An investigation of ethanolic propolis extracts: Their potential inhibitor properties against ACE-II receptors for COVID-19 treatment by Molecular Docking Study

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Conflict of interest

No conflict of interest is declared.

Abstract

The angiotensin-converting enzyme (ACE)-related carboxypeptidase, ACE-II, is a type I integral membrane protein of 805 amino acids that contains one HEXXH-E zinc binding consensus sequence. ACE-II has been implicated in the regulation of heart function and also as a functional receptor for the coronavirus that causes the severe acute respiratory syndrome (SARS). In this study, the potential of some flavonoids present in propolis to bind to ACE II receptors was calculated *in silico*.

Binding constants of ten flavonoids, caffeic acid, caffeic acid phenethyl ester, chrysin, galangin, myricetin, rutin, hesperetin, pinocembrin, luteolin and quercetin were measured using the AutoDock 4.2 molecular docking program. And also, these binding constants were compared to reference ligand of MLN-4760.

The results are shown that rutin has the best inhibition potentials among the studied molecules with high binding energy -8,97 kcal/mol and Ki 0,261 μM, and it is followed by myricetin, caffeic acid phenethyl ester, hesperetin and pinocembrin. However, the reference molecule has binding energy of -7,28 kcal/mol and 4,65 μM. In conclusion, the high potential of flavonoids in ethanolic propolis extracts to bind to ACE II receptors indicates that this natural bee product has high potential for Covid- 19 treatment, but this needs to be supported by experimental studies.

Keywords: Coronavirus, Covid-19, propolis, flavonoids, ACE-II, molecular docking

Introduction

Propolis is a natural mixture that honey bees collect from nature in order to protect their hives. Honey bees use propolis for insulating hives, mummification of dead insects and bees and as an antibacterial, antiviral, antioxidant and anti-inflammatory agent for many biological activities. Crude propolis is a highly viscous, slightly soluble mixture in water and best dissolved in 60-80% ethanol. Propolis has been an indispensable component of apitherapy for centuries and has recently been used as a food additive, or supplementary, under the name of traditional and complementary medicine [1, 2].

Its composition varies according to the flora of the region where it is collected, but the majority of active ingredients of propolis comprise the family of polyphenols. Phenolic acids, flavonoids (flavanones, flavones, flavonols etc.), stilbenes, tannins are the active polyphenols

of propolis [1, 3]. Propolis are not consumed as raw, but their ethanolic and aqueous extracts are widely consumed in different formulations.

It has been reported that polyphenolic agents such as gallic acid, caffeic acid, protocatechuic acid, chrysin, quercetin, rutin, galangin, kaempferol, hesperetin, pinocembrin, pinobanksin, apigenin, luteolin, daidzein, caffeic acid phenyl ester (CAPE) are the most active polyphenols of propolis and these secondary metabolites vary depending on the propolis source [1, 4, 5].

Studies show that propolis extracts have high immunomodulatory effect and inhibition potential for some clinically important enzymes, such as urease, xanthine oxidase (XO), acetylcholinesterase (AChE), α-amylase, α-glucosidase [6, 7]. In addition, *in vivo* and *in vitro* studies show that flavonoids, one of the active ingredients of propolis, have high potential for Angiotensin-Converting Enzyme (ACE) Inhibition [8, 9, 10].

The newly discovered SARS-CoV-2 was characterized as a beta-coronavirus and recognized as the seventh discrete coronavirus species capable of causing human disease. The disease caused by the virus is officially named Coronavirus Disease 2019 (Covid-19) by Word Health Organization (WHO). The emerged global epidemic spread rapidly with 2.246.291 confirmed cases and 152.707 deaths across 213 countries, areas and territories (COVID-19 situation Report WHO, 20 April 2020). Subsequent studies have shown that SARS-CoV-2 has been suggested to recognize human ACE II more strongly than SARS-CoV, thereby increasing the ability to be transmitted from person to person [11]. Therefore, ACE II enzyme inhibition is important for treatments against these virus infections caused by SARS-CoV-2.

The aim of this study was to calculate the inhibition constants of some flavonoids, one of the active ingredients of Anatolian propolis, to the ACE II enzyme by molecular modeling as a positive control (S, S) -2- {1-Carboxy-2- [3- (3,5-Dichloro-Benzyl) -3h-Imidazole-4-Yl] -Ethylamino}-4-Methyl-Pentanoic Acid (MLN-4760). We analyzed therapeutic potential compounds of Turkish propolis extracts tested against ACE-II in experimental studies with *in silico* methods.

