Journal of Plant Process and Function, Vol. 10, No. 46, Year 2022 **page: 11-18**

Research Article

Some biochemical compounds in male and female plants of *Ephedra intermedia* Schrenket

Maryam Ahmadi[1], Babak Delnavaz Hashemloian[1*], Azra Ataei Azimi[1] and Pejman Moradi[2]

[1] Department of Plant Biology, Agriculture Faculty, Saveh Branch, Islamic Azad University, Saveh, Iran.
[2] Department of horticulture, Agriculture faculty, Saveh branch, Islamic Azad University, Saveh, Iran
(Received: 26/01/2021-Accepted: 13/07/2021)

Abstract

***Ephedra intermedia* is a dioecious plant of the Ephedraceae family whose male and female plants are quite similar in shape, but their biochemical differences are not known. The subject was the TLC and GC-MS study of fatty acids, phenols, essential oils and alkaloids in male and female shoots of *E. intermedia*. Male and female shoots were collected from Markazi province in Iran, in spring. Alcoholic extract was obtained by mixing shoot powder with ethanol at 60 °C. Phenols, fatty acids and essential oil were separated from the alcoholic extract with ether and alkaloids with chloroform. Male and female ether extracts contained fatty acids, which appeared male to be a good source of linolenic acid. Kind and amount of organic acids and phenolic of two sexes were different. Large quantity of caprylic acid isoamyl ester was present only in female. 2, 6-Di-Tert-4-Methylphenol was much higher in both sexes than the other compounds. A large number of essential oil compounds were found in the etheric extract of both sexes. The similarity of essential oil compounds between male and female plants was 32.4%. Total essential oils in female and male plants were 9.63 and 11.79 mgg^{-1}DW. Males and females had a large number of alkaloids, but there was no ephedrine and pseudoephedrine. Epherodxan and phendimetrazine in both sexes, ecgonine and aminopyrimidine in the male, and ergotamine and theacrin in the female, were six important medicinal alkaloids. Both sexes of *E. intermedia* were rich in bioactive compounds, but there was no ephedrine or pseudoephedrine.**

Key words: *Ephedra intermedia,* Male, Female, Alkaloid, Phenol, Fatty acid

Introduction

Ephedra intermedia Schrenket., from Ephedraceae family is a shrub (about 45-150 cm tall) with numerous thin branches and nodes, very small leaves and standing or laying on the ground. This plant is dioecious (bisexual). The vegetative form of male and female plants is quite similar and it is not possible to distinguish these plants from each othen until flower formation (Gharaee *et al.*, 2017). Approximately 26 types of alkaloids with at least one ephedrine-based substance, 75 phenolic compounds including aromatic compounds, flavonoids, lignans and protocyanidins and amino acid derivatives have been identified in different species of *Ephedra*. In the essential oils of these species, 75 compounds have been identified (Gönzalez- Joarez *et al.*, 2020). Many Asia -Europeans produce phenylethylamine, ephedrine, and pseudoephedrine, which act on the mammalian nervous system (Caveniy *et al*., 2001). Male and female plants are distributed near or far from each other. Some researchers have reported that the distribution of male and female plants is related to environmental conditions, especially soil moisture, and more female plants grow in soils with higher humidity. But research on *E. trifurca* in the US desert has shown that distribution is random and has nothing to do with soil moisture (Brunnt *et al*., 1988). *Ephedra* species has a morphological diversity depending on environmental conditions and is widely distributed in the world (Gharaee *et al.*, 2017). *Ephedra* species are rich in natural active compounds that can be used in pharmacy. This plant causes weight loss by increasing sweat and basal metabolism and stimulating central nerves (Al-Snafi, 2017). Ephedrine, pseudoephedrine and phenethyl amine compounds identified in some species of *Ephedra* (*Pelati et al*., 2008). *E. sinica* is a source of ephedrine, pseudoephedrine and other compounds and is used as a dietary supplement in some western countries. Morphological, anatomical, biochemical and genetic studies of *E. distachya* have shown that this plant has fiber masses under the epidermis and is genetically different from *E. sinica* and is not suitable for ephedrine content for medicinal use (Siran *et al.*, 2013). Tannins are important constituents of *Ephedra* species that are more condensed tannins. Proanthocyanidins are major tannins in Euro-Asian *Ephedra* species such as *E. californica*, *E. distachya*, *E. fragilis*, *E.alata*, *E. intermedia*, and several others (Al-Snafi, 2017). Comparison of the content of tannins, saponins, flavonoids and alkaloids in aqueous, alcoholic, acetonic and chloroform extracts of *E.*

*Corresponding Author, Email: hashemloian1343@gmail.com

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

altissima has shown that the solvent type has an effect on the separation of these compounds. Alcoholic extract has the most and acetic extract has the least (Edrah *et al*., 2016). Examination of the compounds of several species of *Ephedra* species showed that *Ephedra* species contain a variety of essential compounds such as citronellol, camphen, cymene and others (Gharaee *et al.*, 2017; Boncan *et al*., 2020). The aim and purpose of this study was to identify and compare free fatty acids, phenols, organic acids, essential oils and alkaloids of male and female *E. intermedia* by TLC and GC-MS analysis.

