Received: 15 February 2020 | Revised: 30 March 2020 | Accepted: 30 March 2020

DOI: 10.1002/ffj.3576

RESEARCH ARTICLE

WILEY

Orange peel volatile oil: A green solvent for propolis extraction, enhanced α-amylase inhibition activity

Şaban Keskin

Vocational School of Health Services, Bilecik Şeyh Edebali University, Bilecik, Turkey

Correspondence
Şaban Keskin, Vocational School of Health Services, Bilecik Şeyh Edebali University, Bilecik, Turkey.
Email: sabankeskin61@hotmail.com

Abstract

Orange peel oil contains high amount of d-limonene which is reported to have antidiabetic activity. Propolis contains many biologically active compounds like phenolic acids, flavonoids and terpenes. Because of its rich phenolic composition, antidiabetic activity of propolis has also been shown in literature. Consumption of raw propolis is limited because of its resinous nature. It should be extracted to convert it into consumable form. Ethanol is the most used solvent for this purpose. Ethanol is the limiting factor for propolis consumption either for religious concerns or being harmful for human health. There is an increasing need for new, compatible and healthier solvents for propolis extraction. This is the first paper describing the extraction of propolis with orange peel oil. 1:10 (g/v) ratio was used for extraction. Orange peel oil (OPO), orange peel oil propolis extract (OPOPE) and ethanol propolis extract (EPE) were separately analysed by using GC-MS technique. Total phenolic content of oil and propolis extract was also carried out. Inhibition of α-amylase from barley malt with new extract was also reported. It was found that OPOPE was rich in volatile compounds of propolis, phenolic acid derivatives and free fatty acids. It was observed that OPOPE showed higher inhibition on α-amylase enzyme (IC_{50} 0.196 mg/mL). It can be concluded that orange peel oil could be used as solvent for preparing propolis extract. Propolis extract obtained with orange peel oil could also be used as complementary medicine for the treatment of type 2 diabetes.

KEYWORDS

d-Limonene, inhibition, orange peel oil, propolis, α-amylase

1 | INTRODUCTION

Propolis, a resinous mixture collected by honey bees, contains many biologically active compounds like phenolics and volatiles. More than three hundred compounds have been detected in propolis up to now. Because of its complex composition solubility of propolis depends on the type of solvent. It is stated in literature that 70% ethanol is the best solvent for propolis extraction. Other solvents like ethyl acetate, dichloromethane, dimethyl sulfoxide, diethyl ether, tetra hydro furan, acetone and methanol were used for propolis extraction.[1,2] Most of these solvents are toxic and harmful for human health. That is why these solvents except ethanol have not been found application in the field.

Antioxidant, antimicrobial, antifungal, anticancer activities of propolis have been reported in literature.[3-5] Because of its antimicrobial, antifungal and antiviral activities, propolis could be an alternative for synthetic drugs. Propolis could also be used in food formulations as preservative. Besides such beneficial properties of propolis, there are some limitations of raw propolis usage not only in daily consumption but also in putting it into food formulations.[6] Raw propolis should be extracted with solvents to convert it into consumable form. As mentioned before, 70% ethanol is the best solvent

and the market are dominated by ethanol-based propolis extracts. There is an increasing need for new, compatible and healthier solvents for propolis extraction.

Orange peel oil contains high amount of d-limonene which is considered as natural green solvent.[7] It is stated that chemical composition of orange peel oil depends on the orange cultivar, growing area and distillation technique. Nevertheless, the compounds detected are somehow the same as d-limonene, α-pinene, α-terpineol, β-myrcene, β-pinene, terpinen-4-ol, α-terpinolene and several minor components.[8] The therapeutic effects of d-limonene have been extensively studied, and anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antiviral, antihyperalgesic and gastro protective effects, as well as other beneficial effects in health of d-limonene, have been reported.[9] Although d-limonene has been reported as skin sensitizer in literature, recent studies on animal models have revealed that this action is caused by the air oxidized d-limonene rather than d-limonene itself..[10] Type 2 diabetes is a chronic metabolic disease that is incurable completely. Inhibition of α-amylase coupled with α-glycosidase is one of the strategies in its cure.[11]

Main objective of this study was to carry out propolis extraction by using orange peel oil obtained by hydrodistillation technique. To the best of current knowledge, this is the first paper describing the extraction of propolis with orange peel oil. Chemical composition of orange peel oil propolis extract was compared with 70% ethanol extract composition. Total phenolic contents and antioxidant activity of the extracts were also determined. In vitro α-amylase inhibition activity of both orange peel volatile oil and orange peel oil propolis extract was also carried out. Possibility of using orange peel oil as solvent for propolis extraction was discussed by comparing the obtained data.

