Original Article
Bulletin of Pure and Applied Sciences.
Chemistry , Vol.43 C, No.1
January-June 2024: P.27-38

http://www.bpasjournals.com/chemistry
DOI: https://doi.org/10.52710/bpas-chem.9
Print version ISSN 0970 4620
Online version ISSN 2320 320X

Isolation of Kaempferol, Acute Toxicity Evaluation and Antidiabetic Activity of Methanol Leaf Extract of *Faidherbia albida* on Alloxan-Induced Diabetic Rats

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ABSTRACT
This study aimed at the isolation of 3,5,7,4'-tetrahydroxyflavone (Kaempferol), from the methanol extract of *Faidherbia albida* leaf, determine the acute toxicity (LD_{50}) and antidiabetic efficacy of the extract on alloxan-induced diabetic Wistar rats. The methanol extract was screened for phytochemical and the flavone was isolated by column chromatography on silica gel and Sephadex LH20 via elution with chloroform and ethyl acetate in proportions of increasing polarity as well as methanol respectively. A solvent system of chloroform, methanol and water (7:3:1) was used to preparatively isolated the encoded Compound D. The structure of the compound was elucidated using Fourier Transform Infrared (IR) and Nuclear Magnetic Resonance spectroscopic (NMR) techniques. The acute toxicity study showed that the *i.p* median lethal dose is 3807.8mg/kg and the extract exhibited promising antidiabetic effect against alloxan induced diabetes with protections of 73.3%, 68.1% and 70.8% at doses of 200mg/kg, 400mg/kg and 800 mg/kg, which were higher than insulin (63.1%) at 0.1µg/kg. To the best of our knowledge, this is the first report of isolation of kaempferol from *Faidherbia albida* leaf. This study has also scientific validated the folkloric use of the plant for the treatment of diabetes.

Keywords: *Faidherbia albida*; Kaempferol; Antidiabetic Effect; Acute Toxicity; Isolation

How to cite this article: Khan, I.Z., Mamza U.T., Yakubu, J., Khan, I.S., Baba, A.A., and Maiva, E.J. (2024). Isolation of Kaempferol, Acute Toxicity Evaluation and Antidiabetic Activity of Methanol Leaf Extract of *Faidherbia albida* on Alloxan-Induced Diabetic Rats. *Bulletin of Pure and Applied Sciences-Chemistry*, 43C (1), 27-38.

1. INTRODUCTION

Throughout history, humans have relied on nature to provide them with basic necessities like as food, clothes, housing, flavors, medicines, fertilizers, and modes of transportation. Medicinal plants continue to play a significant part in the healthcare systems of huge segments of the global population. This is especially true in poorer nations where herbal medicine has a long history of usage [1].

Chronic metabolic disorder known as diabetes mellitus (DM) is caused by elevated blood sugar, high blood fat, elevated cholesterol, elevated amino acid content, insufficient insulin, resistance to insulin, or a combination of these factors [2]. Roughly 10% of people worldwide are impacted by it. Modern hypoglycemia medications have made secondary problems less common, but they still pose a serious medical risk. Weight gain and gastrointestinal problems are among the side effects of the majority of contemporary medications [3].

Globally, type 2 diabetes is more common than the other forms [4]. Both macrovascular and microvascular problems are associated with diabetes mellitus. In the development and prognosis of complications, hyperglycemia is a significant risk factor [5]. Beyond cancer and heart attacks, it is the third most common cause of mortality.

According to estimates from the International Diabetes Federation (IDF), 463 million individuals worldwide had diabetes in 2019; by 2045, that number is expected to rise to 700 million [6]. In low- and middle-income countries (LMICs), 79% of people with diabetes reside [7].

The scientific community worldwide is still researching the potential antidiabetic properties of raw materials or separated natural products without side effects, even in the face of the creation of new drugs and their confirmation by scientific standards [8].

Faidherbia albida (Fig. 1), formerly known as *Acacia albida* (Del), is a member of the Fabaceae family Mimosoideae. It originates in Southwest Africa and spreads to Egypt and East Africa via West and North Africa. Common names for this plant include apple-ring acacia and winter thorn. It is known as "Gawo" by the Hausa people of northern Nigeria and "Chayski" in Fulfuldes.

The herb has historically been used to treat skin conditions, asthma, and diarrhea [9, 10]. Additionally, the herb has anti-inflammatory, anti-haemorrhagic, and ophthalmic properties [9]. In certain areas, people use the plant's seeds as sustenance during famines [9, 11], and the powdered pods and seeds are frequently used to kill fish in swimming pools [12]. Pharmacologically, the plant's benefits include antimicrobial [13], anti-diarrheic, anti-pyretic, anti-inflammatory, anti-trypanosomiasis [14], anti-diabetic [15], anti-malarial [16], anti-fungal [17], and nematicidal agent [18]. Phytochemical studies revealed that the plant contains tannins, flavonoids, anthraquinones, alkaloids, reducing sugars, cardiac glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non-protein amino acids, terpenes (including oils essential, diterpenes, phytosterol and triterpene as well as genins and saponins), hydrolyzable tannins, flavonoids and condensed tannins [19].

