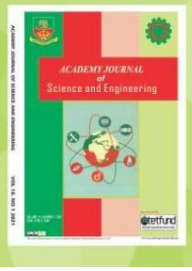
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Original Research

IN VIVO-ANTI-DIABETIC ACTIVITIES OF CRUDE EXTRACTS OF BALINITE AEGYPTICA AND TERMINAIA CATARPA PLANTS

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Abstract

The phytochemical screening and antidiabetic activities of crude extracts of *Balanite aegytica* and *Terminalia catarpa* were investigated and compared with the standard drugs using standard methods. The results of phytochemical screening reveal the presence of alkaloids, flavonoids, phenols, glycosides and Tannins at varying concentrations. The results of antidiabetic test reveal that *Balanites aegytica* and *Terminalia catappa* possess moderate to higher activities with *Balanites aegytica* showing higher activity than the standard drug. Results statistical analysis of the two plants against the standard drugs shows that *Balanites aegytica* posses significant change of 59% reduction of blood sugar at 50 mg on day 7 and 49% reduction on day 14 for Fasting blood sugar as against the standard drugs Metformin which shows 11% reduction at 50 mg on day 7 and 21% at 100 mg on day 14. The results of toxicity test shows that the two crude extracts and the standard drugs were safe at lower concentrations but unsafe at higher concentration with standard drug having higher toxicity that could cause liver damage at 150 mg. The results of this research therefore showed that *Balanites aegytica* plant can be used for the treatment of diabetics.

Keywords: Antidiabetic, In-vivo, *Balanites aegyptica,* and *Terminalia catappa*

Introduction

Diabetes, or diabetes mellitus, is a chronic medical condition where the body either doesn't produce enough insulin or can't effectively use the insulin it produces. Insulin is a hormone that helps regulate blood sugar (glucose) levels. There are three main types of diabetes (Nabarun, *et al.,* 2019). The first type is Type 1 Diabetes which is an autoimmune condition where the body's immune system attacks insulin-producing beta cells in the pancreas. It usually develops in childhood or adolescence, and individuals with Type 1

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diabetes require insulin injections or an insulin pump for life. The second type is the common type, often associated with obesity and a sedentary lifestyle. It occurs when the body becomes resistant to insulin or when the pancreas fails to produce enough insulin. It can often be managed with lifestyle changes, oral medications, and sometimes insulin. The third type is the one that develops during pregnancy and usually resolves after childbirth. However, it increases the risk of developing Type 2 diabetes later in life for both the mother and the child. Managing diabetes typically involves a combination of lifestyle changes, monitoring blood glucose levels, medication, and, in some cases, insulin therapy (Anuforo, 2018). These medications do have drawbacks, too, like their high cost, side effects, and potential for hypoglycaemia (Safet, et al., 2022). As a result, there is a demand for benign alternative diabetic treatments by utilizing plant-derived bioactive antidiabetic compounds. According to the International Diabetes Federation (IDF), an estimated 463 million adults worldwide were living with diabetes in 2019, and this number is expected to rise to 700 million by 2045 (International Diabetes Federation, 2019). The prevalence of diabetes is increasing globally, and it is becoming a significant public health problem, especially in low- and middle-income countries, including Nigeria, where the prevalence of diabetes has been estimated to be 5.5% (International Diabetes Federation, 2019). Medicinal plants are rich sources of biologically active compounds, including alkaloids, flavonoids, tannins, and terpenoids, which have been shown to have antidiabetic properties (Oboh, 2015).

Balanites aegyptiaca commonly known as Desert date, called 'Aduwa' in Hausa language, is a drought tolerant thorny shrub, with ethno-pharmacological relevance for the treatment of various ailments, including diabetes (Kamel, 1991). *B. aegyptiaca* tree produces a pulpy, oval-shaped, yellow edible fruit; contained in the fruits are succulent layers enclosing an inedible hard nut seed (Sule, 2022). Nigeria, particularly Northern Nigerian has a wild distribution of *B. aegyptiaca* in North-East states such as Borno, Yobe states and North-West states such as Kaduna, Katsina and Kano states (Mohammed *et al*., 2013).

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Figure 1: *Balanites aegpytica* fruits

Terminalia catappa, commonly known as Tropical Almond, is a tropical fruit tree native to the tropics of Africa, Asia, Australia, South America, which has been reported to have therapeutics activities including antidiabetic properties (Nonso *et al*., 2020). *Terminalia catappa* tree produces edible fruit with fibrous layers of characteristics bright colour enclosing a hard nut which is mostly discarded after consuming the fleshy layers (Missouri Botanical Garden, 2022). The fruits and nuts of the terminalia species are well recognized for possessing medicinal properties (Nonso *et al*., 2020).

