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Phytochemicals from *Phillyrea latifolia* L. leaves and fruit extracted with various solvents: Their identification and quantification by LC-MS and antihyperglycemic effects

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ABSTRACT

Phillyrea latifolia L. is a type of shrubland, which is widely known as mock privet, and belongs to the Oleaceae family. The objective of this study was to compare and assess the phytochemical composition, antioxidant and antidiabetic activities of ethyl acetate, methanol and aqueous extracts of the fruit and leaves of *P. latifolia* L. Phenolics were analysed by detecting individual bioactive compounds using an LCMS-2020 quadrupole mass spectrometer and by calculating total phenolic content (TPC). For the first time, the antioxidant and antidiabetic activities of both leaves and fruit were determined using DPPH radical scavenging. The aqueous extract was indicated to have higher antioxidant activities than ethyl acetate and methanol extracts. The individual constituents within the different extracts for both fruit and leaves were detected as the luteolin-7-O-glucoside in the ethyl acetate (854 μg · g^{-1} and 1,098 μg · g^{-1}), methanol (1,241 μg · g^{-1} and 2,136.43 μg · g^{-1}) and aqueous (509 μg · g^{-1} and 898.23 μg · g^{-1}) extracts, respectively. Extractions of ethyl acetate and methanol demonstrated stronger inhibitory activity against human salivary α-amylase than the aqueous extract of both parts of the mock privet. Similarly, extraction of ethyl acetate from the leaves and fruit of the mock privet indicated significantly better inhibitory activity than the methanol and aqueous extracts, respectively, for the inhibition of α-glucosidase activity. This study indicates that both fruit and leaves of mock privet may use as a potential source of natural biomolecules to promote healthy activities.

Keywords: antidiabetic, antioxidant, LC-MS, mock privet, phytochemicals

Abbreviations: ANOVA, analysis of variance; DNS, 3,5-dinitrosalicylic acid; DPPH, α-diphenyl-β-picrylhydrazyl; HPAE-PAD, high-performance anion-exchange chromato graphy with pulsed amperometric detection; HPLC-DAD, high-performance liquid chromatography with diode-array detection; IC_{50}, half maximal inhibitory concentration; LC-MS, liquid chromatography-mass spectrometry; MS, mass spectrometry; RT, room temperature; TPC, total phenolic content.

INTRODUCTION

Globally, there were 536.6 million diabetic people in 2021, and this number is expected to increase to 783.2 million people by 2045 (Sun et al., 2022). In 2021, there were approximately 6.7 million reported deaths worldwide attributed to diabetes and its associated complications, and it was reported that type 2 diabetes mellitus primarily affects males at a higher rate (Bhatti et al., 2022). According to the WHO's report (2016), one in eleven people now have diabetes and it is reported that this number will increase every year. These deaths can be prevented by the development of technologies that may prevent an increase in blood glucose levels.

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In the treatment of diabetic patients, 827 trillion US dollars are spent every year worldwide. People are preferring natural treatment methods due to the rapid increase in diabetes mellitus, the expenses of treatment costs and the side effects of medications used in treatment (nausea, vomiting, hypoglycaemia, palpitations, shortness of breath, etc.). Therefore, there is a growing interest in alternative and natural treatment ways for the management of diabetes.

