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Phytochemical constituents found in *Physalis peruviana* L. leaf extracts and their ability to inhibit alpha-glucosidase and scavenge DPPH free radicals *in vitro*

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ABSTRACT

Physalis peruviana L. is widely used in folk medicines to manage diabetes. This study aimed to identify new chemical constituents and explore *in vitro* antioxidant ability and alpha-glucosidase inhibition activity to explain its antidiabetic mechanism. Methanolic extracts and hexane fractions were prepared and analyzed by CG-FID and GC-MS. *In vitro* antidiabetic activity was assessed on α-glucosidase inhibition and DPPH models. The phytochemical analysis detected 29 constituents, manly heptacosane (**25**) and hexadecanoic acid methyl ester (**14**). Aliphatic hydrocarbons and esters were identified as the main classes of secondary metabolites. Compared to the standard, the methanol-water fraction was the most potent fraction on α-glucosidase inhibition (IC_{50} 32.5 ± 0.27 µg/mL). The antioxidant values (IC_{50}) of two fractions, and the reference were quite close (44.5 ± 0.54; 49.5 ± 0.87; 44.2 ± 0.77 µg/mL). Almost 82.75% are recognized for the first time in the leaf hexane fractions of this plant. The observed antioxidant activity was found to be significantly notable.

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1. Introduction

The use of plants in treating diseases in the traditional medicinal system started with the evolution of societies (Bandaranayake, 2006). Medicinal plants and herbal medicines play a crucial role in the life of human and animal beings. Medicinal herbs are essential natural remedies in the conventional health system to manage many diseases. Over the past few decades, several scientific studies have been conducted on a wide spectrum of herbal plants and these attempts have led to the chemical and biological characterizations of a large number of valuable natural compounds in different plant species. Only a little of the fundamental traditional medicines have been scientifically investigated. This situation supports the increasing interest in investigations of medicinal plants (Bailly, 2021; Kemayou et al., 2021; Mohammadhosseini et al., 2021a, 2021b). Antioxidant properties of chemical compounds present in the plant may help prevent oxidative stress in humans and animals (Vega-Gálvez et al., 2014). Oxidative stress is an essential contributing factor to developing chronic diseases, including cancer, cardiovascular diseases, and diabetes mellitus (DM). DM is a significant health problem both in developed and developing countries. Inhibiting enzymatic activities involved in the catalyzing hydrolysis of starch to simple sugars is one of the approaches used to treat diabetes (Medina-Pérez et al., 2019). Alpha-glucosidase inhibition assays are mainly used to study antidiabetic activity *in vitro* investigations (Sathasivampillai et al., 2017). Several side effects of oral hypoglycemic agents include hypoglycemia, gastrointestinal disturbances, skin reactions, hematological disorders, and a rise in hepatic enzyme level (Pillai et al., 2017), prompted extensive studies on medicinal plants to discover safe and alternate natural products for the treatment of diabetes. *Physalis peruviana* L. (Solanaceae) is a semi-upright herbaceous or perennial shrub. Perennial producing branched stems native to the Andean region is

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widely known cultivated worldwide (Kasali et al., 2021). The health benefits of its berries are well documented due to their rich content in bioactive phytochemicals and volatiles that are only responsible for specific flavors and impart various biological activities (Dymerski et al., 2016). The plant is used in traditional medicines to manage several human and veterinary diseases. In Congolese conventional medicine, the treatment of DM (Kasali et al., 2013). According to the literature, studies on the chemical profiling of *n*-hexane sub-fraction of methanol extract of *P. peruviana* L. have not been carried out. This study aims to profile chemical constituents of the *n*-hexane fraction and analyze the *in vitro* antidiabetic and antioxidant properties of fractions from the methanolic extract of *P. peruviana* L.

