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ASSESSMENT OF ANTIOXIDANT, ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE POTENTIAL OF POLYHERBAL FORMULATION

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Keywords:

Gmelina arborea, Basella rubra, Sphaerathus indicus , Alpha amylase, Alpha glucosidase

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ABSTRACT: Diabetes mellitus is a group of disorders that speed up the metabolism of protein, fat, and carbohydrates. It has been forecasting rises in the incidence and prevalence of diabetes mellitus in recent years. Different plant parts have historically been used to treat diabetes mellitus. The Plant *Gmelina arborea* (lamiaceae) treats insecticidal, antidiuretic, anti-diabetic, anti-analgesic, and immunomodulatory activity. *Basella rubra (*Baellaeceae) is used as an Androgenic, diuretic, emollient, demulcent and anticonvulsant. *Sphaeranthus indicus* (Asteraceae) treats ananxiolytic, neuroleptic, antioxidant, anti-inflammatory, bronchodilatory, antihyperglycemic and hepatoprotective. The plants were processed, powdered and extracted with hydroalcoholic solvent. Maceration was processed for 72hrs, 48 hrs and 24 hrs. Solvents were filtered, distilled, and dried in a vacuum desiccator. Polyherbal formulations such as BSG, SBG, BGS were made by taking the required proportion of each powdered drugs. The *in-vitro* anti-oxidant and anti-diabetic potential of plant were determined by DPPH radical scavenging activity, Alpha-amylase and Alpha-glucosidase inhibition assay. The activity reported that hydroalcoholic extract of *Gmelina arborea*, *Basella rubra*, and *Sphaeranthus indicus* exhibits potent activity indose–dependent manner. The Polyherbal formulation is used to treat diabetics with fewer adverse effects.

INTRODUCTION: Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid, and protein metabolism, which causes Hyperglycemia resulting from in sufficient insulin secretion, insulin action or both. It is one of the refractory diseases infections distinguished by the Indian Committee of Clinical exploration for which an elective medication is a requirement for the treatment. Diabetes mellitus has turned into a developing issue in the contemporary world [1].

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India has today become the diabetic capital of the world, with over 20 million diabetes, which is likely to increase to 57 million by 2025. The modern oral hypoglycemic agents produce undesirable side effects; thus, in recent years, considerable attention has been directed toward the antidiabetic potential of Polyherbal formulations based on Indian medicinal plants as antidiabetic [2].

Nowadays, herbal medications are more popular, especially for treating Type 2 diabetes. Although there are several ways to lessen diabetes' negative effects and its subsequent complications, herbal formulations are favoured since they have less side effects and are less expensive [3]. The Plant *Gmelina arborea* (Lamiaceae) is used in the treatment of insecticidal, antidiuretic, anti-diabetic, anti-analgesic, and immunomodulatory activity.

Basella rubra (Baellaeceae) is used as androgenic, diuretic, emollient, demulcent, anticonvulsant. *Sphaeranthus indicus* (Asteraceae) treats anxiolytic, neuroleptic, antioxidant, anti-inflammatory, bronchodilatory, anti-hyperglycemic and hepatoprotective. The Present study is to scientifically evaluate the Polyherbal formulation for the therapeutic management of diabetes.

MATERIALS AND METHODS:

Collection of Plant Materials: In December 2020, the entire plant of *Basella rubra, Sphaeranthus indicus,* and *Gmelina arborea* were collected in Sengottai, Tirunelveli, Tamil Nadu. Mr. V. Chelladurai, Retd.

Research Officer Botany, C. C. R. A. S., Government of India, Tirunelveli, recognized and verified the plant material. The Authentication number of plant material is XCH-40421.

Both diseases and pollution from other plants were absent from the plant material that had been collected.

FIG. 1: ***BASELLA RUBRA***

FIG. 2: ***GMELINA ARBOREA***

FIG. 3: ***SPHAERANTHUS INDICUS***

Preparation of Churna: Formulation was made by taking a required proportion of each powdered drugs. All the procured and authenticated individual drugs were dried in shade and cleaned by hand sorting. The individual drugs were then crushed and passed through mesh no. 80 granules were prepared. Then they were weighed as per the quantity required. The drugs were mixed, weighed, and packed in air tight container.

Preparation of Extracts: 200g of each formulation was macerated by cold maceration with hydroalcohol with occasional shaking. Extract was decanted for (72 hours, 48 hours, 24 hours) filtered, concentrated and kept in a desiccator for complete solvent removal. Extract was then packed in airtight container.

