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Comparative study of polyphenols quantification, total phenolic content, and antioxidant activities of the fruits of three plants of the family of Solanaceae: *Lycium ruthenicum, Lycium barbarum, and Lycium Chinense*

Irma B. Yossa Nzeuwa[1,4,5,7]*, Fatimata Nea[2,3], Junelle Makemteu[1], Gella M. Ngandeu Neubi[4], Florence D. Mabou[5], Jaures A. Noumedem Kenfack[6], Doriane E. Djeussi[6] and Guiju Sun[7]

Abstract

Background: Fruits from *L. ruthenium*, *L. barbarum*, and *L. Chinense*, of the family Solanaceae are well-known in traditional Chinese medicine and have been used as popular functional foods, with a large variety of beneficial health effects.

Methods: In The present study, ethanolic extracts (30%) of lycium fruits were analyzed by high-performance liquid chromatography (HPLC). Total phenolic content was determined together with the quantification of seventeen (17) phenolic compounds. Furthermore, the antioxidant activities of the three plants were investigated *in vitro* through DPPH, ABTS, and FRAP assays.

Results: Results revealed that all three *Lycium* fruits extracts had antioxidant activities. However, *L. ruthenicum* showed the highest radical scavenging capacity. Hydroxycinnamic acid amides (HCAAs) class derivatives including N1, N10-bis(dihydrocaffeoyl)spermidine,N1-bishydrocaffeoyl,N10-caffeoyl spermidine, and N1,N10 -di(caffeoyl) spermidine were dominant in *L. ruthenicum* (15.56-310.80 mg/100g). A significant amount of chlorogenic acid was detected in all the extracts (*L. ruthenicum*: 238.59 mg/100g; *L. barbarum*: 25.76 mg/100g; *L. chinense*: 98.86 mg/100g). Cryptochlorogenic acid was not detected in *L. barbarum,* while protocatechuic acid and neochlorogenic acid were only found in *L. ruthenicum.* The content of caffeoylquinic acid derivatives was particularly high in *L. chinense.* Rutin was detected in all analyzed species, the highest amount being registered for *L.chinense.* (62.56±0.061 mg/100g).

Conclusion: Overall, the results of this study show that Lycium fruit extracts have promising antioxidant potential to be used in food, nutraceutical, and biomedical field. These findings could serve as a scientific foundation for discrimination and quality assessment of the three *Fructus Lycium.*

Keywords: *Antioxidant activities; HPLC; Lycium ruthenicum; Lycium barbarum; Lycium chinense; phenolic content;* Solanaceae.

*Correspondence: Tel.: (237) 698508567 / (032) 465721666; E-mail address: mbelinday@yahoo.fr ; ORCID: https://orcid.org/0000-0002-9972-5532 (Irma Belinda Yossa Nzeuwa)

[1]Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon; [2] Higher Normal School (HNS), Abidjan, Ivory Coast; [3]Laboratory of Constitution and Reaction of the matter, UFR-SSMT, Félix Houphouët-Boigny University, Abidjan, Ivory Coast; [4] Primex Pharma Industries SARL, Douala-Bassa, Cameroon; [5]Department of Chemistry, Faculty of Science, University of Dschang, Dschang, Cameroon; [6]Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon; [7]Key Laboratory of Environmental Medicine Engineering of Ministry of Education, and Department of Nutrition and Food Hygiene, School of Public Health, Southeast University, Nanjing, China

Other authors:
E-mail: neafatima@gmail.com (Fatimata Nea); Email: jinelle3@yahoo.fr, ORCID: https://orcid.org/0000-0002-1109-4079 (Junelle Maemteu); Email: maengandeu@yahoo.fr (Gella M. Ngandeu Neubi); Email: jauresnoume@gmail.com, ORCID: https://orcid.org/0000-0002-5455-2391 (Jaures A. Noumedem Kenfack); Email: princessedoriane@yahoo.fr, ORCID: https://orcid.org/0000-0002-0199-081 (Doriane E. Djeussi); Email: gjsun@seu.edu.cn (Guiju Sun).

