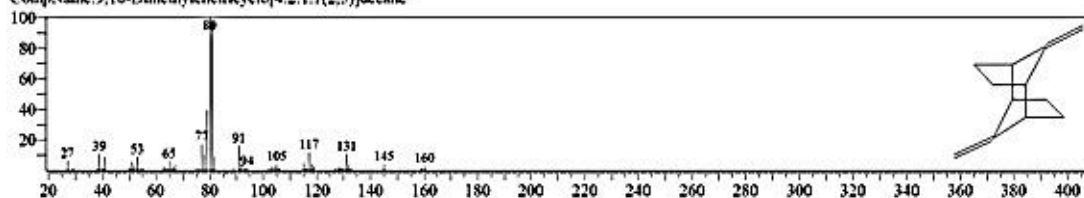
Hit#:1 Entry:18415 Library:NIST17.lib  
 SL84 Formula:C10H16 CAS:488-97-1 MolWeight:136 RetIndex:729  
 CompName:Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-



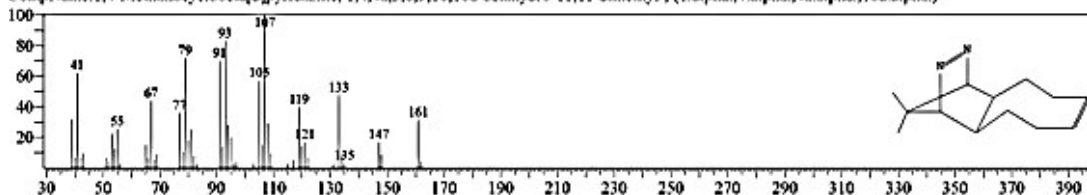
Hit#:1 Entry:38336 Library:NIST17.lib  
 SL89 Formula:C10H16N2 CAS:87143-58-6 MolWeight:164 RetIndex:0  
 CompName:3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl-



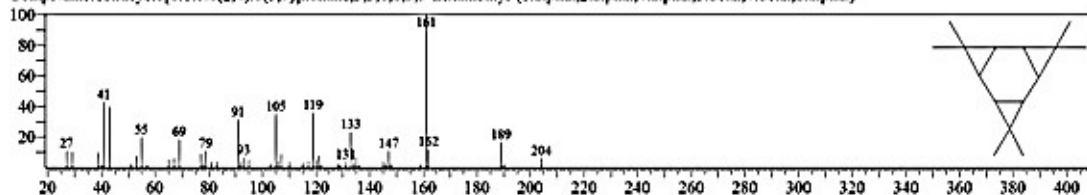
Hit#:1 Entry:35334 Library:NIST17.lib  
SI:68 Formula:C12H16 CAS:0-00-0 MolWeight:160 RetIndex:1014  
CompName:9,10-Dimethylenetricyclo[4.2.1.1(2,5)]decane



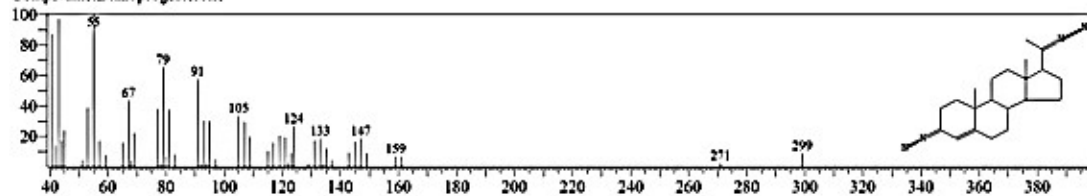
Hit#:1 Entry:74886 Library:NIST17.lib  
SI:85 Formula:C13H20N2 CAS:0-00-0 MolWeight:204 RetIndex:0  
CompName:1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.alpha.,4.alpha.,4a.alpha.,10a.alpha.)-



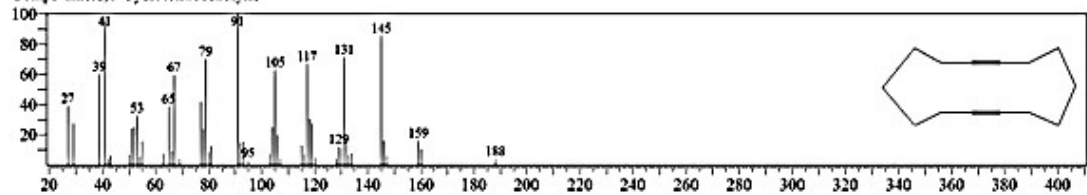
Hit#:1 Entry:75153 Library:NIST17.lib  
SI:79 Formula:C15H24 CAS:51898-92-1 MolWeight:204 RetIndex:1067  
CompName:Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane,3,3,6,6,9,9-hexamethyl-(1.alpha.,2.alpha.,4.alpha.,5.beta.,7.beta.,8.alpha.)-



