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Evaluation of the anti-diabetic activities of crude extracts of *Annona muricata* and *Rutidea parviflora* in alloxan-diabetic rats

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Abstract

Background: Diabetes Mellitus (DM), is a malignant disease that has impacted the globe in astronomical proportions. Chronic hyperglycemia is a major condition suffered by patients with DM. Currently, the cost of managing DM is highly exorbitant and poses a significant obstacle to many living with the disease in developing countries. Thus, the need for affordable alternatives with optimum potency and minimal side effects is justified. Historically, *Annona muricata* is one plant that is used in the treatment of DM and a host of other ailments. *Rutidea parviflora* has been used in combination with other plants in the treatment of DM by some ethnic groups. The aim of this study is to investigate the anti-diabetic activities of these plants.

Method: The anti-diabetic activities of aqueous and organic extracts of *Annona muricata* (leaves), and *Rutidea parviflora* (root bark) at 200 mg/kg and 400 mg/kg, were screened for their anti-diabetic activities in normoglycemic and alloxan-induced diabetic rats (150-200 g) at 1h, 2h, 4h, 6h, 8h, and the 12th hour. DMSO (0.3 ml of 33.3% v/v stock) and Glibenclamide 10 mg/kg were administered to the control and reference groups respectively.

Result: Organic extracts of *A. muricata* at 400 mg/kg significantly decreased FBGL in both normoglycemic and hyperglycemic groups by 65.9% and 62.1% respectively *(P<0.05). R. parviflora* leaves extracts showed less activity. However, it had relatively short acting activity (maximum activity at the 4th hour). In each case, the organic extracts performed better than the aqueous extracts.

Conclusion: *A. muricata* has potentials for the treatment of DM, and merits further research to support the plant's therapeutic application. *R. parviflora* may offer some beneficial effect, and possibly boost the potency of *A. muricata* by synergistic activity when co-administered.

Keywords: *Rutidea parviflora*; *Annona muricata*; hyperglycemia.

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Introduction

Diabetes Mellitus (DM) is a debilitating disorder characterized by severe hyperglycemia owing to insufficient insulin production. The disease is a major contributor to other devastating health conditions which include; kidney failure, stroke, cardiac arrest, blindness and could lead to amputation of lower limbs in complicated cases. In three and a half decades, the number of people living with DM rose from 108 million to 422 million, [1] with a further projection that by 2045, there would be 629 million adults living with DM [2]. As at 2012, the death toll from DM was 1.6 million, which paints a gloomy picture of an enhancing disease with lethal consequences. The economic burden imposed by the disease was 1.31 trillion US dollars in 2015 [3]. The challenge of managing DM is heaviest on developing countries, where affordable healthcare is beyond the reach of many. DM is ranked the seventh killer disease globally and an expensive disease to manage. Thus, the drive for cheaper and more potent therapies; mainly herbal remedies cannot be overemphasized.

A. *muricata* is a tropical shrub, commonly known as soursop or graviola. The fruits of *A. muricata* are highly coveted for their unique flavor and nutritional value. A wide array of ethnomedicinal activities has been attributed to various parts of *A. muricata*. Ethnopharmacological uses of *A. muricata* abound in African and South American communities. Many studies has been carried out on a number of biological activities such as anti-diabetic, anti-cancer, anti-convulsant, anti-parasitic and anti-arthritic, as seen from the review of this plant [4]. Annonaceous acetogenins were found to be the most abundant constituents isolated from the different parts of the plant [4].

There have been documented cytotoxic activities of *A. muricata* against several human cell lines and disease agents in *in vitro* culture and in vivo models designed to specifically target the disease, while exerting little or no effect on normal cell viability [5]. It has been reported also that annonaceous acetogenins found only in the Annonaceae family kill malignant cells of 12 different types of cancer including Breast, Ovarian, Colon, Prostate, Liver, Lung, Pancreatic and Lymphoma [6].

Rutidea parviflora D.C belongs to the genus *Rutidea* in the *Rubiaceae* family. *Rubiaceae* referred to as the coffee family or bedstraw family is the fourth largest family of the angiosperms. *Rubiaceae* has 611 genera and approximately 13500 species distributed in different regions of the world, but with greater diversing in the sub-tropical areas. Two important genera of this family are *Cinchona L.*, from which the drug quinine used in the treatment of malaria was derived and *Coffea L.*, which have provided the world with the most consumed beverage-coffee. Other genera are *Rubia*, a collection of dye plants and used for decorative purposes, *Pavetta L.*, *Uncaria schreb*, *Tarenna Gaertn*, *Oldenlandia L.*, and *Galium L* [7]. This plant is used by the indigenous people of Ethiope East and West Local Government in Delta State, Nigeria for inflammation, infections and a host of other disease conditions, which has necessitated this research to investigate the ethno pharmacological use of this plant for anti-diabetic activities. This plant has scanty reports in literature and has also shown some anti-cancer activity *in vitro*.