2 | MATERIAL AND METHODS

2.1 | Material

Washington navel–type oranges were purchased from a local market. Propolis sample was supplied from a local bee keeper in Bilecik city, Turkey. N-Methyl-N-(trimethylsilyl)-trifluroacetamide (MSTFA) was obtained from Cova Chem, LLC. $FeSO_4.7H_2O$, methanol, gallic acid and ethanol were purchased from sigma Aldrich, USA. All other reagents were analytical grade.

2.2 | Methods

2.2.1 | Extraction of orange peel oil

Orange samples were purchased from a local market. The peels were separated from the oranges and cleaned. Outer coloured part of the peel was manually removed and used for oil extraction. Orange peel oil (OPO) was extracted by using simple distillation method.[12] Obtained oil was kept at +4°C until analysing its chemical composition.

2.2.2 | Preparation of Propolis Extracts

Extraction of propolis with OPO and ethanol was carried out by simple maceration technique. 1:10 (g/v) ratio was used for both of the solvent. Frozen propolis sample was powdered by grinding, and 2 g of this fine powder was mixed with 20 mL of each solvent separately. Extraction was carried out for 48 hours on a magnetic stirrer under constant stirring at 150 rpm. Finally, mixtures were separately filtered and filtrates was labelled as orange peel oil propolis extract (OPOPE), and ethanol propolis extract (EPE).

2.2.3 | Determination of total phenolic and flavonoid contents

Total phenolic content of orange peel oil, orange peel oil propolis extract and ethanol propolis extract was determined by using Folin-Ciocalteu method, gallic acid as standard.[13] Results were expressed as mg GAE/mL. Total flavonoid content of the samples was determined by using aluminium chloride method, quercetin as standard. Results were expressed as mg QE/mL.

2.2.4 | Determination of antioxidant activity

Antioxidant activity of orange peel oil, orange peel oil propolis extract and ethanol propolis extract was measured by using 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH•) free radical scavenging method and ferric reducing antioxidant power (FRAP) test. The radical scavenging capacity of OPO, OPOPE and EPE was separately determined by using DPPH assay described by Molyneux.[14] For this purpose, 0.75 mL of each sample at six different concentrations was mixed with 0.75 mL of 0.1 mmol/L DPPH in methanol, and the absorbance was recorded at 517 nm after incubation of 50 min at room temperature. Results were expressed as SC_{50} (mg sample per mL), which corresponded the concentration of each sample that resulted in 50% scavenging of DPPH. Antioxidant activity of extracts was also measured by using ferric reducing antioxidant power (FRAP) method. The principle of FRAP method is based on the reduction of Fe^{3+} ions within the Fe $(TPTZ)^{3+}$ tripyridyltriazine complex in acidic medium to blue coloured $Fe(TPTZ)^{2+}$ complex by antioxidants.[15] FRAP reagent was prepared by mixing 2.5 mL of 10 mmol/L TPTZ dissolved in 40 mmol/L HCL, 2.5 mL $FeCl_3 \bullet 6H_2O$ and 25 mL of 300 mmol/L pH 3.60 acetate buffer. A 100 µL aliquot of samples was added separately to 3 mL of freshly prepared FRAP reagent and then incubated at 37°C for 4 minutes. The absorbance of the samples was recorded at 593 nm against distilled water blank. Ferrous sulphate solution in different concentrations ranging from 100 to 1000 µmol/L was used to prepare a standard calibration curve. Trolox was used as reference standard. Results obtained by the FRAP assay were given as µM of ferrous equivalent Fe (II) per 100 mL of sample.

