European Journal of Medicinal Plants

31(20): 48-56, 2020; Article no.EJMP.64892
ISSN: 2231-0894, NLM ID: 101583475

Isolation and Characterization of Two New Polyphenols from the Anti-Diabetic Fraction of Pod Extract of *Caesalpinia pulcherrima* Swartz

Kolade Olatubosun Faloye[1], Samson Oluwaseyi Famuyiwa[1*], Marcus Durojaye Ayoola[2] and Derek Tahton Ndinteh[3]

[1]Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Nigeria.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Nigeria.
[3]Department of Chemical Sciences, Faculty of Sciences, Doornfortein campus,University of Johannesburg, South Africa.

Authors' contributions

This work was carried out in collaboration among all authors. Author KOF Conceived, initiated and carried out all the experimental work and wrote the first draft of the manuscript. Author SOF Supervised the work, generated the IR, NMR data of the compounds and did the structure elucidation of the compounds. Author MDA supervised the anti-diabetic aspect of the work. Author DTN Invited SOF to South Africa and provided all necessary tools and space for the spectroscopic aspect of the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i2030356
Editor(s):
(1) Dr. Prem K. Ramasamy, Brandeis University, USA.
(2) Prof. Marcello Iriti, Milan State University, Italy.
Reviewers:
(1) Mahmout Yaya, University of N'Djamena, Chad.
(2) Mohamed Mirghani Mohamed Ahmed Hassan, International University of Africa, Sudan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64892

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

ABSTRACT

This study was planned and executed to validate the anti-diabetic ethno-medicinal claim of *Caesalpinia pulcherrima* (Fabaceae) pods and the isolation of potential chemical compounds responsible for the activity.The anti-diabetic activity of the extract was assayed using oral glucose tolerance test and streptozotocin induced hyperglycaemic rats. The partition fractions of the extract were evaluated for their anti-hyperglycaemic activity using oral glucose tolerance test. The most active fraction was subjected to chromatographic separations that led to the isolation and characterisation of two new polyphenolic compounds. The structures of these compounds were

**Corresponding author: E-mail: oluwaseyi_f@yahoo.com;*

elucidated and characterized using IR, 1D- and 2D-NMR and MS techniques.The extract gave comparable ($p>0.05$) activity to glibenclamide (5 mg/kg) at 100, 200 and 400 mg/kg at 4 h in oral glucose tolerance test and streptozotocin induced diabetic model on day 21. The most active ethyl acetate fraction (200 mg/kg) elicited comparable activity to the positive control at 0.5-4 h with blood glucose reduction of 52.9 % as compared with glibenclamide (5 mg/kg) of 38.9 % at 4 h. The isolated compounds were identified to be 5-(4-hydroxyphenyl)-3-hydroxy-2-methoxyphenol and 3-(4-methanetriol-2,6-dihydroxyphenyl)-3',4',5',5,7-pentahydroxyflavanonol.The significant anti-diabetic property shown by the pods of *C. pulcherrima* justified its anti-diabetic ethno-medicinal use and the two new polyphenolic compounds isolated from its most active fraction could have contributed to the observed activity.

Keywords: Caesalpinia pulcherrima; anti-diabetes; hyperglycaemia; chromatography; polyphenols.

1. INTRODUCTION

Diabetes mellitus is a chronic endocrine and metabolic disorder with deleterious effects on every organ system in the body. It is underlined predominantly by either insulin insensitivity or insulin deficiency or both which results in impaired metabolism of carbohydrates [1,2]. About 451 million people are diabetic globally and this is presumed to increase to 693 million by 2045, with low or middle income countries having the largest percentage of this diabetic population [3]. Although a wide array of options are now available for treating diabetes, including several new pharmacological classes of drugs many patients fail to achieve adequate glycemic control due to several factors among which is poor medication adherence [4]. Poor medication adherence may be due to; the complexity and inconveniences of treatment, cost of treatment, medication beliefs and side effects such as hypoglycemia, heart failure, liver disease, dropsy, and weight gain [4,5,6]. Therefore there is need for novel medications with little or no side effects.

