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Research Article

EVALUATION OF ANTIOXIDANT, A-GLUCOSIDASE AND A-AMYLASE INHIBITORY ACTIVITIES OF *ATALANTIA RACEMOSA* AND *SENNA UNIFLORA* LEAVES

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ABSTRACT

Objective: The current investigation was conducted to investigate the total phenol, total flavonoid content, antioxidant, α-glucosidase, and α-amylase activities in leaves of *Atalantia racemosa* and *Senna uniflora.*

Methods: Different crude solvent extracts were prepared and concentrated using rotary evaporator, these solvent extracts were tested to estimate the antioxidant radical scavenging activity using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2, 2'-azinobis, 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods using spectrophotometer. The extracts were screened by α-amylase inhibition assay and α-glucosidase inhibition assay to estimate the antidiabetic potential of the studied plant with the help of microplate reader.

Results: A potent antioxidant activity, i.e. mean percentage inhibition of DPPH and ABTS radical was observed in *A. racemosa* ethanol (71.5 ± 0.026%) and ethyl acetate extract (97.3 ± 0.076%), respectively, at the concentration of 400 µg/mL. Similarly, highest α-amylase and α-glucosidase inhibitory activities were observed in *A. racemosa* methanolic (82.4 ± 0.016%) and ethyl acetate 91.1±0.018 extracts, respectively.

Conclusions: The present study revealed that the *A. racemosa* extracts possessed good antioxidant, α-amylase and α-glucosidase inhibitory activities; hence, it can be used as a source of natural free radical scavenger and antidiabetic supplement(s). However, further study needs to be carried out to know the active compound and its mode of action.

Keywords: *Atalantia racemosa*, *Senna uniflora*, α-Amylase, α-Glucosidase, Antioxidant activity.

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INTRODUCTION

Diabetes mellitus (DM) has turned out to be one of the major and emerging public health problems of the world. There has been perpetuating increase in the number of diabetic patients almost in all countries, especially in India, which disreputably got nicknamed as the "diabetes capital of the world [1]." DM is not a disease, but a heterogeneous group of symptoms endorsing an endocrine disorder being driven by a defective and deficient process of insulin secretion or it is a group of metabolic alterations characterized by hyperglycemia caused by insulin secretion defects, action, or both and characterized by chronic hyperglycemia or increased blood glucose level with disturbances in carbohydrates, fat, and protein metabolism resulting from absolute or relative lack of insulin secretion [2]. It is estimated approximately 285 million people worldwide or 6.6% adults have diabetes, 70% of whom live in low- and middle-income countries. This number is expected to increase by more than 50% in the next 20 years if prevention programs are not put in place [3]. DM can be classified into two major categories, i.e., Type 1 DM (T1DM) and Type 2 (T2DM). T1DM is often genetically-associated and immune-mediated and often referred to as juvenile diabetes is insulin dependent [4]. Individuals with T1DM have an absolute deficiency in insulin secretion and can be identified by serological evidence of autoimmune-mediated destruction of pancreatic islets or by genetic markers. The most common type of diabetes, T2DM, accounts for 90–95% of those with diabetes. Individuals in this category can either have a predominantly insulin resistance with relative insulin deficiency [5].

Regardless of the type of diabetes, patients are required to control their blood glucose levels with medications and/or by adhering to an exercise program and a diet plan. The conventional available therapies for diabetes include stimulation of endogenous insulin secretion (sulfonylureas and meglitinides), insulin sensitizer (metformin and thiazolidinediones), oral hypoglycemic agents such as biguanides and sulfonylureas, and the inhibition of degradation of dietary starch by glycosidase enzymes such as α-amylase and α-glucosidase by inhibitors (miglitol and acarbose) [6,7]. However, they have prominent side effects and fail significantly to alter the causes of diabetic complications [8]. Synthetic α-glucosidase inhibitors (e.g., voglibose) cause's hepatic disorders and various negative gastrointestinal symptoms at high dose as carbohydrates blocked from absorption in the small intestine are fermented by bacteria in the colon [9].

