Original Research Article

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

ABSTRACT

Smallanthus sonchifolius, 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*. The chloroform extract exhibited the highest phenolic content (21.4 mg GAE/g) and flavonoid content (350.87 mg QE/g) with significant TFC in methanol extract (82.05 mg QE/g). In contrast, the methanol extract showed superior antioxidant activity (IC_{50} = 242.74 µg/mL) and α-amylase inhibition (IC_{50} = 733.83 µg/mL). Antimicrobial testing showed hexane and methanol extracts were effective against *Candida albicans*, with inhibition zones comparable to kanamycin. The methanol extract exhibited moderate lethality in the brine shrimp assay with an LC_{50} value of 216.81 µg/mL. GC-MS analysis identified16 compounds in hexane extract and 4 in methanol extract. Molecular docking demonstrated strong binding affinities for diazoprogesterone and pentadecafluorooctanoic acid, dodec-2-en-1-yl ester with α-amylase, surpassing reference drug miglitol. *In-silico* drug-likeness and ADMET predictions indicated potential drug-like properties of the compound diazoprogesterone but further experimental studies are suggested to explore their pharmacological and safety profiles.

Keywords: Smallanthus sonchifolius, antioxidant, antidiabetic, GC-MS, molecular docking.

1. INTRODUCTION

Smallanthus sonchifolius (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 it's 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 8β-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.

(a) **(b)**

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, and to understand biochemical processes at the atomic level [17] and is useful in drug discovery, including for diabetes, a major global health concern [18]. Inhibiting α-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 integrates *in vitro* screening of crude extracts with analytical and *in-silico* techniques to explore the biological activities and antidiabetic potential of *S. sonchifolius* leaf extracts. Bioactive phytochemicals are identified through GC-MS, while computational docking against α-amylase provides molecular-level insights into protein-ligand interactions and their stability. The research bridges a gap between the plant's traditional use and the biological activity of its compounds, offering strong evidence for its medicinal value in treating various diseases. Notably, the study introduces the novel use of molecular docking alongside traditional phytochemical analysis, highlighting its unique contribution to understanding the *S. sonchifolius* plant's therapeutic potential, especially in α-amylase inhibition and antidiabetic properties.

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_2SO_4, conc. HCl, $AlCl_3$, 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*plant samples 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 taken, soaked 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. Then the marc obtained was used for further extraction with chloroform, ethyl acetate, methanol, and distilled water ina similar patternin the order of increasing polarity of the solvents. After extractions, all dried extracts were collected and stored in airtight vial tubes in the refrigerator until their use.

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 [24]. 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 the standard solution was added in triplicate which was the same for the sample, followed by 100 µL of diluted FC reagent to initiate the reaction and 80 µL of 7.5% Na_2CO_3 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 [25]. 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% $AlCl_3$ 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 [26]. 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:

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

2.7 α-Amylase Inhibition Analysis

The α-amylase inhibition of chloroform and methanol extracts was tested using the 3,5-dinitrosalicylic acid (DNSA) method as mentioned previously [27] 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 µL of it with 200 µL of α-amylase solution. After 10 minutes of incubation at 30°C, 200 µL of 1% starch solution was added and incubated for 3 minutes. The reaction was stopped with 200 µL 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 α-amylase inhibition activity was calculated using the following formula.

$$\text{\% α-amylase inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\% \quad \text{.....(2)}$$

2.8 Antimicrobial Activity

The antimicrobial activity of five *S. sonchifolius* leaf extracts was tested using the agar disc diffusion method [28] 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 µL of microbial seed and 10 µL of each leaf extract (15 mg in 100 µL 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 [29] 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:

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

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[30].

2.11 Computational Methodology

2.11.1 Selection and Preparation of Ligand Database

20 compounds identified bythe GC-MS experiment were used as ligands with their 3D structures retrieved from the PubChem server[31]. It was optimized in the Avogadro (version 1.2.0) program [32] using the UFF force field, 5000 steps, conjugate gradients algorithm, and 10^{-8} kcal/mol convergence. The ligands were converted to PDBQT format with Gasteiger charges via AutoDock Tools[33].

2.11.2 Target Selection and Preparation

The 3D crystal structure of α-amylase (PDB ID 2QV4, X-ray diffraction, 1.97 Å resolution) was obtained from the RCSB database [34] in PDB format. Using the PyMol program [35] 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.

2.11.3 Molecular Docking Calculations

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, withcoordinates 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 [36].

3. RESULTS AND DISCUSSION

3.1 Extracts Yield and Phytochemical Analysis Screening Analysis

The successive extraction was done with the solvents of increasing polarity from non-polar (hexane) to more polar solvent (water). Among five extracts, the aqueous extract showed a high yield followed by hexane and methanol. The high yield of the aqueous extract is due to the high polarity of the water solvent which likely extracts more hydrophilic compounds as

solvent polarity is one of the factors that affect the extract yield [37]. The yield of different extracts in different solvents is presented in Table 1.

The preliminary phytochemical screening of the extracts of the plant demonstrated the presence of alkaloids, carbohydrates, phenolic compounds, flavonoids, and quinones in the leaf extract.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_4SCN, $FeSO_4$ and conc. H_2SO_4	-	+	+	+	+
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.Methanol extract also demonstrated a significant flavonoid content with a TFC value of 82.05 mg QE/g, as reported previously[38].With a TPC value of 2.10 mg GAE/g. The detailed observations during the calculation of the TPC and TPC are illustrated in Table 3.

The bioactivity observed in the extracts can be explained by several phytochemical mechanisms. The high content of phenolic and flavonoid compounds in the methanol and chloroform extracts of *S. sonchifolius* is responsible for their antioxidant and antidiabetic properties. These compounds operate as reducing agentsthat neutralize free radicals by supplying hydrogen atoms, scavenging reactive oxygen species (ROS), chelation of metal ions [20] and are known to interact with enzymes altering their function due to the presence of hydroxyl (OH) groups.

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

Extract	Total phenolic content (mg GAE /g extract)	Total flavonoid content (mg QE/g extract)
Chloroform (mg GAE/g)	21.4	350.87
Methanol (mg GAE /g)	2.10	82.05

3.3 Antioxidant Activity Screening Analysis

The DPPH free radical scavenging assay is a method based on reducing a methanol DPPH solution in the presence of a hydrogen-donating antioxidant, resulting in the non-radical form DPPH-H. The extracts were able to convert the stable DPPH radical into yellow-colored diphenyl picrylhydrazine, with the effect increasing as the concentration increased. From the DPPH assay, the IC_{50} 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_{50} value of 14.38 µg/mL, lower than that of the plant extracts. However, this is expected since the plant extracts consist of a mixture of many compounds, unlike the single compound ascorbic acid. The methanol extract demonstrated notable antioxidant activity with an IC_{50} value of 242.74 µg/mL (<500 µg/mL) [39] as previously reported [38] which is likely due to its flavonoid content. Flavonoids are recognized for their ability to strengthen the body's defense by donating electrons to neutralize free radicals [40]. In this study, the high flavonoid content (82.05 mg QE/g) was directly associated with the observed antioxidant effect [41]. Additionally, the elevated total phenolic content in chloroform and methanol extracts suggests these compounds play a key role in neutralizing free radicals and protecting cells from oxidative damage. Phenolics act as reducing agents, donating hydrogen atoms to interrupt the chain reactions of free radicals and mitigate oxidative stress. These findings are consistent with research on other medicinal plants, where increased phenolic content is linked to greater antioxidant capacity [42]. IC_{50} values of the samples and ascorbic acid standard are listed in Table 4.

Table 4: IC_{50} values of the tested samples and standard drug

Samples	IC_{50} (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_{50} 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. The observed inhibition of α-amylase in this study is due to the interaction of flavonoids and phenolic compounds with the enzyme's active site as mentioned previously [43], which prevents the hydrolysis of starch by binding to its catalytic residues.According to the previous study [44], the methanol extract from our study demonstrated moderate inhibition with an IC_{50} value of 733.83 µg/mL, indicating significant potential for natural diabetes management, even though it is lower than the reference drug acarbose (52.02 µg/mL). The inhibitory effect of the identified compounds likely stems from their specific binding to the enzyme's active site, limiting substrate access.

Table 5:IC_{50} values of the tested samples and standard drug

Samples	IC_{50} (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) [45]against three microbial strains *Candida albicans*, *Bacillus subtilis*, and *Escherichia coli*. At a concentration of 1.5mg/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 mg/mL), indicating potentialantifungal activity. Negative control DMSO did not show any activity. The antimicrobial activity observed in the hexane and chloroform extracts, particularly against *Candida albicans*, is likely due to bioactive phenolic compounds as studied by Oliveira et al in 2007 [46] that disrupt fungal cell membranes. The antifungal effects were comparable to kanamycin, indicating that these extracts may contain potential antifungal agents. The stronger activity against fungal strains than bacterial ones may be attributed to the specific targeting of ergosterol in fungal membranes [47]. The lipophilic nature of the compounds in the hexane and chloroform extracts likely aids their incorporation into fungal cell membranes, leading to disruption and cell death. The enhanced activity against *Candida albicans* relative to bacterial strains suggests a specific interaction between the bioactive compounds and fungal sterols [48].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		ZOIof extracts in mm (7 mm diameter)				
		Kanamycin ZOI (mm)	Negative control	Hex	Chl	EtOAc	MeOH	Aqueous

		(DMSO)						
Bacillus subtilis	(ATCC 6051)	12.0	0.0	4.0	3.0	3.0	2.0	2.0
Escherichia coli	(ATCC 8739)	12.0	0.0	4.0	3.0	3.0	2.0	0.0
Candida albicans	(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.*

3.6 Brine Shrimp Lethality Analysis

From previous studies on plant extracts [49], the degree of brine shrimp lethality was directly proportional to the concentration of the extract. The LC_{50} (lethality concentration) of the chloroform and methanol extract is different in different concentrationsas shown in Table 7.

