ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SELECTED ARMENIAN MEDICINAL PLANTS

Vardapetyan H\(^1\)*, Tiratsuyan S\(^1,2\) and Hovhannisyan A\(^1\)

\(^1\)Russian-Armenian (Slavonic) University, Institute for Mathematics and High Technology, Department of Medical Biology and Bioengineering, H. Emin st.123, Yerevan, 0051, Armenia

\(^2\)Yerevan State University, Faculty of Biology, Department of biophysics, A. Manoogyan 1

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ABSTRACT

The antioxidant potentials of ethanolic and aqueous extracts of *Hypericum perforatum*, *Ocimum basilicum*, *Laurus nobilis* leaves were evaluated by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. Simultaneously total flavonoids content and antibacterial potential of all the tested extract was also monitored. The highest radical scavenging capacity (RSC) was reported in ethanolic extract of *H. perforatum* leaves while the lowest RSC was reported in the aqueous extract of *L. nobilis*. The ethanolic extracts of *L. nobilis* possess more DPPH radical scavenging action, than their aqueous extracts. Ethanolic and aqueous extracts of *H. perforatum* shows significant antioxidant activity, it is near to inhibition capacities while ethanolic extract of *O. basilicum* leaf has exhibited small activity as compared with its aqueous extract. RSC of ethanolic extracts can correlates with flavonoids content. The antiradical activity of ethanolic and aqueous extracts of *L. nobilis* leaf can also correlate with total flavonoids content, while such type of correlation was not reported in the case of *H. perforatum* and *O. basilicum* leaves extracts. RSC of tested extracts can be ranged in the following way: quercetin > rutin > eth. *H. perforatum* > aq. *H. perforatum* > aq. *O. basilicum* > eth. *L. nobilis* > eth. *O. basilicum* > aq. *L. nobilis*. All the ethanolic extracts express more antibacterial activity than aqueous extracts.

* Corresponding author
E-mail: hvardapetyan@mail.ru (Vardapetyan H)

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1 Introduction

Plants have been used by several communities to treat a large number of diseases; also these plants constitute a potential source for the production of new medicines (Trentin et al., 2011; Gunasekar et al., 2012). Plant can also comprise the search of new medicine that should have antimicrobial action and synergism with currently available antimicrobial drugs from medicinal plants (Kavitha & Niranjal 2009; Birdi et al., 2010). Beneficial effects of Armenian plants in prevention of human diseases along with their antioxidant properties is well documented (Martínez-Flores et al., 2002).

Number of disorders, such as cancer, nerve degeneration, inflammation, atherogenesis, autoimmune pathogenesis etc. can be result of oxidative injury (Behl & Mosmann 2002; Gunasekar et al., 2012). Despite the widespread use of plants as medicines and spices, there are some reports on the antioxidant activities of plants, these plants mainly contains flavonoids and terpenes as an active ingredients which might helps in preventing oxidative damage (Middleton et al., 2000; Behl & Mosmann 2002; Sharma et al., 2010), as well as act on gene expression (Soobratte et al., 2005; Dell et al., 2005). The antioxidative capacity of flavonoids confers a therapeutic potential on cardiovascular (Yao et al., 2004), gastric or duodenal ulcers (Moreira et al., 2004) cancer (Yang et al., 2000), viral diseases, allergy, thrombs and inflammations (Nijveldt et al., 2001; Nair et al., 2002; Kwon et al., 2005). Natural antioxidants especially phenolics and flavonoids are already exploited commercially either as additives or as nutritional supplements (Schuler 1990; Salminen & Thorngate 2001; Sharma et al., 2010).

Among many medicinal herbs used throughout Armenia, the use of H. perforatum L. (St. John's wort) has a long history to treat various human diseases like mental disorders, physical ailments, ulcers, burns, wounds, depression, bacterial and viral diseases (Hobbs, 1989; Linde et al., 1996). H. perforatum has unique compounds, such as hypericin that is known as photodynamic (Agostinis et al., 2002) pseudohypericin and hyperforin agents which are responsible for the various medicinal properties.

