https://dx.doi.org/10.4314/ijs.v20i2.3

THE ISOLATION AND CHARACTERIZATION OF LUPEOL-3-ACETATE FROM THE HYPERGLYCAEMIA-LOWERING FRACTION OF THE STEM BARK OF *CRYSTOPHYLLUM ALBIDUM* G. DON (SAPOTACEAE)

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(Received: 19th December, 2017; Accepted: 6th April, 2018)

ABSTRACT

This study investigated the hyperglycaemia-lowering activity of the extract and partitioned fractions of the stem bark of *Chrysophyllum albidum*, and isolation of a bio-active compound from the most active fraction. Powdered stem bark of the plant was extracted with methanol and the extract (**A**) was tested in glucose-induced hyperglycaemic rats at 100, 200 and 400 mg/kg. The extract was partitioned to obtain its *n*-hexane ($\mathbf{B}_1$), dichloromethane ($\mathbf{B}_2$), ethyl acetate ($\mathbf{B}_3$) and mother liquor ($\mathbf{B}_4$) fractions that were tested for blood glucose lowering activity using glucose-induced hyperglycaemic model at the most active dose of the extract. The results were subjected to ANOVA followed by Student–Newman-Keuls post hoc tests and $p< 0.05$ was considered significant. The extract (**A**) gave dose dependent activity with 400 mg/kg giving the highest percentage blood glucose level reduction of 13% at 4 h that was comparable to the standard, glibenclamide at 5 mg/kg. Fractions $\mathbf{B}_1$ and $\mathbf{B}_2$ gave a time-dependent activity up to the fourth hour similar to glibenclamide, while $\mathbf{B}_3$ and $\mathbf{B}_4$ were devoid of hyperglycaemia-lowering activity. Furthermore, $\mathbf{B}_1$ gave 3, 18, 19 and 29% blood glucose levels reduction at 0.5, 1, 2 and 4 h respectively while $\mathbf{B}_2$ gave 12, 28, 33 and 41% blood glucose levels reduction at 0.5, 1, 2 and 4 h respectively. The activity of $\mathbf{B}_2$ was significantly ($p<0.05$) more active than glibenclamide at all-time point. The dichloromethane fraction ($\mathbf{B}_2$), most active, was subjected to column and preparative thin layer chromatography and the fraction afforded lupeol-3-acetate. The structure was validated by using Bruker Ascend 400 NMR instrument and the NMR data was compared with literature. The compound was one of the compounds responsible for the biological activity observed as reported by Lakshmi and his co-workers, 2014.

Keywords: *Chrystophyllum albidum*, Anti-hyperglycaemia, Stem bark, Chromatography, lupeol-3-acetate.

INTRODUCTION

Hyperglycaemia is a metabolic condition in which the blood glucose level is higher than normal. This abnormal condition leads to Diabetes mellitus (DM) if persisted for a long period due to lack of insulin or insulin resistance. It was estimated in 2013 that 382 million people have diabetes worldwide, with type 2 diabetes making up about 90% of the cases (Ayoola *et al*., 2017). Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. It was reported to double the risk of death and World Health Organization (WHO) projected that diabetes will be the seventh leading cause of death in 2030 (Mathers and Loncar, 2006; Hitabchi *et al*., 2009). The number of people with diabetes is estimated to rise to 592 million by 2035 (Ayoola *et al*., 2017)

There is the need to isolate the active principles from medicinal plant extracts as the use of crude extracts has limitations. Isolation of compounds helps greatly in the standardization of dosages and pharmacokinetic studies. In addition, medicinal activities of some plants are rapidly lost on storage, e.g. *Digitalis purpurea* leaf. Furthermore, crude extracts may contain, in addition to the bioactive molecules, other constituents which may have harmful effects. For example aristolochic acids, with nephrotoxic and carcinogenic effects have been isolated from *Aristolochi afangchi* (Loset *et al.* 2001). It is therefore important to isolate and identify the bioactive molecules from the medicinal plant extracts. Indeed, it has been estimated that 25% of prescribed medicines today are substances derived from plants (Rates, 2001) and the most recent example is the antimalarial artemisinin obtained from *Artemisia annua*.