Table 7:LC_{50} values of the tested samples

Samples	**LC_{50} (µg/mL)**
Chloroform extract	92.76
Methanol extract	216.81

From the study, chloroform and methanol leaf extract of *S. sonchifolius* were reported to have LC_{50} values of 92.76 µg/mL and 216.81 µg/mL, respectively. The LC_{50} 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 [50]. The outcomes imply that these extracts are active against the lethality test for brine shrimp as their LC_{50} values were below 1000 µg/mL. According to the previous study, the plant demonstrated a cytotoxic nature which may be attributed to the presence of probable anticancer components and high cytotoxic substances [36]. This offers proof of the plant's historical usage in treating kidney and liver cancer.

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.

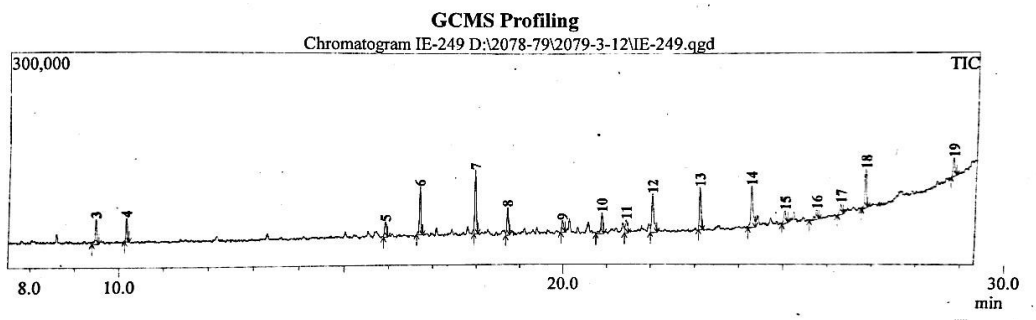


Figure 2: Chromatogram of hexane leaf extract

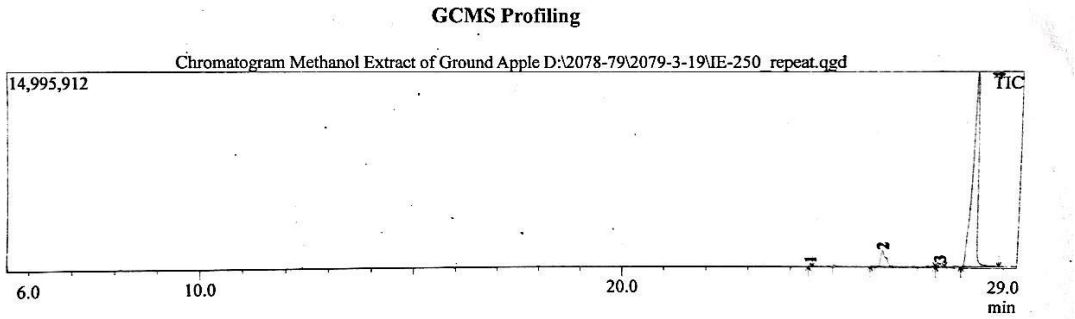


Figure 3: Chromatogram of methanol leaf extract

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 phytocompound identified by GC-MS are presented in the supplementary information (Figures S13 and S14).

Table 8: Components based on GC-MS analysis

Extracts	Name of compounds	Code	PubChem CID	RT	Molecular formula	Peak Area (%)
Hexane	Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl-	**1**	79022	6.300	$C_{10}H_{16}$	2.17
	3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl-	**2**	134900801	7.067	$C_{10}H_{16}N_2$	27.02
	9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane	**3**	556763	15.925	$C_{12}H_{16}$	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	$C_{13}H_{20}N_2$	8.22

	3,3,6,6,9,9-hexamethyltetracyclo [6.1.0.0^{2,4}.0^{5,7}] nonane	**5**	142890	18.726	$C_{15}H_{24}$	3.93
	Diazoprogesterone	**6**	543575	20.583	$C_{21}H_{30}N_4$	7.19
	1.8-Cyclotetradecadiyne	**7**	137070	20.900	$C_{14}H_{20}$	3.67
	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	**8**	8748	23.125	$C_{10}H_{18}O$	5.60
	2-isopropyl-3-methylcyclohexanol	**9**	228304	24.292	$C_{10}H_{20}O$	7.91
	Pentadecafluorooctanoic acid, dodecyl ester	**10**	15918850	24.425	$C_{20}H_{25}F_{15}O_2$	
	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
Methanol	Nonanoic acid, methyl ester	**17**	15606	24.473	$C_{10}H_{20}O_2$	0.38
	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

Only a limited number of studies have explored theGC-MS analysis of this plant's leaf extracts. In a recent study conducted by Wawo et al in 2024 [51] several GC-MS compounds were reported. However, none of those spectral libraries matched with our, suggesting the presence of underreported compounds. These structures of the compounds were further evuluated through computational docking to investigate their potential as therapeutic agentstargeting α-amylase, a crucial enzyme involved in diabetes management.

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[52]. A good RMSD value of 1.3 Å and a binding affinity of -10.4 kcal/mol for α-amylase confirmed the docking protocol's accuracy. A superimposed image of native and docked ligands for α-amylase is shown in Figure 2.

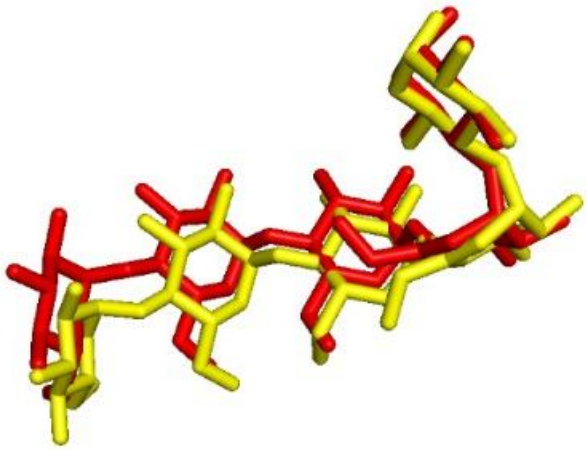


Figure 4: Superimposition of native ligand (yellow) with docked ligand (red) in α-amylase protein (RMSD of heavy ligand atom = 1.3 Å)

The molecular docking analysis conducted in this study revealed significant binding between the bioactive compounds of *S. sonchifolius* and α-amylase, an enzyme involved in diabetes management by reducing postprandial hyperglycemia. The strongest binding affinities were noted for diazoprogesterone and pentadecafluorooctanoic acid dodec-2-en-1-yl ester, with docking score values of -9.1 kcal/mol and -8.0 kcal/mol, respectively, both exceeding the binding affinity of the reference drug, miglitol (-5.8 kcal/mol) which is given in Table 9. This interaction is crucial because inhibiting α-amylase is a key therapeutic approach for managing glucose levels in diabetic patients. The high binding affinities of these natural inhibitors indicate their potential to effectively obstruct the active site of α-amylase, hindering the conversion of starch into glucose and consequently lowering blood sugar levels[53].

The docking scores correlate directly with the experimental findings of the IC_{50} values. A lower IC_{50} value indicates stronger or more efficient binding of the natural product to the α-amylase enzyme, suggesting that the identified compounds have a greater potential to inhibit α-amylase. This is consistent with the experimental results, where the methanol extract, which contains these compounds, exhibited moderate α-amylase inhibition with an IC_{50} of 733.83 µg/mL.

The binding affinity data of GC-MS compounds 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 S15).

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

Extracts	Ligands	Code	Binding Affinities (kcal/mol) with α-amylase protein (PDB ID 2QV4)
Hexane	**Diazoprogesterone**	**6**	-9.1
	pentadecafluorooctanoic acid	**11**	-8.0

	dodec-2-en-1-yl ester	
Reference Drugs	**Miglitol**	-5.8
Native ligand	**QV4**	-10.4

