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PHYTOCHEMICAL SCREENING AND EFFICACY OF ETHANOLIC FRUIT AND SEED EXTRACT OF *Gmelina arborea* IN THE TREATMENT OF DIABETES IN ALBINO RAT (*Ratus norvegicus*)

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ABSTRACT

The present study investigated the potential antidiabetic effects of *Gmelina arborea* fruit, a traditional medicinal remedy utilized for managing diabetes mellitus. Phytochemical screening and assessment of antidiabetic properties were conducted between October 2020 and May 2021. Fruit extraction employed 50% C_2H_5OH, with subsequent vacuum concentration at 40°C. Diabetes induction in overnight-fasted albino rats utilized alloxan (60 mg/kg I.P.). Dose-dependent investigations (100, 200, 400, 600 mg/kg) were performed using fruit and seed extracts to evaluate their impact on alloxan-induced diabetic rats. Blood glucose levels were monitored throughout the study. Phytochemical screening revealed the presence of saponins, terpenes, flavonoids, Salkwosku, Keller-Killian, Cuberman, and glycosides. Conversely, alkaloids, tannins, combined anthraquinone, cyanogenetic glycosides, and free anthraquinone were absent. No significant disparities in phytochemical profiles were observed between *Gmelina arborea* fruit and seed extracts. Oral administration of the extract led to a notable reduction in blood glucose levels at doses of 200, 400, and 600 mg/kg within 24 hours. Notably, treatment with 100 mg/kg of both fruit and seed extracts exhibited a significant difference compared to untreated diabetic rats. Furthermore, doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg of both extracts normalized elevated glucose levels in induced diabetic rats within 14 days. These findings suggest the potential utility of *Gmelina arborea* fruit and seed extracts in the management of heart and diabetes-related ailments

KEYWORDS: *Gmelina arborea* fruit, Phytochemistry, alloxan, diabetes, albino rat

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INTRODUCTION

Herbal medicine, defined as the use of various plant parts for medicinal purposes, has garnered attention for its potential therapeutic effects, including the treatment of diabetes mellitus (Chothani and Patel, 2018). The World Health Organization (WHO) characterizes herbal medicine as preparations derived from one or more plants, possessing therapeutic or health benefits (Soforowa, 1993; WHO, 2005). *Gmelina arborea*, commonly known as the English Candahar tree or white teak, is a rapidly growing deciduous tree found in various regions worldwide, including Nigeria. It is cultivated in gardens and avenues (Lauridesan and Kjaer, 2013; Duke, 1983). The seeds, reddish in color and lenticular in shape, are utilized traditionally for various medicinal purposes. Ripe fruits, after drying, are cooked with cow's milk for urticaria (Khare, 2004) and are employed for alleviating shortness of breath (Heyn, 1990), acting as a cooling agent and exhibiting diuretic and nutritive properties for tuberculosis, promoting hair growth, managing menorrhagia, and addressing burning sensations (Nayak *et al*., 2013; Lauredefin and Kjarr, 2013).

Notably, *Gmelina arborea* fruits possess edible properties (Poorter *et al*., 2004) and are utilized in decoctions to treat body swelling, fever, and bilious disorders. Furthermore, fruit powder, when combined with milk or ghee, is used during pregnancy to aid in the settlement of the fetus. Recent studies have highlighted additional pharmacological properties of *Gmelina arborea* fruits, including antiepileptic, analgesic, antipyretic, antibacterial, antioxidant, antidiabetic, and hepatoprotective activities (Nayak *et al*., 2012, 2015a, 2015b).

While the root and bark of *Gmelina arborea* are employed as stomachics, laxatives, anthelmintics, and appetite stimulants, and are believed to be beneficial for hallucinations, folk medicine recommends their use in combination with other drugs for snakebite treatment. A decoction of the root and bark is administered internally for this purpose. Additionally, the roots and bark are reputed for their efficacy against diabetes (WHO, 2018). Despite the extensive traditional use of *Gmelina arborea*, there is a scarcity of reports on its antidiabetic effects, particularly concerning trials using alloxan-induced diabetic rats in Nigeria and globally. However, some research has focused on the phytochemical screening and antibacterial activities of *Gmelina arborea* extracts (Akyala *et al*., 2018; Chothani and Patel, 2018; Nayak *et al*., 2012, 2013, 2015a, 2015b; Offor, 2014). Therefore, this study aimed to investigate the phytochemical properties and antidiabetic activities of *Gmelina arborea* fruit extracts. The insights gained from this investigation may contribute to the development of drugs or remedies for diabetes treatment.