Figure 2: *Terminalia catarppa* fruits

The *Balanites aegyptiaca and Terminalia catappa* plants have shown promising results in the management of diabetes using its plants parts, and further research is needed to explore the active phytocompounds with the antidiabetic potential contained in the plants. It is in light of this that the study is embarked to

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extract saponins from the hard nuts of *Balanites aegyptiaca and Terminalia catappa* fruits for its antidiabetic activities on albino Wistar rats.

This study is aimed at assessing the anti-diabetic properties of saponins constituents in *Balanites aegyptiaca* and *Terminalia catappa* nuts for medicinal drug use.

EXPERIMENTAL

Balanites aegyptiaca and *Terminalia catappa* fruits was collected from the Botanical Garden of Ahmadu Bello University, Zaria, Kaduna. The fruit samples were identified and given the voucher number at the Herbarium of Biology Department Nigerian Defence Academy. The voucher number is NDA/BIOH/2024/29 and NDA/BIOH/2024/30 for *Balanites aegpytica* and *Terminalia catappa* respectively. The fruits samples were selected and washed thoroughly with tap water followed by distilled water to remove dirts. The flesh of the fruits was peeled off using a microtome knife, till the hard nuts are reached. The hard nuts were then air-dried away from sunlight for two weeks, pulverized using a pulverizer obtainable at National Steel and Raw Materials Agency, Malili, Kaduna to fine powder and stored in an air-tight container for further use (Bindu, 2019).

Extraction crude extracts

The saponins were extracted using the procedure reported by (2009), a portion (100 g) of the pulverized nuts sample was separately percolated in 300 cm^3 of methanol, ethyl acetate, chloroform, and petroleum ether for two weeks. The extracts will be separately filtered and was concentrated using a SCI100 rotary evaporator at 40 ^{0}C. The marc was then re-percolated with recovered solvents for one week and concentrated using a rotary evaporator. The extract was then lyophilized using a freeze dryer (Cole Parmer 79203-00, USA), weighed and stored in the refrigerator for further analysis.

Test for phytochemicals

Phytochemical compounds such as alkaloids, saponins, tannins, glycosides, from the two plants extract were determined using standard methods. (Banu and Catherine, 2015; Sofowora, 1993; Trease and Evans, 1989; Harborne, 1984).

Determination of Anti-diabetic Activity

Albino (Wister Strain) rats weighing between 150 – 180 g (7-10 weeks old) were used for the present study. All the animals were housed and maintained under controlled environment comprising standard conditions of temperature, humidity and a 12 hrs light and 12-hour dark cycle. Animals were allowed free

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access to food and water *ad libitum* (Garba and Salihu, 2008)

Induction of type II diabetes

Rats selected for the study were weighed and marked for the purpose of individual identification. All the animals were allowed to acclimatize and were fed with standard pellet diet (SPD) and water *ad libitum* and maintained on a 12 hrs light and 12-hour dark cycle prior to dietary manipulation for one week. Subsequently, after the acclimatization, the animals were divided into three groups (A, B and C). Group A were fed with standard pellet diet and group B were fed with laboratory prepared high-fat diet, HFD (60.3% fat, 18.4% protein, and 21.3% carbohydrate as percentage total kcal) *ad libitum* for initial 8 weeks. The high-fat diet was replaced with normal standard pellet diet and the animals were given a single intraperitoneal injection of a low dose of Metformin (35 mg/kg body weight; dissolved in 0.1m citrate buffer pH 4.5) while the group A (control) animals were injected with equal volume of citrate buffer. The fasting blood glucose levels were analysed after seven (7) days of the and day 14 metformin administration. Rats showing fasting glucose levels ≥ 199.8 mg/dl (11.1 mmol/lit) according to modified (Zhang *et al*., 2015) method and a significant level of total cholesterol, triglycerides as compared to control were considered as type 2 diabetic and used in the study. The groups were treated as outline below:

Group A: Normal control (these animals will be given distilled water).

Group B: Diabetic control (high fat diet, Metformin 35 mg/kg body weight)

Group C: High fat diet, Metformin 35 mg/kg body weight and extract of selected medicinal plant (dose to be determined after LD_{50}).

Determination of minimum dose of the saponins extract.

Drug dose formulation was determined after testing the toxicity of isolate using Organization for Economic Cooperation and Development (OECD) guidelines for testing chemicals test number 423.

