The Scientific Temper
Vol. 12, No 1-2, January-July, 2021:pp 85-100
ISSN 0976 8653, E-ISSN 2231 6396
A Web of Science Journal
Doc ID: https://connectjournals.com/03960.2021.12.85

Study on the Chemical Composition and Antioxidant Activity of Extracts from Wild and *in vitro* Raised Endangered Medicinal Plant *Ephedra gerardiana*

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ABSTRACT

Ephedra gerardiana is an important medicinal plant known for its tremendous medicinal potential since ages. The plant has been utilized in the treatment of several ailments. The plant possesses several biologically active phytoconstituents which are responsible for its medicinal value. The present study was conducted to identify such phyto-compounds through GC-MS study and also to evaluate the antioxidant potential of the plant. Methanolic extract of wild plant and *in vitro* raised plantlet were utilized for determining antioxidant value and GC-MS analysis. *In vitro* raised plants exhibited enhanced production of flavonoids with prominent antioxidant activity as compared to the mother plant. On the contrary, the level of tannin was found to be higher in the mother plant. GC-MS study revealed the presence of a total of 34 and 43 phyto-compounds in mother and *in-vitro* raised plants respectively. 9-Octadecenoic acid, methyl ester (24.66%), 9-octadecenoic acid, 1,2,3 propanetriyl ester (10.75%), Phytol (8.8%) and 2(5H)- Furanone, 3-hydroxy-4,5-dimethyl (7.41%) were major compounds found in mother plant and Morpholine (28.93%), 9-Octadecenoic acid, methyl ester (20.45%), Hexadecanoic acid, 2-Hexadecen-1-ol (16.16%) were major compounds found to be present in methanolic extract of the wild plant.

Keywords: *Ephedra gerardiana*, Antioxidant, Phytocompounds.

INTRODUCTION

Ephedra gerardiana is an evergreen xerophytic shrub creating a bushy habitat that ascends to altitude up to 5450 m and is known for its importance in medicine and distribution all around the globe including regions of Europe, Asia, South America, Afghanistan and Bhutan (Stevenson, 1993; Sharma and Dhima, 2010; Dar and Khuroo, 2020). Prevalent polyploidy is dynamically found in Ephedra gerandium (Ickert-Bond et al., 2020). Presently, Genus *Ephedra* contains 13 taxa which include 10 species and 3 varieties have been reported in India in the areas of Alpine Himalayas from Kashmir to Sikkim, Chamba, Spiti and Ladakh (Polunin et al., 1987; Chauhan, 1999; Dar and Khuroo, 2020). The plant is known to possess

tremendous medicinal potential. Several studies have reported utilization of the plant in the treatment of ailments and diseases like cold, influenza, asthma, bronchitis, hay fever, arthritis, bone pains, coughing, hyperhidrosis, nasal decongestant, syphilis, rheumatism, syphilis and as CNS stimulant due to its bioactive metabolite ephedrine (Morton, 1977; Sahni, 1990; Leung and Foster, 1996; Porwal et al., 2003; Soni et al., 2004; Chaudhary et al., 2019). Ephedra fruits are especially known for healing dried skin, lip cracking, sunburn, mouth diseases, stomach problems and even treating tuberculosis wounds (Din et al., 2020). Ephedra species also contains some important alkaloids such as norephedrine, methylephedrine, pseudoephedrine, norpseudoephedrine, and methylpseudoephedrine (Uttra and Hasan, 2017). While studying *the Ephedra gerardiana* stem, many phytochemicals have been identified and isolated from it which are widely distributed as flavonoids, alkaloids, terpenoids, saponins and Glycosides (Jamil et al., 2012). The phytochemical profile of different flavonoids in this plant includes leucoanthocyanidin, leucopelargonine, leucodelphinidin, lucenine, vicenin-1 and 2, moreover, tannins and benzylmethylamine are also found in them (Ibragic and Sofić, 2015). Although, it has been a known fact that activated biological components isolated from the plants are dependent on the selected solvent system for their extraction and the exact method used to serve this purpose. As the solvent extract can lead to slowing down the biological activity of any phytochemical compound example preventing the oxidation stress due to free radical production because of insufficient scavenging by anti-oxidants. In this situation, it becomes difficult to fully understand the plant's potential for its therapeutic and medicinal properties (Khan et al., 2017).

The plant has been reported to possess several phytochemical compounds which impart the medicinal value to *Ephedra* out of which Ephedrine is the most important and effective (Finar, 1973; O'Dowd and Richardson, 1994). The plant has been reported to be endangered (Gupta and Sethi, 1983; Aldam, 2002). Several studies have reported rapid and effective methods of tissue culture of *Ephedra* for mass propagation of the plant (Dhiman and Sharma, 2010; Sharma et al., 2012).

The contribution of anti-oxidant property in the Ephedra plant is due to the flavonoids which work against free radicals and possess anti-inflammatory properties in response to it (Mohamad et al., 2016). This anti-oxidant property when combines with the effect of alkaloids, flavonoids, saponins, and *E. gerardiana* hydroalcoholic extract and fractions, can be used in producing anti-arthritic activity (Uttra and Hasan, 2017).

In recent time determination of the antioxidant potential of medicinal plants have gained momentum owing to the significant utilization of antioxidants in medicine. Hence, the present study was conducted to evaluate and compare antioxidant potential and GC-MS analysis of mother and tissue culture-raised plants of *Ephedra gerardiana*.

MATERIAL AND METHODS

Plant Material

Mature plants of *Ephedra gerardiana* were procured from the botanical garden of Delhi University, Delhi, and maintained in the Department of Botany K.L. DAV (PG) College Roorkee. The plants were subsequently utilized to produce tissue culture-raised plants as per the standardized protocol (Dhiman et al., 2010; Rautela et al., 2018).

