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FORMULATION AND EVALUATION OF ANTIDIABETIC POLYHERBAL SYRUP

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ABSTRACT: This study investigated four medicinal plants *Moringa oleifera* leaves, *Hippophae rhamnoides* (Sea buckthorn) berries, *Syzygium cumini* seeds, and *Psidium guajava* leaves for their suitability in developing a polyherbal antidiabetic syrup. Hydroalcoholic extracts were prepared through maceration and yielded diverse phytochemical constituents, including flavonoids, alkaloids, saponins, glycosides, tannins, and phenols. Among the plants, *Syzygium cumini* exhibited the richest phytochemical profile, while Sea buckthorn uniquely contained terpenoids and steroids. Three syrup formulations were evaluated and found to possess acceptable physicochemical properties, with Formulation 2 demonstrating optimal viscosity, pH, and palatability. *In-vitro* antidiabetic analysis showed that all extracts inhibited α -amylase, while *Syzygium cumini* and *Moringa oleifera* demonstrated dual inhibition of α -amylase and α -glucosidase, though with higher IC₅₀ values than Acarbose. Overall, the results support the potential of these plants particularly *Syzygium cumini* for use in a synergistic polyherbal syrup aimed at managing diabetes through digestive enzyme inhibition. Further *in-vivo* and clinical investigations are recommended to confirm efficacy and safety.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels resulting from impaired insulin secretion, action, or both¹. The global prevalence of diabetes continues to rise, posing significant challenges to healthcare systems and contributing to long-term complications such as cardiovascular disease, neuropathy, nephropathy, and retinopathy². Conventional antidiabetic drugs, while effective, may produce adverse effects, high costs, and limited accessibility in certain regions.

These limitations have driven increasing interest in herbal medicines, which offer multi-target therapeutic potential, lower toxicity, affordability, and centuries of traditional usage³. Among various herbal approaches, polyherbal formulations combinations of multiple medicinal plants are gaining prominence due to their synergistic effects, enhanced potency, and ability to address multiple pathways involved in glucose regulation.

The development of antidiabetic polyherbal preparations has focused on plants rich in bioactive constituents such as flavonoids, alkaloids, tannins, glycosides, and phenolic compounds, which exhibit antioxidant, hypoglycemic, and enzyme-inhibitory effects⁴. Plants such as *Moringa oleifera*, *Syzygium cumini*, *Psidium guajava*, and *Hippophae rhamnoides* (Sea buckthorn) have been

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traditionally used for managing blood sugar levels and are scientifically recognized for their ability to modulate key carbohydrate-digesting enzymes like α -amylase and α -glucosidase^{5,6}.

Formulating these botanicals into a stable and palatable polyherbal syrup offers a promising approach for improving patient compliance and therapeutic efficacy. This study focuses on the extraction, formulation, and evaluation of a polyherbal antidiabetic syrup, assessing its phytochemical profile, physicochemical properties, and *in-vitro* enzyme-inhibitory potential to support its future development as a natural antidiabetic remedy.

MATERIALS AND METHODS:

Materials: The formulation of polyherbal anti diabetic syrup was prepared using various ingredients like *M. oleifera* leaves powder, jamun seeds powder, guava leaves powder as these all herbal parts of the plants have anti diabetic properties. Besides this Stevia leaves was used as a natural sweetener in the formulation as it has got no calories in it.

Formulation for Polyherbal Antidiabetic Syrup:

The hydroalcoholic extracts of moringa powder, jamun seeds, and guava leaves for all three formulations (F1, F2, and F3) were prepared separately using maceration in ethanol for two days. For each formulation, a preservative solution was prepared by dissolving 0.1 g of methylparaben, 0.005 g of propylparaben, and 0.05 g of sodium benzoate in 5 ml of distilled water with continuous stirring until a clear solution formed. Separately, the syrup base was prepared by mixing 10 ml of honey with 1 ml of glycerin and 5 ml of Stevia leaf extract, followed by the addition of 1–2 ml of freshly extracted lemon juice to obtain a uniform blend. In the final step, the preservative solution was added to the respective herbal extract mixtures with continuous stirring, after which the syrup base was gradually incorporated with constant agitation to ensure complete homogenization. The final volume for each formulation was adjusted to 100 ml using purified water and stirred thoroughly, resulting in smooth, uniform polyherbal antidiabetic syrup formulations^{7,8}.