Figure 1: *Faidherbia albida* plant in its natural habitat

In an attempt to comprehend the medicinal potential of *Faidherbia albida*, this work presents, for the first time, the isolation and characterization of phytochemicals found in the methanolic extract of the plant's leaf.

2. MATERIALS AND METHODS

Sample collection Preparation and Extraction of *Faidherbia albida* Leaf

The leaf with a Voucher Specimen Number (UMM/FPH/MMS/004) was collected from University of Maiduguri campus, Maiduguri, Borno State, Nigeria and was identified and authenticated by Prof. S. S. Sanusi of Biological Sciences Department, Faculty of Life Sciences, University of Maiduguri, Maiduguri.

The fresh leaves of the plant were cleansed of dirt by hand picking, and rinsing with distilled water, then dried under shade for two (2) weeks. It was then pulverized into powder using wooden a mortar and pestle. The sample was kept in a cool dried place until required for extraction. One hundred and fifty grammes (150 g) of the plant material was introduced into a five litre (5 L) flat bottom flask and 2L of 85 % methanol was added to the flask with occasional shaking. The extraction process lasted for 72 hr (3 days). The solution obtained was filtered using Whatman filter paper no. 1, to remove the debris. The filtrate was poured into an evaporating dish to concentrate on a hot air oven at 40-50 ^{o}C. After evaporation, the extract was stored in an airtight container until required for further analysis.

Reagents/Solvents

The reagents used include, sulphuric acid, distilled water, Dragendorff's reagent, Meyer's reagent, ethanol, Fehling's solutions A and B, ferric chloride solution, magnesium chips and Molisch's reagent, while methanol (analar grade) was used for the extraction.

Phytochemical Analysis

The methanol leaf extract of *Faidherbia albida* was screened for phytochemical constituents using standard methods as described below:

Test for Alkaloids

Half gramme (0.5 g) of the extract was stirred with 5 mL of 1 % aqueous HCl acid on a water bath, then filtered. 3 mL of the filtrate was divided into 3 portions in a test tube:
To the first portion, a few drops of Dragendorff's reagent was added. The occurrence of an orange red precipitate was taken as the indication for the presence of alkaloids.
To the second portion, 1 mL of Meyer's reagent was added and the appearance of a buff-coloured precipitate was an indication of the presence of alkaloids.

To the third portion, a few drops of Wagner's reagent was added and a dark-brown precipitate indicated the presence of alkaloids [22].

Test for Flavonoids

Ferric Chloride Test

Half gramme (0.5 g) of the extract was boiled with distilled water and then filtered. To the 2 mL of the filtrate, a few drops of 10 % ferric chloride solution was added. A green-blue or violet colouration was an indication for the presence of phenolic hydroxyl group [20].

Shinoda's Test

Half gramme (0.5 g) of the extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of conc. HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids [21].

Lead Acetate Test

Half gramme (0.5 g) of the extract was boiled with distilled water and then filtered. To 5 mL of the filtrate, 3 mL of lead ethanoate solution was added and appearance of a buff coloured precipitate was indicative of the presence of flavonoids [22].

Sodium Hydroxide Test

Half gramme (0.5 g) of the extract was dissolved in water and filtered. To the filtrate, 2 mL of 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in the colour from yellow to colourless on addition of dilute hydrochloric acid was an indication of the presence of flavonoids [20].

Test for Carbohydrates

Molish's Test

Half gramme (0.5 g) of the extract was dissolved in distilled water in a test tube. To the mixture, a few drops of the Molisch's reagent were added, and then followed by 1 ml of conc. tetraoxosulphate (VI) acid by the side of the test tube. The mixture was allowed to stand for two minutes and then was diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was considered a positive test [20].

Test for Monosaccharide (Barfoed's Test)

Half gramme (0.5 g) of the extract was dissolved in distilled water and filtered. 1 ml of the filtrate was mixed with 1 ml of Barfoed's reagent in a test tube. This was heated on a water bath for two minutes. Are precipitates of cuprous oxide was considered as a positive test [21].

Test for Combined Reducing Sugar

Half gram (0.5 g) of the extract was mixed with 5 ml dilute hydrochloric acid and boiled. The mixture was neutralized with sodium hydroxide solution. Few drops of Fehling's solution were added to it and then heated on a water bath for two minutes. A reddish-brown precipitate of cuprous oxide was an indication of the presence of combined reducing sugars [20].

