

Formulation and Evaluation of Polyherbal antioxidant Syrup

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Abstract:

Diabetes mellitus is an habitual metabolic complaint characterized by hyperglycemia and altered metabolism of carbohydrates, lipids and proteins. It's a condition that impairs the body's capability to reuse blood glucose as a result of this increased blood glucose position occurs in our body, which causes a diabetes mellitus. The present study reveals to develop an Polyherbal anti-diabetic herbal saccharinity by using an excerpt of leaves of *Gymnema sylvestre* and dried seeds of *Syzygium cumini*. Three expressions of herbal saccharinity were formulated (F1, F2, F3). Herbal shops used in the phrasings show a potent anti-diabetic action over synthetic ones. F1, F2, F3 expressions were prepared and estimated. Evaluation parameters of these expressions were set up to be within the standard limits and *in vitro* studies were performed for discovery of anti-diabetic exertion.

KEYWORDS: Polyherbal syrup, Diabetes mellitus, *Gymnema sylvestre*, *Syzygium cumini*.

• Introduction

The phrase "Herbal and medicinal" describes a range of practices, assertions, therapies, concepts, and claims that are not recognized by the conventional medical community. A specific traditional therapy, such as herbal medicine, may ultimately be recognized as a standard medical practice, provided there is adequate scientific proof and clinical studies. Herbal medicine is gaining increasing popularity among individuals of all ages due to the current positive societal view. A common belief is that herbal remedies enable individuals to engage more actively in their health management, lessen or remove the adverse effects of synthetic medications, and achieve alignment with their cultural or philosophical values. The World Health Organization (WHO) guidelines from 1996 indicate that herbal medicine comprises active final products that contain either underground or above-ground plant parts, or a mix of both, along with plant components.

Herbal Syrup formulation

Natural Saccharinity is described as a decoction that has been produced, combined, and concentrated using honey, sugar, or occasionally alcohol. Strong herbal decoction serves as the foundation of this saccharinity, and adding sugar and honey to a decoction helps to cake and save it. Herbal shops and phrasings are used for numerous types of conditions like cough. multitudinous kinds of herbal shops are employed in cough saccharinity. Herbal drugs have been created for numerous times using constituents like pudina, tulsi, cinnamon, honey, etc. In both developed and developing nations, herbal remedies are most constantly employed in healthcare. saccharinity drug is a liquid cure form with its simplicity of administration to persons with difficulty in swallowing solid tablets of drug. saccharinity is a concentrated result that contains sugar and purified water. The saccharinity may or may not be containing drug or a mixed seasoning agent. When the saccharinity is without drug but the seasoning agent present is known as seasoned or non-medicated saccharinity. seasoned bathos are constantly used as vehicles for the unwelcome test of drug results in treated bathos.

Bathos are impregnated results of sucrose, which acts as a preservative that prevents the growth of microorganisms. saccharinity is a veritably prominent delivery vehicle used for anti-tussive drug because they give a further soothing to swallow (ingest) than the tablet and capsule. This drug is snappily observed. Some available synthetic cough medications beget several adverse goods. A successful

expression of liquid, as well as other lozenge forms requires a mix of scientific perceptivity and medicinal" art. Oral liquid drugs are being supplanted gradually by tablets and capsules because injurious changes take place more readily in result. nonetheless, there are still a large number of liquid oral medications available in the sanctioned books. The verity is that compared to indispensable oral lozenge forms of the same medicinal agent, the immersion of specifics in solution from the GI tract into the systemic circulation may be anticipated to occur more quickly. Oral administration is the preferred method of administering Ayurvedic phrasings, and the maturity of these phrasings are liquid forms of individual medicines or combinations of medicines.

• Types of herbal syrup

1. Flavouredsyrup
2. Medicatedsyrup
3. Artificialsyrup

• Advantages of herbal syrup

- Nonegativeeffects
- No Protection
- Easilyaccessible
- Simpletomodifythedosageas perthechild's weight
- Nonursingisnecessary;thus,the patientcantakeitwithoutassistance
- Productslikecoughmedicationsareadministered inliquiddosageform
- Goodpatient compliance especially paediatric patients as syrup are sweet intest

