Original article

Phytogenic compounds from avocado (Persea americana L.) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes

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Abstract

Diabetes is a worldwide public health disease. Currently, the most effective way to treat diabetes is to mitigate postprandial hyperglycemia by inhibiting carbohydrate hydrolysis enzymes in the digestive system. Plant extracts are rich in bioactive compounds, which can be used in diabetes treatment. This study aims to evaluate the polyphenols content in ethanolic extracts of avocado fruit and leaves (Persea americana Mill.). Additionally, their antioxidant activity using DPPH, while the inhibition ability of α-amylase was examined by reacting different amounts of the extracts with α-amylase compared to acarbose as standard inhibitor. The active compounds were detected in the extracts by LC/MS. The obtained results showed that the leaf extract recorded a significant content of total phenolic compounds compared to the fruit extract (178.95 and 145.7 mg GAE/g dry weight, respectively). The total flavonoid values ranged from 32.5 to 70.08 mg QE/g dry weight of fruit and leaves extracts, respectively. Twenty-six phytogenic compounds were detected in leaf and fruit extract by LC/MS. These compounds belong to fatty acids, sterols, triterpenes, phenolic acids, and flavonoids. The antioxidant activity of the extracts is due to the exist of phytogenic compounds, i.e., polyphenols and flavonoids. The antioxidant activity increased in a concentration dependant manner. Avocado fruit extract (1000 mg/mL) scavenged 95% of DPPH while leaf extract rummaged 91.03% of free radicals compared with Vit C and BHT. Additionally, higher α-amylase inhibitory activity was observed in fruit extract than the leaf extract, where the fruit and leaf extract (1000 μg/mL) inhibited the enzyme by 92.13% and 88.95%, respectively. The obtained results showed that the ethanolic extracts of avocado could have a significant impact on human health due to their high content of polyphenols.

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1. Introduction

The use of natural extracts with pharmacological activity has gain great interest because they have therapeutic potential in treatment of different diseases with low side effects. Hyperglycemia increases the production of different active free radicals, which results in diabetic complications, such as oxidative damage of tissue and nephropathy, neuropathy, retinopathy, and memory disorder (Maritim et al., 2003). Abnormal blood sugar levels are a symptom of diabetes. This is due to the immune system inability to create enough insulin (Petersmann et al., 2018). Hyperglycemia occurs after feeding because of starch hydrolyzes by α-amylase and α-glucosidase to large amounts of glucose. The intestinal enzymes must be inhibited to treat type 2 diabetes (Wang et al., 2013). Glucosidase is a one of digestive enzymes that degrade die- tetic carbohydrates in simple monosaccharides. Glucosidase inhibitors, such as acarbose, reduce the speed of carbohydrate

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digestion and delays the absorption of carbohydrates from the digestive tract. Therefore, they can prevent the start of type 2 diabetes by reducing the level of postprandial glucose (Liu et al., 2011). Avocado (Persea americana Mill.) is subtropical/tropical fruit, a member of Lauraceae and Persea family. It is a Mexican origin. It is extensively cultivated in the Central Region of the United State and consumed worldwide (Melgar et al., 2018). It has become very popular recently and is often sold as a “superfood” due to its unique nutritional content, phytochemical composition, and health benefits. (Segovia et al., 2018). In addition to being used as food, is very popular recently and is often sold as a “superfood” due to its nutritional characteristics. (Wright et al., 2007). Rhamnetin, luteolin, rutin, quercetin, and apigenin are phytochemicals extracted from avocado leaves. (Vajragupta et al., 2010). Avocado wastes are a rich source of several bioactive chemicals with significant nutritional, therapeutic, and medicinal properties. (Vajragupta et al., 2000). Secondary metabolites seem to be the most analysed phytochemical group in fruits and vegetables (Domínguez-Avila et al., 2018). The free radical scavenging activity of ROS is related to health disorders, such as diabetes, hypertension, cancer, neurological illnesses, gastric ulcers, reperfusion therapy, arthritis, and inflammatory diseases. (Vajragupta et al., 2021a). Secondary metabolites seem to be the most analysed phytochemical group in fruits and vegetables (Domínguez-Avila et al., 2018). The free radical scavenging activity of ROS is related to health disorders, such as diabetes, hypertension, cancer, neurological illnesses, gastric ulcers, reperfusion therapy, arthritis, and inflammatory diseases. (Vajragupta et al., 2000). Secondary metabolites seem to be the most analysed phytochemical group in fruits and vegetables (Domínguez-Avila et al., 2018).

