Research Article

Effect of drying on the essential oil traits and antioxidant potential *J. regia* L. leaves from Kumaun Himalaya

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Abstract
Drying is an ancient technique adopted to reduce moisture, preserve aroma profile and increase the shelf life of the food products. During drying, the chemical, nutritional and antioxidant properties of the food can get altered as compared to the fresh material. Natural shade drying is the most accepted storage method for aromatic medicinal herbs because of its low cost and minimum loss of volatile constituents. In the present investigation, the effects of shade drying on the volatile components and antioxidant potential of walnut leaves (*Juglans regia*) have been studied. For this purpose, fresh walnut leaves were shade dried and hydrodistilled using Clevenger apparatus. The extracted oil was analysed by GC and GC/MS. The antioxidant potential of the oils was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging activity. The GC and GC/MS analysis identified 46 and 42 compounds representing 89.29% and 96.38% of the total fresh and dried oils, respectively. The composition of the five major components namely (*E*)-caryophyllene, germacrene D, α-zingiberene, δ-cadinene and (*E*)-β-farnesene was observed to significantly increase after shade drying. Drying caused appearance of four components with complete loss of eight components. Fresh oil showed better antioxidant activity as compared to the shade dried oil.

Keywords *Juglans regia* · Essential oil · Drying · (*E*)-caryophyllene · Germacrene D · Monoterpene · Antioxidant activity

1 Introduction

Juglans regia L., an aromatic indigenous plant of south-eastern Europe, Asia, northern America, North Africa, India and China, belongs to the family Juglandaceae. It is commonly known as walnut "akhrot" (in Hindi) [1]. The essential oil of *J. regia* is an ingredient of dry skin creams, anti-ageing and anti-wrinkle products as it shows moisturizing properties along with free radical scavenging activity [2]. All the plant parts like green husks, leaves, green walnuts, bark and shells have been used in the pharmaceutical and cosmetic industries [3]. Highly nutritious seeds of the walnut tree are consumed as royal food across the globe. These are rich in protein, carbohydrates, unsaturated fatty acids, vitamins, minerals (copper, iron, magnesium, phosphorus, potassium and sulphur) flavonoids, sterols, phenolic acids and fibres [4–6]. The stem bark of the plant is reported to show anthelmintic, astringent, bactericide, detergent, depurative, digestive, diuretic, insecticidal and laxative properties [7]. The leaves have been used as folk medicine due to their anthelmintic, astringent, antidiarrheic, fungicidal and insecticidal properties [4–6]. The oil is a rich source of (*E*)-caryophyllene, caryophyllene oxide, β-pinene, germacrene D and carvacrol which are accountable for various biological activities.

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High demand of preserved and processed food products requires high-quality raw materials. Drying is a post-harvest technique which is used for preservation, microbial decontamination, increase shelf life and disinfections of herbs [8]. It includes natural (sun, shade), hot air, microwave and freeze drying. Drying affects the content of flavour constituents of aromatic and medicinal plants which are considered as antioxidant and antimicrobial agents [9]. Drying may upgrade aroma quality of the plants either by esterification/oxidation/formation of new compounds or loss of volatile components [10]. A number of reports are available on the effect of drying on the essential oil composition of various aromatic species like *Thymus vulgaris* [11], *Murraya koenigii* [12], *Ocimum americanum* [13, 14], *Ocimum gratissimum* [15] and *Origanum vulgare* [16].

Reports are available on the volatile composition of *J. regia* leaves from Egypt [17], India [18–20], Tunisia [21], Iran [22] and the nuts from Pakistan [23]. There are few reports on the antioxidant potential evaluation of leaf oil of *J. regia* [18, 20, 23].

Existing literature data revealed that no work has been reported on the effect of drying on the essential oil composition and antioxidant activity of *J. regia* leave oil. Therefore, the present work aims to explore the impact of natural shade drying on the aroma profile and antioxidant potential of *J. regia* L. oil from the Himalayan region of, Uttarakhand.

2 Materials and methods

2.1 Collection of plant

Fresh leaves of *Juglans regia* were collected from Padli, Ratighat (Bhowali), Nainital (Latitude: 29°23°410′ N; Longitude: 79°30′546″ E; Altitude: 1700 m) in the month of August 2017.

