chemistry

MDPI

Article

Phytochemistry, Anti-Tyrosinase, and Anti-Diabetes Studies of Extracts and Chemical Constituents of *Dicerothamnus rhinocerotis* Leaves

Olusola Ifedolapo Watti [1], Masande Yalo [2], Rajan Sharma [2,†], Masixole Makhaba [2], Ahmed A. Hussein [2] and Wilfred T. Mabusela [1,*]

[1] Department of Chemistry, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa; 3924676@myuwc.ac.za
[2] Chemistry Department, Cape Peninsula University of Technology, Symphony Rd., Bellville 7535, South Africa; yalom@cput.ac.za (M.Y.); rajan.sharma@uct.ac.za (R.S.); makhabam@cput.ac.za (M.M.); mohammedam@cput.ac.za (A.A.H.)
* Correspondence: wmabusela@uwc.ac.za
† Current address: Department of Medicine, Division of Dermatology, Faculty of Health Sciences, University of Cape Town, Cape Town 7700, South Africa.

Abstract: *Dicerothamnus rhinocerotis* (L.f.) Koekemoer, also known as rhinoceros bush and previously called *Elytropappus rhinocerotis* (L.f.) Less., is from the Asteraceae plant family. The plant is traditionally used to treat indigestion, stomach ulcers, influenza, and diarrhea. This study was aimed at investigating the phytochemistry, anti-glucosidase, anti-amylase, and anti-tyrosinase effects of *D. rhinocerotis* as research in this area is limited. The air-dried plant materials were macerated in 80% methanol (MeOH) and fractionated between hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and butanol (BuOH). Column chromatography on silica gel was employed for the isolation of the compounds. A total of six compounds (**1**–**6**) were isolated from the fractions viz. acacetin (**1**), 15-hydroxy-*cis*-clerodan-3-ene-18-oic-acid (**2**), acacetin-7-glucoside (**3**), pinitol (**4**), apigenin (**5**), and β-sitosterol-3-*O*-glycoside (**6**). Compounds **2**–**4** and **6** are reported for the first time from this plant. Among the different fractions, the BuOH and EtOAc fractions had strong tyrosinase inhibitory activities with IC_{50} values of 13.7 ± 1.71 and 11.6 ± 2.68 µg/mL, respectively, while among the isolated compounds, apigenin (**5**) had the strongest inhibitory activity, with an IC_{50} of 14.58 µM, which competes favorably with Kojic acid (17.26 µM). The anti-glucosidase assay showed good activity in three of the fractions and compound **5**, while the anti-amylase assays did not show significant inhibition activity.

Keywords: Asteraceae; *Dicerothamnus rhinocerotis*; phytochemistry; tyrosinase; glucosidase; amylase; diabetes; inhibition

Citation: Watti, O.I.; Yalo, M.; Sharma, R.; Makhaba, M.; Hussein, A.A.; Mabusela, W.T. Phytochemistry, Anti-Tyrosinase, and Anti-Diabetes Studies of Extracts and Chemical Constituents of *Dicerothamnus rhinocerotis* Leaves. *Chemistry* **2024**, *6*, 546–554. https://doi.org/10.3390/chemistry6040032

Academic Editors: Athina Geronikaki, Cosimo D. Altomare and Maria Stefania Sinicropi

Received: 20 April 2024
Revised: 25 June 2024
Accepted: 28 June 2024
Published: 2 July 2024

1. Introduction

Asteraceae or Compositae (commonly referred to as the aster, daisy, composite, or sunflower family) is a very large and widespread family of flowering plants [1,2]. The family has 34,510 accepted species names in 1729 genera [3]. Several species from this family have known medicinal properties. For example, anti-tyrosinase activity was shown in *Bubonium imbricatum*, *Cladanthus arabicus*, *Achyrocline satureioides*, *Artemisia verlotiorum*, *Flourensia campestris*, *Pterocaulon alopecuroide*, and *Tagetes minuta* [4], while *Bubonium imbricatum* and *Cladanthus arabicus* both exhibit good anti-diabetic activity [5].

Dicerothamnus rhinocerotis (L.f.) Koekemoer, also known as rhinoceros bush and renosterbos and previously called *Elytropappus rhinocerotis* (L.f.) Less., is a medicinal plant used in traditional African medicine [6,7]. The plant belongs to the Asteraceae family and is distributed in South Africa and Namibia [6,7]. Rhinoceros bush is a single-stemmed and small shrub averaging 2 m in height. The old branches are rough and have