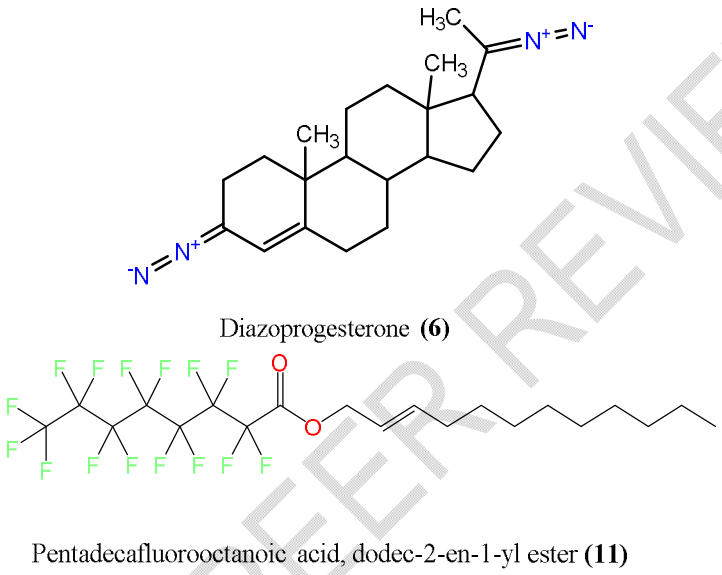


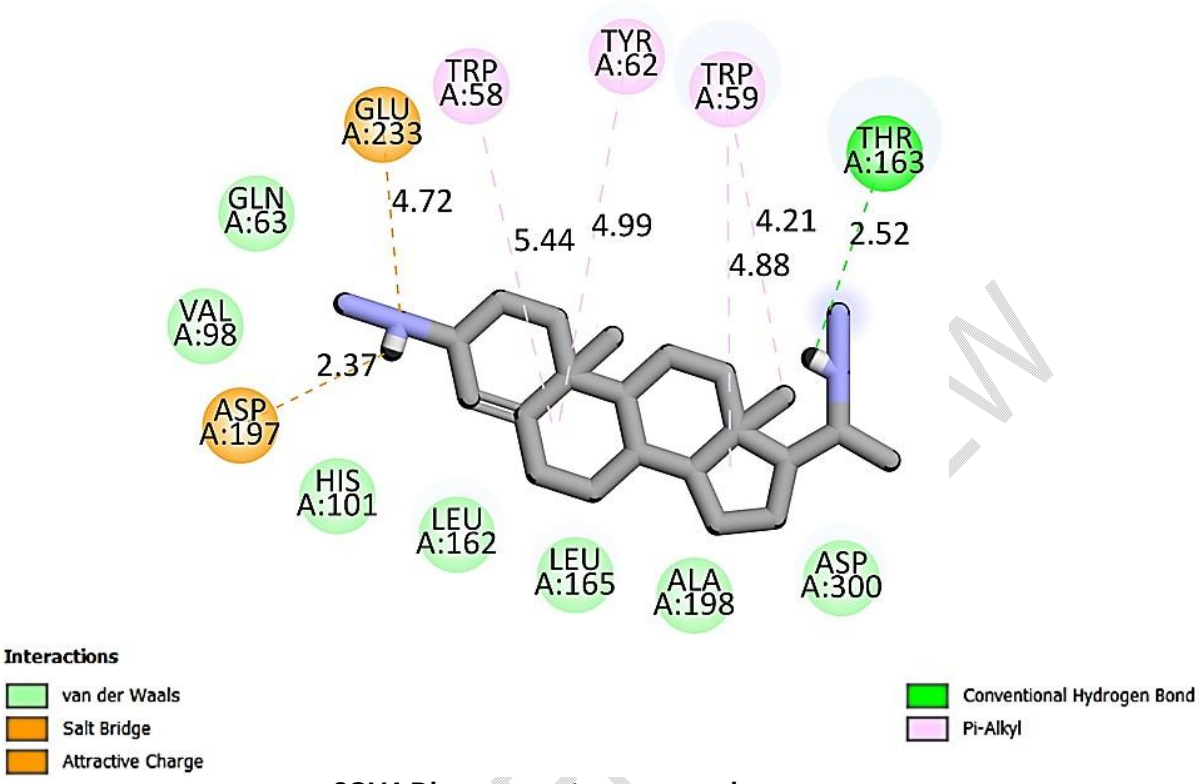
Figure 5: Molecular structures of top two GC-MS compounds with α-amylase

3.8.2 Protein-ligand interaction

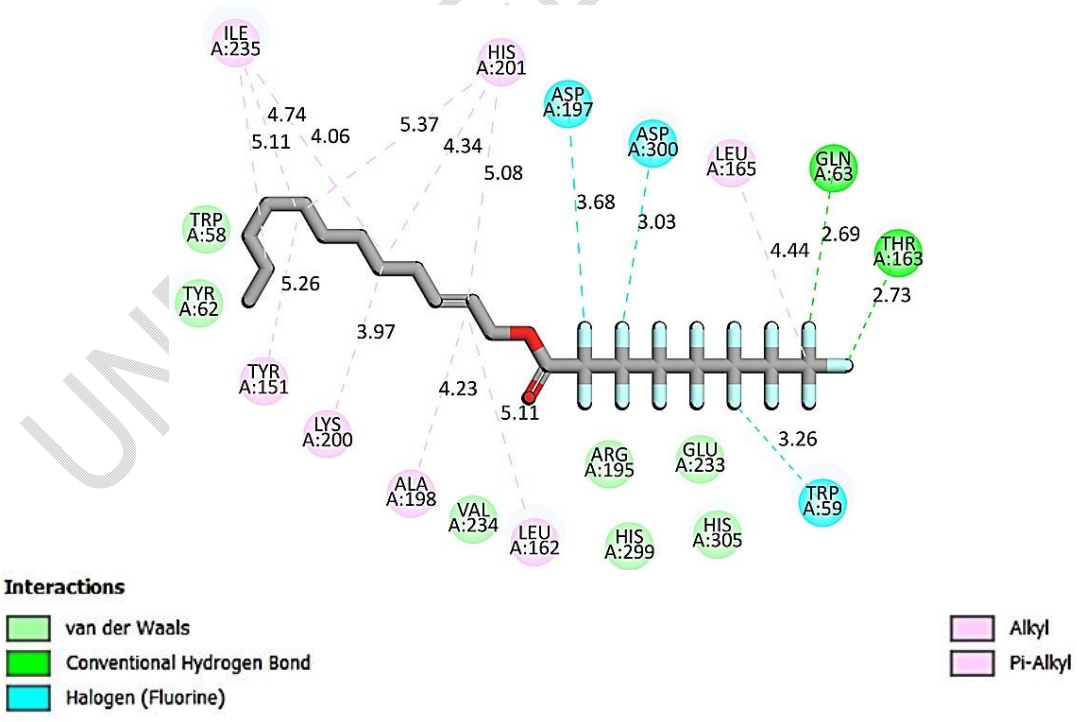
The stability of the ligand-target complex is influenced by effective ligand orientation at the binding site [54].The 2D interaction analysis revealed conventional hydrogen bonds in both the top compounds, with a distance range of 2.20 to 2.73 Å, similar to that of the native ligand, indicating strong 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 the 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 the case of the native ligand. The overall interactions of the top two docked compounds and native ligands with α-amylase are demonstrated in Table 10 and Figure 4.

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 α-amylase protein

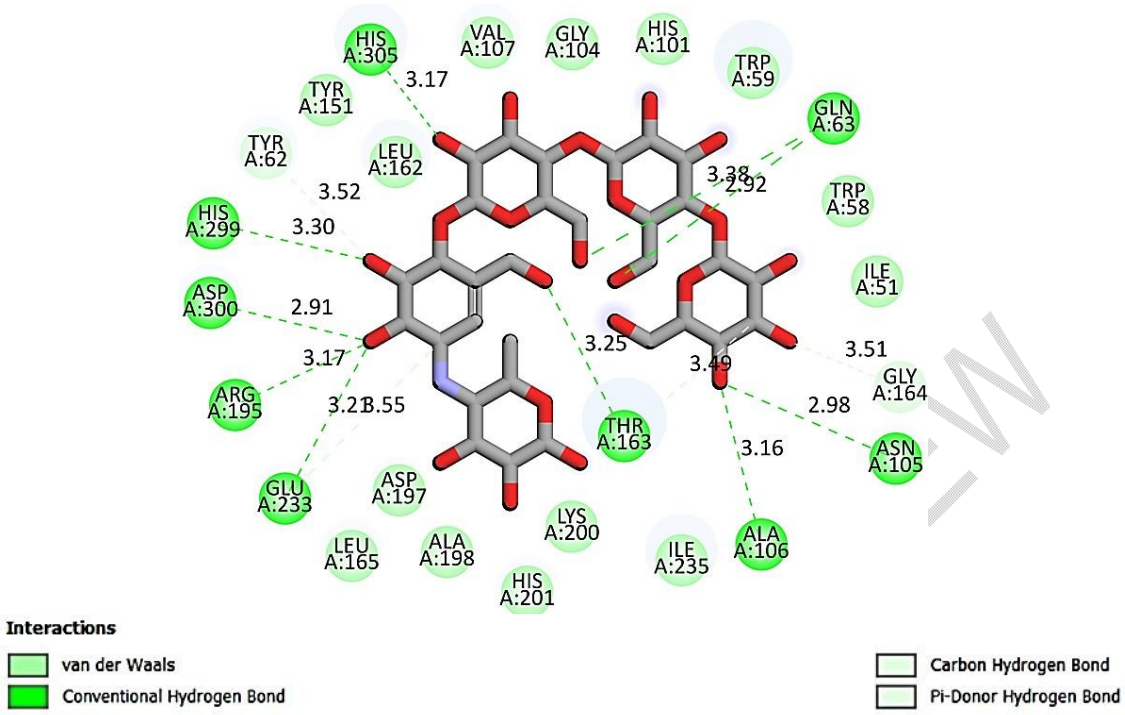
Complex	Type of interactions	Amino acid residues with distance (Å)
2QV4-6	Conventional hydrogen bonds	THR163 (2.52)
	Pi-alkyl	TRP58 (5.44), TRP59 (4.21, 4.88), TYR62 (4.99)
	Salt bridge	ASP197 (2.37)
	Attractive charge	ASP197 (2.37), GLU233 (4.72)
	van der Waals	GLN63, VAL98, HIS101, LEU162, LEU165, ALA198, ASP300
2QV4-11	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
Native-complex	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



2QV4-Diazoprogesterone complex



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



2QV4-QV4 complex

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

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 which may alter the functioning of α-amylase. The findings of this study align with previous research on natural α-amylase inhibitors, which indicates that flavonoids and phenolic compounds often demonstrate considerable enzyme inhibition due to their capacity to establish strong interactions with critical amino acids in the active site. In this instance, the hydrogen bonding observed between the leading ligands and α-amylase residues, such as TRP58, TYR62, and ASP300, illustrates the common binding pattern of natural inhibitors used in diabetes treatment [55].

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 Å², while compound 11 has a TPSA of 26.30 Å², indicating good bioavailability. Compound 6 also has rotatable bonds below 10, unlike compound 11. LogP values were predicted to be 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 [56].

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 (Å^2)	Log P	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 drugs	**Miglitol**	207.11	3	6	5	104.39	-2.27	Accepted

Note: *NRB-Number ofrotatable bonds, NHA-Number of hydrogen acceptor, NHD-Number of hydrogen donor, TPSA-Topological 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 [57]. 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[58].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 [59]. 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_{50} 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_{plasma} (ml/min/kg)**	11.33	5.64	5.36
T	**Toxicity class**	5	5	4
	LD_{50} (mg/kg)	3700	5000	1200
	Hepatotoxicity	Inactive	Inactive	Inactive
	Neurotoxicity	Active	Inactive	Inactive
	Immunotoxicity	Active	Inactive	Inactive
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	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_{plasma}: - Plasma clearance.*

4. CONCLUSION

A detailed quantitative analysis of chloroform and methanol extracts of*S. sonchifolius* plant identified a high concentration of phenolic and flavonoid compounds, believed to contribute to the plant's biological activity. The DPPH assay confirmed strong antioxidant properties, while the α-amylase inhibition assay demonstrated moderate antidiabetic potential for methanol extract. Antimicrobial evaluation of five leaf extracts showed notable antifungal activity. The brine shrimp lethality assay revealed toxic to moderately toxic effects, possibly due to carcinogenic phytochemicals. Molecular docking studies of GC-MS-identified compounds, such as diazoprogesterone and pentadecafluorooctanoic acid, demonstrated strong binding affinities to α-amylase, suggesting their potential in diabetes management. In-silico drug-likeness and ADMET predictions showed that diazoprogesterone exhibits drug-like properties and belongs to toxicity class 5. This study supports the traditional use of *S.sonchifolius* in diabetes management and microbial infection treatment, though further research is required to fully assess its pharmacological properties and safety.

