Study on antibacterial and antioxidant activity of Berberis vulgaris aqueous extracts from Iran

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Abstract

The antioxidant and antibacterial are group from food additive that use on food as preservative. The objective of this study was to determine antioxidant and antibacterial activity of Berberis vulgaris fruit the using different in vitro methodologies. The aquatic and ethanolic extracts, at a concentration from 35to 40 µg/ml, showed a significant antibacterial effect expressed as minimum inhibitory concentration (MIC) against both Gram-negative and Gram-positive bacteria. In particular, Pseudomonas aeruginosa (MIC=16 microg/ml), Proteus vulgaris (MIC=32 microg/ml) and Escherichia coli (MIC=32 microg/ml) were the most inhibited. The antioxidant activity were determined by the 2,2-diphenylpicrylhydrazyl (DPPH) assay and a β-carotene bleaching assay, and compared with that of butylated hydroxyl toluene (BHT). The data were expressed as the mean ± the standard deviation and they were statistically analyzed by SPSS software using ANOVA ($P<0.05$).

Results: The results showed that among all the solvent extracts, water extractof Berberis vulgaris friut had high antioxidant activities as measured by DPPH scavenging (28.62±0.01, 20.58±0.12 and 50.78±0.17, 142.9±1.12, 56.08±2.72, 120.43±0.85 µg/ml) and inhibition of linoleic cid oxidation, respectively (77, 86,70, 58, 72, 62%). These parameters for BHT were 10±0.02 µg/ml, and 95.24%±0.14.

Conclusion: The findings indicated that the water extracts of Berberis vulgaris friut can act both as natural antioxidants and antibacterial and as a possible food supplement or be used in pharmaceutical industry after complementary tests.

Key word: Aqueous extract, Berberis vulgaris, Antioxidant activity, anti bacterial activity.

1. Introduction

Food-borne pathogenic are group of micro-organisms that cause food-borne disease thus, the research for finding effective drugs against this infection is necessary. Prevalence of food-borne disease caused by Food-borne pathogenic has increased worldwide and has become a major cause of mortality in individuals with impaired immune systems in developing countries. Therefore urgent need to monitoring antimicrobial resistance by improved antibiotic use and reduce hospital cross-infection, but the development of new antibiotics (natural) must be continued to maintain the effectiveness of antimicrobial therapy of primary importance. In developing countries, the World Health Organization estimates that about three-quarters of the population relies on plant-based preparations used in traditional medicine for primary health care as a fundamental human needs. Therefore, some herbs have been evaluated for antimicrobial activity may be used to treat a variety of diseases and microbial origin.

Oxidative stress is an important factor in the pathophysiology of pathological conditions, including cardiovascular dysfunction, atherosclerosis, inflammation, cancer, drug toxicity, reperfusion injury and neurological disease.

Different parts of plants, including fruits, leaves; have a wide range of phenolic compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity. By controlling free radicals, antioxidants can reduce fibrosis process. Antioxidants as protective agents may reduce oxidative damage in humans are considered. Antioxidants occur naturally in many fruits and are able to neutralize free radicals by donating an electron and convert them into harmless molecules.

Antioxidants that can quench reactive free radicals can prevent the oxidation of other molecules and may, therefore, have health effects in the prevention of degenerative diseases. In addition, it has been reported that an inverse relationship between dietary intake of foods rich in antioxidants and incidence human disease.

Barberry (Berberis L.) species are used as traditional medicine and food plants. Berberis vulgaris L. Fruits for diseases and disorders of the kidney, urinary tract and gastrointestinal tract, liver disease, bronchial ailments,

and is used as a stimulant for the circulatory system. The root and stem of this plants are used diseases and disorders of gastrointestinal tract, liver, gallbladder, kidney and urinary tract, respiratory tract, heart and circulatory system, as well as refrigerant (Blumenthal et al., 1998). In traditional medicine the extracts of various Berberidaceae (Berberis aquifolium , Berberis vulgaris and Berberis aristata) are used for disorders of rheumatic c and another of chronic inflammations (Ivanovska and Philipov, 1996). The plants of Berberis species were active in many assay employed for antioxidant and antimicrobial activity (Stermitz et al., 2001, Freile et al., 2006, Musumeci et al., 2003, Iauk et al., 2007). (Zovko Končić et al., 2010)

This study was designed to examine antibacterial and antioxidant activities of the extracts obtained from Berberis vulgaris fruit that have been traditionally used as general health supplements.

