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IN-SILICO AND IN-VITRO EVALUATION OF ANTIDIABETIC POTENTIAL OF BLEPHARIS MADERASPATENSIS ETHANOLIC EXTRACT

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ABSTRACT

Blepharis maderaspatensis, a traditionally used medicinal plant, was investigated for its potential antidiabetic activity using both **in-silico** and **in-vitro** approaches. The ethanolic extract of the plant was subjected to **phytochemical screening**, which revealed the presence of flavonoids, alkaloids, tannins, and phenolic compounds. In-vitro antidiabetic activity was evaluated using **alpha-amylase and alpha-glucosidase inhibition assays**, showing significant dose-dependent enzyme inhibition, comparable to standard antidiabetic drugs. In the in-silico analysis, major bioactive compounds identified via GC-MS were docked against key diabetes-related targets such as **alpha-glucosidase, alpha-amylase, and PPAR-γ**, using molecular docking techniques. The results showed good binding affinities, supporting the potential of these compounds in managing hyperglycemia. The combined findings suggest that the ethanolic extract of Blepharis maderaspatensis possesses promising antidiabetic properties, warranting further pharmacological and clinical investigations.

INTRODUCTION

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by high levels of glucose (sugar) in the blood. It occurs when the body is either unable to produce enough insulin or unable to effectively use the insulin it produces. Insulin is a hormone produced by the pancreas that allows glucose from food to enter the body's cells to be used for energy. Without proper insulin function, glucose remains in the blood stream, leading to elevated blood sugar levels, which over time can cause serious health complications.

Diabetes is not a singular disease but a group of metabolic diseases that affect how the body processes blood sugar. It has become one of the most common chronic diseases globally and represents a major public health concern due to its rising prevalence and associated complications. According to the International Diabetes Federation (IDF), as of 2021, approximately 537 million adults were living with diabetes worldwide, a figure expected to rise significantly in the coming decades.

TYPES OF DIABETES

There are several forms of diabetes, each with distinct causes and characteristics. The main types include

Type 1 Diabetes: An autoimmune condition where the immune system attacks the insulin-producing beta cells in the pancreas, leading to little or no insulin production. It is usually diagnosed in children and young adults but can occur at any age.

Type 2 Diabetes: The most common form, accounting for over 90% of all diabetes cases. It occurs when the body becomes resistant to insulin or when the pancreas fails to produce enough insulin. It is often associated with obesity, physical inactivity, and poor dietary habits.

Gestational Diabetes: A form of diabetes that occurs during pregnancy and usually resolves after childbirth. However, It increases the risk of Developing Type2 diabetes later in life for both the mother and child. Other Specific Types: Including monogenic diabetes, cystic fibrosis-related diabetes, and secondary diabetes resulting from other medical conditions or medications.

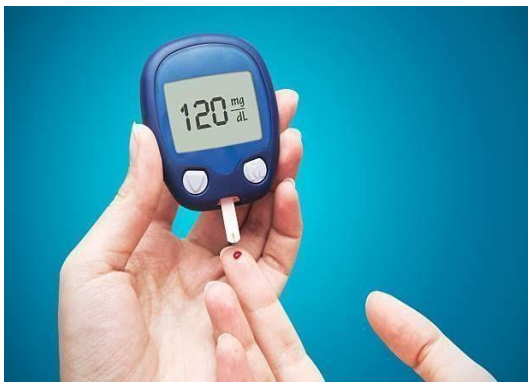
CAUSES AND RISK FACTORS

The exact cause of diabetes varies by type but generally involves a combination of genetic, environmental, and lifestyle factors.

Type 1: Diabetes is primarily due to genetic predisposition and autoimmune destruction of pancreatic beta cells. Environmental triggers like viruses may also play a role.

Type 2: Diabetes has a stronger association with lifestyle factors. Being overweight, leading a sedentary lifestyle, unhealthy eating habits, and family history of diabetes significantly increase the risk. Age and ethnicity also influence susceptibi

Gestational Diabetes is influenced by hormonal changes during pregnancy, genetic.



SYMPTOMS AND CAUSES

Symptoms of diabetes include increased thirst, frequent urination and slow-healing cuts and sores. The severity of symptoms can vary based on the type of diabetes you have. These symptoms are usually more intense in Type 1 diabetes than Type 2 diabetes.

What are the symptoms of diabetes? Symptoms of diabetes include

Increased thirst (polydipsia) and dry mouth.

Frequent urination.

Fatigue.

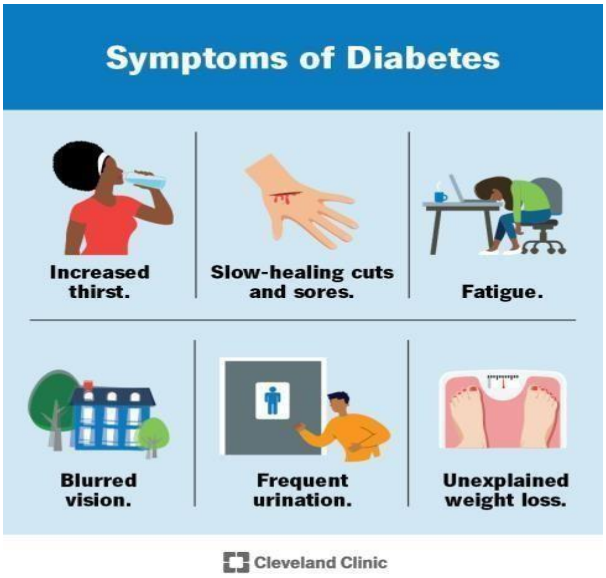
Blurred vision.