Atalantia racemosa weight ex. Hook. is a small evergreen tree, belongs to family Rutaceae. An ethnopharmacological literature survey revealed that the plant has been traditionally used in the treatment snake bite [10], itching of skin, paralysis, and chronic rheumatism [11]. *A. racemosa* leaves decoction is used in the treatment of bronchitis, asthma and cough, bronchi, and blood purifier [12]. *Senna uniflora* (Mill.) H. S. Irwin and Barneby belong to family Caesalpiniaceae. The leaves are used as poultices for wounds. The roots are used for combating dropsy [13]. A decoction of laxative of *A. racemosa* mature leaves is also useful in curing ringworm and skin diseases . The poultice of the leaves is applied to wounds and the extract of the leaves is reported to cure eczema. The roots are used to combat dropsy [15].

Despite being a long tradition of use for the treatment of various ailments, no systematic pharmacological work has not been carried out so far on this potentially useful plant. Thus, the present investigations were planned with an objective to evaluate the antioxidant activity in methanol, ethanol, chloroform, and ethyl acetate solvent extracts of the plant using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2, 2'-azinobis, 3-ethylbenzothiazoline-6-sulfonic (ABTS) methods. Further, α-glucosidase and α-amylase inhibitory assays were performed to evaluate the antidiabetic potential of *A. racemosa* and *S. uniflora*. The present study will be helpful to the species knowledge and due to the

presence of antioxidant and antidiabetic inhibitory activities, in human health applications as well as in pharmaceuticals industries, herbal medical practitioners; bush doctors (Vaidoos) and researchers.

METHODS

Plant materials were collected from in and around Pune district. Efforts were made to collect plant material in flowering and fruiting conditions for the correct botanical identification. The collected plants were identified with the help of the flora of the presidency of Bombay [16], Flora of Maharashtra State Vol I [17]. The identified plants were authenticated from the authorities of Botanical Survey of India (Ref. No. BSI/WRC/IDEN.CER./2017/Dated 24-05-2017), Pune-1, Maharashtra state.

Preparation of extracts

Freshly collected plant samples (50 g) were chopped and placed in the filter paper (No. 89) in a classical Soxhlet apparatus and successively extracted with 170 mL of chloroform, ethanol, methanol, and ethyl acetate solvents for 3 h. Extracts were cooled at room temperature. The extracts were filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure by the rotary evaporator. These extracts were used in the present study.

DPPH free radical scavenging activity

DPPH radical scavenging activity was determined according to the method of Tekao *et al*. [18] adopted with suitable modifications [19]. The DPPH (Hi-Media) stock solution was prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions of plant extracts in various solvents were made to obtain concentrations of 100–400 µg/mL. Diluted plant extracts (1 mL each) were allowed to react with 1 mL of a methanolic solution of DPPH in the concentration of 1 mg/mL at room temperature. After 30 min incubation in darkness at room temperature (23°C), the absorbance values were measured spectrophotometrically at 517 nm against the blank [20]. Control sample contained all the reagents except the extract. Percentage inhibition was calculated using equation= [(A control – A sample)/A control] × 100, where A control is the absorbance of negative control and A sample is the absorbance of the reaction mixture, while inhibitory concentration 50 (IC_{50}) values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. Ascorbic acid was used as positive control. The lower the IC_{50} value indicates high antioxidant capacity. All tests were performed in triplicate.

ABTS free radical scavenging activity

ABTS radical scavenging activity was performed by following the method of Re *et al*. [21] with some modifications. ABTS (Hi-Media) radical cation was produced by reacting ABTS stock solution 7 mM with 2.45 mM potassium persulfate (final concentration) by dissolving in distilled water (1:1) ratio and allowing the mixture to incubate 16-20 h for the formation of ABTS radical cation at room temperature [22]. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 at 734 nm.