2. Experimental

2.1. Instruments, reagents, and chemicals

Gas Chromatography (Shimadzu GC-17A), Gas Chromatograph (7890A, Agilent Technologies, USA), Mass Spectrometer (Agilent Technologies 7000, 5301) equipped with a 72OPTIMA-5 capillary column (30 m x 250 µm x 0.25 µm), and GC-MS Triple Quad (Agilent Technologies 7000, 5301; equipped with flame ionization detector, capillary column SPB-5 (length: 30m, diameter: 0.53mm, film thickness: 0.5 µM; column chromatography on silica gel (70-230 and 240-300 mesh size, E. Merck, Germany), balance (Kern PRS 6200-2), methanol, dichloromethane, and *n*-hexane of commercial-grade, purchased locally and used after distillation.

2.2. Plant materials

The leaves of *P. peruviana* L. were collected from Katana located 50 km from Bukavu city, South-Kivu Province, in the Democratic Republic of Congo in September 2019 (rainy season) and identified by Mr. Gentil IRAGI. A voucher specimen (registered under number LWI113898883) has been deposited at the Department of Biology of the Center for Research in Natural Sciences/ Lwiro (Centre de Recherche en Sciences Naturelles).

2.3. Extraction and fractionation of leaf methanol extract

The diagram in Fig. 1 illustrates the extractive steps. The leaves were air-dried at room temperature and then manually grounded to fine powders. The leaf powder (2,158.5 g) was extracted with methanol (30 L). The crude methanol extract was mixed with silica gel and placed in a vacuum funnel. Then, successively *n*-hexane (10 L), *n*-hexane-dichloromethane (12 L), dichloromethane (14 L), dichloromethane-methanol (16 L), methanol, and methanol-water (5 L) went over vacuum liquid chromatography (VLC) apparatus for fractionation. The different fractions were immediately collected from the Büchner flask connected to a pump, free of solvents on rotavapor, and dried in the fuming hood for one week before submitting to analysis. The combined extract was free of solvent at 40-50 °C on a rotary evaporator and yielded 518.75 g (20.03%) of crude quote.

2.4. Gas chromatographic (GC) analysis

Gas chromatography equipped with flame ionization detector (FID), capillary column SPB-5 (length: 30 m, diameter: 0.53 mm, film thickness: 0.5 µm was used with the following conditions: Helium as the carrier gas (flow/min: 6.830 mL/min); temperatures of injector and detector set at 260 °C and 280 °C. The initial oven temperature was maintained at 75 °C for 5 min., and finally programmed at 250 °C at a rate of 7 °C/min and the final time was 30 min.

2.5. Gas chromatographic-mass spectroscopic (GC-MS) analysis

Equilibration time was estimated at 0.5 min with a maximum temperature of 325 °C. The oven program switched on at 50 °C for 3 min (then 10 °C/min to 200 °C for 20 min, and finally at 7 °C/min to 300 °C for 25 min). Run time was calculated at 77 min. The interpretation of the mass spectrum of GC-MS analysis of the *n*-hexane fraction was carried out using the National Institute Standard and Technology database (NIST Library, version 2.0f. build Oct.22.2009). The mass spectrum of each unknown compound was compared with the spectrum of the known components stored in the NIST database, which has over 62,000 patterns. The compound's name, probability, chemical class, molecular weight, molecular formula, molecular weight, structure, and peak area were ascertained. The relative percentage amount of each analyte was calculated by comparing its average peak area to the area sum (Khan et al., 2016; Wang et al., 2018). The test materials' structures were drawn by using ChemDraw Ultra 8.0 software.

