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Original article

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

Anand Duraiswamy [a], Devanand Shanmugasundaram [a], Changam Sheela Sasikumar [a, *], Sanjay M. Cherian [b], Kotturathu Mammen Cherian [b]

[a] Department of Cellular and Molecular Biochemistry, Frontier Mediville (A Unit of Frontier Lifeline and Dr. K. M. Cherian Heart Foundation), Affiliated to University of Madras, Chennai, Tamil Nadu, India
[b] Department of Cardiothoracic Surgery, Frontier Lifeline Hospital, Chennai, Tamil Nadu, India

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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

* Corresponding author. Frontier Mediville (A Unit of Frontier Lifeline and Dr. K. M. Cherian Heart Foundation), R-30-C, Ambattur Industrial Estate Road, Mogappair, Chennai, Tamil Nadu, India. Tel.: +91 9283143681 (mobile).
E-mail address: sheelsasic@yahoo.co.in (C.S. Sasikumar).

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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