

**RESEARCH ARTICLE**

**Comparative Study of Essential oil, Phenolic content and Antioxidant activity of the *Rosmarinus officinalis* var. prostrates and *Rosmarinus officinalis* plant extracts**

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**ABSTRACT:**

The *Rosmarinus officinalis* var. prostrates and *Rosmarinus officinalis* are important medicinal plant that has been used in folk medicine to treat many diseases, all its parts contain active ingredient but this study focuses on the phenolic content, the anti-oxidant activity, and the chemical composition of the essential oil in The *Rosmarinus officinalis* var. prostrates and *Rosmarinus officinalis* leaves ( in kallamoon region, Syria) This study showed that the phenolic content in the ethanolic extract of *R.officinalis* is(1.6556)mg/1g of dried powder, while the phenolic content in extract of *R.officinalis* var. prostrates is(1.799)mg/1g dried powder and the Determination of Carnosic acid, O-12-methylcarnosic acid, Rosmarinic acid and Ginquanin by HPLC, has shown that the concentration of Carnosic acid, rosmarinic acid and ginquanin in the extract of *R.officinalis* var. prostrates is higher than *R.officinalis*, Additionally the antioxidant capacity value of *R. officinalis* var. prostrates is higher than *R. officinalis* in leaf extract, while the antioxidant capacity value of *R. officinalis* oil is higher than that of *R. officinalis* var. prostrates using PCL assay, The GC- The GC-MS analysis revealed that the major components in *Rosmarinus officinalis* var. prostrates essential oil were 1,8-Cineole (42.18%),  $\alpha$ -Pinene (11.38%), Camphene (5.39%),  $\alpha$ -Humulene (5.63%), p-Cymene (4.98%). Meanwhile the major components in *Rosmarinus officinalis* were 1,8-Cineole (39.19%),  $\alpha$ -Pinene (9.13%), Camphene (4.65%),  $\beta$ -Caryophyllene (4.47%), p-Cymen-8-ol (4.18%),  $\beta$ -Pinene (3.16%)

**KEYWORDS:** *Rosmarinus officinalis*, *Rosmarinus officinalis* var prostrates, Essential oil.

**INTRODUCTION:**

Herbal medicines have been used in medical practices since antiquity<sup>1</sup>, they are still an important part of traditional healing systems<sup>2</sup>, they can also synthesize a wide range of bioactive compounds that performs important biological functions<sup>3</sup>, Therapeutic plants have many different medicinal properties and thereby it helps in finding out a proper health care system<sup>4</sup>

Now a days, traditional medicine is revealed by an extensive activity of research on different plant species<sup>5</sup>, Moreover, only a limited number of medicinal plants have received detailed scientific scrutiny thereby prompting the World Health Organization to recommend that this area be comprehensively investigated<sup>6</sup> Medicinal plants were considered natural resources of bioactive compounds. Secondary metabolites, flavonoids, phenols, and flavanols derived from medicinal plants have been used worldwide to treat many diseases.<sup>7</sup>

Recently, there has been increasing awareness of the importance of the high content of phenolic compounds due to their antioxidant properties, which can prevent oxidative decomposition in food and protect oils and fats<sup>8</sup> They are able to trap free radicals and activate other antioxidants in the body,<sup>9</sup> The three most well-known phenolic compounds important to humans are phenolic acids, flavonoids, and polyphenols<sup>10</sup>.



Rosemary (*Rosmarinus officinalis* L) is a very widely spread shrub in the Mediterranean region. It also exists in many places like Europe, Asia and Africa<sup>11</sup>. *R. officinalis* L. is used as fresh, dried or as an essential oil. In addition, the rosemary extract is now widely used commercially to increase the shelf life of foods<sup>12</sup>. The essential oil of *R. officinalis* L is largely used in traditional medicine, and has a tonic stimulant property; it is used as a pulmonary antiseptic, and stomachic. It also has anti-rheumatic properties<sup>13</sup>. Rosemary essential oil and its composition have been the subject of an important study, reviewed by Lawrence<sup>14,15</sup>. Rosemary extracts can be used as natural alternatives to synthetic antioxidants like Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA), because they have a comparable or even stronger antioxidant activity<sup>16,18</sup>.

