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Bioactive molecules and the antidiabetic efficacy of *Memecylon randerianum* — an ethnomedicinal plant from the Western Ghats

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Diabetes is one of the most chronic diseases and a leading cause of death even though it is preventable. There are multiple therapeutic strategies like daily intake of drugs, insulin treatment and islet transplantation to manage severe diabetic complications. But the plant based therapy with regular exercise can improve diabetic control with reduced medication. The Malabar Memecylon, *Memecylon randerianum* S.M. Almeida & M.R. Almeida, is a medicinal shrub widely distributed in the Western Ghats of Kerala. Leaves are the most utilized plant part in traditional medicine. *In vitro* antidiabetic activity and HR-LCMS-QTOF analysis of the leaves, along with the screening of phytochemicals, has been executed in the present investigation. The methanolic extract showed the highest presence of phytochemicals such as flavonoids, tannins, saponins etc. The quantitative screening revealed that saponins (211.16±1.9 mg/g) and flavonoids (209.74±2.12 mg/g) were highly concentrated, and the leaf extract showed significant dose-dependent inhibition on α-amylase and α- glucosidase. The glucose uptake in L6 cell lines was found to be increased by 25% compared to untreated cells (control) at 100 µg/mL sample concentration. HR-LCMS-QTOF analysis detected nine major compounds and showed that leaves of the plant could be used as an effective therapeutic agent against the most common type 2 diabetes.

Keywords: Antidiabetic, Cosmosiin, Glucose uptake assay, HR-LCMS-QTOF, Malabar Memecylon

Diabetes is a common health problem characterized by elevated blood glucose levels due to defects in the action and secretion of insulin. According to World Health Organization's third global survey report, the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 and diabetes1 directly caused an estimated 1.5 million deaths. Generally, diabetic patients have the poor antioxidant capacity and high metabolic stress. This condition leads to a dramatic shoot up in the level of reactive oxygen species. Sometimes the patients need medication reduction or discontinuation due to ineffectiveness, intolerable side effects, and expense[2]. The financial burden due to diabetes in India is said to be higher in the world, and thereby it is necessary to adopt cost effective measures to control diabetic complications[3]. So the treatment of diabetes should be more effective with reduced side effects and affordability.

Medicinal plants are the major source for the preparation of many ethnomedicinal formulations since ancient times. Herbal therapy is extensively used for the management of many chronic diseases. The consumption of naturally derived products from plants is the most common habit among people all over the country. Researchers are extremely focused on the development of drugs using these natural products for the treatment of these types of diseases. Many of such herbal drugs like Diabecon, Diasulin, Pancreatic tonic 180 cp, Dia-care, Syndrex, Diabecure, Epinsulin, Diabeta, etc. were already formulated from different plants and made available in the market[4]. These drugs were developed by using individual plants, parts of different plants in different combinations. Generally, plant-based drugs have fewer side effects and low costs than synthetic drugs. For standardization of each drug, the main constituent that gives the medicinal property should be analyzed. Clinical studies should be conducted in animal models to use these drugs safely and effectively. It is important to keep a balanced blood glucose level in diabetic patients through sustained improvement in glycemic control[5]. Many medicinal plants and their compounds have been evaluated for their anti-glycemic properties[6]. The scientific evidence of herbal remedies is insufficient for their practical use in diabetes control[7]. Therefore, it is necessary to scientifically validate the plants extensively used in the traditional medicinal systems.

