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An *In-silico* and *In-vitro* Comparative Study of Compounds from *Phoenix sylvestris* Roxb. For Alpha-Amylase Enzyme Inhibition Involved in Diabetes Mellitus

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Abstract: Identification of potential phytocompound from *Phoenix sylvestris* RoxB. For anti-diabetic potential and its validation through computational methods. Partially purified fraction evaluated for Alpha-amylase enzyme inhibition and GC-MS identified fraction validated compounds for potential anti-diabetic activity by Auto docking method. The phytocompounds investigation revealed maximum abundance of total phenol (31.55±0.55 μg/mg equivalent to Gallic acid) and flavonoid (52.90±0.08 μg/mg equivalent to quercetin) content in ethyl acetate extract. Ethyl acetate extract interestingly showed maximum alpha-amylase inhibition (71.15 %, IC50- 98.50±0.10 μg/ml), which was a mixed type of inhibition as compared to acarbose (78.64%, IC50- 88.61±0.50 μg/ml), which showed a competitive type of inhibition analyzed by Line weaver-Burk double reciprocal plot versus 1/v and 1/s. The docking study illustrated that Lupenyl acetate compound was the most active compound that showed maximum binding free energy (-7.16 Kcal/mol) and interacted with the Val64, Asn88, Gly90, Asn87, Arg87, Arg10, Gly9, Gln7, Gln5, Thr5, Trp221, Phe222, Asn5, Pro223, Ser3, Ser226, Lys227 and Gly225 amino acid residues which inhibited the alpha-amylase more efficiently than acarbose (binding energy -4.71 Kcal/mol). The present study concluded that the components of ethyl acetate extract had Alpha enzyme inhibition with reducing potential, and it may be due to the synergistic effect. The study suggested ethyl acetate extract as a natural promising therapeutic compound for the treatment of diabetes.

Keywords: alpha amylase; acarbose; gas chromatography-mass spectrometry; auto dock.

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1. Introduction

Around the globe, there are approx 300 million people being affected by diabetes mellitus. Diabetes is defined by decreased blood glucose homeostasis, including the overture to high blood glucose levels with some lipid parameters modification (International Diabetes Federation, 2013). In the intervening time, a prolonged increase of blood glucose may cause the overproduction of ROS (Reactive Oxygen Species) accompanied by oxidative stress. This causes various diseases, including cardiovascular diseases, diabetes, degenerative diseases, cancer, inflammation, ischemia, and anemia [1-3]. Various synthetic drugs have been developed in the last decades, which help to control oxidative stress. However, these drugs have numerous side effects, toxicity, high cost, and less accessibility. In contrast, herbal medicines gain popularity as they have no side effects, minimal toxicity, easily available, less expensive, and are approximately found in all plants [4]. Plants are a very rich source of

antioxidant compounds, which essentially neutrilizing the free radicals and help in the development of an immune system, and hence, these are very effective in diseases that are caused due to oxidative stress [5-6]. Previous studies have shown that antioxidant property to the plants is provided by flavonoids and phenolics components [7-10] that have tremendous anti-diabetic potential [11-13]. Phenol and flavonoids have been well studied for their antimicrobial, anti-diabetic, anti-cancer, and anti-inflammatory properties, and excellent effects have been observed in *in-vivo* experiments [14].

Arecaceae is a family of dominant perennial trees commonly called palm trees, which are distributed in tropical, subtropical, and warm climates [15-16]. The Indian date palm, *Phoenix sylvestris* is an evergreen plant that grows well in all types of environmental conditions and habitat from rain forest to deserts [17]. Date palms have been reported as a source of various phytocompounds used since prehistoric times for good health and treatment of various ailments. Plants synthesize various types of phytocompounds, which are grouped as primary and secondary metabolites. Primary metabolites include several sugars and fat components, which are essential for plants' growth and development. However, Secondary metabolites are produced in plants as a result of their protective and defensive activities. Mostly biologically active components from plant sources are secondary metabolites. These secondary metabolites included phenolics, flavonoids, trepans, alkaloids, saponins, and sterols, which were practiced to treat various disorders. Extraction of phytocompounds from any source by solvents depended upon the polarity characteristics. The group of extracting compounds is also an important part of any pharmaceutical pieces of evidence. Therefore, in the present study, attempts have been made to investigate the phytochemicals of *Phoenix sylvestris.* Alpha-amylase is a hydrolyzing enzyme involved in the digestion of carbohydrates in glucose, which absorbed and increased the blood glucose level. The alpha-amylase inhibiting formulation can be playing an important role in the prevention of diabetic-like conditions.

