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Research Article

SCREENING OF PHYTOCHEMICALS AND *IN VITRO* ANTIDIABETIC ACTIVITY OF *BAUHINIA RACEMOSA* LAM. LEAVES

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ABSTRACT

Objective: In this study, the leaves of medicinal plant *Bauhinia racemosa* Lam. with different pharmacological activities were subjected to phytochemical screening and assessment of their *in vitro* inhibitory potential with porcine pancreatic α-amylase enzyme to treat and management of diabetes.

Methods: Plant leaves were extracted sequentially with ethanol solvent. A modified 3,5-dinitrosalicylic acid method was adopted to screen α-amylase inhibition assay. The ethanol extract was analyzed qualitatively and gas chromatography–mass spectrometry analysis technique for the active phytoconstituents according to the standard protocols.

Results: A phytochemical screening of leaves extract reveals the presence of carbohydrate, alkaloids, saponin, glycosides, steroids, tannins, flavonoids, triterpenoid, and phenolic compounds. The ethanol extract reported inhibition of α-amylase enzyme activity at IC_{50} value 61.72 ± 0.03 µg/mL and acarbose as a standard drug at IC_{50} value 28.07 ± 0.02 µg/mL.

Conclusion: The results of the study indicate that *B. racemosa* Lam. leaves contain some of bioactive phytochemicals might to be exhibiting *in vitro* antidiabetic activity, which was leading to decreases the rate of starch digestion.

Keywords: *Bauhinia racemosa* Lam., Pharmacological, α-amylase, Phytochemical, Antidiabetic.

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INTRODUCTION

A chronic metabolic disorder of insulin deficiency or ineffectiveness is resulted into diabetes mellitus. It causes a global health burden on public and predictions of it estimate that India, China, and the United States will have the highest number of diabetic people up to the year 2030 [1]. Antidiabetic agents synthetically prepared could be produced serious side effects [2]. The search for plant-based medicine for control of diabetes mellitus continues, and the World Health Organization has also recommended about herbal treatment on diabetes mellitus [3].

Medicinal plants were reporting traditionally antidiabetic activity [4]. These contain active chemical constituents are inhibited α-amylase enzyme, and it prevents dietary starches from being digested and absorbed by the body. Antinutrient acting α-amylase inhibitors are responsible to control rate of starch digestion and absorption. Furthermore, potentially become useful in control of obesity and diabetes like disorders. Hence, they are useful for treating diabetes mellitus Type-II.

The *Bauhinia racemosa* Lam. plant is exhibits various pharmacological activities reported as analgesic, antipyretic, anti-inflammatory [5], antispasmodic [6], anthelmintic, antimicrobial activity [7], and antioxidant [8,9] as well. Due to the presence of several active phytoconstituents [10], this synthesizes primary and secondary metabolites having benefits in traditional system of medicine [11].

Various scientific studies have confirmed the beneficial effect of plants with antidiabetic activity in the traditional management of diabetes mellitus [12] on streptozotocin and alloxan-induced diabetic animal models [13].

The study has been reported that different plant isolates possess vast potentiality of leaves ethanol extract *in vitro* antidiabetic activity. Therefore, the leaves ethanol extract of *B. racemosa* Lam. plant was subjected to study phytochemical screening and assessment of their *in vitro* antidiabetic potential.

METHODS

Collection of plant material

The *B. racemosa* Lam. plant leaves were collected from local area identified and authenticate with the help of botanist from our institute. The plant leaves rinsed with distilled water and dried in shade at room temperature.

Preparation of extract

The air-dried leaves of *B. racemosa* Lam. were finely crushed and powdered. 10 g of powdered plant material was dissolved in 100 mL of ethanol and kept on a magnetic stirrer for 2 h. Thereafter, it was extracted using a Soxhlet apparatus sequentially with ethanol. The extract was collected and the solvent was evaporated out to dryness. The obtained material was stored at 4°C in airtight bottles for further screening studies.

In vitro α-amylase inhibitory assay screening

A modified 3,5-dinitrosalicylic acid (DNSA) method was adopted to screen α-amylase inhibition activity, by quantifying the reducing sugar (maltose) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated [14,15].

Phytochemical screening

The ethanol extract of *B. racemosa* Lam. was screened qualitatively for the active phytoconstituents such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, terpenoids, and phenolic compounds according to the standard protocol [16,17].

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis was carried out on Shimadzu GC–MS model number QP 2010S. The column Rxi-5Sil MS, 30 meter length, 0.25 mm ID, and 0.25 µm thickness, was used. The organic compounds were identified by comparison of mass spectra with the inbuilt libraries NIST-11 and WILEY-8.