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Memecylon randerianum S.M. Almeida & M.R. Almeida (Melastomataceae) is an indigenous medicinal plant used in ethnomedicine for the treatment of diabetes, various bacterial infections, inflammatory and skin disorders, including herpes, chickenpox. The plant is known by various vernacular names like Kaikkathetti, Kaashavu, Koovachekki (Malayalam) Malamthetti, Perungaca, Vacci (Tamil) Dodda nekkare (Kannada) and Olle kodi (Tulu). The plant is traditionally used either singly or in combination with some other medicinal plants[8]. The young shoot tip paste of this plant, along with cumin seeds, is applied externally on the skin for the treatment of herpes which is mainly used in the traditional medicinal system[9], and this plant is reported to have antimicrobial properties against both Gram-positive and Gram-negative bacteria, and fungi[10]. It is also used as root ecbolic[11,12]. Memecylaene, a novel compound was isolated and tested for anti-inflammatory activity in albino rats in acute and sub-acute animal models[13.] Aqueous and ethanol leaf extracts of the plant were evaluated for their anthelmintic activity against *Pheritima posthuma*, which involved the determination of time of paralysis and time of death of the worms [14]. The present study is carried out to investigate the in vitro antidiabetic potential of the leaves of the plant and the major phytochemicals present in it. There are no previous studies conducted on the potential phytochemicals present in the leaves of this plant for its antidiabetic capacity. So HR LC-MS-QTOF analysis of the methanolic leaf extract was also carried out to screen the major bioactive compounds responsible for the antidiabetic potential.

Materials and Methods

Collection and authentication of plant material

The plant material of *Memecylon randerianum* S. M. Almeida & M. R. Almeida were collected from Campus, University of Kerala, Karyavattom. The collected plant was identified and the herbarium specimens were submitted to KUBH (Kerala University Botany Herbarium) for further reference (Voucher No. KUBH 6434). The samples were washed thoroughly with tap water and rinsed with distilled water and air-dried.

Preparation of extract

The leaves of the plant were cut into small pieces, shade-dried and finely powdered by using a pulverizer. The finely powdered sample was then subjected to continuous extraction with organic solvents of increasing polarity such as petroleum ether, chloroform, acetone, methanol, and distilled water by the Soxhlet method for 6 hours. Then the extract was filtered and stored at 4ºC. It is then used for preliminary phytochemical screening, antidiabetic activity studies and HR-LCMS-QTOF analysis.

Qualitative and quantitative phytochemical screening

The presence of different plant metabolites like saponins, flavonoids, alkaloids, phenols, tannins, glycosides, terpenoids, etc. was screened in crude methanolic extract of leaves by using standard procedures for phytochemical screening[15]. Based on the preliminary phytochemical analysis, quantitative determination of the phytoconstituents such as Alkaloids[16], Phenol[17], Flavonoid[18], Saponins[19], Tannins[20], Terpenoids[21], Steroids[22], and Glycosides[23] was carried out using standard spectrophotometric methods.

Estimation of alkaloid

One mg of the plant extract was dissolved in dimethyl sulphoxide (DMSO), 1.0 mL of 2N HCl is added and filtered. Then the solution was transferred to a separating funnel, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added. The mixture was then shaken with 1, 2, 3 and 4 mL chloroform by vigorous shaking and collected in a 10 mL volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm and recorded using UV/Visible spectrophotometer. The total alkaloid concentration is expressed in mg/g of the extract.

Estimation of phenol

To 0.1 mL of the extract, 3.9 mL of distilled water was added followed by 0.5 mL of folin-ciocalteau reagent. The solution was incubated at for 3 min and then added 2 mL of 20% sodium carbonate solution to the mixture. The solution was kept in a boiling water bath for 1 min, and then cooled and the absorbance was read at 650 nm. Gallic acid was used as the standard to express the total phenolic content.

Estimation of flavonoid

Total flavonoid content was measured by the aluminum chloride colorimetric assay. The reaction mixture consists of 1.0 mg/mL of extract, and 4 mL of distilled water was taken in a 10 mL volumetric flask. To the flask, 0.30 mL of 5% sodium nitrite was

treated, and after 5 min, 0.3 mL of 10 % aluminum chloride was mixed. After 5 min, 2 mL of 1M Sodium hydroxide was treated and diluted to 10 mL with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/mL) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as µg/ mg of extract

Estimation of Saponins

In the vanillin-sulphuric acid assay for determining the total saponin content, 1.0 mg/mL of plant extract, standards or reagent blank with 0.25 mL of 8% (w/v) vanillin in ethanol and 2.50 mL of 72% (v/v) sulphuric acid in water were incubated for 15 min at 60°C in a shaking water bath with the standards and the reagent blank made up with the solvent used for extracting the plant samples (extraction solvent). Oleic acid was used as the standard. After cooling in water at the ambient temperature for 5 min, the absorbance of the standards and extracts is measured at 560 nm using a UV–VIS spectrophotometer.