Materials and methods

I. Plant materials and reagents

The leaves of *A. muricata* were sourced from household gardens around the University community, while the root bark of *R. parviflora* were obtained from a bio reserve. The plant materials were air-dried under shade before being milled into fine particles. The following reagents were utilized in the extraction and phytochemical work: Methanol, Dichloromethane, Chloroform, Diethyl Ether, Acetic Anhydride, Glacial acetic acid, Sodium picrate, 2% 3,5-Dinitrobenzoic acid, Picric acid, Iodine solution, Dimethylsulfoxide, (JHD company, Guangdong. Guanghua Sci-Tech. Co. Ltd. China), Hydrochloric acid, Million's reagent, Benedict's solution, Wagner's reagent, Sodium Hydroxide, Ferric chloride solution, Saturated lead acetate solution, Dragendorff's reagent, Kedde reagent, Ammonia solution, 7.5% Potassium Hydroxide, Fehling's solution A and B (Sigma Aldrich Chemicals, St Louis, USA), Distilled water, Deionized water (Pharmaceutical Chemistry Lab, University of Port Harcourt).

II. Assay materials

Alloxan monohydrate (Sigma-Aldrich Merck Germany), Glibenclamide (Actiza Pharmaceutical Private Limited, Surat), ACCU-CHECK glucometer.

III. Extraction of plant materials

The 2 Kg of the pulverized plant materials were extracted according to the American National Cancer Institute (NCI) method of extraction [8]. The pulverized plant material was macerated in a 1:1 mixture of dichloromethane and methanol for 24 h to obtain the extracts. The procedure was repeated thrice. The ratio of pulverized plant material to extraction solvent was 1:5. The residue was further macerated in methanol for another 24 h to yield the methanol extract, which was combined with the dichloromethane/methanol extract to yield the total organic extract. The obtained dry extracts were further dried in a desiccator to remove any trace of solvent. The residue was later macerated in deionized water for 24 hour to yield the aqueous extract, which was dried on a lypholizer. Both organic and aqueous extracts were obtained for both plants.

IV. Phytochemical screening

Phytochemical tests were carried out on the plants' extract by the method described by Trease and Evans, [9] highlighted below.

i. Test for flavonoids

a. Shinoda reduction test

100 mg of the plant extract was dissolved in 5 ml of hydrochloric acid, a few pieces of magnesium metal was added to 5 ml of each plant extract solution. The presence of flavonoids is indicated by a reddish crimson or orange colour.

b. Sodium hydroxide test for flavonoids

A small amount of each of the portions was dissolved in water and filtered; to this, 2 ml of 10% aqueous sodium hydroxide was added to produce a yellow colouration. The addition of dilute hydrochloric acid, effected a colour change from yellow to colourless; indicative of the presence of flavonoids.

ii. Test for tannins

a. Ferric chloride test

100 mg of plant extract was boiled with 5 ml of distilled water for 5 minutes, cooled and filtered, few drops of 5% ferric chloride reagent was added. An observed green to blue black precipitate signified the presence of tannins.

iii. Test for alkaloids

5 ml of 2 M HCl was added to 0.1 g of plant extracts and heated on the steam bath for 2 minutes, the mixture was filtered. 1 ml of the filtered mixture was treated with 2 drops of the following reagents respectively: (i) Dragendorff's reagent; a reddish precipitate signified the presence of alkaloid. (ii) Wagner's reagent; yellow precipitate suggestive of the presence of alkaloid.

iv. Test for saponins

a. Frothing test

100 mg of the plant extract was mixed with 5 ml of water and shaken vigorously for persistent foam, then warmed and observed persistent foaming on warming indicated the presence of saponins.

b. Emulsion test

100 mg of plant extract dissolved in 10 ml of water was mixed with 3 drops of olive oil. The mixture was shaken vigorously. The formation of emulsion signifies a positive test for saponins.

