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Saudi *Rosmarinus officinalis* and *Ocimum basilicum* L. Polyphenols and Biological Activities

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Abstract: Investigating the polyphenolic profile of natural *Rosmarinus officinalis* and *Ocimum basilicum* populations may reveal essential compounds that have biological activities. Natural populations of *R. officinalis* and *O. basilicum* in Northern Riyadh were investigated by HPLC-DAD analyses. Several polyphenols, including rosmarinic acid, gentisic acid, 3,4-dihydroxyphenylacetic acid, rutoside, and others, out of 38 screened were confirmed. Rosmarinic acid was the major polyphenol in both of *R. officinalis* and *O. basilicum*. *R. officinalis* methanolic leaf extracts contained other phenols such as gentisic acid while *O. basilicum* contained also 3,4-dihydroxyphenylacetic acid and rutoside as well as others. *R. officinalis* showed higher antioxidant activities than *O. basilicum* using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and β-carotene bleaching assays. These higher activities are associated with a higher composition of rosmarinic acid in leaf extracts. The antioxidant activities of *O. basilicum* were attributed to identified phenols of rosmarinic acid, 3,4-dihydroxyphenylacetic acid, and rutoside. There were antiproliferative and cytotoxic activities of leaf extracts, as well as identified polyphenols, against several cancer cells. These activities were attributed to the accumulation of necrotic and apoptotic cells in treated cancer cells with leaf extracts as well as identified polyphenols. The antibacterial and antifungal activities of leaf extracts were mainly attributed to 3,4-dihydroxyphenylacetic acid and rutoside in *O. basilicum* and rosmarinic acid and caffeic acid in *R. officinalis*. This study proved that *R. officinalis* and *O. basilicum* natural populations might be considered as promising sources of natural polyphenols with biological activities.

Keywords: *Rosmarinus officinalis*; *Ocimum basilicum*; leaf extract; phenolic acids; flavonoids; antiproliferative; cytotoxicity; antioxidant; antibacterial; antifungal

1. Introduction

Polyphenols are important plant secondary metabolites that are used for the control of aging as antioxidants [1] and the control microbes as antibacterial and antifungal agents [2,3]. Other applications of polyphenols had been discovered, such as anticancer agents [4–7], and food preservatives [8]. The biological activities of polyphenols as antioxidants are related to the ability to scavenge free radicals and the modulation of metal chelation reactions [4,9]. Polyphenols activate the detoxification enzymes and reduce free radicals [4,9]. The antiproliferative and apoptotic activities of polyphenols against cancer cells is attributed to the control of cell cycle arrest, as well as the molecular regulation of cancer related genes [4,9–11].

The Lamiaceae family contains dozens of medicinal plants with important economic output, including *Rosmarinus officinalis* and *Ocimum basilicum*. *R. officinalis* (syn. *R. angustifolius* Mill., *R. flexuosus* Jord. & Fourr., *Salvia rosmarinus* Schleid.)—rosemary—is one of the most widely used herbs in food and cosmetics, as well as in medicine. The fresh or dry stems and leaves are consumed as spice or manufactured for pharmaceutical applications. *R. officinalis* is a perennial herb originated in the Mediterranean region, and contains a large number of ecotypes that differ morphologically and chemically [12]. *R. officinalis* is widely used by desert communities in Saudi Arabia, where it grows wild. Notable is the fact that the phytochemical composition of *R. officinalis* depends on many factors, e.g., site of occurrence, chemotypes, or varieties [13]. The leaf of *R. officinalis* is officially recognized as the raw material [14]. The *R. officinalis* essential oil (*Rosmarini aetheroleum*), is listed in the European Pharmacopoeia [15] and American Pharmacopoeia [16], as well as in the World Health Organization Monographs [17]. The *R. officinalis* essential oil obtained by steam distillation from the leaves (up to 2.5%), which are the most responsible for its biological activity. The main constituents are: camphor (5.0–21%), 1,8-cineole (15–55%), α-pinene (9.0–26%), borneol, camphene, β-pinene, and limonene in changing proportions, depending on growth stage and bioclimatic conditions [18]. Major polyphenols confirmed in *R. officinalis* leaf extracts, are flavonoids: apigenin, luteolin, nepetin, nepitrin, and phenolic acids: rosmarinic acid (c.a. 23%), chlorogenic acid, caffeic acid, and other organic acids like: ursolic acid, betulinic acid, carnosic acid, and carnosol [19]. The extracts obtained from *R. officinalis* are used as a natural antioxidant, in rheumatic complaints, and in circulatory disorders [17]. These plants have antihepatotoxic [20], antimicrobial [21], and antispasmodic [22] activities. Traditionally, *R. officinalis* is used as cholagogue, diaphoretic, digestant, diuretic, emmenagogue, laxative, and a tonic remedy [23].

The *O. basilicum* (syn. *Basilicum citratum* Rumph., *Ocimum album* L., *Ocimum americanum* Jacq.)—sweet basil; common basil—is another medicinal herb that originated in the Mediterranean region and is grown regularly all over the world. The *Ocimum* genus contains a large number of species, which are the subject of hybridization and within-population variation [24]. In addition, large number of commercial cultivars are added to the market every year. The morphological and chemical characteristics of *Ocimum* are highly diverse [24,25]. The *O. basilicum* essential oil obtained from leaves and/or stems and flowers is recognized as raw material [26]. Basil essential oil contains numerous compounds, mainly monotherpenenoids (linalool (57–60%), camphor, limonene, 1,8-cineole, geraniol), and phenylpropanoids (eugenol, methyleugenol, chavicol, estragole, methyl-cinnamate) [27]. Volatile oil composition is strictly dependent on *O. basilicum* chemotypes, cultivars, flower and leaf colours, and aroma, as well as the origin of the plant [27]. The *O. basilicum* leaves also contain several polyphenols, including flavonoids (nevadensin, salvigenin, cirsileol, eupatorin, apigenin, acacetin, cirsimaritin, quercetin, and ladanein) [28], and phenolic acids (rosmarinic acid, caffeic acid, and caftaric acid) [29]. However, natural populations of this species may have a different chemical composition and biological activities. Traditionally, *O. basilicum* is used as a tonic and for the treatment of nausea, flatulence, and dysentery. The *O. basilicum* essential oil has been found to be beneficial for the alleviation of mental fatigue, cold, spasms, and rhinitis [30]. Scientific studies have confirmed the analgesic, anti-inflammatory, antioxidant, antimicrobial, anti-ulcerogenic, chemomodulatory, immunomodulatory, hypolipidaemic, and hypoglycaemic activities of *O. basilicum* [31].

