Phytochemical screening and in vivo antidiabetic evaluation of 1:1 combination of the leaf extracts of Heliotropium indicum L. (Boraginaceae) and Anthocleista djalonensis A. Chev. (Loganiaceae)

Bilqis A. Lawal[1], Rashidat O. Ayanniyi[2], Fatimoh I. Ojuade[2], Sukurat O. Usman[1], Hidayah A. Olumoh-Abdul[2], Kayode M. Salawu[1], Ngaitad S. Njinga[3] and *Abdulkarim Agunu[1]

[1]Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria

[2]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria

[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria

*Deceased

Corresponding author: Bilqis A. Lawal

E-mail: lawal.ba@unilorin.edu.ng; Phone: +234 8033717230

ABSTRACT

Background: Diabetes is a leading cause of death globally. In many part of developing nations herbal medicines are often used singly or in combination for the management of diabetes.

Objectives: This study was designed to investigate the cytotoxicity, anti-diabetic and anti-dyslipidemic effects of *Heliotropium indicum (HI)* and *Anthocleista djalonensis* (AD) used singly and in combination. The qualitative and quantitative phytochemical screening was also carried out.

Methods: The plant extracts were screened for their cytotoxicity using the Brine Shrimp Lethality Assay (BSLA). Hyperglycaemia was induced with streptozotocin (STZ) and confirmed; the hyperglycaemic rats were grouped and various doses of HI and AD extracts administered singly and in combination once a day for 14 days with oral cannular. Blood glucose of various groups was measured on days 0, 1, 4, 7, 10 and 14. The lipid profile was also determined. Qualitative and quantitative phytochemical evaluation was carried out using standard procedures.

Results: The combination of extracts showed more toxic effect compared to individual extracts. The combination of leaf extracts of HI and AD *(*in ratio 1:1) produced significant reduction of blood glucose at 400 mg/kg ($p<0.05$) on day 14 while t*his combination also produced a significant ($p<0.05$) reduction in serum phospholipids at 200 and 400 mg/kg.* Phytochemical screening of the methanol leaf extract of AD revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids and tannins while alkaloids, flavonoids and steroids were absent in HI.

Conclusion: The administration of extract of HI and AD singly produced significant reduction in blood glucose and found to be safe. The methanol leaf extract of HI and AD combination also produced significant reduction in blood glucose and serum phospholipids but was found to be toxic. Hence, combined used of HI and AD should be discouraged because of increased of toxicity.

Keywords: *Heliotropiun indicum*, *Anthocleista djalonensis*, hyperglycaemia, streptozotocin, phytochemistry, cytotoxicity

West African Journal of Pharmacy (2020) 31 (1) 16 - 25

Testphytochimique et évaluation antidiabétique *in vivo* d'une combinaison 1:1 des extraits de feuilles de *Heliotropiumindicum L.* (Boraginaceae) et d'*Anthocleistadjalonensis* A. Chev. (Loganiaceae)

Bilqis A. Lawal[1], Rashidat O. Ayanniyi[2], Fatimoh I. Ojuade[2], Sukurat O. Usman[1], Hidayah A. Olumoh-Abdul[2], Kayode M. Salawu[1], Ngaitad S. Njinga[3] and *Abdulkarim Agunu[1]

[1]Département de pharmacognosie et développement de médicaments, Faculté des sciences pharmaceutiques, Université d'Ilorin, Ilorin, Nigeria

[2]Département de pharmacologie et de toxicologie, Faculté des sciences pharmaceutiques, Université d'Ilorin, Ilorin, Nigéria

[3]Département de chimie pharmaceutique et médicinale, Faculté des sciences pharmaceutiques, Université d'Ilorin, Ilorin, Nigeria

*Décédé

Auteur correspondant :Bilqis A. Lawal

E-mail: lawal.ba@unilorin.edu.ng; Téléphone: +234 8033717230

RESUME

Contexte: Le diabète est l'une des principales causes de décès dans le monde. Dans de nombreuses régions des pays en développement, les plantes médicinales sont souvent utilisées individuellement ou en combinaison pour la prise en charge du diabète.