Unexplained weight loss.

Numbness or tingling in your hands or feet.

Slow-healing sores or cuts.

Frequent skin and/or vaginal yeast infections.



It’s important to talk to your healthcare provider if you or your child has these symptoms.

PLANT PROFILE OF BLEPHARIS MADERASPATENSIS

Synonyms: Acanthusciliaris, Burm. f. Acanthus maderaspatensis L

Biologicalsource: Blepharis maderaspatensis (L.) B. Heyneex Roth

Family: Acanthaceae

GeographicalSource: India, Sri Lanka, Africa, South-east Asia.

Scientific classification: Kingdom : Planate

Order : Lamiales

Family : Acanthaceae

Genus : Blepharis Juss

Species : Maderapatenisis (L.) B. Heyneex Roth

Vernacular names

English : Indian Blephariss

Tamil : Kurunduched

Chemical Components

The alcoholic extract of Utingan revealed the presence of XVII different compounds during GC-MS analysis. Phytoconstituents had been obtained in the mass spectra were authentified with the library of NIST. Chemical constituents which have been presented in the plant are quercitol, quercitrin, tropane, quercetin, indole, 9-eicosyne, kaempferol, myricetin, L-rhamnose, camphor, purine, cholestan-3-ol, diethyl phthalate, palmitic acid, squalene, phytol, rutin, isoquinoline, ursane, lupane, oleanane, gallic acid. The leaf extract ofshowed Caffeic acid, Rutin, Quercetin, and Ferulic acid.

MOLECULAR DESIGN

Molecular design is the process of finding new medicines based on the knowledge of a biological target, it enabled the chemist to predict the structure and then it also allows the medicinal chemist to evaluate the interaction between a compound and its target site before synthesizing a compound so as to increase the ability by reducing the side effects.

Various software used

Chem Sketch

Mol inspiration Swiss ADME Pro Tox 3.0

MOL INSPIRATION

This software is used to calculate the following properties

- Log P
- Molecular weight
- Number of H-bond donor
- Number of H-bond acceptor
- Number of rotatable bonds

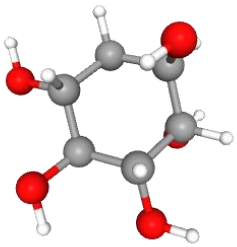
In addition to “LIPINSKI’S RULE” another rule was proposed VEBER he states that the number of rotatable bonds should be less than 10. This rule is more appropriate for oral drug only. According to the vebers rule

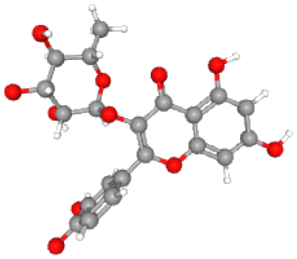
1. The Log P value should not be more than 5

2. The molecular weight of the compound should not more than 500
3. No. of H-bond donor not more than 5
4. No. of rotatable bonds should not be more than 10