Disadvantage of herbal syrup

Solid sedimentation can occasionally give a product a foot. Suspensions must be packaged in unit dosage forms to achieve dosage accuracy. Some microbial contamination may take place if preservation is not added in accurate proportion. Also, herbal medicine has another disadvantage i.e. risk of self-dosing of herbs which is very rare. Fluctuation in storage temperature may cause crystallization of sucrose from saturated syrup

• Material and Methods

Instruments, Chemicals and reagents

Table:List of instruments and model

S.No	Instruments	Model
1.	Boilingwaterbath	Universal
2.	Magneticstirrer	Remi
3.	WeighingMachine	Shimadzu
4.	UV-VisibleSpectrophotometer	Shimadzu1700
5.	Hotairoven	Scientech
6.	ScanningElectron Microscope	HitachiHigh-Tech
7.	DigitalpHmeter	Ei
8.	Brookfieldviscometer	BrookfieldDV-II+Pro(LV)

9.	HumidityChamber	Sentwin
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S. No.	Plant name	Plant part used	Weight
1.	<i>Aeglemarmelos</i>	Leaves	200 gm
2.	<i>Moringaoleifera</i>	Drumstick	213 gm
3.	<i>Hibiscus rosa sinensis</i>	Flower	220 gm
4.	<i>Bombax ceiba</i>	Flower	200gm
			201

➤ Formulation of polyherbal syrup

We selected 2:1:1:1 ratio of *H. rose sinensis*: *M. oleifera*: *B. cieba*: *A. marmelos*(HR: MO: BC: AM) for polyherbal syrup. For preparation of polyherbal syrup, sugar base was prepared by mixing of sucrose and 50 ml of water, heated to boiling point. The liquid was strained and volume made up to 100 ml with distilled water. The preservatives were dissolved in few milliliter of boiled and cooled water and added to a sugar base. Extracts (HR: MO: BC: AM) (2:1:1:1) were dissolved in propylene glycol at 45–50°C and this glycerin and sorbitol were added. Theremaining sweetening agents were added and mixed thoroughly. Adjust the pH between 5.5 and 7.0 with, if necessary.

➤ Experimental Work

Pharmacognosticalevaluation

Ash value:

After been previously fired and weighed, approximately 5 g of the powdered portions were precisely weighed and taken individually in a silica crucible. A thin layer of powder was applied to the crucible's bottom. The powder was burned by progressively increasing the temperature until it was dull red hot and carbon-free. After cooling, the crucible was weighed. To get a constant weight, the process was repeated. The air-dried powder was used as a reference to compute the percentage of total ash

Moisture content

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 °C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the moisture content is determined

$$\text{Moisture content} = \frac{\text{Wt.ofpetridish+crudedrug}-\text{afterdryingwt.ofpetridish+sample}}{\text{Weightofcrudedrug}} \times 100$$

xtractionof plant material:

Dried and powered leaves of *A. marmelos*, drumstick powder of *M. oleifera* and flower powder of *H. rosa sinensis* and *B. ceiba* were successively defatted with petroleum ether and then placed in a thimble of soxhlet apparatus (separately). The extraction was carried out using methanol solvent system at 40-60°C temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tightcontainer Extraction yield of all extracts were calculated using the following equation below:

Preliminary Phytochemical Estimation of Extracts (Qualitative)

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of *A. marmelos*, *M. oleifera*, *H. rosasinensis* and *B. ceiba* by using standard procedure. The extracts were subjected to following tests:

Tests for carbohydrates:

- **Molisch test:** 1-2 drops of an alcoholic -naphthol solution were added to 1 mL of extract. Concentrated sulfuric acid was poured into the test tube's side. It was discovered that a purple ring appeared where two liquids merged, proving that there were carbs in the test samples.
- **Fehling's test:** Fehling's solutions A and B were added in equal amounts to 1 mL of extract, and the mixture was heated for a few minutes in a water bath. It was seen that brick red precipitate was developing.
- **Benedict's test:** A test tube containing an equal volume of extract and Benedict's reagent was heated in a water bath for 5-10 minutes. Depending on the amount of reducing sugar in the test solution, which indicated the presence of reducing sugar, the solution looks green, yellow, or red.
- **Barfoed's test:** A test tube containing one mL of extract and Barfoed's reagent was heated for two minutes on a water bath. The presence of monosaccharide is shown by the red color caused by the production of cupric oxide.

Preparation of DPPH reagent

0.1 mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol will be prepared.

