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Biological and chemical insights of *Morina persica* L.: A source of bioactive compounds with multifunctional properties

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ABSTRACT

Morina persica L. has weak rosy smell flowers that together with aerial parts are infused or decocted and used as a functional tea. It is a representative of the Anatolian traditional medicine and is used for the treatment of cold. Biological and chemical fingerprints of different extracts (methanolic, acetone and water) of *M. persica* were investigated. Antioxidant, enzyme inhibitory, antimicrobial, cytotoxic and mutagenic/antimutagenic effects were evaluated for biological profiling. Rutin, chlorogenic and rosmarinic acids were detected as dominant compounds. Generally, the methanolic and acetone extracts had strong antioxidant, enzyme inhibitory, antimicrobial and antimutagenic effects. Additionally, all extracts showed remarkable inhibitory effects on HeLa cells at 100 μg/mL. Furthermore, possible interactions between rutin, the dominant phenolic compound in *M. persica* and the tested enzymes were assessed by molecular docking. Results indicate that *M. persica* could be considered as a natural source of high-valued functional ingredients for further use in healthful formulations.

1. Introduction

Functional components are non-conventional biomolecules that occur in food and possess the capacity to modulate one or more metabolic processes or pathways in the body, resulting in health promotion and well-being (Xu, Kaur, Dhillon, Tappia, & Dhalla, 2011). Functional foods include phytochemicals which are non-nutritive and biologically active chemicals that contribute in the prevention of certain non-communicable diseases (Abuajah, Ogbonna, & Osuji, 2014). Moreover, modern research has shown a positive correlation between the presence of certain phytochemicals in medicinal plants and foods and health promotion and well-being (Chang, Alasalvar, & Shahidi, 2016; Kowalska & Olejnik, 2016).

As a consequence, in recent years there has been much emphasis on phytochemicals with different biological properties such as antioxidant, antimicrobial, antiviral,

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antimutagenic, and anticancer (Atanasov et al., 2015; Newman & Cragg, 2012; Norberto et al., 2013; Sang, 2014). Among phytochemicals, phenolic compounds have received particular attention and are probably the most investigated molecules of medicinal and nutritional interest due to their antioxidant, antimicrobial, antiviral, antiallergic, anti-inflammatory activities, and their involvement in cell and cutaneous ageing (Lee, 2013; Pereira, Valentão, Pereira, & Andrade, 2009) as well to their wide occurrence in food and herbal products (Embuscado, 2015; Jew et al., 2015). Their antioxidant activities ranging from direct radical scavenging to chelating of transition metals, as well as their indirect antioxidant activity through up-regulating endogenous antioxidant defences are nowadays highly emphasized (Oroian & Escriche, 2015; Prior, 2015; Shahidi & Ambigaipalan, 2015; Shahidi & Zhong, 2015; Velderrain-Rodríguez et al., 2014). Consequently, the interest for developing natural dietary antioxidants is also increasing due to the fact that synthetic antioxidants have been incriminated as endocrine disrupters or even carcinogenic agents (Ito et al., 1986; Mezcua et al., 2012).

Diabetes mellitus (DM) and Alzheimer's disease (AD) are two major health problems affecting generally adult or elder people. Moreover, DM is considered one of the most common chronic diseases in nearly all countries. The disease continues to increase in numbers and significance due to the changing lifestyles that involve sedentariness and obesity. One therapeutic approach for treating early stage diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes (α-amylase and α-glucosidase) in the digestive tract (Tundis, Loizzo, & Menichini, 2010). Therefore, inhibition of these key enzyme activities is an important strategy in the management of diabetes mellitus and a point start in developing new, cost effective, and safe natural inhibitors (Etxeberria, de la Garza, Campión, Martínez, & Milagro, 2012; Yin, Zhang, Feng, Zhang, & Kang, 2014). Concerning AD, in spite its multifactorial nature, currently just the cholinergic hypothesis is followed as a therapeutic approach. Accordingly, at least some of the cognitive decline experienced by patients with AD results from a deficiency in neurotransmitter acetylcholine (ACh) and thus in cholinergic neurotransmission in brain cortical or hippocampal regions, which seems to play a fundamental role in memory (Loizzo, Tundis, Menichini, & Menichini, 2008). Thus, increasing the level of ACh through inhibition of both two major forms of cholinesterase, AChE and BChE, continues to be a useful therapeutic approach to treat not only AD but also other forms of dementia such as vascular dementia and Parkinson's dementia (Loizzo et al., 2008; Paula, Belén, Julia, Paola, & Cavallaro, 2013). However, synthetic cholinesterase inhibitors such as donepezil, galanthamine and tacrin are reported to possess several side effects: nausea, vomiting, cramping, and diarrhoea among others (Qin et al., 2013). Thereupon, the search for effective and safe cholinesterase and carbohydrate enzyme inhibitors from natural sources remains as an aimed objective.

The genus *Morina* L. (Caprifoliaceae) gathers around 12 species growing in different habitats around the world (Kumar & Varshney, 2013; Xue & Wu, 2009). Some species of the genus *Morina* such as *Morina chinensis*, *Morina kokonorica*, *Morina nepalensis* var. *alba*, and *Morina nepalensis* var. *delavayi* have been used as Chinese traditional medicines for the treatment of cerebral apoplexy, arthralgia, lumbago, megrim, and tumours. Additionally, *Morina longifolia* use in traditional systems of medicines is also reported (Kothiyal et al., 2009; Kumar & Varshney, 2013; Kumar, Varshney, Rawat, Martinez, & Stashenko, 2013). Several classes of bioactive compounds such as terpenoids, saponins, alkaloids, phenolic acids, flavonoids, phenylpropanol derivatives and neolignans have been reported in representatives of *Morina* genus (Aynehchi, Salehi Sormaghi, Amin, Khoshkhow, & Shabani, 1985; Kumar & Varshney, 2013; Kumar et al., 2013; Su, Takaishi, Duan, & Chen, 1999; Xue & Wu, 2009).

M. persica L. popularly named "Dikenli adaçayı" is a representative of the Anatolian traditional medicine. Its weak rosy smell flowers together with aerial parts are infused or decocted and used in the treatment of cold, and in some regions the plant is also valorized as melliferous (Baser & Kürkçüoglu, 1998; Demirci & Özhatay, 2012; Tasdemir, Brun, Perozzo, & Dönmez, 2005; Tashev & Pancheva, 2011). Recently, extracts of *M. persica* have shown important antiprotozoal (Tasdemir et al., 2005) and antibacterial activity (Tasdemir, Dönmez, Çalıs, & Rüedi, 2004) as well as weak antimycobacterial potential (Tosun, Akyüz Kızılay, Şener, & Vural, 2005). However, data regarding the chemical composition and further bioactivities of *M. persica* are scarce (Baser & Kürkçüoglu, 1998). Therefore, the purpose of this work was to evaluate the biological (antioxidant, antimicrobial, enzyme inhibitory, and cytotoxic effects) and chemical (individual phenolic components) insights of *M. persica*. This work could provide a new, natural and strong candidate to design new functional food ingredients.