Culinary plants Ocimum basilicum L. (Sweet basil) are used as spices and flavours materials for various food products as well as plant used as an effective drug in many folk medicines. It used to treat feverish illnesses, poor digestion, respiratory diseases, asthma, nausea, gastro-enteritis, migraine, insomnia. O. basilicum has been shown an antioxidant, anti-inflammatory, antimicrobial, relaxant and antitumor activities (Boskabady et al., 2005; Kaya et al., 2008). Active ingredients of basil are thymol, allylthiophiocyanate, oxygenated monoterpene (Chacon et al., 2006; Gunasekar et al., 2012).

Laurus nobilis L. (bay laurel) traditionally used to treat rheumatism, earaches, indigestion, and sprains (Santos & Rao 2000). It also used to treat various disorders like diabetes, migraine (Erler et al., 2006), reduced serum glucose, total and LDL-cholesterol related decreases and to increase of HDL-cholesterol (Alam et al., 2009). Nowadays the bay leaf extracts are of great importance due to their antioxidant properties (Kaurinovic et al., 2011). Bay leaf oil reported very effective against several bacteria (Santos & Rao 2000).

The purpose of this study was to evaluate the antioxidant and antibacterial properties of Armenian H. perforatum, O. basilicum and L. nobilis.

2 Materials and Methods

2.1 Plant materials

Flowers and leaves of H. perforatum, O. basilicum and L. nobilis were collected in September 2011 from various regions of Republic of Armenia. The plant materials were air dried at room temperature and ground in a mixer. Finely powdered material was macerated at room temperature for 24-h period and used for study.

2.2 Total flavonoids determination

Aluminum chloride colorimetric method was used for total flavonoids determination (Vardapetyan et al., 2012a; Vardapetyan et al., 2012b) with known quantities of reference substance of 0-100 μg/ml quercetin and rutin (both Merck) in ethanol. Based on the property of flavonoids and flavones glycosides form internal yellow color complexes (Chang et al., 2002).

2.3 Free radical scavenging activity determination

Radical scavenging capacity (RSC) of tested extracts was assessed in a chemical model i.e. DPPH system (Koleva et al., 2002, Vardapetyan et al., 2012a). DPPH solution plus ethanol was used as a negative control. Quercetin and rutin solutions dissolved in 96% ethanol were used as a positive control. The IC₅₀ values that denote the concentration of sample, required for scavenging the 50% of DPPH free radicals were calculated (Vardapetyan et al., 2012a).

2.4 Antibacterial activity determination

Antibacterial activities of tested plant extracts were investigated by the disc-diffusion method on agar (Sigma- Aldrich). As an indicator of antibacterial activity the inhibition zone (in millimeters) formed after 24 – 48 hours of bacteria E. coli K-12 wild type strain (obtained from Research Laboratory of the Microbiology, Yerevan State University) development was taken (Vardapetyan et al., 2012b). The square (pixel²) was calculated by special program “Image Repair” (Asatryan et al., 2007).

2.5 Statistical analysis
Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by a one-way analysis of variance (ANOVA) and the values of p < 0.05 were considered as significant.

3 Results and discussion

3.1 Flavonoid contents of the extracts

Total flavonoid content of the various samples was determined by the quantified dilution factor, concentration calculated from the standard curve and sample mass (Vardapetyan et al., 2013). Results of flavones determination from leaf extracts of H. perforatum, O. basilicum and L. nobilis are presented in the Table 1. As shown in table ethanolic extracts of all three plants are abundant in flavonoids and it is 5 -15 times higher than the aqueous extracts. Highest amount of flavonoids was reported from the ethanolic extract of O. basilicum. Among the aqueous extracts also highest amount of flavonoids was reported from the same plant. Results of the present study are in conformity with the findings of Zheng & Wang. (2001) who have been reported highest amount of phenolic compounds in basil and it was followed by the H. perforatum and L. nobilis. The main components which identified in the O. basilicum extracts were eugenol, germacrene-δ, epi-α-cadinol, malic acid, tartaric acid, ramnose, caffeic acid, quinic acid, kaempferol, caffeoylquinic acid, and kaempferol 3-O-glucoside (Zheng & Wang., 2001). Phytochemical investigations of H. perforatum extracts have shown the presence of napthodianthrones hypericin and pseudohypericin, tannins, flavonoids, xanthones, benzophenones. Flavonoids and tannins are the major plant compounds with antioxidant activity (Zheleva-Dimitrova et al., 2010).

