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RESEARCH ARTICLE

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Chemical constituents of the essential oil isolated from seed of black pepper, *Piper nigrum* L., (Piperaceae)

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ABSTRACT

Black pepper (*Piper nigrum* L.) is a flowering plant from family Piperaceae. In this research, chemical analysis of the essential oil isolated from this important tropical medicinal plant was performed. For this, seed of this plant was provided, dried under shadow (25 °C), powdered, it's essential oil isolated by Clevenger apparatus, and analyzed by Gas Chromatography related to Mass Spectroscopy device (Agilent 7890). The results showed that there were valuable compounds in its essential oil, total numbers of them reach to 89. Total identification time was estimated to be 38.143 min. Also, nineteen compounds contained more than 1% of total volume; among them, *trans*-caryophyllene bicyclo (peak 44, 19.512 min, 36.43%), L-limonene cyclohexene (peak 13, 7.193 min, 6.75%), 3-carene (peak 9, 6.838 min, 4.97%), cyclohexene, 1-methyl-4-(5-methyl) (peak 60, 22.081 min, 4.93%), and 2-beta-pinene bicyclo (peak 5, 6.220 min, 4.18%) were five dominant constituents, respectively. Moreover, thirty-seven compounds contained lower and equal with 0.1% of total volume and named as the least constituents. In conclusion, this medicinal plant has important secondary compounds which could seriously be focused on them in medical, pharmacology, and toxicology.

1. Introduction

Black pepper (*Piper nigrum* L.) is a tropical medicinal plant belongs to family Piperaceae, cultivated for its fruit known as a peppercorn which is usually used as an important spice (Jaramillo et al., 2001). The fruit is drupe (stone fruit) which when fresh and fully mature is about 5 mm in diameter, dark red, and contains a stone which encloses a single pepper seed. Black pepper is native to present-day Kerala, a state on the southwestern coast of India, and is extensively cultivated there and the other tropical regions of the world (Govindarajan, 1980). Ground, dried, and cooked peppercorns have been used since antiquity, both for flavor and as a traditional medicine. Black pepper is the world's most traded spice and is one of the most common spices added to cuisines around the world (Nirmala Menon, 2000). Its spiciness is due to the chemical compound piperine which is a different kind of spicy from the capsaicin characteristic of chili peppers. The pepper is a perennial woody plant growing up to 4 m in height on supporting trees, poles or trellises. It is a spreading vine, rooting readily where trailing stems touch the ground. Its leaves are alternate, entire, with 5-10 cm long and 3-6 cm width. The flowers are small, produced on pendulous spikes 4-8 cm long at the leaf nodes, the spikes lengthening up to 7-15 cm as the fruit matures (Samsam Shariat, 2007). A single stem bears 20-30 fruiting spikes. The harvest begins as soon as one or two fruits at base of the spikes begin to turn red, and before the fruit is fully mature, and still hard; if allowed to ripen completely, the fruits lose pungency and ultimately fall off and are lost. The spikes are collected and spread out to dry in sun and then the peppercorns are stripped off the spike (Dini, 2005).

Essential oils are volatile and aromatic compounds in plants mostly contain terpenes and terpenoids in their chemical structure. Identifying of these compounds and understanding their roles are very important issues in plant science (Isman, 2000; Isman et al., 2008). Essential oils, known as secondary metabolites, are mainly abundant in Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae families. These compounds mainly have contact, fumigant, repellent, and antifeedant effects and are one of the main components in defense mechanisms of plants against the herbivores (Bakkali et al., 2008; Rafiee-Dastjerdi et al., 2013; Asadi et al., 2018,

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2019). About the isolation method, volatile and aromatic products that are isolated by disstillation process called as the essential oil (Asadi et al., 2019). The essential oils could be used in different aspects due to their important medicinal features. These compounds are different in each plant species and also in each geographical region; therefore, it is not possible to expect same compounds in one plant species from different locations; although, the similarities might be available between their constituets. Therefore, major aim of this study was to identify secondary compounds in the seed of *P. nigrum* as a basic research for their using in future.

