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Volatile Constituents of *Cistanche tubulosa* and Their Antioxidant and Antimicrobial Potentials

Arafa Musa[1,2*], Khaled F. El-Massry[3,4], Ahmed H. El-Ghorab[3,4], Amr Farouk[4], Hazim M. Ali[3], Mohamed A Abdelgawad[5,6], Ibrahim A. Naguib[7] and Ehab M. Mostafa[1,2]

[1]*Department of Pharmacognosy, College of Pharmacy, Jouf University, Sakaka, Aljouf 72341, Saudi Arabia*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, 11371, Egypt*

[3]*Chemistry Department, College of Science, Jouf university, Sakaka, Aljouf 72341, Saudi Arabia*

[4]*Flavour and Aroma Department, National Research Center, Dokki, Giza, Egypt*

[5]*Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf university, Sakaka, Aljouf 72341, Saudi Arabia*

[6]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef university, Beni-Suef 62514, Egypt*

[7]*Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*

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Abstract: The hydrodistilled volatile constituents of *Cistanche tubulosa* (commonly known as Desert Ginseng) have been chemically and biologically investigated. Based on the retention times and mass fragmentation of the obtained GC-MS chromatogram, 106 individual components which representing ≈ 99.29 % of the total volatile constituents have been identified. The major compounds (66.57% of the total composition) were identified as hexanal (15.98%), *trans*-sabinyl acetate (12.22%), allo-aromadendrene (9.30%), nonanoic acid (6.66%), 3*Z*-hexeny-2-methyl butanoate (6.09%), valeranone (5.25%), (*E*, *E*)-α-Farnesene (3.18%), α-pinene (3.06%), linalool isovalerate (3.03%) and α-humulene (1.8%). Estimation of the antioxidant activity of EO showed promising effect at 80 µg/mL concentration, it exerted 62.40, 863.29 and 62.72 % inhibition compared to TBHQ that showed 78.62, 77.56 and 79.23 % inhibition using DPPH, ABTS and *β*-carotene/Linoleic acid, respectively. The antioxidant activity was pronounced at 80 µg/mL than other concentrations. The volatile constituents showed inhibitory activity against gram positive bacteria ranged from 2.23 mg/100 mL (for *staphylococcus aureus*), and 15.68 mg/100 mL (for *Bacillus cereus*) compared to ciprofloxacin which showed inhibitory activity 0.185, and 0.182 mg/100 mL, respectively. Moreover, the MIC of volatiles towards gram negative bacteria are ranged from 18.35 (*Escherichia coli*) to 31.61 mg/100 mL (*Klebsiella pneumonia*) compared to ciprofloxacin with 0.184 to 0.188 mg/mL respectively. Additionally, the antifungal activity against *candida albicans* was rather promising (4.36 mg/mL).

Keywords: *Cistanche tubulosa;* volatile constituents; antioxidant; antimicrobial; Orobanchaceae. . © 2021 ACG Publications. All rights reserved.