Objectifs: Cette étude a été conçue pour étudier la cytotoxicité, les effets antidiabétiques et anti-dyslipidémiques de *Heliotropiumindicum* (HI) et *Anthocleistadjalonensis* (AD) utilisés seuls et en combinaison. Un criblage phytochimique qualitatif et quantitatif a également été effectué.

Méthodes: Les extraits de plantes ont été examinés pour leur cytotoxicité à l'aide du test de létalité des crevettes de saumure (BSLA). L'hyperglycémie a été induite avec la streptozotocine (STZ) et confirmée ; les rats hyperglycémiques ont été groupés et diverses doses d'extraits HI et AD ont été administrées individuellement et en combinaison une fois par jour pendant 14 jours avec une canule orale. La glycémie de divers groupes a été mesurée aux jours 0, 1, 4, 7, 10 et 14. Le profil lipidique a également été déterminé. Une évaluation phytochimique qualitative et quantitative a été réalisée selon des procédures standard.

Résultats: La combinaison d'extraits a montré un effet plus toxique par rapport aux extraits individuels. La combinaison d'extraits de feuilles de HI et AD (dans un rapport 1:1) a produit une réduction significative de la glycémie à 400 mg/kg ($p < 0,05$) au jour 14, tandis que cette combinaison a également produit une réduction significative ($p < 0,05$) du sérum phospholipides à 200 et 400 mg/kg. Le testphytochimique de l'extrait de feuille de méthanol d'ADa révélé la présence d'alcaloïdes, de flavonoïdes, de saponines, de terpénoïdes, de stéroïdes et de tanins tandis que les alcaloïdes, les flavonoïdes et les stéroïdes étaient absents en HI.

Conclusion: L'administration d'extrait de HI et d'AD a produit individuellement une réduction significative de la glycémie et s'est révélée sûre. L'extrait de feuille de méthanol de la combinaison HI et AD a également produit une réduction significative de la glycémie et des phospholipides sériques, mais s'est avéré toxique. Par conséquent, l'utilisation combinée de HI et AD doit être découragée en raison de l'augmentation de la toxicité.

Mots-clés: *Heliotropiunindicum*, *Anthocleistadjalonensis*, hyperglycémie, streptozotocine, phytochimie, cytotoxicité

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by persistent high blood glucose level resulting from defects in insulin secretion, insulin action, or both.[1] The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and derangement in function of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Thus, presence of this disease in a patient poses serious challenges in management.[2] In Africa, it is estimated that about 19.8 million adults have diabetes with Nigeria accounting for about 3.9% of that population; and by 2035, the percentage of diabetic patients in Africa would cross an alarming figure of 58%.[3]

Different types of orthodox medications of synthetic origin have been employed for the treatment of diabetes mellitus. The current pharmacological agents used for the management of diabetes include; sulfonylurea, metformin, alpha-glucosidase inhibitors, and thiazolidinedione. They are not easily accessible and are often associated with adverse effects such as hypoglycaemia, weight gain, gastrointestinal disorders, renal tumours, diarrhea, peripheral oedema and impaired liver function; besides the cost of treatment.[4,5] Hence, majority of people living with diabetes seek alternative therapies, especially from medicinal plants to manage this health problem. The WHO estimated that approximately 80% of the world's population rely mainly on traditional medicines for their primary health care.[6] Thus, search for new drugs which are plant-derived has been receiving renewed interest among researchers throughout the world.[7]

Herbal treatments for diabetes have been used in patients with insulin-dependent and non-insulindependent diabetes, diabetic retinopathy and some other associated complications.[8] The anti-diabetic properties of these plants could be attributed to the presence of their phyto-constituents such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc.[9] The use of crude extracts of medicinal plants in the management of many diseases and disorders, including diabetes mellitus, is widely employed in Nigeria. Some of the medicinal plants in the Nigerian flora that has been exploited for their antidiabetic and anti-hyperglycaemic properties include *Aframomum melegueta*, *Vernonia amygdalina*, O*cimum gratissimum, Moringa oliefera*, *Syzygium aromaticum*, *Momordica charantia*, *Parkia biglobosa*, *Azadirachta indica* and *Picralima nitida*. [10,11]