Materials and methods

Plant materials: In June 2019, shoots of male and female shoots of *Ephedra intermedia* Schrenket., at the time of flowering, were collected from Markazi province of Iran, in latitude 34^0: 45´ north and longitude 49^0: 15´: 30´´ south and altitude 998 meters above sea level. Male and female plants of *E. intermedia* were determined in the central herbarium of Kermanshah University by Dr. Nastaran Jalilian. Identification and complete plants were fixed with the numbers Eph-mMSa07 and Eph-mNSa06. Identification of male and female plants was performed based on the type of sexual flower cone. The shoots of male and female plants were washed separately, dried and powdered in the shade and at room temperature.

Alcohol extract: Shoot powder (10 g) was extracted in 100 ml of 96% alcohol in a hot bath (60°C) for 2 h. The alcoholic extract was dried by a rotary vacuum evaporator at 60°C. The alkaloid, organic acid and phenol were extracted from alcoholic extract by Renaudin (1984) method.

Phenols, fatty acids and organic acids extraction: Alcohol extract was mixed with 100 ml H2SO4 (5%) and an equal volume of ethyl ether. The ethyl ether phase was separated from the acidic phase. The ether phase was derid with a rotary vacuum evaporator at 60°C. The dried extract was dissolved in 5ml absolute ethyl ether and dehydrated with Na_2SO_4. Ether extract was analyzed by Gas Chromatography-Mass Spectrophotometry (GC-MS) to identify fatty acids, organic acids and phenols and thin layer chromatography (TLC) to separate phenols.

Alkaloid extraction: The residual acid phase was used to separate the alkaloids. The pH of the acidic phase was increased to 10 with normal NaOH and mixed with an equal volume of chloroform, and the chloroform phase containing the alkaloids was isolated. The chloroform phase was concentrated with a rotary vacuum evaporator at 60°C. The dried extract was dissolved in 5ml absolute chloroform and dehydrated with Na_2SO_4. Chloroform extract was analyzed by GC-MS and TLC to identify alkaloids.

Essential extract: Shoot powder (20 g) was mixed with 500 ml of distilled water and the essential oil was separated with a Clevenger apparatus for 18 hours. Essential oil dehydrated with Na_2SO_4 and was analyzed by GC-MS and TLC to identify essential oils kind.

Thin layer chromatography (TLC): TLC done according to modified methods of Moriss 1963. 50µl aqueous extract (0.1 gml^{-1}) was applied onto thin layer chromatography (TLC) plates. TLC solvent systems routinely used was ethanol: ethylic ether: chloroform: distilled water (6:3:2:1) and TLC plates (10×15 cm) were formed by coating it to a thickness of 0.2 mm of silica gel G60 (Merck). Isolated phenols and alkaloids appeared on the TLC plate by spraying reagents ferric chloride (2%) and Dragendorff reagent (0.4 g of bismuth subnitrate in 10 ml of concentrated hydrochloric acid (HCl) plus 50 ml potassium iodide 10% and the volume was reached to 100 ml by distilled water), respectively. The Dragendorff reagent, widely used in the visualizing of alkaloid (orange) and ferric salts used in the visualizing of poly phenols (Ataei Azimi and Delnavaz Hashemloian, 2017).

GC-MS condition and specifications: Initial temperature 40°C, initial time 1min, program rate 5°C /min, final temperature 250°C, final time 10min, split ratio ml/min, time 0.5 min, septum purge 5 ml/min and flow rate 1 ml/min.

Instrument specification: Manufacture company Agilent Technologies, GC system 7890A, mass selective mass detector 5975C VL MSD with Triple-Axis detector, Ion source electron impact (EI) 70eV, Analyzer Quadruple, Column Rtx 5MS (length 30m, I.D. 250mm and Film thickness 25µm) and Data processed by Agilent MSD Chem station (Rev E. 02.02.1413).

Condition: Injection port temperature 220°C, Ion source temperature 230°C, Carrier Gas He 99.999% and sample volume 0.5µL.

Results

TLC of alkaloids and phenols of male and female extracts: TLC of alkaloids and phenols of shoot of male and female plants showed that the two sexes were different in terms of alkaloids and phenols type. The repeat of TLC test was showed, the male plant had 6 alkaloids and 3 phenolic substances and the female had 5 alkaloids and 4 phenolic substances (Figure 1). Similarities were observed between the two sexes in terms of phenol 42.86% and in terms of alkaloids 83.33%.

