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The Antihyperglycaemic Effect of the Methanol Stem-Bark Extract of *Ficus capensis* Thunb (Moraceae) and its Fractions on Alloxan-Induced Hyperglycaemia in Wistar Rats

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Abstract:

Background: *The plant Ficus capensis is found in Sub-Saharan Africa, South Africa and South America. It is used for threatened abortion, rheumatism, fever, epilepsy, and traditional cure of diabetes mellitus in south-western Nigeria.*

Objective: *This study was aimed at evaluating the antihyperglycaemic effect of the methanol stem-bark extract of Ficus capensis (Thunb) and its fractions on alloxan induced hyperglycaemia in Wistar rats*.

Method: *Methanol stem bark extract of Ficus capensis (MSFCE) and its Fractions {Ethyl Acetate Fraction (EAF), n-Butanol Fraction (NBF), and Residual Aqueous Fraction (RBF)} were subjected to preliminary phytochemical screening, Oral median lethal dose (LD_{50}) determination. The antihyperglycaemic activity of the extract and its fractions at doses of 100, 200, and 400 mg/kg body weight orally on Alloxan-Induced Hyperglycaemia in Wistar rats was investigated and were carried out. Thirty six (36) were divided into six (6) groups of six (6) rats each, the 1st group (normal rats) served as the negative control received distilled water 1 ml/kg, the 2nd group (hyperglycaemic control) while the 3rd, 4th, 5th (hyperglycaemic rats) received MSFCE and its fractions at doses of (100, 200 and 400 mg/kg respectively) and the 6th group of hyperglycaemic rats received Metformin 100 mg/kg served as positive control. The treatments were given orally and once a day.*

Results: *Preliminary phytochemical constituents of the extract and its fractions revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins, steroids, phenols and triterpenes. LD_{50} of the extract and its fractions in Wistar rats were found to be greater than 5,000 mg/kg bw p.o. The extract at its fractions at tested doses (100, 200 and 400 mg/kg bw) significantly ($p < 0.05$, 0.01 and 0.001) decreased blood glucose level after 2, 4, 8, 16 and 24 hr post administration when compared with diabetic control). On comparison over time from zero hr, the extract and its fractions at doses tested exhibited significant ($p < 0.05$, 0.01 and 0.001) decreased blood glucose levels after the 2nd, 4th, 8th and 16th hr after administration.*

Conclusion: *These findings suggest that the extract and its Fractions possess antihyperglycaemic property which supports the ethno medical use of the plant in the management of diabetes mellitus.*

Keywords: *Phytochemical constituents, Oral median lethal dose (LD_{50}) and blood glucose*

1. INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (American Diabetes Association, 2013) . The effect of diabetes

mellitus includes long-term damage, dysfunction and failure of various organs (Fowler, 2008). The International Diabetes Federation (International Diabetes Federation, 2015) estimated that about 415 million adults, aged 20 to 79 years have diabetes worldwide, or about one in every 11, with type 2 which represents approximately 90% of the cases worldwide and this is expected to increase to 642 million by the year 2040 (Wild *et al*., 2004 and International Diabetes Federation, 2015)

Regardless of the type of diabetes, patients are required to control their blood glucose levels with medications such as insulin and oral antidiabetic agents that include sulphonylureas, biguanides, alpha glucosidase inhibitors, thiozolidinedione derivatives, newer oral antidiabetic agents such as Aldose reductase inhibitors, Amylin agonist, Glucagon like peptide-1 and or by adhering to an exercise programme and a dietary plan (Wolf *et al*., 2004). The problems posed by agents used in management of diabetes mellitus include: expensiveness, adverse effects such as severe hypoglycemia, lactic acidosis, and peripheral edema, abdominal discomfort and require long duration of therapy (Sulaiman *et al*., 2006 and Lorenzati *et al*., 2010). Thus searching for a new class of compound from medicinal plants is essential to overcome side effects (Sulaiman *et al*., 2006).