However, there has been a growing need for the combination therapy for the management of many diseases as is employed in traditional medicine systems. This is based on the assumption that there is a comprehensive synergistic effect of the various ingredients/constituents of the plants to enhance their efficacy and minimize their side effects.[12] It is believed that the synergistic interactions between the constituents are responsible for their therapeutic efficacy.[13] The availability of a readily available natural product that is safe effective, acceptable, assessable and cheap will improve compliance and ensure better management of many cardiovascular diseases.[14]

Heliotropium indicum belongs to the family Boraginaceae and it is commonly known as the “Cock's comb” and “Agogo-igun” in Yoruba (South Western Nigeria). It is an annual, hirsute plant that is a common weed in waste land and settled areas. The plant is native to Asia and some part of Africa.[15] It has been used in different traditional and folklore systems of medicine for curing various diseases. It was reported to possess antibacterial activity, anti-inflammatory activity, antituberculosis activity, anti- proliferative activity and antihyperglycemic activity amongst others. [16] *Anthocleista djalonensis* is commonly known as “Cabbage tree” and is widely used throughout its distribution area, namely tropical Africa, Madagascar and on the Cosmos as a strong purgative and diuretic.[17] *Anthocleista djalonensis* belongs to the family Loganiaceae, it is used in traditional medicine for the treatment of various diseases, such as haemorrhoids, syphilis, female infertility, diabetes, malaria, hernia, hypertension, and is known for its antipyretic, stomachic, analgesic and purgative actions.[18,19]

The selected plants have been widely reported individually to be effective in the management of

diabetes.[16,19] The combination of these two plants was to determine if there would be an enhanced activity of both plants, and thus, propose a co-formulation for therapeutic purposes. This present study was therefore designed to screen phytochemical constituents and investigate antihyperglycemic activity of the combination of *Heliotropium indicum* (HI) and *Anthocleista djalonensis* (AD) in Streptozotocin (STZ) induced diabetic rats. The cytotoxicity profile was also investigated using the Brine Shrimp Lethality Assay.

METHODS Plant collection and processing

The fresh leaves of *Heliotropium indicum* (HI) and *Anthocleista djalonensis* (AD) were collected from the University of Ilorin, Main campus, identified and authenticated at the University of Ilorin Herbarium, where voucher specimens were deposited. The voucher specimen numbers given were HI (UILH/003/968) and AD (UILH/004/1248). The leaves were air-dried at ambient temperature and reduced into powdered form using a milling machine. The powdered plant materials were extracted with n-hexane and 70% methanol successively. The extracts obtained were concentrated using rotary evaporator and the crude extracts obtained were stored at 4 C until use.[o 20]

Animal

Wistar male rats weighing 100 - 120 g were obtained from the University of Ilorin Central Research Laboratory. The rats were housed in an environmentally controlled animal care facility, allowed to acclimatize for 5 days and fed with standard diet and water *ad libitum,* prior to commencement of experiments. All procedures conformed to the University of Ilorin ethical guidelines on treatment of animals under which the current experiments were carried out (UERC Approval Number: UERC/ASN/2018/1112).

Phytochemical investigations

Preliminary phytochemical screening

The methanol extracts of AD and HI were subjected to qualitative tests for identification of different constituents like flavonoids, terpenoids, glycosides, saponins, alkaloids, tannins and amino acids by using standard qualitative methods described by Kokate.[21]

Quantitative analysis

Determination of total phenolics: The estimation of total phenolic content, flavonoids, saponin and alkaloid contents were determined. Total phenolic contents was evaluated with Folin-Ciocalteu's phenol reagent and expressed as mg/g tannic acid equivalent.[22] 1 ml of the extract solution in methanol was mixed with 1 ml FolinCiocalteu reagent previously diluted with water (1:9 v/v). After 5 minutes, 0.8 ml of 7% Na_2CO_3 solution was added with mixing. Absorbance was then measured at 765 nm using the Hewlett Packard UV-vis spectrophotometer.