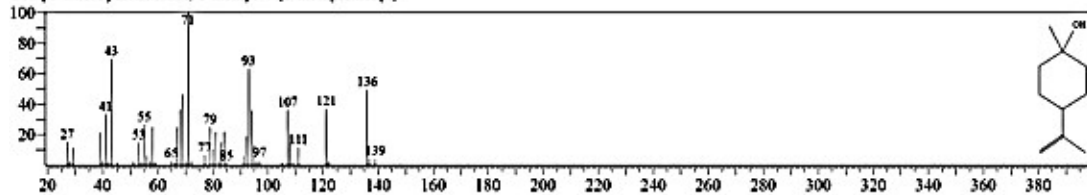
Hit#:1 Entry:217119 Library:NIST17.lib  
SI:66 Formula:C21H30N4 CAS:0-00-0 MolWeight:338 RetIndex:0  
CompName:Dinzoprogestone



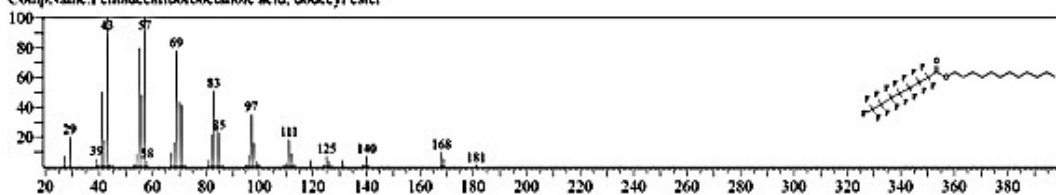
Hit#:1 Entry:59745 Library:NIST17.lib  
SI:68 Formula:C14H20 CAS:1540-80-3 MolWeight:188 RetIndex:0  
CompName:1,8-Cyclotetradecadiyne



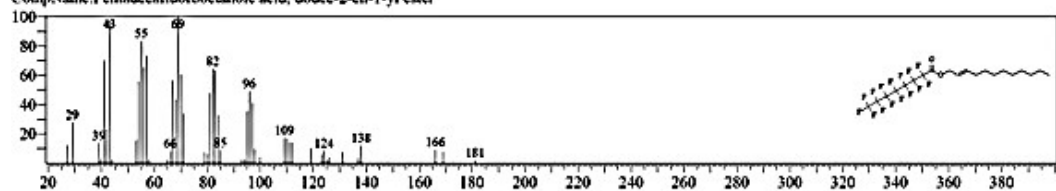
Hit#:1 Entry:30912 Library:NIST17.lib  
SI:69 Formula:C10H18O CAS:138-87-4 MolWeight:154 RetIndex:1158  
CompName:Cyclohexanol, 1-methyl-4-(1-methylethyl)-



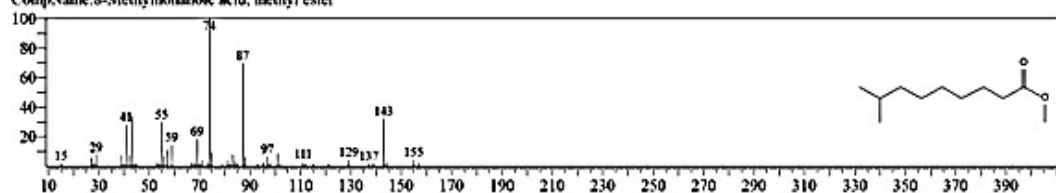
Hit# 1 Entry: 302593 Library: NIST17.lib  
SI: 69 Formula: C<sub>20</sub>H<sub>25</sub>F<sub>15</sub>O<sub>2</sub> CAS: 0-00-0 MolWeight: 582 RetIndex: 1179  
CompName: Pentadecafluorooctanoic acid, dodecyl ester



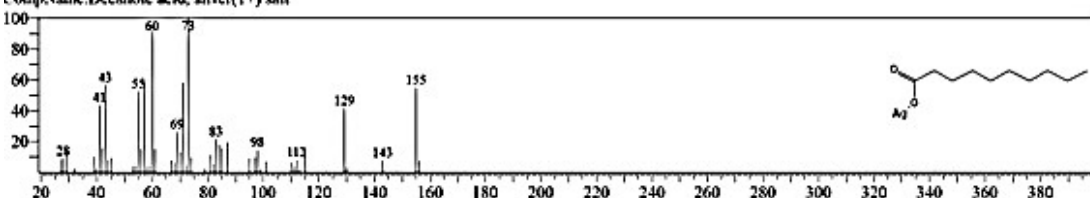
Hit# 1 Entry: 302503 Library: NIST17.lib  
SI: 74 Formula: C<sub>20</sub>H<sub>23</sub>F<sub>15</sub>O<sub>2</sub> CAS: 0-00-0 MolWeight: 580 RetIndex: 1187  
CompName: Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester



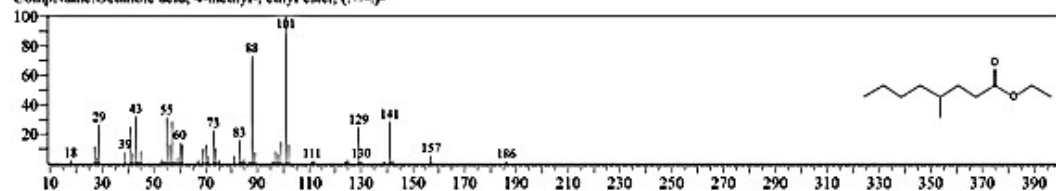
Hit# 1 Entry: 57842 Library: NIST17.lib  
SI: 72 Formula: C<sub>11</sub>H<sub>22</sub>O<sub>2</sub> CAS: 0-00-0 MolWeight: 186 RetIndex: 1218  
CompName: 8-Methylnonanoic acid, methyl ester



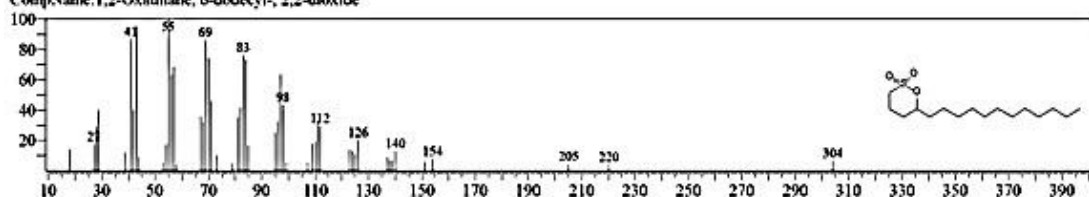
Hit# 1 Entry: 151851 Library: NIST17.lib  
SI: 69 Formula: C<sub>10</sub>H<sub>19</sub>AgO<sub>2</sub> CAS: 13126-67-5 MolWeight: 278 RetIndex: 0  
CompName: Decanoic acid, silver(1+) salt



Hit# 1 Entry: 57877 Library: NIST17.lib  
SI: 75 Formula: C<sub>11</sub>H<sub>22</sub>O<sub>2</sub> CAS: 54831-51-5 MolWeight: 186 RetIndex: 1218  
CompName: Octanoic acid, 4-methyl-, ethyl ester, (+/-)-



Hit# 1 Entry: 180863 Library: NIST17.lib  
SI: 43 Formula: C<sub>16</sub>H<sub>32</sub>O<sub>3</sub>S CAS: 15224-88-1 MolWeight: 304 RetIndex: 0  
CompName: 1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide



Hit#:1 Entry:43887 Library:NIST17.lib  
SI:77 Formula:C<sub>11</sub>H<sub>22</sub>O CAS:41972-59-2 MolWeight:170 RetIndex:1278  
CompName:3,7-Dimethyl-6-nonen-1-ol

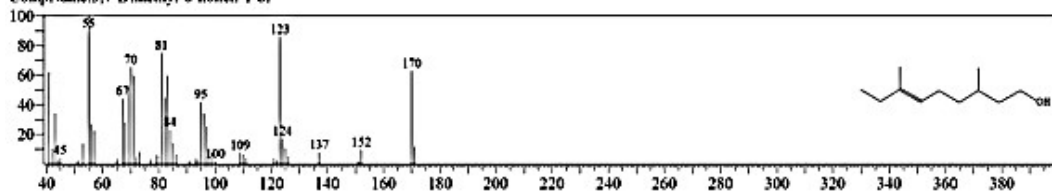
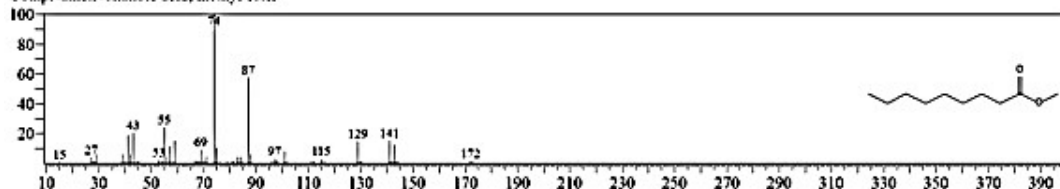


