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Phytochemical Analysis and Anti-diabetic Potential of *Annona muricata* L., *Persea americana* Mill. and *Montrichardia arborescens* L. Schott Utilized by the Residents of Pakuri (St. Cuthbert's Mission) in Guyana

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Authors' contributions

This work was carried out in collaboration among all authors. Author CB conceptualized the research project and the study design. He has also played an integral role in the methodology, data analysis, interpretation and final draft of the paper. Author JR was involved in the data analysis and interpretation and final draft of the paper. Author JS was involved in data interpretation and proof reading the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to assess the utilization of plants with anti-diabetic properties and to conduct preliminary phytochemical analysis and moreover, to assess the anti-diabetic potential of selected plants used to treat and manage Type 2 diabetes mellitus (T2DM) among the residents of Pakuri (St. Cuthbert's Mission) in Guyana.

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Methodology: The leaves of sour sop (*Annona muricata* L.), pear (*Persea americana* Mill.) and Mocou-Mocou (*Montrichardia arborescens* L. Schott) underwent phytochemical testing and α-amylase Inhibition testing to determine their anti-diabetic properties.
Results: This study showed that the leaves of pear, sour sop and mocou mocou contained different chemical constituents including alkaloids, flavonoids, saponins and tannins but the absence of amino acids (proteins), carbohydrates and glycosides. The α-amylase inhibitory studies performed demonstrated that the extracts of *A. muricata* in ethanol and chloroform had significant inhibitory potential.
Conclusion: Considering the promising potential of phytochemicals and the anti-diabetic activity of these species of plant extracts in anti-diabetic drug development, *in vivo* experiments and clinical trials are required for efficacy and safety evaluation. Also, the anti-diabetic phytochemicals may be used in combination with existing orthodox drugs, thereby, reducing the dose of synthetic anti-diabetic drugs, which will help in addressing the toxicity and cost-related issues in chronic use during the management of diabetes mellitus (DM).

Keywords: Traditional medicine; type 2 diabetes mellitus; complementary and alternative medicine; indigenous knowledge; attitude.

1. INTRODUCTION

The beneficial uses of phyto-medicines have a long history in human lives. They are the sum-total of the knowledge, skills and practices based in theories, beliefs and experiences of different cultures. Amerindians, black slaves from Africa, Indian indentured laborers from India, Portuguese from Madeira, Chinese from China, local Amerindians and Caucasians, whether explicable or not, used plants in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses [1]. Most of the plant species in the world have not yet been subjected to phytochemical studies for possible biological active constituents. On the other hand, the majority of secondary metabolites that are identified in medicinal plants show a pleiotropic ability to interact with several targets [2].

Therefore, traditional medicine offers promising solutions to face the global increasing demands for new therapeutic agents, especially with the advent of drug resistance. There is also insufficient data that exist for most plants to guarantee their quality, efficacy and safety. Plants contain hundreds of different bioactive constituents and some of them are very toxic such as the most cytotoxic anti-cancer plant-derived drugs, digitalis and the pyrrolizidine alkaloids [3]. It is worth noting that most active ingredients of most plant-based medicines are destroyed either during cooking or heating. To be effective, they have to be consumed raw or uncooked.

However, the adverse effects of phyto-therapeutic agents are less frequent as compared with synthetic drugs, but well-controlled clinical trials have now confirmed that such effects really exist [4]. WHO Alma-Ata Declaration in 1978 opened the door for a dialogue between traditional and modern health care on the understanding that unsafe practices should be eliminated and that only what is both safe and effective should be promoted. Safety should be the overriding criterion in the selection of phyto-medicines. Screening, chemical analysis, clinical trials and regulatory measures should be undertaken in respect to the medicinal use of phyto-medicines.

The fact, that DM is expected to become a major cause of death by 2030 with increases in prevalence and incidence raises the need to look for more effective agents with fewer side effects and also cost effective. Complications are the major cause of morbidity and mortality in patients with diabetes mellitus [5].