Caesalpinia pulcherrima Swartz (Fabaceae) commonly called Peacock flower or Pride of Barbados is an ornamental plant that is widely grown in tropical gardens [7]. Various parts of the plant are used ethno-medicinally in the treatment of liver disorder, inflammation, bronchitis, ulcer, asthma, tumors, diabetes, malaria and fungal infections [8,9,10,11]. Studies done on the plant have reported its antiulcer [12], anticancer [13], antimicrobial [14] anti-inflammatory and antinociceptive [15], anthelminthic [16], anti-diabetic [17] and antioxidant activities [18]. Isolated compounds from *C. pulcherrima* include; terpenoids, bonducellin and isobonducellin, flavanones, chalcone, benzoquinone, isoliquiritigenin, homoisoflavonoids, pulcherrimins [11,19,20,21,22,23,24]. In spite of the array of biological activities, its activity guided isolation has not been studied. This work was therefore aimed at investigating the anti-diabetic effect of the pods of *Caesalpinia pulcherrima* and the isolation of bioactive compounds from the most active fraction through activity guided isolation.

2. MATERIALS AND METHODS

2.1 Chemicals and Equipment

All solvents used were distilled. Streptozotocin used was obtained from Sigma-Aldrich, Germany. Purified D-glucose (Loba Chemie, India). Accu-Chek Glucometer with Accu-Chek test strips. NMR spectra were measured on Bruker 500 instruments using methanol-d_4 as solvent and internal standard. The mass spectra were determined on a Bruker Compact Q-TOF high resolution Compact mass spectrophotometer. IR analyses were carried out on PerkinElmer Spectrum Version 100. Pre-coated silica gel 60 F_{254} plates were used to view the compounds under the UV lamp and further visualized by spraying with vanillin-sulphuric acid solution.

2.2 Plant Materials and Extraction

The pods of *C. pulcherrima* were collected at Obafemi Awolowo University campus, Ile-Ife, Osun State, Nigeria. It was identified and authenticated by Mr. Ademorriyo at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife. Its voucher specimen (IFE 17513) was prepared and deposited at the same herbarium. The pods were peeled to remove the seeds and air dried for 30 days. The air dried pods were grinded to powder and 6 kg of the powdered pods was exhaustively extracted with methanol and concentrated *in vacuo* to obtain a methanolic extract with 9.6 % w/w yield.

The extract (160.0 g) was suspended in 500 mL of methanol/water (1:1) and successively extracted with *n*-hexane (9×500 mL), DCM (3×500 mL) and ethyl acetate (16×500 mL) to obtain their corresponding *n*-hexane (28.1 g), DCM (47.8 g), ethyl acetate (19.7 g) and aqueous methanol (58.6 g) fractions.

2.3 Animal Study

Healthy albino rats (120–160 g) of both sexes bred under standard conditions (temperature 25 ± 3 °C) at the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used for the experiment. They were fed on a standard pellet diet (Vital Feeds, Nigeria) and water was given ad libitum. All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Academies Press [25].

2.3.1 Glucose lowering activity of the crude extract and the fractions

A glucose tolerance test was performed by giving glucose (10 g/kg, p.o.) to 24 h fasted rats. Those that were hyperglycaemic (blood glucose level ≥ 7 mmol/L (126 mg/dL) after 0.5 h (time point 0 h, To) were divided into groups of five and administered (p.o.) with 1 % tween 80 in normal saline (negative control), extract (100, 200 and 400 mg/kg) and glibenclamide (5 mg/kg) to determine their hyperglycaemic lowering activities. A drop of blood, taken from the tip of the tail of each rat, was dropped onto a glucometer strip and the blood glucose (bg) level read off directly. The blood glucose levels at 0.0 h (To) were normalised to 100 % while those at other times were percentages of these values. Their blood glucose (bg) levels were determined and recorded at 0, 0.5, 1, 2 and 4 h after administration of the normal saline/extract/drug [26].

2.3.2 Anti-diabetic activity of the crude extract using streptozotocin-induced diabetic rats

Rats used for this study were fasted overnight before the induction of diabetes with streptozotocin. The fasted rats were injected intraperitonially with freshly prepared solution of STZ (65 mg/kg) in 0.1 M sodium citrate buffer (pH 4.5). Hyperglycemia was confirmed in induced rats by the elevated fasting blood glucose levels determined at 72 h. Rats with fasting blood glucose levels ≥ 14 mmol/L after 72 h were considered diabetic and divided into three groups of 10 rats each for the study. They were administered with 1 % tween 80 in normal saline, extract (200 mg/kg) and glibenclamide (5 mg/kg) for 21 days. Their fasting blood glucose levels were determined on days 1, 4, 7, 10, 14 and 21 respectively [27].

2.4 Statistical Analysis

Data obtained from this study were expressed as the mean ± SEM for the number (N) of animals in the group. Analysis of variance (ANOVA) was first used followed by Bonferroni t-test to determine the source of significant differences for all determinations and $p<0.05$ was considered to be statistically significant.