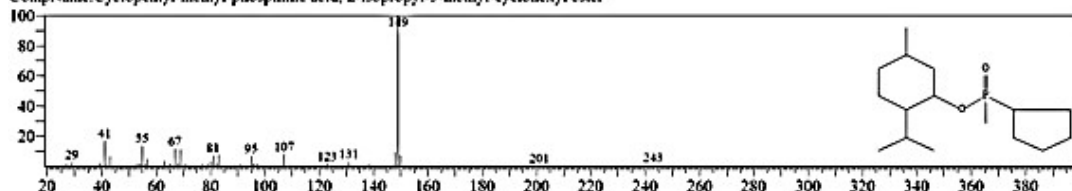
Figure S13: Mass spectral data of constituents identified by GC-MS in hexane extract

### Mass Spectral Data of Constituents Identified by GC-MS in Hexane Extract

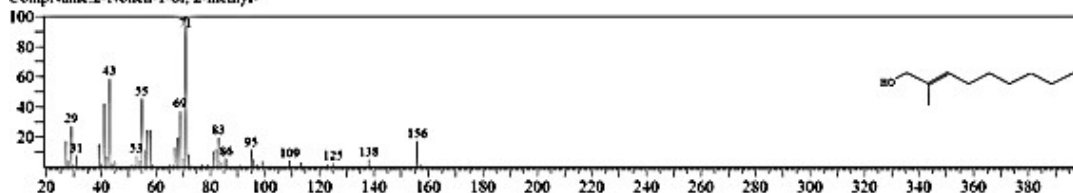
Hit#:1 Entry:45473 Library:NIST17.lib  
SI:85 Formula:C<sub>10</sub>H<sub>20</sub>O<sub>2</sub> CAS:1731-84-6 MolWeight:172 RetIndex:1183  
CompName:Nonanoic acid, methyl ester



Hit#:1 Entry:161458 Library:NIST17.lib  
SI:67 Formula:C<sub>16</sub>H<sub>31</sub>O<sub>2</sub>P CAS:0-00-0 MolWeight:286 RetIndex:0  
CompName:Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester



Hit#:1 Entry:32438 Library:NIST17.lib  
SI:80 Formula:C<sub>10</sub>H<sub>20</sub>O CAS:91008-40-1 MolWeight:156 RetIndex:1243  
CompName:2-Nonen-1-ol, 2-methyl-



Hit#:1 Entry:107374 Library:NIST17.lib  
SI:68 Formula:C<sub>9</sub>H<sub>17</sub>BrO<sub>2</sub> CAS:0-00-0 MolWeight:236 RetIndex:1251  
CompName:4-Bromobutyric acid, 3-methylbut-2-yl ester

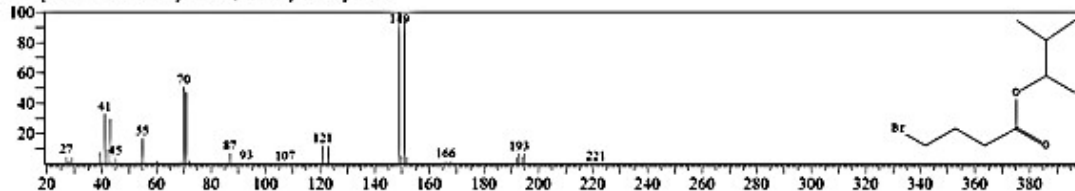
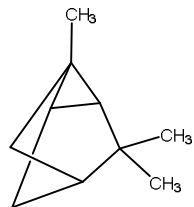
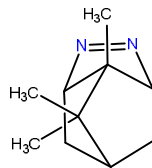


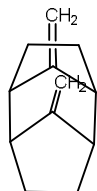
Figure S14: Mass spectral data of constituents identified by GC-MS in methanol extract



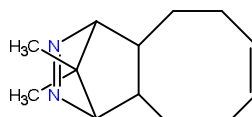
Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl- (1)



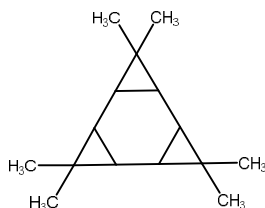
3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl- (2)



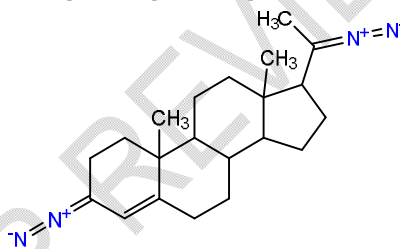
9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane (3)