In this investigation, the polyphenolic composition and biological activities of *R. officinalis* and *O. basilicum* natural populations were explored qualitatively and quantitatively using the HPLC-DAD method. The antioxidant, antiproliferative, cytotoxic, antibacterial, and antifungal activities were examined as a novel investigation of *R. officinalis* and *O. basilicum* natural populations in Northern Saudi Arabia.

2. Materials and Methods

2.1. Plant Material and Preparation

The leaf extracts of *Rosmarinus officinalis* L. and *Ocimum basilicum* L. were obtained from natural populations growing in the Riyadh region. Plants were identified by Hosam Elansary and vouchered at the College of Food and Agricultural Sciences, King Saud University (Hosam0002213–101). Lyophilized dried leaves were powdered then extracted with methanol (0.2 g DW (dry weight) in 10 mL) by sonication twice for 30 min at 30 °C. Purification was conducted using Whatman paper, then the residues were dried at room temperature (to lose methanol). Finally, the residues were frozen at −80 °C. For HPLC analyses, and the residues were dissolved in methanol (1 mL, Merck, Berlin, Germany) while for bioassays, the methanol was totally removed using a rotary evaporator [32]. The experiments were approved by the animal committee of the college of Agriculture, Damietta University (2018-2019-75365). Bacteria, fungi, and cancer cell lines (ATTC collection) were obtained from the Faculty of Agriculture, Alexandria, Egypt.

2.2. Analyses of Phenolic Compounds

The analyses were conducted using HPLC-DAD method on Merck-Hitachi liquid chromatograph (LaChrom Elite, Berlin, Germany) with Purospher RP-18e column (250 × 4 mm; 5 µm, Merck, Berlin, Germany) and DAD detector (L-2455, Berlin, Germany). As mobile phase two solvents were used: methanol (A) and 0.5% acetic acid + methanol 1:4 (v/v). The gradient program was as follows: 0–20′ 100% B; 20–35′ 100%–80% B; 35–55′ 80%–60% B; 55–70′ 60%–0% B; 70–75′ 0%; 0%–100% B for 75–80′; 80–90′ 100% B. Other parameters of analysis were temperature—25 °C, flow rate—1 mL min^{-1}, injection volume—20 µL, wavelength from 210 to 400 nm. Quantitative analysis was measured at 254 nm. The HPLC method was validated earlier by our group [33,34]. Compounds were identified by a comparison of retention times and UV spectra with standards. The quantification was calculated using calibration curve. For qualification and quantification, commercially available standards of the following 22 phenolic acids were used: cinnamic acid and its derivatives (caffeic acid, o-coumaric acid, m-coumaric acid, p-coumaric acid, ferulic acid, hydrocaffeic acid, isoferulic acid, and sinapic acid); benzoic acid and its derivatives (3,4-dihydroxyphenylacetic acid, ellagic acid, gallic acid, gentisic acid, p-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, and vanillic acid), and depsides (chlorogenic acid, neochlorogenic acid, and rosmarinic acid). The standards of 7 flavonoids (cynaroside, myricetin, naringin, quercetin, kaempferol, rhamnetin, and luteolin) and 9 flavonoid glycosides (apigetrin, cynaroside, hyperoside, quercitrin, robinin, rutoside, isoquercetin, trifolin, and vitexin) were used. In total, 38 compounds were screened and all chemicals were obtained from Sigma-Aldrich, Berin, Germany.

2.3. Anticancer Activities

Antiproliferative and cytotoxic effects of *R. officinalis* and *O. basilicum* leaf extracts were tested against Jurkat, MCF-7, HeLa, HT-29, and HEK-293 (normal human cells) (ATTC, Berlin, Germany), [4,5,35]. To determine the changes in cells viability, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed. DMSO (1%) solubilized leaf extracts were added to standard media (MEM) containing (10% FBS, 0.1 mM non-essential amino acids, 17.8 mM $NaHCO_3$, and 1 mM sodium pyruvate) and cancer cells in 75 cm^2 flasks. Cancer cells were prepared in a microtiter plates

using 4×10^{-4} cells per µL in a 270 µL medium for 48 h (37 °C, 5% CO_2). Serial concentrations of leaf extracts were used until reaching a final concentration of 50, 100, 200, 300, and 400 µg/mL.

A washing procedure was performed using PBS, then the MTT dissolved in PBS was added at 12 mM to the medium, then the isoprobanol (0.04 N HCl) was also mixed and the mixture was left for 40 min. Negative control (untreated) and a positive couple (vinblastine sulfate and taxol) were used. The following equation was used to calculate the inhibition activity percentage (*IAA*) obtained from measuring the absorbance at a 570 nm wavelength:

$$IAA = \frac{(AB_{570nm})_C - (AB_{570nm})_s}{(AB_{570nm})_C} \times 100$$

where *AB* is the absorbance, $(AB_{570\ nm})_C$ and $(AB_{570\ nm})_s$ are Abs.570 nm of control and sample, respectively.

The IC_{50} was determined by plotting the percentage of viable cells against extract concentration in µg mL^{-1}. The IC_{50} was applied in the flow cytometry assay to investigate the cytotoxic activities *R. officinalis* and *O. basilicum* leaf extracts, then the apoptotic cell populations were determined (FAC Scan, USA) [4,35,36].