Test for Free Reducing Sugar (Fehling's Test)

Half gramme (0.5 g) of the extract was dissolved in distilled water and filtered. To the filtrate, 5 ml of equal volumes of Fehling's solution A and B were added. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugar [20].

Salivanoff's Test (Standard Test for Ketones)

To the half gramme (0.5 g) of the extract, a few crystals of resorcinol and 2 ml of hydrochloric acid were added and the mixture boiled for 5 minutes. The appearance of a red colouration will indicate the presence of ketones [23].

Test for Cardiac Glycosides

Lieberman Burchard's Test

To the half gramme (0.5 g) of the extract, 2 ml of acetic anhydride was added. The mixture was cooled in ice and then conc. Tetraoxosulphate (VI) acid was added carefully. Colour development from violet to bluish-green indicated the presence of a steroidal ring [24].

Salkowski's Test (Test for Steroidal Nucleus)

To the half gramme (0.5 g) of the extract, 2 ml of chloroform was added. Then, tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish-brown colour or yellow at the inter-phase indicated the presence of steroidal ring [24].

Test for Anthraquinones

Test for free anthraquinones (Borntrager's Test)
To the half gramme (0.5 g) of the extract. 10 ml of benzene was added and shaken. The mixture was filtered. 5 ml of 10 % ammonia solution was added to the filtrate. The mixture was then shaken. The appearance of a pink, red or violet colour in the lower interphase will indicate the presence of anthraquinones [20].

Test for Combined Anthraquinones (Borntrager's Test)
To half gramme (0.5 g) of the extract, 10 ml of aqueous tetraoxosulphate (VI) acid was added and shaken and then filtered while it was still hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was separated and half its own volume was added to 10% ammonia solution. The formation of a pink, red violet colouration in the ammoniacal (lower) phase was an indication of combined anthraquinones [20].

Test for Terpenoids

Half gramme (0.5 g) of the extract was dissolved in ethanol. 1 ml of acetic anhydride was added followed by the addition of conc. tetraoxosulphate (VI) acid. A colour change from pink to violet indicated the presence of terpenoids [24].

Test for Saponins

Frothing Test
One gramme (1 g) of the extract was boiled with 5 ml of distilled water and filtered. The filtrate was divided into 2 portions:
To the first portion about 3 ml of distilled water was added and shaken for about 5 minutes. Frothing which persist on warming was evidence for the presence of saponins [25].
To the second portion, 2.5 ml of a mixture of equal volumes of Fehling's solutions A and B were added. The appearance of a brick-red precipitate was an indication for the presence of saponins glycosides [23].

Test for Tannins
To half gramme (0.5 g) of the extract, 10 ml of distilled water vas added and stirred. The mixture was filtered. The filtered was used for the following test:

Ferric Chloride Test
To 2 ml of the filtrate, a few drops of 1 %ferric chloride solution were added. The occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Lead Ethanoate Test
A mixture of equal volume of 10 % lead ethanoate was added to 2 ml of the filtrate. The formation of white precipitate indicates the presence of tannins. The filtrate was boiled with 3 drops of 10 % HCl, and a drop of methanol. A red precipitate will be taken as indication for the presence of tannins [25, 20].

Test for Cardenolides

Keller-Killiani's Test
Glacial acetic acid (1 ml) was added to about 3 ml of the solution containing 2 mg of the extract in a test tube held at 45^{o}; then two drops of concentrated H_2SO_4 were added along the side of the test tube carefully. Formation of a purple ring colour at the interface indicates the presence of cardenolides [20].

Isolation

Column and Thin Layer Chromatography
The methanol leaf extract of *Faidherbia albida* was fractionated by column chromatography and thin layer chromatography for the isolation of possible bioactive compound.

A glass tube with a diameter of 2.8cm and a height of 90cm with a tap at the bottom was used for the column chromatographic technique. A plug of cotton wool was well placed at the bottom of the column very close to the tap so as to prevent the stationary phase from blocking the column. About 200g of silica gel 60-120 mesh (Quikelem, India) was used to prepare a slurry by wet method. The silica gel was mixed with methanol and stirred with a clean glass rod until a uniform mixture was obtained. It was then packed cautiously and manually to about two third the size of the column tube using a glass funnel. The gel was then allowed to settle and pack for 24 hrs. The air bubbles were avoided and

care was taken not to dry the column by maintaining the level of the methanol to that of the silica gel. The sample fraction of 20g was mixed with 10g of silica gel followed by the addition of 10ml of methanol. The mixture stirred well for proper mixing, allowed to stand overnight and was mounted through a glass funnel using a glass rod to the already equilibrated silica-fixed column on top of the stationary phase. This was topped with a small layer of cotton, then sand to protect the shape of the organic layer from the velocity of newly added eluent (stationary phase). The eluting solvent initially was 100% chloroform and the polarity was gradually increased at 80:20, chloroform: ethyl acetate ratio was used. Twelve (22) sub-fractions were collected. The column fractions were monitored for similarities of the fractions based on retardation factor *(Rf)* values using thin layer chromatographic (TLC) techniques and afforded four (4) pooled fractions. The column fraction encoded FA1 was further subjected to chromatographic purification using Sephadex LH20 as the stationary phase and methanol was used to elude the mixtures. The re-column fractions were also monitored using TLC plates, and re-pooled based on *Rf* similarity.

