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Potential Antidiabetic Effects of Extracts from Four Medicinal Plants Used in Burkina Faso by Inhibition of Alpha-Amylase

Judith N. Semporé [1], Mamounata Diao [1], Lassina Ouattara [2], Paulin Ouoba [2], Windmi Kagambega [2], Hemayoro Sama [1,3], Crépin I. Dibala [1], Kiessoun Konaté [4] and Mamoudou H. Dicko [1,*]

[1] Laboratory of Biochemistry, Biotechnology, Food Technology and Nutrition (LABIOTAN), Université Joseph Ki-ZERBO, Ouagadougou 09 BP. 848, Burkina Faso; semporej@gmail.com (J.N.S.); mamounadiao@yahoo.fr (M.D.); hemayorosama@yahoo.com (H.S.); dibalacrepin@gmail.com (C.I.D.)
[2] Training and Research Unit in Science and Technology, Université Nazi-BONI, Bobo-Dioulasso 01 BP. 1091, Burkina Faso; lasouatt@yahoo.fr (L.O.); ouobapaulin@hotmail.com (P.O.); kagambegawindmi@yahoo.fr (W.K.)
[3] Laboratory of Biochemistry and Applied Chemistry (LABIOCA), University Joseph Ki-Zerbo, Ouagadougou 09 BP. 848, Burkina Faso
[4] Training and Research Unit in Applied Sciences and Technologies, University of Dédougou, Dédougou 09 BP. 176, Burkina Faso; mehekiessoum@yahoo.fr
* Correspondence: mamoudou_dicko2004@yahoo.fr; Tel.: +226-7027-2643

Abstract: Background: The purpose of this study was to evaluate α-amylase inhibitory effects of hydroethanolic extracts of bark from *Daniella oliveri*, *Sclerocarya birrea*, *Maranthes polyandra*, and *Pteleopsis suberosa* to fight type-II diabetes. Methods: Compound extractions were performed by hydroethanol maceration followed by liquid-liquid fractionation with solvents. TLC profiling was carried out with different fractions. The inhibitory effects of plant extracts on α-amylase activity were determined using rice starch as a substrate. Results: TLC profiling of different fractions showed different phytochemical compounds. The hydroethanolic plant extracts exhibited dose-dependent inhibition of α-amylase. *D. oliveri* displayed competitive inhibition, *M. polyandra* and *S. birrea* showed uncompetitive inhibition and *Pteleopsis suberosa* exerted mixed-inhibition. *M. polyandra* extract exerted the highest inhibitory effect (IC50 = 0.5 mg/mL). Conclusions: The barks of *M. polyandra exhibit* a remarkable α-amylase inhibitory effect which can be a novel source of antidiabetic molecules.

Keywords: phenolic compounds; α-amylase inhibition; *Sclerocarya birrea*; *Maranthes polyandra*; *Daniella oliveri*; *Pteleopsis suberosa*

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1. Introduction

In just few years, type-II diabetes has become a real public health problem. Its prevalence is increasing worldwide at an alarming rate due to modern lifestyle changes [1]. According to estimates, 463 million subjects aged 19–79 years had diabetes worldwide in 2019 [2]. About 9.3% of the world's adult population has diabetes (9% for women and 9.6% for men) with increasing level according to age (18.8% after 65 years) and strong regional disparities [2]. Diabetes kills about 1.9 million people per year and it is progressing very rapidly in Africa. Indeed, in 2017, diabetes killed more than 300,000 Africans and it is projected that 41 million people will have diabetes in 2045 in the continent [3]. In 2025, developing countries may count for 75% of World diabetic patients [4].

Indeed, diabetes is a serious, non-communicable and chronic disease characterized by an increase in blood glucose when the pancreas does not produce enough insulin or when the body cannot effectively use insulin in order to allow its anabolism to glycogen [5,6]. Among strategies to combat diabetes, one of therapeutic approaches is to prevent post-prandial hyperglycemia through an inhibition of carbohydrate degrading enzymes such as amylases. α-Amylase (α-1,4-D-glucan 4-glucanohydrolase, EC 3.2.1.1) is an endo-enzyme