2.6. *In vitro* alpha-glucosidase inhibition

The alpha-glucosidase inhibition assay was determined using the method described by Bongmo et al. (2022). The reactants used were α-glucosidase from *Saccharomyces cerevisiae* (Sigma, type III, from yeast) *p*-nitrophenyl-α-D-glucopyranoside (Sigma). In brief, α-glucosidase was dissolved in buffer A (0.1 mol/L of potassium phosphate, 3.2 mmol/L of magnesium chloride, pH = 6.8) (0.1 units/mL), and *p*-nitrophenyl-α-D-glucopyranoside dissolved in buffer A at 6 mmol/L was used as substrate while using 1-deoxynojirimycin (DNJ) as a reference. About 102 µL buffer B (0.5 mol/L potassium phosphate, 16 mmol/L-$MgCl_2$, pH = 6.8), 120 µL sample solution (0.6 mg/mL in dimethylsulfoxide), 282 µL water and 200 µL substrate were mixed. This mixture was incubated in a water bath at 37 °C for 5 min, and then 200-µL enzyme solution was added and mixed. The enzyme reaction was carried out at 37 °C for 30 min, and then 1.2 mL of 0.4 mol/L glycine buffer (pH = 10.4) was added to terminate the reaction (Kurihara et al., 1994). enzymatic activity was quantified by measuring the absorbance at 410 nm with 1-deoxynojirimycin hydrochloride as standard.

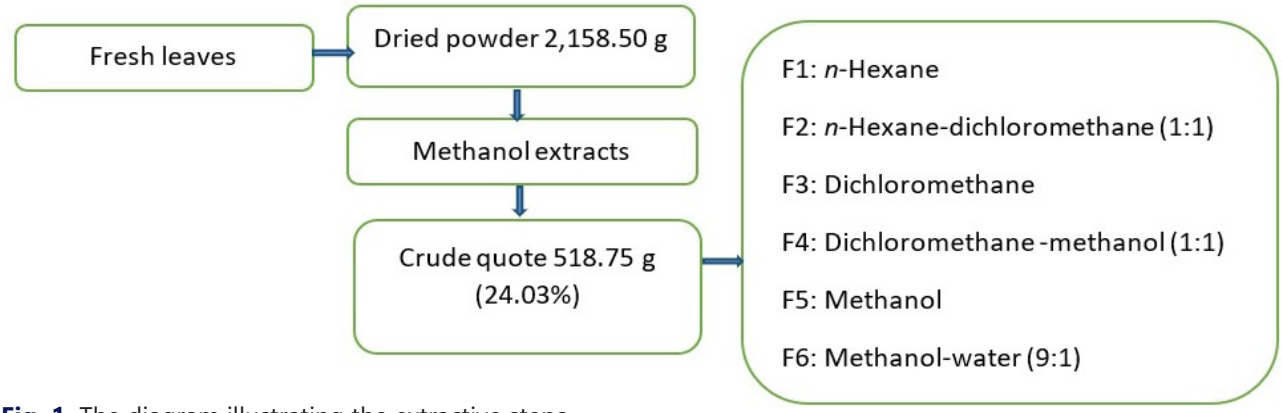

Fig. 1. The diagram illustrating the extractive steps.

$$\text{Percent inhibition (\%)} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100 \quad \text{(Eqn. 1)}$$

2.7. Antioxidant assays

According to the method described by Gülçin et al. (2005), scavenging the free radical activity was measured with 1,1-diphenyl-2-picryl-hydrazil (0.3 mM) solution prepared in ethanol with butylated hydroxyanisole (BHA) as the reference. Five microliters of each sample of different concentration (62.5-500 µg) was mixed with 95 µL of DPPH solution. The mixture was dispersed in 96 plates and incubated at 37 °C for 30 min. A microtitre plate reader measured the absorbance at 515 nm, and percent radical scavenging activity was determined compared to the methanol-treated control. EZFIT-software was used for descriptive statistics to estimate (IC_{50}) mean values and related standard error for each sample.

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100 \quad \text{(Eqn. 2)}$$

3. Results and Discussion

3.1. Yield extraction of leaf methanol extract of *Physalis peruviana* L. and its fractions

The methanol extract of extract weight obtained after extraction (six times) was 518.75 g from 2,158.50 g of the plant material, representing an extractive value of 20.03%. However, different fractions from that methanol extract, *n*-hexane (1.88 g), *n*-hexane-DCM (20.1 g), DCM (11.53 g), methanol-DCM (195.73 g), methanol (74.4 g), and methanol-water (10.76 g), represented yield of, 0.36%; 3.87%; 2.22%; 37.73%; 14.34% and 2.07%, respectively.