The methanol re-columned fraction was subjected to preparative thin layer chromatography (PTLC). The fraction was dissolved in methanol and a band of the mixture was made on a marked spot at the bottom of the plates. The plates were allowed to dry before being placed in a developing tank containing a solvent system of chloroform, methanol and water (7:3:1) respectively. The plates were subsequently removed and were viewed under the UV light (254nm), and also in the tank containing iodine. The isolate encoded D obtained was yellowish and had a melting point of 175-176^{o}C (uncorrected).

Pharmacological Studies

Experimental Animal and Acclimatization

Forty-two (42) adult Wistar rats of both sexes weighing between 120-200g were used for both the acute toxicity studies (LD_{50} determination) and antidiabetic study.

The animals were housed in standard wire meshed plastic cages in the animal section of Physiology, Laboratory of Faculty of Veterinary Medicine, University of Maiduguri, Borno state. At standard condition of temperature, light and humidity for a period of two weeks to allow them acclimatizes to laboratory condition. These animals were allowed free access to drinking water and standard livestock feed (Grand Cereals and Oils Mills Ltd.) Bukuru, Jos, Plateau State, Nigeria. The standard protocol described by ICLAS and CIOMS (2012) was used in handling the rats.

Determination of Acute Toxicity (LD_{50})

The acute toxicity of the methanolic leaf extract of *Faidherbia albida* was determined using standard conventional procedure described by Lorke (1983) [26]. The route of administration used in this study was oral. This comprises two phases which include:

Phase I: The rats were divided into three groups of three rats each; the methanolic leaf extract of *Faidherbia albida* was then administered at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight orally and were observed for signs of acute toxicity and mortality for 24 hrs.

Phase II: Three groups of one rat were used based on the result of phase I after 24 hours. The rats were given the methanolic leaf extract of *Faidherbia albida* at the doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. The rats were then observed for 24 hours for signs of toxicity and death after which the LD_{50} (acute toxicity) was calculated using the formula:

$LD_{50} = \sqrt{a \times b}$.

Where: a = least dose that killed the animal

b = highest dose that did not kill the animal.

Induction of Diabetes Using Alloxan

Wistar rats of both sexes weighing between 100-276 g were used for this study. The rats were fed with standard rat diet purchased from Maiduguri, Borno state, Nigeria. Animals were provided with food and water *ad libitum* and maintained at standard condition of temperature at 25-28 ^{o}C. The study was carried out in the animal section of Physiology Laboratory of Veterinary Medicine Faculty, University of Maiduguri, Maiduguri, Borno State, Nigeria.

Diabetes was induced by injecting alloxan at a dose of 120 mg/kg body weight intra-peritoneally to overnight fasted animals. It was injected as a single dose in a total volume of 0.5 ml freshly prepared distilled water.

Blood was withdrawn from the tail vein at defined time intervals and plasma glucose level was measured using accu-check (Roche Diabetes Care, Inc.,).

Effects of Methanolic Leaf Extract of *Faidherbia albida* on Alloxan-Induced Diabetic Wistar Rats

Method described by Yakubu (2022) [27] as used for the evaluation of the antidiabetic effect of the methanol leaf extract *of Faidherbia albida*. In this study, thirty (30) albino rats of both sexes weighing between 100-276 g were used. The rats were denied feed for 24 hours but were provided with water *ad libitum*. The rats were divided into six groups of five rats each, that is, groups A, B, C, D, E and F. Group A was given 2 ml of distilled water orally and serve as the negative control. Groups B, C and D were treated orally with 200 mg/kg, 400 mg/kg and 800 mg/kg of the extract respectively while group E was given Glibenclamide and it served as the positive control as it is a standard antidiabetic drug. Group F were left as untreated diabetic group.

The glucose level of each rat was measured using glucometer and the test strips at intervals of 0hr, 1hr, 3hrs, 6hrs, 8hrs and 18hr respectively, the results obtained were recorded at the end of the experiment

.

Percentage protection (%) was calculated using the formula:

$$\%\ \textbf{Protection} = \frac{\text{Mean glucose level of control} - \text{Mean glucose level of treated group}}{\text{Mean glucose level of control}} \times 100$$

Data Analysis

The results (data) were analysed using Graphpad Prism software and presented as Mean ± Standard Error of the Mean (SEM). Analysis of variance (one-way ANOVA) followed by Dunnett multiple comparison was used to test between means at which $p < 0.05$ was considered significant.

