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FORMULATION AND EVALUATION OF ANTI DIABETIC SYRUP BY USING JAMUN SEED, GILOY STEM AND BANNANA STEM WATER

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, posing a significant public health concern globally. Traditional medicine systems, particularly Ayurveda, offer potential remedies derived from natural sources. This study aimed to develop an antidiabetic syrup utilizing the synergistic properties of Jamun (*Syzygium cumini*) seed, Giloy (*Tinospora cordifolia*) stem, and Banana (Musa spp.) stem water. The method involved extraction of bioactive compounds from Jamun seeds and Giloy stems through drying for a certain period of time then grinded into powder. Banana stem water was obtained through simple pressing and filtration techniques. The extracts were then blended in appropriate ratios and subjected to physicochemical characterization, including pH, viscosity, and density measurements. Then syrup was gone through analysis the nutrients content like carbohydrates, protein, vitamins, minerals and also identified the bioactive compounds by thin layer pharmacological evaluations were conducted to assess the antidiabetic potential of the syrup formulation. In vitro assays, such as alphaamylase and alpha-glucosidase inhibition assays, pancreatic lipase inhibition assay, glucose uptake assay was performed to evaluate the inhibitory effects on key enzymes involved in carbohydrate metabolism. Furthermore, in vivo studies utilizing diabetic animal models were conducted to assess the efficacy and safety profile of the syrup.

Keywords: Herbal anti-diabetics syrup, Jamun seeds, Giloy stem, Banana stem water

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INTRODUCTION

Herbal drugs play an important role in the development of influential therapeutic agents. Furthermore, it has proven their potential for the obstacle of several ailments. Earlier human beings started their studies on diseases and its treatments, but there was no evidence found that people have prehistoric use of artificial means synthetic medicines for their sickness. Plant medicines are readily used in combination rather than in a single form to acquire maximum benefit from their combined potential to reduce side effects of one another (Maya and Dipak, 2022).

DM develops due to obesity which is also an rising problem worldwide, induces atherosclerosis and other metabolic syndromes like heart diseases, stroke and other health related problems. According to the provisions of insulin DM was classified into two main categories; insulin dependent diabetes mellitus (Type 1), and non-insulin dependent diabetes mellitus (Type 2). Research desires in the field of herbal medicines are huge; the identification of active compounds from the plants source is still remaining a challenge. So, there should be research-based authentication on either whole herbs or on extracted compounds are found to be superior. The issue of herb–herb and herb–drug interactions is also an important, which requires increased awareness and study, as in polypharmacy and polyherbacy. The new technologies, such as nanotechnology and novel emulsification methods are used in the formulation of herbal products, which mainly affect bioavailability as well as efficacy of herbal components and this also needs to study. This can lead to reinvestigation of some agents that failed in earlier trials and can be restudied and redesigned using new technologies to finish whether they can be customized for better efficacy and fewer side effects. Today, there is a vital need to develop safer drugs for the treatment of various disorders. As a result, there is an emergent interest in the pharmacological evaluation of various plants used in traditional systems of medicine (Sahu *et al.*, 2018).

Polyherbal formulations have been prepared and found to have antidiabetic activity. A combination of two or more plants gives better therapeutic effect as compared to a single plant. Hence antidiabetic plants were used to prepare a polyherbal formulation as suspension which is easy, rapid and economical to be formulated (Maya and Dipak, 2022).

Herbal medicine is also known as phyto-medicine or herbalism it is a medicine that use plants or their crude products for the treatment of diseases. It may include also animal fungi or bacteria product. Since ancient era, herbal or plant-based medicines has been used for the prevention, cure & mitigation of diseases and time to time more and more herbal constituents of these natural sources are get enhanced. Herbal medicine has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing. Some herbs have potent (powerful) ingredients and should be taken with the same level of caution as pharmaceutical medications (Mohammed, 2007). In fact, many pharmaceutical medications are based on man-made versions of naturally occurring compounds found in plants. For instance, the heart medicine digitalis was derived from the foxglove plant. Herbal medicine aims to return the body to a state of natural balance so that it can heal itself. Different herbs act on different systems of the body (Mohammed, 2007).

