

## Antioxidant Activity of *Lallemantia royleana* (Benth.) Seed Extract

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### Abstract

*Lallemantia royleana* seeds have been used in Persian traditional medicine during the ages. The seeds are known as “*Balangu*” in Iran and still are widely used as an ethnomedicine for treatment purposes such as in gastrointestinal disease, kidney and urinary disorders and skin complications. In this study, antioxidant and total phenolic content of *L. royleana* seeds were investigated. Seeds of *L. royleana* (50 g) were crushed using a laboratory mill. Ground material (50 g) was extracted by maceration using 500 ml of ethanol-water (80:20). Antioxidant activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays. In addition, Folin-Ciocalteu method was used to determine the total phenolic content. IC<sub>50</sub> value of DPPH scavenging activity was 300 µg/mL. Total phenolic content was 25.3 mg as gallic acid equivalent/g extract. It seems that the phenolic constituents of the seeds are probably responsible for some part of antioxidant activity, while some unsaturated fatty acids (including linoleic and oleic acid) may be responsible for the other part, based on the review of the literature. Due to the limited studies about “*Balangu*” seed, more scientific surveys may be helpful for clarifying other biological properties of this traditionally important medicinal plant.

**Key words:** *Lallemantia royleana*, *balangu*, Antioxidant activity, Persian medicine

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### Introduction

*Lallemantia royleana* (Benth.) from Labiatea family is distributed in different regions of Iran [1]. The vernacular name of this plant is *Balangu* or *Balangu Shirazi* [2]. *L. royleana*, and especially its seed, has been considered as a medicinal herb by traditional Persian practitioners for some centuries. They prescribed oral consumption of the seeds for treatment of various purposes such as gastrointestinal disease, respiratory ailments and kidney and urinary disorders. Administration of seeds depending on the type of disorders could be in row forms or as syrup after maceration in water with or without honey, rose or salix water addition. *Balangu* seeds also applied as one composition of herbal mixtures for anxiety and

depression disorders. Seeds mucilage also applied as a liniment for skin complications [3-4]. There are limited published articles about the pharmacologic properties and phytochemical constituents of *Balangu* seeds. Hypocholesterolemic effect has been observed in rabbits consumed whole seeds as 5, 10 or 20% of diet [5]. Mixture of *Balangu* mucilage with lidocaine possesses more anesthetic effect than commercial lidocaine gel in rats (tail flick experiment assay). *Balangu* gel lonely showed analgesic effect and additionally it may affect the release of drug as well as skin penetration [6]. Different organic extract of seeds showed antibacterial activity [7]. Crude oil, crude protein, and crude ash are determined as 18.27%, 25/6 %

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and 1.29% in *Balangu* seeds [8] and fatty acid including linoleic, oleic, palmitic and stearic acid in addition to beta-sitosterol are the constituents of seed oil [9]. Rhamnose, arabinose, galactose, glucose and xylose are identified as monosaccharides of *Balangu* seed mucilage [10]. Seeds because of their mucilage content are important for food industry and various research have been published in this field [11-12].

In this study antioxidant activity of *Balangu* seeds has been evaluated using DPPH and FRAP assays in addition total phenol content has been determined via Folin-Ciocalteu method.

## Methods

### *Plant material*

Seeds of *L. royleana* were purchased from local market of Tehran, Iran. As the result of authentication, voucher specimen (PMP-713) was prepared and a sample was deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

### *Extraction*

Seeds of *L. royleana* (50 g) were crushed using a laboratory mill. Ground material (50 g) was extracted by maceration using 500 ml of ethanol-water (80:20). Solvent evaporated to dryness. Dried extracts were stored at 2-8 °C with no exposure to light and dissolved in suitable solvents in order to be used in experiments.