3. RESULTS

Chemical Analysis

Extraction Profile of the Extract

The weight, colour, texture and the yield of the ethanolic *Faidherbia albida* methanol leaf extract from maceration extraction are presented in Table 1. The weight was 4g, the colour of the extract was brown, its texture was sticky while the percentage yield was 27.33% w/w.

Phytochemical Screening of the *Faidherbia albida* Methanol Leaf Extract

The phytochemical profile of ethanol extract from the methanol leaf extract is presented in Table 2. The chemical constituents revealed the presence of flavonoids, cardiac glycosides, terpenoids, carbohydrates, tannins, cardenolides and saponins.

Characterization of Isolate

The isolated compound C was structurally analysed using Infrared and nuclear magnetic resonance spectroscopic techniques.

The Fourier transformed Infrared spectra revealed prominent frequencies of absorption at 3233cm^{-1}, 2938cm^{-1}, 1676cm^{-1}, 1620cm^{-1}, 1543cm^{-1}, 1464cm^{-1} and 1020cm^{-1}, which corresponds to hydroxy (-OH), alkene C-H, carbonyl (C=O), three C=C$_{aromatic}$ stretching and C-O.

The nuclear magnetic resonance (1HNMR, 500mHz) revealed proton signals at δ_H 8.11 (2H, d, J=8.0Hz), H-2′6′), δ_H 7.16 ((2H,d, J=8.0 Hz) H-3′5′), δ_H 6.41 (1H, d, J=2.1 Hz, H-8), δ_H 6.32 (1H, d, J=2.0 Hz, H-6), while the 13CNMR (δppm, 500MHz) showed signals at 146.99 (C-2), 137.0

(C-3), 173.20 (C-4), 160.9 (-5), 100.20 (C-6), 166.9 (C-7), 94.31 (C-8), 156.60 (C-9), 104. 34 (C-10), 122.40 (C-1′), 131.0 (C-2′), 116.98 (C-3′), 160.51 (C-4′), 117.20 (C-5′), 130.7 (C-6′). Based on the above spectral data obtained from the FTIR and NMR, the compound is identified as 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chrmomen-4-one as shown in Fig 2 below.

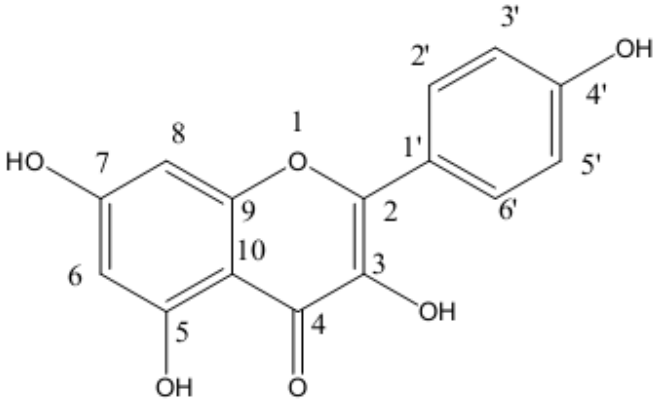

Figure 2: 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chrmomen-4-one

Pharmacological Studies

Acute Toxicity Studies of *Faidherbia albida* Methanol Leaf Extract

The result of the LD_{50} study obtained using Lorke`s method is as shown in Table 3. The *i.p* LD_{50} was calculated a 3807.9 mg/kg as there was no record of mortality.

Antidiabetic Effect of Methanolic Leaf Extract of *Faidherbia albida* on Alloxan Induced-Diabetic Wistar Rats

The result of antidiabetic effect of methanol leaf *Faidherbia albida* obtained after 18 hours of oral administration showed that the control Group A, (negative control) which was pre-treated with distilled water yielded a normal plasma glucose level (random blood glucose test) [51 mg/dl – 90 mg/ dl]. Group B, 200 mg/kg of the *Faidherbia albida* leaf extract had percentage protection of 73.3 %, Group C, 400 mg/kg of the exhibited percentage protection of 69.1 %, Group D, 800 mg/kg of conferred percentage protection of 70.8 %, and finally, Group E, Glibenclamide (standard) [positive control] exerted the least percentage protection of 63.1 % than the other treatment groups (B, C and D).