2. Material and method

Plant materials

The Fruit of Berberis vulgaris were collected from the khorasan, Iran, during Sep 2013. The taxonomic identification of plant materials was confirmed by herbarium of medicinal plants, Tehran University of Medical Sciences.

Preparation extracts

The aqueous extract of the Fruit of Berberis vulgaris were obtained by adding 1 l of boiling water to 500 g of powdered plant material in a glass 2.5-l flask and incubated at room temperature for 8 h on a rotating shaker (200 rpm). The aqueous extracts were filtered using Whatman No. 1 filter paper and then concentrated in vacuum at 40 °C using a rotary evaporator.

In vitro Antimicrobial activity test

Disk Diffusion Test

The aqueous extracts were tested against Staphylococcus aureus ATCC 25913, Escherichia coli ATCC 8739 and bacillus cereus ATCC25730

The microorganisms were cultured in BHI (Brain Heart Infusion) for 18 hours at 37◦ C, and resuspended in 0,5 Mac Farland Standard (5 x 10^8 CFU/mL) and inoculated directly in boards with Mueller-Hinton Agar (Merck). After the inoculation of each microorganism, the diffusion method was used, putting 10µL of essential oil on paper disks (6 mm of diameter) at 37^{o}C/24 hours, after which time the halos of inhibition were measured.

Micro dilution method

Aqueous extracts were diluted by using serial micro dilution method with Mueller Hinton Broth culture medium at a final concentration range from 32 to 0.25 %. Each and every essential oil was assayed for antibacterial activity in triplicate. Before conducting experiments all the conditions were standardized to determine MIC and MBC values in vitro.

In vitro antioxidant activity

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometer assay uses stable radical DPPH (Sigma, Aldrich) as the reagent (Cuendet et al., 1997). Briefly, 50 µL of the extracts (various concentrations) were added to 5 ml of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation: %DPPH radical scavenging = [(control absorbance (blank) -sample absorbance)/ (control absorbance)] ×100

Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration using PHARM/PCS-version 4.All tests were done in triplicate. Values (mean ± SD) of the extracts were compared with those of BHT using student's t-test. A p-value less than 0.05 were statistically considered significant.

β-Carotene–linoleic acid assay

Antioxidant capacity was determined by measuring the inhibition of volatile organic compounds and the conjugated dienehydroperoxides arose from linoleic acid oxidation according to the method of Dapkevicius et al (Dapkevicius et al., 1998).

In this regard, stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene (Merck, K15555836) was dissolved in1 ml of chloroform (HPLC grade) and then 25 µl linoleic acid (Sigma, L1376-500MG) and 200 mg Tween 40 (Merck, 822185) were added. After the evaporation of chloroform, 100 ml of oxygen saturated distilled water was added with vigorous shaking. Then, 2500 µl aliqouts were dispensed into the test tubes, 350 µl of the extract (2 g/L) was added and the emulsion system incubated for 48 h at room

temperature. The same procedure was performed for both BHT (as positive control) and blank. In turn, absorbance spectra of the mixtures were obtained at 490 nm. Afterward, Anti oxidative capacities of the extracts were compared with those of BHT and blank. Further, all inhibition percentages were compared using with 95% confident interval.

Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by Excel software program version 2010.

3. Results and discussion

Berberis aqueous extracts are shown to exhibit widespread antimicrobial activity. Data for berberis aqueous extracts susceptibility testing by broth micro dilution are shown in Table 1. All bacterial strains studied were inhibited by fruits of berberis aqueous extracts, with same degrees of inhibition. The MIC value of berberis aqueous extracts were as follows: Staphylococcus aureus, Escherichia coli and bacillus cereus obtained MIC 40mg/ml. The aqueous extracts of the fruits of berberis showed antimicrobial activity against the all of the bacterial strains, used in this study(Staphylococcus aureus, with an inhibitory zone of 21mm, E. coli, with an inhibitory zones of 15mm, with an inhibitory zone of 22mm). (Table3). Our results showed that higher concentration of berberis aqueous extracts increased its antibacterial effect. Antibacterial susceptibility was evaluated using classical microbiological techniques with disk diffusion, MIC and MBC determination.

