

## ***Lycium barbarum* L. JUICE - NATURAL SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS**

**Adrian ASĂNICĂ<sup>1</sup>, Carmen MANOLE<sup>2,3</sup>, Valerica TUDOR<sup>1</sup>, Andreea DOBRE<sup>2</sup>,  
Răzvan Ionuț TEODORESCU<sup>1</sup>**

<sup>1</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd,  
District 1, 011464, Bucharest, Romania, E-mail: asanica@gmail.com

<sup>2</sup>University of Agronomic Sciences and Veterinary Medicine of Bucharest - Research Center  
for Studies of Food Quality and Agricultural Products, 59 Mărăști Blvd, District 1,  
011464, Bucharest, Romania, E-mail: manolecarmen2000@gmail.com

<sup>3</sup>The National Institute of Research and Development for Biological Sciences,  
296 Splaiul Independenței Avenue, District 6, 060031, Bucharest, Romania,  
E-mail: dobre\_mc\_andreea@yahoo.com

Corresponding author email: manolecarmen2000@gmail.com

### **Abstract**

*The objective of this study was to evaluate the quantity of some phenolic compounds, free radical scavenging activity and antibacterial activity of goji berry fruits (*Lycium barbarum* L.) in order to highlight which of these biotypes fulfills the best nutraceutical qualities. Freshly harvested fruits of three goji berry biotypes ('Biotype 1(B1)', 'Biotype 2' (B2), and 'Biotype Ua' (BUa) were smashed and the juice was subjected to analysis. The phenolic compounds were analyzed as follows: total phenolic content (TPC) expressed as gallic acid equivalents (GAE), total flavonoid content (TFC) expressed as g of rutin equivalents (RE), and free radical scavenging activity expressed as inhibition % (I %). The evaluation of antibacterial activity consisted of using Gram positive and negative bacteria based on the standard agar disk diffusion method. With regard to the TPC, all analysed juices have revealed 'Biotype BUa' with a maximum content of 8.95 mM ± 0.48 GAE / ml juice. Also, the same biotype recorded high levels of free radical inhibition rate of 40%. In terms of flavonoid content, 'Biotype 1' highlighted the best results. With regard to the evaluation of antibacterial activity, all juices showed good results. The most susceptible to all three juices was *S. aureus* and the least, *P. aeruginosa*. The highest inhibitory activity was registered in the case of 'Biotype 1' juice against *E. coli*, showing an average inhibition diameter of 1.84 ± 0.13 and 1.81 ± 0.20 cm.*

**Key words:** antibacterial, biochemical compounds, goji berry fruits, goji berry juice.

### **INTRODUCTION**

*Lycium barbarum* L., also known as goji, is a shrub belonging to the Solanaceae family and is native to Asia.

In Romania, the research studies about *Lycium barbarum* L. are scarce (Mencinicopschi et al., 2012), though given its growing popularity can be a profitable investment.

Over the years, to many regions of the world, *Lycium barbarum* L. has been used in hypotensive and immunomodulatory drugs, or as a potential agent in cancer chemotherapy (Naghbi et al., 2014). Also, is wide known to have beneficial effects against fatigue, aging, glaucoma and to enhance neuroprotection and

cytoprotection (Amagase and Farnsworth, 2011). *Lycium barbarum* L. enhance the immunity due to its content in LBP which stimulates the T lymphocytes synthetisation (Chen et al., 2008). Cong et al. (2005) stated that the extracted polysaccharides have a potential effect in promoting the haematoproteic growth in the recovery of bone marrow injury in the case of animals. The fruit of *Lycium barbarum* L. is abundant in taurine which has been reported as beneficial as treatment for diabetic retinopathy (Song et al., 2011). However, *Lycium barbarum* L. shows a high allergenic potential, being related with the LTPs (plant lipid transfer proteins) presence (Carnés et al., 2013).

Another species, *Lycium chinese* M. was used in traditional Chinese medicine to treat several inflammation-related symptoms, like diabetes mellitus, the main responsible compound for this being the phenolic amides from its Radicis Cortex (Xie et al., 2014).