that plays a key role in the digestion of starch and glycogen into maltodextrins. Its inhibition is considered as a strategy for the treatment of carbohydrate absorption disorders, such as diabetes and obesity [7]. Slow digestion of carbohydrates by inhibition of enzymes such as α-amylase would result in a reduction in blood glucose level and therefore may be efficient as a therapeutic strategy or prevention of diabetes. During digestion, α-amylase splits starch into oligosaccharides which will be further hydrolyzed by R-enzyme (α-dextrin endo-1,6-α-glucosidase) [pullulan 6-α-glucanohydrolase, EC:3.2.1.41], iso-amylase [glycogen 6-α-D-glucanohydrolase], or α-glycosidase [α-1,4-D-glucan-glucosidase, EC:3.2.1.20] to glucose. The latter is absorbed in the enterocytes to reach the liver through the portal vein or pass into the general bloodstream via hepatic veins. Inhibitors of α-amylase delay the digestion and subsequent utilization of starch, thereby lowering postprandial glucose level. Enzymatic inhibitors already used in clinics include acarbose, miglitol, voglibose, etc. However, they have serious side effects such as bloating and abdominal pain [8,9]. In addition, the cost of treatment by modern medicine is very high and beyond the reach of the majority of African populations, who are generally resorted to medicinal plants for their health [7]. Medicinal plants are important sources of chemical constituents, which may have α-amylase inhibitory effects and can be used as a therapeutic for functional food source [7]. Interestingly, some African medicinal plants are important sources of phytochemicals that may inhibit α-amylase activities and may be an alternative source of therapeutic or functional food. Therefore, the intensification of search on medicinal plants is encouraged and supported by health-promoting institutions [10].

The aim of this study was to screen some plants used in traditional medicine in Burkina Faso in order to prevent or challenge type-II diabetes as new sources of α-amylase inhibitors. Thus, the inhibitory effects of hydroethanolic extracts of *Daniella oliveri*, *Sclerocarya birrea*, *Maranthes polyandra*, and *Pteleopsis suberosa* on porcine pancreas α amylase was evaluated.

2. Materials and Methods

Porcine pancreas α -amylase [α-1,4-D-glucan 4-glucanohydrolase, EC 3.2.1.1], *3,5*-dinitrosalicylic acid (DNS), and rice starch were obtained from Sigma-Aldrich (Paris). Double sodium/potassium tartrate, sodium dihydrogeno-phosphate and other chemicals were purchased from Merc (Darmstadt, Germany).

2.1. Plant Materials

The plant materials of our study were barks from *Daniella oliveri*, *Sclerocarya birrea*, *Maranthes polyandra*, and *Pteleopsis suberosa*, which were collected in classified forest of Nasso (west Burkina Faso), located 30 km from Bobo-Dioulasso, in February 2016 in accordance with national guidelines of Burkina Faso. Since used plant materials are from public forest, no permission was required to collect the barks of these plants. In addition, used plant materials for scientific research are in accordance with national legislation according to national law for research (Law 038-AN of 26-12-2013).

Plant identification and authentication were made at the Laboratory of Biology and Ecology of the Department of Biological Sciences of Université Nazi-BONI (Bobo-Dioulasso). The voucher specimens with numbers: UNB 935 for *D.*; UNB 936 for *S. birrea*, UNB 934 for *M. polyandra* and UNB 933 for *P. suberosa* were deposited at the Herbarium of Science and Technology Training and Research Unit, Université Nazi-BONI (Bobo-Dioulasso). Then, samples were packed in air-tight bags, transported to the laboratory, dried at 25 °C with a controlled dryer (1100 VISMARA) to a moisture content of 5–7% (*w*/*w*), and finally ground into flour with a microanalytical mill to pass a screen of 0.5 mm.

2.2. Preparation of Crude Hydroethanolic Extract

The extraction method [11] was carried out as follow: hydro-ethanolic extracts were prepared by soaking 50 g of bark powder in 250 mL of hydro-ethanolic solution (70%, *v*/*v*) at room temperature for 48 h. The extracts were filtered through a 0.2 µm Whatman N°1

paper. The hydroalcoholic extracts were concentrated using rotavapor (Buchi Rotavapor R-200, Berne, Switzerland) with water bath set at 40 °C.