2.5 Isolation and Purification of Compounds

Ethyl acetate fraction (112 g) was adsorbed on 112 g of silica gel (60–200 mesh) and was left overnight to dry. The adsorbed fraction was subjected to column chromatography over silica gel (60–200 mesh) using gradient elution using *n*-hexane, ethyl acetate and methanol. About 30 mL of the eluent was collected into each test tube and 202 column fractions were collected. The fractions were combined into five sub fractions according to their TLC profile. Fractions collected at 100 % EtOAc to EtOAc : MeOH (8:2) was coded as CPEB and was further subjected to column chromatography which yielded a brown solid (1.19 g) at EtOAc:MeOH (8:2). The brown solid obtained was further subjected to column chromatography and eluted with EtOAc:MeOH (9.5:0.5, 9:1, 8.5:1.5, 8:2) to yield 16 sub-fractions. Compound 1 (10.2 mg) was afforded from sub-fraction 15 by purification with Sephadex LH-20 CC (CH_2Cl_2:MeOH, 7:3). Subsequent elution of impure brown solid obtained with EtOAc:MeOH (9:1) followed by purification with Sephadex LH-20 (CH_2Cl_2 : MeOH, 7:3) yielded compound 2 (13.7 mg).

2.5.1 5[4-hydroxyphenyl]-3-hydroxy-2-methoxyphenol (compound 1)

Brown solid, IR (neat): 3253 cm^{-1} (phenolic OH), 1196 cm^{-1} (aromatic C-O-C). ^1H and ^{13}C NMR: Table 4. HRMS-ESI (positive mode): m/z $[M-H]^+$ calcd for $C_{13}H_{13}O_4$, 233.0814; found 233.1141.

2.5.2 3[4-methanetriol-2,6-dihydroxyphenyl]3',4',5',5,7-pentahydroxyflavononol (compound 2)

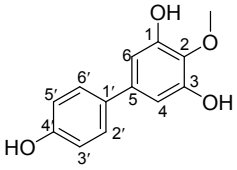
Brown solid, IR (neat): 3282 cm^{-1} (phenolic OH), 1678 cm^{-1} (C=O), 1604 and 1515 cm^{-1} (aromatic C=C), 1234 and 1165 cm^{-1} (C-O-C). ^{1}H and ^{13}C NMR: Table 5. HRMS-ESI (positive mode): m/z $[M\text{-}OH]^{+}$ calcd for $C_{22}H_{27}O_{12}$, 473.0720; found 473.0715.

3. RESULTS AND DISCUSSION

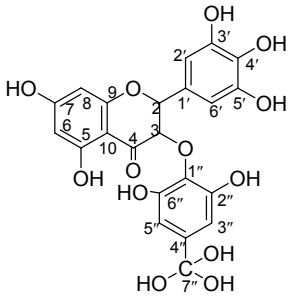
3.1 Glucose Lowering Activity of the Extract

Glibenclamide, a sulphonylurea compound, has been reported to work through major insulin stimulation [28]. It was reported that the glucose-loaded rat model in hyperglycaemia lowering study of plant materials and the use of glibenclamide or other insulin stimulatory drugs as reference could be extrapolated to be type-2 diabetes [29]. Therefore activity profile similar to that of glibenclamide by plant extracts/fractions in glucose-loaded rat model allowed a guess of insulin stimulating mechanisms of action.

In the glucose-loaded rat model (Table 1), there was a time-dependent reduction in blood glucose in the negative control group that lasted up to the fourth hour. This confirmed homeostatic regulatory mechanism of the animals and indicated a healthy state of their pancreas [30]. Glibenclamide also gave a time dependent blood glucose lowering effect from 0.5 – 4 h which established its insulin secretion mechanism of action. The extract at100, 200 and 400 mg/kg was devoid of activity at 0.5-2 h but at 4 h it showed hyperglycaemia lowering effect comparable to glibenclamide (5 mg/kg) thus indicating only insulin stimulation as its mechanism of action (Table 1). Similar effects have been reported for the extracts of *Parquetina nigrescens* and *Uvaria afzelii* [26,31].