2. Material and methods

2.1. Chemicals

Merck Company supplied the ethanol. Sigma-Aldrich Company supplied α-amylase enzyme and acarbose inhibitor. All the other chemicals were of the highest commercial purity.

2.2. Preparation of plant extracts

Avocado leaves and fruits (500 g) were dried in a vacuum oven (45 °C) for 5 h. 100 g of the powder was homogenized in 1000 mL ethanol 70% (1:10, w/v) and stirred for 3 h at room temperature, then filtrated through (Whatman No.1) paper (Saad et al, 2021a). A rotary evaporator was used to recover the solvent in the filtrate and the obtained free solvent extracts were adjusted to a concentration of 100 mg/mL and stored at 4 °C for further analyses.

2.3. Phytochemical analyses

2.3.1. Determination of total phenolics

The total polyphenols (TP) were evaluated according to Saad et al (2021b). In brief, 50 μL of fruit and leaves avocado extracts (500 μg/mL) was mixed with 50 μL of diluted Folin-Ciocalteu reagent and 50 μL Na₂CO₃ (7.5%) in microtiter plate wells, and incubated at room temperature for 60 min, then the absorbance was measured at 760 nm using microtiter plate reader (BioTek ELx808, USA). The total polyphenols content was presented as mg gallic acid equivalent/mL of solutions.

2.3.2. Determination of total flavonoids

Total flavonoids content (TF) was estimated as per Saad et al, (2021c). 20 μL of of fruit and leaves avocado extracts (500 μg/mL) was mixed with 20 μL of sodium nitrite (5%) in microtiter plate wells and 20 μL of ethanol solution of AlCl₃ (10%) was added. The plate was incubated at room temperature for 10 min. 50 μL of NaOH was added to stop the reaction. The absorbance was measured at 450 nm, and TF was expressed as mg quercetin equivalent per mL of each solution.

2.4. DPPH radical scavenging activity

The free radical scavenging activity of polyphenolic compounds was measured using 1, 1-diphenyl-2-picryl-hydrazil (DPPH*) according to the method of (Wang et al., 2008; El-Saadony et al, 2020) DPPH* (0.1 mM) was prepared in methanol. Successive concentration from avocado fruit and leaves extracts and standard materials; BHT and Ascorbic Acid were prepared as 100, 250, 500, 750, and 1000 μg/mL in methanol. 1 mL of DPPH* Solution of 3 mL of each concentration was added, then vigorously stirred up the mixture and permitted to stand 30 min at room temperature. The same procedure was used to test checks, except for samples of methanol. A spectrophotometer read the absorption at 517 nm (Jasco, serial No. C317961148, Japan). According to following equation DPPH* radical scavenging activity was calculated:

Radicals scavenging activity(%) = \frac{(Abs. control - Abs. sample)}{(Abs. control)} \times 100 \tag{1}

Where Abs control was the absorbance of the control and Abs sample was the absorbance in the presence of the sample of Avocado extracts (fruits and leaves).

2.5. Determination of α-amylase inhibitory activity

The hydrolysis of 1.4 glycosidic bonds in starch and other similar carbohydrates is catalysed by -amylase (EC 3.2.1.1). In particular, α-amylase participates in glycolysis digestion and is considered a key enzyme that can control postprandial hyperglycemia. The
The assay’s principle is based on the fact that reducing sugars have the ability to reduce a variety of reagents; chemically, they form an aldehyde or ketone in basic solution, and the aldehyde group of glucose converts DNS to its reduced form (3-amino-5-nitrosalicylic acid), which results in a change in the amount of light absorbed at 540 nm. The proportion of each extract’s α-amylase inhibitory activity values was obtained using this method, as follows:

$$\frac{\text{Amylase activity}}{\text{control sample}} \times 100$$

The extracts inhibitory values were estimated by (El Souda et al., 2017).