In general, flavonoids containing multiple hydroxyl groups have higher antioxidant activities against peroxyl radicals than phenolic acids. Flavonol aglycones such as quercetin, myricetin, kaempferol, have more antioxidant activity in compare with their glycosides such as rutin, naringin, and hesperidin (Robards et al., 1999).
Figure 4. The formation of inhibition zones after injection into the agar holes of *H. perforatum* aqueous - 1, ethanolic extracts - 2, *O. basilicum* aqueous – 3, ethanolic extracts - 4, *L. nobilis* aqueous - 5 and ethanolic extracts - 6. Represented values are means of five independent experiments (mean ± SD, p<0.05).

Table 1. Flavonoids content, IC₅₀ values for DPPH radical scavenging and antibacterial activities (zone inhibition area in pixel²) of tested extracts.

<table>
<thead>
<tr>
<th>Tested samples</th>
<th>Conc. from calib. curve (Cdet) (mg/ml)</th>
<th>Flavonoid content (mg/g)</th>
<th>IC₅₀ (mg/ml)</th>
<th>S (pixel²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>-</td>
<td>-</td>
<td>0.01 ± 0.002</td>
<td>9500 ± 870</td>
</tr>
<tr>
<td>Rutin</td>
<td>-</td>
<td>-</td>
<td>0.03 ± 0.003</td>
<td>3200 ± 299</td>
</tr>
<tr>
<td><em>L. nobilis</em> Et-OH</td>
<td>0.60</td>
<td>26.60 ± 0.15</td>
<td>1.60 ± 0.05</td>
<td>2910 ± 197</td>
</tr>
<tr>
<td><em>O. basilicum</em> Et-OH</td>
<td>0.68</td>
<td>27.2 ± 0.11</td>
<td>3.90 ± 0.08</td>
<td>2177 ± 183</td>
</tr>
<tr>
<td><em>H. perforatum</em> Et-OH</td>
<td>0.59</td>
<td>23.6 ± 0.13</td>
<td>0.68 ± 0.04</td>
<td>7100 ± 568</td>
</tr>
<tr>
<td><em>L. nobilis</em> aq.</td>
<td>0.06</td>
<td>2.40 ± 0.02</td>
<td>13.0 ± 0.10</td>
<td>400 ± 34</td>
</tr>
<tr>
<td><em>O. basilicum</em> aq.</td>
<td>0.13</td>
<td>4.95 ± 0.02</td>
<td>1.30 ± 0.02</td>
<td>369 ± 27</td>
</tr>
<tr>
<td><em>H. perforatum</em> aq.</td>
<td>0.07</td>
<td>2.74 ± 0.04</td>
<td>0.75 ± 0.05</td>
<td>1580 ± 135</td>
</tr>
</tbody>
</table>

Values are the mean of four independent replicates (mean ± SD, p<0.05).

3.2 Antioxidant activity

Evaluation of the RSC of standard flavonoids quercetin and rutin with all the tested ethanolic and aqueous leaves extracts was based on the DPPH consumption elicited by their addition. RSC of tested extracts at different concentrations are presented in the Figure.1 and 2. The obtained quenching activities against DPPH radicals were dose-dependent. Ethanolic extract of *H. perforatum* leaves showed highest RSC activities (Table 1, Figure. 1). The *L. nobilis* leaf extracts expressed more potent antiradical activity than the *O. basilicum* extracts.

Analysis of aqueous extracts RSC (Figure. 2) revealed that maximum antioxidant activity was reported in the leaves extract of *H. perforatum* this was followed by basil and minimal activities was reported from the aqueous extract of bay leaves. Aqueous extract of *L. nobilis* leaves expressed weaker antiradical activity than corresponding ethanolic extracts (Table 1), while aqueous extract of *O. basilicum* leaf shows strong RSC, significantly increasing that of ethanolic extract.