Determination of total flavonoids: The total flavonoid content was determined using colorimetric aluminum chloride method and the total flavonoid content were

calculated as quercetin equivalents (mg/g).[23] Each plant extract in methanol (1.5 ml solution) was separately mixed with 1.5 ml of 2% aluminum chloride. After one hour at room temperature, the absorbance was measured at 420 nm.

Determination of saponin contents: The saponin content in the plant extracts was estimated as described by Kim.[24] About 5g of the powdered sample was placed in 100 ml of 20% ethanol and the suspension was heated in a water bath at 55°C for 4 hours with continuous stirring. This was transferred into a 250 ml separator funnel and 20 ml diethyl ether was added and shaken vigorously. The ether layer was discarded and the extracts were washed twice with 10 ml of 5% aqueous sodium chloride after adding 60ml of butanol. The sample was dried in the oven to a constant weight and the saponin content was calculated according to the equation: amount of saponin (mg/g) = weight of residue / weight of sample.

Determination of alkaloid content: The alkaloid content was determined according to the method of Obadoni and Ochuko and the crude alkaloid was weighed and calculated based on the equation: amount of alkaloid (mg/g) = weight of precipitate/weight of sample.[25] About 25 mg of the powdered sample was weighed into 10 ml of 20% acetic acid in ethanol and allowed to stand for 4 hours. This was filtered and concentrated over water bath to reduce the volume. Concentrated ammonium hydroxide was added drop wise into the extract until precipitation was complete. The precipitate collected was washed with dilute ammonium hydroxide solution and then filtered.

Evaluation of the Brine shrimp lethality (BSL) assay The BSL assay was conducted using a modified method described by Sharififar.[26] The two extracts (10 mg each)

were diluted to 1000 µg/mL by dissolving in 10 mL of 0.5% DMSO. Serial dilutions (5 mL each) of the extracts were made in triplicates. The negative control wells contained 0.5% DMSO in sea water, the positive control contained cyclophosphamide and ten (10) nauplii were suspended in the different concentrations of the extracts. The preparations were incubated at room temperature (RT = 25-33°C) for 24 h, thereafter the numbers of dead nauplii in each well were counted. The eggs of *Artemia salina* were hatched by incubating the eggs in seawater for 48 h.

Evaluation of anti-diabetic activity

Diabetes was induced in rats by intra-peritoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight in 0.1 M cold citrate buffer (pH 4.5). To prevent the STZinduced hypoglycaemia, the rats received 10% dextrose solution after 6 h of STZ administration for the next 24 h. The blood glucose level was measured after 72 h to determine if the animals were diabetic using a glucometer (Accu-Chek® Active, Roche Diagnostic Corporation, Mannheim, Germany). Animals having a blood glucose level higher than 250 mg/dL were considered diabetic and included in the experiments.[27] The extract and glibenclamide were administered orally for 14 days. The fasting body weight and blood glucose level were estimated on days 0, 1, 4, 7, 10 and 14. On day 14, the animals were fasted overnight, administered respective treatment and after 1 hour all animals were anesthetized with ketamine (100 mg/kg, i.p.). Blood sample were collected through retro-orbital plexus puncture and stored.

Determination of lipid profile

Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic methods using commercial assay kits purchased from Fortress Diagnostic United Kingdom according to the manufacturer's protocol. Serum low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's equation.[28] LDL-C = [TC-(HDL-C + (TG/5). Very low density lipoprotein cholesterol (VLDL-C) concentration was calculated by deduction of the sum of HDL-C and LDL-C concentrations from that of TC.

