

Computational approaches to the in vitro antibacterial activity of *Allium hirtifolium* Boiss against gentamicin-resistant *Escherichia coli*: focus on ribosome recycling factor

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Abstract Persian shallot, *Allium hirtifolium* Boiss. (AH), is an Iranian native medicinal plant belongs to Alliaceae family. Here, we investigated in vitro antibacterial activity of hydro-alcoholic extract derived from bulbs of AH. We also employed in silico molecular docking to decipher mechanisms of its antibacterial effects. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) against *E. coli* ATCC 25922 were determined. Molecular docking was performed for major phytochemicals of AH against ribosome recycling factor (RRF). *E. coli* ATCC 25922 was gentamicin-resistant while AH showed MIC ($42 \pm 18 \mu\text{g/ml}$) and MBC ($106 \pm 36 \mu\text{g/ml}$) against *E. coli*. In silico results reported all phytochemicals of AH shown acceptable negative binding affinity (kcal/mol) with RRF. In essence, the binding affinities of alligenin (−11.6), gitogenin (−11.6), kaempferol (−10.2), linoleic acid (−8.4), oleic acid (−8.0), palmitic acid (−7.4), palmitoleic acid (−8.4), quercetin (−10.8), and shallomin (−13.4) with RRF were comparable to that of gentamicin (−12.6). In sum, hydro-alcoholic extract of bulbs of AH could be considered as a commercial phytobiotics if in-depth antibacterial assays employed in future studies. More interestingly, shallomin showed more promising binding affinity with RRF

and can be considered as lead molecule for future drug discovery.

Keywords *Allium hirtifolium* Boiss · *Escherichia coli* · In vitro antibacterial assay · In silico molecular docking · Ribosome recycling factor

Background

Secondary metabolites, phytochemicals, are bioactive compounds derived from medicinal plants and able to destroy pathogenic bacteria via various toolboxes (Monte et al. 2014). In this line, antibacterial phytochemicals, phytobiotics, or synthetic antibiotics block or kill pathogenic microorganisms by targeting proteins, genes, and metabolites that are necessary for survival of microorganisms. For instance, ribosome recycling factor (RRF) is a protein present in *E. coli* responsible for separation of ribosomes from mRNA after the termination of translation and reuse them (Janosi et al. 1994). Indeed, gentamicin is a classic antibiotic that irreversibly binds to 30S subunit of bacterial ribosome and abolishes protein synthesis, however we encounter to the emergence of gentamicin-resistance bacteria for decades (Weinstein 1967). Therefore, the seeking phytobiotics that work on multi-drug resistant bacteria would be a necessity and economic option nowadays.

Allium hirtifolium Boiss. (AH), commonly known as Persian shallot, is a medicinal plant belongs to family Alliaceae and native to Iran which overtly used for the treatment of many diseases (Jafariana et al. 2003). Phytochemical compounds which are reported in the bulbs of AH including linolenic, linoleic, palmitoleic, palmitic, oleic and stearic acid, kaempferol, quercetin, shallomin,

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furostanal, spirostanal, sulphur containing compounds (thiosulfinates), and flavonoids (Jafariana et al. 2003; Amin and Kapadnis 2005; Barile et al. 2005; Ebrahimi et al. 2008; Kazemi et al. 2010; Amin et al. 2012; Asgarpanah and Ghanizadeh 2012). In this continuum, aqueous extracts of bulbs of AH showed considerable inhibitory effect against pathogenic bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* (Gahremani-majd et al. 2012).

Based on (Kurdish) ethnomedicine, this study was aimed firstly to probe in vitro antibacterial activity of the bulbs of AH against *E. coli* and secondly to decipher in silico docking of its major reported phytochemicals (Fig. 1) against RRF as a target.