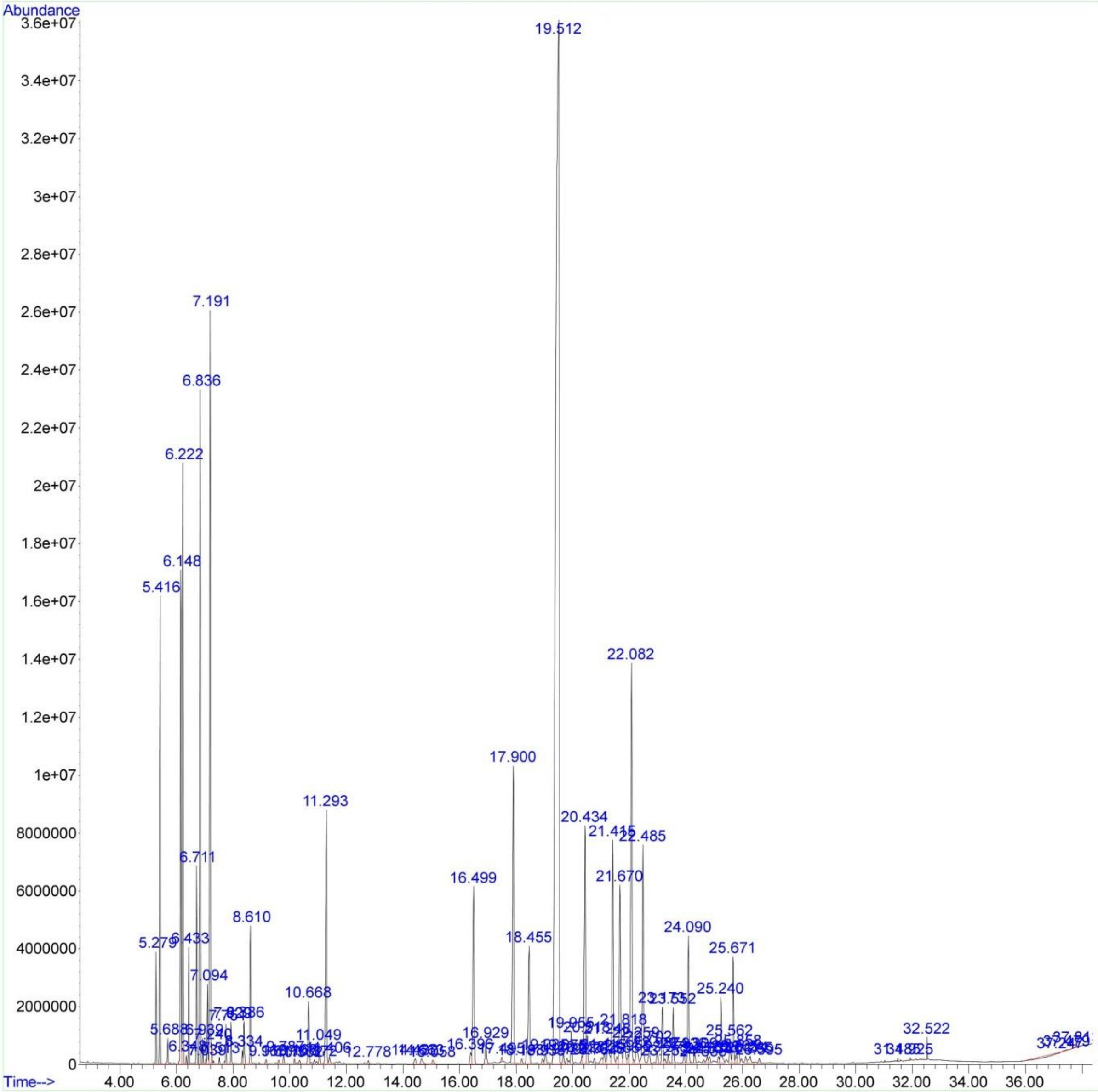

Figure 1. Chromatogram of total compounds in the essential oil of *P. nigrum*

2. Materials and methods

2.1. Identification

Identification of black pepper seed specimen was done by sending of it to Razi University Herbarium (RUHK), Kermanshah, Iran (Code: 1118).