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Background

The genus *Lycium* belongs to the Solanaceae family and includes numerous species growing in arid and semi-arid regions, such as South Africa, South America, Europe, and Asia. In China, there are seven species and three varieties, which are mainly distributed in the north and northwest regions of China [1]. However, only *Lycium ruthenicum, Lycium barbarum,* and *Lycium Chinense* are the species referred to as goji berries in China and have been used as medicine and functional foods for at least 2000 years [2-3]. In many countries around the world, the fruits, commonly known as Fructus Lycii are widely consumed fresh, dried, or transformed into food (juice, wine, or tea preparation, in soups, and added to meat and vegetable dishes), functional food and Traditional Chinese Medicine (TCM).

L. ruthenicum, is a traditional herb used to treat menopause, abnormal menstruation, heart disease, and other ailments [4]. Due to its significant nutritive value and medicinal benefits, it has attracted considerable interest in recent years [3,5]. Its fruits were recently reported to exhibit a wide range of beneficial effects including cardioprotective, antiatherosclerotic, antioxidant, cell-mediated, immune-enhancing, anti-tumor, hepatic-function protector, antifatigue, antiaging, hypolipidemic, and hypoglycemic agent [6-8]. The major constituents associated with these pharmacological effects were mainly polysaccharides, phenolics, and anthocyanins [6-8].

L. barbrarum, widely known as Goji berry has also attracted attention as a superfood in Western countries and has become more popular in the last few years due to its public acceptance as a superfood with highly advantageous nutritive and antioxidant properties. Its fruits were reported to be tonic with the functions of nourishing eyes, liver, and kidneys. Previous research on the fruits reported their anti-aging, anti-cancer, and immunity activities [9-11]. *L. barbarum* has been used mainly as an edible and traditional medicinal plant for a long time in Eastern countries, such as China and Korea [12]. Several studies have reported that their fruits contain flavonoids, and polyphenol compounds [13] compared with *L.ruthenicum* and *L. barbarum* , less attention was given so far to *L.chinense*, especially regarding its phenolic profiles and antioxidant property.

Lycium chinense is an—herb that has antioxidant effects. Experimental studies have demonstrated that fruits have a role in anti-aging, immune modulation, anti-fatigue, anti-tumor, and male fertility-enhancing effects [14-16]. Its fruits have drawn the attention of scientists due to their compounds, such as betaine, cerebrosides, glycolipds, polysaccharides which were known to exhibit several important biological properties including hepatoprotection [17] and antioxidant [18].

The importance of plants belonging to the genus *Lycium* (Solanaceae) has increased rapidly over the past two decades due to their traditional usage in Chinese herbal medicine. An increasing number of researchers have studied the chemical content, nutritional values, and various beneficial properties of *Lycium* fruits as a medicinal plant and as functional food [19-24]. Nevertheless, additional research still needs to be done to confirm the quality of the plant and authenticate each gender accordingly and increase our understanding of the pharmacological and nutraceutical properties of *L. barbarum*, *L. ruthenicum,* and *L. chinense*. To the best of our knowledge, a comparative study of the three different species mentioned above was not yet investigated. Therefore, it is necessary to compare the differences between *L. barbarum*, *L. ruthenicum,* and *L. chinense* berries in terms of phytochemical and antioxidant capacities.

The aim of our study was to investigate the chemical composition of fruits of the three mentioned species, namely *L.ruthenicum*, *L.barbarum*, and *L.Chinense*, from the Solanaceae family. Another purpose of this study was to investigate, in a comparative way, the *in vitro* antioxidant activities of three varieties of Lycium fruits by three different procedures including DPPH, ABTS, and FRAP. This will provide scientific insight into the phenolic and antioxidant functions of goji berries to consumers and the nutraceutical industry.

Methods

Plant materials and extraction

The dry fruits of the three *Lycium* varieties were purchased in the local market in Nanjing, China and further identified by Dr Junelle MAKEMTEU, from the Department of pharmaceutical sciences, Faculty of Medicine and Pharmaceutical Science, as the dried and mature fruits of *Lycium barbarum*, *Lycium ruthenicum* and *Lycium chinense*. Each sample (15 g of whole dried fruits) was cut into small pieces and extracted with 10 mL of 30% methanol (*v/v*) under reflux for 1 h (n=2). The combined extracts were evaporated under reduced pressure at 40°C. Then, the obtained residue was dissolved with 30% methanol to half of the original volume and subsequently centrifuged at 16,000 rpm for 5 minutes and stored at 4°C for further analyses.