Compound **1**



Compound **2**

Fig. 1. The structures of compounds 1 and 2

Table 1. Dose related glucose lowering effect of the pods of *C. pulcherrima* extract

Dose of extract/drug (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_t)				
	0.0 h	**0.5 h**	**1 h**	**2 h**	**4 h**
GLU (10 g/kg)	100	83.79±3.81[a]	85.89±0.50[b]	76.45±1.71[b]	74.18±1.97[b]
CPA (100)	100	92.32±3.60[a] (-10.17%)	85.26 ± 3.51[a,b] (0.73%)	69.51 ± 4.64[a,b] (-1.28%)	55.18±5.00[a] (25.61%)
CPA (200)	100	93.69±2.92[a] (-11.81%)	81.91±6.85[a] (4.56%)	75.46±6.37[a,b] (0.99%)	45.31±3.00[a] (38.92%)
CPA (400)	100	86.24±3.38[a] (-2.92%)	84.27±3.80[a] (1.89%)	76.30±3.02[a,b] (0.22%)	43.05±5.56[a] (41.97%)
GLI (5)	100	75.64±6.73[a] (9.73%)	70.68±6.86[a,b] (17.71%)	58.32±6.44[a] (23.72%)	45.27±6.88[a] (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0h (To), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different ($p < 0.05$). GLU (negative control); GLI: Glibenclamide (positive control).
CPA: Extract of C. pulcherrima pods

Table 2. Anti-diabetic activity of the extract of *C. pulcherrima* pods in streptozotocin induced-diabetic rats

Dose of extract/drug (mg/kg)	Blood glucose level as percentage of T_o (reduction in blood glucose relative to negative control at T_t)					
	Day 1	Day 4	Day 7	Day 10	Day 14	Day 21
Normal saline	100.00	92.53 ± 5.71^{c}	97.22 ± 4.41^{c}	93.52 ± 3.42^{c}	94.65 ± 5.37c	104.21 ± 4.46c
CPA (200)	100.00	43.38 ± 3.98^{b} (53.11%)	33.20 ± 4.74^{b} (65.85%)	29.05 ± 4.08^{b} (68.94%)	25.82 ± 0.82^{b} (72.72%)	22.60 ± 1.62^{a} (78.31%)
GLI (5)	100.00	17.27 ± 2.74^{a}(81.34%)	15.52 ± 2.23^{a} (84.03%)	15.11 ± 1.62^{a} (83.84 %)	15.21 ± 1.04^{a} (83.93%)	17.00 ± 1.91^{a} (83.68%)

Data show the mean ± SEM blood glucose levels at the different time interval expressed as percentages of levels at 0 h (T_o), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different (p<0.05). GLI: glibenclamide, CPA: Extract of C. pulcherrima pods

Table 3. Hyperglycaemia lowering effect of the partition fractions of the crude extract of the pod of *C. Pulcherima*

Dose of Fraction/drug (mg/kg)	Blood glucose levels as percentages of T_o (% percentage reduction in blood glucose relative to negative control at T_t)				
	0 h	0.5 h	1 h	2 h	4 h
GLU (10g/kg)	100.00	83.79 ± 3.81^{a}	85.89 ± 0.50^{b}	76.45 ± 1.71^{c}	74.18 ± 1.97^{c}
n-hexane (200)	100.00	82.11 ± 4.09^{a} (2.00 %)	79.32 ± 3.98^{b} (7.65 %)	73.71 ± 4.01^{b,c} (3.58 %)	62.50 ± 4.58^{b} (15.75 %)
DCM (200)	100.00	80.13 ± 5.12^{a} (4.37 %)	78.26 ± 5.12^{b} (8.88 %)	62.63 ± 3.46^{b} (18.08 %)	46.57 ± 6.71^{a,b} (37.22 %)
EtOAc (200)	100.00	77.20 ± 5.53^{a} (7.87 %)	58.22 ± 3.01^{a} (32.21 %)	49.28 ± 2.07^{a} (35.54 %)	34.92 ± 2.02^{a} (52.92 %)
Aqueous MeOH (200)	100.00	78.82 ± 4.82^{a} (5.93 %)	76.93 ± 4.62^{b} (10.43 %)	66.33 ± 5.37^{b,c} (13.24 %)	58.30 ± 2.87^{b} (21.41 %)
GLI (5)	100.00	75.64 ± 6.73^{a} (9.72 %)	70.68 ± 6.86^{a,b} (17.71 %)	58.32 ± 6.44^{a} (23.71 %)	45.27 ± 6.88^{a} (38.97 %)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at 0 h (T_o), n=5. Values in parentheses represent the percentage reductions in blood glucose levels relative to negative control for each time point. Values with different superscripts within columns are significantly different (p<0.05). GLU (negative control); CPA: Extract of C. pulcherrima pods; GLI: Glibenclamide (positive control)

