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Evaluation of the chemical profile and pharmacological activities of propolis from Tajikistan

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Abstract
The study describes the chemical and pharmacological investigations of propolis collected from Rasht region of Tajikistan. Total polyphenolic and total flavonoid contents resulted in propolis extract were: 0.49 mg gallic acid equivalents (GAE) and 0.11 mg quercetin equivalents (QE) per mg of dried extract, respectively. Chemical investigation of the ethanol extract delivered seven pure phenolic compounds identified as: quercetin (1), caffeic acid (2), ferulic acid (3), luteolin (4), kaempferol (5), apigenin (6) and rutin (7), respectively. Significant activity against *Staphylococcus aureus* and *Candida albicans* were shown by 70% ethanol extract of propolis (PE70), compounds 2 and 3. DPPH free radical scavenging assay yielded in powerful activity of PE70 with IC_{50} value 14.7±1.22 µg/mL while compounds 1, 3, 5, 7 resulted in IC_{50} values: 15.83±0.68 µM, 11.32±0.47 µM, 19.38±0.87 µM and 28.21±1.50 µM respectively. The PE70 and quercetin showed strongest antidiabetic activity with an IC_{50} 7.52±0.21 and 7.54±0.19 µg/mL, respectively. Literature clarified that this is the first report presenting the results regarding the propolis from Tajikistan.

Keywords: Propolis, phytochemistry, polyphenols, flavonoids, antimicrobial, antioxidant, antidiabetic activities

1. Introduction

Propolis is the generic name of the resinous product that is collected by bees from various plant sources. It has been studied extensively with broad spectrum biological and pharmacological properties, such as antioxidant [1-2], anti-inflammatory [3], antiproliferative [4-5], cardiovascular diseases [6], antidiabetic [7], anti-influenza virus activities of flavonoids [8] and hepatoprotective [9] activities. A large number of biological activities of propolis are based on its complex chemical compositions [10-11], which are mainly dependent on the plant sources. It has been shown that poplar propolis possesses stronger antimicrobial activity [12]. Furthermore, propolis has been shown to exert summative immunosuppressive function on T lymphocyte subsets but paradoxically activate macrophage function [11]. On the other hand, they also have potential antitumor properties by different postulated mechanisms [11-13]. The biological activity of propolis is mainly due to the presence of flavonoids and phenolic compounds. The present study has been carried out on propolis collected from the Rasht region of Tajikistan focusing on the determination of total polyphenolic and total flavonoids contents, isolation of major pure compounds and their structures elucidation using 1D, 2D NMR spectroscopy, mass spectrometric techniques and screening pharmacological activities. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, protein tyrosine phosphatase 1B (PTP-1B) inhibition assay, and antimicrobial activity of 70% ethanol extract of propolis (PE70) and pure compounds were determined.

2. Materials and methods

2.1 Chemicals and reagents

Varian NMR-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) and Varian NMRS- 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometers were used to obtain NMR spectra. LC-MS spectrometer (2690-ZQ 4000 Water-Alliance) and BUCHI melting point B-540 apparatus (Germany) were employed to determine the mass spectra and melting points of pure compounds, respectively. Column chromatographic separation was performed on Sephadex LH-20 gel (Amersham Pharmacia Biotech, Sweden) and silica gel (100-200 mesh, Qingdao Haiyang Chemical Factory, China).

Quercetin (98%) and gallic acid (≥ 97%), aluminium chloride, sodium acetate and Folin-Ciocalteau reagent (2 N) were purchased from Sigma-Aldrich GmbH Steinheim. All the chemicals and reagents were of analytical grade and double distilled water was used throughout the experiments.

2.2 Collection of propolis

Propolis was collected representing highlands area (1800 m above sea level) of mountainous Rasht region of Tajikistan in May 2014. Propolis from Rasht region is a bee product which was collected from the area of plants namely as: *Prangos pabularia, Melilotus albus, Fagopyrum dibotrys, Hypericum scabrum, Ferula tadshikorum, Fagopyrum dibotrys* and flowers of apple, pear, peach, cherry etc. Propolis specimen has been deposited in the herbarium (PRGAMS 6059) of the Institute of Gastroenterology Academy of Medical Sciences, Republic of Tajikistan and Xinjiang Technical Institute of Physics and Chemistry Chinese Academy of Sciences, P. R. China.