2.2. Isolation of essential oil

P. nigrum seed was provided from medicinal plants stores in Kermanshah province, Iran, during 2018. After complete drying of the specimen in the temperature about 25 °C, it was transferred to the laboratory and essential oil isolated. For this, the seed of plant was powdered. Then, 30 g of its powder was added with 300 ml of the deionized water in 1 liter balloon of the Clevenger apparatus (Babaee Ghaghelestany et al., 2020). After three hours, its essential oil was separated as pale green layer above the water. In order to remove the water for purifying the essential oil, Na_2SO_4 (Sodium Sulfate) compound was used (Asadi et al., 2019). Finally, the purified essential oil was stored in special microtubes (2 ml) covered with aluminum foil in the refrigerator (about 4 °C) until GC-MS analysis (Negahban et al., 2007; Samsam Shariat, 2007; Shiva Parsia and Valizadegan, 2015; Asadi et al., 2018; Babaee Ghaghelestany et al., 2020).

2.3. Gas Chromatography-Mass Spectroscopy (GC-MS)

Chemical compounds in the isolated essential oil of *P. nigrum* were identified by using chromatographic device related to mass spectroscopy (GC-MS: Agilent 7980, the USA) in the central laboratory, University of Mohaghegh Ardabili, Ardabil, Iran. The device was able to inject samples with dilute split splitless inlet (SSI) ability with mass spectroscopy detector (MSD) to quantitatively and qualitatively recognize of samples. The detector was also equipped with ionization system and four-coupled single analyzer (SQA) (Babaee Ghaghelestany et al., 2020). After injecting of the essential oil by Hamilton syringe, different compounds were detected based on their molar mass in different times and their chromatogram was also drawn by the analyzer device (Figure 1).

Table 1. Total compounds in *P. nigrum* essential oil with their retention time and percentage of total