even greyish barks. The leaves contain feathery white hairs, which gives the plant a greyish general appearance [8]. In traditional medicine, the young tips of the branches are used to treat indigestion, stomach ulcers, dyspepsia, stomach cancer, and fever [9,10]. Additionally, it is used as a bitter tonic to stimulate the loss of appetite and treatment of colic, influenza, diarrhea, and convulsions in children [11]. Furthermore, there are reports regarding the traditional use of the plant to treat type 2 diabetes and reduce high blood pressure [9,10]. Diabetes mellitus is a disease associated with high blood sugar levels, a condition where the body is unable to effectively control the metabolism of glucose, which is the primary source of energy. This high blood sugar presents persistent urination, increased thirst, and hunger as symptoms. Diabetic patients have an increased risk of developing other serious life-threatening health problems, resulting in higher medical care costs, reduced quality of life, and increased mortality [12,13]. Recent data from the International Diabetes Federation (IDF) estimate that 7% of South Africans between the ages of 21 and 79 years have diabetes [14], with the contributing factors being the aging population, economic transition, and urbanization associated with nutrition transition and obesity [15,16]. Diabetes was estimated to cause about 8000 new cases of blindness and 2000 new cases of amputations annually in South Africa [17]. Type 2 Diabetes Mellitus (T2DM) starts with insulin resistance, a condition where the body cells do not respond well to insulin [18]. The common treatment for diabetes includes insulin, pramlintide, and metformin. These drugs have side effects, which include flatulence, fatigue, nausea, weight gain, hypoglycemia, and genitourinary infections [19]. Despite the availability of modern remedies, patients still use alternative medicine (herbs), either alone or in conjunction with conventional medicine, as there is a growing interest in complementary and alternative medicine, especially in the treatment of chronic diseases such as diabetes [20,21]. *Dicerothamus rhinocerotis* has been reported to be used traditionally in treating type 2 diabetes [9,10], which formed the basis of this research, i.e., to determine the activity of this plant and identify the active metabolites that inhibit alpha-amylase and alpha-glucosidase enzymes.

As previously noted, several members of the Asteraceae family are reported to display anti-tyrosinase activities, but reports on *Dicerothamus rhinocerotis* are limited. Tyrosinase is a multi-copper enzyme found in plant and animal tissues and causes the browning of foods and the production of melanin cells in animals. It acts in the catalytic cycle by either hydroxylating monophenols to *o*-diphenols or oxidizing *o*-diphenols to *o*-quinones, which finally form melanin [22,23]. This enzyme is responsible for the browning of fungi, damaged plant tissues, and damaged fruits during handling and processing after harvest. It is also said to be involved in the development and defense mechanism of insects in terms of melanin production, sclerotization, parasite encapsulation, and wound healing [24,25]. The unfavorable actions of tyrosinase in the browning of fruits and vegetables and the hyperpigmentation of the human skin have prompted researchers to find anti-tyrosinase agents, also known as tyrosinase inhibitors, especially in the food and cosmetic industry [24]. These inhibitors target tyrosinase activities, stopping the production of melanin in the melanocyte. Many cosmetic companies producing skin-lightening creams use these inhibitors as they are the most commercially available skin-whitening agents [26,27]. Commonly used anti-tyrosinase, kojic acid, and hydroquinone have been reported to cause oxidative damage of lipids and permanent loss of melanocytes in the skin after long use and have thus been banned in many countries; therefore, the need for safe tyrosinase inhibitors cannot be overemphasized [27].

Regarding the phytochemistry of the plant, cardiac glycosides, tannins, saponins, and reducing sugars were reported [7]. Lipophilic resins were reported to be 20% of its dry mass, while 80% of the raw extract of dried plant materials comprised methoxylated flavones, cirsimaritin, hispidulin, eupafolin, and quercetin [7]. Benzoic acid and its derivatives (protocatechuic acid, veratric acid, and *p*-hydrobenzoic acid), as well as cinnamic acid derivatives (sinapic acid, *p*-coumaric acid, and ferulic acid), were also identified [28,29]. In a more recent study, 6,7-dimethoxycoumarin, 4′,5,7- trihydroxyflavone, 5,7-dihydroxy-4′-

methoxyflavone, 5,7-dihydroxy-4′,6-dimethoxyflavone, kaempferol, 3-methyl ether, (+)-13-epilabdanolic acid, (+)-*ent*-labdanolic acid, (+)-methyl 13-epilabdanolate, and (+)-(8*R*,13*R*)-labdan-8,15-diol were isolated [30]. Also, the isolation of (+)-13-epilabdanolic acid, (+)-ent-labdanolic acid, and *ent*-labd-13-en-8-β-hydroxy-15-oic acid from the leafy stems of the plant was reported [31]. It was observed that the flavonoids isolated from this plant are methoxylated [30], while rhinoterotinoic acid shows anti-inflammatory activity [9–11].

This study aims to investigate the phytochemicals from *D. rhinocerotis* and evaluate their anti-diabetic and anti-tyrosinase activities to substantiate the ethnopharmacological claims.

2. Materials and Methods

2.1. Plant Material

The leaves of *Dicerothamnus rhinocerotis* were collected on 20 May 2019 at Kirstenbosch National Botanical Gardens, Cape Town, South Africa, where it was identified by a taxonomist (Voucher number: OW-2019-01). It was air-dried at room temperature for 10 days and ground into a powder. The resulting plant material was weighed.

