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Original Article

STUDY THE RELATIONSHIP BETWEEN ANTIOXIDANT POTENTIAL AND PHENOLIC CONTENTS OF *JUNIPERUS EXCELSA* FRUIT

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ABSTRACT

Objective: Today's attentions are focused on the finding new natural antioxidant compounds because of their fewer side effects than synthetic antioxidants. The aim of this study was to determine the antioxidant potentials of *Juniperus excelsa* fruit and its fractions by different methods.

Methods: For evaluation antioxidant potentials, DPPH radical scavenging, determination of reducing power and phenolics were used. Gallic acid and quercetin were used as antioxidant standards.

Results: The highest DPPH radical scavenging was observed in n-butanol fraction (IC_{50}= 135.9±2.5 µg/ml) of *Juniperus excelsa* fruit. Also, this fraction possesses the highest reducing power (in 61.4±2.6 µg/ml with absorbance 0.5) and phenolic contents (82. 9±1.1 mg/g).

Conclusion: n-butanol fraction of *Juniperus excelsa* fruit had the highest radical scavenging, reducing power and phenolic compounds. In other words, a relationship between antioxidant potentials and phenolic compounds was found. Anyway, this fraction is a strong source of antioxidant compounds and can be used as a natural antioxidant.

Keywords: *Juniperus excelsa* fruit, Fractions, Reducing power, DPPH radical scavenging. Phenolic contents.

INTRODUCTION

Free radicals are molecules with unpaired electrons in outer orbital which are unstable and very reactive [1]. They attacked macromolecules such as DNA, proteins, lipids and oxidized these molecules which cause different diseases such as diabetes, cancer and atherosclerosis. These oxidant effects [2] of free radicals are neutralized by antioxidants. Synthetic antioxidants in food industrial possessed carcinogenic and toxic effects[3]. Thus today's to find new natural sources of antioxidants is very important. Plants are important sources of antioxidant compounds [3] and these plants decrease diseases depended on aging such as atherosclerosis, diabetes and cancer [3].

In this study, the antioxidant potential of *Juniperus excelsa* (J.e) fruit and its fractions which obtained by liquid-liquid extraction were detected. For evaluation antioxidant potentials, DPPH radical scavenging, reducing power and phenolic contents were determined. Also it was reported that different species of J. e were used as medicinal plants in traditional medicine [4].

MATERIALS AND METHODS

Materials: Gallic acid, DPPH (2, 2- Diphenyl-1-picrylhydrazyl), quercetin were purchased from Sigma (ST. Louis, MO, USA). All other reagents were purchased from Merck Chem. Co.

Preparation Extract and Fractions

The fruits of J.e were collected from Genu mountain (near to Bandar Abbas) and identified by Mr. Zaeifi. 500 g of fruits were dissolved in the ethanol %80 and 90.6 g of the extract was collected. Crude extract of fruit (88g) was dissolved in the methanol 80% (250 CC), and was fractionated by petroleum ether to obtain 9.23 g of the fraction.

The remnant phase was dissolved in distilled water and using chloroform (3×250 CC) to obtain the second fraction (51.9 g). Then fractionation was continued by ethyl acetate and n-butanol respectively. Ethyl acetate (4.85 g) and n- butanol fractions (30.83 g) were collected. All fractions were investigated for antioxidant properties.

DPPH Radical Scavenging

In a modified procedure [5], 100 µl of fruit extract/ fraction (25-800 µg/ml) was added to 100 µl of 50 mM DPPH. Negative controls were prepared with 100 µl of the methanol and 100 µl of the DPPH in triplicate. The microplate was incubated at 25°C for 30 min and the absorbance was measured at 492 nm using a microplate reader model Stat Fax 2100, Awareness technology, Inc. Gallic acid and BHT were used as antioxidant standards. The obtained data were used to determine the concentrations of the extracts required to scavenge 50% of DPPH free radicals (IC_{50} ± SD) in triplicate (Table 1, Figure 1). The DPPH radical scavenging activity was calculated according to the following equation:

% Radical scavenging = 100 – ((A) sample-(A) blank) × 100/ (A) control)

Where "A" is the absorbance of the color formed in microplate wells. DPPH was used as a control (without fruit extract or fraction), blank contains methanol [6].

