Original Research Article

Phytochemical analysis and anti-diabetic potential of *Annova Muricata, Persea Americana* and *Montrichardia Arborescens* utilized by the residents of Pakuri (St. Cuthbert's Mission) in Guyana

ABSTRACT

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

Methodology: The leaves of sour sop (*Annova Muricata L.*), pear (*Persea Americana Mill.*) and Mocou-Mocou (*Montrichardia Arborescens*) underwent phytochemical testing and α-Amylase Inhibition testing to determine their anti-diabetic properties.

Results: This study shows 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 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 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.

Key words: Traditional Medicine, Type 2 Diabetes, Complementary and Alternative Medicine, Indigenous Knowledge, Attitude

1. INTRODUCTION

Phyto-medicines have a long history and 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. There is also insufficient data that exist for most plants to guarantee their quality, efficacy and safety. Plants contain hundreds of 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 during cooking or heating. To be effective, they have to be consumed raw.

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 phytomedicines.

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 diabetes mellitus [5].

The review shows 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 were identified as having pharmacological value 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 pPhytochemical 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 6.36^0 LN, 58.08 LW; the current population is of 200 households, where approximately 1800 persons are currently living.
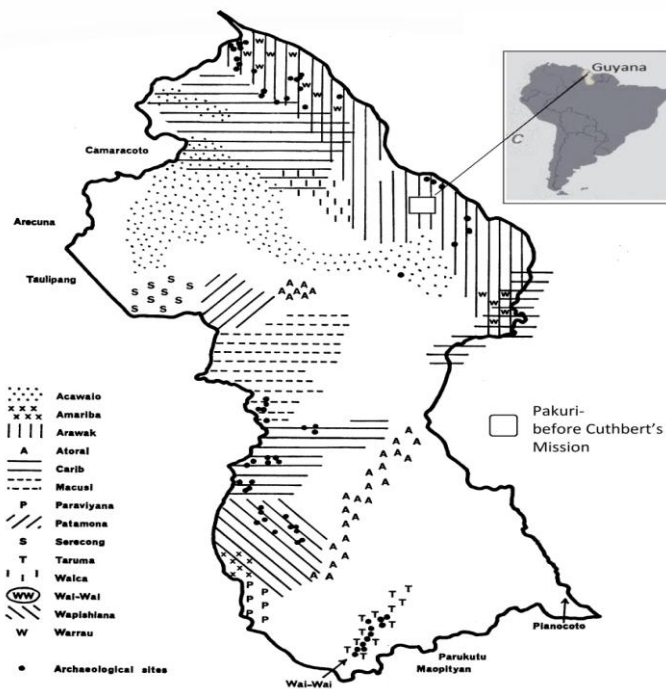
Figure 1. Study Area, modified after Brothwhell (1967)

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 (Figure 1) [6].

The name of the town was given for the abundance of the species named Pakooru *Platonia insignis* from the Botanical Family Guttiferae, an important forestry species with high exploitation since colonial times [7].

2.2 Study sample

Two plants, sour sop (*Annova Muricata L.*) and pear (*Persea Americana Mill.*) were identified as having the most potential since the two were widely used and Mocou-Mocou (*Montrichardia Arborescens*), which is found in the community, was identified in the literature as having anti-diabetic properties.

2.3 Sampling Procedure

Leaves of the three plants identified for testing were collected and preserved for the phytochemical analysis. Specimens collected were in good condition, free of insect damage, rust and/or disease. Pockets were made of newspaper for stem and fruit storage.

Each specimen collected was assigned a unique number (in addition to date, name of collector(s), habitat information and observations(s). Specimens were pressed right away and were marked on the newspaper in a corner with a permanent marking pen. Specimens collected were sprinkled with rubbing alcohol for preservation. Plant specimens were laid between sheets of newspaper separated by corrugated plates, then placed in a pressing frame, which was then tightened with straps/rope. Plant press was cinched daily as press became loose as plants dried. Plants were pressed from the time they were collected from the forest to when they were carried to be dried (approximately one (1) month).

After the specimens were dried, they were mounted on acid-free mounting paper. Large specimens were cut, using a pair of pruning shears, to fit the herbarium sheets (important parts were not cut off/discarded). Specimens were held down with weights on the mounting paper and thin strips of mounting tape were cut and attached from the paper, across part of the plant and back to the paper.
Labels were prepared before identification was made and included information available at that time. Labels were glued to the bottom right of the herbarium sheets after specimens were mounted. The identification of the specimens was done using literature of regional floras, which was then verified by a botanist and several tree spotters [8].