In the present study we also demonstrate the anti oxidant effect of the extract of Berberis Fruit. The extract exhibited a remarkable anti oxidant activity (LC50 = 29.15 µg/ml). Preliminary phytochemical investigation revealed the presence of phenolic compounds and flavanoids which have been reported to be associated with antioxidative action in biological systems acting as scavengers of singlet oxygen and free radicals.

In summary, we conclude that most of the results of this study are in good agreement with the traditional uses of the investigated plant. All the extracts showed significant antibacterial and antioxidant activity. It was postulated that an increase in the antibacterial activity of pure compounds occurred when they are combined with antioxidants. Therefore, we consider that if both antibacterial and antioxidant compounds exist in the extracts, they could interact

and enhance the antibacterial activity.

Motalleb et al (2005) showed that antioxidant activity in Berberis Fruit methanoilc extract more effective than aqueous.

Freile et al. (2006) surveyed antimicrobial effect of berberis aqueous extracts against different Candida species through MIC from 16 µg/ml (C. krusei) to >128 µg/ml (C. haemulonii) (Freile et al., 2006). Iauk et al. reported that Methanolic extract, good activity against C. albicans, C. krusei and C. tropicalis but not against

C. parapsilosis (MICN64 mg/l)(Iauk et al., 2007).

Belofsky et al. [30] showed an increment in the antimicrobial activity of pure compounds when they are combined with antioxidants. Therefore, we consider that if both antimicrobial and antioxidant compounds exist in the extracts, they could interact and enhance the antimicrobial activity. The bioassay-guided fractionation of these extracts in order to isolate and identify the compounds responsible for each of these activities, followed by a study of their interaction, is highly desirable.

Conclusion:

In this study, the antimicrobial activity of aqueous extract of barberry was performanced against food -borne pathogenic bacteria. The aqueous extract may be effective in other gram-positive and gram-negative bacteria. Practical application of aqueous and increase shelf life for food use. Therefore it can be good alternative and satisfactory artificial preservatives used in the food industry today.

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Table 1: Determination of MIC and MBC value (µg/ml) for water and ethanol extract berberis against pathogenic bacterial strains

	S. aureus		V. cholera		Yersinia		E. coli		Salmonella	
Test	**water**	**ethanol**	**water**	**ethanol**	**water**	**ethanol**	**water**	**ethanol**	**water**	**ethanol**
MIC (µg/ml)	80000	1250	40000	1250	160000	2500	320000	10000	320000	5000
MBC (µg/ml)	160000	2500	80000	1250	320000	5000	640000	10000	640000	10000

Table 2- comparison of average inhibitory halo diameter (mm) of various bacterial strains for ethanol extract

Bacterial strain	Samples	Min	Max	Average ± SD
Staphylococcus aureus	3	10	11.5	11 ± 0.87^{a}
Escherichia coli	3	9.5	11	10 ± 0.87^{a}
Vibrio cholera	3	9.5	11.5	10.33 ± 1.04^{a}
Yersinia	3	9	11.5	10 ± 1.32^{a}
Salmonella	3	8.5	10	9 ± 0.87^{a}

Table 3- comparison of average inhibitory halo diameter (mm) of various bacterial strains for water extract

Bacterial strain	Samples	Min	Max	Average ± SD
Staphylococcus aureus	3	13.5	15	14.5 ± 0.87^{b}
Escherichia coli	3	13	14	13.5 ± 0.5^{b}
Vibrio cholera	3	12.5	15	14 ± 1.32^{b}
Yersinia	3	13	15.5	14.5 ± 1.32^{b}
Salmonella	3	11.5	12.5	12 ± 0.5^{b}