Dilutions of plant extracts in various solvents were made to obtain concentrations of 100-400 µg/mL. The plant extract at various concentrations with 1 mL of ABTS solution was homogenized and its absorbance was recorded spectrophotometrically (Sican 2301, Inkarp). At 734 nm. with the help of spectrophotometer(Sican 2301, Inkarp) by keeping ethanol as a blank. Trolox was used as positive control. As for the antiradical activity, ABTS scavenging activity was expressed as IC_{50} (µg/mL). The percent inhibition of ABTS radical scavenging activity was calculated using the following formula: ABTS scavenging activity (%) = (A0-A1)/A0 ×100 where A0 is the absorbance of the negative control, and A1 is the absorbance of the sample.

Determination of α-amylase inhibitory activity

The α-amylase inhibitory activity was measured by following the method of Adisakwattana *et al*. [23] with some modification. Porcine pancreatic α-amylase (4 units/ml) MP Biomedicals (Cat. No: 191239) was dissolved in 0.1 M phosphate buffer saline, pH 6.9 [24]. Plant extracts stock solutions for inhibition assay in various solvents were made to obtain concentrations of 100–400 µg/mL and were added to a solution containing starch (1 g/L) and phosphate buffer (165 µL). The reaction was initiated by adding an enzyme solution (75 µL) to the incubation medium. After 10 min of incubation, the reaction was terminated by adding 250 mL dinitrosalicylic (DNS) reagent (1% 3, 5- DNS acid, 0.2% phenol, 0.05% Na_2SO_3, and 1% NaOH in aqueous solution). The mixtures were heated at 100°C for 10 min to stop the reaction. Thereafter, 250 µL of 40% potassium sodium tartrate solution was added to the mixtures to stabilize the color. After cooling to room temperature, the absorbance was recorded at 540 nm using a microplate reader (EnSpire® Multimode Plate Reader). Acarbose was used as positive control.

Inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\%\text{inhibition of } \pm\text{-amylase} = \frac{(\text{Absorbance of control})-(\text{Absorbance of sample})}{(\text{Absorbance of sample})} \times 100$$

etermination of α-glucosidase inhibitory activity

α-glucosidase inhibitory activity was measured using p-nitrophenyl a-D-glucopyranoside as the substrate [25]. α-glucosidase (Sigma Chemical Co St. Louis M.O. USA) solution (0.006%) was prepared in 0.02 M phosphate buffer (pH 6.3). The enzyme solution (0.13 mL) was incubated with extract (0.13 mL) and 0.02 M phosphate buffer (0.45 mL for 1 h at 25°C). After preincubation, 2 M p-nitrophenyl o-D-glucopyranoside (0.67 mL) was added to the reaction mixture. The mixture was then incubated for another 30 min at 30°C. The reaction was terminated by adding 1 M Na_2CO_3 solution (2 mL). Determination of the amount of p-nitrophenol formed was read using a microplate reader at 405 nm [26].

Inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\%\text{inhibition of } \pm\text{-glucosidase} = \frac{(\text{Absorbance of control})-(\text{Absorbance of sample})}{(\text{Absorbance of sample})} \times 100$$

Determination of IC_{50} values

Regression equations were prepared from the concentrations of the extracts and percentage inhibition in different systems of the assay. IC_{50} values (concentration of inhibitor sample required to inhibit) were calculated from these regression equations. A lower IC_{50} value indicates higher inhibitory activity.

Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA, differences of $p<0.05$ were considered statistically significant. Results were expressed as mean ± SE using the GraphPad prism 5 version (Bonferroni).

