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RAPID COMMUNICATION

Immunomodulatory Activity of Different Polyphenolic Compounds Isolated from *Origanum majorana* L

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Abstract

Growing in many Mediterranean nations since antiquity, *Origanum majorana* L. is an aromatic herb that gained popularity as a spice and medicinal plant during the middle ages. Although *O. majorana* possesses a wide range of pharmacological effects, its immunoreactive components and mechanisms remain unknown. In this study, six compounds were isolated and identified from *O. majorana*. The isolated compounds were identified based on acid hydrolysis, comparative PC, UV, ESI-MS, ^{1}H-, and ^{13}C-NMR spectroscopic analysis and comparing with authentic standards and previously reported data. The compounds were identified as cinnamic acid, gallic acid, caffeic acid, *P*-coumaric acid, apigenin, and luteolin. Proliferation of immune cells was investigated and the effect of the isolated compounds on the growth of raw macrophage 264.7 was estimated using the MTT assay. Immunomodulatory effects of the six compounds were screened showing high immunomodulatory activity on RAW 264.7 cells.

Keywords: ^{13}C-NMR, Immunomodulatory, MTT assay, *Origanum majorana* L, Proliferation, RAW 264

1. Introduction

Herbs and spices have been used since antiquity for their flavor-enhancing, preservative, and medicinal properties. Since BC times, their extracts have been used in the Middle East to cure various disorders as spasmodic gastrointestinal complaints, cough, bronchitis, laryngitis, and tonsillitis They were also used as carminative and diuretic agents. Besides topical applications of their preparations were useful in the treatment of wounds, oral cavity disorders, and oral hygiene. Moreover, they are still used as a condiment to flavor meat, sausages, salads, and soups (Novak et al., 2000).

As a result, both industrialized and developing nations are experiencing an increase in demand for these plants, which drives up their cost. Despite Egypt being one of the countries with favorable conditions for the production of aromatic plants, such as a suitable environment and readily available low-wage manpower (essential for service procedures, agriculture, collection and marketing), the cultivated area of these plants is not satisfactory because the farmers are not interested in growing aromatic plants (Schippmann et al., 2006).

The food industry makes extensive use of synthetic antioxidants, which are less expensive and more effective than natural antioxidants, such as *tert*-butyl hydroquinone (TBHQ), butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT) (Pin-Der & Gow-Chin, 1997). Their safety, however, has been questioned. TBHQ is banned in Japan and certain European countries (Shahidi, 1997), and BHA and BHT are reported to be carcinogenic (Ito et al., 1982). Therefore, numerous natural sources

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are being investigated in an effort to find a safer and more potent natural antioxidant. Antioxidants such as flavonoids, tannins, coumarins, curcumanoids, xanthons, phenolics, lignans, and terpenoids are found in various plant products (such as fruits, leaves, seeds, and oils) (Jeong et al., 2004). For this reason, there is a vast focus in separating these plant antioxidants and using them as natural immune stimulant agents. Among these herbs and spices, marjoram (*Origanum majorana* L.) of family Lamiaceae was known to the ancient Egyptians, Greeks, and Romans (Khohar & Magnusdottir, 2002; Tainter & Grenis, 1993). The Greeks considered this plant to be a symbol of happiness and believed that if grown on tombs that the dead will be happy forever.

Recently, spices have received attention for their useful physiological functions and antimicrobial activity. More research is required on the immunomodulatory activity of herbs and spices.

O. majorana leaves have been proved to have antimicrobial and emmenagogue properties, making them useful for the treatment of respiratory and gastrointestinal problems. They have shown potential in reducing platelet aggregation and as an antihypertensive plant. The essential oil of the plant has been used for pains, gastrointestinal problems, and respiratory tract disorders (Charles, 2013; Erenler et al., 2016; Nahida et al., 2007; Tahraoui et al., 2007; Vogl et al., 2013; Yazdanparast & Shahriyary, 2008). The present work deals with the chemistry and immunomodulatory activity of the ethanolic extract and the isolated polyphenols from leaves of marjoram.

2. Materials and methods

Marjoram, *O. majorana* L. (Lamiaceae) leaves were randomly collected from a farm located at the Fayoum area and stored in a deep freezer at −20 °C until used.