Alloxan-induced hyperglycaemia is a useful model to study the activity of hypoglycaemic agents (Szukudelski *et al*., 2001). Alloxan is well known for its selective pancreatic islet β cell cytotoxity and has been used to induce diabetes in animals. The underlying mechanism of alloxan is still unclear. However, it probably exerts its diabetogenic effect by the production of hydrogen peroxide in intact tissues via generation of reactive oxygen species (Drews *et al*., 2000). It interferes with cellular metabolic oxidative mechanisms forming highly reactive superoxide radicals which destroy the insulin producing cells in the pancreas. The selective uptake of this cytotoxic agent might account for its well known diabetogenic effect (Gorus *et al*., 1982).

Ficus capensis Thunb belongs to the family Moraceae. *Ficus capensis* is found in Sub-Saharan Africa, South Africa and South America mostly along rivers. It is commonly known as fig tree spreading deciduous or evergreen tree with thick bole and spreading roots. It produces fruits throughout the year and the leaves are broad and green. In Nigeria *F. capensis* has been used by Igede people as treatment for dysentery and wound dressing (Igoli *et al*., 2005). It is also used for the traditional cure of diabetes mellitus in south-western Nigeria (Soladoye *et al*., 2012).

There is paucity of scientific information regarding the effect of *Ficus capensis* on blood glucose levels, hence the present study was carried out to investigate the antihyperglycaemic effect of the methanol stem-bark extract of *Ficus capensis* (Thunb) and its Fraction on alloxan induced hyperglycaemia in Wistar rats.

2. MATERIALS AND METHOD

2.1 Animals

Wistar rats (160 - 200 g) of either sex were purchased from and maintained in Animal House, Department of Pharmacology and Therapeutics Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria. They were housed in a well ventilated room, fed with standard Vital Feed® and water *ad libitum*

2.2. Plant material

The stem-bark of *Ficus capensis* was collected in March, 2013 at Basawa village, Samaru Zaria. It was identified and authenticated by Botanist Mal. Umar Shehu Gallah of the herbarium section, Department of Botany, Ahmadu Bello University, Zaria by comparing with voucher specimen number 901459 deposited in the herbarium for future reference.

2.3. Plant extraction

2.3.1 Preparation of the Plant Extract

The method described by (Handa *et al*., 2008) was employed. The stem bark of the plant was washed and cut into small pieces and air dried under shade. The dried plant material was chopped and finely grounded using pestle and mortar. About 500 g dried and powdered stem-bark of *Ficus capensis* was macerated

with 2L of aqueous methanol solution (70%$^{v}/_{v}$ methanol and 30%$^{v}/_{v}$ water) at room temperature for a period of seven (7) days with intermittent agitation. The extract was filtered with the aid of Whatman No. 3 filter paper. The filtrate was concentrated to dryness at room temperature where a dark brown residue referred to as crude methanol stem bark extract was obtained. The crude methanol stem-bark extract obtained was kept in a dessicator until needed for use.

2. 3. 2 Fractionation of crude methanol stem bark extract of Ficus capensis (MSFCE)

The method described by (Handa *et al*., 2008) was employed. 50 g of the crude MSFCE was dissolved in 600 ml distilled water and filtered. The filtrate was successively partitioned three times each with n-hexane, chloroform, ethyl acetate and n-butanol in order of polarity resulting to various fractions. The fractions were kept separately in a dessicator until required for use. Solutions of the fractions were freshly prepared with distilled water for each day of the experiment.

2. 4. Phytochemical screening

Phytochemical Screening of the crude methanol stem bark extract of *Ficus capensis* and its fractions were conducted according to the methods described by (Evans *et al*., 2002)

2. 5. Preparation of extract and treatment

Different stock solutions of MSFCE and its fractions: ethyl acetate fraction (EAF), n-butanol fraction (NBF) and residual aqueous fraction (RAF) were prepared using distilled water followed by serial dilution to obtain the final experimental concentrations. The solutions were freshly prepared daily and orally administered with the aid of oral gavages.

