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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Paeoniflorigenone purified from *Paeonia daurica* roots potently inhibits viral and bacterial DNA polymerases: investigation by experimental validation and docking simulation

Adem Demir[1] · Halbay Turumtay[2] · Mustafa Emirik[1] · Cemal Sandalli[3,4] · Şeyda Kanbolat[5] · Ufuk Özgen[5] · Emine Akyuz Turumtay[1]

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Abstract

The methanolic extracts from fruit, leaf, stem and roots of *Paeonia daurica* subsp. macrophylla (*P. daurica*) were investigated for inhibitory effect on replicative bacterial (PolC and DnaE2) and viral (MMLV-RT from Moloney Murine Leukemia Virus) DNA polymerases by primer extension assay. While all plant parts showed inhibition effect on bacterial and viral DNA polymerases, roots of the plant was focused to purify inhibitory compound(s). The chemical structures of compounds were completely elucidated using a combination of NMR, MS and FT-IR analyses. Five molecules with tree monoterpene glycosides, paeoniflorin (**PD-2**), paeoniflorigenone (**PD-4**), benzoyl paeoniflorin (**PD-5**), and benzoic acid (**PD-3**) with its derivate 2,4,6-trihydroxy-1-methyl benzoate (**PD-1**) were purified and identified. Both DNAdependent and RNA-dependent polymerase activity of MMLV-RT was strongly inhibited by these five molecules. On the other hand, bacterial polymerases PolC and DnaE2 were strongly inhibited by only paeoniflorigenone (**PD-4**). Molecular modeling result suggested that paeoniflorigenone (**PD-4**) interacts with the important residues at active site (palm, fingers and thumb domains) of three polymerases which support our experimental result. Ethyl acetate fraction had smallest SC_{50} value against DPPH and ABTS radicals. It showed also higher scavenging activity than quercetin, trolox and ascorbic acid since its quite high total phenolic content. We proposed that the parts of *P. daurica* might be used to find new antimicrobial agents and generate supplementary material for foods. Furthermore, the isolated molecules with inhibitory effect may be used as new scaffold for the further modification in order to develop novel inhibitors against DNA polymerization.

Keywords *Paeonia daurica* · DNA polymerase inhibition · Molecular docking · Paeoniflorigenone

Introduction

There are a large number of DNA polymerases with the same or different functions involved in protecting, repairing, and replicating the integrity of genomic DNA in viruses, prokaryotes, eukaryotic cells, and organelles such as chloroplast and mitochondria. In recent years, increasing drug resistance has been reported because of mutations in viral genes and resistance genes in pathogenic microorganisms. Therefore, drugs targeting to novel biological mechanisms in drug resistance are needed to be investigated. The multiplication of viruses and bacteria could be prevented by inhibiting the function of DNA polymerases. The development of antimicrobial drugs to inhibit polymerases will provide advantages in the fight

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✉ Emine Akyuz Turumtay
emine.turumtay@gmail.com

[1] Department of Chemistry, Recep Tayyip Erdogan University, 53100 Rize, Turkey

[2] Department of Energy System Engineering, Karadeniz Technical University, 61830 Trabzon, Turkey

[3] Firtina Research Group, 53100 Rize, Turkey

[4] Deparment of Biology, Recep Tayyip Erdogan University, 53100 Rize, Turkey

[5] Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

against disease-affecting microorganisms. One of the excellent resource for identifying molecules having antimicrobial and antiviral effects are plants (Lee et al. 2006; Turumtay et al. 2017).

Paeonia species have been used as traditional medicine especially in traditional Chinese medicine because of their wide range of curative effect for many diseases (He et al. 2010a). It has been claimed that the roots of these species have therapeutic properties due to their anti-inflammatory, antibacterial, antiviral, antioxidant, antispasmodic, tonic, astringent, sedative, and analgesic properties, and flowers have aromatherapatic properties as well (Duan et al. 2009; Magid et al. 2017). The only genus *Paeonia* in Paeoniaceae family comprises 35 species, including three sections as Moutan, Oneapia, and Paeonia. Paeonia is the largest section with 25 species, and widely distributed in the hot regions of Eurasia, especially in the Mediterranean (He et al. 2010b). The studies on the bioactivities of the *Paeonia* species revealed their hepatoprotective and neuroprotective effects (Kim et al. 2009; Lee et al. 2008; Li et al. 2008; Wang et al. 2012), anti-inflammatory (Yang et al. 2011), antioxidant, antiviral, antifungal (Lee et al. 2006; Picerno et al. 2011; Zhang et al. 2018), and antityrosinase activities (Magid et al. 2017). These important bioactivities of *Paeonia* species have been the source of inspiration for phytochemical studies on these plants for advanced drug investigations.

Monoterpenes and monoterpene glycosides such as paeoniflorigenone, paeoniflorin, albiflorin, and their galloyl, benzoyl, vanilloyl, and glycosidic derivatives were considered to be responsible for the therapeutic properties, since they were the major isolated components from *Paeonia* species (Braca et al. 2008; Ding et al. 2012; Duan et al. 2009; He et al. 2010b; Kim et al. 2009; Picerno et al. 2011). These monoterpenes exhibit various medicinal properties such as improvements in memory and blood circulation, amelioration of neuroinflammation (Li et al. 2017), inhibitory effects on nitric oxide (NO) production (Ding et al. 2012; Duan et al. 2009), reduction of inflammatory responses, and prevention of NO hyperfunction such as tissue damage (Parker et al. 2016). Since paeoniflorin was found in all investigated species and varieties of *Paeonia* but not found in other plant species, it was considered to be the characteristic chemotaxonomic marker of the Paeoniaceae family (He et al. 2010b). While the section Moutan has high content of paeonol compounds, Section Paeonia has notably low content of these compounds (He et al. 2010b). *Paeonia daurica* Andrews subsp *macrophylla* Albow (*P. daurica*) is in the section Paeonia and spreads from the western Caucasus in Georgia, grows in Russia, Caucasus mountains, Tbilisi in Georgia, north-eastern Turkey at altitudes from 800 to 2300 m on rocky slopes and in alpine valleys. Its leaves are dark green and flowers are white tinged with yellow bloomed in late spring or early summer (Hong and Zhou 2003).

In this study, methanolic extracts of fruit, fruit barks, leaf, stem, and root from *P. daurica* were investigated for their inhibitory effect on viral reverse transcriptase (RT) (using Moloney Murine Leukemia Virus, MMLV-RT) as the control enzyme and bacterial replicative DNA polymerases (polC from *Bacillus subtilis* and DnaE2 from *Escherichia coli* as the control enzymes). Active molecules were isolated from the roots of *P. daurica* and their structures were elucidated by NMR (Nuclear Magnetic Resonance), MS/MS (Mass Spectrometry/Mass Spectrometry), and FT-IR (Fourier-transform infrared spectroscopy) analyses. Inhibitory effects of isolated compounds were performed on the MMLV-RT, polC, and DnaE2. In-silico-docking approach was also used for modeling the inhibitory effect of isolated compounds on replicative polymerases. Furthermore, the methanol extract of the root was also subjected liquid–liquid fractionation using diethyl ether, ethyl acetate in aqueous solution. Total phenolic content and radical (2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)) scavenging activities of the methanol extract and diethyl ether, ethyl acetate and aqueous fractions of roots were determined using spectrophotometric assays.