The fruits of *Lycium barbarum* L. contain phenolics like caffeic acid, p-coumaric acid, rutin, scopoletin, N-trans-feruloyl tyramine, N-cis-feruloyl tyramine, N-feruloyl tyramine dimer (Forino et al., 2016), kaempferol glycosides, isomers of dicaffeoylquinic acid and coumaric acid (Bondia-Pons et al., 2014). Zhang et al. (2016) detected a series of flavonoids like quercetin-rhamno-di-hexoside and quercetin-3-O-rutinoside and also phenolic acid (chlorogenic acid), a carotenoid (zeaxanthin), etc. It also contains catechins (catechin, epicatechin), monoterpenes (phellandrene, sabinene,  $\gamma$ -terpinene), organic acids (citric acid, malic acid, oxalic acid, quinic acid, tartaric acid) and vitamin C (Donno et al., 2015).

Chung et al. (2015) identified from *Lycium chinese* M. fruit, constituents like labd-3 $\beta$ , arabinofuranosyl-2dp-hydroxybenzoate, and  $\beta$ -sitosterol- $\beta$ -D-glucoside.

According to Mocan et al. (2015), the leaves of *Lycium barbarum* L. contain as polyphenolic compounds, gentisic acid, chlorogenic acid, caffeic acid, p-coumaric acid, sinapic acid, ferulic acid, isoquercitrin, rutin, quercetin, kaempferol and patuletin.

Amagase et al. (2009) determined the *in vivo* antioxidant activity of *Lycium barbarum* L. polysaccharides (LBP) by investigating their effect from LBP-standardized *Lycium barbarum* L. preparation (GoChi) after a trial of 30 days. They registered, on a population formed by 50 Chinese healthy adults aged 55 to 72 years, a significantly increase of the values of antioxidant markers with 9.9% for GSH-Px (glutathione peroxidase) and with 8.4% for SOD (superoxide dismutase), whereas MDA (malondialdehyde) were significantly decreased by 8.7%.

In 2015, Yang et al. isolated the LBPs from *Lycium barbarum* L., having the highest yield by ultrasound-enhanced subcritical water extraction (USWE), 14.1%. The four extracted LBPs showed concentration dependant DPPH radical scavenging activities in a range of 0.5 -

5 mg/ml. Between 2.5 and 5.0 mg/ml, the LBPs produced over 50% suppression of DPPH.

Dahech et al. (2013) studied the antioxidant and antibacterial effects of infusion, macerate in ethanol and fractional extracts with solvents of increasing polarity of another species, *Lycium shawii*. He determined the total flavonoid and phenolic contents which varied from 3.3 to 110.6 mg quercetin/g DW and 100 to 377 mg GAE/g DW. They also showed that the fruits of *Lycium shawii* have antibacterial extract over a series of Gram-positive and Gram-negative bacteria like *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Micrococcus luteus* ATCC 1880, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* CIP 32147 etc.

Also, the same strains of *E. coli* and *S. aureus* were interacted with alcoholic extracts of *Lycium barbarum* L. by Mocan et al. (2014), showing inhibition zones of  $1.31 \pm 0.09$  cm for *S. aureus* ATCC 25923 and of  $1.23 \pm 0.08$  cm for *E. coli* ATCC 25922.

Therefore, this paper aims to study biochemical composition and also antibacterial effect of the goji fruit juice belonging to different biotypes. Also, the study aims to emphasize to the consumer which is the biotype that provide the best nutraceutical qualities.

## MATERIALS AND METHODS

The biological material was represented by the fruits provided from the 3 biotypes of goji berry (*Lycium barbarum* L.). Fruits from the 'Biotype 1' (B1), 'Biotype 2' (B2) and 'Biotype Ua' (BUa) were harvested until the end of September.

The fruits were smashed and the juice was subjected to further analysis.

The total phenolic content of the juices was determined using a method adapted after Singleton et al. (1999). The proper diluted samples were oxidized with the Folin - Ciocálteu reactive and neutralized with sodium carbonate 30%. After 45 minutes, the absorption of the samples was recorded at the wavelength ( $\lambda$ ) of 750 nm. Quantifying the

results was based on the sample curve of the gallic acid, based on the equation 1.

$$\text{Abs} = 0.0204 + 0.0002 \times C_{\text{gallic acid}} \quad (1)$$

The results were expressed as gallic acid equivalents (GAE) / ml juice.

The flavonoid content was determined using a method adapted after Toker et al. (2012) using rutin as standard. The diluted juice was mixed with a sodium nitrite (NaNO<sub>2</sub>) 5%. After 5 minutes was added aluminum chloride (AlCl<sub>3</sub>) 10%. After 6 minutes, NaOH of 1M concentration and water were added too.