GC-MS analysis: 1- Fatty acids: Comparison of fatty acids of male and female showed that there are 4 types of fatty acids in female ethyl ether extract and 6 types in male (Table 1). 4 fatty acids in both sexes were common but two fatty acids including pentadecanoic acid, and linolenic acid were not in the female. The retention time for all and the amount of common fatty acids were equal in both sexes.

2- Phenols and organic acids: 12 types of phenols and organic acids were observed in both sexes (Table 2). Formic acid, glycolic acid, methyl, acetic acid, diglycolic acid, 1h-indole-3-carboxylic acid, 5-hydroxy, glutaconic anhydride and caprylic acid isoamyl ester

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

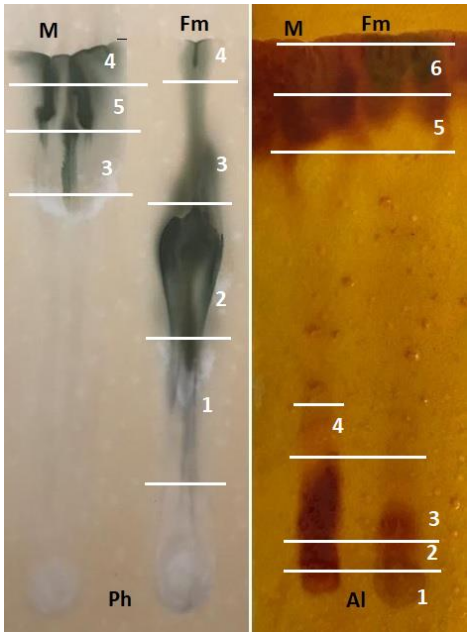

Figure 1. TLC result of phenolic (Ph) and alkaloid (Al) extracts of female (Fm) and male (M) of *E. intermedia*: 4 phenolic substances in female and 3 phenolic substances in male were observed, the bands 3 and 4 of which were common and phenols 1 and 2 in females and 5 in male were observed. 5 alkaloids were observed in the female and 6 alkaloids in the male. Alkaloids 1, 2, 3, 5 and 6 were common in both sexes.

Table 1. Retention time (RT), type and amount of fatty acids in ethyl ether extract of male and female of *E. intermedia*.

N.	RT	Name	Female mgg^{-1}	Male mgg^{-1}
1	25.456	Lauric acid	0.20	0.20
2	31.697	Pentadecanoic acid	-	0.10
3	33.879	Palmitic acid	0.19	0.20
4	37.372	Linolenic acid, ethyl ester	-	1.00
5	37.766	Stearic acid, ethyl ester	0.1	0.11
6	41.163	Adipic acid, dioctyl ester	0.07	0. 07
-	-	Other	0.12	0.23
-	-	Total	0.68	1.900

Table 2. Retention time (RT), type and amount of phenolic compounds and organic acid in ethyl ether extract of male and female of *E. intermedia*.

N.	RT	Name	Female mgg^{-1}	Male mgg^{-1}
1	3.755	Formic acid	0.09	-
2	3.908	Glycolic acid, methyl	0.04	-
3	4.067	Acetic acid	0.09	-
4	5.434	Diglycolic acid	0.01	-
5	7.884	1H-Indole-3-carboxylic acid, 5-hydroxy	0.06	-
6	10.365	Glutaconic anhydride	0.23	-
7	12.108	Propanedioic acid, diethyl ester	-	0. 07
8	12.694	Butanoic acid, 1-methyloctyl ester	-	0.05
9	17.777	Caprylic acid isoamyl ester	3.5	-
10	15.951	Benzoic acid	-	0.08
11	22.364	Coumarin	-	0.09
12	24.094	2,6-Di-Tert-4-Methylphenol	9.6	9.7
-	-	Other	0.23	0.10
-	-	Total	13.88	10.09

were identified in female plants. Propanedioic acid, diethyl ester, Butanoic acid, 1-methyloctyl ester, benzoic acid and coumarin were identified in male plants. Only 2, 6-di-tert-4-methylphenol was the same in both sexes. The highest amount of phenol was related to 2, 6-di-tert-4-methylphenol, in both sexes (9.6 and 9.7 mgg^{-1}DW).