2. Materials and methods

2.1. Plant materials and extraction procedure

M. persica L. was collected from Basarakavak-Altinapa road, Konya-Turkey in the flowering season (July 2014). 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 were grinded to a fine powder using a laboratory mill. Ten grams of powdered samples were separately extracted with acetone and methanol in a Soxhlet apparatus for 6–8 h. Further, the extracts were concentrated under vacuum at 40 °C by using a rotary evaporator. To obtain water extracts, 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. Quantification of phenolic compounds by RP-HPLC-DAD

Phenolic compounds were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Kyoto, 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 autosampler (Shimadzu Scientific Instruments, Columbia, MD, USA). Separation was conducted at 30 °C by using an Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size, Agilent, Santa Clara, CA, USA) and the detection at 278 nm. The phenolic composition of the extracts was determined by a modified method of Caponio, Alloggio, and Gomes (1999). Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (–)-epicatechin, syringic acid, vanilin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, naringin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, naringenin, luteolin, kaempferol and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards in the same chromatographic conditions. The amount of each phenolic compound was expressed as μg per gram of dry material (dw) using external calibration curves obtained for each phenolic standard. Analytical characteristic for the applied method are reported in "Supplementary material" Section S.1.

2.3. Determination of total bioactive compounds

2.3.1. Total phenolic content

The total phenolic content was determined as previously described by Zengin, Sarikurkcu, Aktumsek, and Ceylan (2014) and the results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract).

2.3.2. Total flavonoid content

The total flavonoid content was determined using the methods previously employed by Zengin, Sarikurkcu, Aktumsek, Ceylan, and Ceylan (2014). Rutin was used as a reference standard and the total flavonoid content was expressed as milligrams of rutin equivalents (mg RE/g extract).

2.4. Total antioxidant activity

2.4.1. Phosphomolybdenum method

The total antioxidant activity of the samples was evaluated by phosphomolybdenum method according to Berk, Tepe, and Arslan (2011) with slight modifications, the results being expressed as millimoles of Trolox equivalents (mmol TE/g extract).

2.4.2. β-Carotene bleaching method

In this assay the antioxidant activity (inhibition(%)) is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation as previously reported by Sarikurkcu et al. (2012).

2.5. Radical scavenging activity

2.5.1. DPPH radical scavenging activity

The effect of the samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was estimated according to the method described by Sarikurkcu (2011), the results being expressed as milligrams of Trolox equivalents (mg TE/g extract).

2.5.2. ABTS radical cation scavenging activity

The scavenging activity against ABTS radical cation (2,2′-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid) radical was measured according to the method of Re et al. (1999) with slight modifications, the results being expressed as milligrams of Trolox equivalents (mg TE/g extract).

2.6. Reducing power

2.6.1. CUPRAC assay

The cupric ion reducing activity (CUPRAC) was determined according to the method of Zengin et al. (2014), the results being expressed as milligrams of Trolox equivalents (mg TE/g extract).

2.6.2. FRAP assay

The FRAP (ferric reducing antioxidant power) assay was carried out as previously described by Aktumsek, Zengin, Guler, Cakmak, and Duran (2013) with slight modifications. FRAP activity was expressed as milligrams of Trolox equivalents (mg TE/g extract).

2.7. Metal chelating activity on ferrous ions

The metal chelating activity on ferrous ions was determined by the method previously described by Aktumsek et al. (2013). The metal chelating activity was expressed as milligrams of EDTA (ethylenediaminetetraacetic acid disodium salt) equivalents (mg EDTAE/g extract).

2.8. Enzyme inhibitory activity

2.8.1. Cholinesterase inhibition

Cholinesterase (ChE) inhibitory activity was measured using Ellman's method, as previously reported (Zengin et al., 2014). The cholinesterase inhibitory activity was expressed as milligrams of galanthamine equivalents (mg GALAE/g extract).

2.8.2. α-Amylase inhibition

α-Amylase inhibitory activity was performed using Caraway–Somogyi iodine/potassium iodide (IKI) method (Zengin et al., 2014). The α-amylase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACE/g extract).

2.8.3. α-Glucosidase inhibition

α-Glucosidase inhibitory activity was performed by the previous method of Zengin et al. (2014). The α-glucosidase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACE/g extract).

2.8.4. Tyrosinase inhibition

Tyrosinase inhibitory activity was measured using the modified dopachrome method with L-DOPA as substrate, as previously reported by Zengin et al. (2014) with slight modifications, the results being expressed as equivalents of kojic acid (mg KAE/g extract).

2.9. *Inhibitory effects of* M. persica *extracts on HeLa cells*

HeLa cell lines were cultured in RPMI-1640 medium. The cells were routinely cultured at 37 °C in a humidified atmosphere with 0.5% CO_2. MTT assay is a colorimetric method, which indirectly measures cell viability and/or cell death. To determine cell cytotoxicity, HeLa cells were seeded in 96-well culture plates at a density of 1×10^4 cells/well in RPMI-1640 medium for 24 and 48 h. After 24 h, culture medium was replaced with various concentrations (5, 10, 50, 100 μg/mL) of acetone, methanol and water extracts of *M. persica* for 24 and 48 h. At the end of the incubation period, culture medium was removed and 10 μL of the MTT labelling reagent were added to each well and incubated for 4 h. After 4 h at 37 °C, 100 μL of DMSO was added to each well and incubated overnight. Finally, the absorbance of formazan product in each well was measured by using Elisa reader (BIOTEK) at 590 nm. The cytotoxicity was evaluated as cell viability (expressed as percentage of the untreated control (100% cell viability)) and the experiments were performed in triplicate. Cell inhibition rate was evaluated by the below formula: 1 – (absorbance of sample wells/absorbance of control wells) × 100.

2.10. *Mutagenicity test*

In the present study, mutagenicity was assayed by the Ames test (*Salmonella*/microsome assay) according to Maron and Ames (1983). The test was performed without and/or with metabolic activation. To assay mutagenic activity, three different concentrations of each extract were tested by plate incorporation method. The sample was considered mutagenic when a dose–response relationship was detected and a two-fold increase in the number of revertants was observed for at least one concentration.

2.11. *Antimutagenicity test*

Only extracts considered as non-toxic and non-mutagenic were subjected to this experiment, employing the method of plate incorporation developed by Maron and Ames (1983). Three different concentrations of plant extract (5000, 2500 and 1000 μg/plate) were associated with known mutagens in tests in the absence and presence of metabolic activation, using *Salmonella typhimurium* tester strains TA98 and TA100 (Zengin, Uysal, Gunes, & Aktumsek, 2014). Results were interpreted as having no antimutagenic effect when the inhibition was lower than 25%, a moderate effect for a value between 25% and 40% and strong antimutagenicity for values greater than 40% (Negi, Jayaprakasha, & Jena, 2003).

2.12. *Antimicrobial assay*

The *in vitro* antibacterial activity of *M. persica* extracts was evaluated against a total of six standard bacteria which are human pathogens, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, methicillin sensitive *Staphylococcus aureus* ATCC 25923 (MSSA), *Klebsiella pneumoniae* 70603, methicillin resistant *Staphylococcus aureus* ATCC 43300 (MRSA) and *Salmonella enteritidis* ATCC 13076. Antifungal activity was determined by using *Candida albicans* and *Candida parasilopsis* yeasts isolates. In addition, fourteen methicillin resistant *S. aureus* strains isolated from clinical samples were used to determine anti-MRSA activity of the *Morina* extracts. The methicillin resistances of strains were determined by agar screening, oxacillin disc diffusion and broth microdilution methods. The standard microorganisms were accessed from Microbiology Research Laboratory, Department of Biology, Selcuk University, Konya, Turkey. The broth microdilution method was employed for the determination of minimal inhibition concentration (MIC) values of the extracts. A method described by CLSI (2011) modified by Zengin et al. (2014) was used.