Methods

Plant preparation

The Persian shallot collected from Iran and Iraq since March to October 2016. The collected specie was authenticated by botanist, third author, as *Allium hirtifolium* Boiss (AH). To prepare hydro-alcoholic extract, powdered bulbs (10 g) were extracted twice with 100 ml of 70% ethanol for 48 h at room temperature. The extracted suspensions were filtered and resulting filtrates were dried using a rotary evaporator and stored at -20°C until further use. For the antibacterial assays, dried extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml and stored at 4°C as stock solutions.

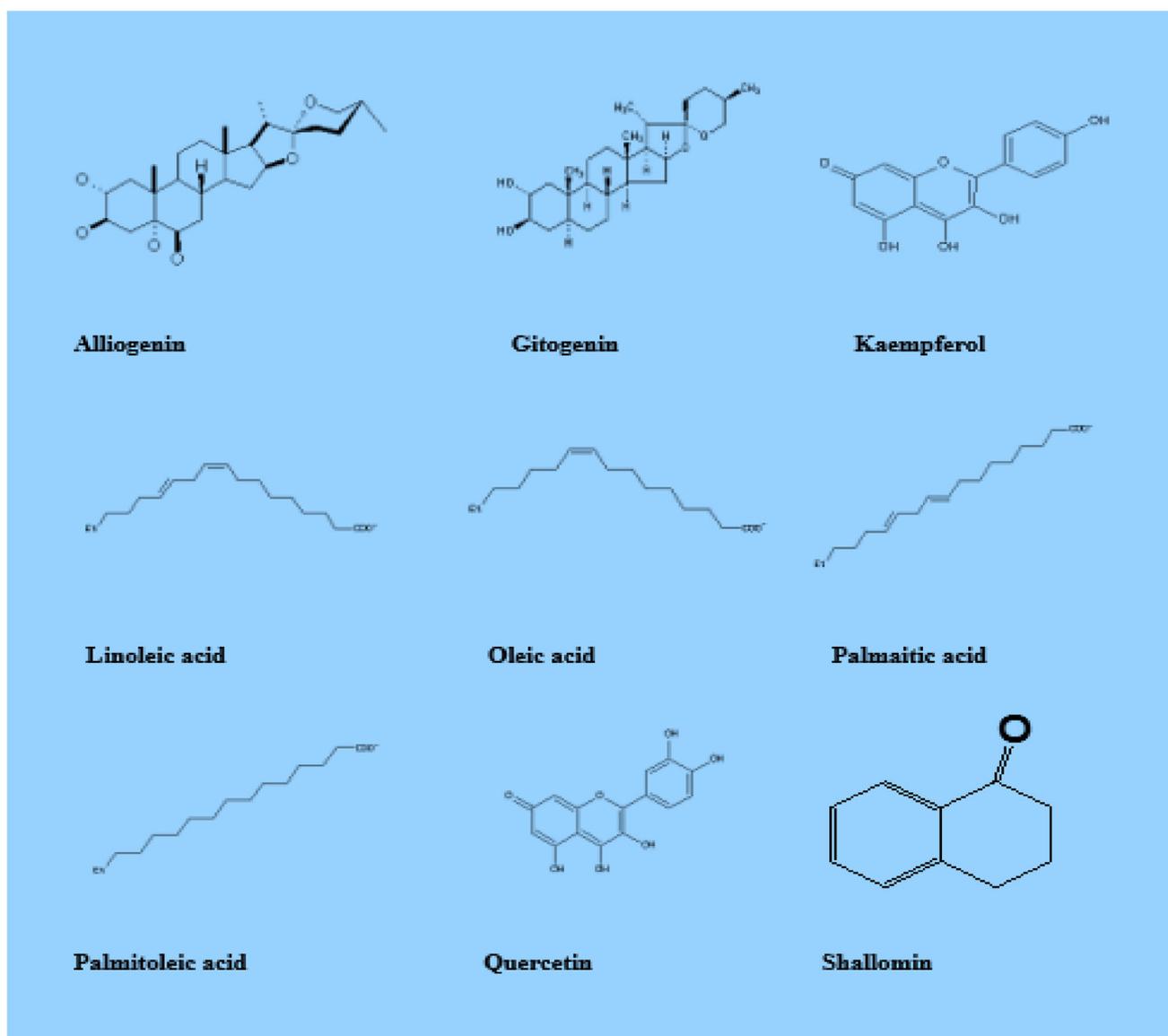


Fig. 1 The phytochemicals that have been reported in the bulbs of *Allium hirtifolium* Boiss