2.2.5 | GC-MS analysis of the samples

Main chemical composition of orange peel oil, orange peel oil propolis extract and ethanol propolis extract was determined by using gas chromatography coupled with mass spectrometry. Derivatization of propolis extracts was carried out by using MSTFA. Briefly, propolis extracts were dried under vacuum. 5 mg of dried extracts was separately dissolved in 50 μL of dry pyridine and 75 μL of MSTFA. This reaction mixture was heated at 80°C for 20 minutes. GC-MS analysis was applied with an Agilent 7890A GC system equipped with HP5-MS capillary column (30 m × 0.25 mm × 0.25 μm) and Agilent 5975C inert MSD with Triple-Axis Detector. Helium was used as carrier gas at 10 mL/min flow rate. The initial temperature of the column was set to 60°C for 2 minutes; then, the temperature was increased to 170°C with 2°C/min rate and held for 3 minutes at this temperature and later increased to 250°C with 3°C/min increment. Analysis was carried out at this temperature. The injection of the samples was carried out at 220°C in splitless mode. MS scan range was applied as 35-450 atomic mass unit. Identification of the compounds was performed with their retention time and mass spectral patterns by comparing them with commercial library as wiley275.L.[16]

2.2.6 | Determination of α-Amylase Inhibition

α-Amylase activity was assayed in the presence of soluble starch as substrate. Reducing ends were determined according to DNS method described by Bernfeld (Bernfeld, 1955) as glucose equivalent. Reaction mixture containing 300 μL of 1% soluble starch and 300 μL of enzyme solution was incubated for 30 min at 35°C. Equal volume of DNS reagent was added into tubes and kept in a boiling water bath. Absorbance of the tubes was recorded at 550 nm against a blank sample. All characterization assays were performed triplicate.

TABLE 1 Total phenolic and total flavonoid contents of the extracts

Sample	Total Phenolics (mg GAE/mL)	Total Flavanoid (mg QE/mL)
EPE	16.46 ± 0.11	2.57 ± 0.06
OPOPE	8.33 ± 0.09	1.86 ± 0.08
OPO	1.2 ± 0.14	nd

Note: Results were expressed as the mean of three separate measurements with standard deviations. nd: not determined.

TABLE 2 Antioxidant activity of the extracts

Sample	DPPH SC_{50} (mg/mL)	FRAP (μmol$FeSO_4 7H_2O$/mL)
EPE	1.43 ± 0.06	26.84 ± 0.08
OPOPE	2.18 ± 0.05	15.81 ± 0.07

Note: Results were expressed as the mean of three separate measurements with standard deviations.

TABLE 3 Chemical content of orange peel volatile oil

No	Component	RT	Area %
1	α-pinene	4.466	2.51
2	tricyclene	5.262	1.53
3	limonene	6.878	64.56
4	bornylene	7.950	1.56
5	linalool	8.168	2.52
6	1,3,8-*p*-Menthatriene	8.289	0.45
7	*t*-*p*-2,8-Menthadien-1-ol	8.349	1.20
8	neral	8.476	0.64
9	*t*-limonene oxide	8.519	0.79
10	2-Cyclohexen-1-one, 4-(1-methylethenyl)	8.758	0.74
11	1,8-menthadien-4-ol	8.842	0.85
12	cyclodecanol	9.116	0.98
13	*cis*-carveol	9.188	0.86
14	*t*-(+)-carveol	9.554	4.34
15	carvone	9.625	3.50
16	α-citral	9.877	0.93
17	isopiperitenone	10.011	0.87
18	perillyl alcohol	10.267	0.51
19	α-fenchene	10.322	0.67
20	β-myrcene	10.571	0.48
21	piperitenone	10.710	0.63
22	camphene	10.879	0.53
23	α-copaene	11.059	0.48
24	octyl butyrate	11.096	0.23
25	germacrene-D	11.187	0.78
26	β-caryophyllene	11.601	0.37
27	Alloaromadendrene	11.886	0.22
28	α-humulene	12.004	0.11
29	α-amorphene	12.177	0.09
30	γ-muurolene	12.226	0.15
31	β-selinene	12.312	0.28
32	valencene	12.471	1.52
33	δ-cadinene	12.667	0.22
34	7-*epi*-α selinene	12.745	0.16
35	oxacyclotridec-10-en-2-one	12.930	0.21
36	elemol	13.020	0.06
37	caryophyllene oxide	13.484	0.36
38	α-limonene diepoxide	13.653	0.06
39	valencene	14.133	0.07
40	γ-gurjunene	14.412	0.20
41	*t*-γ-bisabolene	17.766	0.40
42	tricosane	20.590	0.14