* Corresponding author: E-Mail: akmusa@ju.edu.sa

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1. Introduction

Cistanche tubulosa, family Orobanchaceae, is a perennial parasitic plant, growing in arid areas of Asia and Africa, it has been detected in China, India, Japan, Saudi Arabia (Sakaka desert, Aljouf, KSA) [1]. It possesses various common names in Chinese medicine as Desert Hyacinth, Desert ginseng and Rou Cong Rong, the stem is succulent and fleshy with high water content [1-6]. *Cistanche* is familiar for volatile and non-volatile constituents that may include lignans, phenylethanoid glycosides, oligo- and polysaccharides, alkaloids and iridoids. Due to the great diversity of the phytochemical content and biological activities, *Cistanche* has acquired a high medicinal value in Chinese folk and traditional medicines. Consequently, it has been used as aphrodisiac in case of impotence and infertility, laxative in senile constipation, and found to have neuroprotective effect especially in case of Alzheimer's, Parkinson's and depression, anti-aging, anti-neoplastic, antiplatelet aggregation, antifungal and antibacterial, hepatoprotective, immunostimulant, antioxidant, renal support, and antitumor in colorectal esophageal carcinoma [1, 7-13]. It is also used in treatment of psychroalgia of the knees and back, improvement of immunity and cognitive activities and as antidepressant [14-17]. It has been found to have hypocholesterolemic effect as reported by Shimoda *et al* [18]. The literature survey declared the safe use of *Cistanche* as non-toxic plant for the long run [19]. Although *C. tubulosa* was reputed by the high medicinal values particularly in Chinese traditional medicine, the volatile constituents of which has been scarcely studied, and their chemical compositions haven't been fully characterized. Previous investigation revealed the characterization of 38 components from *C. salsa* essential oil, 25 compounds from *C. deserticola* oil, with the three major components known as methyl 14-methyl pentadecanoate (13.60%), ethyl palmitate (12.40%), and 2,5,6-trimethyloctane (7.61%). The survey also revealed identification of 21 compounds only from the volatile oil *C. tubulosa* [1, 20-22]. Furthermore, the biological activity of the volatile constituents hasn't been fully investigated. Hence, our aim is to identify the chemical compositions of the volatile constituents of *C. tubulosa* flowers and estimate their antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Plant Material

Cistanche tubulosa (Schenk) Hook. f. (Orobanchaceae) was collected in March 2019 from Sakaka desert, Aljouf, KSA. Identification of the plant was done by Mr. Hamdan Al-Hassan, M.Sc. (Camel and Range Research Center), Aljouf, KSA. A voucher specimen (59-CPJU) was archived in the herbarium of the Pharmacognosy Department, Pharmacy College, Jouf University.

2.2. Extraction of the Volatile Constituents

The flowers of *C. tubulosa* were collected in March 2019, carefully washed with running water, and the volatile constituents were extracted by the standard hydro-distillation method with Clevenger apparatus. A 500 g fresh flowers were cut into small pieces and subjected to hydro-distillation for 5 hours until no more yield was produced. the distillates were separated from the aqueous phase by a 500 mL volume separating funnel. NaCl was used to expel the rest of volatile constituents from the aqueous layer by the salting out mechanism. The aqueous phase was shaken several times with CH_2Cl_2 to obtain all distillates. The combined extracts were then filtered through a Whatman filter paper (No.40) after being passed over anhydrous Na_2SO_4 for dehydration. The product was calculated as 0.36 % total volatiles. The obtained constituents were pale yellow liquid with pleasant odor. It was packed in dry clean and tightly closed opaque bottle and kept in dark at 4°C for analysis.

2.3. Gas chromatography and Gas Chromatography - Mass Spectrometry (GC-MS)

The model 6890 of an Agilent gas chromatograph supplied with a 120 m × 0.25 mm i.d. (df= 0.25 µm) cemented phase HP-5MS stuck silica capillary column (Agilent, Folsom, CA) and flame ionization detector (FID) was applied for volatile extract analysis. The temperature of oven, was adjusted from 60 to 240°C at 3°C/min. and kept for 50 min. The carrier gas of linear helium feed ratio was 20 cm/sec. The temperature of injector and detector was 250°C.

The volatile constituents were analyzed by Agilent Technologies model 7890B GC interfaced to Agilent 7000D GC/TQ mass detector (GC/MS), and Agilent 7693A autosampler. Ionization at 70 eV, HP-5MS column (120m x 0.25 mm i.d). The whole process was conducted at 30 cm/s constant velocity of the mobile phase (He) and constant temperature at 250 ºC for both injector and detector. The oven temperature was programmed from 60 to 240 ºC at 3 ºC/min and retained for 50 min.

Concomitant injection of the sample with a solution of homologous n-hydrocarbons (C_8-C_{26}) series under the same conditions were performed determine Kovat's indices values. Identification of the isolated volatiles was done by matching with NIST mass-spectral library data, comparison of Kovat's indices with those of authentic components and with published data. Quantitative determination was carried out according to peak area integration.