3.2. Phytochemical composition of *Physalis peruviana* L.

The analysis showed 52 peaks obtained in *n*-hexane fractions from leaf methanol extract of *Physalis peruviana* L. (Fig. 2 and Table 1), of which 29 phytoconstituents were identified (Fig. 3). Heptacosane (**25**) is the most abundant compound (37.94% obtained at 48.49 min), followed by hexadecanoic acid methyl ester (**14**) (23.67%), 1-hexadecyn-3-ol, 3,7,11,15-tetramethyl- (**20**) (7.94%), tetratetracontane (**29**) (4.96%), hexadecanoic acid (**16**) (3.69%), dodecane, 2,6,10-trimethyl- (**4**) (**16**) (3.69%), dodecane, 2,6,10-trimethyl- (**4**) (3.52%), and 2-(hexadecoxymethyl) oxirane (**18**) (3.43%). Previous studies reported a certain number of compounds in different parts and fractions of *P. peruviana* L. in other countries, particularly 2-undecenal (**3**) and hexadecanoic acid methyl ester (**14**) in Egyptian fruit, leaves, and pulp crude oil (Mayorga et al., 2001; Ramadan and Möersel, 2003, 2007; Rodrigues et al., 2009; Peter et al., 2020). Dihydroactinidiolide (**8**) in Turkish juice (Yilmaztekin, 2014), hexanal (**1**) in Colombian fruit (Dymerski et al., 2016; Majcher et al., 2020), hexahydrofarnesyl acetone (**13**) in leaf dichloromethane extract (Peter et al., 2020). According to our results, a few methyl esters have been identified in the *n*-hexane fraction of the methanolic extract of *P. peruviana* L. The characterized methyl esters include docosanoic acid, methyl ester; eicosanoic acid, methyl ester; heneicosanoic acid, methyl ester; hexadecanoic acid, methyl ester; nonanoic acid, 9-oxo-, methyl ester; octadecanoic acid, methyl ester; and tetradecanoic acid, 5,9,13-trimethyl-, methyl ester. According to Venditti (2018), all the methyl esters identified are possible artefacts due to the extraction with methanol. Nonetheless, some methyl esters have been identified in different parts of *P. peruviana* L. For example, dihydromanoyloxide-7-carboxylic acid methyl ester and methyl-3,7-bis(acetyloxy)cholestan-26-oate in ethanol/ethyl acetate (Ballesteros-Vivas et al.,

2019), methylprednisolone succinate in methanol and 3-methyl butyl butanoate (Yilmaztekin, 2014), capric acid, methyl ester; dodecanoic acid, methyl ester and methyl β-methylcrotonate, octanoic acid, 3-methylbutyl ester (Dymerski et al., 2016), ethyl 2-methyl propanoate in DCM (Majcher et al., 2020), methyl butanoate and trimethyl phenyl butenone in *n*-hexane and ethanol (Ramadan et al., 2015; Yilmaztekin, 2014), etc. In most cases, the solvent of extraction is alcohol (methanol and ethanol), which means probably those methyl esters are artefacts, as suggested in a recently reported paper in literature by Venditti (2018).