MATERIALS AND METHODS

Fresh fruits with seeds of *Gmelina arborea* were collected from the Presidential Villa, Abuja, and were identified by Prof. M. E. Bassey of the Department of Botany and Ecological Studies, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The specimens were deposited in the

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herbarium of the Pharmacognosy Laboratory of the Faculty of Pharmacy, University of Uyo, Nigeria.

Extraction
Extraction of volatile oil from *Gmelina arborea* fruits and seeds was conducted at the Pharmacognosy Laboratory, Faculty of Pharmacy, University of Uyo, Uyo. The method employed was based on Horbone (1976) and Trease and Evans (1985). The fruit (1 kg) was separated from the seeds, dried, powdered, and extracted using n-hexane in a Soxhlet extractor. The seeds were similarly powdered and extracted using methanol for 72 hours. The extracts were filtered, concentrated using a rotary evaporator, and stored in a refrigerator until required.

Phytochemical Screening
Phytochemical screening was performed following standard methods outlined by Trease and Evans (1989), Soforowa (1993), and Harbone (1979). Tests for alkaloids, saponins, flavonoids, tannins, anthraquinones, cardiac glycosides, and terpenes were conducted to determine the presence of these compounds in the extracts.

Animals
Adult albino rats (*Rattus norvegicus*) weighing between 150-225g were procured from the Animal Breeding House of the Department of Animal and Environmental Biology, University of Uyo. The rats were housed in standard polypropylene cages under controlled environmental conditions.

Induction of Experimental Diabetes Mellitus
Diabetes was induced in the rats using alloxan prepared in citrate buffer solution. A dose of 60 mg/kg of alloxan was administered intraperitoneally to overnight-fasted rats. Diabetes was confirmed by blood glucose levels $\geq$ 400 mg/dl measured using a glucometer.

Experimental Design
Diabetic rats were orally administered with fruit and seed extracts of *Gmelina arborea* once daily for 14 days. The rats were divided into six groups and treated accordingly as follows:

Group 1:Normal rats which received normal saline
Group 2:Untreated Diabetic rats
Group 3:Diabetic rats treated with 100mg/kg
Group 4:Diabetic rats treated with 200mg/kg of the extract.
Group 4:Diabetic rats treated with 400mg/kg of the extract
Group 5:Diabetic rats treated with 600mg/kg of the extract
Group 6:Diabetic rats treated with 600mg/kg of the extract

Determination of Blood Glucose Level
Blood samples were collected from the tip of the rat tails at specific intervals, and blood glucose levels were measured using a glucometer. Glucose profiles were plotted for each group.

Statistical Analysis
Data were analyzed using Fisher's Least Significant Difference (FLSD) test, with a significance level set at P < 0.05. One-way analysis of variance (ANOVA) and multiple comparisons were performed using SPSS Version 20 statistical package.

RESULTS

Phytochemical analysis of the fruit and seed of the *Gmelina arborea* (Table 1) revealed the presence of saponins, flavonoids, terpenes, Salkowski, Keller Killian, Liberman and phylobabatamin while cyanogenitic glycosides,alkanoid, tannins, combines anthroquinone and freeanthroguinone were absent (Table 1). There was no significant difference between the phytochemical properties of the seed and fruit of *Gmelina arborea.*