2.4. Antioxidant Activity

2.4.1. DPPH Radical Scavenging Assay

The potential antioxidant activity of the obtained volatile constituents was evaluated by the standard DPPH method, the tert-butylhydroquinone (TBHQ) was applied as a standard antioxidant drug. The measurement of absorption was performed at λ_{max} 517 nm on UV-spectrophotometer (HP 8452, UV-VIS), all tests were conducted in triplicates and average of the results was calculated [23, 24].

2.4.2. β-Carotene Bleaching Assay

The standard β-carotene/linoleic acid method was applied for determination of the antioxidant activity of *C. tubulosa* volatile constituents as previously described, relative to the standard antioxidant tert-butylhydroquinone (TBHQ). All tests were measured in triplicates at λ_{max} 470 nm over 60 minutes starting from the 0 minute, and the average of the results was calculated [23, 24].

2.4.3. ABTS Free Radical Assay

The ABTS [2,2`-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), was applied for the antioxidant determination of *C. tubulosa* volatile constituents as described in the literature [25], in comparison to the standard antioxidant tert-butylhydroquinone (TBHQ). All tests were measured in triplicates at λ_{max} 734 nm over 60 minutes starting from the 0 minute, and the average of the results was calculated [26]. The following equation was applied for calculating the free radical scavenging effect in all methods

% inhibition = A (control) - A (test or standard) / A (control) $\times$ 100,

Where, A = Absorbance

2.5. Antimicrobial Assay

2.5.1. Preparation of Microbial Suspensions

Nine strains of pathogenic microorganisms which are regarded as main source of several diseases and food intoxication were selected for antimicrobial assay including *S. aureus, B. cereus E. fecalis*, and *L. monocytogenes* were employed as G+ve bacteria, *E. coli, P. aeruginosa, K. pneumoniae* and *Salmonella typhimurium* as G-ve bacteria. Additionally, *C. albicans* was employed as fungal strain. The quantitative minimum inhibitory concentration (MIC) method was applied for antimicrobial estimation of the volatile constituents of *C. tubulosa*. The bacterial and fungal suspensions were prepared in the suitable

broth media for each (Muller Hinton Sabaroud Dextrose for bacteria and fungi, respectively). Incubation of each strain with the proper media was done for 24 h at 37 °C for bacteria and 28 °C for fungi. Following the incubation period and the serial dilutions of the prepared suspensions, certain dilutions were selected according to 0.5 Mc-Farlandscale standard for the assay. The standard ciprofloxacin and fluconazole were prepared in 100µg/mL and applied as antimicrobial and antifungal drugs, respectively [28-30].

2.5.2. Minimum Inhibitory Concentration Method (MIC)

A microtiter dilution plate quantitative method was applied, where the minimum inhibitory concentration (MIC) method was applied for assessment of the antimicrobial activity of *C. tubulosa* volatile constituents, against certain microorganisms as mentioned. A sterile 96- micro plate wells was used, where 100µL of the respective microorganisms in concentrations of (0.5 Mc-Farland, about 1×108cfu/mL) were separately mixed with the obtained distillate in different concentrations (100 %, followed by two fold serial dilutions). Ciprofloxacin and fluconazole were applied as positive antibacterial and antifungal standards respectively, while DMSO was applied as negative control. The microplate with the mixed contents in each well was incubated for 24 h at ≈37 °C for bacteria and 28 °C for fungi. The plates were then visualized for any growth precipitation of the tested organisms. All experiments were conducted in triplicates and the MIC was calculated as the lowest concentration that inhibited or hindered the growth of the tested microorganisms [27].

