International Journal of Pharmacognosy and Pharmaceutical Research

ISSN Print: 2664-7168
ISSN Online: 2664-7176
Impact Factor: RJIF 8
IJPPR 2024; 6(1): 09-13
www.pharmacognosyjournals.com
Received: 27-11-2023
Accepted: 01-01-2024

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Pharmacognostical and preliminary phytochemical evaluation of novel antidiabetic polyherbal formulation

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DOI: https://doi.org/10.33545/26647168.2024.v6.i1a.62

Abstract
Background: The goal of this study was to conduct Pharmacognostical testing, preliminary phytochemical testing, standardization, and heavy metal analysis on an antidiabetic polyherbal formulation. *Momordica charantia* (fruits), *Eugenia jambolana* (fruits), *Trigonella foenum graceum* (seeds), *Gymnema sylvestre* (leaves) and *Garcinia cambogia* (fruits) were used to make the formulation, and each herb has a scientific basis in treating diabetes.
Materials and Methods: Standard techniques were used to assess the formulation's pharmacognostical and phytochemical characteristics.
Results: Organoleptic and macroscopic characteristics such as colour, odour, taste, size, and shape are evaluated in pharmacognostical evaluations, while ash levels, extractive values, and loss on drying are considered and documented under standardisation parameters. The extract contained alkaloids, tannins, saponins, flavonoids, steroids, glycosides, and sugars according to phytochemical analysis. The formulation adheres to heavy metal's standard parameters.
Conclusion: The findings of this study support the use of aqueous extracts of polyherbal formulations in ethnomedicine, favouring the extraction of antidiabetic compounds from polyherbal formulation extracts. All of the assessment metrics utilised in the study can be used to standardise the above-mentioned formulation because the study was done in a controlled and authenticated manner.

Keywords: Polyherbal formulation, phytochemical, pharmacognostical, antidiabetic, standardization

Introduction
Medicinal plants have been proven to offer a wide range of medicinal uses for a variety of diseases. The medicinal benefits of plants are due to phytochemicals found in them. Phytochemicals are also responsible for a plant's colour, flavour, and odour. Natural products with unrivalled chemical variety, such as pure phytoconstituents, polyherbal formulations, and plant extracts, provide enormous prospects for novel medication development. A number of active chemicals with varied pharmacological properties have been discovered by phytochemical investigation of plants utilised in folklore [1].

Up to 90% of the population in poor nations uses plants and their products as traditional medicine for basic health care, according to the World Health Organization (WHO). The World Health Organization (WHO) has compiled a list of 21,000 plants that are utilised for therapeutic reasons around the world. India is home to 2500 of these species. Around 800 plants have been identified to have anti-diabetic properties. A large number of plant-derived active principles representing a variety of bioactive substances have been identified as having potential for use in the treatment of diabetes [2].

Diabetes mellitus is a category of metabolic disorders characterized by insulin insufficiency,either absolute or relative, resulting in high blood glucose levels. It has been lin ked to abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only caus e hyperglycemia but also lead to hyperlipidemia, hyperinsulinemia, hypertension, atheroscler osis, and the progression of microvascular (nephropathy, retinopathy, and neuropathy) and m acrovascular complications (Coronary heart disease, stroke, peripheral vascular disease) [3].

Hence, there is a need of safe and potent antidiabetic drug from natural sources. The purpose of the present study was to perform preliminary phytochemical analysis and to investigate

poly herbal formulation, which may lead to find a more effective agent for treating diabetes and managing associated other infections [4]. The beneficial effects of various Indian herbs are reported by various researchers. Hydroxycitrate, the active constituent of *Garcinia cambogia* Gaertn, is reported to enhance glycogen synthesis in exercised human skeletal muscle. The protective effect of Garcinia against renal oxidative stress and biomarkers induced by high fat and sucrose diet has been studied. Gymnemic acid extracted from *Gymnema sylvestre* is reported to have antidiabetic and antioxidant activity, antiobesity and cardioprotective effect in animal models. Methanolic fruit extract of *Momordica charantia* L showed hypoglycemic effect in diabetic rats. Singh *et al*. reviewed the active constituents and modes of actions of the antidiabetic effects of *Momordica charantia.* The oral administration of methanolic extract of fenugreek resulted in hypoglycaemic effect in mice. Administration of *Trigonella foenum-graecum* (fenugreek) seed extract also showed lower blood glucose, glycated hemoglobin, triglycerides and total cholesterol in streptozotocin-induced diabetic rats [1]. The water extract of fruit pulp of *Eugenia jambolana* showed hypoglycemic activity immediately within 30 min after its administration [5].

