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Section B: Herbal Chemistry

Research Article

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Chemical Profiling, Spectroscopic Characterization thereafter Molecular Docking Studies of Phytochemical Constituents of an Antidiabetic Polyherbal Formulation

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Abstract: Ayurveda has a unique concept known as polyherbalism, albeit it can be difficult to define in terms of contemporary standards. The Sarangdhar Samhita, a work of Ayurvedic literature, introduced the concept of polyherbalism to achieve better medicinal efficacy. The amount of active ingredients employed from each plant is insufficient to provide any pharmacological impact. There is evidence to suggest that crude plant extracts frequently have higher potencies than individual ingredients. Due to synergism, polyherbalism confers some benefits which are not accessible in single herbal formulations. This work is to report the method of preparation of Antidiabetic polyherbal formulation, a novel polyherbal formulation for diabetic mellitus, and to find out the active principles in the formulation responsible for the antidiabetic action. To validate the antioxidant activity of the formulation and to study the mechanism of action of the phytochemical constituents in the formulation. The polyherbal formulation is made from

the ethanol extract of Gymnema sylvestre, Berberis aristate, Costus speciosa, Pterocarpus marsupium, Syzygium cumini, Picrorrhiza kurroa, Trigonella foenum, Cinnamomum verum, Alstonia scholaris and Asphaltum pure. Fingerprinting of phytochemical constituents of the antidiabetic polyherbal formulation was performed using spectroscopical (like IR and UV) and chromatographic techniques like LCMS. Biochemical assays like DPPH and SOD radical scavenging assays were done to validate its biological activity. The mode of action of phytochemical constituents against monoamine oxidase B that have a high impact on diabetes management and complications was also investigated using molecular docking. LC-MS/MS analysis of antidiabetic polyherbal formulation ethanolic extract identified 33 compounds, including the popular flavonoids and phenolic acids. Among them, 9 compounds show antidiabetic activity. Besides this, these compounds also show biological activities like anticancerous, anti-inflammatory, antiaging, antiproliferative properties, etc. The absorption bands in the UV spectra and harmonic vibrations in the IR region indicate the presence of phytochemicals like carbohydrates, terpenoids, flavonoids, saponins, and alkaloids. The dose-dependent response of the anti-diabetic polyherbal formulation demands 40 µg/mL concentration of the drug for 50% DPPH radical scavenging and 10 µg/mL for SOD radical scavenging. The docking result points out that many of the active principles in the polyherbal formulation show a significant binding affinity score than the inbuilt ligand, safinamide binding affinity value, the inbuilt ligand safinamide had exhibited a docking score of -9.014 with -74.785 kcal binding energy. While pulmatin showed a docking score of -10.533 with a binding energy of-64.220 kcal, genistein 8-C-glucoside with a docking score of -9.541 with a binding energy of 64.673 kcal and all others have a comparable docking score and binding affinity. These results point out that the polyherbal formulation is rich in antioxidant ingredients and will require less amount for high performance. The findings obtained in the present work indicate that the novel antidiabetic polyherbal formulation, may constitute a safe multi-target remedy to treat diabetes.

Keywords: anticancerous, anti-inflammatory, antiaging, docking.

INTRODUCTION

Diabetes, often known as diabetes mellitus, is a chronic, serious metabolic illness with a variety of etiologies and both acute and long-term effects. One of the most prevalent metabolic disorders in the world, it affects 2.8% of the global population and is expected to reach 4.4% by 2030, reaching an epidemic level that has never been seen before. Diabetes is one of the top five global morbidities despite being a non-communicable disease [1]. Hence, diabetes poses a significant socioeconomic burden, and both individuals in developing and wealthy nations are affected by its complications [2]. The different types of diabetes include type 1, type 2, and gestational diabetes. Type 2 diabetes also known as diabetes mellitus is the most common form of diabetes. It is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [3]. Depending on the pathogenic mechanisms by which hyperglycemia arises, there are several forms of diabetes mellitus. Some are characterized by an insulin deficiency or a genetic defect to defective insulin secretion and some may share insulin resistance

as their underlying etiology. The dysfunction and failure of various organs, particularly the eye(retinopathy), kidney (nephropathy), nerve (neuropathy), Heart (CAD), and blood vessels (PVD) are the long-term complications of diabetes mellitus.The classical symptoms of diabetes are polyuria, polydipsia, polyphagia, blurring of vision, and weight loss[4]. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or nonketotic hyperosmolar syndrome [5].

Many anti-diabetic medications are already on the market to treat hyperglycemia, and they generally function by increasing insulin sensitivity, complementing insulin, increasing insulin secretion, and stimulating glucose uptake [1]. For example, metformin, a class of biguanide, increases the absorption and use of sugar that is triggered by insulin, which reduces hepatic gluconeogenesis and restores the sensitivity of peripheral tissues to insulin [5]. Thiazolidinediones, insulin sensitizers, reduce insulin resistance, increase insulin sensitivity, and lower cardiovascular risks [5]. All these chemical compounds are compromised with unwanted side effects such as hepatic failure, weight gain, and fluid retention, which can result in peripheral edema, heart failure tachycardia, and hypothyroidism. According to recent studies, using plants and plant extracts can have a potential anti-diabetic efficacy, offering an alternative to synthetic medication's adverse effects. Conventional herbal remedies and functional foods are thought to ameliorate diabetic syndromes through six distinct mechanisms of action, including improved insulin secretion and sensitivity, glucose uptake by muscle cells and adipose tissues, inhibition of glucose absorption from the intestine and glucose production from hepatocytes, as well as exhibiting anti-inflammatory properties [1]. There are a few plants having antidiabetic effects, including

(a)Swertiachirayita *(b)Nigella sativa* *(c)Moringa oleifera* *(d)Momordicacharantia* *(e)Curcuma longa L*

Figure 1: A few examples of plants with antidiabetic properties

(a). Swertia chirayita: A well-known medicinal herb endemic to the temperate Himalayan area is Swertia chirayita Buch Ham. (Family: Gentianaceae), also known locally as "Chirayata," "Chirayta," and "Chiretta." Insulin secretion from monolayers of BRINBD11 clonal pancreatic cells was used in the cell line-based assessment approach to demonstrate the promising antidiabetic efficacy of S. chirayita. This species contains various antidiabetic chemicals that have strong antidiabetic efficacy [5].

(b). Nigella sativa: A herbaceous plant known as Nigella sativa L. (family: Ranunculaceae) may be found in a number of southern Mediterranean and Middle Eastern nations. Black seed and black cumin are two names for the seeds of Nigella sativa. Its ethanolic seed extract was discovered to improve glucose absorption in muscle and fat cells, promote pancreatic b-cell proliferation, and increase insulin production. Thymoquinone, dithymoquinone, linoleic acid, and oleic acid may stimulate pancreatic b-cells to secrete insulin, slow down hepatic gluconeogenesis, and increase insulin sensitivity in peripheral tissue, which are thought to be the causes of Nigella sativa's hypoglycemic action [5].