Antidiabetic Effect of the Fruits Extract of *Gmelina arboreal* Fruits
The result on the anti-diabetic effect of the methanol extract of *Gmelina arborea* fruits is shown in Table 2. There was significant increase in the blood glucose levels of the untreated diabetic rats (Group2) in comparison with the normal rats that received normal saline (Group 1). The administration of the different doses of the extract on diabetic rats indicates, a dose dependent effect was observed. With the100 mg/kg dose (Group 3), significant reduction was observed compared with the untreated diabetic rats. The 200 mg/kg dose significantly reduced the blood glucose level from 385 mg/dl to 91 mg/dl at the end of 14 days. There was a sharp gradual reduction from 1st day to 14th day of treatment to 91 mg/dl in comparison with the untreated rats that had a mean glucose level of 411 mg/dl at the end of 14 days. Similar trends were observed with 400 mg/kg and 600mg/kg doses in which the glucose blood level reduces from 392 mg/dl at o hr to 96.3mg/dl at the end of 14 days, and from 351.0 mg/dl at o hr to 100.0 mg/dl at the end of 14 days respectively. There were gradual reductions in blood glucose level of the diabetic rat from day 1 to day 14 after the administration of the fruit extract (Figure 1).

The result of the seed extract (Table 3, Figure 2) also indicated that there was a significant difference between none treated diabetes rat and those administered with normal saline. The result of the doses of 100 mg/kg, 200 mg/kg, 400 mg/kg and 600 mg/kg of the seed extract shows remarkable reduction of the glucose level at the end of 14 days of treatment, for instance, the result of 200 mg/kg shows that at the 24 hours, the mean of 388.7 mg/dl was observed with gradual daily reductions of glucose level up to 121 mg/dl on 9th day. But at the end of 14 days, the glucose level shows a remarkable reduction to 91.0 mg/dl. The result of 400 mg/kg indicates a mean of 102 mg/dl at the end of 14 days with sharp reduction from 1st day 390.3 mg/dl to 102 mg/dl on 14 days.

The result of 600 mg/kg dose had average of 361mg/dl glucose level in the blood at 24 hours with a remarkable reduction to 108.7mg/dl at the end of 14 days. Thus, the study of fruits and seed extracts of *Gmelina arborea* were dose dependent. There was no significant difference between the efficacy of the fruit and seed extract. Both extracts were

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very effective in the treatment of diabetes rats. There were gradual reductions in the blood glucose level of the diabetic rat from 1st day to 14th day after the oral administration of the seed extract (Figure 2).

Table 1: Result of the Phytochemical Properties of Fruit and Seed of *Gmelina arborea*

Plant Name	Alkaloids	Tannins	Saponins	Terpenes	Flavonoids	Salkwosku	Keller-killian	Lieberman	Combined Anthroquinone	Cyanogenetic glycosides	Free Anthroquinon	Phylobabatam in
Gmelina arborea (Gmelina fruits)	-	-	++	+	+++	++	++	++	-	-	-	+
Gmelina arborea (Gmelina seeds)	-	-	+	+	++	++	++	++	-	-	-	+

Table 2: The Antidiabetic Effect of the Method Extract of *Gmelina arborea* Fruit

	1st Day	3rd Day	5th Day	7th Day	9th Day	14th Day
Normal Rat	85	84	82	83	84	86
NIS (Group 1)	90	90	89	90	91	88
Mean	90	91	90	84	90	89
	88.3	88.3	87.7	87.3	88.3	87.7
Untreated Diabetic Rat	310	322	340	344	370	410
UD (Group 2)	311	323	341	343	372	411
Mean	312	324	342	343	374	412
	311	323	341	343.3	372	411
D (Group 3) 100mg/Kg	360	318	287	188	133	101
Mean	361	319	286	189	136	108
	362	320	288	189	133	108
	363	319	287	188.6	134	105.7
D (Group 4) 200mg/Kg	385	217	181	156	124	92
Mean	386	218	180	157	126	90
	384	219	181	158	125	91
	385	218	180.7	151	125	91
D (Group 5) 400mg/Kg	392	250	192	149	131	96
Mean	391	258	194	151	129	98
	390	254	193	152	126	95
	391	254	193	156	128.7	96.3
D (Group 6) 600mg/Kg	350	298	199	141	136	100
Mean	352	297	200	143	131	101
	351	299	194	145	132	99
	351	298	197.7	143	133	100

Table 3: The Antidiabetic Effect of the Method Extract of *Gmelina arborea* Fruit