2.2. Equipment and Chemical Reagents

The NMR spectra (1D and 2D) were recorded on the Avance 400 MHz NMR spectrometer (Bruker, Rheinstetten, Germany) at 400 MHz and 100 MHz for Proton (^{1}H) and carbon (^{13}C), respectively. The chemical shifts (δ) were reported in parts per million (ppm) and coupling constants (*J*) in Hz. The ^{1}H and ^{13}C NMR values were relative to the internal standard tetramehtylsilane (TMS) and were acquired in deuterated solvents (chloroform, water, or DMSO). Column chromatography (CC) was performed using normal-phase silica gel, while thin layer chromatography (TLC) was performed on silica gel aluminum sheets (Silica gel 60 F254, Merck, Rahway, NJ, USA). Visualization of the spots on TLC sheets was achieved using vanillin sulfuric acid reagent and a heat gun.

2.3. Extraction and Fractionation of the Plant Material

Powdered *Dicerothamnus rhinocerotis* (1.065 kg) was macerated in 80% methanol (6.0 L × 2) at room temperature for 24 h, and the mixture was filtered and evaporated under pressure to yield 354.69 g (~33%, dry wt). The yield consisted of a sticky mass (240 g) that stuck to the round bottom flask during evaporation (crude MeOH extract) and the freeze-dried crude extract (144.69 g). The dried crude extract (144.69 g) was suspended in water and successively extracted with hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and butanol (BuOH).

The DCM extract (6 g) was fractionated by pre-adsorbing it on silica gel and using CC by gradient elution, starting with 100% hexane, and gradually increasing the polarity with EtOAc until 100%. EtOAc was used; then, MeOH was introduced to complete the elution. A total of 74 fractions collected from the CC were pooled together based on their TLC profiles to yield three fractions (F_1; F_2, and F_3). Fraction F_2 (2.01 g) was further fractionated using CC, resulting in two compounds: compound 1, which appeared as a yellow needle-like crystal, and compound 2, which appeared as white crystals.

The EtOAc extract (13 g) was pre-adsorbed on silica gel and fractionated using CC by gradient elution initiated with 100% hexane and gradually increasing the polarity with EtOAc until 100% EtOAc was reached. Then, MeOH was introduced to complete the elution. The collection of elutes resulted in 171 fractions, which were pooled together based on their TLC profiles, thereby separating them into 9 fractions. Compound 1 was again obtained from fractions F_{30}–F_{42}, while compound **5** was isolated from fractions F_{66}–F_{73}.

The BuOH extract (59 g) was pre-adsorbed on silica gel and fractionated using CC by gradient elution initiated with 100% DCM and gradually increasing the polarity with MeOH to complete the elution, which resulted in 88 fractions. Compounds 3 (pale-yellow) and **4** (off-white) precipitated directly from the fractions F_{23}–F_{24} and F_{43}–F_{46} of the main column.

The crude methanol extract (120 g) was pre-adsorbed on silica gel and fractionated using CC by gradient elution (hexane: EtOAc, 100:0 → 0:100%). This resulted in the isolation of compound **6** as a white precipitate. Compounds **1**, **2**, and **4** were also re-isolated.

The total yields of the isolated compounds were as follows: compound **1** (171.1 mg), compound **2** (3.13 g), compound **3** (3.4 mg), compound **4** (176.8 mg), compound **5** (24.5 mg), and compound **6** (53.6 g).

2.4. Anti-Tyrosinase Inhibition Assay

The mushroom tyrosinase inhibition of the fractions and isolated compounds was determined by a procedure similar to that described by Yalo et al. [32]. For the fractions, 10 mg/mL concentration was prepared for each sample by dissolving 10 mg of the samples in 1 mL DMSO, while for isolated compounds and the positive control (kojic acid), a 0.02 M stock solution was prepared. The stock solutions were diluted to 100 µg/mL and 200 µM for the fractions and compounds, respectively. In a 96-well plate, 70 µL of the test sample was introduced, after which 30 µL of L-tyrosinase (1000 units/mL) was added. The solution was incubated at room temperature (25 °C) for 20 min. After the first incubation, 100 µL of substrate (2 mM of L-tyrosine) was added and incubated for 30 min at the same temperature. The absorbance was read at 490 nm after the incubation. For the control without enzymes, a background solvent (20 µL DMSO in 1980 µL distilled water) was used instead of the test samples, and phosphate buffer (50 mM, 6.5 pH) was used instead of the enzyme. Kojic acid was used as a positive control at various concentrations, and all experiments were conducted in triplicates. To determine the IC_{50}, various concentrations were used for each test sample, after which the results were plotted on GraphPad Prism 8. Percentage (%) inhibition was calculated using Equation (1).

$$\textbf{Inhibition } (\%) = \frac{(\textbf{CE} - \textbf{C}) - (\textbf{SE} - \textbf{S})}{(\textbf{CE} - \textbf{C})} \times \frac{\textbf{100}}{\textbf{1}} \quad (1)$$

where

CE: absorbance of the control with enzymes.
C: absorbance of the control without enzymes.
SE: absorbance of the test sample with enzymes.
S: absorbance of the test sample without enzyme.