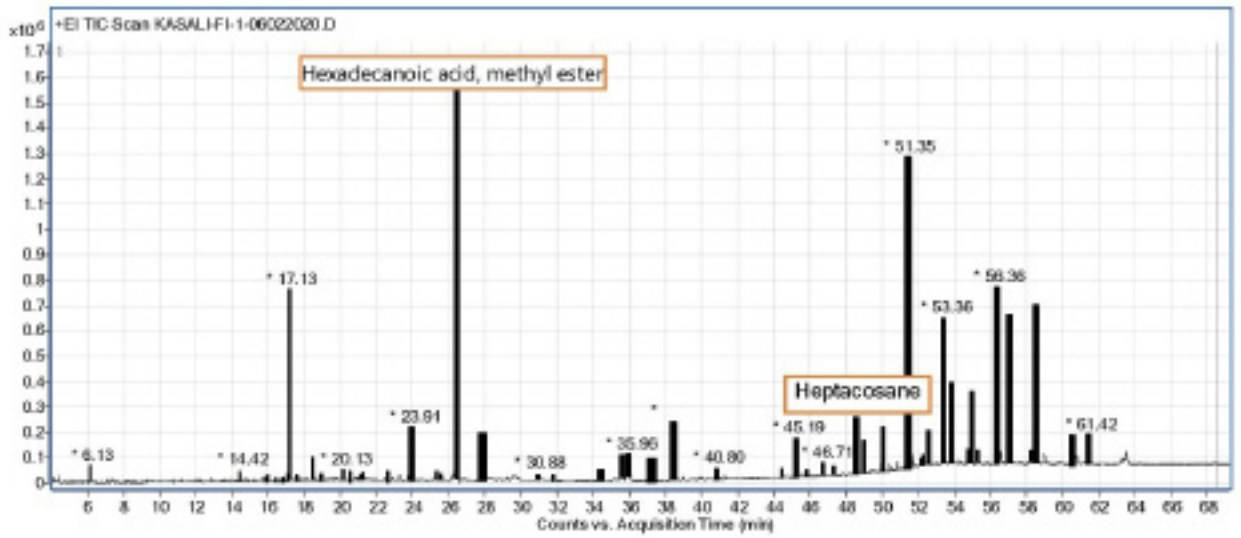

Fig. 2. Typical chromatogram of chemical substances present in *n*-hexane fraction of *P. peruviana* L.

3.3. Pharmacological studies *in vitro*

In vitro antidiabetic evaluation (α-glucosidase) and antioxidant status (DPPH free radical scavenging inhibition) of leaf methanol extract and their fractions from *P. peruviana* L. are presented in Table 2.

Antidiabetic evaluation, methanol-water, and dichloromethane-methanol fractions exhibited the best α-glucosidase activity inhibition than other fractions and methanol extract. With an IC_{50} value of 3.9 ± 0.71 µg/mL, the standard drug (DNJ) was 8.3 and 8.8 times more potent than methanol-water and dichloromethane-methanol fractions, respectively. However, antioxidant status demonstrated that dichloromethane-methanol (IC_{50} = 44.6 ± 0.54 µg/mL) and methanol (IC_{50} = 49.5 ± 0.87 µg/mL) fractions were the most effective and pharmacologically closer to the standard (IC_{50} = 44.2 ± 0.77 µg/mL). *In vitro* antidiabetic results of different fractions from methanolic extract of *P. peruviana* L. have shown α-glucosidase inhibitory activity (Table 2). *n*-Hexane-dichloromethane, dichloromethane-methanol, methanol, and methanol-water fractions demonstrated moderate inhibition, according to the classification of sample based on IC_{50} or CC_{50} (Indrayanto et al., 2021). Methanol-water (9:1) fraction presented the best enzyme inhibition among all fractions with IC_{50} value of 32.5 ± 0.27 µg/mL followed by dichloromethane-methanol (1:1) fraction (IC_{50} = 34.5 ± 0.71 µg/mL). Previously, aqueous fruit extract showed deficient α-glucosidase inhibitory activity (IC_{50} = 37.22 µg/mL) contrary to acarbose, which produced very strong inhibitory effect (IC_{50} = 0.006 µg/mL). That low enzyme inhibition was correlated with the extract's low phenol and flavonoid content (Susmita et al., 2012). Another investigation showed that aqueous and ethanol extracts of the fruits had exhibited no significantly α-glucosidase activity. The plant had the lowest phenolic content (Pinto et al., 2009). The potential of phenolic compounds as key enzymes α-glucosidase and α-amylase of type 2 diabetes was established by inhibiting these enzymes in treating carbohydrate absorption (Lin et al., 2016). Alcoholic solutions extract those compounds, and the presence in fractions 4 and 6 can explain their moderate alpha-glucosidase effect. Natural polyphenols have been reported to possess antidiabetic potential by inhibiting carbohydrate hydrolyzing enzymes like α-glucosidase (Medina-Pérez et al., 2019). They are in our present dichloromethane-methanol, and methanol fractions could justify that antidiabetic potential. On the antioxidant value, DCM and methanol fractions produced the best scavenging activity on DPPH with IC_{50} values of 44.6 ± 0.54 µg/mL and 49.5 ± 0.87 µg/mL, respectively. Pharmacologically that action was closer to butylated hydroxyanisole, used as the standard (IC_{50} = 44.2 ± 0.77 µg/mL). Similar to our results, the fruit methanolic extract revealed DPPH free radical scavenging activity, but antioxidant capacity was lower than gallic acid, ascorbic acid, and BHT, used as standards (Eken et al., 2016). Moreover, Mohammed and Ibraheem (Mohammed and Ibraheem, 2015) demonstrated that the fruit methanolic extract