3- Essential oils: Comparison of essential oils substances (Table 3 and 4), showed that there were 21 types of essential oils in the female plants, and 16 types in male plants. However, 6 types of which were common in both sexes. 1-diethoxyethane, thiophene,

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

Table 3. Retention time (RT), type and amount of essential oil compounds in ether extract of female of *E. intermedia*

N.	RT	Name	Female mgg^{-1}
1	3.386	1,1-Diethoxyethane	0.25
2	4.41	1-Pentyl-3,3-D2 Acetate	0.08
3	4.544	Cis-1-Butyl-2-Methylcyclopropane	0.07
4	4.614	Pentane, 1-Ethoxy	0.05
5	5.326	,2-Ethanediol, Monoacetate	0.05
6	5.651	Chloroethene	0. 08
7	5.842	Glycerose	0. 01
8	6.401	Ethanethiol	0.02
9	7.324	1,3-Dioxolane, 2-Methyl-	0.16
10	11.459	Dipropylene Glycol	0. 08
11	11.924	5-Methyl-4,7,10,13-Tetraoxatetradecan-2-Ol	7.20
12	16.453	Thiophene, 2,5-Dihydro-	0.15
13	16.759	2-Furanone, 3,4-Dihydroxytetrahydro	0.08
14	17.987	Phytane	0.31
15	18.05	Thiuronium	0.05
16	19.622	Undecane, 4,6-Dimethyl-	0.16
17	22.809	Silane, Trimethyl-2-Pentenyl-, Trans	0.04
18	27.39	Cyclooctasiloxane, Hexadecamethyl-	0. 09
19	28.395	Heneicosane	0.37
20	31.118	Neophytadiene	0.02
21	33.777	Cyclodecasiloxane, Eicosamethyl-	0.08
-	-	Other	0.23
-	-	Total	9.63

Table 4. Retention time (RT), type and amount of essential oil compounds in ether extract of male of *E. intermedia*.

N.	RT	Name	Male (mgg^{-1})
1	3.386	1,1-Diethoxyethane	0.14
2	3.895	2,5-Hexanediol, 2,5-Dimethyl	5.40
3	6.077	2-Heptanon, 3-Methyl	3.8
4	11.765	2-(Tetrahydrofurfuryloxy)Tetrahydropyran	0.06
5	12.235	1-Undecene, 7-Methyl	0.28
6	16.453	Thiophene, 2,5-Dihydro	0.16
7	17.351	Linalyl Butyrate	0.06
8	17.987	Phytane	0.31
9	28.395	Heneicosane	0.67
10	30.774	Di-O-Methylisophomazarin Methyl Ester	0. 04
11	31.118	Neophytadiene	0.28
12	33.777	Cyclodecasiloxane, Eicosamethyl	0.09
13	37.2	9,12,15-Octadecatrien-1-Ol, (Z,Z,Z)	0.09
14	37.454	Tetratriacontane	0.09
15	39.019	Cyclononasiloxane, Octadecamethyl	0. 08
16	41.316	Bistrimethylsilyl N-Acetyl Eicosasphinga-4,11-Dienine	0.06
-	-	Other	0.18
-	-	Total	11.79

2,5-dihydro, phytane, heneicosane, neophytadiene and cyclodecasiloxane, eicosamethyl were common essential oil types in the male and female plants of *E. intermedia*.

The amount of 2, 5-hexanediol, 2,5-dimethyl (5.40 mgg^{-1}) and 2-heptanon, 3-methyl (3.8 mgg^{-1}), in the male plant and 5-methyl-4,7,10,13-tetraoxatetradecan-2-Ol (7.20 mgg^{-1}), in the female were the highest essential oil types.

4- Alkaloids: Comparison of identified alkaloids showed that in alkaloid extracts of female and male plants, there were more than 20 and 19 types of alkaloids, respectively (Tables 5 and 6). The main female plant alkaloids were 2-N-propyl-1-D1-aziridine, 5-hydroxyindole, epherodxan, 5-acetyl-2H-(1, 4) benzoxazin-3(4H)-one, phendimetrazine, ergotamine and 3-nitrophthalic acid (0.8- 2.5 mgg^{-1}dw) (Table 5). The highest alkaloid of the female plant was Phendimetrazine (2.5 mgg^{-1}dw (Dry Weight)). Epherodxan (0.83 mgg^{-1} DW), tetramethyluric acid or theacrin is a purine alkaloid (0.46 mgg^{-1} DW), ergotamine (0.93 mgg^{-1} DW) and phendimetrazine is a alkaloid similar to an amphetamine, (2.5 mgg^{-1}dw) are four important medicinal alkaloids that were observed in the alkaloid extract of the female plant.

Epherodxan, iron, tricarbonyl (N-(phenyl-2-pyridinylmethylene) penzenamine-N,N'), Methyl 2,3,4,5-tetrahydro-4-methyl-1,5-dioxo-1h-benz (C) azepine-3-carboxylate and M-nitrophthalic acid were main male alkaloids (Table 6). Comparison of female and male alkaloids showed that most alkaloids were different in the two sexes. Epherodxan (0.83 and 0.24

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

Table 5. Retention time (RT), type and amount of female shoot alkaloids of *E. intermedia*

