Antioxidant and Antimicrobial Activity of Extract of Achillea Millefolium

Bojan Antonic

Department of Plant Origin Foodstuffs Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno

Abstract

The study aimed to prove the assumption that Achillea millefolium has antioxidant and antimicrobial activity. Achillea millefolium, harvested in a suburban area of Banja Luka, was the subject of the study. Two types of extracts were made: one aqueous extract, and one which was made by the separation of aqueous extract with ethyl acetate. For antioxidant activity, DPPH method was used, and IC_{50} values (concentration of antioxidant needed for reduction of 50% of DPPH* radical) for extracts were compared with control antioxidants (BHA and ascorbic acid). Measurements were taken on a spectrophotometer at wavelength 517nm. Values obtained for IC_{50} were: 4.91 µg/mL for BHA, 5.04 µg/mL for ascorbic acid, 31.09 µg/mL for ethyl-acetate extract and 92.51 µg/mL for aqueous extract. Antimicrobial activity was tested by monitoring the impact of the addition of obtained extracts on bacterial growth against the controls by measuring the change in optical density of bacterial suspension over time. For that purpose, Escherichia coli ATCC 25922, cultivated on nutrient agar, was used. Measurements were done on colorimeter during 6 hours of growth on 37ºC. The concentration of water extract was 20mg/mL, and ethyl acetate was 2mg/mL and they inhibited the growth of E.coli for 8.08%, and 28.54% respectively. By results obtained, the extracts of Achillea millefolium showed both antioxidant and antimicrobial activity.

Keywords: Achillea millefolium, antioxidant activity, antimicrobial activity, DPPH, AAI

Introduction

Antioxidants play an important role as a protective biochemical agent. Their presence in food products is important for its stabilization and to help to keep the high-quality properties for a longer period. Nowadays, free radicals are very important for the studies since they cause a lot of unwanted reactions in food products but also in the human body. As highly reactive intermediates they can lead to oxidative tissue damage (Finkel et al. 2000). Every type of molecules may be damaged by this process. Free radicals in the cell may be due to various external factors such as ultraviolet radiation, chemical reactions and some metabolic processes. By accumulating these species cause significant diseases such as cardiovascular diseases, ageing, cancer, inflammatory diseases and other (Visioli et al. 2000).

For synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are known for their ability to stop lipid peroxidation chain reaction, proven is that they are carcinogenic and cause liver damage (Ito et al., 1985). That is the reason why antioxidants from natural sources have big attention and need for analysis and use.

The use of plants in the treatment of a variety of diseases dates back to the distant past. Many plants that have been used in traditional therapies for centuries, are also accepted in modern medicine, where represent important raw materials (Moradi et al., 2013). Yarrow is one of the oldest medicinal plants. In traditional medicine, Yarrow has been used as medicine for the treatment of inflammatory and gastrointestinal disorders. It is also used as an appetite-enhancing substance, for wound healing and against skin inflammations. As a medicine agent, it's mainly used in the form of aqueous or ethanolic extract (Applequist et al. 2013). Aside from use in medicine, it is widespread in some industrial teas blends.

Address for correspondence:
Bojan Antonic
Department of Plant Origin Foodstuffs Hygiene and Technology,
Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého tř. 1946/1, 612 42 Brno
E-mail: H19001@vfu.cz

The name of the genus originates from the ancient use as a wound-healing remedy by the Trojan hero Achilles, whereas millefolium refers to the deeply divided leaves (Benedek et al., 2007). So, the translation of its name Achillea millefolium means Achilles Grass with a Thousand Leaves.

This study was focused on antioxidant activity of extracts of Achillea millefolium in comparison to some known antioxidants and determination of inhibition of microbial growth when being treated with those extracts.

Materials and methods

Collection of plant material

The herbal plant of Achillea millefolium was collected in surrounding of Banja Luka, Bosnia and Herzegovina. For the extraction purposes, there were used flowers and leaves.

Preparation of extracts

The sample of the air-dried plant is boiled in distilled water in extractor on 80ºC for one hour and then left to soak for 24 hours. After that, content is filtered and evaporated for obtaining dry extract for further analysis. From this extract, the water solution is made for testing purposes and it was named aqueous extract. Before evaporation, the part of water extract was separated using the ethyl-acetate to obtain ethyl-acetate extract which is more concentrated with phenols as organic antioxidant substances. After evaporation, this extract was dissolved in methanol for testing purposes.