### *Evaluation of antioxidant activity by DPPH and FRAP methods*

Free radical scavenging activity was determined using DPPH spectrophotometric method which completely described in some previous articles [13]. Based on primary test, four concentrations of extract (100, 200, 300, 500 µg/ mL) were prepared via dissolving in methanol. 1 mL of each samples was added to fresh methanolic solution of DPPH (40 µg/ mL). After 30 minutes incubation in the dark at room temperature, the absorbance was recorded at 517 nm in comparison with

proper blank. Inhibitions percentage was calculated from below equation:

$$I = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

where,  $A_{\text{blank}}$  is the absorbance of control (the DPPH solution without sample solution). The IC<sub>50</sub> value (concentration of examined samples which exhibited 50% scavenging activity) was calculated based on sample concentration against percentage of inhibition and reported as mean ± standard deviation.

FRAP assay for reducing power determination was carried out using FRAP reagent containing 5 mL Tripyridyltroazin (TPTZ) +5 mL FeCl<sub>3</sub>+ 50 mL acetate buffer (pH=3).

This reagent is a labile solution and should be prepared just before the test. Mentioned reagent was added to the tubes and the mixture was heated (37 °C, 5 min). Fifty µL of prepared samples with concentration of 300 µg/ mL and five concentrations of aqueous solutions of FeSO<sub>4</sub>.7H<sub>2</sub>O (125, 250, 500, 750 and 1000 µmol/L (for plotting the calibration curve) were added to the mixture. The change in the absorbance of the sample (in triplicate) was recorded after 30 min at 593 nm against proper blank (in which FRAP reagent was absent). The results were expressed as µmol FeSO<sub>4</sub>.7H<sub>2</sub>O equivalents per mg of the sample [14]. Statistical analyses were performed using ANOVA followed by Tukey post-hoc test for multiple comparisons of means (p < 0.05).

### *Determination of Total Phenolic Content*

Spectrophotometric evaluation with Folin-Ciocalteu method was used for determination of the total phenol content [15]. 1 mL of sample (500 µg/ mL) was mixed with 5 mL of Folin-Ciocalteu's reagent (10-fold diluted with distilled water). After 10 min, 4 mL of sodium hydrogen carbonate solution (7.5% w/v) was added and the mixture was shaken. The absorbance against blank was recorded at 765 nm with an UV-VIS spectrophotometer after 30 min incubation at

room temperature. The total polyphenol content results introduced as (expressed as)  $\mu\text{g}$  of gallic acid equivalents per mg of dried sample, were obtained using a calibration curve of a prepared gallic acid standard solution (75-200  $\mu\text{g}/\text{ml}$ ).

## Results

### Antioxidant activity

*Balangu* seeds hydro- alcoholic extract possessed radical scavenging activity with  $\text{IC}_{50}$  300  $\mu\text{g}/\text{mL}$ . FRAP assay showed no significant results. Based on our investigation, there are no published data for comparing with the result.

### Total phenolic content

Total phenolic content was 25.3 mg as gallic acid equivalent/g extract.

## Discussion

In the present study antioxidant property of *L. royleana* seeds was explored. There is no literature about the antioxidant activity of these seeds and this is the first one. Seeds of another specie, *Lallemantia iberica* (M.Bieb.) may have similar applications to *Balangu* and sometimes used as an alternative [16].

There is also no report on antioxidant activity of *L. iberica* seeds but antioxidant activity of essential oil of its aerial part was investigated using DPPH and FRAP methods. In flowering stage, antioxidant activity was determined as 100  $\mu\text{g}/\text{mL}$  (DPPH assay) and 70  $\mu\text{mol-lfe}+2 \text{ g}^{-1} \text{ DW}$  (FRAP method) [14].

Phenolic compounds such as flavonoides and phenolic acids are considered as one of the main natural antioxidant sources [17].

It seems that phenolic constituents of *Balangu* seeds in addition to unsaturated fatty acids (including linoleic and oleic acid) may be responsible for its antioxidant property. Due to the wide application of *Balangu* seeds in Persian traditional medicine and relatively few published articles about this plant, more scientific surveys may be helpful for clarifying other biological properties.

## Conflict of interests

Authors have no conflict of interests.

## Acknowledgment

None.

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