Up to now, there are very limited studies about Covid-19 and most researchers focused primarily on clinical cases. However, to the authors' knowledge, no study has been made on inhibition of ACE-II known to be associated with Covid-19. Therefore, the paper will encourage further researches about Covid-19 and candidate drug compounds.

Materials and Methods

Materials

Raw propolis samples obtained experienced beekeepers in 2018 from Black Sea Region, Turkey.

Chemicals

All phenolic standard for HPLC analyses of gallic acid, protocathequic acid, *p*-OH benzoic acid, catechin, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, myricetin, resveratrol, daidzein, luteolin, *t*-cinnamic acid, hesperetin, chrysin, pinocembrin, caffeic acid phenethyl ester (CAPE) were purchased from Sigma Chemical Co.(St Louis, MO, USA). All solvent for using mobile phases were analytical grade.

Preparation of propolis extracts

The raw propolis samples were frozen at -20 °C and then grinded to powder. The following method was used to prepare the ethanolic propolis extract: 10 g powdered crude propolis was placed with 100 mL 70% ethanol in a glass flask and stirred with shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h and then filtered with Whatman paper.

Determination of phenolic profiles

In this study, nineteen phenolic standards were used to high-performance liquid chromatography (HPLC) (Elite LaChrom Hitachi, Japan) with a UV detector. Separation was performed on a column with a reverse phase C18 column (150 mmx4.6 mm, 5µm; Fortis), in gradient solvent systems A (2% acetic acid in water) and solvent B (70:30, acetonitrile/water), which was sonicated before stirring and continuously degassed by the built-in HPLC system [12, 13]. The flow rate was kept constant at 1 mL/min using gradient programming, starting the flow of the mobile phase as B (5%) to 3 minutes, gradually increasing (up to 15, 20, 25, 40 and 80% at 8, 10, 18, 25 and 35 minutes, respectively) and decreasing to 5% at 40 minutes, before being left for 10 minutes to equilibrate in the column. The standard phenolic substances chromatogram is given in Figure 1.

Molecular Docking

In the study, some flavonoids detected in the ethanolic propolis extracts and they used as ligand (see in Supplementary File) for ACE II receptors. The crystal structure of a ACE II protein was downloaded from protein data bank web site (http://www.rcsb.org/pdb) (PDB ID:

1R4L: Resolution 3.00 Å). This crystal structure contains the inhibitory bound state of the extracellular metallopeptidase domain of ACE II with MLN-4760 compound. Small compounds of flavonoids used in docking studies were obtained from PubChem as SDF form and were drawn in the Hyperchem software [14] then subjected to conformational search with geometric optimization. Possible docking modes between compounds and the ACE II enzyme were studied using the Autodock 4.2 [15] and Lamarckian genetic algorithm was employed for docking simulations. The selected cavity is the binding site of reference inhibitor MLN-4760 [((S,S)-2-{1-Carboxy-2-[3-(3,5-dichloro-benzyl)-3H-imidazol-4-YL]-ethylamino}-4-methyl-pentanoic acid)]. A grid box dimensions of 52, 34, and 47 points in x, y, and z directions was set with a grid spacing of 0.375 Å. The program was run for a total number of 100 Genetic algorithm runs. The default settings were applied for all other parameters. Results of the molecular docking described the affinity represented by docking score and binding interaction of each ligand on the interested protein target. The visualization of results was performed with the help of the BIOVIA Discovery Studio 2018 [16].

Results and Discussion

Phenolic composition of propolis extract

In this study, a standard HPLC-UV chromatogram prepared with nineteen phenolic standards including some phenolic acids and flavonoids is given in Fig 1. The analysis data of the ethanolic propolis extract carried out according to this chromatogram are summarized in Table 1. Although the hydroxybenzoic acids and catechin derivatives of the propolis sample were found below determination limits, it was found to be rich in hydroxycinnamic acids and flavanoids. Among the hydroxycinnamic acid derivatives, the caffeic acid phenyl ester is the highest amount of phenolic component in the sample and followed it caffeic acid and cinnamic acid. Ferulic acid could not be detected in the sample. Among the flavonoids subclass of flavonoids, the highest amount of myrisetin was detected and rutin followed it.

Among these three flavanons studied, chrysin is the most abundant compounds pinosebrin and hesperetin followed. A smaller amount of flavone derivative of luteolin, was detected, while daidzein is not detected. Of all the studied compounds, chyrisin and pinosembrin were detected as major flavonoids in the propolis sample.