Extract Preparation

Plant extract of mother planet and *in-vitro* raised plantlet of *E. gerardiana* were used for estimation of antioxidant profiling and GC-MS analysis. 10 g of the dry plant was macerated in methanol: water (90:10) for 24 hours. The extract was filtered and then concentrated in a rotary evaporator for 15 min and dried in a lyophilizer. The powder was weighed to calculate the yield and kept at 20^0C for further utilization. For each experiment, the powder was dissolved in 500 µl methanol and diluted as per desired concentration required for analyzing antioxidant activity.

Total phenol content

Total phenolic contents of Plant extract were determined by the Folin–Ciocalteu assay as described previously (McDonald et al., 2001), with slight modifications. A methanolic solution of catechin was utilized as a reference for the estimation of phenol content.

Total flavonoids

The aluminum chloride method was used for determining the total flavonoid content of respective extracts. Absorbance was recorded at 510 nm. The concentrations of flavonoid in the test samples were expressed as mg of quercetin equivalent (QE) per gram of sample (mg QE/g dw).

Total flavonols

Total flavonols were estimated using the modified method (Kumaran and Karunakaran, 2007). Total flavonol content calculated was expressed as mg of quercetin equivalent (QE) per gram of extract of the sample (mg QE/extract).

Tannin content

Tannin estimation was performed according to Folin-Ciocalteu's method (Attarde et al., 2010), The reaction mixture was shaken well, incubated at 20^0C for 30 min and absorbance was measured at 740 nm. The total tannin in the extract was expressed as mg of tannic acid equivalent per gram of extract (TE mg/g extract).

Total antioxidant capacity (TAC)

The total antioxidant capacity was measured by the spectrophotometric method (Aguilar Urbano et al., 1999). Values of the antioxidant activity are expressed as mg of ascorbic acid equivalent per gram of extract.

DPPH free radical scavenging activity

The determination of the free radical scavenging activity of the plant extract of *E. gerardiana* was carried out using DPPH (1,1-diphenyl-2 picrylhydrazyl) assay (Burda and Oleszek, 2001; Ara and Nur, 2009). 5 ml DPPH (0.004%) in methanol was added to 1 ml of extract and standard solution at different concentrations (50, 60, 70, 80 and 90 µg/ml) prepared in methanol. After 30 min absorbance was measured at 517 nm and compared with ascorbic acid taken as standard. A lower absorbance indicates higher radical scavenging power. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

DPPH radical scavenging activity (%)

$= [(Abs_{control} – Abs_{test})/(Abs_{control})] \times 100$

where, $Abs_{control}$ is the absorbance of DPPH radical + Methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract/ standard at 517nm).

Metal chelating activity

The chelating of ferrous ions by the plant extract of *E. gerardiana* was estimated by the modified method (Dinis et al., 1994). The extract samples (50–250 mg/ml) were added to a solution of 2 mmol/ l $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/ l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated from $[(A_0-A_1)/A_0]100$, where A_0 is the absorbance of the control and $A_1$is the absorbance of the extract/standard.

FRAP assay (Ferric Reducing Antioxidant Power)

The determination of the FRAP assay was performed by the modified FRAP method reported by Benzie and Strain (1996). The stock solution included 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution in distilled water. Then acetate buffer (25 ml) and TPTZ (2.5 ml) were mixed with $FeCl_3.6H_2O$ (2.5 ml). The temperature of the solution was raised to 37°C before it was used. Plant extracts (150 µl) were allowed to react with the FRAP solution (2.85 ml) for 30 min under dark conditions. The absorbance was measured at 593 nm. The standard curve was linear between 200 and 1,000 µM $FeSO_4$. Results were expressed in µM Fe (II)/g dry mass and compared with those of standards for ascorbic acid and α-tocopherol.

GC-MS analysis of the sample:

GC-MS analysis of plant extracts was performed using a regular Perkin Elmer Auto System XL GC-MS analyzer. For GC-MS detection, an electron ionization energy system with an ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 2µl was employed. Total GC running time was 22 min. Software adopted to handle mass spectra and chromatograms was Turbo Mass.

Identification of compounds was based on the molecular structure, molecular mass. Interpretation on mass spectrum GC-MS was conducted using the database of NIST (National Institute Standard and Technology) having more than 62,000 patterns and Wiley library. The name, molecular weight and structure of the components of the test material were ascertained by correlating with the library. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas (Sharma et al., 2015).

RESULT AND DISCUSSION

Antioxidant Activity

Assessment of antioxidant properties of medicinal plant has gained importance to find an effective remedy for several diseases and symptoms. There is also a huge demand for natural antioxidants in food and related industries, for replacing synthetic preservatives. Secondary metabolites such as flavonoids from medicinal plants function as effective antioxidants through direct antiradical, chain-breaking of the free radical propagation and interaction with transition metals. Different kinds of Ephedra are known to contain different types of polyphenols, flavonoids, and anthocyanins, whose amount can be quantified by using gallic acid (quercetin or catechin) and cyanidin-3-glucoside as reference or standard components (Alali et al., 2007; Pellati and Benvenuti, 2008; Jaradat et al., 2015; Aghdasi et al., 2016; **Al-**Rimawi et al., 2017; Kallassy et al., 2017; Al-Trad et al., 2018; Hegazy et al., 2019; Mellado et al., 2019; Nasar et al., 2019; Elhadef et al., 2020).

Results obtained in the present study revealed the enhanced production of flavonoids in tissue culture raised plants of Ephedra (0.87±0.11µg/gfwt) as compared to that of the mother plant (0.23±0.02µg/gfwt). Similar trends were also obtained for total antioxidant, 3.97±0.21µg/ gfwt total antioxidants were estimated to be present in micropropagated plant and about 2.33±0.26µg/gfwt in the mother plant. (Figure 1, Table 1).