Material and methods

Chemicals

Methanol, hexane, diethyl ether, ethyl acetate, throlox, ascorbic acid, Folin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Sefadex LH-20 column, VLC column were purchased from Merck Darmstadt, Germany. Na_2CO_3, $K_2S_2O_8$, $MgCl_2$, EDTA, gallic acid, quercetin, bromophenol blue, xylene cyanol, SDS, formamide, polyacrylamide, and urea were provided from Sigma-Aldrich (St. Louis, MO, USA). All used reactants were of high-purity grade (Sigma-Aldrich and Merck) and used without further purification.

Plant materials

P. daurica was collected from Kavrun (2000 m) and Çeymakçur (2300 m) plateus (Rize Province, Turkey). The specimen identification was performed by Prof. Vagif Atamov from the Faculty of Science and Arts, Recep Tayyip Erdogan University in Rize, Turkey. A voucher specimen was deposited in the Herbarium of the Department of Biology (RTEUB: 5103 Vh), Turkey.

Extraction and isolation

The fresh plant samples were stored at −20 °C until extraction. Fruit, green and red fruit barks, leaf, stem, and root parts of the plant was pulverized separately under liquid nitrogen using a mortar and pestle. For enzyme inhibition and antioxidant tests, 10 g of each pulverized parts of *P. daurica* were extracted with 50 mL of methanol at 37 °C for 2.5 h in a thermo-shaker (Heidolph Unimax 1010, Germany) and 25 °C for 1 h in an ultrasonic bath (Elma Clean Box, Elma), and then centrifuged at 4000 rpm for 20 min at 4 °C. Supernatants were transferred into a new Falcon tube and evaporated (EZ-2 Evaporator, GeneVac). Dried extracts of the plant parts were dissolved with methanol for direct analysis of pure extracts with the concentrations of 63.4; 63.7; 70.7; 49.5; 53.1; 77.1 mg/mL for fruit, green and red fruit barks, leaf, stem and root parts, respectively. This study was focused on the root extract of the plant. Therefore, the methanol extract of the roots was subjected to the liquid–liquid fractionation. Dried root extract was re-suspended in ultrapure water and defatted twice with 10 mL n-hexane for 10 min. It is very crucial not to lose partially hydrophobic bioactive compounds during purification of extract from oily and waxy components. Partially hydrophobic compounds can be extracted with chloroform, therefore, hexane was used as a hydrophobic solvent. Diethyl ether and ethyl acetate were used for liquid–liquid fractionation of the root extract. Rest aqueous part and pure methanol extract, which was prepared without further fractionation, were also investigated for their bioactivities. Diethyl ether and ethyl acetate are mostly suitable for flavonoid extraction, while water is preferable for the phenolic acids and glycosidic polyphenols.

For isolation of the main compounds from the root, 300 g of the powdered root was extracted twice using 500 mL of methanol. Extracts were combined and evaporated at 30 °C by rotary evaporator. Seve grams of obtained methanol extract was subjected to Sefadex LH-20 column (Merck) (3 × 25 cm) using methanol as elution solvent. Twelve fractions were collected.

Fractions (Fr.) 5–10 were evaporated (3.9 g). This fraction was subjected to VLC column (LiChroprep RP-18, 25–40 µm, Merck 9303) using by elution solution of water: methanol mixtures (100:0 → 0:100). One-hundred sixteen fractions were collected.

Fr. 26–30 were evaporated and 24.9 mg 2,4,6-trihydroxy-1-methyl benzoate (**PD-1**) was obtained. Fr. 44–46 were evaporated and 55 mg paeoniflorin (**PD-2**) was obtained. Fr. 56–58 were evaporated and 45 mg benzoic acid (**PD-3**) was obtained. Fr. 90–92 were evaporated and 45.1 mg paeoniflorigenone (**PD-4**) was obtained. Fr. 93–95 were evaporated and 8.9 mg benzoylpaeoniflorin (**PD-5**) was obtained. Stock solutions were prepared from the purified molecules (7 mM concentration) and used in experiments at 1 mM final concentration approximately.

Table 1 The DNA substrates used in primer extension assays to investigate inhibition effects of plant parts and the purified molecules

32/20-mer substrate (DNA template/DNA primer): to determine the inhibition effect on the DNA-dependent DNA polymerase activity of polC and DnaE2
3′- GCAATCGGTGAGGCTGACGCCATATTGCGCGT-5′
5′- cy5CGTTAGCCACTCCGACTG-3′
32/14-mer substrate (DNA template/DNA primer): to determine the inhibition effect on the DNA-dependent DNA polymerase activity of MMLV
3′-GCAATCGGTGAGGCTGACGCCATATTGCGCGT-5′
5′-cy5CGTTAGCCACTCCG-3′
32/14-mer substrate (RNA template/DNA primer): to determine the inhibition effect on the RNA-dependent DNA polymerase activity of MMLV
3′- GCAAUCGGUGAGGCUGACGCCAUAUUGCGCGU-5′
5′-cy5CGTTAGCCACTCCG-3′

Preparation of polymerases and DNA templates

Replicative DNA polymerases, PolC and DnaE2, were prepared as described in Turumtay et al. (2017). MMLV-RT was purchased from New England (BioLabs, M0253S). 32-mer DNA and RNA template and 14-mer primer labeled by Cy5 were synthesized by Macrogen (Netherland, Amsterdam) and Iontek (Turkey, Istanbul). The synthetic DNA substrates (Table 1) were prepared as in previous work described by Sandalli et al. (2009) and used in primer extension assays by single or multiple nucleotide addition.

Inhibition effect on DNA polymerases

Inhibition of DNA polymerase activity in the presence and absence of purified molecules was investigated by primer extension assay (single nucleotide or multiple nucleotide addition). The solvent of the extracts/compounds (methanol) was used as a control. The reactions were performed in 20 µL final volume by mixing two reaction mixtures. The mixture 1 was prepared in 13.5 µL final volume with appropriate polymerase (25 nM for bacterial replicative DNA polymerases and 2 units for MMLV-RT), 1 × polymerase activity buffers (harboring 10 and 3 mM of $MgCl_2$ for replicative bacterial polymerases (Turumtay et al. 2017) and MMLV-RT, respectively), and 3 µL of each extract or purified molecules. The mixture 2 was prepared in 6.5 µL final volume with 100 ng synthetic 32/14-mer DNA/DNA substrate (for PolC, DnaE2 and DNA-depended DNA polymerase activity of MMLV-RT) or 32/14-mer RNA/DNA substrate (RNA-depended DNA polymerase activity of

MMLV-RT), and 100 µM of dNTP (both for multiple nucleotide and single nucleotide (dATP) incorporation). The mixture 1 was kept 5 min at room temperature and then the mixture 2 was added and the reactions were carried out for 10 min at 37 °C. The reactions were terminated by addition of 20 µL of 2 × stop solution (20 mM EDTA, 0.2% (w/v) SDS, 80% (v/v) formamide and 0.008% (w/v) each of bromophenol blue and xylene cyanol) and heated at 95 °C for 5 min. Six microliters of reaction mixture was loaded onto a 16% polyacrylamide-8 M urea gel and the products were separated at 1500 V for 3 h. The resulting gels were visualized by Typhoon FLA9500 biomolecular imager (Amersham Biosciences).

Molecular-docking studies

The X-ray crystal structures of studied enzymes were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb) using PDB id (Berman et al. 2000); 4MH8 (Das and Georgiadis 2004), 3F2B (Evans et al. 2008), 3E0D (Wing et al. 2008). Molecular-docking studies were performed using Maestro Molecular Modeling platform (version10.1) by Schrödinger, LLC (Schrödinger 2015). The default Glide/XP (Friesner et al. 2004; Friesner et al. 2006; Halgren et al. 2004) combined with Induced Fit-docking protocol (IFD) (Farid et al. 2006; Sherman et al. 2006a, 2006b) was applied for the prediction of binding poses and interactions between the ligands and active site of the protein. IFD is a robust and accurate docking method that is account for ligand and receptor flexibility for the prediction of binding energies and interactions of small molecule-protein complexes. (Liwo 2013).