Statistical analysis

The experimental tests were performed triplicate in three sets and the results expressed in mean ± SD. Values of $p<0.05$ were considered as statistically significant.

RESULTS

The ethanol extract of *B. racemosa* Lam. leaves results showed that it exhibited dose-dependent porcine pancreatic α-amylase inhibitory activities by *in vitro* assay using potato starch as substrate.

***In vitro* alpha-amylase inhibition assay**

The antidiabetic activity was investigated through the inhibition of α-amylase, an enzyme that made the digestion of starch and so reduced the glucose absorption. Acarbose is a standard drug at a concentration of 20–100 µg/mL showed α-amylase inhibitory activity from 47.17% to 68.81% with an IC_{50} value 28.07 ± 0.02 µg/mL, whereas ethanol extract (20–100 µg/mL) of *B. racemosa* Lam. exhibited potent α-amylase inhibitory activity in a dose-dependent manner from 41.91% to 54.78% with an IC_{50} value of 61.72 ± 0.03 µg/mL (Table 1 and Fig. 1).

Phytochemical screening

The qualitative phytochemical screening of the ethanol extract confirms the presence of alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, and phenolic compounds as shown in Table 2.

GC–MS analysis

A GC–MS of the ethanol extract was carried out up to maximum 44.0 min, which shows six different peaks. The first peak appears at 16.536 min retention time. The maximum peak area was found at retention time 21.276 min as in chromatogram (Fig. 2). The library search shows six compounds (Table 3); out of these, neophytadiene is maximum amount. It will be interesting to investigate further the bioactivity of this compounds when isolated in pure form.

Table 1: % α-amylase inhibition assay of standard and *B. racemosa* Lam. extract

Concentration in µg/mL	% inhibition of standard	% inhibition of extract
20	47.17±0.04	41.91±0.02
40	54.38±0.02	43.86±0.01
60	59.45±0.02	49.90±0.02
80	64.32±0.00	52.05±0.03
100	68.81±0.01	54.78±0.05
IC50 value (µg/mL)	28.07±0.02	61.72±0.03

B. racemosa: Bauhinia racemosa

Table 2: Phytochemical screening of *Bauhinia racemosa* Lam. extract

Phytochemicals	Result
Alkaloid	+
Carbohydrate	+
Protein and amino acids	+
Glycoside	+
Tannin	+
Saponin	+
Flavonoids	+
Steroids	+
Triterpenoids	-
Phenolic compounds	+

+: For present, -: For absent

DISCUSSION

In vitro antidiabetic activity was screened using α-amylase inhibitory assay modified DNSA method. As compared to standard drug ethanol extract of *B. racemosa* Lam. leaves showed significant antidiabetic activity. The ethanol extract reported inhibition of α-amylase at IC_{50} value 61.72 ± 0.03 µg/mL and standard at IC_{50} value 28.07 ± 0.02 µg/mL (Fig. 1).

Leaves extract of *B. racemosa* Lam. has been studying for various pharmacological activities and reported significantly [18]. It contains some isolated coumarins (scopoletin and scopolin) and flavonols (kaempferol and quercetin) compounds [19]. They played major role against various disease treatments. It has been proved by different animal models studies which give many links to develop the future trials [20].

Phytochemical analysis of the extract revealed the presence of various bioactive components such as alkaloids, carbohydrate, protein, amino acids, glycoside, tannins, saponin, flavonoids, steroids, and phenolic compounds [21-24].

B. racemosa Lam. extract possesses significant antidiabetic activity by reducing blood glucose level of STZ-induced diabetic rats [25]. It also exhibits effective normalization in adipose tissues and lipid level. Therefore, the extract showed significant antiadipogenic and hypolipidemic effect. Pharmacologically, it may be useful for the development of new potent herbal medicine for diabetes. These potential effects of extract are used for the treatment of diabetes and related complications [26].

The effective bioactive compounds which are responsible for inhibition were studied by GC–MS analysis technique (Fig. 2). These were reported as phenol,2,4-bis(1,1-dimethylethyl)-, mome inositol, neophytadiene, 6-octen-1-ol,3,7-dimethyl-,propanoate, 16-heptadecenal, and citronellyl butyrate (Table 3). Neophytadiene is previously reported as an enzyme inhibitor [27]. Extract has a potential role in the management of insulin resistance and metabolic disorders that accompany diabetes or obesity.