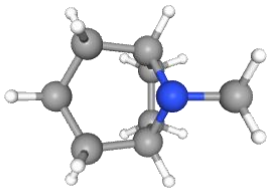
3D VIEW STRUCTIRE OF COMPOUNDS

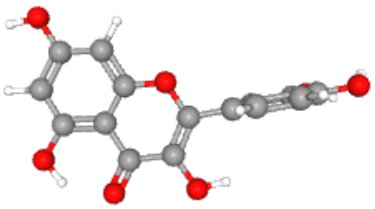
QUERCITOL QUERCITRIN



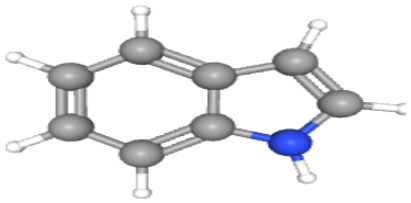


TROPANE QUERCETIN

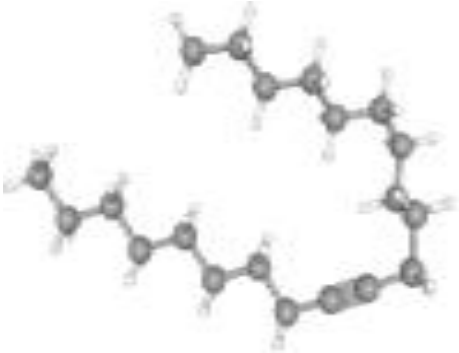




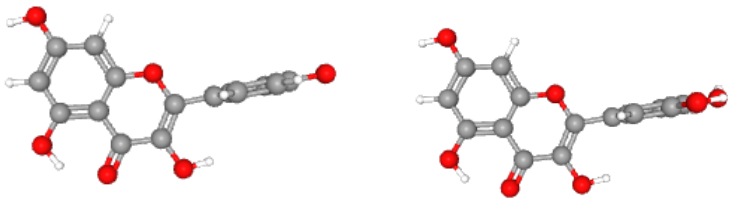
INDOLE



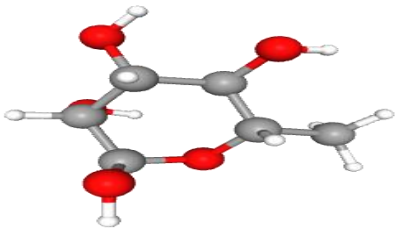
9 –EICOSYNE CAFFEIC ACID



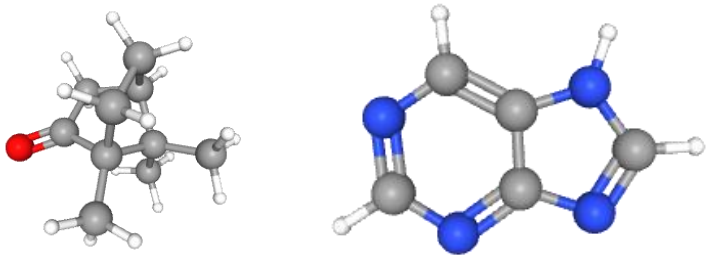
KAEMPEROL MYRICETIN



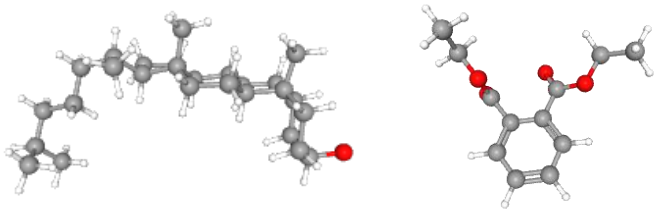
L-RHAMNOSE



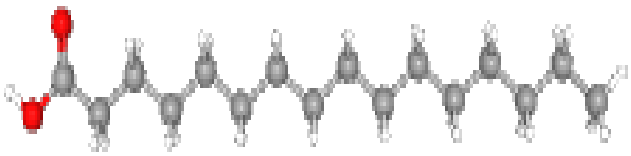
CAMPHOR PURINE



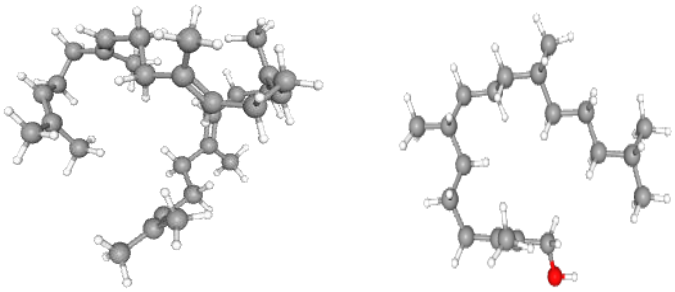
CHOLESTAN-3-OL DIETHYLPHTHALATE



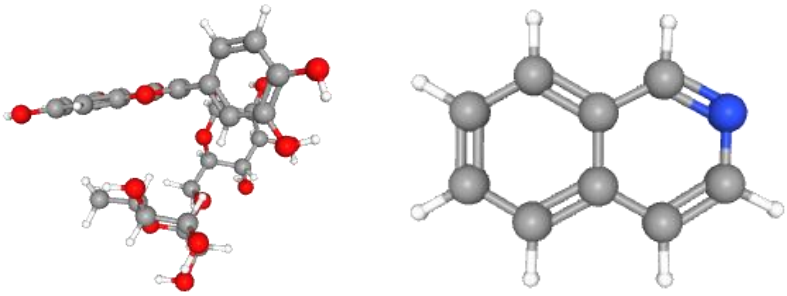
PALMITIC ACID



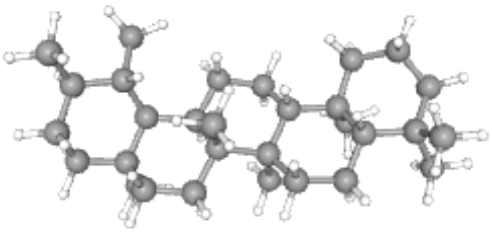
SQUALENE PHYTOL



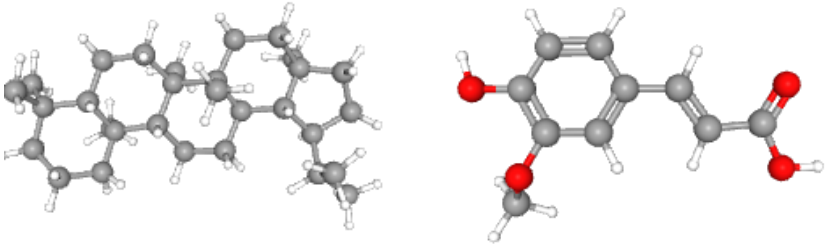
RUTIN ISOQUINOLINE



URSANE



LUPANE FERULIC ACID



OLEANANE GALLIC ACID

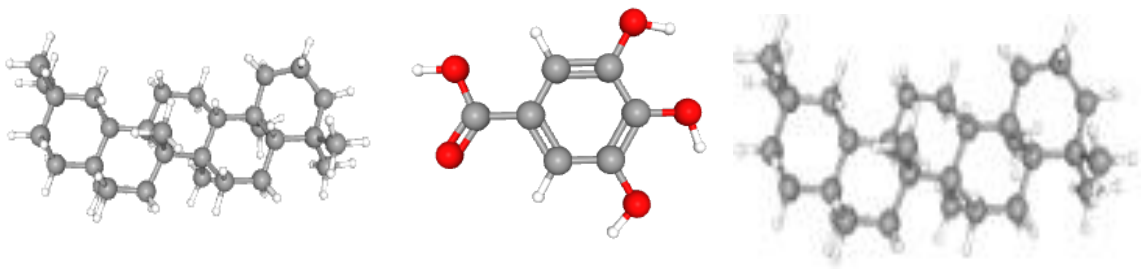


Table 1: Properties of phytoconstituents

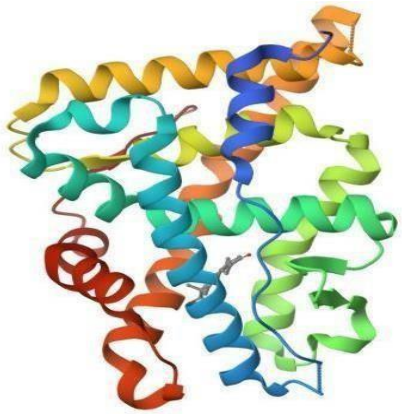
S.NO	PHYTOCONSTITUENTS	PUBCHEM ID	MOLECULAR WEIGHT	MOLECULAR FORMULA
1.	Quercitrin	441437	164.16g/mol	$C_6H_{12}O_5$
2.	Tropane	637986	125.21g/mol	$C_8H_{15}N$
3.	Quercetin	5280343	302.23g/mol	$C_{15}H_{10}O_7$
4.	Indole	798	117.15g/mol	C_8H_7N
5.	9-eicosyne	557019	278.5g/mol	$C_{20}H_{38}$