Studies and research confirm that several extracts, essential oil and chemical constituents of *R. officinalis* L possess a number of interesting biological properties such as anti-mammary tumorigenesis and mutagenesis<sup>19</sup>, anti-hyperglycemic<sup>20</sup>, hepatoprotective<sup>21-22</sup>, antioxidant<sup>23-32</sup>, antiulcerogenic<sup>33</sup>, anticarcinogenic<sup>34</sup>, and antimicrobial<sup>35</sup>. This study is new and had focused on the comparison between *R. officinalis* and *R. officinalis* var. prostrates in syria.

## MATERIAL AND METHODS:

### Material:

- Methanol, Acetone, Hexane, Petroleum ether, Dichloromethane ethyl acetate, hydrochloric acid and anhydrous sodium sulphate were obtained from Sigma- Aldrich,
- Carnosol (>95%) - camphor (>97%) -  $\alpha$ - terpineol (>97%) and linalool (>97%) were purchased from Sigma-Aldrich. Carnosic acid ( $\geq 97\%$ ) - 1,8 cineole(98%) and borneol (>99%).
- Ethanol, Acetonitrile and phosphoric acid (85%) were HPLC grade from Merck.
- Chemical kits used to determine the capacity of antioxidant substances dissolved in water (ACW) and materials dissolved in lipids (ACL) using photochemical illuminating technique were obtained from Analytik Jena AG (Jena, Germany)

### Devises:

- A gas chromatography with mass spectrometry GC-MS is from Agilent with the analytical Chemstation software and Library search Willy - Nist.
- Rotary evaporator with vacuum
- HPLC Device from Agilent with the pump bilateral quadartic with injector automatic Autosampler and PDA detector with analytic software Chemstation
- Photochemical scintigraphy device for determining antioxidants, which is from Ana.

- Ultrasonic device, model 405 from Power Sonic Company
- Centrifuge model 2.0R- A 550 Heraeus from Megafuge

### Plant materials:

Leaves of *R.officinalis* var. prostrates and *R.officinalis* were collected during flowering stage from Deir Atiyah, Syria in September 2023, and they were stored in a dry, dark and cool room.

### Determination of the of total phenolic amount:

#### Extract preparation:<sup>36</sup>

Powder of leaves of *R.officinalis* var. prostrates and *R.officinalis* was dissolved in ethanol 96% and extracted by Soxhlet extraction for four hours, then evaporated until it became dry using a rotary evaporator.

#### Preparation Standard Curve:

The amount of total phenolics in the extract was determined with the folin- Ciocalteu reagent. Tannic acid was used as a standard and a total phenolics were expressed as mg/ g tannic acid equivalents (TAE).

Tannic acid concentrations of 20, 40, 60, 80, and 100 mg/100ml in ethanol. 5ml of sample was introduced into test tubes and mixed with 5ml Folin- Ciocalteu reagent, after three minutes we put 10ml (7.5%) sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30minutes at room temperature before the absorbance at 755nm spectrometrically.

### The Determination of phenols using HPLC with PDA Detector:

#### phenolic Extraction:

The finely dried powdered *R.officinalis* var. prostrates and *R.officinalis* (50g) were extracted with methanol – water (70-30) and placed in ultrasonic bath at 40°C for 1 hour, They were then filtered and the filtrate was acidified with 2N HCl, and the phenols were extracted with 50 ml of ethyl acetate using an ultrasonic bath for 30 minute, Subsequently, the ethyl acetate layer was separated and evaporated to dryness in a rotary evaporator, then dissolved in 2ml of acetonitrile-water acidified with 0.5% acetic acid at a ratio of 80% acetonitrile and 20% acidified water and the extracted phenols were determined by HPLC according to the following analytical conditions.

Mobile phase: first phase (A) acetic acid: water (0.5% v/v), second phase (B) Acetonitrile, according to the table(1) which explains the Proportions of the distribution of the mobile form over time.

**Table 1 Proportions of the distribution of the mobile form over time**

Time	0 min	9 min	15 min	23 min	29 min	37 min
A	100%	80%	70%	55%	75%	100%
B	0%	20%	30%	45%	25%	0%

**Determination of Carnosic acid, O-12-methylcarnosic acid, Rosmarinic acid and Ginquanin by HPLC:**

**Extract preparation:**

100g of powdered of leaves of *R.officinalis* var. prostrates and *R.officinalis* was dissolved in 150ml of methanol and extracted by Soxhlet extraction. the extraction process continued until the organic solvent in the extraction tube turned colorless, then evaporated to dryness using a rotary evaporator, the extract was dissolved in 2ml acetonitrile and determined by HPLC according to the following analytical conditions.

