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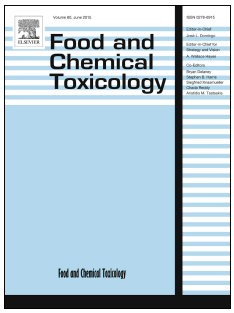

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Biological effects and chemical characterization of *Iris schachtii* Markgr. extracts: A new source of bioactive constituents

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ABSTRACT

This study gathers information about the effects of different extracts (methanol and water) from rhizomes and aerial parts of *Iris schachtii* on selected enzymes (cholinesterases, alpha-amylase and alpha-glucosidase, tyrosinase and lipase) as well as on their antioxidant capacities and antimutagenic properties in relation with their phenolic composition. The chemical composition was assessed by determining total phenolic and flavonoid content as well as individual phenolic compounds by HPLC-DAD. Moreover, antioxidant abilities of the investigated extracts were tested by using different assays including free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating, overall rhizomes being indicated as a superior source of antioxidant compounds. HPLC analysis revealed the abundance of some phenolics including apigenin (2584 µg/g extract) and luteolin (2510 µg/g extract) in aerial parts extracts while rhizomes were rich in apigenin (4734 µg/g extract) and kaempferol (4214 µg/g extract). The methanolic extracts exhibited a high anti-lipase activity while all extracts presented a relatively high inhibition on α-glucosidase. Furthermore, interactions between dominant compounds from extracts and selected enzymes were investigated by molecular modeling studies in order to explain at a molecular level the interactions between selected compounds and active sites of the enzyme/s.

Keywords: *Iris schachtii*; ornamental crops; bioactive compounds; enzyme inhibition; herbal extracts

1. Introduction

There is a new trend around the world in searching of new and safe phytochemicals for pharmaceutical or nutraceutical industries (Magnuson et al., 2013). This trend is in line with a strong consumer demand for safe and high-quality food phytochemicals which can be attributed in part to the widespread availability and accessibility of quality health data and information (Tajkarimi et al., 2010). Moreover, synthetic food additives are passing a difficult season in addition to the great deal of time and money which is required to develop and approve new synthetic preservatives, and considering the high public pressure against them (Ortega-Ramirez et al., 2014; Tajkarimi et al., 2010).

Nonetheless, the excessive use of synthetic food preservatives, some of which are suspected because of their toxicity, increased pressure on food manufacturers to either completely remove these agents or to adopt natural alternatives for the maintenance or extension of a product's shelf life, the so called "clean labeling" (Mocan et al., 2017; Seneviratne and Kotuwegedara, 2010).

Currently, many plant bioactive compounds are considered as excellent alternative candidates to synthetic antioxidants or antimicrobials food additives possessing none side effects in comparison with synthetic additives. In this sense, medicinal plants are seen as an important and feasible alternative, as being used for thousands of years for the treatment and prevention of several health disorders.

Medicinal plant parts (roots, leaves, branches/stems, barks, flowers, and fruits) are rich sources of terpenes (carvacrol, citral, linalool, and geraniol) and phenolics (flavonoids and phenolic acids), and these compounds have been effective pharmacological agents (Ortega-Ramirez et al., 2014; Xu et al., 2017). However, not only medicinal or edible plants are useful from this point of view, but also non-edible plant

materials are usually investigated with this purpose. Currently, many food supplements and pharmaceutical products have been developed recently using plant matrices. (Llorent-Martínez et al., 2017). Hence, the chemical composition of plants commonly used as folk medicines, or from the same genus, is being investigated to look for new alternatives in the pharmaceutical and food industries (Llorent-Martínez et al., 2017).

Iris L. genus (Iridaceae) is widely distributed in Eurasia, North Africa, and North America gathering about 210 species (Rigano et al., 2009). *Iris* rhizomes have been used as diuretics, carminatives, and laxatives as well as for the treatment of coughing and pharyngitis in different folk medicine systems (Hacibekiroğlu and Kolak, 2015; Xie et al., 2014). Additionally, compounds or extracts of *Iris* species have been reported to possess various bioactive properties such as: anti-inflammatory, piscicidal, cytotoxic, hypolipidemic, antibacterial, antiulcer, antioxidant, antimutagenic and anticholinesterase activities (Basgedik et al., 2015; Burcu et al., 2014; Hacibekiroğlu and Kolak, 2015). Besides the medicinal/nutraceutical potential of *Iris* species they have an economic value as being used in perfume and cosmetic industries, and some of them have been cultivated as ornamental plants (Atta-Ur-Rahman et al., 2004; Hacibekiroğlu and Kolak, 2015).

The main purpose of this study was to investigate the phytochemical profile (HPLC-DAD), antioxidant capacity (free radical scavenging assays, reducing power, metal chelating and phosphomolybdenum assays), enzyme inhibitory activity (cholinesterases, a-amylase and a-glucosidase, tyrosinase and lipase) as well as antimutagenic potential of *Iris schachtii* Markgr. aerial parts and rhizomes as novel sources of bioactive compounds with potential pharmaceutical or nutraceutical applications.

2. Materials and Methods

2.1. Plant materials and preparation of extracts

Iris schachtii Markgr. was collected from Yukselen Village-Campus road, Konya-Turkey in the flowering season (July 2015). Taxonomic identification of the plant material was confirmed by senior taxonomist Dr. Murad Aydin Sanda. A voucher specimen was deposited in KNYA Herbarium of the Department of Biology, Selcuk University, Konya-Turkey. The plant material was dried at room temperature in a shaded place.

Dried aerial parts or rhizomes were grinded to a fine powder using a laboratory mill. To produce solvent extracts, the air-dried samples (5 g) were macerated with 100 mL of methanol at room temperature for 24 h. Further, the extracts were concentrated under vacuum at 40 °C by using a rotary evaporator. To obtain water extracts (1:10, w/v), the powdered samples were boiled with 250 mL distilled water for 30 min. The aqueous extracts were filtered and lyophilized (-80°C, 48 h). All samples were stored at + 4°C in dark until use.

2.2. Total phenolics, flavonoids and phenolic composition

The total phenolics content was determined by Folin-Ciocalteu method (Slinkard and Singleton, 1977; Zengin et al., 2016b) with slight modification and expressed as gallic acid equivalents (GAEs/g extract), while total flavonoids content was determined by $AlCl_3$ method with slightly modification and expressed as rutin equivalents (REs/g extract). Total phenolic acid content was determined by the method described by Vladimir-Knežević et al. (2011) and expressed as caffeic acid equivalents (CEs/g extract).

Phenolic compounds were evaluated by RP-HPLC (Shimadzu Scientific Instruments, Tokyo, Japan). Detection and quantification were carried out with a LC-

10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm x4.6 mm length, 5 μm particle size). Phenolic compositions of the extracts were determined by Mocan et al. (2016). Gallic acid, protocatechuic acid, (+)-catechin, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (-)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, luteolin, kaempferol and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as microgram per gram of extract using external calibration curves, which were obtained for each phenolic standard.

2.3. Biological activities evaluation

Antioxidant (radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum, metal chelating (ferrozine method)) and enzyme inhibitory activities (cholinesterase (Elmann's method), tyrosinase (dopachrome method), α-amylase (iodine/potassium iodide method), α-glucosidase (chromogenic PNPG method) and pancreatic lipase (*p*-nitrophenyl butyrate (*p*-NPB) method) determined by the method described by Grochowski et al. (2017). Cupric chelating activity was determined according to Custódio et al. (2012). The antioxidant abilities were expressed as equivalents of trolox (EDTA was used as a standard for evaluating metal chelating activity). The enzyme inhibitory activities of the extracts were obtained as equivalents of standard drugs per gram of the plant sample (galantamine for AChE and BChE, kojic acid for tyrosinase, orlistat for lipase, and acarbose for α-amylase and α-

glucosidase inhibition assays). Mutagenic/antimutagenic properties were determined with Ames method (Zengin et al., 2014b).