Table 1
Compounds identified in n-hexane fraction from *Physalis peruviana* L. methanol crude extract.

Name of the compound	Class	Molecular formula	Molecular weight	Retention time	Area Sum %
(E)-2-Decenal	Aldehydes	$C_{10}H_{18}O$	154	14.42	0.16
(Z)-8-Methyl-9-tetradecenoic acid	Fatty acids	$C_{15}H_{28}O_2$	240	45.8	0.18
1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl-	Alcohols	$C_{20}H_{38}O$	294	38.43	7.18
2-(Hexadecoxymethyl)oxirane	Esters	$C_{19}H_{38}O_2$	226	34.42	3.1
2-Hexadecanol	Alcohols	$C_{16}H_{34}O$	242	52.12	0.09
2-Hexyl-1-octanol	Alcohols	$C_{14}H_{30}O$	214	17.05	0.09
2-Undecanone, 6,10-dimethyl-	Ketones	$C_{13}H_{26}O$	198	16.41	0.04
2-Undecenal	Aldehydes	$C_{11}H_{20}O$	168	15.88	0.07
Cyclohexane, 1,1,3-trimethyl-2-(3-methylpentyl)-	Aliphatic hydrocarbons	$C_{15}H_30$	210	21.12	0.07
Dihydroactinidiolide	Monoterpenes	$C_{11}H_{16}O_2$	180	18.44	0.39
Docosanoic acid, methyl ester	Esters	$C_{23}H_{46}O_2$	354	48.98	0.96
Dodecane, 2,6,10-trimethyl-	Aliphatic hydrocarbons	$C_{15}H_{32}$	212	16	3.18
Eicosanoic acid, methyl ester	Esters	$C_{21}H_{42}O_2$	326	45.19	1.48
Heneicosanoic acid, methyl ester	Esters	$C_{22}H_{44}O_2$	340	47.28	0.18
Heptacosane	Aliphatic hydrocarbons	$C_{27}H_{56}$	380	48.49	34.3
Hexa-hydro-farnesol	Sesquiterpenes	$C_{15}H_{32}O$	228	21.24	0.19
Hexadecane	Aliphatic hydrocarbons	$C_{16}H_{34}$	226	18.9	0.11
Hexadecanoic acid, methyl ester	Esters	$C_{17}H_{34}O_2$	270	26.48	21.4
Hexanal	Aldehydes	$C_6H_{12}O$	100	6.13	0.26
Hexadecanoic acid	Fatty acids	$C_{16}H_{32}O_2$	256	27.79	3.33
Hexahydrofarnesyl acetone	Ketones	$C_{18}H_{36}O$	268	23.91	2.1
Nonadecane	Aliphatic hydrocarbons	$C_{19}H_40$	268	26.59	1.08
Nonanoic acid, 9-oxo-, methyl ester	Diverse functional groups	$C_{10}H_{18}O_3$	186	17.6	0.08
Octadecanoic acid, methyl ester	Esters	$C_{18}H_{36}O_2$	298	37.13	2.2
Phytol	Diterpenes	$C_{20}H_{40}O$	296	20.13	0.36
Tetracontane, 3,5,24-trimethyl-	Aliphatic hydrocarbons	$C_{43}H_{88}$	604	44.48	0.26
Tetradecanoic acid, 5,9,13-trimethyl-, methyl ester	Ethers	$C_{18}H_{36}O_2$	284	30.88	0.36
Tetratetracontane	Aliphatic hydrocarbons	$C_{44}H_{90}$	518	53.36	4.48
Tetratriacontane	Aliphatic hydrocarbons	$C_{34}H_{70}$	478	54.92	2.73

