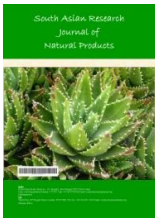

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Anti-diabetic Potential of Methanol Extract of Leaves of Selected Medicinal Plant

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Authors' contributions

This work was carried out in collaboration among all authors. Author VA conceptualized the research, author RAA drafted the proposal for the work, author EO implemented the proposal via laboratory experiments, Author KMR, sourced the materials with which the article was written, author AAK proof read the article. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this work was to determine the anti-diabetic potential of leaves of selected medicinal plants namely; *Carica papaya* (paw-paw), *Psidium guajava* (guava) and *Anacardium occidentale* (cashew). Leaves obtained from each of the aforementioned plants were dried and processed into fine powder which was subsequently extracted with methanol. Phytochemicals present in extracts were quantitatively determined. Thirty (30) adult male albino rats were divided into six groups of five rats each. Group I was the non-diabetic (normal) control, Group II was the untreated (diabetic) control, Groups III-V were separately administered with 200mg/kg b.w of the methanol leaf extract of *Carica papaya* (paw-paw),*Psidium guajava* (guava) and *Anacardium occidentale* (cashew), while Group VI was administered with 2.5 mg/kg glibenclamide. Results on the phytochemical analysis on the extracts showed that saponins was significantly higher (36.89±1.06) in *Psidium guajava* (guava) leaf extract than pawpaw and cashew leaves extracts (25.55±2.36%) and

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(27.03±1.48%) respectively. Antidiabetic studies on the methanol leaf extract of the plants, showed that oral administration of 200mg/kg b.w of *Psidium guajava* (guava) leafextract significantly reduced (121.33±5.78 mg/dl) blood glucose to a level which however was not significantly different from the reduction (121.67±4.4 mg/dl) achieved with 200 mg/kg b.w of *Anacardium occidentale* (cashew) and*Carica papaya* (paw-paw) leaves extract. In conclusion, this study has shown that *Psidium guajava* (guava), *Anacardium occidentale* (cashew) and *Carica papaya* (paw-paw) leaves are all potent anti-diabetic options.

Keywords: Anti-diabetic; medicinal plants; phytochemicals; leaf extract.

1. INTRODUCTION

Diabetes is a metabolic disorder characterized by elevated blood sugar level that result from defects in insulin production and or insulin action as well as impaired function in the metabolism of carbohydrates, lipids and proteins which leads to macro and microvascular complications [1]. Diabetes has attained epidemic status and has persisted as a major burden on the global community. The International Diabetes Federation (IDF) predicts a 552 million global burden of diabetes by 2030 [2].

In spite of new and effective drugs available for the treatment of the disease, numerous pitfalls such as adverse side effects have been associated with them and thus have necessitated the need to consider the age long practice of employing plants as a treatment option. In developing countries especially Africa, about 70-80% of the populace rely heavily on the use of medicinal plants for their primary source of health care [3].

Carica papaya commonly known as paw-paw belongs to the family *Caricacae*[4]. It is mainly cultivated in the tropics. Different parts of the plant are endowed with medicinal values. For instance, the leaf of *C. papaya* has been instrumental in the regeneration of pancreatic beta-cell of diabetic mice. Juice derived from unripe *C. papaya* fruit has also shown an impressive decree of inhibitory effects on type-2 diabetes key enzymes α-amylase and α-glucosidase as well as lipid peroxidation in rat pancreatic cells [5].

Psidiumguajava commonly known as guava and a member of the family *myrtaceae* is cultivated in South Africa [6]. The leaf has been used to treat diverse human diseases. In China, the locals have relied on guava leaf juice to manage diabetic condition [7]. Inhibition study has established a relationship between the inhibitory effect of *Psidiumguajava* leaf extract on intestinal glycosidases and postprandial hyperglycemia [8].

Anacardiumoccidentale is also known as cashew and a member of the family *Anacardiaceae* [9].The tree is native to Brazil, although it is found in tropical countries. Parts of the *Anacardiumoccidentale*have been used to cure diverse human ailments such as bacterial infection and fungal infections as well as oxidative stress related conditions. Specifically, the leaf has been used locally to treat diabetes [9]. Therefore, it is imperative to scientifically validate the local claim on these plants as a potent anti-diabetic remedy.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Leaves of Pawpaw (*Carica papaya*) Cashew (*Anacardiumoccidentale*) and Guava (*Psidiumguajava*) were sourced from Zaria metropolis in the month of January 2012. The leaves were identified and authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, and Zaria, where voucher specimen with numbers 571, 184, and 3253 were respectively deposited.