2.4. Antioxidant Activity

R. officinalis and *O. basilicum* leaf extract antioxidant activities were investigated using β-carotene bleaching, ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays [5,24,37–39]. Lyophilized dried leaves were powdered then extracted with methanol (0.2 g DW (dry weight) in 10 mL) by sonication twice for 30 min at 30 °C. Purification was conducted using Whatman paper, then the residues were dried at room temperature (to lose methanol). Finally, the residues were frozen at −80 °C. The IC_{50} (µg mL^{-1}) was defined as the amount of extracts required to scavenge 50% of β-carotene bleaching/DPPH solution. This value (IC_{50}) was determined by plotting the inhibition percent against extract concentration. The butylated hydroxytoluene (BHT) was used as standard for β-carotene bleaching/DPPH. The absorbance was measured at 470 nm for β-carotene bleaching, at 517 nm for DPPH, and at 593 nm for FRAP. The FRAP reagent was prepared as described before [40–43]. Aliquots (100 µL) of leaf extracts/Trolox (Sigma-Aldrich, Berlin, Germany) were added to the reagent (3 mL), then mixed and incubated for 30 min at 37 °C. The calibration procedure of FRAP was done by applying serial dilutions of Trolox (0–0.5 mmol/L), as standard. Experiments were conducted in triplicated and repeated twice.

2.5. Antibacterial Effect

Bacterial isolates of *L. monocytogenes* (clinical isolate), *S. aureus* (ATCC 6538), *E. coli* (ATCC 35210) *ria*, *B. cereus* (ATCC 14579), *P. aeruginosa* (ATCC 27853), and *M. flavus* (ATCC 10240), were used in this assay. A microtiter plates based protocol (micro-dilution) was used following [7,40–42], by preparing serial concentrations of the extracts that were mixed with bacterial inoculum of 1.0×10^4 CFU in each well with 100 µL tryptic soy broth per well, then incubated for one day at 37 °C on a rotary shaker. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that caused no visible growth by binocular microscope. The MBC was determined using serial subculturing of extracts (2 µL), and the minimal concentration caused eliminating of 99.5% of each inoculum was considered as the MBC value. The optical density (OD) was determined using 655 nm wave length. The positive control (streptomycin) at 0.01–10 mg/mL was used alongside negative one (DMSO, 1%).

2.6. Antifungal Effect

M. × *piperita* and *M. longifolia* antifungal effects were determined against *P. ochrochloron* (ATCC 48663), *A. ochraceus* (ATCC 12066), *C. albicans* (ATCC 12066), *A. niger* (ATCC 6275), *A. flavus* (ATCC 9643), and *P. funiculosum* (ATCC 56755) using a micro-dilution method [36,40,41]. The MIC was determined

by stereomicroscope. The minimum fungicidal concentration (MFC) was determined by preparing serial dilutions of 2 µL extracts in sub-cultures of fungi at 28 °C for 72 h in microtiter plates that contained 100 µL of broth medium. Ketoconazole (KTZ, 1–3500 µg/mL) was used as a positive control. The DMSO (1%) was also used.

2.7. Statistical Analyses

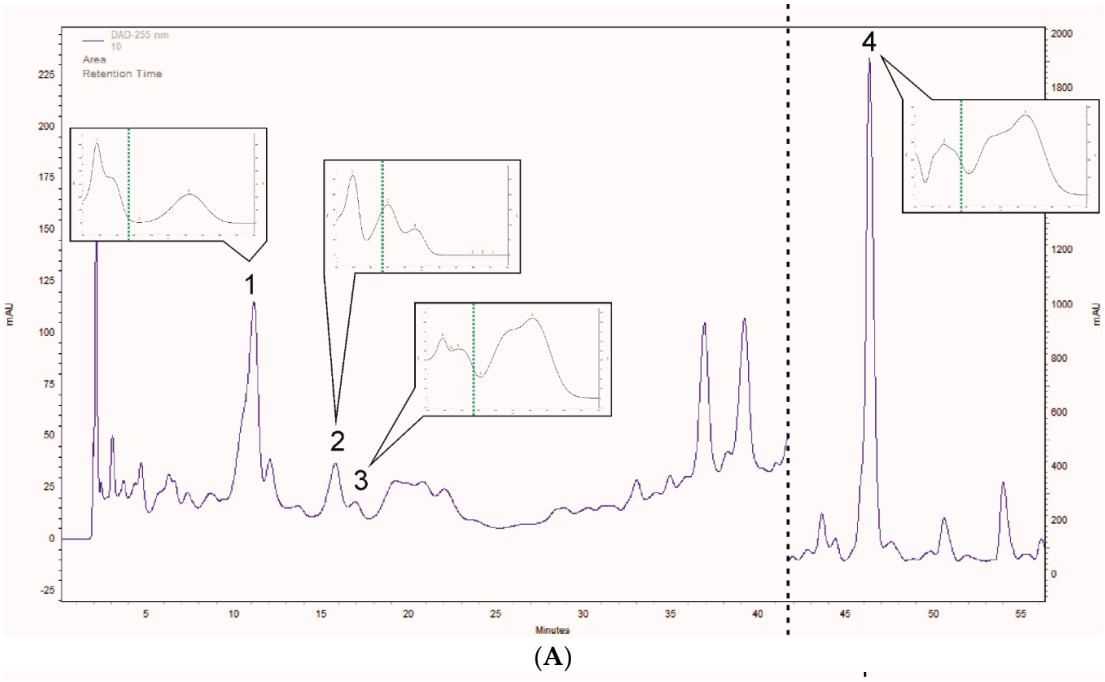
To determine significant differences, the least significance difference (LSD) were determined using SPSS software. The standard deviation (SD) was the mean of three replicates.