N.	RT	Name	Female mgg^{-1}
1	10.23	2-N-Propyl-1-D1-Aziridine	0.93
2	19.42	5-Hydroxyindole	0.97
3	19.72	3-(4-Hydroxyphenyl)propionitrile	0.44
4	20.19	5-Aminobenzimidazole	0.86
5	28.713	Epherodxan	0.83
6	28.97	5-Acetyl-2H-(1,4)benzoxazin-3(4H)-one	0.22
7	29.24	Cyclohexyl nitrite	2.5
8	29.29	Phendimetrazine	0.32
9	29.3	N-Methylazetidine	0.32
10	29.80	Pentyl N,N-dimethylphosphoramidocyanidate	0.44
11	30.02	3H-Pyrazolo(3,4-c)pyridin-3-one, 1,2-dihydro-	0.36
12	33.61	Di-N-Butylphthalate	0.53
13	39.89	1,3,7,9-Tetramethyluric acid= Theacrin	0.46
14	40.02	3,7-Diamino-2-Methylphenazine	0.38
15	40.10	10-methyl-2-aminoacridin-9-one	0.25
16	40.19	Benzenamine, 4-butyl-N-((4-methoxyphenyl)methylene	0.35
17	40.83	Ergotamine	0.93
18	40.99	6-Methyl-6,7,8,9-(5h)-1,2,4-Triazolo(4,3-B)-1,2,4-Triazepine	0.20
19	43.95	3-Nitrophthalic acid	0.91
20	44.2	Iron, Tricarbonyl(N-(Phenyl-2-Pyridinylmethylene)Benzenamine-N,N')	0.64
-	-	Other	0.19
-	-	Total	13.03

Table 6. Retention time (RT), type and amount of alkaloids of male *Ephedra* of *E. intermedia*.

N.	Rt	Name	Male mgg^{-1}
1	20.71	Azetidine, 2-Methyl	0.24
2	21.06	4-Dimethylthiazolidine	0.05
3	28.71	Epherodxan	2.18
4	28.82	1-Tert-Butyl-3-Phenylaziridinone	0.10
5	28.92	Prop-2-Enol	0.05
6	29.92	Phendimetrazine	0.65
7	30,33	Z)-N-Tert-Butyl-1,2-Dicyano-3-Methyl-1-Butenamide	0.14
8	31.15	4-Aminopyrimidine	0.18
9	31.25	Hexyl Isocyanide	0.11
10	31.41	Ecgonine	0.22
11	32.32	Iron, Tricarbonyl(N-(Phenyl-2-Pyridinylmethylene)Benzenamine	4.61
12	32.72	Methyl -2,3,4,5-Tetrahydro-4-Methyl-1,5-Dioxo-1h-Benz(C)Azepine-3-Carboxylate	1.23
13	32.98	Ethene,-1-(4-Diethylaminophenyl)-2-Nitro-	0.38
14	33.08	2,4-Dimethyl-5,6-Dimethoxy-8-Aminoquinoline	0.20
15	34.99	Xylazin	0.68
16	35.00	5-Nitro-6-Methoxy-2,3-Dimethylindole	0.30
17	35.47	Methacrylamide	0.22
18	35.99	Acetamido-4-Hydroxy-2-Butenoic Acid-Y-Lactone	0.15
19	43.98	M-Nitrophthalic Acid	0.91
-	-	Other	0.35
-	-	Total	12.95

mgg^{-1}dw), phendimetrazine (0.32 and 2.18 mgg^{-1}dw), N-methylazetidine (0.32 and 0.65 mgg^{-1}dw) ,3-nitrophthalic acid (0.91 and 4.61 mgg^{-1}dw) and Iron, Tricarbonyl (N-(phenyl-2-Pyridinylmethylene) benzenamine-N,N' (0.64 and 0.91 mgg^{-1}dw) were common in the both sexes. Epherodxan, tricarbonyl (N-(phenyl-2-pyridinylmethylene) benzenamine and methyl -2,3,4,5-tetrahydro-4-methyl-1,5-dioxo-1h-benz (C) azepine-3-carboxylate in the males and cyclohexyl nitrite in the females were much higher than other alkaloids.

Discussion

TLC and total alkaloid and Phenols: The dragendorff reagent, widely used in the visualizing of alkaloid in orange color (Ataei Azimi A and Delnavaz Hashemloian, 2017). TLC analysis showed that male and female *E. intermedia* are different in terms of alkaloids and phenols type. Similarity between the two sexes was 83.33% for alkaloids. Total alkaloids in female and male plants were 13.03 and 12.95 mgg^{-1}, respectively. The total alkaloid content of shoot of *E.*