	1st Day	3rd Day	5th Day	7th Day	9th Day	14th Day
Normal Rat	100	99	98	97	96	95
NIS (Group 1)	101	98	98	99	97	94
Mean	100	98	98	98	96	96
	100.3	98.3	98.3	98	96.3	95.0
Untreated Diabetic Rat	325	336	354	374	385	391
UD (Group 2)	320	329	352	378	387	392
Mean	321	336	356	376	389	395
	323	333.6	354	376	387	392.7
D (Group 3) 100mg/Kg	351	300	268	211	144	119
Mean	353	301	267	210	149	108
	356	302	266	212	147	112
	353.3	301	267	211	146.7	113
D (Group 4) 200mg/Kg	386	230	186	153	121	90
Mean	389	231	189	158	122	91
	391	232	190	160	120	92
	388.7	231	188.3	157	121	91
D (Group 5) 400mg/Kg	391	324	220	163	120	99
Mean	389	323	221	165	129	101
	390	320	222	167	121	106
	390.3	322.3	221	166	123.3	102
D (Group 6) 600mg/Kg	359	286	201	153	137	108
Mean	360	283	206	158	139	109
	365	284	212	150	129	109

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	361.3	284.3	206.3	153.7	135	108.7

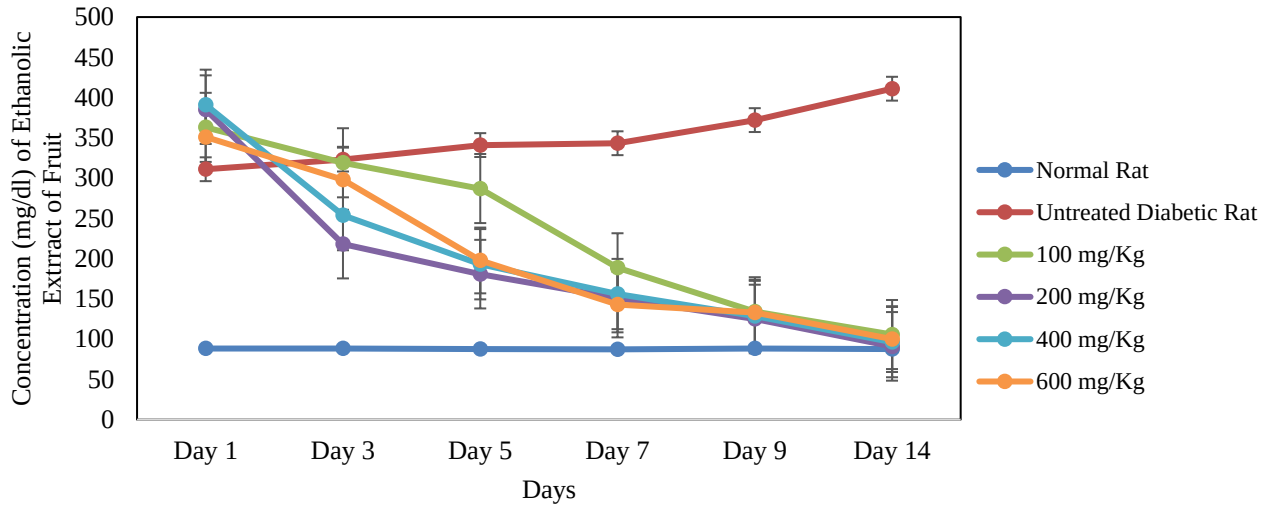

Figure 5: Changes in mg/dl of glucose following repeated injection of different doses of ethanolic extract of *Gmelina aborea* Fruit