2.13. *Molecular docking experiments*

2.13.1. *Ligand preparation*

The 3D structure of rutin was downloaded from the Zinc database (id: zinc_4096846) (Irwin & Shoichet, 2005). The molecule was minimized by AM1 force field (Dewar, Zoebisch, Healy, & Stewart, 1985).

2.13.2. *Enzyme preparation*

Acetylcholinesterase crystal structure (id: 1B41) (Kryger et al., 2000), α-amylase (id: 4GQR) (Williams, Li, Withers, & Brayer, 2012), mushroom tyrosinase (id: 2Y9X) (Ismaya, Rozeboom, Weijn, Mes, & Fusetti, 2011) and α-glucosidase (id: 3W37) (Tagami et al., 2013) were downloaded from the RCSB Protein Data Bank (Berman et al., 2000). The structures were cleaned, the crystallization water, salts and small molecules were removed by chimera (Pettersen et al., 2004) and the structures were neutralized at pH 7.4 by Maestro Schrodinger 2015, and the structure fixed from errors by the Swiss Pdb viewer (Guex & Peitsch, 1997). The receptors were minimized by gromacs 4.6 (Lindahl, Hess, & van der Spoel, 2001). The topology of the enzymes was calculated by pdb2gmx routine included in gromacs, by using the OPLS-AA/L force field, then the enzymes were solvated into a cubic box of water, including the crystallographic ligand where present. The crystallographic ligands were processed by PRODRG2 server available online (Schüttelkopf & Van Aalten, 2004) and the coordinates added manually to those of the respective enzymes. The net charge of the proteins was neutralized by adding Na+ ions. Finally the systems were minimized by gromacs MD engine mdrun, until it reached the minimum potential energy. At this point the minimized receptors were used for docking experiments.

2.13.3. *Docking experiments*

Dockings were carried out by Vina AutoDock (Trott & Olson, 2010). The grids for docking were manually generated by AutoDockTools (Sanner, 1999) by centring the grid on the co-crystallized inhibitors. Only in the case of acetylcholinesterase, where the crystallographic ligand was absent, was the binding pocket centred around His447, covering all the residue reported in the literature: Ser203, Glu334, Tyr124, Trp286, Tyr341, Asp74 (Lushington, Guo, & Hurley, 2006). The routable bonds of rutin were chosen by default. After the docking calculation, the best ranked pose was chosen. In Fig. 2 the best poses are reported for each enzyme–ligand complex. In Fig. 3 are

reported the interactions found between the enzymes and rutin, calculated by the Maestro interface (Schrödinger Release, 2015).

2.14. Statistical analysis

For all the experiments all assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The differences between the different extracts were analysed 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 discussions

3.1. Total bioactive components

Phenolic compounds are nowadays highly emphasized mainly because of their association with a reduction in the incidence of some diseases, particularly for their chemopreventive effects in cancer and metabolic disorders like diabetes and obesity (Heber, 2001; Liu, 2013; Xiao, 2014; Xiao & Högger, 2015). Additionally it is generally known that the yields of chemical extractions depend on the type of solvent with varying polarities. Besides, the solubility of phenolics is highly influenced by the solvent's polarity. Solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone (Naczk & Shahidi, 2004; Teleszko & Wojdyło, 2015). Therefore different approaches must be applied in order to obtain convenient extraction yields. The total phenolic and flavonoid contents of the three *Morina* extracts are presented in Table 1. Hence, the highest level of total phenolic content was measured for the methanolic extract, followed by the water extract and last, the acetone. An identical trend was observed concerning the total flavonoid content, the highest amount being obtained for the methanolic extract. Similarly, our results regarding solvent polarity and extraction yields are in line with the work of Aktumsek et al. (2013) who found higher values of total phenolic and flavonoid contents for methanolic samples.

Table 1 – Total phenolic and flavonoid contents of the solvent extracts from *Morina persica* (mean ± SD).

Assays	Solvent extracts		
	Acetone	Methanol	Water
Total phenolic (mg GAEs/g extract)[a]	32.00 ± 1.59	53.32 ± 087	44.27 ± 0.11
Total flavonoid (mg REs/g extract)[b]	105.40 ± 0.25	122.68 ± 3.06	42.75 ± 1.15

[a] GAEs, gallic acid equivalents.
[b] REs, rutin equivalents.

3.2. Identification of phenolic compounds by HPLC-DAD

The Folin–Ciocâlteu method is a fast and reliable assay in comparing samples of the same type, giving basic information about the amounts of phytochemicals. However, this method does not give a full picture concerning the qualification and quantification of phenolic compounds in complex samples. Therefore, the phenolic profile of *M. persica* extracts was further assessed by HPLC-DAD, and the results are presented in Table 2. Fifteen phenolic compounds were identified by comparing their retention times and UV spectra with standards in the same chromatographic conditions.

The highest amounts of phenolic compounds were detected in the methanolic extract, being in line with the trend from the total phenolic and flavonoid content measurements. The dominant compounds in the methanolic extract were chlorogenic acid (618.18 ± 41.16 μg/g dry plant), rutin (395.80 ± 29.17 μg/g dry plant) and rosmarinic acid (96.50 ± 3.80 μg/g dry plant). Concerning rutin, the beneficial effects of this quercetin glycoside in inflammatory disease and diabetes mellitus are well known (Kim et al., 2005; Sattanathan, Dhanapal, Umarani, & Manavalan, 2011). Besides, chlorogenic

Table 2 – Phenolic components in the solvent extracts (μg/g dry plant) from *Morina persica* (mean ± SD).

No	Phenolic components	Acetone	Methanol	Water
1	Gallic acid	nd	1.40 ± 0.04	nd
2	Protocatechuic acid	1.40 ± 0.13	20.38 ± 0.80	22.73 ± 0.70
3	*p*-Hydroxybenzoic acid	2.83 ± 0.07	19.98 ± 0.40	26.43 ± 0.53
4	Chlorogenic acid	6.83 ± 0.33	618.18 ± 41.16	140.26 ± 2.47
5	Caffeic acid	5.56 ± 0.10	3.00 ± 0.04	26.96 ± 0.53
6	*p*-Coumaric acid	0.55 ± 0.03	5.39 ± 0.18	4.23 ± 0.16
7	Ferulic acid	0.52 ± 0.01	3.80 ± 0.04	3.88 ± 0.04
8	Benzoic acid	1.40 ± 0.01	21.58 ± 0.06	nd
9	*o*-Coumaric acid	1.27 ± 0.03	34.17 ± 0.16	nd
10	Rutin	47.35 ± 4.84	395.80 ± 29.17	nd
11	Rosmarinic acid	1.82 ± 0.03	96.50 ± 3.80	4.58 ± 0.18
12	Eriodictiol	0.33 ± 0.01	3.20 ± 0.04	nd
13	*trans*-Cinnamic acid	1.01 ± 0.02	3.60 ± 0.10	nd
14	Kaempferol	2.86 ± 0.07	31.17 ± 0.60	17.27 ± 0.35
15	Apigenin	11.47 ± 0.23	62.54 ± 1.20	15.15 ± 0.70

nd, not determined.

acid, a ubiquitously found dietary polyphenol, is involved in glucose homeostasis and regulation of skin pigmentation (Fukushima et al., 2015). Rutin was previously identified in *M. kokonorica* while several other quercetin glycosides were found in *M. nepalensis*. Additionally, caffeic acid as well as several caffeoylquinic acid isomers were reported for *M. nepalensis* (Kumar & Varshney, 2013). *p*-Hydroxybenzoic acid, a phenolic derivate of benzoic acid, presented a higher yield in the aqueous extract in comparison with acetone and methanolic samples. Nonetheless, this compound was previously identified in *M. longifolia* (Kumar & Varshney, 2013). All in all, information about the chemical composition of *M. persica* is scarce. With the exception of one paper dealing with essential oil composition, no other data are available up to the present time (Baser & Kürkçüoglu, 1998). Thus, comparison with other researchers' results is impossible.

