INTRODUCTION

Diabetes mellitus (DM) has turned out to be one of the major and emerging public health problems of the world. There has been a 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 glucose blood 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, pananity, 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 (MIL.) H. S. Irwin and Barneby belong to family Caesalpinaceae. 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 acid (ABTS) methods using spectrophotometer. The results were screened by α-amylase inhibition assay and α-glucosidase inhibition assay to estimate the anti-diabetic potential of the studied plant with the help of microplate reader.
presence of antioxidant and antidiabetic inhibitory activities, in human health applications as well as in pharmaceutical industries, herbal medical practitioners; bush doctors (Vaidos) 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 1 [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 methods of Tekoe et al. [18] adopted with suitable modifications [19]. The DPPH (Hi-Media) stocks solution was prepared in methanol to achieve the concentration of 1 mM/L. 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 [% Inhibition = [(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 inhibition percentage 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 production was done by reacting ABTS stock solution 7 mM with 2.45 mM potassium persulphate (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 Z301, Inkarp). At 734 nm, with the help of spectrophotometer (Sican Z301, 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 μL dinitrosalycylic (DNS) reagent (1% 3,5-DNS acid, 0.2% phenol, 0.05% NaSO_{4} 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: % inhibition of α-amylase = ((Absorbance of sample) – (Absorbance of control))/ (Absorbance of control) × 100

**Determination of α-glucosidase inhibitory activity**

α-glucosidase inhibitory activity was measured using p-nitrophenyl α-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). Preincubation, 2 M p-nitrophenyl α-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_{2}CO_{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: % inhibition of α-glucosidase = ((Absorbance of sample) – (Absorbance of control))/ (Absorbance of control) × 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 23.3 ± 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 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
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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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

Table 3: α-amylase inhibitory activity (inhibition %) and IC₅₀ values in different solvent extracts

| Plant name | Extract   | α-amylase inhibition percentage μg/mL | IC₅₀ 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           |

A. racemosa: Atalantia racemosa, S. uniflora: Senna uniflora, IC₅₀ values: Inhibitory concentration 50, each value represents a mean±SE (n=3)

Table 4: α-glucosidase inhibitory activity (inhibition %) and IC₅₀ values in different solvent extracts

| Plant name | Extract   | α-glucosidase inhibition percentage μg/mL | IC₅₀ 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  | 60.1±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, IC₅₀ values: Inhibitory concentration 50, each value represents a mean±SE (n=3)
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.

ACKNOWLEDGMENTS

The authors are thankful to authorities of Savitribai Phule Pune University, for providing facilities and granting research fellowship.

CONFLICTS OF INTERESTS

All authors have none to declare.

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