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Evaluation of volatile phytochemical constituents in cumin (*Cuminum cyminum*) genotypes by gas chromatography-mass spectroscopy

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Abstract
In cumin (*Cuminum cyminum* L.) the aroma is due to the presence of aromatic volatile compounds. The flavor of cumin is judged by its volatile oil content. Cumin seeds of four varieties RZ-19, RZ-223, RZ-341 and RZ-345 collected from western Rajasthan were used for screening of essential oil chemical constituents. Essential oil was isolated by clevenger apparatus. The essential oil content in RZ-19, RZ-223, RZ-341 and RZ-345 was found 1.66%, 2.33%, 3.66% and 3% respectively. Cuminaldehyde was identified as major constituents in cumin essential oil. The GC-MS scan result reveals that RZ-223 variety contains highest Cuminaldehyde (40.88%) followed by RZ-345 (40.69%), Rz-19 (32.59%) and RZ-341 (31.65%). Other phyto-chemical constituents were terpenic compounds, alcohol and aldehydes.

Keywords: Spices, cumin, phyto-chemicals, GC-MS

Introduction
Cumin (*Cuminum cyminum* L), native to the Eastern Mediterranean region and Southwest Asia, is a diploid plant of Apiaceae family. It is the second most popular spice in the world after black pepper [1]. It is a tropical plant and cultivated in winter season in areas where atmospheric humidity remains low during the month of February and March. Cumin is one of the most important seed spice crop produced, consumed and exported from India and occupies significant place in Indian agriculture. There is great demand of value added products of cumin namely seed, powder, essential oil and oleoresin etc. in the domestic as well as in international market [2]. The aroma of cumin is due to the presence of aromatic volatile compounds. The flavor in cumin is judged by its volatile oil content [3]. Now a day's demand for volatile oil in international market is increasing. The advantage in use of volatile oil is that it is 100 times more concentrated than the spice powder and hence is required in very less quantity. The essential oil is responsible for the characteristic cumin odor. This odor and flavor is due to the presence of aldehydes [4]. Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defence system of plant [5]. These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals [6].
In vitro studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial agents [7-8].
The present study was conducted at Sanitary and Phytosanitary Laboratory of Agricultural Research Station, Mandor, (Agriculture University Jodhpur) for evaluation and estimation of phyto-chemicals present in essential oil extracted from four cumin varieties RZ-19, RZ-223, RZ-341 and RZ-345 grown in western part of the Rajasthan.

Materials and Methods
Chemicals and Reagent
The chemicals used in this study were procured from Loba Chemi (India) and Sigma-Aldrich (USA).

Estimation of Essential oil
Seeds of RZ-19, RZ-223, RZ-341 and RZ345 were collected from Agricultural Research Station Mandor Jodhpur. The essential oil was extracted using hydro-distillation (HD) in Clevenger-type apparatus [9]. Hundred gram powdered sample was used to essential oil extraction. The essential oil content was calculated as a relative percentage (v/w). The results were calculated as means of triplicate.

GC-MS scan analysis of essential oil

GC-MS analyses were carried out on a Shimadzu GCMS-QP2010 Ultra system. The injector temperature was 280 °C. The samples were injected in the split mode with split ratio 1:25. Injection volume was 1 µl. A capillary column Rtx-5MS (5% Diphenyl-95% Dimethyl Polysiloxane), 30 m x 0.25 mm x 0.25 µm, was used. Career gas was helium with constant flow of 1.00 ml min^{-1}. The oven temperature was as follows: initial temperature of 60 °C, held for 2 min, increased to 10 °C min^{-1} up to 260 °C and held for 10 min. The MS ionization potential was 70 eV, and the temperatures were as follows: interface 260 °C, Ion source 280 °C. Mass scan range 40-550 [10-12].

Identification of compounds

The chromatograms were analyzed for constituent compounds on the basis of their RI (Retention Index), RT (Retention time) and by comparison of mass spectra with those mentioned in NIST-MS (National Institute of Standards and Technology), mass spectral library of the GC-MS data system and co-injection with reference standard.

