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EVALUATION OF IN-VITRO ANTIDIABETIC ACTIVITY OF ALOE VERA

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ABSTRACT

Diabetes mellitus is a complex, irreversible metabolic illness that affects insulin secretion, function, or both, and raises blood sugar levels. Even now, acarbose and voglibose, either alone or in combination with insulin, are used as inhibitors of the enzymes that break down carbohydrates. Nevertheless, these substances have been linked to adverse side effects. Therefore, this study was conducted with the main purpose to evaluate the in-vitro antidiabetic potential of methanolic leaf extract of Aloe vera. Results depicted that the IC_{50} value of methanolic leaf extract of Aloe vera was found to be 8.94 mg/mL in alpha-amylase inhibition activity which was comparable with that of IC_{50} value standard antidiabetic drug acarbose (5.84 mg/mL). Similarly, IC_{50} value of methanolic leaf extract Aloe vera in alpha-glucosidase inhibition assay was found to be 9.93 mg/mL, and which was also comparable with that of standard antidiabetic drug acarbose (8.56 mg/mL). The total phenolic quantity was found to be highest (36.43 mgGAE/g extract) in methanolic leaf extract of Aloe vera when compared with total flavonoid quantities (1.68 mg QE/g extract). Inconclusion, this study demonstrated the anti-diabetic potential of methanolic leaf extract of Aloe vera. Hence, Aloe vera with the natural properties could be considered in for prevention of diabetes without any side effects.

Keywords: Diabetes mellitus, Allopathy, Aloe vera, Leaf extract, Antidiabetic, Alpha-amylase, Alpha-glucosidase

INTRODUCTION

Since the beginning of human civilization, medicinal plants have played a crucial role in the health and healing of humans. Both the ancient and modern medical systems still mainly rely on plants as a source of medication, despite notable advances in allopathic medicine during the 20th century. Most people on the planet, who live in developing countries, get their primary medical care from traditional medicine and herbal remedies.[1,2]

Diabetes is an important human ailment afflicting many from various walks of life in different countries. Diabetes mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrate, fat and protein. According to estimates from the

World Health Organization, there are 171 million people worldwide who have diabetes mellitus; however, this report projects that number to rise to 366 million by 2030.[3] Allopathic drugs used for the treatment of diabetes have their own side effect and adverse effects like hypoglycaemia, nausea, vomiting, hyponatremia, flatulence, diarrhoea or constipation, alcohol flush, headache, weight gain, lactic acidosis, pernicious anaemia, dyspepsia, dizziness, and joint pain.[4] So instead of allopathic drugs, herbal drugs are a great choice which is having more or less no side effect and adverse effects.[5]

Use of native ethnobotanical medicine is an integral part of Indian tradition particularly for the diabetes mellitus treatment since ages.[6] Furthermore, according to reports, up to 8000 plants can be used in the Ayurvedic, Homeopathic, Siddha, Unani, and Tibetan medical systems.[1,2] The usage of herbal and natural drug products for the treatment of diabetes is growing worldwide.[7] One such medicinal plant is Aloe barbadensis Miller, commonly known as Aloe vera (Family: Aloeaceae) (Figure 1). It has been used for centuries for its health, beauty, medicinal and skin care properties.[8]

Figure 1. Showing Aloe vera plant

Aloe vera has been reported to have significant therapeutic effects such as antidiabetic, antioxidant, anticancer, anti-inflammatory, antibacterial, antifungal properties, and these have been attributed to synergistic effects of numerous bioactive compounds in Aloe vera.[9] Hence, in the current we aimed to evalute the in-vitro anti-inflammatory activity of leaf extract of Aloe vera.

MATERIALS AND METHODS

Collection Leaves of A. vera

The leaves of A. vera were collected in and around district headquarter places of Karnataka. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[10]

Extraction

Approximately 50 g of dried and coarsely powdered leaves of A. vera were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extracts were concentrated by distilling the solvents in a rotary flash

evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.[10]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the methanolic leaf extract of Aloe vera was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[11] The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in methanolic leaf extract of Aloe vera.[12] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay of methanolic leaf extract of Aloe vera was carried out by the method of 13-Miller, (1959).[13] Methanolic leaf extract of Aloe vera and standard drug acarbose (2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL and 10mg/mL) were incubated for 10 minutes at 25°C with 500 µL of 20 mM sodium phosphate buffer (pH 6.8) with 20 µL of amylase (1U/mL). After pre-incubation, each tube was added with 1 mL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 15 min. One mL DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10mL) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation of the control samples. The following formula was used to determine the percent inhibition of alpha-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Alpha-glucosidase inhibition assay

The alpha-glucosidase inhibition assay of methanolic leaf extract of Aloe vera was carried out as described by Matsui et al (1996) with slight modifications.[14] The different concentrations of methanolic leaf extract of Aloe vera and standard drug acarbose (2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL and 10mg/mL) were prepared. Phosphate buffer (1 mL; 100mM, pH 6.8) and 80 µL of test methanolic leaf extract of Aloe vera / acarbose of concentrations (2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL and 10mg/mL) were added to 20 µL of alpha-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG- 50µL (5mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5mL of 0.1 M Na_2CO_3. The absorbance was measured at 400nm to determine the activity of alpha-glucosidase activity. The following formula was used to determine the percent inhibition of alpha-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

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RESULTS

The total phenolic quantity was found to be highest (36.43 mgGAE/g extract) in methanolic leaf extract of Aloe vera when compared with total flavonoid quantities (1.68 mg QE/g extract).

