A comparative study on phytochemical potentials of *Rubus loganobaccus L.*

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Abstract

Raspberries composed of various varieties, are popular ingredients of daily diet with highly distinguished biological activities. In this study, a comparative investigation was conducted on various chemical potential of methanol extracts from *Rubus loganobaccus* L. leaf parts cultured in greenhouse and open-field. Biochemical activity of the extracts obtained from the field cultured leaves were observed to be higher than greenhouse cultured plants. In the antioxidant 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the field cultured leaves were better succeeded in radical scavenging with IC_{50} as low as 1.08 ± 0.75 µg/mL while in reducing power assay, open-field plants against greenhouse plants had a higher EC_{50} (2.82 ± 0.70 and 2.41 ± 0.75 µg/mL, respectively). Considerable antibacterial and antifungal activity were observed in open-field plants and greenhouse plants in minimum inhibitory concentrations (MIC) and minimum bacterial concentration (MBC) tests with a similar pattern, in which the lowest MIC and MBC in open-field and greenhouse plants were 5 mg/mL and 20 mg/mL, respectively, against *Bacillus cereus* and *pseudomonas aeruginosa*. This experiment revealed that leaf parts of *Rubus loganobaccus* cultured in greenhouse and in field possess a number of biological properties including antioxidative and antimicrobial potentials with the superiority of the later source which indicates the criticality of using natural products to improve human health**.**

Keywords: secondary metabolites, DPPH, phytochemicals, biochemical activity, Genus *Rubus*

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Introduction

Of the most diverse and complex genus, *Rubus*, from the *Rosaceae* family and subfamily of *Rosoideae* encompasses roughly 300-700 species, from which blackberries, raspberries and dewberries are known and important members with medicinally and nutritionally critical edible fruits (Debnath, 2016). Their fruits are mainly edible enriched with essential phytochemicals, namely berries contain ascorbic acid, aromatic acids, ellagitannins, and free ellagic acid, anthocyanins, flavones, carotenoids, essential oils with triacylglycerol, fatty acids, and phytoestrogens (Bobinaitė et al., 2013). More often than not, antibacterial potentials of secondary metabolites of *R. loganobaccus* have been found in dietary and medicinal plants, among them, berry fruits, in particular genus *Rubus* could

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Table 1
The antibacterial activity and antioxidant potential of the extracts obtained from *Rubus loganobaccus* leaves cultured in the greenhouse and in the field

Microbial strains		Extracts from *Rubus loganobaccus* leaves cultured in:			
		Greenhouse		Field	
		MIC values mg/mL	MBC values mg/mL	MIC values mg/mL	MBC values mg/mL
Candida albicans	IBRC-M30087	160	160	80	80
Escherichia coli	IBRC-M10871	80	80	20	20
Staphylococcus aureus	IBRC-M10690	40	40	40	40
Pseudomonas aeruginosa	IBRC-M10709	20	20	5	5
Bacillus cereus	IBRC-M10948	20	20	5	5
Staphylococcus epidermis	IBRC-M11151	-	-	-	160
Antioxidant assay					
DPPH*		1.46 ± 0.66		1.08 ± 0.75	
Total antioxidant**		2.41 ± 0.75		2.82 ± 0.70	

***** value expressed as IC50 (µg/mL); ****** value expressed as EC50 (µg/mL)

be an exceptional potential source of antibacterial agents (Puupponen-Pimiä et al., 2005). In this study we aimed to evaluate the crude extracts obtained from *Rubus loganobaccus* leaves cultured in the greenhouse and in the field for possible phytochemical potentials.

Materials and Methods

Plant material and preparation of the extracts

The leaf parts of *R. loganobaccus* cultured in both greenhouse and in the field were collected from Cellul Fanavar Daru in Karaj in the summer of 2017 and were separately air-dried at ambient temperature in the shade. In order to prepare the extract, 10 g of dried powdered leaf parts were mixed with 100 mL of methanol which was used for assays.

Total phenolic contents

Total phenolic content based on Folin–Ciocalteu method (Singleton et al., 1999), and total flavonoid contents using aluminum chloride colorimetric method (Halliwell et al., 2005) were assessed.

Antioxidant activity

Reducing power assay

The antioxidant activity was determined by the reducing power ability following the procedure described by Bondet et al. (1997) and also DPPH assay using Skrovankova et al (2015).