2.3. Extraction, fractionation and isolation

The raw propolis (1 kg) was extracted three times with 70% ethanol (3×5 L) for 48 hours at room temperature and the resulting extract was reduced under vacuum to give the dried residue (456.2 g). The residue was defatted with petroleum ether for several times. About 7 g of the residual extract was chromatographed, employing the silica gel column chromatography (100–200 mesh, 280 g). Gradient elution was initiated with petroleum ether/EtOAc (1:1) and the polarity was gradually increased to 100% EtOAc terminating with EtOAc/MeOH (5:1). Seventy five fractions of 200 mL each were obtained. Similar fractions were combined as: 10-18 (P1), 20-25 (P2), 26-35 (P3), 40-55 (P4) and 58-70 (P5) delivering five main fractions. Fraction P1 (125 mg) was eluted on Sephadex LH-20 using MeOH as mobile phase affording compound 1 (73 mg). Fraction P2 was purified on Sephadex LH-20 using MeOH/H_2O (5:1) as mobile phase affording compound 2 (35 mg) and compound 3 (28 mg). From the fractions P3 and P4 pure compounds 4 (16 mg) and 5 (37 mg) and 6 (23 mg) were obtained through Sephadex LH-20 column chromatography eluting with MeOH/aceton in the ratios of 2:1 and 1:1 respectively. Compound 7 (58 mg) was obtained from the fraction P5 with the eluent MeOH: H_2O (2:1) through Sephadex LH-20 column chromatography. Analysis of the fractions was performed through TLC developed in different mobile phases ($CHCl_3$:MeOH/4:1; CHCl:MeOH:H_2O/65:35:5 and 73:24:4). The spots on the TLC plate were identified by spraying 5% H_2SO_4 solution in ethanol and then heating the plate at about 105°C, and ammonia vapor.

2.4. Determination of total polyphenolic and total flavonoidscontents

Total polyphenolic and total flavonoids contents were determined according to the procedure used by Numonov *et al*. [14].

2.5. Spectral data of the isolated compounds

Quercetin (1):yellow needle crystals (MeOH), m. p. 314–315°C, ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 6.16 (d, 1H, *J* = 1.9 Hz, H-6), 6.38 (d, 1H, *J* = 1.9 Hz, H-8), 6.86 (d, 1H, *J* = 8.5 Hz, H-5'), 7.51 (dd, 1H, *J* = 8.5; 2.1, Hz, H-6'), 7.65 (d, 1H, *J* = 2.1 Hz, H-2'), 12.47 (s, 1H, OH-5), 10.77 (br. s, 1H, OH-7), 9.59 (br. s, 1H, OH-3'), 9.36 (br. s, 1H, OH-3'), 9.30 (br. s, 1H, OH-4'); ^{13}C NMR (100 MHz, DMSO-d_6): δc 156.19 (C-2), 135.72 (C-3), 175.84 (C-4), 160.72 (C-5), 98.18 (C-6), 163.89 (C-7), 93.36 (C-8), 156.13 (C-9), 103.01 (C-10), 121.95 (C-1'), 115.61 (C-2'), 145.06 (C-3'), 147.71 (C-4'), 115.05 (C-5'), 119.97 (C-6').

Ferulic acid (2): Light yellow crystals (MeOH), m. p. 133-134°C, ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 3.78 (3H, s, H-3), 6.33(d, 1H, J = 15.9 Hz, H-8), 6.75 (d, 1H, J = 8.1 Hz, H-6), 7.05 (dd, 1H, J = 8.2; 1.8 Hz, H-5), 7.25 (d, 1H, J = 1.8 Hz, H-2), 7.45 (d, 1H, J = 15.9 Hz, H-7), 13C NMR (100 MHz, DMSO-d6): δc 55.69 (OCH3), 111.10 (C-2), 115.50 (C-8), 115.62 (C-5), 122.87.57 (C-6), 125.77 (C-1), 144.56 (C-7), 147.92 (C-3), 149.09 (C-4), 168.04 (C-9).