(c)Moringa oleifera: The perennial angiosperm plant Moringa oleifera Lam comes under the family of Moringacea, is native to Asia, and is widely distributed in Malaysia and other tropical nations. The English name for M. oleifera is "Drum stick tree," but its local names are "Sajna," "Soanjna," and "Sohanjna". The alcoholic leaf extract of the plant is thought to be useful in treating diabetes Mellitus due to the presence of phyto-compounds, including flavonoids, alkaloids, tannins, steroids, and glycosides. The phytoconstituents including quercetin and kaempferol significantly decrease serum glucose and increase serum insulin levels [5].

(d) Momordicacharantia: *Momordica charantia* L. is a flowering vine cultivated in Asia including Bangladesh, India, and in other regions like East Africa and South America. This plant comes under the family of Cucurbitaceae. The plant known as bitter gourd, also known as "Korolla," "Karela," "Bitter melon," or "Balsam pear," gets its name from the fruit's distinctively bitter flavor. When it ripens, the bitterness increases in intensity. The plant's remarkable antidiabetic and hypoglycemia effects support its adjuvant usage in conjunction with widely used commercial medicines [5].

(e) Curcuma longa L: The plant known as "turmeric," Curcuma longa L, comes under the family of Zingiberaceae. Curcuma longa L is a perennial that grows to a height of about three feet has subterranean rhizomes and is widely cultivated in tropical areas including Pakistan, China, Peru, and India. After being extracted from C. longa, the curcuminoids bisdemethoxycurcumin, curcumin, and demethoxycurcumin were discovered to have a-glucosidase inhibitory action [5]. Bisdemethoxycurcumin had the strongest a-glucosidase inhibition among the three curcuminoids. Moreover, volatile oils isolated from both fresh and dried turmeric rhizomes exhibited robust dose-dependent glucosidase inhibitory activity, and dried rhizomes significantly boosted the glucosidase inhibitory effect. Aromatic-turmerone, the primary volatile component in turmeric rhizome, had strong a-glucosidase and a-amylase inhibitory action.[5].

Although the idea of a polyhedral formulation is clearly described in ancient works of literatures, the conventional system of medicine has several difficulties because of the absence of chemical standardization. Because of the synergistic impact of the chemical components given energy by mechanical stirring and heating during the preparation, polyherbal formulations have better and longer-lasting medicinal potential than a single plant. The degradation of volatile hazardous substances and the creation of new chemical compounds are both potential consequences. This study describes the development of a novel polyherbal formulation for diabetic support, chemical profiling using liquid chromatography-mass spectrometry, and spectroscopic characterization using ultraviolet and infrared spectroscopy. Besides that, the antioxidant activity of the ethanolic extract of the polyherbal formulation was evaluated by DPPH and SOD radical scavenging assay. Moreover, molecular docking studies utilizing Schrodinger Maestro demonstrated the selected phytochemical components' affinity for the MAO-B protein.

2.MATERIALS AND METHODS

2.1. Materials: Based on the documented ethnopharmacological knowledge of the use of medicinal plants, the selected plants (Figure 2) parts were collected (Table 1), washed 2 to 3 times in distilled water then dried in shade for one week, and ground to a fine powder. The obtained powder was stored in closed containers separately with proper labeling for further use.

(a) Gymnema sylvestre

(b) Berberis aristata

(c) Costus speciosa

(d)Pterocarpus marsupium

(e) Syzygium cumini

(f) Picrorrhiza kurroa

(g)Trigonella foenumgraecum

(h) Cinnamomum verum

(i) Alstonia scholaris

Figure 2: Photographic imagesof selected plants for a new formulation

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Table 1: Selected plants and their Traditional uses

Plant Name	Family	Common Name	Parts Used	Traditional Uses
Gymnemasylvestre	Asclepiadaceae	Gurmar	Bark	Antidiabetic, livertonic, diuretic, stomachic, stimulant, laxative, anthelmintic, cardiotonic, expectorant, antipyretic, and uterine tonic[6].
Berberisaristata	Berberidaceae	Berberis	Bark	Diarrhoea, diabetes, ophthalmic diseases, Hepatoprotective [7].
Costusspeciosa	Costaceae	Kabuk	Root	Antidiabetic, anticancerous, antioxidaent, hepatoprotective [8].
Pterocarpus marsupium	Fabaceae	Vijaysar	Bark	Antidiabetic, anticancerous, antioxidaent, hepatoprotective [9].
Syzygiumcumini	Myrtaceae family	Jamun	Seed	antioxidant, anti-inflammatory, analgesic, antipyretic, antimalarial, anticancer, and antidiabetic [10].
Picrorrhizakurroa	Scrofulariaceae	Kutki	Root	Hepatoprotective, Anti-Diabetic, Anti-Asthmatic, Anticancer, Anti-Oxidant [11].
Trigonellafoenum	Fabaceae	Methi	Seed	Antigastriculcer,anticancerous, Hepatoprotective, and Antidiabetic[12].
Cinnamomum verum	Lauraceae	Dalchini	Bark	Antimicrobial, wound healing, antidiabetic, anti-HIV, anti-anxiety, and anti-are Parkinson's [13].
Alstoniascholaris	Apocynaceae	Saptaparni	Bark	Diabetes, Anticancer activity Antifertility activity Analgesic [14].
Asphaltum pure		Shilajeet		bronchial asthma, diabetes, genitourinary infections, and stomach ulcers [15].

2.2. Preparation of Polyherbal formulation from ethanol extract: About 200g of coarsely powdered parts of the plant were extracted with ethanol. The extract of individual plants was concentrated by placing it in a hot air oven at 60-50°C. The Polyherbal formulation (per capsule) contained the ethanolic extracts of Berberis aristata, Gymnema sylvestre, Costus speciosa, Pterocarpus marsupium, Syzygium cumini, Picrorrhiza kurroa, Trigonella foenum, Cinnamomum verum, Alstonia scholaris, and Asphaltum pure.

2.3. Phytochemical screening- LC-MS: LC–MS/MS experiments were performed on Agilent 6520 accurate mass MS Q-TOF coupled with Agilent LC 1200. The MS analysis was performed with dual AJS ESI ion source in positive and negative modes. Mass spectral data analysis was done by Agilent molecular ion extraction algorithm. The general conditions for mass spectrometry were drying gas (nitrogen) flow 13 L/min; nebulizer pressure 35 psig; drying gas temperature 250°C; capillary voltage 3500V; fragmentor volt 750 V; Oct RF Vpp. A gradient of water (95%) and acetonitrile (5%) was used as the mobile phase for ESI ionization mode at a constant flow of 0.3 ml/min. The mobile phase was fixed as a gradient of acidified methanol (A) and water (B) system for ESI ionization mode. Gradient elution was performed at a constant flow rate of 0.9 ml/min and 1200.00 bar pressure.

2.4. UV-Vis Spectroscopy: UV–Visible spectra of polyherbal formulation was recorded from 200 to 800 nm in ethanol solvent on Jasco UV–Visible Spectrophotometer model V-550. The baseline was corrected using ethanol solvent before analysis [16-28].

