Chemical composition of rosemary leaves and black cumin seeds

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ABSTRACT

This investigation aimed to evaluate the chemical constituents of rosemary *(Rosmarinus officinalis* L.) leaves and black cumin (*Nigella sativa* L.) seeds using different chemical analysis methods including moisture, ash, lipid, crude protein, total carbohydrates, crude fiber, total phenols, and essential oil as well as identify the dominant components of essential oils by using GC-MS. The results indicated that the proximate analysis of rosemary leaves achieved significantly the highest values of ash, crude fiber, total phenols, and essential oil, whereas black cumin seeds attained significantly the highest values of moisture, lipid, crude protein, and total carbohydrates. The essential oil content of rosemary leaves and black cumin seeds (1.04 and 0.39%, respectively) was significantly ($p<0.05$) different. Twenty essential oil components of rosemary leaves were identified. The main components of rosemary essential oil were α-pinene (17.13%) and 1.8 cineole (13.69%). On the other hand, eighteen essential oil components of black cumin seeds were identified. The predominant components of black cumin essential oil were Thymoquinone (38.43%) and P-cymene (21.18%).

Keywords: Essential oils; rosemary; black cumin; moisture; ash; lipid; crude protein; total carbohydrates; crude fiber; total phenols.

INTRODUCTION

The medicinal and aromatic plants are the most important non-traditional crops that can contribute to the achievement of the goals of the Egyptian agricultural policy in expanding the export capacity, narrowing their imports, and achieving self-sufficiency in addition to providing job opportunities within the agricultural sector, with the increasing global trend towards using these plants in different industries and the existence of international agreements that Egypt entered as a party to facilitate trade and eliminate the customs barriers of some commodities in certain fungi or throughout the year, especially that Egypt has a great advantage in producing these plants as nontraditional crops (Mohamed *et al.,* 2019).

Rosemary (*Rosmarinus officinalis* L.) is a woody perennial herb belonging to the family Lamiaceae. Due to its essential oils and extracts, use as a spice, and various biological functions, this plant, which is originally from the Mediterranean region, is today grown all over the world (Bozin *et al.,* 2007). It has important chemical constituents, like alkaloids, flavonoids, phenols, rosmarinic acid, mineral constituents, and vitamins. From a medicinal point of view, it is used to strengthen the circulatory system and nervous system. Furthermore, it is used to control cancer, regulate blood pressure, etc. (Tsai *et al.,* 2007).

Black cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranunculaceae. It is cultivated in several parts of the world, especially in the Middle East (Liu *et al.,* 2011). The seeds of black cumin are a rich source of protein, fibers, carbohydrates, vitamins, phenolics, and antioxidants (Kour and Gani, 2021).

Essential oils are fragrant, oily liquids extracted from plant substance that have widely been applied in medicine and the food preservation due to their antibacterial, antifungal, and antiviral properties (Bassole and Juliani, 2012).

This investigation aimed to study the chemical composition of rosemary leaves and black cumin seeds as well as identify the dominant components of essential oils.

MATERIAL AND METHODS

Plant materials

This investigation was carried out in the central laboratory of Horticulture Research Institute, Agricultural Research Center to study the chemical constituents of rosemary *(Rosmarinus officinalis* L.) leaves and black cumin (*Nigella sativa* L.) seeds as well as identify the dominant components of essential oils, which were obtained from the experimental station of Medicinal and Aromatic Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt and they were isolated by hydro-distillation.

The chemical constituents of rosemary leaves and black cumin seeds were adopted as follows:-

Moisture (%): It was calculated on a dry weight basis determined according to A.O.A.C. (2005).

Ash (%): It was calculated on a dry weight basis was determined according to A.O.A.C. (2005).

Lipids (%): It was determined gravimetrically using a soxhlet apparatus and hexane as a solvent (A.O.A.C., 2005).

Crude protein (%): It was calculated by multiplying total nitrogen by 6.25 (A.O.A.C., 2005).