Caffeic acid (3): Light yellow crystals (MeOH), m. p. 223-225°C, ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.36 (1H, d, *J*=15.9 Hz, H-7), 6.97 (1H, d, *J*=2.0 Hz, H-2), 6.91 (1H, dd, *J*=8.2; 2.0 Hz, H-6), 6.70 (1H, d, *J*=8.1 Hz, H-5), 6.12 (1H, d, *J*=15.9 Hz, H-8), ^{13}C NMR (100 MHz, DMSO-d_6): δc 167.95 (C-9), 148.18 (C-4), 145.57 (C-3), 144.63 (C-7), 125.70 (C-1), 121.19 (C-6), 115.75 (C-5), 115.12 (C-8), 114.65 (C-2).

Luteolin(4): Light yellow crystals (MeOH), M. p. 328-329°C, ^{1}H NMR (600 MHz, Py-d_5): δ_H 6.74 (2H, s, H-6, H-8), 6.95 (1H, s, H-3), 7.92 (1H, d, *J*=2.4 Hz, H-2'), 7.3 (1H, d, *J*=8.4 Hz, H-5'), 7.56 (1H, dd, *J*=8.4; 2.4 Hz, H-6'); ^{13}C NMR (150 MHz, Pyridine-*d5*): δc 165.3 (C-2), 104.5 (C-3), 183.2 (C-4), 159.0 (C-5), 104.5 (C-6), 166.3 (C-7), 95.2 (C-8), 163.6 (C-9), 105.5 (C-10), 123.4 (C-1'), 115.1 (C-2'), 148.3 (C-3'), 152.2 (C-4'), 117.3 (C-5'), 120.0 (C-6').

Kaempferol (5): Light yellow crystals (MeOH), m. p. 276-277°C, ^{1}H NMR (400 MHz, CD_3OD): δ_H 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.91 (2H, d, *J* = 8.8 Hz, H-3', H-5'), 8.09 (2 H, d, J = 8.8, H-2', H-6'); ^{13}C NMR (100 MHz, CD_3OD), δc: 148.0 (C-2), 137.1 (C-3), 173.3 (C-4), 162.5 (C-5), 99.3 (C-6), 165.7 (C-7), 94.4 (C-8), 158.2 (C-9), 104.5 (C-10), 123.7 (C-1'), 130.6 (C-2', C-6'), 116.3 (C-3', C-5'), 160.5 (C-4').

Apigenin (6): Light yellow crystals (MeOH), m. p. 346-347°C, ^{1}H NMR (600 MHz, Py-d_5): δ_H 7.81 (2H, d, J=8.4 Hz, H-2' and H-6'), 6.95 (2H, d, J=8.4 Hz, H-3' and H-5'), 6.62 (1H, s, H-3), 6.67 (1H, d, J=2.1 Hz, H-8), 6.88 (1H, d, J=2.1 Hz, H-6); ^{13}C NMR (150 MHz, CD_3OD): δc 164.1 (C-2), 102.8 (C-3), 181.8 (C-4), 161.5 (C-5), 98.8 (C-6), 163.7 (C-7), 93.9 (C-8),157.3 (C-9), 103.7 (C-10), 121.2 (C-1'), 128.4 (C-2', C-6'), 115.9 (C-3', C-5'), 161.1 (C-4').