2.5. FT-IR Spectroscopy: The FTIR spectra of polyherbal formulation was recorded by JASCO FTIR-4100 spectrometer at room temperature. The measurements were taken in the range from 400 to 4000 cm^{-1} using KBr pellet [19,20,23,24,26,29-35].

2.6. DPPH Radical Scavenging Assay: The antioxidant activities of the ethanol extract of the polyherbal formulation against the free radical present in towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) were investigated at various drug concentrations. The diluent part of the DPPH solution in methanol (187 μl) was added to 24 well plates with various drug concentrations to make up 2 mL in each well. The reaction mixture was incubated in the dark condition for 20 minutes and the absorbance was measured at 517 nm. The results were evaluated as percentage scavenging of radical[17,28].

$$\% \text{ Inhibition } = [\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}}] \times 100$$

Results were plotted in a graph of the double integral intensity versus extract concentration. By graphical analysis, it was possible to determine the concentration that reduces the initial signal intensity by 50% (IC_{50}).

2.7.SOD Assay: The superoxide (O_2•−) radical is formed during a normal respiration process, which reduces 1–3% of the oxygen that we breathe into its radical, O_2•−. The reduction of molecular oxygen (O_2) takes place intracellularly in the mitochondria under normal physiological conditions. This enzyme converts O_2•− into H_2O_2, which is further converted into O_2 and water by glutathione peroxidase and catalase. The scavenging activity of AOs towards O_2•− is assessed in terms of their ability to prevent O_2•− generation. The sample solution is treated with 0.05 mL phosphate buffer (250 mmol/L), 0.025 mL NADH (2 mmol/L), and 0.025 mL NBT (0.5 mmol/L). The absorbance of the resulting solution is read as a blank at 560 nm. To the resulting solution, 0.025 mL PMS (0.03 mmol/L) is added and allowed to incubate at room temperature for 5 min. The absorbance is read again at the same wavelength.

Results are expressed as % radical scavenging.

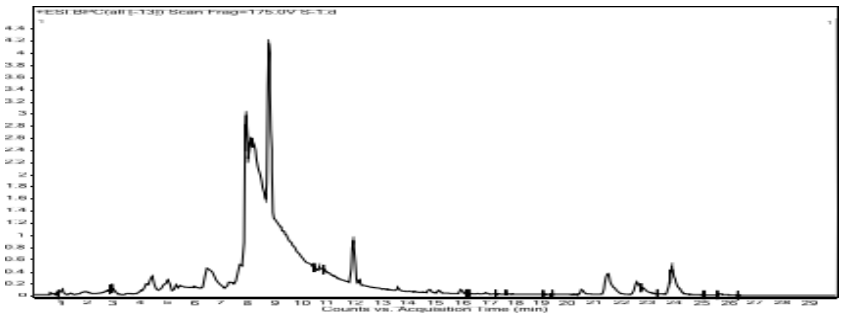
$$\% \text{ radical scavenging } = [\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}}] \times 100$$

2.8. Molecular Docking: Due to its capacity to anticipate the binding conformation of small molecules to the proper target binding site, molecular docking is one of the techniques utilized most frequently in structure-based drug design. Herein, molecular docking of the selected phytochemical compounds identified from LCMS with Monoamine oxidase B, (MAO-B) has been employed to confirm the antioxidant capacity of the polyherbal formulation by studying the underlying binding mechanism of phytochemical compounds. MAO-B is an enzyme that belongs to the Flavin monoamine oxidase family and is found in humans in the outer mitochondrial membrane. It is encoded by the MAO-B gene, which catalyzes the oxidative deamination of biogenic and xenobiotic amines and plays an important function in the peripheral tissues and central nervous system's degradation of neuroactive and vasoactive amines. Benzylamine and phenylethylamine are preferentially degraded by this protein. It also destroys dopamine like MAO-A. Reactive oxygen species are produced by MAO-B on a regular basis, which directly harms cells. Age-related increases in MAO-B levels have been discovered, which raises the possibility that they play a part in both the development of neurological illnesses later in life as well as the normal age-related reduction in cognitive function. Hence, MAO-B protein inhibitors have the potential to be used therapeutically to treat neurological diseases [27].

3.RESULT AND DISCUSSION

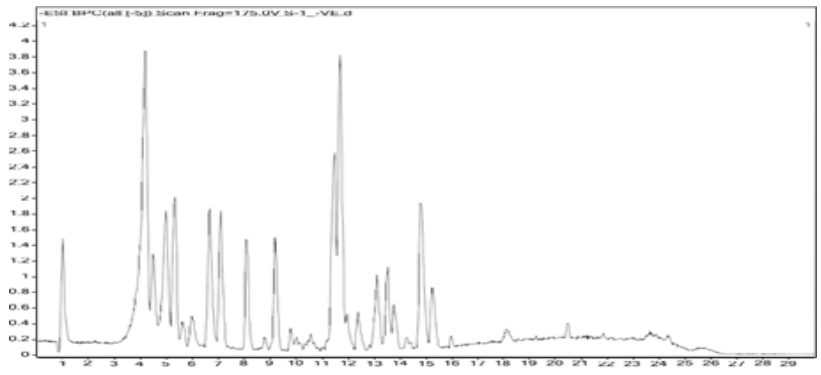
3.1. Chemical Profiling: LC/MS analysis of polyherbal formulation was carried out with ESI ionization in both positive and negative modes and information regarding the presence of chemical compounds was extracted by Agilent Mass Hunter software. 25 molecular ions were detected in negative mode and 8 molecular ion peaks were exhibited in positive mode based on the abundance of ions that were further fragmented in auto ms/ms analysis with varying collision energy. The consistency of fragments was confirmed by targeted ms/ms analysis with fixed collision energy based on the auto ms/ms analysis. The chromatogram in the positive and negative modes were depicted in **Figure 3** and **Figure 4.**

The ESI-MS fingerprint of the sample in positive mode (Table 2) presented the ions of m/z 328.1861-Capsaicin, 336.1188-Muricinine, 336.0821-Avenanthramide 1f, 322.1028- N-cis-Caffeoyltyramine, 366.1654-Gossyrubilone, 274.2703-Sphinganine, 563.2991-Ciclesonide



Acquisition time (Minute)

Figure 3: ESI-MS-base peak chromatogram of polyherbal formulation in positive ESI Mode



Acquisition time(Minute)

Figure 4: ESI-MS-base peak chromatogram of polyherbal formulation in negative ESI Mode