2.4. Molecular Modelling

2.4.1. Receptors preparation

All the crystallographic enzyme structures have been downloaded from the Protein Databank RCSB PDB (Berman et al., 2000): Acetylcholinesterase (pdb:4X3C) (Pesaresi and Lamba, 2016) in complex with tacrine-nicotinamide hybrid inhibitor, α-Amylase (pdb:1VAH) (Zhuo et al., 2004) in complex with r-nitrophenyl-α-D-Maltoside, α-Glucosidase (pdb:3AXI) (Yamamoto et al., 2011) in complex with maltose, and Tyrosinase (pdb:2Y9X) (Ismaya et al., 2011) in complex with tropolone, pancreatic lipase (pdb:1LPB) (Egloff et al., 1995) in complex with C11-Alkyl-3-phosphonate. Waters, inhibitors and all the other molecules present in the pdb files were removed by using Pymol (Schrödinger, 2015) then the enzymes were neutralized at pH 7.4 by PropKa suit implemented in Maestro 9.2 (Maestro, 2011). Seleno-cysteines and seleno-methionines if present, were converted respectively to cysteine and methionine, all the missing fragments and other errors in the crystal structures were automatically solved by the Wizard Protein Preparation implemented in Maestro 9.2 suite (Maestro, 2011).

2.4.2. Ligands preparation

Kaempferol, benzoic acid and rutin, are well-known bioactive compounds and were selected as representative substances present in *Iris schachtii* extracts, for docking studies. The chemical structures are reported in scheme 1 and the 3D representations have been downloaded from Zinc database (Irwin et al., 2012). The 3D structures of the ligands were prepared by the LigPrep tool embedded in Maestro 10.2, neutralized at pH 7.4 by Epik and minimized with OPLS_2005 force field (Shelley et al., 2007).

2.4.3. Molecular Docking

Taking into account the difference of concentration of the selected substances, the docking experiments have been carried out selectively on the enzymes employed for the *in vitro* enzymatic inhibition tests reported in this paper. Gold 6.0 (Jones et al., 1997)with the scoring function GoldScore, which has been previously found to be suitable to produce reliable poses on metal containing enzymes (Mocan et al., 2016) has been employed for all the docking experiments. The enzymatic active pocket was determined automatically by centering the grid on the crystallographic inhibitor, extended in a radius of 10 Angstroms. The poses depicted in Figure 1 are the representation of the lowest energy poses docked to the selected enzymes of kaempferol. In Figure 2, are reported the best and representative enzyme-ligand interactions of the best pose found for benzoic acid and rutin docked respectively to lipase and tyrosinase.

2.5. Statistical Analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The differences between the different extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 14.0 program.

3. Results and Discussion

3.1. Phytochemical composition

Phenolic compounds are considered as being the most abundant antioxidants in the human diet, possessing several important biological activities. Additionally, several studies from the literature have revealed the presence of the original forms of food

phenolic compounds indicating them as well as excellent antioxidants to prevent oxidation as well as exerting several biological activities such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Locatelli et al., 2017; Zengin et al., 2016a). Thus, total phenolic, flavonoid and phenolic acids contents as well as individual phenolic compounds were detected in the present study from different extracts of *Iris schachtii*, and the results are presented in Table 1 and Table 2. In all cases the highest amounts were obtained for the methanolic extracts, and particularly the rhizome extract contained the highest values in terms of total phenolic (74.74 mgGAE/g extract), flavonoids (37.82 mgRE/g extract), and phenolic acids (12.46 mgCE/g) content, while results obtained for extracts from aerial parts were lower (Table 1). A similar trend was previously found by Burcu et al. (2014) who described higher values of total phenolic compounds content for ethanolic extracts from rhizomes of *Iris germanica* in comparison with aerial parts from the same plant. In a similar manner Hacibekiroğlu and Kolak, (2015) found that methanolic extracts from rhizomes of *Iris albicans* contain as well higher values in terms of total phenolic content in comparison with aerial parts.

Besides total bioactive components, individual phenolic compounds were detected using an HPLC-DAD method by comparing their retention times and UV spectra with standards in the same chromatographic conditions (Figure 3). As seen in Table 2, the dominant compounds for the Rhizome-MeOH extract were kaempferol (4214 µg/g extract) followed by (+)-catechin, and benzoic acid, while Rhizome-WAT extract was rich in apigenin (4734 µg/g extract), and kaempferol. Moreover, aerial part-MEOH extract contained high amounts of luteolin (2510 µg/g extract), kaempferol, and benzoic acid while apigenin (2584 µg/g extract), and kaempferol were dominant in Aerial part-WAT. Nonetheless, the results from this present study are in line with previous findings

of Burcu et al. (2014) who detected higher amounts of (+)-catechin, and p-hydroxybenzoic acid in ethanolic extracts of rhizomes from *Iris germanica* in comparison with aerial parts.

3.2. Antioxidant properties

The compounds that can delay or inhibit the effects of oxidation have been considered as antioxidants, including compounds that either inhibit specific oxidizing enzymes or react with oxidants before they damage critical biological molecules (Abeywickrama et al., 2016). The antioxidant abilities of *Iris* extracts were testes using several assays ((free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating)), and the results are presented in Table 3. DPPH and ABTS assays measure the ability of *Iris* phenolic extracts to reduce the *in vitro* formed radicals. In this study, the highest DPPH and ABTS values were exhibited by Rhizome-MeOH extract (107.09 and 534.22 mgTE/g extract, respectively), while the lowest values were shown by Aerial part-Wat extracts (67.13 and 251.00 mgTE/g extract). Moreover, this trend was similarly observed in the total phenolic and flavonoid assays. The reducing power of *Iris* extracts respected a similar trend as in DPPH and ABTS assays, the highest values being obtained in both CUPRAC and FRAP assays for Rhizome-MeOH extract (264.26 and 189.74 mgTE/g extract).

Chelating agents, which bind prooxidant metals, are effective as secondary antioxidants (Abeywickrama et al., 2016). The present study demonstrated significant chelating activity for Aerial part-Wat in both ferrous and cupric chelating assays (17.28 and 31.52 mgEDTAE/g extract, respectively). These results might be ascribed to the presence of non-phenolic chelators including citric acid, polysaccharides, peptides and proteins in the water extracts (Rice-Evans et al., 1996; Zengin et al., 2014a). The phosphomolybdenum assay is considered a useful and simple assay to further estimate

the total antioxidant capacity of samples, and it is widely used to evaluate the antioxidant capacity of plant extracts, herbal dietary supplements or pure compounds (Llorent-Martínez et al., 2017). In this assay both rhizome extracts showed higher total antioxidant capacity than extracts from aerial parts, however in both cases methanolic extracts displayed superior values in comparison with water extracts.

3.3. Mutagenic/antimutagenic properties

In the light of recent studies, some plant and plant products demonstrated notable antimutagenic effects against genotoxic agents. The antimutagenic effect is considered as an important strategy for preventing carcinogenic process. With this in mind, new studies on discovering novel antimutagenic products are great interest in the area of natural products and bioactive compounds (Kinghorn et al., 2004; Zengin et al., 2014b).

The results of the preliminary range finding tests, *Iris* extracts gave no toxic effect to all tester strain *S. typhimurium* TA98 and TA100 at doses of 5000 µg/plate and lower in the presence and absence of S9, respectively. Based on the results of the range finding test, the doses mentioned above were determined as the highest doses. As shown in Table 4, TA98 and TA100 strains did not increase in the number of revertant colonies compared to the negative control when the bacteria were treated with *Iris* extracts at 5000, 2500, and 1000 µg/plate concentrations and below both with and without metabolic activation enzymes (S9). In other words extracts of Iris aerial parts and rhizome were not found to be mutagenic for TA98 and TA100 strains.