2. 6. Acute toxicity study

The method described by (Lorke, 1983) for determining the median lethal dose was used. It was carried out in two phases. In the initial phase, three (3) groups of three (3) rats each were administered with the ethyl acetate fraction of methanol stem bark extract of *Ficus capensis* (EAF) at doses of 10, 100 and 1000 mg/kg body weight orally (*bw p.o*). The rats were observed in the first four (4) hours and 24 hours for signs and symptoms of toxicity such as sniffing, restlessness, hyperactivity, leaking of paws, defaecation, stretching, and protrusion of the eye balls, calmness and death. In the second phase, three (3) fresh rats were divided into three (3) groups of one (1) rat each and administered with EAF at doses of 1600, 2900, and 5000 mg/kg *bw p.o* respectively, based on result obtained in the first phase. The rats were observed for signs and symptoms of toxicity and death over a period of 24 hour. The LD_{50} value was calculated as geometric mean of the highest non lethal dose which the animals survived and lowest lethal dose that caused death.

$$\mathbf{LD_{50}} = \sqrt{\boldsymbol{highest}\ \boldsymbol{nonlethal\ dose} \times \boldsymbol{lowest\ lethal\ dose}}$$

2.7 Effect of Methanol Stem Bark Extract of *Ficus capensis* and its fraction on Alloxan induced hyperglycaemia in Wistar rats

The method described by (Dhandapani *et al*., 2002) was employed in 12 hours overnight fasted normal rats. The rats were injected with alloxan monohydrate dissolved in sterile 0.9% normal saline at a dose of 150 mg/kg intraperitoneally (*i.p*). The rats were kept for the next 24 h on 10% glucose solution in their cages to prevent hypoglycaemia. Seventy two (72) hours post administration of alloxan; rats were examined for hyperglycaemia at intervals of 0, 2, 4, 8, and 24 hours and estimated using a glucose oxidase - peroxidase reactive strips and a glucometer. Rats with blood glucose of 180 mg/dL and above were selected and used in the study. The alloxan induced hyperglycemic Wistar rats were randomly divided into five (5) groups of six (6) rats each as follows:

Group I: Normal rats received distilled water 1 ml/kg

Group II: Hyperglycaemic control received distilled water at dose of 1 ml/kg

Group III: Hyperglycaemic rats received MSFCE 100 mg/kg

Group IV: Hyperglycaemic rats received MSFCE 200 mg/kg

Group V: Hyperglycaemic rats received MSFCE 400 mg/kg

Group VI: Hyperglycaemic rats received Metformin 100 mg/kg

The same procedure was repeated for ethyl acetate fraction (EAF), n-butanol (NBF), and residual aqueous (RAF) fractions of methanol stem bark extract of *Ficus capensis* at doses of 100, 200, and 400 mg/kg *bw p.o* respectively.

2.8 Statistical analysis

All data were expressed as the mean ± SEM. Statistical analysis was carried out using the repeated measures ANOVA followed by benforreni and post hoc test for multiple comparisons and the difference considered significant when ($p \leq 0.05$).

2.9 Results

Crude Methanol Stem Bark Extract of *Ficus capensis*

The maceration of 500 g stem bark of *Ficus capensis* resulted in a dark brown residue which was referred to as crude methanol stem bark extract weighing 85 g which is equivalent to 17% $^{w}/_{w}$ yield.

Fractionation of Crude Methanol Stem Bark Extract of *Ficus capensis* (MSFCE)

The fractionation of crude methanol stem bark extract of *Ficus capensis* yielded five (5) fractions (n-Hexane, Chloroform, Ethyl acetate, n-Butanol and Residual aqueous). The n-Butanol fraction had the highest yield 61. 75 (%$^{w}/_{w}$) while the yield of n-Hexane fraction was negligible

3. Phytochemical Constituents of Methanol Stem Bark Extract of *Ficus capensis* and its Fractions

The Phytochemical Constituents of methanolic stem bark extract of *Ficus capensis* and its Fractions; Ethyl acetate soluble fraction (EAF), n-Butanol soluble fraction (NBF) and Residual aqueous fraction (RBF) revealed the presence of carbohydrate, cardiac glycosides, saponins, steroids, triterpenes, tannins, flavonoids, and alkaloids. Anthraquinone was found to be absent in all the fractions including the methanol stem bark extract of *Ficus capensis*

3.1. Effect of acute administration of MSFCE and its Fractions on general behaviour and mortality

The oral administration of MSFCE and its Fractions produced no visible signs of toxicity and mortality throughout the study period. The LD_{50} was thus found to be greater than 5 000 mg/kg

3.2. Effect of Methanol Stem Bark Extract of *Ficus capensis* on Blood Glucose of Alloxan Induced Hyperglycaemia in Wistar Rats

Methanol Stem Bark Extract of *Ficus capensis* (MSFCE) at doses of 200 and 400 mg/kg significantly ($p<0.05$) decrease blood glucose levels 2 h after administration. Extract also at a dose of 400 mg/kg and at 24 hour produced significant ($p<0.05$) decrease in blood glucose levels when compared with the hyperglycaemic control.