3. Results and Discussion

3.1. Analysis of Volatile Constituents

In our study, hydrodistillation of *C. tubulosa* flowers produced 0.36 % pale yellow distillate, with an aromatic fragrant odor and 106 volatile components (table 1) representing 99.29 % of the volatile content. These components were categorized as 5 monoterpenes, 15 sesquiterpenes, 62 light oxygenated compounds representing the largest group compounds and 24 heavy oxygenated compounds. The major components of the volatile constituents were identified as hexanal (15.98%), *trans*-sabinyl acetate (12.22%), allo-aromadendrene (9.30%), nonanoic acid (6.66%), 3*Z*- hexeny-2-methyl butanoate (6.09%), valeranone (5.25%), (*E*, *E*)-α-Farnesene (3.18%), α-pinene (3.06%), linalool isovalerate (3.03%), α-humulene (1.8%), Jasminol (1.58%), 4-hydroxy benzaldehyde (1.56%), Geosmin (1.44%), 3*Z*- hexenyl isobutanoate (1.39%) and geranyl acetone (1.38%). But in previous literature studies results, which were published by Jiang and Tu 2009, only 21 volatile components were identified in the essential oil of *C. tubulosa*, 38 components were also characterized from *C. salsa* essential oil, while 25 compounds were identified from the oil of *C. deserticola*, with three major components (methyl 14-methyl pentadecanoate; 13.60%, ethyl palmitate; 12.40%, and 2,5,6-trimethyloctane; 7.61%) [22, 28]. Identification of the isolated volatiles was done tentatively by matching with NIST mass-spectral library data, furtherly confirmed by comparison of Kovat's indices with those of authentic components as well as with the published data [29-31]. It was found that the calculated KI of identified compounds fall in the KI range of those published in literature. For instance, the calculated and published KI for trans Sabinyl acetate (1287& 1273-1289), aromadendrene (1444& 1430-1450), valeranone (1678, 1668-1679), allo-aromadendrene (1459& 1458-1470), α-Farnesene (1508& 1505-1520), hexenyl-2-methyl butanoate (1230& 1210-1231), α-humulene (1455& 1452-1570), α-pinene (971& 933-982) [32-36]. According to the mentioned KI values, the identified compounds were consistent with those reported in the literature [37-40].

Table 1. Volatile constituents of *C. tubulosa*

Peak #	[a]Conc.%	Calculated [b]KI	[c]KI Data	Compound Name	Type[d]	Identification Methods
1.	3.06	971	933-982	α-pinene	M	KI&MS[e]&St[f].
2.	15.98	975	932-975	Hexanal	LOC	KI&MS&St.
3.	0.01	976	972-1004	Trans pinane	M	KI&MS
4.	0.06	972	952-1019	Hexanoic acid	LOC	KI&MS&St.
5.	0.02	980	974-993	Octene-2-ol, 3E	LOC	KI&MS&St.
6.	0.01	985	982-1002	Pentyl furan	LOC	KI&MS
7.	0.01	987	962-993	Myrcene	M	KI&MS&St.
8.	0.01	994	988-1009	Butyl butanoate	LOC	KI&MS
9.	0.01	996	987-998	Ethyl hexanoate	LOC	KI&MS&St.
10.	0.02	1007	994-1025	Hexyl acetate	LOC	KI&MS&St.
11.	0.05	1014	1013-1020	Heptadienol (2E, 4E)	LOC	KI&MS
12.	0.05	1025	1020-1043	Cyclo-pentanedione (3-methyl-1,2)	LOC	KI&MS&St.
13.	0.05	1051	1042-1056	γ-hexalactone	LOC	KI&MS
14.	0.01	1058	1049-1060	Pentyl isobutanoate	LOC	KI&MS
15.	0.28	1065	1050-1070	Thiophene (2-butyl)	LOC	KI&MS
16.	0.05	1083	1060-1083	Camphenilone	LOC	KI&MS
17.	0.03	1084	1079-1089	Allyl hexanoate	LOC	KI&MS
18.	0.24	1092	1088-1095	Isobutyl tiglate	LOC	KI&MS
19.	0.04	1093	1080-1101	Linalool	LOC	KI&MS
20.	0.18	1096	1096-1110	Dimethyl styrene (2,5)	M	KI&MS
21.	0.06;2	1105	1103-1140	Phenyl propanol (2)	LOC	KI&MS
22.	0.08	1129	1124-1135	Chrysanthenone	LOC	KI&MS
23.	0.19	1138	1128-1140	Ocimene (allo)	LOC	KI&MS
24.	1.39	1141	1130-1142	Hexenyl isobutanoate (3Z)	LOC	KI&MS
25.	0.05	1149	1145-1455	Myrcenone	LOC	KI&MS
26.	0.02	1151	1149-1165	Thujanol (neoiso-3-)	LOC	KI&MS
27.	0.02	1161	1153-1167	Nerol oxide	LOC	KI&MS
28.	0.04	1163	1159-1170	Hexadienol propanoate (2E, 4E)	LOC	KI&MS
29.	6.09	1230	1210-1231	Hexenyl 2-methyl butanoate (3Z)	LOC	KI&MS&St.
30.	0.62	1236	1234-1250	Linalool acetate (tetrahydro)	LOC	KI&MS
31.	0.98	1239	1234-1250	Citronet	LOC	KI&MS
32.	0.01	1242	1236-1244	Carvotanacetone	LOC	KI&MS
33.	0.02	1259	1240-1265	Sabinene hydrate acetate (trans)	LOC	KI&MS
34.	0.04	1269	1264-1285	Phenol (2-(1E)-propenyl-)	LOC	KI&MS
35.	6.66	1271	1267-1273	Nonanoic acid	LOC	KI&MS&St.