Table 1 In silico results of major phytochemicals of *Allium hirtifolium* Boiss against ribosome recycling factor (PDB:1WIH)

Ligand-code of ZINC ¹²	Binding affinity (kcal/mol)	RMSD/upper bound	RMSD/lower bound
Alliogenin-38140890	-11.6	3.088	1.624
Gitogenin-8234223	-11.6	32.114	1.816
Kaempferol-3869768	-10.2	19.43	18.54
Linoleic acid-13508527	-8.4	17.42	15.243
Oleic-acid-8217338	-8.0	3.476	2.348
Palmitic-acid-6072466	-7.4	9.234	4.534
Palmitoleic-acid-8221009	-8.4	2.348	1.997
Quercetin-3869685	-10.8	20.146	18.732
Shallomin-ChemDraw	-13.4	19.388	18.957
Gentamicin-DrugBank	-12.6	3.65	1.65

RMSD Root mean-square deviation is the measure of the average distance between the atoms

In vitro antibacterial assay

Gentamicin was used to assay the effects of antibiotics on the strains *E. coli* (ATCC[®] 25922TM) at 5 µg/ml for each well of microtiter plate. *E. coli* was cultured on Mueller–Hinton Agar (MHA) and Mueller–Hinton Broth (MHB) media. The antibacterial activity of bulbs of AH on *E. coli* was determined as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using broth microdilution method (Wiegand et al. 2008). Serial dilutions of the extract were prepared in a 96-well microtiter plate. To each well, 100 µl of indicator solution that prepared by dissolving a 10 mg extract in 1 ml of DMSO and 100 µl of MHB were added. Finally, 50 µl of bacterial suspension (10⁶ colony forming unite (CFU)/ml) were added to each well to achieve a concentration of 10⁴ CFU/ml. The microtiter plates were prepared in triplicates and incubated at 37 °C for 18–24 h.

In silico antibacterial assay

Molecular docking of selected bioactive compounds of AH (Fig. 1) and RRF target protein was performed by docking program (VINA WIZARD) installed on PyRx software ver. 0.8 (Dallakyan and Olson 2015). For docking studies, crystal structure of RRF was taken from the Protein Data Bank (PDB) at the Research Collaboratory for Structural Bioinformatics (<http://www.RCSB.org>). The PDB format of target protein has been prepared for docking using Molegro Virtual Docker (Thomsen and Christensen 2006) and Chimera 1.8.1 (<http://www.rbvi.ucsf.edu/chimera>) softwares before submission to PyRx software. The structures of the major phytocompounds of AH were retrieved from ZINC database ver. 12.0 (<http://zinc.docking.org/>) and DrugBank ver. 5.0 (<https://www.drugbank.ca/>) or drawn by ChemDraw (<http://www.cambridgesoft.com/software/overview.aspx>) as required.

The docking results were shown as binding affinity (kcal/mol) values. More negative the binding affinity means better docking of ligand in binding site. The best fitted conformer of ligand has been combined with target protein in Molegro Virtual Docker (Thomsen and Christensen 2006) or Chimera 1.8.1 (<http://www.rbvi.ucsf.edu/chimera>) and its atomic interaction has been analysed with LigPlot⁺ software (Laskowski and Swindells 2011). In LigPlot⁺, purple lines show ligand bonds; orange lines show non-ligand bonds; dotted lines show hydrogen bonds and its length; half red circle show non-ligand residues involved in hydrophobic contacts; and black dots show corresponding atoms involved in hydrophobic contacts (vide infra).