2.2 Extraction of oil

Two-kilogram fresh leaves of *J. regia* were subjected to hydrodistillation using Clevenger apparatus for 5 h. Five kilograms of fresh leaves were shade dried up to the constant weight and 500 g of dried material was hydrodistilled in Clevenger for 5 h. The oils were dried over anhydrous sodium sulphate and kept in Biological Oxygen Demand (BOD) incubator at 4 °C prior to analysis.

2.3 Analysis of oil

The extracted oil was analysed on a Shimadzu 2010 GC, fitted with Rtx-5 column (30 m × 0.25 mm, i.d. 0.25 µm) and flame ionization detector (FID) using Nitrogen/air as the carrier gas. The column was programmed at 50 °C with a hold time of 2 min to 210 °C at a rate of 3 °C/min and increased to 280 °C at 8 °C/min rate and at 280 °C; the sample was hold for 16 min. Nitrogen was adjusted at 30 mL/min column head pressure. The temperature of injector and FID was adjusted to 260 °C and 270 °C, respectively. The split ratio was 1:40, and injection volume was 0.2 µL neat oil. The GC/MS unit was GCMS-QP2010 Ultra consisted of Rtx-5 column (30 m × 0.25 mm, i.d. 0.25 µm). Helium was used as a carrier gas. The mass spectrum was recorded at 70 eV and 40–650 amu. Conditions for GC/MS analysis were similar to that of GC.

2.4 Identification of the components

The identification of the individual constituent was done by comparing the fragmentation pattern of the mass spectral data with literature data [24] and by comparing with NIST (NIST version 2.1) and Wiley (7th edition) mass spectral database. The retention index (RI) of each constituent was calculated by comparing with *n*-alkane series (C_9–C_{33}). The relative percentage of each compound in the oil was attained on the basis of FID response without using a correction factor.

2.5 Antioxidant activity measurement

The antioxidant potential of the oils was determined in terms of their radical scavenging activity by the bleaching of purple coloured methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) [25]. Ascorbic acid was used as standard. One mL solution of oil and standard comprising of different concentrations in methanol (50, 100, 200, 400, 800, 1000 µg/mL for fresh oil; 10, 20, 40, 80, 160 mg/mL for dry oil and 10, 20, 40, 80, 160 µg/mL for ascorbic acid) were mixed with 5 mL methanolic solution of DPPH (0.2 mM). A control was also run without oil under similar conditions. All the samples were incubated for 30 min in the dark at room temperature. The test was performed in triplicate, and the data were represented as mean ± standard deviation (SD) values. The DPPH radical scavenging effect was calculated as "percentage inhibition" according to the equation:

$$\%\text{DPPH scavenging activity} = \frac{(A_o - A_t)}{A_o} \times 100$$

where A_o is the absorbance of control sample at 517 nm and A_t is the absorbance value of oil at 517 nm.

2.6 Statistical analysis

All the analysis of the present study was carried out in triplicate, and the mean ± SD was calculated by MS Excel. Two-tailed paired *t* test was performed to compare mean

content of major essential oil constituents of fresh and dried *J. regia* leaves using MS Excel at probability level of $p < 0.05$ and $p < 0.01$. The data were subjected to one-way ANOVA to evaluate significant difference between different treatment groups (significance level of $p < 0.05$).