The literature reviews reveal that numerous plant species have been tested for their usefulness in treating and managing diabetes worldwide. For Tropical America, including the Guiana Shield and Guyana, there exist identification of native plants used to treat diabetes. However, current data on native phyto-chemistry and pharmacological studies to treat diabetes in Guyana, the Guiana Shield and the Caribbean are lacking.

From the literature review, several phytochemicals from plants were identified as having pharmacological values in treating diabetes. These include alkaloids, glycosides, flavonoids, steroids and polysaccharides among

others. Nevertheless, protecting biodiversity should be at the forefront in Tropical America given that it is well known for their biodiversity value of this region. It seems important to encourage bioprospecting in the pursuit and selection of promising plants, which have both phytochemicals and pharmacological values for diabetes treatment employing from the rich biodiversity of Guyana ecosystems and cultural diversity.

2. MATERIALS AND METHODS

A community based cross-sectional study design was done to assess the utilization of plants with anti-diabetic properties and to conduct phytochemical analysis and assess anti-diabetic potential of two of the most randomly used plants and one plant from the literature.

2.1 Study Area

The study was conducted in Pakuri (previously St. Cuthbert's Mission) located at 6.36° LN, 58.08 LW; the current population is of 200 households, where approximately 1800 persons are currently living.

Pakuri was said to be the "cultural capital" amongst the remaining Arawak Amerindian settlements that according to the map of Brothwell (1967) dominated the coastal areas of Guyana (Fig. 1) [6]. The name of the town was given for the abundance of the species named Pakooru.