Chemicals and reagents

1,1-Dipheny-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (D-9132), while 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), Trolox and the FRAP assay kit were purchased from the Beyotime Institute of Biotechnology (S0119 and S0116, Nantong, China). HPLC-grade acetonitrile, formic acid (J.T.Baker, Phillipsburg, NJ, USA). Protocatechuic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, P-coumaric acid, and N1, N10-bis (P-coumaric acid) spermidine standards were from Shanghai Yuanye Biotechnology (Shanghai, China) while rutin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The other solvents were from Nanjing Chemical Corporation (Nanjing, China). Ultra-pure water was used for all analyses.

HPLC-DAD quantitative analysis

HPLC analysis was carried out on a Shimadzu series 2010 HPLC instrument (Shimadzu Corp., Kyoto, Japan) equipped with a quaternary pump, a diode-array detector, an autosampler, and a Lichrospher C_{18} column (300 mm×4.6 mm, 5µm) from Jiangsu Hanbon Sci. & Tech. Corp. (Nanjing, Jiangsu, China). An aliquot of 20 µL solution was injected for analysis. Chromatograms were obtained at 280 nm. The mobile phase consisted of 1% formic acid (containing 10 mmol/L ammonium acetate) (A) and acetonitrile (B). The gradient elution program was as follows: 5-12% B for 0-25 min, 12-13% B for 25-42 min, 13-25% B for 42-60 min, 25-40% B for 60-70 min, 40-100% B for 70-73 min. The flow rate was 1.0 mL/min, and the column temperature was 30°C.

Determination of total phenolic content (TPC)

The TPC was determined by Folin–Ciocalteu's reagent (FCR) [25]. Briefly, 1.0 mL of each sample or standard solution was combined with 1.0 mL of FCR (1:10, v/v) and 1.0 mL of 7.5% Na_2CO_3 solution (w/v). After 30 min incubation at room temperature in the dark, the fluorescence reading of the incubation against a reagent blank was measured at 765 nm. The TPC values of the tested analytes were expressed as gallic acid equivalent mg per gram of dry weight (mg GAE/g, DW).

Antioxidant capacity assays

Vitamin C equivalent antioxidant capacity (DPPH) assay

The stable radical DPPH assay was carried out at room temperature by the method described by Gouveia-Figueira & Castilho (2015) [26], with slight modifications. In brief, 1 mL of each sample extract and Vitamin C solutions (15-150 µM) was added to 4.0 ml DPPH (0.09 mM in ethanol). The solution was mixed vigorously and allowed to react for 30 min. Absorbance was then read at 517 nm against ethanol as blank. Results were expressed as Vitamin C equivalent µmol per gram of dry weight (VCE µmol/g, DW).

Trolox equivalent antioxidant capacity (ABTS) assay

For ABTS assay, the procedure followed the method of Arnao et *al*. (2001) [27] with some modifications. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h to 16h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 1.1±0.02 units at 734 nm using the spectrophotometer. Fruit extracts (150 µL) were allowed to react with 2850 µL of the ABTS solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed in µM Trolox equivalents (TE)/g dry weight.

Ferric reducing antioxidant power *(FRAP) assay*

The principle of this approach is based on the reduction of Fe (III)-TPTZ (2, 4, 6-tripyridyl-s-triazine) to Fe(II)-TPTZ by the antioxidants and subsequent formation of the blue complex following the residual Fe(III). The FRAP reagent was freshly prepared by mixing TPTZ (2,4,6-tripyridy-s-triazine) dilution, (10 mmol/L) in HCl (40 mmol/L) plus 5 mL of $FeCl_3$ (20 mmol/L) and 50 mL of acetate buffer (0.3 mol/L, pH 3.6) at 37 °C for 1h. 10 µL of test samples or standard solutions were mixed with 180 µL FRAP working solution and the microplate was allowed to warm for 5 min. The calibration curve was constructed using five concentrations of FeSO4 7H2O (1000, 750, 500, 250, 125 µmol ⁄ L) and the absorbance was measured for each sample. The absorbance of the reaction mixture was measured at 593 nm. Results were given as Fe(II) equivalent µmol per gram of dry weight (Fe(II) µmol/g, DW). All the measurements were taken in triplicate and expressed as mean value ± standard deviation (SD)

Statistical analysis

The results are reported as the mean (±) standard deviation (SD) from triplicate analyses. Data analyses were performed using the GraphPad Prism 5.0 software.