Physico-Chemical Parameters:

Ash Value: The ash content of the crude drug was generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for adulteration. Ash values vary with narrow limits in case of individual drugs but vary considerably in different drugs [4].

Total Ash: The pharmacopoeia's approved analytical methods were used to determine total ash (I.P.1998). Weighing 1 gm of the material into a silica crucible that had been burnt and cooled before weighing allowed us to calculate the total amount of ash. Until a steady weight was achieved, the ignition was repeated.

Water Soluble Ash: The ash was filtered through an ash-free filter paper after being heated in 25 cc of water. The next step was hot water washing. The filter paper was fired and cooled in the silica crucible, and the water-insoluble ash was weighed. The water-soluble ash was calculated by subtracting the water-insoluble ash from the total ash.

Acid Insoluble Ash: Acid insoluble ash was determined by boiling with 25 ml of dilute Hydrochloric acid for five minutes and filtered through ashless filter paper. The filter paper was ignited in the silica crucible, cooled and weighed.

Extractive Values: According to the Indian Pharmacopoeia, maceration was used to carry out successive extraction with organic solvents to increase polarity [5].

Water Soluble Extractive: About 5g of air-dried coarse powder of the whole plant was macerated with 100ml of chloroform water (99.5ml water + 0.5ml of chloroform) in closed flasks for 24 hrs, frequently shaking during the first 6 hrs and then allowed to stand for 18 hrs. It was then filtered rapidly. 25ml of the filtrate was evaporated to dryness in three tarred flat-bottomed shallow dishes and dried at 105° Celsius and weighed.

Ethanol Soluble Extractive: About 5g of dried coarse powder of the whole plant was macerated with 100ml of 90% ethanol in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and then allowed to stand for 18 hrs it was then filtered rapidly, 25ml of the filtrate was evaporated to dryness in three tarred flat bottomed shallow dishes and dried at 105°C and weighed.

Loss on Drying: About 1g of each formulation was weighed accurately and dried in instarred china dish at 105°C for 5 hrs. Percent w/w was calculated with reference to initial weight.

Phytochemical Screening of Polyherbal Formulation:

Chemicals used: All of the chemicals and reagents (from the United Scientific Company in Chennai, India) utilised were of analytical quality. Sigma Aldrich's website was used to acquire alpha amylase and alpha-glucosidase.

Preparation of Extracts: Hydroalcohol extract of leaves of *Gmelina arborea, Basella rubra and Sphaerathus indicus.*

Qualitative Phytochemical Evaluation [6]: Different chemical tests were performed for establishing profile of the extract for its chemical composition, the following chemical tests for various phytoconstituents in the hydro alcoholic extracts were carried out as described below. The extracts were hydrolyzed with dil. HCl, the following tests were per formed.

***In-vitro* Antioxidant Activity of Leaves of Polyherbal Formulation:**

DPPH Radical Scavenging Activity: The spectrophotometric method measured DPPH scavenging activity. A stock solution of 8mg of DPPH (200µM) was prepared in 100ml of ethanol, and 0.1ml of test compounds dissolved in ethanol were added at different concentrations (10, 50, 100, 200, 400, 800 and 1000µg/ 0.1ml with 1.9ml DPPH solution. The reaction was allowed to be completed in the dark for about 20 minutes. After 20 minutes, the decrease in absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical [7].

Percentage inhibition = OD of control – OD of test / OD of control x100

***In-vitro* anti-Diabetic Activities of Leaves of Polyherbal Formulation:**

Alpha Amylase Inhibition Assay: The inhibition assay was carried out using the chromogenic DNSA method. A volume of 500µl of enzyme solution was mixed with 1ml of different extract concentrations and incubated at 37°C for 10 minutes. After that 500µl of starch solution was added to each of the test tube and incubated at 37°C for 10 minutes. The reaction was terminated by adding 1ml of DNSA reagent and was incubated in a boiling water bath for 5 minutes. It will be then cooled, diluted with 10ml of water and measured at 540nm. The control without the test sample represents 100% enzyme activity. Absorbance produced by test sample is eliminated by including appropriate sample control without enzyme and starch. Acarbose was used as standard [8].