Result and Discussion

Essential oil yield

Oil estimation studies indicated that the essential oil (EO) yield in varieties RZ-19, RZ-223, RZ-341 and RZ345 was 1.66%, 2.33%, 3.66% and 3% respectively. (Table-1)

Researchers reported a large variation in essential oil yield of cumin. Beis *et al.* [10] from Turkey reported 1.4 to 2.8%. Rebey *et al.* [13] obtained 1.64% EO yield in moderate water deficit area. From China Li and Jiang [14] reported 3.8% EO while from Bulgaria Jirovetz *et al.* [15] estimated upto 5.3% essential oil. An environmental condition, processing technology and genetic constituents influences the yield of essential oil [16-17].

GC-MS scan analysis of essential oil

GC-MS chromatograms profile of the cumin essential oil showed the number of phytochemical constituents. Fifteen compounds were identified and classified respectively in different chemical classes (terpens, sesquiterpens, alcohols and aldehydes etc (Table-2). Aldehydes and terpenic compounds were major constituents in cumin essential oil.

Chemical compounds identification was confirmed on the basis of retention time, peak area and molecular formula from National Institute for Standards and Technology (NIST) mass spectral library, ver. NIST 11. Figure-1 to 4 represents GC-MS chromatogram of cumin varieties. Identified compounds chemical structure was presented in Figure-5.

Cumin genotype RZ-19 essential oil contains several terpenoid compounds i.e. γ.-Terpinene (9.74%), α-pinene (0.12%), β -pinene (2.10%), β-myrcene (0.30%), p-Menthatriene (9.77%) terpinolene (7.95%) and cis-Ocimene (10.26%). Aldehydes found in this genotype were cuminaldehyde (32.59%), Benzaldehyde p-isopropyl (17.27%) myrtenal (8.74%). Unsaturated hydrocarbon was not found in this genotype. RZ-223 essential oil contains terpenoid compounds α-pinene (0.52%), p-Menthatriene (15.81%), terpinolene (14.35 %), 4-terpineol (0.58%) while alcoholic compounds were Verbenol (1.82%) and aldehydes found in this genotype were cuminaldehyde (40.88%), myrtenal (17.86%). Unsaturated hydrocarbon santolina triene (7.54%) was also found in this genotype.

RZ-341 essential oil terpenoid compounds were α-Thujene (0.29%) α-pinene (0.92%), β-Myrcene (0.71%), p-Menthatriene (24.63%), terpinolene (16.51%), 4-terpineol (0.30%) and cis-Ocimene (9.14%). While aldehydes found in this genotype were cuminaldehyde (31.65%), myrtenal (1.78%). Unsaturated hydrocarbon santolina triene (12.52%) found in remarkable amount in this genotype. In RZ-345 genotype essential oil terpenic compounds were α-pinene (0.42%), β-myrcene (0.44%), p-menthatriene (6.51%), terpinolene (13.50%) and cis-Ocimene (4.50%). In this genotype alcoholic compound 2-allylphenol (30.02%) was found in remarkable amount. Aldehydes were myrtenal (1.48%) and cuminaldehyde (40.69). Unsaturated hydrocarbon santolina triene (7.47) was also found. Comparative analyses of phytochemical constituents in cumin essential oil from different genotypes were presented in Figure-6.

Cuminaldehyde was a major constituent in all varieties at varying percent RZ-223 (40.88%) contain highest cuminaldehyde followed by RZ-345 (40.69%), RZ-19 (32.59%) and RZ-341 (31.65%). Cuminaldehyde imparts a pleasant smell and aroma to cumin oil.