2.5. Alpha-Glucosidase Inhibition Assay

The inhibitory activity of the plant extracts and isolated compounds against α-glucosidase from *Saccharomyces cerevisiae* was determined by slightly modifying the method reported by Yamaki and Mori [33]. The reaction mixture of 50 µL of phosphate buffer (50 Mm, pH of 6.8), sample (10 µL, 200 µg/mL), and 50 µL of alpha-glucosidase solution (1 U/mL) was incubated at 37 °C for 15 min in a 96-well plate. After the initial incubation, 20 µL of 5 mM substrate, i.e., *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG), was added to the reaction mixtures in the 96-well plates and incubated at 37 °C for 20 min; then, the enzymatic reaction was terminated by adding 0.1 M sodium carbonate (Na_2CO_3) solution (50 µL). The enzymatic hydrolysis of the substrate was monitored by the amount of *p*-nitrophenol liberated in the reaction mixture at 405 nm using an AccuReader M965 Metertech (V1.11) spectrophotometer. The control without enzymes (C) was prepared by substituting the enzyme with the phosphate buffer, while in the control with the enzyme (CE), i.e., DMSO, which was diluted to the same concentration as the samples, was used instead of the test samples. Acarbose at different concentrations was used as the positive control. All experiments were performed in triplicates, and the % inhibitory activity of the samples on α-glucosidase was calculated using Equation (1). The IC_{50} values of samples with good inhibitions were determined as described earlier.

2.6. Alpha-Amylase Inhibition Assay

In a 96-well plate, 50 µL of phosphate buffer (0.01 M, pH 6.9), 20 µL of porcine pancreatic alpha-amylase (2 U/mL) solution, and the test samples (20 µL) were added and incubated at room temperature for 20 min. Afterward, 20 µL of 1% soluble starch was added to the well plates and incubated further for 30 min at room temperature, after which the reaction was stopped by the addition of 90 µL of 3, 5-dinitro salicylic acid (DNS). The reaction mixture was placed in a boiling water bath for 10 min, and the absorbance was read at 540 nm. Various concentrations of acarbose were used as standards, and each experiment was conducted in triplicate. The percentage inhibitory activity of the α-amylase was calculated using Equation (1).

3. Results and Discussion

Six compounds (Figure 1) were isolated from DCM, EtOAc, BuOH, and the crude lipophilic extracts of *Dicerothamnus rhinocerotis* using CC (Figure 1). The structures were determined by comparing the 1D (^{1}H, ^{13}C, and DEPT-135) and 2D NMR (HSQC and HMBC) spectroscopic data to those from the literature [34–39]. Compounds **2**, **3**, **4,** and **6** are reported for the first time in this plant.

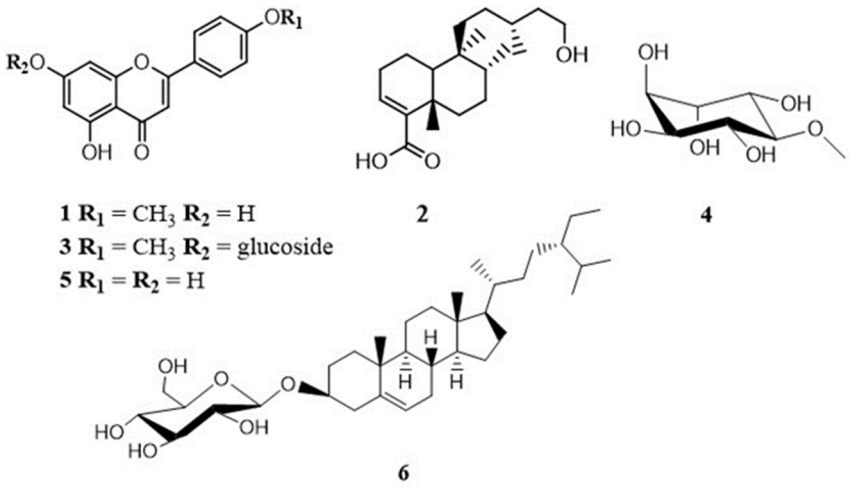

Figure 1. Chemical structure of compounds isolated from *Dicerothamnus rhinocerotis*.

3.1. Structural Elucidation of the Isolated Compounds

The comparison of the NMR spectroscopic data to those formerly published helped elucidate the structure of the isolated compounds as acacetin (**1**) [34], 15-hydroxy-*cis*-clerodan-3-ene-18-oic-acid (**2**) [35], acacetin-7-glucoside (**3**) [36], pinitol (**4**) [37], apigenin (**5**) [38], and beta-sitosterol-3-*O*-glycoside (**6**) [39] (Table S1).