The antimutagenic effect of each extract was assessed from the mean number of revertants/plate, the standard deviation (SD) and the percent inhibition (% I) of the mutagenic activity of 4-NPDA and 2-AF for TA98 strain; SA and 2-AA for TA100 strain on

treatment with the three concentrations of the plant extracts. Inhibition rates of the extracts against well-known mutagens were manifested in Table 5.

According to this table, methanol extracts of both aerial parts and rhizome of *Iris* revealed weak antimutagenicity against 4-NPDA for TA98 strain in the absence of metabolic activation enzymes. Only water extracts exhibited moderate antimutagenicity at the highest doses with rates of 32% and 33%, respectively. After addition of S9 mix, methanol extract of aerial parts of *Iris* ameliorated the mutagenic effects of 2-AF with excellent antimutagenicity ratios (85%, 79%, and 72%) at all test doses for TA98. Rhizome methanol extracts revealed very strong antimutagenicity at doses of 5000 and 2500 µg/plate (88% and 72%, respectively). Although water extracts of rhizome alleviated the mutagenicity with a rate of 63%, considered as strong antimutagenic, water extracts of aerial parts showed weak antimutagenicity against 2-AF in the presence of S9 for TA98.

All extracts of *Iris* had no antimutagenic capacity against SA for TA100 in the absence of metabolic activation. When methanol extract of aerial parts was evaluated, it demonstrated a potential for significant reduction in the numbers of revertants and it was found to be strong antimutagenic at 5000 and 2500 µg/plate doses with rates of 83% and 71% inhibition with S9 mix for TA100 against 2-AA (Table 5). A dose of 1000 µg/plate was considered as moderate antimutagenic with 33% inhibition ratio. The highest inhibition rate (90%) was observed for methanol extract of rhizome at a dose of 5000 µg/plate against mutagenic effects of 2-AA for TA100. Also it had strong antimutagenic capacity at a concentration of 2500 µg/plate, while the lowest concentration (1000 µg/plate) exhibited moderate antimutagenicity.

Associated with 2-AA, the rhizome water extract induced the inhibition of revertants and was determined as strong antimutagenic with a ratio of 58% by adding

S9 mix, while this extract revealed moderate antimutagenicity at a dose of 2500 µg/plate with 33% inhibition for TA100 (Table 5).

Overall, it can be stated from the study that S9 metabolic enzyme system increased the inhibition rate, reaching 90% at some concentrations, of mutagenic effects of known chemicals both for TA98 and TA100 strains. These results suggest that extracts, with high antimutagenic activity in the presence of S9, should be suitable for evaluation concerning CyP450 modulations effects and it could be explained by finding that medicinal plants might contain compounds capable of inhibiting the CyP450 required for activating these mutagens.

3.4. Enzyme inhibitory effects

In the last decade, the prevalence of some diseases has rapidly increased worldwide and thus these diseases (Alzheimer's disease (AD), diabetes mellitus (DM) and obesity) are identified as global health problems. For example, AD is the main type of dementia and affects about 50 million people(ADI, 2015). Similarly, the worldwide prevalence of obesity has nearly doubled between 1980 and 2008 (Stevens et al., 2012). From this point, new strategies are need to decrease the prevalence of these major health problems. Among different therapeutic strategies, key enzyme inhibitory theory is the most popular for the management of these diseases. In this context, several enzymes are considered as targets for alleviating symptoms of these diseases (cholinesterase for AD; amylase and glucosidase for DM; tyrosinase for skin disorders (SD); lipase for obesity). For these purpose, many compounds are used as enzyme inhibitors (galatamine for AD; kojic acid for SD; acarbose for DM; orlistat for obesity), some of which are synthetically produced. However, most of these known inhibitors exhibited several unfavorable effects such as gastrointestinal disturbances, diarrhea and toxicity (Birari and Bhutani, 2007; Etxeberria et al., 2012; Kim and Uyama, 2005; Murray

et al., 2013). From this point, natural products may be considered an important pool to combat these diseases as enzyme inhibitors. Moreover, despite issues regarding absorption and metabolism and precise mechanisms of action *in vivo*, the screening for enzyme inhibitors found in plants is valid in highlighting those compounds with the most promise of biological activity.

Thus, the enzyme inhibitory properties of *I. schachtii* extracts were tested against cholinesterases (AChE and BChE), tyrosinase, α-amylase, α-glucosidase and lipase, and the results are given in Table 6. The methanol extracts exhibited higher AChE inhibition as compared to water extracts, and Rhizome-MEOH was the most active on AChE with 1.43 mgGALAE/g extract. However, the studied extracts were not active on BChE. The observed AChE inhibition may be explained with higher amounts of phenolics found in these methanol extracts and our results are supported by some previous researches who reported a linear correlation between phenolics and AChE inhibition(Bilal et al., 2016; Hasnat et al., 2013). As for tyrosinase, one extract (Rhizome-MEOH) displayed an inhibitory effect with 6.98 mg KAE/g extract. This result might be linked to the presence of kaempferol which was previously reported as a tyrosinase inhibitor(Chang, 2009; Kim and Uyama, 2005). In both amylase and glucosidase inhibition, the methanol extracts exerted higher inhibitory effects than water extracts. Aerial parts-MeOH (0.79 mmol ACAE/g extract) and Rhizome-MEOH (8.59 mmolACAE/g extract) were the most active on amylase and glucosidase, respectively. The higher levels of total bioactive compounds might be responsible for amylase and glucosidase inhibitory effects of the methanol extracts. In accordance with our current findings, correlations between the presence of several bioactive compounds and anti-diabetic properties of different extracts were previously reported (Lin et al., 2016). Similarly, we found the best inhibitory activity in methanol extracts for lipase and the studied water extracts were

not active on this enzyme. To the best of our knowledge, the present study is the first regarding the enzyme inhibitory effects of *I. schachtii.* At this point, the presented results could open new horizons for designing new plant-based nutraceuticals or phytopharmaceuticals.

3.5 Molecular docking

Recently, molecular docking studies have helped increase the knowledge of several key enzymes involved in metabolic processes, regarding the elucidation of their mechanisms and binding modes of inhibitors(Jamila et al., 2015; Picot et al., 2017; Sun et al., 2017). Moreover, in the nutraceutical field, computational techniques have been successfully used both for the prediction of ligand–target binding affinity and to better understand the molecular basis of the biological response (Herrera Acevedo et al., 2017; Zengin et al., 2017).

Enzymatic assays performed on the extracts of *Iris schachtii*, generally evidenced a good inhibitory activity to AChE, and α-glucosidase, α-amylase, pancreatic lipase and tyrosinase with significative differences between methanolic and water extracts. Indeed, the water extracts of aerial part and rhizome were totally inactive on lipase and all the extracts are inactive on BChE, also only the methanolic extract of rhizome showed inhibitory activity to tyrosinase. Among the identified bioactive compounds, we have selected kaempferol for molecular docking studies on AChE, α-glucosidase and α-amylase, being the most abundant in all the four extracts. With regards to lipase, where the activity is significant only for the methanolic extracts and it was plausible to assume that other substances rather than kaempferol would responsible for this activity. In fact, it has been previously reported that caffeic acid, ferulic acid and benzoic acid are good inhibitors of Lipase (Karamać and Amarowicz, 1996). To confirm these data, we have performed the docking of benzoic acid on lipase. Also, an analogous consideration has