Mobile phase: A (acetonitrile), B (0.1% Phosphoric acid in water) as it is explain in Table (2)

**Table 2: Proportions of the distribution of the mobile form over time**

Time	0 min	8 min	25 min	35 min
A	100%	23%	75%	100%
B	0%	77%	25%	0%

The flow rate is constant at 0.9ml/min, the volume of the injection is 20µl, and the signal was stored at a wavelength of 230, 280 and 350nm.

**Determination of the exchange capacity of antioxidants:**<sup>37</sup>

100g of powdered plant leaves were soaked in 200ml of a 50:50 ethanol/water solution and placed in the dark in the water bath at the temperature of 40°C for half an hour, stirring, then subjected to ultrasonic treatment for 25 minutes then filtered evaporated using a rotary evaporator and stored in a refrigerator in preparation for analysis.

**Antioxidant measurement:**<sup>37</sup>

The integral antioxidative capacity of water- soluble substances, was carried out using the ACW protocol. The water-soluble antioxidants present in rosemary leaves plant have been measured. All the required reagent kits were purchased from the company analytic Jena – Germany. ACW protocol can be explained as follows: the first three reagents can be described as follows: reagent 1 (solvent), reagent 2(water buffer solution PH= 10.5) while reagent 3(photosensitizer). Working solution of reagent 3(3-WS) has been prepared by taking reagent 3 stock and then diluted it by 750µl of reagent 2.

The Working solution of reagent 4(4-WS) has been prepared by taking reagent 4 stock, and then adding to it 490µl of reagent 1 and mixed with 10µl of sulfuric acid (95-97% from Merck). the mixture was Stirred for 30 seconds. From the mixture, 10µl was taken and diluted by 990 µl from reagent 1 to obtain a working solution 4 (4-WS). All procedures and volumes used in the analysis are given in table 3.

The ACW calibration and measurements were performed according to the standard kit protocol as menation the table 3, and the measurements were done by Photochem apparatus (Analytic Jena – Germany) device from analytic Jena - Germany. All used volumes were in microliters and the measurements were repeated two times. A light emission curve was recorded in 240 seconds using an inhibitor as a parameter to estimate the antioxidant capacity, and the antioxidant capacity is determined by taking the integration given by the previous curve and expressed in mmol/L ascorbic acid used as the standard to obtain the calibration curve.

**Table 3: Pipetting scheme for sample preparation measurements**

Reagent	1	2	3-WS	4-WS	Sample
Blank	1500□1	1000□1	25□1	0	0
Calibration	1500□1-x	1000□1	25□1	x	0
Measurements	1500□1-y	1000□1	25□1	0	y

x= 10, 15, 20µl, y= 10µl, WS (working solution)

**Distillation of essential oil:**

The essential oil from leaves of *R. officinalis* var. prostrates and *R. officinalis* (100g) were obtained by Clevenger for 3 hour.. The oil was dried over anhydrous sodium sulphate and kept at 4°C until analysis.

**Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Essential oil**

The analysis of the volatile oil was performed using the Agilent GC-6890 with MS model 5973 equipped with DB-35 column (30m × 0.25mm i.d.,0.25µm film thickness) and coupled to a mass selective detector. Helium was used as carrier gas at a flow rate of 0.9 ml/min.

The injection temperature was 275°C, injection volume 1µl, The column temperature was kept for two minutes at 70°C, increased to 170°C at a rate of 2.0°C/min increase, then 3.0°C/min up to 250°C. The injector was splitless. The mass operating parameters were as follow: Ionization potential 70 eV; ion source temperature 250°C; solvent delay 7.0 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. The

components of the oil were identified by matching their fragmentation pattern of mass spectra with those of the spectrophotometer database using the NIST10, NIST21, NIST 69 and Wiley 229 library data, and comparing their retention indices and mass spectra with literature data. The component concentration was obtained by semi quantification by peak area integration from GC peaks and by applying the correction factors.

