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Development of an antidiabetic formulation (ADJ6) and its inhibitory activity against α-amylase and α-glucosidase

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ABSTRACT

There has recently been much advancement in the diagnosis, treatment, and research of metabolic disorders, especially diabetes. Current research around the world is focused on finding an alternative source of treatment from natural resources for diabetic management, apart from the available synthetic medicines. The present study is a preliminary study of a polyherbal formulation using edible natural resources and an assessment of its antidiabetic activity. The formulation was screened for its phytochemical constituents, total phenols, flavonoids, and vitamin C content. It was also analyzed for its inhibitory effect against the digestive enzymes α-amylase and α-glucosidase, compared with the standard drug acarbose. The formulation showed the presence of major constituents such as steroids, cardiac glycosides, phenols, flavonoids, and saponins. It also had a high level of phenols (340 ± 2.5 mg/g), flavonoids (235.4 ± 8.3 mg/g), and vitamin C (470.8 ± 16.6 mg/g), and showed a half-maximal inhibitory concentration (IC_{50}) value of 0.41 ± 0.03 mg/mL and 0.51 ± 0.01 mg/mL for amylase and glucosidase, respectively. The results showed that ADJ6 had a significant inhibitory activity on α-amylase and α-glucosidase; however, its inhibitory activity was less than that of acarbose. The plants that are formulated in ADJ6 possess potent antidiabetic activity. Thus, we found that ADJ6 is a potent lead for effective diabetic management; however, an evaluation of the formulation must be illustrated using an *in vivo* model.

1. Introduction

Type 2 diabetes or hyperglycemia, which is characterized by high blood glucose levels and glycosuria, is an endocrine disorder. It is responsible for the development of various other complications in the human body. It may occur for two main reasons: (1) deficient/insufficient secretion of insulin by the pancreatic beta cells[1]; or (2) decreased or lost sensitivity of the insulin receptors.[2] These two factors are preceded by stress, obesity, and a sedentary lifestyle.[3,4] At present, diabetes management is a prime concern in the medical community. As a result of various factors, diabetes management is a challenging problem.[5–8]

However, many medicinal plants in ancient Indian Medicine (Ayurveda) have been identified and used to reduce the hyperglycemic condition in the body. The herbs control the hyperglycemic condition and effectively maintain normal glucose levels from a long-term perspective. Many medicinal plants have recently been evaluated for their antihyperglycemic property and have been successfully proven for their effects.[9–11] Numerous polyherbal formulations are also being evaluated for effective diabetes management. However, none of the formulations has made significant inroads in treatment alternatives. Thus, a new formulation has been developed that combines six different herbs that are used in daily life as foods and have significant antidiabetic activity. We combined the six plants based on previously available literature, indigenous knowledge, and various preliminary studies. Thus, the polyherbal formulation has been named "ADJ6". Our aim was to investigate the

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antidiabetic potential of the polyherbal formulation (i.e., ADJ6) and prove its synergistic effect against various aspects involved in diabetes management.

2. Materials and methods

2.1. Materials

Chemicals such as soluble starch and porcine pancreatic α-amylase (PPA) were purchased from SRL Pvt. Ltd. (Mumbai, India). α-Glucosidase, 3,5-dinitrosalicylicacid (DNSA), and 2,4-dinitrophenyl hydrazine (DNPH) were obtained from HiMedia Laboratories (Mumbai, India). Para-nitrophenyl α-D-glucopyranoside and standards such as gallic acid, quercetin, and ascorbic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acarbose was purchased from Bayer Scientific (Leverkusen, Germany). All other reagents and chemicals were of analytical grade and procured locally in Chennai.

2.2. Identification of plants

The herbs were collected from the medicinal farm Frontier Mediville (Elavur, Gummidipoondi, India) and were submitted to the Plant Anatomy Research Centre (Tamil Nadu, India) for authentication. The authentication numbers have been provided in an additional file 1. The plant names, families, and parts used and their medicinal properties are listed in Table 1.