Estimation of Tannins

The concentration of tannins was determined by Folin- Denis method. 1.0 mL of the plant extract and standard solution of tannic acid (20-100 µg/mL) was made up to 7.5 mL with distilled water. Then 0.5 mL of Folin- Denis reagent and 1mL sodium carbonate solution were added. The volume was made up to 10mL with distilled water, and the absorbance was measured at 700 nm. Tannic acid was used as the standard.

Estimation of Terpenoids

About 100 µL of the sample was mixed with a vanillin-glacial acetic acid solution (150 µL, 5% w/v) and perchloric acid solution (500 µL). The sample solutions were heated for 45 min at 60°C and then cooled in an ice-water bath to the ambient temperature. After the addition of glacial acetic acid (2.25 mL), each sample solution's absorbance was measured at 548 nm, using a UV-visible-light spectrophotometer. Linalool (0.020–0.1 mg/mL in methanol) was used as a standard.

Estimation of Steroids

One mg/mL of sample extract is taken, and a set of standards (0.1-0.5 mL) were taken and made up to 5 mL with ferric chloride diluting reagent. A blank was prepared simultaneously by taking a 5.0 mL diluting reagent. Then added 4.0 mL of concentrated sulphuric acid to each tube. After 30 min of incubation, intensity of the colour developed was read at 540 nm. Cholesterol is used as a standard in this assay.

Estimation of Glycosides

To the 1.0 mL of the extract, 1.0 mL of Baljet's reagent was added and allowed to stand for 1 h. Then diluted the solution with 2 mL distilled water and mix. Read the intensity of the colour obtained against blank at 495 nm using a spectrophotometer. Digitoxin was used as the standard.

Antidiabetic studies

The methanolic leaf extract is subjected to *in vitro* antidiabetic studies by alpha-amylase and alpha-glucosidase inhibitory assay. Acarbose was used as a positive control for amylase and glucosidase inhibitory assay. The results were expressed in percentage inhibition by using the standard formula.

Alpha-amylase inhibitory activity

The alpha-amylase inhibitory activity was determined as previously described in the Worthington Enzyme Manual[24,25]. A total of 500 mL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 2 u/mL of α amylase and varying concentrations (25,50,100 and 200 µg) of extract as inhibitor were pre-incubated at 25°C for 10 min. After the pre-incubation, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated for 20 min. The reaction was stopped using 1.0 mL of dinitrosalicylic (DNSA) acid colour reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was made up to 10mL by adding distilled water, and the absorbance was measured at 540 nm using UV-Visible light spectrophotometer. The absorbance readings of samples were compared with the negative control that contains buffer and other chemical solutions except for the enzyme. The assay was conducted thrice, and triplicate values were recorded. Varying concentrations of acarbose (1.0 mg/mL stock) were treated as standard (positive control). The results were expressed as a percentage of inhibition.

$$\text{Inhibitory activity (\%)} = ([A - B]/A) \times 100$$

where A = the absorbance of the control reaction mixture and B = the absorbance of the reaction mixture containing enzyme.

Alpha-glucosidase inhibitory activity

The inhibition of the α-glucosidase enzyme was found out as the protocol of Shai *et. al*[26]. 200 µL of α-

glucosidase (0.067 U/mL) was preincubated with different concentrations of the sample for 10 min. Then 200 µL of 3.0 mM (pNPG), which was used as a substrate, was dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was then incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The- glycosidase activity was determined by measuring the yellow-colored para-nitro phenol released from pNPG at 400 nm. The above-said chemicals without the enzyme were taken as a negative control, and the assay was carried out in triplicates. Varying concentrations of acarbose (1mg/ml stock) were treated as standard (positive control). The results were expressed as percentage of inhibition.