2. Materials and Methods

2.1 Collection of plant and authentication.

The leaf Samples of *Phoenix sylvestris* were collected from the Kukrail forest area Lucknow, and the specimens were identified and authenticated as *Phoenix sylvestris* RoxB.In BSIP (Birbal Sahni Institute of Palaeobotany), Lucknow, India. The dried fine powder of leaf was used for the extraction from different individual solvents [18]. The solvent was removed, filtered, and dried at room temperature, and residues were scratched out and stored at −20°C for further use. The percentage yield of different extracts was calculated by using the formula:

$$\%\ \text{Yield} = \frac{Weight\ of\ crude\ extract}{\text{Weight of raw material}} X100$$

2.2. Chemical and reagents.

All the chemicals and reagents were purchased from authenticated suppliers. Solvents like ethyl acetate, chloroform, acetone, butanol, cyclohexane, propanol, ethanol, and benzene were obtained from Merck, India, for extraction. Chemicals used for alpha-amylase assay: dinitrosalicylic acid (DNS), gallic acid, sodium carbonate (Na_2CO_3), aluminium chloride, quercetin, potassium acetate reagents were purchased from HiMedia Laboratories, Mumbai,

India, acarbose (GLUCOBAY™), and a standard drug was purchased from the local market. All other chemicals used were of analytical grade.

2.3. Determination of total phenol content (TPC).

Total phenolic contents in leaf extracts of *Phoenix sylvestris* were determined by the Folin-Ciocalteu method given by McDonald and co-workers [14]. Leaf extract of all solvents was dissolved in methanol (1 mg/ml concentration) and filtered. 5 ml of the mixture contained 0.5 ml of extract or reference (Gallic acid), 2ml (1N aqueous) Na_2CO_3, and 2.5ml of (2 N) Folin–Ciocalteu reagent (Sigma-Aldrich, Germany). The test mixture was incubated at room temperature for 15 minutes. The absorbance at 765 nm was measured using a spectrophotometer (Eppendorf). The standard curve was prepared using 12.5 to 500 µg/ml solution of Gallic acid in methanol. The blank contained 500 µl of double-distilled water, Na_2CO_3, and the Folin–Ciocalteu reagent. Total phenol values are expressed in terms of Gallic acid equivalent (µg/mg of extract).

2.4. Determination of total flavonoid content (TFC).

The quantitative estimation was performed spectrophotometrically by the aluminum chloride method based on complex flavonoid-aluminum formation [18]. *Phoenix sylvestris* of all solvents was dissolved in methanol (1 mg/ml concentration) and filtered. Aliquots of 0.5 ml of each sample were mixed with 1.5 ml of methanol, 0.1 ml of 10 % Aluminium Chloride, 0.1 ml of (1M) Potassium acetate, and 2.8 ml of distilled water. It was incubated for 30 minutes at room temperature. The absorbance of the test mixture was observed at 415 nm. The calibration curve was prepared by Quercetin standard solutions at various concentrations 12.5 to 500 µg/ml in methanol. Total flavonoid values are expressed in terms of Quercetin equivalent (µg/mg of extract).