**RESULTS:**

**Determination of the amount of total phenols:**

The table (4) shows the mean absorbance of various concentrations of tannic acid and figure 1 shows the standard tannic acid curve and regression equation used to calculate total phenolic content of the extract

The total phenolic content of the plant leaves extract, determined by Folin- Ciocalteu, the phenolic content in the extract of *R.officinalis* is 82.78 mg/100 ml while the phenolic content in extract of *R.officinalis* var. *prostrates* is 89.95mg/100ml.

**Table (4): Absorbance of standard (tannic acid)**

Concentration mg/ 100 ml tannic acid	20	40	60	80	100
Absorbance	0.192	0.493	0.558	0.764	0.967

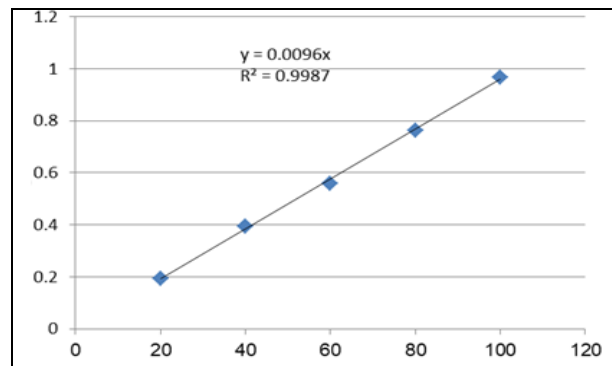


Figure 1: Standard tannic acid curve (mg/l)

**Determination of Carnosic acid, O-12-methylcarnosic acid, Rosmarinic acid and Ginquanin by HPLC:**

The figure 2 shows chromatogram separated phenolate by HPLC, the table 2 shows concentration Carnosic acid, 12O-methylcarnosic acid, rosmarinic acid and ginquanin in the extract of *R.officinalis* and *R.officinalis* var. *prostrates*, the results shows that concentration Carnosic acid, rosmarinic acid and ginquanin in the extract of *R.officinalis* var. *prostrates* is higher than *R.officinalis* while the concentration of 12O-methylcarnosic acid in the extract of *R.officinalis* is higher than *R.officinalis* var. *prostrates*.

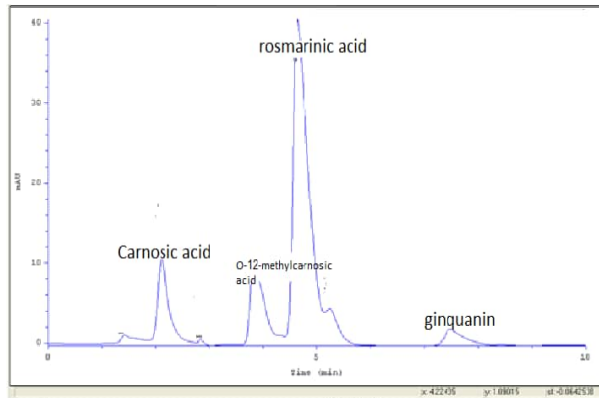


Figure 2: Chromatogram separated phenolate by HPLC

**Table 2: Concentration Carnosic acid, 12O-methylcarnosic acid and ginquanin in the extract of *R.officinalis* and *R.officinalis* var. *prostrates*,**

Phenolate	Retention time	Concentration µg/ml	
		<i>R.officinalis</i>	<i>R.officinalis</i> var. <i>prostrates</i>
Carnosic acid	7.3	12.45	16.72
12O-Methylcarnosic acid	13.2	17.91	15.67
Rosmarinic acid	23.4	33.49	37.82
Ginquanin	29.7	11.87	16.48

**Determination of the exchange capacity of antioxidants:**

The table 3 shows the antioxidant capacity value of *R.officinalis* var. *prostrates* and *R.officinalis* leaves extract and oil, the antioxidant capacity value of *R.officinalis* var. *prostrates* is higher than *R.officinalis* leaves extract, while the antioxidant capacity value of *R.officinalis* oil is higher than *R.officinalis* var. *prostrates*,

**Table 3: Presents the Integral Antioxidant capacity as ascorbic acid equivalent using PCL assay**

samples	<i>R.officinalis</i> leaves extract	<i>R.officinalis</i> var. <i>prostrates</i> leaves extract	<i>R.officinalis</i> oil	<i>R.officinalis</i> var. <i>prostrates</i> oil
Integral Antioxidant as ascorbic acid equivalent nmol/g	114.7	120.08	123.50	120.49

**Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Essential oil of *Rosmarinus officinalis*:**

Chemical compositions of the essential oil of *Rosmarinus officinalis* are given in table 4, thirty two compounds were identified representing 99.03 of the oil, 1,8-Cineole (39.19%), α-Pinene (9.13%), Camphene (4.65%), β-Caryophyllene (4.47%), p-Cymen-8-ol (4.18%), β-Pinene (3.16%), were founds as a major compounds.