2.4 Data collection

Preparation of Extracts

The leaves for testing of the sour sop plant (*Annova Muricata*), pear (*Persea Americana*) and Mucuo-Mucuo (*Montrichardia Arborescens*) were collected in paper bags and air dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve no.40 and stored in an airtight container for the extraction. The collected, cleaned and powdered leaves were used for the extraction purpose. 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.

Qualitative phytochemical analysis

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

Determination of Alkaloids

Few quantity of the each portion 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; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [9].

Determination of Flavonoids

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

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

Determination of Saponins

Foam Test: One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 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].

Determination of Tannins

Braemer's test: About 0.5 g each portion 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 occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [10].

Determination of Amino acids

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

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 colour at the interphase of the two layers was a positive test [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].

α-Amylase Inhibition Activity [13]

The assay was carried out following the 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 was dissolved in DMSO in order 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:

The α-amylase inhibitory activity = (Ac +) − (Ac −) − (As − Ab)/(Ac +) − (Ac −) × 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

Phytochemical analyses of the three plant extracts

Table 3.1 shows the different classes of chemical compounds present in pear, sour sop and mucou mucou for comparison.

CLASS OF COMPOUNDS	TESTS PERFORMED	RESULTS					
		PEAR		SOUR SOP		MUCOU MUCOU	
		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

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 pear, sour sop and mocou mocou 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. Flavanoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals. Flavanoids 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]. Studies have also shown that saponins stimulate secretion, the action of insulin, regeneration of beta cells islets and activate the enzymes, which are responsible for glucose utilization [20].

Within this study alkaloids were present in all the chloroform extracts and in the ethanol extract of *P. americana* (pear). A large number of alkaloids have been isolated from numerous medicinal plants and investigated by the researchers for their possible anti-diabetic activity [21]. Glycolysis is the hub of carbohydrate metabolism because virtually all sugars ultimately can be converted to glucose *via* a series of 10 reactions with three regulatory steps catalysed 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 *Catharanthus 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 dispiutase, catalase, reduced glutathione and glutathione-S-transferases [25]. Therefore, the importance of phytochemicals cannot be understated in the fight against diabetes mellitus.

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α - Amylase Inhibition Assay of Plants extracts

Table 3.2: α- amylase inhibition of Ethanol extract of *P. americana* leaves

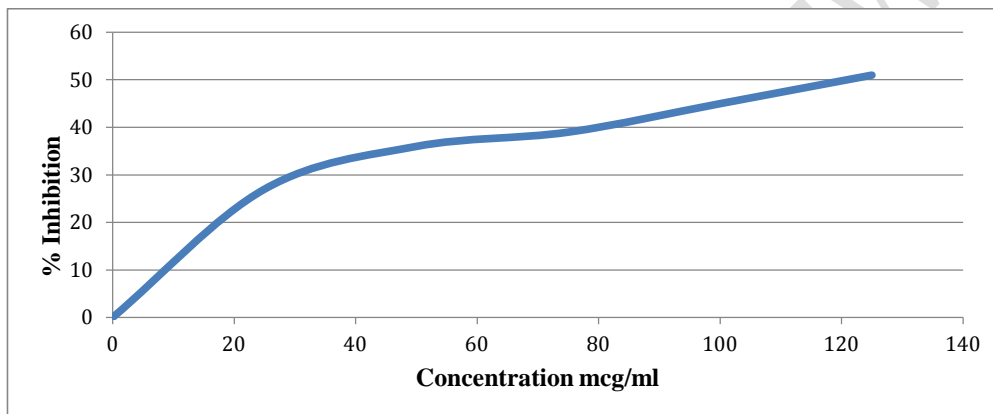| Concentration (μg/ml)	Percentage Inhibition (%)
0	0
25	27
50	36
75	39
100	45
125	51