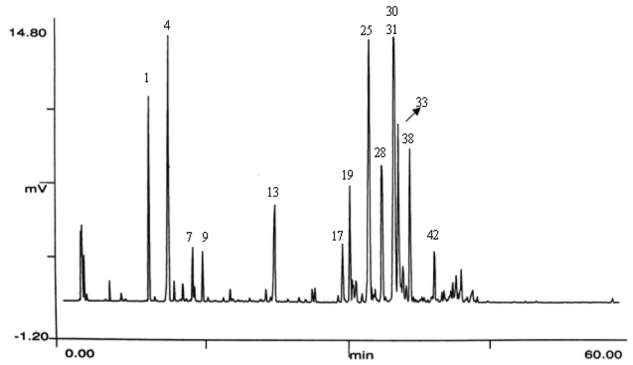

Fig. 1 Gas chromatogram of fresh *J. regia* oil

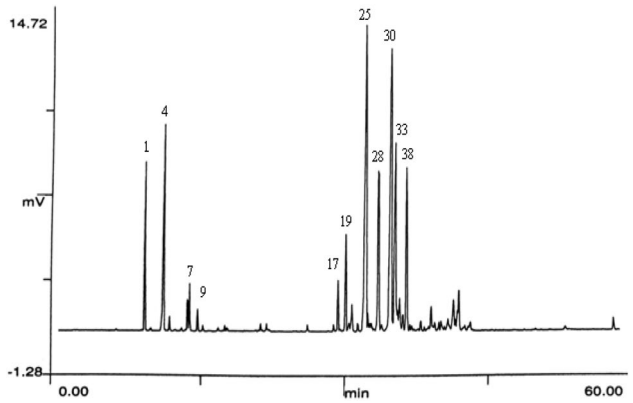

Fig. 2 Gas chromatogram of dried *J. regia* oil

3 Results and discussion

3.1 Essential oil composition

A total of 46 and 42 compounds were identified on the basis of GC (Figs. 1, 2) and GC/MS analysis which represented 89.29% and 96.38% of the total fresh and dried oils, respectively. The essential oil yield was 0.02% (v/w). The major components in the fresh leave oil of *J. regia* were (*E*)-caryophyllene (13.30%), β-pinene (11.63%), germacrene D (9.31%), β-selinene (7.77%), α-zingiberene (6.74%), α-pinene (6.22%) and δ-cadinene (5.38%) (Table 1; Fig. 3).

Previous study on *J. regia* fresh leaf oil from Indian Western Himalaya also reported the high percentage of (*E*)-caryophyllene, β-pinene, germacrene D and α-zingiberene [19] (Table 1; Fig. 4). Another report on the leaf essential oil of *J. regia* from USA suggested the presence of germacrene D and methyl salicylate as major constituents [17]. Furthermore, the leaf essential oil from Kashmiri akhrot was predominated by β-pinene, α-pinene, β-caryophyllene and germacrene D [18]. A previous study from Pakistan reported the high percentage of benzyl alcohol, nerolidol, globulol and p-cymene in the nuts oil of *J. regia*. Moravej et al. [22] examined the essential oil composition of walnut hydrosol from Iran and observed the composition to be entirely different from leaf oil. The main components of the hydrosol oil were oxygenated monoterpenes such as thymol and carvacrol (Table 1).

In the present study, the major volatile components in the shade dried oil were (*E*)-caryophyllene (18.88%), germacrene D (18.53%), α-zingiberene (9.88%), β-pinene (8.27%), (*E*)-β-farnesene (7.73%), δ-cadinene (6.81%) and α-pinene (5.04%). In a previous report from Tunisia [21], caryophyllene oxide, (*E*)-caryophyllene, germacrene D and β-pinene were obtained as major components in the shade dried *J. regia* leaf oil (Table 1; Fig. 4). Eight components including camphene, (3*E*)-hexenyl acetate, methyl salicylate, bornyl acetate, β-selinene, *trans*-muurola-4(14),

Table 1 Previous reports on *J. regia* essential oil

S. no.	Collection site	Plant part	Major constituents	References
1.	Kentucky, USA	Leaf	Germacrene D (28.6%) and methyl salicylate (16.8%)	[17]
2.	Kashmir	Leaf	β-Pinene (30.5%), α-pinene (15.1%), β-caryophyllene (15.5%) and germacrane D (14.4%)	[18]
3.	India (Western Himalaya)	leaf	(*E*)-Caryophyllene (47.9%), β-pinene (39.5%), germacrene D (23.3%), α-pinene (18.1%), α-humulene (11.8%), α-zingiberene (11.3%), α-copaene (10.1%), limonene (8.6%), caryophyllene oxide (8.6%), ar-curcumene (7.2%), δ-cadinene (6.7%), (*E*)-β-farnesene (5.9%) and 1,8-cineole (5.4%)	[19]
4.	Tunisia	Leaf	Caryophyllene oxide (27.4%) β–caryophyllene (22.5%), germacrene D (9.4%) and β-pinene (9.5%)	[21]
5.	Iran	Walnut hydrosol	Carvacrol (33.21%), thymol (16%) and homoveratrole (15.83%)	[22]
6.	Pakistan	Nuts oil	Benzyl alcohol (18.14%), nerolidol (13.54%), globulol (10.95%) and p-cymene (10.94%)	[23]