been made for tyrosinase, where the active Rhizome- MeOH was the only one to contain rutin. As previously reported in our work (Mocan et al., 2016), rutin is capable to bind to tyrosinase in an effective mode. We have repeated also this docking experiment by using the same condition of the other dockings. The model obtained for rutin is well representative of the interaction of the glucoside moiety of the molecule with the copper atoms present in the bottom of the enzymatic pocket of this enzyme. For AChE, α-amylase and α-glucosidase there is a good correspondence with the inhibition activity found and the concentration of kaempferol contained in the four extracts. Indeed, it has been previously reported that kaempferol is a good inhibitor of all these enzymes. The best pose found for kaempferol docked to AChE showed several interactions with the residues surrounding the enzymatic pocket such as Gly117, Asp72, Tyr334, His440 with hydrogen bonds and the residues Trp84 and Phe330 with π–π interactions. The best pose found for the complex of kaempferol with α-amylase is stabilized by two hydrogen bonds with Asp197 and His299 and two π–π interactions to Tyr62 and Trp59. Kaempferol established also interactions with α-glucosidase, to the residues Arg213 and Glu411 with two hydrogen bonds and one π–π interaction to Phe303. The molecular docking experiments reported for α-amylase, α-glucosidase and AChE may explain how kaempferol can interact in the binding pocket of these enzymes, involving a mechanism of action compatible with a competitive-type inhibition. Benzoic acid was also found to fit well in the lipase enzymatic pocket and we found two hydrogen bonds to residues Leu153 and Phe77 and a p-p interaction with tyr114, also the carboxylate group is perfectly superimposed with the phosphonate group of the crystallographic ligand. Rutin was docked to tyrosinase; and in this experiment the lowest energy pose found was able to chelate directly both the copper atoms present in the enzyme, (this finding is in agreement with a previous reported binding mode of rutin to tyrosinase (Si et al.,

2012)) and the pose is stabilized by several interactions established to residues His85, His296, Arg268, Asn260 and Glu256. Thus, rutin is able to enter efficaciously inside the enzymatic pocket of tyrosinase and acting as a competitive inhibitor.

4. Conclusion

In summary, our findings proved that *Iris schachtii* extracts exhibited important biological activities having as well interesting chemical profiles. The extracts contain important biologically-active compounds including kaempferol, luteolin, catechin and rutin. To the best our knowledge, the present study is the first concerning *I. schachtii* phenolic chemical profile and related biological activities. On the basis of our results, *I. schachtii* might be considered as a valuable source for designing of novel phytopharmaceuticals or nutraceuticals. However, further studies are necessary in order to elucidate the mechanisms of *in vivo* antioxidant and enzyme inhibition action, bioavailability and involved metabolic pathways.

References

Abeywickrama, G., Debnath, S.C., Ambigaipalan, P., Shahidi, F., 2016. Phenolics of Selected Cranberry Genotypes (*Vaccinium macrocarpon* Ait.) and Their Antioxidant Efficacy. J. Agric. Food Chem. acs.jafc.6b04291. doi:10.1021/acs.jafc.6b04291

ADI, 2015. The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. World Alzheimer Report, Alzheimer's Disease International (ADI). London, UK,.

Atta-Ur-Rahman, Nasim, S., Baig, I., Sener, B., Orhan, I., Ayanoglu, F., Choudhary, M.I., 2004. Two new Isoflavanoids from the Rhizomes of *Iris Soforana*. Nat. Prod. Res. 18, 465–471. doi:10.1080/14786410310001608136

Basgedik, B., Ugur, A., Sarac, N., 2015. Antimicrobial, antioxidant and antimutagenic properties of *Iris albicans*. Ind. Crops Prod. 69, 480–484. doi:10.1016/j.indcrop.2015.02.060

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The Protein Data Bank. Nucleic Acids Res. 28, 235–242. doi:10.1093/nar/28.1.235

Bilal, S., Khan, A.L., Waqas, M., Shahzad, R., Kim, I.-D., Lee, I.-J., Shin, D.-H., 2016. Biochemical Constituents and in Vitro Antioxidant and Anticholinesterase Potential of Seeds from Native Korean Persimmon Genotypes. Molecules 21, 893.

Birari, R.B., Bhutani, K.K., 2007. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov. Today 12, 879–889.

Burcu, B., Aysel, U., Nurdan, S., 2014. Antimicrobial, antioxidant, antimutagenic activities, and

phenolic compounds of *Iris germanica*. Ind. Crops Prod. 61, 526–530. doi:10.1016/j.indcrop.2014.07.022

Chang, T.S., 2009. An updated review of tyrosinase inhibitors. Int. J. Mol. Sci. 10, 2440–2475. doi:10.3390/ijms10062440

Custódio, L., Justo, T., Silvestre, L., Barradas, A., Duarte, C.V., Pereira, H., Barreira, L., Rauter, A.P., Alberício, F., Varela, J., 2012. Microalgae of different phyla display antioxidant, metal chelating and acetylcholinesterase inhibitory activities. Food Chem. 131, 134–140.

Egloff, M.-P., Marguet, F., Buono, G., Verger, R., Cambillau, C., van Tilbeurgh, H., 1995. The 2.46 A resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. Biochemistry 34, 2751–2762.

Etxeberria, U., de la Garza, A.L., Campión, J., Martínez, J.A., Milagro, F.I., 2012. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin. Ther. Targets 16, 269–297. doi:10.1517/14728222.2012.664134

Grochowski, D.M., Uysal, S., Aktumsek, A., Granica, S., Zengin, G., Ceylan, R., Locatelli, M., Tomczyk, M., 2017. In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of *Potentilla thuringiaca*. Phytochem. Lett. doi 10.1016/j.phytol.2017.03.005.

Hacibekiroğlu, I., Kolak, U., 2015. Screening antioxidant and anticholinesterase potential of *Iris albicans* extracts. Arab. J. Chem. 8, 264–268. doi:10.1016/j.arabjc.2012.04.051

Hasnat, M.A., Pervin, M., Lim, B.O., 2013. Acetylcholinesterase inhibition and in vitro and in vivo antioxidant activities of Ganoderma lucidum grown on germinated brown rice. Molecules 18, 6663–6678.

Herrera Acevedo, C., Scotti, L., Feitosa Alves, M., Formiga Melo Diniz, M.D.F., Scotti, M.T., 2017. Computer-aided drug design using sesquiterpene lactones as sources of new structures with potential activity against infectious neglected diseases. Molecules 22, 79.

Irwin, J.J., Sterling, T., Mysinger, M.M., Bolstad, E.S., Coleman, R.G., 2012. ZINC: a free tool to discover chemistry for biology. J. Chem. Inf. Model. 52, 1757–1768.

Ismaya, W.T., Rozeboom, J., Weijn, A., Mes, J.J., Fusetti, F., 2011. Crystal Structure of *Agaricus bisporus* Mushroom Tyrosinase: Identity of the Tetramer Subunits and Interaction with Tropolone. Biochemistry 50, 5477–5486.

Jamila, N., Yeong, K.K., Murugaiyah, V., Atlas, A., Khan, I., Khan, N., Khan, S.N., Khairuddean, M., Osman, H., 2015. Molecular docking studies and in vitro cholinesterase enzyme inhibitory activities of chemical constituents of *Garcinia hombroniana*. Nat. Prod. Res. 29, 86–90.

Jones, G., Willett, P., Glen, R.C., Leach, A.R., Taylor, R., 1997. Development and validation of a genetic algorithm for flexible docking. J. Mol. Biol. 267, 727–748.

Karamać, M., Amarowicz, R., 1996. Inhibition of pancreatic lipase by phenolic acids-examination in vitro. Zeitschrift für Naturforsch. C 51, 903–906.

Kim, Y.J., Uyama, H., 2005. Tyrosinase inhibitors from natural and synthetic sources: Structure, inhibition mechanism and perspective for the future. Cell. Mol. Life Sci. 62, 1707–1723. doi:10.1007/s00018-005-5054-y

Kinghorn, A.D., Su, B.-N., Jang, D.S., Chang, L.C., Lee, D., Gu, J.-Q., Carcache-Blanco, E.J., Pawlus, A.D., Lee, S.K., Park, E.J., 2004. Natural inhibitors of carcinogenesis. Planta Med. 70, 691–705.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., 2016. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Molecules 21, 1374.