Chrysophyllum albidum G. Don (Sapotaceae), that is called "agbalumo" and "udara" in South-Western and South-Eastern Nigeria respectively, is a small to medium buttressed tree that is widely distributed and grown in the low land rain forest

zones of West Africa (Madubuike and Ogbonnaya, 2003, Onyeka *et al*., 2012). It is used ethno-medicinally in the treatment of malaria, anaemia, ulcer, intestinal worm, asthma, cough, yellow fever and diabetes (Laurent *et al*., 2012; Chukwuma *et al*., 2015). Also, its barks and leaves extracts are reported to have anti-diabetic activity (Engwa *et al*., 2016). The stem bark and the leaf extracts of the plant have been reported for antioxidant, antiplatelet and hypoglycaemic properties (Adebayo *et al*., 2010 &2011), anti-plasmodia (Adewoye *et al*., 2010), antimicrobial (Idowu *et al*., 2016) activities while the ethanolic extract of *C. albidum* seed cotyledon has been shown to elicit anti-hyperglycaemia activity (Olorunnisola *et al*., 2008). Stigmasterol, Eleagnine, myricetin-3- rhamnoside, procyanidin B5, epigallocatechin and epicatech in (Idowu *et al*., 2003, 2016, Adebayo *et al*., 2011) have been isolated from various parts of the plant. The present work isolated lupeol acetate from the most active hyperglycaemic fraction of the stem bark extract of the plant, *Chrysophyllum albidum*.

MATERIALS AND METHODS

Plant Material

The stem bark of *C. albidum* was collected at Modomo area, along Ibadan road, Ile-Ife. It was authenticated at Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife. The voucher specimen IFE-NO.17604 was prepared and deposited at the Herbarium. The stem bark was air-dried for six weeks at room temperature and powdered.

Extraction and Partitioning

Three kilograms (3 kg) of the powdered sample was extracted with methanol (32 L) and filtered. The filtrate was concentrated *in-vacuo* to give a methanolic extract with (377.01 g). The extract was then retaken into 50% aqueous methanol (1000 mL) and partitioned between n-hexane (12×400 mL), DCM (20×400 mL) and EtOAc (10×400 mL) in a 5 L separating funnel to obtain n-hexane (35.43 g), DCM (14.19 g), EtOAc (22.10 g) and aqueous methanol (258.21 g) fractions.

Bioassays

Animals

Forty healthy albino rats weighing between 120-170 g were used for the experiment. All the rats were given a period of acclimatization of three weeks before the commencement of the experiment. The animals were housed in well-ventilated cages and kept under controlled environment conditions of temperature (25 ± 5 °C), relative humidity (50 ± 5 °C) and 12 hour light /dark circle at animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile- Ife. They were fed *ad libitum* everyday with standard chow diet and were allowed free access to water (Onyeka *et al.,* 2013).

Hyperglycaemia-Lowering Assay Using Glucose–Loaded Rats

A glucose tolerance test was performed by oral administration of glucose (10 g/kg) to 24 h fasted rats. Hyperglycaemic rats with blood glucose level 7.0 mmol/L (126 mg/dL) after 0.5 h of administration were divided into five groups of five rats each and administered orally with 1% Tween 80 (negative control), extract (100, 200, 400 mg/kg) and glibenclamide (5 mg/kg) (positive control). A drop of blood taken from the tip of the tail of each rat at 0.0, 0.5, 1.0, 2.0 and 4.0 h 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 taken as 100% while those at other times were expresses as percentage of these values (Adebajo *et al*., 2013a). All values were normalised to blood glucose at To taken as 100% (Adebajo *et al*., 2013a, 2013b). Blood glucose (bg) levels were determined at 0 h, 0.5, 1, 2, and 4 h after administration of the normal saline/ extract/fractions/drugs (Adebajo *et al.,* 2009).

Statistical Analysis

All values were express as Mean ±SD. The differences were compared using one-way Analysis of Variance (ANOVA) followed by student t-test p values <0.05 were considered as a significant.

Isolation and purification of the compound

Dichloromethane fraction (13.22 g) was subjected to column and thin layer chromatography for isolation and purification of the compound. The fraction was dissolved in a suitable organic solvent and the solution was adsorbed on silica gel (13.22 g) which was left to dry. The adsorbed fraction was

packed into glass column dry and gradient elution from n-hexane (HEX) through dichloromethane (DCM) to methanol (MeOH) was carried out. About 30 mL of eluent was collected into each beaker, 174 column fractions collected were bulked based on their TLC profile to obtain 15bulked fractions.