Besides cumin, cuminaldehyde is also reported in essential oils of *Eucalyptus robusta*, *Commiphor myrrha*, *Cinnamomum, cassia* and other spices. It contains pleasant fragrance responsible for the aroma of these oils [18-20]. It is used commercially in perfumes and other cosmetics. Cuminaldehyde content is associated with characteristic spicy fragrance of cumin seeds. Cumin seeds with more cuminaldehyde content have preference in domestic and international market over the seeds containing higher amount of terpinene compounds [21]. Chemical composition of cumin oil has also been reported by some researchers. EL-Manylawi *et al.* [22] reported higher cuminaldehyde (43.32%) while Baser *et al.* [23] reported 19.25-27.02% cuminaldehyde in essential oil of Turkish cumin. Patil *et al.* [24] reported 36.67% cuminaldehyde in cumin essential oil. An experimental result of Ramasamy *et al.* [25] showed the presence of low cuminaldehyde content (8–17%) in eight cumin samples. Aćimović *et al.* [26] analyzed Serbian cumin seed and reported 19-36% cuminaldehyde. Current study reveals that western Rajasthan growing prominent cumin genotypes were having higher cuminaldehyde content over previous reported studies.

Table 1: Volatile oil content of cumin genotype

Variety	Volatile Oil %
RZ-19	1.66 ± 0.04
RZ-223	2.33 ± 0.12
RZ-341	3.66 ± 0.41
RZ-345	3.0 ± 0.28

Values represents mean of triplicates ± S.D.

Table 2: Essential Oil Composition of Cumin Genotypes identified by GC-MS

SN	Compound Name	R. Time	Mol. Weight	Mol. Formula	CAS No.	Area %			
						RZ-19	RZ-223	RZ-341	RZ-345
1.	γ.-Terpinene	4.363	136	$C_{10}H_{16}$	99-85-4	9.74	-	-	-
2.	α-Thujene	4.716	136	$C_{10}H_{16}$	2867-05-2	-	-	0.29	-
3.	α-Pinene	4.842	136	$C_{10}H_{16}$	80-56-8	0.12	0.52	0.92	0.42
4.	β -Pinene	5.574	136	$C_{10}H_{16}$	127-91-3	2.10	-	-	-
5.	Santolina triene	5.668	136	$C_{10}H_{16}$	2153-66-4	-	7.54	12.52	7.47
6.	β-Myrcene	5.747	136	$C_{10}H_{16}$	123-35-3	0.30	-	0.71	0.44
7.	p-Menthatriene	6.344	134	$C_{10}H_{14}$	18368-95-1	9.77	15.81	24.63	-
8.	2-Allylphenol	6.433	134	$C_9H_{10}O$	1745-81-9	-	-	-	30.02
9.	Terpinolene	6.968	136	$C_{10}H_{16}$	55956-33-7	7.95	14.35	16.51	13.50
10.	4-Terpineol	8.750	154	$C_{10}H_{18}O$	562-74-3	-	0.58	0.30	-
11.	Verbenol	9.000	152	$C_{10}H_{16}O$	1845-30-3	-	1.82	-	-
12.	Benzaldehyde p-isopropyl	9.431	148	$C_{10}H_{12}O$	122-03-2	17.27	-	-	-
13.	Cuminaldehyde	9.960	148	$C_{10}H_{12}O$	122-03-2	32.59	40.88	31.65	40.69
14.	Myrtenal	10.577	150	$C_{10}H_{14}O$	564-94-3	8.74	17.86	1.78	1.48
15.	cis-Ocimene	10.248	150	$C_{10}H_{14}O$	0-00-0	10.26	-	9.14	4.50