Llorent-Martínez, E.J., Ortega-Barrales, P., Zengin, G., Mocan, A., Simirgiotis, M.J., Ceylan, R., Uysal, S., Aktumsek, A., 2017. Evaluation of antioxidant potential, enzyme inhibition activity and phenolic profile of *Lathyrus cicera* and *Lathyrus digitatus*: Potential sources of bioactive compounds for the food industry. Food Chem. Toxicol. doi:10.1016/j.fct.2017.03.002

Locatelli, M., Zengin, G., Uysal, A., Carradori, S., De Luca, E., Bellagamba, G., Aktumsek, A., Lazarova, I., 2017. Multicomponent pattern and biological activities of seven *Asphodeline* taxa: potential sources of natural-functional ingredients for bioactive formulations. J. Enzyme Inhib. Med. Chem. 32, 60–67.

Maestro, S., 2011. Version 9.2. LLC, New York.

Magnuson, B., Munro, I., Abbot, P., Baldwin, N., Lopez-Garcia, R., Ly, K., McGirr, L., Roberts, A., Socolovsky, S., 2013. Review of the regulation and safety assessment of food substances in various countries and jurisdictions. Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 30, 1147–220. doi:10.1080/19440049.2013.795293

Mocan, A., Zengin, G., Simirgiotis, M., Schafberg, M., Mollica, A., Vodnar, D.C., Crişan, G., Rohn, S., 2017. Functional constituents of wild and cultivated Goji (*L. barbarum* L.) leaves: phytochemical characterization, biological profile, and computational studies. J. Enzyme Inhib. Med. Chem. 32, 153–168. doi:10.1080/02699931.2011.628301

Mocan, A., Zengin, G., Uysal, A., Gunes, E., Mollica, A., Degirmenci, N.S., Alpsoy, L., Aktumsek, A., 2016. Biological and chemical insights of *Morina persica* L.: A source of bioactive compounds with multifunctional properties. J. Funct. Foods 25, 94–109.

Murray, A.P., Faraoni, M.B., Castro, M.J., Alza, N.P., Cavallaro, V., 2013. Natural AChE inhibitors from plants and their contribution to Alzheimer's disease therapy. Curr. Neuropharmacol. 11, 388–413. doi:10.2174/1570159X11311040004

Ortega-Ramirez, L.A., Rodriguez-Garcia, I., Leyva, J.M., Cruz-Valenzuela, M.R., Silva-Espinoza, B.A., Gonzalez-Aguilar, G.A., Siddiqui, M.W., Ayala-Zavala, J.F., 2014. Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: A hypothesis. J. Food Sci. 79, 129–137. doi:10.1111/1750-3841.12341

Pesaresi, A., Lamba, D., 2016. Torpedo Californica acetylcholinesterase in complex with a tacrine-nicotinamide hybrid inhibitor. Protein Data Bank. doi10.2210/pdb4x3c/pdb 10.

Picot, M.C., Zengin, G., Mollica, A., Stefanucci, A., Carradori, S., Mahomoodally, M.F., 2017. In vitro and in silico studies of mangiferin from *Aphloia theiformis* on key enzymes linked to diabetes type 2 and associated complications. Med. Chem. doi:doi: 10.2174/1573406413666170307163929.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Rigano, D., Conforti, F., Formisano, C., Menichini, F., Senatore, F., 2009. Comparative free radical scavenging potential and cytotoxicity of different extracts from *Iris pseudopumila* Tineo flowers and rhizomes. Nat. Prod. Res. 23, 17–25. doi:10.1080/14786410701740237

Schrödinger, L., n.d. The PyMOL Molecular Graphics System, version 1.8; Schrödinger, LLC, 2015. There is no Corresp. Rec. this Ref.

Seneviratne, K.N., Kotuwegedara, R.T., 2010. Antioxidant Activities of the Phenolic Extracts of Seed Oils and Seed Hulls of Five Plant Species. Food Sci. Technol. Int. 15, 419–425.

doi:10.1177/1082013209352718

Shelley, J.C., Cholleti, A., Frye, L.L., Greenwood, J.R., Timlin, M.R., Uchimaya, M., 2007. Epik: a software program for pKa prediction and protonation state generation for drug-like molecules. J. Comput. Aided. Mol. Des. 21, 681–691.

Si, Y.-X., Yin, S.-J., Oh, S., Wang, Z.-J., Ye, S., Yan, L., Yang, J.-M., Park, Y.-D., Lee, J., Qian, G.-Y., 2012. An integrated study of tyrosinase inhibition by rutin: progress using a computational simulation. J. Biomol. Struct. Dyn. 29, 999–1012.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28, 49–55.

Stevens, G.A., Singh, G.M., Lu, Y., Danaei, G., Lin, J.K., Finucane, M.M., Bahalim, A.N., McIntire, R.K., Gutierrez, H.R., Cowan, M., Paciorek, C.J., Farzadfar, F., Riley, L., Ezzati, M., 2012. National, regional, and global trends in adult overweight and obesity prevalences. Popul. Health Metr. 10, 22. doi:10.1186/1478-7954-10-22

Sun, H., Wang, D., Song, X., Zhang, Y., Ding, W., Peng, X., Zhang, X., Li, Y., Ma, Y., Wang, R., 2017. Natural Prenylchalconaringenins and Prenylnaringenins as Antidiabetic Agents: α-Glucosidase and α-Amylase Inhibition and in Vivo Antihyperglycemic and Antihyperlipidemic Effects. J. Agric. Food Chem. 65, 1574–1581.

Tajkarimi, M.M., Ibrahim, S.A., Cliver, D.O., 2010. Antimicrobial herb and spice compounds in food. Food Control 21, 1199–1218. doi:10.1016/j.foodcont.2010.02.003

Vladimir-Knežević, S., Blažeković, B., Bival Štefan, M., Alegro, A., Kőszegi, T., Petrik, J., 2011. Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. Molecules 16, 1454–1470.

Xie, G.Y., Zhu, Y., Shu, P., Qin, X.Y., Wu, G., Wang, Q., Qin, M.J., 2014. Phenolic metabolite profiles and antioxidants assay of three Iridaceae medicinal plants for traditional Chinese medicine "She-gan" by on-line HPLC-DAD coupled with chemiluminescence (CL) and ESI-Q-TOF-MS/MS. J. Pharm. Biomed. Anal. 98, 40–51. doi:10.1016/j.jpba.2014.05.008

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.-J., Li, H.-B., 2017. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int. J. Mol. Sci. 18, 96. doi:10.3390/ijms18010096

Yamamoto, K., Miyake, H., Kusunoki, M., Osaki, S., 2011. Steric hindrance by 2 amino acid residues determines the substrate specificity of isomaltase from *Saccharomyces cerevisiae.* J. Biosci. Bioeng. 112, 545–550. doi:10.1016/j.jbiosc.2011.08.016

Zengin, G., Ceylan, R., Katanić, J., Mollica, A., Aktumsek, A., Boroja, T., Matić, S., Mihailović, V., Stanić, S., Aumeeruddy-Elalfi, Z., 2017. Combining in vitro, in vivo and in silico approaches to evaluate nutraceutical potentials and chemical fingerprints of *Moltkia aurea* and *Moltkia coerulea*. Food Chem. Toxicol.

Zengin, G., Menghini, L., Malatesta, L., De Luca, E., Bellagamba, G., Uysal, S., Aktumsek, A., Locatelli, M., 2016a. Comparative study of biological activities and multicomponent pattern of two wild Turkish species: *Asphodeline anatolica* and *Potentilla speciosa*. J. Enzyme Inhib. Med. Chem. 31, 203–208.

Zengin, G., Nithiyanantham, S., Locatelli, M., Ceylan, R., Uysal, S., Aktumsek, A., Selvi, P.K., Maskovic, P., 2016b. Screening of in vitro antioxidant and enzyme inhibitory activities of different extracts from two uninvestigated wild plants: *Centranthus longiflorus* subsp. *longiflorus* and *Cerinthe minor* subsp. *auriculata*. Eur. J. Integr. Med. 8, 286–292.

Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., 2014a. *Sideritis galatica* Bornm.: A source of

multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. J. Funct. Foods 11, 538–547. doi:10.1016/j.jff.2014.08.011

Zengin, G., Uysal, A., Gunes, E., Aktumsek, A., 2014b. Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch. et Mey.): A potential source for functional food ingredients and drug formulations. PLoS One 9. doi:10.1371/journal.pone.0113527

Zhuo, H., Payan, F., Qian, M., 2004. Crystal structure of the pig pancreatic α-amylase complexed with ρ-nitrophenyl-α-D-maltoside-flexibility in the active site. Protein J. 23, 379–387. doi:10.1023/B:JOPC.0000039552.94529.95

Table 1. Yields (%), total phenolic, flavonoid and phenolic acid contents of *Iris schachtii* extracts (mean±SD)

Samples	Yields (%)	Total phenolic content (mgGAE/g extract)[a]	Total flavonoid content (mgRE/g extract)[b]	Total phenolic acid (mgCE/g)[c]
Aerial part-MeOH	9.11	52.52±0.45	33.56±0.45	7.64±0.35
Aerial part- Wat	15.17	48.47±0.06	13.78±0.19	4.94±0.17
Rhizome-MeOH	16.26	74.74±0.39	37.82±0.03	12.46±0.24
Rhizome-Wat	12.36	71.17±0.92	25.00±0.16	6.92±0.24

[a] GAE, gallic acid equivalents.

[b]RE, rutin equivalents.

[c]CE, caffeic acid equivalents

Table 2. Phenolic components of *Iris schachtii* extracts (µg/g extract) (mean±SD)

Phenolic Components	**Aerial part-MeOH**	**Aerial part-Wat**	**Rhizome-MeOH**	**Rhizome-Wat**
Gallic acid	32±0.6	152±8	nd	12±0.4
Prorocatecheuic acid	38±0.4	76±8	54±8	110±8
(+)-Catechin	290±20	nd	2326±46	nd
p- hydoxybenzoic acid	122±4	nd	214±4	708±20
Chlorogenic acid	nd	nd	nd	nd
Caffeic acid	44±4	nd	nd	nd
Epicatechin	nd	nd	nd	nd
Syringic acid	14±0.2	58±0.2	24±0.2	90±4
Vanilin	nd	44±2	nd	48±2
p- coumaric acid	40±1.6	40±1.6	30±1.6	nd
Ferulic acid	78±0.4	158±6	132±6	144±1
Sinapic acid	nd	nd	292±2	nd
Benzoic acid	1028±24	nd	1014±24	nd
o- coumaric acid	nd	nd	nd	nd
Rutin	nd	nd	896±16	nd
Hesperidin	nd	nd	nd	nd
Rosmarinic acid	nd	nd	nd	nd
Eriodictyol	nd	nd	nd	nd
Cinnamic acid	nd	nd	24±1	nd
Quercetin	nd	nd	nd	nd
Luteolin	2510±8	nd	556±10	448±8
Kaempferol	1748±16	1152±6	4214±94	1568±88
Apigenin	nd	2584±50	nd	4734±160

nd: not detected

Table 3. Antioxidant properties of *Iris schachtii* extracts (mean±SD)

Samples	DPPH (mgTE/g extract)[a]	ABTS (mgTE/g extract)[a]	CUPRAC (mgTE/g extract)[a]	FRAP (mgTE/g extract)[a]	Ferrous chelating activity (mgEDTA/g extract)[b]	Cupric chelating activity (mgEDTAE/g extract)[b]	Phosphomolybdenum (mgTE/g extract)[a]
Aerial part-MeOH	78.89±0.97	278.02±4.14	214.56±4.51	144.02±3.97	14.37±2.54	25.09±0.58	0.68±0.01
Aerial part- Wat	67.13±1.79	251.00±7.93	170.33±1.30	133.67±1.91	17.28±0.81	31.52±0.42	0.52±0.02
Rhizome-MeOH	107.09±2.84	534.22±7.66	264.26±1.71	189.74±8.09	4.56±0.01	20.68±1.02	0.87±0.02
Rhizome-Wat	96.03±2.63	490.22±6.92	225.38±1.81	168.52±4.05	7.76±0.02	33.25±0.92	0.72±0.03

[a]TE, trolox equivalents

[b]EDTA, EDTA equivalents

Table 4. Mutagenic activity expressed as mean number of revertants/plate ± standard deviation of *Iris* rhizome and aerial part extracts towards *S. typhimurium* TA98 and TA100 strains with and without S9

	Concentration (µg/plate)	TA 98		TA 100	
		S9 (-)	S9 (+)	S9 (-)	S9 (+)
†Negative Control		34±7	39±6	151±13	140±9
®Positive Control		917±72	3965±184	1995±110	5019±288
Control plate		30±5	31±6	135±9	142±16
Aerial part-MeOH	5000	41±4	45±7	178±14	140±16
	2500	33±2	40±2	165±12	119±22
	1000	29±1	46±4	147±25	130±5
Aerial part-Wat	5000	43±7	49±5	154±6	144±9
	2500	41±2	41±2	162±18	147±20
	1000	40±7	41±1	143±11	139±5
Rhizome-MeOH	5000	34±6	28±1	153±7	141±16
	2500	36±1	35±7	140±28	120±8
	1000	37±3	32±2	156±14	128±4
Rhizome-Wat	5000	39±3	37±2	143±15	136±10
	2500	40±7	41±4	143±34	130±12
	1000	24±5	43±2	161±5	120±10

† Negative control: DMSO (100 µl/plate) was used as negative control for *S. typhimurium* TA98 and TA100 both in the presence and absence of S9

® Positive controls:

2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*O*-fenilendiamine (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA98 strain.

2-Aminoanthracene (5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA100.

Table 5. Anti-mutagenicity and inhibition ratios of *Iris* extracts towards *S. typhimurium* TA98 and TA100 strains with and without metabolic activation (S9) against direct and indirect mutagens.

	Concentration (µg/plate)	TA 98				TA 100			
		S9 (-)	I%	S9 (+)	I%	S9 (-)	I%	S9 (+)	I%
†Negative Control		34±7		39±6		151±13		140±9	
®Positive Control		917±72	**0**	3965±184	**0**	1995±110	**0**	5019±288	**0**
Control plate		30±5		31±6		135±9		142±16	
Aerial part-MeOH	**5000**	683±92	**23**	635±22	**85**	1827±104	**9**	965±56	**83**
	2500	799±172	**13**	852±112	**79**	1979±75	**1**	1539±52	**71**
	1000	864±110	**6**	1142±152	**72**	1988±25	**0**	3417±45	**33**
Aerial part-Wat	**5000**	634±69	**32**	3774±155	**5**	1859±100	**7**	4904±156	**2**
	2500	763±72	**17**	3896±156	**2**	1888±114	**6**	4999±313	**0**
	1000	845±39	**8**	3944±79	**1**	1995±96	**0**	5015±14	**0**
Rhizome-MeOH	**5000**	714±140	**23**	492±84	**88**	1733±125	**14**	607±41	**90**
	2500	779±164	**16**	1135±76	**72**	1731±109	**14**	2113±360	**60**
	1000	723±63	**22**	3913±372	**1**	1974±336	**1**	4930±105	**2**
Rhizome-Wat	**5000**	624±32	**33**	1495±185	**63**	1849±188	**8**	2211±364	**58**
	2500	900±98	**2**	3495±210	**12**	1926±297	**4**	3422±120	**33**
	1000	914±45	**0**	3971±101	**0**	1992±145	**0**	4921±212	**2**

I%: Inhibition

† Negative control: DMSO (100 µl/plate) was used as negative control for *S. typhimurium* TA98 and TA100 both in the presence and absence of S9

® Positive controls:

2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-*O*-fenilendiamine (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA98 strain.