Although composition of propolis varies according to the flora of the region where it is produced, these flavonoids were also reported in propolis samples of different countries [17, 18, 19]. There are many scientific studies showing that propolis, a natural bee product, is a very rich mixture of flavonoids and is an important agent of apitherapy. Polyphenolic profile

of propolis varies according to the flora of the region where it is collected, caffeic acid, CAPE, rutin, quercetin, polyphenols such as myricetin, kampherol, hesperetin, galangin are the active substances of Anatolian propolis [3, 4, 5, 20, 21]. Barbarić et al. (2011) studied chemical composition of the ethanolic propolis extracts and determined ferulic acid, p-coumaric acid, caffeic acid, tectochrysin, galangin, pinocembrin, chrysin, apigenin, kaempferol, quercetin as phenolic compound in Croatia, Bosnia and Hercegovina and Macedonia propolis [22]. Major compounds of red propolis samples from Brasilia were found as luteolin (1.75 mg/g), naringenin (0.96 mg/g), kaempferol (0.59 mg/g), pinocembrin (0.41 mg/g) and biochanin A (0.39 mg/g) [23]. There are some differences between the findings because the chemical composition of propolis varies according to the geographical region, climate, environmental conditions and collection seasons [23, 24, 25]. The findings show that propolis are a good source of phenolic substances. The literature states that propolis samples from different geographical origins have a good antioxidant antimicrobial, antifungal and antiviral (Avian influenza virus) activity [26, 27, 28, 29, 30].

Binding affinity analysis for proteins and ligands with molecular docking

We focus here on the Anatolian propolis compounds used by people to treat infections against ACE II with molecular docking methods. For this purpose, we made docking analysis with the compounds and found that quercetin, rutin, myrisetin and hesperetin have a better affinity against ACE II enzyme than natural inhibitor MLN-4760 with low μM K_i values among the evaluated compounds (Table 2).

Furthermore, these compounds interacted with *Arg273, Thr371, His345, Pro346, Tyr515, Glu402* and *Glu375* in ACE II binding site. Especially, our *in silico* study showed that, rutin has the best binding affinity to the ACE II enzyme (Binding energy: -8.98 kcal/mol, K_i 0.261 μM). This compound was observed to bind to the residues *Asn149, His345, Asp269, Glu375, Glu406, Thr371, Tyr127* and *Asp368* of ACE II protein with the stronger hydrogen bond (Figure 2). It can be suggested that these residues can contribute to the enhancement of ligand affinity for ACE II enzyme. In addition, this compound has the pi-cation interaction with ***Arg273,*** pi-pi T shape interaction with ***His374,*** alkyl interaction with ***Cys344*** and pi-alkyl interaction with ***Tyr 127*** residues (Figure 2).

Therefore, in this study, *in silico* effects of Anatolian propolis on ACE II enzyme inhibition was investigated with the ten flavonoids as major substances. The results of this study showed that quercetin, rutin, myricetin and hesperetin compounds effectively inhibit the ACE II enzyme. These compounds can be clinically tested and used for the treatment disease

role of ACE II. Furthermore, *Arg273, Thr371, His345, Pro346, Tyr515, Glu402 and Glu375* are potential inhibitor targeting sites for the ACE II enzyme. Based on this information, we propose guidelines to develop novel and specific inhibitors that target ACE II enzyme.

Guerrero et al. (2012) experimentally demonstrated that some flavonoids have a relatively high inhibition potential for ACE-I [31]. With the molecular docking studies, we have shown that some of these flavonoids inhibit ACE-II. ACE-I and ACE-II enzymes are metalloproteases, both of which contain similar zinc fingers (HEXXH) in their active sites. Molecular docking studies indicated that there are bond interactions between rutin and zinc finger residues of ACE II. Because of similar active sites of ACE I and II, rutin may functionally bind both ACE I and II similar way.

It is revealed that Covid-19 binds to human angiotensin-converting enzyme 2 (ACE2) to enter the host cells. Rutin may compete with Covid-19 for ACE II and may prevent or delay the entry of Covid-19 into the cell.

In recent years, flavonoids have gained a great amount of interest with regards to their potential for cardiovascular protection. In fact, many epidemiological studies associate an increased consumption of foods and beverages rich in flavonoids with a reduced risk of CVD death [32, 33, 34]. Additionally, several of these flavonoids or their derivatives (i.e., diosmin, rutin and quercetin) are widely used as pharmaceutical agents for their vasoprotective properties (i.e., Daflon 500, cantaining flavonoid derivatives hesperedin and diosmin) [35]. Therefore, rutin and other flavonoids used in this study can be used for prophylactic purposes as ACE II inhibitors and competitor [36, 37].