was inhibited at 95.33 ± 2.52% of the DPPH radicals at 0.500 mg/mL concentration, significantly higher vitamin C (64.67 ± 5.03%), used as standard. On the other hand, the authors have revealed that the plant also contained a high level of flavonoids. It is naturally known that antioxidant activity is the critical biological activity of flavonoids (Kumar and Pandey, 2013). Previous antioxidant studies have focused mainly on different extracts from the fruit (Kasali et al., 2022). This is the first research on the α-glucosidase inhibition and scavenging property of the methanol extract of leaves and its fractions.

4. Concluding remarks

This first report of phytochemical profiling of leaf *n*-hexane fraction of *Physalis peruviana* L. indicated that the plant possesses chemical compounds from various groups, including aliphatic hydrocarbons (50.67%), esters (32.54%), alcohols (8.07%), fatty acids (3.85%), ketones (2.35%), diverse functional groups (0.95%), aldehydes (0.54%), monoterpenes (0.43%), diterpenes (0.39%), and sesquiterpenes (0.21%). Even though the substances described are not new molecules, almost 82.75% are recognized for the first time in the

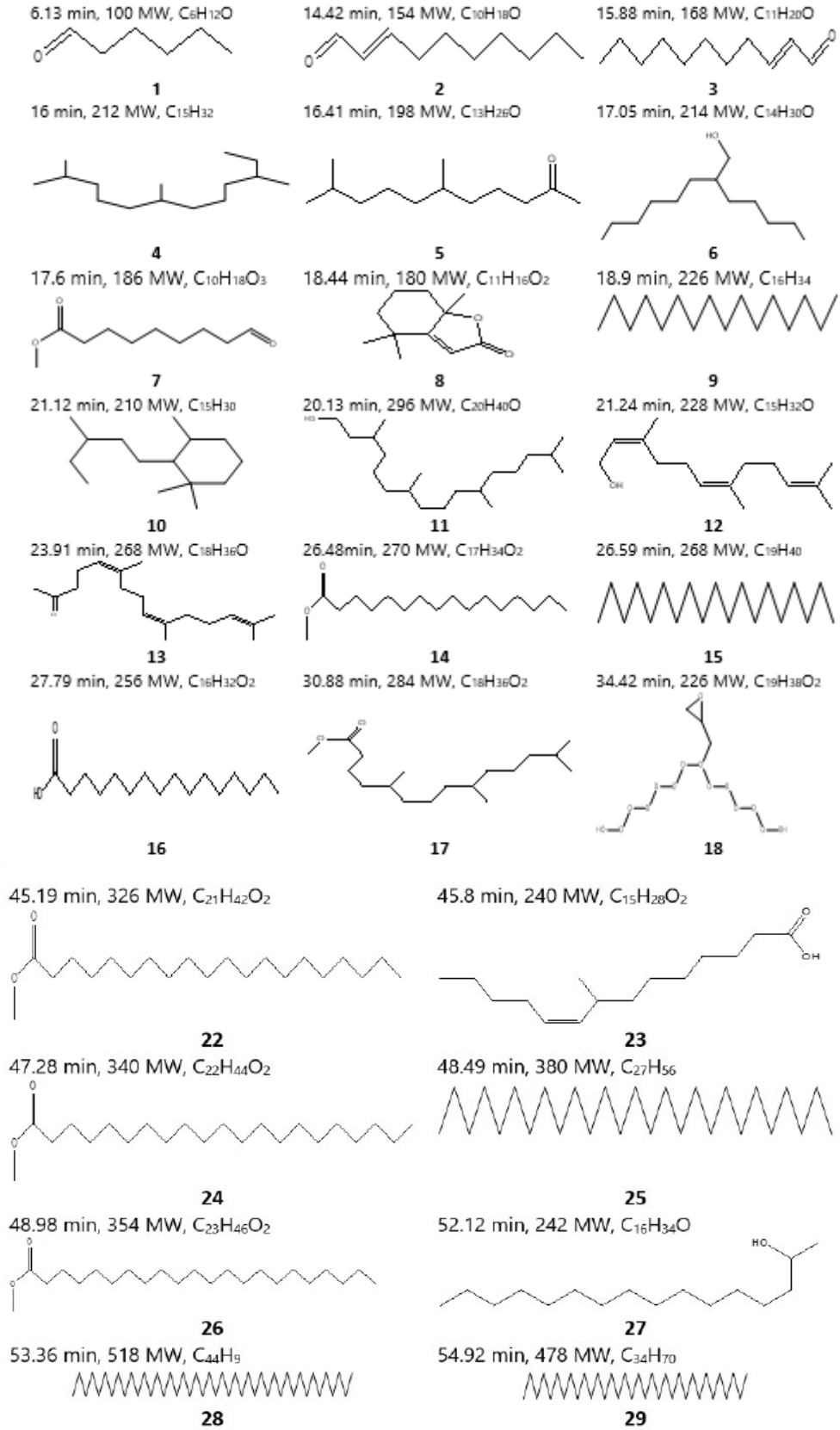

Fig. 3. Compounds identified in *n*-hexane fraction from *P. peruviana* L. methanol crude extract.

Table 2
In vitro antidiabetic and antioxidant activities of methanol extract and their fractions.