2.3. Preparation of ADJ6

The plant parts were minced individually using a mixer. The individual plants were then freeze-dried using a lyophilizer to minimize the loss of bioactive components. The freeze-dried powder was stored in an air-tight container at room temperature until further use. The traditional Ayurvedic methods of preparing herbal formulations are primarily aqueous (it is likely that the peptides, proteins and glycans possessing antidiabetic activity would be denatured in organic solvents.). The powders of each plant were mixed together in a specific proportion, soaked in water for 24 hours, and filtered. The filtrate was used for further analysis. Fresh extracts were prepared when needed using the specific proportion.

2.4. Pancreatic α-amylase inhibition assay

The inhibition assay was performed using the DNSA method.[43] The assay mixture consisted of 500 μL of 0.02M sodium phosphate buffer [containing 6mM sodium chloride (NaCl), pH 6.9], 0.05 units of PPA solution, and ADJ6 at a concentration of 0.1–1.5 mg/mL (w/v). The assay mixture was preincubated at 37°C for 20 minutes. After incubation, 250 μL of 0.5% (v/v) starch solution in the aforementioned buffer was added to the tubes and incubated for 15 minutes at 37°C. The reaction was terminated by adding 1 mL of dinitrosalicylic acid reagent and then incubated in a boiling water bath for 10 minutes. The tubes were cooled and the absorbance was measured at 540 nm (Shimadzu UV-VIS 1800 spectrophotometer; Shimadzu Corporation, Kyoto, Japan). A tube with PPA but without ADJ6 served as the control with 100% enzyme activity. Acarbose, an amylase inhibitor, was the positive control.

2.5. α-Glucosidase inhibition activity

The assay was performed with slight modifications using α-glucosidase from *Saccharomyces cerevisiae*.[44] The assay mixture consisted of 150 μL of 0.1M sodium phosphate buffer (containing 6mM NaCl, pH 6.9), 0.1 unit of α-glucosidase, and ADJ6 at a concentration of 0.1–1.5 mg/mL (w/v). The assay mixture was preincubated at 37°C for 10 minutes. After incubation, 50 μL of 2mM para-nitrophenyl α-D-glucopyranoside in 0.1M sodium phosphate buffer was added to the mixture and incubated at 37°C for 25 minutes. The reaction was terminated by adding 50 μL of 0.1M sodium carbonate (Na_2CO_3). The yellow color that developed was measured at 405 nm (Bio-Rad microplate reader; Bio-Rad Laboratories, California, USA). The tube with α-glucosidase but without ADJ6 served as the control with 100% enzyme activity, and acarbose served as the positive control.

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

2.6. Qualitative phytochemical analysis

The formulation ADJ6 was analyzed for the presence of amino acids, steroids, cardiac glycosides, phenols, tannins, terpenoids, alkaloids, flavonoids, saponins, carbohydrates, reducing sugar, and anthrones.[45]

2.7. Evaluation of bioactive constituents

2.7.1. Estimation of total phenolic content

Total phenolic content was determined using gallic acid as the reference standard.[46] One milliliter of sample (0.2–1 mg/mL) was

Table 1
Plants and their parts used for the polyherbal formulation.

Serial Number	Binomial name	Common name	Family name	Part used	Activity	Reference no.
1	*Momordica charantia*	Bitter gourd	Cucurbitaceae	Whole fruit	Antihyperglycemic effect, analgesic, antipyretic, hypotriglyceridemic, hypocholesterolemic, antiulcerative, antiproliferative, wound healing	12–19
2	*Psidium guajava*	Guava	Myrtaceae	Whole fruit	Hypolipidaemic, hepatoprotective, antihyperglycemic, renal protective, antioxidant	20–24
3	*Phyllanthus emblica*	Amla	Phyllanthaceae	Pulp	Antihyperglycemic, hypolipidemic, antiapoptotic, anti-inflammatory, chondroprotective, endothelial dysfunction	25–29
4	*Trigonella foenum-graecum*	Fenugreek	Fabaceae	Seeds	Antihyperglycemic, neuroprotective, immunomodulatory,	30–33
5	*Syzygium cumini*	Jamun	Myrtaceae	Seeds	Antihyperglycemic, antiproliferative, chemoprotective	34–38
6	*Gymnema sylvestre*	Gymnema	Asclepiadaceae	Leaves	Antihyperglycemic effect	15,39–42

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added with 0.5 mL of Folin–Ciocalteau reagent and incubated at room temperature for 10 minutes. Furthermore, 2.5 mL of saturated Na_2CO_3 solution was added and incubated at room temperature for 30 minutes. The resultant color was measured at 750 nm versus a blank containing distilled water and Folin–Ciocalteau reagent. Total phenolic content was expressed in gallic acid equivalents using the equation T = CXV/M (T = Total Phenolic Content (mg/g) of extract as gallic acid equivalents, C = Concentration of Gallic acid established from the calibration curve, V = Volume of the extract solution in ml, M = weight of extract in grams).