Data Analysis

The data obtained from this study were expressed as Mean ± Standard error of mean (SEM) and analyzed using One–Way Analysis of Variance (ANOVA). A value of $p \leq 0.05$ was considered significant. The difference between the means of treated groups and the control group was evaluated by the Dunnett's Multiple Comparison Test. The statistic analysis was carried out with GraphPad Prism version 6.0.

RESULTS Phytochemical Evaluation of *A. djalonensis* and *H. indicum* leaves

Qualitative analysis

The methanol leaf extract of AD tested positive to alkaloids, flavonoids, saponins, terpenoids, steroids and tannins while HI was observed to contain cardiac glycosides, terpenoids and tannins (Table 1).

Table 1: Preliminary phytochemical screening of *A. djalonensis* and *H. indicum* Leaves

Constituents	**Test**	**Observation**	**Inference**	
			AD	**HI**
Alkaloids	Mayer's reagent	Creamy white precipitate	++	-
	Hager's reagent	Yellow precipitate	++	-
	Wagner's reagent	Reddish brown precipitate	++	-
Anthraquinones	Borntrager's	Pink colour	+	-
Cardiac glycosides	Keller-killiani	Reddish brown at interphase	+	+
Flavonoids	NaOH test	Yellow colouration turns colourless with HCl	++	-
Saponins	Frothing test	Persistent froth	+	+
Terpenoids	Salkowski's test	Brown colour at interphase	+	+
Steroids	LiebermannBurchard	Greenish colouration	+	-
Tannins	$FeCl_3$ test	Greenish black precipitate	+++	++

+++ (copiously present), ++ (moderately present), + (slightly present), - (absent)

AD- *Anthocleista djalonensis*; HI- *Heliotropium indicum*

Quantitative phytochemical analysis

The quantitative estimations of total phenolics, flavonoids, saponins and alkaloidal contents from methanol extract of AD and HI revealed that AD has higher flavonoid content (0.862 mg/g quercetin) than HI (0.376 mg/g quercetin) such that the total flavonoid contents obtained from AD was about two folds greater than that of HI. The difference was statistically at $P<0.05$.

significant The total phenolic content of AD (0.289 mg/g gallic acid) was not significantly higher ($P < 0.05$) than that of HI (0.268 mg/g gallic acid) as shown in Figure 1. The result also shows that the quantities of alkaloid obtained in AD were significantly higher (18.75 % alkaloid) than HI (7.25 % alkaloid); however, the saponin content in AD was significantly lower (1.63 % saponins) than that of HI (9.25 % saponins) as shown in Figure 2.

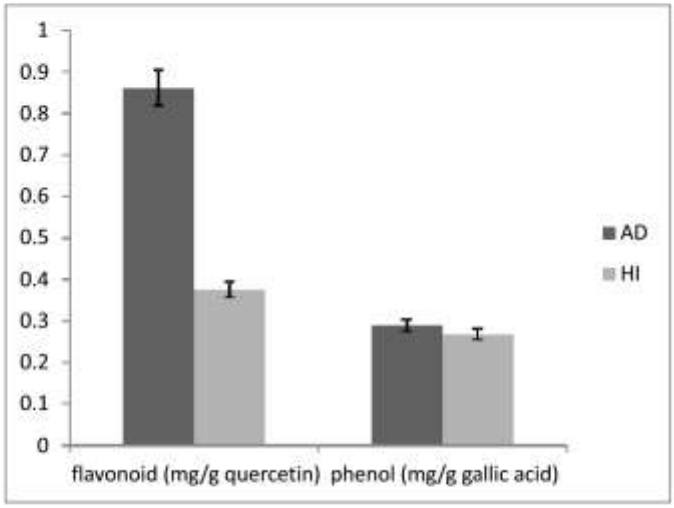

Figure 1: Total phenol (mg/g gallic acid) and flavonoid (mg/g quercetin) in AD and HI

