Asian Journal of Chemistry; Vol. 31, No. 12 (2019), 3012-3016

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.22369

Chemical and Biological Characteristics of Ethanolic Extract of *Tussilago farfara* L. Flowers: Composition, Antimicrobial and Antioxidant Activities

D. TERENZHEV[1], N. SHARONOVA[1,*], A. ERMAKOVA[1], S. GUMEROVA[1], K. BUSHMELEVA[1], G. SHUMATBAEV[1], I. FITSEV[2] and A.S. PONOMAREVA[3]

[1]Laboratory for Plant Raw Material Conversion for Organic Farming, Federal State Budgetary Institution of Science Federal Research Center, Kazan Scientific Center of Russian Academy of Sciences, 2/31 Lobachevskogo Ul., Kazan 420111, Russia
[2]Distributed Spectral-Analytical Center of Shared Facilities for Study of Structure, Composition and Properties of Substances and Materials, Federal State Budgetary Institution of Science Federal Research Center, Kazan Scientific Center of Russian Academy of Sciences, 2/31 Lobachevskogo Ul., Kazan 420111, Russia
[3]Pryanishnikov All Russian Scientific Research Institute of Agrochemistry, 31 A Pryanishnikova St, Moscow 127550, Russia

*Corresponding author: E-mail: sharonovanl1979@gmail.com

Received: 3 August 2019; *Accepted*: 21 September 2019; *Published online*: 16 November 2019; AJC-19656

Tussilago farfara L. (Asteraceae) has a wide spectrum of biological reactivity, widely used in herbal medicine and has the potential for use in the agricultural sector. The ethanolic extract was obtained by maceration of freshly harvested flowers, followed by filtration of the extract and concentration with the help of a rotary evaporator. The chemical composition of ethanolic extract of *Tussilago farfara* L. was studied with the help of gas chromatography/mass spectrometry (GC/MS) method. It was found that the ethanolic extract of *Tussilago farfara* L. had antimicrobial reactivity against human pathogens and phytopathogens (bacteria and fungi). The values of the minimal inhibiting, bactericidal and fungicidal concentrations varied in the range of 2500-5000 µg/mL. The phytopathogenic fungus *Alternaria solani* St108 was the most sensitive to the components of ethanolic extract of *Tussilago farfara* L. Moderate antioxidant properties of ethanolic extract of *Tussilago farfara* L. at concentrations of 0.001 mg/mL and higher were revealed with the help of chemiluminescence analysis.

Keywords: *Tussilago farfara* L., Ethanolic extract, Chemical compositions, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Plant diseases directly or indirectly cause significant losses of crops worldwide, estimated at billions of dollars every year. According to some estimates, 20-40 % of crop losses are caused by pathogenic infections [1]. The main means of plant protection worldwide are chemical pesticides. However, their long term and improper use leads to the accumulation of preparations and metabolites in ecosystem components, food chains, and foodstuffs, respectively [2]. In recent years, an alternative strategy for the control of agricultural pests, bacterial and fungal diseases, based on the use of biopesticides, has been actively introduced. Biopesticides have some advantages; *e.g.*, they are biodegradable and in most cases, do not lead to the formation of resistant strains of bacteria and fungi [3]. Studies of bactericidal and fungicidal reactivity of plant components and metabolites are extremely important in terms of their potential use as biopesticide resources. The extracts of many plants, in particular, those used in folk medicine, have reactivity against human pathogens [4,5], and their chemical composition varies depending on species and growing conditions [6]. It is important to study the reactivity of plant extracts against phytopathogens - causative agents of common bacterial and fungal infections. Asteraceae is a large family of flowering plants, consisting of about 1,100 genera and 20,000 species. Many species of plants of the Asteraceae family have therapeutic potential and contain a wide range of biologically active compounds [7,8]. *Tussilago farfara* L. is a common perennial herbage plant. In European countries, the leaves of *Tussilago farfara* L., also known as coltsfoot, are eaten as a vegetable crop and traditionally used to treat bronchial infections [9,10].