Peak #	[a]Conc.%	Calculated [b]KI	[c]KI Data	Compound Name	Type[d]	Identification Methods
36.	0.79	1273	1268-1298	Decen-1-ol (2E)-	LOC	KI&MS
37.	12.22	1287	1273-1289	Sabinyl acetate (trans)	LOC	KI&MS&St.
38.	1.05	1292	1270-1293	Verbinyl acetate (trans)	LOC	KI&MS&St.
39.	0.06	1299	1298-1320	Pinocarvyl acetate	LOC	KI&MS
40.	0.14	1300	1299-1310	Terpinen-4-ol acetate	LOC	KI&MS
41.	0.86	1302	1270-1315	Necrodol acetate (cis-□)	LOC	KI&MS
42.	0.05	1319	1318-1330	Pinanediol (cis-2,3)	LOC	KI&MS
43.	0.34	1321	1282-1325	Verbenol acetate (neo)	LOC	KI&MS
44.	0.95	1326	1305-1332	Myrtenyl acetate	LOC	KI&MS
45.	1.58	1331	1315-1340	Jasminol	LOC	KI&MS&St.
46.	0.15	1334	1330-1350	Menth-1-ene-7-al (3-oxo-p)	LOC	KI&MS
47.	0.06	1337	1333-1441	Hasmigone (E)	LOC	KI&MS
48.	0.16	1339	1334-1442	Linalool propanoate	HOC	KI&MS
49.	0.86	1341	1335-1345	Elemene (δ)-	S	KI&MS
50.	0.95	1342	1339-1350	Carvyl acetate (trans)	LOC	KI&MS
51.	0.01	1344	1342-1370	Verbenol acetate	LOC	KI&MS
52.	0.02	1347	1343-1367	Piperitol acetate (trans)	LOC	KI&MS
53.	1.56	1353	1353-1355	Hydroxybenzaldehyde (4)	LOC	KI&MS&St.
54.	0.06	1360	1301-1370	Undecanol (2E)	LOC	KI&MS
55.	0.03;2	1366	1366-1371	Piperitenone oxide	LOC	KI&MS
56.	0.05	1368	1367-1373	Furfuryl hexanoate	LOC	KI&MS
57.	0.25	1375	1373-1385	Linalool isobutanoate	HOC	KI&MS
58.	0.06	1380	1377-1380	Ethyl-(4E)-decenoate	LOC	KI&MS
59.	0.14	1387	1382-1387	Hexyl hexanoate	HOC	KI&MS
60.	0.01	1390	1385-1398	Myrtanol acetate (trans)	LOC	KI&MS
61.	0.10	1392	1389-1398	Damascene (Z) -β	LOC	KI&MS
62.	0.08	1395	1399-1407	Neptalactone (4aα, 7α, 7aβ)	LOC	KI&MS
63.	0.5	1398	1393-1405	Vanillin	LOC	KI&MS&St.
64.	1.44	1399	1399-1402	Geosmin	LOC	KI&MS
65.	0.45	1404	1400-1410	*n*-Tetradecane	M	KI&MS&St.
66.	0.74	1409	1407-1440	Decyl acetate	HOC	KI&MS
67.	0.06	1411	1409-1420	Gurjunene (α)	S	KI&MS.
68.	0.08	1420	1417-1460	Ethyl-(2E)-decenoate	LOC	KI&MS&St.
69.	0.56	1433	1430-1450	Isoamyl 3-(2-furan) propionate	HOC	KI&MS
70.	0.05	1434	1417-1445	Caryophyllene (E)	S	KI&MS&St.
71.	0.53	1438	1420-1450	Cascarilladiene	S	KI&MS
72.	0.92	1440	1438-1460	Methyl butyl benzoate	LOC	KI&MS&St.
73.	2.80	1444	1430-1450	Aromadendrene	S	KI&MS&St.
74.	1.8	1455	1452-1570	Humulene (α)	S	KI&MS&St.
75.	1.38	1456	1453-1570	Geranyl acetone	LOC	KI&MS&St.