2.5. Alpha-amylase inhibition assay.

The *in-vitro* alpha-amylase inhibition assay of different solvent leaf extracts of *Phoenix sylvestris* was performed following the standard protocol with some modification [19]. Alpha-amylase was dissolved in 20mM ice-cold phosphate buffer with pH 6.7, containing 6.7mM sodium chloride to maintain a 0.15 unit/ml concentration. Each test tube containing 250 µl of the enzyme was mixed with 100 µl of all extracts except the blank. The mixture was vortexes and incubated in a water bath for 20 minutes at 37°C. After incubation, 250 µl of 0.5% w/v starch in 20 mM phosphate buffer (pH 6.7) was added into each tube to start the reaction, vortexed, and incubated for 15 minutes at 37°C. 2 ml of DNS (40 mM DNS, 1M K-Na tartrate, and 0.4M sodium hydroxide) was added vortexed and boiled for 10 minutes in a water bath at 100°C. After that, the mixture was cooled down, and absorbance was observed at 540 nm. Acarbose was used as standard. Inhibition was calculated by using the formula:

$$\text{Percent inhibition} = 100 - \text{percent reaction}$$

Where percent reaction = (mean product in sample/mean product in control) × 100.

Further, IC_{50} value represents the concentration of the extract exhibiting 50% inhibition of alpha-amylase.

2.6. Determination of mode of inhibition.

Determination of mode of inhibition of α-amylase against the extract was determined by the standardized protocol of Mogale and co-workers [20]. In the analysis, two sets of 6 duplicate test tubes were set to analyze the enzyme activity in the presence and absence of an inhibitor (standard acarbose/extract). In the presence of inhibitor, 100 µl of inhibitor (plant extract or acarbose, 1mg/ml) solution was added in each test tube except the blank; this was followed by the addition of 100 µl of the porcine enzyme α-amylase (0.15 units/ml). In another set of tubes, 100 µl of 20 mM phosphate buffer at pH 6.7 and 6.7 mM sodium chloride was added in each test tube, followed by 100 µl of the enzyme solution. All the test tubes were thoroughly mixed in a vortex mixer and incubated in a water bath at 37°C for 20 minutes. Serial dilutions of the substrate solution were added in all the test tubes with concentrations ranging between 2.5 µg/ml and 0.156 µg/ml and incubated for 15 minutes at 37°C. 2ml of colored reagent DNS was added to each tube and then boiled for 10 minutes. Absorbance was recorded at 540 nm. Double reciprocal curve (1/ $[V]$ *vs.* 1/$[S]$) with inhibitor and without inhibitor was plotted to analyze the effect of the extract and standard acarbose on Vmax and Km of the enzyme, where V and S are, respectively, the velocity of the reaction and substrate concentration.

2.7. Gas chromatography-mass spectrometry analysis.

The potential extract was homogenized in the appropriate solvent, and GC-MS analysis of the sample was carried out by the following process in the GC-MS machine (model GC-MS-QP-2010 plus, Shimadzu Make). The test sample (1 µl) was used as an injector in RTX-5 column of GC-MS. The Carrier gas helium was used at a regular flow of 1.2 ml per minute. The temperature was maintained in the range of 100°C to 200°C. The temperature for the ion source and injector were 250°C and 270°C, respectively. Further, mass spectra investigation carried out at fragments ranges from 40 to 950 Dalton. The phytoconstituents were finally confirmed by using the reference compound data present in the Wiley and NIST (National Institute Standard and Technology) mass spectral database.

2.8. Docking analysis.

Molecular docking of major GC-MS identified compounds and standard acarbose with alpha-amylase enzyme was carried out using Auto Dock Tools [21] to find the preferred binding conformations of the ligands in the receptor. The binding conformation analysis of the protein-ligand complex was performed using a scoring function based on the free energy of binding [22]. The 3D structure of porcine alpha-amylase (PDB ID: 1DHK) for Auto-docking study was obtained from Research Collaboratory for Structural Bioinformatics (RCSB), and 3D structure of each GC-MS identified compound was retrieved from Pubchem database, which was further converted to compatible pdb file with the help of Discovery Studio 2.5 visualize. Among the stochastic search algorithms offered by Auto Dock suite, the Lamarckian Genetic Algorithm (LGA), which combines global search (Genetic Algorithm alone) to local search Solis and Wets [23] algorithm was chosen. The grid parameter file of the receptor was generated using Auto Grid. The number of grid points in x, y, and z-axes was 50 × 50 × 50˚ A. The distance between the two connecting grid points was 0.603˚ A. Auto Dock 1.5.6 and Lamarckian Genetic Algorithm (LGA) were used for docking calculations. Ten search attempts (GA run parameter) were performed for each ligand. The maximum number of energy evaluations before the termination of LGA run was 2,500,000, and the maximum number of