The objective of the present paper was to obtain modern knowledge about the phytochemical composition and biological activity of the flower extract *Tussilago farfara* L. to assess the potential effectiveness of its use for plant protection from phytopathogenic infections. To achieve this goal, the several

tasks are undertaken *viz*. (i) to investigate the phytochemical composition of ethanolic extract of *Tussilago farfara* L.; (ii) to evaluate antimicrobial reactivity of the ethanolic extract of *Tussilago farfara* L. against human and agricultural plant pathogens; and (iii) to study the antioxidant properties of ethanolic extract of *Tussilago farfara* L.

EXPERIMENTAL

Tussilago farfara L. plants were collected from Komsomolsk District of the Chuvash Republic of Russian Federation in early May 2019. The plants were identified by Dr. F.M. Khazieva, All-Russian Scientific Research Institute of Medicinal and Aromatic Plants, Moscow, Russia. The plant samples were deposited in the herbarium of the same institution. The aerial parts of plants were harvested at the flowering stage.

Preparation of ethanolic extract of *Tussilago farfara* L. flowers: The flowers were separated from the stems and cut in a laboratory mill (LM 202, Russia). A 120 mL of 85 % ethanol was added to the cut the subsample of flowers (15 g) and macerated with continuous stirring for 1.5 h at 45 ºC. The mixture was filtered (Whatman No. 1), then the filtrate was concentrated with the help of a rotary evaporator (LabTex RE100-Pro).

GC-MS analysis: Mass spectra (EI, 70 eV, *m/z* = 30-550; CI, 30 eV, *m/z* = 100-550) of ethanolic extract of *Tussilago farfara* L. was recorded on GC/MS "Agilent 6890N" with a mass selective detector 5973 N (Agilent Technologies, USA) using a silica capillary column Restek-5 MS (5 % biphenyl, 95 % dimethylpolysiloxane, 30 m × 0.25 mm, film thickness 0.25 µm (Restek, Germany).

GC separation: Evaporation temperature: 250 ºC, interface temperature: 290 ºC, initial temperature of the thermostat: 75 ºC (holding time 2 min); rate of rise of the column temperature: 10 ºC/min; final temperature of the column: 280 ºC; flow rate of the column carrier gas (He, 99.999 %): 0.9 mL/min, separate injection: 40:1; sample volume: 1 ml/min. Isobutane (99.999 %) was used as a reagent gas for CI.

Analysis of mass spectral data was performed with the help of the software "TurboMass Ver. 6.0" (Perkin-Elmer, USA), MS Interpreter. Ver. 2.0" (NIST, USA), "AIPS IN" (BelHard Group, Belarus).

Microbial strains and culture media: The following strains were used: standard bacterial strains of human pathogens: *Escherichia coli* F50, *Staphylococcus aureus* 209P, *Bacillus cereus* 8035 and fungi: *Candida albicans* 885653, obtained from the state collection of pathogenic microorganisms of Tarasevich State Institute of Standardization and Control of Biomedical Preparations; while the phytopathogenic strains *viz*. *Agrobacterium tumefaciens* A-47, *Erwinia amylovora* S59/5, *Erwinia carotovora* spp. *carotovora* SCC3193, *Xanthomonas arboricola* S3 and phytopathogenic fungi *Alternaria solani* St108, *Fusarium graminearum* PH-1, *Fusarium culmorum* 3288, *Phytophthora* sp.

Microorganisms were incubated in standard sterile nutrient broths. The concentration of bacteria was determined using a den-1B densitometer (Biosan, Latvia) according to standard protocols. As reference compounds, norfloxacin (Sigma-Aldrich Co., USA), ketoconazole (Sigma-Aldrich Co., USA), chloramphenicol (JSC "Tatchempharmpreparaty", Russia) and difenoconazole (Score250 EC, Syngenta, USA) were used in the experiments.