Results and discussion

In numerous developing countries, traditional medicine is still the mainstay of therapeutics. Even in developed countries, raw materials for industrialization of base drugs are extracted from medicinal plants. In this regard, (reverse) pharmacognosy has played a pivotal role in the detection and development of novel drugs and therapies (Satyajit 2012). Medicinal plants are rich in a wide array of natural components which have antimicrobial properties and could be utilized as a valuable source for phytobiotics (Cowan 1999). Meanwhile, *E. coli* causes diverse diseases in digestive tract in human and animals (Kaper et al. 2004). Strain of *E. coli* ATCC 25922 employed in this study was resistant to gentamicin. Gentamicin is a bactericidal and massively used antibiotic that causes mRNA decoding mistake, prevents transfer RNA and mRNA translocation, and blocks ribosome recycling in bacteria (Borovinskaya et al. 2007). Our results showed that this strain of *E. coli* ATCC 25922 was resistant to gentamicin while sensitive to AH. In essence, bulbs of AH have inhibitory at MIC

Fig. 2 The molecular docking of phytochemicals of *Allium hirtifolium* Boiss (in yellow) against ribosome recycling factor (PDB:1WIH; in cyan).

Alliogenin Corresponding atoms and non-ligand residues Ile74, Asn60, Pro75, Pro33, Pro73, Lys76, Gln34, Val35, and Lue61 involved in hydrophobic interactions.

Hydrogen bonds between alliogenin and Arg71 shown by dotted red lines.

Gitogenin Corresponding atoms and non-ligand residues Ile74, Pro33, Pro73, Lys76, Gln34, and Val35 involved in hydrophobic interactions. Hydrogen bonds between gitogenin and Arg71 and Glu64 shown by dotted red lines.

Kaempferol Hydrogen bonds between kaempferol and Thr12, His10, and Gly58 shown by dotted red lines.

Linoleic acid Hydrogen bonds between linoleic acid and Ala41 and Ser42 shown by dotted red line.

Oleic acid Hydrogen bonds between oleic acid and Ala41 shown by dotted red line.

Palmitic acid Hydrogen bonds between palmitic acid and Ala16 shown by dotted red line.

Palmitoleic acid Hydrogen bonds between palmitoleic acid and Ala41 shown by dotted red line.

Quercetin Corresponding atoms and non-ligand residues Ala41, Met40, Gly26, Gln27, and Asn23 involved in hydrophobic interactions.

Hydrogen bonds between quercetin and Gln24, Ile25, Asn39 and Ser42 shown by dotted red lines.

Shallomin Corresponding atoms and non-ligand residues Thr12 and Ile11 involved in hydrophobic interactions. Hydrogen bonds between shallomin and Glu56, Ala53, and Ser57 shown by dotted red lines.

Gentamicin Corresponding atoms and non-ligand residues Ile11 involved in hydrophobic interactions. Hydrogen bonds between gentamicin and Ser57, Thr12 and His10 shown by dotted red lines