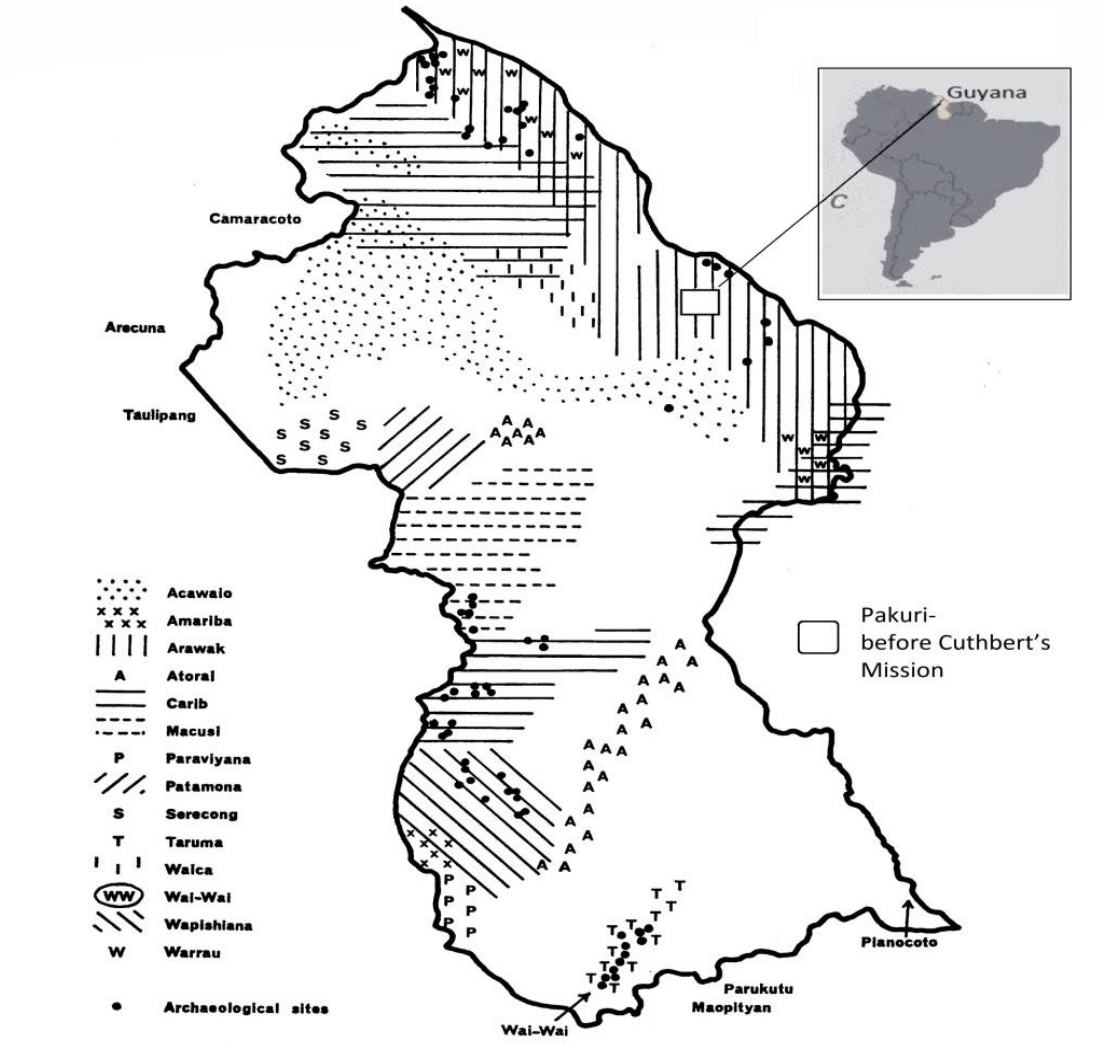

Fig. 1. Study Area, modified after Brothwhell (1967)

Platonia insignis from the Botanical Family Guttiferae, an important forestry species with high exploitation since colonial times [7].

2.2 Study Sample

Two tree species of plants, sour sop (*Annona muricata* L.) and pear (*Persea americana* Mill.) which are widely cultivated in the gardens of Pakuri residents, as well as, a wild wetland tall herb species, Mocou-Mocou (*Montrichardia arborescens* L. Schott), which is found in the river and creeks of the black water rivers, swamps, estuarine and deltaic environments with brackish waters of the community (Mahaica river watershed) were selected as they are widely used by the community against diabetes [8].

2.3 Sampling Procedures for Identification of the Species

Vouchers 1 to 6 of botanical specimens were collected amongst plants in good condition, free of insect damage, rust and/or disease using traditional botanical procedures. Verification and identification were done in the Herbaria of the Center of Biodiversity, University of Guyana.

At least 20 g each of fresh leaves of the three species were collected from 3 different individuals of each species in paper bags and transported the same day to the lab of technology.

2.4 Data Collection

2.4.1 Preparation of extracts

The leaves for testing of the sour sop plant (*Annona muricata*), pear (*Persea americana*) and Mucuo-Mucuo (*Montrichardia arborescens*) were air dried under shade and then coarsely powdered with the help of a mechanical grinder. The powder from each species leave was passed through sieve no.40 and stored in an airtight container for extraction. It was then extracted with chloroform (55-56°C) and ethanol 90% v/v. (75-78°C) solvents. The solvents used were purified before use. The extraction process was done by soaking the weight powdered leaves (Sour Sop- 4.1 g, Pear- 6.164 g and Mocou-Mocou- 5.3) in each solvent and filtering after 24 hrs. This process was repeated for three consecutive days. Extracts obtained were evaporated under reduced pressure using rotovac evaporator.

2.4.2 Qualitative phytochemical analysis

The extracts obtained (chloroform and ethanol) were subjected to the following preliminary phytochemical studies.

2.4.3 Determination of alkaloids

For each essay, a sample of 0.5 g was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added until occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-colored precipitate will be an indication for the presence of alkaloids [9].

2.4.4 Determination of flavonoids

Shinoda's test: About 0.5 g of each portion from the three different species was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl until a pink, orange, or red to purple coloration indicates the presence of flavonoids [10].

Lead acetate test: Extracts were treated with few drops of lead acetate solution until formation of yellow color indicates the presence of flavonoids [10].

2.4.5 Determination of saponins

Foam test: One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins [9].