Reducing Power Assay

The reducing power of the fruit extract and its fractions were evaluated according to the method of Moein [7]. To 1.0 ml of the extract suspended in distilled water, 2.5 mL of 0.2 M phosphate buffer (pH 6.6), which contained 1% $K_3Fe(CN)_6$ was added. The mixture was incubated at 50°C for 20 min and then 2.5 ml of TCA (10%) was added. The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml ($FeCl_3$ 0.1%).

Then the absorbance was measured at 700 nm against a blank sample. Increasing in the absorbance of the reaction mixture indicated more reducing power (Figure 2).

Determination of Total Phenolic Contents

Total phenolic content was determined according to the method of Miliauskas [8]. Briefly; For the preparation of calibration curve, 0.5 ml aliquots of (0.024, 0.075, 0.105 and 0.3 mg/ml) gallic acid solutions were mixed with 2.5 ml of the Folin- ciocalteu reagent

(diluted ten –fold) and 2 ml (75 g/l) sodium carbonate. The absorption at 765 nm was measured by a UV-Vis spectrophotometer (T80 plus, PG Instrument, UK). Half of one ml plant extract (10 g/l) was mixed with the same reagents as described above, and after 1 hour the absorption was measured. All determinations were performed in triplicate. Total content of phenolic compounds in plant extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = c.v/m$$

Where: C is the total content of the phenolic compounds, mg/g plant extract, in GAE; c is the concentration of gallic acid established from the calibration curve, mg/ml; v is the volume of the extract, ml; m is the weight of plant extract, g.

Statistical Analysis

Means ± SD were calculated. The IC_{50} values were calculated by linear regression. The data were analyzed for statistical significance using one way ANOVA followed by Tukey post test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

In present study, the yield of fruit extraction is 18.1% which similar to raw fruit of J.e. In other research, the yield of raw fruit and ripe fruit is reported 19.3 and 33.2% respectively [4]. In present study, the antioxidant potentials of fruit extract (and fractions) are evaluated using DPPH radical scavenging, determination of reducing power and phenolic compounds.

In other research, the antioxidant potential of *Juniperus communis* was reported by detection reducing power, scavenging of free radicals, anion superoxide and hydrogen peroxide [9].

In DPPH radical scavenging, single electron of DPPH reacts with hydrogen of antioxidant compounds and purple color of DPPH changes to yellow. This change of color becomes as an indicator of antioxidant activity [8, 10,11]. In DPPH radical scavenging, the IC_{50} of J.e fruit extract and their fractions are increased as butanol fraction> ethyl acetate fraction> chloroform fraction= Petrolium ether fraction =crude extract. In other words, butanol fraction possesses the highest DPPH radical scavenging (IC_{50}=135.9±2.5 µg/ml, p<0.001) in comparison with other fractions. The least IC_{50}, (IC_{50}=21.2 ± 0.015 µg/ml) was observed in gallic acid (p<0.001).

In other research, it is reported that that all fractions of *Juniperus dropacea* fruit possess

radical scavenging activity except n-hexane fraction [12]. Also it is reported that in radical scavenging the IC_{50} of *Juniperus sibirica* fruit extract was 15.2 µg/ml [13].

Increase in reducing power, decreases the destructive effects of free radicals [14]. The reducing power of J.e fruit extract and its fractions are decreased as n-butanol fraction > ethyl acetate fraction > crude extract > Petroleum ether fraction (Figure 2). It means that n-butanol fraction posesses more reducing power (in 61.4±2.6 µg/ml with absorbance 0.5), p<0.00.1, Table 1. All of the samples show less reducing power than gallic acid (p<0.001, Figure 2).

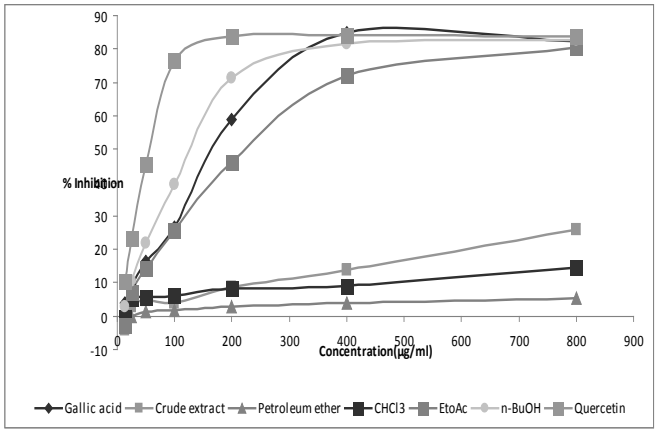

Fig. 1: DPPH radical scavenging of fruit extract and its fractions in comparison with gallic acid and quercetin