3. Results

3.1. Polyphenol Profiling of Rosmarinus Officinalis and Ocimum Basilicum Leaf Extracts

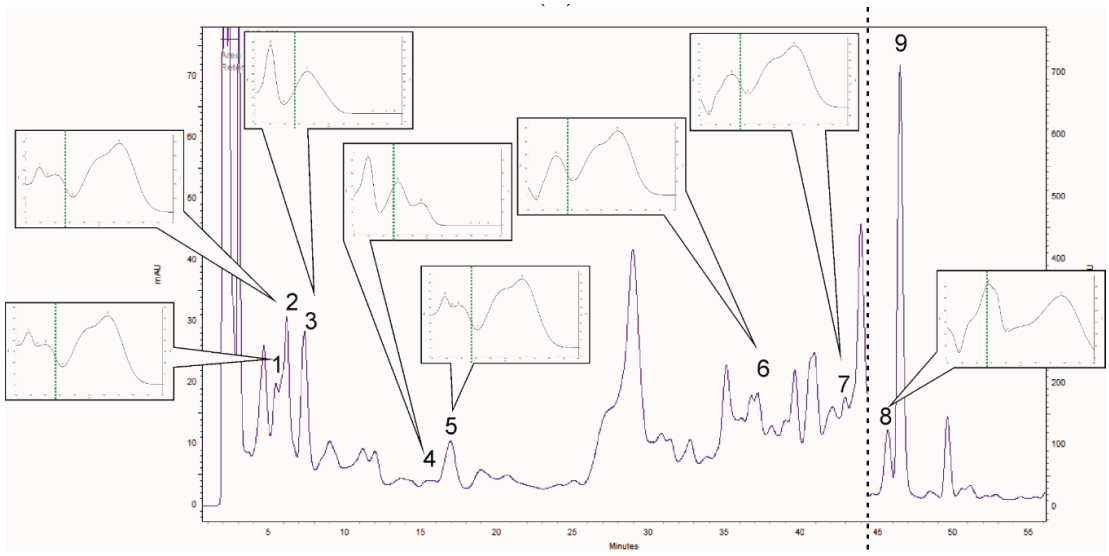
In the *R. officinalis* methanolic leaf extract, the HPLC-DAD qualitative and quantitative analyses of selected phenolic compounds confirmed very high amount of rosmarinic acid (4040.00 mg/100 g DW (dry weight)). Moreover, other polyphenols, including genistic aicd (199.9 mg/100 g DW), vanilic acid (36.6 mg/100 g DW), and caffeic acid (27.6 mg/100 g DW) were determined (Figure 1A, Table 1).

In *O. basilicum* leaf extracts, a larger number of polyphenols were determined (8 phenolic acids and 1 flavonoid). In phenolic acids, the major one was rosmarinic acid (1128.5 mg/100 g DW), followed by 3,4-dihydroxyphenylacetic acid (312.3 mg/100 g DW), caftaric acid (60.3 mg/100 g DW), neochlorogenic acid (38.6 mg/100 g DW), isochlorogenic acid (30.0 mg/100 g DW), caffeic acid (17.6 mg/100 g DW), ferulic acid (14.4 mg/100 g DW), and a small amount of vanilic acid (1.1 mg/100 g DW) (Figure 1B, Table 1).



(A)

Figure 1. *Cont.*



(**B**)

Figure 1. The examples of HPLC-DAD chromatographic separation (λ = 254 nm) of leaf extracts of: (**A**) *R. officinalis* (1—gentisic acid, 2—vanillic acid, 3—caffeic acid, 4—rosmarinic acid); and (**B**) *O. basilicum* (1—neochlorogenic acid, 2—caftaric acid, 3—3,4-dihydroxyphenylacetic acid, 4—vanillic acid, 5—caffeic acid, 6—ferulic acid, 7—isochlorogenic acid, 8—rutoside, 9—rosmarinic acid).

Table 1. The contents of polyphenols in *R. officinalis* and *O. basilicum* leaf extracts (mg/100g DW ± SD).

Compound	*Rosmarinus officinalis*	*Ocimum basilicum*
Caffeic acid	27.6 ± 3.6	17.6 ± 1.3
Caftaric acid	nd *	60.3 ± 4.5
3,4-Dihydroxyphenylacetic acid	nd	312.3 ± 20.4
Ferulic acid	nd	14.4 ± 1.7
Gentisic acid	119.9 ± 2.0	nd
Isochlorogenic acid	nd	30.0 ± 3.3
Neochlorogenic acid	nd	38.6 ± 2.7
Rosmarinic acid	4040.0 ± 189.2	1128.5 ± 70.4
Vanillic acid	36.6 ± 1.7	1.1 ± 0.0
Rutoside	nd	139.4 ± 18.8

* nd—not detected.

3.2. Antioxidant Effects

R. officinalis and *O. basilicum* leaf extracts, as well as major compounds, showed significant antioxidant effects by several methods (Table 2). *R. officinalis* showed high antioxidant effects compared to *O. basilicum*, as measured by all methods. Further, *R. officinalis* showed a comparable antioxidant effect to rosmarinic acid. The highest antioxidant values (lowest IC_{50}) were found in 3,4-Dihydroxyphenylacetic acid and rosmarinic acid. Rutoside showed relatively low antioxidant effects as compared to other identified compounds.

Table 2. 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-Carotene bleaching and ferric reducing antioxidant power (FRAP) measures of *R. officinalis* and *O. basilicum* leaf extracts, rosmarininic acid, 3,4-dihydroxyphenylacetic acid, gentisic acid, and rutoside.

	DPPH (IC_{50}, μg mL^{-1})	β-Carotene-Bleaching Assay (IC_{50}, μg mL^{-1})	FRAP (IC_{50}, mM TEAC/g Extract)
R. officinalis	2.9 ± 0.1c	3.6 ± 0.1c	4.1 ± 0.2e
O. basilicum	4.7 ± 0.1cd	5.1 ± 0.3d	5.5 ± 0.1e
Rosmarinic acid	2.6 ± 0.1d	3.0 ± 0.1d	3.3 ± 0.9f
3,4-Dihydroxyphenylacetic acid	2.1 ± 0.1d	2.7 ± 0.1d	3.2 ± 0.1d
Gentisic acid	5.8 ± 0.1d	7.3 ± 0.2d	9.5 ± 1.0f
Rutoside	14.3 ± 1.1d	16.5 ± 0.7d	18.6 ± 0.9d
BHT	2.6 ± 0.2e	3.1 ± 0.1e	-
Trolox	-	-	3.1 ± 0.1g

Different letters within a column indicates significant differences ($p \leq 0.05$). TEAC: Trolox equivalents antioxidant.