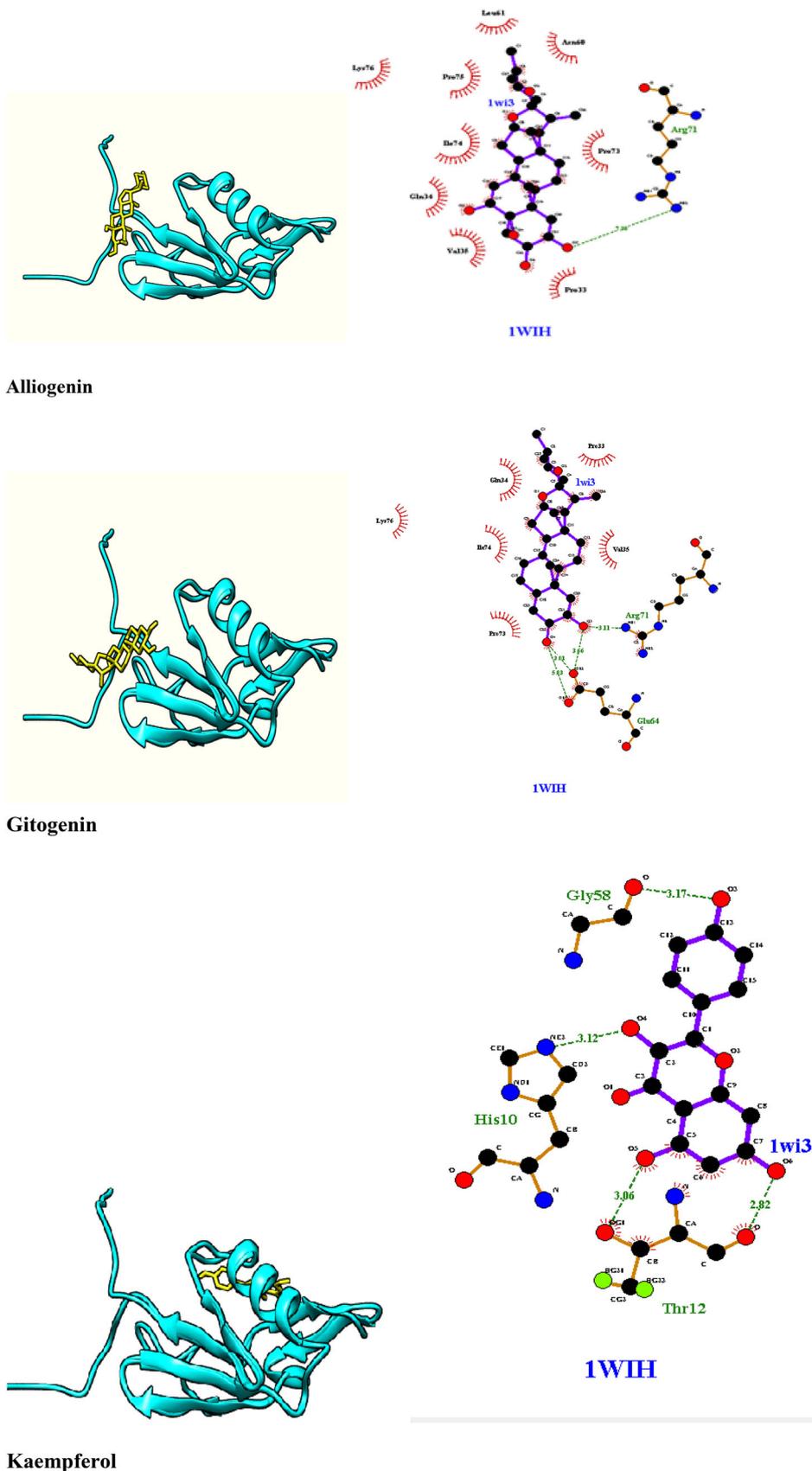
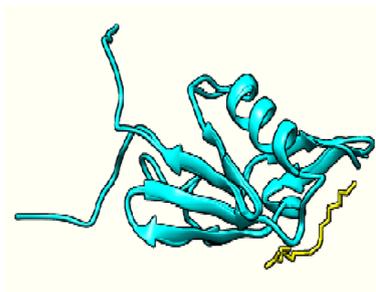
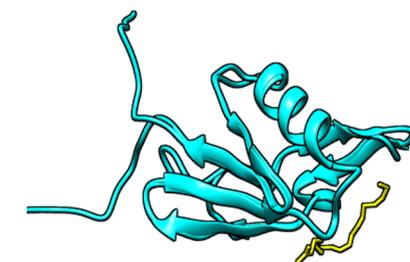
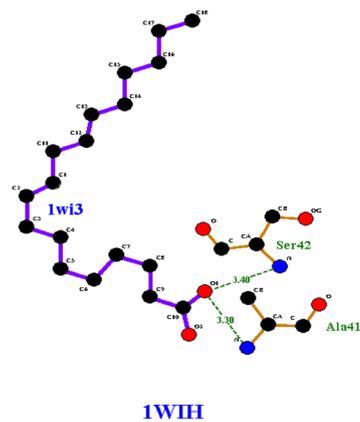