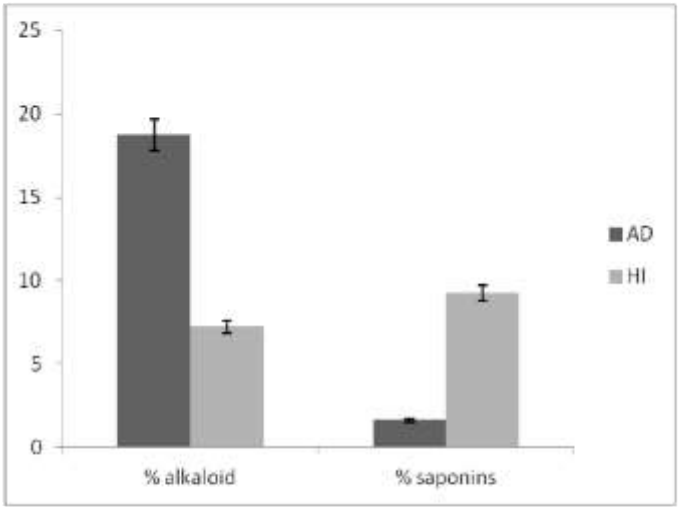

Figure 2: Percentage saponin and alkaloid in AD and HI

Brine shrimp lethality bioassay

The leaf extracts showed a concentration dependent BSL effect as shown in Table 2. The combination of the extracts elicited more toxic effect compared to the individual toxic effect of each extract as shown in Table 3. The extract of HI and AD in 1:1 combinations elicited 60% inhibition compared to (1:3 and 3:1) combinations that had 70% inhibition respectively.

Table 2: Brine Shrimps Lethality of *Heliotropium indicum and Anthocleista djalonensis* extracts

	Percentage mortality		
Conc. (µg/mL)	**HI**	**AD**	**CYP**
1000	30.00±0.00	20.00±0.00	100.00±0.00
500	20.00±0.00	10.00±0.00	70.00±0.00
100	10.00±0.00	0.00±0.00	50.00±0.00
10	0.00±0.00	0.00±0.00	36.67±3.33
1	0.00±0.00	0.00±0.00	6.67±3.33
IC_{50} (µg/mL)	3662	2886	52.26

AD: *Anthocleista djalonensis*, HI: *Heliotropium indicum*, CYP: cyclophosphamide, n=3

Table 3: Brine Shrimps Lethality of combination of HI and AD Extracts at 1000 µg/mL

Ratio	Percentage mortality
HI (4:0)	30.00±0.00
HI:AD (3:1)	70.00±0.00
HI:AD (1:1)	60.00±0.00
HI:AD (1:3)	70.00±0.00
AD (0:4)	20.00±0.00

Anti-diabetic activity in streptozotocin-induced diabetic rats

Fasting blood glucose level was measured on days 0, 1, 4, 7 and 14 in the diabetic control and this was observed to be significantly ($p<0.05$) higher compared to the normal control (Table 4). The leaf extract of HI at 200 mg/kg and 400 mg/kg significantly decreased ($p<0.05$) blood glucose level of the rats on day 14 (98.68±18.19 mg/dL and 107.70±26.89 mg/dL) respectively when compared to the diabetic control values of the same day (457.00±18.48 mg/dL). Similarly, the extract of AD (400 mg/kg) produced a significant reduction ($p<0.05$) in blood glucose on day 14 (200.00±1.73 mg/dL) (Table 4).

Effect of *Heliotropium indicum + Anthocleista djalonensis (1:1)* on blood glucose level in streptozotocin-induced diabetic rats

The combination of leaf extract of HI and AD at 200 mg/kg on day 14 (125.30±36.97 mg/dL) when compared with in ratio 1:1 did not produce any significant difference. the diabetic control on the same day (457.00±18.48 However, the combination at (400 mg/kg) in ratio 1:1 mg/dL). produced significant ($p<0.05$) reduction of blood glucose

Table 4: Effect of methanol leaf extract of *Heliotropium indicum* and *Anthocleista djalonensis* on blood glucose level of streptozotocin-induced diabetic rats