v. Test for phlobatannins

Hydrochloride Acid Test 100 mg of plant extract was dissolved in water and filtered. The filtrate was boiled with 1% hydrochloric acid. Deposition of a red coloured precipitate indicates a positive test

vi. Test for sterols/triterpenoids

a. Liebermann-buchard test

100 mg of plant extract is dissolved in chloroform and acetic anhydride is added followed by sulphuric acid. Green colour indicates the presence of steroids and pink colour indicates terpenoids.

b. Salkwoski's test

100 mg of plant extract was dissolved in 2 ml chloroform, then conc. H_2SO_4 was carefully added to form a layer. An observed reddish brown colour at the interface strongly indicates a steroidal nucleus.

vii. Test for Cardiac glycosides

a. Keller killiani test

100 mg of plant extract was dissolved in about 5 ml of water, followed by the addition of glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown colour that appears at the junction of the two liquid layers and an upper layer that appears to be bluish-green confirms the presence of glycosides.

b. Kedde test

About 5 ml of dissolved extract was treated with a small amount of Kedde reagent (which was prepared by combining the same volumes of 2% solution of 3, 5 dinitrobenzoic acid in menthol to a 7.5% aqueous solution of KOH). The formation of a blue or violet colour that fades out in 1-2 hours indicates the presence of cardiac glycoside.

viii Test for carbohydrates

a. Fehling's test

A few drops of Fehling's solution A + B was added to 0.1 g of the plant extracts and boiled for a few minutes. The presence of deep blue to green; yellow to red colour change indicates a positive reduction test.

b. Molish's test

100 mg of plant extract was dispersed in 5 ml of distilled water and the resulting mixture allowed to boil for 15 minutes and filtered. Molish reagent (1 ml) was added to the filtrate (1 ml) and agitated, followed by addition of 1 ml of concentrated H_2SO_4. A reddish ring was observed indicative of carbohydrate.

ix. Test for proteins

About 100 mg of the sample was extracted with distilled water by boiling and subsequent filtration.

a. Million's test

To a little portion of the filtrate in a test tube, 2 drops of Million's reagent was added. Formation of white Precipitate which indicates the presence of protein was watched out for.

b. Picric acid test

To a little portion of the filtrate were added drops of Picric acid solution. A yellow Precipitate indicates the presence of protein.

x. Tests for fixed oils and fats

Oil Stain test: The extracts were placed between two filter papers and pressure exerted on the filter papers. The detection of oil stains on the papers indicated the presence of fixed oils.

V. Experimental design

This study was designed in line with the ethically approved experimental protocols adopted by the department of Experimental Pharmacology and Toxicology, of the Faculty of Pharmaceutical Sciences, University of Port Harcourt. Adult mixed-gender wistar rats weighing between 150-200 g, bred by the animal house unit of the department of Experimental Pharmacology and Toxicology were used for the study. The rats were housed in spacious cages, to allow for free movement at room temperature, sufficient humidity and 12 hourly cycles of light and darkness. The animals, had access to standard laboratory animal feed and water prior to the commencement of the experiment.

The rats were randomly divided into 14 groups of 6 rats each: 8 test groups and 6 control groups and were fasted overnight. The 14 groups were sub-divided into two categories, A and B. Category A consist of normoglycemic rats that received the extracts and the controls. While category B were alloxan-induced rats that were treated with the extracts and the controls respectively.

Freshly prepared alloxan solution (in 0.9% w/v normal saline) was administered at a dose of 150 mg/kg Intraperitoneally to normoglycemic rats. Those with FBGL>200 mg/dL after 72hrs were considered to have developed experimental diabetes. The FBGL of the animals were determined to ensure that they were

not diabetic before carrying out the actual experiment. ACCU-CHECK glucometer was used in BGL determination. The blood analyzed was gotten by pricking the tail of the animals.

4 of the 14 test groups were treated with intraperitoneal injection of organic extract, one dose per group (200 mg/kg and 400 mg/kg) respectively. Another 4 group received intraperitoneal injection of aqueous extract, one dose per group (200 mg/kg and 400 mg/kg) respectively. The 6 control groups, were treated as follows: One was untreated, 0.3 ml of normal saline was administered to 1 group; two other groups were treated with I. P. injection of 0.3 ml of the DMSO stock while the last two group were treated with Glibenclamide 10 mg/kg (I.P.)