Peak #	[a]Conc.%	Calculated [b]KI	[c]KI Data	Compound Name	Type[d]	Identification Methods
76.	9.30	1459	1458-1470	Aromadendrene (allo)	S	KI&MS&St.
77.	3.03	1470	1466-1495	Linalool isovalerate	HOC	KI&MS
78.	0.26	1473	1465-1483	Jasmone lactone	LOC	KI&MS
79.	0.27	1474	1472-1515	Terpenyl isobutanoate (α)	HOC	KI&MS
80.	0.01	1479	1474-1499	Acoradiene (-10-epi-β-)	S	KI&MS.
81.	0.25	1483	1481-1499	Curcumene (γ)	S	KI&MS&St.
82.	0.04	1485	1481-1520	Menthyl lactate	HOC	KI&MS
83.	0.14	1490	1487-1516	Ionone (E)-β-	LOC	KI&MS
84.	0.42	1491	1486-1515	Menthyl lactate (iso)	HOC	KI&MS
85.	0.05	1496	1494-1523	Maltyl isobutyrate	LOC	KI&MS
86.	0.01	1498	1496-1524	Valencene	S	KI&MS
87.	0.39	1504	1501-1525	Aciphyllene	S	KI&MS
88.	3.18	1508	1505-1520	Farnesene (E, E)-α-	S	KI&MS&St.
89.	1.07	1514	1513-1518	Cadinene (γ)	S	KI&MS&St.
90.	0.15	1518	1514-1520	Italicene epoxide (iso)	HOC	KI&MS
91.	0.18	1520	1518-1526	Silphiperfolan-6-β-ol (7-epi-)	HOC	KI&MS
92.	0.06	1522	1519-1528	Dihydroagarofuran (cis)	HOC	KI&MS
93.	0.59	1524	1520-1529	Ionone (6-methyl-α-)	HOC	KI&MS
94.	0.34	1532	1529-1535	Kessane	HOC	KI&MS
95.	0.30	1537	1533-1540	Cadina-1,4-diene (trans)	S	KI&MS
96.	0.20	1539	1533-1545	Cubebol (10-epi)	HOC	KI&MS
97.	0.02	1540	1537-1546	Cadinene (α)	S	KI&MS
98.	0.03	1545	1539-1549	Copaen-11-ol (α)	HOC	KI&MS
99.	0.56	1549	1547-1555	Elemol	HOC	KI&MS&St.
100.	0.02	1638	1632- 1638	Isoborneol (8-isobutyryloxy)	HOC	KI&MS
101.	0.53	1647	1639-1455	Aromadendrene epoxide (allo)	HOC	KI&MS
102.	0.08	1648	1639-1650	Cedrenal (1,7-diepi-α)	HOC	KI&MS
103.	0.12	1649	1467-1654	Methyl jasmonate (Z)	HOC	KI&MS
104.	0.86	1662	1660-1675	Intermedeol (neo)	HOC	KI&MS
105.	1.24	1675	1668-1683	Bisabolol (epi-β-)	HOC	KI&MS&St.
106.	5.25	1678	1668-1679	Valeranone	HOC	KI&MS&St.
	Total			99.29		