5-diene, α-bulnesene and elemol were present only in fresh oil. Out of these, β-selinene was present as major component. In addition, α-terpineol, spathulenol, globulol and salvia-4(14)-en-1-one were present only in the dried sample. The mean percentage of six components namely β-pinene (11.63–8.27% at $p < 0.05$), β-selinene (7.77–0.00%), α-pinene (6.22–5.04% at $p < 0.05$), methyl salicylate (4.02–0.00%), caryophyllene oxide (1.56–0.84% at $p < 0.01$) and 1,8-cineole (1.47–1.15% at $p < 0.01$) significantly decreased on drying. It was observed that drying led to the significant increase in the percentage of five major compounds including (*E*)-caryophyllene (13.30–18.88% at $p < 0.01$), germacrene D (9.31–18.53% at $p < 0.01$), α-zingiberene (6.74–9.88% at $p < 0.01$), δ-cadinene (5.38–6.81% at $p < 0.05$) and (*E*)-β-farnesene (4.57–7.73% at $p < 0.05$). Four common constituents present in less than 0.20% amount were neryl acetate (0.02–0.04%), linalool (0.06–0.14%), β-sesquiphellandrene (0.09–0.12%) and α-cubebene (0.16–0.19%) (Table 1). The present study identified β-selinene (7.77%), α-bisabolol (0.99%), geranyl acetate (0.57%), 2-undecanone (0.36%), β-gurjunene (0.29%), humulene epoxide II (0.23%), 1,10-di-*epi*-Cubenol (0.25%) and 1-*epi*-cubenol (0.17%) in significant amount for the first time in the dried oil of *J. regia*.

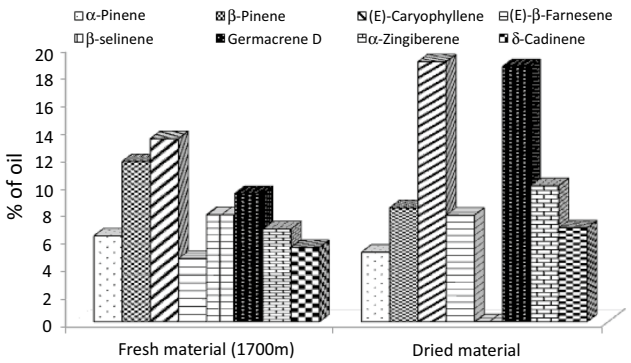

Fig. 3 Variation in the major constituents of fresh and dried *J. regia*

Sesquiterpene hydrocarbon was the predominating class of volatile compounds in the fresh and dried oil collected from Bhowali (Fig. 5). The result was in good agreement with those obtained by Verma et al. [19]. Kashmiri akhrot oil was rich in monoterpene hydrocarbons (α-pinene and β-pinene) and sesquiterpene hydrocarbons (β-caryophyllene and germacrene D) [18]. Furthermore, the oil from Tunisia was found to be rich in oxygenated sesquiterpene (caryophyllene oxide) and sesquiterpene hydrocarbon (β-caryophyllene) [21] (Fig. 6). The difference in the percentage of the compounds depends upon environmental (seasonal, geographical, climatic), genetic factors, distillation and post-harvest technique (drying, storage conditions) [12–15, 26]

3.2 Antioxidant activity

The DPPH radical scavenging activity of *J. regia* leaf oils and standard (ascorbic acid) is shown in Tables 2, 3 and Table 4. The results revealed that the samples exhibited concentration-dependent DPPH radical scavenging activity. The antioxidant activity of the fresh oil (IC_{50}: 923.49 µg/mL) was found to be ten times less than the standard (IC_{50} (92.78 µg/mL) (Tables 3, 5). The results were in good agreement with the previous report on *Ocimum gratissimum* in which the fresh oil showed better DPPH radical scavenging activity as compared to the dried oil [15]. The dried oil showed moderate antioxidant activity with IC_{50} value of 48.77 mg/mL (Table 4). Similar to the present study, fresh material of *Camellia sinensis* was observed to have higher antioxidant content as compared to the dried plant material. This could be attributed degradation caused by the process of drying [27]. On the contrary, study by Pinela et al. has shown that the dried plant material has higher antioxidant content than the fresh plant material [28].