Acute oral toxicity of the saponins isolates was performed on Wister rats, according to OECD (organization of economic cooperation and development) guideline 423. Two groups of three rats each were used for the study. Group I served as control and was treated with only distilled water. Group II were given single oral dose of extract (200 mg/kg). The animals were observed for gross behavioural, neurological, autonomic and toxic effects at short intervals of time for 24h and then daily for 14th days. During the experimental period food consumption were monitored

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daily and body weights were recorded weekly (Sule, 2022).

Result and Discussion

The results of phytochemical screening of the crude extract is presented in Table 1, the results indicated the presence of all the tested phytocompounds except Anthraquinone revealing the great potentials inherent in the targeted plants.

Table 1: Results of Phytochemical Screening

	Balanite Aegytiaca	*Terminalia Catappa*
Alkaloid	+	+
Flavonoid	+	+
Phenols	+	+
Saponins	+	+
Steroids	+	+
Terpenoids	+	+
Glycosides	+	+
Tannins	+	+
Anthraquinone	-	-

The presence of alkaloids and saponins in the crude extracts of the targeted fruits shows that the two fruits possess the potentials of being used for the treatment of diabetic Meletus. Similar results were reported by Mujeeb, 2014; Wang et al., 2015; Shi, et al., 2013 and Chen, et al., 2010 from the crude extracts of *Momordica charantia*, *Trigonella foenum*, *Astragalus membranaceus* and *Panax notoginseng* respectively.

The result of activities of *Balanite aegyptiaca* crude extracts on the induced albino rats from day zero through day 7 to day 14 and the statistical presentation of the data is presented on Table 2. There is significant decrease in the level of sugar at Day 7 when the induced rats were treated with 50 mg solution of the crude extracts of *Balanite aegyptiaca*, the decrease increases sightly when the concentration of the extracts were increased. The results reveals that the crude extract of the plants at all the administered concentration were found to be active compared the standard drugs at similar concentrations (see Table 2). This activity could be attributed the presence of various phytoconstituents such as flavonoids, terpenoids, saponins, alkaloids, and glycosides, which were reported by many researchers to possess antidiabetic activities [Afrisham, et al., 2015; Durazzo, et al., 2017 and 2018]. Also, the combined action of biologically active compounds could be responsible for the potential beneficial properties of this plant matrix, and this can represent the first step for understanding their biological actions and beneficial activities.

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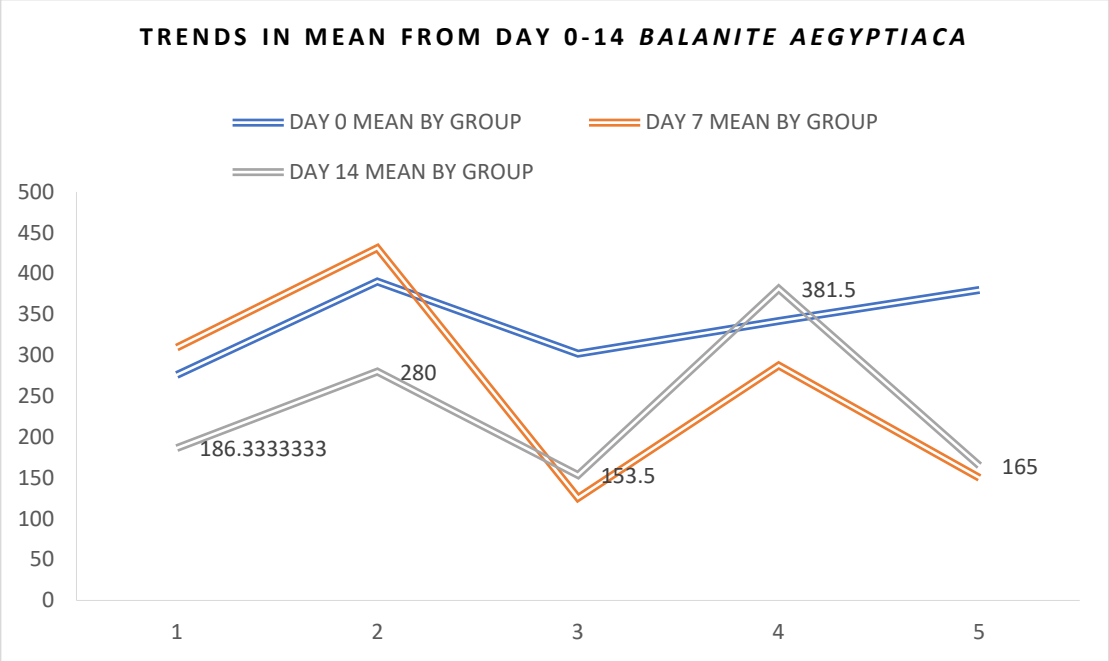