**Table 4. Chemical composition of essential oil of *Rosmarinus officinalis***

Peak	Compounds	RT	RI	Area%
1	a-Pinene	6.23	1 072	9.13
2	β-Pinene	6.52	1 096	3.16
3	Camphene	6.71	1 105	4.65
4	α-Thujene	7.85	1 133	1.11
5	Carene	8.17	1 165	0.76
6	β- Myrcene	8.68	1 176	1.92
7	α-Phellandrene	8.93	1 179	0.49
8	Fenchene	9.32	1 193	0.89
9	Limonene	10.21	1 211	2.18
10	1,8-Cineole	11.54	1 223	39.19
11	Linalool	12.39	1 257	1.18
12	p-Cymene	14.98	1 283	2.96
13	α-Terpinene	15.84	1 295	2.47
14	Cubene	20.14	1 513	1.14
15	Camphor	21.84	1 546	1.29
16	Borneol	22.38	1 584	1.78
17	Bornyl acetate	23.14	1 616	0.87
18	1-Terpinen-4-ol	24.97	1 619	1.18
19	α-Copaene	25.17	1 624	0.34
20	β-Caryophyllene	28.91	1 639	4.47
21	E-pinocarveol	31.01	1 693	0.56
22	a-Humulene	35.34	1 704	2.93
23	Δ-Cadinene	36.11	1 713	1.96
24	a-Terpineol	37.45	1 725	1.48
25	Citronellol	38.62	1 737	2.24
26	p-Cymen-8-ol	40.21	1 771	4.18
27	Berbonone	41.28	1 809	1.47
28	Eugenol methyl ether	42.89	1 904	0.84
29	Eugenol	43.66	2 048	0.62
30	Thymol	44.57	2 099	0.24
31	Carvacrol	45.98	2 306	0.51
32	Verbenone	46.17	2 327	0.54
				99.03
<b>Monoterpenes</b>		<b>91.12</b>		
<b>Sesquiterpenes</b>		<b>7.91</b>		

12	p-Cymene	14.98	1 284	4.98
13	α-Terpinene	15.84	1 296	0.37
14	-Cubene	20.14	1 515	0.20
15	Camphor	21.84	1 547	2.21
16	Borneol	22.38	1 580	1.52
17	Bornyl acetate	23.14	1 612	0.87
18	1-Terpinen-4-ol	24.97	1 618	0.15
19	α-Copaene	25.17	1 627	0.90
20	β-Caryophyllene	28.91	1 637	3.22
21	E-pinocarveol	31.01	1 692	0.21
22	a-Humulene	35.34	1 702	5.63
23	Δ-Cadinene	36.11	1 710	1.14
24	a-Terpineol	37.45	1 723	0.24
25	Citronellol	38.62	1 736	3.19
26	p-Cymen-8-ol	40.21	1 796	0.24
27	Berbonone	41.28	1 808	1.14
28	Eugenol methyl ether	42.89	1 904	0.84
29	Eugenol	43.66	2 047	0.23
30	Thymol	44.57	2 095	0.16
31	Carvacrol	45.98	2 305	0.17
32	Verbenone	46.17	2 324	1.14
				98.5
Monoterpenes		91.12		
Sesquiterpenes		7.91		

**DISCUSSION:**

The total phenolic content in *Rosmarinus officinalis* var prostrates (1.799mg/1g of the dried powder) is greater than its content in *Rosmarinus officinalis* (1.6556mg/1g of the dried powder), and this is consistent with the amount of phenols separated according to the method HPLC, as the amount of separated phenols was higher in *Rosmarinus officinalis* var prostrates than in *Rosmarinus officinalis*. One study showed The highest amount of phenolics (49mg/ml rosmarinic acid) was determined in the tincture produced with 50% ethanol, while 96% ethanol extracted the lowest value (19.5mg/ml rosmarinic acid) As for the antioxidant action, the ability of the *Rosmarinus officinalis* var prostrates leaves extract was higher *Rosmarinus officinalis* leaves extract, while the ability of the essential oil *Rosmarinus officinalis* was greater than essential oil *Rosmarinus officinalis* var prostrates.