2.3. Fractionation of Crude Hydroethanolic Extracts

Crude extracts were fractionated using 2.5 g of hydroethanolic extract in 50 mL of distilled water. The resulting mixture was subjected to liquid-liquid separation with solvents in order of increasing polarity (hexane, ethyl acetate and butanol) for 20 min. The two organic phases (ethyl acetate and butanol) and the aqueous phase were dried in oven at 40 °C.

2.4. Separation and Analysis of Chemical Compounds by Thin-Layer Chromatography (TLC)

Silica gel on aluminum plates were used for TLC. After sample applications, elution by capillarity was performed using mobile phase solvent system of chloroform/ethyl acetate/formic acid/acetic acid, at 2.5/1.5/0.2/0.8 ($v/v/v/v$).

After migration, compounds were revealed with NEU reagent (mixture of 1%, w/v diphenyl-boryl-oxy-ethylamine in methanol and 5%, w/v poly-ethylene glycol in ethanol) with or without the visualization with 254/365 nm UV lamp to identify their indicative chemical nature [12]. For phenolic compounds, gallic acid, caffeic acid, kaempferol and catechin were used as standards.

2.5. In Vitro α-Amylase Inhibitory Effects

α-Amylase inhibitory effect was determined by measuring the reduction power of dinitrosalicylate (DNS) on released reducing oligosaccharides during starch splitting by the endo-enzyme α-amylase with slight modifications. Reaction mixtures containing 200 µL of increasing concentrations of plant bark extracts and 200 µL of enzymatic solution were incubated at 25 °C for 10 min. Thereafter, 0.80 mL of gelatinized rice starch solution (1% in 20 mM phosphate buffer pH 6.9) was added in each mixture and incubated at 40 °C for 30 min. The reaction was stopped by addition of 1 mL of DNS reagent (43.8 mM of 3,5-dinitrosalicylic acid, 1.06 M of potassium sodium tartrate tetrahydrate and 0.40 M of NaOH) followed by heating in boiling water bath for 10 min. After cooling, the reaction mixtures were diluted with 10 mL of distilled water and absorbances were read at 540 nm with UV-visible spectrophotometer (Thermo scientific Helios Epsilon). The amount of released reducing carbohydrates was determined using maltose as standard. Blanks were similarly prepared by replacing enzyme solution with buffer. A control of enzyme activity was prepared in parallel by replacing plant extract with 200 µL of phosphate buffer pH 6.9. α-Amylase inhibitory effects were expressed according to the following formula:

$$\text{Inhibition } (\%) = \frac{[\text{Control} - (\text{control} + \text{bark extract})]}{\text{Control}} * 100$$

IC50 (concentration of inhibitors that produces 50% inhibition of the initial rate of reaction) values were calculated using regressing analysis.

2.6. Determination of α-Amylase Inhibition Model

The inhibition type was determined by analysis of Lineweaver and Burk double reciprocal plots [13]. For this, initial velocities (V_0) were determined with rice starch concentrations ranging from 0.5 to 2.5 mg/mL with or without plant extract. Roughly, 200 µL of plant extract (0.5 mg/mL) were incubated with 0.20 mL of porcine α-amylase solution at 25 °C for 10 min. In parallel experiment 200 µL of phosphate buffer (pH 6.9) was also incubated with 200 µL of α-amylase. Afterwards, 0.8 mL of increasing concentrations of starch solution (0.5–2.5 mg/mL) were added to both sets of reaction mixtures to start the reaction. Enzymatic reactions were followed using routine assays described above by converting released reducing sugars into Vo. Double reciprocal (1/Vo) versus 1/[So] of Lineweaver–Burk were plotted. These data were used to determine the enzyme kinetic parameters (Km, Vm and Ki) and kinetic inhibition models.

2.7. Statistical Analyses

Data analyses were performed using SPSS version 25. All assays were carried out in triplicate and values were presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate the statistical differences between different inhibitory concentrations followed by the Student's *t*-test. The level of significance for analyses were set to $p < 0.05$. Regression modelling was used to estimate the inhibitory concentration 50 (IC50). Both Kendall's τ (*tau*) and Spearman's ρ (*rho*) were calculated as non-parametric correlation tests to measure ordinal association between levels of extracts and proportion of inhibition.