3.2 Anti-Diabetic Activity of the Extract on Streptozotocin-Induced Diabetic Rats

Since there was no significant difference between the activity of the extract at 200 and 400 mg/kg at 4 h in glucose-loaded rats (Table 1), 200 mg/kg was therefore used to evaluate its anti-diabetic effect in streptozotocin induced rats. Rats in the negative control group showed consistent hyperglycaemia throughout the duration (21 days) of the experiment which showed that the induced hyperglycaemia was permanent (Table 2). The extract demonstrated a time dependent anti-diabetic activity that was significantly less active than glibenclamide on days 1-14 but gave comparable (p<0.05) effect on day 21 (Fig. 2). This result further confirmed insulin release as the major mechanism of action of the extract, as was earlier suggested from its anti-hyperglycaemic action with the glucose-loaded rat model (Table 1). Furthermore, the results were in agreement with the reported anti-diabetic activity of the pod extract on alloxan-induced diabetic rats [17].

The result of the hyperglycaemia lowering activity of the partition fractions of the extract showed that solvent partitioning of the crude extract increased the activity in which ethyl acetate fraction showed 52.92 % blood glucose reduction at 4 h compared to glibenclamide of 38.97 % suggesting the constituents responsible for the activity were concentrated in this fraction (Table 3).

3.3 Structure Elucidation of Isolated Compounds

3.3.1 Compound 1.

The HRESIMS (positive mode) of the compound showed a peak of $(M+H)^+$ at m/z 233.1141 which is consistent with a molecular formula $C_{13}H_{13}O_4$. The ^{1}H-NMR of the compound showed resonances for aromatic protons at δ 6.84 (d, J = 8.7), δ 7.06 (s) and δ 7.90 (d, J = 8.7) each integrated for two protons with signals at δ 6.84 and δ 7.90 suggesting protons of AA'BB' system of an aromatic ring. And signal at δ 7.06 suggesting protons of AA' system of an aromatic ring. The only non-aromatic signal at δ 3.84 (s) integrated for three protons suggested methoxy protons. The ^{13}C-NMR showed very distinct signals for nine carbon atoms at δ 50.8, 108.7, 114.6, 120.1, 131.6, 138.4, 145.1, 161.8 and 167.6. DEPT135 spectrum showed three signals for six methine carbon atoms at 131.6, 114.6 and 108.7, and one methyl carbon atom at 50.8. COSY experiment showed cross peaks between protons at δ 6.84 and δ 7.90. The HSQC experiment showed cross peaks between carbon atoms at 50.8, 108.7, 114.6 and 131.6 ppm with protons at 3.84, 7.06, 6.84 and 7.90 ppm respectively. Thus the compound was identified as 5[4-hydroxyphenyl]-3-hydroxy-2-methoxyphenol (Fig. 1).

3.3.2 Compound 2.

The HRESIMS (positive mode) of the compound showed a pseudo-molecular ion peak at m/z 473.0715 of the molecular ion peak calculated to be 490.0797 which is consistent with a molecular formula $C_{22}H_{18}O_{13}$. The pseudo-molecular ion observed with pseudo-molecular ion formula of $C_{22}H_{17}O_{12}$ at m/z = 473.0715 was as a result of loss of water molecule from the protonated molecular ion $[M+H]^+$ as shown in Fig. 2. The ^{1}H-NMR of the compound showed resonances for aromatic protons at δ 5.98 (s), 6.54 (s) and 7.07 each integrated for two protons. Signals at δ 5.34 (d, J = 11.5) and δ 5.88 (d, J = 11.5) each integrated to be one proton suggesting protons on oxygenated methine groups. The ^{13}C-NMR showed very distinct signals for eighteen carbon atoms at δ 192.0, 167.7, 165.4, 164.1, 162.8, 145.6, 145.0, 138.8, 133.8, 126.6, 119.1, 109.1, 106.4, 100.8, 96.3, 95.2, 81.5 and 72.8. DEPT135 spectrum showed six signals for eight methine carbon atoms at 72.8, 81.5, 95.2, 96.3, 106.4 and 109.1. COSY experiment showed cross peaks between protons at δ 5.34 and δ 5.88. The HSQC experiment showed peaks correlations between carbon atoms at 72.8, 81.5, 95.2, 96.3, 106.4 and 109.1 ppm with protons at 5.88, 5.34, 5.98, 5.98, 6.54 and 7.07 ppm respectively suggesting protons on positions 6 and 8 of ring A of a typical flavonoid for cross peaks between carbon atoms at 95.2, 96.3 ppm and protons at 5.98, 5.98 ppm respectively.. The cross peaks between carbon atoms at 106.4, 109.1 and protons at 6.54, 7.07 ppm suggested two distinct AA' systems. The aforementioned analysis together with HMBC analysis, Table 3. made the compound to be identified as 3[4-methanetriol-2,6-dihydroxypheny l]3',4',5',5,7pentahydroxyflavononol (Fig. 1).