No.	Extract/Fractions	Glucosidase inhibition activity IC_{50} value (µg/mL)	Antioxidant activity IC_{50} value (µg/mL)
1	MEPh	51.2 ± 0.23	74.2 ± 0.34
2	F1	55.6 ± 0.75	70.7 ±0.12
3	F2	48.9 ± 0.42	75.3 ± 0.56
4	F3	65.7 ± 0.28	75.7 ± 0.78
5	F4	34.5 ± 0.39	44.6 ± 0.54
6	F5	43.5 ± 0.86	49.5 ± 0.87
7	F6	32.5 ± 0.27	53.4 ± 0.64
8	DNJ	3.90 ± 0.71	-
9	BHA	-	44.2 ± 0.77

MEPh (methanol extract of *P. peruviana* L.); F1 (*n*-hexane); F2 (*n*-hexane-dichloromethane); F3 (dichloromethane); F4 (dichloromethane-methanol); F5 (methanol); F6 (methanol-water), IC_{50} values [the means (95% confidence interval) of three measurements]. Data are expressed as the mean ± standard deviation (n=3).

leaf *n*-hexane fractions of this plant. Furthermore, this is the first investigation to establish the potential methanol extract and its fractions in the α-glucosidase and scavenging activity 2,2-diphenyl-1-picrylhydrazyl mechanism. Thus, dichloromethane-methanol, methanol, and methanol-water fractions exhibited interesting bioactivity, explaining the antidiabetic property locally attributed to *P. peruviana* L. leaves. Antioxidant activity is significantly noble contrarily to α-glucosidase inhibition. Further in-depth studies are needed to isolate bioactive compounds and expand the possibility of exploiting that plant.

Conflict of interest

The authors declare that there is no conflict of interest.

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