3.3. Antiproliferative and Cytotoxic Effects

The MTT assay of methanolic leaf extracts and identified phenols revealed obvious antiproliferative effects against T-cell lymphoblast like (Jurkat), breast adenocarcinoma cultures (MCF-7), colon adenocarcinoma (HT-29), cervical adenocarcinoma (HeLa), and HEK-293 (normal human cells), as shown in Table 3. The 3,4-Dihydroxyphenylacetic acid and rutoside had the lowest IC_{50} values compared to other compounds as well as leaf extracts.

The flow cytometry assay (Figure 2) showed the apoptotic cell accumulation the upper and lower right quadrant, following 48 h of exposure.

Table 3. Antiproliferative activity (IC_{50} (μg mL^{-1})) of *R. officinalis* and *O. basilicum* leaf extracts, rosmarinic acid, 3,4-dihydroxyphenylacetic acid, gentisic acid, and rutoside on cancer cells.

	HeLa	HT-29	MCF-7	Jurkat	HEK-293
R. officinalis	39.52 ± 2.3	36.59 ± 2.7	38.29 ± 1.7	64.39 ± 3.1	>400
O. basilicum	45.26 ± 3.2	48.31 ± 2.9	47.23 ± 3.1	75.13 ± 3.6	>400
Rosmarinic acid	36.30 ± 1.3	24.27 ± 2.1	25.61 ± 2.5	47.43 ± 3.1	>400
3,4-Dihydroxyphenylacetic acid	3.11 ± 0.1f	20.41 ± 0.7	7.12 ± 0.9	5.63 ± 1.1	>400
Gentisic acid	5.7 ± 0.5	24.26 ± 0.9	10.13 ± 0.7	7.4 ± 0.6	>400
Rutoside	3.9 ± 01	17.5 ± 0.8f	5.0 ± 0.3f	4.0 ± 0.5	>400
Vinblastine sulfate	2.1 ± 0.04	16.3 ± 0.7	-	0.11 ± 0.01	42.7 ± 1.3
Taxol	-	-	0.07 ± 0.004	-	-

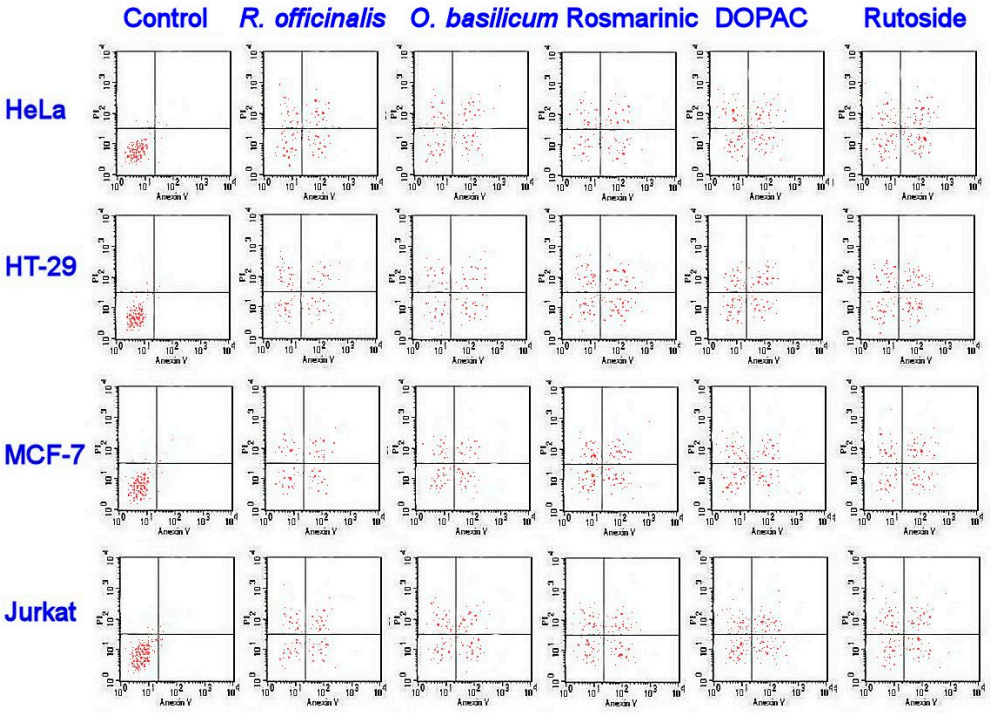


Figure 2. Cytotoxicity of *R. officinalis* and *O. basilicum* leaf extracts, and rosmarinic acid, 3,4-dihydroxyphenylacetic acid (DOPAC), and rutoside, as estimated with flow cytometry. There was an accumulation of apoptotic cells in the early (lower right quadrant) and late apoptotic (upper right quadrant).

3.4. Antibacterial Effects

R. officinalis and *O. basilicum* methanolic leaf extracts had strong antibacterial effects against screened bacteria (Table 4). *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Micrococcus flavus* were the most sensitive to leaf extracts. 3,4-dihydroxyphenylacetic acid, rutoside and caffeic acid were the most active isolated compounds against most bacteria. Gentisic acid was not active against all bacterial except *Escherichia coli*. Rosmarinic acid was weak against most bacteria and showed MBC > 500 mg mL^{-1}.

Table 4. *R. officinalis* and *O. basilicum* leaf extracts, rosmarinic acid, 3,4-dihydroxyphenylacetic acid, gentisic acid, and rutoside antibacterial activities by means of minimum inhibitory (MIC) and bactericidal concentration (MBC) in (mg mL^{-1}).