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.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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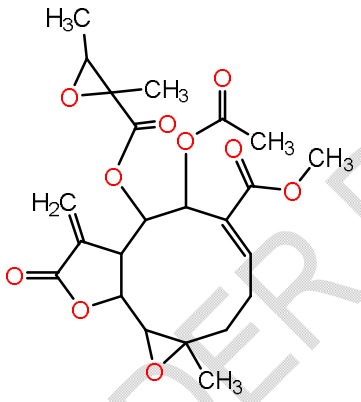
SUPPLEMENTARY INFORMATION



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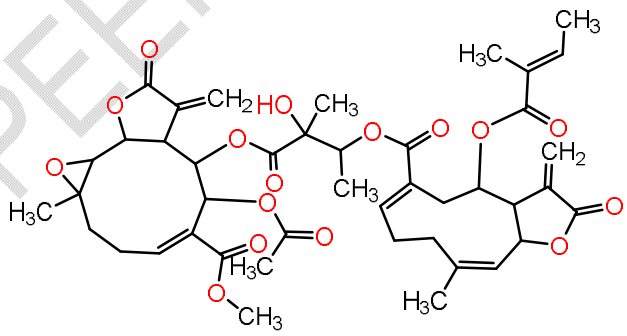

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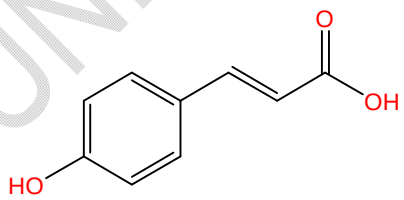

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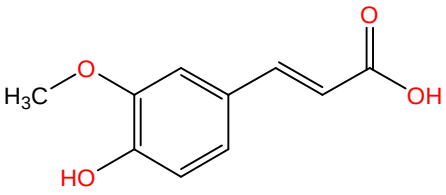

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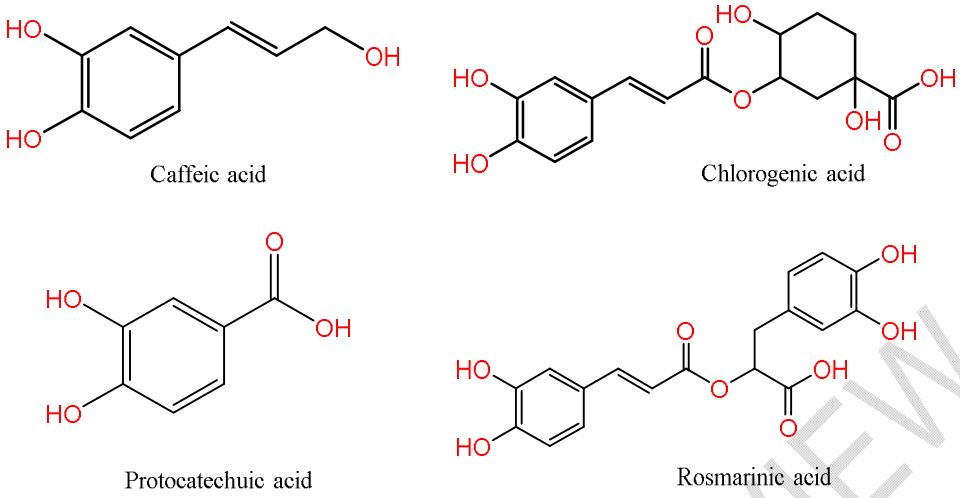


Figure S1: Structures of some chemical compounds found in the leaf of *S. sonchifolius*

Table S1: Gallic acid standard

Conc. (µg/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
Control	**0.235**	**0.231**	**0.266**	**0.244**	

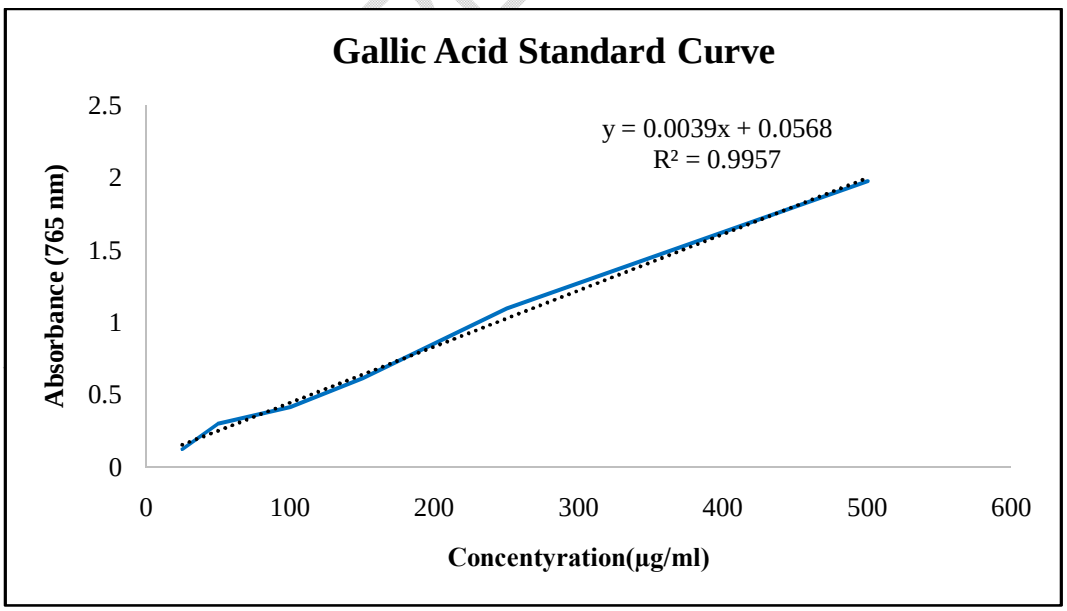


Figure S2: Calibration curve for standard gallic acid

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

Extracts	Concentration (µg/mL)	$Absorbance_{Sample}$	$Absorbance_{control}$	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
Control	**0.054**	**0.066**	**0.065**	**0.062**	

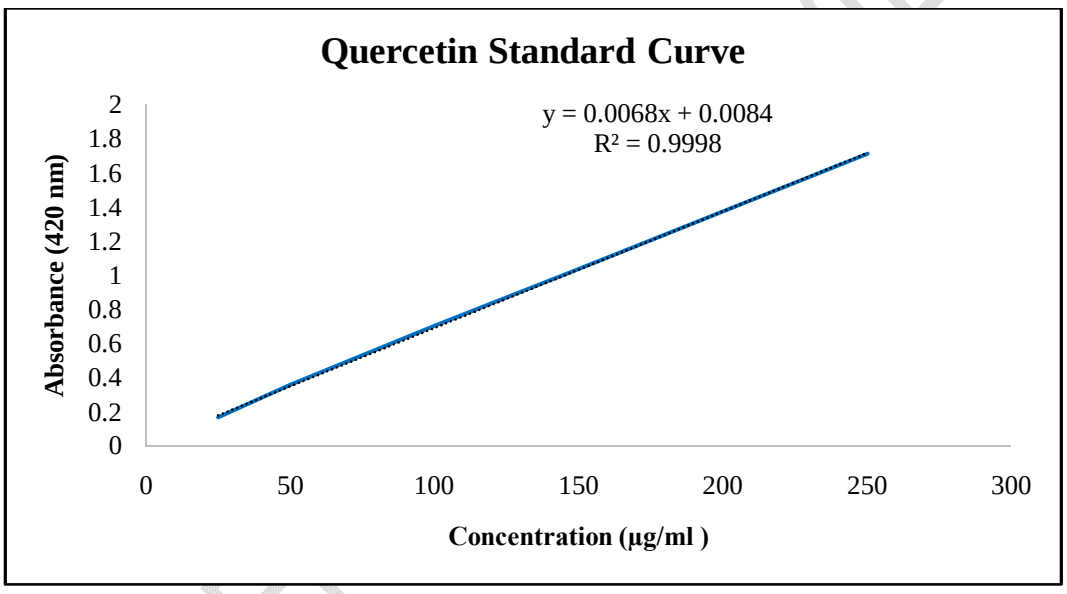


Figure S3: Calibration curve for standard quercetin

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

Extracts	Concentration (µg/mL)	$Absorbance_{Sample}$	$Absorbance_{control}$	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
Control	0.48	0.524	0.47	0.491	18.33
IC_{50} = 14.38 µg/mL					