Herbal formulation a most commonly used a development as well as developing countries as health care. The cough syrup medication is a liquid dosage form use of oral liquid pharmaceutical has been confirm on basic ease of administration to those people who have the problem in the swallowing of solid dosage from medication. Syrup is a concentrated solution contains sugar and purified water. In syrup from the other type of syrup solutions. The syrup may be or may not be containing medication or mixed flavoring agent. When the syrup without a medication but the flavoring agent present are known as flavored or nonmedicated syrup (Butler et al., 1943). Flavored syrup are frequently used as vehicle for the unpleasant test of medications results (found as) is medicated syrups (Jadhao *et al.*, 2021).

Syrup are present in syrup in high amount predisposes then to the bacteria infection so they often. Use as preservative (Kaushik *et al.*, 2016). Syrup are very prominent delivery vehicle use for the anti tissue medication because they give a more soothing to swallow (ingest)then the tablet and capsule. This medication is quickly observed. There are same available synthetic cough preparations they cause several adverse effect. So the present study was show to enlarge and in violet herbal cough syrup carry natural element having no any side effect (Akula *et al.*, 2017) in general health professionals having difficulties of accessing effectiveness

and safety natural treatment (therapy). Number of instance allopathic medication product has not been studied in large scale and generally they solid without in knowledge of there mechanism of action or side effect. Even so the use of complementary medication is sometime helpful and the confirmation is same time helpful and the confirmation the effectiveness of some this all medication literature is limited, they frequently sold with the drug store (Mujawa *et al.,* 2016).

A successful formulation of liquid , as well as other dosage forms , requires a blend of scientificacuity and pharmaceutical "art" (Lachman *et al.*, 1987). Oral liquid medicines are being superseded gradually by tablets and capsule because of deleterious changes take place more readily in solution (Carte, 2000). Nevertheless there are still a large number of liquid oral preparations are available in the official books. The fact is that the absorption of medicaments in solution from the GI tract into the systemic circulation may be expected to occur more rapidly than other oral dosage forms of the same medicinal agent8 .Ayurvedicformulations are preferentially administered by oral route (Ansel and Allen, 2000), and most of the orally administered Ayurvedic formulations belong to liquid form of drug or drug combination. However herbal medicinal combination (Stability Testing for new Dosage forms QIC, 1996).

Although allopathy has been the most acceptable system of medicine over the years, people are now shifting back to the utilization of herbal medicine. This is due to the setbacks of allopathic medicine like it is very expensive, it has serious and frustrating side effects, its relief from ailments is only symptomatic and fear of toxicity to allopathy drugs. Herbal medicine like Ayurveda and Homeopathy are preferred in the treatment of chronic diseases because of the characteristic features of Ayurveda like it is less costly and more sensible, exactly aligns with the patient's thoughts, more easily accessible, time tested, it's said to be more natural and safer and it is thought to have fewer or no negative effects (Priya *et al.*, 2019).

The aim of the present study to preparation of anti-diabetic syrup from mixture of Jamun seed, Guduchi and Banana stem water, and its evaluation of anti-diabetic activity.

MATERIALS AND METHODS

Collection of raw materials and preparation of anti-diabetic syrup

The Jamun seed and Guduchi powder were purchased in February 2024 from Siddha medical Shops in Thanjavur, and Banana stem water collected from home garden from Thanjavur, Thanjavur district, Tamil Nadu, India.

