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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY ANALYSIS OF BLACK CURRANT

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ABSTRACT

The aim of this study was to categorize and calculate phenolic compounds and antioxidant activity in *Ribes nigrum* fruit extract. Antioxidant properties were investigated using different methods such as hydroxyl radicals scavenging activities , inhibition of lipid peroxidation and DPPH. The amount of phenolics (14.4±1.53 mg GAE/g) and flavonoids (6.32±0.32 mg QE/g) were found in black currant. Methanolic extracts of black current was showed strong scavenging activity against DPPH radicals (IC_{50} value 58.18±1.34 µg/ml). Black currant fruit extract has rich phenolic compounds and higher antioxidant activity could be used as natural antioxidant in food and pharmaceutical industries.

INTRODUCTION

Ribes nigrum L.(Blackcurrant) is a shrub generally grown in temperate climate. Its rich source of vitamin C and other health favourable substances like: essential oils, routine, organic acids, micro- and macronutrients and pectins (P.H.Mattila et al., 2011). Plants have the facility to produce chemical compounds which are correlated with the biochemical mechanisms of human metabolism. Polyphenol is general name of the compounds with numerous hydroxyl phenols in a single molecule (Zheng J et al., 2009; Callemiena D et al., 2010). Previous studies investigated that fruits of black currant contains high level of anthocyanins, polyphenol compounds, phenolic acid, proanthocyanidins as well as flavonols comparing to the another berries (Karjalainen et al. 2009; Mattila et al. 2011). Phenolic acids and flavonoids have higher biological activity such as antiviral antioxidative, anti-aging effect, antiallergic, antiinflammatory and antibacterial (Liu, J., *et al.*, 2011). The beneficial effect of numerous medicines could be determined by the occurrence of these natural antioxidants. For that reason, a significant awareness is paid to study of plant extracts with possible antioxidant effect. In *R. nigrum* has many polyphenolic antioxidants including phenolic acids, flavonol glycosides, anthocyanidines and proanthocyanidines (Aguié-Béghin V et al., 2008). The major active biological compounds in blackcurrant are the catechins: EGCG, EGC, ECG, and CE, found in fruit from where they could be isolated with comparatively high yields [20]. EGCG is the most important component of the

polyphenolic expressive about 10–50% of total catechins in blackcurrant (Coutinho D et al., 2008). The *R. Nigrum* large chemical composition diversity and high antioxidant properties keep on to create a centre of attention of researchers in the field of phytochemistry and pharmacogenesis (Callemiena D et al., 2010). The aim of this study is to investigate the chemical composition and antioxidative activity of black currant. Furthermore, total phenol and flavonoids content analysis of black currant fruit. Due to high antioxidant capacity black currant may used as a dietary supplement and food.

MATERIALS AND METHODS

Materials: Black current fruit was purchased by local medicinal herbs supplier. Fruits were dried and made it fine powder and stored at laboratory condition.

Reagents: Folin-Ciocalteu reagent in sodium carbonate, gallic acid, catechin, DPPH (2,2-diphenyl-1-picrylhydrazyl), aluminum chloride, were purchased from Sigma-Aldrich.

Extract Preparation: Fruit samples (20.0g) were extracted by 100ml methanol as a solvent. The extraction process was done using an ultrasonic bath at room temperature for 1h. After filtration, solvent was removed by a rotary evaporator under vacuum and the dried extract was stored in glass bottles.

Determination of Total Phenols: Total phenolics content were assessed spectrophotometrically using the Folin–Ciocalteu method (Singleton et al. 1999) with some modification. For the analysis, 0.5mL of extract, 2.5mL of 10% Folin–Ciocalteu's reagent and 2.5mL 7.5% NaHCO3, and kept for incubation at room temperature for 1 hour. Blank was concurrently set, containing 0.5mL methanol, 2.5mL 10% Folin–Ciocalteu's reagent and 2.5mL of of NaHCO3(7.5%). The absorbance was calculated at 765nm. Gallic acid was used as a standard. Each tests were done in triplicate.

Determination of total flavonoids : The flavonoid content of black currant fruit methanolic extracts were calculated using Garcia-Alonso (2004) method. 0.5 ml of extract was added to a 10 ml volumetric flask and make up volume up to 5 ml by water. Then 0.3 ml of 5 % NaNO2 was added to the flask. After 5 min, 0.6 ml of (10 %) $AlCl_3$ was added and after 6 min, 2 ml of NaOH (1M) was added to the reaction mixture followed by the addition of 2.1 ml distilled water. Absorbance was recorded at 350 nm. Total flavonoid content was expressed as mg quercetin equivalents in 100 g of fresh material.