On comparison overtime, the extract at the doses tested did not produce significant ($p>0.05$) decrease blood glucose level throughout the period of the study as compared to zero hour. Metformin significantly ($p<0.05$ and $p<0.01$) decreased blood glucose levels from the 0 hour and when compared with the diabetic control at 2, 8 and 24 h (Table 1).

Table1. *Effect of Methanol Stem-Bark Extract of Ficus capensis on Blood Glucose Levels of Alloxan-Induced Hyperglycaemia in Wistar Rats*

Treatment	Dose mg/kg	Mean Blood Glucose Level (mg/dL)				
		0 h	2 h	4 h	8 h	24 h
D/W	1 ml/kg	74.6 ± 5.0	82.3 ± 5.6	81.2 ± 5.1	82.8 ± 4.3	102.4 ± 5.6
ALX	150	370.1 ± 14.8	324.0 ± 5.7	314.2 ± 9.5	317.5 ± 11.6	300.3 ± 17.6
MSFCE	100	296.4 ± 12.5	315.3 ± 10.5	297.1 ± 19.9	289.2 ± 19.1	279.6 ± 25.7
MSFCE	200	298.3 ± 12.9	279.4 ± 19.2*	390.5 ± 8.5	320.3 ± 54.8	347.2 ± 53.8

MSFCE	400	326.7 ± 11.5	292.2 ± 18.8*	304.3 ± 26.5	321.0 ± 19.9	290.5 ± 17.6*
MTF	100	308.4 ± 4.8	253.5 ± 10.7^{*a}	292.0 ± 9.9^{a}	257.3±15.8^{**b}	258.9 ± 11.2^{**b}

Data were presented as Mean ± SEM; superscripts *, **, and $^{a, b}$ represents the level of significance at $p<0.05$ and $p<0.01$ when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test, n=6. D/W=Distilled water, ALX=Alloxan, MSFCE= Methanol Stem Bark Extract of *Ficus capensis* and MTF= Met

3.3. Effect of Ethyl Acetate Fraction of *Ficus capensis* on Blood Glucose of Alloxan Induced Hyperglycaemia in Wistar Rats

Single administration of ethyl acetate fraction of methanol stem-bark extract of *Ficus capensis* (EAF) at a doses of (100, 200 and 400 mg/kg *bw*) produced significant ($p<0.05$) decrease in blood glucose levels of rats at different time intervals (4, 8, and 16 hour respectively) compared with diabetic control. Metformin at a dose of 100 mg/kg produced significant ($p<0.001$) decrease in blood glucose level when compared with the diabetic control. The greatest change of blood glucose level (38.8%) was seen with EAF at 100 mg/kg *bw* then followed by EAF at doses of 200, 400 mg/kg *bw* (29%) and (23.7%) respectively in decreasing manner while metformin produced glycaemic change of (27.8%) at first 4 hour post administration. EAF at doses tested (100, 200 and 400 mg/kg) significantly ($p<0.05$) decreased blood glucose levels after the 4th, 8th and 16th hour after administration as compared to 0 hour (Table 2)

Table2. *Effect of Ethyl Acetate Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

		Mean Blood Glucose Level (mg/dL)					
Treatment	mg/kg	0 h	2 h	4 h	8 h	16 h	24 h
D/W	1ml/kg	77.0±3.8	74.0±2.3	67.2±3.5	67.8±3.7	68.4±1.4	81.2±1.8
ALX	150	439.0±58.7	367.4±26.2	343.4±29.2	358.6±26.9	356.8.5±20	432.8±35.3
EAF	100	353.6±62.6	344.6±56.2	364.4±47.7	303.4±43.3	216.6±46.6^{*a}	328.2±59.5
EAF	200	276.0±25.4	317.8±11.7	271.6±35.3	235.8±28.0*	196.0±22.7^{**a}	227.0±28.5^{a}
EAF	400	308.0±14.4	243.2±25.7	221.0±52.4^{*a}	222.6±58.5^{*a}	235.2±51.6^{*a}	331.0±55.3
MTF	100	228.4±29.1	179.0±36.3**	164.8±27.5^{**a}	198.8±67.2**	247.2±28.2	353.7±32.8