Enzyme structures were prepared using Protein Preparation Wizard (Sastry et al. 2013). Missing side chains of the proteins were filled using the Prime tool (Jacobson et al. 2002, 2004). The protein was further refined using restrained optimization by removal of water molecules except within 5 Å from ligand. After protein preparation, all molecules are docked into the receptor grid of radii 20 Å at the binding sites of receptor structures.

LigPrep tool was used for ligand optimization, which produced lowest energy 3D structures at neutral pH and under OPLS_2005 force field (Harder et al. 2016; Jorgensen et al. 1996). A maximum of 80 poses were generated for each ligand. Prime refinement was carried out for residues within 5 Å of the ligand. Glide re-docking was carried out for structures within 30 kcal/mol of the best structure. IFD score for each of these compounds was generated and reported as:

$$\begin{aligned}\text{IFDScore} = {} & 1.0 * \text{Prime Energy} + 9.057 \\ & * \text{GlideScore} + 1.428 * \text{Glide Ecoul}\end{aligned}$$

The more negative the IFD score means the more favorable the binding interactions. This score accounts for both the protein–ligand interaction energy and the total energy of the system. The final ligand–protein interactions were visualized.

In order to estimate approximate free energies of binding (reported in kcal/mol) for the enzyme–ligand complexes, the Molecular Mechanics Generalized Born and Surface Area (MM/GBSA) method was used implemented in Prime module of the Schrodinger's molecular modeling platform (Genheden and Ryde 2014; Jacobson et al. 2002, 2004). Prime utilizes the VSGB 2.0 solvation model and the OPLS_2005 force field to simulate enzyme–ligand interactions (Li et al. 2011a). More negative value of the MM-GBSA binding energies indicates stronger binding affinity (Mentese et al. 2019).

Determination of total phenolic content

Methanolic extract of the plant root and its fractions were dried and dissolved in methanol prior to the spectrophotometric assays. Total phenolic content of the extracts was measured by Folin–Ciocalteu's phenol reagent (Singleton 1985). Although it is not very specific for phenolics, this protocol gives a good correlation of the total phenolic contents. Gallic acid and quercetin were used as phenolic standards to generate standard curves in a range of 0.019 and 0.600 mg/mL at five concentration levels ($r^2 = 0.999$). The extracts were diluted with methanol to provide optical density in the dynamic range of calibration curves. Twenty microliters of the extracts, 400 µL of 0.5 M Folin–Ciocalteu reagent, and 680 µL of distilled water were strongly mixed and incubated for 3 min at room temperature before the addition of 400 µL of Na_2CO_3 (10%). The optical density of the mixture was measured at 760 nm with a UV–Vis detector (Thermo Scientific Multiskan Go, USA) after 2 h. The results were expressed in µg of gallic acid equivalent (GAE) and µg of quercetin equivalent (QE), respectively, per gram of extracts. All concentration point of the standards and extracts were analyzed in triplicate.

DPPH activity assay

The free-radical scavenging activity of the root extracts was estimated against the DPPH radical, which is accepting a hydrogen (H) atom from the scavenger molecule, i.e., antioxidant. The purple color of this stable radical changes to yellow with concomitant decrease in absorbance at 517 nm due to the reduction of DPPH to $DPPH_2$ (Mishra et al. 2012). Briefly, 0.15 mL of plant extract was mixed with 0.15 mL of freshly prepared DPPH (0.1 mM) in

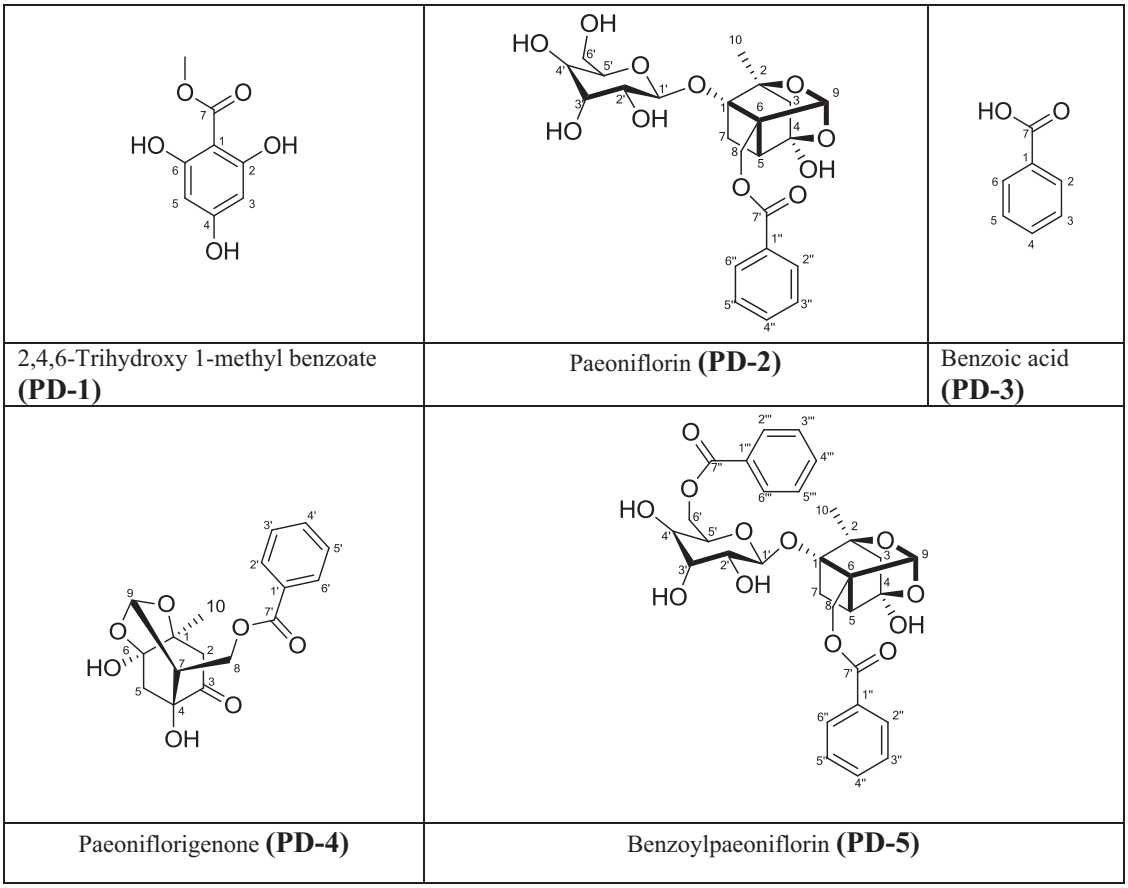

Fig. 1 Molecular structures of the isolated compounds

methanol and incubated for 30 min in the dark. Gallic acid and quercetin were used as standards. Results are reported as SC_{50} values, demonstrating the concentration of extract (μg extract per mL) necessary to scavenge 50% of DPPH radical. All concentration point of the extracts was analyzed in triplicate. Calculations for statistic have been performed as described in Willemse (2009).