An impure solid (51.1 mg) was collected from the fraction eluted with HEX-DCM 60:40. This was further purified on Sephadex LH-20 with isocratic elution using $CHCl_3$/MeOH 7:3 solvent system (200 mL) and 19 column fractions (5 mL each) were obtained which was again bulked based on their TLC profile to obtain 9 bulked fractions. And fraction 3 (8.0 mg) was the only fraction that gave a single spot on analytical TLC.

RESULTS AND DISCUSSION

Glucose Lowering Effect of the Extract and Partition Fractions

The glucose-induced hyperglycaemic rats administered with normal saline (negative control) demonstrated significant time dependent reduction in their blood glucose levels up to the fourth hour (Table 1). This is due to homeostatic regulatory mechanism in the normal animals. The reductions also confirmed that their pancreases were functioning well (Kar *et al.,* 1999; Adebajo *et al.,* 2009, 2013a). Glibenclamide at 5 mg/kg gave 10, 18, 24 and 39% blood glucose levels reduction at 0.5, 1, 2 and 4 h respectively, indicated time-dependent activity and this confirmed its early extra-pancreatic and late insulin stimulating mechanisms of action in glucose-induced hyperglycaemic rats (Murray, 2006; Adebajo *et al.*, 2013a,b). The extract showed dose-dependent and time-dependent activity with significantly lower activity than that of glibenclamide (5 mg/kg) (Table 1). However, its 400 mg/kg gave a better activity that was comparable to glibenclamide (5 mg/kg) at 0.5-4 h indicating similar mechanism of action at this dose, Table 1.

Table 1: Dose related hyperglycaemia lowering effect of the crude extract of *C. albidum* stem bark in glucose-loaded rats.

Drug/extract doses (mg/kg)	Blood glucose levels as a percentage of T0 (% reduction in blood glucose relative to negative control Tt)				
	0 h	0.5 h	1 h	2 h	4 h
GLU, 10 g/kg	100	83.8±3.81^{a}	82.9±0.50^{a}	76.5±1.71^{b}	74.2±2.07^{b}
CA (100)	100	82.14±12.23^{b} (1.74%)	81.62±12.87^{b} (3.82%)	75.23±12.80^{b} (5.64%)	73.73±6.69^{b} (7.17%)
CA (200)	100	81.83±0.38^{b} (2.79%)	79.83±0.38^{b} (4.08%)	75.76±3.76^{b} (6.18%)	71.80±4.49^{b} (8.23%)
CA (400)	100	79.22±8.41^{a} (5.45%)	76.63±7.19^{a} (7.78%)	70.44±6.32^{a,b}(10.86%)	64.50±5.45^{a,b} (13.05%)
GLI (5)	100	75.64±6.73^{a} (9.73%)	70.68±6.86^{a} (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 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 ($p<0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test). GLU: Glucose in 1% of Tween 80 in normal saline (negative control); CA: Stem bark extract of *C. albidum*; Gli: Glibenclamide.

The most active dose of the crude extract, 400 mg/kg (Table 1), was used for the fractions in which the ethyl acetate fraction **(B_3)** and the mother liquor **(B_4)** were totally devoid of hyperglycaemia-lowering activity at 0.5-4 h apart from **(B_4)** that gave 15% activity at 4 h indicating that the hyperglycaemia lowering compounds of the extract were not in these fractions (Table 2). However, both $\mathbf{B}_1$ and $\mathbf{B}_2$ fractions gave a time-dependent activity up to the fourth hour showing similar mechanism of activity with glibenclamide (5 mg/kg). Fraction $\mathbf{B}_1$ gave 3, 18, 19 and 29% blood glucose levels reduction at 0.5, 1, 2 and 4 h respectively and Fraction $\mathbf{B}_2$ gave 12, 28, 33 and 41% blood glucose levels reduction at 0.5, 1, 2 and 4 h respectively. The activity of $\mathbf{B}_1$ and $\mathbf{B}_2$ were comparable to that of glibenclamide (5 mg/kg) at all time points but $\mathbf{B}_2$ was significantly ($p<0.05$) more active than glibenclamide (5 mg/kg). This indicated that the constituents responsible for the observed activity were non-polar to moderate polar compounds that were distributed into fractions $\mathbf{B}_1$ and $\mathbf{B}_2$ respectively but were more concentrated in fraction $\mathbf{B}_2$ (Table 2). The results also showed that partitioning improved the activity of the extract in which the constituents that masked the activity in the crude extract were polar constituents and these were distributed in ethyl acetate fraction and mother liquor.

Table 2: Hyperglycaemia lowering effect of the partition fractions of *C. albidum* stem bark in `glucose-loaded rats.