3. Results

3.1. Phytochemistry Analysis of Extracts

Several groups of chemical compounds (Figure 1) from *Sclerocarya birrea* (Figure 1A), *Maranthes polyandra* (Figure 1B), *Daniella oliveri* (Figure 1C), *Pteleopsis suberosa* (Figure 1D), were detected by TLC after revelation with NEU reagent and visualization at 365 nm with UV lamp. In order to optimize the extraction of chemical compounds, different solvents were specifically used for each plant (Table 1). The presence of chemical compounds was assessed by final coloration in green, blue and yellow, that indicate the presence of multiple classes of secondary metabolites.

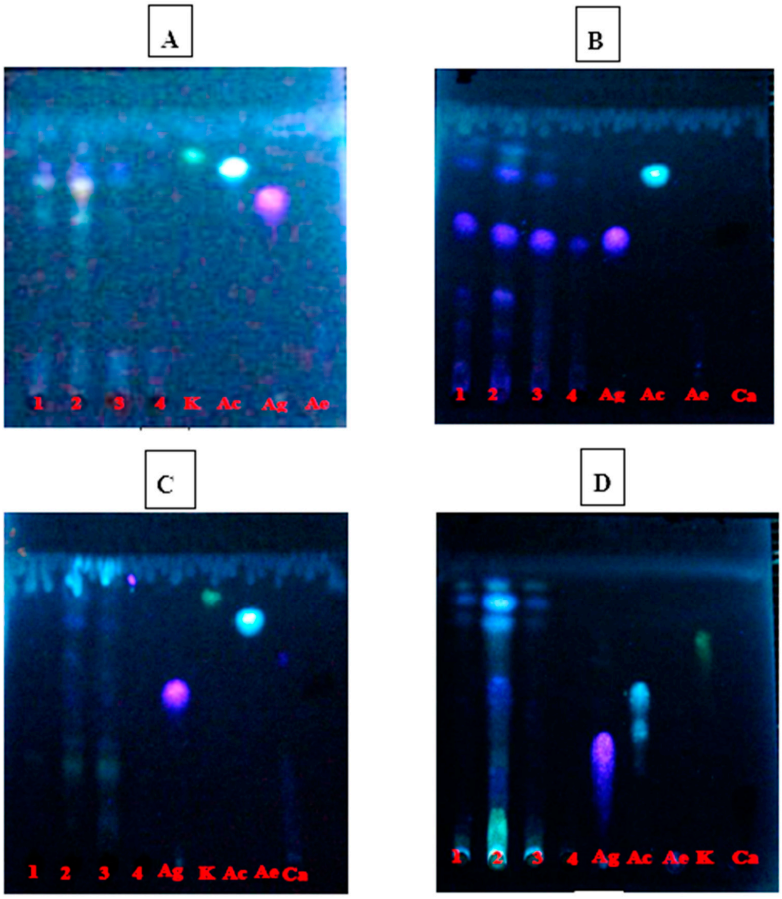

Figure 1. Thin Layer Chromatography (TLC) of extracts from *S. birrea* (**A**), *M. polyandra* (**B**); *D. oliveri* (**C**); and *P. suberosa* (**D**). TLC plates were revealed with UV radiation at 365 nm after spraying of NEU reagent. Applied samples or standards were (1) crude extract, (2) ethyl acetate fraction, (3) butanoic fraction, (4) aqueous fraction. Phenolic standards were kaempferol (K), caffeic acid (Ac), gallic acid (Ag) and ellagic acid (Ae).

Table 1. Eluent composition.

Plants	Eluant
Sclerocarya birrea	Chloroform/Ethyl acetate /Methanol/ formic acid (3.5/0.5/0.8/0.2)
Maranthes polyandra	Chloroform/Ethyl acetate/Methanol/formic acid (3.5/0.5/0.8/0.2)
Daniella oliveri	Chloroform/Ethyl acetate/formic acid/glacial acetic acid (2.5/1.5/0.2/0.8)
Pteleopsis suberosa	Dichloromethane/ethyl acetate/glacial acetic acid (3.8/1/0.2)