The IC_{50} value of fresh *J. regia* oil from Kashmir was 34.5 µg/mL [18]. The compounds present in the Kashmiri

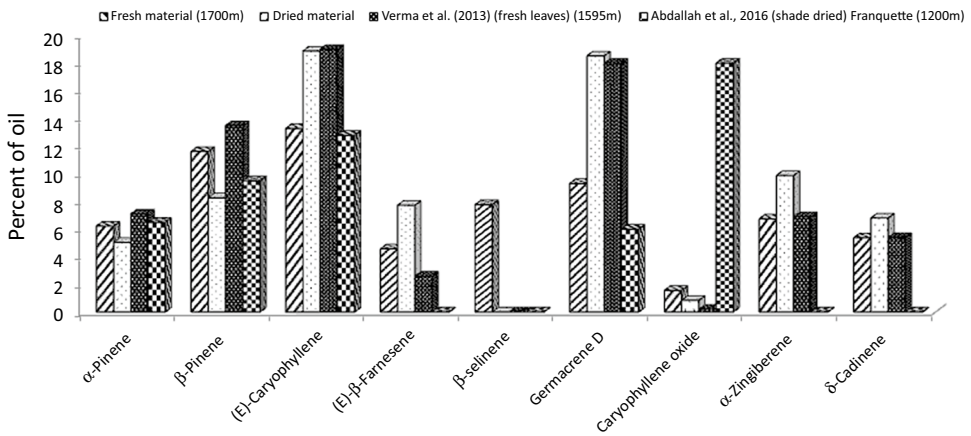

Fig. 4 Comparative essential oil composition of *J. regia* oil

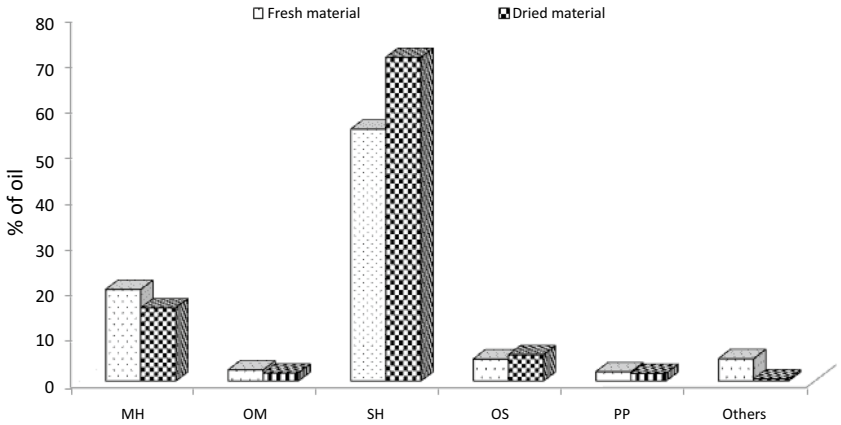

Fig. 5 Variation in the class of terpenoids in fresh and dried *J. regia*

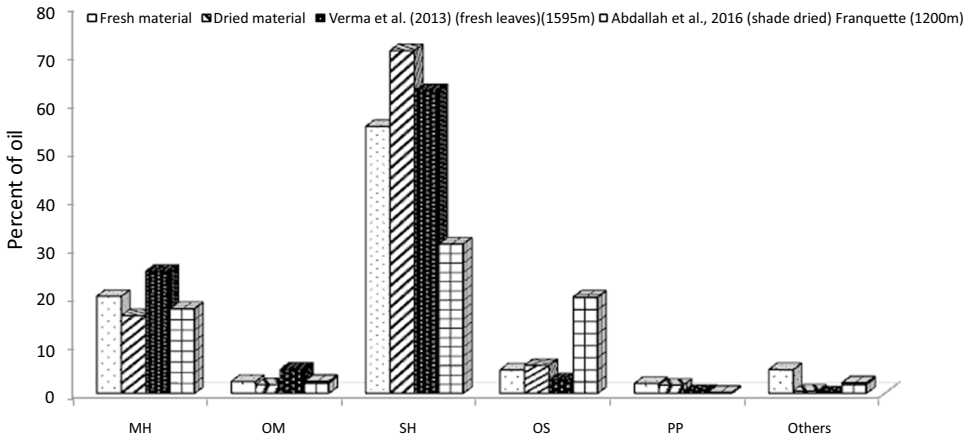

Fig. 6 Comparative class of compounds in *J. regia*

Table 2 Variation in the essential oil composition of fresh and dried *J. regia*

S. no.	Calculated retention index	Retention index [24]	Name of component	Retention time of components in fresh *Juglans regia* leaves (minutes)	Mean per cent ± SD (fresh *Juglans regia* leaves)	Retention time (GC) of components in dried *Juglans regia* leaves (minutes)	Mean per cent ± SD (dried *Juglans regia* leaves)
1.	929	932	α-Pinene	9.19	6.22 ± 0.26	9.17	5.04* ± 0.44
2.	945	946	Camphene	9.78	0.07	–	ND
3.	969	969	Sabinene	10.93	0.14	10.97	0.47
4.	974	974	β-Pinene	11.21	11.63 ± 0.92	11.17	8.27* ± 0.31
5.	987	988	Myrcene	11.77	0.48 ± 0.14	11.78	0.40
6.	1003	1001	(3*E*)-Hexenyl acetate	12.66	0.49	–	ND
7.	1029	1026	1,8-Cineole	13.70	1.47 ± 0.06	13.68	1.15** ± 0.05
8.	1044	1044	(*E*)-β-Ocimene	13.89	0.29 ± 0.01	13.88	1.25** ± 0.09
9.	1082	1086	Terpinolene	14.75	1.19 ± 0.33	14.73	0.58 ± 0.10
10.	1099	1095	Linalool	15.27	0.06	15.28	0.14
11.	1178	1174	Terpinen-4-ol	21.34	0.24	21.31	0.17
12.	1185	1186	α-Terpineol	–	ND	21.94	0.17
13.	1190	1190	Methyl salicylate	22.29	4.02 ± 0.17	–	ND
14.	1281	1284	Bornyl acetate	25.50	0.06	–	ND
15.	1291	1293	2-Undecanone	26.22	0.36 ± 0.10	26.20	0.17 ± 0.01
16.	1344	1345	α-Cubebene	28.94	0.16	28.91	0.19