IC_{50} value of the extracts was determined at five different extract concentrations at standard assay condition and dose-response curve was generated. Acarbose was used as reference inhibitor.[11]

3 | RESULTS AND DISCUSSION

3.1 | Determination of total phenolic and flavonoid contents

After the preparation of extracts, the amount of total phenolic and total flavonoid contents was determined in propolis extracts separately. The amount of these components in extracts was calculated, and results are summarized in Table 1. It is obvious from the results that total phenolic and total flavonoid concentration of EPE is two times more than OPOPE. This result could be explained by the fact that ethanol is the best solvent for the resin of propolis. This result also showed that nearly half part of propolis resin could be solved by OPO. It is stated in literature that total phenolic content of propolis samples differ from each other depending on many factors like resin source and harvesting season. It was reported that Bulgarian propolis samples collected from different parts of the country contained different amount of total phenolic content over the range of 11.2%-42.0%.[17] Ristivojević et al studied 47 different Turkish propolis samples and they reported that total phenolic content changed in the

TABLE 4 Identified compounds of the extracts by GC-MS

	OPOPE		EPE	
Identified Compound	**Ret. Time min.**	**Area %**	**Ret. Time min.**	**Area %**
terpinene-4-ol	8.174	1		
β-Pinene	8.375	1		
decyl aldehyde	8.471	0.3		
thymol	9.836	3.1		
eugenol	11.891	0.3		
β-eudesmol	14.281	0.4	47.745	8.29
4-vinyl-2-methoxy phenol			27.284	3.51
octanol	8.046	4.2		
glycerol	9.299	2.7		
2-methyl 2-butenoic acid	5.575	1	9.882	3.51
caproic acid	6.483	0.3		
palmitic acid	18.251	4.1	68.118	0.55
oleic acid	19.893	1	72.828	0.59
stearic acid	20.327	1		
benzoic acid	8.998	4.7	23.350	30
hydrocinnamic acid	11.249	0.7	33.920	7.6
cinnamic acid	12.864	1.2	41.740	9.88
p-coumaric acid	17.172	1	63.385	5.02
3,4 dimethoxycinnamic acid	18.091	0.4		
ferulique acid	18.692	0.4	70.068	4.45
caffeic acid	19.114	2.6	71.966	5.32
3,4 dihydroxybenzoik acid	15.862	0.2		
1,3,5 trihydroxybenzene	13.899	0.3		
vanillin			40.815	0.85
cinnamylcinnamate			80.494	3.05
dehydroabietic acid			79.425	2.1
chalcone	21.942	3.7		
aloeemodin	23.485	1.6		
lactone Derivatives	14.945	0.2	56.947	3.05
pinostrobine Chalcone			79.675	5.21
tectochrysin			84.861	4.32
d-fructose	15.639	4.9		
β-DL-arabinopyranose	16.102	1.1		
d-glucose	16.346	1		
ribitol	16.536	0.3		

range of 11.5%-48.6%.[18] Findings of the present study are in good agreement with these literature data.

3.2 | Determination of antioxidant activity

FRAP and DPPH methods were frequently used antioxidant tests for mostly natural extracts. The results are summarized in Table 2. It was found that EPE showed two times more antioxidant activity than OPOPE. This result could be explained by the total phenolic and total flavonoid contents of these extracts. It is also clear from the results that antioxidant activity of OPOPE is related to extracted active compounds of propolis since no antioxidant activity of OPO was detected under tested experimental conditions. Similar results were reported earlier.[19-21]