Antioxidant activity

The antioxidant activity was done using DPPH method. Measurements were taken on a spectrophotometer at a wavelength of 517 nm which is a maximum of the absorbance spectrum of DPPH* (1,1-diphenyl -2-picrylhydrazyl) free radical solution (Zhu et al., 2002). Incubation of the DPPH solution was performed with different concentrations of extracts and for controls antioxidants, ascorbic acid and BHA (butylated hydroxyanisole) were used. It was carried out at room temperature for 30 minutes, and then read absorbance (A). The reaction mixture contained 2 mL 150μM solution of DPPH and 2 mL different concentration of test substances, (A_{test}). For blank, (A_{blank}), 2mL of methanol was added to 2mL of DPPH (Liyana-Pathirana 2005). Percent of the inhibition of the free radical DPPH (I%) is calculated according to the formula:

$$I\% = \frac{A_{blank} - A_{test}}{A_{blank}} \times 100$$

Inhibition concentration of 50% of the free radical DPPH (IC_{50}), is then obtained from the linear formula. The results were also presented as an antioxidant activity index (AAI) (Scherer et al., 2009) by using as a concentration of DPPH in the reaction mixture and obtained IC_{50}:

$$AAI = \frac{C_{DPPH}^{final}}{IC_{50}}$$

Antimicrobial activity

A pure Escherichia coli ATCC 25922 culture grown on nutrient agar was used to determine the antimicrobial activity. It was determined by measuring the bacterial optical density of suspensions of bacteria during the time with extract added and for corresponding controls.

Growth curves were made for microorganisms over 6 hours, for tests with added aqueous and ethyl acetate extract, and for tests with the addition of appropriate aqueous and methanolic control. Sterile tubes were prepared with 5 mL of substrate each (nutrient broth), 0.5 mL of culture, and 0.5 mL of extract or corresponding control. The blank contained 5.5 mL of the substrate and 0.5 mL of the appropriate extract or water. After component dosing, the absorbance was measured on a colorimeter for every hour, starting with the zero (initial) hour, for the next six hours. The concentrations of extract solutions were 20 mg/mL for aqueous extract and 2 mg/mL for ethyl-acetate extract. The antimicrobial activity is followed using the inhibition growth rate (L) for bacterial suspension with and without extract added (Pavicic et al. 2009):

$$L\% = (1 - T_g / T_{ge}) \times 100$$

Where T_g is mean bacterial growth time for bacterial culture without added extract and T_{ge} is mean bacterial growth time for bacterial culture with extract added.

Results and discussion

The DPPH* method is based on the reaction between the stable radical DPPH* and hydrogen donors. The fact that DPPH* radical is very stable (She et al. 2010) makes this method suitable for the determination of the antioxidant activity of extracts from natural products.

The antioxidant and antimicrobial activity of herbal extracts depends to a large extent on the way the extract is obtained and the composition of the extracts and their activity rely on that. The chemical composition of the extract also depends on the geographical origin of the plants, and on the maturity of the plant itself (Candan et al., 2003).

Since water is playing important and not interchangeable role in every biological system, food and beverages people ingest, main focus in this study was to determine antioxidant and antimicrobial activity of aqueous extract of plant without any isolation of essential oils, for which is proven to have strong antioxidant (Gharibi et al., 2015) and effective antimicrobial (Maz et al., 2013) activity. In this way, it can be seen that when added as a raw material to food, beverages or some mixtures of teas, this herb can improve to some extent the quality and value of such a product and extend its shelf life. The ethyl-acetate extract was made to show the potential of concentrating the organic compounds which are marked as strongest antioxidants in this plant.

For antioxidant activity the following results were obtained:

Table 1. Percentage of inhibition of DPPH for different concentrations of extracts

Aqueous extract		Ethyl-acetate extract	
c(μg/mL)	I(%)	c(μg/mL)	I(%)
40.00	22.78 ± 0.00	9.58	16.27 ± 0.90
80.00	47.26 ± 0.73	19.15	33.73 ± 0.68
120.00	60.76 ± 0.00	38.30	63.53 ± 0.00
160.00	83.97 ± 0.73	57.45	86.67 ± 0.68

Table 2. Percentage of inhibition of DPPH for different concentrations of control substances

BHA		Ascorbic acid	
c(μg/mL)	I(%)	c(μg/mL)	I(%)
1.50	21.54 ± 0.93	1.00	11.23 ± 0.63
3.00	38.62 ± 0.70	2.50	22.10 ± 0.63
6.00	67.89 ± 0.70	5.00	60.14 ± 0.63
10.00	83.33 ± 0.70	10.00	91.30 ± 0.00
12.00	87.80 ± 0.70		