Inhibitory activity (%) = (B-T/B-C) ×100

where T is the absorbance of a test sample, C is the absorbance of control and B is the absorbance of blank.

Glucose uptake assay

Glucose uptake activity in L6 cells was estimated by the methods described by Guptha *et al.* [27] with slight modifications. Cells were cultured on 48-well plates and incubated for 48 h at 37°C in a CO_2 incubator. When a semi-confluent monolayer was formed, the culture was renewed with serum-free DMEM containing 0.2% BSA and incubated for 18 h at 37°C in the CO_2 incubator. After 18 h, the medium was discarded, and cells were washed with PBS (pH 7.4) buffer once and treated with 1000 µg/ml glucose along with the extract (25, 50 and 100 µg/mL) for 1 h. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium. The final glucose concentration was estimated by the anthrone method with the aid of a glucose standard graph. The glucose uptake in L6 cells treated with test compounds was compared with that of control cells (untreated). If the treated cells showed improved glucose uptake compared to that of control cells, we could assume that the compound has medicinal value.

Statistical analysis

All the experiments were done in triplicate and results are expressed in terms of mean ± standard error using SPSS Software.

High resolution liquid chromatography and mass spectrometry (HR-LCMS Q-TOF) analysis

HR-LCMS was done to detect the chemical components present in the leaf extract of the plant and was performed at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Pawai, Mumbai. The extract was prepared in methanol and then subjected to HR-LCMS analysis. It was prepared through Agilent high resolution liquid chromatography and mass spectrometry model-G6550A with 0.01% mass resolution. The acquisition method was set to be MS- minimum range 50 (M/Z) and maximum 1000 dalton (M/Z) with scanning rate for each spectrum per second. Gas chromatography had maintained at 250°C with a gas flow 13 psi/min. Hip sampler with model G4226A was used with auxiliary speed 100 µL/min, ejection speed 100 µL/min, flush out factor of 5 and 8 µL injection volume. Within 30 min of acquisition time in the initial 2 min the flow of solvent composition A: B was 95: 5 (100% Water and 100% Acetonitrile) in the HR-LCMS analysis. This instrument has high-resolution analytical power due to the high-quality mass analyser (time of flight). It is very helpful to improve the precision of the chemical formula and library matching for detection of unknown compounds in a particular crude sample.

Results

Qualitative phytochemical analysis

The phytochemical screening confirmed that different solvent extracts of leaf contain different types of phytochemicals. The presence and absence of the various phytoconstituents were listed in Table 1. The majority of the phytochemicals except iridoids were present in the methanolic extract. Glycosides, terpenoids, and steroids were observed in all the solvent extracts except petroleum ether. Coumarins were detected in all the extracts except aqueous extract. Flavonoids, tannins, and phenols were noticed only in acetone and methanolic extracts. Quinones were present in acetone, methanolic, and aqueous extracts. Alkaloids and saponins were found only in the methanolic extract.

Quantitative phytochemical analysis

The quantitative determination of major bioactive components in the leaves of *Memecylon randerianum* was investigated in the methanolic extract based on the preliminary phytochemical screening. The results of this quantitative screening were listed in Table 2. Saponins (211.16±1.9 mg/g) and flavonoids (209.74±2.12 mg/g) were highly concentrated in the methanolic leaf extract. Alkaloid and tannin content was found to be 145.38±3.09 mg/g and 111.3±3.1 mg/g, respectively. The amount of glycosides

(3.79±0.09 mg/g) was very less in the leaf extract. The phenolic content was found to be 96.75±7.29 mg/g. The related phytocompounds such as terpenoids (21.42±0.15 mg/g) and steroids (21.88±0.2 mg/g) were present in equal amounts.

Alpha-amylase inhibitory activity

The methanolic leaf extract of the plant showed significant alpha-amylase inhibitory activity in a dose-dependent manner. The percentage inhibition of α-amylase was noted in the concentration ranging from 25 to 200 µg/mL. Acarbose was used as the standard drug for amylase inhibitors. The inhibitory activity of the extract ranges from 9.39±0.06 at 25 µg/mL to 76.08±0.07 at 200 µg/mL with an IC_{50} value of 107.648 µg/mL. Acarbose showed inhibitory activity with an IC50 value of 85.659 µg/ml. The result of alpha-amylase inhibitory activity in the methanolic leaf extract is depicted in Table 3.