Table 1: Extraction Profile of *Faidherbia albida* Leaf

Parameter	Methanol Leaf Extract
Weight	41g
Colour	Greenish
Texture	Sticky
% yield (w/w)	27.22

Weight of pulverized leaf = 150 g
Weight of the extract = 41 g
Percentage (%) yield = $\frac{41g}{150\ g} \times 100 = 27.33$ % w/w

Table 2: Phytochemical Constituents of *Faidherbia albida* Leaf Extract

Phytochemical	Inference
Alkaloids	-
Flavonoids	+
Saponins	+
Carbohydrates	+
Cardiac glycosides	+
Terpenoids	+
Tannins	+
Anthraquinones	_
Cardenolides	+

Keys: + = present
- = absent

Table 3: Intraperitoneal Acute Toxicity (LD_{50}) Test of the Crude Methanol Leaf Extract on Wistar Rats of *Faidherbia albida*

S/N	Phase	No. of Rats	Dose (mg/kg)	Mortality
1	1	3	10	0/3
2	1	3	100	0/3
3	1	3	1000	0/3
4	2	1	1600	0/1
5	2	1	2900	0/1
6	2	1	5000	**1/1**

LD_{50} = $\sqrt{a \times b}$
i.p LD_{50} = 3807.9 mg/kg
Where: a = least dose that killed the animal
b = highest dose that did not kill the animal

Table 4: Anti-diabetic Effect of Methanol Leaf Extract of *Faidherbia albida* on Alloxan Induced Diabetic Rats

s/n	Treatment (mg/dL)	Fasting Blood Glucose (FBG) Concentration (mg/dL) time(hr) after treatment						% Inhibition of Glycaemia
		0	1	3	6	8	18	
1	Normal Saline (Control)	85.25±24.2	84.50±.21.67	85.00±41.34	84.00±43.12	83.50±23.41	82.50±12.52	-
2	Diabetic Untreated	243.10±21.68	250.20±41.95	260.40±34.53	300.10±31.57	300.20±27.98	290.80±04.34	-19.6
3	200	238.30 ± 31.54^{a}	146.50 ± 30.21^{b}	101.80 ± 45.75^{c}	85.50 ± 22.73^{d}	71.80 ± 55.23^{e}	63.50 ± 10.54^{f}	73.3
4	400	240.50 ± 41.12^{a}	200.80 ± 23.42^{b}	150.50 ± 34.74^{c}	93.750 ± 23.42^{d}	82.75 ± 12.45^{e}	74.25 ± 14.97^{f}	69.1
5	800	280.10 ± 12.51^{a}	260.80±12.65	170.80 ± 23.41^{b}	130.30 ± 25.78^{c}	90.30 ± 32.76^{d}	81.80±12.64	70.8
6	Insulin (0.1µg/kg)	210.50 ± 34.16^{a}	133.00 ± 21.42^{b}	107.50 ± 32.41^{c}	90.00 ± 41.63^{d}	82.00 ± 44.32^{e}	77.50 ± 42.87^{f}	63.1

N=5 Number of rats in each group within columns
Values with alphabetical superscripts across columns are significantly different statistically ($p<0.05$) when compared with normal saline treated rats.

4. DISCUSSION

The present study revealed that, the methanolic leaf extract of *Faidherbia albida* contains flavonoids, glycosides, tannins, saponins and terpenoids. Studies have shown the antidiabetic and antioxidant activity of flavonoids [28]. The presence of flavonoids and tannins may trigger insulin secretion, and demonstrated significant lowering of blood glucose level, serum sugar level, and biochemical parameters, and statistical improvement in the body weight of animals in a dose-dependent manner by enhanced peripheral glucose utilization by direct stimulation of glucose uptake and reduced blood glucose level [29].

Chromatography has been one of the best techniques for the purification of natural products. This technique was used to isolate the yellowish amorphous compound. The structural elucidation was achieved through the use of FTIR and NMR. The FTIR aided in the identification of the functional groups. The absorption band at 3233cm-^{1} is typical of hydroxy group (O-H) 2938cm^{-1} corresponds to stretching signal of C-H, 1676cm^{-1} is due to C=O, stretching at 1620cm^{-1}, 1543cm^{-1}, 1464cm^{-1} are typical of aromatic C=C and 1020cm^{-1} corresponds to C-O linkage in the chromen nucleus, typical of flavonoids [30]. This result is in good agreement with the previous literature for the molecular structure of kaempferol [31, 32]. The Nuclear magnetic resonance (^{1}HNMR) which showed resonating signals of meta coupled protons at δ_H 6.41 (1H, d, J=2.1 Hz, H-8) and δ_H 6.32 (1H, d, J=2.0 Hz, H-6). Signals of ortho-coupled protons at δ_H 7.16 (2H, d, J=8.0 Hz) H-3′5′) and δ_H 8.11 (2H, d, J=8.0Hz), H-2′6′) is of a 1′4′-disubstituted benzene ring of a flavonol nucleus of kaempferol [33].

The ^{13}CNMR revealed the presence of 15 carbon atoms. Carbon chemical shifts of quaternary carbons at showed signals at 146.99 (C-2), 137.0 (C-3), 160.9 (C-5), 166.9 (C-7), 156.60 (C-9), 160.51 (C-4′). 160.51 (C-4′). 173.20 (C-4) is a downfield chemical shift, which is due to carbonyl carbon. Other signals at 100.20 (C-6), 94.31 (C-8), 104. 34 (C-10), 122.40 (C-1′), 131.0 (C-2′), 116.98 (C-3′), 117.20 (C-5′), 130.7 (C-6′) are all of aromatic carbons. These spectral data of FTIR and NMR has confirmed that the compound is kaempferol [32].