Recent analyses have observed that inhibiting digestive enzymes have an effect to delay glucose transport in the small intestinal epithelium and decrease postprandial plasma glucose (Nyambe-Silavwe et al., 2015; Villa-Rodriguez et al., 2017; Barber et al., 2022). The interest in the inhibition of these enzymes with phenolic-rich foods is growing worldwide for the management of diabetes. *Phillyrea latifolia* L. commonly known as mock privet grows in the Mediterranean region of Turkey. Mock privet is a type of shrub that play a vital role in terrestrial biomes, particularly within Mediterranean ecological contexts (Yazici-Tutunis et al., 2016). These shrubby vegetation types exhibit a compact growth habit and possess well-developed subterranean root systems, enabling efficient nutrient and water uptake from the soil (Parlak et al., 2011; Barbeta et al., 2013). In different studies, it was reported that mock privets' resilience to drought is a testament to their intricate physiological mechanisms, which encompass osmotic regulation, antioxidant synthesis and anatomical features that contribute to water storage and efficient resource utilization (Ogaya and Peñuelas, 2003; Parlak et al., 2011; Barbeta et al., 2013). Therefore, mock privet may be highlighted for its crucial role in maintaining ecological stability and biodiversity under challenging environmental conditions within Mediterranean ecosystems. The economic importance of mock privet is related to its potential medicinal and traditional use (pharmaceutical and culinary; Pieroni et al., 2000; Gori et al., 2020; Emre et al., 2021), ease of growing conditions (resistance to salt and drought; Tattini et al., 2002) and utilization as firewood and timber (has a dense and durable wood; Poggiali et al., 2017). In this study, the importance of mock privet for both the food and pharmaceutical industries was examined due to its bioactive compounds and health-promoting activities. Some studies have found that both the fruit and leaf of mock privet are rich in phenolic compounds (Ayranci and Erkan, 2013; Selmi et al., 2020; Gori et al., 2021). In addition, it was reported that apigenin, caffeic acid, kaempferol, luteolin, myricetin, quercetin tannin and their derivatives were detected in the leaf of mock privet. In another study, the anthocyanins content of the fruit was analysed, and cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside were detected as the most abundant (Longo et al., 2007). Both leaf and fruit of the mock privet are used for their health-promoting activities such as antioxidant activity, the treatment of mouth ulcers and inflammations, and reduced the bilirubin level (Lanza et al., 2001; Janakat and Al-Merie, 2002; Ayranci and Erkan, 2013; Yazici-Tutunis et al., 2016). Arıtuluk (2012) reported that both leaf and fruit are traditionally used for their antidiabetic activities. The purpose of this study was to analyse the effect of leaf and fruit of mock privet on human salivary α-amylase and α-glucosidase enzymes using a more sensitive method, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). Analysis of carbohydrates is available with several methods in the literature. But the detection of carbohydrates with pulsed electrochemical detection at a gold working electrode is a reproducible and sensitive method. This is the first report for the detection of mock privet antidiabetic activity using HPAE-PAD. In addition, for the first time, the phytochemical composition of mock privet leaves and fruit was analysed using an LCMS-2020 quadrupole mass spectrometer.

MATERIALS AND METHODS

Plant material

Mock privet leaves and fruit were collected from Antalya, Turkey, in December. Fruits were at the mature stage. The plant material was identified by Agriculturist Yavuz Çetin, TropikHal. Both the leaves and fruit were dried at 50 °C using an oven (Azaizeh et al., 2013). Next, the dried fruits and leaves were ground and tightly packed in polyethene bags and were stored at −20 °C for further experiments.

Extract preparation

According to Sarikurkcu et al. (2018), phenolics were extracted from the dried mock privet leaves and fruit using ethyl acetate, methanol and water. Twenty grams of air-dried samples were extracted using ethyl acetate and methanol through a Soxhlet extractor for a duration of 5 hr. For water extract, 20 g of air-dried sample were mixed with 400 mL of deionized water and boiled for 15 min. Following, the ethyl acetate and methanol were removed using a rotary evaporator. The resulting water extract was subsequently freeze-dried. The extracts were stored at 4 °C.

Characterization of phenolic compounds in mock privet leaves and fruit

Chromatographic analysis of mock privet leaves and fruit compounds was conducted using the LCMS-2020 quadrupole mass spectrometer (linked with Shimadzu LC-2010 HT) fitted with an electrospray ionization source (Milton Keys, UK). It was performed for the identification of phenolics in the extracts and confirmed with reference standards. A Kinetex C18 analytical column (2.10 mm × 150 mm, 2.6 µm, 35 °C) was used for chromatographic separation. The conditions were determined as a flow rate of 0.25 mL · min^{-1}, an injection volume of 5 µL, mobile phase A (0.1% formic acid, 95% demineralized water and 5% acetonitrile) and mobile

phase B (95% acetonitrile, 5% demineralized water and 0.1% formic acid). Elution was started at 0% solvent B and increased to 10% at 5 min, afterwards solvent B rose to 25% and 35% at 10 min and 20 min, respectively. Following, solvent B reached 50% at 25 min and held up until 30.5 min. Finally, this gradient increased to 100% for a further 5 min and reduced to 0% at 36 min. The column was reequilibrated for 41 min. The mass-to-charge ratio in negative mode (m/z(–)) was used to identify each compound, and it was compared with their respective true standard. Cyanidin 3-O-glucoside, rutin, myricetin, quercetin-3-O-glucuronide, quercetin-7-O-glucoside, luteolin 7-O-glucoside and cyanidin 3-O-rutinoside were purchased from Extrasynthese (Lyon, France). 5-Caffeoylquinic acid was purchased from Acros Organics (Geel, Belgium). Ferulic acid, caffeic acid kaempferol aglycone, apigenin aglycone, rosmarinic acid and oleuropein were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method (T70 + UV/VIS spectrophotometer, PG Instruments, UK; Li et al., 2006). TPC was expressed as mg gallic acid equivalents (GAE) per 100 g of extract. In addition, the TPC of GFE and FDM was detected using the above procedure. Three replicates were performed for each sample.