2.4.6 Determination of tannins

Braemer's test: About 0.5 g each portion from the different species of the plant leaves was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate until the occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [10].

2.4.7 Determination of amino acids

Ninhydrin test: To the extract from the, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of proteins and amino acid [11].

2.4.8 Determination of carbohydrates

Molish's test: Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water. This was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was a positive test [9].

2.4.9 Determination of glycosides

Modified Borntrager's test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides [12].

2.4.10 α-amylase inhibition activity [13]

The assay was carried out following a standard protocol with slight modifications [14]. Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution were boiled for 5 min and then pre-incubated at 37°C for 5 min. Each extract from the different plant species was dissolved in DMSO to obtain concentrations of 0, 25, 50, 75, 100 and 125 µg/ml. Then, a volume of 0.2 mL of plant extract of particular concentration was added to the tube containing the substrate solution. In addition, 0.1 mL of porcine pancreatic amylase in Tris–HCl buffer (2 units/mL) was added to the tube containing the plant extract and substrate solution. The reaction was carried out at 37°C for 10 min. Adding 0.5 mL of 50% acetic acid in each tube stopped the reaction. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer. Same procedure was followed for other plants extracts (chloroform and hexane) to test their α-amylase inhibitory effects. Acarbose, a known α-amylase inhibitor, was used as a standard drug. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula:

$$\text{The α-amylase inhibitory activity} = (Ac +) - (Ac -) - (As - Ab)/(Ac +) - (Ac -) \times 100,$$

where Ac+, Ac−, As and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively. The concentration of acarbose and plant extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of plant extracts and acarbose were calculated and its IC_{50} values were determined.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analyses of the Three Plant Extracts

Table 1 shows the different classes of chemical compounds present in *A. muricata*, *P. americana* and *M. arborescens* for comparison.

Generally, plants and their different parts including roots, leaves, stems and fruits contain different chemical compounds and elements in the form of anions and cations. These different compounds play major roles in their medicinal value as therapy for different diseases. This study has shown that the leaves of *A. muricata*, *P. americana* and *M. arborescens* contained different chemical constituents including alkaloids, flavonoids, saponins and tannins. However, the data reveal the absence of any amino acids (proteins), carbohydrates and glycosides.

The presence of flavonoids represents biological activity in the ethanol extract of *P. americana.* Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [15,16]. The presence of tannins represents the possibility of biological activity such as antidiarrheal, hemostatic, antihemorrhoidal, anti-inflammatory, astringent and anti-infective. Flavonoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals. Flavonoids and tannins have been reported to produce anti-diabetic activity [17]. Similarly, saponins have been reported to possess a wide range of biological activities, For instance, saponins were known to be bioactive against diabetes [18,19]. A previous study has also shown that saponins can exert numerous biological effects including the stimulation of insulin secretion, the action of insulin, regeneration of beta cells islets and activate the enzymes, which are responsible for glucose utilization [20].

Table 1. Phytochemical analysis of the three plant extracts

Class of compounds	Tests performed	Results					
		P. americana		*A. muricata*		*M. arborescens*	
		Ethanol	Chloroform	Ethanol	Chloroform	Ethanol	Chloroform
Amino Acids	Ninhydrin Test	_	_	_	_	_	_
Alkaloids	Dragendorff's Test	+	+	+	_	+	_
Carbohydrates	Molish Test	_	_	_	_	_	_
Flavanoids	Shinoda Test	+	_	_	_	_	_
	Lead acetate Test	+	_	+	_	+	_
Glycosides	Modified Borntrager's test	_	_	_	_	_	_
Saponins	Foam Test	+	_	+	_	+	_
Tannins	Braemer's Test	+	_	+	_	+	+