Data were presented as Mean ± SEM; superscripts *, **, and $^{a, b}$ represents the level of significance at $p<0.05$ and $p<0.01$when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test,.n=6. D/W= Distilled water, ALX= Alloxan, EAF= Ethyl acetate Fraction, and MTF =Metformin

3.4. The Effect of n-Butanol Fraction of *Ficus capensis* on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats

Single administration of n-butanol fraction of methanol stem-bark extract of *Ficus capensis* (NBF) at doses of 100 and 200 mg/kg *bw* produced significant ($p<0.05$) decrease in blood glucose levels of rats and at the 2nd , 4th, 8th and 16th hour respectively post administration. Unlike the NBF, metformin at a dose 100 mg/kg *bw* produced significant ($p<0.01$) decrease in the blood glucose levels of rats at the 2nd, 4th, and 8th hour post administration when compared with the diabetic control (Alloxan 150 mg/kg *bw*). The highest glycaemic change of blood glucose level (BGL) of (52.3%) was observed with NBF 200 mg/kg *bw* of the extract after 16 hour after treatment. On comparison overtime, NBF at doses tested (100, 200 and 400 mg/kg) significantly ($p<0.05$ and $p<0.01$) decreased blood glucose levels at the 2nd, 4th, 8th and 16th after administration as compared to 0 hour. Metformin significantly ($p<0.05$ and $p<0.01$) decreased blood glucose levels at the 2nd and 4th hour respectively after administration as compared to 0 hour (Table 3)

Table3. *The Effect of n-Butanol Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

		Mean Blood Glucose Level (mg/dL)					
Treatment	Dose	0 h	2 h	4 h	8 h	16 h	24 h

	mg/kg						
D/W	1ml/kg	77.0±3.8	77.4±2.1	73.6±1.0	67.2±3.4	71.6±3.4	63.4±1.5
ALX	150	439.0±58.7	367.8±26.2	343.4±29.2	358.6±26.9	356.6±20.2	432.8±35.3
NBF	100	347.2±63.7	315.0±89.5*	267.4±72.8^{*a}	264.6±70.0^{*a}	273.4±76.5^{a}	326.6±78.9
NBF	200	375.2±25.8	309.8±15.7^{*a}	275.6±18.2^{*a}	235.2±22.0^{*a}	209.4±25.9^{*b}	404.2±36.0
NBF	400	461.6±67.4	346.0±38.5^{a}	330.4±37.8^{a}	321.8±39.4^{a}	384.6±69.8	520.8±41.2
MTF	100	228.4±29.1	179.0±36.3^{**a}	164.8±27.5^{**b}	198.8±67.2**	247.2±28.2	353.7±32.8

Data are presented as Mean ± SEM; superscripts *, ** and $^{a, b}$ represents the level of significance at $p<0.05$,and $p<0.01$ when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test,n=6. D/W = Distilled water, ALX = Alloxan, NBF= n-Butanol Fraction, and MTF= Metformin

3.5. The Effect of Residual Aqueous Fraction of *Ficus capensis* on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats

Residual aqueous fraction of methanol stem-bark extract of *Ficus capensis* (RAF) significantly ($p<0.05$) decreased blood glucose levels of rats at a dose of 100 mg/kg *bw* at the 4th hour post administration only. Also it significantly ($p<0.05$) decreased blood glucose levels of rats at a dose of 200 mg/kg *bw* and at the 2nd, 8th and 16th hour post administration. Metformin at a dose 100 mg/kg *bw* produced significant ($p<0.01$) decrease in the blood glucose levels of rats at the 2nd, 4th, and 8th hour post administration when compared with the diabetic control. RAF at doses tested significantly ($p<0.05$ and $p<0.01$) decreased blood glucose levels of rats and metformin significantly ($p<0.05$ and $p<0.01$) decreased blood glucose levels at the 2nd and 4th hour after administration as compared to day 0 (Table 4).