Rutin (7): Needle yellow crystals (MeOH), m. p. 212-214°C, ^{1}H NMR (600 MHz, DMSO-d_6): δ_H 6.18 (d, 1H, *J* = 1.7 Hz, H-6), 6.37(d, 1H, *J* = 1.7 Hz, H-8), 6.84 (d, 1H, *J* = 8.4 Hz, H-5'), 7.54 (d, 1H, *J* = 8.4; 2.0 Hz, H-6'), 7.53 (d, 1H, *J* = 1.9 Hz, H-2'), 12.60 (s, 1H, OH-5), 5.34 (d, 1H, *J* = 7.2 Hz, H-1''), 3.23-2.06 (m, 8H, H-2'', H-3'', H-4'', H-5'' and H-2''', H-3''', H-4''', H-5'''), 3.38 (m, 1H, H-6a''), 3.70 (d, 1H, *J* = 10.5 Hz, H-6b''); 4.38 (br. s, 1H, H-1'''), 0.99 (d, 3H, *J* = 6.2 Hz, H-6'''); ^{13}C NMR (150 MHz, DMSO-d_6): δc 158.61 (C-2), 133.32 (C-3), 177.30 (C-4), 156.56 (C-5), 98.82 (C-6), 161.23(C-7), 93.71(C-8), 156.39 (C-9), 103.78 (C-10), 121.60 (C-1'), 116.20 (C-2'), 144.83 (C-3'), 148.53(C-4'), 115.21 (C-5'), 121.0 (C-6'), 101.32 (C-1''), 74.10 (C-2''), 75.91 (C-3''), 71.84 (C-4''), 76.43 (C-5''), 67.0(C-6''), 100.80 (C-1'''), 70.51 (C-2'''), 70.36 (C-3'''), 70.0 (C-4'''), 68.3 (C-5'''), 17.89 (C-6''')

2.6. Antimicrobial, antidiabetic and antioxidant activities

Procedures adopted by Jiang *et al*. [15] for the measurement of antimicrobial and antidiabetic activities, were used while antioxidant activity was determined using the method of Zhao *et al.* employing the DPPH scavenging assay [16].

3. Results & Discussion

3.1. Spectral elucidation of the isolated compounds

^1H NMR spectrum of 1 (in DMSO-d_5; 400 MHz) showed three aromatic protons signals at 7.65 (1H, d, *J*=2.1 Hz, H-2′), 6.86 (1H, d, *J*=8.5 Hz, H-5′) and 7.51 (1H, dd, *J*=8.5; 2.1 Hz, H-6′) in the form of an ABC spin-system suggesting a flavonol with 3′,4′- disubstituted B-ring and showed a pair of meta coupling proton signals at 6.16 (d, *J*=1.9 Hz, H-6) and 6.38 (d, *J*=1.9 Hz, H-8) for the A-ring. The ^{13}C NMR spectra supported this hypothesis and showed 15 signals including carbonyl signal at (175.84 (C-4)). It revealed chemical shifts of carbon nucleus at 135.72 (C-3), 160.72 (C-5), 163.89 (C-7), 145.06 (C-3′), 147.71 (C-4′) that suggested the 3,5,7,3′,4′-oxygenated flavone nucleus. ESI-MS of 1 in positive mode presented molecular ion at *m/z* 303.04 $[M-H]^+$, suggesting the molecular formula $C_{15}H_{10}O_7$ after confirmation from ^1H NMR and ^{13}C NMR spectral data. The ^1H NMR and ^{13}C NMR spectral data of compound 1 were consistent with the reported literature [17].

^1H NMR spectrum of 2 displayed the characteristic signal for a methoxy group at 3.78 (3H, s, H-3). The compound spectrum also showed three aromatic proton at 6.75 (1H, d; *J*=8.1 Hz), 7.05 (1H, dd; *J*=8.2 and 1.8 Hz) and 7.25 (1H, d; *J*=1.8 Hz), characteristics for the H-6, H-5 and H-2 of aromatic part of isolated compound. The presence of further two proton doublets with *J*=15.9 Hz at 6.33 and 7.45 indicated the presence of H-8 and H-7 respectively in the side chain of compound. The ^{13}C NMR spectrum showed the presence of ten signals (six aromatic carbon and four aliphatic chain) in agreement with the proposed structure of ferulic acid (4-hydroxy-3-methoxycinnamic acid) [18].

