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GC-MS analysis and antidiabetic potentials of *Bridelia Micrantha* crude extracts

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Abstract

Background: The quest for continuous search for the use of plants for the cure of diabetes mellitus necessitated the research work.

Objective: The search for total and complete remedy for the treating of diabetes mellitus has necessitated the beaming of search light on *bridelia micrantha* for its potential anti-diabetic inhibitors.

Materials and method: The bark of the tree plant was obtained, air dried and turned to powdered. Extraction was done using methanol and chloroform solvents and the in-vitro studies of the inhibitory potentials of the crude extracts were done using Gao *et.al* method with slight modification. The IC_{50} values were calculated using Graph pad prism 5.0software.

Results: Methanolic crude extract was found to be the most potent inhibitor of α-glucosidase with 1.06 ± 0.1 µg/mL IC_{50} value and the chloroform crude extract had 2.84 ± 0.1 µg/mL IC_{50} which was better than standard inhibitor acarbose (234.6 ±2.01µM)). The IC_{50} (7.35 ± 1.0µg/mL) of chloroform extract showed less potent inhibitor against glucoamylase than that of methanolic extract with IC_{50} (1.12 ± 0.10 µg/mL). The *β*-glucosidase screening of the extracts showed that they do not potent and selective inhibitors.The high inhibitory effects of the extracts against glucoamylase and *α*-glucosidase were confirmed by the presence of bioactive compounds present. The OSIRIS drug properties of some identified compounds was screened using online OSIRIS property explorer server and were found to possess various drug properties.

Conclusion: The *in vitro* assays of these extracts indicated that they are therapeutically interesting and could serve as important tools for treatment of diabetes.

Introduction

Diabetes mellitus disease has been a major ailment around the world and Nigeria not being exempted from the dreaded disease. The searching for the complete eradication of the disease through the use of plants has consistently been a major task for the pharmaceutical chemists. Diabetes mellitus is said to be a group of metabolic disorders manifested by innate or acquired failure to transport glucose from blood stream to cells [1]. There are many anti-diabetes inhibitors and among which are *α*-glucosidase, *β*-glucosidase. *α* -glucosidase inhibitors are oral drugs made for DM type 2 and it works by disallowing the digestion of carbohydrates and thereby reducing the impact of carbohydrate on blood sugar. Generally, plants are considered to be a source for the most active, potent hypoglycaemic properties [2,3]. Natural drugs from plants are considered to be nontoxic with lesser side effects than synthetic drugs. It has been reported that medicinal plants possessing anti-diabetic activities could be a useful tool for the discovery of safer hypoglycaemic agents [4]. These plants are said to be the major source for discovering new compounds with therapeutic value for drug development against most common and very prevalent disease, diabetes mellitus. The plants which have therapeutic application possess bioactive composites viz., alkaloids, glycosides, tannins, flavonoids, saponins, phenolics and vitamins [5]. *B. micrantha* is a medium sized semi-deciduous to deciduous tree that grows up to 20 m tall and belongs to the family Euphorbiaceae [6]. In Nigeria, different parts of the plant are used traditionally in the treatment of some ill-health by different cultural groups [7]. Pharmacological properties such as antidiabetic, antioxidant [6,8], anti-inflammatory [7], hepatoprotective [9] and abortifacient [10] activities of the plant have been reported. Bark of *B. micrantha* plant has been previously evaluated for its phytochemicals; Saponins Alkaloids Tannins Phytosterols Glycosides Flavonoids [11].

Research Methodology

Research laboratory

The research work was carried out at the centre for the Advanced Drug Research (CADR), Department of Pharmacy, COMSATS Institute of Information and Technology, Abbottabad, Pakistan in the month of July, 2017.

Materials and instruments

All the chemicals, solvents used are of analytical grade, α-glucosidase (from Saccharomyces cerevisiae), substrate p-nitrophenylα-D-glucopyranoside (pNPG), *β*-glucosidase (from sweet almonds) and 96 well plates were purchased from Sigma Aldrich. ELIZA micro plate reader.

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Plant source

B. micrantha* belong to the family of *Euphorbiaceae. The barks of the plant were obtained from a small farm in Akure South Local Government Area of Ondo State in Nigeria on the 5th of April, 2017 and identified at the Department of crop and soil science, Federal University of Technology Akure, Nigeria.