Table 1: Preparation of anti-diabetic syrup

S. No	Materials	Quantity
1	Jamun seed powder	10 gm
2	Guduchi powder	10 gm
3	Banana stem water	300 ml
4	Barley extract	100ml
5	Sugar free sugar	0.75 packet

Take 10 gms of Jamun seed powder and 10gms of Guduch powder, then mix it with 300ml of Banana stem water. Boil the mixture until total volume becomes one fourth of intial volume and then cool the decoction and filter with filter press. Use the filtrate to prepare final herbal syrup. Mix Sugar free sugar in required quantity prepare a concentrated solution of syrup. Filtrate was taken and added to simple syrup to yield final syrup. For the preparation of final herbal syrup, mix 3 part of syrup with 1 parts of Barley extract (3:1), then finally make up the value to 100ml (Raju *et al.*, 2020).

Physical properties of prepared anti-diabetic syrup

Physicochemical parameters were performed according to the method described in WHO guidelines (WHO, 1998).

Proximate analysis

Crude fibre content was determined by following the method of Sadasivam and Manikam (1992). Dry Ashing (Ranganna, 1986). Protein was estimated by the method of Lowry *et al.* (1951). Total lipids in tissues were estimated by the method of Folch *et al* (1957). To estimate the amount of carbohydrate present in the given sample by using Anthrone method. Amino acid in tissues were estimated by the method of Rosen *et al.*, (1959).

Determination of Energy Value Sample

The energy value of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 (AOAC, 1990).

Energy Value = (Crude protein × 4) + (Total carbohydrate × 4) + (Crude fat × 9) Kcal/100 g

Qualitative analysis of Inorganic elements and vitamins

Sample (2gm) was prepared and treated with HNO_3 and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the following tests (Khandelwal, 2006). Qualitative analysis of vitamins was followed by the methods by Pearson (1976) and Patel (2005).

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

***In vitro* anti-diabetic activity**

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis *et al.,* (2007). The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis *et al*., (2007). Yeast cells were prepared according to the method of Gupta daksha *et al*., (2013).

Pancreatic lipase inhibitory activity

Antilipase activity carried out by Shivanna *et al.,* (2017).

***In silico* Molecular docking**

Computational drug discovery technique in the recent day of Pharmaceutical research has successfully molecular modeling with different algorithm based programing software's been used. The ligand and protein binding scores according to algorithm based program thereby may use any software for protein and ligand interactions for best results (Velavan *et al.,* 2020).

The ligands are Gallic acid was obtained from Pubchem database, ligands were converted in to PDB format using Open bable software and Protein obtained from PDB database. The anti-diabetic target Human pancreatic alpha-amylase (PDB ID: 2qv4) preparation was generally to have a remove of all water molecules and any other Ligand molecules prior to docking, using Pymol software prepared protein was saved as PDB formed. The grid map was centered at particular residues of the protein and was generated with grid dimension prepared. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters (Ghose and Crippen, 1987; Binkowski *et al*., 2003; Vidya *et al*., 2012). Complex structures were modeled using modeling software's Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for biocomputing visualization and informatics, NIH, CA, USA) and 2D pose viewed using Discovery Studio Visualizer (Trot and Olson, 2010).

RESULTS AND DISCUSSION

The prevalence of diabetes in the world was estimated to be 2.8% for all ages in 2000, and that is expected to increase to approximate 4.4% in 2030 (WHO, 2016). Diabetes causes of death will increase to 366 million by 2030 (Suganthi, 2018). The World Health Organization (WHO) estimates that 415 million people will be affected by diabetes in 2015 (IDF, 2015), and this is expected to rise to 642 million by 2040, worldwide (Kerru *et al.,* 2018). Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) (Bilal *et al.,* 2018). It is caused by the deficiency or ineffective production of insulin by pancreas which result in increase or decrease in concentration of glucose in the blood (Ellappan *et al.,* 2013; Choo *et al.,* 2012). Due to various drawbacks of synthetic antidiabetic drugs there is a continuous search for alternative therapy in diabetes. Many herbal syrup with hypoglycemic properties are known for a long time and they are used traditionally in India.

In the present study, the anti-diabetic syrup prepared from Jamun seed, Guduch and Banana stem water and evaluated the anti-diabetic potential.