+ Present in moderate amount _ Absence

Within this study alkaloids were present in all the chloroform extracts and in the ethanol extract of *P. americana* (pear). Aniszewski (21) stated that a large number of alkaloids have been isolated from numerous medicinal plants and investigated in several studies for their possible anti-diabetic activity [21]. The process of glycolysis converts sugars to glucose *via* a series of 10 reactions with three regulatory steps catalyzed by the enzymes hexokinase, phosphofructokinase and pyruvate kinase. For example, the alkaloid berberine, extracted from *Tinospora cordifolia*, enhances the activity of hexokinase and phosphofructokinase, resulting in glucose transport, carbohydrate digestion and absorption [22]. Also, catharanthine, vindoline and vindolinine, obtained from C*atharanthus roseus* lower the blood sugar level and show free radical scavenging action [23,24]. Glucose takes part in the glycation of the membrane lipid and its peroxidation to produce free radicals. In DM, the glucose concentration is very high and so is the amount of free radicals in the body, which are highly reactive. To prevent their deleterious effect, our body has a defense system comprising several enzymes, which include superoxide disputase, catalase, reduced glutathione and glutathione-S-transferases [25]. Therefore, the importance of phytochemicals cannot be understated in the fight against diabetes mellitus.

3.2 α - Amylase Inhibition Assay of Plants Extracts

Fig. 2 shows the dose–dependent curve for the inhibitory action of α- amylase on an ethanol extract of the plant, *P. americana* leaves. The data are presented in Table 2. The results show that α- amylase can inhibit the ethanolic extract of *P. americana* leaves in a dose –dependent manner.

Table 2. α- amylase inhibition of ethanol extract of *P. americana* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	27
50	36
75	39
100	45
125	51

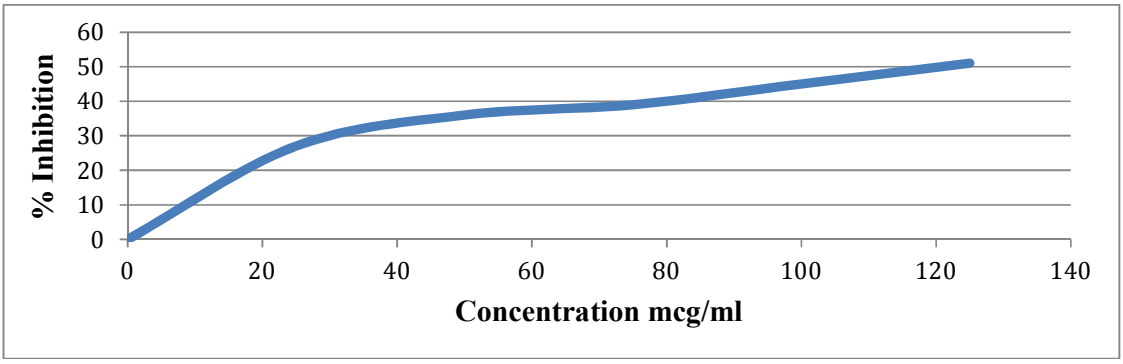

Fig. 2. Dose-dependent of α- amylase inhibition of ethanol extract of *P. americana* leaves

Table 3. α- amylase inhibition of ethanol extract of *A. muricata* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	31
50	42
75	47
100	52
125	58