2.1. Extraction and isolation

A powdered, air-dried leaf of *O. majorana* (500 g) was exhaustively extracted with hot 80% EtOH (4 × 3 l) under reflux. The dry residue obtained (80 g) was fractionated on a polyamide column (Ø 5.5 × 120 cm) and was eluted with water followed by water/methanol mixtures of decreasing polarities to afford several fractions. Those fractions were concentrated under vacuum and purified on cellulose columns followed by Sephadex LH-20 columns using different eluting systems (Abu-Izneid et al., 2018).

3. Instruments and materials

The UV analyses of pure samples were recorded on a Shimadzu UV240 spectrophotometer, separately as solutions in methanol and with different diagnostic UV shift reagents (Mabry et al., 1970) and with sprayed Naturstoff reagent (Brasseur & Angenot, 1986). Fractionation of the extract was done by column chromatography using polyamide 6 S (Riedel-De Han Ag, Seelze Hannover, Germany) and Sephadex LH-20 (Fluka, Switzerland); isolation and purification of compounds were done on either cellulose (Pharmacia, Uppsala, Sweden) or Sephadex LH-20 columns of different dimensions and eluted with different solvent systems.

Separation processes were followed up by 2D-PC and CoPC using Whatmann No. 1 paper with *n*-BuOH-AcOH-H_2O (BAW) (4 : 1: 5, top layer) and 15% aqueous AcOH as solvent systems. Authentic reference phenolic compounds were kindly supplemented by Prof. Dr Mohamed Abdelwahab of Faculty of Pharmacy, Menia University, Egypt (Biopharma of purity 95—99%). NMR (^{1}Hand ^{13}C-NMR) spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury 300. The δ-values are reported as ppm and *J*-values are in Hz. Negative ESI-MS Spectrometer: Finnigan LCQ-deca, Finnigan.

3.1. Proliferation of immune cells

The effect of the isolated compounds on the growth of Raw macrophage 264.7 (Cells were purchased from the American Type Culture Collection, ATCC, Rockville, MD, USA) was estimated using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure according to the method of Rashad et al. (2012) and El Malah et al. (2016). Briefly, raw macrophage 264.7 cells were inoculated in a 96-well microtiter plate (104 cells/well) for 24 h. Tested extracts and doxorubicin (as standard reference drug) were dissolved in DMSO at 1 mg/ml and diluted to appropriate volumes and then added to the cells (three wells were prepared for each individual dose). Cells were incubated for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 24 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) MTT dissolved in DMSO. Unbound dye was removed by washing with DMSO. Color intensity was measured in an ELISA reader (Asys Hitech, Austria) at 570 nm. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated Rashad et al. (2012); El Malah et al. (2016).

The yellow tetrazolium salt of MTT is reduced by mitochondrial dehydrogenase in metabolically

active cells to form insoluble purple formazan crystals, which are solubilized by the addition of DMSO. The relative cell viability was determined by the amount of MTT converted to the insoluble formazan salt. The data were expressed as the mean percentage of viable cells as compared with DMSO-treated cells Rashad et al. (2012); El Malah et al. (2016).

3.2. Investigation of polyphenolic contents

Ethanolic extract of *O. majorana* leaves was fractionated on a polyamide column followed by separation on sephadex LH-20 columns using 50% methanol as the eluting system. The isolated compounds were identified on the basis of acid hydrolysis, comparative PC, UV, ESI-MS, ^{1}H-, and ^{13}C-NMR spectroscopic analysis and comparing with authentics and the previous reported data (Mabry et al., 1970; Markham et al., 1978; Smith et al., 1985; Brasseur & Angenot, 1986; Balasubramanian et al., 2010; Abdelhady et al., 2012; Rashad et al., 2012; El Malah et al., 2016; Shaheen et al., 2017; Abu-Izneid et al., 2018; Balata et al., 2018; Mansour et al., 2018). The compounds are identified as cinnamic acid, gallic acid, caffeic acid, *P*-coumaric acid, apigenin, and luteolin (Fig. 1).

3.3. Statistical analysis

Data were analyzed using two-factorial analysis of variance, including first-order interactions (two-way analysis of variance), for multiple comparisons. *P* value more than 0.05 indicates statistical significance.

Viability results are represented as a percentage of control cells (n = 4).