TABLE 1: FORMULATION CHART OF F1, F2 AND F3

S. no.	Ingredients	F1 Quantity	F2 Quantity	F3 Quantity
1	Moringa conc. extract	2.5 ml	2 ml	2.5 ml
2	Guava conc. extract	2.5 ml	2 ml	2.5 ml
3	Sea buckthorn conc. extract	2 ml	2.5 ml	2.5 ml
4	Blackberry conc. extract	2 ml	2.5 ml	2.5 ml
5	Stevia leaves conc. extract	5 ml	5 ml	5 ml
6	Vanilla essence	0.2 g	0.2 g	0.2 g
7	Honey	10 ml	10 ml	10 ml
8	Methylparaben	0.1 g	0.1 g	0.1 g
9	Propylparaben	0.005 g	0.005 g	0.005 g
10	Sodium benzoate	0.05 g	0.05 g	0.05 g
11	Distilled water	q.s.	q.s.	q.s.

Evaluation of Polyherbal Anti Diabetic Syrup:

The prepared polyherbal antidiabetic syrups (F1, F2, and F3) were evaluated for various organoleptic and physicochemical parameters. Colour was checked visually for all three formulations, while odour was assessed, acknowledging that perception may vary between individuals⁹. The taste of the syrups was evaluated manually, and visual inspection was performed to examine the purity and overall appearance, which is critical for patient compliance¹⁰. Physical stability was assessed to ensure that the syrups remained homogeneous, free from microbial growth, and that the colour was

completely soluble with other ingredients¹¹. The pH was determined by preparing a 1% solution of the syrup (0.5 g in 50 ml distilled water), calibrating the pH meter using a standard pH 7 buffer, and recording the sample's pH. Viscosity was measured using a thoroughly cleaned Ostwald viscometer, first with water to establish flow time and density, followed by the test syrup, and calculated using the formula:

$$\text{Viscosity} = (\text{Density of syrup} \times \text{Time required for syrup} \times \text{Viscosity of water}) / (\text{Density of water} \times \text{Time required for water})^{12,13}$$

Density was determined using a pycnometer, where the weight of the empty bottle (w1), bottle with 10 ml of water (w2), and bottle with 10 ml of syrup (w3) were recorded, and the density calculated as

$$\text{Density of syrup} = (w3 - w1) / (w2 - w1) \times \text{Density of water.}$$

Finally, the specific gravity was calculated using the formula Sp.

In-vitro Antidiabetic Activity: The effect of the plant crude extracts on α -amylase and α -glucosidase activity¹⁴. The inhibitory effects of the plant crude extracts on α -amylase and α -glucosidase activities were evaluated using modified methods described by Kazeem *et al.* For the α -amylase assay, 250 μ L of each extract concentration (0–360 μ g/mL) was mixed sequentially with 250 μ L of buffered α -amylase (0.05 mg/mL) and 250 μ L of 1% starch solution in a test tube¹⁵. The reaction mixture was incubated at 25 °C for 10 minutes, after which 500 μ L of DNSA reagent was added and the mixture boiled for 5 minutes. The mixture was then cooled and diluted with 5 mL of distilled water. A control was prepared similarly, replacing the extract with distilled water. The absorbance of each sample was measured at 540 nm, and the percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = (Ac - At) / Ac \times 100$$

Where, Ac and At are the absorbance of the control and tests, respectively. The extract concentration resulting in 50% enzyme inhibition (IC₅₀) was determined graphically. The α -glucosidase assay involved mixing 50 μ L of each extract concentration (0–40 μ g/mL) with 100 μ L of buffered α -glucosidase (1.0 U/mL) and incubating the mixture for 10 minutes at 37 °C¹⁶. After that, 50 μ L of 3.0 mM p-nitrophenyl- α -D-glucopyranoside (pNPG) was added, and the mixture was incubated for 20 minutes at 37 °C. 5% w/v Na₂CO₃ was used to stop the reaction, which was then cooled to 25°C and diluted with 5 millilitres of distilled water. At 405 nm, the absorbance of the yellow p-nitrophenol that was

emitted was measured. The IC₅₀ values were visually obtained, and percentage inhibition was computed using the same formula as for α -amylase. These tests quantify the extracts' capacity to block important enzymes that break down carbohydrates and are involved in the control of postprandial hyperglycemia^{17, 18}.