Alpha-glucosidase inhibitory activity

The antidiabetic activity of methanolic leaf extract using alpha-glucosidase inhibitory assay also showed a dose-dependent increase in inhibition (Table 3). The percentage inhibition of the extract varied from 43.06±0.29 to 87.60±0.51 µg/mL with an IC_{50} value of 63.279 µg/mL, and acarbose showed inhibitory activity with an IC_{50} value of 63.279 µg/mL.

Glucose uptake assay

The glucose utilization in L6 cell lines was studied in the present study. The plant extract showed significant glucose uptake in L6 cells compared to untreated cells (Control). The glucose uptake was found to be increasing in a concentration dependant manner, as seen in Fig. 1. At 100 µg/ml concentration of the leaf extract, the glucose uptake was found to be increased by 25% compared to the untreated control.

High resolution liquid chromatography and mass spectrometry (HR-LCMS) Analysis

The bioactive compounds identified from HR-LCMS-QTOF analysis were cosmosiin, apigenin, cinnamic acid, ursolic acid, rutin, isorhamnetin, gallic acid, quercetin and catechin. The retention time, abundance, and other mass spectral details were noted in Table 4.

Discussion

The plants and its product play an important role in the environment and the entire living system. The phytochemicals present in the plants give numerous medicinal activities as well as defencing properties. They have a wide range of functions, and they especially provide protection of human health through proper diet[28]. They are applicable for the control and management of many chronic degenerative diseases such as diabetes and cancer [29]. Many research studies have been focused on the health benefits of

Table 1 — Phytochemical screening of different solvent extracts of the leaves of *Memecylon randerianum*

Phytochemicals	PE	CH	AC	ME	WA
Alkaloids	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	+	+	+	+
Terpenoids	-	+	+	+	+
Tannins	-	-	+	+	-
Steroids	-	+	+	+	+
Coumarins	+	+	+	+	-
Quinones	-	-	+	+	+
Phenols	-	-	+	+	-
Saponins	-	-	-	+	-
Iridoids	-	-	-	-	-

[Here (+), presence of constituents; (−), absence of constituents; PE, Petroleum ether; CH, Chloroform; AC, Acetone; ME, Methanol; ET, Ethanol; and WA, Water]

Table 2 — Quantitative analysis of phytochemicals in the leaves of *Memecylon randerianum*

Phytochemicals	Concentration (mg/g)
Alkaloids	145.38±3.09
Flavonoids	209.74±2.12
Glycosides	3.79±0.09
Terpenoids	21.42±0.15
Tannins	111.3±3.1
Steroids	21.88±0.2
Phenols	96.75±7.29
Saponins	211.16±1.9

[Values are expressed in Mean ± Standard error]

Table 3 — *In vitro* Alpha-amylase and Alpha- glucosidase inhibitory activity in the methanolic leaf extract of *Memecylon randerianum*

Alpha-amylase	Alpha- glucosidase
Percentage of inhibition	
9.39±0.06	25.72±0.29
30.56±0.06	41.26±0.51
63.86±0.05	57.90±0.59
76.08±0.07	67.36±0.51

[Values are represented in Mean ± Standard error]

Table 4 — Bioactive Compounds detected in the methanolic leaf extract of *Memecylon randerianum*

Compound	RT	Abundance	Mass	Formula	DB Diff (ppm)
Cosmosiin	6.485	6255.17	432.1056	$C_{21}H_{20}O_{10}$	0.12
Apigenin	8.799	4744	270.0544	$C_{15}H_{10}O_5$	-5.69
Cinnamic acid	6.678	1735	148.0485	$C_9H_8O_2$	26.65
Ursolic acid	9.434	1131	456.3642	$C_{30}H_{48}O_3$	-8.4
Rutin	14.241	851	610.1523	$C_{27}H_{30}O_{16}$	1.82
Isorhamnetin	4.116	663	316.048	$C_{16}H_{12}O_7$	32.45
Gallic acid	8.799	303	170.0322	$C_7H_6O_5$	-62.61
Quercetin	20.136	265	302.0385	$C_{15}H_{10}O_7$	13.73
Catechin	11.536	97	290.0752	$C_{15}H_{14}O_6$	13.31