2.2 Preparation of Plant Extracts

Leaves obtained from the three different plants were separately dried at room temperature after which they were separately ground to powder with the aid of an electric blender. The resulting powder was sieved to obtain fine powder. 500 g of the powdered plant sample was separately soaked in 2 Litres of 70% methanol for about 72 hours and stirred intermittently. The extracts were filtered and the filtrates concentrated at 40^0C.

2.3 Quantitative Phytochemical Analysis

2.3.1 Determination of alkaloids

About 5.7 g of the methanol leaf extract of each of *Carica papaya* (paw-paw),*Psidiumguajava*(guava) and

Anacardiumoccidentale (cashew) was introduced into a 250 ml beaker, after which 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 48 hr. After filtration, the extracts were concentrated on a water bath to 1/4thof initial weight of extract. The whole solution was collected,washed using dilute ammonium hydroxide prior to being filtered. The residue obtained was dried and weighed [10].

2.3.2 Determination of saponins

Precisely 20 g of methanol leaf extract of each of *Carica papaya* (paw-paw), *Psidiumguajava* (guava) and *Anacardiumoccidentale* (cashew) was separately placed into conical flask, this was followed by the addition of 100 ml of 20% aqueous ethanol. The samples were subjected to heating over a hot water bath for 4 hr with continuous stirring at 55°C. The mixture was filtered and the residue extracted again using another 200 ml of 20% of ethanol. The combined extracts were reduced to 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was introduced and shaken vigorously. The aqueous layer was recovered while the ether layer was disposed. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in the water bath. After evaporation, the samples were dried in the oven to a constant weight and saponins content of the extracts was determined [10].

2.3.3 Determination of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, after which aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg each of the pawpaw, guava, cashew leaf extract was dissolved in 10 ml methanol and filtered; three ml (1mg/ml) of this extract was used for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ methanol solution was added to 3 ml of extract standard and allowed allowed to stands for 15 minute at room temperature; absorbance was measured at 420 nm [11].

2.3.4 Determination of tannins

Precisely 10 ml of standard solution was made up to 100 ml with distilled water. 100 µl aliquots of the methanol leaf extract each of *Carica papaya* (paw-paw), *Psidiumguajava* (guava) and *Anacardiumoccidentale* (cashew) was introduced into test tubes. This was followed by the addition of 0.5 µl of Folin-Denis reagent and 1 ml of sodium carbonate solution was introduced into each tube. The contents of each test tube were mixed thoroughly and allowed to stand for about 30 min and read at 760 nm against reagent blank [12].

2.3.5 Determination of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol.Various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of Extract: 10 mg each of the pawpaw, guava, cashew leaf extract was dissolved in 10 ml methanol and filtered, 2 ml (1mg/ml) of each leaf extract was used for the estimation of phenols.

Procedure: 2 ml each of the extracts and standard was mixed with 1 ml of FolinCiocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15sec and allowed to stand for 15 minute at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Labindia, 3000+) [13].

2.4 Animals

Adult male albino rats (150-200g) were obtained from the animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were housed in well-ventilated cages and fed with commercial laboratory diet and water *ad libitum.*

2.5 Acute Toxicity Study

This was carried out using the method described by Lorke [14]. In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with 10mg, 100mg and 1000mg of the extract per kg body weight orally. They were

observed for 24 hours for signs of toxicity, including death. In the absence of observable sign of toxicity, the second phase was set up and constituted of 4 rats which were divided into 4 groups of 1 rat each. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

2.6Induction of Diabetes

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg b.w of streptozotocin (dissolved in 0.1mol fresh cold citrate buffer, (pH 4.5) into experimental rats which had been starved for 24hr [15]. Glucose solution (5%) was administered 6hr after induction. After 3 days the blood sugar levels was determined with glucometer (Acccheek Advantage Roche diagnostics GmbH, Germany) and the rats with fasting blood glucose level more than 200mg/dl (11.1mmol/L) were considered diabetic hence selected for experiment. The extracts and glibenclamide were administered on daily basis to the experimental rats for 28 days by gastric intubation. At the end of 28 days, the rats were anaesthetized using chloroform and sacrificed 24hrs after the last treatment. Blood samples were collected in specimen bottles, allowed to clot, centrifuged and serum was generated.