Peak	Compound	Retention time (min)	Percentage of total
1	Thujene	5.276	0.65
2	1R-alpha-Pinene	5.413	2.93
3	Camphene bicyclo [2.2.1] heptan	5.688	0.17
4	Sabinene bicyclo [3.1.0] hexane	6.146	3.81
5	2-beta-Pinene bicyclo	6.220	4.18
6	3-Octanone (cas) eak	6.346	0.05
7	beta-Myrcene 1, 6-octadiene	6.432	0.70
8	1-Phellandrene	6.712	1.28
9	3-Carene	6.838	4.97
10	alpha-Terpinene para-menth	6.941	0.15
11	alpha-Terpinene 1, 3-cyclohexene	7.038	0.03
12	Benzene, 1-methyl-2-(1-methylethyl)	7.096	0.56
13	L-Limonene cyclohexene	7.193	6.75
14	1, 8-Cineole 2-oxabicyclo	7.239	0.10
15	1, 3, 6-Octatriene, 3, 7-dimethy	7.513	0.05
16	1, 4-Cyclohexadiene, 1-methyl-4	7.748	0.26
17	*cis*-Sabinenehydrate	7.931	0.29
18	alpha-Terpinolene cyclohexe	8.332	0.09
19	(+)-4-Carene	8.389	0.34
20	1, 6-Octadien-3-ol, 3, 7-dimethyl	8.612	1.03
21	gamma-Terpinene 1, 4-cyclohexen	9.161	0.05
22	beta-Phellandrene cyclohexen	9.619	0.05
23	Bicyclo [2.2.1] heptan-2-one	9.785	0.08
24	*p*-Mentha-1, 5-dien-8-ol	10.168	0.05
25	Isoborneol	10.351	0.03
26	3-Cyclohexen-1-ol, 4-methyl-1	10.666	0.62
27	Benzenemethanol	10.872	0.04
28	Beta fenchyl alcohol	11.050	0.20
29	Estragole	11.290	2.65
30	Benzene, butyl	11.404	0.08
31	2-Cyclohexen-1-one, 2-methyl-5	12.778	0.04
32	Bicyclo [2.2.1] heptan-2-ol	14.431	0.09
33	Phenol, 5-methyl-2-(1-methylethyl)	14.660	0.10
34	Phenol, 2-methyl-5-(1-methylethyl)	15.061	0.06
35	1, 3-Cyclohexadiene, 1-methyl-4	16.394	0.14
36	Cyclohexene, 4-ethenyl-4-methyl	16.497	2.32
37	alpha-Cubebene	16.932	0.29
38	gamma-Cadinene naphthalene	17.492	0.05
39	alpha-Copaene tricyclo	17.899	3.71
40	Cyclohexane, 1-ethenyl-1-methyl	18.196	0.06
41	beta-Elemene	18.454	1.63
42	Benzene, 1, 2-dimethoxy-4	18.934	0.07
43	1H-Cycloprop *[E]* azulene	19.037	0.16
44	*trans*-Caryophyllene bicyclo	19.512	34.63
45	Germacrene-D	19.673	0.12
46	gamma-Elemene cyclohexane	19.793	0.07
47	Azulene, 1, 2, 3, 4, 5, 6, 7, 8-octahyd	19.953	0.37
48	alpha-Cubebene 1h-cyclopent	20.331	0.10
49	alpha-Humulene	20.434	2.85
50	*trans*-beta-Farnesene	20.519	0.30
51	gamma-Gurjunene azulene	20.765	0.06
52	6, 10, 11-Tetramethyl-tricyclo	21.029	0.11
53	alpha-Amorphene	21.114	0.15
54	Germacrene D 1, 6-cyclodecadie	21.246	0.45
55	Eudesma-4(14), 11-diene	21.418	2.61
56	Isoledene	21.567	0.09
57	alpha-Selinene	21.669	2.23
58	Naphthalene, 1, 2, 4a, 5, 6, 8a	21.818	0.57
59	Germacrene A (CAS)	21.956	0.12
60	Cyclohexene, 1-methyl-4-(5-methyl)	22.081	4.93
61	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a	22.259	0.38
62	delta-Cadinene naphthalene	22.482	2.25
63	Cadina-1, 4-diene	22.699	0.23
64	*cis*-alpha-Bisabolene	22.986	0.14
65	Cyclohexanemethanol, 4-ethenyl	23.174	0.60
66	Caryophyllene oxide	23.260	0.06
67	gamma-Elemene	23.375	0.10
68	1, 6, 10-Dodecatrien-3-ol, 3, 7, 11	23.552	0.56
69	1H-Cycloprop[E]azulen-7-ol	23.935	0.13
70	*(-)*-Caryophyllene oxide	24.090	1.40
71	*(-)*-Dehydroaromadendrene	24.307	0.13
72	Cyclohexene, 6-ethenyl-6-methyl	24.633	0.05
73	*cis*-Z-alpha-Bisabolene epoxide	24.748	0.09
74	beta-Selinene naphthalene	24.834	0.09
75	Epizonarene	25.148	0.07
76	7-Tetracyclo [6.2.1.0 (3.8) 0 (3.9)]	25.240	0.80
77	Bicyclo [4.4.0] dec-1-ene	25.560	0.27
78	1-Naphthalenol	25.669	1.11
79	beta-Eudesmol 2-naphthalene	25.766	0.10
80	beta-Panasinsene	25.858	0.22
81	*ar*-Tumerone	26.115	0.10
82	Cyclohexane, 1, 5-diethenyl-3	26.264	0.16
83	1H-Benzocycloheptene	26.596	0.07
84	Oxirane, hexadecyl	31.483	0.02
85	Eicosane	31.923	0.01
86	4H-1, 3, 5-Thiadiazin-4-one	32.524	0.08
87	Silicone grease, siliconfett	37.245	0.31
88	Gibberellin A3 gibb-3-ene	37.479	0.02
89	Silane, 1, 4-phenylenebis[trimethyl]	37.811	0.03

3. Results and discussion

The chromatogram of available chemical compounds in *P. nigrum* seed essential oil by GC-MS is shown in Figure 1. In the chromatogram, longitudinal axis (X) contained retention time and transverse axis (Y) was the amount of each compound. According to the chromatogram, compounds with higher and lower peaks indicated high and low values in the essential oil, respectively.