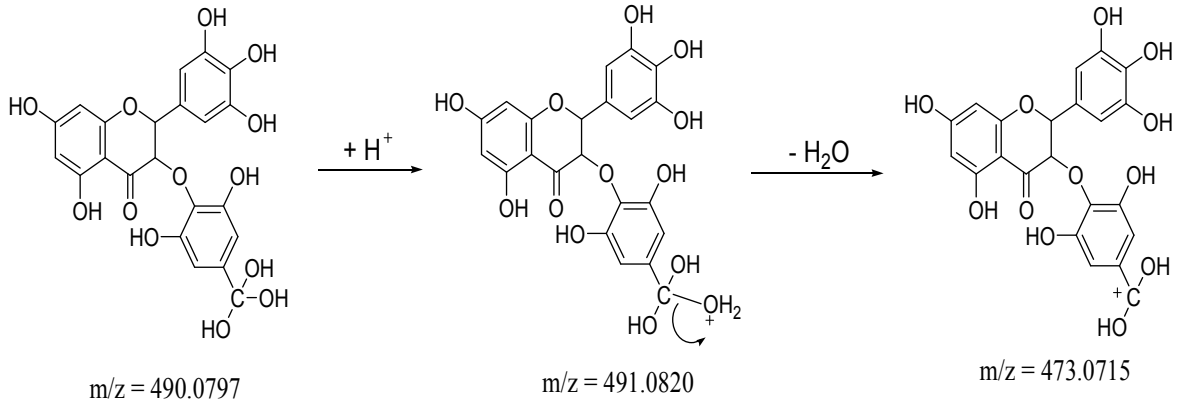


Fig. 2. The proposed fragmentation of $[M+H]^+$ of compound 2 to form the pseudo-molecular ion

Table 4. NMR data of compound 1 in MeOD (multiplicities and J values are given in Hz in parenthesis)

Position	Proton (multiplicity)	Carbon-13	Hmbc (^{1}H to ^{13}C)
1	-	145.1	
2	-	167.6	
3	-	145.1	
4 & 6	7.06 (s)	108.7	108.7, 120.1, 138.4, 145.1, 167.6
5	-	138.4	
1'	-	120.1	
2' & 6'	7.90 (d, J=8.7)	131.6	131.6, 161.8
3' & 5'	6.84 (d, J=8.7)	115.4	115.4, 120.1, 161.8
4'	-	161.8	
2-OCH_3	3.84 (s)	50.8	167.6

Table 5. NMR data of compound 2 in MeOD (multiplicities and J values are given in Hz in parenthesis)

Position	Proton (multiplicity)	Carbon-13	Hmbc (^{1}H to ^{13}C)
1	-	-	
2	5.34 (d, J=11.5 Hz)	81.5	72.8, 106.4, 126.6, 192.0
3	5.88 (d, J=11 Hz)	72.8	81.5, 126.6, 165.4, 192.0
4	-	192.0	-
5	-	164.1	-
6	5.98 (s)	95.2	96.3, 100.8, 164.1, 167.7
7	-	167.7	-
8	5.98 (s)	96.3	95.2, 100.8, 167.7
9	-	165.4	-
10	-	100.8	-
1'	-	126.6	-
2' & 6'	6.54 (s)	106.4	81.5, 106.4, 126.6, 133.8, 145.6
3' & 5'	-	145.6	-
4'	-	133.8	-
1"	-	138.8	-
2" & 6"	-	145.0	-
3" & 5"	7.07 (s)	109.1	109.1, 119.1, 138.8, 145.0, 162.8
4"	-	162.8	-
7"	-	119.1	-

4. CONCLUSION

Purification of the extract led to increase in the activity of the fractions in which two new polyphenols were isolated from the most active fraction. These compounds are expected to contribute to the spectrum of potential compounds that could be drugs and/or lead drugs in the treatment of diabetes. It was also observed that DCM fraction (in Table 3) had percentage reduction at 4 h quite similar to the percentage reduction of glibenclamide at 4 h, 37.22% and 38.97% respectively, so it is expected that DCM fraction may also contain active compound(s) either different or similar to the active compounds present in EtOAC fraction. Therefore, purification and isolation of compound(s) from the DCM fraction is being considered for further work on this study for comparison with the constituents from EtOAC fraction.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal experiments conformed to the guide for the care and use of laboratory animals published by the national academies press [25].