In conclusion, *in silico* study is shown that the high binding constants for the ACE II receptors of flavanones in the ethanolic propolis extract make it a good competitive inhibitor and protective natural agents for the treatment of Covid-19. However, this study should be supported with further *in vivo* studies.

References

[1] Anjum, S.I., Ullah, A., Khan, K.A., Attaullah, M., Khan, H., Ali, H. and Adgaba, N. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences*, *26*(7), 1695-1703.

[2] Pobiega, K., Kraśniewska, K. and Gniewosz, M. (2019). Application of propolis in antimicrobial and antioxidative protection of food quality–A review. *Trends in food science and technology*, *83*, 53-62.

[3] Graikou, K., Popova, M., Gortzi, O., Bankova, V. and Chinou, I. (2016). Characterization and biological evaluation of selected Mediterranean propolis samples. Is it a new type?. *LWT-Food Science and Technology*, *65*, 261-267.

[4] Aliyazıcıoglu, R., Sahin, H., Erturk, O., Ulusoy, E., and Kolayli, S. (2013). Properties of phenolic composition and biological activity of propolis from Turkey. *International Journal of Food Properties*, *16*(2), 277-287.

[5] Yang, L., Yan, Q.H., Ma, J.Y., Wang, Q., Zhang, J.W. and Xi, G.X. (2013). High performance liquid chromatographic determination of phenolic compounds in propolis. *Tropical Journal of Pharmaceutical Research*, *12*(5), 771-776.

[6] Baltas N., Yildiz O. and Kolayli S. (2016) Inhibition properties of propolis extracts to some clinically important enzymes, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31:sup1, 52-55.

[7] Catchpole, O., Mitchell, K., Bloor, S., Davis, P. and Suddes, A. (2018). Anti-gastrointestinal cancer activity of cyclodextrin-encapsulated propolis. *Journal of functional foods*, 41, 1-8.

[8] Hussain, F., Jahan, N., Rahman, K.U., Sultana, B. and Jamil, S. (2018). Identification of hypotensive biofunctional compounds of coriandrum sativum and evaluation of their Angiotensin-Converting Enzyme (ACE) inhibition potential. *Oxidative medicine and cellular longevity*, 2018. Article ID 4643736

[9] Silveira, M.A.D., Teles, F., Berretta, A.A., Sanches, T.R., Rodrigues, C.E., Seguro, A.C. and Andrade, L. (2019). Effects of Brazilian green propolis on proteinuria and renal function in patients with chronic kidney disease: a randomized, double-blind, placebo-controlled trial. *BMC nephrology*, 20(1), 140.

[10] Wang, Q., Sui, X., Sui, D. J. and Yang, P. (2018). Flavonoid Extract from Propolis Inhibits Cardiac Fibrosis Triggered by Myocardial Infarction through Upregulation of SIRT1. *Evidence-Based Complementary and Alternative Medicine*, 2018.

[11] Wan, Y., Shang, J., Graham, R., Baric, R. S. and Li, F. (2020). Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *Journal of virology*, 94(7).

[12] Cakir, H.E., Şirin, Y., Kolayli, S. and Can, Z. (2018). Validation methods for phenolic components with RP-HPLC-UV in various bee products. *Apiterapi ve Doğa Dergisi*, *1*(1), 13-19.

[13] Malkoç, M., Çakır, H., Kara, Y., Can, Z. And Kolaylı, S. (2019). Phenolic composition and antioxidant properties of Anzer honey from black sea region of Turkey. *Uludağ Arıcılık Dergisi*, *19*(2), 143-151.

[14] Froimowitz, M. (1993). HyperChem: a software package for computational chemistry and molecular modeling, *BioTechniques*, 14(6), 1010–1013.

[15] Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J. (2009). AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J Comput Chem.*, 30, 2785–2791.

[16] Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.

[17] Can, Z., Yıldız, O., Şahin, H., Asadov, A. and Kolaylı, S. (2015). Phenolic profile and antioxidant potential of propolis from Azerbaijan. *Mellifera*, *15*(1), 16-28.

[18] Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, *10*(3).

[19] Sun, C., Wu, Z., Wang, Z. and Zhang, H. (2015). Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing propolis extracts. *Evidence-Based Complementary and Alternative Medicine*, 2015, Article ID 595393.