Table 2. Nineteen dominant constituents in *P. nigrum* essential oil

Peak	Compound	Retention time (min)	Percentage of total
44	*trans*-Caryophyllene bicyclo	19.512	34.63
13	L-Limonene cyclohexene	7.193	6.75
9	3-Carene	6.838	4.97
60	Cyclohexene, 1-methyl-4-(5-methyl)	22.081	4.93
5	2-beta-Pinene bicyclo	6.220	4.18
4	Sabinene bicyclo [3.1.0] hexane	6.146	3.81
39	alpha-Copaene tricyclo	17.899	3.71
2	1R-alpha-Pinene	5.413	2.93
49	alpha-Humulene	20.434	2.85
29	Estragole	11.290	2.65
55	Eudesma-4(14), 11-diene	21.418	2.61
36	Cyclohexene, 4-ethenyl-4-methyl	16.497	2.32
62	delta-Cadinene naphthalene	22.482	2.25
57	alpha-Selinene	21.669	2.23
41	beta Elemene	18.454	1.63
70	*(-)*-Caryophyllene oxide	24.090	1.40
8	1-Phellandrene	6.712	1.28
78	1-Naphthalenol	25.669	1.11
20	1, 6-Octadien-3-ol, 3, 7-dimethyl	8.612	1.03
	Total volume		87.27

The results showed that *P. nigrum* essential oil contained 89 different chemical compounds (Table 1) with a total retention time about 38.143 min. The retention time in first and last compounds (thujene and silane, 1, 4-phenylenebis [trimethy l]) were determined as 5.276 and 37.811 min, respectively. As shown in Table 2, among the identified compounds, nineteen compounds contained more than 1% of total volume and known as the dominant compounds. By comparing of the percentage for each compound on total volume in the essential oil, it was found that *trans*-caryophyllene bicyclo at peak of 44 (19.512 min and 34.63%) (Table 2) and Eicosane at peak 85 (31.923 min and 0.01%) (Table 3) were the highest and lowest constituents in the essential oil, respectively. Approximately, it can be said that nineteen major compounds have been identified by the detector in middle peaks; In contrast, the least compounds of the essential oil have been distinguished in initial and last peaks. Looking closely at the composition of the essential oil, there were seventy different compounds with percentages of lower and equal with 1% in total volume which totally contained 13.03% of the essential oil; nevertheless, nineteen dominant compounds make up 87.27% of total volume, indicating that most of the essential oil volume was occupied by these nineteen compounds (Table 2).

Table 3. Thirty-seven constituents in *P.nigrum* essential oil that had the lowest percentage (≤0.1 in total)

Peak	Compound	Retention time (min)	Percentage of total
14	1, 8-Cineole 2-oxabicyclo	7.239	0.10
33	Phenol, 5-methyl-2-(1-methylethyl)	14.660	0.10
48	alpha-Cubebene 1h-cyclopent	20.331	0.10
67	gamma-Elemene	23.375	0.10
79	beta-Eudesmol 2-naphthalene	25.766	0.10
81	*ar*-Tumerone	26.115	0.10
18	alpha-Terpinolene cyclohexe	8.332	0.09
32	Bicyclo [2.2.1] heptan-2-ol	14.431	0.09
56	Isoledene	21.567	0.09
73	*cis*-Z-alpha-Bisabolene epoxide	24.748	0.09
74	beta-Selinene naphthalene	24.834	0.09
23	Bicyclo [2.2.1] heptan-2-one	9.785	0.08
30	Benzene, butyl	11.404	0.08
86	4H-1,3,5-Thiadiazin-4-one	32.524	0.08
42	Benzene, 1, 2-dimethoxy-4	18.934	0.07
46	gamma-Elemene cyclohexane	19.793	0.07
75	Epizonarene	25.148	0.07
83	1H-Benzocycloheptene	26.596	0.07
34	Phenol, 2-methyl-5-(1-methylethyl)	15.061	0.06
40	Cyclohexane, 1-ethenyl-1-methyl	18.196	0.06
51	gamma-Gurjunene azulene	20.765	0.06
66	Caryophyllene oxide	23.260	0.06
6	3-Octanone (cas) eak	6.346	0.05
15	1, 3, 6-Octatriene, 3, 7-dimethy	7.513	0.05
21	gamma-Terpinene 1, 4-cyclohexen	9.161	0.05
22	beta-Phellandrene cyclohexen	9.619	0.05
24	*p*-Mentha-1, 5-dien-8-ol	10.168	0.05
38	gamma-Cadinene naphthalene	17.492	0.05
72	Cyclohexene, 6-ethenyl-6-methyl	24.633	0.05
27	Benzenemethanol	10.872	0.04
31	2-Cyclohexen-1-one, 2-methyl-5	12.778	0.04
11	alpha-Terpinene 1, 3-cyclohexene	7.038	0.03
25	Isoborneol	10.351	0.03
89	Silane, 1, 4-phenylenebis[trimethyl]	37.811	0.03
84	Oxirane, hexadecyl	31.483	0.02
88	Gibberellin A3 gibb-3-ene	37.479	0.02
85	Eicosane	31.923	0.01

