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Induction of Rosmarinic Acid Synthesis in *Origanum majorana* by Seaweeds Extracts and Its Antioxidant Activity

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Abstract: Rosmarinic acid (RA) was extracted and determined from *Ocimum basilicum, Origanum majorana, Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris. R. officinalis* expressed the highest content (186.4 mg/DW) followed by *O. majorana* (140 mg/DW). However, *O. basilicum* and *T. vulgaris* and *S. officinalis* showed 80.7 mg/DW, 66.7 mg/DW, and 50.4 mg/DW of RA, respectively. Treatment of *O. majorana* seedlings with the various seaweed extract from *J. rubens, C. prolifera and C. sinuosa* increased RA content with 7.7%, 21.6% and 50% , respectively. RA from *O. majorana* exhibited appreciable antioxidant activity using DPPH and ABTS compared to ascorbate as standard antioxidant.

Key words: Rosmarinic acid , *Origanum majorana* , Seaweeds , Antioxidant, DPPH.

1.Introduction

Rosmarinic acid (RA) is a secondary compound found in various plants (Sik *et al*., 2020) [1] and it has various applications such as cosmetics (Caleja *et al*., 2017) [2] and pharmaceutical industry (Serrano *et al*., 2021) [3] . It has been described that RA has different health promoting properties including neuroprotective (Costa *et al*., 2013) [4] and hepato-protective (Wang *et al*., 2019) [5] . RA is an antioxidant and it is applied in food industry (Li *et al*., 2021) [6] . The chemical structure of RA is shown in Fig. 1

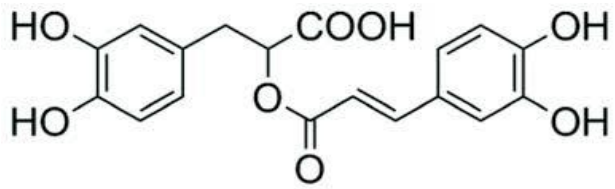

Fig. 1: Chemical structure of rosmarinic

RA has been described in Lamiaceae, Anthocerotaceae, Boraginaceae and Blechnaceae families (Sik *et al*. 2020**)** [1] **.** Among the sources of RA are *Salvia officinalis* (Celano *et al*., 2017) [7] , *Rosmarinus officinalis* (Sik *et al*.2020) [1] , *Ocimum basilicum* (Saad et al., 2021) [8] , *Salvia miltiorrhiza* (Deng *et al*., 2020) [9] .

Consumers are concerned about the synthetic antioxidants safety. Therefore, RA is one of the most consumed antioxidants in food industry (Choi *et al*., 2019) [10] . Therefore, the present investigation aimed to isolate RA from various plants, study the influence of various seaweed extracts on RA content in these plants and measure its antioxidant activity.

2. Materials and methods

2.1. Plant Seeds

Seeds of pure strain for each tested plant (*Ocimum basilicum, Origanum majorana, Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris*) were obtained from Egyptian Ministry of Agriculture.

2.2. Seed germination

Seeds of *Ocimum basilicum, Origanum majorana, Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris* were sterilized using 95% commercial bleach. Broken seeds were abandoned and the remaining seeds were imbibed in sterile water for 24 h, germinated and

grown for 14 days according to El-Shora and Abo-Kasem (2001) [11] .

2.3. Seaweed collection

The three seaweeds were *Jania rubens, Caulerpa prolifera* and *Colpomenia* sinuosa collected from the coast of Hurghada Red sea coast of Egypt and identified according to Aleem (1993) [12] .

2.4. Preparation of seaweeds extracts

The three extracts of seaweeds were prepared according to Khelil-Radji *et al*. (2017) [13] .

2.5. Extraction of RA and its assays

The extraction of RA and its assay were carried out according to the method of Öztürk *et al*. (2010) [14] .

2.6. Preparation of plant extract

The fresh leaves of each tested plant (2 g) were macerated in 25 ml of pre-chilled 150 mM potassium phosphate buffer (pH 7.0) including 1mM cysteine. The homogenate was centrifuged at 5,000 rpm at 25^{o}C for 20 min. The resulting supernatant was used as crude extract for subsequent analysis El-Shora *et al*.

(2021) [15] .

2.7. DPPH & ABTS scavenging activity

Radical DPPH and ABTS radical scavenging activity was determined according to Baliyan *et al*. (2022) [16] .

3. Results

The content of rosmarinic acid was determined in leaves of five plants namely *Ocimum basilicum, Origanum majorana, Salvia officinalis, Rosmarinus officinalis* and *Thymus vulgaris.*

The obtained results are shown in Table 1 and indicate that three tested plants contained variable contents of rosmarinic acid. *R. officinalis* showed the highest content (186.4 mg/DW) followed by *O. majorana* (140 mg/DW), *O. basilicum* (80.7 mg/DW), *T. vulgaris* (66.7 mg/DW) and *S. officinalis* (50.4 mg/DW).

Table 1: Rosmarinic acid content in various non- treated plants.