3.3. *Radical scavenging properties*

Antioxidants have become an indispensible group of food additives mainly because of their unique properties of extending the shelf-life of food products without any adverse effect on their sensory or nutritional qualities. Phenolic compounds are classified as primary antioxidants which are mainly free radical scavengers that delay or inhibit the initiation step or interrupt the propagation step of lipid oxidation, thus decreasing the formation of volatile decomposition products (e.g., aldehydes and ketones) that cause rancidity in foods (Shahidi & Ambigaipalan, 2015; Shahidi & Naczk, 2004; Shahidi & Wanasundara, 1992). Additionally, their antioxidant potential depends on the number and arrangement of the hydroxyl groups in the molecules of interest (Shahidi & Zhong, 2015).

Thus, the radical scavenging activities of the different solvent extracts of *M. persica* are presented in Table 3, the results being expressed as Trolox equivalents (TEs/g extract). Two synthetic free radicals were used: DPPH and $ABTS^{\bullet+}$. For instance, in the DPPH assay, the colour of the reaction changes from purple to yellow, the difference in absorbance being quantified at 517 nm. In the DPPH and $ABTS^{\bullet+}$ assays the methanol extract displayed superior scavenging activity (48.09 and 146.05 mg TEs/g extract, respectively). This can be attributed to the higher yields of phenolic compounds presented by this extract. Nonetheless, it is worthy to mention that besides major compounds like rutin and chlorogenic acid, even compounds with rather low amounts like gallic acid may influence the antioxidant activity of the extracts due to prerequisite structural features.

3.4. *FRAP and CUPRAC assays*

The reducing capacity of an extract is considered an important indicator of its potential antioxidant properties. For measurements of reducing activity, the Fe^{3+}–Fe^{2+} and Cu^{2+}–Cu^{+} transformation was investigated in the presence of antioxidants from *Morina* extracts. In the FRAP assay, the presence of antioxidants in the samples causes the reduction of $TPTZ/Fe^{3+}$ to $TPTZ/Fe^{2+}$. The ferrous complex can be monitored by measuring the formation of a blue complex at 595 nm (Huang, Boxin, & Prior, 2005). As can be seen in Table 3, the methanolic extract (82.85 mg TEs/g extract), which contains the highest values in terms of total phenolic and flavonoid contents, also exhibited the highest ferric reducing activity amongst the studied samples.

The CUPRAC assay is based on the reduction of Cu(II) to Cu(I) by reductants/antioxidants which are present in the studied samples using copper(II)-neocuproine reagent as the chromogenic oxidant (Huang et al., 2005). The CUPRAC values of the *M. persica* samples ranged between 89.26 and 131.51 mg TEs/g extract for the acetone and methanolic samples, respectively (Table 3). A similar trend, showing good correlation between cupric and ferric reducing power assays was reported by Aktumsek et al. (2013).

3.5. *Chelating effect on ferrous ions*

Iron is regarded as the most important pro-oxidant and increases formation of the hydroxyl radicals via Fenton reaction (Flora, 2009). Consequently, ferrous chelating ability might be considered a good indicator of the antioxidant activity of *M. persica*. Table 3 gathers the chelating effects of the extracts on the ferrous ions. In this case, the water extract presented the highest chelating effects (42.41 mg EDTAEs/g extract), while the methanolic and acetone samples rather low values (18.36 and 9.45 mg EDTAEs/g extract, respectively). Interestingly, water extract which contain lower phenolics levels comparing with methanolic extract exhibited the highest chelating effects. Rice-Evans, Miller, and Paganga (1996) stated that metal chelating plays a minor role in the overall antioxidant activities of some phenolic compounds. Therefore, the highest metal chelating activity of water extract might be caused by

Table 3 – Radical scavenging, reducing power and metal chelating activities of the solvent extracts from *Morina persica* (mean ± SD).

Extracts	Radical scavenging activity (mg TEs/g extract)[a]		Reducing power (mg TEs/g extract)[a]		Metal chelating (mg EDTAEs/g extract)[b]
	DPPH radical	ABTS radical cation	CUPRAC	FRAP	Ferrous chelating
Acetone	44.69 ± 1.20	76.46 ± 1.31	89.26 ± 3.28	59.17 ± 1.15	9.45 ± 1.82
Methanol	48.09 ± 0.86	146.05 ± 6.84	131.51 ± 2.98	82.85 ± 2.53	18.36 ± 0.95
Water	44.75 ± 1.00	100.97 ± 3.69	109.35 ± 1.75	74.67 ± 5.81	42.41 ± 1.58

[a] TEs, Trolox equivalents.
[b] EDTAEs, ethylenediaminetetraacetic acid disodium salt equivalents.

non-phenolic chelators with a high solubility in water, including polysaccharides, peptides and proteins.

3.6. Total antioxidant activities by phosphomolybdenum and β-carotene/linoleic acid assays

Total antioxidant capacity of *M. persica* extracts was evaluated by phosphomolybdenum and β-carotene/linoleic acid assays, and the results are shown in Table 4. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidants, and the subsequent formation of green phosphate/Mo (V) complex with an absorption maxima at 695 nm (Prieto, Pineda, & Aguilar, 1999). Surprisingly, in this assay the acetone extract presented the highest total antioxidant activity (1.44 mmol TEs/g extract), and the aqueous extract the lowest (0.94 mmol TEs/g extract).

The antioxidant activity measured as the inhibition of oxidation of linoleic acid can simulate the oxidation of the membrane lipid components and also measures the capacity of inhibition of conjugated diene hydroperoxide arising from the linoleic acid oxidation. Thus, this assay measures the antioxidant activity towards linoleic acid relatively to β-carotene. This one is not affected due to the presence of a strong antioxidant. In this case the solution shall remain with the same initial colour, meaning that β-carotene was not necessary to prevent the oxidation of linoleic acid as the antioxidant present in the extract was able to do so (Ferreira, Proenca, Serralheiro, & Araujo, 2006). When compared to standard synthetic antioxidants (BHA and, BHT), all extracts were found to be less effective. Additionally, the highest inhibition was presented by the acetone extract (80.20%), while the water and methanolic extracts presented similar values, but lower. Nevertheless, a similar trend was observed towards β-carotene/linoleic acid assay in our previous works (Zengin et al., 2014).

Table 4 – Total antioxidant activity (by β-carotene bleaching and phosphomolybdenum methods) of the solvent extracts from *Morina persica* (mean ± SD).

Samples	Phosphomolybdenum (mmol TEs/g extract)[a]	β-carotene bleaching (%)[b]
Acetone	1.44 ± 0.02	80.20 ± 1.46
Methanol	1.25 ± 0.16	78.51 ± 0.46
Water	0.94 ± 0.05	78.04 ± 0.85
BHA	–	94.83 ± 2.07
BHT	–	98.69 ± 0.69

[a] TEs, Trolox equivalents; nd, not determined.
[b] At 2 mg/mL concentration.