ABTS radical scavenging assay

Seven micromolar of ABTS solution and 2.4 mM of potassium persulfate solution were mixed in equal quantities and allowing to oxidation reaction of ABTS by $K_2S_2O_8$ for 18 h at room temperature in the dark to form the $ABTS^{\bullet+}$ radical. This solution was then diluted by mixing 30 times with methanol to obtain an $ABTS^{\bullet+}$ radical solution has optical density of 0.700 ± 0.01 at 734 nm using a spectrophotometer (Arnao et al. 2001). Fifty microliters of plant extracts were allowed to react with 250 μL of the $ABTS^{\bullet+}$ radical solution and the absorbance was measured at 734 nm after 30 min using a spectrophotometer. The $ABTS^{\bullet+}$ scavenging capacity of the extracts were compared with that of trolox and ascorbic acid and reported with SC_{50} values (μg extract/mL). All concentration point of the extracts were analyzed in triplicate ($n = 3$).

Results and discussions

Identification of the five isolated compounds from methanolic extract

Isolated compounds were identified using NMR, FT-IR, and ESI-MS/MS spectra and their molecular structures were given at Fig. 1.

2, 4, 6-Trihydroxy 1-methyl benzoate (PD-1)

It was obtained as a cream-pale yellow powder; mp 157 °C; Rf $[\alpha]_D^{25} + 1.631$ (c 0.037, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.45) nm (Duan et al. 2009); IR (ATR) υ_{max}: 3354, 3258 (OH); 1693 (C=O); 1258, 1195 (C–O) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): $\delta = 7.03$ (2H, s, H-3, H-5), 3.80 (3H, s, $-O\underline{C}H_3$); ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 167.6$ (C, C = O), 145.1 (C, C-2, -6), 138.3 (C, C-4), 120.0 (C, C-

1), 119.6 (CH, C-3, -5), 50.9 (CH_3, OCH_3); ESI-MS *m/z* $[M-H]^-$ calcd. for $C_8H_7O_5$ 183.07, found 182.90, MS^2 fragments *m/z* $[M-CH_3]^-$ 167.97 and $[M-CH_3COO]^-$ 123.87; ^1H and ^{13}C-NMR spectrum of this compound were given at Supp. Fig. 1.

Paeoniflorin (PD-2)

It was obtained as a white amorphous powder; mp 157–158 °C; Rf $[\alpha]_D^{25} + 17$ (c 1.00, MeOH); UV(MeOH) λ_{max} (logε) 229 (3.68) nm (Kaneda et al. 1972); IR (ATR) υ_{max}: 3390 (OH); 1714 (C=O); 1279 (C–O–C); 1178, 1116, 1074 (C–O), 715 (mono Subs. Ar.) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): $\delta = 8.05$ (2H, d, $J = 8.0$, H-2'', H-6''), 7.61 (1H, m, H-4''), 7.48 (2H, t, $J = 6.8$, H-3'', H-5''), 5.42 (1H, s, H-9), 4.74 (1H, s, H-8), 4.53 (1H, d, $J = 7.6$, H-1'), 3.85 (1H, d, $J = 11.6$, H-6'α), 3.61 (1H, d, $J = 10$, H-6'β), 3,34–3,19 (4H, sugar protons), 2.58 (1H, d, $J = 6$, H-5), 2.49 (1H, m, H-7α,), 2.19 (1H, d, $J = 12.4$, H-3α), 1.95 (1H, d, $J = 10.4$, H-7β), 1.81 (1H, d, $J = 12.4$, H-3β), 1.36 (1H, s, H-10); ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 166.6$ (C, C-7'), 133.0 (CH, C-4''), 129.8 (C, C-1''), 129.2 (CH, C-2'', -6''), 128.3 (CH, C-3'', -5''), 105.0 (C, C-4), 100.9 (CH, C-9), 98.8 (CH, C-1'), 87.9 (C, C-1), 85.9 (C, C-2), 76.6 (CH, C-5'), 76.5 (CH, C-3'), 73.6 (CH, C-2'), 70.8 (CH, C-4'), 70.3 (C, C-6), 61.5 (CH_2, C-6'), 60.3 (CH_2, C-8), 42.5 (CH, C-5), 22.0 (CH_2, C-7), 18.2 (CH_3, C-10); ESI-MS *m/z* $[M+Na]^+$ calcd. for $C_{23}H_{28}O_{11}Na$ 502.41, found 502.88; MS^2 fragments *m/z* $[M- C_6H_5COO]^+$ 380.98 and $[M\text{-glucosyl}+H]^+$ 340.88; ^1H and ^{13}C-NMR spectrum of this compound were given at Supp. Fig. 2.

Benzoic acid (PD-3)

It was obtained as a white amorphous powder; mp 122 °C; Rf $[\alpha]_D^{25} + 1.54$ (c 1.00, MeOH); UV(MeOH) λ_{max} (logε) 225 (3.98) nm (Wang et al. 2012); IR (ATR,) υ_{max}: 3389 (OH); 1698 (C=O); 1178 (C–O) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): $\delta = 8.02$ (2H, d, $J = 7.9$, H-2, H-6), 7.56 (1H, t, $J = 6.8$, H-4), 7.45 (2H, d, $J = 7.5$, H-3, H-5); ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 132.5$ (CH, C-4), 129.3 (CH, C-2, -6), 128.2 (C, C-1), 128.0 (CH, C-3, -5); ESI-MS *m/z* $[M-H]^-$ calcd. for $C_7H_6O_2$ 121.04, found 121.14; MS^2 fragments *m/z* $[M\text{-}COO]^-$ 76.96 and $[M\text{-}OH]^-$ 105.08; ^1H and ^{13}C-NMR spectrum of this compound were given at Supp. Fig. 3.

Paeoniflorigenone (PD-4)

It was obtained as a white amorphous powder; mp 121 °C; Rf $[\alpha]_D^{25} + 4.3$ (c 0.069, MeOH); UV(MeOH) λ_{max} (logε) 220 (3.94) nm (Shimizu et al. 1983); IR (ATR) υ_{max}: 3418 (OH); 1722, 1715 (C=O); 1278, 1250 (C–O–C); 1102, 1071 (C–O); 713 (mono subs. Ar.) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): $\delta = 7.99$ (2H, d, $J = 7.6$, H-2', H-6'), 7.60 (1H, t, $J = 6.8$, H-4'), 7.46 (2H, t, $J = 7.6$, H-3', H-5'), 5.40 (1H, s, H-9), 4.25 (1H, m, H-8α), 4.04 (1H, m, H-8*β*), 2.82 (1H, bd, H-4), 2.78 (1H, d, $J = 17.4$, H-2*α*), 2.53 (1H, d, $J = 17.4$, H-2*β*), 2.40 (1H, m, H-7), 2.35 (1H, d, H-5*α*), 2.24 (1H, d, $J = 12.5$, H-5*β*), 1.24 (1H, s, H-10); ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 211.1$ (C, C-3, C=O), 166.3 (C, C-7', C=O), 133.0 (CH, C-4'), 129.7 (C, C-1'), 129.2 (CH, C-2', -6'), 128.2 (CH, C-3', -5'), 101.4 (C, C-6), 99.5 (CH, C-9), 78.7 (C, C-1), 62.8 (CH_2, C-8), 48.3 (C, C-4), 47.0 (CH_2, C-2), 43.0 (CH, C-7), 34.1 (CH_2, C-5), 20.4 (CH_3, C-10); ESI-MS *m/z* $[M+Na]^+$ calcd. for $C_{17}H_{18}O_6Na$ 341.31, found 340.98; MS^2 fragment *m/z* $[M- C_6H_5COO]^+$ 219.17; ^1H and ^{13}C-NMR spectrum of this compound were given at Supp. Fig. 4.