Results and discussion

HPLC-DAD Quantitative analysis

The assay results are summarized in Table 1. The results indicated that there were distinctive differences in the chemical constituents and corresponding contents of the seventeen compounds among the three extracts. hydroxycinnamic acid amides (HCAAs) class derivatives including N1, N10-bis(dihydrocaffeoyl)spermidine, N1-bishydrocaffeoyl, N10-caffeoyl spermidine, and N1, N10 -di(caffeoyl)spermidine were dominant in *L. ruthenicum* (15.56-310.80 mg/100g). Important amount of chlorogenic acid was detected in all extracts (*L. ruthenicum*: 238.59 ± 0.088 mg/100g; *L.barbarum*: 25.76 ± 0.202 mg/100g; *L. barbrarum. Var.*: 98.86 ± 0.176 mg/100g). Cryptochlorogenic acid was not detected in *L. barbarum,* while protocatechuic acid and neochlorogenic acid were only found in *L. ruthenicum.* Noteworthy, *p*-coumaric acid derivatives were the predominant components of *L. barbarum,* but the content of caffeoylquinic acid derivatives was particularly high in *L. barbrarum.Var*. Rutin, as the dominant flavonoid, was detected in all analyzed species, the highest amount being registered for *L. barbrarum.Var.*(62.56 ± 0.061 mg/100g). Our results were in fair accordance with the reported literature. Donno et *al*. [28], Wang et *al*. [20] and Mocan et *al*. [29] showed that *Lycium barbarum* contains chlorogenic acid with values of 113.18 g/100g of fresh weight, 12.40 mg/g of extract and 5899.29 µg/g of plant material, Coumaric acid 111.32 g/100g of fresh weight, 6.06 mg/g of extract and 30.29 µg/g of plant material. Chlorogenic acid content of *L. ruthenicum* was relatively in accordance with the results reported in our previous study (within the range 0.57-2.15 mg/g), while rutin content was three times lower (between 0.1-0.21 mg,g) [9]. This difference observed might be due to the harvest time and the difference in soil composition.

Total phenolic content (TPC)

Total phenolic content (expressed as mgGAE/g) of all *Lycium* dry fruits samples is shown in Table 2. From the results, we can clearly observe that *L. ruthenicum* sample had the highest amount of TPC (69 ± 2.64 mg GAE/g), compared to 20 ± 1.72 mg GAE/g for *L.barbarum* and 14 ± 2.98 mg GAE/g for *L.chinense, L. ruthenicum* TPC value was found to be 5 times higher than the value of *Lycium chinense* sample.The TPC sequence was as followed: *L.ruthenicum* > *L.barbarum* > *L.chinense.* The considerable amount of TPC indicated that *L.ruthenicum* fruits extract might have a high potential antioxidant activity.

Antioxidant capacity assays

Results in Table 2 showed that *L.ruthenicum* extract obtained the highest antioxidant activity (212 ± 3.02 VCE µmol/g) in the DPPH assay, followed by *L.chinense* (98 ± 2.35 VCE µmol/g) and *L. barbrarum.Var.* (90 ± 1.69 VCE µmol/g). The results of ABTS and FRAP were similar, both showed that *L.ruthenicum* possessed the maximal scavenging activity (248 ± 2.98 TE µmol/g for ABTS, 400 ± 8.02 Fe(II) µmol/g for FRAP), followed by *L.chinense* (60 ± 1.11 TE µmol/g for ABTS, 65 ± 3.58 Fe(II) µmol/g for FRAP) and *L.*

barbrarum.Var. (56 ± 4.72 TE µmol/g for ABTS, 63 ± 1.89 Fe (II) µmol/g for FRAP). Results of the current study corroborate those of a previous study in which it was proven that the phenolics and antioxidant capacities are much higher in black goji berry than in red goji berry [30]. Nevertheless, studies previously reported are not totally consistent with our results, this might be due to the different methodology used [23-24]. The linear correlation between the TPC data and the antioxidant assay (DPPH, ABTS, FRAP) values demonstrated strongly positive relationships (R^2 = 0.9983, 0.9934, 0.9911, respectively), which indicated that the antioxidant abilities of these species are caused particularly from polyphenols. Kulczyński and Gramza-Michałowska from an analysis of previous studies related the scavenging activity against free radicals (superoxide anion, hydroxyl radicals) and antioxidative activity of *L. barbarum* to carotenoid pigments, flavonoids, polysaccharide fraction and vitamin analog C-2-O-(beta-D-glucopyranosyl) ascorbic acid [31].