***in vitro* Antimicrobial analysis:** The minimal inhibitory concentration was determined by the method of double sequential dilution [11] through slight modification [12]. The fungistatic activity of the ethanolic extract was determined by the method of serial dilution [13] in a liquid medium.

Liquid broth with spores of microorganisms was prepared on standard nutrient media: Hottinger broth for bacterial pathogens of human diseases, Sabouro medium for fungi pathogens of human diseases and broth of potato-glucose extract for phytopathogenic microorganisms from 24 h bacterial cultures, and for fungal spores – 7-14-day cultures, respectively. The final size of inoculates was 10^5 CFU/mL in the case of bacteria analysis and $1.1\text{-}1.5 \times 10^2$ CFU/mL in the case of fungi analysis.

As a control, tubes containing only nutrient media were used. To identify the minimal bactericidal and fungicidal concentrations (MBC and MFC, respectively), 10 µL of innoculate (or a piece of mycelium of fungi) taken from test tubes without visible growth was added to petri-dishes with agarized nutrient medium using a bacteriological loop.

The results were recorded every day for 5 days at 37 ºC for *Escherichia coli* F50, *Staphylococcus aureus* 209P, *Bacillus cereus* 8035, 30 ºC for *Agrobacterium tumefaciens* A-47, *Erwinia amylovora* S59/5, *Erwinia carotovora* spp. *carotovora* SCC3193 and 25 ºC for *Xanthomonas arboricola* S3, respectively. The time of incubation of fungi in a thermostat at 26 ºC with the appropriate substance made up to 14 days. The growth of microorganisms was determined visually. All analyses were carried out in triplicate.

Evaluation of antioxidant activity: Antiradical properties of ethanolic extract were evaluated using chemiluminescent (CHL) analysis [14] using a chemiluminometer "Lum-100" (DISoft, Russia). A solution of luminol (Alfa Aesar, UK) 1 mmol/L was prepared by dissolving in 0.1 M NaOH; before analysis, it was diluted with distilled water four times. The composition of the reaction mixture included: 400 µL of 250 µm luminol, 500 µL 0.5 M tris-buffer solution (Fisher Chemical, UK) of pH 8.6 and 100 µL of 40 mm AAPH solution, 2,2'-azo-bis(2-methylpropionamidine)dihydrochloride (Acros Organics, USA) in distilled water. The reaction mixture was incubated at 30 ºC. The main CL level was measured during 10 min, then, 10 µL of the test compound solution was added to the reaction mixture and finally the CL level was measured during 20-30 min. The ethanolic extract was diluted in distilled water to a concentration of 1, 0.5, 0.1, 0.01 and 0.001 mg/mL.

Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma-Aldrich Co., USA) and quercetin were used as standard antioxidants. To estimate the CL value of the studied samples, TAR (total antioxidant reactivity) and TRAP (total reactive antioxidant potential) were calculated [15].

The relative inhibitory activity of each sample was estimated based on CL curve area measurement. The inhibition coefficient was calculated according to eqn. 1:

$$\text{Inhibition } (\%) = \frac{100 \cdot AUC_1}{AUC_0} \quad (1)$$

where AUC_0 and AUC_1 are the area under the curve observed for control and in the presence of the test solution, respectively.

The results were processed with the help of the programs PowerGraph (http://www.powergraph.ru) and OriginLab.

RESULTS AND DISCUSSION

Phytochemical composition of ethanolic extract of *Tussilago farfara* L. flowers: The phytochemical composition of ethanolic extract of *Tussilago farfara* L. flowers was studied with the GC-MS method (Table-1), where 20 compounds were identified. The spectrum of chemical components was represented by cyclic ketone (50.1 %), compound ethers (14.7 %), furans (8.5 %), pyrans (6.5 %), sterol (6 %), polyatomic alcohol (4.3 %), alkanes (3.61 %), amino acid ether (2.6 %), carboxylic acid (2.2 %) and hydroxy ketone (1.5 %) (Table-1).