Percentage Inhibition = (Abs control-Abs sample) / Abs control x 100

Alpha-glucosidase Inhibition Assay:

Procedure: 200µl of the α-glucosidase solution was pre-incubated with the test sample/ standard and controlled for 5 min. The reaction was started by adding 200µl of sucrose, and it was terminated after 30min incubation at 37°C by adding 5% TCA. The precipitate was removed by centrifugation at 3500rpm for 10 minutes. The glucose liberated in the supernatant was determined. The enzyme activity was directly proportional to liberated glucose and was measured by the GOD-POD method. To 10 µl of supernatant, 1ml of glucose reagent was added, and the absorbance was measured at 405nm.1ml of glucose reagent alone severed as blank. The percentage of inhibition of the test sample / standard was calculated from control sample. The α- glucosidase inhibitory activity was calculated as follows [9].

Percentage inhibition = (AbsControl-Abs Sample) / AbsControl x 100

RESULTS AND DISCUSSION:

TABLE 1: FORMULATION INGREDIENTS OF CHURNA

S. no.	Ingredients	Partused	Formulation (200g)		
			BSG	SBG	GBS
1	*Gmelina arborea*	Leaves	100	50	50
2	*Sphaeranthus indicus*	Leaves	50	100	50
3	*Basella rubra*	Leaves	50	50	100

TABLE 2: PHYSICOCHEMICAL EVALUATION OF THE POLYHERBAL FORMULATION

S. no.	Parameters	Observation		
		BSG(%w/w)	SBG(%w/w)	GBS(%w/w)
I1	Ash Values Totalash	9.6	9.2	9.7
2	Acid in soluble ash	1.036	1.363	1.421
3	Water in soluble ash	4.56	5.15	5.12
II1.	Extractive Values Hydroalcohol	3.56	4.01	4.45
1	Solubility Water soluble extractive	12.21	13.10	12.34
2	Ethanol soluble extractive	10.1	6.03	9.2
III	Loss on Drying	1.3	1.27	1.0

The recent study on physicochemical screening provides useful information which may help authenticate the genuine plant along with the nature of its phytoconstituents.

TABLE 3: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF HYDROALCOHOLIC EXTRACTS OF POLYHERBAL FORMULATION

S. no.	Particulars	BSG	SBG	GBS
1	Phenols	+	+	+
2	Flavonoids	+	+	+
3	Quinones	-	-	-
4	Steroids	-	-	-
5	Terpenoids	+	+	+
6	Coumarins	+	+	+
7	Alkaloids	+	+	+
8	Tannins	+	+	+
9	Saponins	+	+	+
10	Proteins	-	-	-
12	carbohydrates	+	+	+

The Hydro alcoholic extract showed the presence of Phenols, Flavonoids, Alkaloids, Tannins, Saponins and Carbohydrates. The result of different phytochemical tests for hydroalcoholic extract were given in table. (+) Indicates presence of compounds (-) Indicates absence of compounds.

In-vitro Antioxidant Activities of Leaves of Polyherbal Formulation:

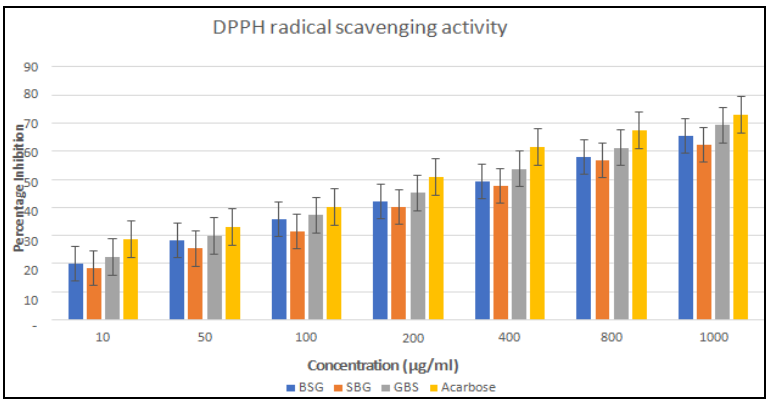

FIG. 4: DPPH RADICAL SCAVENGING ACTIVITY OF LEAVES EXTRACTS OF POLYHERBAL FORMULATION

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either hydrogen or electron donation. The IC_{50} is represented as follows GBS>BSG>SBG. The IC_{50} of DPPH radical scavenging activities of hydroalcoholic extract from the leaves of polyherbal formulation in the order of the IC_{50} values were found to be BSG=140.8µg/ml the IC_{50} values were found to be SBG = 152.6µg/ml. The IC_{50} values were found to be GBS=125.4µg/ml