[20] Lu, Y., Wu, C. and Yuan, Z. (2004). Determination of hesperetin, cinnamic acid and nicotinic acid in propolis with micellar electrokinetic capillary chromatography. *Fitoterapia*, *75*(3-4), 267-276.

[21] Erdogan, S., Ates, B., Durmaz, G., Yilmaz, I. and Seckin, T. (2011). Pressurized liquid extraction of phenolic compounds from Anatolia propolis and their radical scavenging capacities. *Food and Chemical Toxicology*, 49(7), 1592-1597.

[22] Barbarić, M., Mišković, K., Bojić, M., Lončar, M.B., Smolčić-Bubalo, A., Debeljak, Ž. and Medić-Šarić, M. (2011). Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells. *Journal of ethnopharmacology*, *135*(3), 772-778.

[23] Andrade, J.K.S., Denadai, M., de Oliveira, C.S., Nunes, M.L. and Narain, N. (2017). Evaluation of bioactive compounds potential and antioxidant activity of brown, green and red propolis from Brazilian northeast region. *Food Research International*, *101*, 129-138.

[24] López, B.G.C., Schmidt, E.M., Eberlin, M.N. and Sawaya, A.C. (2014). Phytochemical markers of different types of red propolis. *Food Chemistry*, *146*, 174-180.

[25] Sawaya, A.C.H.F., da Silva Cunha, I.B. and Marcucci, M.C. (2011). Analytical methods applied to diverse types of Brazilian propolis. *Chemistry Central Journal*, *5*(1), 1-10.

[26] Saral, Ö., Kilicarslan, M., Şahin, H., Yildiz, O. and Dincer, B. (2019). Evaluation of antioxidant activity of bee products of different bee races in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, *43*(4), 441-447.

[27] Saral, Ö., Yildiz, O., Aliyazicioğlu, R., Yuluğ, E., Canpolat, S., Öztürk, F. and Kolayli, S. (2016). Apitherapy products enhance the recovery of CCL4-induced hepatic damages in rats. *Turkish journal of medical sciences*, *46*(1), 194-202.

[28] Pratsinis, H., Kletsas, D., Melliou, E. and Chinou, I. (2010). Antiproliferative activity of Greek propolis. *Journal of medicinal food*, *13*(2), 286-290.

[29] Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of natural products*, *63*(7), 1035-1042.

[30] Yildiz, O., Karahalil, F., Can, Z., Sahin, H. And Kolayli, S. (2014). Total monoamine oxidase (MAO) inhibition by chestnut honey, pollen and propolis. *Journal of enzyme inhibition and medicinal chemistry*, *29*(5), 690-694.

[31] Guerrero, L., Castillo, J., Quiñones, M., Garcia-Vallvé, S., Arola, L., Pujadas, G., Muguerza, B. (2012). Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies, *PLoS One,* 7(11):e49493

[32] Zhou, B., Yang, L., Liu, Z.L. (2000). Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chem Phys Lipids*, 106: 53–63.

[33] Joshipura, K.J, Hu, F.B., Manson, J.E., Stampfer, M.J., Rimm, E., Frank, E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D. and Willett, W. (2001) The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann Intern Med.,* 134: 1106–1114.

[34] Kris-Etherton, P., Keen, C. (2002) Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr Opin Lipidol*., 13: 41–49.

[35] Muhammad, S. A. and Fatima, N. (2015). In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. *Pharmacognosy magazine*, 11(Suppl 1), S123.

[36] Mishima, S., Yoshida, C., Akino, S. and Sakamoto, T. (2005). Antihypertensive effects of Brazilian propolis: identification of caffeoylquinic acids as constituents involved in the hypotension in spontaneously hypertensive rats. *Biological and Pharmaceutical Bulletin*, *28*(10), 1909-1914.

[37] Maruyama, H., Sumitou, Y., Sakamoto, T., Araki, Y. and Hara, H. (2009). Antihypertensive effects of flavonoids isolated from brazilian green propolis in spontaneously hypertensive rats. *Biological and Pharmaceutical Bulletin*, *32*(7), 1244-1250.