Table 2: ESI-LC-MS/MS analysis of polyherbal formulation in positive ESI Mode

Sl. No:	m/z	MS/MS	Tentative Identification	Type of Compound	Molecular Formula	Biological Activity
1	328.1861	327.1861	Capsaicin	Alkaloids	$C_{18}H_{27}NO_3$	Antidiabetic
2	336.1188	335.1188	Muricinine	Alkaloid	$C_{18}H_{19}NO_4$	
3	336.0821	335.0821	Avenanthramide 1f	Polyphenolic alkaloids	$C_{17}H_{15}NO_5$	(Anticancer, antioxidant,anti-inflammatory
4	322.1028	321.1028	N-cis-Caffeoyltyramine	Alkaloid	$C_{17}H_{17}NO_4$	immunomodulatory, antimicrobial, antiviral, larvicidal, insecticidal and antioxidant
5	366.1654	365.1654	Gossyrubilone	Sesquiterpenoids	$C_{20}H_{25}NO_4$	
6	274.2703	273.2703	Sphinganine	aminoalcohols	$C_{16}H_{35}NO_2$	Anti-fungal, anti-cancerous
7	563.2991	562.2991	Ciclesonide	Glucocorticoid	$C_{32}H_{44}O_7$	Antiinflammatory, antiviral

The ESI-MS fingerprint of the sample in negative mode (Table 3) presented the ions of m/z 191.0558-Quinic acid, 182.996-Sulfoacetaldehyde, 178.0504-Methyl n-formylanthranilate, 433.115-2-O-Caffeoylarbutin, 435.1299-Phenethyl 6-galloylglucoside, 421.078-Tripteroside, 447.0932-8-C-Galactosylluteolin, 435.1295-Phlorhizin, 431.0987-Genistein 8-C-glucoside, 197.045-Vanillylmandelic acid, 301.0714-4,2',4',6'-Tetrahydroxy-3- methoxychalcone, 403.0674-Urolithin A-3-O-glucuronide, 477.1407-Eugenol O-[3,4,5-Trihydroxybenzoyl-(->6)-b-D-glucopyranoside], 415.1044-Chrysophanol 8-O-β-Dglucoside, 681.3867-Madlongiside C, 665.3915-Cyclopassifloside V, 763.4289-Digitoxin, 766.4482-beta-Solanine, 765.4451-28-Glucosylsiaresinolate 3- arabinoside, 331.2493-Phloionolic acid, 805.4382-Gymnemic acid I, 807.4542-Calenduloside G methyl ester, 847.4852-Vinaginsenoside R14, 479.2808-Tingenone and 601.3542-Isoxanthochymol.

Most of the compounds characterized by LCMS shows antidiabetic activity. Besides this these compounds also show biological activities like anticancerous, anti-inflammatory, antiaging, antiproliferative properties etc.

Table 3: ESI-LC-MS/MS analysis of polyherbal formulation in positive ESI Mode

Sl.No:	m/z	MS/MS	Tentative Identification	Type of Compound	Molecular Formula	Biological Activity
1	191.0558	190.558	Quinic acid	Cyclohexanecarboxylic acid.	$C_7H_{12}O_6$	antioxidant, antidiabetic, anticancer activity, antimicrobial, antiviral, aging, protective, anti-nociceptive and analgesic effects
2	182.996	181.996	Sulfoacetaldehyde	Organosulfonic acid	$C_2H_4O_4S$	
3	178.0504	177.0504	Methyl n-formylanthranilate	Amidobenzoic acid.	$C_9H_9NO_3$	
4	433.115	432.115	2-O-Caffeoylarbutin	Glycoside	$C_{21}H_{22}O_{10}$	
5	435.1299	434.1299	Phenethyl6-galloylglucoside	Galloyl esters	$C_{21}H_{24}O_{10}$	
6	421.078	420.078	Tripteroside	Xanthone glycoside	$C_{19}H_{18}O_{11}$	
7	447.0932	446.0932	8-C-Galactosylluteolin		$C_{21}H_{20}O_{11}$	antidiabetic
8	435.1295	434.1295	Phlorhizin	Dihydrochalcone	$C_{21}H_{24}O_{10}$	antidiabetic
9	431.0987	430.0987	Genistein 8-C-glucoside	Isoflavone	$C_{21}H_{20}O_{10}$	Anti-proleferative and apoptotic, Antidiabetic
10	197.045	196.045	Vanillylmandelic acid	Catecholamines	$C_9H_{10}O_5$	
11	301.0714	300.0714	4,2',4',6'-Tetrahydroxy-3-methoxychalcone	Chalcones	$C_{16}H_{14}O_6$	antidiabetic
12	403.0674	402.0674	Urolithin A-3-O-glucuronide	Phenolic glycosides	$C_{19}H_{16}O_{10}$	
13	477.1407	476.1407	Eugenol O-[3,4,5-Trihydroxybenzoyl-(->6)-b-D-glucopyranoside]	Methylated flavonoids	$C_{23}H_{26}O_{11}$	antidiabetic
14	415.1044	414.1044	Chrysophanol 8-O-β-Dglucoside	Beta-D-glucoside	$C_{21}H_{20}O9$	antidiabetic
15	681.3867	680.3867	Madlongiside C	Triterpenoids	$C_{35}H_{56}O_{10}$	

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16	665.3915	664.3915	Cyclopassifloside V	Triterpene Saponins	$C_{36}H_{58}O_{11}$	Antidiabetic
17	763.4289	762.4289	Digitoxin	cardiac glycoside	$C_{41}H_{64}O_{13}$	Antidiabetic
18	766.4482	765.4482	beta-Solanine	Steroidal saponins	$C_{39}H_{63}NO_{11}$	
19	765.4451	754.4451	28-Glucosylsiaresinolate 3- arabinoside	Triterpenoids	$C_{41}H_{66}O_{13}$	antidiabetic
20	331.2493	330.2493	Phloionolic acid		$C_{18}H_{36}O_5$	antidiabetic
21	805.4382	804.4382	Gymnemicacid I	Triterpene glycoside	$C_{43}H_{66}O_{14}$	anti-sweet compounds
22	807.4542	806.4542	Calenduloside G methyl ester	Triterpenoid Saponin	$C_{43}H_{68}O_{14}$	
23	847.4852	846.4852	Vinaginsenoside R14		$C_{41}H_{70}O_{15}$	antidiabetic
24	479.2808	478.2808	Tingenone	Pentacyclic triterpene	$C_{28}H_{36}O_3$	
25	601.3542	600.3542	Isoxanthochymol		$C_{38}H_{50}O_6$	

3.2. Spectroscopic characterization

3.2.1. UV-Visible spectroscopy: The UV spectrum of the polyherbal formulation extract was obtained in ethanol. The spectrum was obtained in the 200–800 nm range. The spectrum exhibits 3 absorption peaks in the UV region and a small single absorption in the visible region as shown in **Figure 5**. The peaks observed at 237and 272 nm may be attributed to π-$\pi^*$and n-π^* transitions of conjugated C=C and C=O groups present in terpenoids, tannins, and flavonoids in polyherbal formulation. The band at 380 nm may be due to π -π^* transitions of the azomethine group present in Gossyrubilone. The absorption bands in the UV spectra indicate the presence of phytochemicals like carbohydrates, terpenoids, flavonoids, saponins, and alkaloids.