Plant preparation

The barks were collected and air dried for one month and later grinded into powdered sample using grinder. The commercial grinder is made in China, brand MPN, having the following specifications; power (2.2kw), dimension (34×38×75cm), roller length (26cm), roller diameter (14cm), voltage (220v/50Hz).

Crude extract preparation

Two hundred grams of powdered *B. micrantha* were soaked in 1000 ml of Chloroform and methanol for five days and filtered through whatman filter paper. The extract was concentrated using a rotary evaporator at 35°C and the dried extract was stored at room temperature for further use. Ten milligram (10 mg) of dried crude extracts were dissolved in 1 ml of 100% Dimethyl sulfoxide (DMSO) and labelled as stock (10 mg/ml), working solution was made as 1 mg/ml**.**

α-glucosidase inhibition study

Assay for α-glucosidase inhibition was performed by slight modification of a previously published method [12]. Briefly, solutions of *α*-glucosidase (from Saccharomyces cerevisiae) and its substrate p-nitrophenyl α-D-glucopyranoside (pNPG) were prepared in phosphate buffer (70 mM, pH 6.8). Buffer was used for the preparation of inhibitor solutions. The inhibition assays were conducted by adding inhibitor solution (10 µL) to 70 µL buffer and 10 µL of enzyme solution (2.5unit/mL) in 70 mM phosphate buffer (pH 6.8) followed by pre-incubation at 37 °C for 5 min. After pre-incubation, 10 µL of 10 mM substrate (pNPG) prepared in phosphate buffer was added to the mixture to initiate enzymatic reaction. The reaction mixture was incubated at 37 °C for 30 minutes. Acarbose was used as a positive control. The *α*-glucosidase activity was determined by measuring the p-nitrophenol released from pNPG at 405 nm using an Eliza micro plate reader. The experiment was performed in triplicates.

β- glucosidase inhibition study

The evaluation of inhibitory activity against *β*-glucosidase was performed with slight modification of the previously published method [13]. Briefly, *β*-glucosidase (from sweet almonds) enzyme and *p*-nitrophenyl *β*-D-glucopyranoside (pNPG) as substrate were prepared in 0.07 M phosphate buffer (pH 6.8). The inhibition assays were conducted by adding inhibitor solution (10 µL) to 70 µL buffer and 10 µL of enzyme solution (2.0 unit/mL) in 70 mM phosphate buffer (pH 6.8) followed by pre-incubation at 37 °C for 5 min. After pre-read, 10 µL of substrate was added to the mixture and then incubated at 37 °C for 30 min and final reading was obtained. Negative control contained 10 µL of 10% DMSO instead of inhibitor. The experiment was performed in triplicates and the % inhibition was calculated.

Maltase glucoamylase inhibition study

Assay for glucoamylase inhibition was carried out by slight modification of a previously published method [12]. Shortly, solutions of glucoamylase (from maltase) enzyme and its substrate p-nitrophenyl α-D-glucopyranoside (pNPG) were prepared in phosphate buffer (70 mM, pH 6.8). Buffer was used for the preparation of inhibitor solutions. The inhibition assays were conducted by adding inhibitor solution (10 µL) to 70 µL buffer and 10 µL of enzyme solution (2.5unit/mL) in 70 mM phosphate buffer (pH 6.8) followed by pre-incubation at 37 °C for 5 min. After pre-incubation, 10 µL of 10 mM substrate (pNPG) prepared in phosphate buffer was added to the mixture to initiate enzymatic reaction. The reaction mixture was incubated at 37 °C for 30 min. Acarbose was used as a positive control. The glucoamylase activity was determined by measuring the p-nitrophenol released from pNPG at 405 nm using an Eliza micro plate reader. Each experiment was performed in triplicates. The % inhibition was calculated.

Statistical analysis: The total percentage inhibitions were calculated by method of [12]:

$$\%Inhibition = [100 - (\frac{absorbancetestwell}{absorbancecontrol}] \times 100$$

IC_{50} (i.e., the concentration of sample inhibiting 50%) values of potent inhibitors were determined by testing the serial dilutions of inhibitors and were calculated by using the program PRISM 5.0 (GraphPad, San Diego, California, USA).