3.7. Inhibitory activities on AChE, BChE, α-amylase and α-glucosidase and tyrosinase

In this study, the *in vitro* inhibitory effects of different solvent extracts of *M. persica* on α-glucosidase, α-amylase, cholinesterases and tyrosinase were investigated. The results are presented in Table 5.

In traditional practices of medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as AD and other memory related disorders. Cholinesterase inhibitors are clinically used as the first therapeutic approach in treatment for AD (Howes, Perry, & Houghton, 2003). Except the water extract, the other extracts possessed a certain level of inhibitory activity against AChE and BChE. The best inhibition was shown on AChE by the methanolic extract (1.34 mg GALAEs/g extract), the same extract being not active towards BChE. Additionally, the inhibitory effects of the methanolic extract on AChE might be related to the high contents in chlorogenic acid. This compound was recently suggested as a potent neuroprotective agent by Kwon et al. (2010) and Oboh, Agunloye, Akinyemi, Ademiluyi, and Adefegha (2013) towards its inhibitive properties on AChE and BChE.

Post-prandial hyperglycaemia is characterized by high circulating blood glucose levels, particularly after fasting and consumption of meals. Pancreatic glucosidase and amylase play important roles due to their hydrolysis of starch and oligosaccharides. Thus, an important strategy for managing hyperglycaemia is to inhibit these two key enzymes (Krentz & Bailey, 2005). Recently, attention has been focused on natural products especially food plants, as possible sources of potent and safer antidiabetic therapies. Several studies have reported the antidiabetic and insulin modulatory potentials of food plants (Kazeem & Davies, 2016). Additionally, compounds like gallic or chlorogenic acids were found to reduce plasma glucose level or to enhance insulin sensitizing (Kazeem & Davies, 2016; Shahidi, 2011). Consequently, the application of dietary therapy in the treatment of diabetes mellitus is one of the options aimed at controlling diabetes-induced hyperglycaemia not only by enzyme inhibition mechanisms but by modulation of further involved metabolic pathways.

Table 5 – Enzyme inhibitory activity of the solvent extracts from *Morina persica* (mean ± SD).

Assays	Acetone	Methanol	Water
Acetylcholinesterase inhibition (mg GALAEs/g extract)[a]	1.26 ± 0.36	1.34 ± 0.12	na
Butyrylcholinesterase inhibition (mg GALAEs/g extract)[a]	1.19 ± 0.29	na	na
α-Amylase inhibition (mmol ACEs/g extract)[b]	0.47 ± 0.02	0.25 ± 0.01	0.06 ± 0.01
α-Glucosidase inhibition (mmol ACEs/g extract)[b]	0.43 ± 0.04	0.81 ± 0.09	0.56 ± 0.12
Tyrosinase inhibition (mg KAEs/g extract)[c]	-	-	6.36 ± 1.35

na, not active.
[a] GALAEs, galanthamine equivalents.
[b] ACEs, acarbose equivalents.
[c] KAEs, kojic acid equivalents.

As seen in Table 5, all extracts were able to inhibit the two tested enzymes. Particularly, the highest inhibition rate on α-amylase was presented by the acetone extract (0.47 mmol ACEs/g extract), while the best inhibition on α-glucosidase was exhibited by the methanol extract (0.81 mmol ACEs/g extract). As a peculiarity, the good inhibitory effect of the acetone extract towards α-amylase might be related to the high yield of cinnamic acid (Chethan, Sreerama, & Malleshi, 2008). Nonetheless, chlorogenic and rosmarinic acids are also suggested as inhibitory agents of porcine amylases (McCue & Shetty, 2004; Narita & Inouye, 2009).

Tyrosinase is a copper-containing enzyme that catalyses melanin synthesis. Therefore, tyrosinase inhibitors are of great interest in medical and cosmetic products as they may be used to prevent or treat pigmentation disorders (Kim & Uyama, 2005). However, the only tyrosinase inhibitory potential was exhibited by the water extract with a value of 6.36 mg KAEs/g extract suggesting the involvement of more hydrophilic compounds (i.e. polysaccharides, proteins) (Chang, 2009; Rout & Banerjee, 2007).

In summary, previous information about enzyme inhibitory properties of *M. persica* extracts is limited. In a previous work of Tasdemir et al. (2004) an extract from the aerial parts of *M. persica* was found exhibiting AChE inhibitory activity by TLC bioautographic method. However, concerning all the other enzymes tested in this study, no previous data are available, and thus comparison with other researchers' results is not possible.

3.8. *Inhibitory effects of* M. persica *extracts on HeLa cells*

There is increasing evidence from population as well as laboratory studies that support an inverse relationship between regular consumption of fruits, vegetables, and herbs and the risk of specific cancers. Moreover, several organizations – such as the world health organization (WHO) – have established dietary guidelines to help people reduce cancer risk. Besides, many clinical trials on the use of nutritional supplements and modified diets to prevent cancer are ongoing.

According to MTT results, *M. persica* extracts have inhibitory activities on HeLa cell line. Inhibition rates of methanolic extract in all concentrations for 24th and 48th hours were higher than acetone and water extracts, which exhibited similar inhibition rates on HeLa cells, as seen in Fig. 1. Depending on the concentration of *M. persica* extracts, inhibitory activities were increased. The most effective concentration was 100 μg/mL for all extracts. Up to date, there is no study in the literature about inhibitory effects of extracts of *M. persica* on cancer cell lines. The inhibitory activities of *M. persica* extracts may come from its bioactive components such as flavonoids and phenolic acids. According to total bioactive components and HPLC-DAD assays, the highest amounts of phenolic compounds were detected in the methanolic extract. Nevertheless, previously, many researchers showed that phenolic compounds have an inhibitory effect on cancer invasion and metastasis (Weng & Yen, 2012). Particularly speaking, in this case the high yields of rutin, chlorogenic and rosmarinic acids might be responsible for the inhibitory effects of *Morina* on HeLa cells; all these phytochemicals have documented effects as chemopreventive agents via several mechanisms (Feng et al., 2005; Hur, Yun, & Won, 2004; Lee & Zhu, 2006; Szliszka, Czuba, Jernas, & Król, 2008; Vidya Priyadarsini et al., 2010).

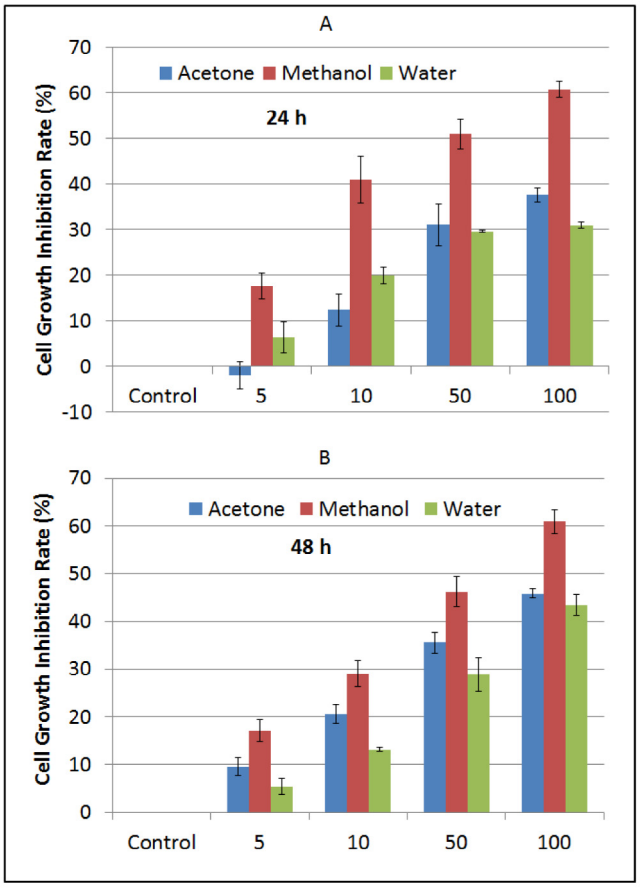

Fig. 1 – The cell growth inhibition at different concentrations of acetone, methanol and water extracts of *M. persica* on HeLa cells for 24 (A) and 48 (B) hours. The differences between the groups are significant statistically.