generations was 27,000, and other parameters were set to the software's default values. After complete Auto Dock execution, ten conformations of the ligand in a complex with the receptor were obtained, which were finally ranked based on interaction energies and inhibition constant (Ki). The enzyme-ligand interaction analysis and illustration were performed by discovery studio visualize.

3. Results and Discussion

3.1. Phytochemical analysis.

All the extracts of *P. sylvestris* leaf were extracted using various types of solvents in which the maximum percent yield (5.22%) was observed in ethyl acetate solvent extract. All the samples were analyzed to evaluate potential extract having a maximum amount of total phenolic and flavonoid contents. Among all the solvent extracts, ethyl acetate extract was identified to contain a higher amount of total phenolic (31.55±0.55 µg/mg of extract equivalent Gallic acid) and total flavonoid (52.90±0.08µg/mg of extract equivalent to quercetin) contents followed by other extracts described in table 1.

Table 1. Percent yield and quantitative phytochemical analysis.

Extracts	Percent yield	TPC µg/mg of extract equivalent gallic acid	TFC µg/mg of extract equivalent to quercetin
ethyl acetate	5.22	31.55±0.55	52.90±0.08
butanol	2.50	11.804±0.52	20.65±0.30
chloroform	2.10	13.20±0.04	20.235±0.5
acetone	2.30	10.75±0.35	17.65±0.40
ethanol	4.20	22.60±0.4	33.70±0.5
cyclohexane	3.90	17.30±0.07	24.90±0.40
2-propanol	3.50	15.65±0.5	23.00±0.60
benzene	2.40	12.65±0.4	21.75±0.5

All experiments were performed in a triplicate manner and expressed as ±S.D. values, n=3.

3.2. Alpha-amylase assay.

In-vitro anti-diabetic activity of the *P. sylvestris* leaf extracts was carried out by the inhibitory analysis of the alpha-amylase enzyme. Acarbose was used as a standard drug to compare the inhibitory effects. Among all extracts, ethyl acetate extract showed maximum inhibition (71.15%) against alpha-amylase enzyme whereas, standard acarbose showed 78.64% inhibition at 500 µg/ml(table 2). Also, the ethyl acetate extract showed a concentration-dependent increase in percent inhibition of alpha-amylase and showed lower IC_{50} value (98.50±0.10 µg/mL) as compared to acarbose (88.61±0.50 µg/mL), which clearly showed the potential of extract for an anti-diabetic property (fig. 1).

3.3. Determination of mode of inhibition.

In-vitro results of alpha-amylase inhibition demonstrated the significant potential of ethyl acetate leaf extract of *P. sylvestris* comparable to the standard drug acarbose. The mode of inhibition of both was analyzed by Lineweaver-Burk double reciprocal plot between $1/[v]$and $1/[S]$. Ethyl acetate extract showed a mixed type of enzyme inhibition, whereas acarbose competitively showed inhibition. Maximum velocity of the inhibition reaction was observed V_{max}=0.751 and 0.510 for ethyl acetate extract (at $Km=0.138$) and acarbose (at $Km=0.500$), respectively (fig. 2). Maximum velocity indicated the requirement of low substrate

concentration for enzyme inhibition. As the substrate concentration increased, the reaction rate decreased, so inhibition of alpha-amylase directly depends on the substrate concentration.

Table 2. Alpha-amylase inhibition assay.