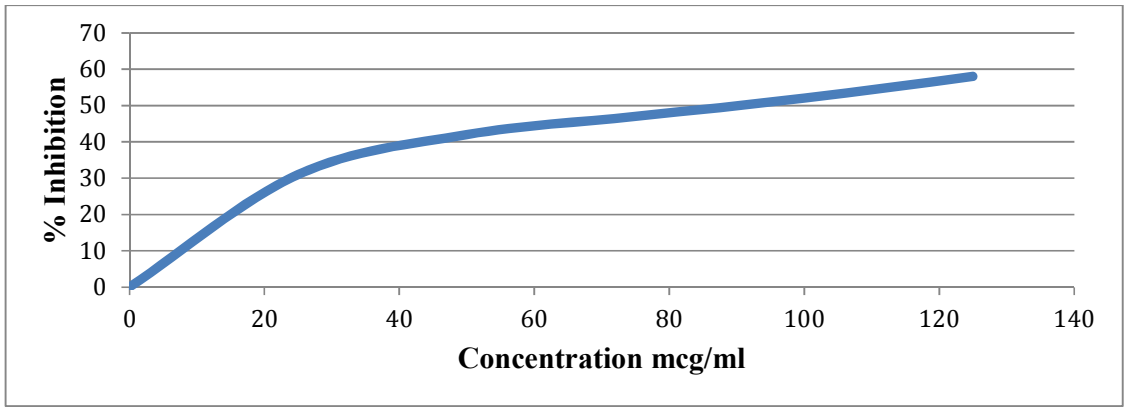

Fig. 3. Dose-dependent of α- amylase inhibition of ethanol extract of *A. muricata* leaves

Table 4. α- amylase inhibition of ethanol extract of *M. arborescens* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	29
50	31
75	37
100	43
125	50

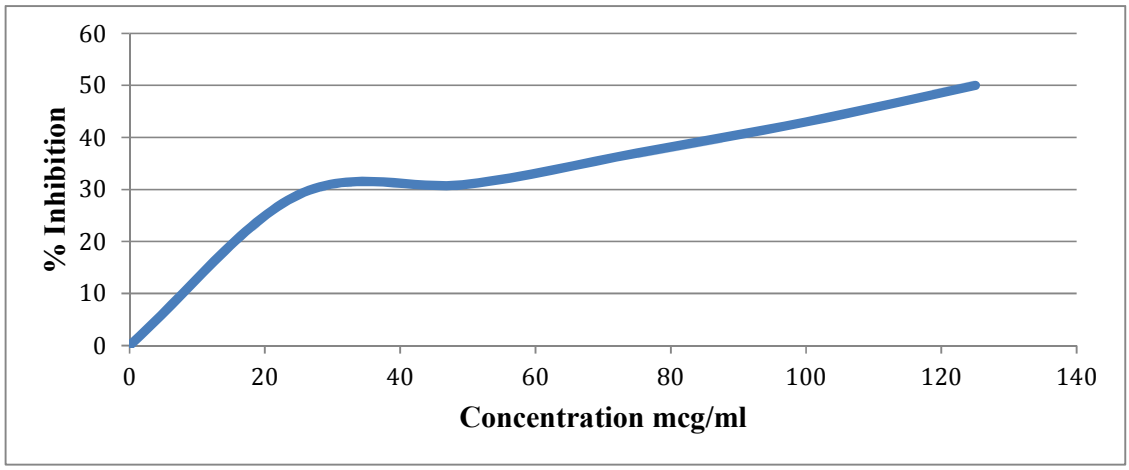

Fig. 4. Dose-dependent of α- amylase inhibition of ethanol extract of *M. arborescens* leaves

Table 5. α- amylase inhibition of chloroform extract of *P. americana* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	27
50	35
75	41
100	46
125	52