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

intermedia was 12.9 mgg^{-1} (Ataei Azimi A and Delnavaz Hashemloian *et al*., 2015). Comparison of total alkaloids, phenols, flavonoids and tannins and total ephedrine and pseudoephedrine of male and female sexes have shown that recent compounds are different in male and female and different months of the year (Bojnordy *et al*., 2016; Aghdasi *et al*., 2016). The proanthocynidins (codensed tannins) are polyflovoniods of some plants in nature. The hydrolyzable tannins are usually subdivided into gallotnnins and ellagitannins. Ferric salts used in the visualizing of poly phenols on TLC page. The hydrolysable tannins (gallitannins and ellagitannins) give blue-black precipitates and condensed tannins greenish-brown ones (Ataei Azimi A and Delnavaz Hashemloian, 2017). The polyphenols of male and female *E. intermedia* were hydrolysable tannins, because presented in TLC black after visualizing with ferric chloride spray. Similarities were observed between the two sexes in terms of phenol 42.86%. Total phenols and organic acids in female and male plants were 13.88 and 10.09 mgg^{-1}, respectively.

1- Fatty acids: Palmitic acid and linolenic acid are the main fatty acids of *E. sinica* (Gonzalez- Joarez *et al.*, 2020). Comparison of fatty acids of male and female plants of *E. intermedia* showed that there were 4 types of fatty acids in female and for the male 6 types. 4 fatty acids in two fatty acids pentadecanoic acid and linolenic acid, ethyl ester acid were not in the female. Total fatty acids in female and male plants were 0.68 and 1.9 mgg^{-1} DW, respectively.

2- Phenols: 12 types of organic acids and phenols were observed in both sexes. Formic acid, Glycolic acid, methyl, acetic acid, diglycolic acid, 1h-indole-3-carboxylic acid, 5-hydroxy, glutaconic anhydride and caprylic acid isoamyl ester were in female plants and for the male propanedioic acid, diethyl ester, butanoic acid, 1-methyloctyl ester, benzoic acid and coumarin in male plants. Only 2,6-di-tert-4-methylphenol was the same in both sexes. The highest amount of phenol was related to 2, 6-di-tert-4-methylphenol, in both sexes (9.6 and 9.7 mgg^{-1}DW). Coumaric acid, furanofuran lignan, digalloyl-glucose and nilocitin are among the compounds identified by mass analysis in *E. alata* (Nawar *et al*., 1985). Analysis by GC-MS of dichloromethane extract of shoots and flowers of *E. alata* identified 52 and 65 compounds, respectively, containing phenol, fatty acids and organic acids such as propionic acid, guanidine acid and benzoic acid (Chebouat *et al*., 2016). Phenolic compounds of benzoic acid and coumaric acid have been isolated from *E. equisetina*. From *E. lomatolepis*, protocyanidins have been identified. In *E. major*, coumaric acid and epicatechin have been found (Elhadef *et al*., 2020). The phenol content of *E. alata* methanolic extract was 47.62 mgg^{-1} DW. The phenols identified in *E. sinica* were catechins, coumaric acid and apigenin (Gul *et al*., 2017). Gas chromatography showed that the main constituents in the aqueous extract of E. major were quinic acid, cinnamic acid and naringin, and for methanolic extract of E. sinica, alanine, malonic acid, cinnamic acid, proline, glutamic acid, benzoic acid as well as citric acid (Lev *et al.*, 2015)

3- Essential oil: In essential oils of *E. sinica*, alpha-terpinol and p-vinyl anisole, phytol gamma-iodesmanol and octadecadiene-enoyc-N 1 have been isolated (Gonzalez- Joarez *et al.*, 2020). Two main compounds of Tetramethylenpyrazine and a-terpineol have been identified in shoot and root essential oils of *E. sinica* (Abourashed *et al*., 2003). According to previous reports, analysis of essential oils of several species of *Ephedra* in Iran showed that *E. major* contains only citronellol and hexenyl benzoate, while *E. intermedia* contains camphen, cymene, limonene, sainol, cineol, camphor, terpineol, and myrtenol (Gharaee *et al.*, 2017). Total essential oils in female and male plants were 9.63 and 11.79 mgg^{-1}DW. 21 and 16 types isoprenoid, and sesquiterpenoid compounds were identified in the essential oils of male and female plants of *E. intermedia* shoots, respectively. 1-Diethoxyethane, Thiophene, 2,5-Dihydro, Phytane, Heneicosane, Neophytadiene and Cyclodecasiloxane, Eicosamethyl were common to both sexes. The amount of 2, 5-hexanediol, 2,5-dimethyl and 2-heptanon, 3-Mhethyl, in the male and 5-hethyl-4,7,10,13-tetraoxatetradecan-2-Ol, in the female were the highest essential oil types.