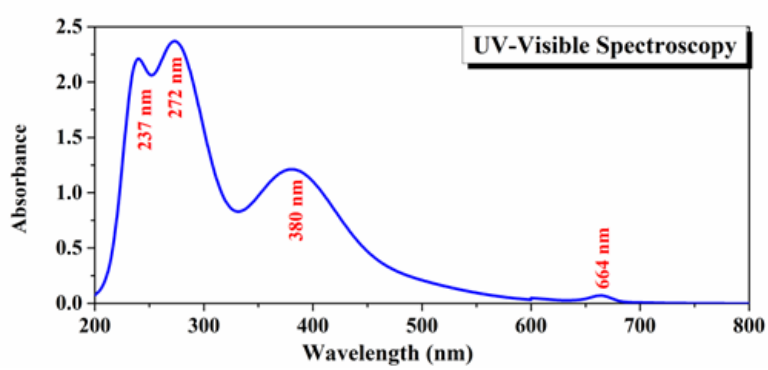

Figure 5: Showing the UV-Visible spectrum of polyherbal formulation.

3.2.2. Vibrational Analysis: The FTIR spectrum of the polyherbal formulation extract was obtained in ethanol as shown in **Figure 6.** The spectrum was obtained in the range 400–4000 cm^{-1}. The polyherbal formulation shows IR peaks at 3312, 2935, 1653, 1453, 1007, 862 and 605 cm^{-1}. The broad peak that appeared at 3312 cm^{-1} corresponds to the –OH stretching of the phenolic and alcoholic compounds present in the formulation. The band at 2935 cm^{-1} corresponds to the C-H stretching vibrations present in $-CH_3$ and $-CH_2-$ groups present in the compounds. The peak at 1653 cm^{-1} is also assigned to the stretching vibration of the C=O groups of the conjugated and aromatic rings, it indicates the presence of terpenoids, tannins, and flavonoids in polyherbal formulation. The C-H bending vibrations of $-CH_3$ and $-CH_2-$ groups present in the compounds appeared at 1453 cm^{-1}. The peak at 1007 cm^{-1} corresponds to C–O stretching vibrations present in primary and secondary alcohols and esters present in it. Peaks at 862 and 605 cm^{-1} correspond to C-H bending vibration substituted alkanes and benzene rings and O-H wagging in alcoholic groups present in carbohydrates. All the IR peaks originate from the phytochemicals like carbohydrates, terpenoids, flavonoids, saponins, and alkaloids.

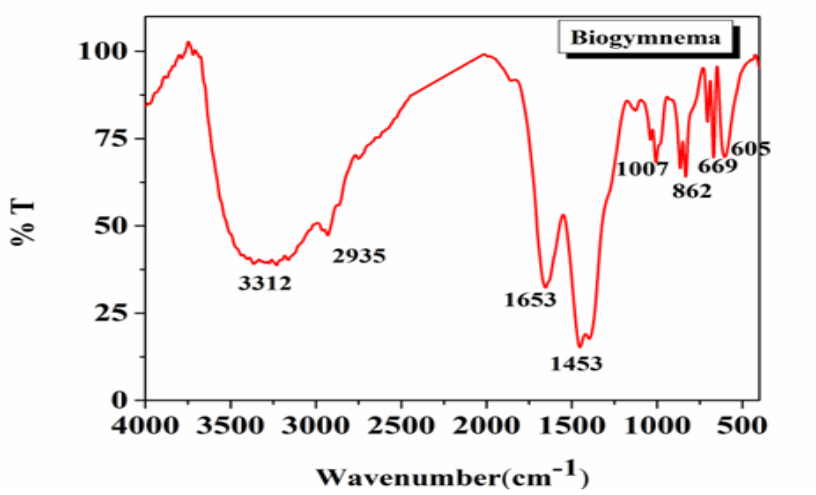

Figure 6: Showing the FTIR spectrum of Antidiabetic polyherbal formulation

Table 4: Assignments of characteristic peaks of vibration of polyherbal formulation and their comparison with ingredient herbs

Wavenumber (cm^{-1})											Bond Assigned	Functional groups
Polyherbal formulation	Gymnemasylvestre[36]	Berberisaristata[37]	Costusspeciosa[38]	Pterocarpus marsupium[39]	Syzygiumcumini[40]	Picrorhizakurroa[41]	Trigonallafoenumgree[42]	Cinamomumverum[43]	Alstoniascholaris[44]	Shilajeet		
	3433			3437	3425	3435	3422	3481	3412		O-H (SV)	Alcohol, phenol
3312			3367							3243	-OH (S)	
2935	2917	2826	2902	2924			2927		2929	2925	CH	Aromatic

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											(S)	functional group
1653	1613		1602	1618	1620		1651	1642	1609		C=C (S) C=O (S) NH (B)	Carboxylic, polyphenols
		1597		1529			1541	1553	1518	1546	C (S) C=O N-O (S)	Nitro compounds Aromatic ring
1453	1460	1442					1456		1442	1400	C-H (B) C-C (S)	Aromatics, Nitro
1007	1034	1032	1047		1057	1045	1073	1060	1059	1069	C-O (S) C-N (SV)	Alcohols, Ethers, esters, Aliphatic amines
						997			926		C-H C-Br (SV)	Alkyl halides
862	822								828		C-H	Alkenes, amines
669												
605								594			O-H (SV)	Pyranoside ring

3.3. Antioxidant activity

3.3.1. DPPH Assay:A dose response pattern of DPPH confirms the free radical scavenging activity of the polyherbal formulation. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance**.** The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability. The components with higher donating capacity have shown higher DPPH free radical scavenging activity[45]. The dose dependent response of DPPH radical scavenging activity of the anti-diabetic polyherbal formulation is depicted in **Figure 7**.

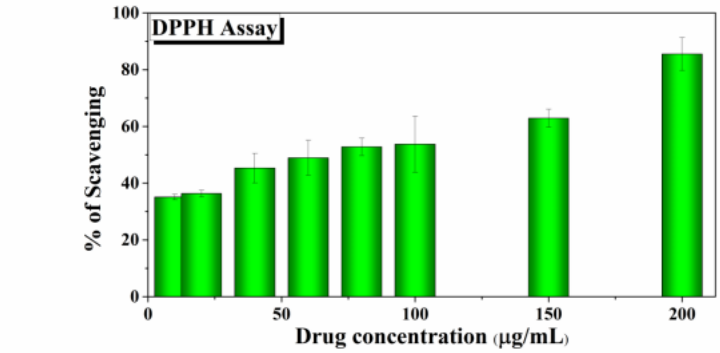

Figure 7: Showing the antioxidant activity of Antidiabetic polyherbal formulation by DPPH assay

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Figure 7exhibits an increasing trend, indicating that the percentage of radical scavenging of DPPH radical increases with increasing concentration of the polyherbal formulation. This suggests that the polyherbal formulation contains a high concentration of antioxidant molecules that can effectively scavenge the DPPH radical. The data were fitted with the Hill Langmuir Equation to calculate the IC_{50} value, which represents the concentration of the plant extract required to scavenge 50% of the DPPH radical. The IC_{50} value of the polyherbal formulation under study was found to be 40 µg/mL, indicating a strong antioxidant activity of the formulation. As the concentration of the formulation increases the absorbance at 517nm decreases indicating the transformation of DDPH radical to DPPH colourless by proton transfer mechanism from the active components of polyherbal formulation, leading to the formation of a stable diamagnetic molecule and resulting in a colorless methanol solution.