Preparation of anti-diabetic syrup from Jamun seed, Guduch and Banana stem water

Method of preparation of decoction

Take 10 gms of Jamun seed powder and 10gms of Guduch powder, then mix it with 300ml of Banana stem water. Boil the mixture until total volume becomes one fourth of initial volume and then cool the decoction and filter with filter press. Use the filtrate to prepare final herbal syrup.

Method of preparation of syrup

Mix Sugar free sugar in required quantity prepare a concentrated solution of syrup

Method of preparation of herbal syrup

Filtrate was taken and added to simple syrup to yield final syrup. For the preparation of final herbal syrup, mix 3 part of syrup with 1 parts of Barley extract (3:1), then finally make up the value to 100ml.

The prepared herbal syrup was further evaluated in terms of organoleptic

characteristics, physical characterization, proximate composition, bioactive compounds, and *in vitro* anti-diabetic activity. The Organoleptic and Physical Characters were observed and represent in table 2 and 3. Carbohydrates, proteins, and amino acids are the principal natural products acquired from plants, animals and microorganisms. In this present study the carbohydrate, protein and lipids amino acid content was investigated in herbal anti-diabetic syrup. The herbal anti-diabetic syrup exhibited fiber (1.34%), protein (31.43%), ash (0.21%), carbohydrate (5.81%), fat (0.82%) and energy (156.34 Kcal/100ml) respectively (Table 4).

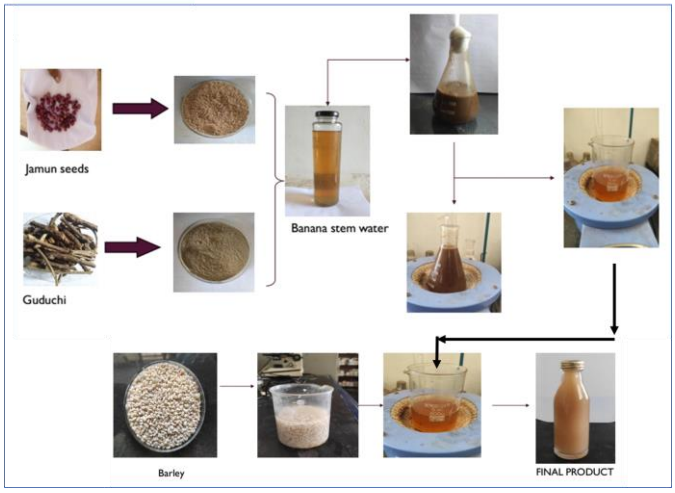


Plate 1: Preparation of herbal anti-diabetic syrup

Table 2: Organoleptic Characters of herbal anti-diabetic syrup

Characters	Results
Colour	Ash brown
Odor	Aromatic
Taste	Sweet
Appearance	Liquid

Table 3: Physical characterization of herbal anti-diabetic syrup

Characters	Results
pH	7
Viscosity	3.413 cp
Density	0.96
Specific gravity	1.04
Solubility Test	Partially soluble

Table 4: Proximate composition of herbal anti-diabetic syrup

S. No	Analysis	Syrup
1.	**Fiber (%)**	1.34
2.	**Protein (%)**	31.43
3.	**Fat (%)**	0.82
4.	**Total Ash (%)**	0.21
5.	**Carbohydrates (%)**	5.81
6.	**Energy (Kcal/100 ml)**	156.34
7.	**Amino acid (µg/ml)**	67.31

Screening of Bioactive compounds from herbal anti-diabetic syrup

In the present study was carried out on the herbal anti-diabetic syrup revealed the presence of bioactive constituents. The phytochemical screening of herbal anti-diabetic syrup showed that the presence of tannin, saponin, flavonoids, terpenoids, alkaloids, steroids, polyphenol, anthroquinone, coumarins and glycosides (Table 5). Secondary metabolites are reported to have many biological and therapeutic properties. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al*., 2012). Nutritional minerals and vitamins are qualities of herbal anti-diabetic syrup was summarized table 6 and 7.