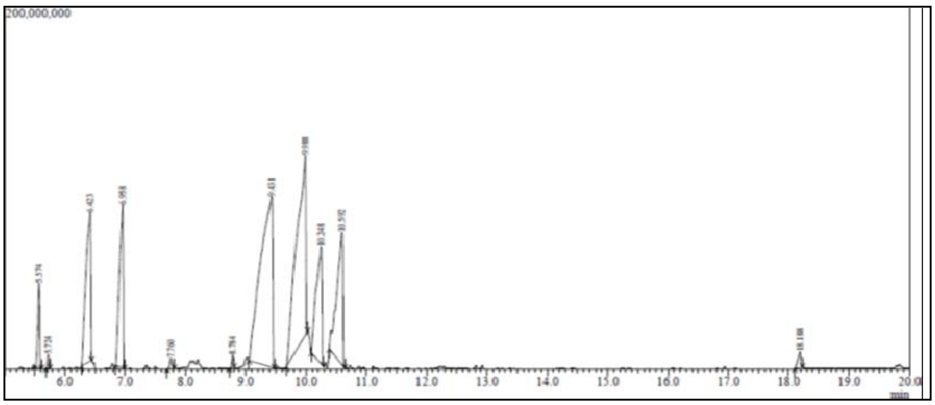


Fig 1: GC-MS Chromatogram of Cumin Genotype RZ-19

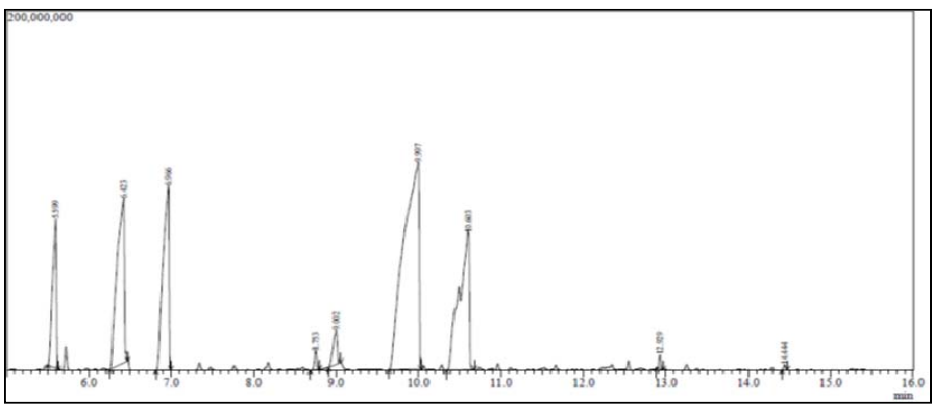


Fig 2: GC-MS Chromatogram of Cumin Genotype RZ-223

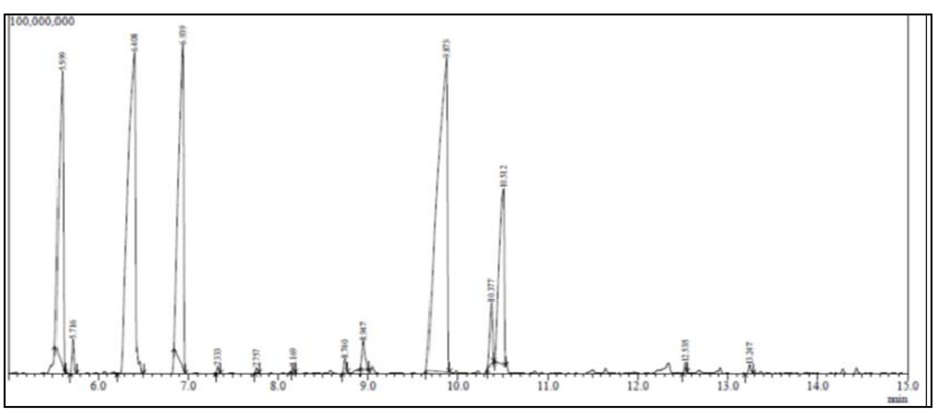


Fig 3: GC-MS Chromatogram of Cumin Genotype RZ-341

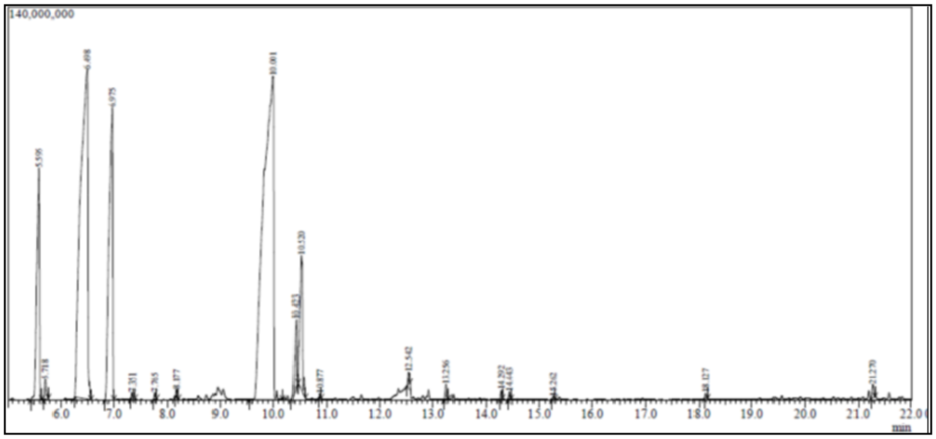
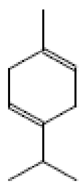
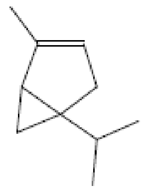


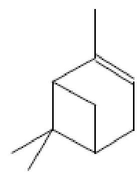
Fig 4: GC-MS Chromatogram of Cumin Genotype RZ-345