3.9. *Mutagenic and antimutagenic evaluation*

Mutations have been reported to be an important cause of initiation and progression of many diseases including atherosclerosis, heart diseases, and cancer. Hence, antimutagens from natural dietary sources which can counteract the promutagenic and carcinogenic effects of mutagens have immense significance (Kumar, Gautam, & Sharma, 2013). Current research suggests that the use of antimutagens in everyday diets can be an effective method for preventing cancer and genetic diseases (Bhagavathy, Sumathi, & Madhushree, 2011; Zhao et al., 2014). Antimutagenic compounds (i.e. carotenoids) disturb the metabolic activation of mutagen, and they may act as mutagen scavengers, or they may inhibit either the initiation or promotion phase of the carcinogenic process (Abdillahi, Verschaeve, Finnie, & Van Staden, 2012). Additionally, in this case, phenolic compounds might have a positive contribution; in fact, previous findings have reported that these compounds play an important role in the antimutagenic activity of beverages, fruits, vegetables or herbs (Edenharder, Sager, Glatt, Muckel, & Platt, 2002; Monente et al., 2015). Although an antimutagenic effect

Table 6 – Mutagenic activity expressed as mean number of revertants/plate ± standard deviation of extracts of *Morina* towards *Salmonella typhimurium* TA98 and TA100 strains with and without S9.

Samples	Concentration (µg/plate)	Number of His$^+$ revertants/plate			
		TA 98		TA 100	
		S9 (–)	S9 (+)	S9 (–)	S9 (+)
[a]Negative control	100 µL/plate	23 ± 3	35 ± 3	139 ± 9	135 ± 7
[b]Positive control		637 ± 37	2866 ± 31	1835 ± 45	2632 ± 33
	0	32 ± 4	40 ± 4	156 ± 24	170 ± 11
Morina acetone	5000	28 ± 0	32 ± 3	163 ± 3	171 ±
	2500	38 ± 4	34 ± 5	151 ± 8	148 ±
	1000	25 ± 4	29 ± 2	151 ± 1	159 ±
Morina methanol	5000	38 ± 5	27 ± 4	204 ± 9	185 ± 10
	2500	29 ± 6	42 ± 2	190 ± 23	176 ± 8
	1000	20 ± 0	33 ± 2	146 ± 1	179 ± 11
Morina water	5000	24 ± 6	33 ± 4	157 ± 3	161 ± 6
	2500	24 ± 1	34 ± 5	141 ± 3	154 ± 9
	1000	30 ± 4	32 ± 1	144 ± 5	149 ± 4

[a] 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.
[b] Positive controls:
2-Aminofluorene (7.5 µg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-O-phenylenediamine (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.

found in a plant extract does not automatically mean that it is an anticarcinogen, it is however an implication of the possibility of acting as one (Abdillahi et al., 2012).

Tables 6 and 7 present the mutagenic and antimutagenic properties of *Morina* extracts on *S. typhimurium* strains TA98 and TA100 both with and without metabolic activations. In the mutagenicity assays, no significant differences in the revertant colonies were observed when the bacterial plates were treated with different concentrations of *Morina* extracts up to the concentration of 5000 µg/plate. No toxicity was observed even for the highest dose tested in this experiment. As shown in Table 6, TA98 and TA100 strains did not increase in the number of revertant colonies compared to the negative control when the bacteria was treated with *Morina* extracts at 5000, 2500, and 1000 µg/plate concentrations both with and without metabolic activation enzymes (S9). Thus all extracts tested were found to be non-mutagenic neither at highest doses nor at lowest on *S. typhimurium* TA98 and TA100 without metabolic activation.

In the antimutagenicity assay a significant decrease in the revertant colonies was observed for the plates treated with positive mutagen and *Morina* extracts (Table 7). For TA98 strain all extracts of *Morina* exhibited strong antimutagenicity against 4-NPDA (4-nitrophenylene diamine) at doses of 5000, 2500 and 1000 µg/plate in the absence of S9 mix. The inhibition ratios ranged from 49% to 66% and showed the extracts as very strong antimutagenic agents. Addition of S9 mix decreased the inhibition rates of extracts for TA98, although a 5000 µg/plate dose of acetone and methanol extracts revealed excellent antimutagenicity against 2-AF (2-aminoflourene) with rates of 91% and 86%, respectively, in the presence of metabolic activation enzymes (Table 7). Concerning the plates associated with SA (sodium azide), the three different extracts of *Morina* were ineffective at all tested doses against positive mutagen (SA) and they exhibited inhibition rates lower than 25% (ranging from 4% to 24%), ranking them as weak antimutagens for TA100 strain in the absence of S9 mix. Additionally, acetone and methanol extracts of *Morina* showed an inhibition exceeding 89%, indicating the extracts as very strong antimutagens at a concentration of 5000 µg/plate in the presence of metabolic activation system for TA100 against 2-AA (2-amino anthracene). Also 2500 µg/plate dose of methanol extract manifested moderate antimutagenicity with a rate of 36%. With the exception of these mentioned doses, all remaining concentrations of the extracts did not ameliorate the base pair substituent mutations induced by 2-AA for TA100 strain with S9 mix (Table 7).

Based on these results, it can be stated from this study that S9 metabolic enzyme system increased the inhibition rate, reaching 91% at higher concentrations for acetone and methanol extracts, of mutagenic effects of standard chemicals both for TA98 and TA100 strains. These results suggest that acetone and methanol extracts of *Morina*, with high antimutagenic activity in the presence of S9, should be suitable for evaluation concerning CyP450 modulations effects. This could be explained by the fact that medicinal plants might contain compounds capable of inhibiting the CyP450 required for activating these mutagens (Del-Toro-Sánchez et al., 2014). On the contrary, all extracts revealed strong antimutagenicity against mutagens in the absence of S9 mix for TA98 strain. This means that *Morina* extracts strongly alleviated the frame shift mutations originating from mutagenic action of 4-NPDA. Addition of S9 mix decreased the inhibition rate of all extracts except for acetone and methanol, at a dose of 5000 µg/plate. Hence, *Morina* extracts can be protective against frame shift mutations induced by direct mutagens.

3.10. *Antimicrobial evaluation*

In this study antimicrobial and anti-MRSA activities of methanol, acetone and water extracts of *M. persica* were investigated

Table 7 – Antimutagenicity and inhibition ratios of *Morina* extracts towards *Salmonella typhimurium* TA98 and TA100 strains with and without metabolic activation (S9) against direct and indirect mutagens.