The ^1H NMR spectrum of 3 displayed of *ortho* coupled doublet (*J*=8.1 Hz) and double doublet (*J*=8.2; 2.0 Hz) each for 1H, at 6.70 and 6.91 and doublet (*J*=2.0 Hz) for 1H at 6.97 in the aromatic region indicated the presence of a trisubstituted aromatic ring in the molecule. The chemical shifts of these signals indicated the presence of catechol moiety in the molecule, which was confirmed by ^{13}C NMR chemical shifts of the carbon atoms at 114.65 (C-2), 115.75 (C-5) and 121.19 (C-6). The ^1H NMR spectrum also displayed two doublets (*J*=15.9 Hz), each for 1H, at 7.36 (H-7) and 6.12 (H-8). The large value of coupling constant indicated the presence of *trans* disubstituted ethylene moiety in the molecule. The ^{13}C NMR spectrum of 3 exhibited presence of nine carbon atoms in the molecule. The ^{13}C chemical shifts of a carbon at 167.95 indicated the presence of carboxylic functional group in the molecule. The up field chemical shifts of one of the ethylenic carbon (C-8) at 115.12 and proton (H-1) indicated that the carboxylic group is located at C-8 position. The ^{13}C NMR chemical shifts of carbon atoms at 145.57 (C-3), 148.18 (C-4), indicated that the hydroxyl groups are attached at C-3 and C-4 positions. The position of ethylene function was determined by chemical shift of C-1 carbon at 125.70 and the downfield chemical shifts of C-7 (δ 144.63) carbon and H-7 proton of ethylene moiety. On the basis of these spectral data compound 3 was characterized as caffeic acid [19].

Compound 4 was obtained as yellow needle like crystals. ^1H NMR spectrum showed typical signals of flavonoids. They showed two singlets at 6.95 (1H, s, H-3) and 6.74 (2H, s, H-6, H-8) indicated the single methine signals. Also, the proton resonances at 7.92 (1H, d, *J*=2.4 Hz, H-2'), 7.3 (1H, d, *J*=8.4 Hz, H-5'), and 7.56 (1H, dd, *J*=8.4; 2.4 Hz, H-6') were aromatic protons, suggested the splitting of signals of the skeleton in the B-ring structure. The ^{13}C NMR of compound 4 showed 15 signals, where ten were of aromatic carbons, four oxygenated aromatic carbons and one was of carbonyl carbon. The carbonyl carbon signal of the C-ring in 4 was observed at 183.2. Compound 4 showed a negative molecular ion peak at *m/z* 285.3 $[M-H]^-$ in the ESI-MS, corresponding to a molecular formula of $C_{15}H_{10}O_6$. Accordingly, compound 4 was identified as luteolin (3', 4', 5, 7-tetrahydroxyflavone) [20].

A compound 5 was isolated as yellow, needle crystals from methanol. Two sets of symmetric proton signals at 8.09 (2H, d, *J* = 8.8 Hz, H-2', H-6') and at 6.91 (2H, d, *J* = 8.8 Hz, H-3', H-5') showed the presence of an AA′BB′ coupling system, which indicated that the substituent is attached to the 4th carbon of the B-ring. ^1H NMR also showed a pair of *meta* coupled proton signals at 6.19 (d, *J*=2.0 Hz, H-6) and 6.40 (d, *J*=2.0 Hz, H-8) for the A ring. The ^{13}C NMR spectra supported this hypothesis and showed 15 signals including carbonyl signal at 173.3 (C-4). It revealed chemical shifts at 137.1 (C-3), 162.5 (C-5), 165.7 (C-7), 130.6 (C-3', C-5'), 116.3 (C-2', C-6') that suggested the 3,5,7,4′-oxygenated flavone nucleus, suggesting the molecular formula $C_{15}H_{10}O_6$ [17].

Compound 6 was obtained as yellow powder and its molecular formula was established as $C_{15}H_{10}O_5$ from its ESI-MS spectral data that showed $[M+H]^+$ ion at m/z 271. The molecular formula of 6 was further supported by its ^{13}C NMR spectral data. The ^1H NMR spectrum of 6 showed the presence of two *meta* coupled aromatic doublets at 6.67 and 6.88 correspond to H-6 and H-8 protons respectively, two doublets at 6.95 and 7.81 for H-3' and H-5', H-2' and H-6' protons of ring B, and a singlet at 6.62 corresponding to H-3 proton; characteristic for a 5,7,4'-trisubstituted flavone. A search in literature suggested the spectral data of 6 was consistent to 4',5,7-trihydroxyflavone, also known as apigenin [21].