Materials and Methods

Plant Materials

All the plant materials used in the study were collected from herbal store and individual drugs were identified and authenticated. The list of plants used for the preparation of the formulation are provided in Table 1.

Chemicals, Drugs and Instruments

All the chemicals used in pharmacognostical and phytochemical analysis like hydrochloric acid, α-napthol, Sulphuric acid, Fehling A & B, Benedict reagent, sodium hydroxide, nitric acid, ninhydrin, acetic anhydride, ferric chloride, zinc, Dragendroff's reagent, Wagner's reagent, Folin-Ciocalteu reagent, Sodium carbonate and sodium chloride were collected from the store of Anuradha College of Pharmacy, Chikhli. All the chemicals used in the study were of analytical grade.

Formulation of Polyherbal Formulation

All of the crude medications listed in Table 1 were obtained in good condition and thoroughly cleaned to exclude the possibilities of extraneous materials. Herbal medications were washed and dried in the shade. Individual crude medications were roughly powdered before being mixed in equal quantities. To avoid moisture exposure, the powdered mixture was stored in sealed containers.

Pharmacognostical Evaluation

Organoleptic Evaluation

The Organoleptic properties such as colour, odor, and taste of *Garcinia cambogia Gaertn, Eugenia Jambolana, Gymnema sylvestre Retz, Momordica charantia* L and *Trigonella graecum* were evaluated and reported.

Macroscopical Evaluation

The macroscopical studies such as size and shape of *Garcinia cambogia Gaertn. Eugenia jambolana, Gymnema sylvestre Retz, Momordica charantia* L and *Trigonella graecum* were evaluated and reported.

Standardization Parameters: The total ash value including acid insoluble and water soluble ash values, extractive values, loss on drying value and heavy metal contents of polyherbal formulation investigations were determined using pre-existing methodologies.

Phytochemical Analysis

Systematic qualitative tests for identification of various plant constituents such as carbohydrates, amino acids, proteins, glycosides, fats, oils, phenolic compounds, tannins, saponins, steroids, flavanoids, and alkaloids were performed using standard procedures in accordance with Kokate [6] Trease and Evans [7] and Harborne [8] with minor modifications. The formation of colour or precipitate, depending on the end point of the respective test, was noted after the addition of the reagent and the outcome of the test was represented as present (+) or absent (-). All the tests except those required the powder form of the formulation were carried out with freshly prepared stock solution of the formulation with a concentration of 1 mg/mL.

Test for carbohydrates

a. Molisch's Test

To one millilitre test solution was added a few drops of Molisch's reagent (5% alcoholic α- naphthol) followed by 2 mL of concentrated sulphuric acid along the inner side of the test tube.

b. Benedict's test for reducing sugar

To one millilitre of test solution was added 5 mL of Benedict's reagent and kept boiling in a water bath for 5-7 minutes.

c. Fehling's test for reducing sugar

To two millilitres of prior mixed equal volume Fehling's solution A and B was added 1 mL of test solution and kept in boiling water bath for 5-10 minutes.

Test for Saponins

Froth test

The test solution (2 mL) was shaken well in test tube and observed for froth (Foam) formation.

Test for Flavonoids

a. Shinoda Test

To one millilitre of test solution were added fragments of magnesium ribbon and a few drops of concentrated hydrochloric acid.

b. Zinc-Hydrochloride test

To one millilitre of test solution were added zinc dust and a few drops of hydrochloric acid.

Test for Steroids

a. Sulphur powder test

To one millilitre of test solution was added a little amount of sulphur powder and mixed well.

b. Liberman Burchard's test

To one millilitre of test solution were added a few drops of acetic anhydride, boiled in boiling water for 3-5 minutes and after cooling added 1 ml of concentrated sulphuric acid.