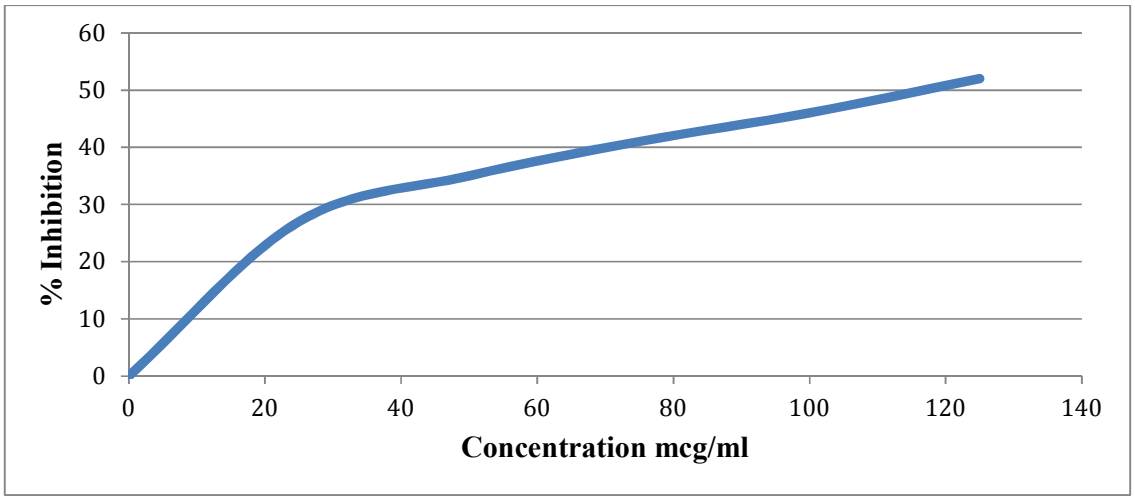

Fig. 5. Dose-dependent of α- amylase inhibition of chloroform extract of *P. americana* leaves

Table 6. α- amylase inhibition of chloroform extract of *A. muricata* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	30
50	39
75	45
100	50
125	56

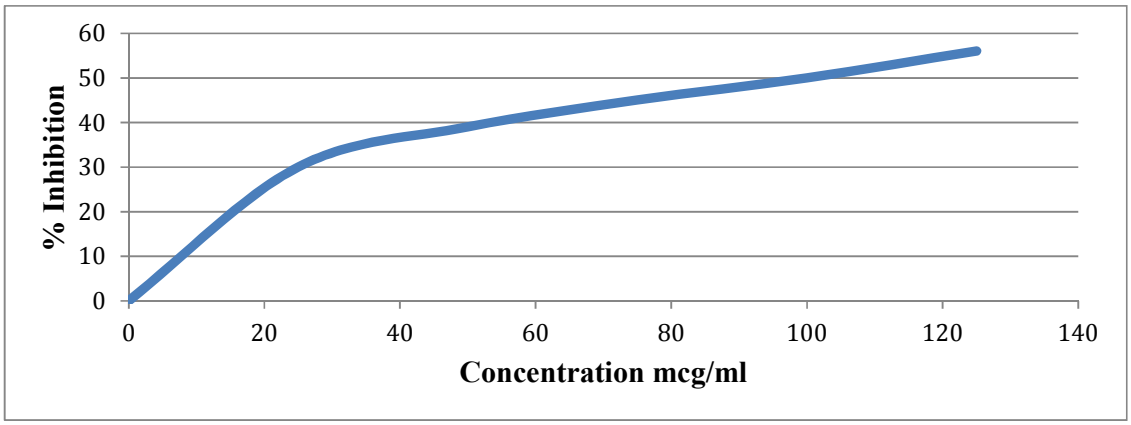

Fig. 6. Dose-dependent of α- amylase inhibition of chloroform extract of *A. muricata* leaves

Table 7. α- amylase inhibition of chloroform extract of *M. arborescens* leaves

Concentration (µg/ml)	Percentage inhibition (%)
0	0
25	28
50	37
75	42
100	47
125	50

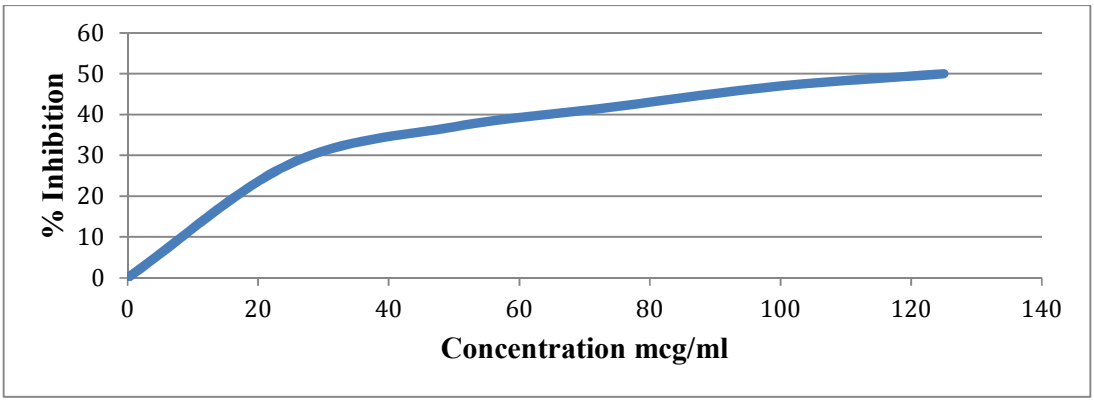

Fig. 7. Dose-dependent of α- amylase inhibition of chloroform extract of *M. arborescens* leaves