ACKNOWLEDGEMENTS

SOF is grateful to the Department of Chemistry, Obafemi Awolowo University and Obafemi Awolowo University, Nigeria for granting him three months research visit to University of

Johannesburg, South Africa. SOF and DTN are grateful to Mr Mutshinyalo Nwamadi of the Department of Spectrau, University of Johannesburg, APK campus, South Africa for his assistance in the acquisition of NMR data. SOF and DTN are also grateful to Eric Morifi of the Department of Chemistry, University of Witwatersrand, South Africa for his assistance in the acquisition of MS data. DTN is grateful to University of Johannesburg for grant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kibiti CM, Afolayan AJ. Herbal therapy: A review of emerging pharmacological tools in the management of diabetes mellitus in Africa. Pharmacogn. Mag. 2015;11(44):258-274. Available:https://doi.org/10.4103/0973-1296.166046
2. Ighodaro OM. Molecular pathways associated with oxidative stress in diabetes mellitus. Biomed. Pharmacother. 2018;108:656–662. Available:https://doi.org/https://doi.org/10.1016/j.biopha.2018.09.058
3. Cho NH, Shaw JE, Karuranga S, Huang Y da, Rocha Fernandes JD, Ohlrogge AW et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res. Clin. Pract. 2018;138:271–281. Available:https://doi.org/10.1016/j.diabres.2018.02.023
4. Polonsky WH, Henry RR. Poor medication adherence in type 2 diabetes: Recognizing the scope of the problem and its key contributors. Patient Prefer. Adherence. 2016;10:1299–1307. Available:https://doi.org/10.2147/PPA.S106821
5. Tahrani AA, Piya MK, Kennedy A, Barnett AH. Glycaemic control in type 2 diabetes: Targets and new therapies. Pharmacol. Ther. 2010;125(2):328–361. Available:https://doi.org/https://doi.org/10.1016/j.pharmthera.2009.11.001
6. Widyawati T, Yusoff NA, Asmawi MZ, Ahmad M. Antihyperglycemic effect of methanol extract of Syzygium polyanthum (Wight.) Leaf in streptozotocin-induced diabetic rats. Nutrients. 2015;7(9):7764–7780. Available:https://doi.org/10.3390/nu7095365
7. Roach JS, McLean S, Reynolds WF, Tinto WF. Cassane diterpenoids of Caesalpinia pulcherrima. J. Nat. Prod. 2003;66(10):1378–1381. Available:https://doi.org/10.1021/np0302955
8. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. II. Lalit Mohan Publication, Allahabad. 1935;1347-1348.
9. Quisumbing E. Medicinal plants of the Philippines. Dep. agric. commer. Philipp. Islands Tech. Bull;1951.
10. Chiang LC, Chiang W, Liu MC, Lin CC. In vitro antiviral activities of Caesalpinia pulcherrima and its related flavonoids. J. antimicrob. chemother. 2003;52(2):194–198. Abaliable: https://doi.org/10.1093/jac/dkg291
11. Srinivas KVNS, Koteswara Rao Y, Mahender I, Das B, Rama Krishna KVS, Hara Kishore et al. Flavanoids from Caesalpinia pulcherrima. Phytochemistry. 2003;63(7):789–793. Available: https://doi.org/10.1016/s0031-9422(03)00325-x
12. Takawale H, Mute V, Awari D, Hukkeri VI, Mehta P, Vawhal P. Screening of antiulcer activity of Caesalpinia pulcherrima L. bark. against aspirin induced ulcer in Rats. World J. Med. Sci. 2011;6(4):168–172.
13. Nainwal P, Dhamija K, Tripathi S. Study of antihyperlipidemic effect on the juice of the fresh fruits of Lagenaria siceraria. Int. J. Pharm. Pharm. Sci. 2011;3(1):88–90.
14. Sudhakar M, Rao CV, Rao PM, Raju DB, Venkateswarlu Y. Antimicrobial activity of Caesalpinia pulcherrima, Euphorbia hirta and asystasia gangeticum. Fitoterapia. 2006;77(5): 378–380. Abaliable:https://doi.org/https://doi.org/10.1016/j.fitote.2006.02.011
15. Kumbhare M, Sivakumar T. Anti-inflammatory and antinociceptive activity of pods of *Caesalpinia pulcherrima*. J. Applied Pharmaceutical Science, 2011;1(7):180-184.
16. Kumbhare M, Sivakumar T, Kalantri M, Mahajan V. Investigation of anthelmintic activity of pods of Caesalpinia pulcherrima. J. Pharm. Res. And Opin. 2012;2(7):63-65.
17. Kumbhare MR, Sivakumar T. Caesalpinia pulcherrima extracts on blood glucose in normal and alloxan monohydrate-induced Diabetic Rats. J. Biol. Sci. 2019;19(1):34–39. Available:https://doi.org/10.3923/jbs.2019.34.39