3.2. Effects of Extracts on α-Amylase Activity

Hydroethanolic extract of four selected plants were investigated for their ability to inhibit α-amylase activity at different doses ranging from 50 µg/mL to 5 000 µg/mL. The magnitude of inhibition depended on the nature of the extract and concentrations used with group comparisons including negative control (NC) exhibiting a p value < 0.05 for all extracts. It appeared that hydroethanolic extracts of *D. oliveri*, *S. birrea*, *M. polyandra* and *P. suberosa* exhibited remarkable α-amylase inhibition (Table 2). However, according to their IC50 values, they exhibited different degree of α-amylase inhibitory effects using rice starch as substrate. *M. polyandra* extract exerted the highest inhibitory effect (IC50 = 0.5 mg/mL) followed by *D. oliveri* and *S. birrea* with the IC50 values of 0.75 mg/mL and 0.98 mg/mL, respectively. In addition, *M. polyandra* extract gave total inhibition of the enzyme at 2.5 mg/mL in the reaction medium. The lowest inhibitory effect was obtained with *P. suberosa* which displayed IC50 above 1.6 mg/mL. Data also showed that the inhibition of α-amylase was positively correlated to the different concentrations of the extracts tested with significant correlations for the concentrations of 250 µg/mL, 500 µg/mL, 1000 µg/mL, and 2500 µg/mL extracts (Table 3).

Table 2. Alpha amylase inhibitory effects and IC50 values.

Extract	Extract Concentration (µg/mL)							Calculated IC50 (µg/mL)
	50	250	500	1000	2500	3300	5000	
	Proportion of Inhibition (%)							
Daniella oliveri	32.52± 1.72 *	36.33 ± 0.34 **	47.05 ± 1.62 ***	78.61 ± 2.02 ***	100 ± 00 ***	100 ± 00 **	100 ± 00 **	757.75 ± 38.96 **
Sclérocarya birrea	27.4 ± 8.43 *	35.27 ± 1.69 **	41.99 ± 0.34 **	64.04 ± 16.93 **	85.38 ± 6.74 **	100 ± 00 **	100 ± 00 **	981.78 ± 227.63 **
Maranthes polyandra	37.18 ± 3.54 **	38.89 ± 5.06 **	58.33 ± 0.34 ****	81.14 ± 16.81 ****	100 ± 00 ***	100 ± 00 **	100 ± 00 **	495.69 ± 40.95 *
Pteléopsis suberosa	40.63 ± 5.06 **	29.69 ± 3.22 *	30.67 ± 8.6 *	46.76 ± 3.47 *	67.95 ± 8.36 *	60.94 ± 5.13 *	88.78 ± 10.42 *	1605.42 ± 152.93 ***

Each value represents the mean ± SEM (Standard Error of the Mean). IC50 refers to the concentration of inhibitors that produces 50% inhibition of the initial rate of reaction. Values in the same column with different superscripts are significantly different (p < 0.05). Values with the same symbol *, **, ***,**** in the columns are not statistically different.

Table 3. Correlations tested using two non-parametric correlation tests: Kendall and Spearman.

Plant Extract	Correlation Coefficients		Significance (p Value)
	Kendall's τ (*tau*)	Spearman's *rho*	
Daniella oliveri	0.926	0.964	0.01
Sclerocarya birrea	0.976	0.991	0.01
Maranthes polyandra	0.976	0.991	0.01
Pteleopsis suberosa	0.714	0.857	0.05

3.3. Type of Inhibition of Hydroethanolic Extracts on α-Amylase

Lineweaver Burk's representations (Figure 2) allowed to calculate kinetic parameters such as Vm, Km, and Ki (Table 4). Using rice starch as substrate, end-point kinetic studies revealed that control α-amylase had Km of 2.64 M and Vm of 4.52 M/min. Kinetic studies in presence of plant extracts revealed that not only the extracts exhibited inhibition by different fashions and that extract of *P. suberosa* affected the enzyme velocity (2.83 M/min) proportionally to extract concentration with a Ki value of 1.45 mg/mL, but did not affect the Km value, indicating a non-competitive inhibition model. The Vm of α-amylase remained unchanged in the presence of *D. oliveri* extract, whereas its Km increased from 2.64 M to 9.86 M. This type of inhibition fits with competitive-type fashion on α-amylase with calculated Ki of 0.44 mg/mL. Calculated kinetic parameters gave values of Km (0.76 M and 1.03 M) and Vm (1.32 M/min and 1.76 M/min) of the inhibited enzyme by *S. birrea* and *M. polyandra* extracts, respectively. Extracts of *S. birrea* and *M. polyandra* all showed an uncompetitive-type inhibition fashion with Ki of 0.43 mg/mL and 0.62 mg/mL, respectively.