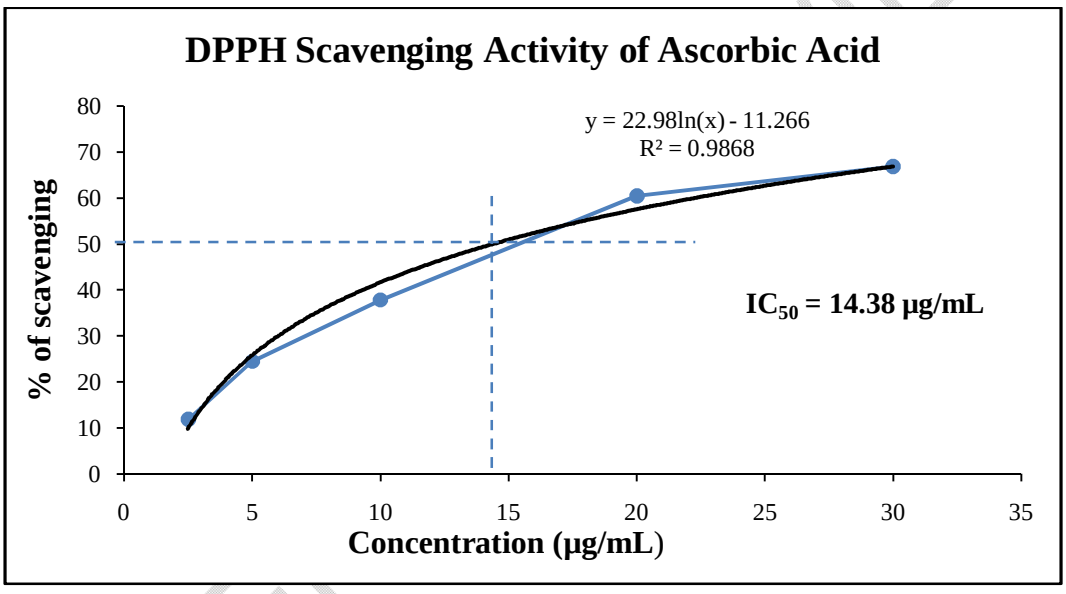


Figure S4: Antioxidant activity of ascorbic acid

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 µg/mL		

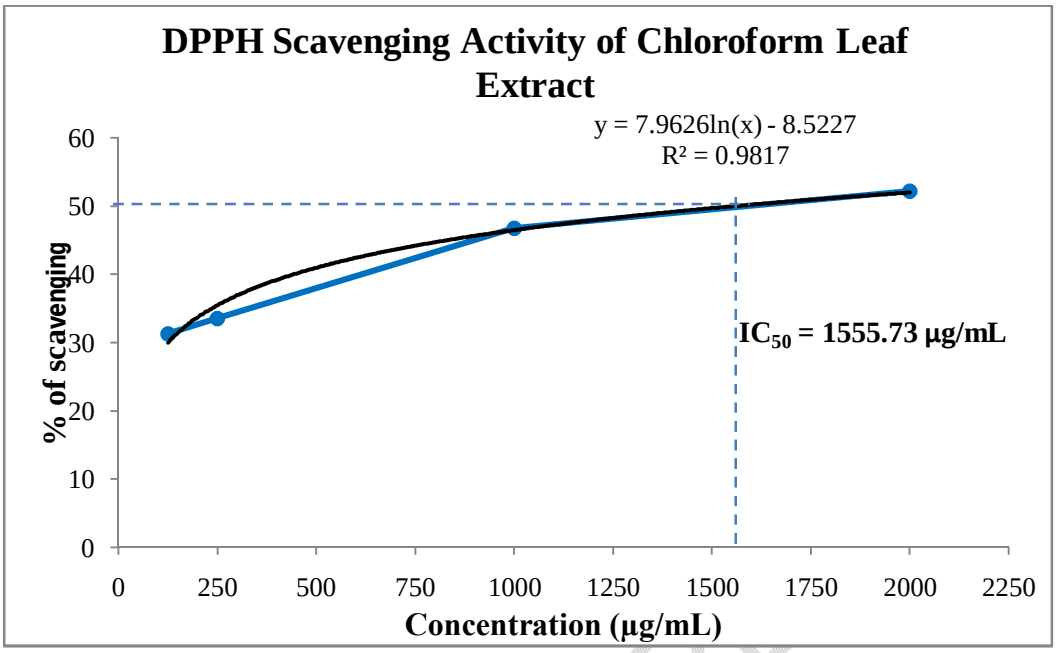


Figure S5: Antioxidant activity of chloroform leaf extract

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 µg/mL		

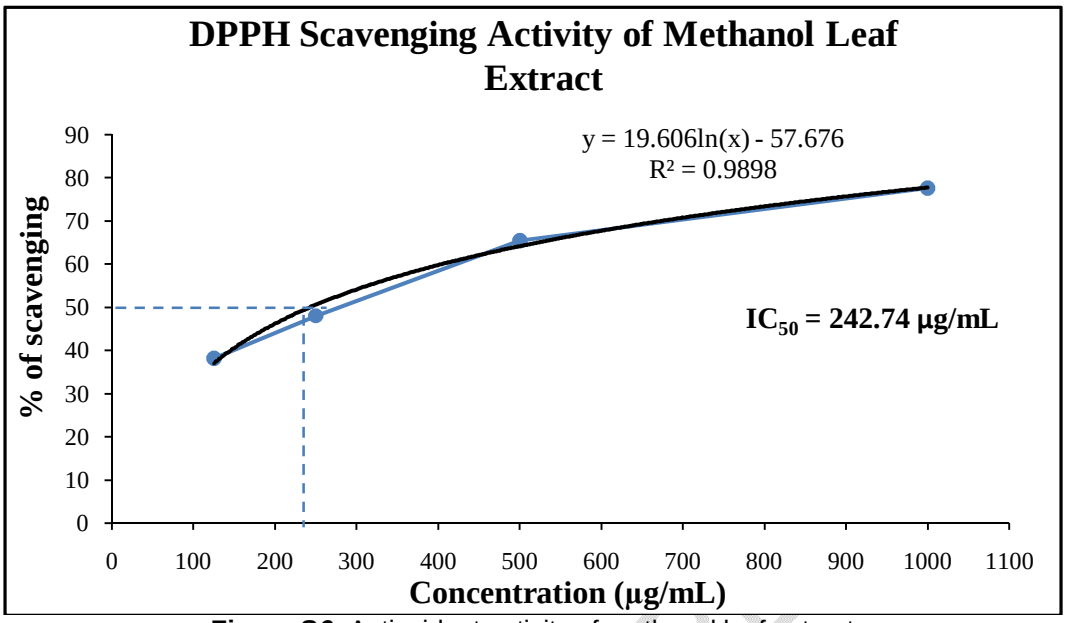


Figure S*6*: Antioxidant activity of methanol leaf extract

Table S8:α-amylase inhibition of acarbose

Concentration (µg/mL)	% of Inhibition
62.5	52.48
31.25	45.7
15.625	35.02
7.8125	29.08
IC_{50} = 52.019 µg/mL	

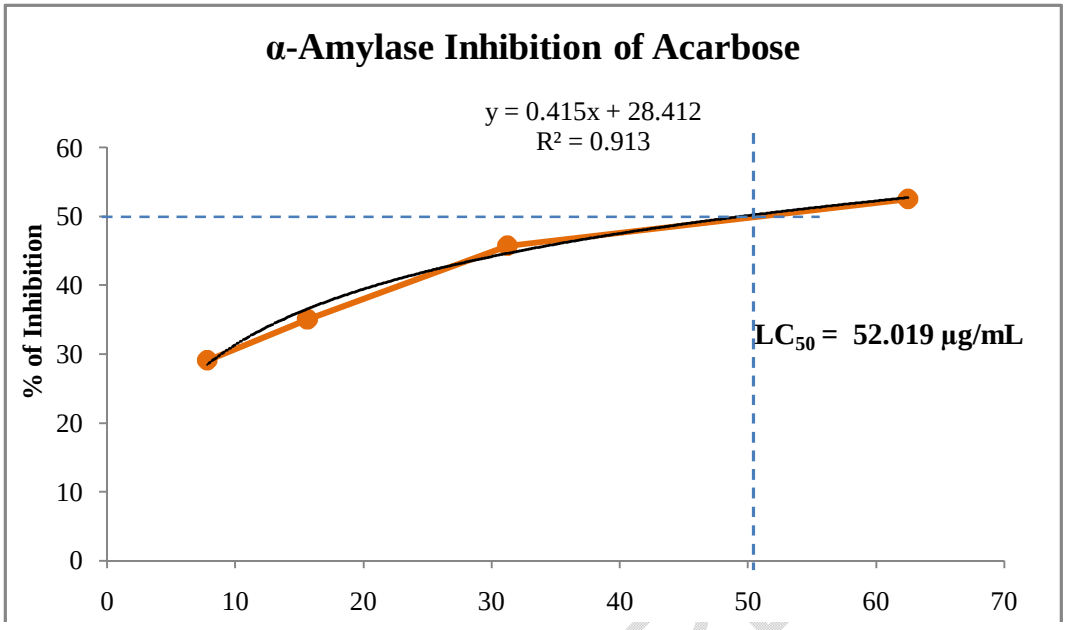


Figure S7:α-amylase inhibition of standard acarbose

Table S9:α-amylase inhibition of chloroform leaf extract

Concentration (µg/mL)	% of Inhibition
2000	55.918
1000	30.642
500	19.582
250	5.760
IC_{50} = 1819.11 µg/mL	

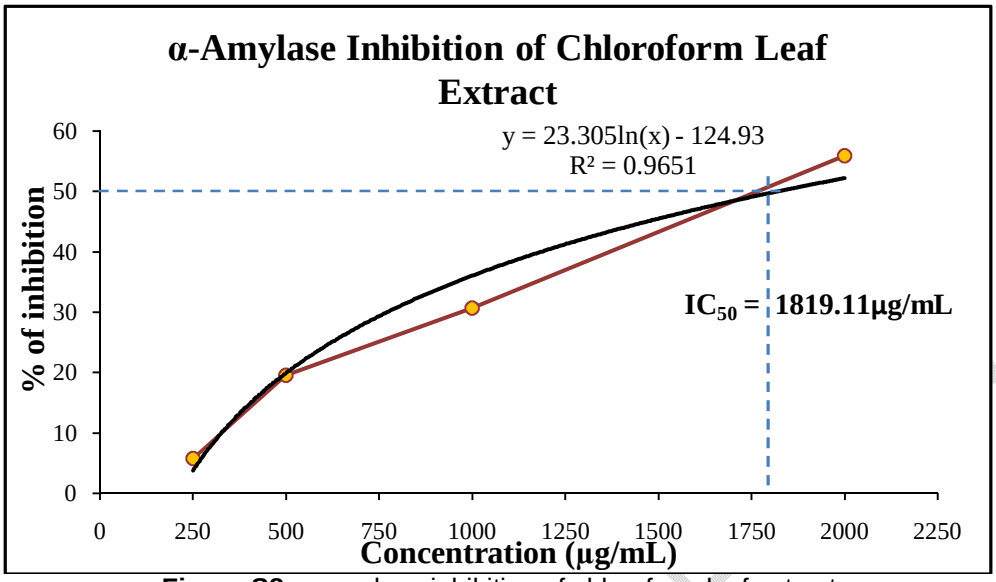


Figure S8: α-amylase inhibition of chloroform leaf extract

Table S10:α-amylase inhibition of methanol leaf extract

Concentration (µg/mL)	% of Inhibition
1000	58.959
500	36.383
250	37.260
125	25.589
IC_{50}= 733.83 µg/mL	