As for the composition of the essential oil, the ingredients were almost similar between the two plants, but the concentration of the active ingredients in the *Rosmarinus officinalis* var prostrates was higher than *Rosmarinus officinalis*

One study about the essential oil of aerial parts *R.Officinalis* var. prostrates was conducted in four provinces in Morocco<sup>38</sup> By analyzing these results, we conclude that the majority of compounds in Rosemary from WadLaou are: α-Pinene (36.15%), 1,8-Cineole (33.93%), Camphene (6.1%), Camphor (5.08%) and Sabinene (3.4%). The majority of compounds in Rosemary in Agadir’s Garden Jacky: Bornyl Acetate

**Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of Essential oil of *Rosmarinus officinalis* var prostrates:**

Chemical compositions of the essential oil of *Rosmarinus officinalis* var prostrates are given in table 5, thirty two compounds were identified representing 98.5 of the oil, 1,8-Cineole (42.18%), a-Pinene (11.38%), Camphene (5.39%), a-Humulene (5.63%), p-Cymene (4.98%), were founds as a major compounds.

**Table 5. Chemical composition of essential oil extract of *Rosmarinus officinalis* var prostrates**

Peak	Compounds	RT	RI	Area%
1	a-Pinene	6.23	1 075	11.38
2	β-Pinene	6.52	1 097	4.23
3	Camphene	6.71	1 102	5.39
4	α-Thujene	7.85	1 132	1.86
5	Carene	8.17	1 164	0.78
6	β- Myrcene	8.68	1 174	1.24
7	α-Phellandrene	8.93	1 178	0.57
8	Fenchene	9.32	1 191	0.51
9	Limonene	10.21	1 210	1.19
10	1,8-Cineole	11.54	1 221	42.18
11	Linalool	12.39	1 256	1.42

(31.21%),  $\alpha$ -Pinene (15.79%), Camphene (13.06%), Sabinene (7.11%), 3-Carene (4.54%), Caryophyllene Oxide (4.71%),  $\beta$ -Caryophyllene (4.09%), and Borneol (3.59%). The majority of compounds in Rosemary in AyounCharquia are: 1,8-Cineole (35.91%),  $\alpha$ -pinene (16.61%), Camphene (13.67%), Camphor (6.4%), sabinene (6.04%), Borneol (4.81%) and Caryophyllene Oxide (3.8%). In Sefrou the majority of compounds in Rosemary are: Camphor (22.1%), 1,8-Cineole (18.35%),  $\alpha$ -Pinene (12.19%), Camphene (6.81%), Caryophyllene Oxide (6.3%)<sup>38</sup>

Moreover, the compounds in essential oils in *R. officinalis* L in three locations of mount Lebanon are  $\alpha$ -pinene (18.8–38.5%) and 1,8-cineole (19.1–25.1%), camphene (2.1–6.5%),  $\beta$ -pinene (1.8 - 6.5%)<sup>39</sup>. The hydro-distilled essential oils of three samples of aerial parts of *R. officinalis* L. grown in Tunisia have been analyzed by GC-MS. The main compounds in essential oil are 1,8-cineol (33.08, 37.75 and 36.75%), camphor (18.13, 13.55 and 15.57%),  $\alpha$ -pinene (9.23, 9.32 and 8.58%),  $\alpha$ -terpineol (8.17, 6.79 and 6.98%), borneol (5.48, 4.08 and 4.49 %)<sup>40</sup>

One study was conducted in 2023 that had been compared the amount of phenols in the aqueous extract, methanol, and ethanol of rosemary leaves. It showed that the amount of phenols in the ethanolic extract is the highest, followed by the aqueous extract, and then the methanol with 39.71±6.77 (mg/g GAE), 24.91±5.15 (mg/g GAE), and 24.91±7.30 (mg/g GAE)<sup>41</sup> Another study in 2012 study the amount of phenols in the ethanolic extract.<sup>42</sup>