Benzoylpaeoniflorin (PD-5)

It was obtained as a white amorphous powder; mp 131–132 °C; Rf $[\alpha]_D^{25} + 42$ (c 0.67, MeOH); UV(MeOH) λ_{max} (logε) 229 (4.42) nm (Kaneda et al. 1972); IR (ATR) υ_{max}: 3406 (OH); 1703, 1644 (C=O); 1278 (C-O-C); 1072 (C-O), 714 (mono subs. Ar.) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): $\delta = 8.03$ (1H, m, H-2'', H-2''', H-6'', H-6'''), 7.60 (1H, t, $J = 6.8$, H-4'', H-4'''), 7.47 (1H, t, $J = 7.2$, H-3'', H-3''', H-5'', H-5'''), 5.37 (1H, s, H-9), 4.70 (1H, s, H-8), 4.62 (1H, m, H- 6'α), 4.56 (1H, d, $J = 7{,}6$, H-1'), 4.48 (1H, m, H-6'β), 3.59–3.24 (4H, m, sugar protons), 2.49 (1H, m, H-7α), 2.49 (1H, m, H-5), 1.84 (1H, d, $J =$ 12.8, H- 3α), 1.68 (1H, m, H-7*β*), 1.68 (1H, m, H-3*β*), 1.23 (1H, s, H-10); ^{13}C-NMR (CD_3OD, 100 MHz): $\delta = 166.2$ or 166.5 (C, C=O, C-7', -7''), 133.0 or 133.1 (CH, C-4'', -4'''), 129.8 or 129.9 (C, C-1'', -1'''), 129.1 or 129.2 (CH, C-2'', -2''', -6'', -6'''), 128.2 or 128.3 (CH, C-3'', -3''', -5'', -5'''), 100.8 (CH, C-9), 104.8 (C, C-4), 98.6 (CH, C-1'), 87.9 (C, C-1), 85.6 (C, C-2), 76.4 (CH, C-3'), 73.8 (CH, C-2'), 73.5 (CH, C-5'), 70.6 (C, C-6), 70.6 (CH, C-4'), 63.7 (CH_2, C-6'), 60.2 (CH_2, C-8), 43.0 (CH_2, C-3), 42.4 (CH, C-5), 21.6 (CH_2, C-7), 18.1 (CH_3, C-10); ESI-MS *m/z* $[M+Na]^+$ calcd. for $C_{30}H_{32}O_{12}Na$ 606.60, found 606.73; MS^2 fragment *m/z* $[M- C_6H_5COO]^+$ 484.94; ^1H and ^{13}C-NMR spectrum of this compound were given at Supp. Fig. 5.

Paeoniflorin **(PD-2)**, characteristic monoterpene glycoside for the *Paeonia* section, was the most abundant isolated compound from the methanol extract of *P. daurica* roots (55 mg in 7 g of extract). The paeoniflorin **(PD-2)** structure comprises a benzoyl and a glycosyl group attached to the "cage-like" pinane skeleton. Benzoylpaeoniflorin **(PD-5)** has an extra benzoyl group attached to the glycosyl group of the paeoniflorin **(PD-2)**. Paeoniflorigenone **(PD-4)** is structurally very similar to the paeoniflorin aglycone and it contains a

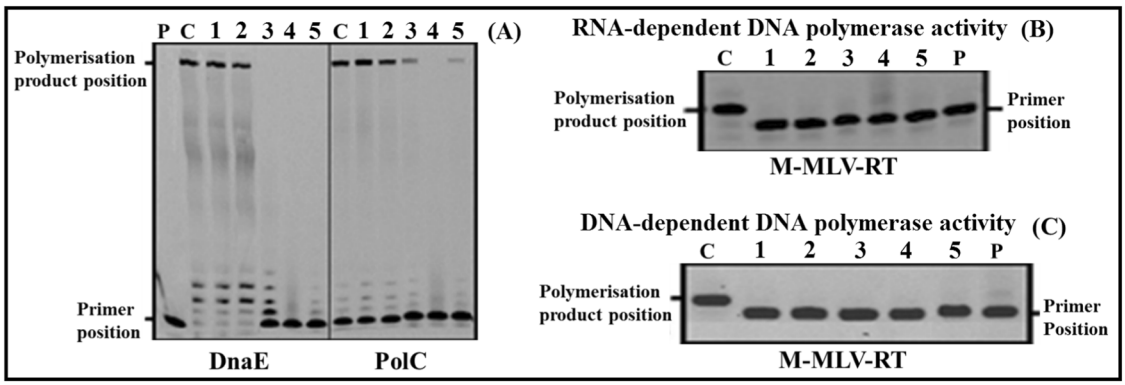

Fig. 2 Primer extension assays were performed in the presence or absence of purified molecules to detect their inhibitory effect. (**a**), Primer extension assay with multiple nucleotides (four dNTPs) to show the inhibition of polymerization on DnaE and PolC (Gram (−) and Gram (+) bacterial polymerases). **b** and **c** Primer extension assay with single nucleotide (just dATP) to show the inhibition on both RNA-dependent DNA and DNA-dependent DNA polymerase activity of MMLV-RT, respectively. **P:** primer, **C:** control, **1**: 2,4,6-trihydroxy-1-methyl benzoate, **2**: paeoniflorin, **3**: benzoic acid, **4**: paeoniflorigenone, **5**: benzoylpaeoniflorin

benzoyl group attached to the "cage-like" skeleton, which has a small structural modification of the paeoniflorin monoterpene skeleton. Other two isolated compounds were identified as benzoic acid **(PD-3)** and 2,4,6-trihydroxy 1-methyl benzoate **(PD-1)**.

Paeonia species contain "cage-like" monoterpene glycosides as major compounds along with the other types of monoterpene and monoterpene glycosides. Chemotaxonomic studies for the discrimination of species or taxa of plants are usually based on qualitative and quantitative analyzes of secondary metabolites (He et al. 2014). Different species in the same genus contain more likely major secondary metabolites in the same class of compounds or common compounds with different quantity (Zhao et al. 2016). Geographical distributions and climatic variations should also be considered in taxonomic studies based on secondary metabolites. Because of missing points about *P. daurica* in the literature, comparison of the chemical content of this species could only be possible way with other species in the *Paeonia* genus.

The chemical composition of *Paeonia rockii* root has been investigated and some polyphenols, monoterpenes, and triterpenes in their methanol extract, and butanol fractions were isolated (Picerno et al. 2011). Extracts, which revealed strong radical scavenging activity, had high content of gallic acid derivatives and paeoniflorin (the major compound in the methanolic extracts of *P. daurica* roots). Paeoniflorin, isolated from *Paeonia lactiflora* roots, decreased the cholesterol level in the experimentally induced hyperlipidemic rats and showed anti-inflammatory and immunomodulatory effects (He and Dai 2011; Yang et al. 2004). Paeoninol (resveratrol trimmer) and paeonin C, oligostilbene and monoterpene galactoside, have been isolated from the methanolic extract of the fruits of *Paeonia emodi* (Riaz et al. 2004). The molecular structure of paeonin C has only one difference from that of paeoniflorin, which has the benzoyl group in its structure, instead of *o*-hydroxy benzoyl group at paeonin C.