After an incubation time of 45 minutes, the absorption was measured at the wavelength (λ) of 510 nm. The results were then obtained based on the sample curve of the rutin, equation 2.

$$\text{Abs} = 0.004 + 0.0001 \times C_{\text{rutin}} \quad (2)$$

The results were then shown as rutin equivalents (RE) /ml juice.

The free radical scavenging activity of the extracts was determined using stable radical 2,2 diphenyl-1-picrylhydrazyl (DPPH•), after a method adapted after Marinova et al. (2011). The inhibitory effect (I %) of DPPH was calculated using the equation 3.

$$I (\%) = \frac{[\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}]}{\text{Absorbance}_{\text{control}}} \times 100 \quad (3)$$

The statistical significance was considered for the probability value of difference p<0.05. The obtained results were expressed as mean values ± standard deviation (SD).

#### *Biological materials, growth conditions and inoculation*

They were used two Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, and also 2 Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923 and *B. cereus* (B5). The strains were isolated from *Allium cepa* L. rhizosphere and identified with Biolog GEN III (data not shown). The bacteria were precultivated at 30°C on Nutrient Agar (Liofilchem) for 24 h. The inoculum required for screening was realized at the value of 0.5

for McFarland Scale (less than 300 CFU x 10<sup>6</sup>/mL).

#### *Screening for susceptibility determination of bacteria to the action of goji juices*

The screening method was derived from standard agar disk diffusion method by using wells instead of discs (NCCLS, 2008). The culture medium used for screening was Mueller Hinton Agar (Liofilchem), 12 ml being poured in each Petri dish (Ø 90 mm). After polymerisation, 1 ml of bacteria inoculum was spread on each Petri dish and the surplus was removed. After the culture medium surface has dried, they have been made, with a corkscrew, three wells with a diameter of 6 mm, arranged equidistant from one another and half way between center and edge of the Petri plate. In each well they were poured 75 µl of juice. The control plates used for the experiments followed a similar protocol using standardized antibiotic discs. The antibiotics used were Gentamicin (CN10) and Ampicilin (AM10) (Comuzzi et al., 2001; Braga et al., 2005; NCCLS, 2008). The bacteria inoculum was cultivated at 30°C for 18 h in interaction with the crude vegetal extracts. The experiments were realised in triplicate.

The results were expressed as mean values ± standard deviation, representing two perpendicular diameters (D1 and D2).

## RESULTS AND DISCUSSIONS

The results of the biochemical examination of the goji juices are shown in Table 1.

According to them, it can be seen as the highest phenolic content was reported for the BUa biotype.

Table 1. Total phenolic and flavonoid contents values of *Lycium barbarum* L. biotypes

Name	Mean mM GAE / ml juice	Mean mM RE / ml juice	Inhibition rate %
BUa	8.95 ± 0.48	12.58 ± 0.15	40.08 ± 0.21
B1	6.77 ± 0.27	14.00 ± 0.40	31.32 ± 0.00
B2	6.97 ± 0.18	11.36 ± 0.39	24.50 ± 0.33

In terms of the flavonoid content was revealed the B1, with a value of 14.00 mM RE/ml juice. With regard to the flavonoids, there are several studies (Amagase and Farnsworth, 2011)

confirming their goji fruits (fresh or dried). In this regard, Le et al. (2007) presented the research study about *Fructus lycii*. The results showed that *Fructus lycii* have antioxidative activities and is rich in flavonoids.

The scavenging activity of the juices against free radical DPPH, highlighted BUa with a value of 40% rate of inhibition. The values

obtained in this study are lower than those obtained by Istrati et al. (2013).

The antimicrobial effect of *Lycium barbarum* L. juice over a series of Gram positive and Gram negative bacteria was studied and the results are showed in the Table 2.

Table 2. The inhibitory effect of *Lycium barbarum* L. juices over a series of bacteria

	Biotype						Control			
	BUa		B1		B2		Gentamicin		Ampicillin	
	D1 (cm)	D2 (cm)	D1 (cm)	D2 (cm)	D1 (cm)	D2 (cm)	D1 (cm)	D2 (cm)	D1 (cm)	D2 (cm)
<i>B. cereus</i>	1.48 ± 0.24	1.36 ± 0.12	1.40 ± 0.24	1.31 ± 0.15	1.18 ± 0.11	1.13 ± 0.18	2.3 ± 0.1	2.27 ± 0.81	0.8 ± 0.00	0.8 ± 0.00
<i>E. coli</i>	1.43 ± 0.14	1.28 ± 0.10	1.84 ± 0.13	1.81 ± 0.20	1.32 ± 0.08	1.37 ± 0.08				
<i>P.aeruginosa</i>	0.00	0.00	1.28 ± 0.16	1.29 ± 0.11	1.07 ± 0.09	0.95 ± 0.05				
<i>S. aureus</i>	1.79 ± 0.23	1.69 ± 0.25	1.79 ± 0.10	1.74 ± 0.19	1.63 ± 0.21	1.59 ± 0.29				

The most susceptible bacteria to BUa juice was *S. aureus* showing average inhibition diameters of  $1.79 \pm 0.23$ , respectively  $1.69 \pm 0.25$  cm. *P. aeruginosa* was resistant to BUa juice.