3.4. Immunomodulatory activity (proliferation of immune cells)

Results of the effect of incubation with the tested samples (80% EtOH leaf extract, compounds 1, 2, 3, 4, 5, and 6) for 48 h on the proliferation of RAW 264.7 macrophage cells which was estimated by the MTT assay, indicated that the incubation of macrophages with ethanolic extract, compounds 1–6 resulted in a highly significant increase ($P < 0.05$) in cell proliferation at the highest tested dose and that this dose-dependent increase started from the lower tested dose and reached 114, 150, 154, 157, 142, 147, 112 folds of the control, respectively, at the highest tested dose indicating immunomodulatory activity (Smith et al., 1985). Treatment of macrophages with compound 2 and 3 showed no significant increase ($P > 0.05$) in the macrophage proliferation at any of the tested doses (Fig. 2).

4. Discussion

O. majorana L. family *Lamiaceae* is widely used in Egyptian cuisine as a seasoning and flavor enhancer. It is also a well-known traditional medicine in Egypt and is widely used for treating

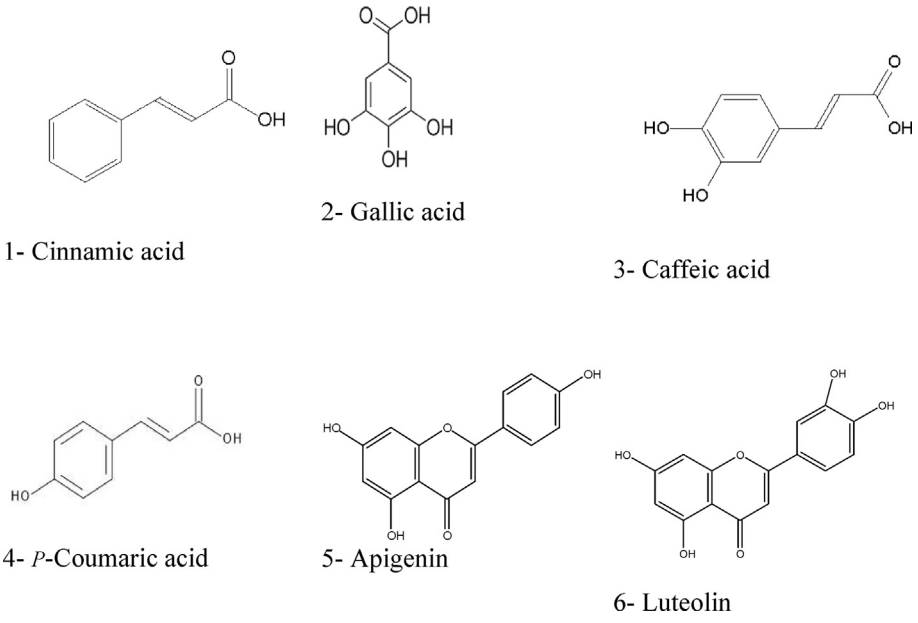

Fig. 1. Structures of the isolated compounds.

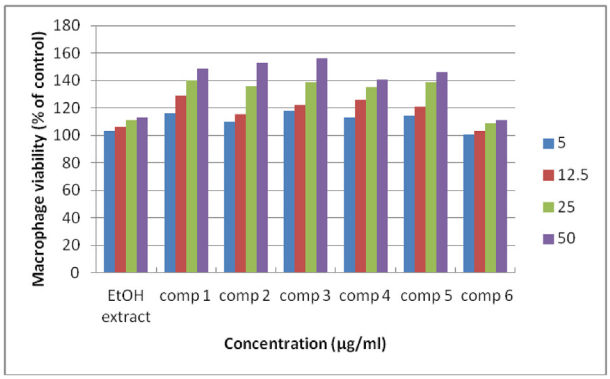

Fig. 2. The effect of different doses of 80% EtOH leaf extract of Origanum majorana and compounds 1, 2, 3, 4, 5, and 6 on the growth of RAW 264.7 macrophages, as measured by MTT assay.

abdominal colics due to its antispasmodic properties (Taha et al., 2023).

In this study, the six identified compounds isolated from *O. majorana* showed high immunomodulatory activity on raw macrophage 264.7, which may be attributed to the presence of cinnamic acid that acts as an anti-inflammatory agent, has an immunomodulatory effect and affect toll-like receptor downstream pathways and cytokines such as NF-κB, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α (Niknejad et al., 2023).