The ^1H NMR spectrum of compound 7 exhibited a characteristic proton signal at 12.60 corresponding to a free hydroxyl at C-5. The aromatic protons exhibited one ABX coupling system at 7.54 (1H, dd, *J*=8.4, 2.0 Hz) for H-6', 7.53 (1H, d, *J*=1.9 Hz) for H-2′, and 6.84 (1H, d, *J*=8.4 Hz) for H-5′. The other AX coupling system at 6.18 (1H, d, *J*=1.7 Hz) and 6.37(1H, d, *J*=1.7 Hz) was assigned to H-6 and H-8 protons, respectively. The ^1H NMR spectrum also supported the presence of rhamnose and glucose moieties with the rhamnose anomeric proton signal at 4.38 (1H, br.s) and glucose H-1" signals at 5.34 (1H, d, *J*=7.2 Hz) correcondending to the molecular formula $C_{27}H_{30}O_{16}$. The coupling constant of anomeric protons (*J* = 7.2 Hz) confirmed the β-linkage of glucose [22].

3.2. Total polyphenolic contents

Total polyphenolic contents were estimated as gallic acid equivalents (GAE), using the reaction of Folin-Ciocaltaeu with polyphenolic compounds resulting in blue colored complex having a wave length maximum at 740 nm. Seven point calibration curve was constructed with the help of the Microsoft Excel sheet passing the curve through zero. The curve delivered R^2 value 0.9956 and a straight line equation (y=10.909x+X). The amount of total polyphenolic compounds was calculated as 0.49 mg GAE/mg of the dried extract (Table 1).

3.3. Total flavonoids contents

The quantification of contents of total flavonoids was based on the complex formation between $AlCl_3$ and flavonoids present in the propolis. Seven point calibration curve was obtained with an R^2 value as 0.9971 producing a straight line equation (y=9.157x+X). Using this equation, quantity of total flavonoids (x) was determined as 0.11 mg quercetin (1) equivalents (QE)/mg of the dried extract (Table 1). The Tajikistan's propolis showed a high polyphenolic contents compared with some European propolis [23].

3.4. Antimicrobial activity
The antimicrobial activity using the PE70 and isolated compounds against the three microorganisms: *Staphylococcus aureus* (SA) ATCC6538 (Gram positive bacteria), *Escherichia coli* (EC) ATCC11229 (Gram negative bacteria) and *Candida albicans* (CA) ATCC10231 (Fungi) strains were determined (Table 2). The PE70 showed activity with zones of inhibitions as 13 mm, 8 mm and 13 mm against CA, EC and SA respectively. All the pure compounds, except 1 and 4, showed activity against SA. Among the active compounds, compounds 2 and 3 delivered highest zones of inhibition as 9.5 mm and 10.5 mm respectively. Inhibition zones of compounds 5, 6 and **7** against the SA were 7 mm. Ferulic acid (2) and caffeic acid (3) were active against EC yielding inhibition zones as 7.5 mm and 8 mm respectively. Compounds 2 and 3 delivered zones of inhibition against CA as 11 mm and 8.5 mm respectively (Table 1). These results suggest that the PE70, ferulic acid and caffeic acid showed significant activity against Gram positive bacteria and fungi, while average mild activity against Gram negative bacteria were observed for these samples in comparison to the reference drugs Ampicillin and Amphotericin B. The results on Gram negative and positive bacteria are consistent with the literature [15].

3.5. Antioxidant capacity
The free radical scavenging activity of the PE70 and pure compounds were measured *in vitro* by DPPH assay. A lower IC_{50} value confirms a significant antioxidant activity of the tested samples. The PE70 showed good antiradical activity with IC_{50} value 14.7±1.22 µg/mL. This significant activity proved the synergistic relationship of various compounds mainly flavonoids and phenolic compounds in the PE70. Pure compounds quercetin (1), caffeic acid (3), kaempferol (5) and rutin (**7**) were the most active, with an IC_{50} value of 15.83 µM 11.32 µM, 19.38 µM and 28.21 µM respectively, which were even better than that of vitamin C (IC_{50} 30.32 µM). Remaining three isolated compounds also showed potent antiradical activities. The results of the antioxidant activity of all the tested samples with IC_{50} values are presented in Table 1.