Table 5: Qualitative phytochemical analysis of herbal anti-diabetic syrup

S. No	Phytochemicals	Aqueous extract
1	Tannin	+
2	Saponin	++
3	Flavonoids	++
4	Steroids	++
5	Terpenoids	+
6	Alkaloids	+
7	Antroquinone	+
8	Polyphenol	++
9	Glycosides	++
10	Coumarins	++

(+) Presence, (++) High concentrations and (–) Absences

Table 6: Qualitative analysis of minerals in herbal anti-diabetic syrup

S. No	Inorganic elements	Herbal anti-diabetic syrup
1	Calcium	++
2	Magnesium	+
3	Sodium	+
4	Potassium	++
5	Iron	++
6	Sulphate	+
7	Phosphate	++
8	Chloride	+
9	Nitrate	+

(+) Presence, (++) High concentration and (-) Absence

Table 7: Qualitative analysis of vitamins in herbal anti-diabetic syrup

Vitamins	Herbal anti-diabetic syrup
Vitamin A	-
Vitamin C	+
Vitamin D	-
Vitamin E	++

(+) Presence, (++) High concentration and (-) Absence

Identification of polyphenolic using thin layer chromatography (TLC) from herbal anti-diabetic syrup

TLC plate showed the presence of gallic acid as compared with the standard as gallic acid (GA), herbal anti-diabetic syrup contain gallic acid compound. Gallic acid as a potential anti-diabetic agent for DM and its complications reported by Zu *et al*., (2021).

Table 8: TLC analysis of herbal anti-diabetic syrup

Sample	Rf
Herbal anti-diabetic syrup	0.86
Gallic acid (GA)	0.88
Quercetin (Q)	0.92
Rutin (R)	0.91
Tannic acid (TA)	0.79

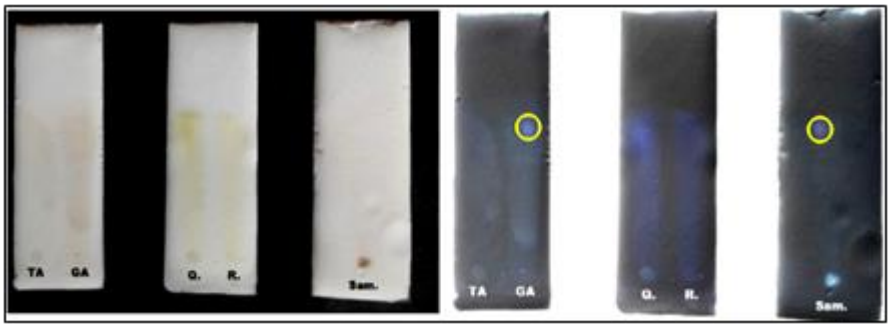


Plate 2: Identification of Gallic acid using thin layer chromatography (TLC) from herbal anti-diabetic syrup

Overall herbal anti-diabetic syrup was rich source secondary metabolites like phenols and flavonoids while vitamins and minerals were qualified using phytochemical techniques. Secondary metabolites and vitamins has a good potential anti-oxidant agent, further confirmation of gallic acid compound was present, playing impartment role of anti-diabetic activity.

Self-life analysis of herbal anti-diabetic syrup, through total viable bacterial colony count (TVC)

A viable cell is defined as a cell which is able to divide and form a population (or colony). A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions onto an appropriate culture medium, then incubating the plates under proper conditions so that colonies are formed. After incubation, the colonies are counted and, from a knowledge of the dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers.

The herbal anti-diabetic syrup bacteria was quantified by calculating Colony Forming Unit (C.F.U), numbers of bacterial colonies were observed in 264 homologues

colonies per ml and control was nil. The present homologue colonies revealed may be beneficial bacterial, which results may be probiotic.