2-Aminoanthracene (5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; Sodium azide (5 µg/plate) was used as positive direct mutagen in the absence of S9 mix for *S. typhimurium* TA100.

Table 6. Enzyme inhibitory effects of *Iris schachtii* extracts (mean±SD)

Samples	AChE (mgGALAE/g)[a]	BChE (mgGALAE/g)[a]	Tyrosinase (mgKAE/g)[b]	α-Amylase (mmmol ACAE/g)[c]	α-glucosidase (mmmol ACAE/g)[c]	Lipase (mgOE/g)[d]
Aerial part-MeOH	1.35±0.04	na	na	0.79±0.03	5.37±0.05	23.14±1.19
Aerial part- Wat	0.17±0.02	na	na	0.35±0.01	4.44±0.13	na
Rhizome-MeOH	1.43±0.08	na	6.98±0.01	0.66±0.03	8.59±0.24	26.55±2.33
Rhizome-Wat	0.53±0.03	na	na	0.43±0.01	6.95±0.05	na

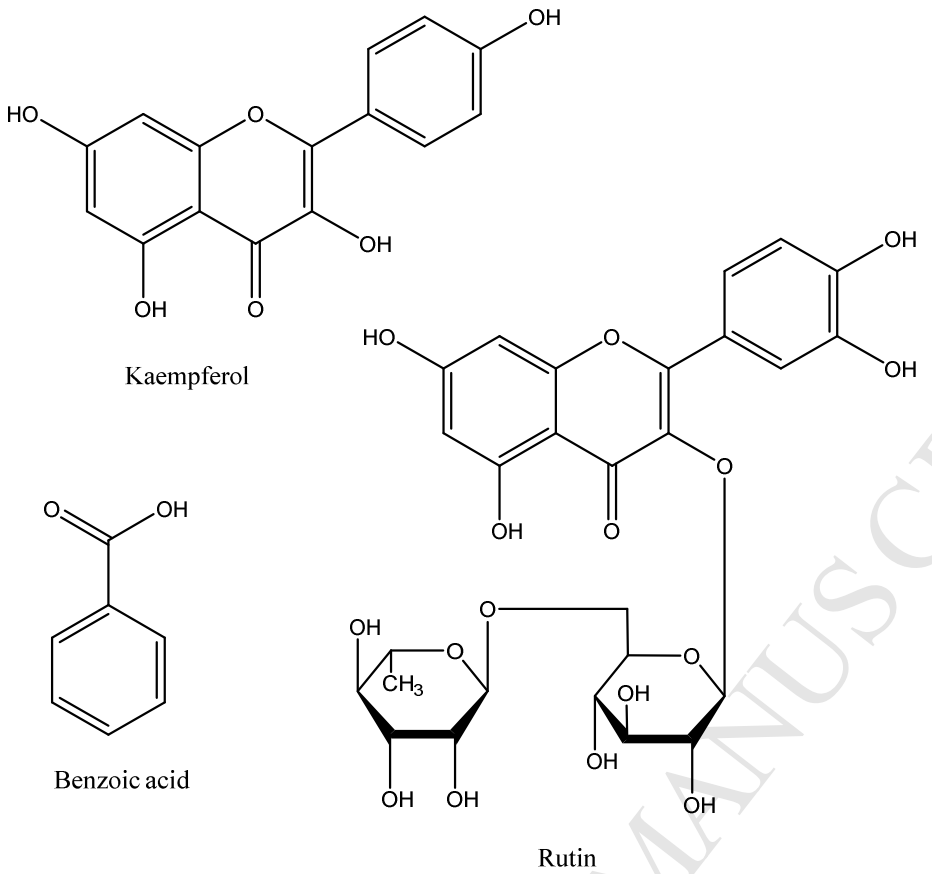
[a]GALAE, galanthamine equivalents

[b]KAE, Kojic acid equivalents

[c]ACAE, Acarbose equivalents

[d]OE, Orlistat equivalents

na: not active



Scheme 1. Chemical structures of compounds kaempferol, benzoic acid and rutin employed for docking experiments.

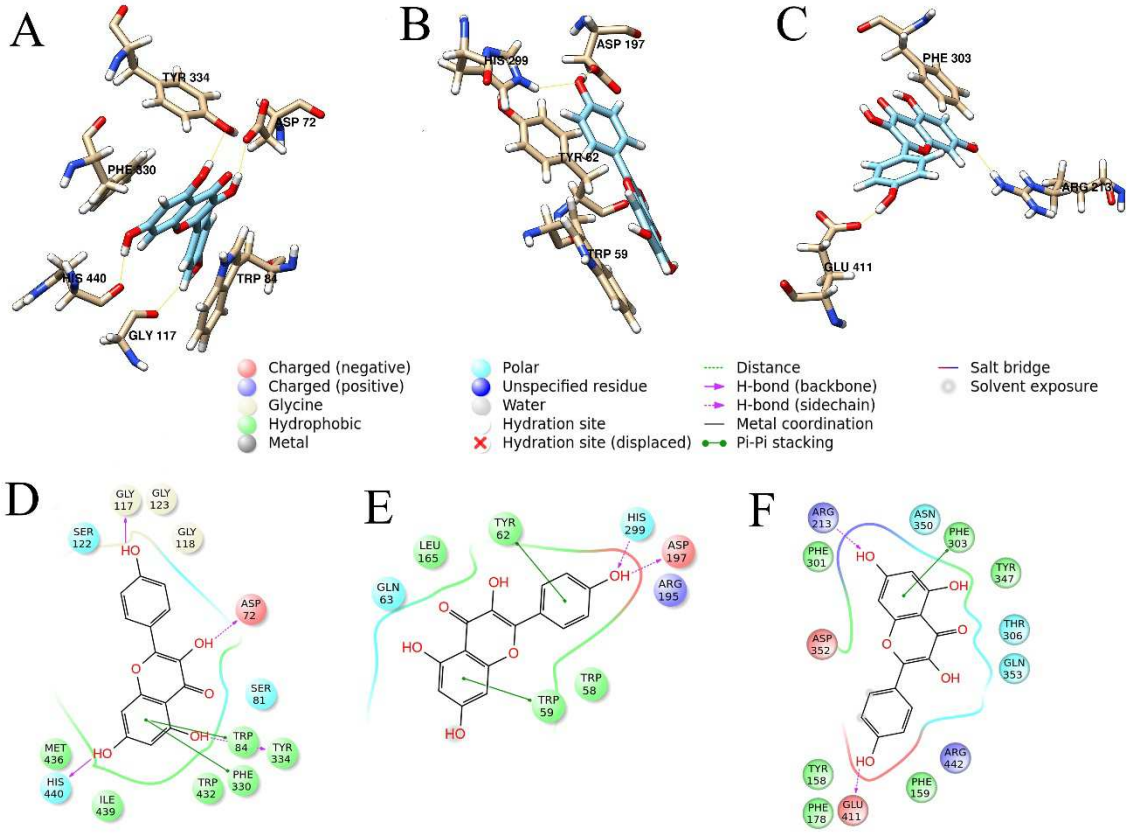


Figure 1. Representation of the best poses found for kaempferol in the docking with (A and D) AChE, (B and E) α-amylase, (C and F) α-glucosidase.