Total carbohydrates (%): It was assayed using the phenol sulfuric acid method and calculated as glucose (%) (Dubois *et al.,* 1956).

Fiber (%): It was calculated on a dry weight basis and was determined according to A.O.A.C. (2005).

Total phenols (%): It was extracted according to the method described by Daniel and George (1972). Determination of phenolic compounds was determined by the colorimetric method of Folin-Denis as described by Larsen (1962).

Essential oil content: A known weight of Rosemary leaves (200g) and Black Cumin seeds (100g) were placed in the distillation flask of the Clevenger apparatus essential oils were separated by water distillation for 3-4 h. according to the method of Guenther (1961). The essential oil was separated by a Clevenger apparatus which was modified to extract the oil without facing any difficulties of foaming. This modification was made according to El-Deeb *et al.* (1993). The percentage of the essential oils was calculated and expressed as volume / weight (ml/100g) according to A.O.A.C. (2005).

Physical properties of essential oil

Specific gravity: The specific gravity of the essential oils was determined by using a pycnometer according to Guenther (1961).

Optical rotation: The optical rotation of the oil is determined according to Guenther (1961).

Refractive index: The refractive index was determined according to Guenther (1961).

Chemical components of essential oil

Identification of essential oil: The analysis of essential oils was carried out using the gas chromatography instrument GC-MS ion trap. Hewlett Packard (hp) 5890 series II was used to separate and identify the chemical constituents of the essential oils under study.

Experimental design and statistical analysis

A completely randomized design was performed for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Cochran (1980). Averages were compared using the T-test at 5% level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical constituents of rosemary leaves and black cumin seeds

Data presented in Table (1) and Figure (1) indicated that the proximate analysis of rosemary leaves significantly achieved the highest values of ash, crude fiber, total phenols, and essential oil, whereas black cumin seeds significantly attained the highest values of moisture, lipid, crude protein, and total carbohydrates.

There are many factors which include the regionality, seasonality, environmental conditions, agronomic conditions and varieties affect chemical profile (Bajalan *et al.,* 2017).

These data were similar to the results of the proximal analysis reported by Ngan *et al.* (2019) and Shetti and Altae (2019) for rosemary leaves and also by Suri *et al.* (2019) and Barkah *et al.* (2021) for black cumin seeds.

Physical properties of rosemary and black cumin essential oils

The physical properties are usually determined for essential oils to assess their values and application, therefore, the most important physical characteristic of rosemary and black cumin essential oils of such values involved specific gravity, optical rotation, and refractive index which are revealed in Table (2) and Figure (2).

With respect to specific gravity, it was noticed that rosemary essential oil significantly had the lowest values of specific gravity as compared to black cumin essential oil.

Concerning optical rotation, it was obvious that the optical rotation of rosemary essential oil was +6.94°, while the optical rotation of black cumin essential oil was +4.27°.

Regarding the refractive index, it was noticed that rosemary essential oil significantly achieved the highest values of the refractive index compared to black cumin essential oil.

These results are in accordance with those mentioned by Ngan *et al.* (2019) who mentioned that the specific gravity, optical rotation and refractive index of rosemary essential oil were found to be 0.8978, +6.25° and 1.4680 at 20°C, respectively. On the other hand, Abd-Allah (2018) revealed that the specific gravity and refractive index of black cumin essential oil were found to be 0.9230 and 1.4675 at 25°C, respectively.

Chemical components of rosemary leaves and black cumin seeds essential oils

The chemical composition was identified by using GC and GC/MS, which their fragmentation, as well as, their retention times with those of authentic samples were held in the computer library and through the value recommended in new low of literature in this field concentration of essential oil components; they can be divided in three categories i.e. trace (<1.0%), minor (<1.0% -> 1.0 %) and major (>10 %).

As shown in Table (3) and Figure (3), chemical constituents of rosemary and black cumin essential oils were affected by the type of oil.