17.	1350	1356	Eugenol	29.47	2.01 ± 0.09	29.40	1.65 ± 0.18
18.	1357	1359	Neryl acetate	29.58	0.04	29.56	0.02
19.	1373	1374	α-Copaene	30.24	3.71 ± 0.26	30.18	3.54 ± 0.05
20.	1377	1379	Geranyl acetate	30.46	0.57	30.43	0.10
21.	1379	1387	β-Cubebene	30.57	0.35	30.53	0.18
22.	1386	1389	β-Elemene	30.85	0.84 ± 0.05	30.82	1.07 ± 0.06
23.	1401	1390	7-*epi*-Sesquithujene	31.46	0.18	31.44	0.24
24.	1411	1411	(*Z*)-α-Bergamotene	31.96	0.29	31.94	0.35
25.	1417	1417	(*E*)-Caryophyllene	32.33	13.30 ± 0.26	32.30	18.88** ± 0.92
26.	1425	1430	β-Copaene	32.51	0.28	32.49	0.43
27.	1432	1431	β-Gurjunene	32.85	0.29	32.82	0.24
28.	1453	1454	(*E*)-β-Farnesene	33.59	4.57 ± 0.31	33.56	7.73* ± 0.87
29.	1473	1478	γ-Muurolene	33.64	0.02	33.64	0.70
30.	1479	1484	Germacrene D	34.89	9.31 ± 0.60	34.94	18.53** ± 1.01
31.	1485	1489	β-Selinene	35.01	7.77 ± 0.20	–	ND
32.	1488	1493	*trans*-Muurola-4(14),5-diene	35.11	0.07	–	ND
33.	1494	1493	α-Zingiberene	35.39	6.74 ± 0.67	35.37	9.88** ± 0.34
34.	1498	1505	(*E,E*)-α-Farnesene	35.82	1.29 ± 0.29	35.79	1.32 ± 0.16
35.	1505	1509	α-Bulnesene	35.97	0.05	–	ND
36.	1509	1513	γ-Cadinene	36.14	0.46	36.13	0.63
38.	1516	1521	β-Sesquiphellandrene	36.89	0.09	36.87	0.12
39.	1521	1522	δ-Cadinene	36.57	5.38 ± 0.13	36.53	6.81* ± 0.27
40.	1545	1548	Elemol	37.08	0.10	–	ND
41.	1559	1561	(*E*)-Nerolidol	38.00	0.09	37.99	0.32
42.	1573	1577	Spathulenol	–	ND	38.38	0.09
43.	1576	1582	Caryophyllene oxide	39.13	1.56 ± 0.10	39.07	0.84** ± 0.05
44.	1581	1590	Globulol	–	ND	39.25	0.05
45.	1587	1594	Salvial-4(14)-en-1-one	–	ND	39.46	0.28
46.	1603	1608	Humulene epoxide II	39.90	0.23	39.89	0.22
47.	1611	1618	1,10-di-*epi*-Cubenol	40.13	0.25	40.11	0.23