Figure 1: **Trend in the mean from Day 0 -14 *Balanite aegyptiaca***

Table 2: Result of Activities of *Balanites aegyptiaca* on diabetic induced albino rats

Group	FBS Day 0	FBS Day 7	%CHANGE IN FBS 0-7	FBS Day 14	%CHANGE IN FBS 0-14
Normal control	423	420	-0.709219858	129	-69.5035461
Normal control	248	328	32.25806452	256	3.225806452
Normal control	157	180	14.64968153	174	10.82802548
Metformin	432	448	3.703703704	168	-61.11111111
Metformin	319	371	16.30094044	472	47.96238245
Metformin	420	475	13.0952381	200	-52.38095238
50mg	332	145	-56.3253012	150	-54.81927711
50mg	272	106	-61.02941176	157	-42.27941176
100mg	432	450	4.166666667	527	21.99074074
100mg	252	125	-50.3968254	236	-6.349206349
150mg	433	132	-69.51501155	169	-60.96997691
150mg	327	168	-48.62385321	161	-50.76452599

Figure 1 below shows the trend in the mean value of the activity of *Balanite aegyptiaca* from Day 0 -14, this trend clearly indicates that the crude extracts from the plant is highly active at Day 7 and the activity slightly increases at Day 14 (see Figure 1).

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The result of antidiabetic activity of the crude extract of *Terminalia catappa* also show similar trends although the activity of the crude is less than that of *Balanite aegpytiaca* and that of the control drugs metformin. Despite the low reduction in the blood sugar, the plant also shows a promising the results (See Table 2).

Table 2: Result of Activities of *Terminalia catappa* on diabetic induced albino rats

Group	FBS Day 0	FBS Day 7	% CHANGE IN FBS 0-7	FBS Day 14	% CHANGE IN FBS 0-14
Normal control	423	420	-0.709219858	129	-69.5035461
Normal control	248	328	32.25806452	256	3.225806452
Normal control	157	180	14.64968153	174	10.82802548
Metformin	432	448	3.703703704	168	-61.11111111
Metformin	319	371	16.30094044	472	47.96238245
Metformin	420	475	13.0952381	200	-52.38095238
50mg	416	427	2.644230769	311	-25.24038462
50mg	268	148	-44.7761194	147	-45.14925373
100mg	210	169	-19.52380952	185	-11.9047619
100mg	208	130	-37.5	172	-17.30769231
150mg	452	488	7.96460177	555	22.78761062
150mg	211	177	-16.11374408	215	1.895734597

Trends in the activity of crude extract of *Terminalia catappa* is represented in form Bar Chart in Figure 2, the results show clearly how the extracts reduce the fasting blood sugar in the body of Wister rats. The extract shows significant reduction the blood sugar at 100 mg suddenly the activity reduces at 150 mg.The data presented in Tables 1 and 2 above shows a percentage change in FBS at all points for both plants and standard drug. The mean % change for each group shows how the groups changed over the 14 days period. For *Balanite aegyptiaca* there is a statistically significant change of 59 % reduction at 50 mg on day 7 and 49 % change at day 14 for the FBS. As against the standard drug metformin which increases by 11 % at day 7 and reduces by 21 % on day 14. The plant extract for *Balanite aegyptiaca* is significantly more effective than the standard drug.

Terminalia catappa shows statistically significant change of 29% on day 7 and 15% on day 14 for 100mg concentration, as against the standard drug metformin which increases by 11 % at day 7 and reduces by 21 % on day 14. For all other concentrations and days there is no statistically significant difference in the activities of the *Balanite aegyptiaca* plant extract in comparison to the standard drug.

The t-Test result for *Balanite aegyptiaca* against metformin at 50mg for 7 days has a P-value of 0.00028 (one tail) and 0.00057(two tail) which is lower than α of 0.05. Also, the t-Test for *Terminalia catappa* against metformin at 100 mg for 7 days has a P-value of 0.00875 (one tail) and

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0.01749 (two tailed) which is lower than α of 0.05.