α-diphenyl-β-picrylhydrazyl radical scavenging activity

α-diphenyl-β-picrylhydrazyl (DPPH) was analysed according to the method developed by Dorman et al. (2003). The mixture of 50 µL extract, 450 µL tris-HCl buffer (50 mM, pH 7.4) and 1.00 mL of fresh methanolic solution DPPH (0.10 mM) was well-shaken and kept in dark condition at room temperature for 30 min. The percentage of DPPH was calculated using the following equation after the samples' absorbance was read (517 nm): Inhibition % (DPPH) = $[(Abs_{Control} - Abs_{Sample})/Abs_{Control}] \times 100$. The half maximal inhibitory concentration (IC_{50}) value of the extract concentration, to inhibit 50% of the free radicals, was determined based on the graph produced from DPPH (%) activity, and the results were shown as IC_{50} = mg · mL^{-1}. All analyses were performed in triplicate.

In-vitro antidiabetic activity

A buffer solution of 20 mM sodium phosphate and 6.7 mM sodium chloride (pH 6.9) was used to dissolve human salivary α-amylase (S/3160/53 from Fisher Scientific, Loughborough, UK). On the other hand, 10 mM sodium phosphate buffer with pH 7.0 was used to dissolve intestinal acetone rat powder (I1630). Following, both enzyme solutions were vortexed for 30 s and then centrifuged at 17,000 *g* for 10 min. The supernatants were removed to conduct analysis, and these enzyme solutions were prepared freshly before each experiment (Aydin, 2015).

Determination of α-amylase activity

The developed method by Nyambe-Silavwe et al. (2015) was used. Twelve grams of sodium tartrate were added to 8 mL of 2M sodium hydroxide and heated until dissolved. It was included to stabilize the colour and to protect the product from oxidizing. The DNS solution was produced by combining the DNS powder with deionized water (20 mL) before placing the mixture immediately on a heating plate to dissolve. The colorant employed in the α-amylase reaction was DNS. The reducing sugars are produced as a by-product of the hydrolysis of starch by human salivary α-amylase. In an alkaline environment, DNS reacts with the free carbonyl group of the reducing sugars to form 3-amino-5-nitrosalicylic acid, which can be detected at 540 nm. As the reducing sugars are released, DNS altered the colour. DNS and prepared sodium tartrate were combined with deionized water (40 mL) to create the colour reagent solution.

A total of 200 mL of the sucrose solution, 50 mL of buffer (pH 7.0–10 mM), 50 mL of plant material and 200 mL of enzyme solution at various concentrations were combined for the experiment. Following, the mixture was vortexed (10 s) and incubated at 37 °C (10 min). As it was mentioned by Nyambe-Silavwe et al. (2015) study, a cartridge (Waters Oasis MAX-003036349A) was applied to eliminate the polyphenol's potential to interfere with colour development, the samples were left for 10 min incubation in a boiling water bath to stop the enzyme activity before the addition of colour reagent solution. Following the incubation, to measure the production of reduced sugar amount, a colour reagent solution was added to each sample (1 mL). After that, the samples were immediately placed on ice for cooling down to RT after being placed in a boiling water bath (10 min) to prevent enzyme activity. Following this, the samples were transferred to a boiling water bath for 10 min again. Then they were placed in vials for electrochemical detection using HPAE-PAD.