Due to high importance of *P. nigrum* in traditional and modern medical, different researches have been performed on this valuable medicinal plant worldwide that we will briefly review them. Sumathykutty et al. (1999) studied the essential oil composition from some *Piper* species by capillary GC and GC-MS methods and stated that elemol (11.5%) and beta-caryophyllene (13%) were the highest constituents of leaf essential oils from *P. nigrum* and *P. attenuatum*, respectively. Moreover, beta-cubebene (10%) and cubebol (23.6%) were the major constituent of *P. attenuatum* and *P. cubeba* berry essential oils. In another study, Sasidharan and Menon (2010) studied the chemical composition of *P. nigrum* and concluded that the main compounds of its leaf essential oil were alpha-bisabolol (24.3%), alpha-cubebene (20%), elemol (15%), bisabolene (15%), and alpha-guaiene (15%) that was completely different with my results about the seed essential oil. Parts of plants differ in terms of essential oil constituents and this is the main reason for different results in these researches. Singh et al. (2013) studied the chemical properties of *P. nigrum* essential oil and concluded that there were 40 different components approximately contained 97.7% of the essential oil while beta-caryophyllene (16.0%), sabinene (12.6%), limonene (11.9%), and torreyol (9.3%) were the major components. Plants from different geographical areas have different constituents; so, we can not expect similar compounds in different regions. For this reason, the plant essential oils science is very interesting and complex. Generally, climate is one of the most affecting factors in changing of secondary metabolites from plants. Bagheri et al. (2014) studied *P. nigrum* essential oil extracted by supercritical carbon dioxide (SC-CO_2) technique and stated that the main components isolated by this method under optimal conditions were beta-caryophyllene (25.38%), limonene (15.64%), sabinene (13.63%), 3-carene (9.34%), beta-pinene (7.27%), and alpha-pinene (4.25%). This technique is new compared to the hydro-distillation method which could be developed in future. Jeena et al. (2014) studied black pepper (*P. nigrum*) essential oil and stated that that its main constituents were caryophyllene (23.98%) and limonene (14.36%). Another subject that must be mentioned is the collection conditions, type of plant part, and analyzer device which are very effective in changing the results of plant essential oils. Morshed et al. (2017) studied the physicochemical features of isolated essential oil from *P. nigrum* cultivated in Chittagong from Bangladesh and concluded that major components of its essential oil were caryophyllene (19.12%), limonene (9.74%), and camphene (8.44%) which is different with my results. One of the main reasons for this difference is the geographical changes between Iran and Bangladesh, which are responsible for variable secondary compounds in this plant from two regions.

4. Conclusions

The medicinal plants are considered as god-given natural resources; so, identification, classification, and study of their chemical properties are very important issues in plant science. Author of this article hopes that next researchers will take more steps to identify the compounds of the other medicinal plants belonging to different species and determine their properties for usage in medical, pharmacology, and toxicology.

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Conflict of interest

The author confirms that there is no known conflict of interest.

CRediT authorship contribution statement

Mohammad Asadi: M. Asadi performed all parts of the article alone.

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Supplementary File

None.

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