The acute *i.p* LD_{50} was calculated as 3807.9 mg/kg. Report by Clarke and Clarke (1979) [34] suggest that the extract is safe for use as food or medication. The extract exerted significant antidiabetic activity by lowering the blood glucose levels of the rats induced with diabetes. Since alloxan is a known inducer of diabetes, which act by destroying the β cells produced by the islet of Langerhans, the extract must have contained compounds with possesses the potentials of reviving, revitalizing or regenerating the cells [35], thus having an antidiabetic property.

5. CONCLUSION

In conclusion, the present study revealed the tremendous anti-diabetic effect of the extract and this could be due to the presence of phytochemicals such as flavonoids, tannins, cardenolides saponins, found in the extract. The acute toxicity evaluation study has revealed that the plant is relatively safe for use as a drug. The antidiabetic effect exerted by all the various doses used for the study has justified the folkloric use of the plant for the treatment of diabetes. Thus, the plant could be said to a good antidiabetic agent. To the best of our knowledge, this is the first research, reported on the isolation and characterization of a kaempferol from the leaf of *Faidherbia albida*.

REFERENCES

1. Dar, R.A., Shahnawaz, M., and Qazi, P.H. (2017). Natural product medicines: A literature update. *Journal of Phytopharmacology*, 6(6), 349-351.
2. Punthakee, Z., Goldenberg, R., and Katz, P. (2018). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Canadian Journal of Diabetes*. 42, S10–5.
3. Fattaheian-Dehkordi, S., Hojjatifard, R., Saeedi, M., Khanavi, M., and Mancianti, F. (2021). A review on antidiabetic activity of Centaurea spp.: a new approach for developing herbal remedies. *Evidence Based*

Complementary and Alternative Medicine. 2021:1–23.

4. Baynest, H.W. (2015). Classification, pathophysiology, diagnosis and management of diabetes mellitus. *Journal of Diabetes and Metabolism*. 06(05).

5. Skyler, J.S., Bakris, G.L., Bonifacio, E., Darsow, T., Eckel, R.H., Groop, L., Groop, P.H., Handelsman, Y., Insel, R.A., Mathieu, C., McElvaine, A.T., Palmer, J.P., Pugliese, A., Schatz, D.A., Sosenko, J.M., Wilding, .JP., and Ratner, R.E. (2017). Differentiation of diabetes by pathophysiology, natural history, and prognosis. *Diabetes*. 2017; 66(2), 241–255.

6. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., et al. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical Practice*. 157, 107843.

7. IDF (2019). International Diabetes Federation. Diabetes Atlas, 9th edn. Brussels. Belgium: 2019. https://www.diabetesatlas.org, accessed 18 November 2020: 2019.

8. Yakubu, J., Maina, C.A., Medugu, A.N., Dawa, S.I., Abdulrahman, F.I., Sodipo, O.A. and Wampana, B. (2023): Bioassay-guided Antidiabetic Study of Chromatographic Fractions of Boswellia Dalzielii Hutch. Leaf Extract, Pharmaceutical and Biomedical Research, 9(1), 53-62

9. Wickens, G.E., Seif, E., Din, A.G., Guinko, S., and Ibrahim, N. (1995). Role of Acacia species in the rural economy of dry Africa and the near East, FAO-conservation guide, 1995, (27).

10. Muhammad, S., Mushtaq, A., and Ashfaq, A. (1998). Chemistry of the medicinal plants of the genus Acacia. *Hamdardmedicus*. 1998; 4(1):63-67.

11. Murunda, C.T. (1992). Use of seed of *Faidherbia albida* (syn *Acacia albida*.) for human consumption during famine periods in the Gokwe communal lands of Zimbabwe, Australian dry zone Acacias for human food, CSIRO, Canberra, Australia, pp. 93-98.

12. Timberlake, J. (1990). Field guide to the Acacias of Zimbabwe, CBC Publishing, Harare, pp. 160.

13. Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D.A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology*, 6(14), 1690-1696.

14. Tijani, A.Y., Uguru, M.O., Salawu, O.A., Abubakar, A., Onyekwelu, N.O., Akingbasote, J.A., (2009). Effect of *Faidherbia albida* on biochemical parameters of rats infected with *Trypanosoma brucei brucei*. *African Journal of Pharmacy and Pharmacology*, 3(1), 26-30.

15. Salisu, Y., Agunu, A., Abubakar, M.S., and Ibrahim, G. (2009). Hypoglycemic effects of *Acacia albida* del. (Mimosaceae) methanolic root bark extract. *Nigerian Journal of Pharmaceutical Science*, 8(1), 66-67.