Thus, due to the existence of potent bioactive compounds in extract [28], it showed α-amylase inhibitory activity.

CONCLUSION

The α-amylase inhibitory activity showed by *B. racemosa* Lam. leaves ethanol extract has a significant role in management of diabetes. These overall activities due to bioactive phytochemicals were present in the extract. The leaves of *B. racemosa* Lam. could be a source of natural antidiabetic agent, which has contribution of most significant

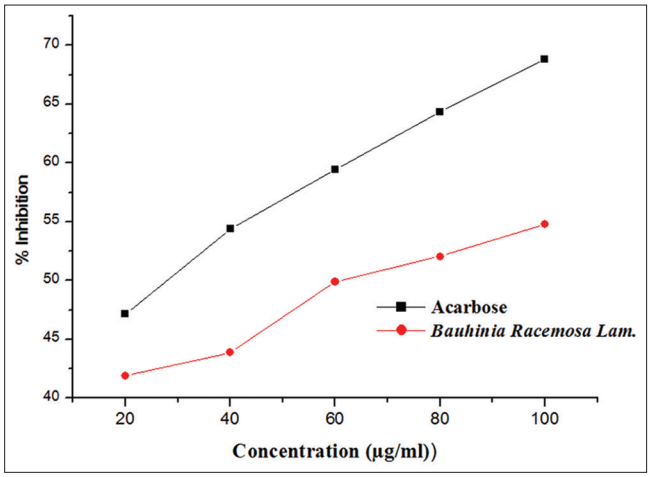

Fig. 1: α-amylase inhibition assay of Bauhinia racemosa Lam. leaves ethanol extract

Table 3: Phytochemicals detected in GC–MS analysis of *B. racemosa* Lam. extract

Peak #	R. time	Area	Area%	Height	Height %	Name	Base m/z
1	16.536	67449	9.02	40237	11.74	Phenol, 2,4-bis (1,1-dimethylethyl)-	191.10
2	19.026	209769	28.04	40409	11.79	Mome inositol	87.00
3	21.276	293491	39.23	157931	46.08	Neophytadiene	68.05
4	21.592	43257	5.78	26964	7.87	6-Octen-1-ol, 3,7-dimethyl-, propanoate	82.05
5	21.834	80268	10.73	47747	13.93	16-Heptadecenal	82.05
6	24.633	53838	7.20	29467	8.60	Citronellyl butyrate	43.00
		748072	100.00	342755	100.00		

GC–MS: Gas chromatography–mass spectrometry, *B. racemosa: Bauhinia racemosa*

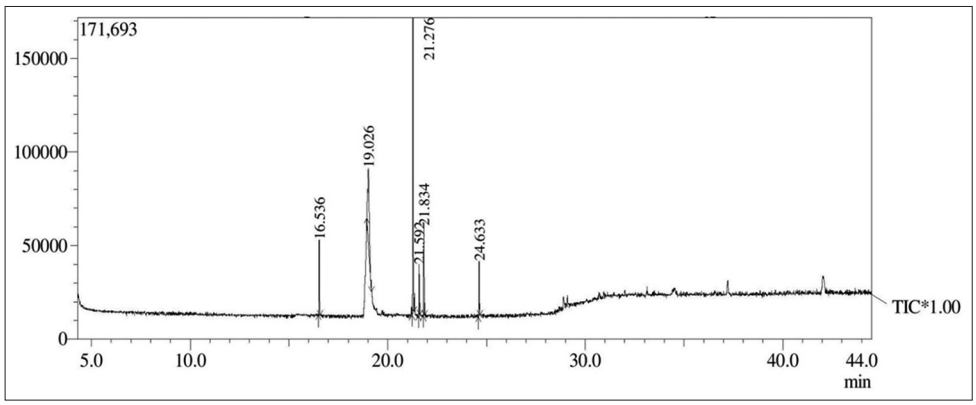

Fig. 2: Gas chromatography–mass spectrometry chromatogram of *Bauhinia racemosa* Lam. leaves crude extract

therapeutic agents responsible for prevent and management of Type-II diabetes. Thus, it was concluded that *B. racemosa* Lam. leaves ethanol extract showed *in vitro* antidiabetic activity. The more investigative study is proposed to validate these claims by separation, isolation, and identifying bioactive components with potential therapeutic value.

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AUTHOR'S CONTRIBUTION

Performed collection of sample, extraction, analysis, interpreted data, and wrote the manuscript. Supervised the progress of work, helped in the evaluation of the manuscript, and acted as corresponding author.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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