	B. cereus	*P. aeruginosa*	*L. monocytogenes*	*E. coli*	*M. flavus*	*S. aureus*
	MIC	**MIC**	**MIC**	**MIC**	**MIC**	**MIC**
	MBC	**MBC**	**MBC**	**MBC**	**MBC**	**MBC**
R. officinalis	0.56 ± 0.03 0.97 ± 0.05	0.43 ± 0.03 0.85 ± 0.03	0.54 ± 0.03 0.96 ± 0.05	0.46 ± 0.02 0.91 ± 0.03	0.36 ± 0.03 0.82 ± 0.03	0.29 ± 0.03 0.79 ± 0.02
O. basilicum	0.43 ± 0.03 0.85 ± 0.05	0.32 ± 0.02 0.75 ± 0.03	0.41 ± 0.01 0.89 ± 0.03	0.35 ± 0.01 0.68 ± 0.03	0.27 ± 0.02 0.59 ± 0.03	0.23 ± 0.01 0.56 ± 0.03
Rosmarinic acid	39.31 ± 1.13 >500	37.2 ± 1.64 >500	46.21 ± 3.12 >500	42.1 ± 3.42 >500	31.11 ± 1.53 >500	23.31 ± 0.96 >500
Gentisic acid	N.D. N.D.	N.D. N.D.	N.D. N.D.	23.00 ± 0.02 >100	N.D. N.D.	N.D. N.D.
3,4-Dihydroxyphenylacetic acid	0.07 ± 0.01 0.15 ± 0.01	0.06 ± 0.01 0.13 ± 0.01	0.07 ± 0.01 0.17 ± 0.01	0.08 ± 0.01 0.19 ± 0.02	0.14 ± 0.02 0.30 ± 0.02	0.13 ± 0.01 0.27 ± 0.02
Rutoside	0.10 ± 0.01 0.20 ± 0.01	0.06 ± 0.01 0.11 ± 0.01	0.10 ± 0.01 0.20 ± 0.02	0.10 ± 0.01 0.24 ± 0.01	0.13 ± 0.01 0.24 ± 0.02	0.12 ± 0.01 0.24 ± 0.02
Caffeic acid	0.12 ± 0.01 0.29 ± 0.03	0.06 ± 0.01 0.12 ± 0.01	0.25 ± 0.01 0.55 ± 0.03	0.10 ± 0.01 0.23 ± 0.02	0.12 ± 0.01 0.29 ± 0.02	0.21 ± 0.01 0.43 ± 0.03
Streptomycin	0.07 ± 0.01 0.16 ± 0.01	0.09 ± 0.01 0.19 ± 0.01	0.10 ± 0.01 0.23 ± 0.01	0.10 ± 0.01 0.21 ± 0.01	0.10 ± 0.01 0.21 ± 0.01	0.15 ± 0.01 0.31 ± 0.01

3.5. Antifungal Activities

R. officinalis and *O. basilicum* methanolic leaf extracts showed antifungal activities against all fungi screened (Table 5). *R. officinalis* showed lower IC_{50} values than *O. basilicum*. 3,4-Dihydroxyphenylacetic acid, gentisic acid, rutoside, and caffeic acid showed strong antifungal activities against examined bacteria. Rosmarinic acid showed the weakest antifungal effects compared to other identified polyphenols.

Table 5. Minimum inhibitory (MIC) and fungicidal concentration (MFC) of *R. officinalis* and *O. basilicum* leaf extracts, rosmarinic acid, 3,4-dihydroxyphenylacetic acid, gentisic acid and rutoside. Values are means of 3 replicates in (mg mL^{-1}).

	Aspergillus flavus	*Aspergillus ochraceus*	*Aspergillus niger*	*Candida albicans*	*Penicillium funiculosum*	*Penicillium ochrochloron*
	MIC	**MIC**	**MIC**	**MIC**	**MIC**	**MIC**
	MFC	**MFC**	**MFC**	**MFC**	**MFC**	**MFC**
R. officinalis	0.49 ± 0.05 0.96 ± 0.05	0.56 ± 0.03 1.18 ± 0.13	0.69 ± 0.03 1.23 ± 0.07	0.86 ± 0.03 1.59 ± 0.15	0.42 ± 0.03 0.87 ± 0.05	0.49 ± 0.05 0.93 ± 0.05
O. basilicum	0.31 ± 0.02 0.74 ± 0.03	0.38 ± 0.05 0.89 ± 0.03	0.54 ± 0.05 1.01 ± 0.09	0.63 ± 0.09 1.16 ± 0.08	0.35 ± 0.03 0.76 ± 0.05	0.38 ± 0.01 0.89 ± 0.03
Rosmarinic acid	181.92 ± 8.47 >1000	222.32 ± 9.18 >1000	231.65 ± 8.69 >1000	385.7 ± 15.76 >1000	218.86 ± 11.78 >1000	349.9 ± 11.45 >1000
Gentisic acid	0.20 ± 0.03 0.53 ± 0.05	0.15 ± 0.01 0.49 ± 0.03	0.24 ± 0.03 0.58 ± 0.04	0.29 ± 0.03 0.65 ± 0.05	0.23 ± 0.01 0.52 ± 0.03	0.18 ± 0.02 0.43 ± 0.04
3,4-Dihydroxyphenylacetic acid	0.19 ± 0.01 0.41 ± 0.03	0.21 ± 0.02 0.45 ± 0.03	0.25 ± 0.02 0.51 ± 0.04	0.28 ± 0.03 0.63 ± 0.05	0.27 ± 0.03 0.63 ± 0.04	0.21 ± 0.01 0.45 ± 0.05
Rutoside	0.20 ± 0.02 0.45 ± 0.03	0.19 ± 0.01 0.56 ± 0.03	0.29 ± 0.03 0.64 ± 0.05	0.24 ± 0.03 0.53 ± 0.03	0.31 ± 0.02 0.73 ± 0.04	0.21 ± 0.03 0.47 ± 0.05
Caffeic acid	0.21± 0.02 0.43 ± 0.03	0.23 ± 0.01 0.46 ± 0.03	0.20 ± 0.02 0.40 ± 0.03	0.31 ± 0.01 0.63 ± 0.03	0.26 ± 0.01 0.61 ± 0.04	0.20 ± 0.01 0.42 ± 0.03
KTZ (Ketoconazolum)	0.22 ± 0.02 0.45 ± 0.03	0.20 ± 0.01 0.41 ± 0.03	0.10± 0.01 0.21 ± 0.02	0.22 ± 0.01 0.43 ± 0.03	2.00 ± 0.11 3.54 ± 0.10	0.22 ± 0.01 0.44 ± 0.03