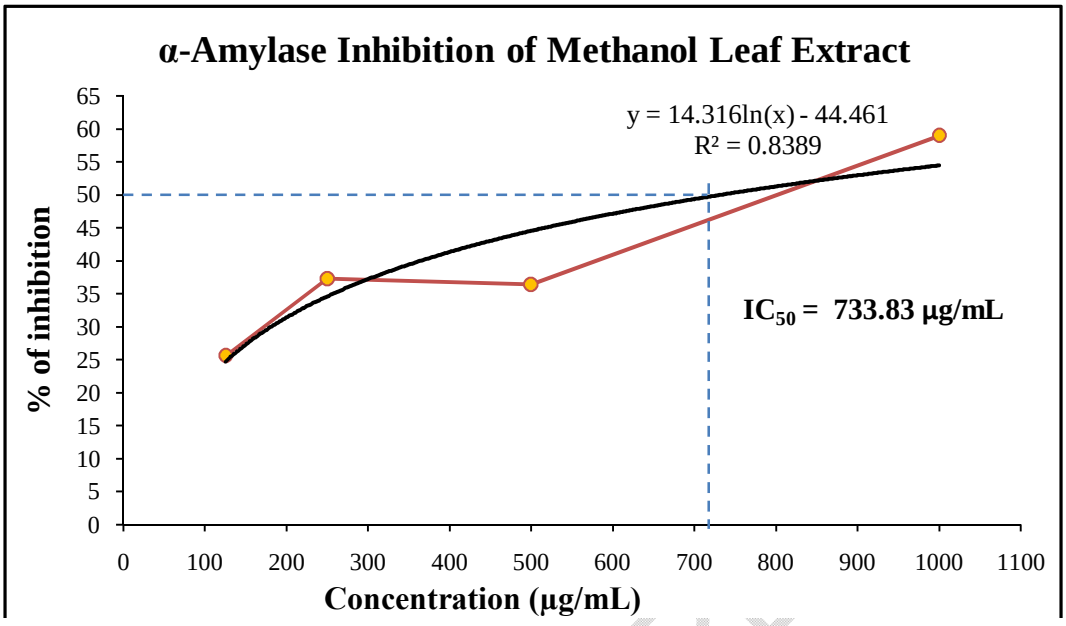


Figure S9: α-amylase inhibition of methanol leaf extract

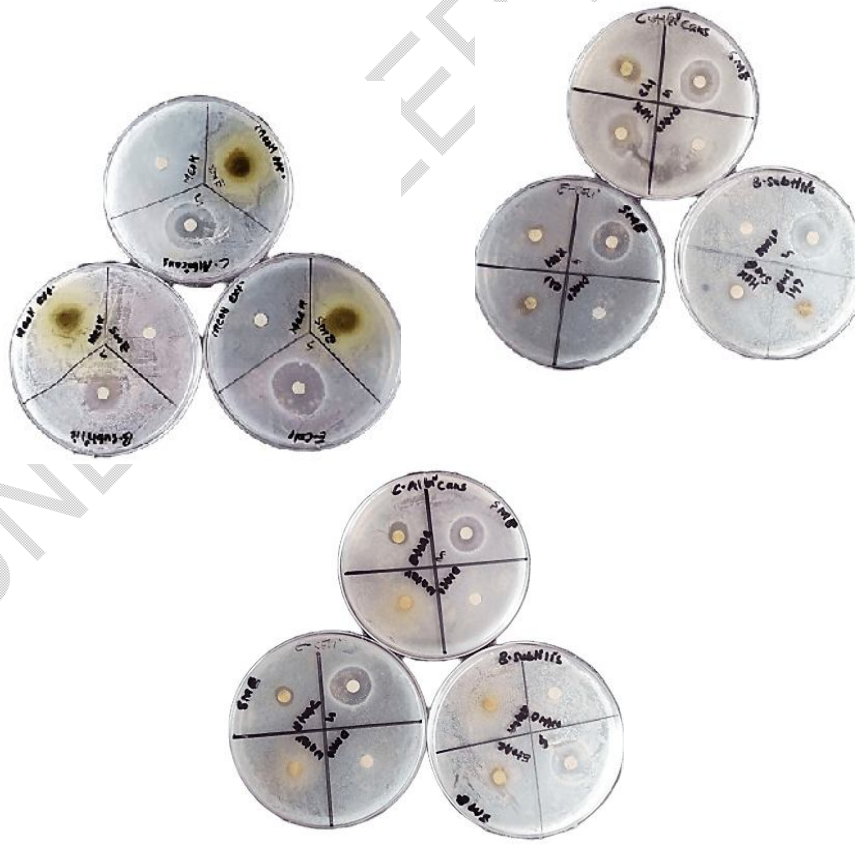


Figure S10:ZOIof leavesextracts of *S. sonchifolius*

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
LC_{50} = 92.76 µg/mL				

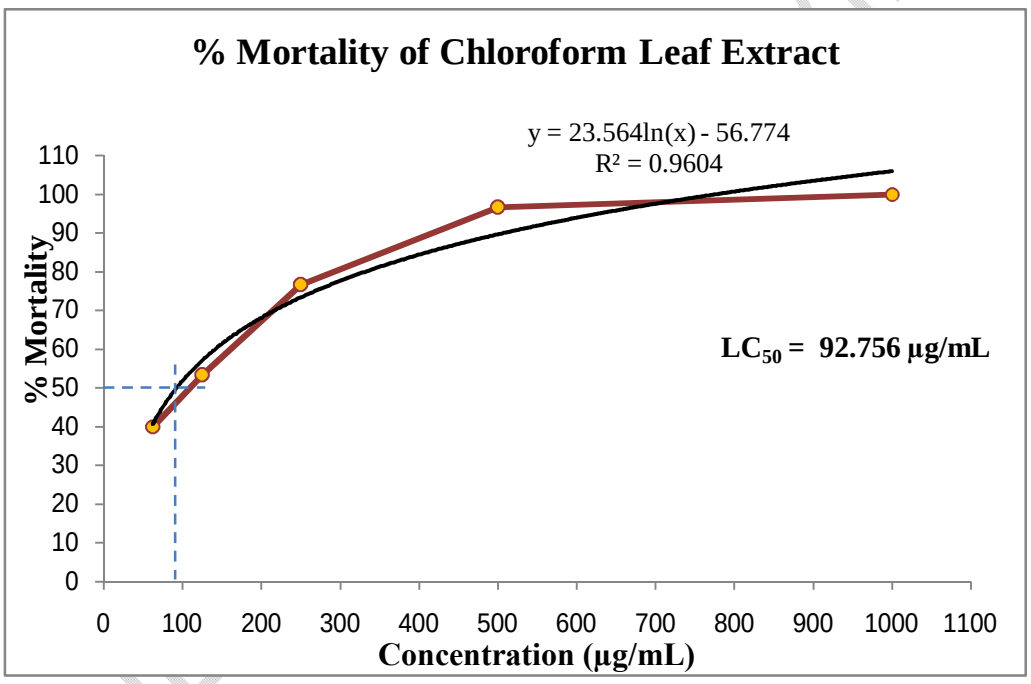


Figure S11: Cytotoxicity activity of chloroform leaf extract

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
LC_{50} = 216.81 µg/mL				

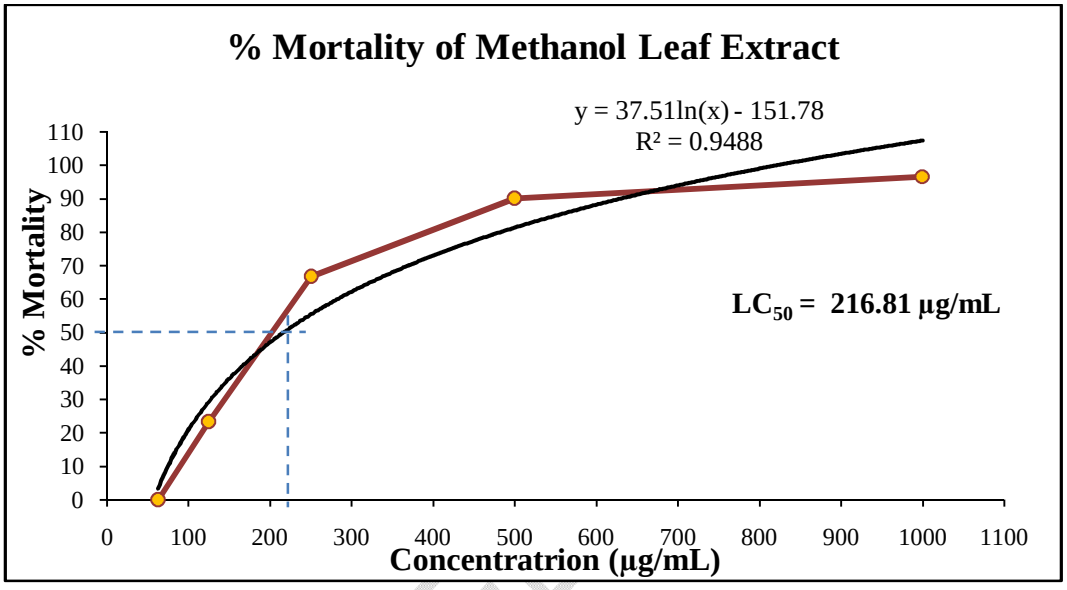
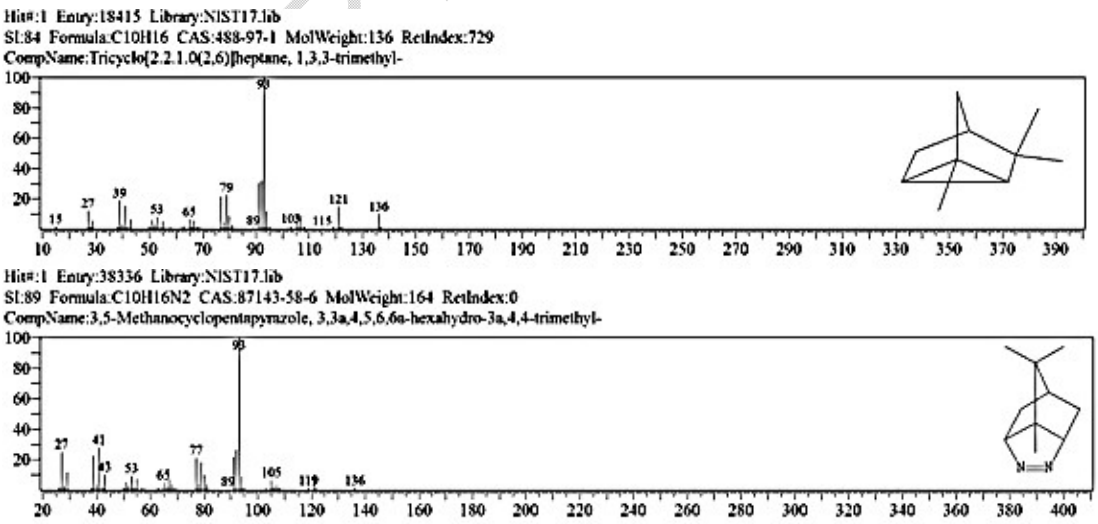