Table 1: Quantitative estimation of phytochemicals in methanolic leaf extract of Aloe vera

Phytochemicals	Methanolic leaf extract of Aloe vera
Total Phenolics	36.43 mgGAE/g extract
Total flavonoids	1.68 mgQE/g extract

Values are expressed mean; n=3

The methanolic leaf extract of Aloe vera at a concentration range of 2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL and 10mg/mL, shown inhibition effect of 6.67%, 17.17%, 28.72%, 40.38%, and 53.60% respectively in alpha-amylase inhibition activity (Table 2). The IC_{50} value of methanolic leaf extract of Aloe vera was found to be 8.94 mg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 5.84 mg/mL.

Table 2: Effect of methanolic leaf extract of Aloe vera on alpha-amylase inhibition activity

Conc. of methanolic leaf extract of Aloe vera (mg/mL)	Inhibition (%)	Conc. of Acarbose (mg/mL)	Inhibition (%)
2	6.67	2	30.46
4	17.17	4	42.37
6	28.72	6	51.17
8	40.38	8	62.76
10	53.60	10	77.23
IC_{50} (mg/mL) = 8.94		IC_{50} (mg/mL) = 5.84	

Values were expressed Mean; n=3

The methanolic leaf extract of Aloe vera at a concentration range of 2mg/mL, 4mg/mL, 6mg/mL, 8mg/mL, and 10mg/mL, shown inhibition effect of 9.69%, 19.72%, 28.52%, 35.63%, and 54.29% respectively in alpha-glucosidase inhibition activity. The IC_{50} value of methanolic leaf extract of Aloe vera was found to be 9.93 mg/mL in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 8.56 mg/mL (Table 3).

Table 3: Effect of methanolic leaf extract of Aloe vera on alpha-glucosidase inhibition activity

Conc. of methanolic leaf extract of Aloe vera (mg/mL)	Inhibition (%)	Conc. of Acarbose (mg/mL)	Inhibition (%)

2	9.69	2	20.83
4	19.72	4	31.91
6	28.52	6	36.63
8	35.63	8	51.62
10	54.29	10	64.04
IC_{50} (mg/mL) = 9.93		IC_{50} (mg/mL) = 8.56	

Values were expressed Mean; n=3

DISCUSSION

Several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. The greatest bioresource for pharmaceutical intermediates, modern and traditional medicine, nutraceuticals, food supplements, folk remedies, and chemical entities for synthetic drugs is found in medicinal plants.[15,16] The therapeutic claim of Aloe vera covers a wide range of diseases. Diabetes is an important human ailment afflicting many from various walks of life in different countries. A more practical approach would involve a series of in-vitro prescreens before testing a potential new hypoglycaemic agent in animals. This is because there are no perfect models for type 2 diabetes, and there are social and financial constraints on the extensive use of animals in experimentation. Furthermore, alpha-amylase and alpha-glucosidase inhibitors have become a new treatment strategy to combat diabetes mellitus.[17] Hence the present study was conducted with the main purpose to evalute in-vitro antidiabetic activity of methanolic leaf extract of Aloe vera.

Alpha-amylase and alpha-glucosidase are the enzymes responsible for digestion of carbohydrates and increasing the postprandial glucose levels in diabetic patients. Inhibiting their activity could help in controlling postprandial hyperglycemia.[18] Results of our study revealed IC_{50} value of Aloe vera 8.94 mg/mL in alpha- amylase inhibition activity which was comparable with that of IC_{50} value standard antidiabetic drug acarbose (5.84 mg/mL). Similarly, IC_{50} value of methanolic leaf extract of Aloe vera in alpha-glucosidase inhibition assay was found to be 9.93 mg/mL, and which was also comparable with that of standard antidiabetic drug acarbose (8.56 mg/mL). In concurrence with our study findings literature reports evidenced that Aloe vera leaf extract is effective in lowering hyperglycemia.[19,20] Another study reported the prevention of hyperglycemia in alloxan-treated rabbits by Aloe vera extract.[21] Moreover Mohamed et al., also reported that treatment with Aloe vera juice filtrate was also confirmed to have led to considerable improvements in diabetic rats serum glucose compared with nondiabetic control.[22] The data of a clinical experiment on the antidiabetic activity of Aloe vera juice by Yongchaiyudha et al., depicts the antidiabetic property of Aloe vera gel as the considerable decrease was seen in the patients treated daily with one table spoonful of Aloe juice twice a day for 42 days consecutively.[23] Furthermore, treatment with Aloe vera juice filtrate was also confirmed to have led to considerable improvements in diabetic rats' serum glucose compared with nondiabetic control.[22]

The Rajasekaran et al., demonstrated that extracts of Aloe vera gel can not only normalize blood glucose and serum insulin but also decrease levels of triglycerides, cholesterol, and free fatty acids in the liver, urine, and streptozotocin-induced kidney diabetes rats.[24] Multiple mechanisms have reported antidiabetic effects of Aloe vera plant parts. Mechanism includes that alloxan acts as a cytotoxic agent on the insulin secreting β cells of pancreas and effectively induces diabetes mellitus in a wide variety of animal models which share many features with that of human type. The influence of pseudoprototinosaponin AIII as well as prototinosaponins AIII on the uptake of glucose and release of insulin states that hypoglycemic properties exist because of the activities on hepatic gluconeogenesis or glycogenolysis.[25] Furthermore, frequent quantities of Aloe vera doses in diabetic mice have shown the hypoglycemic influence by stimulating the synthesis of insulin in the beta cells of pancreas.[26]

CONCLUSION

In conclusion, results of the present study demonstrated the anti-diabetic potential of methanolic leaf extracts of Aloe vera. Hence, Aloe vera with the natural properties could be considered for prevention of diabetes without any side effects.

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