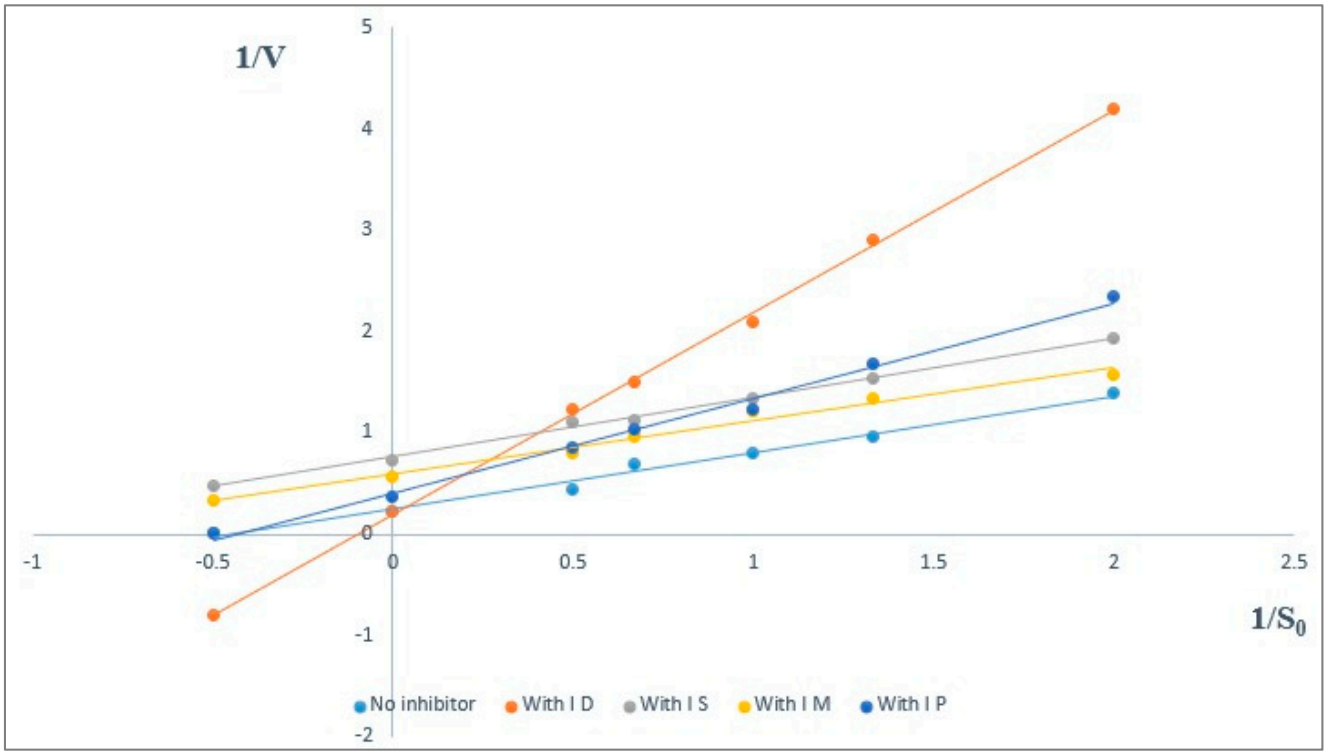

Figure 2. Lineweaver–Burk plots of α-amylase alone or with inhibiting extracts from *S. birrea* (IS); *M. polyandra* (IM); *D. oliveri* (ID); and *P. suberosa* (IP).

Table 4. Inhibition type of the four medicinal plants.