Inhibition of α-glucosidase enzyme

A previously reported technique by Gao et al. (2007) was modified to detect the activities of sucrase in an acetone extract of rat intestinal tissues by analysing sugars [sucrose and its products (glucose and fructose)] via a Dionex system running Chromeleon 6.5. Ion-exchange-LC combined with electrochemical detection permits the direct quantification of low-level (pM) carbohydrates <10,000 Da without the need for derivatisation or intensive sample preparation. To optimise the assay conditions, first Michaelis Constant (K_m/V_{max}) was detected and K_m was found as 18 mM and V_{max} as 0.09 µmoL sucrose hydrolysis/min. The amount of enzyme at different concentrations and the hydrolysis of sucrose were carried out at 37 °C for different time intervals to determine the optimum incubation time and the amount of enzyme. Based on preliminary studies, 18 mM of sucrose (200 µL) and 15 mg · mL^{-1} of acetone rat intestinal powder (200 µL) were mixed

with 100 µL of sodium phosphate buffer as the control sample. To analyse the antidiabetic activity of the extract, as a test sample 100 µL of sodium phosphate buffer was changed with 100 µL of extract/solution. To analyse the antidiabetic activity of leaf and fruit extracts, as a test sample 100 µL of sodium phosphate buffer was changed with 100 µL of samples. After that, the samples were incubated at 37 °C for 15 min. To stop enzyme activity, 750 µL of acetone was added to mixtures and immediately vortexed for 10 s and hold in ice to cool down at RT. The acetone was removed under nitrogen gas and centrifugation was repeated. Finally, the supernatant was filtered, and the amount of sucrose and its products (glucose and fructose) were determined using HPAE-PAD.

Detection of carbohydrates with HPAE-PAD chromatography system

Carbohydrate molecules are often separated using anion-exchange chromatography but considering they are weak acids, it may be more sensitive to identify them using amperometric detection, due to its speciality to depend on the oxidation of carbohydrates in the presence of sodium hydroxide at the gold electrode. HPAE-PAD (Dionex DX500, Sunnyvale, CA) comprised of a GP40 gradient pump, PAD system, an LC20 column oven and electrochemical detectors [ED 40, e.g., gold working, titanium and silver (reference) electrode]. The analytical column for α-glucosidase assay was used as Carbopac PA20 [Dionex, 3 mm × 150 mm and guard column (3 mm × 30 mm)] and for human salivary α-amylase assay, Carbopac PA200 (Dionex, 3 mm × 250 mm) with guard (3 mm × 50 mm). In addition, the mobile phase was 200 mM NaOH (flow rate: 0.4 mL × min^{-1} and injection volume: 10 µL). Finally, the elution was achieved using a gradient from 0% to 30% 200 mM NaOH in 10 min, 50% 200 mM NaOH from 10 min to 15 min and re-equilibration at 30%, 200 mM NaOH for 15 min.

Statistical analysis

The data analysis process was carried out using IBM SPSS Statistics 22. The homogeneity of the mean groups was assessed using Levene's test. The one-way analysis of variance (ANOVA) was followed by the Dunnett *C* test unless the condition was achieved, in which case the Tukey HSD post hoc test was used. If $p \leq 0.05$, differences were regarded as statistically significant unless otherwise stated.

RESULTS AND DISCUSSION

The interest in the therapeutic potential of plant species is growing worldwide. There is limited information about the phytochemical composition of mock privet fruit and leaves. For the first time, this study analysed the phytochemical profiles and antidiabetic activities of both fruit and leaves of mock privet using different solvents for the extraction.

Extraction efficiency and TPC

The type of solvents, such as ethyl acetate, chloroform, methanol, water, hexane, ethanol and acetone, is considerable for the extraction of bioactive compounds and these solvents have been widely used in the literature (Altemimi et al., 2017). Based on the type of solvent used, mock privet leaves and fruit extracts showed different efficiency rates (Table 1). For the leaves, the aqueous extract (18.02%) had the highest yield, followed by the methanol extract (16.98%) and the ethyl acetate extract (2.05%). Similar to leaves, for the extraction yield of the fruit, the aqueous extract had the maximum efficiency (19.02%), followed by the methanol extract (13.83%) and the ethyl acetate extract (1.47%). According to Altemimi et al. (2017), the extraction yield is influenced by the solvents, besides the temperature and time, the chemical characteristics of the samples have effects. Considering current findings, different studies indicated that water showed better extraction efficiency (EE) than methanol, thus these findings collaborated with what was reported in the literature (Kuo et al., 2015; Sarikurkcu et al., 2020). In addition, another recent study also reported that the polarity of solvents had a direct impact on the extraction yield for most of the plants, and water, methanol and ethyl acetate had polarity indexes of 9.0, 6.6, and 4.3, respectively (Ng et al., 2020). Therefore, water may show better EE than methanol and ethyl acetate.