Regarding the chemical components of rosemary essential oil, twenty essential oil components of rosemary leaves were identified. The main components of rosemary essential oil were α-pinene (17.13%) and 1.8 cimeole (13.69%), while the minor components of rosemary essential oil were α-thugone (9.23%), β-pinene (8.67%), β-phellandrene (7.89%), Camphene (4.91%), p-cymene (4.89%), β-caryophllene (4.68%), Bronyl Methylether (4.63%), Verbenone (4.51%), Terpinylacetate (3.61%), Limonene (3.09%), Terpinolene (3.02), γ-terpinene (2.04%), Traieyclene (1.92%), Limalool (1.89%), Cis-ocimene (1.47%) and Eucarvone (1.12%).

These results were in agreement with those of Kassahun and Feleke (2019) who identified 17 components that were in *Rosmarinus officinalis* L. oil. The main components were α-pinene followed by 1,8-cineole, camphene, camphor, β-caryophyllene and β-pinene. Also, Ngan *et al.* (2019) identified 23 components that were in *Rosmarinus officinalis* L. oil. The major components were α-pinene followed by eucalyptol, camphene, bicycle, hept-3-en-2-one, caryophyllene, endo-borneol and bornyl acetate.

Concerning the chemical components of black cumin essential oil, eighteen essential oil components of black cumin seeds were identified. The main components of black cumin essential oil were Thymoquinone (38.43%) and P-cymene (21.18%), while the minor components of black cumin essential oil were D-Limonene (7.68%), Linalool (4.71%), Phellandrene (4.42%), Eugenol (3.87%), α-Terpinene (3.23%), Myrcene (2.21%), Borneol (2.19%), 1.8 cineole (1.59%), Nerol (1.54%), β-pinene (1.37%), Carvacrol (1.32%) and Thymol (1.09%).

Such results are in accordance with those of Singh *et al.* (2014) who revealed that the major components in black cumin essential oils were Thymoquinone followed by P-cymene, α-thujene, Thymohydroquinone, and Longifolene.

CONCLUSION

In conclusion, it is clear from the results that rosemary and black cumin plants succeeded under Egyptian conditions, which are characterized by the high quality of essential oil components. So, we recommend expansion in the cultivation of these cultivars under Egyptian conditions.

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Table 1 : Chemical composition of rosemary leaves and black cumin seeds

Character	Moisture (%)	Ash (%)	Lipid (%)	Crude protein (%)	Total carbohydrates (%)	Crude fiber (%)	Total phenols (mg GAE /g DE)	Essential oil (%)
Rosemary	4.67	5.71	11.9	6.53	9.27	15.23	94.7	1.04
Black Cumin	6.78	3.59	32.7	22.79	23.81	7.39	39.1	0.39
T. test	1.41	1.27	13.2	11.27	9.74	5.23	37.4	0.47

Table 2: Physical properties of rosemary and black cumin essential oils

Characteristics	Specific gravity	Optical rotation	Refractive index
Rosemary	0.8967	+6.94°	1.4751
Black Cumin	0.9139	-4.27°	1.4590
T. test	0.0117		0.0107

Table 3: Chemical constituents of rosemary leaves and black cumin seeds essential oils

Peak No.	Rosemary		Black Cumin	
	Component	%	Component	%
1	Camphene	4.91	α-pinene	0.87
2	β-phellandrene	7.89	β-pinene	1.37
3	β-pinene	8.67	Phellandrene	4.42
4	Limonene	3.09	μunknown	0.26
5	α-pinene	17.31	Myrecene Myrcene	2.21
6	1.8 cineole	13.69	*P*-cymene	21.18
7	Verbenone	4.51	α-Terpinene	3.23
8	Terpinolene	3.02	1.8 cineole	1.59
9	*p*-cymene	4.89	D-Limonene	7.68
10	α-thujone	9.23	Unknown	1.47
11	Eucarvone	1.12	Linalool	4.71
12	Valencene	0.64	Thymoquinone	38.43
13	Limalool	1.89	Unknown	2.57
14	γ-terpinene	2.04	Borneol	2.19
15	Bronyl Methylether	4.63	Nerol	1.54
16	Tricyclene	1.92	Eugenol	3.87
17	β-sclinene	0.79	Carvacrol	1.32
18	β-caryophyllene	4.68	Thymol	1.09
19	Terpinyl Acetate	3.61		
20	Cis-ocimene	1.47		

