Saudi Journal of Biological Sciences 25 (2018) 811–815

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

High-efficient extraction of principal medicinal components from fresh *Phellodendron* bark (cortex phellodendri)

Keqin Xu [1], Gongxiu He [1], Jieming Qin, Xuexiang Cheng, Hanjie He, Dangquan Zhang *, Wanxi Peng *

Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees (Ministry of Education) & Hunan Provincial Key Laboratory of Forestry Biotechnology, Central South University of Forestry and Technology, Changsha 410004, China

ARTICLE INFO

Article history:
Received 16 August 2017
Revised 25 September 2017
Accepted 3 October 2017
Available online 5 October 2017

Keywords:
Phellodendron
Cortex phellodendri
Extraction methods
Medicinal components

ABSTRACT

There are three key medicinal components (phellodendrine, berberine and palmatine) in the extracts of *Phellodendron* bark, as one of the fundamental herbs of traditional Chinese medicine. Different extraction methods and solvent combinations were investigated to obtain the optimal technologies for high-efficient extraction of these medicinal components. Results: The results showed that combined solvents have higher extracting effect of phellodendrine, berberine and palmatine than single solvent, and the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction. Conclusion: The hydrochloric acid/methanol-ultrasonic extraction has the best effect for three medicinal components of fresh *Phellodendron* bark, providing an extraction yield of 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine.

© 2016 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Phellodendron bark has been widely known as one of the fundamental herbs of traditional Chinese medicine. *Phellodendron* bark is also called cortex phellodendri, which is the bark of two *Phellodendron* trees: *P. amurense* and *P. chinense*. *Phellodendron* bark is characterized by humorism, as bitter and cold, affecting the kidney, urinary bladder and large intestine meridians in a traditional Chinese medicine counterpart (Zhang et al., 2016). *Phellodendron* bark is also medically used to clear heat, reduce fire, dry dampness and release toxins (Li et al., 2014). Modern pharmacological researches indicated that *Phellodendron* bark has the functions of anti-pathogenic microorganism, anti-ulcer, antihypertensive and anti-arrhythmic, etc. *Phellodendron* bark can reduced blood uric acid levels in mice with hyperuricemia by inhibiting xanthine oxidase activity (Yang et al., 2015).

Many scientific findings had revealed the biomedical activities of extracts from *Phellodendron* bark. The further researches indicated that there are three key bioactive components (phellodendrine, berberine and palmatine) in the extracts of *Phellodendron* bark. Ethanol extracts of *Phellodendron* bark displayed antidiarrheal activity by attenuating ion transport by intestinal epithelium (Tsai et al., 2004; Guo et al., 2017). Medicinal extracts of *Phellodendron* bark reduced the rate of growth of Candida, which has been ascribed to berberine and palmatine content (Park et al., 1999). Different extracts of *Phellodendron* bark showed multiple functions, especially including: protect against airway inflammation in response to lipopolysaccharide treatment of mice (Mao et al., 2010; Gao et al., 2017), reduce blood glucose levels and slow the development of diabetic nephropathy in mice treated with streptozocin to induce diabetes (Kim et al., 2008; Muhammad et al., 2017a), reduce contractions of smooth muscle of isolated rat prostate glands (Xu and Ventura, 2010), and reduce cell replication of tumors in mice (Park et al., 2004).

Therefore, the efficient extraction of medicinal components from *Phellodendron* bark was focused by researches (Boost et al., 2016). The widely used extraction methods were soxhlet extraction, distillation and ultrasonic extraction, and the extraction solvents were water, organic solvents and their combinations (Wang et al., 2015; Chen et al., 2014). To obtain the optimal technologies for high-efficient extraction of principal medicinal components (phellodendrine, berberine and palmatine) from fresh *Phellodendron* bark, the three extraction methods and several solvent combinations were systematically analyzed.