Extract	Km (mol/L)	Vm (mol/L/min)	Ki (mg/mL)	Inhibition Type
Without Extract	**2.64**	**4.52**		
With *D. oliveri*	9.86	4.52	0.44	Competitive
With *S. birrea*	0.76	1.32	0.43	Uncompetitive
With *M. polyandra*	1.03	1.76	0.62	Uncompetitive
With *P. suberosa*	2.64	2.83	1.45	Mixed

4. Discussion

Many plants are traditionally used in the management or prevention of diabetes. These species are believed to inhibit hydrolytic enzymes and decrease glucose level in the blood, thus ensuring human prevention against hyperglycemia [14–17]. Therefore, it was interesting to investigate on experimental basis some medicinal plants traditionally used to prevent of combat diabetes mellitis. Indeed, *D. oliveri*, *S. birrea*, *M. polyandra*, and *P. suberosa* used in folk medicine were never screened for this purpose. The present work was the first to show that the hydroethanolic extracts of the bark of these plants have α-amylase inhibitory effects. The magnitude of inhibition of α-amylase are close to *Salacia oblonga* aqueous stem extract effect on α-amylase and α-glucosidase inhibition [18]. However, different results were observed with the hexane and chloroform fractions of *Swertia longifolia* aerial parts [19]. *M. polyandra* extract from our study exerted the highest inhibitory effect (IC50 = 495.69). However, the IC50 values were vastly higher than those (61.17 µg/mL) obtained with ethanol extract of *Abrus precatorius* leaves [20] and those (17.22 µg/mL) of *Croton bonplandianum* leaves [21]. The differences in inhibitory strengths observed with the four extracts can be justified by the botanical differences among these plants and also the purity of the extracts. The study also revealed a correlation between inhibitory effects and plant extract concentrations as previously found elsewhere [22]. The dose-dependent effect observed in our study. suggested uncompetitive type of inhibition for extract from *M. polyandra*. Reversible enzyme inhibitors can act according to various mechanisms, combining either with the enzyme (competitive with the substrate), or with the enzyme-complex substrate (uncompetitive) or with both cases (mixed) [23,24]. This hypothesis is confirmed by the Lineweaver–Burk plots which showed three different types of inhibition for the four extracts.

TLC qualitative analysis showed a diversity in phenolic acid and flavonoid profiles depending on the extract. *M. polyandra* showed the presence of several blue spots indicating a high presence of simple phenolics and yellow and green colors are characteristic of flavonoids. TLC showed an abundant content of several phenolic acids and flavonoids in *D. oliveri*. These results are similar to those found by other authors [25] who showed the presence of phenols and flavonoids in *D. oliveri* extracts. Other authors found the abundance of flavonoids in the extracts of *M. polyandra* [26]. The inhibitory effect of α-amylase by these plant extracts may be undoubtedly due to the presence of these detected bioactive compounds. The difference in the mechanism of action of extracts may be related to their chemical composition. Many authors have attributed inhibitory effect by the presence of phytochemicals such as polyphenols, saponins, triterpene, steroids, palmitic acid ester, etc. [27]. Indeed, the inhibitory activity of an extract may depend on several factors including its chemical composition which determine its mechanism of action. The chemical medicinal compounds, such as acarbose, which are similar in form to oligosaccharides derived from starch digestion, can bind to the sites of pancreatic α-amylase and inhibit it potently in a competitive and dose-dependent manner. Some plants may have enzyme inhibitory activity including polyphenolic compounds and glycoproteins [28]. Many of polyphenols such as tannins are able to bind to and inhibit digestive enzymes.

5. Conclusions

The hydroethanolic extracts of the four medicinal plants, i.e., *Daniella oliveri*, *Sclerocarya birrea*, *Maranthes polyandra*, and *Pteleopsis suberosa* plants displayed dose-dependent α-amylase inhibitory effects. The analysis of extracted chemical groups indicated that the inhibitors are mainly phenolic compounds. The kinetic study of enzymatic reactions in the presence of the extracts of these plants shows that these extracts exhibit different types of inhibition models on α-amylase. In particular, the barks of *M. polyandra* may be candidate sources for end-use, not only to cure but also to prevent diabetes mellitis through inhibition of starch degrading enzymes such as α-amylase.

Author Contributions: M.H.D. and L.O. designed the idea. P.O. and K.K. selected the plants. J.N.S. W.K., H.S. and M.D. performed the experiments. P.O. identified and authenticated the plants. J.N.S. analyzed the data, interpreted the data, drafted and edited the manuscript. M.H.D. and C.I.D. supplied the chemicals and reagents. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: All authors mentioned no conflict of interest in this research.

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