It can be seen from the obtained values (Table 3.) that control antioxidants have approximately equal value for antioxidant activity. For the results of the obtained extracts, satisfactory values were obtained, and as expected ethyl-acetate extract has significantly higher antioxidant activity. The assumption was that ethyl-acetate extract mainly consists of phenols, and phenol-like compounds, which are known as ones that have good antioxidant properties (Zheng et al., 2001). Since ethyl-acetate extract was obtained from aqueous, the aqueous extract contains dissolved phenols as well, but at a much lower concentration,

Table 3. The calculated values of IC_{50} and AAI

Tested substance	IC_{50} (μg/mL)	AAI
Aqueous extract	92.51	0.32
Ethyl-acetate extract	31.09	0.95
BHA	4.91	6.02
Ascorbic acid	5.04	5.87

so it required about 3 times higher concentration of aqueous extract to inhibit 50% of DPPH radical. Obtained values for AAI showed that control antioxidants BHA and ascorbic acid can be classified as strong antioxidants, while the ethyl-acetate extract is a moderate antioxidant and aqueous extract can be classified as a weak antioxidant (Scherer et al. 2009). Using the AAI extracts activities from different studies which used the DPPH method can be compared. In a study from Kukric et al. 2012, the extract of Urtica dioica L. had AAI of 0.85, and in the study from Teixeira et al. 2012, the extract of Mentha pulegium had AAI of 0.45 for cold water extract and 0.80 for ethanolic extract. According to those results, the ethyl-acetate extract of Achillea millefolium from this study showed better antioxidant activity than the ones form Urtica dioica and Mentha pulegium. On the other hand, the aqueous extract of Achillea millefolium tested in this study showed lower values for AAI than the above mentioned ones.

For antimicrobial activity the following results were obtained:

The linear formula for calibration curve of dependence of a logarithmic number of microorganisms (E.coli) on absorbance was obtained and it was: y = 9.963x + 7.0127; with regression, R2 = 0.921.

Using this formula, the new linear curves for growth of the E.coli over time were made from the experiments with the addition of extracts and corresponding controls (Table 4.). Constant k is taken from the linear formula and then calculated mean generation time using formula (Table 4.):

$T_g = \ln(2) / k$ (Herries, 1972).

Table 4. Overview of linear formulas for microbial growth, constants of growth and mean generation growth time

Experiment setup	Linear formula ln(n)/h	k (1/h)	T_g (h)
Aqueous control	$y = 0.4209x + 7.1721$; $R^2 = 0.9541$	0.4209	1.65
Aqueous extract	$y = 0.3861x + 7.3514$; $R^2 = 0.9229$	0.3861	1.795
Methanol control	$y = 0.0996x + 7.2568$; $R^2 = 0.9829$	0.0996	6.96
Ethyl-acetate extract	$y = 0.0712x + 7.5192$; $R^2 = 0.9197$	0.0712	9.74

Table 5. The calculated inhibition growth rate

Extract type	L (%)
Aqueous extract	8.08
Ethyl-acetate extract	28.54

According to the results (Table 5.), both extracts obtained from plant Achillea millefolium inhibited the growth of E.coli bacteria. By analogy to antioxidant activity, the ethyl-acetate extract showed much higher antimicrobial effect, and it was more than 3 times greater than one obtained from aqueous extract. Because of different ways of extraction and different concentrations of extracts used to test the inhibition of microbial growth, it‘s not easy to compare the results obtained for some other extracts. In study Kukric et al. 2013, methanolic extract of Equisetum arvense L. in concentrations ranges from 0.21 to 1.61 mg/L showed inhibition growth rates in the range from 51 to 81% on growth of E.coli. Study of Pavicic et al. 2009, for antimicrobial activity of methanolic extract of Reynoutria japonica with a concentration of 0.81mg/mL, showed 59.3% growth inhibition of culture

E.coli. In comparison to above mentioned studies, both extracts used in this study showed significantly lower antimicrobial activity.

Conclusion

Based on the tests, it can be concluded that both aqueous and ethyl-acetate extract show antioxidant and antimicrobial activity. The ethyl-acetate extract showed three times more potent antioxidant activity than the aqueous extract, which is consistent with the assumption that phenols and phenol-like compounds were more concentrated in that extract. Relative to control antioxidants, ethyl acetate extract showed 6 times lower antioxidant activity. Analogous to its antioxidant effect, the ethyl-acetate extract was with much stronger antimicrobial activity in comparison to aqueous extract.

It's recommended for some future experiments to isolate the essential oils of this plant in different plant maturity stages and to test their activity to get data on the best period for doing the harvest, and to have a better comparison to the same plant from different geographical regions.

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