Moreover, gallic acid and caffeic acid which is immune-stimulatory functions by enhancing the phagocytes and lymphocytes, the major innate immune cells (Ahmed et al., 2020; Shruthi et al., 2018).

However, *p*-coumaric acid was proved to have anti-oxidative, antimicrobial, anti-inflammatory and immunomodulatory, antiviral, fungicidal, antimutagenic, neuroprotective, anticancer, antidiabetic, and anti-hypercholesterolemic properties. In addition, recent studies have reported its protective effects against oxidative stress and lipid peroxidation (Mehdi et al., 2022).

Also, apigenin and luteolin show immunomodulatory effect through natural killer cell cytokine secretion (Oo et al., 2022).

4.1. Conclusion

Finally, the ethanolic leaf extract of *O. majorana* contains many phenolic compounds, some of which were isolated and showed significant immunomodulatory activity, and thus may be used in immunocompromised patients to improve their immunity against various bacterial infections, giving *O. majorana* great potential as a natural healthcare product.

Conflicts of interest

No conflict of interest disclosure.

References

Abdelhady, M. I. S., Kamal, A. M., Tawfik, N. F., & Abdelkhalik, S. M. (2012). Polyphenolic constituents of the methanolic extract of Callistemon viridiflorus leaves and its antimicrobial activity. *Pharmacognosy Journal, 4*, 47–53.

Abu-Izneid, T., Rauf, A., Shah, S. U. A., Mansour, N., & Patel, S. (2018). In vivo study on analgesic, muscle-relaxant, sedative activity of extracts of hypochaeris radicata and in silico evaluation of certain compounds present in this species. *BioMed Research International, 2018*, Article 3868070.

Ahmed, I., Lin, H., Xu, L., Li, S., Costa, J., Mafra, I., et al. (2020). Immunomodulatory effect of laccase/caffeic acid and transglutaminase in alleviating shrimp tropomyosin (Met e 1) allergenicity. *Journal of Agricultural and Food Chemistry, 68*, 7765–7778.

Balasubramanian, T., Chatterjee, T. K., Sarkar, M., & Meena, S. L. (2010). Anti-inflammatory effect of Stereospermumsuaveolens ethanol extract in rats. *Pharmaceutical Biology, 48*, 318–323.

Balata, G. F., Abdelhady, M. I. S., Mahmoud, G. M., Matar, M. A., & Abd El-Latif, A. N. (2018). Formulation of saudi propolis into biodegradable chitosan chips for vital pulpotomy. *Current Drug Delivery, 15*, 97–109.

Brasseur, T., & Angenot, L. (1986). Reagents for densitomeric determination of flavonoids. *Journal of Chromatography, 351*, 351–355.

Charles, D. J. (2013). Marjoram sweet. In *Antioxidant properties of spices, herbs and other sources* (pp. 393–399). New York: Springer.

El Malah, T., Nour, H., Nayl, A., Elkhashab, R., Abdel-Megeid, F., & Ali, M. (2016). Anticancer evaluation of Tris (triazolyl) triazine derivatives generated via click chemistry. *Australian Journal of Chemistry, 69*, 905–910.

Erenler, R., Sen, O., Aksit, H., et al. (2016). Isolation and identification of chemical constituents from Origanum majorana and investigation of antiproliferative and antioxidant activities. *Journal of the Science of Food and Agriculture, 96*, 822–836.

Ito, N., Hagiwara, A., Shibata, M., Ogiso, T., & Fukushima, S. (1982). Induction of squamous cell carcinoma in the forestomach of F344 rats treated with butylated hydroxyanisole. *Gann, 73*, 332–334.

Jeong, S. Y., Kim, D. R., Kim, K. C., Nam, D. U., & Ahn Lee, S. C. (2004). Effect of seed roasting conditions on the antioxidant activity of defatted sesame meal extracts. *Food and Chemical Toxicology, 69*, 377–381.

Khohar, S., & Magnusdottir, S. G. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry, 50*, 565–570.

Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The systematic identification of flavonoids*. Berlin: Springer-Verlag.

Markham, K. R., Terani, B., Stanley, R., Gieger, H., & Mebry, T. J. (1978). Carbon-13 NMR studies of flavonoid glycosides and their acylated derivatives. *Tetrahedron, 34*, 1389–1397.