Extracts	Percent Inhibition	IC_{50} value (µg/mL)
ethyl acetate	71.15	98.50±0.10
butanol	58.90	298.55±0.5
chloroform	60.40	142.65±0.6
acetone	58.50	302.25±0.7
ethanol	63.80	124.9±0.3
cyclohexane	60.62	173.23±0.4
2-propanol	59.00	290.66±0.8
benzene	50.40	405.52±0.72
acarbose	78.64	88.61±0.45

Percent inhibition was calculated at a maximum concentration of 500 µg/mL. All experiments were performed in a triplicate manner and expressed as ±S.D. values, n=3

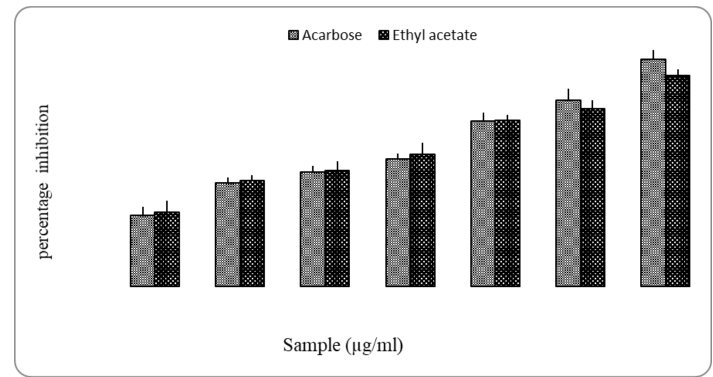
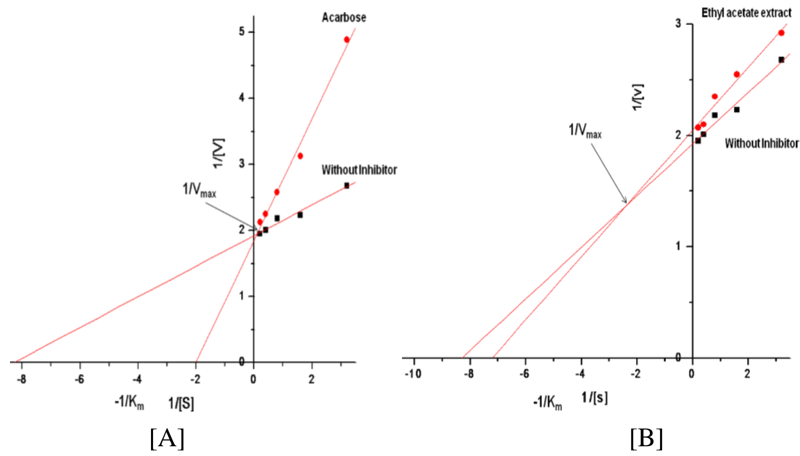


Figure 1. Concentration-dependent inhibition of alpha-amylase enzyme by ethyl acetate extract and standard compound acarbose. The results are expressed as mean ± S.D., n=3.



[A] [B]

Figure 2. Lineweaver-Burk double reciprocal plot versus 1/v and 1/s against alpha-amylase of acarbose [A] and ethyl acetate extract [B].

3.4. GC-MS analysis.

The major identified compounds of the GC-MS analysis of ethyl acetate leaf extract *P. sylvestris* are shown in table 3. The spectrum shows peaks with percent area is shown in figure 3. According to their elution order, the eight major compounds identified were arranged with their Retention Time and Percent Area. The GC–MS result was identified for over 35 compounds like fatty acid flavonoid and terpenoid in nature. Out of them, all the eight compounds had a relatively maximum percent area. These major compounds were identified as hexadecanoic acid (17.28%), 4H-pyran-4-one, 2-hydroxy-3 methyl (10.02%), 9,12-

octadecadienoic acid (9Z,12Z)-ethyl ester (8.32%), tetradecanoic acid (8.18), lupenyl acetate (8.05%), phthalic acid, mono-(2-ethylhexyl) ester (5.89%), stigmast-5-en-3-ol (3.87%), 9-12, octadecadienoic acid (2.11%). These phytocompounds are well-known plant-derived antibacterial, antioxidant, and anti-inflammatory agents.

Table 3. Major compounds identified from ethyl acetate extract.