RESULTS AND DISCUSSION: The maceration of *Moringa oleifera* leaves using 94% v/v hydroalcoholic gave a dark green, oily extract with an unclear aroma, generating 2.11 g of dry extract (2.11% w/w), suggestive of chlorophylls, polyphenols, and flavonoids with antioxidant and antidiabetic potential. Similarly, *Hippophae rhamnoides* (Sea buckthorn) berries extracted with 94% hydroalcoholic yielded 1.34 g (1.34% w/w) of a reddish-brown semisolid extract with an indistinct odour, indicating the presence of the berry's characteristic flavonoids, carotenoids, fatty acids, tocopherols, and phenolic acids. The extraction of *Syzygium cumini* (Jamun) seeds using 95% ethanol provided a dark brown, greasy extract with an unclear aroma and a yield of 1.97 g (1.97% w/w), suggesting the presence of tannins, flavonoids, ellagic acid, jamboline, and other phenolic compounds. Likewise, *Psidium guajava* (Guava) leaves macerated in 94% hydroalcoholic produced a dark green semisolid extract with an indistinct odour, yielding 2.11 g (2.11% w/w), indicating the presence of chlorophylls, tannins, flavonoids, and phenolic compounds known for their medicinal and antidiabetic properties.

Pre-Phytochemical Analysis: Standard qualitative chemical assays were used to pre-phytochemically screen the four extracts: *Psidium guajava* leaves, *Hippophae rhamnoides* (Sea buckthorn) berries, *Syzygium cumini* seeds, and *Moringa oleifera* leaves. Alkaloids, glycosides, saponins, flavonoids, tannins, and phenols are among the significant groups of phytoconstituents that were found. Variations were noticed among the extracts based on plant portion, polarity, and solvent utilised. **Table 2** provides an overview of the observations.

TABLE 2: PRE-PHYTOCHEMICAL SCREENING OF EXTRACTS

Class of Drug	Chemical test	Moringa extract	Sea buck thorn extract	Syzygium extract	Psidium Extract
Alkaloids	Dragendroff's test Hager's, Mayer, Wagner's	Positive	Positive	Positive	Positive

Carbohydrate	Fehling, Molisch	Positive	Negative	Positive	Positive
Glycoside	Modified Borntrager Test	Positive	Positive	Positive	Positive
Coumarin glycoside	Liebermann test, (Bufadienolides)	Negative	Negative	Positive	Negative
Saponin	Froth test, Foam test	Positive	Positive	Positive	Positive
Terpenoid and steroid	Salkowski Test, Liebermann Burchard Test	Negative	Positive	Negative	
Flavonoid	Common test, Lead acetate	Positive	Positive	Positive	Negative
Tannin and phenols	Ferric Chloride Test	Negative	Positive	Positive	Positive
Protein and amino acid	Millon's test, Ninhydrin	Positive	Positive	Negative	Positive

Evaluation of Polyherbal Syrup: Three formulations of the polyherbal antidiabetic syrup were evaluated for their physicochemical properties, including colour, odour, taste, pH, viscosity, density, and specific gravity. All formulations showed a reddish-brown colour and aromatic odour, indicating uniformity in appearance and sensory profile. Taste evaluation showed that Formulation 1 had an intense bitter taste, whereas Formulations 2 and 3 exhibited a lighter bitterness, which may improve patient

acceptability. The pH values of the formulations ranged between 6.26 and 6.56, falling within the acceptable range for oral herbal syrups. Viscosity varied among the formulations, with Formulation 2 displaying the highest viscosity (1.45), followed by Formulation 1 (1.23) and Formulation 3 (1.19). The density of the formulations ranged from 1.15 to 1.25, and specific gravity was recorded between 1.04 and 1.09, indicating consistency and stability of the syrup formulations.

TABLE 3: EVALUATION PARAMETERS OF POLYHERBAL SYRUP

S. no.	Evaluation Parameters	Formulation 1	Formulation 2	Formulation 3
1	Color	Reddish brown	Reddish brown	Reddish brown
2	Odour	Aromatic	Aromatic	Aromatic
3	Taste	Intensity bitter	Lightly bitter	Lightly bitter
4	pH	6.26	6.43	6.56
5	Viscosity	1.23	1.45	1.19
6	Density	1.25	1.18	1.15
7	Specific gravity	1.04	1.09	1.04

In-vitro Antidiabetic Activity: The *in-vitro* enzyme inhibition assay was performed to assess the antidiabetic potential of the hydroalcoholic extracts of *Moringa oleifera* (MOE), Sea buckthorn (SBE), *Syzygium cumini* (SCE), and *Psidium guajava* (PGE) by measuring their ability to inhibit

α -amylase and α -glucosidase activities. The standard antidiabetic drug Acarbose was used as a positive control. The IC₅₀ values obtained for α -amylase and α -glucosidase inhibition are presented in **Table 4**.