Preparation of Sample/Standard

Freshly prepared 1 mg/ml methanol solution of Ascorbic acid will be used as standard. 1 mg of test sample/standard will be taken with methanol to make 1 mg/ml stock solution. Different volumes of extract/standard (20 – 100 µl) will be taken from stock solution in a set of test tubes and methanol will be added to make the volume to 1 ml. To this, 2 ml of 0.1 mM DPPH reagent will be added mixed thoroughly and absorbance will be recorded at 517 nm after 30 minutes' incubation in dark at room temperature.

Preparation of control

$$\% \text{ Inhibition} = \frac{A_{\text{of control}} - A_{\text{of sample}}}{A_{\text{of control}}} \times 100$$

Spectrophotometric Quantification of Total Phenolic Content:-

The total phenolic content of extract was determined using the Folin-Ciocalteu Assay. The extracts (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2 mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Gallic Acid Equivalent (GAE) mg/gm standard solutions were used to create calibration curves. Gallic acid was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL. The reagent Folin-Ciocalteu is susceptible to reducing agents, such as polyphenols. When they react, they turn blue. Spectrophotometric measurements were made of this blue hue.

Anti-bacterial Activity Preparation of Nutrient Agar Media

1L of distilled water was used to dissolve 28 g of nutritional media. The media's pH was measured prior to sterilization. The medium was autoclaved for 15 minutes at 121°C and 15 lbs pressure. After adding

nutritional media to plates, they were put in a laminar air flow until the agar solidified.

Evaluation parameter of Polyherbal syrup

Color and appearance: Syrup was taken in a watch glass and viewed against a white light for its color and appearance by naked eye.

Odor: The syrup was tested for its odor by smelling 2 ml of the syrup individually with an interval of 2 minutes.

Taste: A drop of syrup was placed on the tongue and examined for its taste.

pH: pH of the formulation was determined by using Digital pH meter (EI). The meter was allowed to stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper. Immerse the sensing tip of the probe in the sample and record the pH reading and Rinse the probe, blot dry and repeat step 2 on a fresh portion of sample. The two readings should agree to within the accuracy limits of the meter. The samples were analyzed in triplicate.

Density: Density is a physical parameter that provides information on the mass of a sample or body divided by its volume: in other words, how tightly a substance's molecules are packed together in space. Density is determined using a 25ml density bottle. Firstly, the weight of an empty bottle was noted. The liquid whose density is to be determined was filled in the 25ml density bottle and weighed. Then the weight of the syrup was calculated by subtracting the weight of the empty bottle from the weight of the filled bottle. The density of the syrup was calculated using the formula.

$$\text{Density} = \text{Mass/Volume}$$

Viscosity: A clean Ostwald viscometer was taken which was previously cleaned. Place the viscometer in vertical position and fill it with water up to the mark. Note the time required in seconds for the water to flow from mark A to mark B. Repeat this step for at least 3 times to get accurate reading. Now fill the viscometer with the test solution (syrup) up to mark A and note the time required for the liquid to flow to mark B. Density of the liquid can be obtained from the density determination experiment. The viscosity of the syrup was calculated.

• Result and Discussion

Processing of collected Plant material

Table: Description of plant material

S. No.	Plant name	Plant part used	Weight
1.	<i>Aegle marmelos</i>	Leaves	200 gm
2.	<i>Moringa oleifera</i>	Drumstick	213 gm
3.	<i>Hibiscus rosa sinensis</i>	Flower	220 gm
4.	<i>Bombax ceiba</i>	Flower	200 gm

Pharmacognostical evaluation

Table: Pharmacognostical evaluation of plant

Parameters	Value in percentage (% w/w)			
	<i>Bombax ceiba</i> (BC)	<i>Hibiscus rosa</i> (HR)	<i>Moringa oleifera</i> (MO)	<i>Aegle marmelos</i> (AM)

Ashvalue	4.61	7.45	7.86	9.14
Extractivevalue (Aqueous)	8.48	10.36	7.21	9.29
Extractivevalue (Alcoholic)	7.62	8.75	6.73	7.56
Moisture content	6.89	5.44	5.69	6.04

According to the given results, total ash value of *B. ceiba*, *H. rosa sinensis*, *M. oleifera* and *A. marmelos*, were found to be 4.61, 7.45, 7.86 and 9.14%, Water and alcoholic extractive value were found to be 8.48, 10.36, 7.21 and 9.29% and 7.62, 8.75, 6.73 and 7.56%. Moisture content of *B. ceiba*, *H. rosa sinensis*, *M. oleifera* and *A. marmelos* were also calculated, it was found to be 6.89, 5.44, 5.69 and 6.04%

Physical properties of plant extract

Soxhlet extraction process is used to extract active constituents from plant materials. Petroleum ether and methanol were used as solvents for extraction. Shows the various properties of plant extract.