The Maximum Reduction was calculated using the formula:

(Initial reading after Induction) – (Lowest reading obtained after treatment) While the percentage Maximum Reduction was calculated using the formula:

$$\frac{Maximum\ Reduction}{Initial\ reading\ after\ Induction}\ x\ 100$$

VI. Statistical analysis

The analytical tool used was Graph pad Prism version 8 for calculation of the mean values ± Standard Error of Mean (SEM). The one-way ANOVA was used to determine statistical difference between means. A $p<0.05$ was considered statistically significant.

Results

Results from the phytochemical screening

Table 1 displays the result for the phytochemical screening of the aqueous and organic extracts of the plants. The results showed that the organic extracts contained most of the plants constituents than the aqueous extracts. Previous studies on *A. muricata* showed that methanol and water were the most used solvents of extraction, resulting in higher yield of phenolic compounds [10,11]. This may suggests the reason for the organic extracts giving more potent anti-diabetic activities than the aqueous extracts. Organic extracts of *A. muricata* also showed heavy Presence of lipids and moderate presence of carbohydrates, flavonoids, tannins alkaloids, phlobatannins and proteins while that of *R. parviflora* showed heavy Presence of alkaloids. In all the plant extracts having moderate to heavy Presence of tannins and flavonoids and alkaloids showed relatively high anti-diabetic activities. This is affirmed by several research works including those by Yogesha Mohan *et al*. and Kossouoh C, *et al* [12,1].

Table 1: The results of the qualitative phytochemical tests of extracts of the plant materials.

Test	A. muricata Extracts		R. parviflora Extracts	
	Aqueous	Organic	Aqueous	Organic
Flavonoids	++	+	+	++
Tannins	+	++	+	++
Alkaloids	+	+++	Trace	+++
Saponins	++	++	++	+
Phlobatannins	Trace	++	-	-
Sterols/Terpenoids	_	+	Trace	+
Cardiac Glycosides	+	++	+	++
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Fats and oils	Trace	++	Trace	++

KEY: -: Absent; +: Slightly present; ++: Moderately present; +++: Heavily present.

Effect of *Annona muricata* leaves aqueous extracts on blood glucose levels (mg/kg) in normoglycemic and alloxan-treated diabetic rats.

Table 2.1: Effect of *A. muricata* aqueous extract on blood glucose levels (mg/kg) in normoglycemic rats.

Treatment Groups	Before Treatment	After Treatment							Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h		
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3±5	91.6 ± 6	91.7±4	76.7 ± 7	0.7	0.9
A. muricata										
200 mg/kg	74.7 ± 2		140.7 ± 5	131.7 ± 2	108.0 ± 1	96.3 ± 4	77.3 ± 5	63.3 ± 3	11.3	15.2
400 mg/kg	77.7 ± 6		86.0 ± 4	96.0 ± 4	91.3 ± 4	97.7 ± 6	84.7 ± 8	57.3 ± 1	20.3	26.2
DMSO (0.3 ml	65.7 ± 1		70.0 ± 2	70.0 ± 1	66.7 ± 2	68.3 ± 2	69.0 ± 1	65.0 ± 2	0.7	1.2
Glibenclamide (10 mg/kg)	79.0 ± 4		73.0 ± 4	63.3 ± 1	43.3 ± 4	31.3 ± 3*	13.3 ± 1*	Lo	65.7	83.3

*: $p<0.05$ ≥ 200 mg/dL FBGL = Diabetic. Lo: Too low to be detected.

Table 2.2: Effect of *A. muricata* aqueous extract on blood glucose levels (mg/kg) in diabetic rats.

Treatment Groups	Before Treatment	After Treatment							Maximal Reduction	% Maximal Reduction
			1h	2h	4h	6h	8h	12h		
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
Alloxan (150 mg/kg)	69.0 ± 6	475.5 ± 2	47.3 ± 2	514.8 ± 1	509.3 ± 8	495.3 ± 4	477.0 ± 1	467.1 ± 8	8.4	1.8
A. muricata										
200 mg/kg	80.0 ± 3	506.7.0 ± 7	547.8 ± 3	546.0 ± 2	579.8 ± 4	530.0 ± 5	482.8 ± 5	439.8 ± 4	73.0	13.3
400 mg/kg	82.0 ± 2	475.0 ± 7	494.3 ± 1	483.8 ± 6	434.3 ± 4	383.0 ± 3	400.8 ± 3	382.3 ± 5	112.0	23.4
DMSO (0.3 ml)	76.3 ± 4	398.5 ± 6	400.5 ± 8	397.3 ± 6	383.8 ± 1	398.3 ± 9	398.3 ± 7	401.0 ± 6	14.8	3.7
Glibenclamide (10 mg/kg)	85.5 ± 2	446.0 ± 2	311.8 ± 3	231.8 ± 7	204.3 ± 7*	135.3 ± 1*	104.0 ± 3*	86.0 ± 7*	360.0	80.7

*: $p<0.05 \geq 200$ mg/dL FBGL: Diabetic.