2.6. Detection bioactive compounds by LC/MS

Electrospray Ionisation Mass Spectrometry (ESI-MS, XEVO TQD, Waters Corporation, Milford, MA01757 U.S.A) was used to detect the bioactive compounds in avocado extracts. The device occupied with C18 column (ACQUITY UPLC – BEH); 1.7–2.1 mm, packed with 50 mm C18 with C18 column (ACQUITY UPLC – BEH); 1.7–2.1 mm, packed with 50 mm. The sample (100 μg/mL) solution was prepared using methanol (HPLC analytical grade), filtered using a membrane disc filter (0.2 μm) then subjected to LC-ESI-MS analysis. 10 μL of sample was injected into the UPLC instrument equipped with reverse phase C-18 column. The sample was eluted using gradient elution. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. The data were detected in 100–1000 m/z mass spectra range and processed using the Masslynx 4.1 software, then identified by comparing its retention time (Rt) and mass spectrum with reported data (Bakr et al., 2021).

2.7. Statistical analysis

One way ANOVA in SPSS 16 was used to conduct statistical analysis. The results were obtained as a mean ± standard deviation (SD). The significance of differences between means was assessed at p < 0.05 using post hoc Duncan’s test.

3. Results

The higher polyphenol and flavonoids contents were detected in fruit and leaves ethanolic extracts. It can be seen from the data that the leaves’ extract reported significantly more than Fruit extract it was 178.95 and 145.7 mg GAE/ g DW, respectively. The values of flavonoids contents ranged from 32.5 to 70.08 mg QE/ g DW for fruit and leaves ethanol extracts, respectively.

Table 1 and Fig. 1 illustrate the free radical scavenging activities of Persea americana fruits and leaves. DPPH value of concentration (1000 μg / mL) of avocado fruit extract was 95.00 ± 0.68 %, while bring down at lower concentrations 100, 250, 500 and 750 μg/mL causes 63.70 ± 1.6, 66.47 ± 0.82, 72.32 ± 0.74 and 83.43 ± 0.76 %. The results of avocado leaves extract were 91.03 ± 0.19 %, At 1000 μg/mL, which also reduces at concentrations 100, 250, 500 and 750 μg/mL the inhibition percent were 36.11 ± 0.27, 43.07 ± 0.30, 52.66 ± 0.05, and 71.03 ± 0.19 %, respectively. These results are compared with V.C and BHT as standard the outcome at high concentrate 1000 μg/mL was 99.84 ± 0.15 and 98.18 ± 0.17 %, respectively although the reduction of the different concentrations 100, 250, 500 and 750 μg/mL caused 73.00 ± 1.15, 90.09 ± 0.51, 94.42 ± 0.54 and 96.62 ± 0.23 %, respectively for vitamin C and it’s were 80.33 ± 0.29, 95.99 ± 0.07, 96.16 ± 0.18, and 98.05 ± 0.08 % for BHT butylated hydroxytoluene, respectively. 

Table 2 shows the values of the α-amylase inhibitory activity affected by avocado fruit and leaves ethanolic extracts compared with acarbose inhibitor, both fruit and leaves extracts recorded lower values as 21.00 ± 0.30, 37.98 ± 0.52, and 51.38 ± 0.21%, and 18.67 ± 0.36, 30.69 ± 0.20 and 42.51 ± 0.51 %, respectively at concentrations 100, 250, and 500 μg/mL, while the avocado fruit extract had more inhibitory effects on α-amylase at high concentrations of (750 and 1000 μg/mL) than the avocado leaves and fruits.

| Conc. (μg/mL) | AFE | ALE | V.C | BHT |
|---------------|-----|-----|-----|-----|
| 100           | 21.00 ± 0.30 e | 18.67 ± 0.36 e | 23.35 ± 0.28 e |
| 250           | 37.98 ± 0.52 e | 30.69 ± 0.20 e | 51.38 ± 0.21 e |
| 500           | 51.38 ± 0.21  e| 42.51 ± 0.51  e| 69.54 ± 0.19 e |
| 750           | 82.60 ± 0.36  e| 75.52 ± 0.24  e| 96.62 ± 0.23 e |
| 1000          | 92.13 ± 0.03 e | 88.95 ± 0.34 e | 98.05 ± 0.08 e |

The results are presented as a mean ± standard Error (SEM). Significant differences (p < 0.05) are indicated by different superscripts within the same column.