GC/MS analysis

GC-MS analysis of methanol and chloroform extracts of *B.Micrantha* barks was performed using TurboMass GC System, fitted with an Elite-5 capillary column (30 m, 0.25 mm inner diameter, 0.25 µm film thickness; maximum temperature 350 °C, and coupled to a Perkin Elmer Clarus 600C MS. Helium was used as gas carrier at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were 280 °C. The ionizing energy was 70 eV. The oven temperature was programmed from 70 °C (hold for 2 min) to 280 °C (hold for 10 min) at a rate of 5 °C/min. The crude extract was solubilised with chloroform and filtered with syringe filter (Corning, 0.45 µm). Volumes of 1 µL of the crude extracts were injected with a split ratio 1:20. The data were obtained by collecting the mass spectra within the scan range 50-550 m/z. The identification of chemical compounds in the extracts was based on GC retention time; the mass spectra matched those of standards available at NIST library.

Results

Tables 1-4, Figures 1, 2 and 3.

Discussion

It is worthy to note that from the result of table 1, the IC_{50} of methanolic extract (1.06+0.1µg/mL) showed high inhibitory potential against *α*-glucosidase than that of chloroform extract IC_{50} (2.84+0.1µg/mL). However, the two results were better than the acarbose standard (234.6 ±2.01µM) against *α*-glucosidase. The IC_{50} both for the extracts of *B.micrantha* against *α*-glucosidase were better than the ethanolic extracts of *Andrographis paniculata* and *andrographolide* as reported by Rammohan [15] when compared. The extract of *A. Paniculata* showed *α*-glucosidase inhibitory effect in a concentration-dependent manner (IC_{50} of 17.2 ± 0.15 mg/mL) and *andrographolide* demonstrated a similar (IC_{50} of 11.0 ± 0.28 mg/mL) against *α*-glucosidase. Furthermore, the *α*-glucosidase of the *B.micrantha* extracts were better than the methanolic extracts of *Artocarpus altilis* (IC_{50} 129.85+10.29µg/mL), *A.heterophyllus* (IC_{50} 76.90+9.55µg/mL), *Cinnamomus zeylanicum* (IC_{50} 140.01+0.08 µg/mL) and *piper betel* (IC_{50} 96.56+12.93µg/mL) at concentrations ranged from 20 to 100 µg/mL as reported by Sindhu [16]. In addition, the methanolic extract of *B.micrantha* (IC_{50} 1.06+0.1µg/

Table 1. Inhibition potency of crude extracts against *α*-glucosidase, maltase glucoamylase and *β*-glucosidase

Extracts	*α*-glucosidase IC_{50} ± SEM (µg/mL)	*β-glucosidase % inhibition± SEM*	Maltase glucoamylase IC_{50}±SEM (µg/mL)
$CHCl_3$ extract	2.84 ± 0.08	24.53±3.64	7.35 ± 1.02
MeOH extract	1.06 ± 0.11	24.16±1.54	1.12±0.10
Acarbose[a]	234.6 ±2.01(µM)	Not tested	234.6 ±2.01(µM)
Castonospermine[b]	Not tested	59.98%[14]	Not tested

[a] *α*-glucosidase standard
[b] *β*-glucosidase standard
± SEM: standard Error mean

Table 2. Identified compounds in the chromatogram of methanolic extract

Compound name	Molecular formula	CAS	Molecular Retention time weight ($g.mol^{-1}$) (minutes)
Valeric acid	$C_{21}H_{42}O_2$	125164-54-7	326 11.822
L-Ascorbic acid,6-octadecanoate	$C_{24}H_{42}O_7$	10605-09-1	442 31.442
Oxalic acid ,butyl-6-ethyloct-3-yl-ester	$C_{16}H_{30}O_4$	900309-34-3	286 13.608
Menthol	$C_{10}H_{20}O$	1490-04-6	156 42.89
Phytol	$C_{20}H_{40}O$	150-86-7	296 28.639
Stearic acid,2-phenyl-M-dioxan-5-yl ester trans	$C_{28}H_{46}O_4$	10564-35-9	446 10.842
Phenol3.5-Bis(1,1-dimethyl ethyl)	$C_{14}H_{22}O$	1138-52-9	206 20.241
Myristic acid vinyl ester	$C_{16}H_{30}O_2$	5809-91-6	254 49.638
Vitamin A Aldehyde	$C_{20}H_{28}O$	116-31-4	284 16.784
3-Methyl-2-(2-oxopropyl)Furan	$C_8H_{10}O_2$	87773-62-4	138 20.841
1H-Imidazole,1-(1ioxooctadecyl)	$C_{21}H_{88}ON_2$	17450-32-7	334 20.526
D-mannitol, 1-decylsulfonyl	$C_{16}H_{34}O_7S$	900154-76-1	370 50.274