Mehdi, S., Ahmad, F-ud-D, Lodhi, A. H., Khurshid, U., Khalid, A. A., Sidiq, S. S., et al. (2022). Protective Effects of p-CA against acute liver damage induced by LPS/D-GalN in Wistar Albino Rats. *Drug Design, Development and Therapy, 16*, 3327–3342.

Nahida, J., Wissal, D., Thouraya, D., et al. (2007). Essential oil composition of Origanum majorana leaves. *INIST-CNRS, 1*, 190–193.

Niknejad, A., Razavi, S. M., Hosseini, Y., Arab, Z. N., A, Abdolghaffari, H., & Momtaz, S. (2023). Cinnamon modulates toll-like receptors: A new therapeutic approach for diabetes. *Revista Brasileira de Farmacognosia, 34*, 223–235.

Novak, J., Bitsch, C., Langbehn, J., Pank, F., Skoula, M., & Gotsiou, Y. (2000). Ratios of cisand trans-sabinene hydrate in

Origanum majorana L. and Origanum microphyllum. *Biochemical Systematics and Ecology, 28*, 697−704.

Oo, A. M., Nor, M. N. M., Lwin, O. M., Simbak, N., Mohd Adnan, L. H., & Mahadeva Rao, U. S. (2022). Immunomodulatory effects of apigenin, luteolin, and quercetin through natural killer cell cytokine secretion. *Journal of Applied Pharmaceutical Science, 12*, 121−127.

Pin-Der, D., & Gow-Chin, Y. (1997). Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. *Journal of the American Oil Chemists Society, 74*, 745−748.

Rashad, A. E., Shamroukh, A. H., Yousif, N. M., Salama, M. A., Ali, H. S., Ali, M. M., et al. (2012). New pyrimidinone and fused pyrimidinone derivatives as potential anticancer chemotherapeutics. *Arch Pharm Chem Life Sci, 345*, 729−738.

Schippmann, U., Leaman, D., & Cunningham, A. (2006). A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. *Medicinal & Aromatic Plants, 17*, 1−6.

Shaheen, U., Shoeib, N. A., Temraz, A., & Abdelhady, M. I. S. (2017). Flavonoidal constituents, antioxidant, antimicrobial, and cytotoxic activities of *Dipterygium glaucum* grown in Kingdom of Saudi Arabia. *Pharmacognosy Magazine, 13*, S484−S488.

Shahidi, F. (1997). Natural antioxidants: An overview. In F. Shahidi (Ed.), *Natural antioxidants, chemistry, health effects and applications* (pp. 1−10). Champaign, IL, USA: AOCS Press.

Shruthi, S., Vijayalaxmi, K. K., & Bhaskar Shenoy, K. (2018). Immunomodulatory effects of gallic acid against cyclophosphamide- and cisplatin-induced immunosuppression in swiss albino mice. *Indian Journal of Pharmaceutical Sciences, 80*, 150−160.

Smith, P. K., Krohn, R., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry, 150*, 76−80.

Taha, M., Elazab, S. T., Abdelbagi, O., Saati, A. A., Babateen, O., Baokbah, T. A. S., et al. (2023). Phytochemical analysis of Origanum majorana L. extract and investigation of its antioxidant, anti-inflammatory and immunomodulatory effects against experimentally induced colitis downregulating Th17 cells. *Journal of Ethnopharmacology, 317*, Article 116826.

Tahraoui, A., El-Hilaly, J., Israili, Z. H., & Lyoussi, B. (2007). Etshnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *Journal of Ethnopharmacology, 110*, 105−117.

Tainter, D. R., & Grenis, A. T. (1993). *Spices and seasonings: A food technology hand book*. New York: VCH, Publishers, Inc.

Vogl, S., Picker, P., Mihaly-Bison, J., et al. (2013). Ethnopharmacological in vitro studies on Austria's folk medicine—an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *Journal of Ethnopharmacology, 149*, 750−771.

Yazdanparast, R., & Shahriyary, L. (2008). Comparative effects of Artemisia dracunculus, Satureja hortensis and Origanum majorana on inhibition of blood platelet adhesion, aggregation and secretion. *Vascular Pharmacology, 48*, 32−37.