4. Discussion

The rosmarinic acid in *R. officinalis* leaf extracts has been reported by several authors. However, the qualitative estimations are limited. Borrás-Linare et al. [43] studied 20 rosemary plants (harvested from different geographical zones of Serbia) by HPLC–ESI-QTOF-MS (high performance liquid chromatography coupled to electrospray quadrupole-time of flight mass spectrometry) method. The amount of rosmarinic acid varied from 50 to 2500 mg/100 g DW, depending on the place of harvest. Interestingly, the amount of rosmarinic acid quantified in their study was 1.6 times lower than that found in the North Saudi Arabia natural population origin samples in the current study. The rosmarinic acid was the unique phenolic acid confirmed by Kontogianni et al. [44] in the extracts of *R. officinalis* leaf commercial samples (Greece) using liquid chromatography/diode array detection/electrospray ion trap tandem mass spectrometry (LC/DAD/ESI-MS) method. The amount of rosmarinic acid from Greece [44] was equal to 1160 mg/100 g DW, which is 3.5 times lower than in the *R. officinalis* plants of Saudi origin of this study. The rosmarinic and caffeic acids in the *R. officinalis* leaf extracts samples obtained from United Kingdom ranged from 1000.0 to 1100.0 and from 10.0 to 20.0 mg/100 g DW, respectively [45]. These amounts were respectively c.a. 3.8 and 1.8 times lower than in the Saudi extracts of the present study. The vanillic acid was quantified (4 mg/100 g DW) by Luis et al. [46] in the leaf extracts of *R. officinalis* from United Kingdom. That amount was 9 times lower than in the samples of our collection. In addition, the same researchers detected rosmarinic acid (2150 mg/100 g DW) and caffeic acid (12 mg/100 g DW) in amounts respectively 1.9 and 2.3 times lower than the North Saudi Arabia natural population origin samples. Zgórka and Głowniak studied the compositions some phenolic acids in *R. officinalis* leaf extracts of Polish origin [47]. They detected relatively low amount of

rosmarinic acid—c.a. 700 mg/100 g DW (5.8 times lower than in Saudi origin samples), caffeic acid—c.a. 25 mg/100 g DW (similar to Saudi origin samples) and vanillic acid—c.a. 15 mg/100 g DW (1.8 times lower Saudi origin samples). They also confirmed the gentisic acid in the extracts in amount 2 times lower than the current study samples (c.a. 50 mg/100 g DW).

In *O. basilicum* leaf extracts, the HPLC-DAD analyses revealed high amount of rosmarinic acid (1128.5 mg/100 g DW). This compound is generally recognized as typical for the Lamiaceae family species. For example, there are many studies describing the endogenous synthesis of high amounts of this compound of in vitro cultured biomass of *O. basilicum* of a different origin [48]. The phenolic composition of *O. basilicum* leaf extracts has not been studied deeply. One study investigated 16 different *O. basilicum* cultivars from North America [49]. There were considerable differences between tested cultivars and the main compound was rosmarinic acid (varied from 6 to 609 mg/100 g DW), followed by caftaric acid (from 9 to 49 mg/100 g DW) and caffeic acid (from 4 to 77 mg/100 g DW). These North American plants compositions of rosmarinic acid and caftaric acid were respectively 1.8 and 1.2 times lower than in the samples of Saudi origin, also the caffeic acid was 4.4 times higher. In *O. basilicum* plants from Iraq, the rosmarinic acid composition was equal to 306 mg/100 g DW, which is several times lower than Saudi one [50]. Zgórka and Głowniak [47] studied leaf extracts of Polish *O. basilicum* and they detected rosmarinic acid—c.a. 1100 mg/100 g DW, which is comparable to Saudi origin samples. Moreover, they confirmed also: caffeic acid—c.a. 270 mg/100 g DW (15 times more than in Ssaudi origin samples) and vanillic acid—c.a. 8 mg/100 g DW (7 times more than Saudi origin samples). The rosmarinic acid is the ester of caffeic acid with 3,4-dihydroxyphenylacetic acid (α-hydroxydihydrocaffeic acid). In the Saudi *O. basilicum* leaf extracts samples, we confirmed a high amount of 3,4-dihydroxyphenylacetic acid (α-hydroxydihydrocaffeic acid) (312.3 mg/100 g DW) and caffeic acid (17.6 mg/100 g DW). Vlase et al. studied the phenolic composition of *O. basilicum* leaf extracts of Romanian origin [51], and the major compound was rutoside (42.57 mg/100 g DW). The amount of rutoside was 3 times higher in the Saudi Samples than those of Romanian origin. In the Romanian samples, they also confirmed the availability of ferulic acid (0.7 mg/100 g DW), p-coumaric acid (2.1 mg/100 g DW), and trace amounts (below 0.02 mg/1000 g DW) of caffeic acid, caftaric acid, chlorogenic acid and rosmarinic acid. In the Saudi plants, we detected considerably high amounts of these compounds, especially for chlorogenic acid derivatives—caftaric acid (60.3 mg/100 g DW), neochlorogenic acid (38.6 mg/100 g DW)—as well as isochlorogenic acid (30.0 mg/100 g DW).