2.6.1 Determination of fasting blood glucose

Exactly 1 ml of glucose working reagent containing glucose oxidase, peroxidase and 4-amino antipyrine as chromogen was introduced into test tubes after which 20µL of the test samples were added into the samples in test tubes. Carefully, 20µL of standard reagent was introduced into the standard test tube. They were all incubated at room temperature for 30 minutes and absorbance was read with the aid of a spectrophotometre at 510nm Barham and Trinder [16].

2.6.2 Experimental design

Thirty adult male rats albino were divided into six groups of five rats each. Rats were treated for 28 days.

Group I: Normal control was fed with only normal rat diet and water *ad libitum.*

Group II: Negative control was induced with diabetes but not given any treatment.

Group III: Rats induced with diabetes and treated with 200 mg/kg b. w of methanol extract of *Carica papaya* (pawpaw) leaf orally.

Group IV: Rats induced with diabetes and treated with 200 mg/kg b. w of methanol extract of *Anacardiumoccidentale*leaf orally.

Group V: Rats induced with diabetes treated with 200 mg/kg b. w of methanol extract of *Psidiumguajava*(guajava)leaf orally.

Group VI: Rats were induced with diabetes and treated with 2.5 mg/kg b. w glibenclamide orally

2.7 Statistical Analysis

Data were expressed as mean ± standard deviation. The data were analyzed using analysis of variance (ANOVA). The difference in mean was compared using Multiple Range Test. $P<0.05$ was considered significant

3. RESULTS AND DISCUSSION

Phytochemicals are biologically active compounds in plants with medicinal properties [17].Table 1. shows the quantity of phytochemicals in methanol leaf extract of *Carica papaya* (paw-paw), *Psidiumguajava*(guava) and *Anacardiumoccidentale* (cashew). Results on the quantitative phytochemical analysis on the extracts showed that the saponins content of guava leaf extract was significantly ($P<0.05$) higher (36.89±1.06) compared to pawpaw leaf (25.55±2.36) and cashew leaf (27.03±1.48%). This may be as a result of the variation that characterizes the responses of the plants to abiotic environmental factors [18]. Studies on the anti-diabetic potential of paw-paw, guava and cashew leaves indicated that oral administration of 200mg/kg b.w of methanol leaf extract of guava caused a significant ($P<0.05$) reduction (121.33±5.78mg/dl) in the blood glucose level compared to value reported on the untreated diabetic control group (268.53±1.64mg/dl) which however was not significantly ($P<0.05$)different (121.67±4.41mg/dl) from the reduction observed with 200mg/kg methanol leaf extract of cashew but was significantly ($P<0.05$) (125.67±2.96mg/dl) different from the reduction

Table 1. Quantitative phytochemical composition of methanol leaf extracts of *carica papaya*, *psidium guajava* and *anacardium occidentale*

	% composition		
Phytochemicals	**Pawpaw**	**Gauva**	**Cashew**
Flavonoids	47.55±2.29[a]	32.73±1.25a	29.36±1.05[a]
Alkaloids	48.17±1.89[a]	16.98±5.07c	25.08±2.02[b]
Phenols	7.83±0.35[d]	6.21±0.54d	6.21±0.54[c]
Saponins	25.55±2.36[c]	36.89±1.06[a]	27.03±1.48
Tannins	6.85±0.76	5.76±0.45[d]	3.44±0.39[d]

Values are expressed as mean ± SD from three determinations. Values with the different superscript in column are significantly different (P<0.05)

Table 2. Fasting blood glucose levels of the extract treated rats in comparison with the controls

Group	Treatments	Blood sugar level (mg/dl)
Group I	Normal control (NC)	77.33±3.71[e]
Group II	Diabetic control (DC)	268.53±1.64[a]
Group III	D+*C. papaya* 200mg/kg	125.67±2.76[b]
Group IV	D+ *P.guajava* 200mg/kg	121.33±5.78[c]
Group V	D + *A. occidentale* 200mg/kg	121.67±4.41[d]
Group VI	D + standard drug 2.5kg	97.33±3.71[d]

Values are expressed as mean ± SD from three determinations. Values with the different superscript in column are significantly different (P<0.05)

in blood glucose level obtained with 200 mg/kg methanol leaf extract of paw-paw. This may be attributed to its comparatively higher saponin content, this finding which is consistent with the work of Amira et al. [19] which confirms that saponins regulate blood glucose level and prevent diabeticcomplications due to their antioxidant activity.

5. CONCLUSION

This study clearly reveals that leaves of *Psidiumguajava* (guava), *Anacardiumoccidentale* (cashew) and *Carica papaya* (paw-paw) can be instrumental in the regulation of blood sugar levels and further consolidates claims by other researcher on the viability of saponinsin normalizing elevated blood levels.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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