3.3.2.SOD Assay: Superoxide scavenging activity is a crucial antioxidant activity that may be assessed using the McCord and Fridovich, 1969, nitrobluetetrazolium (NBT) reduction technique. The test is predicated on a drug's capacity to prevent NBT from being reduced by superoxide, which is produced by the system's photoreduction of riboflavin. In this work, the antioxidant activity of a polyherbal mixture was assessed using the SOD radical scavenging test. The results of the assay were plotted as a graph of the percentage of radical scavenging versus the concentration of the formulation as shown in **Figure 8**. The graph obtained showed an increasing trend, indicating the antioxidant property of the polyherbal formulation. The IC_{50} value was calculated using the Hill Langmuir Equation and was found to be 10 µg/mL. A higher percentage of inhibition activity indicates a higher SOD activity, and thus a higher antioxidant capacity. The IC_{50} value obtained in this study suggests that the polyherbal formulation has significant SOD radical scavenging activity and thus, a potent antioxidant capacity

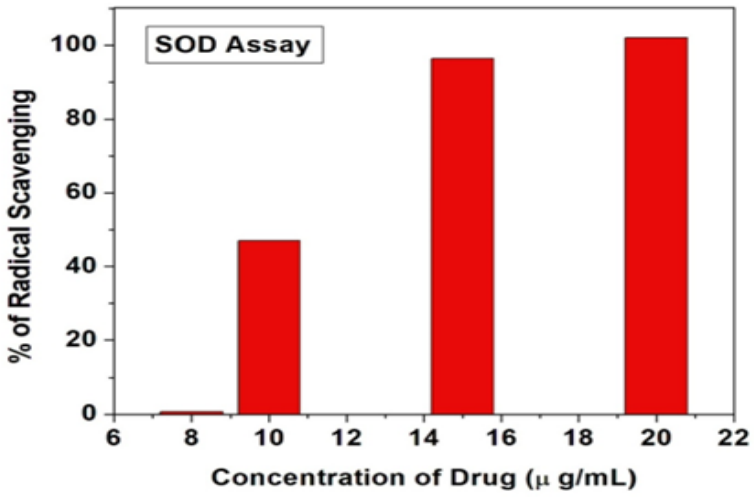

Figure 8: Showing free radical scavenging activity of Antidiabetic polyherbal formulation

3.4. Molecular Docking: LC-MS/MS analysis of antidiabetic polyherbal formulation ethanolic extract identified 33 compounds, including the popular flavonoids and phenolic acids. Based on pharmacokinetic screening based on literature, 9 compounds with antidiabetic action were chosen for subsequent *in-silicon* analysis, with monoamine oxidase B target targets from various databases and literature.The chemical structure of possible 9 compounds is depicted in **Figure 9.**

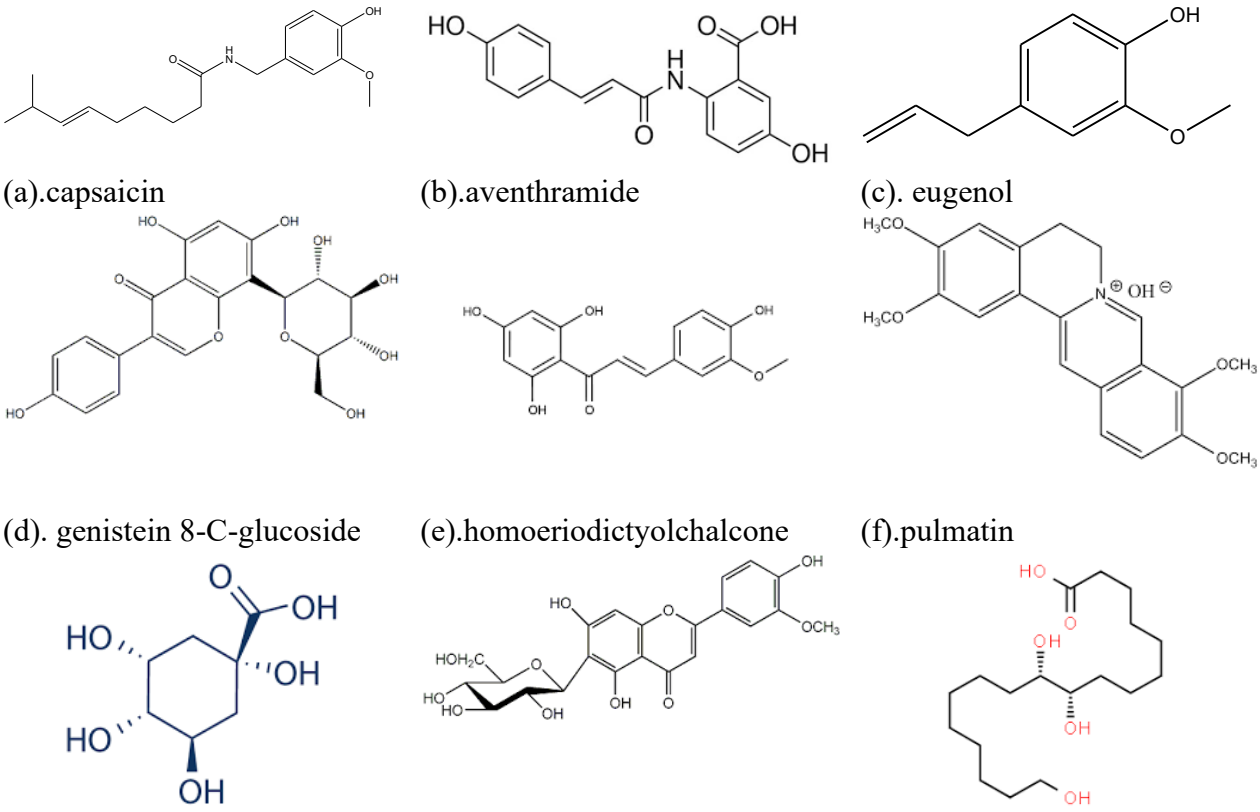

Figure 9: The chemical structure of chemical compounds with antidiabetic property.

Here, molecular docking of the screened 9 compounds with Monoamine oxidase B, (MAO-B) has been employed to confirm the antioxidant capacity of polyherbal formulation. MAO-B as shown in **Figure 10**, is an enzyme that in humans located in the outer mitochondrial membrane, belongs to the Flavin monoamine oxidase family encoded by the MAO-B gene. MAO-B catalyzes the oxidative deamination of biogenic and xenobiotic amines and plays anessential role in the catabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues. Benzylamine and phenyl ethylamine are preferentially degraded by this protein. It also destroys dopamine like MAO-A. Reactive oxygen species are produced by MAO-B on a regular basis, which directly harms cells. Age-related increases in MAO-B levels have been discovered, which raises the possibility that they play a part in both the development of neurological illnesses later in life as well as the normal age-related reduction in cognitive function. Therefore, MAO-B protein inhibitors have the potential to be used therapeutically to treat neurological diseases.

The docking scores and binding free-energies of the lowest energy pose of inbuilt ligand, safinamide, and the selected active principles in the polyherbal formulation in active sites on chain B of the MAO-B X-ray crystal structures have been computed using Schrodinger Maestro Software and are given in **Table 5**. The docking result points out that many of the active principles in the polyherbal formulation show a significant binding affinity score than the inbuilt ligand, safinamide binding affinity value, the inbuilt ligand safinamide had exhibited a docking score of -9.014 with -74.785 kcal binding energy. While

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pulmatin showed a docking score of -10.533 with a binding energy of -64.220kcal, genistein 8-C-glucosidewith a docking score of-9.541with binding energy of -64.673 kcal and all others have a comparable docking score and binding affinity. These results point out that the polyherbal formulation is rich in antioxidant ingredients and will require less amount for high performance.

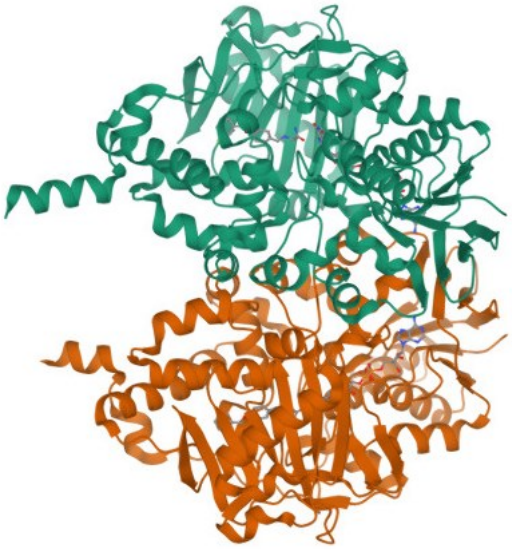
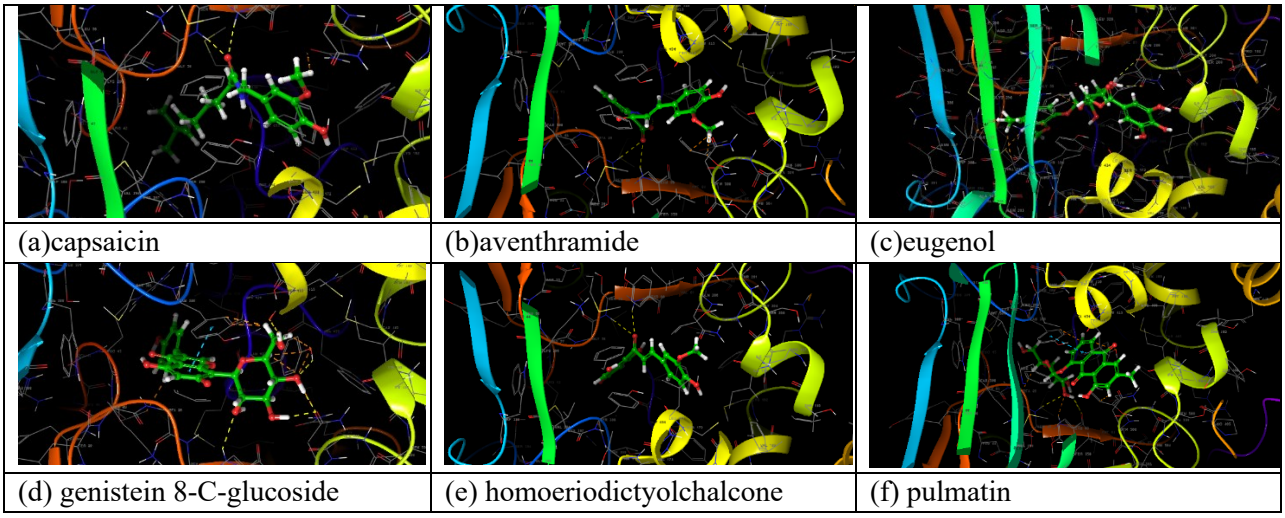
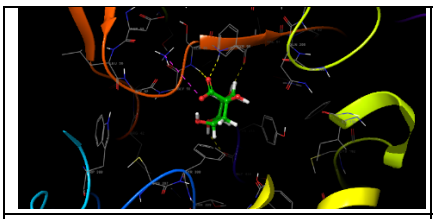


Figure 10: The structure of monoamine oxidase B enzyme taken from protein data bank

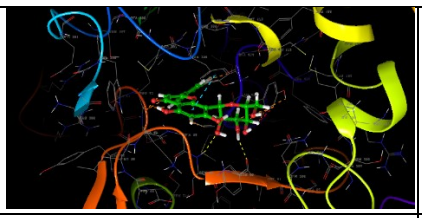
Figure. 11 demonstrates the 3-dimensional protein-ligand interaction of all selected compounds in the dynamic site of MAO-B obtained from the graphical interface Maestro. All the selected active principles and the co-crystalized ligand safinamide are found to be buried in the deep binding pocket of MAO-B in an indistinguishable way. All the ligands interact with the active site of amino acids of the protein by H-bonding and π bonds, which are depicted in red and dotted lines.



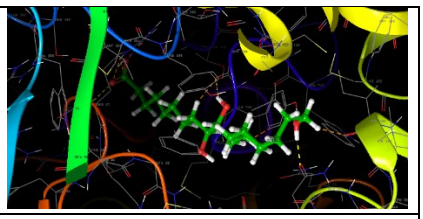
(a)capsaicin	(b)aventhramide	(c)eugenol
(d) genistein 8-C-glucoside	(e) homoeriodictyolchalcone	(f) pulmatin