Figure 3.1: α- amylase inhibition of Ethanol extract of *P. americana* leaves

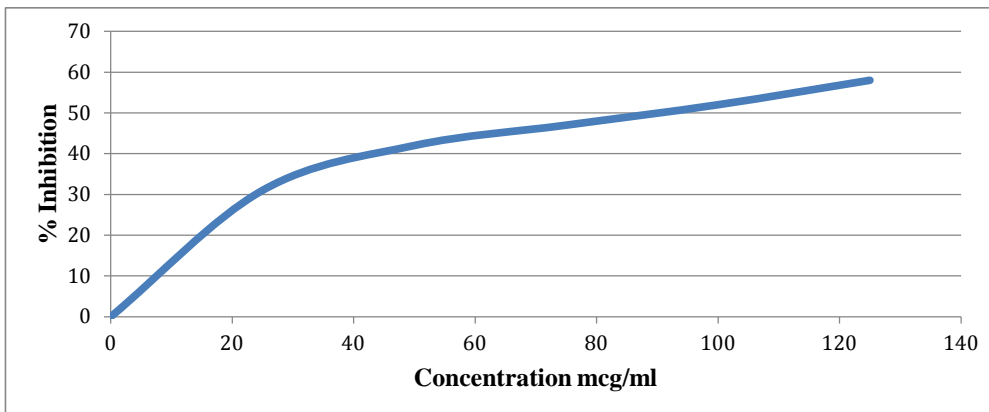

Figure 3.1 shows the concentration –dependent curve for the inhibitory action of α- amylase on an ethanol extract of the plant, *P. americana* leaves. The data are presented in table 3.2. The results show that α- amylase can inhibit the ethanolic extract of *P. americana* leaves in a dose –dependent manner.

Table 3.3: 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

Figure 3.2: α- amylase inhibition of Ethanol extract of *A. muricata* leaves

Figure 3.2 shows the concentration –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 3.3. The results show that α- amylase can inhibit the ethanolic extract of *A. muricata* leaves in a dose –dependent manner.

Table 3.4: 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

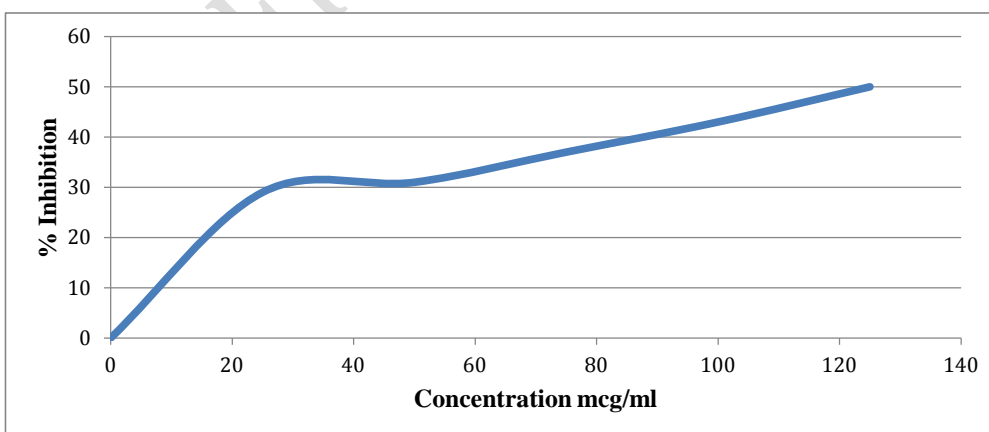Figure 3.3: α- amylase inhibition of Ethanol extract of *M. arborescens* leaves

Figure 3.3 shows the concentration –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 3.4 for comparison.

The results show that α- amylase can inhibit the ethanolic extract of *M. arborescens l*eaves in a dose – dependent manner

Table 3.5: 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

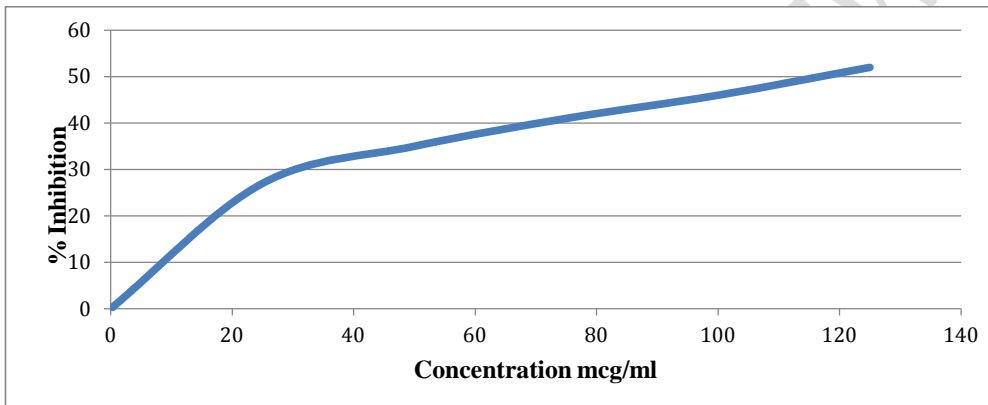Figure 3.4: α- amylase inhibition of Chloroform extract of *P. americana* leaves