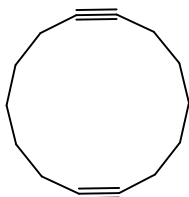
1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl- (1.alpha.,4.alpha.,4a.alpha.,10a.alpha.)- (4)



3,3,6,6,9,9-hexamethyltetracyclo [6.1.0.02,4.05,7] nonane (5)



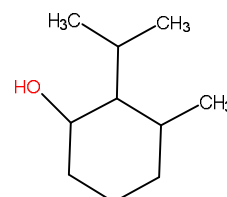
Diazoprogestrone (6)



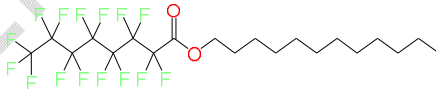
1,8-Cyclotetradecadiyne (7)



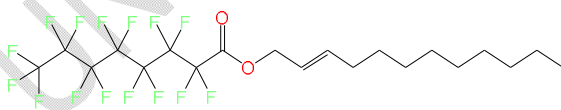
Cyclohexanol, 1-methyl-4-(1-methylethenyl)- (8)



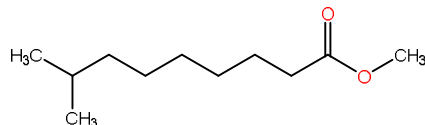
2-isopropyl-3-methylcyclohexanol (9)



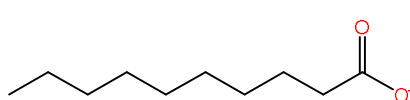
Pentadecafluorooctanoic acid, dodecyl ester (10)



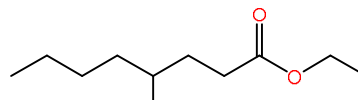
Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester (11)



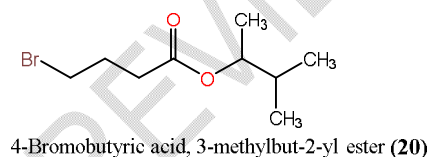
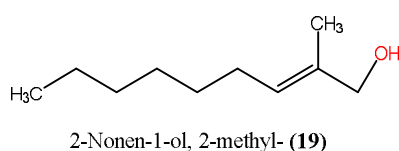
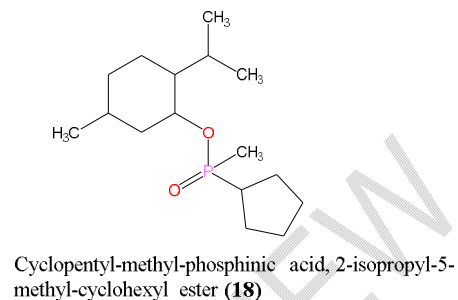
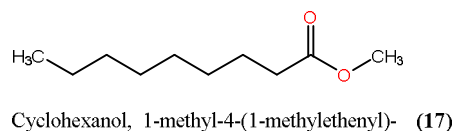
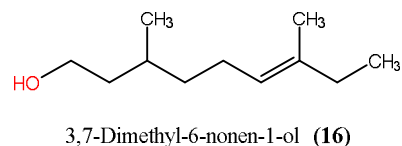
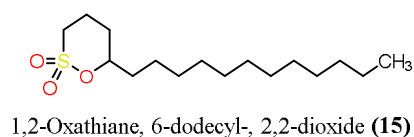
8-Methylnonanoic acid, methyl ester (12)



Decanoic acid, silver (1+) salt (13)



Ethyl-4-methyl-octanoate (14)



**Figure S15:** Molecular structures 20 GC-MS compounds from hexane and methanol leaf extracts of *S. sonchifolius*

**Table S13:** Binding affinities of GC-MS identified compounds from molecular docking calculations against  $\alpha$ -amylase protein along with those of native and reference drugs

Extracts	Ligands	Binding Affinities (kcal/mol) with $\alpha$ -amylase protein (PDB ID 2QV4)
Hexane	1	-5.4
	2	-5.7
	3	-6.4
	4	-6.9
	5	-6.2
	6	<b>-9.1</b>
	7	-7.1
	8	-5.8
	9	-5.2
	10	-7.1
	11	<b>-8</b>
	12	-5

	13	-5.2
	14	-4.7
	15	-6
	16	-5.6
<hr/>		
	17	-4.7
	18	-6.7
Methanol	19	-5.4
	20	-5
<hr/>		
Reference Drugs	Miglitol	-5.8
Native ligand	QV4	-10.4
<hr/>		

UNDER PEER REVIEW