Tables 2.1 and **2.2** show the effect of aqueous extracts of *A. muricata* on FBGL+- SEM in normoglycemic and hyperglycemic rats respectively. The anti-diabetic effects of the aqueous extract dosed at 200 mg/kg and 400 mg/kg separately, were less potent, giving (15.2% and 26.2%) and (13.3% and 23.4%), in normoglycemic and hyperglycemic groups respectively, 12 hour post administration. This can be explained by the low secondary metabolites' concentration of the aqueous extract and possibly by the high carbohydrate and protein-secondary metabolites ratio which may serve as diluent to the active anti-diabetic compounds.

Effect of *Annona muricata* leaves organic extracts on blood glucose levels (mg/kg) in normoglycemic and alloxan-treated diabetic rats.

Table 3.1: Effect of *A. muricata* organic extracts on blood glucose levels (mg/kg) in normoglycemic rats.

Treatment Groups	Before Treatment	After Treatment							Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h		
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
A. muricata										
200 mg/kg	86.0 ± 6		77.3 ± 1	78.7 ± 8	76.0 ± 3	68.3 ± 5	72.0 ± 1	55.7 ± 6	30.3	35.3
400 mg/kg	70.3 ± 5		83.7 ± 7	78.7 ± 9	75.7 ± 1	64.7 ± 5	48.3 ± 3	24.0 ± 3*	46.3	65.9
DMSO (0.3 ml)	65.7 ± 1		70.0 ± 2	70.0 ± 1	66.7 ± 2	68.3 ± 2	69.0 ± 1	65.0 ± 2	0.7	1.0
Glibenclamide (10 mg/kg)	79.0 ± 4		73.0 ± 4	63.3 ± 1	43.3 ± 4	31.3 ± 3*	13.3 ± 1*	Lo	65.7	83.3

*: $p<0.05 \geq 200$ mg/dL FBGL: Diabetic

Table 3.2: Effect of *A. muricata* organic extracts on blood glucose levels (mg/kg) in diabetic rats.

Treatment Groups	Before Treatment	After Treatment							Maximal Reduction	% Maximal Reduction
			1h	2h	4h	6h	8h	12h		
Untreated	77.3 ± 5		77.7 ± 3	79.67 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
Alloxan (150mg/kg)	69.0 ± 6	475.5 ± 2	47.3 ±2	514.8 ± 1	509.3 ± 8	495.3 ± 4	477.0 ± 2	467.1 ± 8	8.4	1.8
A. muricata										
200mg/kg	62.5 ± 3	401.8 ± 5	404.8 ± 2	382.3 ± 1	340.0 ± 20	312.0 ± 4	323.0 ± 9	266.3 ± 2	135.5	33.7
400mg/kg	82.3 ± 2	502.8 ± 4	445.0 ± 5	397.0 ± 4	321.0 ± 3	271.0 ± 3	250.5 ± 4*	190.8 ± 5*	312.0	62.1
DMSO (0.3ml)	76.3 ± 4	398.5 ± 6	400.5 ± 8	397.3 ± 6	383.8 ± 1	398.3 ± 9	398.3 ± 7	401.0 ± 6	14.8	3.7
Glibenclamide (10mg/kg)	85.5 ± 2	446.0 ± 2	311.8 ± 3	231.8 ± 7	204.3 ± 7*	135.3 ± 1*	104.0 ± 3*	86.0 ± 7*	360.0	80.7

*: $p<0.05 \geq 200$ mg/dL FBGL: Diabetic

The results in Tables 3.1 and 3.2 show the anti-diabetic activity of the organic extracts of *A. muricata* in normoglycemic and hyperglycemic groups respectively. It was revealed that the organic extract has better potency than the aqueous extracts in a dose-dependent manner. In normoglycemic groups, the activity was found to be 35.3% and 65.9% while in hyperglycemic rats the activity was 33.7% and 62.1% dosed at 200 mg/kg and 400 mg/kg respectively. The above effects were observed at the 12th hour post-administration. The heavy presence of alkaloids, tannins and some lipids in this extract suggests its activity which is consistent with the report presented by Yogesha Mohan *et al*. [12] The effects at 400 mg/kg dose had significant anti-diabetic effect (P<0.05). Several *in vitro* studies and one *in vivo* experiment observed that *A. muricata* inhibited the activities of α-amylase and α-glucosidase; the enzymes responsible for the breakdown of starch in the body. This indicates the suppression of postprandial hyperglycemia, [14-16] which is consistent with a low BGL usually observed after consumption of *A. muricata* byman.