Test for Glycosides

General test

Test A: Two hundred milligram of the formulation was mixed with 5mL of dilute sulphuric acid by warming in a water bath, filtered, and neutralized by adding of 5% of sodium hydroxide solution until it became alkaline (tested with pH paper). Added 0.1 mL of Fehling's solution A and B and heated in a water bath for 2 minutes.

Test B: Repeated 'Test A, procedure by using 5mL of water instead of dilute sulphuric acid. Compared whether the intensity of precipitation showed in Test A was more than Test B.

Test for Alkaloids

a. Dragendroff's test

To two millilitre of test solution was added 1 mL of Dragendroff's reagent.

b. Wagner's test:

To two millilitre of test solution was added a few drops of Wagner's reagent.

Test for proteins

Biuret test

To one millilitre of test solution was added 1 mL of Biuret reagent.

Test for Tannins

To one millilitre of test solution was added a few drops of 5% ferric chloride.

Test for insulin

To one millilitre of test solution was added the solution of α-naphthol and sulphuric acid.

Test for Anthocynidine

To two millilitre of test solution was added concentrated H_2SO_4.

Test for Terpenoids

To five milliliters of test solution was added 2 mL of chloroform and 3 mL of concentrated sulphuric acid.

Test for amino acid

To one millilitre of test solution was added a few drops of 0.25% of ninhydrin reagent and kept in a boiling water bath for 2-5 minutes.

Test for Phenols

To one millilitre test solution was added 1 mL of Folin-Ciocalteu reagent and 0.5 ml of Na_2CO_3.

Results and Discussion

The results of the pharmacognostical analysis as organoletic, macroscopic and physicochemical, screening for heavy metals of polyherbal formulation described below.

Morphological features

The morphological features of selected medicinal herbs viz., leaves of *Gymnema sylvestre*, fruits of *Momordica charantia*, fruit pulp of *Garcinia cambogia*, seeds of *Eugenia jambolana* and seeds of *Trigonella foenum graceum* were studied and were presented in Table No.2 and 3.

Standardization Parameters

Physico- chemical evaluation of Polyherbal Formulation

The total ash value including acid insoluble and water soluble ash values, extractive values and loss on drying value and heavy metal contents of polyherbal formulation are listed in Table 4 and 5.

Phytochemical Analysis

Carbohydrates, amino acids, proteins, phenols, flavonoids, tannins, terpenoids, steroids, Wagner's test, anthocyanine, and insulin were found in the extract of the antidiabetic polyherbal formulation, but glycosides and saponins were not present, as shown in Table 6. Strong antioxidants, phenol and flavonoid are linked to a variety of beneficial biological effects, Polyphenol rich diets offer significant protection against the cellular damage that occurs as a result of many chronic pathological illnesses, such as cancer, diabetes, cardiovascular disease, and ageing. The therapeutic potential of flavonoids is due to their antioxidative, anti-atherosclerotic, antitumor and anti-inflammatory effects. Tannins have an antioxidative characteristic that protects cells from oxidative damage, such as lipid peroxidation, whereas plant sterols inhibits cholesterol absorption, resulting in lower LDL-cholesterol levels in the blood [1].

Table 1: Ingredients of the antidiabetic polyherbal formulation

Sr. No.	Botanical Name	English Name	Family	Used part
1	*Garcinia cambogia Gaertn.*	Gamboge	Clusiaceae	Fruit pulp
2	*Eugenia Jambolana*	black plum	Myrtaceae	Fruit pulp
3	*Gymnema sylvestre Retz.*	Periploca of the woods	Asclepiadaceae	Leaves
4	*Momordica charantia* L.	Bitter gourd	Cucurbitaceae	Fruit pulp
5	*Trigonella foenum graecum* L.	Fenugreek	Fabaceae	Seeds

Table 2: Organoleptic evaluation of selected Medicinal herbs used in the treatment of Diabetes [9-11].

