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PHYTOCHEMICAL SCREENING, ANTIOXIDANT POTENTIAL AND ALPHA AMYLASE INHIBITORY ACTIVITY OF AN ANTIDIABETIC POLYHERBAL FORMULATION

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ABSTRACT

The objective of this study was to evaluate the phytochemical profile, antioxidant activity and α- amylase inhibitory activity of an antidiabetic polyherbal formulation (PHF) that included *Eugenia Jambolana* L., *Gymnema sylvestre* Retz, *Momordica charantia* L, and *Trigonella foenum-graecum* L. Methods. Standard procedures were used to determine the total phenolic content, flavonoids, and flavonols in the formulation and to evaluate α-amylase inhibitory activity of the formulation. Total antioxidant activity, ferric reducing antioxidant power, and 2-diphenylpicrylhydrazyl (DPPH) assays were used to determine antioxidant potential. Results. The PHF contained 213.4 ± 0.72 mg gallic acid equivalent/g of total phenolic content. The PHF had 152.6 ± 0.45 mg quercetin equivalent/g of total flavonols and 126.2 ± 0.34 mg quercetin equivalent/g of total flavonoids. The reducing power of the PHF was 18.4 mg ascorbic acid equivalent/g. The total antioxidant capacity of 126.2 mg ascorbic acid equivalent/g was discovered. The PHF had a ferric reducing antioxidant power of PHP was 1024.4 (µM Fe (II)g). The formulation had a DPPH radical scavenging activity value was 1.8µg/mL for gallic acid and 2.6 µg/mL for the formulation. The IC_{50} value of α-amylase inhibition for water extract and hydroalcohol extract was found to be 8.425 and 10.242 mg/ml respectively Conclusions. The findings indicate that the polyherbal formulation has high antioxidant activity and could be a promising option for anti-diabetic research.

KEYWORDS: Polyherbal formulation, Phytochemical screening, antioxidant activity, α- amylase.

INTRODUCTION

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 linked to abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only cause hyperglycemia but also lead to hyperlipidemia, hyperinsulinemia, hypertension, atherosclerosis and the progression of microvascular and macrovascular complications.[1] 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, antioxidant potential and alpha-amylase inhibitor activity of polyherbal formulation to find a more effective agent for treating diabetes and managing associated other infections.[2]

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:

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

MATERIALS AND METHODS

Chemicals, Drugs and Instruments

All the chemicals used in phytochemical screesning, antioxidant potential and alpha amylase Inhibitory Activity of an Antidiabetic Polyherbal Formulation 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 Table1 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.[3,4,5,9]

MATERIALS AND METHODS

Phytochemical Screening

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 1mg/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 1mL of test solution and kept in boiling water bath for 5-10 minutes.

Test for Saponins

Froth test

The test solution (2mL) 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. LibermanBurchard'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 millilitres 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.

Estimation of phytochemical constituents[12,13]

Estimation of total Phenolic content

Total phenolic content in aqueous extracts of PHF was evaluated with **modified Folin-Ciocalteu (FC) method.** Gallic acid was used as reference standard for total phenolic estimation. PHF sample (0.2–1 mg/mL) was added in different aliquots of 20–100 µL in graduated tubes. To each tube 0.5mL of 1:1 FC reagent was added and incubated for 10 min at RT. Further, 2.5 mL of saturated sodium carbonate solution was added and incubated at RT for 30 min. Phenols in PHF extract were reduced by FC reagent and the absorbance was measured at 750nm. Reaction mixture involving FC reagent and Na_2CO_3 solution alone served as blank. Total phenol content was expressed in terms of gallic acid equivalents (GAE) using the equation

$$T = C \times V/M$$

Where,

T = total phenolic content (mg/g) of extract as GAE,

C = concentration of gallic acid established from the calibration curve,

V = volume of the extract solution in mL,

M= weight of extract in grams.

Estimation of total flavonoid content

Total flavonoid content in aqueous extract of PHF was evaluated with minor modifications. Quercetin was used as reference standard. PHF sample (0.2–1 mg/mL) was added in different aliquots of 20–100 µL in graduated tubes. 0.5mL of 10% AlCl3 in 10% methanol and 0.5mL sodium potassium tartrate were added and the final volume was made up to 3mL with water. The reaction mixture was incubated for 30 min in the dark and the absorbance was read at 415nm. $AlCl_3$ and sodium potassium tartrate alone served as blank. The total flavonoid content in the PHF extract was expressed in terms of quercetin equivalents.