Effect of *R. parviflora* root bark aquoeus extracts on blood glucose levels (mg/kg) in normoglycemic and alloxan-treated diabetic rats.

Table 4.1: Effect of *R. parviflora* aquoeus extract on blood glucose levels (mg/kg) in normoglycemic rats.

Treatment Groups	Before Treatment	After Treatment						Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h	
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
R. parviflora										
200 mg/kg	85.3 ± 3		130.7 ± 3	119.7 ± 2	78.7 ± 4	86.0 ± 5	88.3 ± 5	107.0 ± 4	6.6	7.8
400 mg/kg	64.3 ± 2		79.3 ± 12	87.0 ± 6	50.3 ± 4	52.0 ± 3	59.3 ± 3	59.0 ± 5	14.0	21.8
DMSO (0.3 ml)	65.7 ± 1		70.0 ± 2	70.0 ± 1	66.7 ± 2	68.3 ± 2	69.0 ± 1	65.0 ± 2	0.7	1.0
Glibenclamide (10 mg/kg)	79.0 ± 4		73.0 ± 4	63.3 ± 1	43.3 ± 4	31.3 ± 3*	13.3 ± 1*	Lo	65.7	83.3

Table 4.2: Effect of *R. parviflora* aqueous extract on blood glucose levels (mg/kg) in diabetic rats.

Treatment Groups	Before Treatment	After Treatment						Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h	
Untreated	77.3 ± 5		77.7 ± 3	79.7± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
R. parviflora										
Alloxan 150 mg/kg	69.0 ± 6	475.5 ± 2	47.3 ± 2	514.8 ± 1	509.3 ± 8	495.3 ± 4	477.0 ± 2	467.1 ± 8	8.4	1.8
200 mg/kg	60.8 ± 4	371.8 ± 12	354.8 ± 3	340.0 ± 7	332.3 ± 6	354.5 ± 14	346.3 ± 10	401.0 ± 11	39.5	10.6
400 mg/kg	64.5 ± 5	425.3 ± 11	359.3 ± 16	338.3 ± 9	331.3 ± 7	337.0 ± 7	379.8 ± 12	394.5 ± 12	94.0	22.1
DMSO (0.3 ml)	76.3 ± 4	398.5 ± 6	400.5 ± 8	397.3 ± 6	383.8 ± 1	398.3 ± 9	398.3 ± 7	401.0 ± 6	14.8	3.7
Glibenclamide (10 mg/kg)	85.5 ± 2	446.0 ± 2	311.8 ± 3	231.8 ± 7	204.3 ± 7*	135.3 ± 1*	104.0 ± 3*	86.0 ± 7*	360.0	80.7

*: p<0.05 ≥ 200 mg/dL FBGL: Diabetic

Tables 4.1 and 4.2 demonstrates the effect of aqueous extracts of *R. parviflora* on the FBGL +- SEM in normoglycemic and hyperglycaemic rats hyperglycemic rats respectively. Anti-diabetic effects of 7.8% and 21.8% were shown in normoglycemic rats whereas in hyperglycemic rats, 10.6% and 22.1% activity were shown, at 200 mg/kg and 400 mg/kg respectively in each treatment group. The observed effects at different doses yielded a non-consistent dose-activity relationship and minimal anti-diabetic activity as well.

Effect of *Rutidea parviflora* root bark organic extracts on blood glucose levels (mg/kg) in normoglycemic and alloxan-treated diabetic rats.

Table 5.1: Effect of *R. parviflora* root bark organic extract on blood glucose levels (mg/kg) in normoglycemic rats.