6.	Caffeic acid	689043	180.16g/mol	$C_9H_8O_4$
7.	Kaempferol	5280863	286.24g/mol	$C_{15}H_{10}O_6$
8.	Myricitin	5281672	318.23g/mol	$C_{15}H_{10}O_8$
9.	L-Rhamnose	25310	164.16g/mol	$C_6H_{12}O_5$
10.	Camphor	2537	152.23g/mol	$C_{10}H_{16}O$
11.	Purine	1044	120.11g/mol	$C_5H_4N_4$
12.	Cholestan-3-ol	11036532	388.7g/mol	$C_{27}H_{48}O$
13.	Diethyl phthalate	6781	222.24g/mol	$C_{12}H_{14}O_4$
14.	Palmitic acid	985	256.42g/mol	$C_{16}H_{32}O_2$
15.	Squalene	638072	410.7g/mol	$C_{30}H_{50}$
16.	Phytol	5280435	296.5g/mol	$C_{20}H_{40}O$
17.	Rutin	5280805	610.5g/mol	$C_{27}H_{30}O_{16}$
18.	Isoquinoline	8405	129.16g/mol	C_9H_7N
19.	Ursane	9548870	412.7g/mol	$C_{30}H_{52}$
20.	Lupane	9548715	412.7g/mol	$C_{30}H_{52}$
21.	Ferulic acid	445858	194.18g/mol	$C_{10}H_{10}O_4$
22.	Oleanane	9548717	412.7g/mol	$C_{30}H_{52}$
23.	Quercitol	441437	164.16g/mol	$C_6H_{12}O_5$
24.	Gallic acid	370	170.12g/mol	$C_7H_6O_5$
25.	Cyanidin-3,5-diglucoside	5158757	610.2g/mol	$C_{27}H_{30}O_{16}$

ANTI-DIABETIC ACTIVITY

PREPARATION OF PROTEIN

The protein target, obtained from the RCSB protein Data Bank with the PDB accession code ISQN, functions as a docking receptor. The active site of the receptor was cleared of all sound lignds and water molecules.