RESULTS AND DISCUSSION

DPPH free radical scavenging activity

The DPPH radical scavenging assay was done for all the four solvent extracts of ethanol, methanol, chloroform, and ethyl acetate. In the present study, the inhibition percentage and IC_{50} values ranges from 24.3 ± 0.015 to 71.5 ± 0.026 and 0.9 ± 0.015 to 13.3 ± 0.036 µg/mL, respectively, inhibition percentage and IC_{50} values are depicted in Table 1. Among the four different concentrations of standard ascorbic acid (100, 200, 300, and 400 µg/mL) used in the study showed 70.3%, 87.1%, 89.6%, and 96.5% scavenging activity, respectively, where highest scavenging activity was recorded as 96.5 ± 0.025% at 400 µg/mL concentration Fig. 1. Both plants, *A. racemosa* as well as *S. uniflora*, exhibited an antioxidant activity in a dose-dependent manner. The ethanolic leaf extract of *A. racemosa* exhibited higher antioxidant activity 71.5 ± 0.026% as compared to *S. uniflora* leaf.

ABTS free radical scavenging activity

The four solvent extracts and standard tested for in antioxidant activity using the ABTS method. In the present study, the inhibition percentage and IC_{50} values ranges from 20 ± 0.075 to 97.3 ± 0.076 and 2.0 ± 0.022 to 3.8 ± 0.020 µg/mL, respectively, inhibition percentage and IC_{50} values are depicted in Table 2. Among the four different concentrations of standard trolox (100, 200, 300, and 400 µg/mL) used in the study showed 72.7%, 78.0%, 83.8%, and 91.7% scavenging activity Fig. 2, respectively, where highest scavenging activity was recorded as 91.7 at 400 µg/mL concentration (Table 2). Both plants, *A. racemosa* as well as *S. uniflora*, exhibited an antioxidant activity in a dose-dependent manner. The ethyl acetate extract of *A. racemosa* exhibited higher antioxidant activity 97.3 ± 0.076% with IC_{50} value 2.0 ± 0.022 as compared to *S. uniflora* leaf. However, the *S. uniflora* ethyl acetate extract was found to be more active than the standard trolox.

α-amylase inhibitory activity

In the present study, the inhibitory activities of the selected plant species extract on the rat intestinal α-glucosidase were determined with various concentrations (100-400 µg/mL). Acarbose was used as a standard reference drug Fig. 3, which showed α-amylase inhibitory activity 83 ± 0.017% µg/ml with an IC_{50} value 0.7 ± 0.015 at 400 µg/mL concentration (Table 3). The methanol extracts of *A. racemosa* (at a concentration 400 µg/mL) exhibited highest α-amylase inhibitory activity 82.4 ± 0.016% with IC_{50} value 0.5 ± 0.015 µg/mL compared with *S. uniflora* extracts and standard acarbose. It was also observed that the proportionate increases in the percentage of α-amylase inhibition by an