Figure 3.4 shows the concentration –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 3.5 for comparison. The results show that α- amylase can inhibit the chloroform extract of *P. americana l*eaves in a dose –dependent manner.

Table 3.6: 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

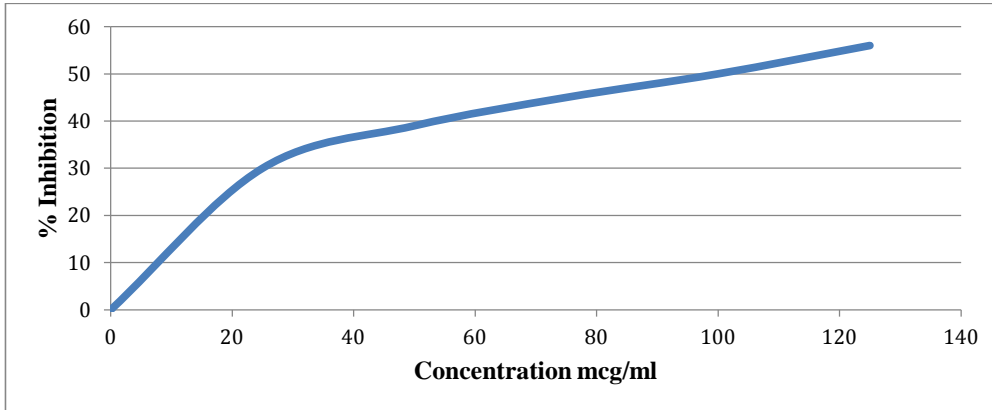Figure 3.5 α- amylase inhibition of chloroform extract of *A. muricata* leaves

Figure 3.5 shows the concentration –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 3.6 for comparison. The results show that α- amylase can inhibit the chloroform extract of *A. muricata* leaves in a dose – dependent manner.

Table 3.7: 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

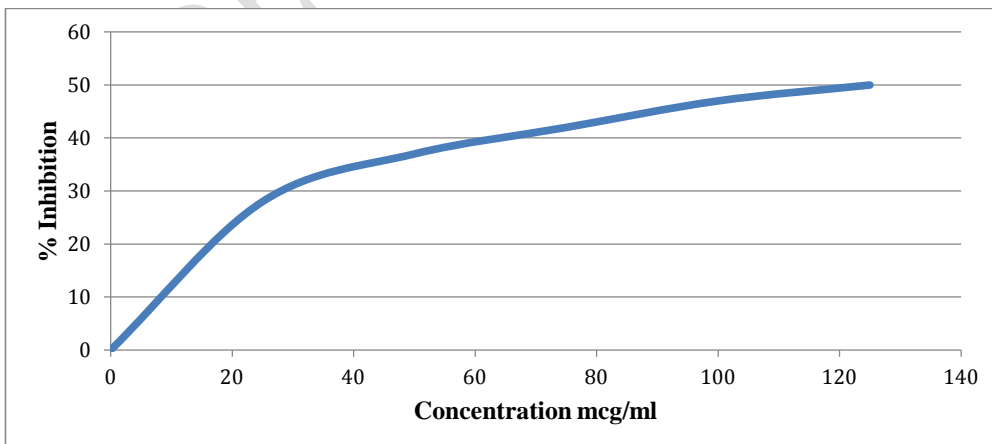Figure 3.6: α- amylase inhibition of Chloroform extract of *M. arborescens* leaves

Figure 3.6 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 3.7 for comparison. The results show that α- amylase can inhibit the chloroform extract of *M. arborescens* leaves 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 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 these plants 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 drugs, thereby, reducing the dose of 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.

ETHICAL APPROVAL

Formal letter of approval was obtained from the 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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