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The results are presented as a mean ± standard Error (SEM). Significant differences (p < 0.05) are indicated by different superscripts within the same column.
acarbose, where values were 82.60 ± 0.36 and 92.13 ± 0.03 % and 75.52 ± 0.24 and 88.95 ± 0.34 % and 78.50 ± 0.22 and 88.34 ± 0.23 %, respectively.

3.1. LC/MS analysis of Persea americana fruit extract

Table 3 shows that LC/MS analysis of fruit extract identified 26 chemical peaks for several classes such as fatty acids, sterols, triterpenes, phenolic acids, and flavonoids. In the MS spectrum of the tested extract, strong peaks of flavonoids fractions: M–162 and M–146, M–132, indicating the extense of flavonoids O-glycosides; hexose or O-rhamnoside (dehyoxyllose), O-pentoside. Isoflavonoid derivatives allocated in peak no (6) showed molecular ion at (M–H)+ at m/z 461.3723 with a main fragment ion at m/z 315, 300, 176 corresponding to (M–H-)glucuronide group 194 where values were 82.60 ± 0.36 and 92.13 ± 0.03 % and 75.52 ± 0.24 and 88.95 ± 0.34 % and 78.50 ± 0.22 and 88.34 ± 0.23 %, respectively.

Table 3 shows that LC/ESI-MS analysis of the ethanolic extract of Persea americana Mill tree fruit revealed the presence of 26 chemical peaks for different classes of secondary metabolites such as fatty acids, sterols, triterpenes, phenolic acids, and flavonoids. The molecular ion and fragment ions of these compounds were detected in the LC/MS of Persea americana Mill fruit extract. Anthocyanins related to Peak 3, 4 showed a molecular ion peak at m/z 431.2636 and main fragment ions at 315, 289, 245 and 300 corresponding to (M–H-)glucuronide group (Sharifi-Rad et al., 2018). Many plants are considered a basic source of powerful anti-diabetic medications. In developing countries, particularly medicinal plants deal with diabetes and exceed the burden of traditional drugs to the population (Arumugam et al., 2013). Recently, it is recommended to treat diseases such as diabetic containing diabetes using medicinal plants (Mansouri et al., 2015), which including bioactive compounds, flavonoids, terpenoids, saponins, carotenoids, alkaloids, glycolic, etc. as anti-diabetic agents (Durazzo et al., 2018). Natural products of plant origin are rich in bioactive compounds with antioxidant (Saad et al., 2020a; El-Saadony et al., 2020; Abdel-Moneim et al., 2021) and antimicrobial activity (El-Tarabily et al., 2021). They are used as food additives for preserving juices (El-Saadony et al., 2021a), meat (Saad et al., 2020b; El-Saadony et al., 2021b), bakery (Saad et al., 2015; Saad et al., 2021d), and dairy (El-Saadony et al., 2021b).

