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Original Article

# Effect of Salicylic acid on Phenols and flavonoids content and DPPH scavenging activity in cell suspension culture of Iranian sodab (Ruta graveolens)

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Ruta graveolens, locally called sodab or sadab, is a known medicinal plant in north of Iran. The plant is a source for the production of secondary metabolites such as Glycosides, lignins, alkaloids, Coumarins, flavonoids and phenolic acids. In this work, the effect of salicylic acid on phenols and flavonoids contents of R. graveolens were investigated in the cell suspension culture. A significant enhancement in total phenolic and flavonoids contents were observed in salicylic acid treated samples. The results revealed that salicylic acid (10 mg/ml) showed as maximum as 3.14 fold enhancement in total flavonoid content (40.35 mg/g) and salicylic acid (20 mg quercetin equivalent/g of extract powder) showed as maximum as 18.33 fold improvement in total phenolic content (438.75 mg gallic acid equivalent/g of extract powder). Also in this study, salicylic acid (10 mg/ml) showed as maximum as 3.55 fold increase in DPPH radical-scavenging activity compared to control. These results suggest that the positive effect of salicylic acid on phenols and flavonoids content and DPPH scavenging activity in cell suspension culture. This effect was dose dependent.

**Keywords:** Salicylic Acid, Phenolic Content, Flavonoids, DPPH, Cell Suspension Culture, Ruta Graveolens, Sodab.

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## Introduction

Iranian sodab (Ruta graveolens) is an evergreen erect, glabrous and glaucous perennial plant with 30 - 80 cm in height, which belongs to the botanical family of Rutaceae [1]. Rue, locally called "sodab or sadab", is a valuable medicinal and culinary plant that grows in Mediterranean zone and north of Iran [1, 2]. The plant is a real factory to produce secondary metabolites such as alkaloids, flavonoids, lignins, Glycosides, Coumarins and phenolics compounds [3]. Rue has more than 120 valuable compounds belonging to four different classes have been identified [4,5]. It has known for its high pharmaceutical value such as Antioxidant activity, anti-inflammatory, cytotoxic, antitumor, anti-arrhythmic, anti-androgenic, contraception and fertility [6]. Historical informations show that the herb has been used since ancient as medicine. In Iran and China Ruta used to treat fibril disease, flue, rheumatism, infantile convulsions, dysuria, diarrhea, hernia, amenorrhea, traumatic injuries, and suppurative infections of skin, snake bites, miasma, epistaxis, toothache and furuncle [1,7]. Production of secondary metabolites notably phenols and flavonoids has gained significant interest in the medical and pharmaceutical community, and there are available data which [8].

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The development of plant cell and tissue culture can effectively facilitate propagation of medicinal herbs for pharmaceutical production and other applications would require its mass propagation to be improved to reduce the cost of its production [9]. One of the problems of industrial synthesis by plant cell culture is the low productivity because of low concentration of the natural compounds. To enhance the productivity, addition of enhancer include elicitors has been investigated [10, 11].

Salicylic acid (2-hydroxybenzenecarboxcylic acid) is a crystalline organic acid. It has important role to play in growth and development, photosynthesis, transpiration, ion uptake and solute transport. SA (Salicylic acid) has been reported to induce gene regulation related to biosynthesis of secondary metabolites in plants [12,13]. The effect of SA on secondary metabolite production has been investigated in plant systems like Andrographis paniculata and Jatropha curcas [13,14]. We have recently reported the effect of salicylic acid on phenols and flavonoids content in callus culture of R. graveolens [15].

There is no report of pharmaceutical studies in the literature about effect of Salicylic acid on phenols and flavonoids content in cell suspension culture of Iranian sodab (Ruta graveolens). The present study seeks to improvement of phenols and flavonoids content production and DPPH scavenging activity of R. graveolens through cell suspension culture.

## Materials and Methods

*Chemicals*

Gallic acid and Quercetin were purchased from Merck and Fluka companies. All other chemicals and reagents used were of the highest commercially available purity.

*Plant material preparation*

The whole parts of Ruta were collected from Noshahr (Kheirudkenar forest) in Mazandaran in July of 2015.

*Inoculation and culture condition*

For surface Sterilization, stem explants were rinsed with running tap water for 20 minutes, placed at 70% ethanol for 30 seconds, and then washed with sterilized distilled water three times. Later on, they were submerged in 1.5% sodium hypochlorite for 20 minutes and repeatedly washed three times with sterilized distilled water. Callus formation was observed from the stem explants, when they were inoculated on MS supplemented with NAA (3 mg/l) within six days of culture. The developed callus was transferred to a flask in MS medium supplemented NAA (3 mg/l) 3 with various concentration of salicylic acid (10 and 20 mg/ml). Cell suspension culture was kept in a rotary shaker at 100 rpm for 15 days under dark condition maintained at 27 ± 1°C. The control and treated callus were removed from culture and dried under laminar flow without light [14].