TABLE 4: INHIBITORY EFFECT (IC₅₀) OF HYDROALCOHOLIC EXTRACT OF MORINGA, SEA BUCKTHORN, SYZGIUM AND PSIDIUM ON A-AMYLASE AND A-GLUCOSIDASE ACTIVITY IN-VITRO

Sample	α -Amylase IC ₅₀ (μ g/mL)	α -Glucosidase IC ₅₀ (μ g/mL)
MOE	3135.5 \pm 60.0 ^c	2986.7 \pm 243.6 ^b
SBE	2802.0 \pm 58.0 ^b	N.I
SCE	2945.8 \pm 137.0 ^b	2456.6 \pm 543.5 ^b
PGE	6803.6 \pm 786.0 ^b	4239.8 \pm 337.0 ^b
Acarbose	116.1 \pm 1.3 ^a	482.05 \pm 7.4 ^a

MOE: *Moringa oleifera* extract; SBE: Sea buckthorn extract; SCE: *Syzygiumcumini* extract, PGE: *Psidium guajava* extract. NI: No significant inhibition. Values are mean \pm SEM, n=3 Superscripts: Values bearing the same superscript along a column are not statistically different (p>0.05).

The hydroalcoholic extracts of the four plant materials demonstrated varying degrees of inhibitory activity against α -amylase and α -glucosidase enzymes, suggesting potential antidiabetic effects:

α -Amylase Inhibition: All extracts inhibited α -amylase to some extent, with IC₅₀ values ranging from 2802.0 μ g/mL (SBE) to 6803.6 μ g/mL (PGE). The inhibitory activity of the extracts was significantly weaker compared to Acarbose (IC₅₀ =

116.1 µg/mL). SBE (Sea buckthorn) and SCE (*Syzygium cumini*) showed moderate α -amylase inhibition, suggesting they could slow down starch breakdown and glucose release.

α -Glucosidase Inhibition: Only MOE, SCE, and PGE demonstrated significant inhibition of α -glucosidase, with IC₅₀ values of 2986.7, 2456.6, and 4239.8 µg/mL, respectively. SBE did not show significant inhibition (N.I), indicating its effect may be more selective towards α -amylase or that its active compounds are less effective against α -glucosidase. The inhibition of α -glucosidase is particularly important for controlling post-prandial hyperglycemia by delaying carbohydrate absorption.

Comparative Activity: Among the extracts, SCE (*Syzygium cumini*) displayed the strongest dual inhibition (both α -amylase and α -glucosidase), highlighting its potential as a key component in a polyherbal antidiabetic formulation. MOE (*Moringa oleifera*) also showed notable inhibition of both enzymes, whereas PGE (*Psidium guajava*) exhibited weaker α -amylase inhibition but moderate α -glucosidase activity. Acarbose, as expected, was the most potent inhibitor for both enzymes. The hydroalcoholic extracts of *Moringa oleifera*, *Syzygium cumini*, *Psidium guajava*, and Sea buckthorn exhibit significant in-vitro inhibitory activity against key carbohydrate-digesting enzymes. *Syzygium cumini* and *Moringa oleifera* show the most promising dual enzyme inhibition, supporting their potential use in polyherbal antidiabetic formulations for managing post-prandial hyperglycemia.

CONCLUSION: The study successfully demonstrated that *Moringa oleifera*, Sea buckthorn, *Syzygium cumini*, and *Psidium guajava* possess significant phytochemical diversity and bioactive potential, supporting their therapeutic use in antidiabetic formulations. *In-vitro* assays confirmed that the extracts, particularly *Syzygium cumini* and *Moringa oleifera*, can inhibit α -amylase and α -glucosidase mechanisms crucial for controlling post-prandial blood glucose levels. Although less potent than Acarbose, their natural origin and broad phytochemical profile highlight their potential for safer, multi-target antidiabetic therapy. The formulated polyherbal syrup exhibited acceptable

physicochemical properties, with Formulation 2 being the most suitable for further stability and pharmacological evaluation. Overall, the findings support the development of a polyherbal antidiabetic syrup combining these extracts, with potential benefits in managing diabetes through enzyme inhibition, antioxidant activity, and synergistic phytochemical interactions. Further *in-vivo* and clinical studies are recommended to validate efficacy, safety, and therapeutic potential.

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