Table 1. Contents (mg/100g, DW) of seventeen marker compounds in three Fructus lycii.

Compound	Contents (mg/100g) (mean ± SD)		
	L . ruthenicum Murr.	***L. barbarum***	***L. chinense.***
Caffeoylquinic acid hexose[a]	107.63 ± 0.010	21.57 ± 0.125	78.60 ± 0.007
Protocatechuic acid	23.29 ± 0.046	ND	ND
Neochlorogenic acid	14.20 ± 0.102	ND	ND
Caffeoylquinic acid hexose[a]	ND	17.92 ± 0.179	21.86 ± 0.231
Chlorogenic acid	238.59 ± 0.088	25.76 ± 0.202	98.86 ± 0.176
Caffeoylquinic acid hexose[a]	ND	17.92 ± 0.034	9.07 ± 0.003
Coumaric acid hexose[b]	19.51 ± 0.131	38.24 ± 0.006	16.98 ± 0.201
Cryptochlorogenic acid	33.01 ± 0.095	ND	7.25 ± 0.096
Caffeic acid	12.24 ± 0.004	24.01 ± 0.075	11.44 ± 0.011
N^1,N^{10}-bis(dihydrocaffeoyl)spermidine[c]	107.95 ± 0.022	6.76 ± 0.009	8.76 ± 0.129
N^1-caffeoyl,N^{10}-dihydrocaffeoylspermidine[c]	84.39 ± 0.139	9.39 ± 0.057	7.45 ± 0.042
N^1-dihydrocaffeoyl,N^{10}-caffeoylspermidine[c]	310.80 ± 0.037	7.14 ± 0.013	4.16 ± 0.008
p-Coumaric acid	12.15 ± 0.006	13.98 ± 0.092	8.84 ± 0.053
N^1,N^{10}-bis(caffeoyl)spermidine[c]	44.92 ± 0.051	ND	ND
p-Coumaric acid[b]	ND	8.69 ± 0.065	5.10 ± 0.276
N^1-dihydrocaffeoyl,N^{10}-coumaroyl spermidine[c]	15.56 ± 0.276	ND	ND
Rutin	59.85 ± 0.032	31.35 ± 0.033	62.56 ± 0.061

ND, not detected. [a] Semi-quantified using the calibration curve of chlorogenic acid; [b] Semi-quantified using the calibration curve of p-coumaric acid; [c] Semi-quantified using the calibration curve of N^1, N^{10}-bis(p-coumaroyl) spermidine.

Table 2. Results of the TPC and DPPH, ABTS, FRAP free radical-scavenging activity of three Lycii fructus.

Assays	***L. ruthenicum***[a]	***L. barbarum***[a]	***L. chinense***[a]	Standard curve
TPC (mg GAE/g)	69 ± 2.64	20 ± 1.72	14 ± 2.98	y= 0.0348x + 0.0386 R^2 =0.9993
DPPH (VCE µmol/g)	212 ± 3.02	98 ± 2.35	90 ± 1.69	y=−0.0047x + 0.8447 R^2 =0.9991
ABTS (TE µmol/g)	248 ± 2.98	60 ± 1.11	56 ± 4.72	y=−0.0014x + 0.6302 R^2= 0.9994
FRAP (FeII µmol/g)	400 ± 8.02	65 ± 3.58	63 ± 1.89	y= 0.5703x−0.0825 R^2= 0.9998

GAE/g: gallic acid equivalent/g; VCE/g; TE/g: trolox equivalent/g; [a] Data are represented as the mean±SD from triplicate analyses (n=3).

Conclusion

In this paper, we clearly showed effective (DPPH, ABTS, and FRAP) antioxidant capacity properties of the three studied Lycium species. Furthermore, the antioxidant assessments demonstrated that reducibility varied remarkably across three species, and *L.ruthenicum* possessed the highest performance in all antioxidant tests. The current study does not only mark a new insight into the phenolic components in three Fructus Lycii but also provides quite valuable information for their authentication and their quality assessment.