Alkaloid: Comparison of identified alkaloids showed that in alkaloid extracts of female and male, there were more than 20 and 19 types of alkaloids, respectively. Approximately 26 types of alkaloids with at least one ephedrine-based substance have been identified in different species of *Ephedra* (Gonzalez- Joarez *et al*., 2020). *Ephedra* species contain very alkaloids including ephedrine, pseudoephedrine and similar substances to serotonin and amphetamine (Hagel *et al*., 2012). Both species of *E. intermedia* lacked ephedrine and pseudoephedrine. Comparison of ephedrine and pseudoephedrine in shoots of several ephedra species showed that ephedrine was the main alkaloid in *E. major*, *E. fragilis* and *E. distachya*. *E. monosperma* was the source of pseudoephedrine and *E. foemina* is free of ephedrine and pseudoephedrine. The amount of ephedrine in the analysis by HPLC was 2.5-1.2 mgg^{-1} dry weight (Elhadef *et al*., 2020). Ephedrine is one of the main alkaloids of *E. sinica* and *E. intermedia* and pseudoephedrine is the main alkaloid of *E. lomatolepis* (Gonzalez- Joarez *et al.*, 2020). Ephedrine and peseduephedrin in organs of *E. intermidia* was reported 0.45- 0.92% and and 0.65-1.93%, respectively (Elhadef *et al*., 2020). Gas chromatography has identified the methanolic extract of *E. sinica*, ephedrine and its derivatives and pseudoephedrine and its derivatives (Lev *et al*., 2015).

The main alkaloids were phendimetrazine, 5-hydroxyindole, epherodxan and ergotamine and 3-nitrophthalic acid in the females and for the male, epherodxan, tricarbonyl (n- (phenyl-2-pyridinyl methylene) benzenamine and tetrahydro-4-methyl-1,5-dioxo-1h-benz-azepine-3-carboxylate. Ergotamine is an

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

alkaloid produced by ascomycete fungi (plant parazites) but has been found in some plant families such as Convolvulaceae, Poaceae and Polygonaceae but has not been reported in *Ephedra* genus (Mantle, 2020). Ecgonine in *E. intermedia*, is a valuable alkaloid tropane found in coca trees (*Erythroxylum*) (Albert *et al*., 2018) but not reported for *Ephedra*. The highest alkaloid of the female was phendimetrazine and for the male tricarbonyl (n-(phenyl-2-pyridinyl methylene) benzenamine. Epherodxan, phendime trazine, N-methylazetidine, 3-nitrophthalic acid and tricarbonyl (N-(phenyl-2-pyridinyl methylene) benzenamine were common in the both sexes.

Dimethyl-5,6-dimethoxy-8-aminoquinoline was quinoline alkaloid in the male *E. intermedia*. Quinoline alkaloids are produced in species of *Ephedra*. Transtorine is a quinoline alkaloid from *E. transitoria* (Gonzalez- Joarez *et al.*, 2020; Edrah *et al*., 2016). Imidazole alkaloids such as freulyol histamine and the alkaloids methyl benzylamine and tetramethylpyrazine have been isolated from *E. sinica* (Gonzalez- Joarez *et al.*, 2020). 5-Aminobenzimidazole in female and 4-dimethylthiazolidine in male *E. intermedia* were imidazole alkaloids.

Conclusion

Ephedra intermedia is an evergreen, dioecious plant found in many deserts of Iran. Both sexes are rich in alkaloids and phenolic compounds, each of which is different from the other. Males and female plants of *E. intermedia* had the highest alkaloids. They haven't ephedrine and pseudoephedrine (two important alkaloids in the Ephedra genus). Some compounds such as linolenic acid, ecgonine, and aminopyrimidine were found only in male plants. Caprylic acid isoamyl ester, ergotamine, and theacrin were found only in the female plants. Based on this, it can be concluded that each of these compounds can be used as a marker to identify the male and female sexes of *E. intermedia* without waiting until the flowering stage.

Acknowledgmant: We, the authors, thank Mr. Esharti for helping us with this research.

References

Abourashed, E. A., El-Alfy, A. T., Khan, I. A. and Walker, L. (2003) Ephedra in perspective: Acurrent review. Phytotherapy research 17: 703-712.

Aghdasi, M., Bojnordy, M., Mianabadi, M. and Naddaf, M. (2016) Chemical components of the *Ephedra major* from Iran. Natural Product Research 30: 369-371.

Albert, P. S. F., Daniel, M., Morris, A. A. S., Baranenko, D. A. and Myer, J. J. M. (2018) Seasonal analysis of the tropane alkaloid ecgonine methyl ester and occurrence in *Erythroxylum* trees. Acta Physiologiae Plantarum 40: 1-23.

Al-Snafi, A. S. (2017) Therapeutic importance of *Ephedra alata* and *E. foliata*: A review. Pharmacological of Medicinal Plants 4: 160-168.

Ataei Azimi, A. and Delnavaz Hashemloian, B. (2015) *In vitro* shoot and callus induction and alkaloid content of *Ephedra intermedia* of Iran. Journal of Plant Sciences 3: 1-8.