3.3 | GC-MS Analysis of the Samples

Chemical composition of the oil and propolis extracts was determined by gas chromatography coupled with mass spectrometry. Main compounds found in orange peel oil are listed in Table 3 as d-limonene (65%), *t*-carveol (4.5%), carvone (3.5%), alpha-pinene (2.5%), linalool (2.5%), bornylene (1.5%), valencene (1.5%), tricyclene (1.5%), alpha-citral (1%) and about thirty minor components. The composition of the oil was similar with some literature data.[22,23] Chemical composition of propolis extracts is summarized in Table 4. It was determined that orange peel oil propolis extract contained certain class of compounds found in propolis like volatiles, phenolic acid derivatives, cinnamic acid derivatives, flavonoids and some chalcone derivatives. When compared with the EPE, OPOPE contained more compound class of propolis nevertheless the concentration of phenolic compounds in EPE was higher. This result could be explained by the resinous nature of the propolis. Thymol, β-pinene and terpinene 4-ol were the most abundant volatiles in OPOPE, while β-eudesmol and 4-vinyl-2-methoxy phenol were most abundant in EPE. Benzoic acid, caffeic acid, cinnamic acid and *p*-coumaric acid were the main aromatic acids found in OPOPE, whereas benzoic acid, hydrocinnamic acid, cinnamic acid, cinnamyl cinnamate and dehydroabietic acid were the most abundant aromatic acids found in EPE. Tectochrysin and pinostrobin chalcone were only detected in EPE. Our findings of propolis composition for both of the extracts are quite similar with given literature data. Ristivojević et al (2018) studied 47 different Turkish propolis samples, and they reported that Turkish propolis samples were rich in benzoic acid derivatives, phenolic acid derivatives and flavonoids.[18] It was reported in another study that propolis samples from Turkey were rich in phenolic acid derivatives and flavonoids.[21]

3.4 | Determination of α-amylase inhibition

Inhibition effect of the extract on α-amylase was firstly investigated. The results were expressed as IC_{50} and are summarized in Table 5. It was found that OPOPE exhibited better inhibition activity than OPO and acarbose, a standard inhibitor of α-amylase. Better inhibitory activity could be explained by the synergistic action of limonene and propolis phenolic compounds. There are some papers reporting α-amylase inhibitory properties of both limonene and propolis.[24,25] Inhibitory activity of propolis is explained by its rich polyphenol composition. A wide range of IC_{50} values could be found from literature both for limonene and propolis on α-amylase. These differences could be explained by the source of enzyme.

TABLE 5 Amylase inhibition properties of OPO and OPOPE

	OPO	OPOPE	Acarbose
IC_{50} Values (mg/mL)	0.782 ± 0.05	0.196 ± 0.03	1.18 ± 0.06

Note: Results are the mean value of three separate measurements with standard deviations.

4 | CONCLUSION

Propolis should be extracted to convert it into consumable form since its resinous nature. It is stated in literature that 70% ethanol is the best solvent for propolis extraction. Other solvents like water, olive oil, glycerol and glycols were also used for propolis extraction. Antioxidant and antimicrobial activities of propolis have been revealed. On the other hand, there are some limitations of propolis usage in the aspect of its ethanol solubility, strong scent, bitter taste and aroma.[6] There has been an increasing demand for natural, healthier and green solvents for propolis extraction. Orange peel oil contains mainly d-limonene which is considered as natural green solvent and considered as a GRAS (Generally Recognized as Safe) material by the US Food and Drug Administration.[7] In this study, the extraction of propolis by using orange peel oil was described. Chemical composition of orange peel oil, orange peel oil propolis extract and ethanol propolis extract was determined by using GC-MS technique. When compared to EPE, OPOPE contained more compound class but in lower quantities. OPOPE also showed better inhibitory activity than OPO on α-amylase. Obtained results showed that orange peel oil could be a new solvent for propolis extraction. Further study should be carried out to show that OPOPE could be used as complementary medicine for the treatment of type 2 diabetes mellitus.

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work.

ORCID

Şaban Keskin https://orcid.org/0000-0002-0287-4268

REFERENCES

1. Bankova V, Popova M, Trusheva B. Plant sources of propolis: An update from a chemist's point of view. *Nat Prod Commun*. 2006;1:1023-1028.