Abbreviations

DPPH: 2.2-diphényl 1-pycrilhydrazyl
FRAP. Ferric Reducing Antioxidant Power
HCAAs: Hydroxycinamic Acid Amids
HCAs: Hydoxycinamic acids
HPLC: High Perfomance Liquid Chromatography
TCM: Traditional Chinese Medicine
TPC: Total Phenolic Content
ANOVA: Analysis of Variance
FCR: Folin Ciocalteu reagent
TE: Trolox Equivalent
TPTZ: 2, 4, 6-tripyridyl-s-triazine
DW: Dry Weight
SD: Stabtard Deviation
DAD: Diode Array Detector

Authors' Contribution

IBYN, FN, and FDM contributed to total phenolic content, and polyphenols quantification. JM identified the three plants. GMNN, DED and JANK carried out the antioxidant activity of samples. IBYN, FN, FDM, and GMNN wrote the original draft. JM, DED and JANK methodology. GJS supervised the work. All authors have read and agree to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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References

1. Yao R, Heinrich M, Zou Y, Reich E, Zhang X, Chen Y, Weckerle SC. 2018. Quality Variation of Goji (Fruits of *Lycium* spp.) in China: A Comparative Morphological and Metabolomic Analysis. *Front Pharmacol*. 9:151-62.
2. Ulbricht C, Bryan JK, Costa D, Culwell S, Giese N, Isaac R, Nummy K, Pham T, Rapp C, Rusie E, Weissner W, Windsor RC, Woods J, Zhou S. 2015. An Evidence-Based Systematic Review of Goji (*Lycium* spp.) by the Natural Standard Research Collaboration. *J Diet Suppl*. 12: 184-240.
3. Zheng J, Ding CX, Wang L S, Li G L, Shi Y, Li H, Wang H L, Suo YR. 2011. Anthocyanins composition and antioxidant activity of wild *Lycium ruthenicum Murr.* from Qinghai-Tibet Plateau. *Food Chem.* 126: 859-65.
4. Lu W Y, Jiang Q Q, Shi H M, Niu Y, Gao B, Yu LL. 2014. Partial Least-Squares-Discriminant Analysis Differentiating Chinese Wolfberries by UPLC-MS and Flow Injection Mass Spectrometric (FIMS) Fingerprints [J]. *J Agric Food Chem*.62: 9073−80
5. Liu Z, Dang J, Wang Q, Yu M, Jiang L, Mei L, Shao Y, Tao Y. (2013). Optimization of polysaccharides from *Lycium ruthenicum* fruit using RSM and its antioxidant activity. *Int J Biol Macromol.* 61, 127-134
6. Nzeuwa IBY, Guo B, Zhang T, Wang L, Ji Q, Xia H, Sun G. 2019. Comparative metabolic profiling of lycium fruits (*Lycium barbarum* and *Lycium chinense*) from different areas in China and from Nepal. *J Food Qual.* 2019: 4396027.
7. Peng Q, Lv X, Xu Q, Li Y, Huang L, Du Y. 2012. Isolation and structural characterization of the polysaccharide LRGP1 from Lycium ruthenicum. *Carbohydr Polym.* 90(1), 95–101.
8. Yossa NIB, Xia H, Shi Y, Yang C, Waseem SM, Guo B, Wang L, Sun G. 2020. Fatty acid and mineral contents of *Lycium ruthenicum* Murr. and antioxidant activity against isoproterenol-induced acute myocardial ischemia in mice. *Food Sci Nutr.* 8(2): 1075-81.
9. Amagase H., Farnsworth N.R. 2011. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). *Int Food Res J.* 44: 1702-17.
10. Su CX, Duan XG, Liang LJ, Wang F, Zheng J, Fu XY, Yan Y M, Huang L, Wang NP. 2014. *Lycium barbarum* polysaccharides as an adjuvant for recombinant vaccine through enhancement of humoral immunity by activating Tfh cells. *Vet Immunol Immunopathol.* 158: 98-104.
11. Tang W M, Chan E, Kwok CY, Lee Y K, Wu JH, Wan C W, Chan R Y, Yu PH, Chan S W. 2012. A review of the anticancer and Immunomodulatory effects of *Lycium barbarum* fruit. *Inflammopharmacology*. 20:307-14.
12. Mocan A, Vlase L, Vodnar DC, Bischin C, Hanganu D, Gheldiu A-M, Oprean R, Silaghi-Dumitrescu R, Crișan G. 2014. Polyphenolic Content, Antioxidant and Antimicrobial Activities of *Lycium barbarum* L. and *Lycium chinense* Mill. Leaves. M*olecules 19*(7): 10056-73.