Fig. 3 shows dose–dependent curve for the inhibitory action of α- amylase on an ethanol extract of the plant, *A. muricata* leaves. The data are presented in Table 3. The results show that α- amylase can inhibit the ethanolic extract of *A. muricata* leaves in a dose –dependent manner.

Fig. 4 shows the dose–dependent curve for the inhibitory action of α- amylase on an ethanol extract of the plant, *M. arborescens* leaves. The data are presented in Table 4 for comparison. The results show that α- amylase can inhibit the ethanolic extract of *M. arborescens l*eaves in a dose –dependent manner.

Fig. 5 shows the dose–dependent curve for the inhibitory action of α- amylase on a chloroform extract of the plant, *P. americana* leaves. The data are presented in Table 5 for comparison. The results show that α- amylase can inhibit the chloroform extract of *P. americana l*eaves in a dose –dependent manner.

Fig. 6 shows the dose–dependent curve for the inhibitory action of α- amylase on a chloroform extract of the plant, *A. muricata* leaves. The data are presented in Table 6 for comparison. The results show that α- amylase can inhibit the chloroform extract of *A. muricata l*eaves in a dose –dependent manner.

Fig. 7 shows the concentration –dependent curve for the inhibitory action of α- amylase on a chloroform extract of the plant, *M. arborescens* leaves. The data are presented in Table 7 for comparison. The results show that α- amylase can inhibit the chloroform extract of *M. arborescens l*eaves in a dose –dependent manner.

The α-amylase inhibitory studies performed demonstrated that the extracts of *A. muricata* in ethanol and chloroform had significant inhibitory potential. This, however, is worth investigating further in order to isolate pure active compounds.

4. CONCLUSION

A number of phytochemicals have been isolated from medicinal plants exhibiting anti-diabetic activity. Many of these phytochemicals have shown equal hypoglycemic/anti-diabetic activity equal and sometimes even more potent than currently used drugs. In this study alkaloids, flavonoids, saponins and tannins have all been qualitatively identified in the crude plant extracts. In addition, the α-amylase inhibitory activity of *A. muricata* shows remarkable promise.

Therefore, considering the promising potential of phytochemicals and the anti-diabetic activity of the extracts of these plants in anti-diabetic drug development, further *in vivo* experiments and clinical trials are required to determine the efficacy and safety evaluation. Also, the anti-diabetic phytochemicals may be used in combination with existing orthodox drugs, thereby reducing the side effects synthetic anti-diabetic drugs. This in turn will help in addressing the toxicity and cost-related issues in chronic use during the management of diabetes.

Bioprospecting wild species with important medicinal value for local populations has its potential for sustainability of local communities. *Annona muricata* and *Persea americana* were once only found as wild species in the Neotropical forest of Central America. Amerindian communities selected those species

with importance value as medicines and more than 10.000 years of indigenous occupation in America have resulted in a dissemination of those species amongst all villages in the indigenous gardens. However Neotropical wild species of wetlands such as *Montrichardia arborescens* is not widely cultivated, although it is of value for the control of hypertension [26].

CONSENT AND ETHICAL APPROVAL

Formal letter of approval was obtained from the Pakuri Village Council and the Ministry of Indigenous People's Affair. Each participant of the study was informed about confidentiality. Each participant of the study agreed to participate voluntarily. Participants were allowed to discontinue the interview when they needed. All participants of the study declared their willingness to participate and approved by their verbal consents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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