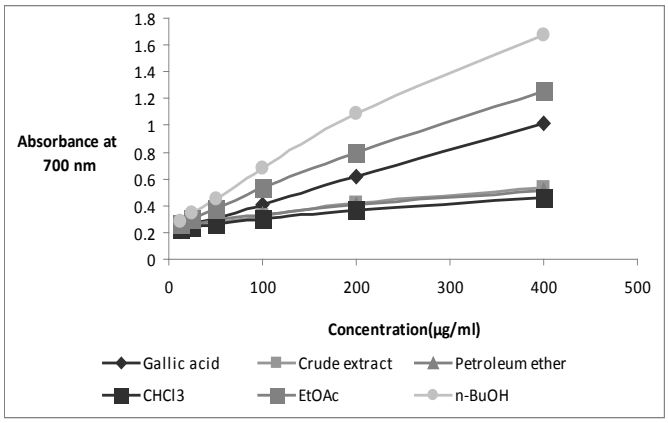

Fig. 2: Reducing power of fruit extract and its fractions in comparison with gallic acid and quercetin

In the determination of phenolic compounds, hydroxyl group of phenolics reacts with foline ciocaltu and complex of these two compounds becomes blue. More phenolics, more blue complex is formed [15].

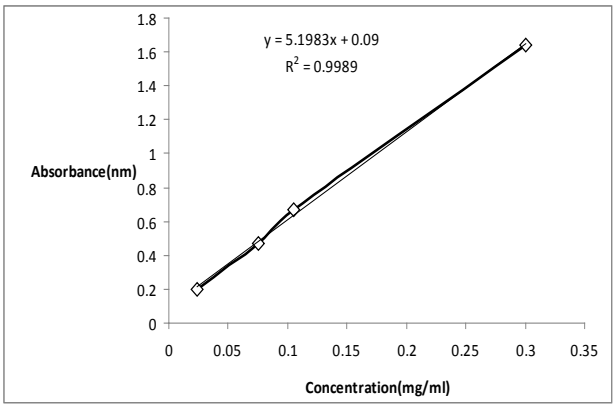

Fig. 3: Standard curve of gallic acid for determination phenolic compound

The amonut of phenolic compounds are decreased as n- butanol fraction > ethyl acetate fraction > Petroleum ether fraction > chloroform fraction, Table 1. Thus, in n- butanol fraction of the fruit the most amounts of phenolics (82.9± 1.1 mg/g) is detected, p<0.001.

Table 1: IC_{50}, phenolic compounds and concentrations of *Juniperus excelsa* fruit extract (or fraction) at absorbance 0.5 compared with gallic acid in reducing power assay

Samples	IC_{50} µg/ml ±SD	Concentration µg/mL (absorbance 0.5)±SD	Phenolic compounds mg/g ±SD
Crude extract	>800	347.72±21.5	1.85±0.02
Petroleumether fraction	>800	357.7±5.8	0.51±0.01
Chloroform fraction	>800	ND	0.295±0.01
Ethylacetate fraction	257.1±0.1	96.7±12.2	24.69±0.4
Butanol fraction	135.9±2.5	61.39±2.5	82. 9±1.1
Gallic acid	21.2±0.015	14.7±0.93	ND

Values are means ± SD, P<0.05 significant as compared to standard (gallic acid).

ND: Not determined

In present study, n-butanol fraction possesses the highest DPPH radical scavenging and reducing power (Figures 1, 2). In this research, a correlation between DPPH radical scavenging and phenolic compound is found. It means that phenolic compounds may be involved in radical scavenging [16-17]. In other study, a correlation between DPPH radical scavenging and phenolic compound is not observed [19]. Also in this research, n-butanol fraction has the highest amounts of phenolics and reducing power. In other words, phenolic compounds may be involved in reducing power potential.

CONCLUSION

Polar fractions of J.e fruits specially n-butanol fraction showed interesting antioxidant potential and can be used as a source of the antioxidant compounds. This result confirms the other results which showed that the heart wood of J.e could scavenge free radicals and its n- butnol fraction showed the highest radical scavenging activity [18].

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CONFLICT OF INTEREST

The authors declare that they have not any conflict of interest.

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