Inhibition of plant parts and isolated compounds on DNA polymerases

The method based on the extension of 5'-end fluorescent labeled-primer by DNA polymerases (Primer Extension Assay, PEA) and the imaging of the result by Typhoon FLA9500 offers a strong approach to investigate inhibitory effect of plant crude extracts and their purified molecules. It gives very sensitive and repeatable results to describe the inhibitory affect against DNA polymerases. The inhibition of single-nucleotide addition activity of DNA polymerases could be measured by this approach too. We used this approach to investigate the inhibitory effect on MMLV-RT and bacterial replicative DNA polymerases (polC and DnaE2) in fruit, green and red fruit barks, leaf, stem, and root extracts of *P. daurica*. In all experiments, we used the primer position (shown by P and it means there is no synthesis) as negative control and the C position as positive control (P and C) (Fig. 2). All methanol extracts prepared from *P. daurica* plant parts showed the inhibitory effect on bacterial DNA polymerases. Because of both polymerases were inhibited in crude extract of root, it was clearly seen that new inhibitory molecules on DNA polymerase would have been purified from the root of *P. daurica*. Therefore, methanol extract of the roots was selected to isolation and identification of main secondary metabolites and five isolated compounds were tested for their inhibition effect on bacterial and viral replicative DNA polymerases.

The inhibition effect of the isolated molecules on both RNA-dependent and DNA-dependent DNA polymerase activities of MMLV-RT was investigated by single nucleotide addition assay (Fig. 2). It was determined that the solvent did not inhibit the polymerization activity of MMLV-RT and it elongated the 14-mer primer to the 15-mer position using a single nucleotide (dATP). MMLV-RT could not add a single nucleotide to the 14-mer primer in the

presence of the isolated molecules, which revealed that all isolated molecules completely inhibited the polymerization activity of MMLV-RT with both RNA (Fig. 2b) and DNA-dependent (Fig. 2c) substrates. Since all molecules have strong inhibitory effect on MMLV-RT, all reaction products were located at the primer position.

The inhibitory effect of the isolated molecules on DNA polymerase activity of polC and DnaE was investigated by multiple nucleotide addition assays. Methanol did not inhibit the polymerization activity of both polymerases and enzymes elongated the 14-mer primer to the 32-mer position (Fig. 2a). Paeoniflorigenone (**PD-4**) strongly inhibited the polymerase activity of both enzymes, which the enzymes could not add a single nucleotide to the primer terminus (Fig. 2a). Benzoylpaeoniflorin (**PD-5**) had also showed inhibitory effect on both polymerases and but it was relatively more effective on DnaE than PolC (Fig. 2a). Benzoic acid (**PD-3**) had a relatively low inhibitory effect on PolC. DnaE could not complete the polymerization and it could not elongate the 14-mer primer into the 32-mer product position in the presence of **PD-3**. It was concluded that the polC was less inhibited than DnaE by **PD-3** and polC was completed the polymerization and elongated the 14-mer primer into the 32-mer product position in the presence of this molecule (Fig. 2a). Paeoniflorin (**PD-2**) and 2,4,6-Trihydroxy-1-methyl benzoate (**PD-1**) were found to be ineffective against both polC and DnaE (Fig. 2a).

According to the primer extension results, paeoniflorigenon (**PD-4**) was found the main active compound since it strongly inhibited all polymerases (viral and bacterial). The same inhibitory effect on viral and bacterial polymerases means that paeoniflorigenon (**PD-4**) affects the common part (such as invasion of the replication active site) of the polymerases. Furthermore, a different degree of inhibitory effect was observed on both DNA polymerases. A strong inhibitory effect was observed on MMLV-RT by all purified molecules. That means *P. daurica* may have many chemicals as inhibitors of the polymerization activity of viral reverse transcriptase. The reverse transcriptase (RT) is a multi-functional enzyme and it carries out both RNA- and DNA-dependent DNA polymerase activities required for the synthesis of double-stranded DNA from viral single-stranded RNA genome. Because of MMLV-RT synthesizes DNA by using both DNA and RNA template, it is classified as DNA polymerase.

Therapies for viral infections such as HIV or hepatitis B/C consist of the combinations of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), and integrase inhibitors (INSTIs). HIV-1 reverse transcriptase (RT) is an extremely attractive pharmaceutical target for the identification of new inhibitors possibly active on drug resistant strains. MMLV-RT can be used as a model to investigate nucleoside and non-nucleosite inhibitory molecules in plants. There are some studies showing some purified molecules from plants, which have inhibitory effect on DNA polymerases. Mizushina et al. (2000) have investigated the inhibitory effect of benzoic acid derivatives obtained from *Myrsine seguinii* on DNA polymerases. They also reported that terpeno-benzoic acid derivatives containing carboxylic acid and phenolic hydroxyl showed a strong inhibitory effect in accordance with our study. Benzoic acid derivatives show inhibition effect by selectively binding to the enzyme at its hydrophobic region, i.e., the alkyl chain region. It was shown that epigallocatechin gallate mostly found in tea inhibited the HIV-1 replication (Li et al. 2011b). Another study showed that natural coumarins widely found in the plant kingdom had a similar inhibitory effect on DNA polymerases (Garro and Pungitore 2015). It was also identified some new scaffolds from the traditional Chinese medicine plant *Schisandra chinensis* with inhibitory effect on HIV-1 RT (Xu et al. 2015). *P. daurica* has a rich source of secondary metabolites and can be used to identify novel scaffolds to inhibit polymerization activity of viral and bacterial polymerases.

The commercial antibiotics affect different metabolic pathways in bacterial cells and bacteria may possess an alternative mechanism to overcome the effect of antibiotic. Since, growing antibiotic resistance in bacteria, it is urgently needed to perform ongoing studies to discover and develop new antibiotics. Therefore, there is an urgent medical need for potent and safe antibacterial compounds with novel mechanisms of action. Based on our recent review of the literature, antibiotics that target bacterial replication enzymes have not yet been reported. The DNA polymerases have been considered targets for antibiotic development for a long time (Wright and Brown 1990). To generate new antibacterial drugs, the inhibition of DNA polymerase activity in living bacteria is considered an excellent target. The structures of replicative DNA polymerase IIIs are strongly conserved in both Gram (+) positive and Gram (–) negative pathogens. Based on their function, DNA polymerases broadly belong to two groups as known replicative and non-replicative. Replicative DNA polymerases are required only during cell division to replicate the genome, while the non-replicative DNA polymerases are needed throughout the life-cycle of the cell. Secondary molecules with inhibitory effect on bacterial and viral DNA polymerases described in plants will definitely be useful for the development of new antibiotics.

In earlier study, we showed that some species from *Sorbus* genus had inhibitory molecules against both replicative and non-replicative DNA polymerases in Gram (+) and Gram (−) bacteria (Turumtay et al. 2017). In this current study, we showed that *P. daurica* may be evaluated as a potential

Table 2 Docking scores, IFD scores, and relative-binding free energy of isolated compounds

Code	Compounds	MMLV-RT			DNA polymerase DNAE2			DNA polymerase polC		
		[a]Docking score	[a]IFD score	[a]ΔG binding	[a]Docking score	[a]IFD score	[a]ΔG binding	[a]Docking score	[a] IFD score	[a]ΔG binding
PD-1	2,4,6-Trihydroxy 1-methyl benzoate	−5.646	−15683.3	−21.21	−2.303	−2336.9	−24.35	*	*	*
PD-2	**Paeoniflorin**	**−7.122**	**−15649.4**	**−60.98**	**−9.138**	**−2337.0**	**−57.91**	**−13.188**	**−833.9**	**−60.19**
PD-3	Benzoic acid	−5.232	−15489.9	−11.65	*	*	*	*	*	*
PD-4	**Paeoniflorigenone**	**−6.994**	**−15676.2**	**−48.93**	**−9.91**	**−2354.9**	**−34.59**	**−11.252**	**−839.9**	**−48.86**
PD-5	**Benzoylpaeoniflorin**	**−7.188**	**−15685.8**	**−61.96**	**−9.39**	**−2344.1**	**−54.91**	**−13.096**	**−844.4**	**−56.96**

*Not determined

[a]All values were reported in kcal/mol, Bold rows indicates compounds has better docking scores

reservoir for inhibitory molecules against bacterial DNA polymerases. This is the first report about the inhibitory effect of paeoniflorigenon **(PD-4)** on DNA polymerases. It can be used directly as antibiotic or antiviral and also it can be used as scaffold to generate its different variants.