Table 2 (continued)

S. no.	Calculated retention index	Retention index [24]	Name of component	Retention time of components in fresh *Juglans regia* leaves (minutes)	Mean per cent ± SD (fresh *Juglans regia* leaves)	Retention time (GC) of components in dried *Juglans regia* leaves (minutes)	Mean per cent ± SD (dried *Juglans regia* leaves)
48.	1623	1627	1-*epi*-Cubenol	40.83	0.17	40.81	0.64
49.	1640	1644	α-Muurolol	41.06	0.51	41.01	0.04
50.	1652	1652	α-Cadinol	41.40	0.90 ± 0.10	41.41	1.57 ± 0.06
51.	1684	1685	α-Bisabolol	41.94	0.99 ± 0.09	41.95	1.68** ± 0.17
Total				–	89.29	–	96.38

ND, Not detected

Mean values ± SD (standard deviation) followed by ** and * indicated significance difference between pairs (fresh and dried) at $p < 0.01$ and $p < 0.05$, respectively

Table 3 Antioxidant activity of *J. regia* fresh oil

S. no.	Oil concentration (µg/mL)	% Inhibition ± SD
1	50	$13.77^{a} \pm 0.41$
2	100	$16.04^{b} \pm 0.30$
3	200	$18.39^{c} \pm 0.31$
4	400	$23.98^{d} \pm 0.35$
5	800	$47.14^{e} \pm 0.31$
6	1000	$53.29^{f} \pm 0.40$
IC_{50}		923.49 µg/mL

The mean values followed by different alphabets (a–f) at superscript are significantly different at $p < 0.05$ according to Duncan's Test

Table 4 Antioxidant activity of dried *J. regia* oil

S. no.	Concentration (mg/mL)	% Inhibition by oil ± SD
1	10	$26.73^{a} \pm 0.87$
2	20	$40.35^{b} \pm 0.56$
3	40	$57.37^{c} \pm 0.33$
4	80	$66.43^{d} \pm 0.60$
5	160	$80.43^{e} \pm 0.40$
IC_{50}		48.77 mg/mL

The mean values followed by different alphabets (a–e) at superscript are significantly different at $p < 0.05$ according to Duncan's Test

Juglans were also present in our collection in significant amounts. Some authors have observed the antioxidant properties of monoterpenes in essential oils. The antioxidant potential of *Retama raetam* oil could be attributed to the relatively high percentage of monoterpenes [29]. Rather et al. [18] reported that the antimicrobial and antioxidant activity of *J. regia* could be attributed to the synergetic effect of the bioactive constituents. The antioxidant capacity of *Schinus molle* L. could be explained by the presence of monoterpenes (β-carotine) [30].

Table 5 Antioxidant activity of ascorbic acid

S. no.	Concentration (µg/mL)	% Inhibition by ascorbic acid ± SD
1	10	$4.78^{a} \pm 0.48$
2	20	$27.33^{b} \pm 0.15$
3	40	$38.61^{c} \pm 0.17$
4	80	$47.45^{d} \pm 0.43$
5	160	$72.73^{e} \pm 0.25$
IC_{50}		92.78 µg/mL

The mean values followed by different alphabets (a–e) at superscript are significantly different at $p < 0.05$ according to Duncan's Test

4 Conclusion

From the study, it can be concluded that drying has impact on the essential oil composition, number of components and the antioxidant potential of *J. regia*. The plant could be a potential natural resource for high value aroma chemicals such as (*E*)-caryophyllene, germacrene D, β-pinene, β-selinene and α-zingiberene. The results showed that the shade drying of *J. regia* decreased its antioxidant power.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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