Based on previous studies in plant extracts *Tussilago farfara* L., sesquiterpenes [16-18], phenolic compounds [19], polysaccharides [18,20], flavonoids [21,22], chromones [22], phenylpropanoids [23], pyrrolizidine alkaloids [24,25] and tussfarfarins [26,27] were identified.

Antimicrobial activity: The minimal inhibitory concentration of ethanolic extract of *Tussilago farfara* L. against human pathogens was 2500-5000 µg/mL (Table-2). The greatest sensitivity was noted for *S. aureus*. The minimal bactericidal/fungicidal concentrations were practically the same.

The reactivity of ethanolic extract of *Tussilago farfara* L. concerning plant pathogens also did not differ significantly. The minimal inhibitory and minimal bactericidal concentrations in the case of phytopathogenic bacteria ranged from 2500-5000 µg/mL, for *Xanthomonas arboricola* was more than 5000 µg/mL.

Minimal inhibitory and minimal fungicidal concentrations for phytopathogenic fungi *Fusarium graminearum*, *Fusarium culmorum*, *Phytophthora* sp. were 5000 µg/mL and more. *Alternaria solani* was the most sensitive to the components of ethanolic extract, where MIC/MFC values were 625 µg/mL.

TABLE-1
PHYTOCHEMICAL COMPOSITION OF ETHANOLIC EXTRACT OF *Tussilago farfara* L. FLOWERS

Component	RR_t	Yield (%)
1,2-Ethanediol	3.613	4.329
2-Hydroxy-2-cyclopenten-1-one	3.897	1.474
(+)-2,4-Dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one	4.544	1.150
2*H*-Pyran-2,6(3*H*)-dione	4.852	1.654
l-Alanine, *n*-propargyloxycarbonyl-, ethyl ester	5.978	2.559
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	6.927	4.873
5-Hydroxymethylfurfural	8.024	4.775
Acetin	8.273	1.668
(-)-2,4-Dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one	8.836	2.588
2-(2,6-Dimethylheptyl)-cyclobutanone	12.986	50.057
n-Hexadecanoic acid	15.180	2.162
Hexadecanoic acid, ethyl ester	15.370	1.877
Linoleic acid ethyl ester	16.716	3.424
(Z,Z,Z)-9,12,15-Octadecatrienoic acid, ethyl ester	16.781	2.628
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	19.437	1.628
Pentacosane	20.949	1.612
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.293	2.164
(Z,Z,Z)-Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	21.411	1.352
Nonacosane	23.735	1.999
β-Sitosterol acetate	36.506	6.026

TABLE-2
ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *Tussilago farfara* L. FLOWERS AGAINST HUMAN AND PLANT PATHOGENS

Microbial strains	MIC	MBC/MFC	MIC	MBC/MFC
	EtOH extract (µg/mL)		Norfloxacin (µg/mL)	
Staphylococcus aureus	2500	2500	2.4 ± 0.25	2.4 ± 0.0019
Bacillus cereus	5000	> 5000	7.8 ± 0.78	15.6 ± 1.25
Escherichia coli	5000	5000	1.5 ± 0.15	1.5 ± 0.14
			Ketoconazole (µg/mL)	
Candida albicans	5000	5000	3.9 ± 0.37	3.9 ± 0.33
			Chloramphenicol (µg/mL)	
Agrobacterium tumefaciens	2500	2500	250 ± 22.5	500 ± 42.2
Pantoea agglomerans	5000	5000	250 ± 21.5	250 ± 20.0
Erwinia carotovora	5000	5000	125 ± 12.3	125 ± 11.5
Xanthomonas arboricola	> 5000	> 5000	250 ± 23.1	500 ± 35.6
			Difenoconazole (µg/mL)	
Alternaria solani	625	625	1.9 ± 0.15	31.3 ± 3.1
Fusarium graminearum	5000	5000	3.9 ± 0.35	62.5 ± 5.9
Fusarium culmorum	5000	5000	3.9 ± 0.22	125 ± 11.1
Phytophthora sp.	> 5000	5000	7.8 ± 0.75	7.8 ± 0.68