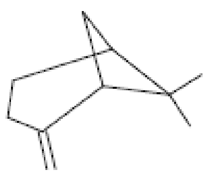
γ.-Terpinene



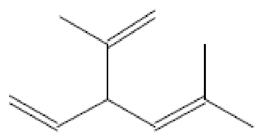
α-Thujene



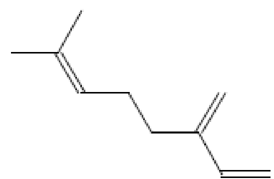
α-Pinene



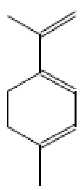
β -Pinene



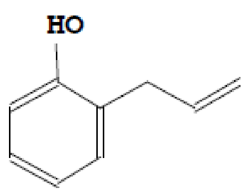
Santolina triene



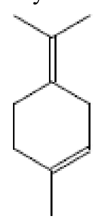
β-Myrcene



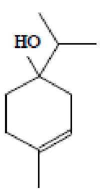
p-Menthatriene



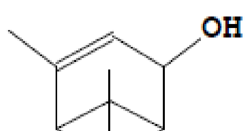
2-Allylphenol



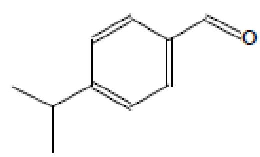
Terpinolene



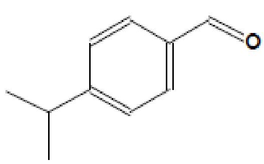
4-Terpineol



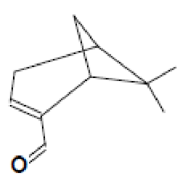
Verbenol



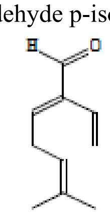
Benzaldehyde p-isopropyl-



Cuminaldehyde



Myrtenal



cis-Ocimene

Fig 5: Chemical Structure of Essential Oil constituents in Cumin Genotype

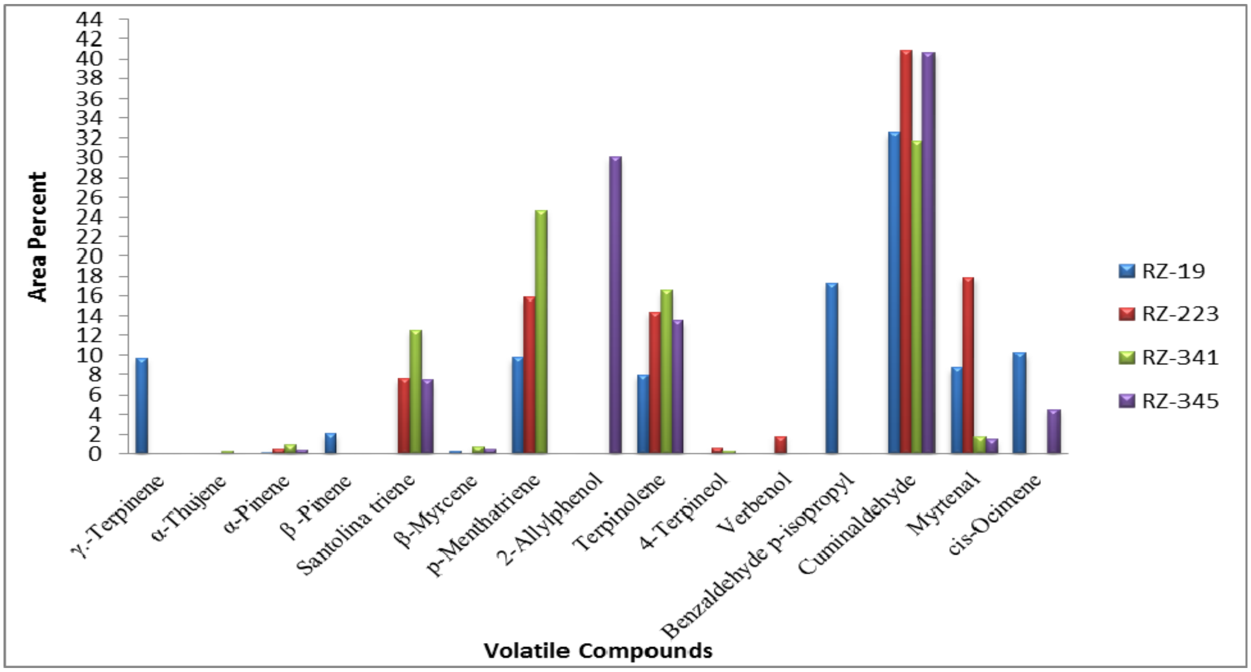


Fig 6: Essential oil phytochemical constituents in different genotypes of cumin

Conclusion

Cumin is prominent seed spice crops produced, consumed and exported from India and occupies significant place in Indian agriculture. Aromatic volatile compounds are responsible for pleasant aroma of cumin. The flavour of cumin is judged by its volatile oil content. Now a day's demand for volatile oil in international market is go on increasing. The advantage of use of volatile oil is that it is 100 times more concentrated then the spice powder and hence is required in a very less quantity.
The essential oil is responsible for the characteristic cumin odor. In present study evaluation of fragrance and flavour profile in essential oil of four cumin genotype from western Rajasthan of India has been identified. The result reveals that both genotypes were contain higher terpenoids and aldehydes content as compare to other cumin growing area of world.

Conflict of Interest

There is no Conflict of Interest

References

1. Hasan AM, Sakure AA, Fougat RS, Kumar S. Estimation of nuclear DNA content of cumin. International Journal of Seed Spices. 2016; 6(1):96-99.
2. Kumar S, Saxena SN, Mistry JG, Fougat RS, Solanki RK, Sharma R. Understanding *Cuminum cyminum*: An important seed spice crop of arid and semi arid regions. International Journal of Seed Spices. 2015; 5(2):1-19.
3. Hirasa K, Takemasa M. Cooking with spices. In: Spice Science and Technology. Marcel Dekker, New York, 1998, 70.