Table 1: Antioxidant profiling of Plant extract of mother plant and *in vitro* plant *E. gerardiana.*

S.NO	Assay	Mother Plant	*Invitro* Plant
1	Protein	12.5±0.20 (µg/gfwt)	23.9±0.93(µg/gfwt)
2	Phenolic	45.9±0.56(µg/gfwt)	62.61±0.48(µg/gfwt)
3	Flavonoid	0.23±0.02(µg/gfwt)	0.87±0.011(µg/gfwt)
4	Total Antioxidant	2.33±0.26(µg/gfwt)	3.97±0.21(µg/gfwt)
5	Tannin	0.15±0.02(µg/gfwt)	0.12±0.01(µg/gfwt)
6	Flavanols	15.21±0.80(µg/gfwt)	19.21±0.45(µg/gfwt)
10	FRAP	278.89±0.08(µmol/gfwt)	387.54±0.02(µmol/gfwt)
11	DPPH	25.67%	30%
12	Chelating	18.78%	38%

In vitro regenerated plants also exhibited enhanced DPPH and chelating activity as compared to the mother plant (Table 1, Figure 1). A significant amount of increase was obtained in proline content estimated to be present in tissue cultured plant (97.66±0.09 µg/gfwt) as compared to that found in the mother plant (55.67±0.11µg/gfwt). Flavanol and phenols present also exhibited an enhanced production in micropropagated plants as compared to mother plants.

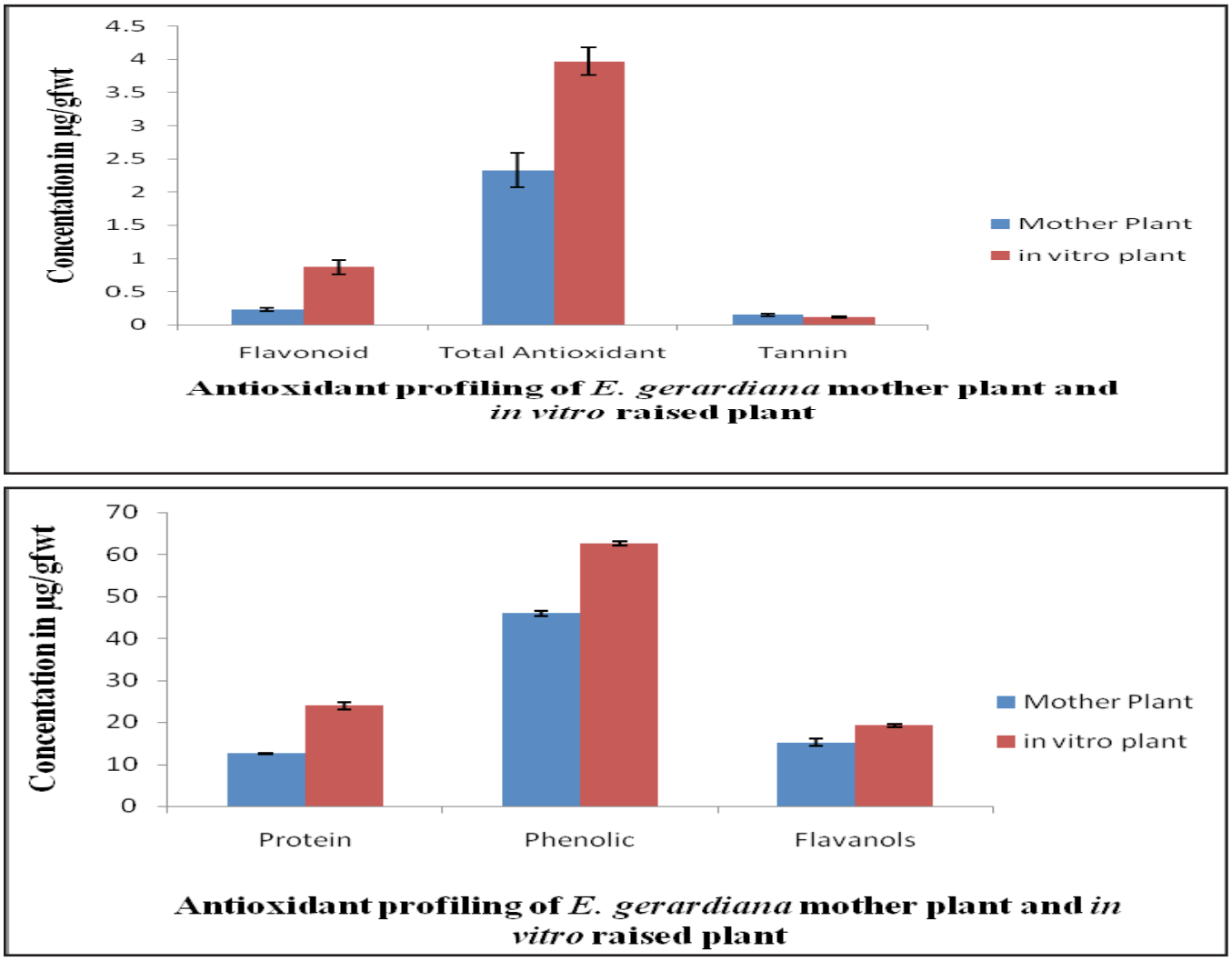

Figure 1: Comparative analysis of antioxidant profiling of mother plant and *in vitro* raised *Ephedra gerardiana*

Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Free radical scavenging and antioxidant activity of polyphenolics (e.g. flavonoids, phenolic acids) mainly depends upon the number and position of hydrogen donating hydroxyl groups on aromatic rings of the phenolic molecules, and is also affected by other factors, such as glycosylation of glycones, other H- donating groups (-NH, -SH) etc. For example flavonols such as quercetin, myricetin and kaempferol, containing multiple hydroxyl groups had higher antioxidant activity than their glycosides such as rutin and astragalin. Thousands of such phenolic compounds are present in medicinal herbs with similar properties (Iwashina, 2000; Xiao et al., 2000).