Samples	Concentration (μg/plate)	Number of His+ revertants/plate							
		TA 98				TA 100			
		S9 (–)	% I	S9 (+)	% I	S9 (–)	% I	S9 (+)	% I
[a]Negative control	100 μL/plate	36 ± 9		37 ± 3		162 ± 26		127 ± 14	
[b]Positive control		504 ± 38	0	3622 ± 139	0	1428 ± 88	0	4430 ± 181	0
	0	27 ± 3		32 ± 5		160 ± 9		139 ± 10	
Morina acetone	5000	191 ± 14	66	359 ± 12	91	1128 ± 41	24	605 ± 7	89
	2500	234 ± 8	57	2531 ± 71	30	1254 ± 53	14	3512 ± 31	21
	1000	267 ± 26	50	3329 ± 108	8	1259 ± 45	13	4167 ± 94	6
Morina methanol	5000	220 ± 16	60	552 ± 40	86	1180 ± 72	20	608 ± 56	89
	2500	243 ± 6	55	3113 ± 51	14	1191 ± 28	19	2878 ± 37	36
	1000	270 ± 12	49	3224 ± 101	11	1242 ± 14	15	4356 ± 106	2
Morina water	5000	250 ± 15	53	3120 ± 187	14	1258 ± 38	13	3674 ± 39	18
	2500	200 ± 26	64	3542 ± 131	2	1270 ± 42	12	3902 ± 109	12
	1000	232 ± 10	57	3569 ± 145	1	1379 ± 65	4	4012 ± 127	10

% I: % Inhibition.
[a] 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.
[b] Positive controls:
2-Aminofluorene (7.5 μg/plate) was used as positive indirect mutagen in the presence of S9 mix; 4-nitro-O-phenylenediamine (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.

by broth microdilution method according to Zengin et al. (2014). The obtained results are presented in Table 8.

When the methanol extract was evaluated, this extract showed antibacterial and antifungal activity at a dose of 6.25 mg/mL on standard bacteria and yeasts strains (Table 8). *M. persica* acetone extract was the most effective against standard bacteria compared with methanol and water extracts, at a dose of 3.125 mg/mL. Additionally, it was found that acetone extract had antifungal activity against *C. albicans* and *C. parasilopsis* at a dose of 6.25 mg/mL. Besides, the water extract displayed antimicrobial activity only against *S. aureus* ATCC 25923 (MSSA) and *S. aureus* ATCC 43300 (MRSA) standard strains. Besides, it was determined that the water extract presented antifungal activity against *C. albicans* and *C. parasilopsis* at 6.25 mg/mL.

Table 8 – MIC values of *M. persica* extracts against standard bacteria and MRSA strains isolated from clinical samples.

Tested microorganisms	MIC values of *Morina persica* extracts (mg/mL)			MIC value of gentamicin (μg/mL)	MIC value of oxacillin (μg/mL)
	Methanol	Acetone	Water		
Escherichia coli ATCC 25922	6.25	3.125	–	2.44	–
Pseudomonas aeruginosa ATCC 15442	6.25	3.125	–	9.76	–
Staphylococcus aureus ATCC 25923 (MSSA)	6.25	3.125	6.25	2.44	0.25
Klebsiella pneumoniae ATCC 70603	6.25	3.125	–	2.44	–
Staphylococcus aureus ATCC 43300 (MRSA)	6.25	3.125	3.125	78.12	64
Salmonella enteritidis ATCC 13076	6.25	3.125	–	4.88	–
Candida albicans	6.25	6.25	6.25	312.5	–
Candida parasilopsis	6.25	6.25	6.25	312.5	–
MRSA strain 1 (ES 16)	6.25	3.125	3.125	156.25	16
MRSA strain 2 (ES 25)	6.25	3.125	3.125	312.5	≥128
MRSA strain 3 (ES 29)	6.25	3.125	3.125	312.5	32
MRSA strain 4 (ES 67)	6.25	3.125	3.125	156.25	32
MRSA strain 5 (ES 68)	3.125	3.125	6.25	156.25	≥128
MRSA strain 6 (ES 69)	3.125	3.125	–	312.5	≥128
MRSA strain 7 (ES 75)	6.25	3.125	3.125	156.25	≥128
MRSA strain 8 (ES 93)	6.25	3.125	6.25	78.12	≥128
MRSA strain 9 (ES 100)	6.25	3.125	–	78.12	≥128
MRSA strain 10 (ES 107)	6.25	3.125	3.125	156.25	8
MRSA strain 11 (ES 110)	3.125	3.125	–	156.25	≥128
MRSA strain 12 (ES 123)	6.25	3.125	3.125	78.12	16
MRSA strain 13 (ES 124)	6.25	3.125	3.125	78.12	≥128
MRSA strain 14 (ES 128)	6.25	3.125	3.125	78.12	≥128

Furthermore, extracts of *M. persica* were tested against clinical isolates of MRSA. MIC values of the extracts against MRSA strains are presented in Table 8. *Morina* extracts displayed antimicrobial activity against both *S. aureus* ATCC 43300 and all of the 14 tested MRSA strains as shown by the MIC values in the broth microdilution method. Thus the methanolic extract was effective at doses of 6.25–3.125 mg/mL on MRSA isolates. Additionally, it was determined that *Morina* acetone extract showed anti-MRSA activity at a dose of 3.125 mg/mL against all MRSA isolates. However, water extracts of *Morina* exhibited anti-MRSA activity at doses of 6.25–3.125 mg/mL against MRSA isolates 1, 2, 3, 4, 5, 7, 8, 10, 12, 13, and 14 but showed no anti-MRSA activity against MRSA isolates 6, 9 and 11.

Previous research provided ample evidence which established the association among certain bacteria and cancer (Mager, 2006; Živković et al., 2014). Mechanisms by which bacteria have been associated with cancer are induction of chronic inflammation and production of carcinogenesis. For instance, in the development of hepatocellular carcinoma the staphylococcal α-toxin can activate simultaneous production of several cytokines connected to all steps of tumour development and nuclear factor κB, the known tumour promoter (Kullander, Forslund, & Dillner, 2009). Thus, the interest of consumers and food producers is towards consuming and developing functional foods with antibacterial properties and chemopreventive effects.

3.11. *Molecular docking experiments*

The flavonoid rutin has been selected as representative molecule to perform the docking experiments, being one of the dominant compounds in the acetone and methanol extracts. As reported previously (Cao, Xia, Chen, Xiao, & Wang, 2013; Lai, Lin, & Chiang, 2014; Oboh, Ademosun, Ayeni, Omojokun, & Bello, 2014), rutin is able to strongly inhibit several enzymes such as α-amylase, α-glucosidase, acetylcholinesterase and tyrosinase. By molecular docking experiments we were able to show a possible way of interaction of rutin and the enzymes listed above. As depicted in Figs. 2 and 3, rutin fits well in the binding pockets of all selected enzymes with the glycoside part, as previously shown by Bader et al. (2015). Rutin also displays a series of interactions with the amino acid side chains present in the enzymatic pockets by establishing several hydrophobic interactions, pi–pi stacks and few key hydrogen bonds. It has also been reported that rutin is more active than quercetin, and this is possibly explained by the further multiple interactions found between the amino acid residues surrounding the enzymatic pocket on the external edge and the molecule of rutin. In the case of tyrosinase, where two copper atoms are present in the enzymatic pocket, our lowest energy pose of rutin–tyrosinase complex did not show a direct bond to the metal ions, in an analogous way of tropolone. However, other authors have reported that the interaction with one copper atom of rutin is possible and may contribute to stabilizing the interaction pose with the enzyme (Si et al., 2012). In the lowest energy binding pose we found that the complex is stabilized by one hydrogen bond, a pi-pi interaction between the quercetin portion and His244, and a cation-pi interaction between Arg268 and the aromatic ring of quercetin. In the case of acetylcholinesterase, two hydrogen bonds and a large hydrophobic interaction contribute to stabilize the docking complex. In case of α-glucosidase, the complex is stabilized by a large hydrophobic interaction and one hydrogen bond with Asp630, whereas for α-amylase, the main interactions are very effective and involve the glycoside part with the presence of three hydrogen bonds. These data are in agreement with the previous studies (Cao et al., 2013; Lai et al., 2014; Oboh et al., 2014) and with the *in vitro* experiments reported in this paper. Molecular docking has shown a possible way of interaction of rutin with the target enzymes, showing that it can effectively bound by forming multiple stable interactions.