Treatment	Blood	Glucose	Concentration	(mg/dL)	
	Day 0	Day 1	Day 4	Day 7	Day 14
I	102.70±6.23	107.70±4.49	106.00±7.22	104.00±6.03	107.00±17.00
II	406.70±8.82	398.00±12.29	399.00±1.44	396.30±16.29	457.00±18.48
III	440.00±50.00	401.00±56.50	386.00±46.50	316.50±4.50	272.50±68.50
IV	209.00±3.97	204.00±4.42	212.00±57.73	154.70±44.21	98.68±18.19*
V	317.00±4.67	310.00±2.03	375.00±73.53	211.00±67.99	107.70±26.89*
VI	406.00±5.50	395.70±0.88	502.00±31.18	372.50±50.50	305.00±0.00
VII	430.00±0.00	438.00±10.97	514.00±8.66	404.00±2.31	200.00±1.73*
VIII	516.00±7.64	509.70±8.88	519.70±34.71	417.00±24.95	311.00±30.94
IX	378.00±31.67	340.30±7.84	431.00±20.52	208.00±10.49	125.30±36.97*

I: Normal control, II: Diabetic control, III: Streptozotocin+glibenclamide, IV: *Heliotropium indicum 200 mg/kg, V: Heliotropium indicum 400 mg/kg,* VI: *Anthocleista djalonensis 200 mg/kg, VII: Anthocleista djalonensis 400 mg/kg,* VIII: *Heliotropium indicum+Anthocleista djalonensis 200 mg/kg (1:1),* IX: *Heliotropium indicum+Anthocleista djalonensis*

*400 mg/kg (1:1) *p*<0.05, when each group is compared with diabetic control

Effect of methanol leaf extract of *Heliotropium indicum* and *Anthocleista djalonensis* on lipid profile of streptozotocin-induced diabetic rats

The leaf extract of AD (400 mg/kg) produced a significant (*p<0.05)* decrease in serum cholesterol, similar to the effect produced by 5 mg/kg glibenclamide (Table 5). The extracts of HI and AD had no significant effect on serum triglyceride and low density lipoproteins. An increase in high density lipoprotein was observed which was also not significant. Furthermore the leaf extracts of HI and AD (200 mg/kg) significantly (*p<0.05, p<0.01) decreased serum phospholipids respectively. The combination 1:1 of HI and AD (200 and 400 mg/kg) also produced a significant (p<0.05) reduction in serum phospholipids* (Table 5).

Table 5: Effect of methanol leaf extract of *Heliotropium indicum* and *Anthocleista djalonensis* on lipid profile of streptozotocin-induced diabetic rats

Treatment	Serum Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum HDL (mg/dl)	Serum LDL (mg/dl)	Serum Phospolipids (mg/dl)
I	80.02 ± 1.92	53.26 ± 5.07	10.89 ± 1.44	47.85 ± 4.69	124.29 ± 47.71
II	98.75 ± 6.37	63.93 ± 2.49	11.44 ± 2.62	64.26 ± 4.00	163.04 ± 31.95
III	69.11 ± 2.51*	50.41 ± 1.11	17.09 ± 2.89	54.79 ± 2.64	99.30 ± 7.18*
IV	75.31 ± 3.55	49.82 ± 3.45	20.38 ± 2.69	44.26 ± 1.51	72.68 ± 18.67**
V	81.06 ± 1.58	54.72 ± 2.90	16.30 ± 4.98	54.44 ± 8.71	111.67 ± 21.60
VI	75.16 ± 1.12	51.65 ± 2.29	17.93 ± 2.16	51.16 ± 8.71	94.55 ± 11.63*
VII	75.79 ± 2.51	52.59 ± 0.02	10.78 ± 1.91	33.53 ± 8.97	96.11 ± 7.68*

VIII	69.77 ± 0.00*	42.77 ± 0.00	8.55 ± 0.00	37.69 ± 0.00	95.83 ± 0.00*
IX	72.47 ± 1.64	54.84 ± 2.35	12.71 ± 5.42	29.09 ± 4.72*	94.55 ± 17.72*