Table 3. Identified compounds in the chromatogram of chloroform extract

Compound name	Molecular formula	CAS	Molecular Retention time weight ($g.mol^{-1}$) (minutes)
3-methyl-2-(2-oxopropyl) Furan	$C_8H_{10}O_2$	87773-62-4	138 11.707
Z,Z-6,28-Heptatriactontadiene-2-one	$C_{37}H_{70}O$	133530-21-9	538 51.313
1,10-Hexadecanediol	$C_{16}H_{34}O_2$	39516-54-6	258 19.895
(Z)-14-tricosenyl formate	$C_{24}H_{46}O_2$	77899-10-6	366 21.176
3-T-Butyl-oct-6-yn-1-ol	$C_{12}H_{24}O$	900185-34-1	184 24.527
Cis-1-chloro-9-octadecene	$C18H_{35}Cl$	16507-61-2	286 20.576
4-N-Hexylthiane,s,s-dioxide	$C_{11}H_{22}O_2S$	70928-52-8	218 27.183

OSIRIS drug properties and toxicity profile

Table 4. Drug properties of some of identified compounds in the crude extracts determined by OSIRIS property explorer

Compound	Drug likeness	Mutagenic	Tumorigenic	cLogS	CLogP	Polar surface area(Å²)	%Absoption	H-bond Acceptor	H-bond Donor	Irritability
Valeric acid	-7.0646	None	None	-1.269	1.0641	37.3	96.13	2	1	None
Menthol	-25.216	High	None	-2.501	2.4112	20.23	102.02	2	1	None
Phytol	-3.7661	None	High	-4.633	7.4212	20.23	102.02	1	1	High
1H-imidazole (identified compound] having imidazole ring)	0.44659	None	None	-0.431	-0.1802	28.68	99.11	2	1	None
Ascorbic acid (molecular aspect)	0.023806	None	None	-0.349	-2.8448	90.15	77.9	6	4	None