The results of the toxicity test conducted for both the extracts and the standard drugs is presented in Figure 3:

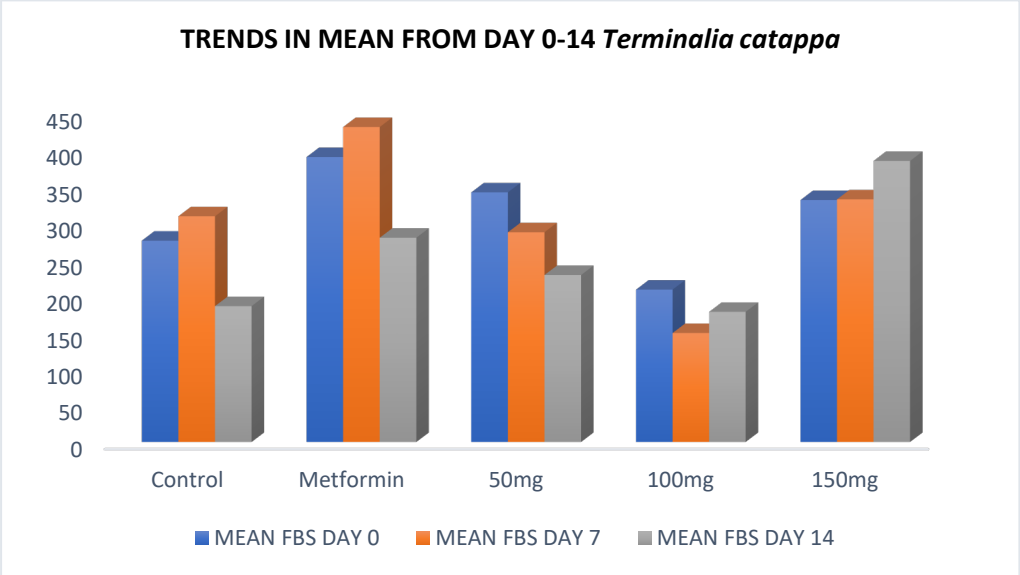

Figure 2: Trends in the activity of crude extract of *Terminalia catappa*

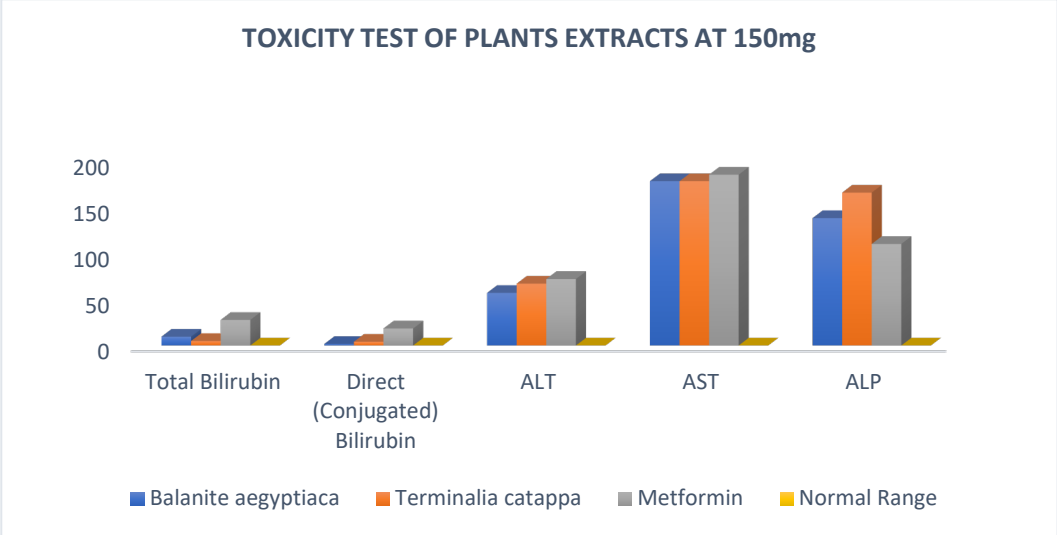

Figure 3: Results of Toxicity Test of Plant extract at 150 mg

The above results shows that toxicity of the plants and standard drug reveals that at 150 mg metformin has higher levels of total Bilirubin, Direct (Conjugated) Bilirubin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) than both plants. Presence of higMMher levels of those substance could indicate potential risk of liver damage (toxicity). All the tested extracts and the standard drug are

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relatively safe at lower concentration but both can be toxic at higher concentration with Metformin being potentially more toxic than the two plant extracts.

Conclusion

The results of this research, therefore shows that *Balanite eagyptiaca* crude extracts possess a great potential as antidiabetic agents, its activity is higher than the standard drug and therefore could be a promising plant for the treatment of diabetic mellitus type II.

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