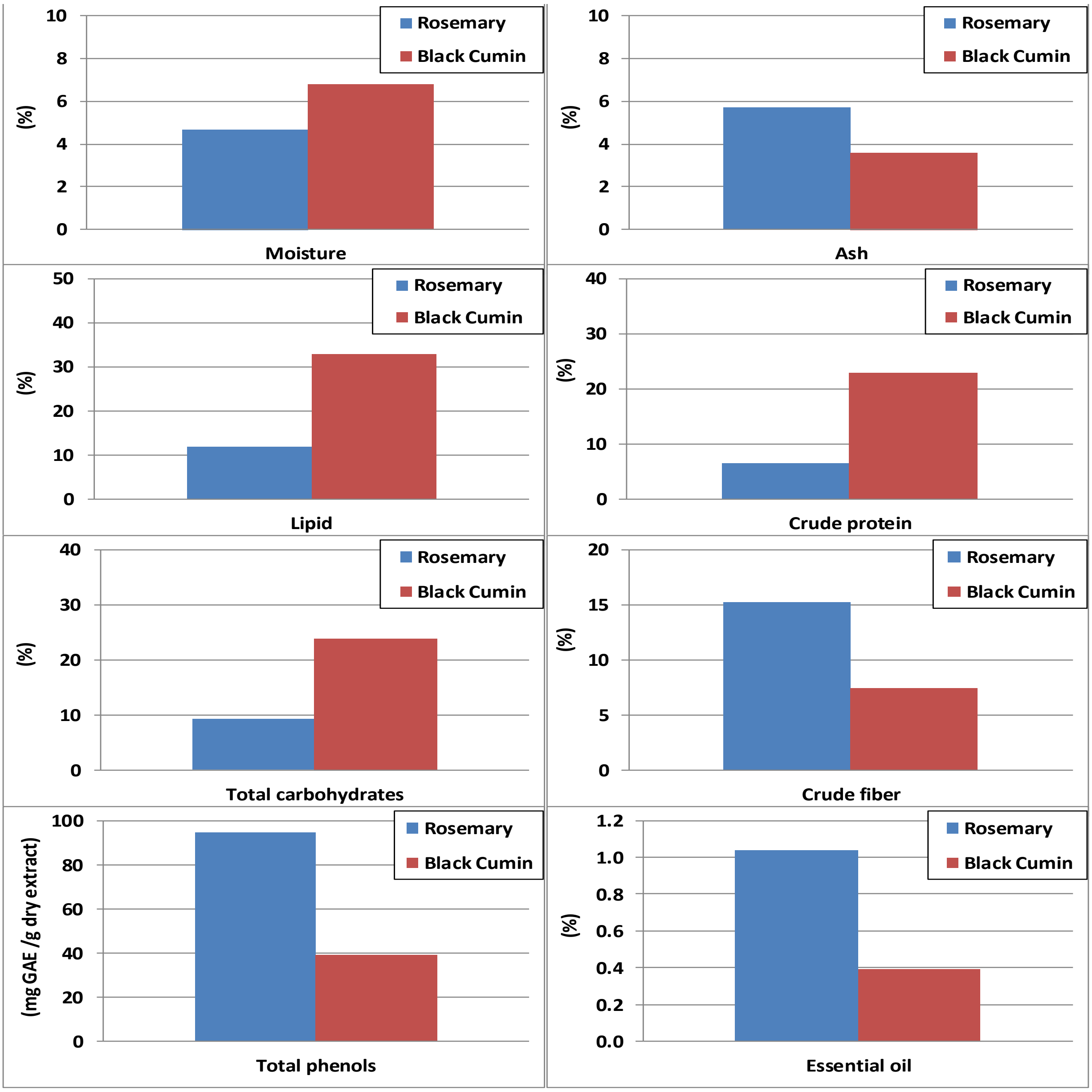

Figure (1) : Chemical composition of rosemary leaves and black cumin seeds

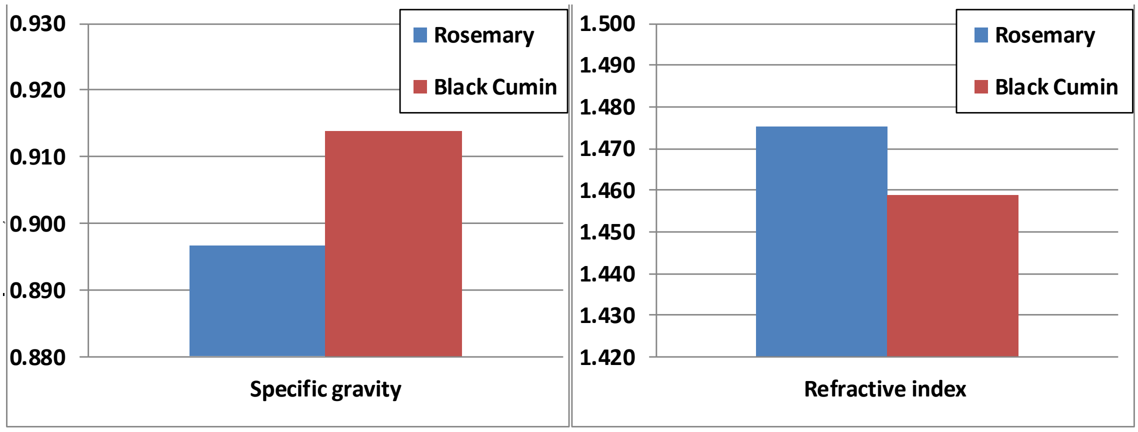

Figure (2) : Physical properties of rosemary and black cumin essential oils

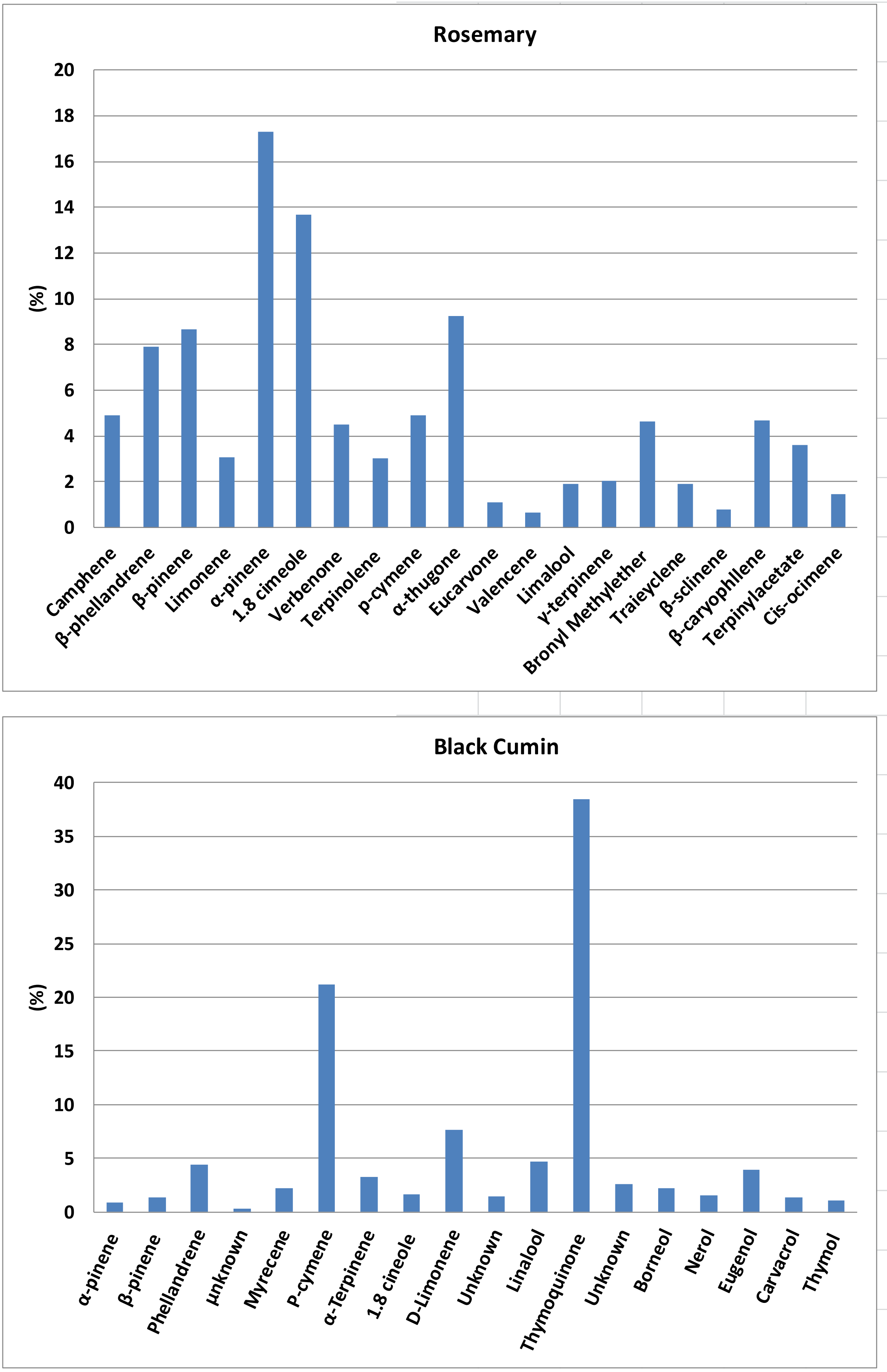

Figure (3): Chemical constituents of rosemary leaves and black cumin seeds essential oils

المكونات الكيميائية لأوراق الحصالبان وبذور حبة البركة

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الملخص العربى