Also there were a study about antioxidant potential of six herbs extracts showed that DPPH radicals scavenging activity exhibited ethanol extracts of rosemary (0.46mg Trolox/g dry weight)<sup>43,44</sup>. Another study in Alecrim about the ethanolic extracts of the leaves of *Rosmarinus officinalis* which were extracted through supercritical fluid extraction (SFE), and Soxhlet extraction was IC<sub>50</sub> (15.73µg/ml)<sup>45</sup>

Daiane Pereira and his Colleagues determined the antioxidant activity and applied the Rosemary lyophilized extract (RLE) in chicken burger, to assess their ability to reduce the lipid oxidation that IC<sub>50</sub> in 80% ethanolic extract was (EC<sub>50</sub>=127.33±0.12µg/ml)<sup>46</sup>.

Rodríguez-Rojo and his group studied (DPPH) for Rosemary leaves from Spain and obtained values of IC<sub>50</sub> of 45 and 17µg.ml<sup>-1</sup> for ethanolic<sup>47</sup>. The ability of the extract to scavenge free radicals in 70% ethanolic extract *Rosmarinus officinalis* from Tunisia (2.23±0.14 µg/ml)<sup>48</sup>, and 50% ethanolic extract Rosemary leaves

(*Rosmarini folium*) from Romania was IC<sub>50</sub> (4.63±0.30 µg/ml)<sup>49</sup>. The percent inhibition of DPPH was 90.14% for rosemary alcoholic extract 75% from Brazil<sup>50</sup>. Dry rosemary leaf powder was subjected to Soxhlet extraction with pure methanol and was studied through the radical scavenging method IC<sub>50</sub> (24±0.005µg/ml)<sup>51</sup>. The IC<sub>50</sub> values of methanol extract of rosemary (*Rosmarinus officinalis* L.) growing wild in Hammam Dalâa (Algeria) was (11.741±0.004µg/ml)<sup>52</sup>, and values of methanol extract *Rosmarinus officinalis* L was 54.0 µg/ml<sup>53</sup>

There was a study indicated the presence of phenolic acids after they were separated by HPLC (gallic, protocatechuic, caffeic, ferulic, and rosmarinic acid). The highest concentration was for rosmarinic acid, and this is similar to our study<sup>54</sup>

The bottom line is that The total phenolic content in *Rosmarinus officinalis* var prostrates was higher than in *Rosmarinus officinalis*, aligning with other studies such as one that identified the highest phenolic content (49 mg/ml rosmarinic acid) in tinctures made with 50% ethanol. In contrast, 96% ethanol extracted the lowest amount (19.5mg/ml). This confirms the variation in phenolic content based on extraction methods, a finding consistent with a 2023 study which found the highest phenolic content in ethanolic extracts compared to aqueous and methanolic extracts.

In terms of antioxidant potential, the extract from *R. officinalis* var prostrates demonstrated stronger activity than *R. officinalis*, but the essential oil from *R. officinalis* showed greater efficacy. These results correspond with studies like the one that assessed DPPH scavenging activity, showing ethanol extracts of rosemary with values of IC<sub>50</sub> as low as 2.23µg/ml for 70% ethanol extracts, further emphasizing the potent antioxidant capabilities of rosemary extracts depending on the solvent used.

Lastly, the essential oil composition was similar between the two plants, though *R. officinalis* var prostrates exhibited a higher concentration of key compounds. Studies from different regions, including Morocco, Tunisia, and Lebanon, confirm the variability of oil composition in rosemary, with dominant compounds like  $\alpha$ -pinene, 1,8-cineole, and camphor appearing across locations, but in differing concentrations, further validating the findings of this study.

## CONCLUSION:

The results showed that the antioxidant capacity and phenolic content of The *Rosmarinus officinalis* var. prostrates extracts was higher than that of *Rosmarinus*

*officinalis*. This is consistent with the fact that the phenolic acids that were separated by HPLC had a higher concentration in *Rosmarinus officinalis* var. *prostrates* extracts than *Rosmarinus officinalis*, and rosmarinic acid was in its the highest concentration, and by comparing the essential oil of both plants, it was noted that the same compounds are present, but the concentration of these compounds in the *Rosmarinus officinalis* var. *prostrates* is higher than the other plant extract, the same thing was for the essential oil, This study underscores the importance of *Rosmarinus officinalis* var. *prostrates* as a source of valuable compounds, suggesting that further research should focus on this less-studied variety

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