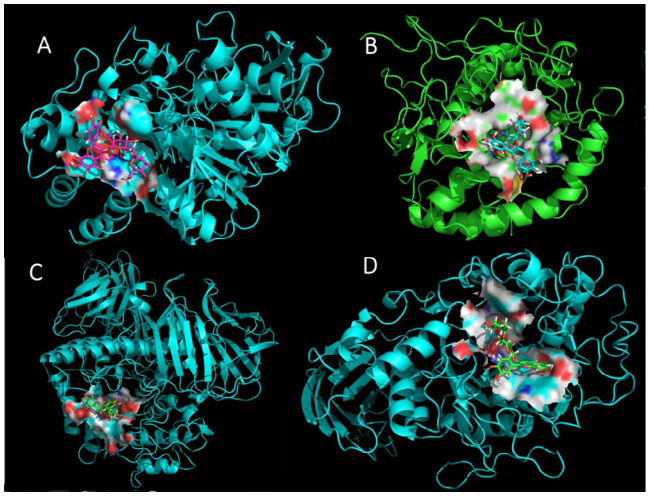

Fig. 2 – Graphical representation of the docking of rutin with enzyme: (A) acetylcholinesterase, (B) tyrosinase, (C) α-glucosidase, (D) α-amylase. The ligand is represented in the best scored pose. The surface is calculated by the residues in 3 Angstrom. Image realized by Pymol (PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC.).

4. Conclusion

The present study was designed to assess the chemical composition and potential health beneficial properties of *M. persica*, a functional tea used in the traditional Anatolian medicine. *M. persica* aerial parts proved to be a rich source of phenolic compounds, particularly chlorgenic acid, rutin and rosmarinic acid and possessed significant antioxidant capacity. Furthermore, acetone and methanolic extracts of *M. persica* revealed good inhibitory properties on acetylcholinesterase and HeLa cells. Besides, the interactions between rutin, the dominant flavonoid and the tested enzymes were further assessed by molecular docking. Additionally, the same extracts exhibited a strong, dose-dependent antimutagenicity against 4-NPDA. Nonetheless, the acetone extract of *M. persica* presented strong

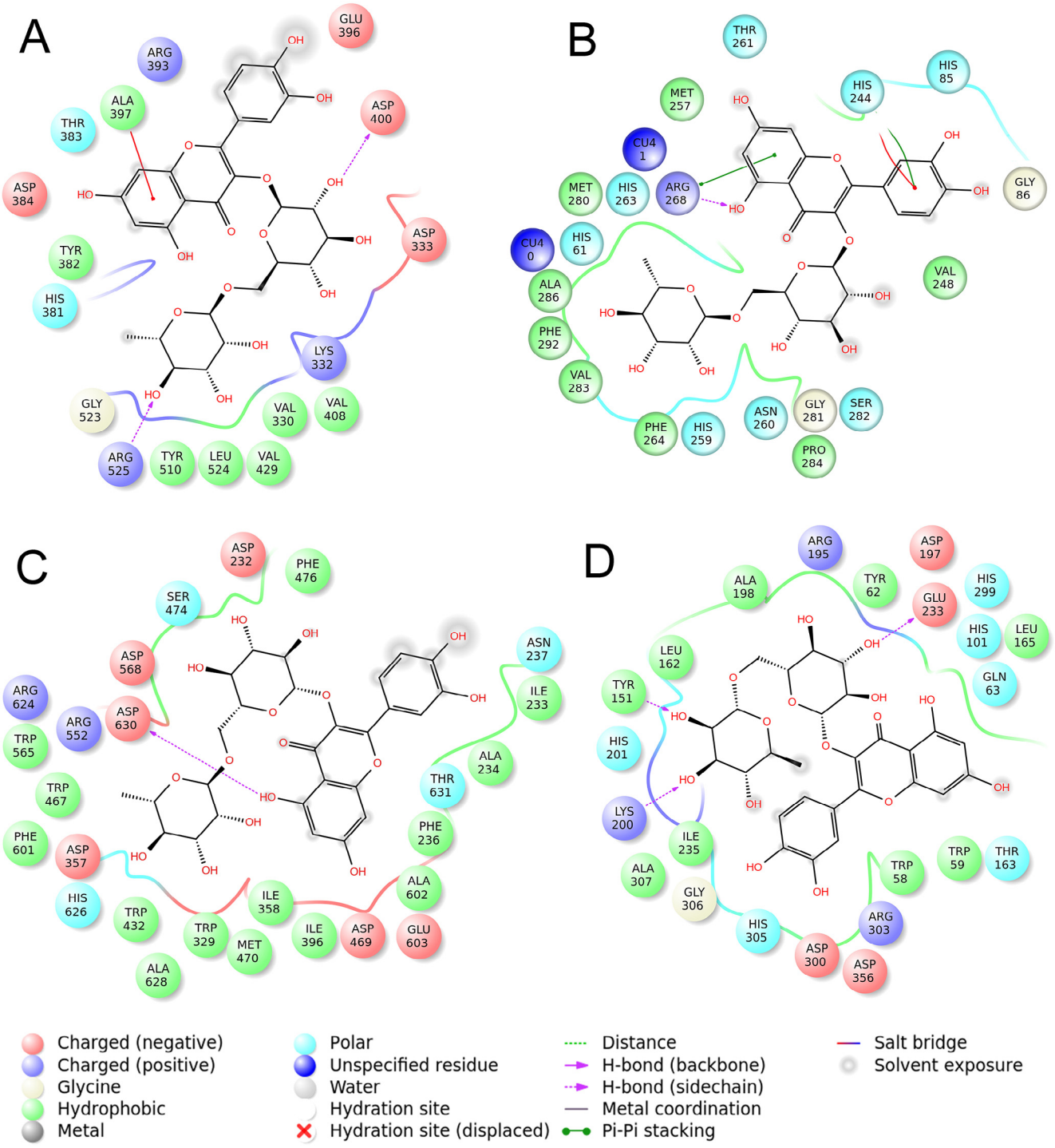

Fig. 3 – Overall binding interactions calculated in 3 Angstrom distance from the ligand. Enzyme–rutin complex interactions with: (A) acetylcholinesterase, (B) tyrosinase, (C) α-glucosidase, (D) α-amylase. The ligand is represented in the best scored pose. Image realized by Maestro (Schrödinger, 2015).

antibacterial and antifungal properties not only against all tested microorganisms, but also on MRSA. Results indicate that *M. persica* could be considered a natural source of high-value functional ingredients which may exert several health benefits and can be valorized in functional foods applications. However, further studies are necessary in order to elucidate the mechanisms of *in vivo* action, bioavailability and involved metabolic pathways.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2016.05.010.

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