In-silico-docking study

To screen for potential inhibition of the MMLV-RT and bacterial replicative DNA polymerases (polC and DnaE2), experimentally determined compounds were docked against the crystal structure of MMLV-RT (Pdb id: 4MH8), bacterial replicative DNA polymerase polC (Pdb id: 3F2B) and DNAE2 (Pdb id: 3E0D). The receptor–ligand complexes obtained after docking study of the experimentally determined phenolic compounds were analyzed in terms of docking scores, IFD scores, and orientation of the docked compounds in the active site of target enzyme structures. The docked poses of the compounds were ranked based on the docking scores and IFD scores in Table 2.

According to in-silico prediction, the compounds paeoniflorin (**PD-2**), paeoniflorigenone (**PD-4**), and benzoylpaeoniflorin (**PD-5**) could be considered as stronger inhibitors of MMLV-RT, PolC, and DnaE2 polymerases than the other compounds. Since paeoniflorigenone (**PD-4**) demonstrated the best binding interactions in the active site of studied proteins based on the primer extension assays, the best fitted conformations and ligand interactions of the paeoniflorigenone (**PD-4**) was analyzed and illustrated in Fig. 3. According to the in-silico results, it is clearly observed that paeoniflorigenone (**PD-4**) could form hydrogen bond interactions with SER426, ARG452, and ARG767 and π–π stacking interaction with HIS817 residue of DnaE2 enzyme (Fig. 3). The corresponding docking score of paeoniflorigenone (**PD-4**) is −9.91 kcal/mol with IFD score −2354.88 kcal/mol. The docked pose of paeoniflorigenone (**PD-4**) at active site of PolC enzyme showed the H-bond interactions between the paeoniflorigenone (**PD-4**) and ARG893 and HIS1186 with linking H_2O molecules and π-π stacking interaction of TYR1269 residue. The corresponding docking score of paeoniflorigenone (**PD-4**) in binding site of PolC protein is −11.25 kcal/mol with IFD score −839.92 kcal/mol. The docked pose of paeoniflorigenone (**PD-4**) and MMLV-RT protein complex showed that three H-bond interactions with ASN131, TYR222 and ARG284 residues. The corresponding docking score is −6.99 kcal/mol with IFD score −15676.2 kcal/mol.

MMLV-RT, PolC, and DnaE polymerases resemble to a right hand and they have three common domains. These are the fingers (bind the incoming nucleotide) domain, the thumb (binds DNA) domain, and the palm (catalytic) domain. The fingers domain interacts with the

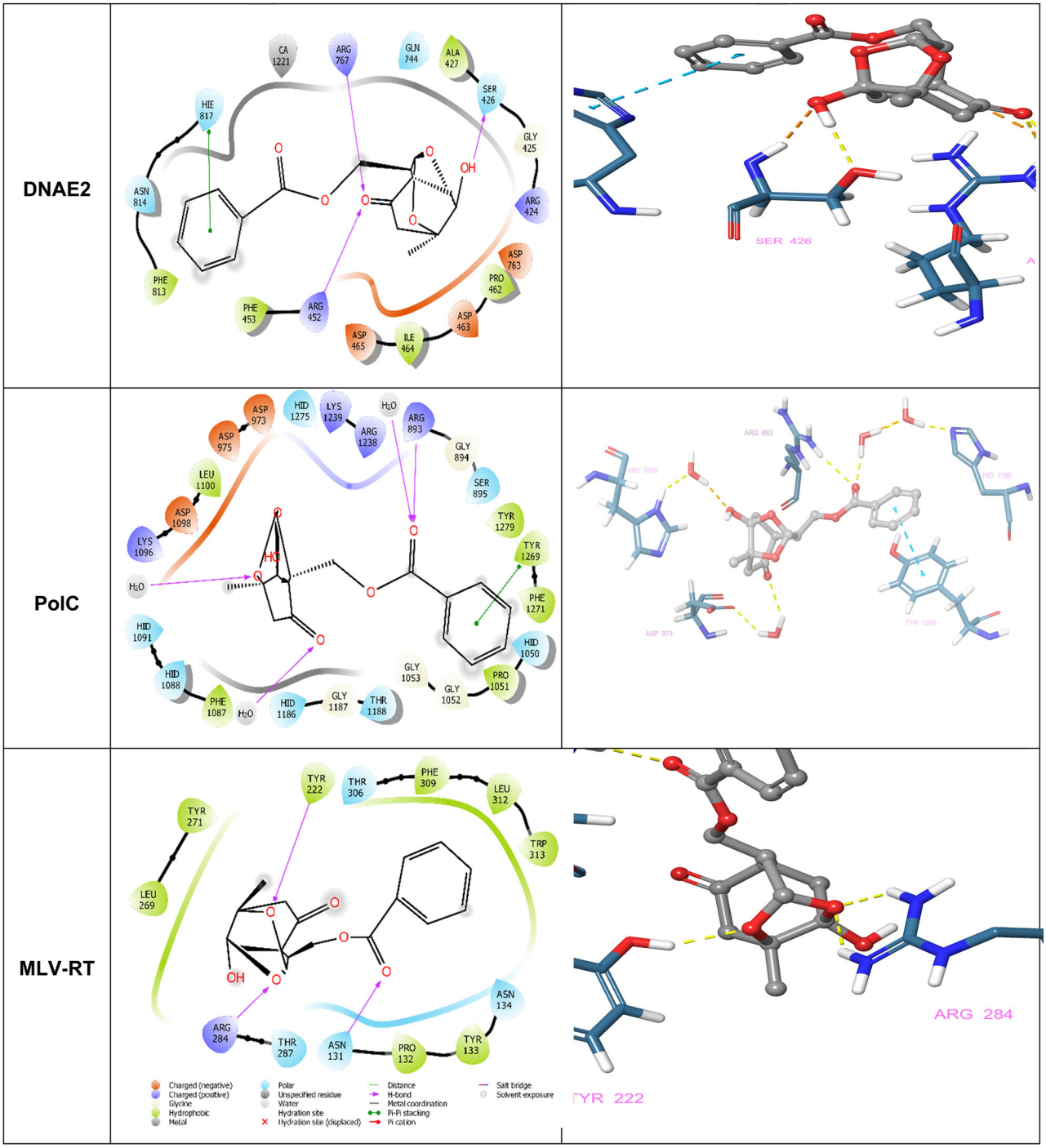

Fig. 3 Ligand interaction diagrams and the binding pose of Paeoniflorigenon (**PD-4**) at the active site of studied enzyme structures

incoming nucleotide and adds it to the template strand to generate the phosphodiester bond in DNA synthesis. The thumb domain ensures the DNA template is correctly aligned with the active site and involves the processivity of the polymerases. Finally, the active site is located in the palm domain and it catalyzes the formation of the phosphodiester bond between the incoming nucleotide and the 3′ end of the DNA strand. All these polymerases use two metal ions for catalytic activity (Steitz 1999).