Figure 7: Cytotoxicity activity of methanol leaf extract

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



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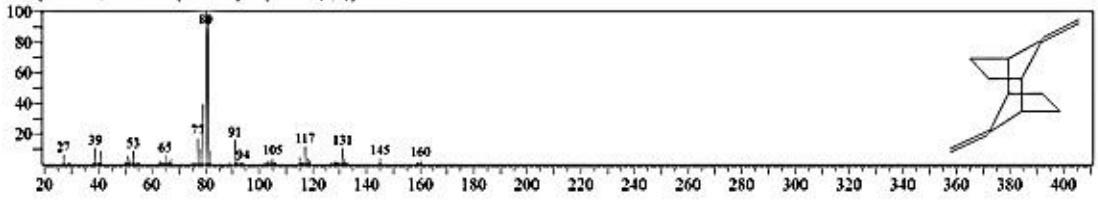

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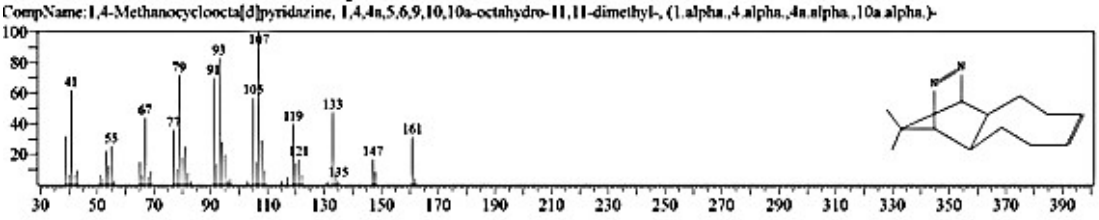

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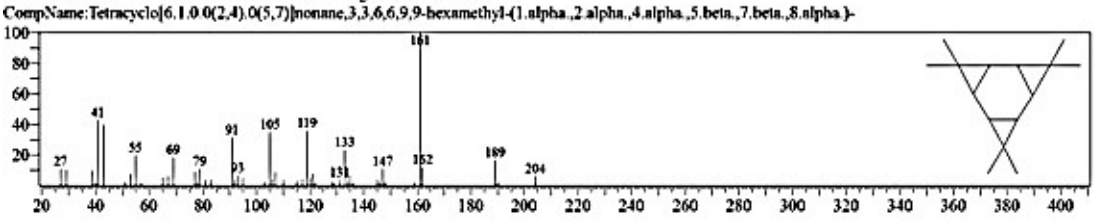

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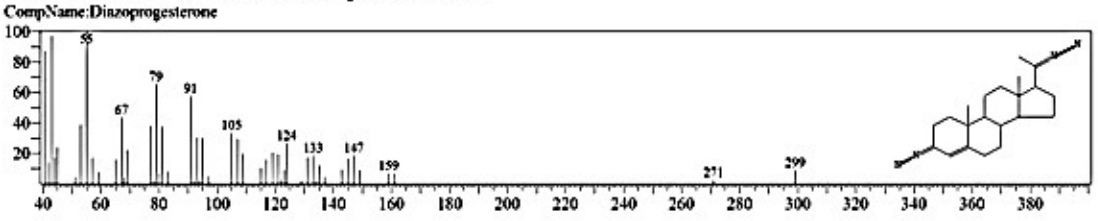

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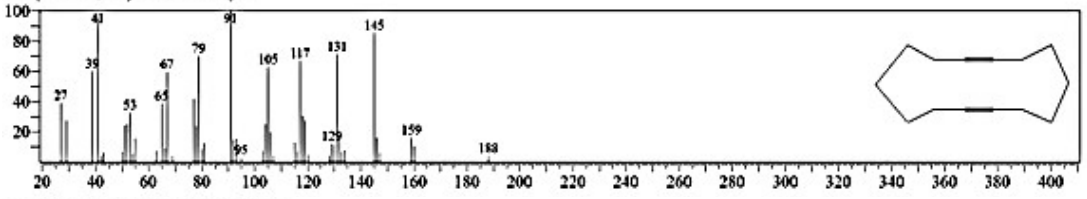

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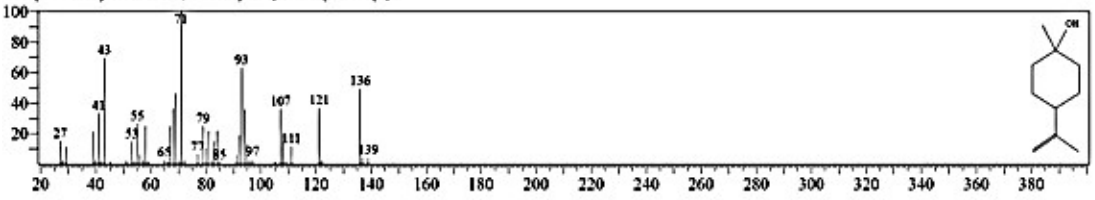

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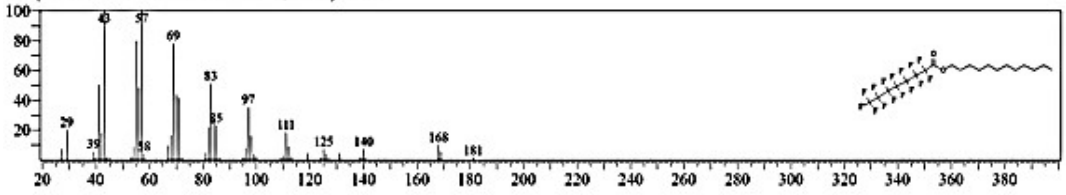

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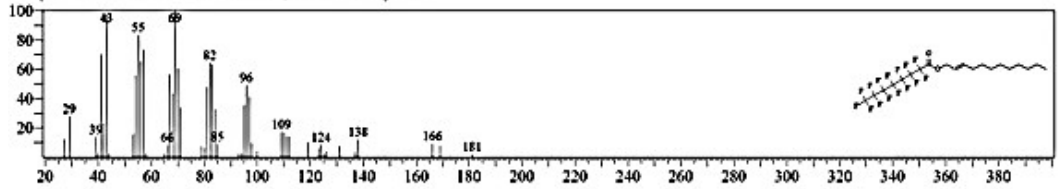

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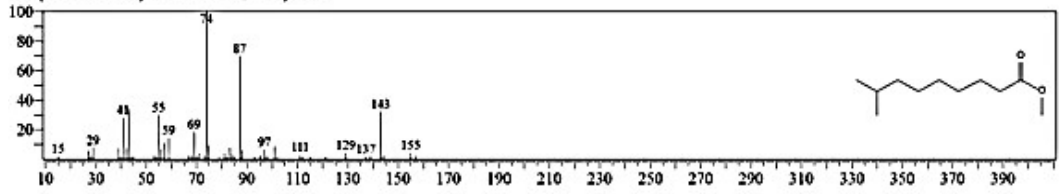

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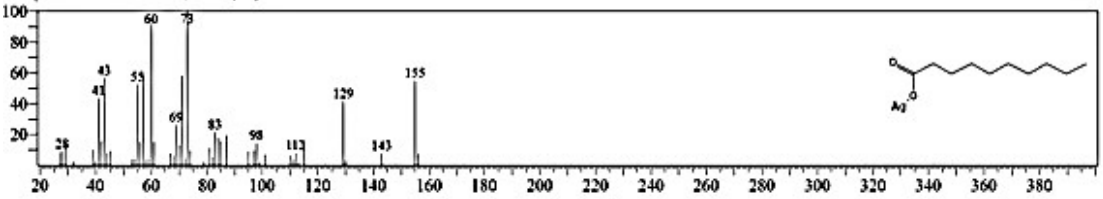

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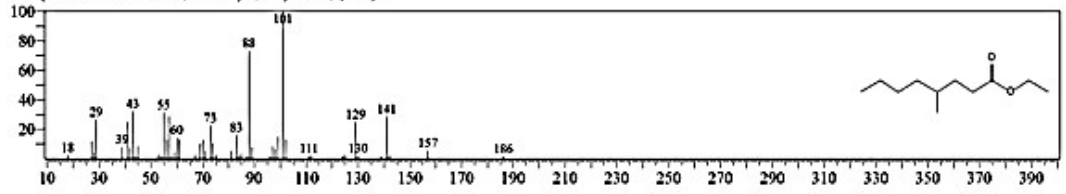