Table: Physical properties of the plant extracts

Sr. No.	Plant name	Solvent	Colour of extract	Initial weight(gm)	Yield (gm)	% Yield
1.	<i>Aegle marmelos</i>	Petroleum Ether	Green	200	0.27	0.13
		Methanol	Dark Green	200	18.61	9.68
3.	<i>Moringa oleifera</i>	Petroleum Ether	Green	213	0.20	0.09
4.		Methanol	Brown	213	7.84	3.68
5.	<i>Hibiscus rosa sinensis</i>	Petroleum Ether	Orange	220	0.24	0.11
		Methanol	Brown	220	17.35	7.88
7.	<i>Bombax ceiba</i>	Petroleum Ether	Brown	200	0.44	0.22
		Methanol	Brown	200	8.500	2.43

Preliminary phytochemical evaluation of Extracts

Table: Preliminary phytochemical evaluation in Methanolic extracts

S.No.	Experiment	Result			
		<i>Aegle marmelos</i>	<i>Moringa oleifera</i>	<i>Hibiscus rosa sinensis</i>	<i>Bombax ceiba</i>
Test for Carbohydrates					

1.	Molisch's Test	+	+	+	+
2.	Fehling's Test	+	+	+	+
3.	Benedict's Test	+	+	+	+
4.	Bareford's Test	+	+	+	+
Test for Alkaloids					
1.	Mayer's Test	+	+	+	+
2.	Hager's Test	+	+	+	+
3.	Wagner's Test	+	+	+	+
4.	Dragendroff's Test	+	+	+	+
Test for Triterpenoids and steroids					
1.	Salkowski Test	+	+	+	+
2.	Libermann-Burchard's Test	+	+	+	+
Test for Flavonoids					
1.	Lead Acetate Test	+	+	+	+
2.	Alkaline Reagent Test	+	+	+	+
3.	Shinoda Test	+	+	+	+
Test for Tannins and Phenolic Compounds					
1.	FeCl ₃ Test	+	+	+	+
2.	Lead Acetate Test	+	+	+	+
3.	Gelatine Test	+	+	+	+
Test for Saponins					
1.	Foam Test	+	+	+	+
Test for Protein and Amino acids					
1.	Ninhydrin Test	+	+	+	+
2.	Biuret's Test	+	+	+	+
3.	Million's Test	+	+	+	+

Test for Glycosides					
1.	Legal's Test	-	-	-	+
2.	Keller Killani Test	-	+	-	+
3.	Borntrager's Test	-	-	-	+

+ = Present; - = Negative

Quantitative analysis of extracts:

In Polyherbal formulation, various phytochemical constituents were found, from which total phenolics and flavonoids in methanolic extract were estimated to be taken as parameter.

Table : Total Phenolic Content in extracts

Total Phenolic content (mg/gm equivalent to Gallic acid)				
Extracts	Methanol (BC)	Methanol (HR)	Methanol (MO)	Methanol (AM)
Absorbance Mean \pm SD	0.204	0.277	0.346	0.359
TPC	45.75	64.00	81.25	84.5

Table: Total Flavonoid Content in extracts

Total Flavonoid content (mg/gm equivalent to Rutin)				
Extracts	Methanol (BC)	Methanol (HR)	Methanol (MO)	Methanol (AM)
Absorbance Mean \pm SD	0.080	0.106	0.133	0.146
TPC	38.5	52	65	69.5

Formulation of Syrup

Public health issues include the lack of oral liquid medication formulations that are suitable for usage in children and adults who have swallowing issues. These research highlighting the need for liquid formulations to facilitate dose adjustment, administration, and swallowing. The present study aimed to develop polyherbal syrup formulation containing *A. marmelos*, *B. ceiba*, *H. rosa sinensis* and *M. oleifera* extracts, optimize them through the quality by design approach, and evaluate their physicochemical, anti oxidant and microbiological quality.