3.6. Antidiabetic activity
PTP-1B inhibition experiment of propolis extract (PE70) resulted in potent inhibition activity with IC_{50} value 7.52±0.21 µg/mL. The individual isolated compounds except quercetin (IC_{50} value 7.54±0.19 µg/mL) have not shown good effect for PTP-1B inhibition. IC_{50} values of the all the samples are presented in Table 1. On the basis of the activities of isolated compounds, we inferred that the relationship between structure and activity may be associated with the OH and OCH_3 groups of substances in the PE70 (Figure 1). This significant activity in the PE70 with lower activity in the individual isolated compounds proved the synergistic relationship among the compounds in the PE70, which weakened after the separation and isolation of the synergistically bound analytes.

4. Conclusions
From the study it can be confirmed that propolis collected from Tajikistan has a number of pharmacologically active compounds which suggest its use in various pharmaceutical formulations as raw material. The results of the study can be used by the industrial sector dealing with propolis for establishing their quality control profile. Highest extraction yield was obtained by using 70% aqueous ethanol. The experimental results from our investigations suggest the use of PE70 for the development of phytopharmaceuticals and food formulations against free-radical-associated oxidative damage, microbial and diabetes. In the future, work will be done to isolate more bioactive constituents of PE70 to locate potential pharmacological agents. From our literature review it can be confirmed that propolis collected from Republic of Tajikistan has not been chemically explored yet and this is the first time to report all the results presented in this manuscript.

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Table 1: Results of the total polyphenolic compounds, total flavonoids, antimicrobial, antidiabetic and antioxidant activities of Propolis 70% ethanol extract and isolated pure compounds (1-7).

Samples	Antimicrobial Activity: Inhibition Zone Diameter (mm)			PTP-1B Inhibition; IC_{50} Values ($\mu g \cdot mL^{-1}$)	DPPH Scavenging Effects; IC_{50} Values ($\mu g \cdot mL^{-1}$) or µM
	SA	EC	CA		
PE70	13	8	13	7.52±0.21	(14.7±1.22)*
Quercetin (1)	-	-	-	7.54±0.19	15.83±0.68
Ferulic acid (2)	9.5	7.5	11	>50	82.81±3.94
Caffeic acid (3)	10.5	8	8.5	>50	11.32±0.47
Luteolin (4)	-	-	-	>50	40.50±1.03
Kaempferol (5)	7	-	-	>50	19.38±0.87
Apigenin (6)	7	-	-	>50	57.68±2.98
Rutin (7)	7	-	-	>50	28.21±1.50
Vitamin C	-	-	-	-	(5.34±0.42)* 30.32±2.4
PTP-1B	-	-	-	1.97±0.40	-
Ampicillin	19	12	-	-	-
Amphotericin B	-	-	11.5	-	-
Parameter				**Results**	
Yield of extract				45.62% of raw propolis	
Total polyphenolic compounds				0.49 mg GAE/mg of the dried extract	
Total flavonoids				0.11 mg QE/mg of the dried extract	

*The values within brackets are in $\mu g \cdot mL^{-1}$. SA: *Staphylococcus aureus*; EC: *Escherichia coli*; CA:*Candida albicans*.

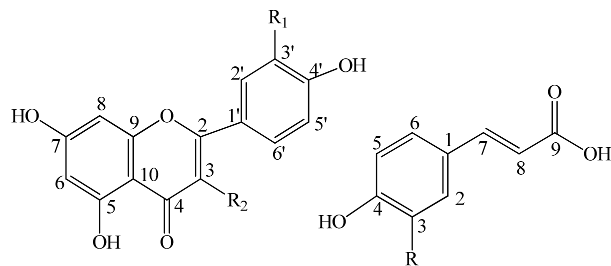

Fig 1: Chemical structures of flavonoids and phenolic compounds isolated from PE70. Quercetin (1), ferulic acid (2), caffeic acid (3), luteolin (4), kaempferol (5), apigenin (6) and rutin (7).

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