In DnaE, the palm domain is located between the residues 271–432 and 511–560. It has strictly conserved aspartate residues (ASP401, ASP403, and ASP555) that are essential for catalysis. The fingers domain (residues 561–911) is located on the left side of the palm domain. The thumb domain (residues 433–510) is located at the right

Table 3 The total phenolic content and radical scavenging activities of *P. daurica* root extracts

Samples and standards	TPC[a]		RSA[b]	
	mg GAE/g	mg QE/g	DPPH SC_{50}	ABTS SC_{50}
Methanolic extract	130.86 ± 4.49	87.54 ± 3.04	10.03 ± 0.48	16.78 ± 0.91
Ether fraction	217.77 ± 3.65	147.02 ± 2.49	6.47 ± 0.39	12.00 ± 0.27
Ethyl acetate fraction	671.63 ± 18.28	462.11 ± 12.64	3.00 ± 0.09	7.39 ± 0.16
Aqueous fraction	192.64 ± 5.53	128.58 ± 3.83	28.57 ± 1.87	33.81 ± 1.86
Gallic acid	–	–	1.45 ± 0.04	
Quercetin	–	–	7.35 ± 0.10	10.48 ± 0.37
Trolox	–	–	6.71 ± 0.24	14.59 ± 0.31
Ascorbic acid	–	–		11.71 ± 0.29

GAE gallic acid equivalent, *QE* quercetin equivalent, SC_{50} value of the concentration of sample required to scavenge 50% of DPPH and ABTS radicals (μg extract per mL methanol)

[a]Total phenolic contents are expressed in mg GAE/g extract and mg QE/g extract

[b]Radical scavenging activity data represent the mean ± SD of three independent experiments, –, not determined

side of the palm domain (Lamers et al. 2006). According to in-silico analysis, four residues were determined in contact to paeoniflorigenone (**PD-4**) at the active site of enzyme. The paeoniflorigenone (**PD-4**) locates near to these four residues found in the palm (SER426), the fingers (ARG767 and HIS817) and the thumb (ARG452). In PolC, the fingers, palm, and thumb domains are located between the residues 828–1293. The triphosphate of the incoming dNTP is held into position by direct and water-mediated hydrogen bonds to ARG1213, ARG1238, and TYR1269 in the fingers and to SER895 and LYS970 in the palm (Evans et al. 2008). The paeoniflorigenone (**PD-4**) locates near to ARG893 residue and directly interact with TYR1269 residue in PolC. In MMLV-RT, the conserved aspartate residues for catalytic activity are ASP150, ASP224 and ASP225 residues. The paeoniflorigenone (**PD-4**) has interactions with TYR222 at active site. This residue is so close to conserved ASP224 and ASP225 residues and it may inhibit the catalytic activity of MMLV-RT (Georgiadis et al. 1995). In-silico results support that paeoniflorigenone (**PD-4**) locates in active site of the polymerases and directly interacts with the active amino acids or interacts with amino acids close to these amino acids. This also explains the inhibition observed in experimental results.

The estimation of binding free energies of studied compounds reported in Table 2 were performed by using MM/GBSA method, which is based on the molecular dynamics simulations of the receptor–ligand complexes (Genheden and Ryde 2014). The isolated compounds PD-2, PD-4, and PD-5 have more negative-binding free energy for all three enzymes structures than that of the other studied compounds, meaning formation of more stable enzyme-ligand complexes. Binding free energy calculations also supported the experimental results.

Spectrophotometric determination of antioxidant potential of *P. daurica* root extracts

The total phenolic content (TPC) of the *P. daurica* root extracts was estimated as mg gallic acid and quercetin equivalent per gram extract. DPPH scavenging activity of the extracts was compared with that of standard gallic acid, quercetin and trolox, which had SC_{50} values of 1.45 ± 0.04, 7.35 ± 0.10, and 6.71 ± 0.24 μg/mL, respectively (Table 3). Standard quercetin, trolox, and ascorbic acid were tested for their ABTS radical scavenging activities to compare with that of the extracts. Ethyl acetate fraction of the methanol extract had the highest TPC in all extracts, which is correlated with antioxidant test results with lowest SC_{50} values against to DPPH and ABTS with the values of 3.00 ± 0.09 and 7.39 ± 0.16 μg extract/mL methanol.

Methanolic extract and its diethyl ether and ethyl acetate fractions had strong radical scavenging activity. The best radical scavenger, ethyl acetate fraction, showed also higher scavenging activity than quercetin, trolox and ascorbic acid, well-known antioxidant standards, with the highest phenolic content. Ether fraction had the second highest total phenolic content and antioxidant potential, therefore, it can be concluded that the ethyl acetate and ether had the best solvent characters to extract the potent antioxidant phenolic compounds from the methanolic extract of *P. daurica* root. Bioactive compounds of the crude methanolic extract had been enriched using these extraction solvents.

Paeonia species have been recognized as rich sources for bioactive compounds due to their well-known curative effects. Therefore, they have been tested for their bioactivities and their active compounds are clarified thanks to the chromatographic separation techniques. For instance, the methanolic extract, its n-butanol soluble fraction, and

some isolated compounds of *Paeonia rockii* were extensively investigated for their antioxidant activities. Both extracts and some of their compounds revealed strong DPPH free-radical scavenging activity with the SC_{50} value between 1.2 and 13.3 µg extract per mL methanol due to the presence of gallic acid derivatives and paeoniflorin (Picerno et al. 2011). Methanolic extract of root bark of *Paeonia suffruticosa* was screened for antioxidant activity versus resveratrol (Lee et al. 2003). The SC_{50} value of plant extract was found as 5.9 µg/mL against DPPH while that of standard resveratrol was 4.8 µg/mL. The extract prevented normal cells against H_2O_2-induced oxidative damage probably due to its high radical scavenging activity. *Paeonia lactiflora* aqueous extract showed strong antioxidant effect against ABTS and DPPH radicals and had high total phenolic content with 222.01 mg GAE/g dried weigh of flower (Chen et al. 2015). This flower extract had highest amount of quercitrin following with gallic acid and epicatechin among the studied phenolic acids and flavonoids.

Conclusion

There has been a huge demand for natural products because of adverse toxicological reports on many synthetic compounds. Natural products have been extracted from different plant parts like leaves, roots, stems, fruits, seeds, and bark. Inhibitory affect was firstly found on bacterial and viral replicative DNA polymerase in methanol extracts of fruit, fruit barks, leaf, stem, and root parts of *P. daurica*. Because of these properties, they can be promising sources of natural bioactive compounds in the pharmaceutical industry. Methanol extract and its diethyl ether and ethyl acetate fractions from the plant's root part had strong radical scavenging activity. Inhibitory effects of isolated and purified five molecules from methanol extract of root part were verified by primer extension assay sensitively. Especially, the strong inhibitory effect of paeoniflorigenon (**PD-4**) on the bacterial and viral replicative polymerases was identified by experimental and in-silico approach. All isolated compounds from *P. daurica* have a common benzoyl group, while paeoniflorin (**PD-2**), paeoniflorigenone (**PD-4**), and benzoylpaeoniflorin (**PD-5**) have the monoterpene skeleton as the second structural similarity. Either benzoyl group or monoterpene skeleton of these compounds or both should have played effective role in the mechanism of DNA polymerase inhibition. In-silico results support that paeoniflorigenone (**PD-4**) locates in active site of the polymerases and directly interacts with the active amino acids or interacts with amino acids close to these amino acids. The occupation of the active site by paeoniflorigenone (**PD-4**) might be the reason of inhibition against three polymerases. In addition to further investigation of the inhibition effects of the molecules described in this study, comparative studies involving other species of *Paeonia* species will produce useful results. The identification of novel molecules that inhibit DNA polymerases from *Paeonia* species may have potent to control the increasing resistance rate in bacterial or viral diseases. Considering the enzyme inhibition effects of the compounds in the context of correlation between structure and activity might indicate the development of a new convenient, in vitro assay method to screen for novel anti-chronic inflammatory compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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