Table: Measure Polyherbal formulations

Formulation	Factor 1 A: Propylene glycol (ml) X1	Factor 2 Sugar base (gm) X2	Factor 3 Sorbitol (gm) X3	Response 1 pH (Degree) Y1	Viscosity (Poise) Y2

F1	10	70	3	6.6	0.03
F2	10	65	1	5.1	0.029
F3	5	70	3	6.9	0.031
F4	7.5	65	3	6.2	0.028
F5	7.5	65	3	6.3	0.021

Table: Data of Independent variables

S.No.	Coding	Variables	Category
1.	X1	Propyleneglycol	Solublizer
2.	X2	Sugar base (Sucrose)	Sweetningagent
3.	X3	Sorbitol	Stablizer

Table: Data of Dependent variables

S.No	Coding	Variables
1.	Y1	pH
2.	Y2	Viscosity

Table: Physico-chemical parameter of herbal syrup

S.No	Evaluation parameter	Observation
1.	Colour	Slightly yellowish to brown
2.	Odour	Characteristic
3.	Taste	Sweet
4.	Appearance	yellowish to brown liquid
5.	Viscosity	0.024
6.	Density	1.09
7.	pH	6.2

Antibacterial activity of formulation

Antibacterial activity of selected 18 formulations was done *in vitro* against *S. mutans* using well diffusion method. Figure shows zone of inhibition in selected formulations.

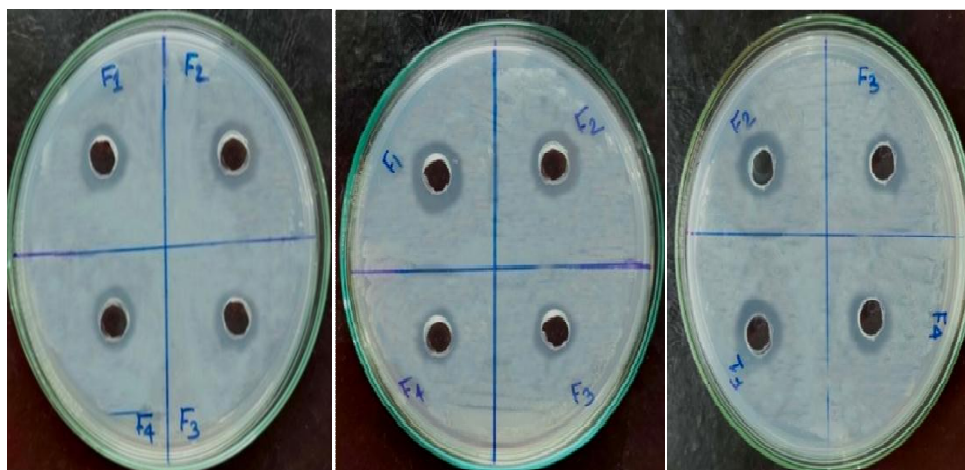


Fig:Zone of inhibition in Formulations 1, 2, 3 and 4

Table: Antibacterial activity formulations

Concentration (mg/ml)	Zone of inhibition			
	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean ± SD
F1	11	12	10	11 ± 1
F2	12	11	11	11.33 ± 0.577
F3	9	8	9	8.66 ± 0.577
F4	10	8	9	9 ± 1
F5	8	9	9	9.33 ± 0.577

➤ Conclusion

- Based on the scientific literature, the ingredients of the polyherbal formulation were selected that possess the preservative and curative role. The Poly-herbal syrup formulation was prepared by incorporating herbal extracts into simple syrup.
- Based on the extensive literature, four raw materials (*Aegle marmelos*, *Moringa oleifera*, *Hibiscus rosa sinensis*, and *Bombax ceiba*) were selected for the formulation of polyherbal syrup whose *in vitro* antioxidant and anti-microbial potency were evaluated.
- The present study aimed to develop a polyherbal syrup formulation containing *marmelos*, *B. ceiba*, *H. rosa sinensis*, and *M. oleifera* extracts, optimize them through the quality-by-design approach, and evaluate their physicochemical, antioxidant, and microbiological quality.
- Preformulation and preliminary stability studies were carried out with 18 polyherbal syrup formulations stored at room temperature, evaluating aspects, color, odor, pH, viscosity, etc..
- The formulation studies of all these formulations were within the specifications. Also, the physicochemical properties of the prepared syrup like appearance, odor, taste, pH, specific gravity, viscosity, and stability studies were satisfactory.

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