S. No	Name of the plant/drug	Plant Part	Colour	Odour	Taste
01.	*Garcinia cambogia*	Fruit plup	yellow, orange or red	Characteristic	Slightly Bitter and Astringent
02.	*Gymnema sylvestre*	Leaves	Green	characteristic	Slightly bitter
03.	*Mormodica Charantia*	Fruit	Green	Characteristic	Bitter
04.	*Eugenia jambolana*	Fruit plup	Dark purple or black	Astringent	Sweet/atringent flavor
05.	*Trigonella foenum graecum*	Seed	Yellow	Characteristic	Bitter

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Table 3: Morphological characteristics of selected Medicinal herbs used in the treatment of Diabetes [9-11].

S. No	Name of the plant/drug	Plant Part	Size	Shape
01.	*Garcinia cambogia*	Fruit plup	5 cm in diameter	Ovoid
02.	*Gymnema sylvestre*	Leaves	2-6 cm length 1-4cm width	simple, petiolate, rounded to cordate base
03.	*Mormodica Charantia*	Fruit	2.5-25 cm long 2-7 cm diameter	Elongated, fusiform, longitudinally grooved, ridged
04.	*Eugenia jambolana*	Fruit plup	1/2 to 2 in. long	round or oblong or ellipsoid
05.	*Trigonella foenum graecum*	Seed	3-5 mm long, 1.5-3 mm wide	oblong, flattened or irregularly rhomboidal

Table 4: Physicochemical parameters and their found values

Sr. No.	Physicochemical parameters	Value (% w/w)
1	Total Ash value	9.12 ± 0.82
2	Water soluble ash value	3.42 ± 0.42
3	Acid insoluble ash value	2.15 ± 0.94
4	Water soluble extractive value	25.82 ± 0.24
5	Alcohol soluble extractive value	8.45 ± 0.46
6	Ether soluble extractive value	2.12 ± 0.38
7	Loss on drying Ash value	6.72 ± 0.15

Note: The values are presented as mean ± SD (N=3)

Table 5: Heavy metal contents of Polyherbal Formulation [12]

Sr. No.	Parameters	Observed value (mg/kg)	WHO permissible limit (ppm)	Detectable limit (mg/L)
1	Arsenic	< BDL	5	0.078
2	Cadmium	< BDL	0.3	0.006
3	Lead	< BDL	10	0.062
4	Mercury	< BDL	0.2	0.124

Table 6: Result of qualitative analysis of the antidiabetic polyherbal formulation:

Test	Method Used	Observation	Grading of Observations
Carbohydrates	Molisch test	Red violet ring appeared at the junction of two reagents	+
	Fehling test	Brownish red precipitate	+
	Benedict;s test	Reddish colour	+
Saponins	Foam Test	No froth formation	-
Flavanoid	Shinoda Test	Green to blue colour	+++
	Zinc-Hydrochloride test	Red colour	+++
Steroids	Liebermann Burchard test	Green colour at upper layer	++
	Sulphur powder test	The Sulphur powder sank to the bottom	++
Glycosides	General test	No red precipitation	-
Alkaloids	Dragendroff's test	Orange or orange red precipitate	+
	Wagner's test	Reddish brown colour	+
Proteins	Biuret test	Violet colour	++
Tannins	Ferric chloride test	Green colour	+
Test for insulin	α -Naphtholand sulphuric acid test	Brownish red colour	++
Anthocynidine	H_2SO_4 test	yellow colour	+
Terpenoids	Salkowski's test	Reddish brown precipitate	++
Amino acids	Ninhydrin test	Blue colour	++
Phenols	Folin-Ciocalteu test	Blue colour	+++

Conclusion

It was concluded from this study that the extract of polyherbal formulation is rich in phytochemicals such as flavonoids, tannins, cardiac glycosides, alkaloids, saponins and steroids. These phytochemicals have been reported to be of pharmaceutical importance. This supports the use of this formulation in folklore medicine for the herbal treatment of diabetes. The extract and fractions of polyherbal formulation used in this work revealed that the product does not possess any heavy metal traces.

Acknowledgement

The authors are grateful to the authorities of Anuradha College of Pharmacy, Chikhli Dist. Buldana Maharashtra – 442 201 for providing necessary facilities to carry out the research work.

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