Determination of total flavonols content

The total flavonols content were determined using the $AlCl_3$ method of Kumaran and Karunakaran. One millilitre of the PHF solution was mixed with 1.0 mL of 2% AlCl3 prepared in distilled water and 1.5 mL of 5% sodium acetate solution was added. The mixture was incubated at 20°C for 2.5 hours and the absorption was read at 440 nm on a spectrophotometer. Concurrently, the same procedure was used for the standard solutions of quercetin (concentrations: 20-100 µg/mL) for calibration curve. Total flavonols contents were calculated as mg quercetin equivalent (mg QUE/g of the PHF).

Reducing power assay

The reducing power capacity of the PHF was determined using the method described by Oyaizu. An aliquot (200 µL) of the PHF solution was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. The mixture was incubated at 50°C for 30 minutes. Thereafter, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 minutes at 4000 rpm.

Reducing power assay

Finally, 2.5 mL of the upper layer was taken and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous FeCl3 and the absorbance was recorded at 700 nm using a spectrophotometer. Ascorbic acid was used as reference standard. The result was expressed as mg ascorbic acid equivalent/g of PHF.

Ferric reducing antioxidant power assay

The ferric reducing activity was determined using the modified method of Benzie and Strain. The stock solutions included, 300 mM acetate buffer, 10 mM TPTZ (2, 4, 6-tripyridyl-striazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL $FeCl_3 \cdot 6H_2O$ and incubated at 37°C for 10 min.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The DPPH radical scavenging assay of the PHF was measured in accordance with the procedure described by Blios. The PHF solution (10-50 µL) was mixed with 0.5 mL of 0.1 mM methanolic solution of DPPH and incubated in dark for 30 minutes at room temperature. After incubation, the absorbance of each solution was measured at 517 nm using spectrophotometer (Shimadzu UV-1800). The same procedure was used for standard solution of gallic acid at different concentrations (1-5 µg/mL). The scavenging ability of the plant extract was calculated using the equation

The scavenging ability of the plant extract was calculated using the equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100$$

Where,
Abscontrol is the absorbance of DPPH + methanol;
Abssample is the absorbance of DPPH radical + sample extract or standard.

The result of DPPH radical scavenging was expressed as IC50 calculated by using GraphPad software.

Ferric reducing antioxidant power assay
Two hundred microliter of FeSO4 at different concentrations (20 - 100 μM) was allowed to react with 1800 μL of the working solution for 30 min in the dark condition. The same procedure was used for the comparative standard solution of ascorbic acid as well as the PHF. Readings of the colour product (ferrous tripyridyltriazine complex) were taken at 593 nm on a Spectrophotometer (Shimadzu UV-1800). FRAP (Ferric Reducing Antioxidant Power) values were expressed in μM Fe (II)/g of PHF or ascorbic acid.

***in-vitro* anti-diabetic assay[13]**
α-Amylase inhibitory assay
The estimation of inhibition of α-amylase was done by determining the amount of reducing sugar liberated under the assay conditions, in terms of maltose equivalent i.e, decrease in units of maltose liberated. Dinitrosalicylic acid (DNS) method with slight modification was used for the estimation of maltose equivalent. Test samples [PHF] (125, 250, 500, 1000, 1250 μg/ml)] and allopathic drug Acarbose (125, 250, 500, 1000, 1250 μg/ml)] were added to 500 μl of sodium phosphate (0.02 M) buffer (pH adjusted to 6.9 with 1% NaCl) having 0.5 mg/ml of α-amylase enzyme solution. All the tubes were incubated at 25ºC for 10 min. Then to each tube, 500 μl of 1% starch solution was added at specified intervals. Incubation was continued for another 10 min. The reaction was arrested by the addition of 1 ml of 3, 5-dinitro salicylic acid (colouring reagent). This was followed by incubation of the tubes in boiling water bath for 5 mins; cooled to room temperature and diluted with distilled water to get a total of 10 ml. The absorbance of the diluted solution was then measured at 540 nm.

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of extract}}{\text{absorbance of control}} \times 100$$

This was followed by incubation of the tubes in boiling water bath for 5 mins; cooled to room temperature and diluted with distilled water to get a total of 10 ml. The absorbance of the diluted solution was then measured at 540 nm.