Treatment Groups	Before Treatment	After Treatment						Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h	
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
R. parviflora										
200 mg/kg	76.0 ± 4		73.3 ± 7	70.3 ± 5	55.7 ± 7	59.3 ± 5	66.0 ± 5	68.7 ± 3	20.3	26.8
400 mg/kg	90.3 ± 1		82.3 ± 2	72.7 ± 5	43.0 ± 2*	60.3 ± 1	65.0 ± 4	74.1 ± 2	47.3	52.4
DMSO (0.3 ml)	65.7 ± 1		70.0 ± 2	70. 0± 1	66.7 ± 2	68.3 ± 2	69.0 ± 1	65.0 ± 2	0.7	1.0
Glibenclamide (10 mg/kg)	79.0 ± 4		73.0 ± 4	63.3 ± 1	43.3 ± 4	31.3 ± 3*	13.3 ± 1*	Lo	65.7	83.3

*: p<0.05 ≥ 200 mg/dL FBGL: Diabetic

Table 5.2: Effect of R. parviflora root bark organic extract on blood glucose levels (mg/kg) in diabetic rats.

Treatment Groups	Before Treatment	After Treatment							Maximal Reduction	% Maximal Reduction
		0h	1h	2h	4h	6h	8h	12h		
Untreated	77.3 ± 5		77.7 ± 3	79.7 ± 5	83.3 ± 5	91.6 ± 6	91.7 ± 4	76.7 ± 7	0.7	0.9
Alloxan 150 mg/kg	69.0 ± 6	475.5 ± 2	47.3 ± 2	514.8 ±1	509.3 ± 8	495.3 ± 4	477.0 ± 2	467.1 ± 8	8.4	1.8
R. parviflora										
200 mg/kg	63.3 ± 2	560.0 ±12	587.0 ± 4	508.8 ± 4	422.0 ± 7	488.0 ± 6	499.0 ± 14	516.0 ± 9	138.0	24.6
400 mg/kg	68.5 ± 4	531.5 ± 13	471.0 ± 9	390.5 ± 6	286.8 ± 5*	302.5 ± 3	311.3 ± 9	363.0 ± 16	244.8	46.1
DMSO (0.3 ml)	76.3 ± 4	398.5 ± 6	400.5 ± 8	397.3 ± 6	383.8 ± 1	398.3 ± 9	398.3 ± 7	401.0 ± 6	14.8	3.7
Glibenclamide (10 mg/kg)	85.5 ± 2	446.0 ± 2	311.8 ± 3	231.8 ± 7	204.3 ± 7*	135.3 ± 1*	104.0 ± 3*	86.0 ± 7*	360.0	80.7

*: $p<0.05 \geq 200$ mg/dL FBGL: Diabetic

The results in Tables 5.1 and 5.2 reflect the anti-diabetic activity of the organic extract of *R. parviflora* on the FBGL+-SEM in the normoglycemic and hyperglycemic groups respectively. In the normoglycemic groups, the blood glucose levels were reduced by 26.8% and 52.4%, 4 hours post administration of the extract at doses of 200 mg/kg and 400 mg/kg respectively. Effects of 24.6% and 46.1% were recorded in the diabetic rats, 4 hours post-administration of the organic extracts at 200mg/kg and 400 mg/kg respectively.

It was observed that the performance of the extracts in the normoglycemic and hyperglycemic groups where not significantly different and the maximum effects were recorded at the 4th hour. There was a slight activity of *R. parviflora* aqueous extracts; however a moderate activity was recorded in the organic extract. It may be suggested that *R. parviflora* could boost the activity of *A. muricata* by synergism when co-administered and thus provide better therapeutic outcomes.

Comparing the anti-diabetic activity of *A. Muricata* and *R. Parviflora*

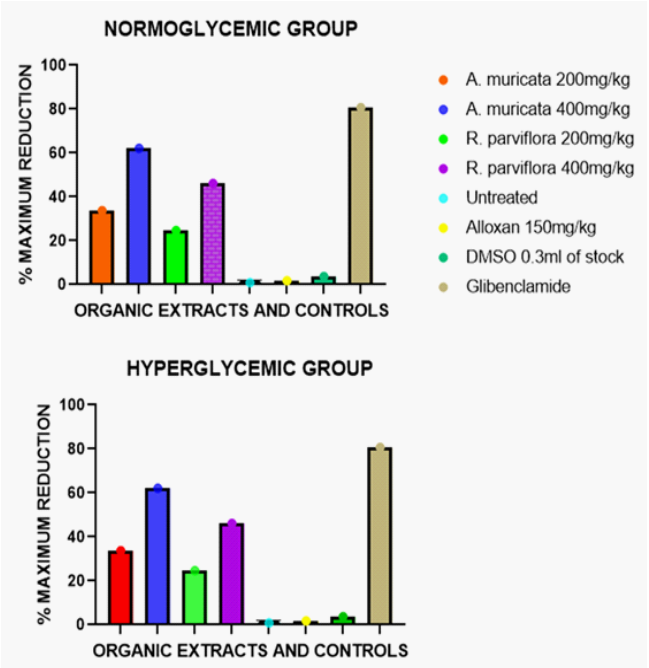

Figure 1: Percentage maximum reduction in FBGL in normoglycemic and hyperglycemic rats by the plants organic extracts at different the doses and the controls.