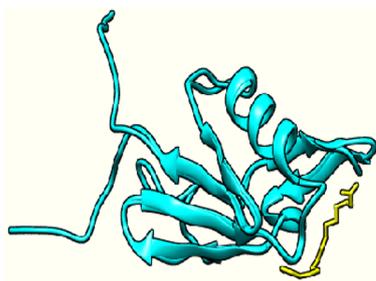
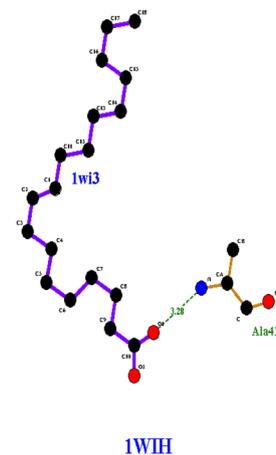
Fig. 2 continued



Linoleic acid



Oleic acid



Palmitic acid

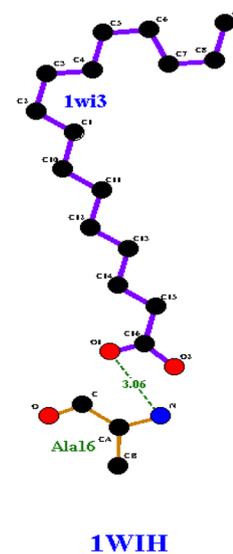
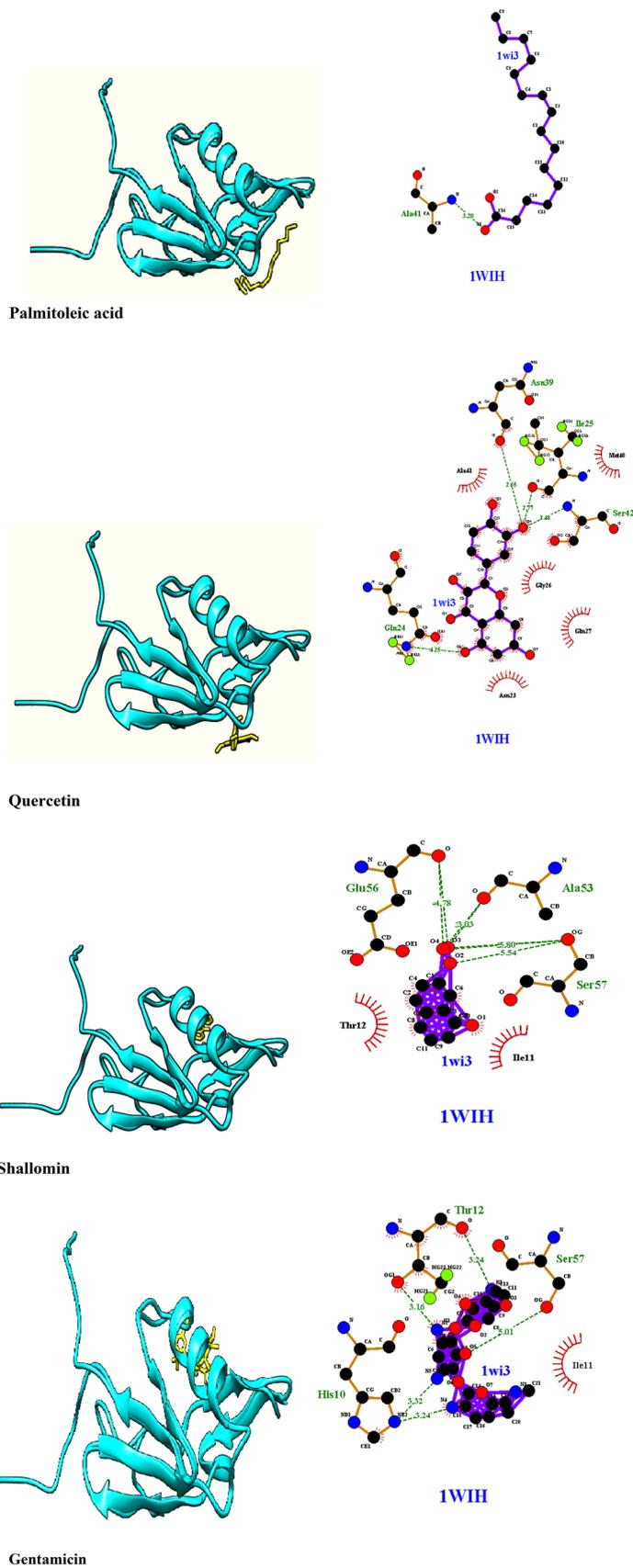