4. Nadeem M, Riaz A. Cumin (*Cuminum cyminum*) as a potential source of antioxidants. Pakistan Journal of Food Sciences. 2012; 22(2):101-107.
5. Lako J, Trenerry V, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R. Phytochemical Flavonols, Carotenoids and the Antioxidant Properties of a Wide Selection of Fijian Fruit, Vegetables and Other Readily Available Foods. Journal of Food Chemistry. 2007; 102:777-783.
6. Rathore SS, Saxena SN, Singh B. Potential health benefits of major seed spices. International Journal of Seed Spices. 2013; 3:1-12.
7. AL Juhaimi FY, Ghafoor K. Extraction optimization and *in vitro* antioxidant properties of phenolic compounds from Cumin (*Cuminum cyminum* l.) seed. International Food Research Journal. 2013; 20(4):1669-1675.
8. Senevirathne M, Kim S, Siriwardhana N, Ha J, Lee K, Jeon Y. Antioxidant potential of Ecklonia cava on reactive oxygen species scavenging metal chelating, reducing power and lipid peroxidation inhibition. Food Science and Technology International. 2006; 12:27-38.
9. Clevenger JF. Apparatus for the determination of volatile oil. Journal of the American Pharmacists Association. 1928; 17(4):345-349.
10. Beis SH, Ayas N, Ozek T, Kara M, Baser KHC. The production of essential oil from cumin seed. Chemistry of Natural Compounds. 2000; 36(3):265-268.
11. Benrejdal A, Dridi F, Nabiev M. Extraction and Analysis of Essential Oil of Cumin. Asian Journal Chemistry. 2012; 24:1949-1951.
12. Chaudhry AH, Tanveer A, Shar A, Akhtar S. Physico-Chemical Investigation and Antimicrobial Activity of Essential Oil of *Cuminum cyminum* L. World Applied Sciences Journal. 2012; 19:330-333.
13. Rebey IB, Karoui IJ, Sellami IH, Bourgou S, Limam F, Marzouk B. Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Industrial Crops & Products. 2012; 36:238-245.
14. Li R, Jiang ZT. Chemical composition of the essential oil of (*Cuminum cyminum* L.) from China. Flavour and Fragrance Journal. 2004; 19:311-313.
15. Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV, Damianova ST. Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years. International Journal of Food Science & Technology. 2005; 40:305-310.
16. Msaada K, Hosni K, Taarit MB, Hammami M, Marzouk B. Effects of growing region and maturity stages on oil