Ataei Azimi, A. and Delnavaz Hashemloian, B. (2017) Allelopathy and Anti-mitotic effects of *Cuscuta campestris* and *C. monogyna* extracts on plant cell division. Journal of Medicinal Plants and By-products 2: 131-138.

Bojnordy, M. M., Aghdasi, M., Mian Abadi, M. and Naddaf, M. (2016) Chemical compounds study on some secondary metabolites of male and females stems of *Ephedra major* Host. Iranian Journal of Medicinal Plants 32: 290-300.

Boncan, D. A. T., Tsang, S. S. K., Li, C., Lee, I. H. T., Lam, H. M., Chan, T. F. and Hu, J. H. L. (2020) Terrenes and terpenoids in plants. International Journal of Molecular Science 21: 7382.

Brunnt, J. W., Conli, M. R. and Coningham, G. L. (1988) Sex in *Ephedra trifurca* with relation to Chihuahuan desert habitats. The American Midland Naturalist 119: 137-142.

Caveniy, S., Charlet, D. A., Frietag, H., Stolti, M. M. and Starat, A. N. (2001) New observations on the secondary chemistry of world *Ephedra*. American Journal of Botany 88: 1199-1208.

Chebouat, E., Gheraf, N., Damousa, B., Alaoui, M., Chirite, A. and Zellagoi, A. (2016) Chemical composition of the dichloromethane extract of *Ephedra alata* leaves and flowers. Der Pharmacia Letter 8: 10-13.

Edrah, S. M., Aljenkavi, A., Omeman, A. and Alafid, F. (2016) Qualitative and quantities analysis of phytochemicals of various extract for *Ephedra altissima* from Liba. Journal of Medicinal Plants Studies 4: 119-121.

Elhadef, K., Smauoi, S., Foorati, M., Helima, H. B., Mtibba, A. C., Selam, I., Enouri, K. and Melouli, L. (2020) A review on worldwide *Ephedra* history and story. Hindawi 1-22.

Gharaee, M. E., Hosaini, B. A., Khayiat, M. H., Emami, S. A., Asyli, J., Shakeri, A., Hasani, M., Ansary, A., Arazade, S., Kasian, J. and Behravani, J. (2017) Essential oil diversity and molecular characterization of *Ephedra* genus using rapid analysis. Research Journal of Pharmacognosy 4: 261-270.

Gonzalez-Joarez, D. G., Moratiila, A. E., Floores, J., Figoerua, S. H., Taguena, N. N., Jimenz, J. M., Ramyrez, A. M., Palcios, G. P., Mirand, S. P., Hernandz, A. R., Trojilo, J. and Baotista, E. (2020) A review of the *Ephedra* species. Molecules 25: 3283.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S. and Jahan, S. (2017) Preliminary phytochemical screening, quantitative of total phenols and antioxidant activity of crud extracts of *Ephedra intermedia*, indigenous of baluchistane. The Scientific World Journal 5873648: 1-7.

[DOR: 20.1001.1.23222727.1400.10.46.1.1]

Hagel, J. M., Krizevski, R., Marsolais, F., Lewinsohn, E. and Facchini, P. J. (2012) Biosynthesis of amphetamine analogs in plants. Trends in Plant Science 17: 404-412.

Lev, M., Chen, J., Gao, Y., Sun, J., Zhang, Q., Zhang, M., Xo, F. and Zhang, Z. (2015) Metabolomics based on liquid chromatography with mass spectrometry reveals the chemical difference in the stems and roots from *Ephedra sinica*. Journal of Separation Science 38: 3305-3486.

Mantle, P. (2020) Comparative ergot alkaloid elaboration by selected plectenchymatic mycelia of *Claviceps purpurea*. Biology 9: 1-14.

Moriss, L. J. (1963) Fraction of cholesterol esters by Thin Layer Chromatography (TLC). Lipid Research 4: 357-359.

Nawar, M. A. M., Barakat, H. H., Bodrust, J. and Linsched, M. (1985) Alkaloid, lignin and phenolic constiuents of *Ephedra alata*. Phytochemistry 24: 878-879.

Pelati, F. and Benvenoti, S. (2008) Determination of epiderine alkaloids in *Ephedra*, natural products using HPLC on a pentaflourophenylpropyl stationary phase. Journal of Pharmaceutical and Biomedical Analysis 48: 254-268.

Renaudin, J. P. (1984) Reversed-phase high perfomance liquid chromatographic characteristic indole alkaloids from *Catharantus roseus*. Journal of Chromatography A 291: 165-174.

Siran, N., Matsumato, M., Shimoiama, Y., Alaian, N., Coskon, M., Yilmas, T. and Mikhage, M. (2013) Antimicrobial, chemical and molecular genetic study of *Ephedra distachya*. The Journal of Japanases Botany 88: 144-155.

[DOR: 20.1001.1.23222727.1400.10.46.1.1]