3.2. Tyrosinase Inhibitory Activities of Fractions and Isolated Compounds

The average tyrosinase inhibitory activities of the fractions and isolated compounds were determined at 100 µg/mL and 200 µM, respectively (Table 1). DRE (EtOAc fraction) and compound **5** had strong inhibition values of 67.87% and 67.51%, respectively. The other fractions, as well as compound **6,** had moderate inhibition values, ranging from 30.24% to 50.11%, while compound **2** had a weak inhibition of 12.72%. Compound **1** had a negligible inhibition (0.02%) against mushroom tyrosinase despite having a similar structure to compound **5**; this could be attributed to the activity of the hydroxyl group in the B ring of compound **5** compared to the methoxy group in compound **1**. DRE, the fraction with the most activity, exhibited a similar percentage inhibition as compound **5,** which can potentially be because compound **5** was isolated from the DRE fraction. All the samples were investigated further to determine their IC_{50} values. Compound **5**, DRB (BuOH fraction), and DRE with IC_{50}

values of 14.58 µM, 13.7 ± 1.71 µg/mL, and 11.66 ± 2.68 µg/mL, respectively, and had comparable IC_{50} values to kojic acid, a known strong inhibitor of tyrosinase with an IC_{50} value of 17.26 µM. Compound **5** had an activity similar to those reported in the literature, i.e., 17.3 µM [40] and 38.5 µM [41]. Compound **1** was reported to have an IC_{50} value greater than 2000 [42] and 700 µM [43], while the experimental IC_{50} observed was 1011 µM.

Table 1. Anti-tyrosinase activity screening * and IC_{50} values of samples from *D. rhinocerotis*.

Extracts/Compounds	% Inhibition	IC_{50}	
		(µg/mL)	µM
DRC (Crude)	43.41	42.2	
DRH (Hexane)	36.26	200.1	
DRD (DCM)	40.36	35.1	
DRE (EtOAc)	67.87	11.6	
DRB (BuOH)	44.04	13.7	
DRM (crude MeOH)	50.11	57 ± 2.48	
1	nd	-	1011
2	12.72	-	1552
3	30.42	-	583.3
4	30.24	-	995.6
5	67.51	-	14.58
6	37.24	-	273
Kojic acid	100	-	17.26

nd: not determined due to negative or non-replicable values. The data are presented as means ± SDs (standard deviations). * The screening was performed at 100 µg/mL for fractions and 200 µM for compounds.

3.3. Alpha-Glucosidase Assay

Table 2 shows the inhibition of fractions and compounds isolated from *D. rhinocerotis* against alpha-glucosidase at 200 µg/mL. Compound **5** had the strongest percentage inhibition (94.17%), while the inhibition of DRC and compound **3** were not determined. Their negative values indicate that they did not inhibit alpha-glucosidase at this concentration. The DRD, DRE, and DRM fractions had moderate inhibition values, while DRH, DRB, and compounds **1**, **4,** and **6** had weak inhibitions. The inhibitory activity of compounds isolated from *D. rhinocerotis* ranged from 3.74% to 94.17% for compounds **2** and **5**, respectively. Compound **5**, due to hydroxylation on A and B rings had higher activity than compound **1,** with a methoxy group substitution on its B ring. The lower activity of compound **3** compared to compound **1** may be due to its glycosylation on position 7. These activities based on structural differences agree with those reported in [44]. Although compound **4** has been reported to reduce blood glucose by increasing the secretion of insulin in mice, this research does not support this claim [45,46]. Samples with the highest inhibition values were analyzed further to determine their IC_{50} values. Compound **5** had a higher IC_{50} (83.01 ± 2.16 µg/mL) than acarbose (130.2 ± 1.84 µg/mL). Our value for acarbose is similar to that reported by Le Nguyen et al. [47].

Table 2. Inhibitory activities of samples from *D. rhinocerotis* on alpha-glucosidase and alpha-amylase.

Extracts/Compounds	% Inhibition	Alpha-Glucosidase IC_{50} (µg/mL) ± SD	% Inhibition	Alpha-Amylase IC_{50} (µg/mL) ± SD
DRC (Crude)	nd	-	nd	-
DRH (Hexane)	6.13	-	nd	-
DRD (DCM)	41.49	201.8 ± 2.12	nd	-
DRE (EtOAc)	44.45	199.8 ± 2.57	3.44	-
DRB (BuOH)	9.33	-	nd	-
DRM (crude MeOH)	53.50	198.4 ± 2.48	5.59	-

Table 2. *Cont.*

Extracts/Compounds	% Inhibition	Alpha-Glucosidase IC_{50} (µg/mL) ± SD	% Inhibition	Alpha-Amylase IC_{50} (µg/mL) ± SD
1	13.88	-	nd	-
2	3.74	-	nd	-
3	nd	-	nd	-
4	7.05	-	0.67	-
5	94.17	83.0 ± 2.16	7.04	-
6	3.86	-	28.56	-
Acarbose	63.94	130.2 ± 1.84	88.86	20.25 ± 1.23

nd: not determined due to negative or non-replicable values. SD: standard deviation.