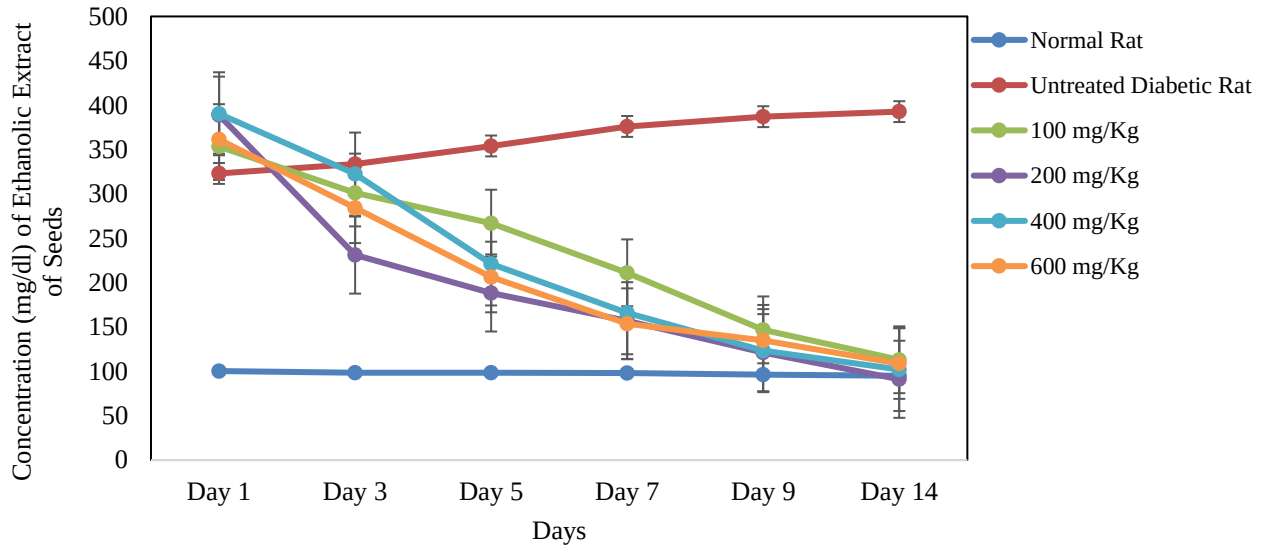

Figure 6: Changes in mg/dl of glucose following repeated injection of different doses of ethanolic extract of *Gmelina aborea*seed

DISCUSSION

This research demonstrates that the ethanolic extracts derived from the fruits and seeds of *Gmelina arborea* exhibit dose-dependent anti-diabetic properties. The presence of flavonoids and saponins, as reported previously by Akyala, David, and Simon (2013), was confirmed through phytochemical analysis. Additionally, the identification of bioactive compounds aligns with previous findings by Chothani and Patel (2018), Nayak *et al*. (2015a, 2015b, 2013b). Cardiac glycosides, known for their therapeutic potential in heart-related diseases (Trease and Evans, 1989), were also detected in the extract. Flavonoids, known for their diverse therapeutic activities such as antihypertensive, antimicrobial, antioxidant, and anti-inflammatory properties (Kako*et al*., 1997; Nayak *et al*., 2012; Pietta, 2002; Soforowa, 1993), are believed to contribute to the observed anti-diabetic effects. Secondary metabolism, as indicated by Harbone (1979), plays a role in the anti-diabetic activity of plants.

Untreated diabetic rats exhibited significantly higher blood glucose levels compared to non-diabetic rats. The dose-dependent effect observed in the extract suggests a hypoglycemic effect, comparable to glibenclamide, the reference drug (Nayak *et al*., 2015a, 2015b). The reduction in glucose levels in alloxan-induced diabetic rats over 14 days by *Gmelina arborea* fruit and seed extracts aligns with the findings of Qinna and Badwan (2015) regarding the impact of insulin in reducing high glucose levels in streptozotocin-induced diabetic rats. Interestingly, increasing the concentration of *Gmelina arborea* fruit and seed extracts from 200 mg/kg to 400 mg/kg and 600 mg/kg did not significantly influence the reduction in blood glucose

levels. This suggests a potential saturation point or maximal efficacy of the extract at higher doses, warranting further investigation into its pharmacokinetics and mechanism of action, as indicated by Lenson (2008).

CONCLUSION

This study underscores the dose-dependent anti-diabetic activity of *Gmelina arborea* fruits and highlights the presence of various phytochemicals. Medicinal plants offer affordable and potentially safer alternatives to synthetic drugs for diabetes treatment. However, further research is recommended to isolate and identify the specific constituents responsible for the observed activities, particularly in relation to antihelminthic effects.

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