CRYSTAL STRUCTURE OF ISQN



PDBDOI: https://doi.org/10.2210/pdbISQN/pd6

Classification: HORMONE/GROWTH FACTOR RECEPTOR

Organism (S): homo sapiens

Expression System: Escherichia coli

Mutations (S): No

Experimental Data Snapshot Method: X-RAY DIFFRACTION

Resolution: 1.45A^0

R-value Free: 0.216(depositor), 0.190(DCC)

R-value Work: 0.180(depositor), 0.190(DCC)

R-value Observed: 0.188 (depositor)

Table 2: ADME Properties

S.No	Phytoconstituents	No of Rotatable Bonds	No of H-Bond Acceptors	No of H- Bond Donors	Logpc/W9 (Liogl)	Molar Refractivity	Solubility	Gi Absroption
1.	Quercitrin	5	7	5	78.03	78.03	Soluble	High
2.	Tropane	0	0	3	71.0	91.0	Soluble	Low
3.	Quercetin	4	1	1	135.14	5.16	Soluble	Low
4.	Indole	1	8	1	122.43	7.16	Soluble	High
5.	9-eicosyne	8	4	2	119.66	2.51	Soluble	High
6.	Caffeic acid	3	4	2	119.66	2.51	Soluble	High
7.	Kaempferol	1	6	4	76.01	1.70	Soluble	High
8.	Myricitin	1	8	4	34.57	1.14	Soluble	High
9.	L-Rhamnose	0	5	4	34.57	1.14	Soluble	High
10.	Camphor	0	5	4	39.47	0.21	Soluble	High
11.	Purine	0	3	1	31.68	0.31	Soluble	High
12.	Cholestan-3-ol	7	15	10	144.25	-7.41	Soluble	Low
13.	Diethyl phthalate	4	1	1	135.14	5.16	Soluble	Low
14.	Palmitic acid	5	0	0	32.22	7.91	Soluble	High
15.	Squalene	0	1	1	132.75	5.08	Soluble	Low
16.	Phytol	7	6	5	96.61	2.96	Soluble	High
17.	Rutin	7	1	1	132.75	5.08	Soluble	Low
18.	Isoquinoline	0	2	4	145.25	6.01	Soluble	Low
19.	Ursane	0	0	0	134.45	5.02	Soluble	Low
20.	Lupane	1	0	0	132.45	5.08	Soluble	Low
21.	Ferulic acid	0	0	0	34.22	5.11	Soluble	High
22.	Oleanane	0	0	0	134.19	5.05	Soluble	Low
23.	Quercitol	0	7	5	78.03	1.63	Soluble	High
24.	Gallic acid	0	5	4	31.47	0.21	Soluble	High
25.	Cyanidin-3,5diglucoside	0	15	10	144.25	-2.51	Soluble	Low

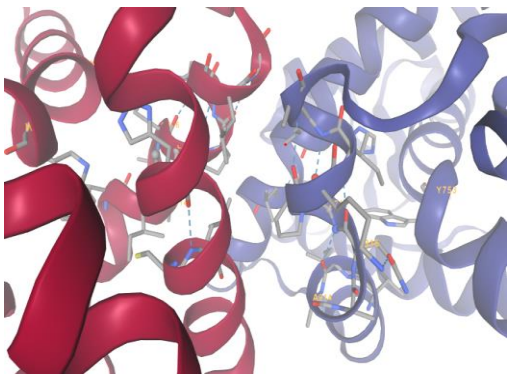
Table 3: Toxicity of phytoconstituents

S.No	Phytoconstituents	Neurotoxicity	Nephro Toxicity	Cardio Toxicity	Immuno Toxicity	Cytotoxicity	Clinical Toxicity	Nutrirional Toxicity	Aryl Hydrocarbon Receptor
1.	Quercitrin	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
2.	Tropane	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
3.	Quercetin	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
4.	Indole	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
5.	9-eicosyne	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
6.	Caffeic acid	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
7.	Kaempferol	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
8.	Myricitin	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
9.	L-Rhamnose	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
10.	Camphor	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
11.	Purine	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
12.	Cholestan-3-ol	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
13.	Diethyl phthalate	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
14.	Palmitic acid	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
15.	Squalene	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
16.	Phytol	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
17.	Rutin	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
18.	Isoquinoline	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
19.	Ursane	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
20.	Lupane	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
21.	Ferulic acid	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
22.	Oleanane	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
23.	Quercitol	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
24.	Gallic acid	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive
25.	Cyanidin- 3,5diglucoside	inactive	inactive	inactive	inactive	inactive	inactive	Inactive	inactive