* Corresponding authors.
E-mail addresses: zhangdangquan@163.com (D. Zhang), pengwanxi@163.com (W. Peng).
[1] These authors contributed equally to this work.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2017.10.008
1319-562X/© 2016 Production and hosting by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

2. Methodology

2.1. Preparation of Phellodendron barks

The fresh *Phellodendron* barks were collected from the average 15a trees in *Phellodendron* plantation in Cili country, Hunan province, China (Choi et al., 2014; Xian et al., 2011). The barks were treated by drying at 50 °C for 10 h, then meshed sieved by 60 meshes. The bark powders obtained were stored 4 °C.

2.2. Preparation of standard samples

Three standard samples (phellodendrine hydrochloride, berberine and palmatine hydrochloride) were dissolved in solution of acetonitrile/0.1% phosphoric acid to form sample solutions of gradient concentration (Hu et al., 2010; Garazd et al., 2017).

2.3. Distillation extraction

Accurately weigh 5.0 g powders of *Phellodendron* barks and put them into the round bottom flask, then add 40 times of ultrapure water (200 mL), followed by refluxing extraction in 100 °C. The extracts were collected finally by centrifugation (1000r/min) for 1 min (Jung et al., 2009).

2.4. Ultrasonic extraction

Weigh 0.50 g powders (by 20 mesh sieve) for 3 copies, then put them in the conical flask of 25 mL and add different extraction solvents: hydrochloric acid/methanol (1:100 v/v), hydrochloric acid/ethanol (1:100 v/v) and hydrochloric acid/water (1:100 v/v) in 50 mL (Kim et al., 2008). Powders were extracted with ultrasonic 20 min under the condition of 40 kHz and 250 W. The extracts were collected by centrifugation (1000 r/min) 1 min.

2.5. Soxhlet extraction

Ethanol extraction. Weigh 10.0 g powders, wrapped with filter paper and bounded with string, then put them into the drawer tube, then add 20 times amount of 70% ethanol (Chan et al., 2007). The extraction condition: extraction temperature 80 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts.

Acid-water extraction. Weigh 5.0 g powders, rapped with filter paper and bounded with string, then soaked in 2% hydrochloric acid for 1 h. Put them in the drawer tube and add 10 times amount of water (Li et al., 2006). The extraction condition: extraction temperature 100 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts (Muhammad et al., 2017b).

Double solvents extraction. Weigh 5.0 g powders of *Phellodendron* barks for 3 copies, wrapped with filter paper and bounded with string, then put them into the drawer tube (Liu et al., 1993; Wu et al., 2017). Then add 200 ml double solvents: hydrochloric acid/methanol (1:100 v/v), hydrochloric acid/ethanol (1:100 v/v) and hydrochloric acid/water (1:100 v/v). The extraction condition: extraction temperature 90 °C, 3 siphoning treatment (each for 1 h). Finally, combine the 3 extracting solutions to form extracts.

2.6. HPLC condition

Phellodendrine and berberine were gradient eluted with Kromasil RP-C18 chromatographic column (4.6 mm RP-C18), and palmatine is gradient eluted with Waters symmetry C18 chromatographic column (4.6 * 250 mm, 5 μm).

Mobile phase for berberine: Acetonitrile/0.1% phosphoric acid (50:50 v/v) (plus 0.1 g sodium dodecyl sulfonate per 100 mL), and the detection wavelength is 265 nm.

Mobile phase for Phellodendrine: Acetonitrile/0.1% phosphoric acid (36:64 v/v) (plus 0.2 g sodium dodecyl sulfonate per 100 mL), and the detection wavelength is 284 nm.

Mobile phase for Palmatine gradient eluting: 0.05% TFA ddH_2O (A)/0.05% TFA acetonitrile (B), and the detection wavelength is 270 nm.

3. Results & discussion

3.1. Component identification of Phellodendron barks extracts by HPLC

According to the peak area integral (mAU) and concentrations (μg/mL) of three standard samples (phellodendrine hydrochloride, berberine and palmatine hydrochloride), their regression equations were established (Fig. 1).