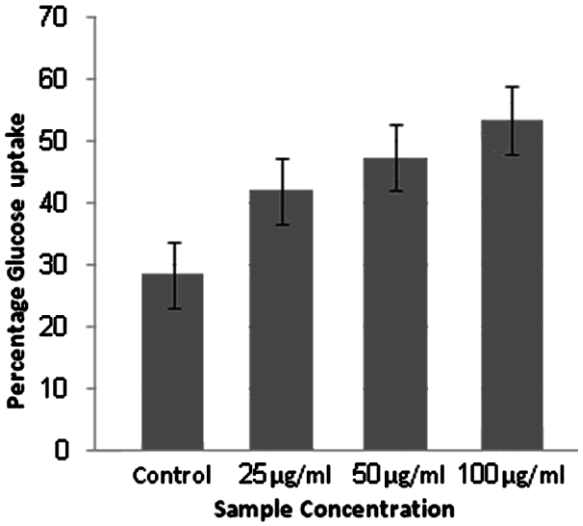

Fig. 1 — Percentage Glucose uptake of control and different concentration of extracts

phytochemicals in different plants.

In the present study, phytochemical screening of the leaves confirmed the presence of valuable secondary metabolites such as alkaloids, flavonoids, glycosides, terpenoids, tannins, phenolics, saponins etc. These phytoconstituents have been reported to display potent hypoglycemic activity. Saponins and flavonoids were found higher in the quantitative phytochemical analysis of the methanolic leaf extract of *M. randerianum*. Saponins are well-known bioactive compounds with potent antidiabetic properties[30]. Many medicinal plants such as *Anabasis articulate*, *Astragalus membranaceous*, *Dioscorea rotundata*, *Enteda phaseoloides*, *Garcinia kola*, *Helicteres isora*, *Polygonatum adoratum and Terminalia arjuna* owe their antidiabetic property to saponins[31]. El Barky *et al.*[32] made a detailed review on the fundamental role of saponin as an antidiabetic agent and its various sources for diabetic treatment. Flavonoids are one of the accepted antidiabetic phytochemicals, among others. They help to reduce the pathogenesis of diabetes and its complications[33].

The prevalence rate of diabetes is estimated to reach 10.2% by 2030 and by 2045 to 10.9%[34] since it is a non-infective disease[35]. Many enzymes and their changes are linked to diabetes. The study of these changes is very significant in the complexity of the disease. Alpha-amylase and alpha-glucosidase are thoroughly studied carbohydrate digesting enzymes in the human metabolic system and play a key role in controlling diabetes. The dysfunction of pancreatic enzymes like alpha-amylase may lead to diabetes mellitus[36]. The alpha-amylases are the calcium containing digestive enzymes present in the human pancreas and saliva responsible for starch digestion. Alpha glucosidases are another class of digestive enzymes responsible for the absorption of glucose in the gastrointestinal system. The inhibition of these enzymes helps to reduce the diabetic problems by decreasing the glucose absorption. This inhibition of the two enzymes is one of the most widely followed remedial approaches for controlling diabetes. Majority of the *Memecylon* species show good to moderate *in vitro* antidiabetic activity. Here the phytochemicals present in the leaves of *Memecylon randerianum*, such as saponins, flavonoids, tannins, alkaloids, phenols, offer prospective remedies for controlling diabetic complications. The antidiabetic studies conducted by Bharathi *et al.*[37] reveals that the leaves of *Memecylon talbotianum* inhibited both alpha-amylase and the standard acarbose with a corresponding IC_{50} value of 160 and 120 µg/reaction at 240 µg/reaction for the methanolic crude extract, respectively. The alpha-glucosidase inhibitory activity was also studied in this plant with an IC_{50} value of 9.1 for the methanolic extract and 7 µg/reaction for acarbose at 15 µg/reaction. In the present study, the methanolic extract of *M. randerianum* leaf revealed a considerable inhibitory effect on alpha-amylase with an IC_{50} value of 107.648 µg/mL when compared with standard Acarbose (IC_{50} value of 85.659 µg/mL). The inhibitory effect of alpha glucosidase also shows a concentration-dependent effect with an IC_{50} value of 102.597 µg/mL for the sample and 63.279 µg/mL for the standard. Therefore, this inhibitory potential of the leaf extract might be attributed to the presence of the phytochemicals (Table 2).