3.4. Alpha-Amylase Inhibition Assay

As seen in Table 2, the inhibitory activities for all the fractions against alpha-amylase ranged from 0% to 5.59%, with only the ethyl acetate fraction and the lipophilic crude extract showing activities of 3.44 and 5.593%, respectively, at a concentration of 100 µg/mL, which was used for the preliminary screening. The other fractions did not show any activity against the enzyme. The inhibitory activity of the six compounds isolated from *D. rhinocerotis* against alpha-amylase was investigated at 200 µM. Compound **6** was the most active, with an inhibition of 28.563%, followed by compound **5,** with an activity of 7.040%. This supports the claim that compound **6** is an anti-hyperglycemic that works by releasing insulin to regulate blood sugar [48]. Compounds **1** and **5**, despite having similar structures, have significantly different inhibitory activities, which may be due to the methoxy group in compound **1** [44]. Acarbose, which was used as a positive control, had an inhibitory activity of 88.86%. Although *D. rhinocerotis* was reported to be used traditionally for treating diabetes [10], preliminary screening of the fractions, as well as six isolated compounds, do not support this claim; therefore, no further assay was carried out.

4. Conclusions

Six known compounds were isolated from *Dicerothamnus rhinocerotis*, of which four were reported for the first time in the plant. EtOAc and BuOH fractions and the crude MeOH extract showed stronger anti-tyrosinase activities than other fractions. Of the isolated compounds, compound **5** showed the strongest inhibition against L-tyrosine. These strong activities suggest that this plant can be investigated further for its use in the cosmetic industry as this is the first research reported on its anti-tyrosinase potential. The DRD, DRE, and DRM fractions had good alpha-glucosidase activities. Compound **5** also had an inhibitory activity stronger than acarbose, which supports the ethno-medicinal use of the plant for treating diabetes. Although the plant showed good activity in inhibiting alpha-glucosidase, this was not the case for alpha-amylase as all the samples showed low activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry6040032/s1, Table S1: Comparison of 13C-NMR experimental data with literature at 100 MHz.

Author Contributions: Conceptualization, O.I.W. and W.T.M.; Formal analysis, O.I.W., M.Y. and M.M.; Investigation, O.I.W., M.Y., R.S. and M.M.; Methodology, O.I.W., A.A.H. and W.T.M.; Supervision, A.A.H. and W.T.M.; Writing—original draft, O.I.W., M.Y. and R.S.; Writing—review and editing, M.Y., R.S., M.M., A.A.H. and W.T.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation of South Africa grant numbers 116101, 139209 and 106055 and the APC was funded by the University of the Western Cape (UWC) and Cape Peninsula University of Technology (CPUT).

Data Availability Statement: The experimental data generated during this research are available upon request from the corresponding author.