Drug/fractions (mg/kg)	Blood glucose level as percentage of T0 (reduction in blood glucose relative to negative control at Tt)				
	0 h	0.5 h	1 h	2 h	4 h
GLU, 10 g/kg	100	$83.8\pm3.81^{b,c}$	$82.9\pm0.50^{b,c}$	76.5 ± 1.71^{c}	74.2 ± 2.07^{c}
CA (400)	100	$79.22\pm8.41^{a,b,c}$ (5.45%)	$76.63\pm7.19^{b,c}$ (7.78%)	70.44 ± 6.32^{c} (10.86%)	64.50 ± 5.45^{b} (13.05%)
B1 (400)	100	$81.76\pm6.52^{a,b}$ (2.42%)	$70.72\pm7.31^{a,b}$ (17.66%)	61.75 ± 4.72^{b} (19.23%)	52.49 ± 5.37^{a} (29.24%)
B2 (400)	100	73.46 ± 7.65^{a} (12.33%)	61.91 ± 4.55^{a} (27.92%)	51.01 ± 5.71^{a} (33.28%)	43.71 ± 0.58^{a} (41.08%)
B3 (400)	100	$84.95\pm5.43^{b,c}$ (-1.38%)	81.09 ± 7.29^{c} (-5.59%)	79.57 ± 6.36^{c} (-4.08%)	74.01 ± 8.72^{c} (0.23%)
B4 (400)	100	92.43 ± 2.24^{c} (-10.31%)	87.53 ± 2.10^{c} (-1.91%)	81.14 ± 4.26^{c} (-6.14%)	63.10 ± 1.34^{b} (14.94%)
GLI (5)	100	75.6 ± 6.73^{a} (9.73%)	70.7 ± 6.86^{b} (17.71%)	$58.3\pm6.44^{a,b}$ (23.72%)	45.3 ± 6.88^{a} (38.97%)

Data show the mean ± SEM blood glucose levels at the different time points expressed as percentages of levels at h (T_0), $n = 5$. 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 ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keuls' test). Tween 80: < 1 % of Tween 80 in normal saline (negative control). CA**:** Extract of *Chrysophyllum albidum;* B_1 – n-Hexane fraction, B_2 – DCM fraction, B_3 – EtOAc fraction, B_4 – Aq-MeOH fraction, GLI – Glibenclamide (positive control).

Structural elucidation of isolated compound and its spectroscopic data

The compound (8 mg) was isolated as a white solid from dichloromethane fraction eluted with HEX-DCM 60:40. The ^{1}H-NMR ($CDCl_3$, 400 Mhz) spectrum showed high field signals with signals for eight methyl groups at δ_H 2.03 (s), 1.68 (s), 1.00 (s), 0.99 (s), 0.91 (s), 0.88 (s), 0.86 (s) and 0.79 (s). A pair of singlet at δ_H 4.69 and 4.54 (1H, each) indicated olefinic protons. The ^{13}C-NMR (100 MHz) spectrum showed some characteristic signals at δ_C 171.0, 152.8, 109.1 and 80.7 ppm. The signal at δ_C 171.0 was diagnostic of carbonyl of ester, signal at δ_C 152.8 and 109.1arethe quaternary and methylene of olefinic carbon atoms. The signal at δ_C 80.7 was assigned to the oxygenated sp^3

carbon atom. The analysis of the ^{13}C- (Table 3) and Dept-35 NMR spectra in which seven quaternary, six methine, eleven methylene and eight methyl carbon atoms were identified, gave a molecular formula of $C_{32}H_{52}O_2$. The ^{1}H-NMR and ^{13}C-NMR data of the compound were compared with the ^{1}H-NMR and ^{13}C-NMRdata of lupeol-3-acetate (Nesrin *et al.*, 2013), and the data agreed well with that of lupeol-3-acetate (Tables 1 and 2). Thus, the isolated compound was identified as lupeol-3-acetate (Nesrin *et al.,* 2013).