In vitro Antidiabetic activity of herbal anti-diabetic syrup

A comparison of α-amylase, α-glucosidase, lipase and glucose uptake by yeast cell and inhibitory activity between the standard drugs has been depicted. Our results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit pancreatic α-amylase (Sincy Joseph *et al.*, 2016). The isolated compounds were tested for their antidiabetic potential in vitro by inhibition of α-amylase enzyme. Phenol, flavonoids, saponins and terpenoids showed higher alpha amylase inhibitory activity which confirms its antidiabetic potential was reported (Mai *et al.*, 2007). The plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase. The inhibition of this enzyme delay carbohydrate digestion and protract overall carbohydrate digestion time, resulting in the reduction in glucose absorption rate and consequently dulling the postprandial plasma glucose rise.

In this study *in vitro* α-amylase, α-glucosidase and lipase inhibitory activities of the herbal anti-diabetic syrup were investigated. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α-amylase, α-glucosidase and lipase inhibitory enzyme. Acarbose is a standard drug for α-amylase and α-glucosidase inhibitor, while Orlistat is a standard drug was lipase inhibitor. On the basis of the amylase inhibition activity showed the anti-diabetic activity of the herbal anti-diabetic syrup is evidenced (Figure 1 to 2).

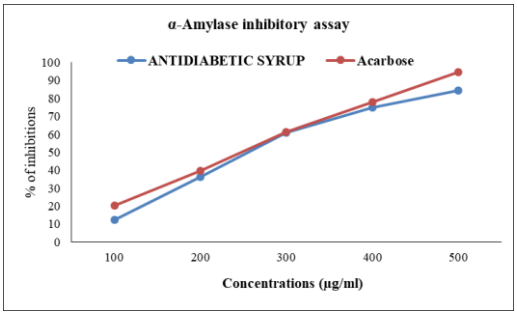


Figure 1: *In vitro* Alpha-amylase inhibitory activity of herbal anti-diabetic syrup

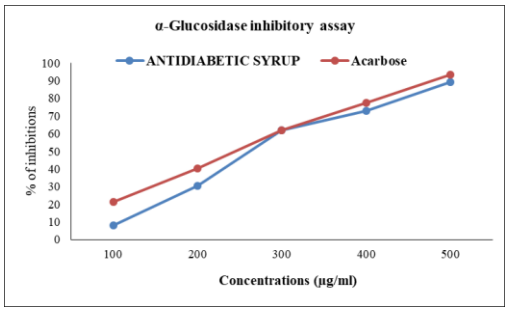


Figure 2: *In vitro* Alpha-glucosidase inhibitory activity of herbal anti-diabetic syrup

This inhibition involve pancreatic lipase enzyme, the principle lipolytic enzyme synthesized and secreted by the pancreas. Pancreatic lipase is important enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerol to monoacylglycerol and fatty acid. Herbal anti-diabetic syrup significantly inhibited lipase with low concentration was 8.23 % of inhibition while the higher concentration was 62.35 % of inhibitions as compared about orlistat as standard drug, represent in Figure 3.

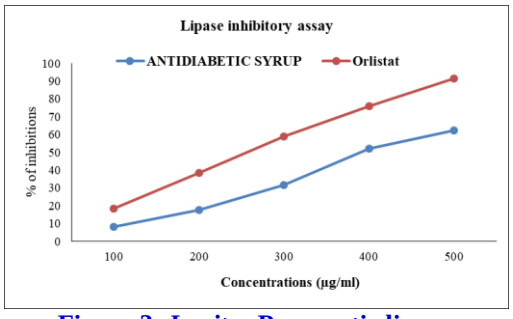


Figure 3: *In vitro* Pancreatic lipase inhibitory activity of herbal anti-diabetic syrup

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycaemic effect of various compounds medicinal plants. It is stated that transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized). The data obtained clearly suggests that the plant extract is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing effective glucose utilization thereby controlling blood glucose level (Ahmed et al., 2009). The herbal anti-diabetic syrup showed

inhibitory activity from 19.82 to 90.22 % of inhibitions at concentration 100 and 500µg/ml respectively (Figure 4). Metformin at a concentration of (100-500 µg/ml) showed glucose uptake activity from 21.43 to 96.19 % of inhibitions at the same concentrations 100 and 500µg/ml respectively.