With regard to *B. cereus* and *E. coli*, the results were similar. Thus, in the case of first one was registered an average inhibition diameter of  $1.48 \pm 0.24$  and  $1.36 \pm 0.12$  cm. In the case of *E. coli*, the average was  $1.43 \pm 0.14$  and  $1.28 \pm 0.10$  cm.

The most susceptible bacteria to B1 juice was *E. coli* with the values of  $1.84 \pm 0.13$  and  $1.81 \pm 0.20$  cm. The second most susceptible bacteria was *S. aureus* which showed values as  $1.79 \pm 0.10$  and  $1.74 \pm 0.19$  cm. The lowest susceptibility to the juice was recorded by *P. aeruginosa*.

The B1 juice had a higher inhibitory effect than BUa juice over the entire series of bacteria.

The B2 juice registered the highest inhibitory activity in the case of *S. aureus* strain and the lowest in the case of *P. aeruginosa* (Table 1).

*B. cereus* is the most susceptible to BUa juice. B2 juice is the one that had the lowest inhibitory effect, recording the average diameters (cm) of halos  $1.18 \pm 0.11$  and  $1.13 \pm 0.18$  cm.

*S. aureus* was the most susceptible to all *Lycium barbarum* L. juices, showing similar results for BUa and B1. B2 juice had the lowest inhibitory effect on *S. aureus* than the other two (B1 and BUa). The results obtained are encouraging compared with the results

obtained by Fiş et al. (2013) were the extracts (aqueous and a macerate), obtained from rehydrated fruits didn't present any inhibitory activity against *S. aureus*.

The same fruit extracts were tested by Fiş et al. (2013) on *E. coli* showing halos of inhibition with diameters ranging in size between 2.2 and 2.5 cm. In the case of our experiments, *E. coli* registered the highest susceptibility with the values of  $1.84 \pm 0.13$  cm and  $1.81 \pm 0.20$  cm interacting with B1 juice. The B2 and BUa juices had a lower inhibitory effect on *E. coli* than B1 juice.

The second Gram negative bacteria, *P. aeruginosa* was the most resistant one to all *Lycium barbarum* L. juices. BUa biotype has no effect, at the opposite being B1 juice, which recorded  $1.28 \pm 0.16$  and  $1.29 \pm 0.11$  cm.

Alassadi et al. (2015) tested the same strains of *E. coli* and *S. aureus*, with a *Lycium barbarum* L. ethanolic extract (80%). The obtained results using ethanolic extracts were 1.5 cm in case of *S. aureus* and 1.2 cm in case of *E. coli*. Also, the same strains of *E. coli* and *S. aureus* were interacted with alcoholic extracts of *Lycium barbarum* L. by Mocan et al. (2014), showing inhibition zones of  $1.31 \pm 0.09$  cm for *S. aureus* and  $1.23 \pm 0.08$  cm for *E. coli*.

The results demonstrate that the juices obtained from the three biotypes of *Lycium barbarum* L. have inhibitory effect on bacteria taken into the study.

## CONCLUSIONS

Biochemical analysis of goji juice showed that BUa have a high content of phenols, and an ability to inhibit free radicals.

Also, B1 was emphasized by the flavonoid content.

All four bacteria showed susceptibility towards the action of *Lycium barbarum* L. juices. *B. cereus* was most inhibited by BUa and *S. aureus*, *E. coli* and *P. aeruginosa* by B1. The highest inhibition was registered in the case of B1 against *E. coli* which recorded average values of  $1.84 \pm 0.13$  and  $1.81 \pm 0.20$  cm. The only case that did not generate an inhibition halo was *P. aeruginosa* in the presence of BUa. The biochemical results have a certain connection with those obtained by evaluating the antibacterial activity. Therefore, it requires a deeper research on the biochemical compounds that are found in juice.

## ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-0749.

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