Acknowledgments: We thank Kirstenbosch Gardens, Cape Town, South Africa for providing the plant used for this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Stevens, P. Angiosperm Phylogeny Website. 2001. Available online: http://www.mobot.org/MOBOT/research/APweb/ (accessed on 12 April 2024).
2. Jeffrey, C. Compositae: Introduction with key to tribes. *Fam. Genera Vasc. Plants* **2007**, *8*, 61–87.
3. WFO 2024: Asteraceae Giseke. Published on the Internet. Available online: http://www.worldfloraonline.org/taxon/wfo-7000000146 (accessed on 25 June 2024).
4. Chiari, M.E.; Joray, M.B.; Ruiz, G.; Palacios, S.M.; Carpinella, M.C. Tyrosinase inhibitory activity of native plants from central Argentina: Isolation of an active principle from *Lithrea molleoides*. *Food Chem.* **2010**, *120*, 10–14. [CrossRef]
5. Aghraz, A.; Gonçalves, S.; Rodríguez-Solana, R.; Ait Dra, L.; Di Stefano, V.; Dugo, G.; Cicero, N.; Larhsini, M.; Markouk, M.; Romano, A. Antioxidant activity and enzymes' inhibitory properties of several extracts from two Moroccan *Asteraceae* species. *S. Afr. J. Bot.* **2018**, *118*, 58–64. [CrossRef]
6. Pool, E.; Klaasen, A.; Shoko, Y. The environmental toxicity of *Dicerothamnus rhinocerotis* and *Galenia africana*. *Afr. J. Biotechnol.* **2009**, *8*, 4465–4468.
7. Proksch, P.; Proksch, M.; Rundel, P.; Rodriguez, E. Ecological significance of the chemistry of the leaf resin of *Elytropappus rhinocerotis*. *Biochem. Syst. Ecol.* **1982**, *10*, 49–53. [CrossRef]
8. Levyns, M. A revision of *Elytropappus Cass*. *S. Afr. J. Bot.* **1935**, *1*, 89–103.
9. Mitchell-Watt, J.; Breyer-Brandwijk, M. *The Medical and Poisonous Plants of Southern and Eastern Africa*, 2nd ed.; Livingston: London, UK, 1962; p. 226.
10. Davids, D.; Gibson, D.; Johnson, Q. Ethnobotanical survey of medicinal plants used to manage high blood pressure and type 2 diabetes mellitus in Bitterfontein, Western Cape Province, South Africa. *J. Ethnopharmacol.* **2016**, *194*, 755–766. [CrossRef]
11. Bremer, K. *Asteraceae, Cladistic and Classification*; Timber Press, Inc.: Portland, OR, USA, 1994; pp. 435–458.
12. Wadkar, K.; Magdum, C.; Patil, S.; Naikwade, N. Antidiabetic potential and Indian medicinal plants. *J. Herb. Med. Toxicol.* **2008**, *2*, 45–50.
13. Baena-Díez, J.; Peñafiel, J.; Subirana, I.; Ramos, R.; Elosua, R.; Marín-Ibañez, A.; Guembe, M.; Rigo, F.; Tormo-Díaz, J.; Moreno-Iribas, C.; et al. Risk of cause—Specific death in individuals with diabetes: A competing risks analysis. *Diabetes Care* **2016**, *39*, 1987–1995. [CrossRef] [PubMed]
14. International Diabetes Federation (IDF). *Diabetes Atlas*, 7th ed.; International Diabetes Federation: Brussels, Belgium, 2015.
15. Kengne, A.P.; Echouffo-Tcheugui, J.B.; Sobngwi, E. New insights on diabetes mellitus and obesity in Africa-part 1: Prevalence, pathogenesis and comorbidities. *Heart* **2013**, *99*, 979–983. [CrossRef] [PubMed]
16. Peer, N.; Kengne, A.; Motala, A.; Mbanya, J. Diabetes in the Africa Region: An update. *Diabetes Res. Clin. Pract.* **2014**, *103*, 197–205. [CrossRef] [PubMed]
17. Bertram, M.; Jaswal, A.; Van Wyk, V. The Non-Fatal Disease Burden Caused by Type 2 Diabetes in South Africa. *Glob. Health Action* **2013**, *6*, 19244. [CrossRef] [PubMed]
18. Shouip, H. Diabetes mellitus: Signs and symptoms. *Open J. Nurs.* **2014**, *10*, 1–9.
19. America Diabetes Association. Pharmacologic Approaches to Glycemic Treatment. *Diabetes Care* **2017**, *40*, S64–S74. [CrossRef] [PubMed]
20. Keskin, A.; Bilge, U. Mental disorders frequency alternative and complementary medicine usage among patients with hypertension and type 2 diabetes mellitus. *Niger. J. Clin. Pract.* **2014**, *17*, 717–722. [PubMed]
21. Bhalerao, M.; Bolshete, P.; Swar, B.; Bangera, T.; Kolhe, V.; Tambe, M.; Wade, M.; Bhowate, S.; Sonje, U.; Gogtay, N.; et al. Use of and satisfaction with complementary and alternative medicine in four chronic diseases: A cross-sectional study from India. *Natl. Med. J. India* **2013**, *26*, 75–78. [PubMed]
22. Zolghadri, S.; Bahrami, A.; Khan, M.; Munoz-Munoz, J.; Garcia-Molina, F.; Garcia-Canovas, F.; Saboury, A. A comprehensive review on tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 279–309. [CrossRef] [PubMed]
23. Kumar, C.; Sathisha, U.; Dharmesh, S.; Rao, A.; Singh, S. Interaction of sesamol (3, 4-methylenedioxyphenol) with tyrosinase and its effect on melanin synthesis. *Biochimie* **2011**, *93*, 562–569. [CrossRef] [PubMed]
24. Chang, T. An Updated Review of Tyrosinase Inhibitors. *Int. J. Mol. Sci.* **2009**, *10*, 2440–2475. [CrossRef]
25. Chai, W.; Wei, M.; Wang, R.; Deng, R.; Zou, Z.; Peng, Y. Avocado Proanthocyanidins as a Source of Tyrosinase Inhibitors: Structure Characterization, Inhibitory Activity, and Mechanism. *J. Agric. Food. Chem.* **2015**, *63*, 7381–7387. [CrossRef]
26. Pillaiyar, T.; Manickam, M.; Namasivayam, V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *J. Enzyme. Inhib. Med. Chem.* **2017**, *32*, 403–425. [CrossRef] [PubMed]
27. Liu, J. Natural products in cosmetics. *Nat. Prod. Bioprospect.* **2022**, *12*, 40. [CrossRef] [PubMed]
28. Germishuizen, G.; Meyer, N. *Plants of Southern Africa: An annotated Checklist*; National Botanical Institute: Pretoria, South Africa, 2003; Volume 14, p. 186.