R. Time	Percent area	Compound name	Compound nature	Activity**
7.053	10.02	4H-pyran-4-one, 2-hydroxy-3-methyl	Flavonoid	Anti-inflammatory, Antifungal, antioxidant
15.567	8.32	9,12-octadecadienoic acid (9Z,12Z)-methyl ester	Fatty acid ester	Bactericide, Fungicide
21.584	8.18	Tetradecanoic acid (Myristic acid)	Fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor
25.601	17.28	Hexadecanoic acid (Palmitic acid)	Fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor
28.715	2.11	9-12,Octadecadienoic acid	Fatty acid	Antioxidant, antibacterial activity, anti-inflammatory, antiarthritic, antiasthma, diuretic
35.446	5.89	Phthalic acid, mono-(2-ethylhexyl) ester	Fatty acid ester	Antioxidant, Antimicrobial
45.776	3.87	stigmast-5-en-3-ol	Terpenoid	Antioxidant, Anti-microbial
46.550	8.05	Lupenyl acetate	Terpenoid	Antioxidant, Antimicrobial

(**activity was confirmed by Dr. Dukes medicinal plant compounds reference book.)

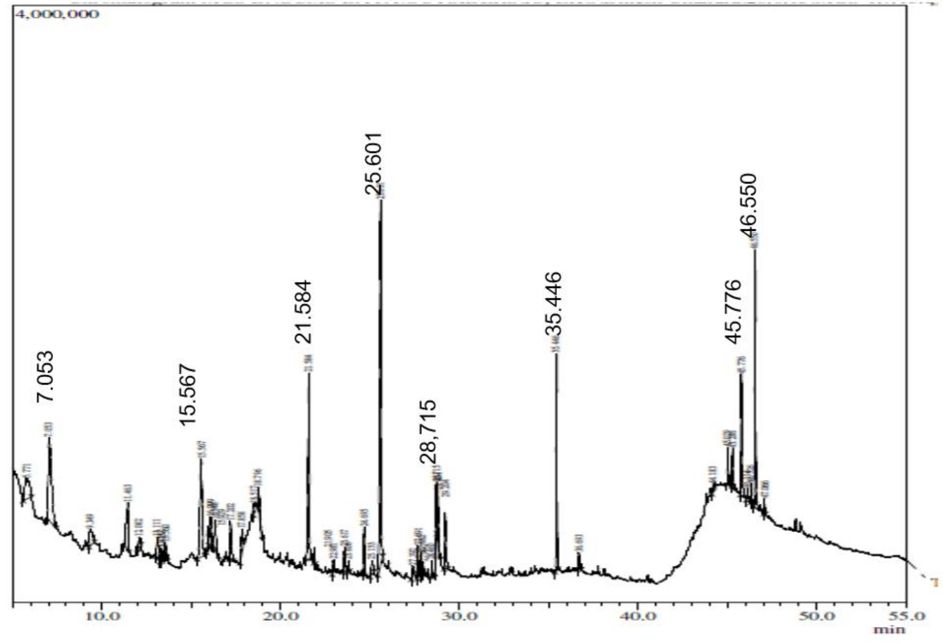


Figure 3. GC-MS graph of Ethyl acetate extract.

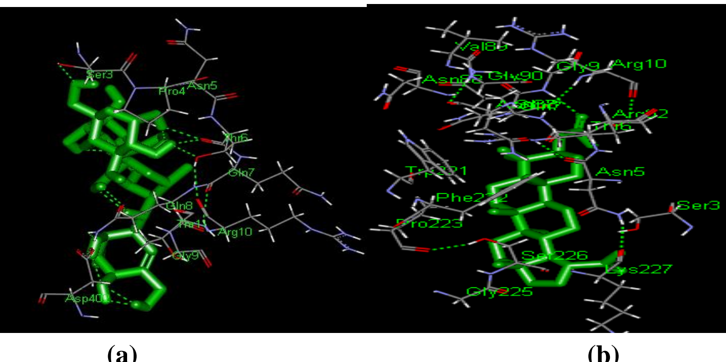
3.5. Docking results.