Nine combinations of three extraction methods and solvent combinations were used to extract *Phellodendron* barks, and their principal medicinal components were identified by HPLC (High Performance Liquid Chromatography) and verified by three different standard samples (Fig. 2).

3.2. Extraction yield of medicinal components from Phellodendron barks

The extraction effects of principal medicinal components in fresh *Phellodendron* barks were investigated using five extracting

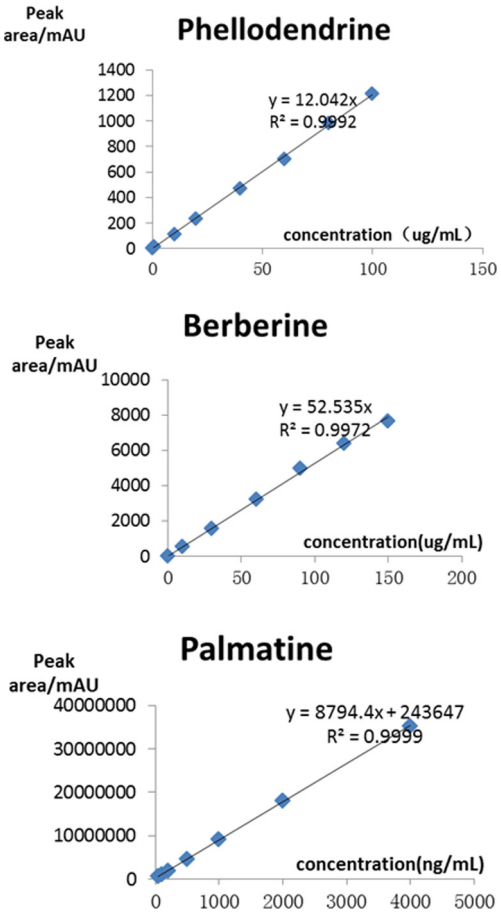

Fig. 1. Regression equations of phellodendrine hydrochloride, berberine and palmatine hydrochloride.

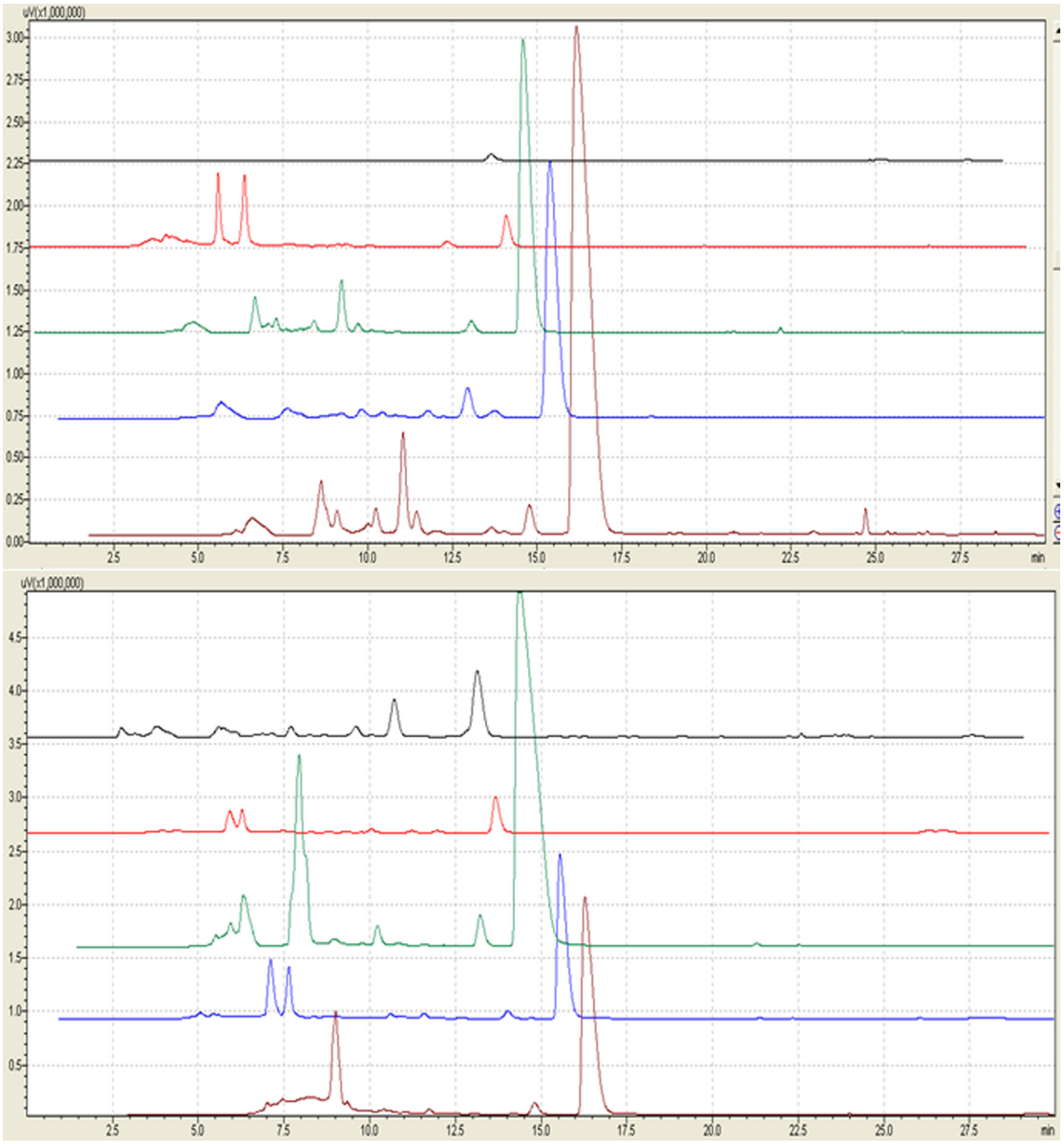

Fig. 2. HPLC chromatograms of Palmatine in nine extracts of *Phellodendron* barks.

solvents (distilled water, methanol, ethanol, hydrochloric acid/methanol, hydrochloric acid/ethanol, hydrochloric acid/distilled water) and three extracting methods (soxhlet extraction, distillation and ultrasonic extraction).

The analytical result showed that the extraction effects of hydrochloric acid/methanol, hydrochloric acid/ethanol, hydrochloric acid/distilled water are better than those of hydrochloric acid, methanol, ethanol and distilled water, suggesting that combined solvents have higher extracting effect of phellodendrine, berberine and palmatine than single solvent (Mustafa et al., 2017). Further, the hydrochloric acid/methanol (1:100 v/v) display the highest extracting efficiency.

The three-good extraction yield of phellodendrine were achieved by hydrochloric acid/methanol-ultrasonic, hydrochloric acid/distilled water-ultrasonic and hydrochloric acid/ethanol-ultrasonic. The three-good extraction yield of berberine were achieved by hydrochloric acid/methanol-ultrasonic, hydrochloric acid/distilled water-ultrasonic and hydrochloric acid/ethanol-ultrasonic. The three-good extraction yield of palmatine were achieved hydrochloric acid/ethanol-ultrasonic, hydrochloric acid/methanol-ultrasonic and hydrochloric acid/methanol-Soxhlet (Nawaz et al., 2017).

Among the three extraction methods, the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction.

According to the analytical result, it is confirmed that the hydrochloric acid/methanol-ultrasonic extraction has the best effect, providing an extraction yield of 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine (see Fig. 3).

It is reported in the literatures that the results of the experiment are different because of raw material origin, variety, pretreatment methods and data analysis methods. The findings here focused on the exploration of the best extraction technology of medicinal components from fresh *Phellodendron* barks, which maybe have high quality than those of long-term stored barks (Sarfraz et al., 2017).

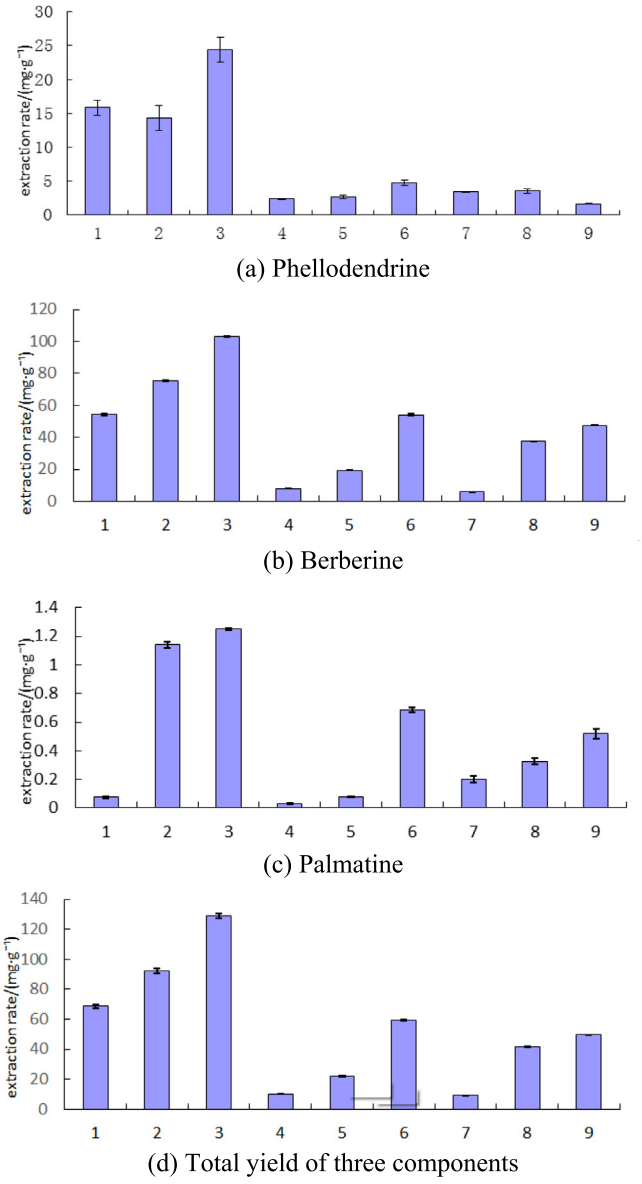

Fig. 3. Extraction yields of principal medicinal components by different solvents and extraction methods (mg/g).

The phellodendrine, berberine and palmatine both are quaternary ammonium type alkaloid with good water solubility, and are generally insoluble in lipophilic organic solvent but soluble in water, alcohol and acid water. Free alkaloids and their salts are soluble in methanol and ethanol. Therefore, methanol and ethanol were used as the extraction solvents.

The optimal extraction method of three medicinal components is hydrochloric acid/methanol-ultrasonic. The extraction yield of berberine reaches to 16.59 mg/g, and the drying degree of raw materials may affect extraction. The highest extraction yield of phellodendrine is 24.41 mg/g, which is close to the Pharmacopoeia provided. The highest extraction yield of palmatine is 1.25 mg/g, which is higher than reported in the literatures.

Three extraction methods including distillation, ultrasonic extraction and soxhlet extraction were used here. The effect of ultrasonic extraction is significantly higher than those of distillation and soxhlet extraction. The ultrasonic extraction method has advantages of short extraction time, less material consumption, simple operation and low cost of materials. Therefore, it is priority to use the ultrasonic extraction as the best extraction method for medicinal components from fresh *Phellodendron* barks.

4. Conclusion

Combined solvents have higher extracting effect of than single solvent for the extraction of medicinal components from fresh *Phellodendron* barks, and the effect of ultrasonic extraction is distinctly better than those of distillation and soxhlet extraction. The hydrochloric acid/methanol-ultrasonic extraction has the best effect for three medicinal components of fresh Phellodendron bark, and the extraction yield reaches to 103.12 mg/g berberine, 24.41 mg/g phellodendrine, 1.25 mg/g palmatine.

Acknowledgements

This research was financially supported by Science and Technology project of Hunan Province (Grant No. 2016NK2154).

References

Boost, M., Yau, P., Yap, M., Cho, P., 2016. Determination of cytotoxicity of traditional Chinese medicine herbs, Rhizoma coptidis, Radix scutellariae, and Cortex phellodendri, by three methods. Cont. Lens. Anterior. Eye. 39 (2), 128–132.

Chan, C.O., Chu, C.C., Mok, D.K., Chau, F.T., 2007. Analysis of berberine and total alkaloid content in cortex phellodendri by near infrared spectroscopy (NIRS) compared with high-performance liquid chromatography coupled with uible spectrometric detection. Anal. Chim. Acta 592 (2), 121–131.

Chen, G., Li, K.K., Fung, C.H., et al., 2014. Er-Miao-San, a traditional herbal formula containing Rhizoma Atractylodis and Cortex Phellodendri inhibits inflammatory mediators in LPS-stimulated RAW264.7 macrophages through inhibition of NF-κB pathway and MAPKs activation. J. Ethnopharmacol. 154 (3), 711–718.

Choi, Y.Y., Kim, M.H., Han, J.M., et al., 2014. The anti-inflammatory potential of Cortex Phellodendron in vivo and in vitro: down-regulation of NO and iNOS through suppression of NF-κB and MAPK activation. Int. Immunopharmacol. 19 (2), 214–220.

Gao, W. et al., 2017. The first multiplication atom-bond connectivity index of molecular structures in drugs. Saudi Pharm. J. 25 (4), 548–555.

Garazd, Y., Garazd, M., Lesyk, R., 2017. Synthesis and evaluation of anticancer activity of 6-pyrazolinylcoumarin derivatives. Saudi Pharm. J. 25 (2), 214–223.

Guo, J.J., Han, J., et al., 2017. An ethnobotanical survey of medicinal plants used for the ailment of diabetes mellitus in Changzhi city of Shanxi province, China. Biomed. Res. – India 28 (3), 1370–1377.

Hu, Y.M., Su, G.H., Sze, S.C., Ye, W., Tong, Y., 2010. Quality assessment of Cortex Phellodendri by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry. Biomed. Chromatogr. 24 (4), 438–453.

Jung, H.W., Jin, G.Z., Kim, S.Y., Kim, Y.S., Park, Y.K., 2009. Neuroprotective effect of methanol extract of Phellodendri Cortex against 1-methyl-4-phenylpyridinium (MPP+)-induced apoptosis in PC-12 cells. Cell. Biol. Int. 33 (9), 957–963.

Kim, H.J., Kong, M.K., Kim, Y.C., 2008. Beneficial effects of Phellodendri Cortex extract on hyperglycemia and diabetic nephropathy in streptozotocin-induced diabetic rats. BMB Rep. 41 (10), 710–715.

Li, C.Y., Lu, H.J., Lin, C.H., Wu, T.S., 2006. A rapid and simple determination of protoberberine alkaloids in cortex phellodendri by 1H NMR and its application for quality control of commercial traditional Chinese medicine prescriptions. J. Pharm. Biomed. Anal. 40 (1), 173–178.

Li, S., Liu, C., Guo, L., et al., 2014. Ultrafiltration liquid chromatography combined with high-speed countercurrent chromatography for screening and isolating potential α-glucosidase and xanthine oxidase inhibitors from Cortex Phellodendri. J. Sep. Sci. 37 (18), 2504–2512.

Liu, Y.M., Sheu, S.J., Chiou, S.H., Chang, H.C., Chen, Y.P., 1993. A comparative study on commercial samples of phellodendri cortex. Planta Med. 59 (6), 557–561.

Mao, Y.F., Li, Y.Q., Zong, L., You, X.M., Lin, F.Q., Jiang, L., 2010. Methanol extract of Phellodendri cortex alleviates lipopolysaccharide-induced acute airway inflammation in mice. Immunopharm. immunotoxic. 32 (1), 110–115.

Muhammad, G., Rashid, I., Firyal, S., 2017a. Practical aspects of treatment of organophosphate and carbamate insecticide poisoning in animals. Matrix Sci. Pharma 1 (1), 10–11.

Muhammad, G., Rashid, I., Firya, S., Saqib, M., 2017b. Successful treatment of idiopathic generalized subcutaneous emphysema in kajli a ram by large bore injection needle. Matrix Sci. Med. 1 (1), 01–02.

Mustafa, G., Arif, R., Atta, A., Sharif, S., Jamil, A., 2017. Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. Matrix Sci. Pharma 1 (1), 17–26.

Nawaz, S., Shareef, M., Shahid, H., Mushtaq, M., Sajid, S., Sarfraz, M., 2017. Lipid lowering effect of synthetic phenolic compound in a high-fat diet (HFD) induced hyperlipidemic mice. Matrix Sci. Pharma 1 (1), 12–16.

Park, K.S., Kang, K.C., Kim, J.H., Adams, D.J., Johng, T.N., Paik, Y.K., 1999. Differential inhibitory effects of protoberberines on sterol and chitin biosyntheses in Candida albicans. J. Antimicrob. Chemother. 43 (5), 667–674.

Park, S.D., Lai, Y.S., Kim, C.H., 2004. Immunopontentiating and antitumor activities of the purified polysaccharides from Phellodendron chinese SCHNEID. Life Sci. 75 (22), 2621–2632.

Sarfraz, M., Ashraf, Y., Ashraf, S., 2017. A Review: Prevalence and antimicrobial susceptibility profile of listeria species in milk products. Matrix Sci. Med. 1 (1), 03–09.

Tsai, J.C., Tsai, S., Chang, W.C., 2004. Comparison of two Chinese medical herbs, Huangbai and Qianniuzi, on influence of short circuit current across the rat intestinal epithelia. J. Ethnopharm. 93 (1), 21–25.

Wang, L., Yan, G., Zhang, A., Shi, H., Sun, H., Wang, X., 2015. Fingerprinting and simultaneous determination of alkaloids and limonins in Phellodendri amurensis cortex from different locations by high-performance liquid chromatography with diode array detection. J. Chromatogr. Sci. 53 (1), 161–166.

Wu, J., Qu, Y., et al., 2017. Molecular docking studies of kirenol a traditional Chinese medicinal compound against rheumatoid arthritis cytokine drug targets (TNF-alpha, IL-1 and IL-6). Biomed. Res. – India 28 (5), 1992–1995.

Xian, Y.F., Mao, Q.Q., Ip, S.P., Lin, Z.X., Che, C.T., 2011. Comparison on the anti-inflammatory effect of Cortex Phellodendri Chinensis and Cortex Phellodendri Amurensis in 12-O-tetradecanoyl-phorbol-13-acetate-induced ear edema in mice. J. Ethnopharmacol. 137 (3), 1425–1430.

Xu, Y., Ventura, S., 2010. Extracts of bark from the traditional Chinese herb Phellodendron amurense inhibit contractility of the isolated rat prostate gland. J. Ethnopharmac. 127 (1), 196–199.

Yang, C., Zhu, J.X., Wang, Y., Wen, Y.L., Kong, L.D., 2015. Effects of processing Phellodendron amurense with salt on anti-gout. China J. Chinese Mater. Med. 30 (2), 145–148.

Zhang, Z., Zhang, Y., Zhang, Z., et al., 2016. Comparative analysis of DNA barcoding and HPLC fingerprint to trace species of Phellodendri Cortex, an important traditional Chinese medicine from multiple sources. Biol. Pharm. Bull. 39 (8), 1325–1330.