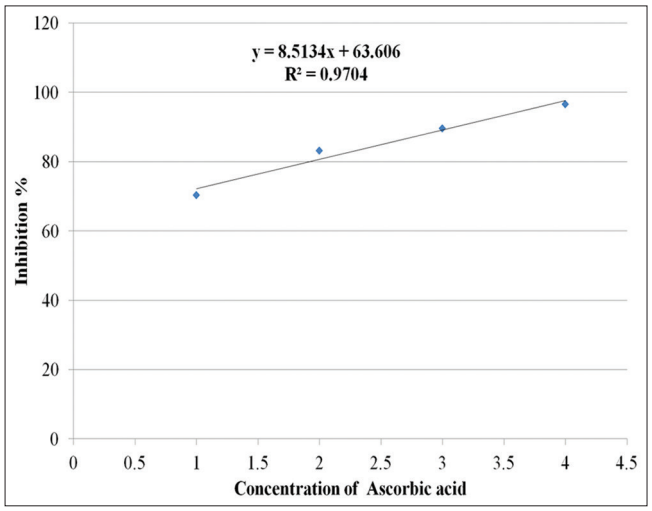

Fig. 1: Standard curve of extinction against ascorbic acid concentration

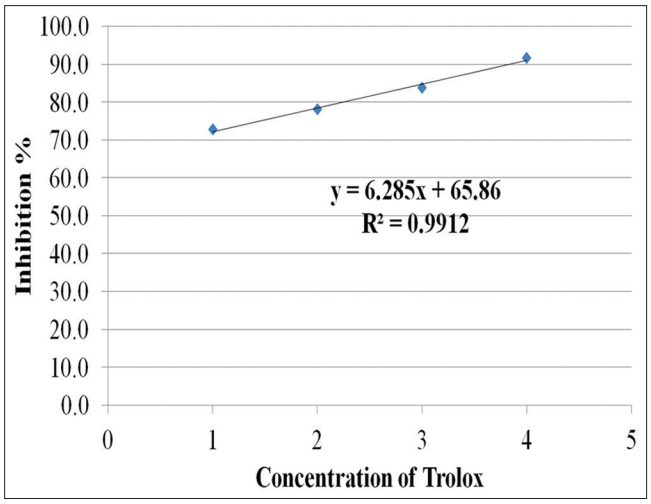

Fig. 2: Standard curve of extinction against trolox concentration

Table 1: DPPH free radical scavenging activity and IC_{50} values in different solvent extracts

Plant name	Extract	DPPH inhibition percentage µg/mL				IC_{50} values (µg/mL)
		100	200	300	400	
A. racemosa	Methanol	32.4±0.023	35.4±0.020	37.6±0.012	40.4±0.026	7.6±0.025
	Ethanol	57.4±0.017	65.2±0.029	68.3±0.023	71.5±0.026	0.9±0.015
	Chloroform	30.4±0.026	32.1±0.026	34.9±0.020	37.9±0.026	8.8±0.032
	Ethyl acetate	28.0±0.038	31.1±0.017	32.9±0.012	35.8±0.023	9.6±0.035
S. uniflora	Methanol	28.7±0.029	32.3±0.020	35.1±0.020	38.0±0.020	8.0±0.030
	Ethanol	24.3±0.015	26.9±0.015	29.4±0.018	38.4±0.025	7.0±0.026
	Chloroform	40.9±0.023	50.6±0.023	66.4±0.032	70.2±0.023	1.8±0.017
	Ethyl acetate	28.5±0.020	30.2±0.015	31.8±0.015	33.7±0.017	13.3±0.036
Ascorbic acid		70.3±0.017	87.1±0.012	89.6±0.018	96.5±0.025	1.5±0.025

**A. racemosa: Atalantia racemosa, S. uniflora: Senna uniflora.* IC_{50} values: Inhibitory concentration 50, DPPH: 1,1-diphenyl-2-picryl-hydrazyl, each value represents a mean±SE (n=3)

Table 2: ABTS free radical scavenging activity and IC_{50} values in different solvent extracts.

Plant name	Extract	ABTS inhibition percentage µg/mL				IC_{50} values (µg/mL)
		100	200	300	400	
A. racemosa	Methanol	73.0±0.029	84. 9±0.023	91.8±0.017	96.5±0.020	2.2±0.020
	Ethanol	75.8±0.023	81.5±0.010	90.1±0.017	93.3±0.023	3.3±0.030
	Chloroform	73.0±0.072	85.2±0.019	91.4±0.088	96.3±0.012	2.2±0.019
	Ethyl acetate	74.1±0.068	81.5±0.07	90.9±0.023	97.3±0.076	2.0±0.022
S. uniflora	Methanol	75.7±0.072	83.9±0.07	88.6±0.074	94.7±0.051	3.2±0.012
	Ethanol	75.4±0.096	80.2±0.010	86.7±0.021	93.9±0.055	3.0±0.018
	Chloroform	20.3±0.075	30.9±0.08	42.0±0.011	50.9±0.049	3.8±0.020
	Ethyl acetate	74.2±0.062	80.6±0.06	87.9±0.065	91.2±0.052	3.2±0.023
Trolox		72.7±0.016	78.0±0.018	83.8±0.017	91.7±0.015	2.5±0.027

**A. racemosa: Atalantia racemosa, S. uniflora: Senna uniflora.* IC_{50} values: Inhibitory concentration 50, ABTS: 2, 2'-azinobis, 3-ethylbenzothiazoline-6-sulfonic acid, each value represents a mean±SE (n=3)