The calculated IC₅₀ values indicate that the antioxidant activity of ethanolic extract of *H. perforatum* is higher than its aqueous extract. It is reported that flavonoids content of the extracts played a significant role in their antioxidant capacity (Middleton et al., 2000; Silva et al., 2005). The aqueous extract of *H. perforatum* has very low content of flavonoids that suggests that the expressed antiradical activity is more likely caused by water-soluble non-flavonoid origin antioxidant compounds (Figure. 3). The high RSC value of ethanolic extracts is connected with quantity of flavonoids and quercetin in extract, particular the content of extracts was reported to be 8 times more in ethanolic extracts (Table 1) (Vardapetyan et al., 2013). Similar type of relationship between the total flavonoids content and the antiradical activity of *L. nobilis* leaf extracts was also reported in present study and it certifies the potent role of flavonoids in RSC (Khanduja & Bhardway., 2003; Martínez-Flores., 2002). The opposite picture was observed in extracts from *O. basilicum*, in which a large part of the components with antioxidant activity and were water-soluble.
The IC50 values provide information about the reaction kinetics, in terms of velocity (Silva et al., 2005; Khanduja & Bhardway 2003). Therefore, an additional parameter, reduction index (RI) was also used for this study (Silva et al. 2005, Vardapetyan et al., 2012b, ) and it gave an idea of the reactivity of tested extracts in bleaching the DPPH free radical. Both extracts of H. perforatum induced a moderate decrease in the DPPH radical (when compared with quercetin reactivity). It was observed that inserted RI values were significantly differing for quercetin, ethanol (0.26 ± 0.017) and aqueous (0.08 ± 0.005) extracts. Thus, despite the practically similar IC50 values, the ethanolic extract of H. perforatum was more reactive. It is possible that high velocity of DPPH bleaching is due to antioxidant compounds of flavonoid origin, whereas slow velocity of DPPH decolorization by aqueous extract is due to antioxidant compounds of non-flavonoid origin (Lee et al., 2005). Besides, synergism of phenol compounds in an extract may contribute to the overall antioxidant activity.

The lowest activity was found in ethanolic extract of O. basilicum. A major aroma constituent, 4-allylphenol present in extracts of basil leaves O. basilicum, is found to demonstrate strong antioxidant activities comparable to those of the known antioxidants such as α-tocopherol and butylated hydroxy toluene (Lee et al., 2005; Sharma et al., 2010).

3.3. Antibacterial activity of extracts

The results of the antibacterial activity (via the disc diffusion method) of the ethanolic and aqueous plant extracts, quercetin and rutin are presented in the Table1, Figure. 3 and 4. Quercetin and ethanolic extract of H. perforatum showed practically equal antibacterial activities against E. coli K-12. Considering the fact that the flavonoids content in ethanolic extract is equal to quercetin content expressed in equivalents (Table1), it could be concluded that the main input in antibacterial activity of this extract belongs to quercetin, although rutin can introduce antibacterial activity of extract (Pimentel et al., 2013; Hendra et al., 2011). The aqueous extract performed a weak antibacterial activity. The differences in antibacterial activities of ethanolic and aqueous extracts within the same concentrations could be explained by the low quantity of flavonoids in the latter and agree well with the published data on antimicrobial activities of H. perforatum extracts on various bacteria (Milošević, 2006). The antibacterial activity of H. perforatum extracts can also be due hypercin, its derivatives and hyperforin (Vardapetyan et al., 2012b). It was revealed that hypercin binds adhesins, complexes with cell wall and inactivates enzymes (Silva & Fernandes, 2010). It was shown correlation between RI and antibacterial activity of H. perforatum ethanol extract, that certify both antibacterial activity and velocity of DPPH bleaching is due to the same antioxidant compounds (Vardapetyan et al., 2012a).