Plants	Rosmarinic acid (mg/ g DW)
Ocimum basilicum	80.7 ± 1.9
Origanum majorana	140.0 ± 1.7
Salvia officinalis	50.4 ± 1.0
Rosmarinus officinalis	186.4 ± 2.5
Thymus vulgaris	66.7 ± 1.8

Since *origanum majorana* expressed appreci- able a content of RA, it was used to investigate the influence of seaweed extracts on RA synthesis. The effect of the three seaweed extracts on rosmarinic acid content in *Origanum majorana* was investigated at different concentrations (20, 40, 60, 80 and 100 µg/ml).

The results in Table 2 indicated that the three extracts induced rosmarinic acid synthesis in the plant. The control value was 150.0 mg/DW which increased under treatment to 161.6, 182.2 and 225 mg/DW and the percent of increase reached 7.7%, 21.6% and 50% in presence of the three extracts in the same order

Table 2: Rosmarinic acid content in *Origanum majorana* after treatment with seaweeds extracts.

Treatment	RA content (mg/ g DW)	% increase
Control	150.0 ± 1.8	-
J. rubens	182.2 ± 1.4	21.6
C. prolifera	161.6 ± 2.1	7.7
C. sinuosa	225.0 ± 1.9	50.0

The antioxidant activity of RA from *Origanum majorana* was estimated by DPPH. RA was examined at various concentrations (10-50 µg/ml). Ascorbate was used as standard at the same concentrations.

The results in Table 3 indicated that any increase of RA concentration resulted in the increase of DPPH scavenging activity from 32% at 10µg/ml to 87.4% at 50µg/ml. However, ascorbate as standard antioxidant expressed the higher scavenging activity (43.4% to 96%) in presence of the various RA concentrations

Table 3: DPPH scavenging activity of RA from *Origanum majorana*

Concentration of RA (µg/ml)	DPPH radical scavenging (%)	
	Ascorbate	Rosmarinicacid
10	43.4 ± 0.9	32.0 ± 0.8
20	56.7 ± 1.0	40.0 ± 0.8
30	75.7 ± 1.3	50.9 ± 1.2
40	86.2 ± 1.6	69.8 ± 1.4
50	96.0 ± 1.8	87.4 ± 1.7

The antioxidant activity of rosmarinic acid from *Origanum majorana* was estimated by a second method of ABTS. RA was examined at the same concentrations used for DPPH (10-50 µg/ml). Ascorbate was used as standard at the same concentrations.

The results in **Table 4** reveal that increasing the rosmarinic acid concentration resulted in the increase of ABTS scavenging activity from 27.6% at 10µg/ml to 80.4% at 50µg/ml. However, ascorbate as standard expressed higher scavenging activity (48.8 % to 95.5 %) in presence of the various rosmarinic acid concentrations.

Table 4: ABTS scavenging activity of RA from *Origanum majorana.*

Concentration of RA (µg/ml)	ABTS radical scavenging (%)	
	Ascorbate	Rosmarinic acid
10	48.8 ± 0.9	27.6 ± 0.6
20	64.7 ± 1.3	47.0 ± 0.8
30	78.4 ± 1.4	60.2 ± 1.2
40	88.6 ± 1.8	69.8 ± 1.4
50	95.5 ± 1.9	80.4 ± 1.7

4. Discussion

RA is found in plants of Boraginaceae and Lamiaceae with infrequent reports of its occurrence in other mono- and dicotyledonous plants. RA also found in plants of hornworts and the fern families such as Dennstaedtiaceae and Blechnaceae (Petersen *et al*., 2009; Petersen, 2013) [17-18]

Seaweeds encompass various growth regulators, vitamins, amino acids, polysaccharides, nutrients, and polyamines which control the metabolism of cells (Khan *et al*., 2009) [19] and accordingly increase rosmarinic acid synthesis. It has been found that the polyphenols, the polysaccharides including fucoidan, alginate, carrageenans and laminaran found in seaweed extracts enhanced the growth of many plants (Drira *et al*., 2021) [20] and increase in RA.

Other findings indicated that biostimulation is produced by proteins, the carbohydrates, minerals, phytohormones, vitamins and fatty acids in algal extracts (Hernández-Herrera *et al*., 2018) [21] . The spinach treated with *Ecklonia maxima* extracts expressed an increase of endogenous auxins, cis-zeatin, isopentyladenine, cytokinins, abscisic acid, dihydrozeatin, gibberellins in plants and all these compounds have been connected with the positive plant growth as well as synthesis of RA (Jayaraj, 2018) [22]

RA in the present investigation exhibited appreciable antioxidant activity using DPPH and ABTS methods and these results are in harmony with the results of Suthar *et al*. (2021) [23] who reported the antioxidant capacity of RA from *Ocimum tenuiflorum*. Also, these results are in aagreement with those of Aldoghachi *et al*. (2021) [24] who found antioxidant activity of RA from *Mentha piperita* using DPPH.

Antioxidants are identified as substances that react with and neutralize free radicals. Thus, Antioxidants prevent or decrease the harmful effects of free radicals on the human body. It has been found that the antioxidant activity of RA is higher than that of α-tocopherol (vitamin E) (Park *et al*., 2008) [25] . Cuvelier (1996) [26] reported that RA is the plentiful caffeic acid dimer and the main phenolic compound accountable for the antioxidant activity exhibited by *Salvia* extract.

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