تهدف هذه الدراسة إلى تقييم المكونات الكيميائية لأوراق الحصالبان وبذور حبة البركة باستخدام طرق التحليل الكيميائي المختلفة والتى تشمل نسبة الرطوبة والرماد والدهون والبروتين الخام والكربوهيدرات الكلية والألياف الخام والفينولات الكلية والزيوت الطيارة وكذلك تحديد المكونات الكيميائية للزيوت الطيارة بواسطة جهاز الفصل الكروماتوجرافي الغازي مع جهاز قياس طيف الكتلة. أشارت النتائج إلى أن أوراق الحصالبان سجلت أعلى قيمة من الرماد والألياف الخام والفينولات الكلية والزيوت الطيارة ، بينما سجلت بذور الكمون الأسود أعلى قيمة من الرطوبة والدهون والبروتين الخام والكربوهيدرات الكلية. سجلت أوراق الحصالبان معنويا أعلى نسبة مئوية للزيوت الطيارة وهى1,04% مقارنة ببذور حبة البركة حيث حققت 0,39%. من جهة أخرى، تم تحديد عشرين مركب من مكونات الزيت الطيار لأوراق الحصالبان، وقد وجد أن المكونات الرئيسية لهذا الزيت هى ألفا بينين (17.13%) و 1.8 سينيول (13.69%). من ناحية أخرى، تم تحديد ثمانية عشر مكونًا من مكونات الزيت الطيار لبذور حبة البركة. وقد وجد أن المكونات الرئيسية لهذا الزيت هى ثيموكوينون (38.43%) و بيتا-سيمين (21.18%).

الكلمات الاسترشادية: الزيوت الطيارة، الحصالبان، حبة البركة، الرطوبة، الرماد، الدهون، البروتين الخام، الكربوهيدرات الكلية، الألياف الخام، الفينولات الكلية.