Table4. *Effect of Residual Aqueous Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

Mean Blood Glucose Level (mg/dL)							
Treatment	Dose mg/kg	0 h	2 h	4 h	8 h	16 h	24 h
D/W	1 ml/kg	77.0±3.8	77.4±2.1	73.6±1.0	67.2±3.4	71.6±3.4	63.4±1.5
ALX	150	439.0±58.7	367.8±26.2	343.4±29.2	358.6±26.9	356.6±20.2	432.8±35.3
RAF	100	316.2±20.3	309.4±11.1	254.6±9.4^{*a}	240.2±17.4^{*a}	216.4±11.4^{b}	360.0±8.3
RAF	200	313.0±17.5	270.8±5.5*	269.2±4.0	205.6±4.0^{*b}	205.0±14.1^{*b}	391.6±14.3
RAF	400	313.4±23.5	287.6±36.3	262.4±15.7*	227.0±21.7^{*b}	211.8±11.9^{b}	375.2±32.0
MTF	100	228.4±29.1	179.0±36.3^{**a}	164.8±27.5^{**b}	198.8±67.2**	247.2±28.2	353.7±32.8

Data were analysed using Repeated Measures ANOVA followed by Bonferroni post hoc test and presented as Mean ± SEM, superscripts *, ** and $^{a, b}$ the level of significance at $p < 0.05$ and $p < 0.01$ when compared to diabetic control and day zero respectively. n=6. D/W = Distilled water, ALX = Alloxan, RAF = Residual Aqueous Fraction, and MTF = Metformin

4. DISCUSSION

The oral median lethal dose (LD_{50}) of the methanol stem bark extract of *Ficus capensis* in rats was found to be greater than 5000 mg/kg body weight which is relatively safe because no death was recorded even at higher dose of 5000 mg/kg. The Organization for Economic Cooperation and Development (OECD), Paris, France (Walum, 1998) recommended chemical labeling and classification of acute systemic toxicity

based on oral LD_{50} values as: very toxic, $\leq$ 5 mg/kg; toxic, > 5 $\leq$ 50 mg/kg; harmful, > 50 $\leq$ 500 mg/kg; and not toxic or harmful, > 500 $\leq$ 2,000 mg/kg. However, the doses selected used in this work were lower than 30% of the LD_{50} which have been shown to be relatively safe for ethnopharmacological research (Voungtau, *et al*., 2004).

In the present study, methanol stem bark extract of *Ficus capensis* (MSFCE) and its fractions: ethyl acetate fraction (EAF), n-butanol fraction (NBF), and residual aqueous fraction (RBF) significantly decreased the blood glucose levels (BGL). Significant reduction in BGL followed the pattern in group treated with metformin. The significant decrease in blood glucose levels is an indication that the extract and its fractions have antihyperglycaemic activity which could be attributed to the presence phytochemical constituent's steroids, triterpenes, glycosides, alkaloids, flavonoids, phenols, and saponins. This is in agreement with earlier report by (Roy *et al*., 2006), the presence of secondary metabolites such as saponins, alkaloids and flavonoids in *Mangifera indica* are responsible for the antihyperglycaemic effect. Studies have also shown that the administration of *Gymnema sylvestre* extract, saponin fraction or isolated triterpene glycosides is responsible for prolong antidiabetic effect of insulin and extended reduced blood glucose levels (Di Fabio *et al*., 2013 and Alqantani *et al*., 2013).

The phytochemical constituents of methanol stem bark extract of *Ficus capensis* revealed the presence of phenols flavonoids, alkaloids, tannins, saponins, steroids and triterpenes which may be responsible, in part or in combination for the observed pharmacological activities of the plant extract. Researchers have found that they are implicated as having antidiabetic activities (Malviya *et al*., 2010). Literature has also shown that the biological activities of alkaloids and flavonoids to include hypoglycaemia, hypolipidemia, hypoazotemia, hypotension among other biological effects (Sani *et al*., 2009). The antidiabetic activities could be obtained from several parts of the plants such as the aerial parts, bark, flower, root, seeds, leaves, bulb, tubers and/or whole plant (Maroo *et al*., 2002).

Also the possible mechanism by which MSFCE and its fractions bring about its blood glucose lowering action is by potentiating the insulin effect by increasing the pancreatic secretion of insulin from β cell. The findings also suggest the extract and its fractions may act by promoting insulin secretion from the remnants of β cells and improving insulin sensitivity.