However, H_2O_2 levels were found to be much higher in the mother plant (28.95±0.10µg/gfwt) as compared to tissue-cultured plant (12.25±0.13µg/gfwt). Similarly, the amount of tannins produced were also higher in the mother plant (0.15±0.02 µg/gfwt) as compared to that of tissue cultured plant (0.12±0.01µg/gfwt) **(Table1)**. Besides the enhanced production of the above-mentioned metabolites in vitro regenerated plants also exhibited enhanced DPPH and chelating activity. Comparatively enhancement in chelating activity was much more (18.78% in mother plant and 30% in tissue cultured plant) as compared to DPPH activity (25.67% in mother plant and 30% in tissue cultured plant).

GC-MS ANALYSIS

GC-MS analysis of methanolic plant extract obtained from mother and *in vitro* raised plant *Ephedra gerardiana* revealed the presence of 34 and 43 phytochemical compounds in mother and tissue cultured plant respectively (Table 2, Table 3, Figure 2, 3).

Table 2: Identified compound, Area and Retention time of peak of *in virto* plant extract of *E. gerardiana.*

S.No	R.Time	Area	Area%	Name
1	5.617	594740	0.70	Undecane
2	7.597	400289	0.47	2,3-Dihydro Benzofuran
3	8.975	237535	0.28	2-Methoxy-4-vinylphenol
4	9.648	772770	0.91	Borinic acid, diethyl-
5	9.839	24455859	28.93	Morpholine, 3-Methyl-2-Phenyl
6	10.348	1620107	1.92	Benzyl alcohol, alpha.-(1-(dimethyl aminoethyl)
7	12.179	609522	0.72	Phneol, (1,1-Dimethylethyl)-4-Methoxy
8	12.413	255095	0.30	Benzothiazole
9	12.506	167264	0.20	Diethyl Phthalate
10	12.636	1051674	1.24	3A(1H)-Azulenol
11	13.026	631623	0.75	3,4-Altrosan
12	13.525	146766	0.17	2-Propenoic acid, tridecyl ester
13	13.644	328408	0.39	1-tetraadecanamine,N,N-dimethyl
14	14.150	544146	0.64	6-Isopropenyl-4,8A-dimethyl
15	14.443	344510	0.41	d-Glycero-d-ido-heptose
16	14.588	593172	0.70	8-Hydroxy-4-isopropylidene-7-methyl
17	14.846	164065	0.19	Morpholine, 3,4-Dimethyl-2-phenyl
18	15.101	267223	0.32	Oxirane, hexadecyl-
19	15.334	281088	0.33	Cyclo hexane carboxamide, N-(4-fluorophenyl)-
20	15.541	296812	0.35	2,4,6, tribromoaniline
21	15.625	153891	0.18	Kauren-19-yl-acetate
22	15.823	1255476	1.48	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-met
23	15.985	6314866	7.47	Hexadecanoic acid, methyl ester
24	16.338	162905	0.19	9-Octadecenoic acid
25	16.449	280943	0.33	1,2-Benzenedicarboxylic acid
26	16.823	534221	0.63	1-Methyl-5-Phenylbiocyclo heptane
27	17.500	232986	0.28	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)

S.No	R.Time	Area	Area%	Name
28	17.692	17293734	20.45	9-Octadecenoic acid, methyl ester,
29	17.818	13660639	16.16	2-Hexradecen-1-ol, 3,7,11,15-Tetramethyl
30	17.903	3724332	4.41	6,9-Octadecadienoic acid
31	18.079	616433	0.73	Spiro(2,5)octane, 5,5-dimethyl-4-(3-oxobutyl)
32	18.168	288757	0.34	6,6-Dimethyl-2-vinyl-bicyclo [3,1]Hept-2-ene
33	18.322	218873	0.26	Widdrol
34	18.467	1228157	1.45	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
35	19.258	121598	0.14	Methyl 5,11,14-eicosatrienoate
36	19.345	529666	0.63	2H-Pyran, 2-(2-heptadecynyloxy) tetra hydroxy
37	19.425	183016	0.22	9-octadecensaeure1,2 hydroxy
38	19.711	727006	0.86	Eicosanoic acid
39	22.055	1118109	1.32	Docosanoic acid
40	22.467	531197	0.63	1,2-Benzenedicarboxylic acid,
41	25.230	625656	0.74	Tetracosanoic acid, methyl ester
42	26.390	178720	0.21	Cyclopropane butanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)m
43	35.067	803110	0.95	Stigmast-5-en-3-ol

Table 3: Identified compound, Area and Retention time of peak of mother plant extract of *E. gerardiana.*

Peak	Retention Time	Area	Area%	Name
1	5.443	468576	0.73	2-cyclopenten-1-one
2	5.773	4782810	7.41	2(5H)-furanone, 3-hydroxy-4,5-dimethyl-
3	6.342	219112	0.34	2-acetyl-2-hydroxy-.gamma.-butyrolactone
4	6.491	2988514	4.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
5	6.945	658078	1.02	5-Methoxypyrrolidin-2-one
6	7.369	527000	0.82	But-3-enoic acid methyl ester
7	7.719	889217	1.38	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
8	9.766	802151	1.24	5-oxo-pyrrolidine-2-carboxylic acid methyl
9	11.266	374538	0.58	1-(3-methyl-2-pyrazinyl)-1-ethanone
10	11.849	347529	0.54	Hexanedioic acid, dimethyl ester
11	12.961	1183648	1.83	.Alpha.-d-galactopyranoside, methyl
12	13.152	825985	1.28	.Alpha.-d-glucopyranoside, .alpha.-d-glucopyranosyl
13	14.267	337403	0.52	Tetradecanoic acid
14	14.375	224738	0.35	Octahydro-2(1H)-quinolinone
15	14.466	496589	0.77	D-Glycero-d-ido-heptose
16	15.312	331864	0.51	2-Pentadecyn-1-ol
17	15.500	492256	0.76	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
18	15.984	5005994	7.76	Hexadecanoic acid, methyl ester
19	16.349	3074394	4.77	N-Hexadecanoic acid
20	16.450	367203	0.57	Dibutyl phthalate
21	17.517	282054	0.44	N-Nonadecanol-1
22	17.690	15905695	24.66	9-Octadecenoic acid, methyl ester, (E)-
23	17.815	5731446	8.88	Phytol
24	17.903	3731188	5.78	Octadecanoic acid, methyl ester
25	18.073	6935309	10.75	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
26	18.471	1212492	1.88	Methyl 9-cis,11-trans-octadecadienoate

Peak	Retention Time	Area	Area%	Name
27	19.342	800880	1.24	Cyclopentanone, 2-(2-nitro-2-heptenyl)-
28	19.714	741856	1.15	Eicosanoic acid, methyl ester
29	22.058	1626495	2.52	Docosanoic acid, methyl ester
30	22.473	596605	0.92	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
31	23.650	180728	0.28	Triacontanoic acid, methyl ester
32	25.233	814381	1.26	Tetracosanoic acid, methyl ester
33	32.953	308242	0.48	Ergost-5-en-3-ol, (3.beta.,24r)-
34	35.063	1246838	1.93	Stigmast-5-en-3-ol, (3.beta.)-

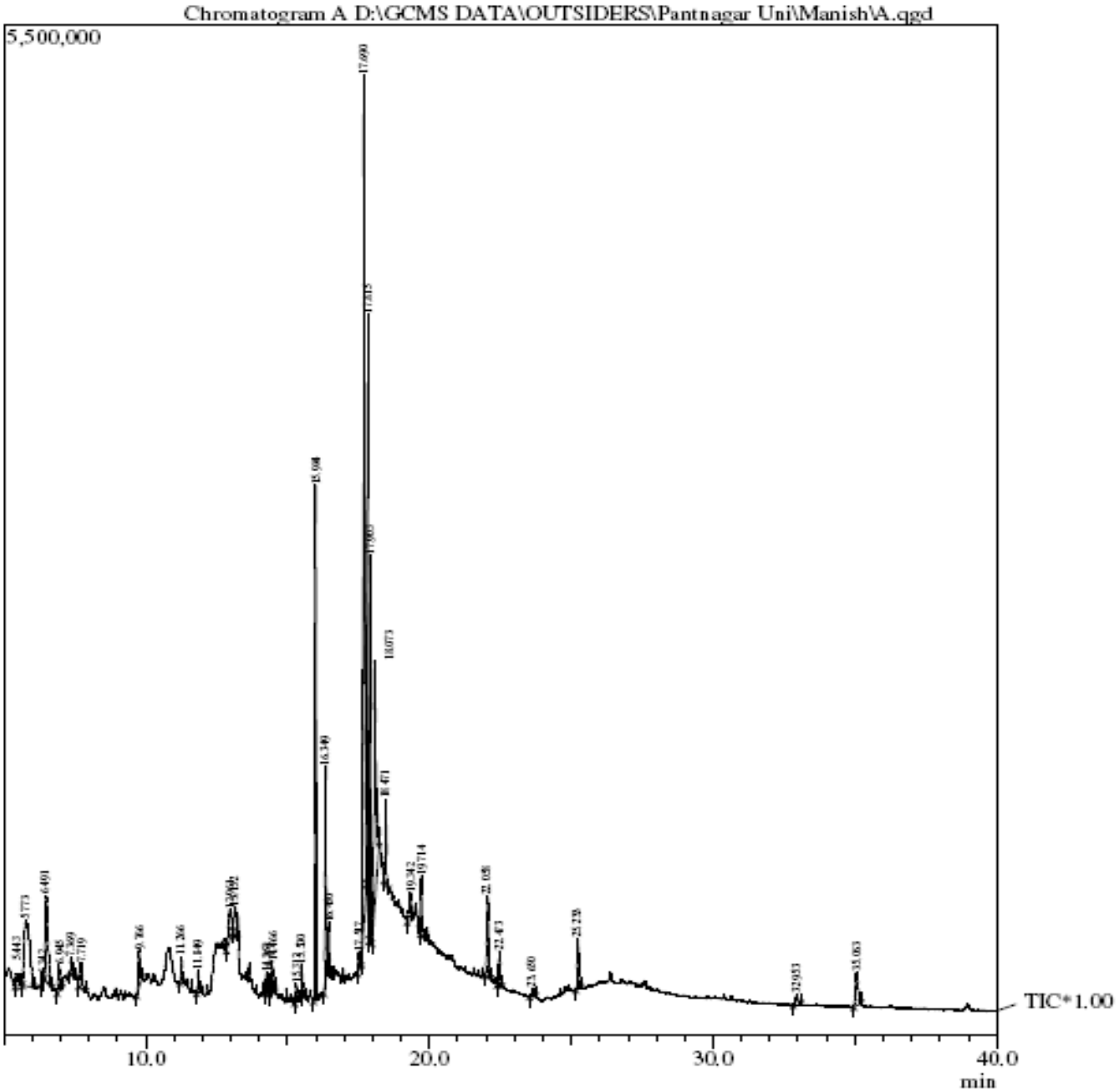

Figure 2: GC-MS chromatography of methanolic extract of Mother Plant of *Ephedra gerardiana*

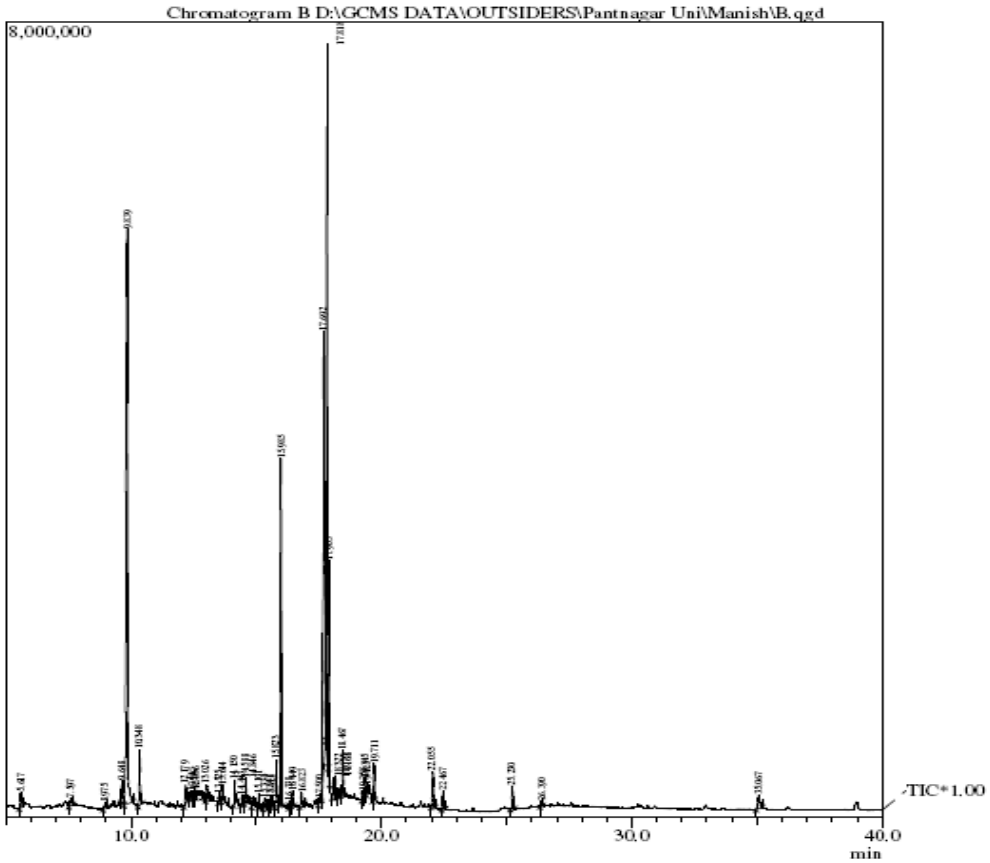

Figure 3: GC-MS analysis of methanolic extract of *in vitro* raised plant of *Ephedra gerardiana.*

In extract of mother plant major phytochemical compounds identified were 9-Octadecenoic acid, methyl ester (24.66%), 9-octadecenoic acid, 1,2,3 propanetriyl ester (10.75%), Phytol (8.8%) and 2(5H)- Furanone, 3-hydroxy-4,5-dimethyl (7.41%) with retention time 17.69,18.07,17.81 and 5.77 respectively. Among these 9-octadecenoic acid was most abundant. Compound such as hexanedioic acid (0.54%), Octahydro-2(1H)-quinolinone (0.35%), Ergost-5-EN-3-ol (0.48%) and 2-Acetyl-2-hydroxy, gamma-butyrol acetone (0.34%) with retention time 11.84, 14.37, 32.95 and 6.34 respectively were found to be present in comparatively lesser amount into plant extract of mother plant. On the other hand extract prepared from *in vitro* raised plant was found to contain Morpholine (28.93%),

Table 4: Biological activities of the identified compound in methanolic plant extract of mother plant *Ephedra gerardiana.*

S.No	Compound	Biological activity	Reference
1.	1,3,5-Triazine-2,4,6-triamine	Antibacterial activity	Baldaniya, 2010
2.	4,5-diaryl-3-hydroxy-2(5*H*)-furanones	Antioxidant	Bailey et al., 2007
3.	Butyric acid	Anticancer	Prasad, 1980
4.	5-(hydroxymethyl, azidomethyl,	Antiviral (HSV-1)	Shiau et al., 1980
5.	L-Histidine (H_2his) and (*S*)-(−)-2-Pyrrolidone-5-carboxylic Acid (H_2pyrrld)	Antibacterial and Antifungal Activities	Nomiya et al., 2000
6.	4-oxo-butenoic acid	Treatment of breast cancer	Miles et al., 2004
7.	5-Hydroxymethylfurfural	food additives	Abraham et al., 2011
8.	2-Pyrrolidone-5-carboxylic acid, methyl ester	Antiviral	Seeger et al., 2003
9.	1-(6-Methyl-2-pyrazinyl)-1-ethanone	volatile flavor components	Ba et al., 2010
10.	alpha.-D-Glucopyranoside, alpha-D-glucopyranosyl	pancreas protective, antioxidant and hepatoprotective effects	Kumar et al., 2013

11.	Myristic acid	Help in Cellular Transforming activity	Buss et al., 1984
12.	2-Amino-3-Hydroxypyridine	Used in Cosmetics	Belsito et al., 2014
13.	alpha.-D-Glucopyranoside, .alpha.-D-glucopyranosy	Enhanced glycemic control, pancreas protective, antioxidant and hepatoprotective effects	Kumar et al., 2013
14.	1,2-benzenedicarboxylic acid	Insecticidal, pesticide, Antitumor	Jain et al., 2012
15.	n-Hexadecanoic acid	Antioxidant,Hypocholesterolemic Nematicide, Pesticide, Anti androgenic, Flavor Hemolytic,5-Alpha reductase inhibitor	Jananie et al., 2011
16.	Palmitic acid	Antitumor activity	Harada et al., 2002
17.	Dibutyl phthalate	Dibutyl phthalate	Roy et al., 2006
18.	1-Docosanol	Antiviral activity	Katz et al., 1991
19.	Methyl elaidate	Useful in the transformation of fatty acids	Dhopeshwarkar and Mead, 1962
20.	Phytol	Activate the Nuclear Receptor RXR	Kitareewan et al., 1996
21.	Oleic Acid	Treatment of Skin Papillomas	Gustafsson et al., 2004
22.	Methyl linoleate	Antioxidant Activity	Hopia and Heinonen, 1999
23.	14-Methyl-8-hexadecenal Z	Antipodes of the khapra beetle pheromone	Mori et al., 1982
24.	Mono(2-ethylhexyl) phthalate	Testis toxicity	Dalgaard et al., 2001
25.	Tetracosanoic acid, methyl ester	Antibacterial and insecticidal activity	Khan Nadeem et al., 2012
26.	Ergost-5-EN-3-OL, (3, BETA)	Liver diseases, Jaundice, atherosclerosis	Kumar et al., 2014
27.	Stigmast-5-en-3β-ol	Antihyperlipidemic and Anti-tumor agent	Iyer and Patil, 2012

9-Octadecenoic acid, methyl ester (20.45%), hexadecanoic acid, 2-hexadecen-1-ol (16.16%) and methyl ester (7.47%) by way of retention time 9.8, 17.69, 17.81 and 15.98 respectively as the major compounds. Compounds such as Cyclopropanebotanoic acid (0.21%), 9-octadecensaeure, 12-hydroxy-methylester (0.22%), Methyl 5,11,14-eicosatrienoate (0.14%) and Diethyl Phthalate (0.20%) with retention time 26.39,19.42, 19.25 and 12.50 respectively were present in small amounts. The compounds identified were found to belong to different classes such as steroids, acid, phytosterols, alkaloids, ketones, ester, etc.

Table 5: Biological activities of the identified compound in methanolic plant extract of *in vitro* plant *Ephedra gerardiana*.

S.No.	Compound	Biological activity	Reference
1.	2-Methoxy-4-vinyl phenol	Antioxidant activity	Jeong et al., 2011
2.	Morpholine, 3-methyl-2-phenyl-	biosurfactant activity	Shubhrasekhar et al., 2013
3.	Methylephedrin	Treatment of cough and cold	Imai et al., 2010
4.	Butyl hydroxyanisole	Use as an Antioxidant	Williams et al., 1999
5.	1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl	Cytotoxic activity	Jalill et al., 2014
6.	Diethyl phthalate	Antimicrobial Antifouling	Miller et al., 2004
7.	3,4-Altrosan	Bacteriostat Fungicide	Ravikumar et al., 2012
8.	9-Octadecenoic acid (z)	Antiandrogenic, Lipoxygenase inhibitor, Allergenic, Flavour, Hypocholesterolemic, Insectifuge, Irritant, Percutaneo-stimulant,	Omotoso et al., 2014
9.	Trehalose	Preservation of Membranes in Anhydrobiotic Organisms:	Crowe et al., 1983

S.No.	Compound	Biological activity	Reference
10.	Spathulenol	Antibacterial and Antioxidant Activities	Wang et al., 2011
11.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Cancer-preventive	Ponnamma and manjunath, 2012
12.	Cyclohexanecarboxamide, N-(4-fluorophenyl)	Chelating extractants	Özer et al., 2009
13.	n-Hexadecanoic acid	Antiinflammatory	Aparna et al., 2012
14.	Stearic acid	lowering plasma cholesterol levels	Bonanome and Grundy, 1988
15.	9-tetradecenal	Neurotransmitter	(Mathur and Kamal, 2011)
16.	Oleic Acid	Treatment of Skin Papillomas	(Gustafsson et al., 2004)
17.	9,12-Octadecanoic acid (z, z)-methyl ester	Anti-inflammatory, Hypercholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamine, Anti-eczemic, Anti-acne, 5-Alpha reductase inhibitor, Antiandrogenic, Anti-arthritic, Anti-coronary	(Omotoso et al., 2014)
18	1,2-benzenedicarboxylic acid	Insecticidal, pesticide, antitumor	(Jain et al., 2012)

Irrespective of the amount or concentration (high or low) in which these compounds were found to be present, almost all these compounds have been reported to possess some pharmacological or biological activity (Table 4, 5, 6). Nearly, all the compounds identified have been reported to exhibit antibacterial, antifungal, antioxidant and antiviral activities against several pathogenic bacteria, fungal and viral species (Hopia and Heinonen, 1999; Seeger et al., 2003; Bailey et al., 2007; Jananie et al., 2011; Khan et al., 2012). Besides the antioxidant activity of n-hexadecanoic is also reported to possess hypocholesterolemic and anti-androgenic activity (Jananie et al., 2011). The antioxidant property is one of the crucial properties possessed by this plant and this study focus on its compounds such as Methyl linoleate, 2-Methyl-4-vinyl phenol, 4,5-diaryl-3-hydroxy-2(5H)-furanones, Spathulenol and n hexadecanoic acid identified to be present in both plant extract of *E. gerardiana* have been reported to possess potent antioxidant activity (Hopia and Heinonen, 1999; Bailey et al., 2007; Okada et al., 2010; Jananie et al., 2011; Wang et al., 2011). Identified compounds Butyric acid, 4-oxo-butenoic acid, 1,2-benzene dicarboxylic acid, Palmitic acid, stigmast-5-en-3β-ol have been reported as anticancer and are utilized in the treatment of breast cancer and antitumor agent (Prasad et al., 2001; Harada et al., 2002; Jain et al., 2012; Patil and Paikrao, 2012). Compounds like Cyclohexanecarboxamide, N (4-fluorophenyl), 9-tetradecenal, Morpholine, 3-methyl-2-phenyl have been reported to possess chelating and biosurfactant activity respectively (Ozer et al., 2008; Shubhrasekhar et al., 2013).

Table 6: Total compound present in mother and *in vitro* plant of *Ephedra gerardiana.*

S. No.	Chemical Compound	Mother plant	*In vitro* plant
1	Undecane		+
2	2,3-Dihydro-benzofuran		+
3	2-methoxy-4-vinylphenol		+
4	Borinic acid, diethyl		+
5	Morpholine, 3-methyl-2-phenyl		+
6	Benzyl alcohol, alpha-(1-(dimethylamino) ethyl)		+
7	Phenol, (1,1-Dimethylethyl)-4-Methoxy		+
8	Diethyl Phthalate		+
9	3A(IH)-Azulenol 2,3,,4,5,8,8A-Hexahydro-6,8A-Di		+
10	3,4-Altrosan		+
11	2-Propenoic acid, tridecyl ester		+
12	1-Tetradecanamine, N-N-dimethyl		+
13	6-isopropenyl-4-8 A-dImethyl-1,2,3,5,6,7,8,8A-OCT		+
14	d-Giycero-d-ido-heptose	+	+
15	7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5,3,1]		+

16	Morpholine 3,4-dimethyl-2-phenyl-(2R Trans)		+
17	Oxirane, hexadecyl		+
18	Cyclohexanecarboxamide,N-(4-fluorophenyl)		+
19	Kauren-9-yl-acetate		+
20	1H-Cycloprop(e)azulen-7-ol,decahydro-1,1,7-trimethyl-4-methyl		+
21	Hexadecancic acid, methyl ester	+	+
22	9-Octadecenoic acid (z)	+	+
23	1,2-Benzenedicarboxylic acid, Bis (2-Methoxy)		+
24	1-Methyl-5-Phenylbicyclo(3,2,0) Heptane		+
25	9-Octacecenoic acid, methyl ester, (E)		+
26	2-Hexadecen-1-ol,3,7,11,15-Tetramethyl-(R-(R		+
27	6,9-Octadecadienoic acid, methyl ester		+
28	6,6-Dimethyl-2-viniyl-bicyclo(3,1,1) Hept-2-ene)		+
29	9.12-Octadecadienoic acid (Z,Z)-methyl ester		+
30	Methyl 5,11,14-eicosatrienoate		+
31	2H-Pyran,2-(2-heptadecynyloxy) tetrahydro		+
32	9-Octadecensaeure,12-hydroxy-methylester		+
33	Eicosanoic acid, methylester	+	+
34	Docosanoic acid, methylester	+	+
35	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	+	+
36	Tetracosanoic acid, methyl ester	+	+
37	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)		+
38	Stigmast-5-en-3-ol,(3,beta)	+	+
39	2(5H)-Furanone, 3-hydroxy-4-5-dimethyl	+	
40	2-acetyl-2-hydroxy-gamma-butylrolactone	+	
41	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methy	+	
42	5-methoxypyrrolidin-2-one	+	
43	But-3-enoic acid methyl ester	+	
44	2-farancarboxaldehyde,5-(hydroxymethyl)	+	
45	5-oxo-pyrrolidine-2-carboxylic acid methyl	+	
46	1-(3-methyl-2-pyrazinyl)-1-ethanone	+	
47	Hexanedioic acid, dimethyl ester	+	
48	Alpha-D-Galactopyranoside methyl	+	
49	Alpha-D-Glucopyranoside, alpha-D-glucopyranosy;	+	
50	Tetradecanoic acid	+	
51	octahydro-2(1H)-quinolinone	+	
52	2-Pentadecyn-1-ol	+	
53	n-hexadecanoic acid	+	
54	Dibutyl phthalate	+	
55	Phytol	+	
56	Octadecenoic acid, methyl ester	+	
57	9-Octadecenoic acid, 1,23-propanetriyl ester,(E,E,E)	+	
58	Methyl 9-cis,11-trans octadecadienoate	+	
59	Cyclopentanone,2-(2-nitro-2-heptenyl)	+	
60	1-2-Benzenedicarboxylic acid, Mono (2-ethylhexyl) ester	+	
61	Triacontanoic acid, methyl ester	+	
62	Ergost-5-en-3-ol.(3,beta 24R)	+	

Besides these, oleic acid is known for treating skin papillomas and n-hexadecanoic acid provides an anti-inflammatory function (Gustafsson et al., 2004; Aparna et al., 2012). Some studies have reported that the anti-androgenic, allergenic and hypercholesterolemic activity possessed by 9-octadecanoic acid and 1-(6-Methyl-2-pyrazinyl)-1-ethanone works as potent volatile flavour components, whereas 9-tetradecenal is reported to work as Neurotransmitter (Iwahashi et al., 1991; Mathur and Kamal, 2011).

CONCLUSION:

Results obtained provide an insight into the potential antioxidant activity of *Ephedra*. Also, the identification of several phytochemical compounds found to be present in extracts of mother and tissue culture-raised plants with several biological properties reveals the immense medicinal potential of the plant. Although tissue-cultured plants contain more phytochemicals than the mother plants, some of the phyto-compounds are found to be more prominent in the mother plant rather than the tissue-cultured ones. The anti-oxidant activities are found to be more ubiquitous in the case of tissue cultured plants.

The results of the present study indicate that there is a great potential of using *Ephedra gerandium* in the field of disease treatments and medicines, but also its antioxidant

property can provide a better food lifestyle of having the richer source of proper nutrition. It is a giver trend nowadays to focus not only on the best clothing and sheltering but also to ensure to have the best nutrition supplements in regards of avoiding any specific medications related to any disease prevalent or not. It is not a hidden fact that the morbidity rates have been increased in the past few decades, which brings special attention in the field of the antioxidant property providing species of any kind. Antioxidants remove free radicals and activated oxides from the human body which are broadly responsible for developing several diseases and abnormalities which can be as serious as mutations and cancers. Moreover, if diseases are not concerned, antioxidants have been linked with inner body cleansing and anti-aging properties, which are enough for researchers to make their crude focus in this particular area of study and research. Also, each and every part of *Ephedra gerandium* has some therapeutic and medicinal property and its abundance subsidized more to use this plant species in the concern of humankind benefit.

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