Flavonoid contents of ethanolic extract of O. basilicum and H. perforatum are almost equal with the lowest RSC was found in O. basilicum extract, but revealed the action against the E. coli K12 bacteria. It could be concluded that antibacterial actions may be posses due to other compounds of basil extract, such as carvacrol and thymol (Souza et al., 1998). These are natural products that have received attention as possible food antioxidants. Their structure disintegrates the external membrane of Gram- negative bacteria and increasing the permeability of the cytoplasmic membrane to ATP (Ultee et al., 2002). Thymol has been shown to be active against viruses, bacteria, and fungi, biofilms (Braga et al., 2008). The other antibacterial compounds of O. basilicum extracts can be rosmarinic acid - a caffeic acid ester (Ultee et al., 2002) which has been shown to inhibit planktonic growth of some Pseudomonas spp., and interferes with quorum sensing activities and biofilm development (Walker et al., 2004; Nostro et al., 2007). Cinnamaldehyde a main component of O. basilicum extracts has been shown to decrease E. coli biofilm formation (Niu & Gilber, 2004), and it interferes with C4- and C6-homoserin lactones (Niu et al., 2006).

It was recently reported that the leaf extracts of L. nobilis promote healing activities on incision, burn and induced by E. coli purulence wound models (Rukhkyan et al., 2013a; Rukhkyan et al., 2013b). It is shown that an earlier differentiation of the extracellular matrix and the intensification collagenogenesis occur if purulent wounds are treated with the L. nobilis ointment (Rukhkyan et al., 2013a).

Flavonoids are efficient antioxidants with holding the capability of scavenging free radical species. This compound plays an important role in augmenting the wound-healing process. Flavonoids and flavons also bind with adhesins that have been termed as the most important determinant of pathogenicity (Silva & Fernandes 2010; Croxen & Finlay 2010). Terpens and terpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction (Nayak et al., 2005).

Significant antimicrobial activity of bay extract against E. coli strains virulence was observed, and it is connected with the adhesion activity (Sun et al., 1988). Flavonoids content of ethanol extract of L. nobilis is more than H. perforatum, in the same time the RSC is less (Figure. 3, Table 1) that can be connected with different flavonoids composition of both plants extracts. The antibacterial activity of bay can be also due compounds non-flavonoid origin or flavonoids without antioxidant properties, such as p-cymene - a precursor of carvacrol (Ultee et al., 2002; Braga et al., 2008). The high contents of eugenol, methyl eugenol and fatty acid methyl esters together with other active components (Marzouki et al., 2008) could contribute to its overall antibacterial activity (Ivanović et al., 2010).

4 Conclusion

In present study a comparative analysis of the RSC of ethanolic and aqueous extracts of selected plant species, which are being used traditionally i.e. H. perforatum, O. basilicum
and L. nobilis, and their antibacterial activities was carried out. The results revealed that the H. perforatum ethanolic extract which contain the lowest amount of flavonoids, exhibited the greatest RSC, while the L. nobilis aqueous extract has the lowest RSC. Ethanolic and aqueous extracts of H. perforatum express significant antiradical activity with near inhibition capacities that vary significantly in their RI mean. Ethanolic extract of O. basilicum leaf has exhibited significantly small activity as compared with its aqueous extract. The ethanolic extract of L. nobilis leaves posses more RSC, than aqueous extract. There is obtained correlation between flavonoids content and RSC only for L. nobilis leaf extracts. Although, H. perforatum has low content of flavonoids, it showed high total antioxidant activity probably due to the presence of other compounds. This fact clearly shows that the antioxidant capacity is a consequence of the joint action of numerous substances which manifest themselves as antioxidants, such as phenol acids, flavonoids, tannins, coumarins and more recently procyanidins (Zheleva-Dimitrova et al., 2010).

RSC of tested extracts can be ranged in the following way: quercetin > rutin > eth. H. perforatum > aq. H. perforatum > aq. O. basilicum > eth. L. nobilis > eth. O. basilicum > aq. L. nobilis.

A significant correlation was reported between RI and antibacterial activity of H. perforatum ethanolic extract, determined by disc-diffusion method that certify both antibacterial activity and velocity of DPPH bleaching is due to the same antioxidant compounds. Antibacterial activity of plant extracts can be a promising ally in the development of medicines necessary to combat the increasing number of bacterial strains that become resistant to conventional antibiotics.

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References