In our study, treatment with MSFCE and its fractions proved effective in managing hyperglycaemia because blood glucose levels was found to have normalized or near normal. As this was only observed in alloxan treated hyperglycaemic rats.

Thus, the results obtained from this study suggest that the crude methanol stem bark of *Ficus capensis* and its fractions contained bioactive constituents that may have relevant antidiabetic properties used in the management of diabetes mellitus.

5. CONCLUSION

The methanol stem bark extract of *Ficus capensis* and its fraction were found to possess significant antihyperglycaemic activity. This justifies the traditional use of the plant in the management of diabetes mellitus.

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APPENDIX

Appendix I: *Crude Methanol Stem Bark Extract of Ficus capensis*

The maceration of 500 g stem bark of *Ficus capensis* resulted in a dark brown residue which was referred to as crude methanol stem bark extract weighing 85 g which is equivalent to 17% $^{w}/_{w}$ yield.

Appendix II: *Fractionation of Crude Methanol Stem Bark Extract of Ficus capensis (MSFCE)*

The fractionation of crude methanol stem bark extract of *Ficus capensis* yielded five (5) fractions (n-Hexane, Chloroform, Ethyl acetate, n-Butanol and Residual aqueous). The n-Butanol fraction had the highest yield 61. 75 (%$^{w}/_{w}$) while the yield of n-Hexane fraction was negligible

Appendix III: *Oral LD_{50} determination in rats for Methanol Stem Bark Extract of Ficus capensis using Lorke method 1983*

Phase I

Group	Treatment	Dose mg/kg	Mortality %
I	MSFCE	10	0/3 (none died)
II	MSFCE	100	0/3 (none died)
III	MSFCE	1000	0/3 (none died)

Phase II

Group	Treatment	Dose mg/kg	Mortality %
I	MSFCE	1600	0/1 (no death)
II	MSFCE	2900	0/1 (no death)
III	MSFCE	5000	0/1 (no death)

LD_{50} = √ Geometric mean of lethal dose (X) and highest non lethal dose (Y)

Therefore, LD_{50} in both mice and rats was found to be greater than 5000 mg/kg.

The oral LD_{50} of Ethyl acetate, n- Butanol and Residual Aqueous Fractions in both mice and rats was greater 5000 mg/kg

AppendixIV: *Effect of Methanol Stem-Bark Extract of Ficus capensis on Blood Glucose Levels of Alloxan-Induced Hyperglycaemia in Wistar Rats*

Treatment	Dose mg/kg	Mean Blood Glucose Level (mg/dL)				
		0 h	2 h	4 h	8 h	24 h
D/W	1 ml/kg	74.6 ± 5.0	82.3 ± 5.6	81.2 ± 5.1	82.8 ± 4.3	102.4 ± 5.6
ALX	150	370.1 ± 14.8	324.0 ± 5.7	314.2 ± 9.5	317.5 ± 11.6	300.3 ± 17.6
MSFCE	100	315.3 ± 10.5	289.2 ± 19.1	279.6 ± 25.7		
MSFCE	200	296.4 ± 12.5	279.4 ± 19.2*	297.1 ± 19.9	320.3 ± 54.8	347.2 ± 53.8
MSFCE	400	321.0 ± 19.9	290.5 ± 17.6*			
MTF	100	298.3 ± 12.9	292.2 ± 18.8*	390.5 ± 8.5	257.3±15.8**b	258.9 ± 11.2**b
		326.7 ± 11.5	253.5 ± 10.7*a	304.3 ± 26.5		
		308.4 ± 4.8		292.0 ± 9.9a		

Data were presented as Mean ± SEM; superscripts *, **, and [a, b] represents the level of significance at $p<0.05$ and $p<0.01$ when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test, n=6. D/W=Distilled water, ALX=Alloxan, MSFCE= Methanol Stem Bark Extract of *Ficus capensis* and MTF= Met

AppendixV. *Effect of Ethyl Acetate Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