Fig. 2 continued



42 ± 18 ($\mu\text{g/ml}$) and bactericidal effects at MBC 106 ± 36 ($\mu\text{g/ml}$) on *E. coli*. With respect to antimicrobial activity, the extracts of bulbs AH showed moderate to good inhibitory activities against pathogenic bacteria including *Bacillus cereus*, *Listeria monocytogenes*, *Proteus vulgaris*, and *Salmonella typhimurium* (Ghasemi et al. 2016).

In this study, we selected gentamicin as reference antibiotic to compare its in silico binding affinity with RRF (PDB:1WIH) to those of ligands of bulbs of AH. Here we decided to decipher computationally phytochemicals which may involve in anti-*E. coli* effects of AH (Table 1, Fig. 2). More specifically, we selected RRF as target protein since it is product of the *frz* gene in *E. coli* and is responsible for separation of ribosomes from mRNA after the termination of translation and recycling of ribosomes (Janosi et al. 1994).

Based on docking results, gentamicin showed negative binding affinity with RRF at -12.6 kcal/mol (Table 1) and docked by hydrophobic interactions and hydrogen bonds (Fig. 2). In silico findings showed that among phytochemicals found in bulbs of AH, shallomin has been docked with lower binding affinity with RRF with respect to gentamicin. Shallomin is a flavonoid and would be a new lead molecule in AH and has a wide range of antibacterial properties (Bouktaib et al. 2002). Shallomin showed the lowest negative binding affinity against RRF among phytochemicals of AH. It docked with RRF utilizing both hydrophobic interactions and hydrogen bonds. Shallomin has been docked with amino acid residues (Thr12 and Ser57) in RRF which gentamicin also employed these amino acids to dock with RRF (Fig. 2). In a recent study, a preliminary in vivo toxicological estimate of escalating doses of shallomin was made and showed that shallomin was a relatively safe agent when administered at its standard antimicrobial concentration via intraperitoneal route (Amin et al. 2012).

Quercetin is a major representative of the flavonol subclass and often found as its glycoside form which has antibacterial activity (Kumar et al. 2016). Quercetin showed in vitro antibacterial activity against *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* as major food-borne microorganisms (Mertens-Talcott and Percival 2005). In the present study, quercetin has been suitably docked with RRF via both hydrophobic interaction and hydrogen bonds. The hydroxyl groups of quercetin participated in four hydrogen bonds with various amino acids of RRF (Fig. 2).

Kaempferol is a type of flavonoid found in various plants and plant-derived formulations and used in traditional medicine. Numerous preclinical researches have shown that kaempferol has antimicrobial, antioxidant, anticancer, anti-inflammatory, antidiabetic, cardioprotective, neuroprotective, anti-osteoporotic, anxiolytic,

analgesic, and anti-allergic activities (Calderón-Montaña et al. 2011). In this study, kaempferol like its congener, quercetin, has been docked with RRF by using four hydrogen bonds (Fig. 2). More surprisingly, kaempferol like gentamicin has been docked with identical amino acids (Thr12 and His10) of RRF. In the other word, kaempferol mimics the strategy of gentamicin to dock RRF (Fig. 2).