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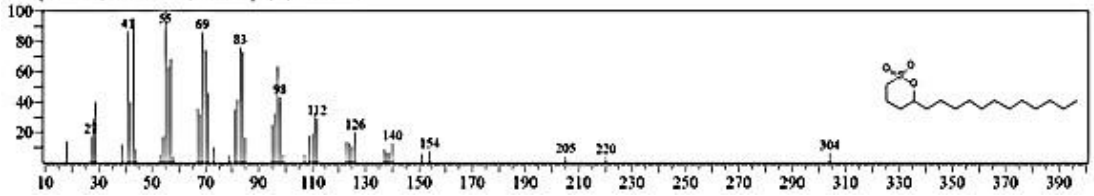
CompName:1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide



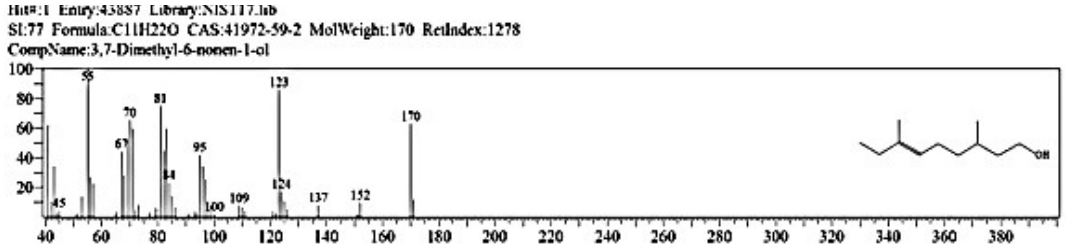


Figure S13: Mass spectral data of constituents identified by GC-MS in hexane extract

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

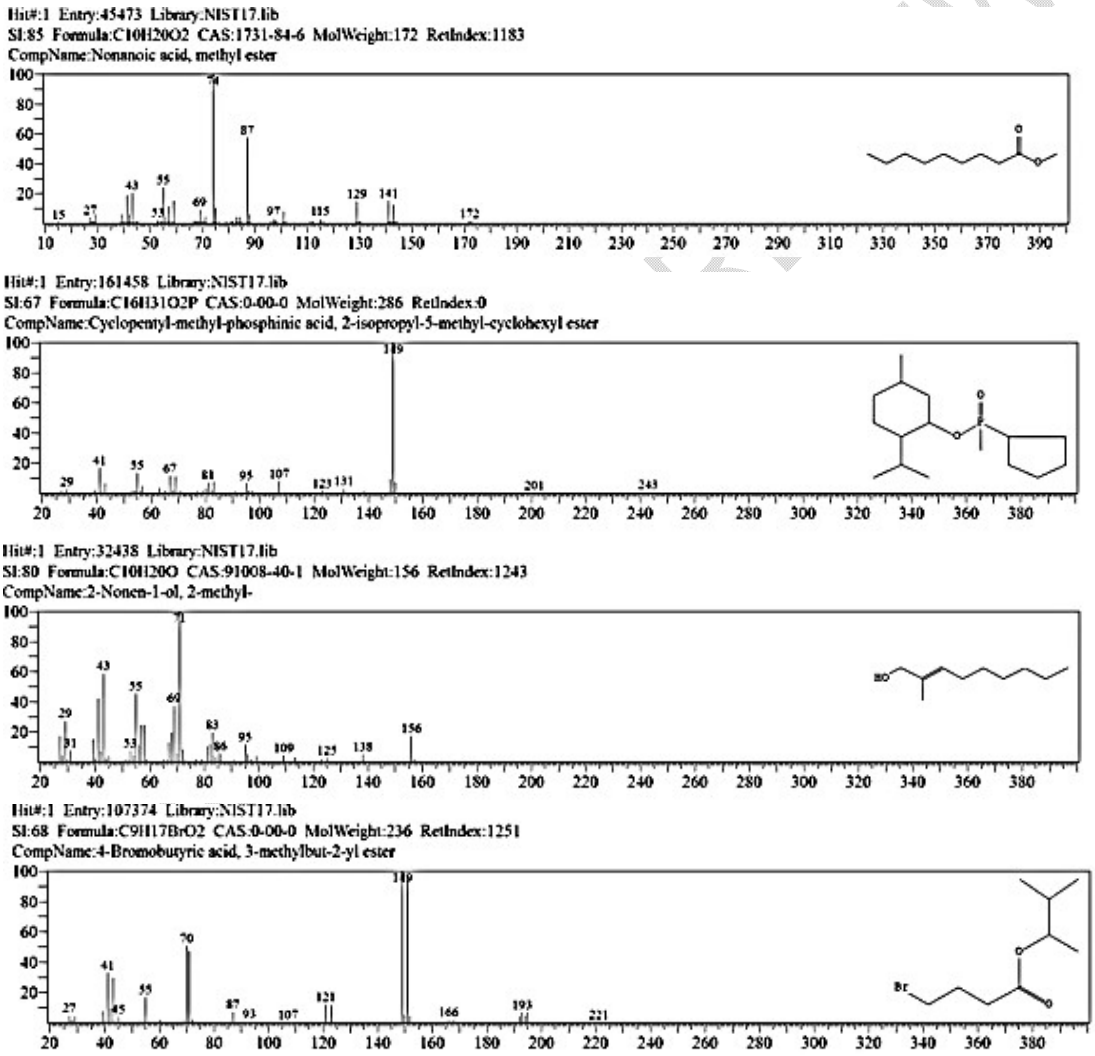
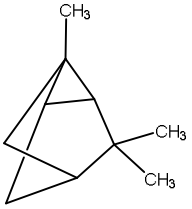
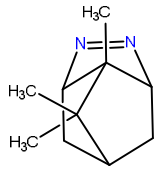


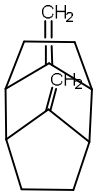
Figure S14: Mass spectral data of constituents identified by GC-MS in methanol extract



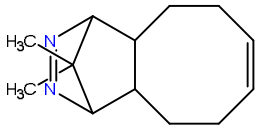
Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl- **(1)**



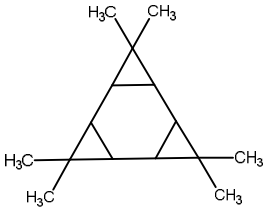
3,5-Methanocyclopentapyrazole, 3,3a,4,5,6,6a-hexahydro-3a,4,4-trimethyl- **(2)**



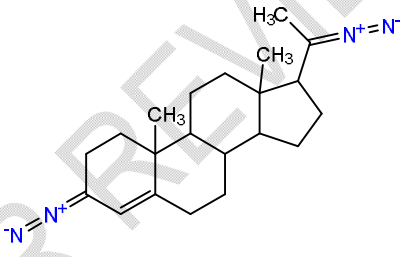
9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane **(3)**



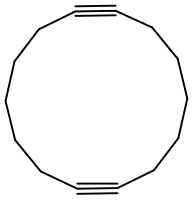
1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-(1. alpha.,4. alpha.,4a. alpha.,10a. alpha.)- **(4)**



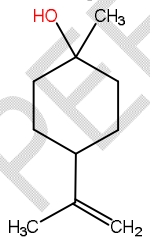
3,3,6,6,9,9-hexamethyltetracyclo [6.1.0.02,4.05,7] nonane **(5)**



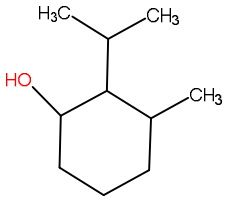
Diazoprogesterone **(6)**



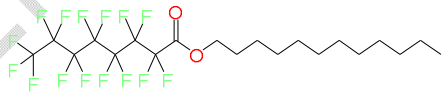
1.8-Cyclotetradecadiyne **(7)**



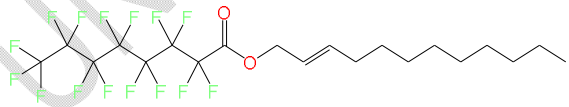
Cyclohexanol, 1-methyl-4-(1-methylethenyl)- **(8)**



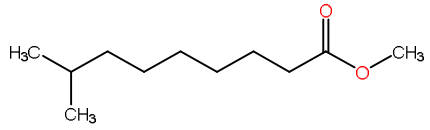
2-isopropyl-3-methylcyclohexanol **(9)**



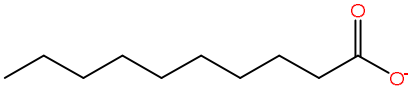
Pentadecafluorooctanoic acid, dodecyl ester **(10)**



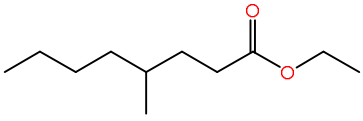
Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester **(11)**



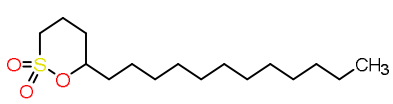
8-Methylnonanoic acid, methyl ester **(12)**



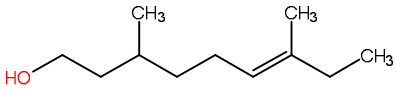
Decanoic acid, silver (1+) salt **(13)**



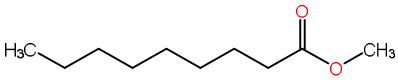
Ethyl-4-methyl-octanoate **(14)**



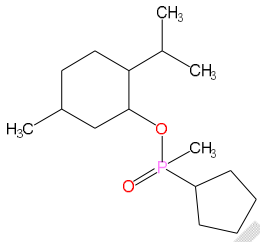
1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide **(15)**



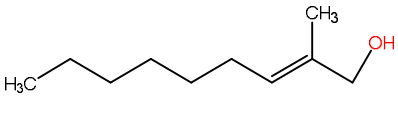
3,7-Dimethyl-6-nonen-1-ol **(16)**



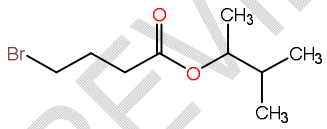
Cyclohexanol, 1-methyl-4-(1-methylethenyl)- **(17)**



Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester **(18)**



2-Nonen-1-ol, 2-methyl- **(19)**



4-Bromobutyric acid, 3-methylbut-2-yl ester **(20)**

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 α-amylaseprotein along with those of native and reference drugs

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

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