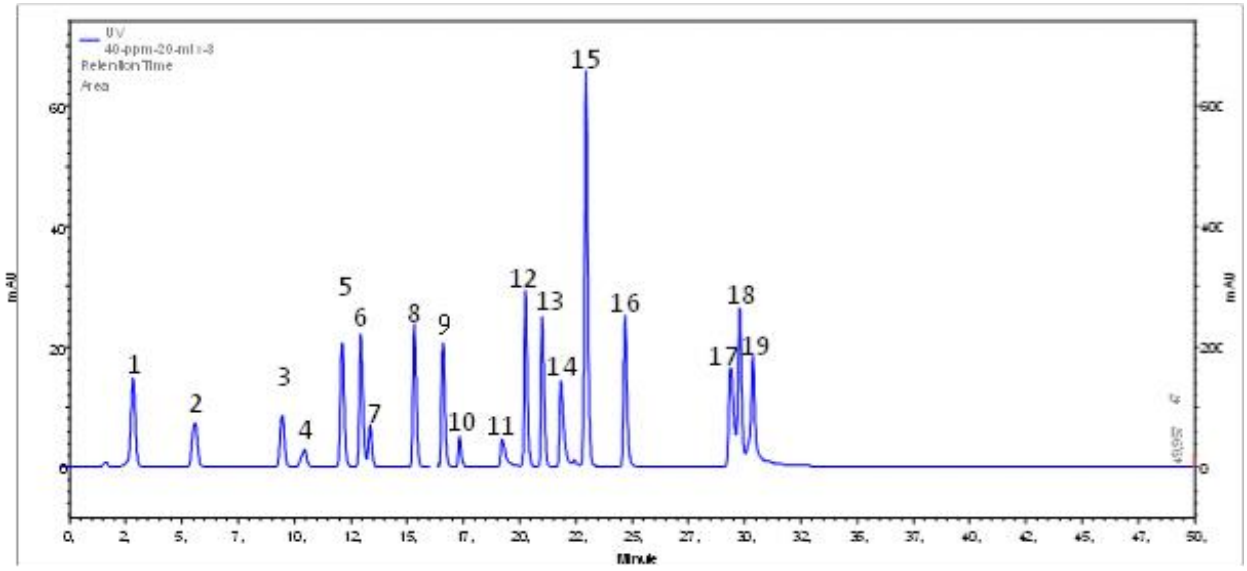


Figure 1. Chromatogram of nineteen phenolic compounds of HPLC-UV; (1): Gallic acid, (2): Protocathequic acid, (3): p-OH benzoic acid, (4):catechin, (5): caffeic acid, (6): syringic acid, (7): epicatechin, (8): p-coumaric acid, (9): ferulic acid, (10): rutin, (11): myricetin, (12): resveratrol, (13).daidzein, (14); luteolin, (15): t-cinnamic acid, (16): hesperetin, (17): chrysin, (18):pinocembrin, (19): Caffeic acid phenethyl ester (CAPE).

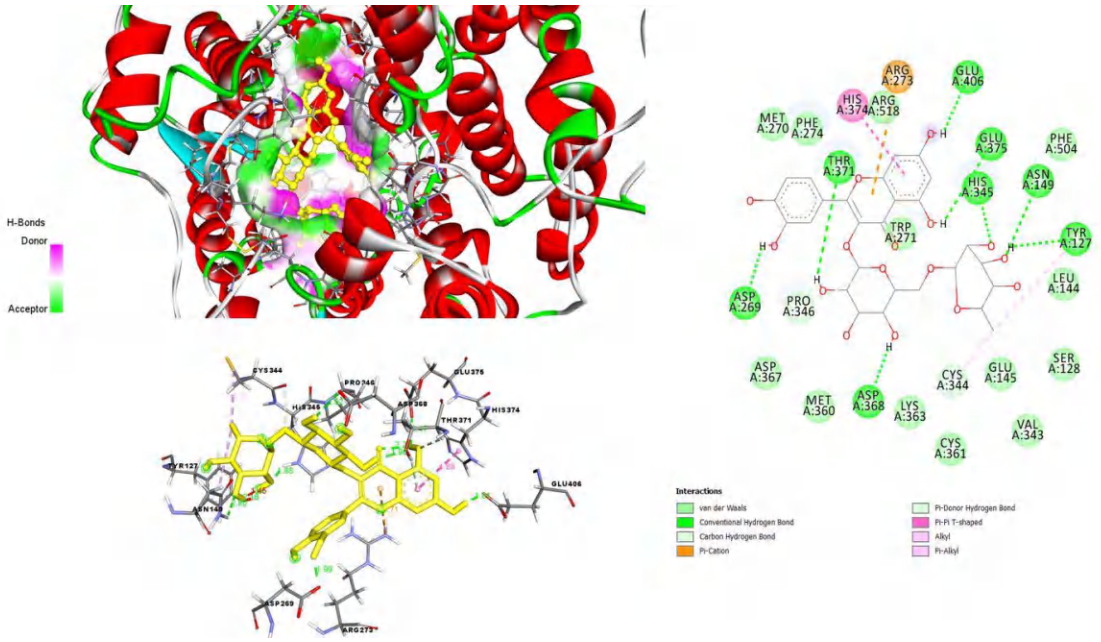


Figure 2. The two-dimension (2D) and three-dimension (3D) interaction analysis of ACE II with rutin