*Methanolic extracts preparation*

Materials dried under laminar flow without light before extraction. Each part was extracted at room temperature by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained.

*Determination of total flavonoid content*

Colorimetric aluminum chloride method was used for flavonoid determination [16]. Briefly, 0.5 ml solution of each plant extracts in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Total flavonoid contents were calculated as quercetin from a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg/ml in methanol.

*Determination of total phenolic content*

Total phenolic compound contents were determined by the Folin-Ciocalteau method [16] .The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na2CO3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

*DPPH radical-scavenging activity*

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of the free radical-scavenging activity of the extracts [17]. Different concentrations of extract were added, at an equal volume, to a methanolic solution of DPPH (100 mM). After 15 min at room temperature,



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the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamin C, BHA and quercetin were used as standard controls. IC50 values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals. The IC50 values were calculated from a linear regression analysis.

*Statistical analysis*

Experimental results are expressed as means. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p<0.05$) and the means separated by Duncan's multiple range tests (by SAS software).

## Results and Discussion

Phenols and polyphenolic compounds, such as flavonoids, are extensively found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [17, 18]. Investigations have shown that enhancing levels of flavonoids in the diet could decrease certain human diseases [16,19].

Total phenol compounds, as determined by Folin-Ciocalteau method, are reported as mg gallic acid equivalent/g of extract powder, by reference to standard curve (y = 0.0063x, r2 = 0.987). The total flavonoid contents are reported as mg quercetin equivalent/g of extract powder, by reference to standard curve (y = 0.0067x + 0.0132, r2 = 0.999). The IC50 values for Ascorbic acid, quercetin and BHA were 1.26 ± 0.11, 1.32 ± 0.07 and 13.49 ± 1.04 mg/ml, respectively. DPPH radical-scavenging activity decreased with increasing concentration of salicylic acid from 10 mg/ml (8.206 mg/ml) to 20 mg/ml (7.630 mg/ml) (Table 1.).

**Table 1.** Effect of salicylic acid on synthesis in total phenolic and flavonoids content and DPPH scavenging activity in callus culture of Ruta graveolens.

| Treatment (mg/ml) | Phenolic content (mg/g) | Flavonoids content (mg/g) | $IC_{50}$ (mg/mL, d.w.) of DPPH scavenging activity |
|---|---|---|---|
| 0 mg/ml SA | 23.93 | 12.81 | 2.308 |
| 10 mg/ml SA | 54.95 | 40.35 | 8.206 |
| 20 mg/ml SA | 438.75 | 23.16 | 7.630 |

The results revealed that salicylic acid (10 mg/ml) showed as maximum as 3.14 fold increase in total flavonoid content (40.35 mg/g) and salicylic acid (20 mg quercetin equivalent/g of extract powder) showed as maximum as 18.33 fold increase in total phenolic content (438.75 mg gallic acid equivalent/g of extract powder). Also, in this study, salicylic acid (10 mg/ml) showed as maximum as 3.55 fold increase in DPPH radical-scavenging activity compared to control.

Flavonoids content decreased at higher concentration of salicylic acid treatment but increased at lower concentration. Phenolic contents increased with increasing concentration of salicylic acid (Table 1.). Phenolic contents production increased to higher level at 10 mg/ml SA followed by 20 mg/ml SA treatments compared to control. Flavonoids content decreased with increasing salicylic acid concentration ranging from 40.35 to 23.16 mg quercetin equivalent/g of extract powder. The study shows that the elicitation depends on elicitor dose and type of compound to be elicited.

Mahalakshmi et al (2013) reported production of secondary metabolites from cell suspension culture of Jatropha curcas using salicylic acid elicitation and have important industrial applications [14].

The enhancement of andrographolide content in cell suspension culture of Andrographis paniculata (Burm. f.) Nees. was observed when treated with 0.05 mM SA for 24 hours treatments, it yielded maximum as 18.5-fold increment in andrographolide content (37.0 μg/g) against control [13].

## Conclusion

In present study, a significant enhancement in total phenolic and flavonoids contents and DPPH radical-scavenging activity observed in salicylic acid treated samples. Also this study shows that the elicitation depends on elicitor dose and type of compound to be elicited.

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***Conflict of interests***

There is no conflict of interests.

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