Antibacterial and fungal activity

The leaf crude extracts of *R. loganobaccus* cultured both in the greenhouse and in the field were used to evaluate antimicrobial activity based on the macro dilution method based on Thompson (1995). The microorganisms evaluated in this study were obtained from Iranian Biological Resource Centre (IBRC), Iran (Table 1).

Statistical Analysis

All analyses were run in triplicate and expressed as means ± SD. Statistical analyses were performed using SPSS version 24.0 software.

Results

Content of secondary metabolites and antioxidant capacity

Total phenol content was measured by Folin-Ciocalteu reagent in terms of Gallic acid equivalent. As shown in Table 1, total phenolic contents in *R. loganobaccus* leaves cultured in the field were higher than those cultured in the greenhouse (66.63 ± 1.31 and 65.30 ± 2.56 mg GAE/g, respectively). Moreover, leaves of *R.*

loganobaccus cultured in the field had a higher level of flavonoid (29.35 ± 8.53 mg of QE/g) compared with greenhouse-cultured plants (22.44 ± 3.32 mg QE/g, Table 1).

Employing reducing power test, antioxidant capacity in terms of ascorbic acid equivalent (Table 1) showed that the EC_{50} of *R. loganobaccus* leaves cultured in the field were higher than those cultured in the greenhouse (2.82 ± 0.70 vs. 2.41 ± 0.75 µg/mL, respectively). As displayed in Table 1 the extracts of *R. loganobaccus* leaves cultured in the field showed a higher antioxidant activity against DPPH radical in comparison with greenhouse-cultured plants.

Antibacterial and antifungal activity

Results of antimicrobial activity for extracts of the leaf parts of *R. loganobaccus* cultured in the greenhouse and in the field are given in Table 1. Both plant sources to some extend followed a similar reaction pattern in either MIC or MBC, with difference in concentration in which open-field grown plant concentrations used were strikingly lower than the greenhouse plant leave extract except for *Staphylococcus aureus*. A relatively wide range of concentration from 5 to 160 mg/mL. The maximum antibacterial potential was obtained from the extract of the field cultured leaves and the highest level of sensitivity of the bacterial strain *Bacillus cereus* was observed with the MIC value of 5 mg/mL.

Discussion

Berries are known as a rich source of phenolic compounds (Vuorela et al., 2005). Plants containing high level of Gallic acid which is a markedly potent antioxidant are raspberries, black tea, and red wine which possesses 3-fold higher antioxidant activity then either vitamin C or E which confers a significant potential against cancerous cells and its preventative impacts on cell proliferation and cell death in prostate cancer cell lines has been proven (Yokozawa et al., 1998).

Further, using various spectrophotometric antioxidant assays is highly recommended since the reaction mechanism and methodology from an assay to another vary significantly (Mafakheri and Hamidoghli, 2015; Nasiry et al., 2017; Bakhshipour et al., 2019). Correspondingly, effects of light intensity, photoperiod, and temperature on the biosynthesis of many secondary metabolites (Ahmadvand et al., 2013) have been established. Jorge et al. (2017) observed a higher concentration of bioactive substrates in field-grown plants compared to greenhouse plants of *Amaranthus hypochondriacus* L.

Consistent with our results, the previous studies similarly indicated the strong antimicrobial activity of raspberries possibly owing to their high ellagitannin content and their total phenolic content (Hood et al., 2004). Also, it was suggested by Cavanagh et al. (2003) and Puupponen-Pimiä et al. (2005) that berry extracts inhibit gram-negative and not gram-positive bacteria, possibly due to their differences in cellular wall structures. Recently, Shibu and Prata (2017) evaluated the antibacterial potential of ethanolic leaf extract from some species of Rubus (*R. ellipticus*, *R. niveus*, *R. racemosus*, and *R. rugosus*) cultivated in open-field condition and their findings indicated the high sensitivity of different bacteria strains to the extracts in concentrations range from 13.5 to 27.5 mg/mL.

Conclusions

Overall, the field-cultured leaves from *R. loganobaccus* showed higher phytochemical activities in our study and in accordance with the official standard values of berries, it gives support to the general trend in promoting sustainable use of natural resources. Considering the capability of genus *Rubus,* further studies are highly required particularly testing the potency of extracts on more bacterial species exclusively the renown resistant species in addition to using various solvents to optimize the extraction process.

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