Table 3: α-amylase inhibitory activity (inhibition %) and IC_{50} values in different solvent extracts

Plant name	Extract	α-amylase inhibition percentage µg/mL				IC_{50} values (µg/ml)
		100	200	300	400	
A. racemosa	Methanol	63.6±0.012	72.9±0.013	80.8±0.012	82.4±0.016	0.5±0.015
	Ethanol	45.7±0.013	57.8±0.012	66.6±0.011	77.0±0.059	1.3±0.015
	Chloroform	41.4±0.012	57.9±0.015	62.1±0.015	66. 0±0.019	1.6±0.011
	Ethyl acetate	60.2±0.015	69.9±0.015	74.4±0.017	79. 0±0.017	1.0±0.018
S. uniflora	Methanol	61.7±0.012	68.1±0.012	71.4±0.066	80.7±0.015	0.9±0.015
	Ethanol	41.2±0.014	55.3±0.09	59.1±0.025	61.9±0.012	1.8±0.018
	Chloroform	50.6±0.013	54.4±0.013	60.4±0.011	74.7±0.015	1.2±0.021
	Ethyl acetate	62.7±0.010	67.1±0.010	74.8±0.017	79.6±0.09	1.1±0.013
Acarbose		52.0±0.014	64.0±0.016	70.0±0.012	83.0±0.017	0.7±0.015

**A. racemosa: Atalantia racemosa, S. uniflora: Senna uniflora,* IC_{50} values: Inhibitory concentration 50, each value represents a mean±SE (n=3)

Table 4: α-glucosidase inhibitory activity (inhibition %) and IC_{50} values in different solvent extracts

Plant name	Extract	α-glucosidase inhibition percentage µg/mL				IC_{50} values (µg/mL)
		100	200	300	400	
A. racemosa	Methanol	69.1±0.07	73.6±0.020	78.9±0.012	84.6±0.012	2.6±0.013
	Ethanol	69.3±0.07	73.7±0.018	79.1±0.014	85.8±0.017	2.4±0.020
	Chloroform	68.5±0.014	82.5±0.015	87.1±0.015	88.9±0.015	2.3±0.022
	Ethyl acetate	65.6±0.023	78.9±0.015	88.4±0.016	91.1±0.018	1.1±0.020
S. uniflora	Methanol	68.1±0.015	77.4±0.015	81.8±0.014	87.4±0.013	2.1±0.023
	Ethanol	67.7±0.010	78.2±0.015	81.7±0.015	87.0±0.016	2.1±0.012
	Chloroform	67.6±0.07	76.9±0.012	79.2±0.013	82.8±0.018	2.9±0.011
	Ethyl acetate	69.5±0.011	79.5±0.012	87.1±0.024	88.9±0.021	2.2±0.017
Acarbose		40.0±0.013	64.3±0.015	70.3±0.014	83.0±0.012	1.4±0.018

**A. racemosa: Atalantia racemosa, S. uniflora: Senna uniflora*. IC_{50} values: Inhibitory concentration 50, each value represents a mean±SE (n=3)