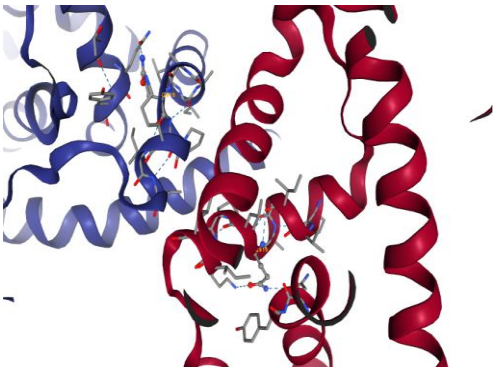
Table 4: Affinity of phytoconstituents

S.NO	PHYTOCONSTITUENTS	BINDING ENERGY
1.	Quercitrin	-7.1917
2.	Tropane	-5.9969
3.	Quercetin	-7.4582
4.	Indole	-6.0213
5.	9-eicosyne	-7.6015
6.	Caffeic acid	-6.5595
7.	Kaempferol	-7.3218
8.	Myricitin	-7.2396
9.	L-Rhamnose	-5.9587
10.	Camphor	-5.8697
11.	Purine	-5.9399
12.	Cholestan-3-ol	-6.8879
13.	Diethyl phthalate	-6.6529
14.	Palmiticacid	-7.1123
15.	Squalene	-8.1716
16.	Phytol	-7.5193
17.	Rutin	-7.8178
18.	Isoquinoline	-8.2096
19.	Ursane	-6.9619
20.	Lupane	-7.0080
21.	Ferulic acid	-6.9783
22.	Oleanane	-7.5650
23.	Quercitol	-6.095
24.	Gallic acid	-5.9362
25.	Cyanidin-3,5-diglucoside	-6.5140

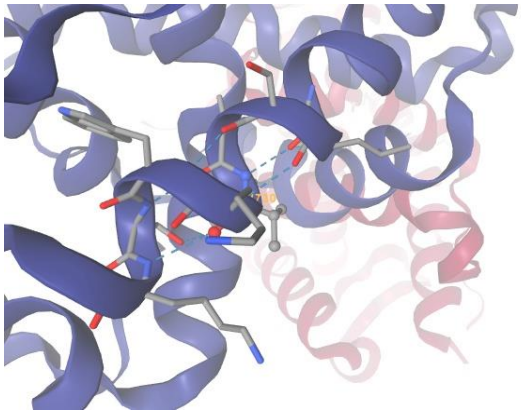
PROTEIN BINDING STRUCTURE



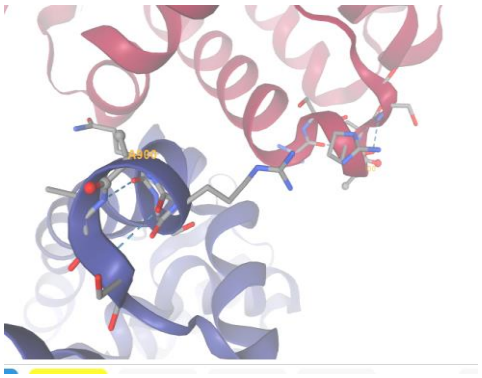
INDOLE



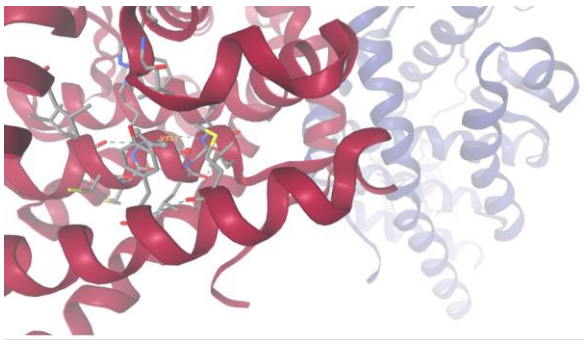
TROPANE



KAEMPFEROL



L-RHAMNOSE



PURIENE

MATRIALS AND METHOD

Processing of plant

The collected plant has identified by Dr.V.Suresh kumar, Assistant Professor, Dept of Botany, Government Arts College, Tiruvannamalai (District), Tamilnadu, India. The plant was washed with tap water 3 times and sterilized by spraying with 70% alcohol.

The purified plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was dried entirely (figure1a), it has subjected to prepare fine powder with help of mixer grinder.

The fine material powder is collected and used for extraction of the crude drug in solvent by soxhlet extraction method.

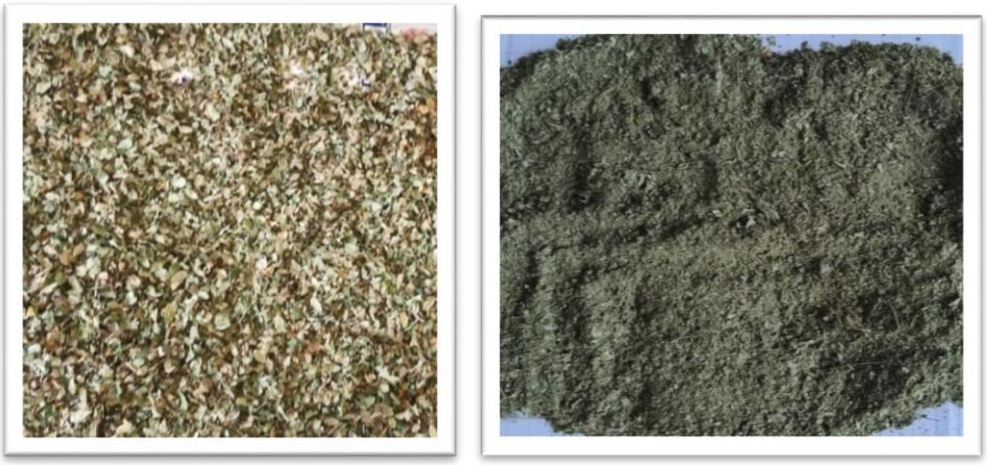


Figure 1a: (Driedleaves) Figure 1b: (finepowder) Extraction by Soxhlet.