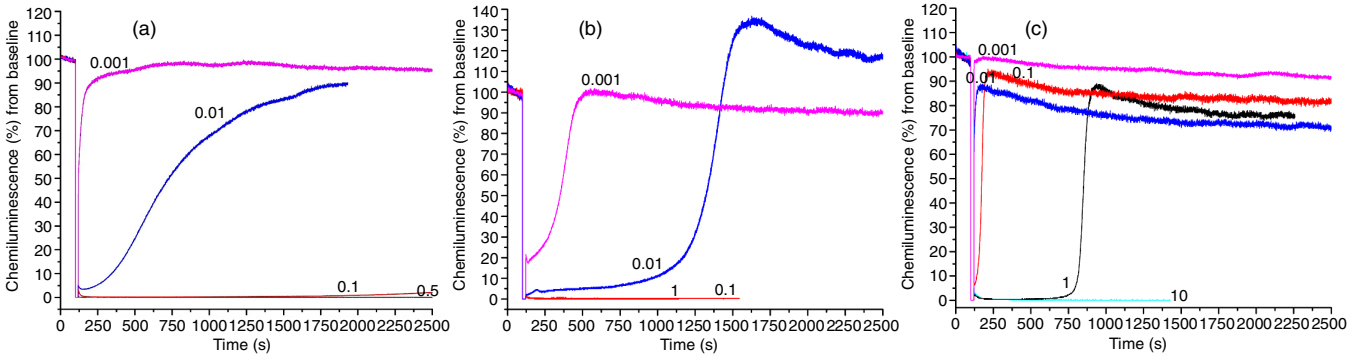

Fig. 1. Chemiluminescence of ethanolic extract of *Tussilago farfara* L. flowers (A), quercetin (B) and Trolox (C) intensity of light emission *vs.* time. The numbers beside the curves are the concentrations of *Tussilago farfara* L. ethanolic extract, quercetin and Trolox (mg/mL), time (s) is plotted on the abscissa axis and chemiluminescence intensity (a.u.) is plotted on the ordinate axis

Based on the data of phytochemical composition, the main contribution to the antimicrobial activity of ethanolic extract of *Tussilago farfara* L. is made by furan and pyran derivatives *viz.* 2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one (2 isomers) and 5-hydroxymethylfurfural-2*H*-pyran-2,6-(3*H*)-dione present in the extract. Similar findings are reported by some authors [28,29].

Antioxidant activity: The analysis of chemiluminescent activity revealed the antioxidant properties of ethanolic extract of *Tussilago farfara* L. flowers, where the level of TAR in concentrations of 0.1 mg/mL and 1 mg/mL reached 96% or higher, and the level of TRAP, similar to bioflavonoid quercetin, revealed a long latent period, which apparently indicates the ability to bind free radicals in the system (Table-3, Fig. 1).

TABLE-3
VALUES OF THE TAR AND TRAP OF THE ETHANOLIC EXTRACT OF *Tussilago farfara* L. FLOWERS

Method	Conc. (mg/mL)	A*	Quercetin	Trolox
TAR (%)	0.5–1	99.7	98.1	10.8
	0.1	96.8	98.0	4.9
	0.01	9.7	-36.7	11.1
	0.001	0.3	-1.8	-0.3
TRAP (s)	0.5–1	1500+	1500+	555.3
	0.1	1263.5	1500+	8.4
	0.01	117.5	136.4	0.8
	0.001	0	19.9	0

A* = Ethanolic extract of *Tussilago farfara* L. flowers

At a concentration of 0.01 mg/mL, ethanolic extract of coltsfoot had a short latent period comparable to that of quercetin and was characterized by a slow increase in the chemiluminescent glow, *i.e.* had a low rate constant of interaction with radicals. Ethanolic extract of coltsfoot flowers did not have pro-oxidant properties and went to the plateau, binding radicals and reducing the degree of TAR to 9 % of the initial level. Quercetin in the same concentration increased the intensity of glow, and the degree of TRAP at the same time exceeded the initial level by 36 %. At a concentration of 0.001 mg/mL, ethanolic extract of coltsfoot flowers had no significant antioxidant effect and had no latent period, unlike quercetin at a similar concentration. The ethanolic flower extract showed higher antioxidant properties compared to Trolox.