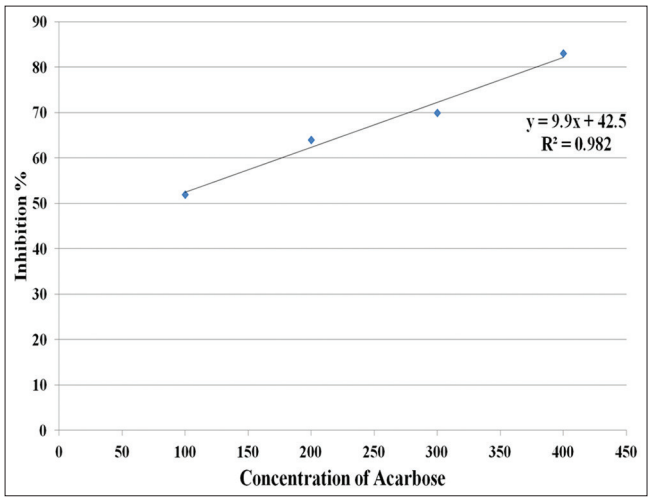

Fig. 3: Standard curve of extinction against acarbose concentration

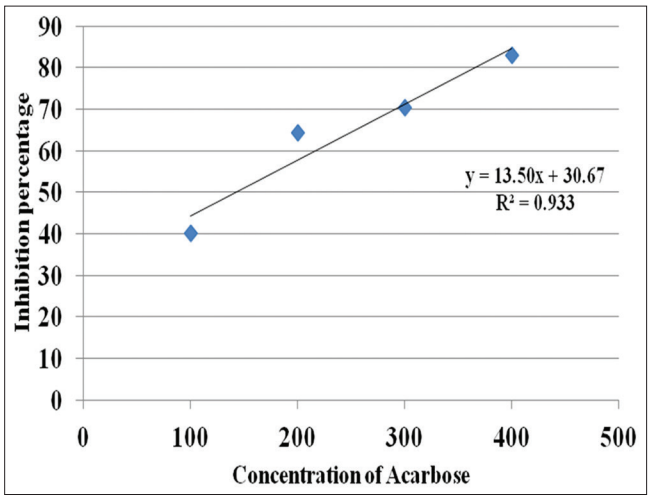

Fig. 4: Standard curve of extinction against acarbose concentration

increase in the concentration of methanolic extract of *A. racemosa* and standard acarbose.

α-glucosidase inhibitory activity

In the present study, the inhibition percentage and IC_{50} values ranges from 40.0 ± 0.013 to 91.1 ± 0.018% and 1.1 ± 0.020 to 2.9 ± 0.011 µg/mL, respectively. The inhibition percentage and IC_{50} values are depicted in Table 4. Among the plants studied, it was observed that ethyl acetate extract of *A. racemosa* showed the highest inhibitory activity 91.1 ± 0.018% against the enzyme with IC_{50} value 1.1 ± 0.020 µg/mL compared with *S. uniflora* and standard acarbose. The same pattern was observed in the α-glucosidase assay that proportionate increases in the percentage of α-glucosidase inhibition by an increase in the concentration of ethanolic extract of *A. racemosa* and standard acarbose Fig. 4.

DISCUSSION

The results of this study revealed that all the tested solvent extracts possess antioxidant activity. *A. racemosa* exhibited high antioxidant activity with a low IC_{50} value. Antioxidants derived from medicinal plants provide protection to cells by scavenging the excessive free radicals through offsetting ROS. This has been made possible due to the presence of certain bioactive substances; these compounds are might be responsible for antidiabetic activity in *A. racemosa*. Strong positive and significant correlation between radical scavenging activities with antidiabetic activities was observed. It has been also observed that the radical scavenging and antidiabetic activities increased with the increasing concentration of the plant extracts to a certain extent. Hence, it can be concluded that the antioxidant antidiabetic activities are said to be strongly dependent on the extract concentrations of the

studied plants. There is no previous literature available so far about the antioxidant and antidiabetic activities of the studied plant so the mechanism by which *A. racemosa* exerted action may be due to its action on carbohydrate binding regions of α-glucosidase enzyme, α-amylase, and endoglucanases that catalyze hydrolysis of the internal α-1, 4 glycosidic linkages in starch and other related polysaccharides have also been targets for the suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which then breaks down to glucose before absorption.

CONCLUSIONS

The present work shows that *A. racemosa* is a medicinal plant with antioxidant and antidiabetic activities which could be utilized in several medicinal applications because of its effectiveness. Hence, the results of the present study may be useful to traditional healers and pharmaceutical industries. The results from the present study also indicated that it would be highly economical for the production of potential antioxidant and antidiabetic supplement(s). Authors are now involved in the identification and isolation of active compounds responsible for the antioxidant and antidiabetic activities of the plant.

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CONFLICTS OF INTERESTS

All authors have none to declare.

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