18. Pawar CR, Mutha RE, Landge AD, Jadhav RB, Surana SJ. Antioxidant and cytotoxic activities of Caesalpinia pulcherrima wood. Indian J. Biochem. Biophys. 2009;46:198-200.
19. McPherson DD, Cordell GA, Soejarto DD, Pezzuto JM, Fong HHS. Peltogynoids and homoisoflavonoids from Caesalpinia pulcherrima. Phytochemistry. 1983;22(12):2835–2838. Available:https://doi.org/https://doi.org/10.1016/S0031-9422(00)97708-2
20. Islam AKMN, Ali MA, Sayeed A, Syed M, Abdus Salam AI, Rahman M et al. An antimicrobial terpenoid from Caesalpinia pulcherrima Swartz : Its characterization , antimicrobial and Cytotoxic Activities. Asian J. Plant Sci. 2003;2(17):1162–1165. Available:https://doi.org/10.3923/ajps.2003.1162.1165
21. Rao YK, Fang SH, Tzeng YM. Anti-inflammatory activities of flavonoids isolated from Caesalpinia pulcherrima. J. Ethnopharmacol. 2005;100(3):249–253. Available:https://doi.org/https://doi.org/10.1016/j.jep.2005.02.039
22. Das B, Thirupathi P, Ravikanth B, Aravind Kumar R, Sarma AVS, Basha SJ. Isolation, synthesis, and bioactivity of homoisoflavonoids from Caesalpinia pulcherrima. Chem. Pharm. Bull. (Tokyo). 2009;57(10):1139–1141. Available:https://doi.org/10.1248/cpb.57.1139
23. Pranithanchai W, Karalai C, Ponglimanont C, Subhadhirasakul S, Chantrapromma K. Cassane diterpenoids from the stem of Caesalpinia pulcherrima. Phytochemistry 2009;70(2):300–304. Available:https://doi.org/10.1016/j.phytochem.2008.12.006
24. Yodsaoue O, Karalai C, Ponglimanont C, Tewtrakul S, Chantrapromma S. Pulcherrins D-R, potential anti-inflammatory diterpenoids from the roots of Caesalpinia pulcherrima. Tetrahedron. 2011;67(36): 6838–6846. Available:https://doi.org/https://doi.org/10.1016/j.tet.2011.06.087
25. Committee for the update of the guide for the care and use of laboratory animals, institute for laboratory animal research, division on earth and life studies, national research council of the national academies. Guide for the care and use of laboratory animals, 8th ed. The National Academies Press, Washington, DC; 2011.
26. Ayoola MD, Balogun JO, Famuyiwa FG, Yeboah SO, Famuyiwa SO. Isolation and characterization of 2-hydroxy-3-[4-hydroxyphenyl]-2-propenoic acid and 4-bromophenol from anti-diabetic extract of the root bark of Uvaria afzelii. South African J. Bot. 2017;112:527–532. Available:https://doi.org/https://doi.org/10.1016/j.sajb.2017.05.006
27. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Verdi AA, et al. Induction of diabetes by streptozotocin in rats. Indian J. Clin. Biochem. 2007;22:60–64. Available:https://doi.org/10.1007/BF02913315
28. Luzi L, Poza G. Glibenclamide: An old drug with a novel mechanism of action? Actadiabetologica. 1997;34:239–244.
29. Verspohl EJ. Recommended testing in diabetes research. Planta Med. 2002;68(7):581–590. Abailable: https://doi.org/10.1055/s-2002-32894
30. Kar A, Choudhary BK, Bandyopadhyay NG. Preliminary studies on the inorganic constituents of some indigenous hypoglycaemic herbs on oral glucose tolerance test. J. Ethnopharmacol. 1999;64(2):179–184. Available:https://doi.org/https://doi.org/10.1016/S0378-8741(98)00118-4
31. Faloye KO, Ayoola MD, Amos-Tautua BM, Famuyiwa SO. Anti-diabetic activity of convallatoxin isolated from the root bark of Parquetina nigrescens (Afzel.) Bullock (Asclepiadaceae). European J. Med. Plants. 2018;25(4):1–9. Available:https://doi.org/10.9734/ejmp/2018/45129

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64892