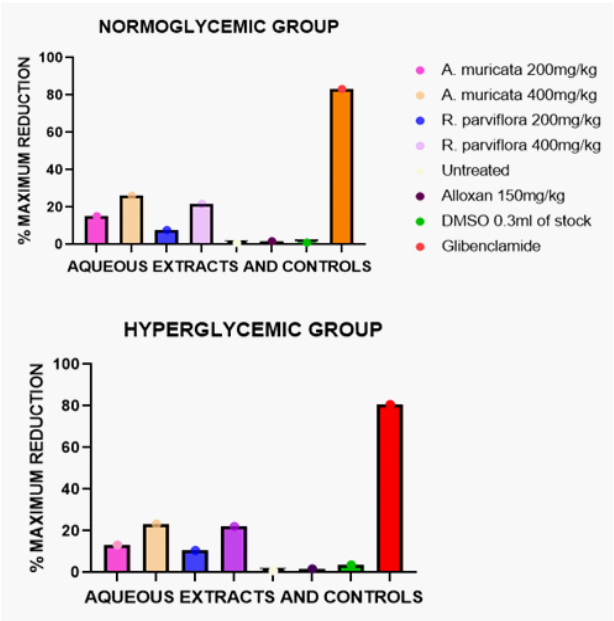

Figure 2: Percentage maximum reduction in FBGL in normoglycemic and hyperglycemic rats by the plants aqueous extracts at different doses and the controls.

A close look at figures 1 and 2 revealed that the maximum activities of the individual plants were found to be effected by their organic extracts: It was observed that the organic extract contained the bulk of most of the secondary metabolites hence; activity can be related to this trend. This observation is in the affirmative with the works done by other researchers who reported tannins, alkaloids, flavonoids and saponins to have BGL reduction activities [17,18].

Their anti-hyperglycemic activities were more pronounced in the normoglycemic groups than in the hyperglycemic groups. Extracts of *R. parviflora* showed its maximum effect at the 4th hour compared to the extracts of *A. muricata* having their maximum effect at the 12th hour: this indicates that the plant is short acting, relatively. This phenomenon may partly be explained by the relatively low concentrations of tannins, flavonoids and saponins in the extracts. Also, other studies have reported a number of encouraging metabolic parameters on the leaf extracts of *A. muricata in vivo*. The documented findings include significant decrease in BGL as observed from this study, better serum lipid

profile and increased body weight [19]. The possibility of preventing the destruction of pancreatic β-cells and their regeneration by *A. muricata* has been reported [14]. This potential benefit of *A. muricata* could boost insulin production in the body.

The results of toxicity studies conducted by two independent research teams demonstrated that *A. muricata* had good safety profile. LD_{50} of 1918.33 mg/kg and >5000 mg/kg were reported for the leaf extracts. Sub-chronic toxicity indicated diarrhea and increased heart rate as the only adverse effects observed [10, 20]. Sherif and his colleagues suggested a potential hepato-protective and neuphro-protective activity displayed by the methanol leaf extract investigated [20].

Conclusion

According to WHO reports, in the last four decades, the burden of DM has almost quadrupled resulting from obesity, overweight and sedentary lifestyles, which has led to more adults in the age bracket of 20-79 years living with DM. Furthermore, in 2012, an estimated 2.2 million deaths associated with the increased risk of cardiovascular diseases were linked to high BGL. The astronomical cost of managing diabetes can be catastrophic to developing nations, where affordable healthcare is beyond the reach of many. This underscores the relevance of researches into medicinal plants which are cheaper, readily available and efficacious in the management of DM [1].

A. muricata, is one plant that has demonstrated significant anti-diabetic activity, *in vitro*, *in vivo* and *in silico studies* [21] as documented in literature. This current study reported significant $p>0.05$ from the 400 mg/kg leaf extract. The plant has also shown promise as a hepato-protective and neuphro-protective agent with good safety profile. However, further studies are required to identify the compounds responsible for the demonstrated activity and clinical data to support its use.

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