Table 3

| No. | Rt (26 to 30 min) | Mol. ion (+) | Mol. ion (-) | formula | fragment | Compounds |
|-----|------------------|-------------|-------------|---------|----------|-----------|
| 1   | 0.87             | 191.1228    |             | C₉H₁₄O₁   | 191      | Quinic acid |
| 2   | 1.97             | 163.0880    |             | C₉H₁₂O₂   | 163      | p-Coumaric acid |
| 3   | 2.15             | 577.5127(-) |             | C₁₂H₁₈O₁₂ | 425      | Procyanidin dimer B |
| 4   | 2.15             | 433.3954    |             | C₁₂H₁₄O₁₁ | 289, 245 | Peonidin 3-O-pentoside |
| 5   | 2.15             | 289.6310    |             | C₁₁H₁₄O₇  | 315, 300, 176 | Isorhamnetin-O-glycoside |
| 6   | 2.15             | 461.3723    |             | C₂₉H₃₈O₁₀ | 311      | Vitexin |
| 7   | 2.15             | 431.2636    |             | C₂₁H₂₀O₁₀ | 329, 475, 607 | Tricin-O-rhamnosylpentoside |
| 8   | 2.15             | 461.3723    |             | C₂₁H₂₀O₁₀ | 315, 300, 176 | Isorhamnetin-O-glycoside |
| 9   | 2.15             | 461.3723    |             | C₂₁H₂₀O₁₀ | 311      | Vitexin |
| 10  | 2.15             | 461.3723    |             | C₂₁H₂₀O₁₀ | 329, 475, 607 | Tricin-O-rhamnosylpentoside |
| 11  | 595.5849         |             |             | C₁₇H₁₄O₇  | 329      | Tricin aglycone |
| 12  | 2.15             | 329.55(-)   |             | C₁₇H₁₄O₇  | 329      | Tricin aglycone |
| 13  | 2.15             | 281.3471    |             | C₁₇H₁₄O₇  | 281      | Apigenin aglycone |
| 14  | 2.15             | 269.3015    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 15  | 2.15             | 425.3806    |             | C₁₇H₁₄O₇  | 289, 245 | Isorhamnetin-O-rhamnoside |
| 16  | 2.15             | 413.3064    |             | C₁₇H₁₄O₇  | 269, 433, 595 | Apigenin-O-dihexoside |
| 17  | 2.15             | 401.4063    |             | C₁₇H₁₄O₇  | 329      | Tricin aglycone |
| 18  | 2.15             | 399.3789    |             | C₁₇H₁₄O₇  | 281      | Apigenin aglycone |
| 19  | 2.15             | 391.3430    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 20  | 2.15             | 430.3855    |             | C₁₇H₁₄O₇  | 289, 245 | Isorhamnetin-O-rhamnoside |
| 21  | 2.15             | 277.3062    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 22  | 2.15             | 273.3136    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 23  | 2.15             | 279.3407    |             | C₁₇H₁₄O₇  | 289, 245 | Isorhamnetin-O-rhamnoside |
| 24  | 2.15             | 255.3120    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 25  | 2.15             | 281.3320    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |
| 26  | 2.15             | 283.3519    |             | C₁₇H₁₄O₇  | 269      | Apigenin aglycone |

4. Discussion

The natural products of plant origin are mainly quarries to find promising reading candidates and play an ordering role in the next drug development program (Sharifi-Rad et al., 2018). Availability, low cost, and minimal side effects are especially the main key player of all treatments available in rural areas (Mansouri et al., 2015). In addition, many plants are released from undesirable side effects and provide a rich biological source of biologically active chemical products having a powerful pharmacological behavior (Sharifi-Rad et al., 2018). Many plants are considered a basic source of powerful anti-diabetic medications. In developing countries, particularly, medicinal plants deal with diabetes and exceed the burden of traditional drugs to the population (Arumugam et al., 2013). Recently, it is recommended to treat diseases such as diabetic containing diabetes using medicinal plants (Mansouri et al., 2015), which including bioactive compounds, flavonoids, terpenoids, saponins, carotenoids, alkaloids, glycolic, etc. as anti-diabetic agents (Durazzo et al., 2018). Natural products of plant origin are rich in bioactive compounds with antioxidant (Saad et al., 2020a; El-Saadony et al., 2020; Abdel-Moneim et al., 2021) and antimicrobial activity (El-Tarabily et al., 2021). They are used as food additives for preserving juices (El-Saadony et al., 2021a), meat (Saad et al., 2020b; El-Saadony et al., 2021b), bakery (Saad et al., 2015; Saad et al., 2021d), and dairy (El-Saadony et al., 2021b).
These activities are attributed to biologically active compounds (i.e., polyphenols, carotenoids, lignans, coumarin, glycosinolates, etc.), resulting in possible beneficial properties of each plant matrix, which can be represented as a first step for biological understanding. The antigenic effects resulting from behavioral and beneficial activity plants generally occur by increasing insulin secretion, or due to the ability to improve pancreatic tissue performance carried out by reducing the intestinal absorption of glucose. The adverse effects of these episodes are significant and include subsequent Diabetes mellitus (DM) problems. Because ROS has been linked to the disease’s aetiology, it is possible to use antioxidants for the logical technique for managing and expanding its potential burden (Swelum et al., 2020). It was therefore proven that antioxidants prevent the demise of the pancreatic beta cells through inhibition of automated oxidation reactions and therefore regulate diabetes progression towards complications (Liu et al., 2007). Synthetic antioxidants, on the other hand, are restricted in their usage due to their carcinogenicity, while plant antioxidants degrade at a much lower level of toxicity. The plants containing a natural antioxidant retain the function of the beta cell and prevent the activity of alpha-amylase inhibitory activity of avocado fruit and leaves extracts. Alpha-amylase is a key enzyme for starch digestion and plays an important role in determining the amount of glucose released. Inhibition of enzyme activity is considered a potential method to control starch digestion and regulate postprandial hyperglycemia in diabetic patients. It is crucial in the creation of nutritional health products as well as the treatment of diabetes. The pathogenesis of diabetes is linked to oxidative free radicals (Baynes and Thorpe, 1999).