2.7.2. Determination of total flavonoid content

Total flavonoid content was determined with slight modifications using quercetin as the reference standard.[47] One milliliter of sample (0.2–1 mg/mL) was added with 0.5 mL of 1.2% aluminum chloride in 10% methanol, 0.5 mL of 1M potassium acetate, and made up to 3 mL with distilled water. The mixture was incubated for 30 minutes in the dark, and the absorbance was read at 415 nm. Aluminum chloride without the sample alone served as the blank. Total flavonoid content was expressed in gram quercetin equivalents.

2.7.3. Determination of vitamin C

Vitamin C was determined using the method described by Roe,[48] but with modification, using ascorbic acid as the reference compound. Fifty microliters of sample (0.2–1 mg/mL) was added with 150 μL of 5% TCA (Trichloroacetic acid). The tubes were centrifuged at 3500 g for 5 minutes. The mixture was added with 500 μL of 10mM DNPH and 500 μL of 4% thiourea and incubated at room temperature for 3 hours. Postincubation 2.5 mL of 85% sulfuric acid was added and the absorbance was measured at 530 nm. The result was expressed in milligrams per gram of ascorbic acid.

2.8. Statistical analysis

All experiments were performed in three different sets with each set duplicated. The data are expressed as the mean ± the standard deviation (SD).

3. Results and discussion

3.1. Inhibition of α-amylase and α-glucosidase

α-Amylase (EC 3.2.1.1) randomly cleaves the α-(1–4) glycosidic linkages of amylase to yield dextrin, maltose, or maltotriose,[49] whereas α-glucosidase (EC 3.2.1.20) hydrolyzes the terminal nonreducing 1-4 linked α-glucose to release glucose molecules.[50] Incubation of the α-amylase with the substrate leads to the generation of maltose; however, the addition of the ADJ6 [inhibitory effect (IC_{50}), 0.41 ± 0.03 mg/mL] significantly inhibited the liberation of maltose in a dose-dependent manner, compared with acarbose (IC_{50}, 0.2 ± 0.01 mg/mL) (Fig. 1). The IC_{50} of α-glucosidase was 0.51 ± 0.01 mg/mL, whereas that of acarbose was 0.39 ± 0.02 mg/mL (Fig. 2).

3.2. Phytochemical analysis

The aqueous extract of the formulation also showed the presence of major phytochemical constituents such as phenols, flavonoids, steroids, cardiac glycosides, and saponins (Table 2).

3.3. Total phenol, flavonoid, and vitamin C content

The aqueous extract of the formulation was estimated for total phenol, flavonoids, and vitamin C content. The amount of phenols

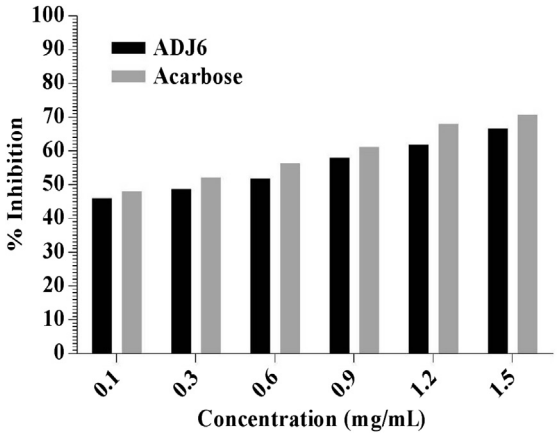

Fig. 1. α-Amylase inhibition assay.

was 340 ± 2.5 mg/g equivalents of gallic acid. The amount of flavonoids was 235.4 ± 8.3 mg/g of quercetin equivalents, whereas the level of vitamin C was the highest at 470.8 ± 16.6 mg/g of ascorbic acid equivalents (Fig. 3).