2. Berretta AA, Arruda C, Miguel FG et al. Functional properties of Brazilian propolis: From chemical composition until the market, Chapter 4. In: Waisundara VY, Shiomi N, eds. *Superfood and Functional Food - An Overview of Their Processing and Utilization*, London: Intech Open, 2017:55–85.
3. Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis from different geographic origin. *J Ethnopharmacol*. 1999;64:235-240.
4. Sforcin JM, Bankova V. Propolis: is there a potential for the development of new drugs? *J Ethnopharmacol*. 2011;133:253-260.
5. Katircioğlu H, Mercan N. Antimicrobial activity and chemical compositions of Turkish propolis from different region. *Afr J Biotechnol*. 2006;5:1151-1153.
6. Busch VM, Gonzalez PA, Sepatin N, Santagapita PR, Ulrih PN. Propolis encapsulation by spray drying, characterization and stability. *LWT-Food Sci Technol*. 2017;75:227-235.
7. Aissou M, Chemat-Djenni Z, Yara-Varon E, Fabiano-Tixier A, Chemat F. Limonene as an agro-chemical building block for the synthesis and extraction of bioactive compounds. *C R Chim*. 2017;20:346-358.
8. Ayala JR, Montero G, Campbell HE et al. Extraction and Characterization of Orange Peel Essential Oil from Mexico and United States of America. *J Essent Oil Bear Pl*. 2017;20:897-914.
9. Murali R, Saravanan R. Antidiabetic effect of d-limonene, a monoterpene in streptozotocin-induced diabetic rats. *Biomed Prev Nutr*. 2012;2:269-275.
10. Ravichandran C, Badgujar PC, Gundev P, Upadhyay A. Review of toxicological assessment of *d*-limonene, a food and cosmetics additive. *Food Chem Toxicol*. 2018;120:668-680.
11. Keskin Ş, Şirin Y, Çakir HE, Keskin M. An investigation of *Humulus lupulus* L.: Phenolic composition, antioxidant capacity and inhibition properties of clinically important enzymes. *S Afr J Bot*. 2019;120:170-174.
12. Golmohammadi M, Borghei A, Zenouzi A, Ashrafi N, Taherzadeh MJ. Optimization of essential oil extraction from orange peels using steam explosion. *Heliyon*. 2018;4:1-18.
13. Slinkard K, Singleton V. Total phenol analysis, automation and comparison with manual methods. *Am J Enol Vitic*. 1977;28:49-55.
14. Molyneux P. The use of the stable free radical diphenylpicrylhyrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*. 2004;26:211-219.
15. Benzie IFF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. 1999;299:15-27.
16. Afrouzan H, Tahghighi A, Zakeri S, Es-haghi A. Chemical composition and antimicrobial activities of Iranian propolis. *Iran Biomed J*. 2018;22:50-65.
17. Popova M, Trusheva B, Bankova V. Content of biologically active compounds in Bulgarian propolis: A basis for its standardization. *Bulg Chem Commun, Special Edition B*. 2017;49:115-120.
18. Ristivojevića P, Dimkić I, Guzelmeric E et al. Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities. *LWT- Food Sci Technol*. 2018;95:367-379.
19. Can Z, Yıldız O, Şahin H, Asadov A, Kolaylı S. Phenolic profile and antioxidant potential of propolis from azerbaijan. *Mellifera*. 2015;15(1):16-28.
20. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. *Food Chem*. 2004;84:329-339.
21. Aliyazıcıoglu R, Sahin H, Erturk O, Ulusoy E, Kolayli S. Properties of phenolic composition and biological activity of propolis from Turkey. *Int J Food Prop*. 2013;16:277-287.
22. Bozkurt R, Gülnaz O, Aka Kaçar Y. Chemical composition of the essential oils from some citrus species and evaluation of the antimicrobial activity. *IOSR J Environ Sci Toxicol Food Technol*. 2017;11:1-8.
23. Gültepe N. How the use of orange (*Citrus sinensis*) peel essential oil affected the growth performance of rainbow trout (*Oncorhynchus mykiss*)? *Bull Univ Agric Sci Vet Med Cluj Napoca*. 2018;75:16-20.
24. Murali R, Saravanan R. Anti-diabetic effect of d-limonene a monoterpene in streptozotocin-induced diabetic rats. *Biomed Prev Nutr*. 2012;2:269-275.
25. Zhang H, Wang G, Beta T, Dong J. Inhibitory properties of aqueous ethanol extracts of propolis on alpha-glucosidase. *Evid Based Complement Alternat Med*. 2015;2015:1-7.

How to cite this article: Keskin Ş. Orange peel volatile oil: A green solvent for propolis extraction, enhanced α-amylase inhibition activity. *Flavour Fragr J*. 2020;35:411–416. https://doi.org/10.1002/ffj.3576