LC/MS analysis of the fruit extract of the avocado Persea americana Mill was conducted because of its highest inhibitory effect as antioxidants and its ability to inhibit the activity of the enzyme alpha-amylase the results revealed presence of unsaturated and saturated fatty acids, sterol, triterpenes, flavonoids, and phenolic compounds. Flavonoids have dominated in Lauraceae which Persea americana is a member. Kaempferol, apigenin and isorhamnetin glycosides were the main flavanoids in this family (Owolabi et al., 2010) and (Tremoccoli et al., 2018) reported that Persea americana Millis contains Anthocyanins and catechin. (Wang et al., 2020) reported saturated and unsaturated fatty acids i.e., linolenic acid, Palmitoleic acid, linoleic acid, Palmitic acid, Oleic acid, Stearic acid in avocado plants at Rts (26–30 min). Cycloartenol (Peak 15), Avenasterol (16), Campasterol (18); γ-Tocopherol (17); α-Tocopherol (20) all these compounds were previously reported in the plant (Ramos-Aguilar et al., 2019). The presence of these compounds may be a reasonable cause of the hypoglycemic potential observed in this study. It has been shown to have anti-diabetic characteristics by regenerating pancreatic cells and suppressing carbohydrate metabolism enzyme activity (Kwon et al., 2008); (Chika and Bello, 2010). C. Drosferifolia aqueous extract is traditionally used to treat hyperglycemia, according to (Abdel Motaal et al., 2020), Multiple in vitro and in vivo investigations have revealed anti-diabetic potential. The flavonol glycosides accounted for 78 % of the water extract, and there were five main peaks. These 5 flavonol glycosides have been demonstrated to inhibit intestinal enzymes involved in the hydrolysis of polysaccharides to glucose in vitro tests. Furthermore, by inhibiting aldehyde reductase and reducing oxidative stress, which is linked to the aetiology of diabetes complications, these flavonol glycosides can prevent insulin resistance. Generally, the attributes exhibited of avocado fruit extract are related to its phenols and flavonoids, which are recognized for having antioxidant capabilities. Flavonoids can be a natural phenolic compound of the highest diversity and widely distributed nature compounds (Ward, 1950). Its wide spectrum chemical and biological activities have been reported by (Prasad et al., 2009), including free radical scavenging. Flavon has similar characteristics (Millaukskas et al., 2004), and it has been found to be an effective hypoglycemic agent (Kim et al., 2004) and had high alpha-glucosidase and alpha-amylase inhibitory effects (Li et al., 2018).

5. Conclusion

The general clarification of this study was LC/MS, antioxidants, total phenols, and total ethanol extract flavonoids, from avocado extracts, which made its application as an antidiabetic agent credible. In the treatment of diabetes, it is very important to highly use affinity plant inhibitors to regulate the activity of α-amylase. It promotes the use of a therapeutically significant natural plant because of its availability and relative safety (Persea americana Millis).

Declaraton of Competing Interest

The authors declare that they have no known conflicting financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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