Oleic acid is a monounsaturated ω -9 fatty acid widely distributed in nature (De Silva et al. 2014). Oleic acid is a bactericidal agent against important pathogenic microorganisms including methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* (Farrington et al. 1992). Oleic acid has been moderately docked with Ala41 residue of RRF as compared to other fatty acids of AH (Fig. 2).

Linoleic acid is a polyunsaturated omega-6 fatty acid occurring widely in plant glycosides (Pariza et al. 2000). Linoleic acid has antibacterial activities against *Bacillus megaterium*, *Pseudomonas phaseolicola* and *Streptococcus mutans* (Dilika et al. 2000). Linoleic acid has been bound with amino groups of Ala41 and Ser42 residues of RRF through its carboxyl group. Its binding affinity was more suitable as compared with other fatty acids reported in this study.

Palmitic acid is the most prevalent saturated fatty acid present in plants, animals and microorganisms (Gunstone et al. 2007). This fatty acid destroys the structure and function of bacterial cell walls and membranes (McGaw et al. 2002). Palmitic acid showed the weakest binding affinity with RRF among fatty acids and all ligands of AH in this study (Table 1). It also has been docked through its carboxyl group with amino group of Ala16 in RRF (Fig. 2).

Palmitoleic acid is a class of unsaturated fatty acid synthesized from the breakdown of triglyceride. Palmitoleic acid has bactericidal properties toward *Staphylococcus aureus*, *Helicobacter pylori*, *Streptococcus salivarius*, *Fusobacterium nucleatum* and *Neisseria gonorrhoeae* (Yamamoto et al. 2015). Palmitoleic acid and linoleic acid showed identical binding affinity to RRF (Table 1) although palmitoleic acid has been solely docked with Ala41 of RRF (Fig. 2). In sum, all carboxylic groups of fatty acids reported in AH have been docked via hydrogen bonds to amino group of amino acid Ala or Ser of RRF (Fig. 2). To the best of our knowledge, this study for the first time shows that interaction of fatty acids and RRF. Further investigations are welcomed to decipher the role of Ala41, Ala16, and Ser42 in RRF that interact with carboxyl groups of fatty acids.

Alliogenin and gitogenin are type of spirostanol saponins (Amin and Kapadnis 2005). Alliogenin and gitogenin shows identical and very powerful binding affinity against RRF (Table 1). Based on the similar amino acids of RRF which participated in hydrophobic interactions and hydrogen bond

formation with alligenin and gitogenin, we can conclude that these two ligands would be docked in identical site of RRF. Alligenin also has been isolated from the bulbs of *Allium minutiflorum* and has antifungal activity (Barile et al. 2007). These spirostanol saponins make hydrogen bonds between hydroxyl groups of their spirostan rings and guanidinium group of Arg71 of RRF. Gitogenin additionally formed hydrogen bond with delta carboxyl group of Glu64 residue of RRF. The antimicrobial activity of gitogenin has been not reported previously, while our in silico results show that this bioactive compound would be a lead molecule in antibacterial investigations.

Conclusions

The emergence of antibiotic-resistant bacteria and hard access to antibiotics in distinct parts of the world motivated researchers to seek alternate therapeutics. In this continuum, hydro-alcoholic extract prepared of AH bulbs showed promising antibacterial activity against *E. coli*. In silico findings showed that among phytochemical compounds found in bulbs of AH, shallomin has more negative binding affinity to RRF compared to gentamicin and would be considered as a lead molecule for further attempts to design phytobiotics.

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Authors contributions statement NK gathered data of Kurdish ethnomedicine and authenticated plants. SEA, KC, IK, and NK carried out the experiments. SEA and IK analyzed data and carried out the molecular docking work. SEA and IK prepared the manuscript while all authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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