The preserved rat skeletal myoblast (L6) cell lines are widely used to explicate the process of glucose uptake in antidiabetic studies. The results from the present study clearly point out that the sample may have type 2 antidiabetic properties and can be considered as a promising drug candidate against insulin-resistant hyperglycemia. The glucose utilization has increased with the increase in the concentration of leaf extract. The methanolic leaf extract showed significant glucose uptake (53%) with an average absorbance (630 nm) of 0.46 at 100 µg/mL concentration.

More interestingly, the HR-LCMS-QTOF analysis of the leaf extract could find out the major bioactive compounds trustworthy for the suppression of diabetes. Apigenin, the natural flavonoid found from

the majority of the plants, has been reported to have strong hypoglycemic activity[38]. Based on the abundance of the compounds identified from HR-LCMS-QTOF analysis, cosmosiin was dominant over other compounds. Cosmosiin is an apigenin derivative and named as apigenin 7-O-glucoside or apigetrin[39]. The insulin mimetic action of cosmosiin may be useful to develop potent lead compounds for the treatment of diabetes through promoting the adiponectin secretion and tyrosine phosphorylation of insulin receptor-β and GLUT4 translocation[40]. The antidiabetic potential of all the bioactive compounds detected from the leaf extract such as cosmosiin, apigenin, cinnamic acid, ursolic acid, rutin, isorhamnetin, gallic acid, quercetin, and catechin, has been well demonstrated in the previous studies[41]. Among these compounds cosmosiin, apigenin, rutin, isorhamnetin, quercetin and catechin are belongs to the group of flavonoids. Generally, flavonoids have many therapeutic properties with special mention to type 2 diabetes[42]. Some of the flavonoids are reported to have an inhibitory effect against the digestive enzymes like intestinal alpha-glucosidase and pancreatic alpha amylase[43]. Patel *et al.* [44] evaluated the antihyperglycemic, antihyperlipidemic and antioxidant activities of Dihar, a polyherbal formulation containing drugs from eight different herbs. The strong antioxidant potential of these eight other plants is associated with correcting hyperglycemia and helps to reduce diabetic complications. The antidiabetic potential of the compounds such as apigenin[45], cinnamic acid[46], ursolic acid[47], rutin[48], isorhamnetin[49], gallic acid[50], quercetin[51] and catechin[52] has been evaluated in previous research investigations. Therefore with the presence of the bioactive compounds in the present study, the leaf extract of *M. randerianum* might be an excellent source for the discovery of new antidiabetic drug.

Conclusion

In the present study, the methanolic leaf extract of *M. randerianum* shows the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins, phenolics, and saponins. The leaves of the plant possess dose-dependent antidiabetic activity when compared to the widely used antidiabetic drug acarbose. These results specified that the abundance of saponins and flavonoids in the leaf extract have the capacity to reduce the blood glucose level and induce the production of insulin. As shown in the current study, LCMS analysis of the leaf extract could find out the compounds responsible for a number of therapeutic disorders. The antidiabetic efficiency of the compound, cosmosiin should be subjected to further clinical investigation using *in vivo* experimental models for its scientific validation. This study is also advantageous in the sense that its leaves can be used for development of effective antidiabetic drug formulation.

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Conflict of interest

Authors declare no competing interests.

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