The analyses suggested that the IC_{50} values of ADJ6 against α-glucosidase and α-amylase at various concentrations were less than those for the standard drug acarbose. Acarbose is a potent inhibitor of α-amylase and α-glucosidase, but adverse effects have occurred in the gastrointestinal tract because of excessive inhibition of the amylase enzyme that results in flatulence and diarrhea.[51] Use of acarbose for the treatment of diabetes by dietary control has reportedly resulted in hepatitis.[52–54] Acarbose treatment also elevates liver enzyme levels, whereas its withdrawal normalizes levels.[55]

There has recently been a push to find an alternative source of treatment for type 2 diabetes, especially from medicinal plants that have high potency and fewer adverse effects than preexisting drugs.[56–61] Excessive inhibition of pancreatic amylase can lead to abnormal bacterial fermentation of carbohydrate foods in the colon, which may lead to adverse digestive disorders.[51,62] Plant-based compounds and products derived from natural sources have been reported for their potent inhibitory activities of the enzymes, but have fewer or no side effects.[56–61] The polyherbal formulation ADJ6, although it has slightly less inhibitory activity than acarbose, will likewise have fewer or no adverse effects because it is completely derived from plant sources. Because ADJ6 is rich in phytochemical constituents (i.e., phenols, flavonoids, and vitamin C), it will be helpful in the management of diabetes and will

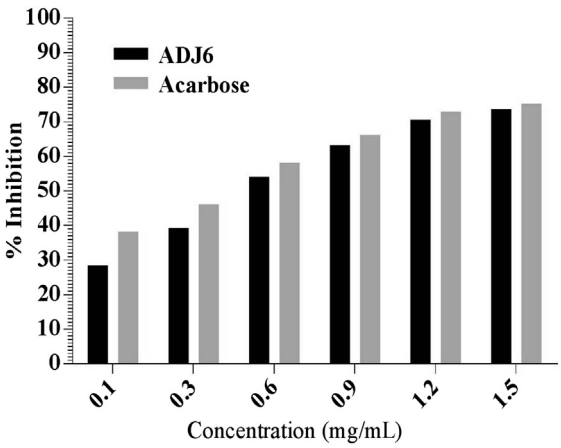

Fig. 2. α-Glucosidase inhibition assay.

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Table 2
Phytochemical analysis.

Name of the constituent	Inference
Amino acids	–
Steroids	+
Cardiac Glycosides	+
Phenols	+
Tannins	–
Terpenoids	–
Alkaloids	–
Flavonoids	+
Saponins	+
Carbohydrates	–
Reducing sugar	+
Anthrones	–

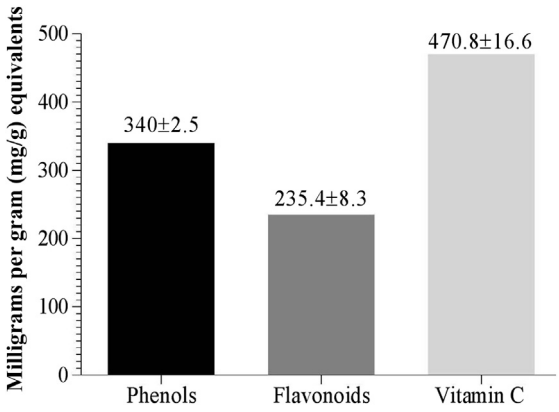

Fig. 3. Estimation of phenols, flavonoids, and vitamin C.

decrease the risk of other chronic disorders that are associated with diabetes.

The present study elucidates that the polyherbal formulation ADJ6 can be used for effectively managing type 2 diabetes mellitus. The activities of the individual components of the plants are well known for their insulin mimetic role and for their role in reducing insulin resistance.

4. Conclusion

The plants that are used in the formulation have been long used for food and medicinal purposes. Our results suggest that ADJ6 inhibits the enzymes α-amylase and α-glucosidase and contains a high amount of phytochemical constituents (i.e., total phenols, flavonoids, and vitamin C). Thus, ADJ6 may be used in the management of type 2 diabetes mellitus with few or no side effects. However, further studies of ADJ6 using *in vitro* and *in vivo* models need to be performed to elucidate its insulin mimetic activity and reduction of insulin resistance.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtcme.2014.12.006.

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