16. Salawu, O., Tijani, A.B., Nwaeze, A.A., and Agbakwuru, A. (2010). Antimalarial activity of ethanolic stem bark of *Faidherbia albida* (Del.) a. Chev (Mimosidae) in mice. *Scholars Research Library Archives of Applied Scientific Research*, 2(5), 261-268.

17. Bazie, S., and Ayalew, A. (2014). Woldetsadik K. Antifungal activity of some plant extracts against *Colletotrichum musae* the cause of post-harvest banana Anthracnose. Journal of Plant Pathology and Microbiology, 5, 2-6.

18. Umar, I., and Mamman, A. (2014). Nematicidal potential of *Faidherbia albida* fruit against Meloidogyne javanica on cowpea. *Pakistan Journal of Nematology*, 32(1), 77-83.

19. Tawfiq, U.A., Yusha'u, M., Bashir, M., Adamu, S., and Umar, P.M. (2017). Synergistic antibacterial effect of stem bark extracts of *Faidherbia albida* and *Psidium guajava* against methicillin resistant *Staphylococcus aureus*. *Bayero Journal of Pure and Applied Sciences,* 10(1), 112 – 115.

20. Evans, W. C. (2009). *Trease and Evans Pharmacognosy* 16th edition. Elseviers Ltd., UK, pp. 45-50, 560-562, 568-570.

21. Markham. K. R. (1982). *Techniques of Flavonoids Identification*. Academic Press London: pp. 38-39.

22. Brain, K.R. and Turner, T.D. (1975). *The Practical Evaluation of Phytopharmaceuticals*. Wright Science Technical, Bristol Britain, Vol. 1, pp.144-152.

23. Vishnoi, N. R. (1979). *Advanced Practical Chemistry*. Ghazaibadindia: pp. 447-449.

24. Silva, G.L., I. Lee and K.A. Douglas, (1998). Special problems with extraction of plants. In: Cannel, J.P.R. (ed.). Natural Products Isolation. Humana press publishers, New Jersey (USA). Pp 356-358.

25. Sofowora, A. (2008). *Medicinal and Traditional Medicine in Africa* 2nd Ed. Spectrum books Ltd. Sunshine House, Ibadan Nigeria: p. 130.

26. Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. Archives Toxicology, 5, 275-287.

27. Yakubu, J., Isah, T. A., Medugu, A.N., Andema K.A., Abdulrahman, F.I., Sodipo O.A. and Balami, V. M. (2022). Toxicity evaluation and antidiabetic efficacy of *Senna siamea* Lam. methanol leaf extract in mice, *Open Journal of Bioscience Research*, 3(1), 18-27.

28. Chua, L.S., Abdulah, F.I., and Awang, M.A. (2020). Potential of natural bioactive C-glycosyl flavones for antidiabetic properties. *Studies in Natural Product Chemistry*, 64, 241-261.

29. Kuntal, D.A.S., Khan, S.M., Sounder, J., Umohan, U., Prasad V.S. (2020). Phytochemical screening and establishment of the antidiabetic potential of aqueous leaf extract of the endangered plant *Decalepis nervosa* in rats with alloxan-induced diabetes. *Turkey Journal of Pharmaceutical Sciences*, 17(3), 319-328.

30. Heneczkowski, M., Kopacz, M., Nowak, D., and Kuźniar, A. (2001). Infrared spectrum analysis of some flavonoids. Acta Poloniae Pharmaceutica, 58(6), 415-420.

31. Sambandam, B., Thiyagarajan, D., Ayyaswamy, A., Raman, P., Kulasekaran, J., and Venkatasamy, H. (2016). Extraction and isolation of flavonoid quercetin from the leaves of *Trigonella foenum-graecum* and their antioxidant activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8, 120-124.

32. Ali, A., Osman, A., Abo Eita, A.M., and Sitohy, M.Z. (2019). Isolation and characterization of quercetin and kaempferol from *Ginkgo biloba* leaves grown in Egypt. *Zagazig Journal of Agricultural Research,* 46(5), 1562-1571.

33. Fleming, I., and, Williams, D. (2019). Spectroscopic Methods in Organic Chemistry. 7th Edition. Springer Publication.

34. Clarke, E.G.C. and Clarke, M.L. (1977). *Veterinary Toxicology*. Cassel Collier Macmillan Publishers; London; pp.268-271.

35. Yakubu, J., Abdulrahman, F.I. and Sodipo, O.A. (2020). Evaluation of the toxicity profile and antidiabetic potentials of the methanol extracts of *Boswellia dalzielii* (Frankincense Tree) in alloxan-induced diabetic rats. *Tropical Journal of Natural Product Research*, 4(5), 190 – 194.

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