Apparatus

Extraction by soxhlet apparatus the extraction procedure for the isolation of crude drug from plants has been practiced for a long time. The mode of extraction process depends on the presence of water content of the plant materials that have been extracted.

Usually, the crude extract has taken from the Soxhlet apparatus with the aqueous solvent. This apparatus mainly consists of three parts, a round bottom flask in which the solvent has taken, the main jar in which the material from which the compounds to been extracted has kept loaded, and a condenser in which condensation of vapours of solvents takes place.

Approximately 40 g of the powder of plant material from which the extract has to take into packed into Soxhlet main jar. The solvent is poured in the round bottom flask and extract condensation under reduced pressure, and a controlled temperature of 60-80^{o}C has set to boil through the regulated heating mantle (figure 2). The vapour of the solvent pass-through drive tubes enters the condenser through the main jar and gets condensed where there is a continuous flow of water in the condenser.

Figure 2: Soxhlet Apparatus Extraction.

The condensed solvent falls back on packed material in the main jar before collecting in a jar itself. The collection and extraction of material take place simultaneously in the main jar, as seen by the colouring of the solvent as a compound of material gets dissolved in the solvent. Thus, the crude plant material extract has been obtained, and it usually takes 28h to complete an extraction. The solvent has evaporated, and finally, it yields green extract; this has been stored in the refrigerator for further usage.

MACERATION

Procedure

1. **Preparation**

- The plant material (80gm) is first prepared, often by washing and removing any unwanted material.
- It can be used dried, depending on the desired outcome.
- The material is then typically a coarse powder to increase surface are a for better extraction.

2. **Soaking**

- The prepared plant material is placed in a suitable container, such beaker
- A solvent, chosen based on the desired compounds to be extracted, is poured over the plant material, ensuring it is completely submerged.

- Common solvents include ethanol(1000ml)
- The container is sealed to prevent evaporation and contamination.

3. Time and Agitation

- The mixture is left to soak at room temperature for a specified period, often atleast Seven days
- Some procedures may recommend intermittent Shaking or agitation to ensure good contact between the solvent and plant material.
- The duration of maceration can vary depending on the plant material and the specific compounds being extracted.

4. Separation

- After the maceration period, the mixture is filtered or pressed to separate the liquid extract from the solid plant material.
- Filtration is a common method for removing the plant debris, while pressing can be used to extract more liquid.

5. Further Processing

- The extracted liquid may be further processed,such as by evaporation to concentrate the extract or by distillation to separate specific compounds.

CONFORMATION TESTS FOR CRUDE EXTRACT

S.NO	CHEMICAL CONSTITUENTS	TEST	ETHANOL
1.	Alkaloids	Dragendroff's test	+ve
		Mayer's test	+ve
		Wagner's test	+ve
		Hager's test	+ve
2.	Flavonoids	Alkaline reagent test	+ve
		Lead acetate test	+ve
3.	Saponins	Foam test	-ve
		Emulsification test	+ve
4.	Tannins	Ferric chloride test	+ve
		Lead acetate test	+ve
5.	Glycosides	Keller-killiani test (For cardiac glycosides)	+ve
		Borntrager's test (For anthraquinone glycosides)	+ve
6.	Phenols	Ferric chZoride test	+ve
		Lead acetate test	+ve

METHODS

Diabetic activity by α-amylase

Alpha-amylase is an enzyme that plays a crucial role in breaking down starch into simple Sugars like glucose and maltose. It is found in various organisms, including plants, animals, and microorganism. Alpha-amylase activity can be influenced by factors like temperature, pH and the presence of certain metal ions. It is also target for inhibitors, which can be used to manage conditions like diabetes by slowing down the digestion of carbohydrates.

Evaluation of in-vitro anti-diabetic activity by α-amylase inhibition assay Principle

Inhibiting alpha-amylase slows the break down of starch in to glucose, thereby Reducing postprandial blood glucose spikes.

BLANK

Add 0.5ml of phosphate buffer solution

↓

Add 0.5ml of starch solution

↓

Incubate at 37˚C for 10minutes

↓

1ml of DNS reagent

↓

Boil at 100˚C for 5minutes

↓

Make up for 10ml with distilled water

↓

Measure absorbance at 540nm

CONTROL

Add 0.5ml of α-amylase solution

↓

Add 0.5ml of phosphate buffer solution

↓

Incubate at 37˚C for 10minutes

↓

Add 0.5ml of starch solution

↓

Incubate at 37˚C for 10minutes

↓

Add 1ml of DNS reagent

↓

Boil at 100˚C for 5minutes

↓

Make up for 10 ml with distilled water

↓

Measure absorbance at 540nm

STANDARD

Add0.5 ml of Acarbose solution

↓

Add 0.5ml of α-amylase solution

↓

Add 0.5ml of phosphate buffer solution

↓

Incubate at 37˚C for 10minutes

↓

Add 0.5ml of starch solution

↓

Incubate at 37˚C for 10minutes

↓

Add 1ml of DNS reagent

↓

Boil at 100˚C for 5 minutes

↓

Make up for 10ml with distilled water

↓

Measure absorbance at 540nm

TEST: FOR 50/100/200/300/400/500 mcg/ml

Add 0.5ml of plant extract (Blepharis maderaspatensis)