yield and fatty acid composition of coriander (*Coriandrum sativum* L.) fruit. Scientia Horticulturae. 2009; 120:525-531.

17. Saxena SN, Sharma YK, Rathore SS, Singh KK, Barnwal P, Saxena R *et al*. Effect of cryogenic grinding on volatile oil, oleoresin content and anti-oxidant properties of coriander (*Coriandrum sativum* L.) genotypes. Journal of Food Science and Technology. 2015; 52:568-573.
18. Hanuša LO, Řezankab T, Dembitskya VM, Moussaieffa A. Myrrh-Commiphora Chemistry. Biomed Papers, 2005; 149(1):3-28.
19. Rao BRR, Rajput DK, Bhattacharya AK. Essential oil composition of petiole of Cinnamomum verum Bercht. & Presl. Journal of Spices and Aromatic Crops. 2007; 16(1):38-41.
20. Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima LEM, Moreno PRH. Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. Phytotheraphy Research. 2007; 21(3):231-233.
21. Dubey PN, Saxena SN, Mishra BK, Solanki RK, Vishal MK, Singh B *et al*. Preponderance of cumin (*Cuminum cyminum* L.) essential oil constituents across cumin growing Agro-Ecological Sub Regions, India. Industrial Crops & Products. 2017; 95:50-59.
22. EL-Manylawi MA, Ali HFM. Gas Chromatography-Mass Spectroscopy Analysis and Evaluate Cumin Seeds and Their Essential Oil as Growth Promoters of New Zealand White Rabbits. International Journal of Agricultural Research. 2009; 4:107-115.
23. Baser KHC, Kurkcuoglu MT, Ozen J. Composition of Turkish cumin seed oil. Journal of Essential Oil Research. 1992; 4:133-138.
24. Patil SD, Maknikar PP, Wankhade SKJ, Ukesh CS, Rai MK. Chemical composition, antimicrobial and antioxidant activity of essential oils from cumin and ajowan. Nusantara Bioscience. 2016. doi: 10.13057/nusbiosci/n080111
25. Ramasamy R, Prakash M, Bhat KK. Characterization of Aroma Active Compounds of Cumin (*Cuminum cyminum* L.) by GC-MS, E-Nose, and Sensory Techniques. International Journal of Food Properties. 2013; 16:1048-1048,
26. Aćimović MG, Tešević V, Mara D, Cvetković M, Stanković J, Filipović V. The Analysis of Cumin Seeds Essential Oil and Total Polyphenols from Postdestillation Waste Material. Advanced Technologies. 2016; 5(1):23-30.