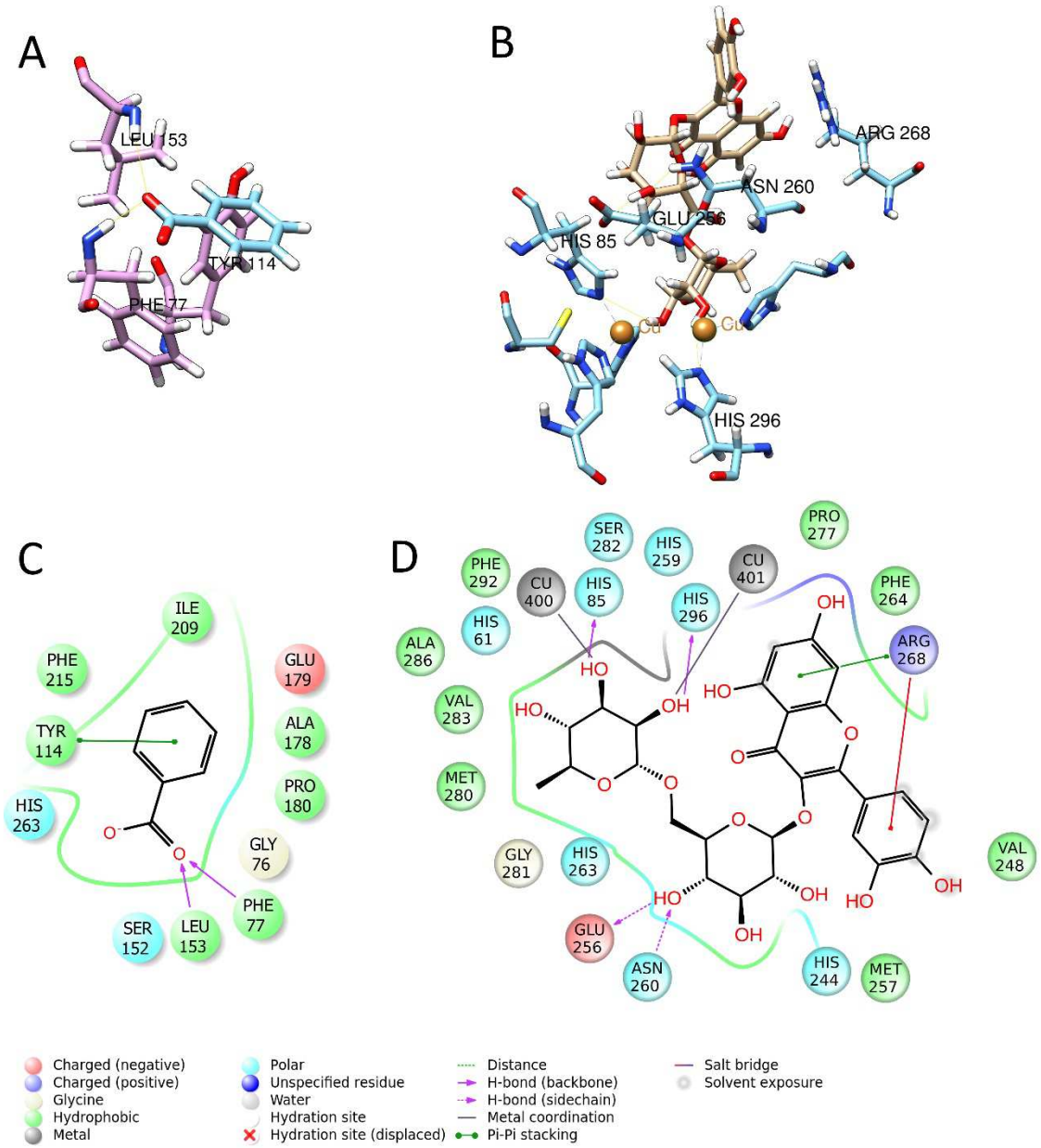


Figure 2. Representation of the best poses found for benzoic acid with lipase (A and B) and for rutin docked to tyrosinase (C and D).

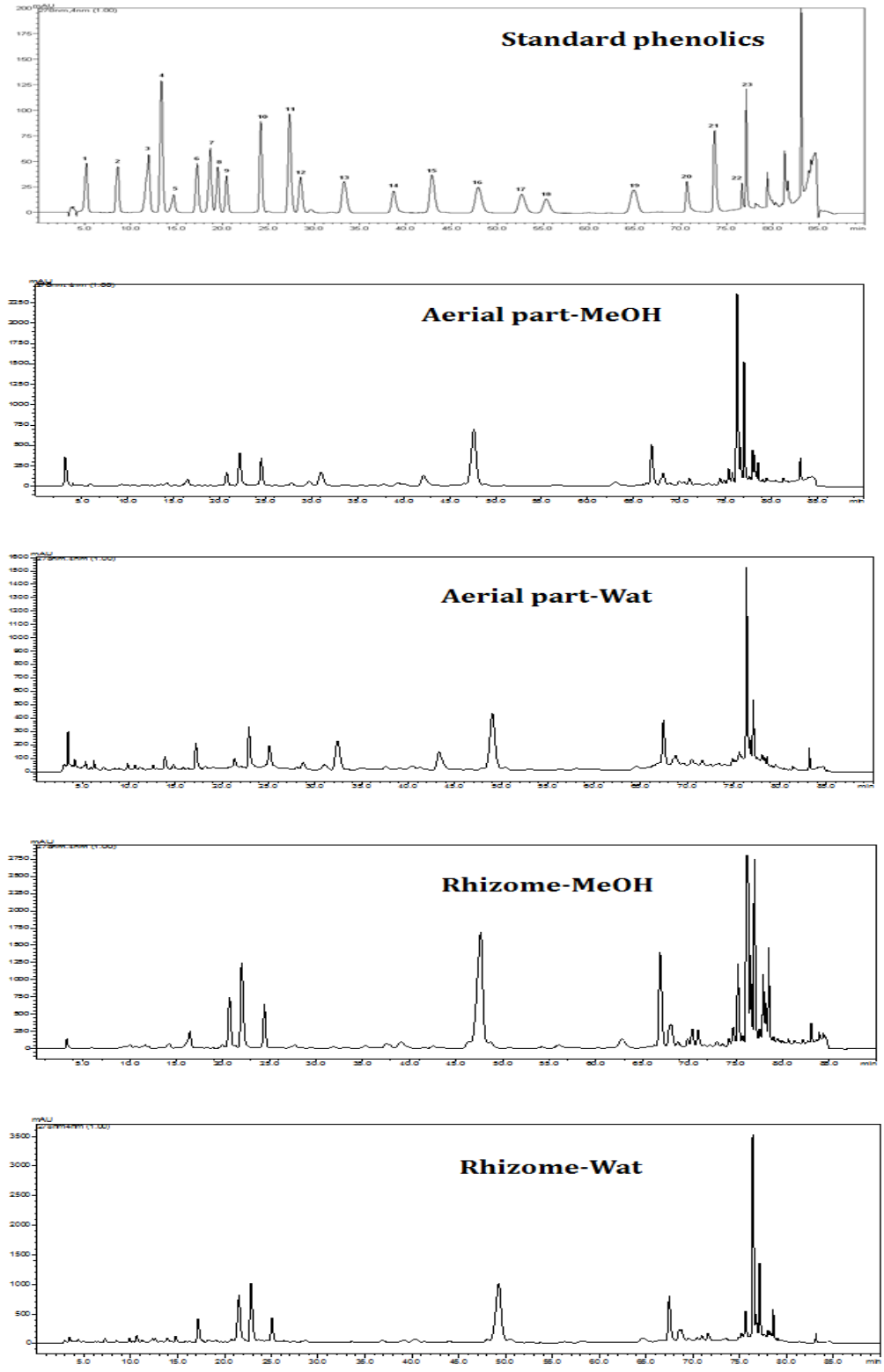


Figure 3. HPLC chromatograms of the studied extracts (1:gallic acid 2:protocatechic acid 3: (+)-catechin 4:*p*-hydroxybenzoic acid 5:chlorogenic acid 6:caffeic acid 7:epicatechin 8:syringic acid 9:vanilin 10:*p*-coumaric acid 11:ferulic acid 12:sinapic acid 13:benzoic acid 14:*o*-coumaric acid 15:rutin 16:hesperidin 17:rosmarinic acid 18:eriodictyol 19:cinnamic acid 20:quercetin 21:luteolin 22:kaempferol 23:apigenin)