(g)quinic acid



(h)c-galacosylluteolin



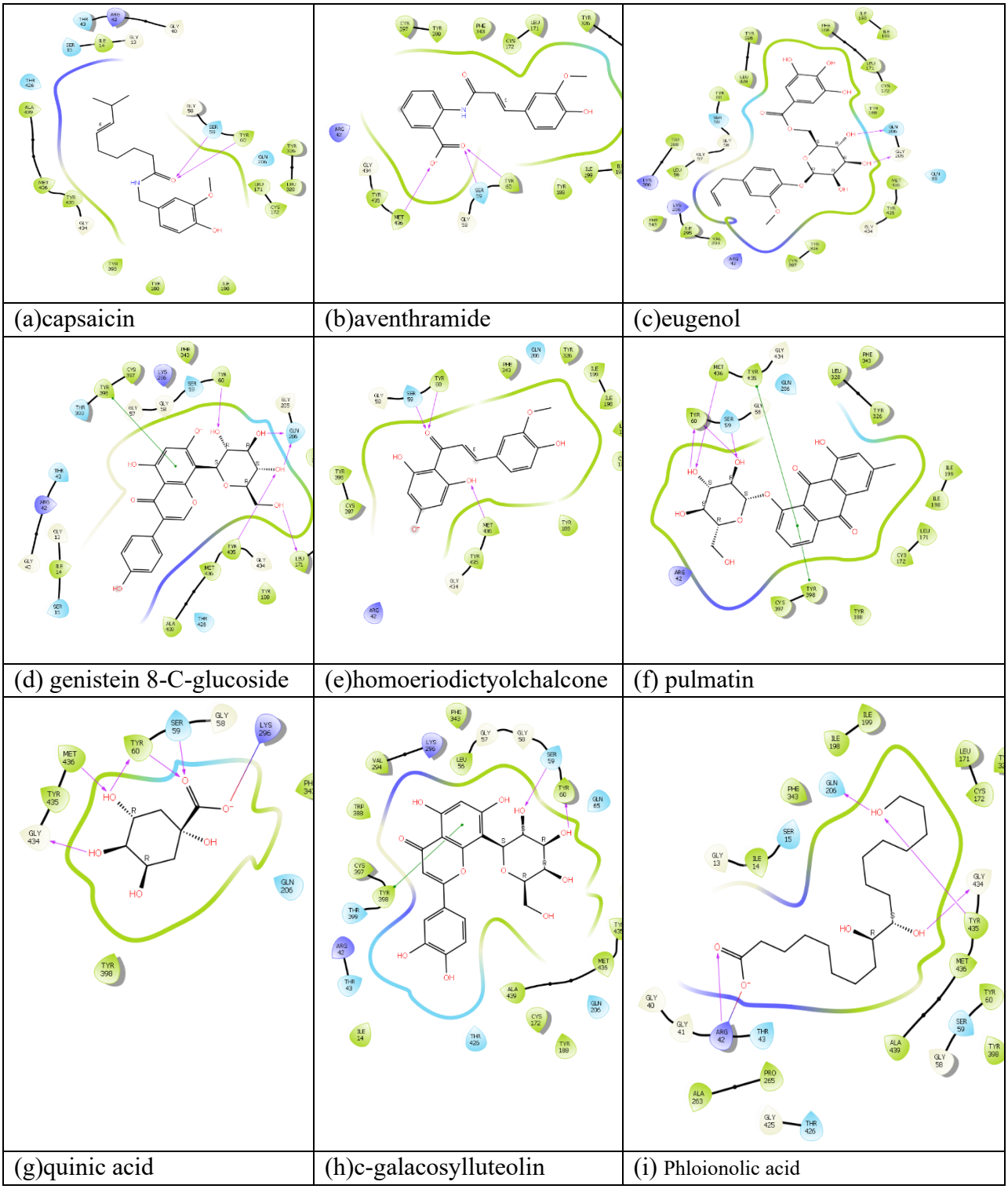
(i) phloionolic acid

Figure 11: Three-dimensional (3D), protein-ligand interactions of selected compounds in polyherbal formulation (stick model) with the X-ray crystal structure of MAO-B using. The red dashed lines represent the weak bonding of ligand with protein.

Table 5: Docking score and binding affinity of inbuilt ligand and 9 possible compounds with antioxidant property

Sl No	Ligand Name	Docking Score	Binding Energy
1	capsaicin	-8.141	-70.525
2	Aventhramide	-8.719	-74.569
3	Eugenol	-8.494	-42.312
4	Genistein 8-C-glucoside	-9.541	-64.673
5	Homoeriodictyolchalcone	-8.356	-68.207
6	pulmatin	-10.533	-64.220
7	Quinic acid	-6.506	-51.637
8	c-galacosylluteolin	-7.676	-52.319
9	Phloionolic acid	-8.633	-88.147
10	Inbuilt ligand	-9.014	-74.785

In addition to the 3D binding orientations of the ligands in the protein, the docking results provided further insights into selective interactions of the ligand with the human MAO-B in 2D image as shown in **Figure 12.** The ligands were encompassed by active site amino acids PHE343, LEU171, CYS172, GLN206, ILE198, SER59, TYR398, and TYR435 of MAO- B. The co-crystallized ligand safinamide occupied the deep cavity by forming only a single hydrogen bond with amino acid GLN206, while the ligand AMB under study, interacted with MAO-B via two hydrogen bonds and three π-π interaction with active site amino acids. Capsaicin forms hydrogen bond interaction with SER 50 and TYR 60, aventhramides showed 3 hydrogen transfer mechanism from MET 436, SER 49, and TYR 60, eugenol exhited tendency to donate a proton to GLN 206 and GLY 200, genistein 8-C-glucoside formed 5 hydrogen bonds with TYR 60, TYR 398, GLN 206,IEU 171,and TYR 435 with an additional π bond to TYR 398,homoeriodictyol chalcone have 3 hydrogen bonds with SER 59, TYR 60 and MET 436,pulmatin docked with 2 π–π interactionswith TYR 435 and TYR 398 in addition to 4 hydrogen bonds with TYR 60, MET 436, SER 59 by proton transfer mechanism in two waysbetween the ligand and the amino acids quinic acid formed 6 hydrogen bond GLY 434, MET 436, TYR 60, SER 59 and LYS 296, c-galacosylluteolin forms p bond with TYR 398 and 2 hydrogen bond interaction with SER 59 and TYR 398 and Phloionolic acid 4 hydrogen bonds with ARG 42,GLN 205 and TYR 425.



(a)capsaicin	(b)aventhramide	(c)eugenol
(d) genistein 8-C-glucoside	(e)homoeriodictyolchalcone	(f) pulmatin
(g)quinic acid	(h)c-galacosylluteolin	(i) Phloionolic acid

Figure 12: Two-dimensional (2D), protein-ligand interactions of selected compounds in polyherbal formulation (stick model) with the X-ray crystal structure of MAO-B using. The red dashed lines represent the weak bonding of ligand with protein.

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CONCLUSION

A novelpolyherbal formulation, antidiabetic polyherbal formulation used for the treatment of diabetes mellitus has been investigated in this article. Several shreds of scientific evidence have proved that the synergetic action of the phytochemicals in the polyherbal formulation possess antihyperglycemic potentials and can be effectively implicated in the management of diabetic and metabolic complications avoiding notable side effects exerted by conventional drugs.Identification and estimation of bioactive phytochemical constituents in the formulations were done using different chromatographic and spectroscopic techniques. Results of the analytical report suggest that Antidiabetic polyherbal formulation is rich in phytochemicals like carbohydrates, terpenoids, flavonoids, saponins, and alkaloids. This study screened the most significant 9 phytochemicals responsible for antidiabetic activity and their complex mechanism was studied using molecular docking after evaluating their biological potency through biochemical assays. Regarding the role of traced phytochemicals in Diabetes, this formulation could be an appropriate candidate for reducing blood sugar levels with respect to its traditional use in Ayurveda.

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