29. Dekker, T.; Fourie, T.; Matthee, E.; Snyckers, F.; Van der Schyf, C.; Boeyens, J.; Denner, L. Studies of South African medicinal plants: Pt. 7. Rhinocerotinoic acid: A labdane diterpene with anti-inflammatory properties from *Elytropappus rhinocerotis*. *S. Afr. J. Chem.* **1988**, *41*, 33–35.
30. Mshengu, B.; Gakuba, E.; Van Heerden, F. Chemical constituents from *Elytropappus rhinocerotis* (Lf) Less. *Biochem. Syst. Ecol.* **2017**, *75*, 18–20. [CrossRef]
31. Hulley, I.; Van Vuuren, S.; Sadgrove, N.; Van Wyk, B. Antimicrobial activity of *Elytropappus rhinocerotis* (Asteraceae) against micro-organisms associated with foot odour and skin ailments. *J. Ethnopharmacol.* **2019**, *228*, 92–98. [CrossRef]
32. Yalo, M.; Makhaba, M.; Hussein, A.A.; Sharma, R.; Koki, M.; Nako, N.; Mabusela, W.T. Characterization of Four New Compounds from *Protea cynaroides* Leaves and Their Tyrosinase Inhibitory Potential. *Plants* **2022**, *11*, 1751. [CrossRef]
33. Yamaki, K.; Mori, Y. Evaluation of a-glucosidase inhibitory activity in colored foods: A trial using slope factors of regression curves. *J. Jpn. Soc. Food. Sci.* **2006**, *53*, 229–231. [CrossRef]
34. Miyazawa, M.; Hisama, M. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2091–2099. [CrossRef] [PubMed]
35. Kalpoutzakis, E.; Aligiannis, N.; Skaltsounis, A.L.; Mitakou, S. *cis*-Clerodane type diterpenes from *Cistus monspeliensis*. *J. Nat. Prod.* **2003**, *66*, 316–319. [CrossRef]
36. Sofrenić, I.; Anđelković, B.; Gođevac, D.; Ivanović, S.; Simić, K.; Ljujić, J.; Tešević, V.; Milosavljević, S. Metabolomics as a Potential Chemotaxonomical Tool: Application on the Selected Euphorbia Species Growing Wild in Serbia. *Plants* **2023**, *12*, 262. [CrossRef]
37. Raya-Gonzalez, D.; Pamatz-Bolanos, T.; Rio-Torres, R.; Martinez-Munoz, R.; Ron-Echeverria, O.; Martinez-Pacheco, M. D-(+)-pinitol, a component of the heartwood of *Enterolobium cyclocarpum* (Jacq.) Griseb. *Z. Naturforschung C* **2008**, *63*, 922–924. [CrossRef] [PubMed]
38. Mariappan, G.; Sundaraganesan, N.; Manoharan, S. The spectroscopic properties of anticancer drug Apigenin investigated by using DFT calculations, FT-IR, FT-Raman and NMR analysis. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2012**, *95*, 86–99. [CrossRef] [PubMed]
39. Flamini, G.; Antognoli, E.; Morelli, I. Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. *Phytochemistry* **2001**, *57*, 559–564. [CrossRef] [PubMed]
40. Kim, D.; Lee, J. Comparative evaluation of phenolic phytochemicals from perilla seeds of diverse species and screening for their tyrosinase inhibitory and antioxidant properties. *S. Afr. J. Bot* **2019**, *123*, 341–350. [CrossRef]
41. Shimmyo, Y.; Kihara, T.; Akaike, A.; Niidome, T.; Sugimoto, H. Flavonols and flavones as BACE-1 inhibitors: Structure–activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. *Biochim. Biophys. Acta* **2008**, *1780*, 819–825. [CrossRef] [PubMed]
42. Roh, J.; Han, J.; Kim, J.; Hwang, J. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. *Biol. Pharm. Bull.* **2004**, *27*, 1976–1978. [CrossRef] [PubMed]
43. Cespedes, C.; Balbontin, C.; Avila, J.; Dominguez, M.; Alarcon, J.; Paz, C.; Burgos, V.; Ortiz, L.; Penaloza-Castro, I.; Seigler, D.; et al. Inhibition on cholinesterase and tyrosinase by alkaloids and phenolics from *Aristotelia chilensis* leaves. *Food. Chem. Toxicol.* **2017**, *109*, 984–995. [CrossRef] [PubMed]
44. Hui, C.; Xiaoqing, C. Structures required of flavonoids for inhibiting digestive enzymes. *Anti-Cancer Agents Med. Chem.* **2012**, *12*, 929–939.
45. Narayanan, C.; Joshi, D.; Mujumdar, A.; Dhekne, V. Pinitol—A new anti-diabetic compound from the leaves of *Bougainvillea spectabilis*. *Curr. Sci.* **1987**, *56*, 139–141.
46. Abo-Elghiet, F.; Ahmed, A.; Aly, H.; Younis, E.; Rabeh, M.; Alshehri, S.; Alshahrani, K.S.; Mohamed, S. D-Pinitol Content and Antioxidant and Antidiabetic Activities of Five *Bougainvillea spectabilis* Willd. Cultivars. *Pharmaceuticals* **2023**, *16*, 1008. [CrossRef]
47. Le Nguyen, T.T.; Pham, T.T.; Hansen, P.E.; Nguyen, P.K. In vitro α-glucosidase inhibitory activity of compounds isolated from mangrove *Lumnitzera littorea* leaves. *Sci. Tech. Dev. J.* **2019**, 106–113. [CrossRef]
48. Ivorra, M.; D'ocon, M.; Paya, M.; Villar, A. Antihyperglycemic and insulin-releasing effects of beta-sitosterol 3-beta-D-glucoside and its aglycone, beta-sitosterol. *Int. Pharmacodyn. Ther.* **1988**, *296*, 224–231.