Conclusion

This study confirms the possibility of using ethanolic extract of *Tussilago farfara* L. flowers as a biobactericide and biofungicide in concentrations of more than 2500 µg/mL. The most promising use of the extract is against the phytopathogenic fungus *Alternaria solani*. The ethanolic extract showed strong antioxidant properties. Ketones, derivatives of furans and pyrans should be considered as the main biologically active components of ethanolic extract of *Tussilago farfara* L. flowers.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Distributed Spectral Analytical Center of Shared Facilities for Study of Structure, Composition and Properties of Substances and Materials, Laboratory of Chemical and Biological Research, Laboratory of Microbiology of Federal Research Center of Kazan Scientific Center of Russian Academy of Sciences for their assistance in discussing the results.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Savary, A. Ficke, J.-N. Aubertot and C. Hollier, *Food Security*, **4**, 519 (2012); https://doi.org/10.1007/s12571-012-0200-5.
2. H.X. Xu, X.S. Zheng, Y.J. Yang, J.C. Tian, Y.H. Lu, K.H. Tan, K.L. Heong and Z.X. Lu, *Crop Protection*, **72**, 144 (2015); https://doi.org/10.1016/j.cropro.2015.03.017.
3. P.C. Stevenson, M.B. Isman and S.R. Belmain, *Ind. Crop Prod.*, **110**, 2 (2017); https://doi.org/10.1016/j.indcrop.2017.08.034.
4. L.G. Copping and S.O. Duke, *Pest Manag. Sci.*, **63**, 524 (2007); https://doi.org/10.1002/ps.1378.
5. N.E. El-Wakeil, *Gesunde Pflanzen*, **65**, 125 (2013); https://doi.org/10.1007/s10343-013-0308-3.
6. K.D. Gwinn, B.H. Ownley, S.E. Greene, M.M. Clark, C.L. Taylor, T.N. Springfield, D.J. Trently, J.F. Green, A. Reed and S.L. Hamilton, *Phytopathology*, **100** (2010); https://doi.org/10.1094/PHYTO-100-5-0493.
7. S. Koc, B.S. Isgor, Y.G. Isgor, N.S. Moghaddam and O. Yildirim, *Pharm. Biol.*, **53** (2015); https://doi.org/10.3109/13880209.2014.942788.
8. B. Singh, P.M. Sahu and M.K. Sharma, *Phytomedicine*, **9**, 355 (2002); https://doi.org/10.1078/0944-7113-00143.