↓

Add 0.5ml of α-amylase solution

↓

Add 0.5ml of phosphate buffer solution

↓

Incubate at 37˚C for 10minutes

↓

Add 0.5ml of starch solution

↓

Incubate at 37˚C for 10 minutes

↓

Add 1ml of DNS reagent

↓

Boil at 100˚C for 5 minutes

↓

Make up for 10ml with distilled water

↓

Measure absorbance at 540nm

UV-VISIBLE SPECTROPHOTO METER

RESULT

ALPHA-AMYLASE INHIBITION ASSAY REPORT

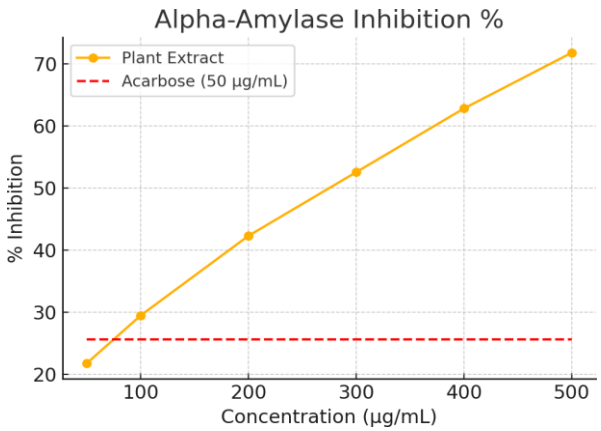
Table: Inhibition of Alpha-Amylase by Acarbose and Plant Extract.

SAMPLE	CONCENTRATION (µg/ml)	ABSORBANCE (540nm)	% INHIBITION
Control	----------	0.78	0%
Acarbose	50	0.58	25.64%
Plant extract	50	0.61	21.7%
Plant extract	100	0.55	29.4%
Plant extract	200	0.45	42.3%
Plant extract	300	0.37	52.5%
Plant extract	400	0.29	62.8%
Plant extract	500	0.22	71.7%

Observation

- Acarbose at 50µg/mL showed 25.64% inhibition, validating the assay.
- The plant extract showed dose-dependent inhibition, reaching 71.79% at 500µg/mL.
- This suggests that the extract contains active phytochemicals capable of inhibiting alpha-amylase, potentially beneficial for managing postprandial hyperglycemia

Graph



% of inhibition = Absorbance of control – Absorbance of test X 100

Absorbance of control

DISCUSSION

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostatis. Lack of insulin affects carbohydrates, fats and protein metabolism. Management of diabetes without side effects is still Challenge to the medical community. It was proposed that inhibition of the activity of

such alpha-amylase Would delay the degradation of carbohydrates, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. In the present study, research has been carried out to evaluate the preliminary Phytochemical investigation and the potential of ethanol extract of Blepharis maderaspatensis leaf in Inhibiting alpha-amylase.

The present finding of phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like quercitol, quercitrin, Tropane, quercetin, indole, 9-eicosyne, caffeic acid, kaemperol, myricetin, L-rhamnose, camphor, purine, cholestan-3-ol, diethyl phthalate, palmitic acid Which could be responsible for the versatile medicinal properties of this plant.

The present finding reveals that Blepharis maderaspatensis efficiently inhibits alpha-amylase enzymes Invitro in a dose dependent manner. The ethanolic extract of Blepharis maderaspatensis showed a dose Dependent inhibitory effect on alpha-amylase activity. The anti-diabetic action of Blepharis maderaspatensis can also be attributed to the intestinal alpha-amylase inhibitory activity.

Further studies are required to elucidate whether Blepharis maderaspatensis have anti-diabetic Potential by in-vivo for validating the traditional claim of the plant.

In this present study we evaluated in-vitro alpha-amylase activity of crude ethanol extract of Blepharis maderaspatensis leaves.

CONCLUSION

The anti-diabetic properties of plants can be evaluated in-vitro by several methods such as study of glucose uptake, effect on glycosylation of the haemoglobin and inhibition of alpha amylase enzymes. The mechanism of glucose transport across the yeast cell membrane has been gaining significant importance as an in-vitro screening method for evaluating the hypoglycemic effects of various medicinal plants .The above conducted in vitro studies depict an appreciable increase in the glucose uptake by the yeast cells in combination with the plant extracts. It was observed that the plant extracts inhibited glycosylation of hemoglobin and thereby helps in the inhibition of the formation of glycated end products. We can therefore conclude from this study that the presence of the phytochemicals in these plants might be the reason for these inhibitions and that the plants may essentially contain herbal

bioactive compounds which require further structural elucidation and characterization methodologies to identify the bioactive constituents. The plant extracts understudy can serve as therapeutic agents and can be used as potential sources of novel bioactive compounds for treating Diabetes mellitus type 2.

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