Figure 1. Bark of *Bridelia micranth*

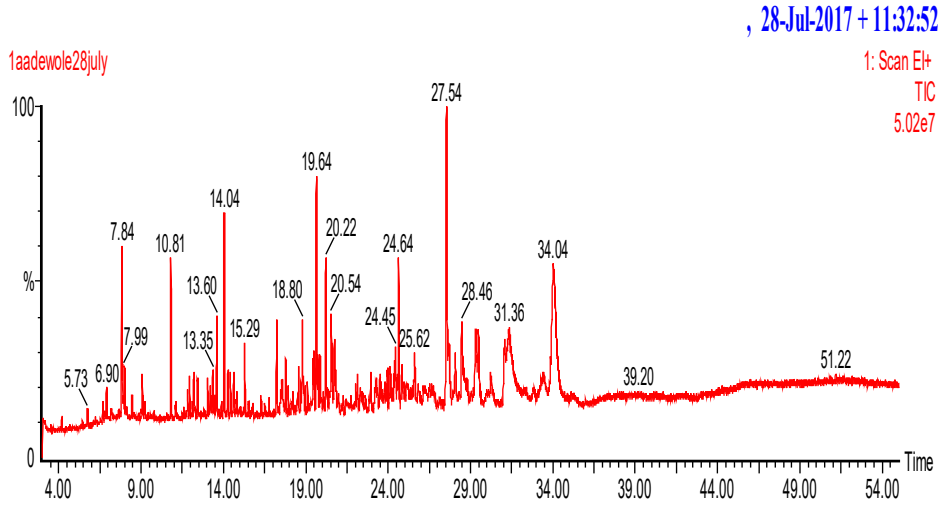

Figure 2. Chromatogram of Methanolic extract of *B. micrantha*

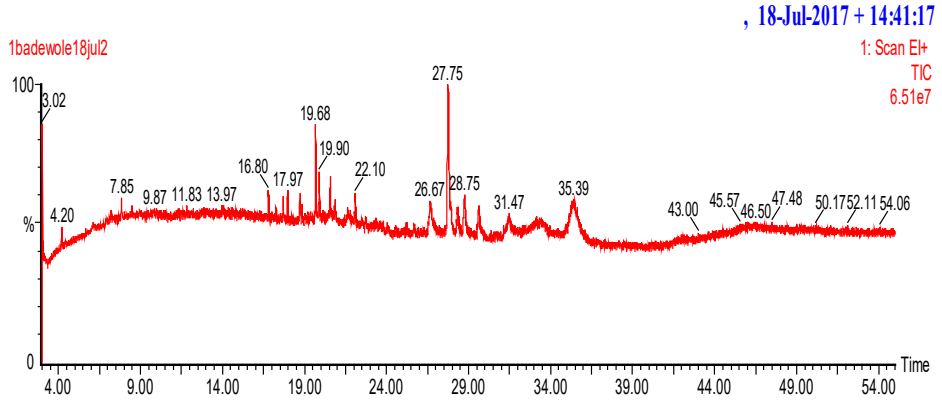

Figure 3. Chromatogram of chloroform extract of *B. micrantha*

mL) showed good potent and selective inhibitor than *A.calamus* (IC_{50} 1.26mg/mL) and *N.sativa* (IC_{50} 1.53mg/mL) as reported by Balaji [17] against α-glucosidase. The IC_{50} (1.12+0.10 µg/mL) of methanolic extract of *B.micrantha* was better than the chloroform extract of IC_{50} (7.35+1.02 µg/mL) when tested against maltase glucoamylase and the results were better than the standard acarbose (IC_{50} 234.6 ±2.01µM). However, the IC_{50} both for the extracts of *B.micrantha* against maltase glucoamylase showed good inhibitory potentials than the methanolic extracts of *Artocarpus altilis* (IC_{50} 118.88+11.14µg/mL), *A.heterophyllus* (IC_{50} 70.58+9.66 µg/mL), *Cinnamomus zeylanicum* (IC_{50} 130.55+10.5 µg/mL) and *piper betel* (IC_{50} 84.63+13.09 µg/mL) at concentrations ranged from 20 to 100 µg/mL as reported by Sindhu [16]. The β-glucosidase screening of the *B.micrantha* extracts showed that they do not potent and selective inhibition, the methanolic extract had 24.16+1.54% and chloroform extract had 24.53+3.64% inhibitory potential against β-glucosidase and these values were less than the Castonospermine standard (59.98%) as reported by Verma [14].

Moreover, the good potent and inhibitory potentials of the extracts of *B.micrantha* against α-glucosidase and maltase glucoamylase are good indication that the plant possesses therapeutic properties. The identification of bioactive compounds as revealed by gas chromatography Mass spectrophotometer has shown that the efficacy of the plant being used for the treatment of diabetes mellitus may not be unconnected to the presence of the these bioactive compounds both in the chloroform and methanolic extracts as many heterocyclic compounds have been found to possess various pharmacological activities against different ailments.

In addition, in the characterization, the results of GC-MS profile can be employed as a tool for the identification of novel compounds [18] as revealed in tables 2 to 3**.** It is interesting to note that some of the identified compounds in the crude extracts were screened computationally using online OSIRIS property explorer server [19] and were found to possess various drug properties as shown in Table 4.It has been reported that molecular properties which include bioavailability, hydrophobicity and membrane permeability are linked with some molecular descriptors as cLog P (partition coefficient),cLogS (solubility) number of H-bond acceptors and H-bond donors and molecular weight. It is documented that Lipinski's rule of five [20] is widely used to predict molecular drug-likeness. According to the rule, a drug like molecule has log P≤5, molecular weight <500 g/mol, hydrogen bond acceptors ≤10, hydrogen bond donors ≤5 and molar refractivity between 40-130. Molecules violating more than one of these rules are not expected to be viable drug candidates. The solubility parameter, log S, is another important parameter for determining drug likeness. The absorption of a compound is considerably influenced by its solubility. Generally, high log S values correspond to good absorption. Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties (PSA must be ≤140 Å^2). It is used to

estimate the percentage of absorption using the expression %ABS=109-0.345PSA [21]. The online OSIRIS property explorer server has revealed the relevance and various drug properties of some of the identified compounds in the crude extracts using GC-MS and this could serve as a tool for the pharmaceutical chemists to further research on the plant as a potential anti-diabetic agent.

Statement of significance

This study discovered the potent anti-diabetic inhibitory potentials of the methanolic and chloroform crude extracts of *B. micrantha* against α-glucosidase and maltase glucoamylase which can be highly beneficial for the treatment of diabetes mellitus type 2. Also, the online OSIRIS server explorer has revealed the drug properties of some of the identified compounds which will be beneficial to the scientists for further investigation. This study could be explored by researchers and a new anti-diabetic agent may be arrived at.

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