The *in-vitro* results showed ethyl acetate extract as a potential alpha-amylase inhibitor compared to standard acarbose. Furthermore, the *in-silico* docking study was carried out to assume high docking values, which will help predict the potential component of extract for

alpha-amylase inhibition. All major GC-MS identified compounds' docking results demonstrated their strong binding with the enzyme, and the binding free energies range between -3.23 to -7.16 Kcal/mol (Table 4). The docking study illustrated that the Lupenyl acetate compound was the most active compound, followed by stigmast-5-en-3-ol, Phthalic acid, mono-(2-ethylhexyl) ester, Tetradecanoic acid, 4H-pyran-4-one, 2-hydroxy-3-methyl 9,12-octadecadienoic acid (9Z,12Z)-methyl ester, Hexadecanoic acid, and 9,12-octadecadienoic acid. Lupenyl acetate compound showed maximum binding free energy (-7.16 Kcal/mol) and interacted to the Val64, Asn88, Gly90, Asn87, Arg87, Arg10, Gly9, Gln7, Gln5, Thr5, Trp221, Phe222, Asn5, Pro223, Ser3, Ser226, Lys227 and Gly225 amino acid residues which inhibited the alpha-amylase more efficiently than acarbose (binding energy -4.71 Kcal/mol) (Figure 4). The docking results validated our *in-vitro* analysis of alpha-amylase inhibition.

Table 4. Binding energies of GC-MS identified compounds with the alpha-amylase enzyme.

Compound name	Binding energy (Kcal/mol)	Involved Residues
4H-pyran-4-one, 2-hydroxy-3-methyl	-3.64	Asp402, Val401, Arg398, Gly403, Gly334, Pro332,Arg44
9,12-octadecadienoic acid (9Z,12Z)-methyl ester	-3.88	Gly9, Arg10,Thr11, Gln8, Phe335, Gln7, Thr6, Pro6, Arg92,Asn5, Ser3, Arg252,Tyr2, Gly251
Tetradecanoic acid (Myristic acid)	-4.18	Arg291, Arg252, Asp290, Tyr2, Ser3, Pro4, Lys227, Asn5, Thr6, Arg10, Gly9, Gln8, Gln7
Hexadecanoic acid (Palmitic acid)	-3.81	Arg252, Tyr2, Ser3, Pro4, Lys227, Gly225, Ser226, Asn5, Thr6, Gln7, Arg92, Gly90, Asn87, Trp271, Phe222, Pro223
9-12,Octadecadienoic acid	-3.23	Phe222, Arg92, Pro223, Thr6, Ala224, Asn5, Ser226, Gly225, Pro4, Pro228, Lys227, Phe229, Ser3, Ile230, Tyr2, Arg252
Phthalic acid, mono-(2-ethylhexyl) ester	-4.33	Arg398, Val401, Asp402, Thr11, Gly9, Gly403, Phe375, Gly334, Arg252, Ser289
stigmast-5-en-3-ol	-6.74	Gly90, Phe222, Pro223, Ser226, Lys227, Arg92, Gln7, Thr6, Gln8, Gly9,Asn5, Pro4, Thr1, Arg398, Asp402, Phe335, Gly334
Lupenyl acetate	-7.16	Val69, Asn88, Gly90, Asn87, Arg10, Gly9, Gln5, Thr5, Trp221, Phe222, Asn5, Pro223, Ser3, Ser226, Lys227, Gly226



(a) **(b)**

Figure 4. Binding patterns of Acarbose (a) and GC-MS identified compound Lupenyl acetate (b) With alpha-amylase enzyme.