Table 1 also demonstrated that the solvents that were most effective at extracting had the greatest phenolic compounds. The methanol extract (1,974 mg GAE · 100 g^{-1} extract), ethyl acetate extract (1,497 mg

Table 1. EE (%) and TPC (mg GAE · 100 g^{-1} extract) of the mock privet leaves and fruit.

Extracts	EE		TPC	
	Leaves	Fruit	Leaves	Fruit
Ethyl acetate	2.05 ± 0.003 x	1.47 ± 0.004 x	1,497 ± 1.04 x	1,004 ± 1.19 x
Methanol	16.98 ± 0.010 y	13.83 ± 0.011 y	1,974 ± 3.90 y	1,492 ± 0.23 y
Water	18.02 ± 0.012 y	19.02 ± 0.010 z	2,531 ± 2.75 z	2,260 ± 3.16 z

The values represent the mean ± SD of three replicates.
Means that are shared by different letters within each column indicate a Tukey's test comparison between the extracts at $p < 0.05$.
EE, extraction efficiency; GAE, gallic acid equivalents; SD, standard deviation; TPC, total phenolic content.

GAE · 100 g^{-1} extract) and aqueous extract (2,531 mg GAE · 100 g^{-1} extract) had the maximum phenolic content of leaves, respectively. Similar to leaves' TPC, fruit's ethyl acetate extract (1,004 mg GAE · 100 g^{-1} extract) has the lowest phenolic content whereas aqueous extract (2,260 mg GAE · 100 g^{-1} extract) has the highest. Statistical analyses showed significant differences between TPC values for both samples ($p < 0.05$). In addition, due to the presence of polar phenolic hydroxyl groups, phenolics, including numerous flavonoids, exhibit a strong tendency to be extracted into methanol and water. Similarly, the hexanic extract with lowest TPC can also be elucidated based on the same principles. The significant role of retrieving polyphenols from diverse sources has been suggested to be influenced by the varying polarity of solvents (Teruel et al., 2015). The data obtained in our study showed that the total phenolic of aqueous extracts was higher than that of methanol and ethyl acetate extracts. Similarly, Romero-Diez et al. (2018) and Wang et al. (2019) observed that the aqueous extract of the sample plant exhibited superior extraction efficacy for phenolic compounds when compared with the methanol extract. The results obtained regardless of the solvent revealed that mock privet leaves and fruit contain a significant amount of phenolic compounds, with leaves having a higher phenolic content than fruit. Teleszko and Wojdyło (2015) also informed that the leaves of different plants (chokeberry, quince, cranberry or apple) contain significantly more polyphenols than the fruit.

Phytochemical composition of mock privet leaf and fruit

For the first time, analysis of mock privet leaf and fruit phenolics was revealed using LC-MS and demonstrated a great diversity of compounds in both samples for all solvents used during extraction. A total of 14 phenolic compounds for the extractions of leaves and fruit of mock privet were identified and quantified, including 7 flavonols, 2 anthocyanidins, 4 hydroxycinnamic acids and 1 secoiridoid by using LC-MS (Table 2). The results demonstrated that the fruit and leaves of the plant have different phenolic component profiles and concentrations. The individual constituents within the different extracts for both fruit and leaves were detected as the luteolin-7-O glucoside in the ethyl acetate (854 µg · g^{-1} and 1,098 µg · g^{-1}), methanol (1,241 µg · g^{-1} and 2,136.43 µg · g^{-1}) and aqueous (509 µg · g^{-1} and 898.23 µg · g^{-1}) extracts, respectively. Similar to this study, Yazici Tutuinis et al. (2016) also reported the major phenolic of mock privet leaves as luteolin-7-O glucoside. Followed by caffeic acid was investigated in the ethyl acetate (202.75 µg · g^{-1} and 562 µg · g^{-1}), methanol (1,094.04 µg · g^{-1} and 1,596 µg · g^{-1}) and 615.33 µg · g^{-1} and 1,367 µg · g^{-1} in order of aqueous extracts of fruit and leaves. Rutin was found in fruit and leaves extracts of the ethyl acetate (430.84 µg · g^{-1} and 24.90 µg · g^{-1}), methanol (632.16 µg · g^{-1} and 254.34 µg · g^{-1}) and aqueous extracts (822.09 µg · g^{-1}

Table 2. Analytical characteristics of aqueous extract of mock privet fruit and leaf and their concentration for various solvents used.