In-vitro Antidiabetic Activities of Leaves of Polyherbal Formulation:

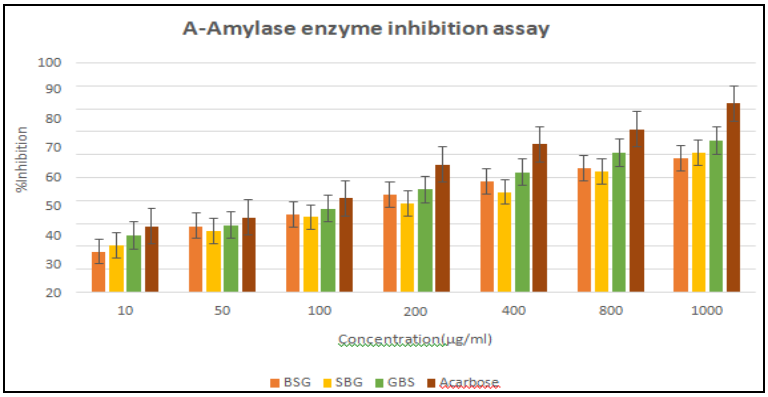

FIG. 5: PERCENTAGE INHIBITION OF ALPHA-AMYLASE OF HYDROALCOHOLIC EXTRACTS OF DIFFERENT FORMULATIONS

In-vitro anti-diabetic activities of different polyherbal formulation formulations were evaluated using the α-amylase inhibition method.GBS exhibited more potent activity than BSG and SBG. The IC_{50} of BSG, SBG and GBS were found to be 205.2µg/ml, 230.6µg/ml, 165.4 µg/ml, respectively. This effect might be due to alkaloids, flavonoids, saponins, and tannins, which were observed by preliminary phytochemical screening. This study revealed that the *in-vitro* anti-diabetic activity of leaves of polyherbal formulation may be used to control Hyperglycemia.

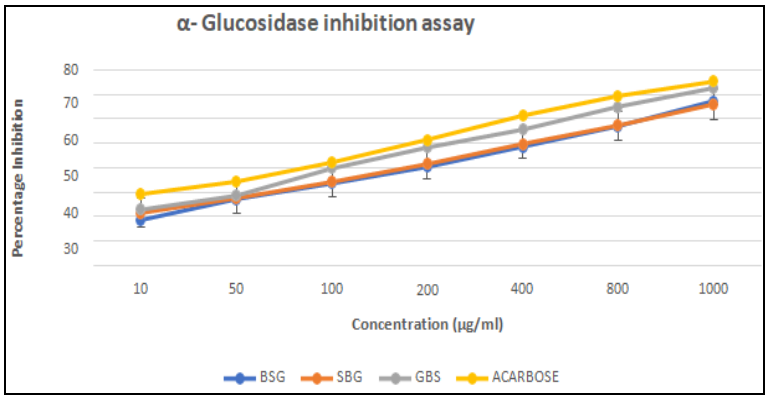

FIG. 6: PERCENTAGE INHIBITION OF ALPHA-GLUCOSIDASE OF HYDRO ALCOHOL EXTRACTS OF DIFFERENT FORMULATIONS

The alpha glucosidase inhibition method evaluated *In-vitro* Anti-diabetic activities of different formulations. GBS exhibited more activity than BSG and SGB. The IC_{50} of BSG, SBG GBS & Acarbose were found to be72.3µg/ml, 80.6µg/ ml, 63.43µg/ ml and 50.23µg/ ml, respectively. The results of the present study conclude that solvent hydroalcoholic extract with polyherbal formulation possesses a significant alpha-glucosidase inhibitory effect. The significance of α- glucosidase inhibitory activity compared with different formulations. The GBS formulation was more effective than BSG and SBG formulations by alpha-glucosidase inhibitory assay. This effect might be due to the result of IC_{50} values of the formulations. This study revealed that the *in-vitro* anti-diabetic activity of leaves of the

polyherbal formulation may be used to control Hyperglycemia.

CONCLUSION: The Phytochemical, *in-vitro* antioxidant, and antidiabetic activities of leaves of polyherbal formulation have been studied. The hydroalcoholic extracts of the GBS formulation showed significant antidiabetic activity. The Principle compounds responsible for inhibiting alpha-amylase and alpha-glucosidase need further identification and characterization. The developed polyherbal formulation might be a safe alternative to antidiabetic synthetic drugs. This may be useful for developing new antidiabetic agents from plant resources.

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