Table 1. Phenolic composition of propolis

Group name	**Compound name**	**mg/100g**
Hydroxybenzoic acids	Gallic acid	n.d.
	Protocateuic acid	n.d.
	p-OH Benzoic acid	n.d.
	Syringic acid	n.d.
Catechin	Catechin	n.d.
	Epicatechin	n.d.
Hydroxycinnamic acids	Caffeic acid	254.501
	p-Coumaric acid	63.871
	Ferulic acid	n.d.
	t-Cinnamic acid	145.455
	CAPE	541.213
Flavanons	Rutin	770.970
	Myricetin	1567.750
Flavones	Hesperetin	258.010
	Chrysin	4678.423
	Pinocembrin	1467.260
Isoflavons	Luteolin	684.752
Stilbands and Lignans	Daidzein	n.d
	Resveratrol	2.372

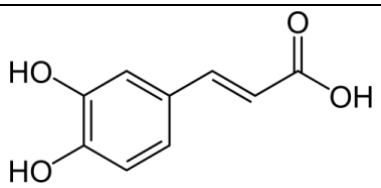
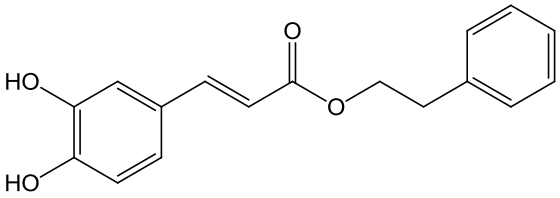
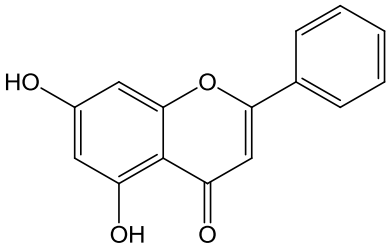
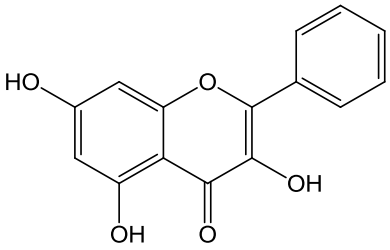
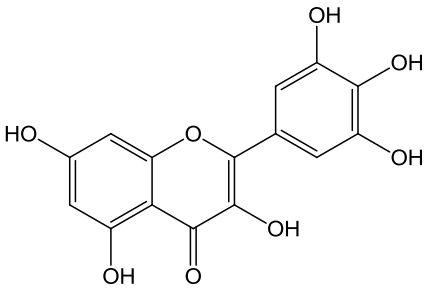
n.d: not detected

Table 2: Summary of reference and flavonoids compounds aganist ACE II with binding energy, Ki and interacted residues in the ACE II binding site.