Compound name	Morphological part	Ethyl acetate (µg · g^{-1} extract)	Methanol (µg · g^{-1} extract)	Aqueous (µg · g^{-1} extract)	Precursor ion (m/z)	Production (m/z)	Fragmentor energy (V)	Collision energy (V)	Retention time (min)
Flavonols									
Rutin	Leaves	430.84 ± 3.18	632.16 ± 21.78	822.09 ± 4.02	609	301	135	35	12.3
	Fruit	24.90 ± 0.10	254.34 ± 4.09	361.94 ± 1.12	609	301	135	35	12.5
Myricetin	Leaves	98 ± 1.63	136.43 ± 2.06	n.d	479	317	135	25	15.30
	Fruit	64 ± 1.08	91 ± 1.38	n.d	479	317	135	25	15.31
Quercetin-3-O-glucuronide	Leaves	38 ± 1.03	146.43 ± 2.11	34.26 ± 1.03	477	301	135	35	13.2
	Fruit	22 ± 1.08	109 ± 0.38	28 ± 0.08	477	301	135	35	13.2
Quercetin	Leaves	29 ± 0.94	204.08 ± 1.06	31.7 ± 0.04	463	301	135	25	12.5
7-O-glucoside	Fruit	26 ± 1.08	121 ± 0.38	30.01 ± 0.08	463	301	135	25	12.5
Luteolin	Leaves	1,098 ± 1.63	2,136.43 ± 12.06	898.23 ± 6.04	447	285	135	25	13.5
7-O-glucoside	Fruit	854 ± 1.08	1,241 ± 1.38	509 ± 0.78	447	285	135	25	13.5
Kaempferol aglycone	Leaves	35.12 ± 1.47	148.75 ± 0.32	108.20 ± 2.94	285.2	151	135	20	17.7
	Fruit	22 ± 0.03	91 ± 0.82	32 ± 0.08	285.2	151	135	20	17.7

(*Continued*)

Table 2. Continued.

Compound name	Morphological part	Ethyl acetate (μg · g^{-1} extract)	Methanol (μg · g^{-1} extract)	Aqueous (μg · g^{-1} extract)	Precursor ion (m/z)	Production (m/z)	Fragmentor energy (V)	Collision energy (V)	Retention time (min)
Apigenin aglycone	Leaves	12 ± 0.07	116.04 ± 2.01	673.91 ± 4.14	269.2	151	135	20	20.6
	Fruit	n.d	n.d	n.d	269.2	151	135	20	20.6
Anthocyanidins									
Cyanidin 3-O-rutinoside	Leaves	95 ± 0.50	377.43 ± 6.0	583.17 ± 9.51	595	449	162	30	9.12
	Fruit	101 ± 3.18	641 ± 7.51	1,009 ± 0.78	595	449	162	30	8.99
Cyanidin 3-O-glucoside	Leaves	46.61 ± 0.07	239.40 ± 1.91	398.23 ± 6.04	449	287	162	23	8.07
	Fruit	354 ± 1.08	278 ± 0.26	414 ± 11.43	449	287	162	23	8.07
Hydroxycinnamic acids									
Ferulic acid	Leaves	233 ± 1.57	316.12 ± 1.45	38.23 ± 1.32	193	149	135	10	13.16
	Fruit	279 ± 1.89	341 ± 1.38	40.92 ± 0.95	193	149	135	10	13.18
Caffeic acid	Leaves	562.17 ± 1.01	1,596 ± 11.37	1,367.40 ± 4.02	179	135	135	15	9.85
	Fruit	202.75 ± 0.70	1,094.04 ± 15.29	615.33 ± 5.42	179	135	135	15	9.85
Rosmarinic acid	Leaves	47 ± 0.54	77.03 ± 1.06	68.23 ± 0.06	359	161	90	20	11.08
	Fruit	29.75 ± 0.68	43.27 ± 0.57	39.87 ± 0.31					
5-caffeoylquinic acid	Leaves	498 ± 11.3	636.23 ± 9.84	1,298.23 ± 15.04	353	191	135	30	9.5
	Fruit	106 ± 1.08	364 ± 2.31	609 ± 7.91	353	191	135	30	9.4
Secoiridoids									
Oleuropein	Leaves	947.93 ± 10.02	571.19 ± 3.16	123.98 ± 2.49	539	275	165	20	6.3
	Fruit	322.05 ± 0.88	149 ± 1.42	87.49 ± 0.91	539	275	165	20	6.3

Based on this study, luteolin-4-O-glucoside was not detected in the leaves and this may be related to the plant cultivars, growing conditions and collecting time of the material. The findings of this study showed that both fruit and leaves of the mock privet contain flavonols more than anthocyanidins, hydroxycinnamic acids and secoiridoids. Also, this is the first study that indicates the oleuropein content of the mock privet fruit.

and 361.94 μg · g^{-1}), respectively. Apparently, both leaves and fruit of mock privet comprised of oleuropein content. In addition, ethyl acetate extraction of both parts indicated the highest concentration of oleuropein, which was followed by methanol and aqueous extracts. Also, the oleuropein content of the leaves was higher than the fruit. Only one study reported the oleuropein content of the ethanolic extraction of mock privet leaves with an amount of 160 mg · L^{-1} (Azaizeh et al., 2013). This is the first report about the oleuropein content of mock privet fruit. The results of phenolic composition indicated that leaves of mock privet contain more polyphenols than fruit. While apigenin aglycones were only detected for all extraction types of the leaves, the fruit did not have it. Ayranci and Erkan (2013) analysed the phenolic composition of methanolic extraction of the mock privet fruit using HPLC-DAD. It was reported that the fruit extracts included cyanidin, rosmarinic acid, cyanidin 3-O-glucoside, ferulic acid, cyanidin-3-O-rutinoside and caffeic acid at amounts of 191.4 mg · 100 g^{-1}, 190.1 mg · 100 g^{-1}, 90.4 mg · 100 g^{-1}, 289.1 mg · 100 g^{-1}, 225.2 mg · 100 g^{-1} and 221.2 mg · 100 g^{-1} fresh weight of fruit, respectively. Chlorogenic and *p*-coumaric acids were also detected in lower amounts (Ayranci and Erkan, 2013). Longo et al. (2007) investigated the phenolic composition of mock privet fruit, and it was found that cyanidin 3-O-rutinoside was the major polyphenol of the fruit whereas cyanidin 3-O-glucoside have found in low quantities. In this study, the aqueous extract of the fruit contains a higher amount of cyanidin 3-O-rutinoside than cyanidin 3-O-glucoside. In addition, fruit contains higher cyanidin 3-O-rutinoside and cyanidin 3-O-glucoside than the leaves extracts. Recently another study demonstrated that leaves of mock privet contain luteolin and quercetin derivatives as major polyphenols followed by caffeic acid, rutin and kaempferol derivatives (Gori et al., 2020). In addition to this study, Gori et al. (2021) analysed the phytochemical content of mock privet leaves' different tissues (e.g., adaxial and abaxial epidermis, mesophyll parenchyma) during different seasons. The HPLC-DAD analyses showed that different leaf tissues have similar phytochemical content and the identified peaks were quercetin, kaempferol, luteolin-7-O-glycosides, hydroxycinnamic acid, apigenin and luteolin-4-O-glycoside derivatives. It was concluded that the evergreen habit of mock privet may affect flavonoids' enhanced biosynthesis from spring to summer and the summer season contains higher phenolic content compared with other seasons (Gori et al., 2021).

Functional properties of mock privet leaves and fruit extracts

Antidiabetic activity and DPPH radical scavenging of fruit and leaves of mock privet were presented in Table 3. The leaves for all extraction methods showed higher antioxidant and antidiabetic activities compared with fruit. This may be due to the higher amount of TPC and phenolic composition and their synergist effects on the leaves.

The extracts' antioxidant capacity for both leaves and fruit were ranked using the IC_{50} values (mg · mL^{-1}) as follows: aqueous extract, methanol extract and ethyl acetate. Table 3 shows that the aqueous extract was more effective than the methanol and ethyl acetate extracts for the DPPH radical scavenging activity whereas lowest for the antidiabetic activities. The phenolic and flavonoid content of the extracts, as well as individual phenolic compounds found in the extracts, such as luteolin-7-O glucosidase, which was effective in antioxidant and antidiabetic activities, may be related to the extracts' functional potential (Park et al., 2016; Caporali et al., 2022). These findings are in agreement with the literature demonstrating that the antioxidant and antidiabetic activities are correlated to the phenolic content, which is also related to the solvent applied (Mokrani and Madani, 2016; Ismail et al., 2019; Venkatachalam et al., 2020). It is challenging to compare antioxidant assays due to the differences between the synergy of the antioxidant compounds in the mixture (e.g., some phenolic compounds respond slowly and take longer to react). Also, some compounds' antioxidant activity responses may effect by the factors such as structure and concentration (Sarikurkcu et al., 2020). Ethyl acetate and methanol extractions indicated better inhibitory activity compared with α-amylase than the aqueous extract for both parts of the mock privet. Similarly, ethyl acetate extraction of leaves and fruit of the mock privet indicated significantly better inhibitory activity than the methanol and aqueous extracts, respectively, for the inhibition of α-glucosidase activity. A positive control (acarbose,

Table 3. Antioxidant, α-amylase and α-glucosidase activities (IC_{50} mg · mL^{-1}) for mock privet leaf and fruit extracts and acarbose 1 mg · mL^{-1}.

Functional properties	DPPH		α-amylase		α-glucosidase	
	Leaves	Fruit	Leaves	Fruit	Leaves	Fruit
Ethyl acetate	19.3 ± 4.21 x	23.2 ± 1.92 x	2.6 ± 0.07 x	3.1 ± 0.08 x	1.8 ± 0.14 x	2.3 ± 1.02 x
Methanol	12.9 ± 2.13 x	15.1 ± 1.27 x	2.8 ± 1.39 x	4.1 ± 1.59 y	2.4 ± 1.17 y	3.0 ± 1.24 y
Water	8.6 ± 0.94 x	10.3 ± 1.31 x	11.3 ± 0.94 z	13.4 ± 1.41 z	10.9 ± 0.14 z	12.1 ± 3.15 z
Acarbose	–		2.2 ± 0.001		1.3 ± 0.001	

The values represent the mean ± SD of three replicates.
Means that are shared by different letters within each column indicate a Tukey's test comparison between the extracts at $p < 0.05$.
DPPH, α-diphenyl-β-picrylhydrazyl; IC_{50}, half maximal inhibitory concentration; SD, standard deviation.

1 mM) inhibited both α-amylase and α-glucosidase activities with IC_{50} values of 2.2 mg · mL^{-1} and 1.3 mg · mL^{-1}, respectively. The higher ethyl acetate and methanol extracts activity in the results can be explained for various reasons. First, these solvents extracted the substance(s) more successfully with higher α-amylase inhibitory activity than water. Secondly, interactions are possible between the substance(s) in the extracts and the enzymes (Zengin et al., 2018). Finally, the observed effects may also have been caused by the synergistic interaction of phenolics with one another and with other extract constituents (Abdillahi et al., 2011). The antioxidant and antidiabetic outcomes of this study also revealed the mock privet's leaves and fruit as a potential natural source for the food and pharmaceutical industries, regardless of solvent. Additionally, some studies reported important antioxidant capacities of mock privet leaves and fruit (Ayranci and Erkan, 2013; Gori et al., 2020, 2021). Therefore, both leaves and fruit of the mock privet may be used in the management of diabetes.

CONCLUSION

LC-MS is a powerful analytical technique for the identification and quantification of the polyphenol composition of a matrix under investigation. The use of the MS detector allows for higher accuracy and confidence in identifying analytes, even when phenolic compounds are present in trace amounts. Additionally, compared with the existing literature, it has been observed that LC-MS provides excellent selectivity for phenolic compound analysis in mock privet leaves and fruit extracts, utilizing various solvents such as ethyl acetate, methanol and water. This selectivity enables accurate quantification of phenolic compounds due to the technique's high sensitivity, linearity and low detection limits. The phytochemical composition of mock privet leaves and fruit was analysed for the first time using the LCMS-2020 quadrupole mass spectrometer. The results obtained have also shown that mock privet leaves and fruit extract had significant antioxidant potential and attractive inhibitory activity against the enzymes α-amylase and α-glucosidase. The extracts with different solvents possess significant differences in both antioxidant and antidiabetic activities. Besides, these results suggested that mock privet leaves and fruit may be considered as a source of beneficial polyphenols for the food and pharmaceutical industries. However, further verifications are needed to determine and assess the hyperglycaemic effect of mock privet leaves and fruit extract using cell culture and *in vivo* studies.

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EA contributed extensively to the work presented in this paper.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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