Treatment	mg/kg	Mean Blood Glucose Level (mg/dL)					
		0 h	2 h	4 h	8 h	16 h	24 h
D/W	1ml/kg	77.0±3.8	74.0±2.3	67.2±3.5	67.8±3.7	68.4±1.4	81.2±1.8
ALX	150	439.0±58.7	367.4±26.2	343.4±29.2	358.6±26.9	356.8.5±20	432.8±35.3
EAF	100	353.6±62.6	344.6±56.2	364.4±47.7	303.4±43.3	216.6±46.6^{*a}	328.2±59.5
EAF	200	276.0±25.4	317.8±11.7	271.6±35.3	235.8±28.0*	196.0±22.7^{**a}	227.0±28.5^{a}
EAF	400	308.0±14.4	243.2±25.7	221.0±52.4^{*a}	222.6±58.5^{*a}	235.2±51.6^{*a}	331.0±55.3
MTF	100	228.4±29.1	179.0±36.3**	164.8±27.5^{**a}	198.8±67.2**	247.2±28.2	353.7±32.8

Data were presented as Mean ± SEM; superscripts *, **, and $^{a, b}$ represents the level of significance at $p<0.05$ and $p<0.01$when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test,.n=6. D/W= Distilled water, ALX= Alloxan, EAF= Ethyl acetate Fraction, and MTF =Metformin

AppendixVI: *The Effect of n-Butanol Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

Treatment	Dose mg/kg	Mean Blood Glucose Level (mg/dL) 0 h	2 h	4 h	8 h	16 h	24 h
D/W	1ml/kg	77.0±3.8	77.4±2.1	73.6±1.0	67.2±3.4	71.6±3.4	63.4±1.5
ALX	150	439.0±58.7	367.8±26.2	343.4±29.2	358.6±26.9	356.6±20.2	432.8±35.3
NBF	100	347.2±63.7	315.0±89.5*	267.4±72.8^{*a}	264.6±70.0^{*a}	273.4±76.5^{a}	326.6±78.9
NBF	200	375.2±25.8	309.8±15.7^{*a}	275.6±18.2^{*a}	235.2±22.0^{*a}	209.4±25.9^{*b}	404.2±36.0
NBF	400	461.6±67.4	346.0±38.5^{a}	330.4±37.8^{a}	321.8±39.4^{a}	384.6±69.8	520.8±41.2
MTF	100	228.4±29.1	179.0±36.3^{**a}	164.8±27.5^{**b}	198.8±67.2**	247.2±28.2	353.7±32.8

Data are presented as Mean ± SEM; superscripts *, ** and $^{a, b}$ represents the level of significance at $p<0.05$,and $p<0.01$ when compared to diabetic control and day zero respectively. Repeated Measures ANOVA followed by Bonferroni post hoc test,n=6. D/W = Distilled water, ALX = Alloxan, NBF= n-Butanol Fraction, and MTF= Metformin

AppendixVII. *Effect of Residual Aqueous Fraction of Ficus capensis on Blood Glucose Levels of Alloxan Induced Hyperglycaemia in Rats*

		Mean Blood Glucose Level (mg/dL)					
Treatment	Dose mg/kg	0 h	2 h	4 h	8 h	16 h	24 h
D/W	1 ml/kg	77.0±3.8	77.4±2.1	73.6±1.0	67.2±3.4	71.6±3.4	63.4±1.5
ALX	150	439.0±58.7	367.8±26.2	343.4±29.2	358.6±26.9	356.6±20.2	432.8±35.3
RAF	100	316.2±20.3	309.4±11.1	254.6±9.4^{*a}	240.2±17.4^{*a}	216.4±11.4^{a}	360.0±8.3
RAF	200	313.0±17.5	270.8±5.5*	269.2±4.0	205.6±4.0^{**a}	205.0±14.1^{**a}	391.6±14.3
RAF	400	313.4±23.5	287.6±36.3	262.4±15.7*	227.0±21.7^{**a}	211.8±11.9^{a}	375.2±32.0
MTF	100	228.4±29.1	179.0±36.3^{**a}	164.8±27.5^{**a}	198.8±67.2**	247.2±28.2	353.7±32.8

Data were analysed using Repeated Measures ANOVA followed by Bonferroni post hoc test and presented as Mean ± SEM, superscripts * ** and $^{a, b}$ the level of significance at $p < 0.05$ and $p < 0.01$ when compared to diabetic control and day zero respectively. n=6. D/W = Distilled water, ALX = Alloxan, RAF = Residual Aqueous Fraction, and MTF = Metform

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