I: Vehicle, II: Streptozotocin 50 mg/kg, III: Glibeclamide 5 mg/kg, IV: *Heliotropium indicum* (200 mg/kg), V: *Heliotropium indicum* (400 mg/kg) VI: *Anthocleista djalonensis* (200 mg/kg) VII: *Anthocleista djalonensis* (400 mg/kg), VIII: *Heliotropium indicum+Anthocleista djalonensis 200 mg/kg (1:1), IX: Heliotropium indicum+Anthocleista djalonensis 400 mg/kg (1:1)* ***p<0.05, ****p<0.01 compared to control, n=5.*

DISCUSSION

Diabetes is a metabolic disorder and can be complicated by high serum lipid profile which is a risk factor for development of cardiovascular diseases.[29] The use of conventional orthodox medicines for the management of this disorder has been reported to be associated with numerous side effects. More recently, there has been a huge focus on seeking alternative therapy from medicinal plants which has been reported to have advantages of lower cost, availability, accessibility and safety.[30]

The phytochemical analysis of *A. djalonensis* showed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids and tannins in the leaves of *A. djalonensis* while alkaloids, flavonoid and steroids were found to be absent in *H. indicum.* Quantitatively, the estimated amount of flavonoids and alkaloids present in AD was statistically more significant than that of HI, however, the amount of phenols was not significantly different and saponin was even higher in HI than AD. Phytochemical studies of medicinal plants are important in other to ensure reproducible quality of herbal medicines which contributes to their efficacy and safety.[31] Most of these phytochemicals such as alkaloids, flavonoids and
saponins have also been reported to have anti-hyperglaemic potentials.[31,32]

The Brine shrimp assay result revealed that the individual extracts were non-toxic to the brine shrimp compared to the positive control (cyclophosphamide) while the combination of the extracts elicited more toxic effect compared to the individual effect of each extract. These results suggest that the two extracts are non-toxic but their combinations elicit an increased but varying toxicity. Brine Shrimp Lethality Assay (BSLA) has been applied as an alternative bioassay technique to screen the toxicity of plant extracts as it is inexpensive and very minimal amount of amount of test material is often used.[33]

The leaf extract of *H. indicum* produced a significant reduction in blood glucose level in STZ-induced diabetic rats and this is in tandem with other reports.[34-36] *A. djalonensis* leaf extract also exhibited a significant reduction in blood glucose level in STZ-induced diabetic rat. The root extract of *A. djalonensis* was reported to produce significant decrease in blood glucose level in alloxan-induced diabetic rats.[37]

This present study shows the combination of *H. indicum* and *A. djalonensis* leaf extract produced a significant reduction in blood glucose on day 14 compared to the effect produced by each of the extracts. However, a more toxic effect was observed with the combination of HI and AD when compared to the individual extracts.

Furthermore, the two extracts produced a significant reduction in serum phospholipids when administered singly and in combination. The combination of the two extracts also produced a significant reduction in low density lipoproteins and cholesterol. This effect may indicate plausible hydrolysis, selective uptake and metabolism of certain lipoproteins which may reduce complication of diabetes such as artherosclerosis.[38] There are also evidences which suggest adequate management of hyperlipidemia can reduce cardiovascular complications associated with diabetes.[39] Thus, the ability of the crude extracts of HI and AD alone and in combination in reducing serum lipids may be beneficial in their hypoglycaemic activity.

The study of the plausible mechanisms of action of the extracts singly and in combination would further establish their anti-hyperglycaemic activities *in vivo*.

CONCLUSION

The crude extracts of *Heliotropium indicum* and *Anthocleista djalonensis* significantly reduced blood glucose and serum lipids concentration individually and in combination. However, the combination of both extracts should be avoided because of increased toxicity.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Tertiary Education Trust Fund; Institutional-based Research 20142016 (TETFUND-IBR), Nigeria for funding of this research.

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