RESULTS AND DISCUSSION

Preliminary phytochemical screening
Table 7: 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 pricipitate	+
	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_4test	Yellow colour	+
Terpenoids	Salkowski's test	Reddish brown precipitate	++
Amino acids	Ninhydrin test	Blue colour	++
Phenols	Folin-Ciocalteu test	Blue colour	+++

Table: Result of quantitative estimation of the polyherbal formulation.

Sample	Total phenolic content (mg gallic acid equiv. /g	Total flavonoid content (mg quercetin equivalent/g)	Total flavonols content (mg quercetin equiv./ g)
PHF	213.4 ± 0.72	126.2 ± 0.34	152.6 ± 0.45

Table: Antioxidant Activity.

Sr. No.	Sample	Total Antioxidant Power (mg AscAE/g)	Reducing Power (mg AscAE/g)	FRAP value@ (µM Fe (II)/g)
1.	PHP	126.2	18.4 ± 0.42	1024.4 ± 22.43
2.	Ascorbic acid	--	--	2268.5 ± 18.35

Table: DPPH Radical Scavenging Activity.

Sr. No.	Sample	DPPH IC50 (µg/mL
1.	PHP	2.6
2.	Gallic acid	1.8

α-Amylase inhibitory assay

The IC_{50} value of α-amylase inhibition for water extract and hydroalcohol extract was found to be 8.425 and 10.242 mg/ml respectively.

COLCLUSION

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 and also posses potent antioxidant activity and alpha amylase inhibitory activity. 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.

REFERENCES

1. S. I. Rizvi and N. Mishra, "Traditional Indian Medicines Used for the Management of Diabetes Mellitus," *J. Diabetes Res.*, 2013; 1–11. doi: 10.1155/2013/712092.
2. P. Kumar and M. Michael, "Preliminary phytochemical investigation and HPTLC finger printing of an antidiabetic polyherbal formulation," *Int. J. Pharm. Sci. Res.*, 2015; 6(7): 2955.
3. G. R. Kokil, P. V. Rewatkar, A. Verma, S. Thareja, and S. R. Naik, "Pharmacology and Chemistry of Diabetes mellitus and Antidiabetic Drugs: A Critical Review," *Curr. Med. Chem.*, 2010; 17(35): 4405–4423. doi: 10.2174/092986710793361225.
4. T. K. Ujwala, S. Celine, S. Tomy, and P. Arulraj, "Preliminary Pharmacognostical and Phytochemical Screening of DiaKure-A Polyherbal Antidiabetic Formulation," *J. Young Pharm.*, 2016; 8(4): 385.
5. S. B. Sharma, A. Nasir, K. M. Prabhu, and P. S. Murthy, "Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus," *J. Ethnopharmacol.*, 2006; 104(3): 367–373. doi: 10.1016/j.jep.2005.10.033.
6. C. K. Kokate, *Handbook of Practical Pharmacognosy*, 4ed ed. Vallabh Prakashan, 1994.
7. G. Trease and W. Evans, *Textbook of Pharmacognosy*, 12th Edn. Balliere, Tinadl London, 1989.
8. J. Harborne, *Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall, 1998.
9. M. S. Baliga, H. P. Bhat, B. R. V. Baliga, R. Wilson, and P. L. Palatty, "Phytochemistry, traditional uses and pharmacology of *Eugenia jambolana* Lam.(black plum): a review," *Food Res. Int.*, 2011; 44(7): 1776–1789.
10. R. Pothuraju, R. K. Sharma, J. Chagalamarri, S. Jangra, and P. Kumar Kavadi, "A systematic review of *Gymnema sylvestre* in obesity and diabetes management," *J. Sci. Food Agric.*, 2014; 94(5): 834–840.
11. R. B. Semwal, D. K. Semwal, I. Vermaak, and A. Viljoen, "A comprehensive scientific overview of *Garcinia cambogia*," *Fitoterapia*, 2015; 102: 134–148. doi: 10.1016/j.fitote.2015.02.012.
12. D. Shanmugasundaram, A. Duraiswamy, A. Viswanathan, C. S. Sasikumar, C. M. Cherian and K. M. Cherian, "Development of an antidiabetic polyherbal formulation (ADPHF6) and assessment of its antioxidant activity against ROS-induced damage in pUC19 and human lymphocytes – an in vitro study," *J. Complement Integr Med.*, 2016; 1-8. doi: 10.1515/jcim-2015-0028.
13. P. Kumar, P. Pravinkumar, S. S. Iqbal, K. S. Pillai, and M. Michael, "Phytochemical profile, heavy metals contents and antioxidant activities of an antidiabetic polyherbal formulation, 9.