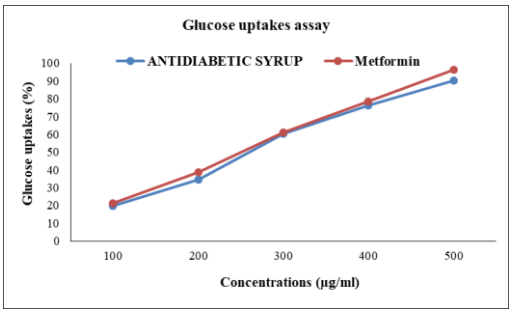
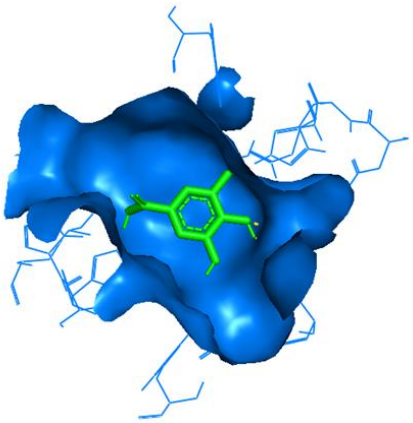


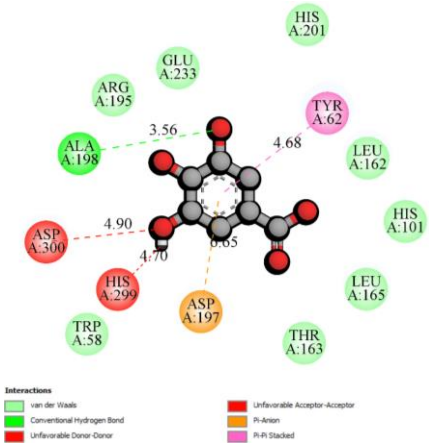
Figure 4: *In vitro* glucose uptake activity of herbal anti-diabetic syrup

In silico anti-diabetic activity

The α–amylase is one of the major secretory products of the pancreas and salivary glands and plays a pivotal role in the digestion of starch and glycogen. α-amylase catalyzes the hydrolysis of starch via a double displacement mechanism involving the formation and hydrolysis of a covalent β-glycosyl enzyme intermediate using active site carboxylic acids for it (Brayer et al. 2000). Gallic acid were subjected to *in silico* docking studies using Auto dock vina software and the human pancreatic alpha-amylase (PDB ID: 2qv4). The docking results has shown that the binding strength of Gallic acid binding affinity -6.40 kcal/mol. This indicates that Gallic acid can act as a better inhibitor of the anti-diabetic target human pancreatic alpha-amylase (Plate 3). Zu *et al*., (2021) reported that Gallic acid as a potential anti-diabetic agent for DM and its complications.



3D view of Gallic acid binding with target



2D view of Gallic acid interaction with target

Plate 3: *In silico* anti-diabetic activity of Gallic acid identified from anti-diabetic syrup, against Human pancreatic alpha-amylase (PDB ID: 2qv4)

CONCLUSION

The present study concluded that syrup prepared from Jamun seed, Guduch and Banana stem water is required in order to generate better, safer, and more cost-effective anti-diabetic syrup to treat Diabetes. The *in vitro* anti-diabetic activity confirmed through inhibition of alpha amylase and glucosidase enzymes which increased the blood glucose level. *In silico* study also further supported the *in vitro* anti-diabetic activity. This study shows that the active compounds from Jamun seed, Guduch and Banana stem water have Phenolic compound as gallic acid which create good anti-diabetes agent.

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