Leaf extracts of *R. officinalis* and *O. basilicum* showed antioxidant effects, as revealed by several methods. The antioxidant activity of Northern Riyadh *R. officinalis* leaf extracts is mainly attributed to major identified polyphenols including rosmarinic acid. The rosmarinic acid showed comparable antioxidant activities to *R. officinalis* leaf extracts. Previous investigation on *R. officinalis* from Morocco revealed antioxidant activities of ethanolic leaf extracts, and this was attributed to the major identified polyphenols, including carnosol and carnosic acid [52]. Other reports indicated that the major leaf extract polyphenols responsible for antioxidant activities might be rosmarinic acid, hesperidin, and rosmanol as well [53]. The rosmarinic acid itself has strong antioxidant activities, as found in this study, as well as in previous investigations [54]. Other compounds that were also present in the extracts of rosemary in this study, including gentisic acid, showed antioxidant activities and have been associated with antioxidant activities against gamma irradiation [25] and as an antioxidant agent food industries [32]. In *O. basilicum*, there were also antioxidant activities in leaf extracts, and this might be attributed to major identified polyphenols including rosmarinic acid, DOPAC and rutoside. DOPAC, has known antioxidant activities as revealed in previous studies [55]. Rutoside (rutin) is known also as potent antioxidant [56]. Previous studied revealed antioxidant activities of leaf extracts of *O. basilicum* [24,25,57].

Antiproliferative and cytotoxic activities against Jurkat, MCF-7, HT-29, and HeLa were found in the methanolic leaf extracts of *R. officinalis* in the current study. It was attributed to DOPAC and rosmarinic acid, as found in the MTT assay and flowcytometry. A previous report found that rosemary leaf extracts (from Germany) may have antiproliferative activities against human ovarian cancer A2780,

and this activity might be attributed to main detected polyphenols such as carnosol, carnosic acid, and rosmarinic acid [58]. They described the perturbation of cell cycle progression in different phases of the cell cycle in response to leaf extract treatment. In the current study, there was an accumulation of necrotic and apoptotic cells in treated samples with the Saudi rosemary leaf extracts. The effects of rosmarinic acid and DOPAC on cancer cells had been described before. A previous investigation showed that rosemary leaf extracts had antiproliferative activities against HT-29 cancer cells by inhibiting cell proliferation, increasing cell cycle arrest, and increasing apoptosis [59]. The major composition of this extract was the rosmarinic acid [60]. The rosmarinic acid has strong anticancer activities against MCF-7 [61] and HT-29 [62]. The DOPAC has antiproliferative activities against few cancer cells, such as CHO-K1 [63]. In the current study, O. basilicum leaf extracts showed noticeable antiproliferative and cytotoxic activities against cancer cells, and this could be attributed to the polyphenolic composition, including rosmarinic acid, DOPAC, rutoside, ferulic acid, caffeic acid. Egyptian *O. basilicum* showed leaf methanolic extracts showed cytotoxic activities against HCT116 and HEBG2 cancer cells, and this was attributed to the phenolic composition, including caffeic acid, ferulic acid, catechin, quercetin, and others [64]. These polyphenols had known antiproliferative and cytotoxic activities, for example the rutoside obtained from *Ferocactus* species showed strong antiproliferative and cytotoxic activities against cancer cells [39].

The leaf extracts obtained from Saudi *R. officinalis* and *O. basilicum* had strong antibacterial and antifungal effects against screened bacteria and fungi. *S. aureus*, *P. aeruginosa*, and *M. flavus* were the most sensitive to leaf extracts. The antibacterial activity of leaf extracts is associated with some major polyphenols including DOPAC and rutoside. However, other polyphenols, including gentisic acid and rosmarinic acid were not relatively active. The antibacterial activity of DOPAC had been reported before against several bacteria [39]. Rutoside also has antibacterial activities against *B. cereus* and *Salmonella enteritidis* [65], *S. aureus* [66], and other bacteria [39]. *R. officinalis* and *O. basilicum* (from different regions) leaf extracts showed antibacterial activities in previous investigations [67]. Caffeic acid showed strong antibacterial activities, which is in agreement with previous studied confirming the antibacterial activities against different bacteria [5].

The antifungal activities of *R. officinalis* and *O. basilicum* methanolic leaf extracts had been reported before against several fungi [68]. However, this is the first report on the antifungal activities of *R. officinalis* from Northern Saudi Arabia. These antifungal activities are strongly associated with identified polyphenols, including 3,4-dihydroxyphenylacetic acid, gentisic acid, and rutoside. Gentisic acid is a dihydroxybenzoic acid that have known antifungal activities [69]. Similarly, DOPAC, rutoside, and caffeic acid had strong antifungal activities [5,39].

5. Conclusions

This is a novel report exploring the polyphenolic profile of natural populations of *R. officinalis* and *O. basilicum* in Northern Riyadh in Saudi Arabia. The HPLC-DAD analyses confirmed several polyphenols, including rosmarinic acid, gentisic acid, 3,4-dihydroxyphenylacetic acid (α-hydroxydihydrocaffeic acid), rutoside, and others out of 38 screened polyphenolic compounds. Rosmarinic acid was the major polyphenol in both studied species. *R. officinalis* methanolic leaf extracts contained rosmarinic acid and gentisic acid as major polyphenols, while *O. basilicum* contained rosmarinic acid, 3,4-dihydroxyphenylacetic acid, and rutoside, as well as others. *R. officinalis* showed higher antioxidant activities than *O. basilicum* using DPPH, FRAP, and β-carotene bleaching assay. These higher activities are associated with a higher composition of rosmarinic acid in leaf extracts. The antioxidant activities of *O. basilicum* were attributed to identified phenols of rosmarinic acid, 3,4-dihydroxyphenylacetic acid, and rutoside. There were antiproliferative and cytotoxic activities of leaf extracts, as well as identified polyphenols against several cancer cells. These activities were attributed to the accumulation of necrotic and apoptotic cells in treated cancer cells, with leaf extracts as well as identified polyphenols. The antibacterial and antifungal activities of leaf extracts were mainly attributed to 3,4-dihydroxyphenylacetic acid and rutoside in *O. basilicum* and rosmarinic acid and

caffeic acid in *R. officinalis*. This study proved that *R. officinalis* and *O. basilicum* natural populations might be considered as promising sources of natural polyphenols with biological activities.

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