9. R. Lebada, A. Schreier, S. Scherz, C. Resch, L. Krenn and B. Kopp, *Phytochem. Anal.*, **11**, 366 (2000); https://doi.org/10.1002/1099-1565(200011/12)11:6<366::AID-PCA538>3.3.CO;2-T.
10. S.Y. Xue, Z.Y. Li, H.J. Zhi, H.F. Sun, L.Z. Zhang, X.Q. Guo and X.M. Qin, *Biochem. Syst. Ecol.*, **41**, 6 (2012); https://doi.org/10.1016/j.bse.2011.11.003.
11. CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Wayne, Pennsylvania, USA (2018).
12. V. Kanagarajan, E.M. Ramanathan and M. Gopalakrishnan, *Org. Med. Chem. Lett.*, **1**, article no. 8 (2011); https://doi.org/10.1186/2191-2858-1-8.
13. NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, In: Approved Standard, Wayne, Pennsylvania, USA, edn 2 (2002).
14. A.B. Vyshtakalyuk, V.E. Semenov, I.A. Sudakov, K.N. Bushmeleva, L.F. Gumarova, A.A. Parfenov, N.G. Nazarov, I.V. Galyametdinova and V. Zobov, *Russ. Chem. Bull.*, **67**, 705 (2018); https://doi.org/10.1007/s11172-018-2126-3.
15. C. Desmarchelier, M. Repetto, J. Coussio, S. Llesuy and G. Ciccia, *Int. J. Pharmacogn.*, **35**, 288 (1997); https://doi.org/10.1076/phbi.35.4.288.13303.
16. H. Jang, J.W. Lee, C. Lee, Q. Jin, J.Y. Choi, D. Lee, S.B. Han, Y. Kim, J.T. Hong, M.K. Lee and B.Y. Hwang, *Arch. Pharm. Res.*, **39**, 127 (2016); https://doi.org/10.1007/s12272-015-0667-7.
17. H.J. Lim, G.Z. Dong, H.J. Lee and J.H. Ryu, *J. Enzyme Inhib. Med. Chem.*, **30**, 852 (2015); https://doi.org/10.3109/14756366.2014.965701.
18. K. Qin, C.H. Liu, Y.X. Qi and K. Li, *Asian J. Chem.*, **26**, 3073 (2014); https://doi.org/10.14233/ajchem.2014.16685.
19. S. Uysal, I. Senkardes, A. Mollica, G. Zengin, G. Bulut, A. Dogan, J. Glamoèlija, M. Sokovic, D. Lobine and F.M. Mahomoodally, *J. Biomol. Struct. Dyn.*, **37**, 3269 (2019); https://doi.org/10.1080/07391102.2018.1506361.
20. P. Zhao, L. Li, Z.S. Tang and W.H. Li, *Asian J. Chem.*, **24**, 2707 (2012).
21. M.R. Kim, J.Y. Lee, H.H. Lee, D.K. Aryal, Y.G. Kim, S.K. Kim, E.R. Woo and K.W. Kang, *Food Chem. Toxicol.*, **44**, 1299 (2006); https://doi.org/10.1016/j.fct.2006.02.007.
22. D. Wu, M. Zhang, C.F. Zhang and Z.T. Wang, *Biochem. Syst. Ecol.*, **36**, 219 (2008); https://doi.org/10.1016/j.bse.2007.07.003.
23. H. Gao, Y.-N. Huang, B. Gao, P.-Y. Xu, C. Inagaki and J. Kawabata, *Food Chem.*, **106**, 1195 (2008); https://doi.org/10.1016/j.foodchem.2007.07.064.
24. A. Nedelcheva, N. Kostova and A. Sidjimov, *Biotechnol. Biotecnol. Equip.*, **29**, S1 (2015); https://doi.org/10.1080/13102818.2015.1047149.
25. N. Smyrska-Wieleba, K.K. Wojtanowski and T. Mroczek, *Phytochem. Lett.*, **20**, 339 (2017); https://doi.org/10.1016/j.phytol.2016.11.009.
26. A.M. Yang, Q. Shang, L. Yang, C.L. Li and H.J. Yuan, *Chem. Nat. Compd.*, **53**, 584 (2017); https://doi.org/10.1007/s10600-017-2058-4.
27. A.M. Yang, A. Zhao, Z.S. Zheng, Q. Shang, F.L. Zhang, N. Han, L. Yang and C.L. Li, *Chem. Nat. Compd.*, **54**, 978 (2018); https://doi.org/10.1007/s10600-018-2527-4.
28. S.D. Joshi, J. Ashwini, H.M. Vagdevi, V.P. Vaidya and G.S. Gadaginamath, *Indian J. Pharm. Educ. Res.*, **44**, 148 (2010).
29. M. Ramasamy and U. Balasubramanian, *Int. J. Sci. Nat.*, **3**, 263 (2012).