3.6. Discussion.

The preliminary phytochemical screening was performed to authenticate the occurrence of biologically active plant metabolites in different solvent extracts, which confirmed the presence of total phenol and flavonoid contents. Many *in-vitro* studies have confirmed that anti-diabetic efficacy and other biologically useful medicinal plants' activities could be characterized by their high phenolic and flavonoid contents [24]. Based on their reducing properties, these secondary metabolites can neutralize free radicals, quenching singlet and triplet oxygen, or decompose peroxides to act as an antioxidant [11]. In the present study, the effects of different polarity solvents were analyzed for the percent yield of TPC and TFC in leaf extract of *P. sylvestris.* The TPC determined in different solvent extracts of *P. sylvestris* was expressed as µg/mg of extract equivalent to Gallic acid and ranged from 10.75 to 31.55 µg/mg, whereas TFC ranged between 17.65 to 52.90 µg/mg of extract equivalent to quercetin. The compounds were extracted from plant samples by using polarity based solvents. However, the respective solvent's polarity plays a key role in extracting more specific bioactive compounds (phenols and flavonoids) for potential antioxidant activity [25-27].

About 300 million people have been suffering from a chronic metabolic disorder, diabetes mellitus. One of the curing strategies of diabetes is to minimize the absorption of glucose from the hydrolysis of carbohydrates by the inhibition of alpha-amylase enzymes [28]. A recent finding supposed alpha-amylase enzyme as a potentially targeted for the management of glucose level as well as diabetes mellitus and natural secondary metabolites (phenolics and flavonoids) were also reported for the inhibition activity of alpha-amylase [29-30]. Interestingly, ethyl acetate extract was identified as a potential alpha-amylase inhibitor among all the extracts. Maximum inhibition (71.15%) was estimated in ethyl acetate extract at 500 µg/ml concentration, comparable to acarbose (78.64%). Although, previous reports of *P. sylvestris* have shown a significant presence of phytocompounds in other extracts with a satisfactory anti-diabetic approach in terms of hyperglycemic activity [31-32]. Ethyl acetate extract showed a concentration-dependent mixed type of alpha-amylase inhibition, whereas acarbose showed competitive enzyme inhibition, which is clearly demonstrated in fig. 2. These observations strongly suggest that ethyl acetate extract constituents structurally resemble the substrate of the alpha-amylase enzyme, which interacts accordingly and inhibit the enzyme activity.

To identify the phyto-constituents of most potent ethyl acetate extract, the GC-MS examination was performed. Various major and minor components of hydrocarbons, ethers, phenols, terpinoids, flavonoids, alkaloids and steroids etc., were identified. Among all the present constituents, hexadecanoic acid (17.28%), 4H-pyran-4-one, 2-hydroxy-3 methyl (10.02), 9, 12-octadecadienoic acid (9Z, 12Z)-methyl ester (8.32%), tetradecanoic acid (8.18), lupenyl acetate (8.05%) phthalic acid, mono-(2-ethylhexyl) ester (5.89%), stigmast-5-en-3-ol (3.87%), 9-12, octadecadienoic acid (2.11%) was recognized which possess maximum percent area as found in the spectrum. Based on all activities, we can conclude that all major components are synergistically responsible for the activities because all identified components individually have a diverse type of medicinal and inhibitory properties, as earlier discussed by Duke [33]. Further, to validate the *in-vitro* alpha-amylase inhibition of all major components identified by GC-MS analysis were examined for *in-silico* analysis, which showed significant interactions in terms of binding energy and ligand efficiency. The observed binding energy of GC-MS components confirmed that ethyl acetate extract has much more potential to minimize the diabetic condition than the acarbose. Therefore, our results suggest that alpha-amylase inhibition of ethyl acetate extract might be due to GC-MS identified compounds' synergistic

effect. Ethyl acetate extract needs to be further explored to isolate new and novel compounds for more potential anti-diabetic property which may prove to be more significant than synthetic drugs.

4. Conclusions

The present investigation concluded that *P. sylvesteris* leaf has several phyocompounds that will be incorporated with the anti-diabetic property. Based on *in-vitro* and *in-silico* studies, it was concluded that the lupenyl acetate compound present in the leaf extract has more affinity to inhibit the alpha-amylase enzyme activity and be considered for an effective role in counter the diabetes conditions. The present findings will be beneficial to the researchers to find out new directions in diabetes research.

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Conflicts of Interest

The authors declare no conflict of interest.

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