DPPH radical scavenging activity: The scavenging activity of black currant fruit was calculated by Mathew, S. and T.E. Abraham (2006) method. Briefly, 1.0 ml of extract was added to 2.0 ml of DPPH solution at room temperature. The absorbance was measured at 515 nm against a blank. The results were expressed as percentage of reduction of the initial DPPH adsorption:

% of reduction of the initial DPPH adsorption = ADPPH (t)-Asample (t)/ADPPH (t)-100, ADPPH(t) = absorbance of DPPH at time t , Asample (t) = absorbance of sample at t

Determination of Lipid Peroxidation: Investigation of inhibitory activity against lipid peroxidation was determined using the thiocyanate method (Hsu et al. 2008). 0.5mL of extract was added to 2.5mL linoleic acid emulsion (0.2804g of linoleic acid and 0.2804g of Tween- 20 in 50mL of 40mM phosphate buffer). The reaction mixture was incubated at 37°C for 72 hours.

Table 1.- Total Phenol and Flavonoid content analysis of Ribes nigrum.

	Total Phenol Content (mg GAE/g)	**Total Flavonoid Content (mg QE/g)**
Methanol Extract	14.4±1.53	6.32±0.32

Table 2. DPPH, Hydroxyl Radical Scavenging and Lipid Peroxidation Activity of Ribes nigrum

	DPPH (IC_{50})	**Hydroxyl Radical Scavenging Activity (IC_{50})**	**Lipid Peroxidation Activity (IC_{50})**
Methanol Extract	58.18±1.34 µg/ml	49.86±1.65 µg/ml	38.68±1.23 µg/ml

After incubation, 0.1mL of reaction solution was mixed with 4.7mL of ethanol (75%), 0.1mL of iron (II)chloride (20mmol/L) and 0.1mL of ammonium thiocyanate (30%). The absorbance was measured at 500 nm. BHT was used as reference. The control was prepared the test sample without extract and reference compound was used. All tests were done in triplicates and the results were expressed IC_{50} values.

Determination of Hydroxyl Radical Scavenging Activity: The capability to inhibit non-site-specific hydroxyl radical-mediated peroxidation was performed using Hinneburg (2006) method. Extract was mixed with 500µL of (5.6mM) 2-deoxy-D-ribose in sodium phosphate buffer (50mM, pH= 7.4), 200µL of (100µM)of iron (III) chloride and (104mM) EDTA solution, 100µL of 1.0mMH2O2, and 100µL of (1.0mM) aqueous ascorbic acid. The mixture was incubated at 50°C for 30min. After then, 1mL of trichloroacetic acid (2.8%) and 1mL of thiobarbituric acid (1.0%) were added to each tube. The samples were mixed well and heated in water bath at 50°C for 30min. The oxidation of 2-deoxyribose level was estimated from the absorbance at 532 nm. The values are presented as the triplicate analyses. The results were expressed as IC_{50} values.

RESULTS

The total phenolic content: A high antioxidant activity of black currant was determined by total phenol content analysis. The phenolic compound content of black current fruit 14.4±1.53 mg GAE/g was recorded. (Table 1)

The total flavonoid content: The flavonoids have potent antioxidant and free radical scavenging properties in human body. The flavonoids content of black currant fruit 6.32±0.32 mg QE/g was recorded. Results were presented in table 1.

Antioxidant Activity:
DPPH Assay

DPPH is the one of the method to analysis of antioxidant activity is colorimetry of free radicals, based on the reaction of DPPH. Results are showing of DPPH by antioxidant a blue purplish. DPPH scavenging activity of black current was IC_{50} value (58.18±1.34 µg/ml) recorded.

Hydroxyl Radical Scavenging and Lipid Peroxidation Activity: Black currant extract was expressed well inhibitory activity against DPPH and hydroxyl radical. On the other hand, black currant also showed inhibition of lipid peroxidation activity. Hydroxyl radical scavenging activity of black currant fruit was recorded (IC50 value 49.86±1.65 µg/ml) and Inhibitory activity against lipid peroxidation was shown (IC_{50} 38.68±1.23 µg/ml). Results were presented in Table 2.

CONCLUSION

The growing significance of useful ingredients in food provides new challenges for plant sciences to get better health. The results offered in this paper specify a high antioxidant and Total Phenol and Flavonoid content. For that

reason, black currant can be considered as a good source of natural antioxidants as well as their consumption can bring health profit. The results of this study designate that this fruit requires a additional classification of its biologically active antioxidant compounds.

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