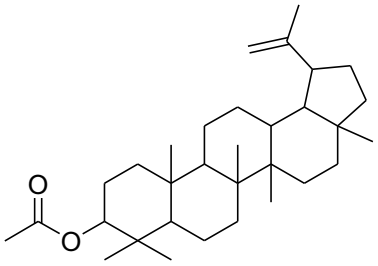

Fig.1: Structure of Lupeol-3-acetate

Table 3: The comparison ^{1}H-NMR data between lupeol-3-acetate and the isolated compound

Lupeol-3-acetate		Isolated compound	
H-atom	ppm (multiplicity)	H-atom	ppm (multiplicity)
H-3	4.51 (m)	H-3	4.50 (m)
3H-23	0.79 (s)	3H-23	0.79 (s)
3H-24	0.86 (s)	3H-24	0.86 (s)
3H-25	0.88 (s)	3H-25	0.88 (s)
3H-26	0.95 (s)	3H-26	0.91 (s)
3H-27	0.99 (s)	3H-27	0.99 (s)
3H-28	1.04 (s)	3H-28	1.00 (s)
2H-29	4.70 (s)	2H-29	4.69 (s)
	4.58 (s)		4.54 (s)
3H-30	1.70 (s)	3H-30	1.68 (s)
3H-32	2.05 (s)	3H-32	2.03 (s)

Table 4: The comparison ^{13}C-NMR data between lupeol-3-acetate and the isolated compound

Lupeol-3-acetate				Isolated compound			
C-atom	ppm	C-atom	ppm	C-atom	ppm	C-atom	ppm
1	38.4	17	43.0	1	38.0	17	43.0
2	21.1	18	48.3	2	23.7	18	48.0
3	81.0	19	48.0	3	80.7	19	49.0
4	38.1	20	150.9	4	35.5	20	152.8
5	55.4	21	29.9	5	55.3	21	29.8
6	18.2	22	40.0	6	18.3	22	40.0
7	34.3	23	28.0	7	35.0	23	27.4
8	41.0	24	16.5	8	40.8	24	18.0
9	50.4	25	16.2	9	50.3	25	18.0
10	37.8	26	16.0	10	37.8	26	16.0
11	21.0	27	14.5	11	20.9	27	16.0
12	25.2	28	18.0	12	26.0	28	19.3
13	37.1	29	109.3	13	36.1	29	109.1
14	42.9	30	19.3	14	43.0	30	22.0
15	28.7	31	170.9	15	28.1	31	171.0
16	35.6	32	28.1	16	35.5	32	31.3

CONCLUSION

Lupeol-3-acetate, a triterpenoid ester was previously isolated from the fruits of *Manikara zapota* (Fayek *et al*., 2013), leaves of *Alstonia scholaris*, *Phyllanthus reticulatus*, *Pouteria torta*, *Ficus ulmifolia*, *Ficus odorata*, *Ficus carica* and *Macaranga barteri* (Gupta *et al*., 2005; Jamal *et al*., 2008; Perfeito *et al*., 2005; Ragasa *et al*., 2009; Tsai *et al*., 2012; Saeed and Sabir, 2002; Fred-Jaiyesimi and Ibukunoluwa, 2016), seeds of *Caesalpinia bonducella* (Saeed and Sabir, 2001), root and stem barks of *Crataeva nurvala*, *Crataeva nurvala*, *Vernonia cinerea* and *Hemidesmus indicus*, *Himatanthus sucuuba*, latex of *Himatanthus drasticus* (Lakshmi and Chauhan, 1975; Lucetti*et al*., 2010; Mistra *et al*., 1993; Wood *etal*., 2001; Chatterjee *et al*., 2006), stem of *Diospyros rubra* (Prachayasittikul *et al*., 2010) and aerial parts of *Plumbago zeylanica* (Nguyen *et al.,* 2004) but not from *Chrysophyllum albidum*. It is noteworthy to report its isolation for the first time from this plant, *Chrysophyllum albidum*. The compound was reported to possess anti-fertility (Gupta *et al*., 2005), anti-inflammatory (Lucetti *et al*., 2010; Chen *et al*., 2012), anti-nociceptive (Chen *et al*., 2012), anti-arthritic (Kweifio-Okai and Caroll, 1993), anti-venom (Chatterjee *et al*., 2006) and anti-diabetic (Lakshmi *et al*., 2014; Fred-jaiyesimi and Ibukunoluwa, 2016) properties. The presence of lupeol-3-acetate appears to account for the hyperglycaemia lowering activity of dichloromethane fraction.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Ademoriyo of the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria for botanical identification and collection of the plant. The authors are grateful to Dr (Miss) Adetokunbo Famojuro for her assistance in linking the authors with Mr. Gershon Amenuvor. SOF is grateful to Mr. Gershon Amenuvor of the Department of Chemistry, University of Johannesburg for his assistance in the acquisition of the NMR data.

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