13. Hempel J, Schädle CN, Sprenger J, Heller AR, Reinhold C, Schweiggert RM. 2017. Ultrastructural deposition forms and bioaccessibility of carotenoids and carotenoid esters from goji berries (*Lycium barbarum* L.). *Food Chem*. 218: 525-33.
14. Amagase, H., Sun, B. & Borek, C. 2009. *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nut Res*. 29: 19-25.
15. Li XM., Ma YL, Liu XJ. 2007. Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *J Ethnopharmacol.* 111: 504-11.
16. Lin C L, Wang CC, Chang SC, Inbaraj BS, Chen BH. 2009. Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. *Int J Biol Macromol.* 45:146-51.
17. Zhang R, Kang KA, Piao MJ, Kim KC, Kim AD, Chae S, Park JS, Youn UJ, Hyun JW. 2010. Cytoprotective effect of the fruits of *Lycium chinense* Miller against oxidative stress-induced hepatotoxicity. *J. Ethnopharmacol*. 130: 299–306.
18. Chung I M, Ali M, Praveen N, Yu B R, Kim S H, Ahmad A. 2014. New polyglucopyranosyl and polyarabinopyranosyl of fatty acid derivatives from the fruits of *Lycium chinense* and its antioxidant activity. *Food Chem*. 151 : 435–43.
19. Gao K, Ma D, Cheng Y, Tian X G, Lu YY, Du XY, Tang H F, Chen J Z. 2015. Three New Dimers and Two Monomers of Phenolic Amides from the Fruits of *Lycium barbarum* and Their Antioxidant Activities. *J Agric Food Chem.* 63: 1067-75.
20. Wang, C. C., Chang, S. C., Stephen, I. B. & Chen, B. H. (2010). Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum L.* and evaluation of antioxidant activity. *Food Chem.* 120: 184-192.
21. Yan YM, Ran LW, Cao Y L, Qin K, Zhang X, Luo Qi, Jabbar S, Abid M, Zeng XX. 2014. Nutritional, Phytochemical Characterization and Antioxidant Capacity of Ningxia Wolfberry (*Lycium barbarum L.*). *J Chem Soc Pak.* 36: 1079-1087.
22. Zhang JX, Guan SH, Yang M, Feng RH, Wang Y, Zhang YB, Chen X, Chen XH, Bi KS, Guo DA. (2013). Simultaneous determination of 24 constituents in Cortex Lycii using high-performance liquid chromatography triple quadrupole mass spectrometry. *J Pharm Biomed Anal.* 77: 63-70.
23. Le K, Chiu F, Ng K.2007. Identification and quantification of antioxidants in *Fructus lycii*. *Food Chem.* 105: 353-63.
24. Osman NI, Awal A, Sidik NJ, Abdullah S. 2012. Antioxdant activities of *in vitro* seedlings of *Lycium barbarum* (Goji) by diphenyl picrylhydrazyl (DPPH) assay. *Int J Clin Pharmacol.* 4:137-41.
25. Spínola V, Pinto J, Castilho PC. 2015. Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD–ESI-MS^n and screening for their antioxidant activity. *Food Chem*. 173:14-30.
26. Gouveia-Figueira SC, Castilho PC. 2015. Phenolic screening by HPLC-DAD-ESI/MS^n and antioxidant capacity of leaves, flowers and berries of *Rubus grandifolius Lowe*. *Industrial Crops and Products*. 73: 28-40.
27. Arnao MB, Cano A, Acosta M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem*. 73: 239-44.
28. Donno D., Beccaro G.L., Mellano M.G., Cerutti A.K., Bounous G. 2015. Goji berry fruit (Lycium spp.): antioxidant compound fingerprint and bioactivity evaluation. *J Funct Food.* 18: 1070-1085.
29. Mocan A., Vlase L., Vodnar D.C., Bischin C., Hanganu D., Gheldiu A., Operan R., Silaghi-Dumitrescu R., Crisan G. 2014. Polyphenolic content, antioxidant and antimicrobial activities of Lycium barbarum L. and Lycium chinense Mill. leaves. Molecules. 19: 10056–10073.
30. Islam T, Yu X, Badwal TS, Xu B. 2017. Comparative studies on phenolic profles, antioxidant capacities and carotenoid contents of red goji berry (Lycium barbarum) and black goji berry (Lycium ruthenicum). *Chem Cent J.* 11(1):59.
31. Bartosz Kulczyński, Anna Gramza-Michałowska. 2015. Goji Berry (Lycium barbarum): Composition and Health Effects – a Review. *Polish J Food Nutr Sci.* 66(2): 67–75.