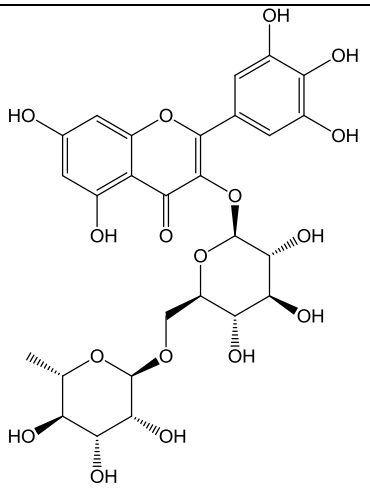
Ligand Name	Binding Energy (kcal/mol)	Ki (uM)	ACE II residues interacting with ligands
MLN-4760 ((S,S)-2-{1-Carboxy-2-[3-(3,5-Dıchloro-Benzyl)-3h-Imıdazol-4-Yl]-Ethylamıno}-4-Methyl-Pentanoic Acid)*	-7.28	4.65	Tyr127, Arg273, Phe274, Trp271, Arg273, Phe274, His345, Pro346,Cys361, Thr371, His374, Glu375, His378,Glu406, Phe504, His505, Tyr515, Arg518
Caffeic acid (3,4-dihyroxycinnamic acid)	-5.53	89.08	Arg273, His345, Pro346, Thr347, Ala348, His374, Glu375, His378, Phe504, His505, Tyr515
Caffeic acid phenethyl ester (Caffeic acid 2-phenylethyl ester; β-Phenylethyl caffeate) (2-Phenylethyl (2E)-3-(3,4-dihydroxyphenyl)acrylate)	-7.76	2.04	Tyr127, Ser128, Leu144, Glu145, Asn149, Trp271, Val343, Cys344, His345, Pro346, Met360, Cys361, Thr362, Lys363, Asp368, Phe504
Chrysin (5,7-Dihydroxyflavone)	-7.08	6.41	Tyr127, Ser128, Glu145, Asn149, Cys344, His345, Pro346, Met360, Cys361, Thr362, Lys363, Asp368, Phe504
Galangin (3,5,7-Trihydroxyflavone)	-7.18	5.41	Arg273, Phe274, His345, Pro346, Thr347, Ala348,Thr371, His374, Glu375, His378, Glu406, Phe504, His505, Tyr515, Arg518
Myricetin (3,3′,4′,5,5′,7-Hexahydroxyflavone)	-7.70	2.28	Arg273, Phe274, His345, Pro346, Thr347, Ala348, Thr371, His374, Glu375, His378, Glu406, Phe504, His505, Tyr515, Arg518,
Rutin (Quercetin-3-rutinoside hydrate)	-8.98	0.261	Tyr127, Ser128, Leu144, Glu145, Asn149, Asp269, Met270, Trp271, Arg273, Phe274, Val343, Cys344, His345, Pro346, Met360, Cys361, Lys363, Asp367, Asp368, Thr371, His374, Glu375, Glu406, Phe504,Arg518
Hesperetin (3',5,7-Trihydroxy-4'-methoxyflavanone)	-7.40	3.79	Arg273, His345, Pro346, Thr347, Ala348, Trp349, Thr371, His374, Glu375, His378, Glu406, Phe504, His505, Tyr510, Tyr515, Arg518
Pinocembrin (5,7-Dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one)	-7.46	3.38	Tyr127, Ser128, Leu144, Glu145, Asn149, Trp271, Val343, Cys344, His345, Pro346, Met360, Cys361, Thr362, Lys363, Asp368
Luteolin (2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone)	-6.93	8.36	Arg273, Phe274, His345, Pro346, Thr347, Ala348, Trp349, His374, Glu375, His378, Asp382, Glu402, Phe504, His505, Tyr510, Tyr515, Arg518
Quercetin	-7.62	2.62	Arg273, Phe274, His345, Pro346, Thr347, Ala348, Thr371, Glu375, His374, His378, Glu406, Phe504, His505, Tyr515, Arg518

*Reference compund

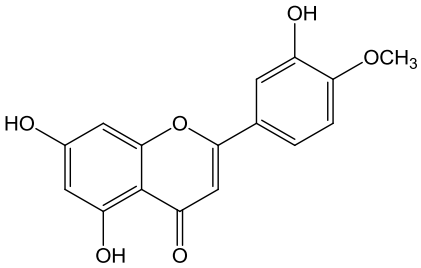
Supplementary Table: Chemical compounds used for the molecular docking screening

Chemical compound	2D-Structure
Caffeic acid (3,4-dihyroxycinnamic acid)	
Caffeic acid phenethyl ester (Caffeic acid 2-phenylethyl ester; β-Phenylethyl caffeate) (2-Phenylethyl (2E)-3-(3,4-dihydroxyphenyl)acrylate)	
Chrysin (5,7-Dihydroxyflavone)	
Galangin (3,5,7-Trihydroxyflavone)	
Myricetin (3,3′,4′,5,5′,7-Hexahydroxyflavone)	

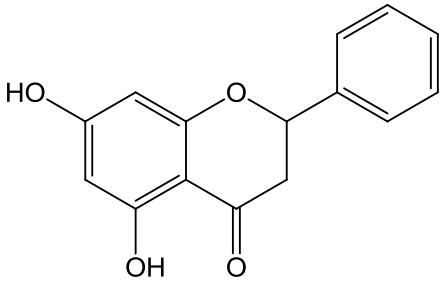
Rutin (Quercetin-3-rutinoside hydrate)



Hesperetin (3',5,7-Trihydroxy-4'-methoxyflavanone)



Pinocembrin (5,7-Dihydroxy-2-phenyl-2,3-dihydro-4H-chromen-4-one)



Luteolin (2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone)