Bangladesh J. Bot. **49**(3): 709-717, 2020 (September) Special

CHINESE MEDICINAL MATERIAL-METABOLITE PROFILEING OF *LYCIUM RUTHENICUM* MURR. AND EVALUATION OF ANTIOXIDANT ACTIVITY

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Keywords: Lycium ruthenicum, Antioxidant activity, Metabolic product

Abstract

Analysis of the volatile metabolites and the antioxygenation ability of *Lycium ruthenicum* Murr. were investigated. Dynamic headspace collection and automated thermal desorption-gas chromatography-mass spectrometry (ATD-GC-MS) were used to analyze the volatile metabolites of *L. ruthenicum*h. Twenty five metabolites were identified and derived from methanol extraction. These metabolites exhibited good antioxidant effect which was determined by DPPH, ABTS, and ferric reducing antioxidant power (FRAP). The main metabolites of *L. ruthenicum* were revealed by GC-MS to be alcohols, hydrocarbons, terpenoids, aromatic and ester compounds. Among 25 identified metabolites, trans-2,3-butanediol and methylene chloride were the most abundant. This test established a rapid assay method for the analysis of volatile components of *L. ruthenicum.* The results laid a foundation for further research and application of these compounds in antioxidant drugs and health products.

Introduction

Black goji berries/black wolf berries (*Lycium ruthenicum* Murr.) is mainly distributed in Qinghai, Xinjiang, Ningxia, Gansu, Tibet in China, and Central Asia, Caucasus and Europe. However, they are widely distributed in the lower reaches of Tarim Basin in Xinjiang and the salt deserts in Qaidam Basin in Qinghai. It is often used in folk to nourish and strengthen the eyes and reduce blood pressure. *L. ruthenicum* has been widely utilized as a restoratives for 1000s of years and it has widespread pharmacological activities, including anti-ductal adenocarcinoma of the pancreas (Zhang *et al*. 2019), anti-oxidation (Wang *et al*. 2018), anti-radiation (Duan *et al*. 2015), improvement of memory impairment, neuroprotective effect (Gao *et al*. 2019), immuno-enhancement effects (Gong *et al*. 2015), lowering of blood glucose and blood lipid levels, and anti-inflammatory (Peng *et al*. 2014). Black wolfberry is rich in carotenoids, vitamins and other inorganic elements, such as Fe, Zn, Se, etc., and its extract also has strong antioxidant effect. Goji berries/wolf berries (*Lycium barbarum* L.) have been reported to have effective bioactivity, and in comparison, the same types of constituents are also found in *L. ruthenicum*, but with a high content of nutritionally active constituent (Wu *et al*. 2016).

Headspace injection can be utilized to authenticate the natural biomaterial ingredients and provide different and wider outline the sense of olfactory (Psillakis *et al*. 2019). ATD is an effective method to volatile components of fractionated plants, and it can be performed online with gas chromatography analysis. ATD-GC-MS is widely used in the study of plant metabonomics to identify and describe volatile metabolites in different plants (Qin *et al*. 2015). Therefore, it is feasible to use ATD-GC-MS detection to predict the volatile metabolites of *L. ruthenicum.*

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At present, the research on *Lycium barbarum* mainly focuses on the extraction, separation, identification and biological activity of polysaccharides, flavonoids, pigments and other small molecular compounds, and the volatile components of *L. ruthenicum.* There are a few reports on the sub research, only the volatile components in the essential oil were studied. In order to provide a theoretical basis for the development and utilization of wolfberry fruit, the volatile components and antioxidant activities of wolfberry fruit were studied by GC-MS.

Materials and Methods

The mature fruit of *Lycium ruthenicum* were collected from the Turpan Desert Botanical Garden of the Chinese Academy of Sciences, Xinjiang province in July. The external standard alpha-pinene (> 99% purity) solution was diluted with methanol to 2 mg/ml (HPLC purity), and then standard solutions of 0.4, 0.8, 1.2, 1.6, and 2.0 mg/ml were prepared. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-etlhylbenzothiazoline-6-sulphonic acid) (ABTS); 2,4,6-tripyridyl-s-triazine (TPTZ), and Folin-Ciocalteu's phenol reagent all were purchased from Sigma-Aldrich (St. Louis, MO, USA).

For ATD (350, PerkinElmer Corp., Norwalk, CT, USA), the sample adsorbed in the sample tube was completely eluted by two-stage desorption process. Thermal desorption GC with direct injection, flow rate of carrier gas was 2.5 ml/min, temperature of sample tube was 250°C, heating for 10 min; temperature of cold well in primary thermal desorption process: –30°C, temperature of cold well in primary to secondary thermal desorption process: 40°C/S; temperature of secondary thermal desorption: 300°C, holding for 5 min; then the analyte was injected into capillary column through heating transmission line (250°C); inlet shunt flow 10 ml/min, the split ratio so that 50% of the sample enters.

The 7890A Network Gas Chromatographic System was connected with 5975c Network MSD (Agilent Technologies, Santa Clara, CA, USA). The MS detector adopts full scan mode ion monitoring (SIM) mode. The chromatographic column is a capillary column (Agilent DB-5ms, 60 m × 0.25 mm × 0.25 μm); the temperature rise procedure of GC column temperature chamber: kept at 40°C for 2 min, raised to 120°C at 3°C/min, kept for 5 min, and then raised to 220°C at 6°C/min, kept for 3 min.

Temperature of ion source was 250°C; interface temperature was 250°C; the helium flow rate was 1.5 ml/min; impact voltage 70ev; scanning speed was 0.2S, recovery time was 0.1s; scanning mass load ratio ranged (M/z) from 29 - 600.

The method of DPPH radical scavenging experiment was adopted (Skenderidis *et al.* 2019). In short, methanol extract (2 ml) of *L. ruthenicum* and 2 ml of DPPH (200 μmol/l) were mixed, and extracted after 30 min at room temperature, the absorbance determined by an ultraviolet spectrophotometer in 517 nm. The effective concentration (EC_{50}) value was determined for antioxidants. The FRAP assay was performed according to a previously published method (Li *et al.* 2012). That is, 0.5 ml of sample extract was drawn into 10 ml of PV tube, 3.0 ml of FRAP reagent was added, and then mixed well, put the tube at 37°C for reaction, after 75 min, read the absorbance at 593 nm with ultraviolet spectrophotometer. The ABTS assay was done according to previous study (Benchennouf *et al.* 2017). In the dark at room temperature, ABTS reagent was produced by reacting 10 ml of 7 mM ABTS solution with 178 μl of 140 mM potassium persulfate (*aq*), which has been placed for 13 hrs. The ABTS solution was diluted to the appropriate absorbance, and then, in the dark at room temperature, 1 μl sample was added to 799 μl diluted ABTS solution to react for 10 min. The absorbance at 732 nm was subsequently recorded.

SPSS 20 software was used for one-way ANOVA analysis. All the significance analysis was at the level of $p < 0.05$. Metabolites have been used for principal component analysis (PCA) and mapped by origin 8.5 software.

Results and Discussion

Using the peak area as the ordinate and concentration as the abscissa, a standard curve was drawn using the software of the Agilent GC-MS MSD (Fig. 1). In the range of 0.4 - 2.0 mg/ml, the calibration curve showed a linear relationship, and R^2 was 0.999. The LOD was 1.0 ng/kg, and the LOQ was 2.5 ng/kg. Repeatability was performed according to Briand *et al.* (Raeppel *et al.* 2015). Satisfactory repeatability (n = 5) was determined and its RSD was 2.1%. The calibration range was between 0.2 and 2.0 µg. According to the standard curve, the amounts of the volatile components obtained from *L. ruthenicum* are presented in Table 1.

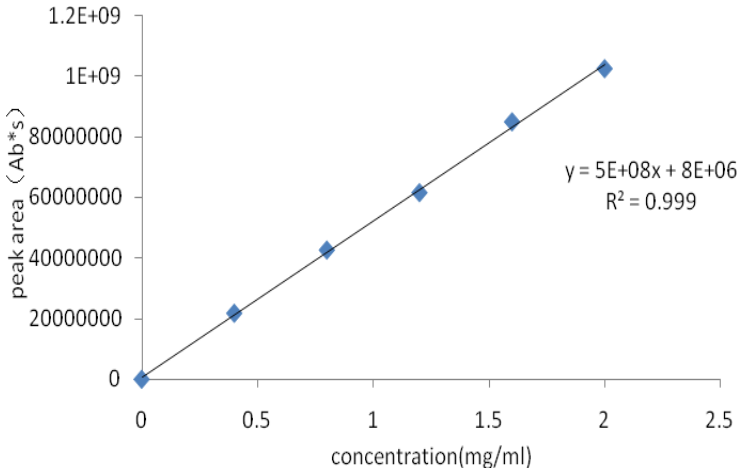

Fig. 1. The alpha-pinene standard.

According to the TIC (Fig. 2) of the wolfberry volatile, the main chromatographic peaks in the wolfberry volatile were analyzed qualitatively and quantitatively by mass spectrometry (Table 1). The results showed that 25 compounds were detected, including 10 hydrocarbons, 4 alcohols, 3 terpenes, 2 benzenes, 2 ketones, 1 ester and 3 others (Table 1). The main volatile components of *L. ruthenicum* are alcohols, ketones, terpenes, aromatic compounds, hydrocarbons and esters, among which alcohols and hydrocarbons were dominant, while other compounds were typically present in smaller amounts.

The volatile metabolites of *L. ruthenicum* are shown in Table 1. The relative content of the volatile compounds anti-form 2,3-butanediol, cis-form 2,3-butanediol, methylene chloride (MC), n-hexane, ethyl acetate, 1,3,5-cycloheptatriene, benzene, *o*-xylene, and undecane were the most common components, and anti-form 2,3-butanediol, cis-form 2,3-butanediol, methylene chloride (MC), n-hexane, and ethyl acetate were present at particularly high levels. The amount of compound alcohols (67%, 4.035 µg) was the highest in the volatile components of *L. ruthenicum* (Figs 3 and 4 (No. 1), followed by hydrocarbons (19%, 1.129 µg), terpenoids (2%, 0.109 µg), aromatic compounds (2%, 0.117 µg), ketones (1%, 0.096 µg), and ester compounds (3%, 0.354 µg). The relative content and content of alpha-pinene were 0.333% and 0.02 µg, respectively.

Table 1. Volatile compounds released from *Lycium ruthenicum.*

No.	Retention time (min)	Compounds	Molecular formula	Relative molecular mass	Relative content (%)	Content (μg)
1	4.066	Methyl alcohol	CH4O	32.026	0.48	0.029
2	4.266	Ethanol	C2H6O	46.042	0.297	0.018
3	4.472	Acetone	C3H6O	58.042	0.824	0.05
4	4.791	Methylene chloride	CH2Cl2	83.953	12.116	0.729
5	4.975	Furan, tetrahydro-2-methyl-	C5H10O	86.073	0.96	0.058
6	5.137	Pentane, 3-methyl-	C6H14	86.11	0.478	0.029
7	5.338	*n*-hexane	C6H14	86.11	2.116	0.127
8	5.505	Acetic acid	C2H4O2	60.021	4.539	0.273
9	5.63	Ethyl acetate	C4H8O2	88.052	2.894	0.174
10	5.857	Cyclohexane	C6H12	84.16	0.363	0.022
11	6.599	Benzene	C6H6	78.047	1.766	0.106
12	7.854	Acetoin	C4H8O2	88.052	0.76	0.046
13	9.727	1,3,5-cycloheptatriene	C7H8	92.063	1.183	0.071
14	10.539	2,3-butanediol (R,R)	C4H10O2	90.068	23.466	1.411
15	11.026	2,3-butanediol (S,S)	C4H10O2	90.068	42.867	2.577
16	14.231	o-xylene	C8H12	106.078	0.19	0.011
17	14.436	n-butyl ether	C8H18O	130.136	0.377	0.023
18	15.205	Nonane	C9H20	128.157	0.341	0.02
19	17.186	Alpha-pinene	C10H16	136.125	0.333	0.02
20	20.379	Decane	C10H22	142.172	0.961	0.058
21	25.592	Undecane	C11H24	156.188	1.23	0.074
22	30.961	Azulene	C10H8	128.063	0.297	0.018
23	34.263	Tridecane	C13H28	184.219	0.218	0.013
24	37.245	Tetradecane	C14H30	198.235	0.736	0.044
25	39.773	Pentadecane	C15H32	212.25	0.208	0.013

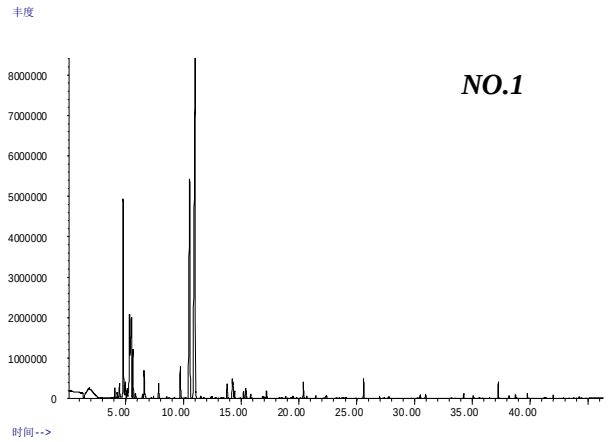

Fig. 2. Typical TIC of the volatile metabolites in *Lycium ruthenicum.*

From the different alcoholic compounds released from *L. ruthenicum* (Fig. 5, No. 2), the relative content and content of the volatile compound trans-2,3-butanediol (63%) were the highest, and cis-2,3-butanediol (35%) appeared with a consecutive retention time. 2,3-butanediol (2,3-BD) is an important bio-based platform chemical that has garnered increasing interest because of its extensive industrial applications in the production of fuel additives, synthetic rubber, solvents,

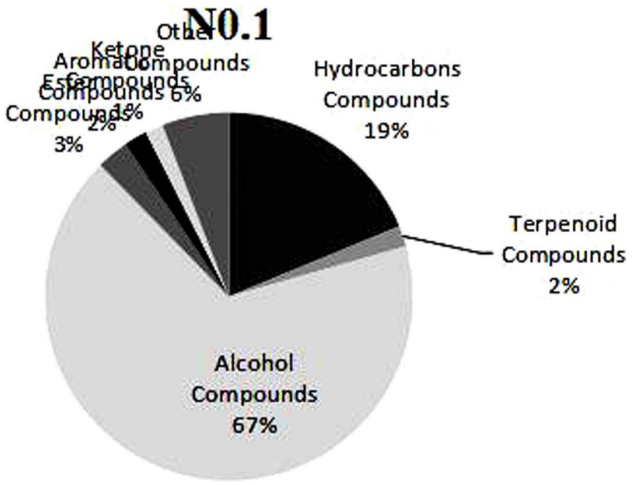

Fig 3. Percentage of alcohols, hydrocarbons, and so on released from *Lycium ruthenicum.*

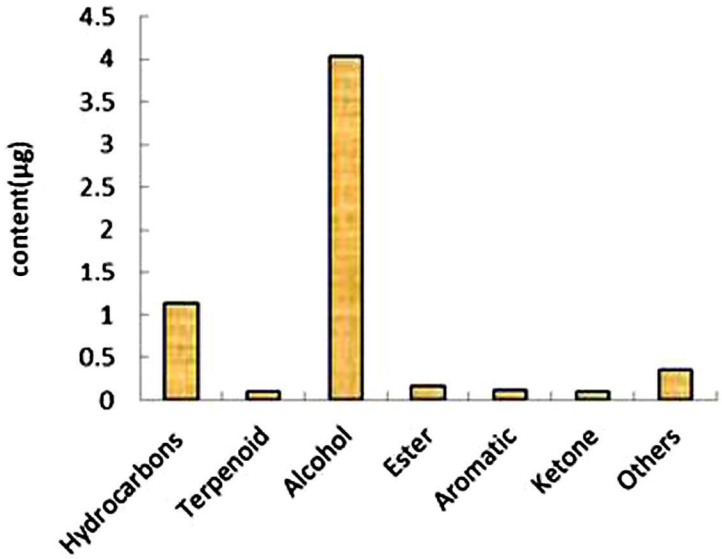

Fig. 4. Content of alcohols, hydrocarbons, and other compounds released from *Lycium ruthenicum.*

food additives, plasticizers, cosmetics, and pharmaceutical carriers (Kim *et al*. 2013, Oliver *et al*. 2014). Moreover, from the different hydrocarbons' compounds released from *L. ruthenicum* in Fig. 6 (No. 3), the relative content and content of the volatile compound methylene chloride (MC)

(12.116%) was the most abundant, and other predominant components were n-hexane, undecane, and decane.

The metabolic profiling of volatile metabolites from *L. ruthenicum* was analysed by unsupervised PCA. This model explained 73.27% of the variance. PCA of metabolic characteristics generated six principal components (PCs) (Eigenvalues >1, representing the main variables), and each of the first three accounts for more than 10% of the variance (41.764, 17.503 and 14.000%). Fig. 7 shows the PCA analysis of hydrocarbons, terpenes, aromatic compounds,

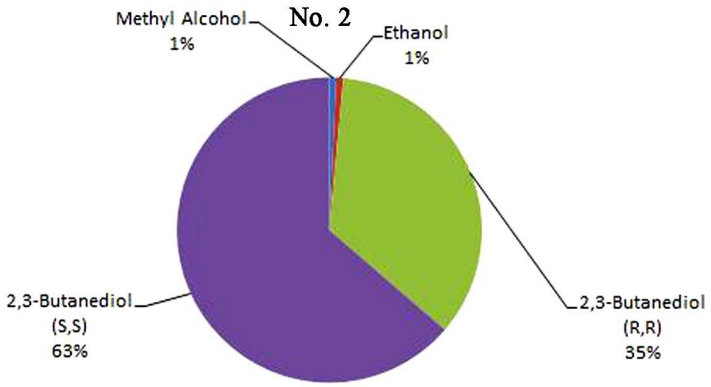

Fig. 5. Proportions of alcohol metabolites from *L. ruthenicum.*

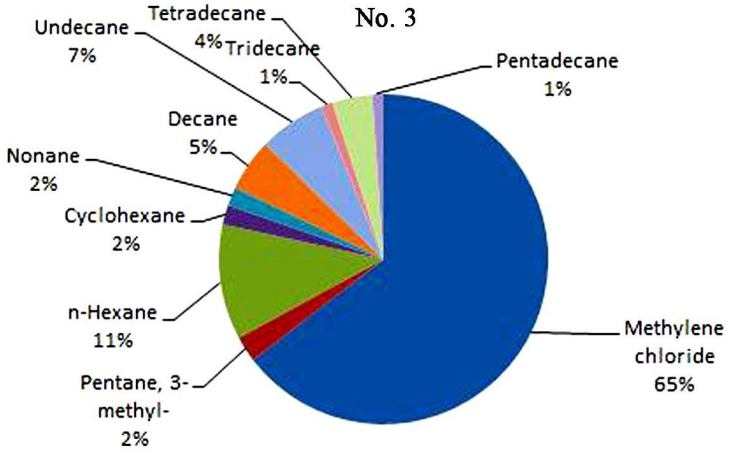

Fig. 6. Hydrocarbon metabolite proportions from *L. ruthenicum.*

alcohols, ketones, and esters and their contributions. The Fig. 7 shows the maximum contribution values in PC1 and PC2 were hydrocarbon and alcohol volatile compounds, respectively. This is consistent with the proportion of the compound type content in Fig. 3. The results showed that the difference between hydrocarbon- and alcohol compounds was large, and combined with the content analysis, this difference could be attributed to dichloromethane and butanediol.

The antioxidative activity of *L. ruthenicum* was evaluated by three kinds of common antioxidative experiments *in vitro*. The results showed that the methanol extract of *L. ruthenicum* had significant antioxidative activity (Table 2). In the DPPH assay, all extracts exhibited sufficient radical scavenging activity and therefore could be applied as antioxidant agents. According to

several reports, *L. ruthenicum* possesses excellent antioxidant activity (Rjeibil *et al*. 2018, Xia *et al*. 2019, Ali *et al*. 2020) and from the data obtained in this experiment, the same conclusion may be reached.

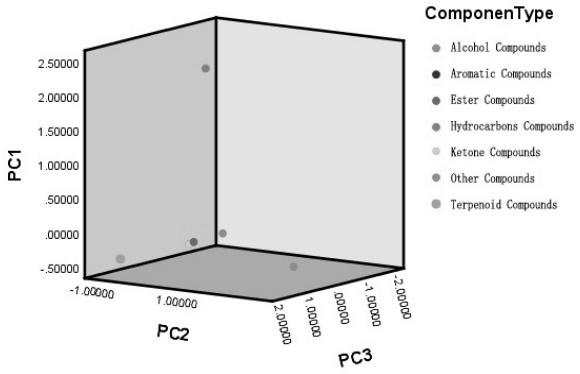

Fig. 7. PCA of volatile metabolites.

Table 2. The antioxidant activity of the methanol extracts of *Lycium ruthenicum.*

Samples	DPPH	FRAP	ABTS
Methanol extracts of *L. ruthenicum*	0.091 ± 0.008**	2.810 ± 0.012*	0.82 ± 0.021*

Values significantly different are marked *with "*";* *p <0.05, **p < 0.01. Antioxidant activity is expressed as mmol $FeSO_4$ equivalents per 1 g sample.

Dynamic headspace collection and ATD-GC-MS were used to analyze qualitatively and quantitatively the volatile components of *L. ruthenicum* collected on Tenax-TA resin, and 25 volatile compounds were identified. The ATD-GC-MS method provides rapid screening for the analysis of volatile components. Recently, GC/MS has been employed in the study of various volatile substances, especially in Chinese medicinal materials, but different extraction methods used in these studies might affect the measured volatile compositions. In the present study, the main volatile metabolites of *L. ruthenicum* were shown by GC/MS to be alcohols, hydrocarbons, terpenoids, aromatic compounds, and ester compounds.

The present analysis reveals only a glimpse into the chemical composition of *L. ruthenicum.* The relative content and content, calculated by the external method, of emission from *L. ruthenicum* are presented in Table 1. The most abundant constituent was the volatile compound trans-2,3-butanediol (42.867%, 2.577 μg), followed by cis-2,3-butanediol (23.466%, 1.411 μg) and methylene chloride (12.116%, 0.729 μg). In this study, the antioxidant activity of *L. ruthenicum* was verified by the common method of detecting antioxidant activity. The study of its biological effects is not enough, and more lateral extension studies are needed. An initial determination of *L. ruthenicum* was conducted in this study, and further investigation is needed, which reflected in to simplify volatile matter extraction procedures and to improve the prediction model through increase more key variables. All in all, this study is valuable for further full development of Chinese medicinal materials derived from *L. ruthenicum.*

In this paper, dynamic headspace sampling technology was used to collect the hair components of fresh black wolfberries, and ATD-GC-MS technology was used for qualitative and quantitative analysis. A total of 25 volatile components were identified, including alcohols,

hydrocarbons, terpenes, aromatic compounds and esters. The main volatile compounds are trans-2,3-butanediol, methylene chloride (MC), n-hexane, etc. PCA analysis of the volatile metabolites of *L. ruthenicum* showed that the main components were hydrocarbons and alcohols, which constituted the typical aroma of *L. ruthenicum*. DPPH, FRAP and ABTS were used to detect the antioxidant activity *in vitro*, which showed that the methanol extract of *L. ruthenicum* had significant antioxidant activity.

Acknowledgments

The first (Pan) and the author for correspondence acknowledges the financial grant from the special fund for basic research of forest and fruit industry (XJLG 201906, XJLG 202010) and Fund project of Scientific Research Institute of Xinjiang Academy of Forestry (KY2019052).

References

Ali SS, El-Zawawy N.A., Al-Tohamy R, El-Sapagh S, Mustafa AM and Sun J 2020. *Lycium shawii* Roem. & Schult.: A new bioactive antimicrobial and antioxidant agent to combat multi-drug/pan-drug resistant pathogens of wound burn infections, Journal of Traditional and Complementary Medicine **10**(1): 13-25.

Benchennouf A, Grigorakis S, Loupassaki S and Kokkalou E 2017. Phytochemical analysis and antioxidant activity of *Lycium barbarum* (Goji) cultivated in Greece. Pharmaceutical Biology. **55**(1): 596-602.

Chen S, Zhou H, Zhang G, Meng J, Deng K, Zhou W, Wang H, Wang Z, Hu N and Suo Y 2019. Anthocyanins from *Lycium ruthenicum* Murr. ameliorated d-galactose-induced memory impairment, Oxidative stress, and neuroinflammation in adult rats. J. Agricultural and Food Chemistry. **67**(11): 3140-3149.

E Psillakis, N Koutela, AJ Colussi 2019. Vacuum-assisted headspace single-drop microextraction: Eliminating interfacial gas-phase limitations. Analytica Chimica Acta 1092: 9-16.

Gao H, Yuan X, Wang Z, Gao Q and Yang J 2019. Profiles and neuroprotective effects of *Lycium ruthenicum* polyphenols against oxidative stress-induced cytotoxicity in PC12 cells. J. Food Biochem. 44(1): e13112.

Gong Y, Wu J and Li ST 2015. Immuno-enhancement effects of *Lycium ruthenicum* Murr. polysaccharide on cyclophosphamide-induced immunosuppression in mice. International J. Clinical and Experimental Medicine **8**(11): 20631-20637.

Kim S-J, Seo S-O, Jin Y-S and Seo J-H 2013. Production of 2,3-butanediol by engineered saccharomyces cerevisiae. Bioresource Technology **146**: 274-281.

Li H., Deng Z and Zhu H 2012. Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. Food research International **46**(1): 250-259.

Oliver JW, Machado IM, Yoneda H and Atsumi S 2014. Combinatorial optimization of cyanobacterial 2, 3-butanediol production. Metabolic Engineering **22**: 76-82.

Peng Q, Liu H, Shi S and Li M 2014. *Lycium ruthenicum* polysaccharide attenuates inflammation through inhibiting TLR4/NF-κB signaling pathway. International J. Biol. Macromolecules **67**: 330-335.

Qin Y, Yang X, Leng P and Hu Z 2015. Analysis of floral volatile components from 6 Syringa plants collected by dynamic headspace sampling by ATD-GC/MS. Acta Bot. Boreal Occident Sin. **35**: 2078-2088.

Rjeibi I, Feriani A, Ben A, Sdayria J, Saidi I, Ncib S, Souid S, Allagui M and Hfaiedh N 2018. *Lycium europaeum* extract: A new potential antioxidant source against cisplatin-induced liver and kidney injuries in mice. Oxid. Med. Cell. Longev. **5**: 9.

Skenderidis P, Lampakis D, Giavasis I, Leontopoulos S, Petrotos K, Hadjichristodoulou C and Tsakalof A 2019. *Lycium barbarum* chemical properties, fatty-acid composition and antioxidant activity of goji berry (L. and Mill.) fruits. Antioxidants (Basel, Switzerland) **8**(3).

Wang Y, Luan G, Zhou W, Meng J, Wang H, Hu N and Suo Y 2018. Subcritical water extraction, UPLC-Triple-TOF/MS analysis and antioxidant activity of anthocyanins from *Lycium ruthenicum* Murr. Food Chem. **249**: 119-126.

Wu T, Lv H, Wang F and Wang Y 2016. Characterization of polyphenols from *Lycium ruthenicum* fruit by UPLC-Q-TOF/MSE and their antioxidant activity in Caco-2 cells. J. Agricultural and Food Chem. **64**(11): 2280-2288.

Xia Z, Xu TQ, Xu W, Zhang HX. and Zhou GX 2019. *Lycium yunnanense* lyciyunin, a new dimer of feruloyltyramine and five bioactive tyramines from the root of Kuang. Natural Product Res. **1**: 1-8.

Zhu J, Wang L, Xiao Z and Niu Y 2018. Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography-olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC-MS) and flame photometric detection (FPD). Food Chem. **245**: 775-785.

Zhang S, He F, Chen X and Ding K 2019. Isolation and structural characterization of a pectin from *Lycium ruthenicum* Murr and its anti-pancreatic ductal adenocarcinoma cell activity. Carbohydrate Polymers **223**: 115104.

(Manuscript received on 4 June 2020; revised on 12 October 2020)