[a]Conc.%: the percent of concentrations based on peak area integration.
[b]KI Confirmed by comparison with Kovat's index on DB5 column (Adams, 2017).
[c]KI data -Kovats index data reported in plants essential oils on DB5 (Ref. 31-40) and non-polar column (www.webbook.nist.gov)
[d]M, monoterpene; LOC, light oxygenated compounds; HOC, heavy oxygenated compounds.
[e]Tentative identification by comparison with data obtained from NIST mass spectra library.
[f]Confirmed by comparison with mass spectrum of authentic compound.

3.2. Results of Antioxidant Assay

DPPH, ABTS and *β*-carotene assays were applied to investigate the antioxidant activity of *C. tubulosa* volatile constituents, the results exhibited reliable antioxidant activity of the tested volatiles (Table 2). The current study indicated that the scavenging ability of *C. tubulosa* volatile constituents at various concentrations (μg/mL) ranged from 26.08 % to 62.40 % for the DPPH assay, while from 25.71 to 63.29% and 27.31 to 62.72% at (20-80) μg/mL for the ABTS and *β*-carotene testing systems, respectively, compared to the standard TBHQ antioxidant drug, that showed 43.15 to 78.62%, 41.32 to 77.56% and 42.21 to 79.23% at (20-80) μg/mL for the DPPH, ABTS and *β*-carotene testing systems, respectively. The promising antioxidant activity may be attributed to the presence of highly active complex mixture in the distillate as allo-aromadendrene (9.3%), valeranone (5.25%), (E, E)-α-Farnesene (3.18%) and α-pinene (3.06%) which were supposed to have pronounced impact on the antioxidant activity. According the literature survey, the target biological activity of the volatile constituents may be attributed to the existing mixture of terpenoid and phenolic components, which known to have antimicrobial and antioxidant activities and can enhance or synergizes the target activities [32].

Table 2. Antioxidant activity of hydro-distilled (HD) *C. tubulosa* volatile constituents determined by DPPH, ABTS and by β carotene/ Linoleic acid assays compared to the synthetic antioxidant TBHQ

Sample concentration	**% Inhibition by DPPH**		**% Inhibition by ABTS**		**% Inhibition by *β* carotene/ Linoleic acid**	
	EO	TBHQ	EO	TBHQ	EO	TBHQ
20 (μg/mL)	26.08 ± 1.8	43.15 ± 2.1	25.71 ± 1.9	41.32 ± 2.0	27.31 ± 1.9	42.21 ± 2.1
40 (μg/mL)	39.56 ± 2.0	64.41 ± 1.8	38.43 ± 2.0	63.63 ± 1.9	38.02 ± 2.0	65.46 ± 2.2
60 (μg/mL)	51.22 ± 2.1	71.47 ± 2.0	52.14 ± 1.8	70.73 ± 2.1	51.86 ± 2.2	72.34 ± 1.9
80 (μg/mL)	62.40 ± 2.0	78.62 ± 2.2	63.29 ± 2.1	77.56 ± 2.3	62.72 ± 2.1	79.23 ± 2.1

[a]Values characterize averages ± standard deviations for triplicate experiments. Means with the same letter within the same row are not significantly different ($P > 0.05$).

3.3. Results of Antimicrobial Assay

The MIC (minimum inhibitory concentration) method was applied to test the antimicrobial activity of the volatile constituents of *C. tubulosa* against nine pathogenic microorganisms of animal origin was investigated. The distillate exhibited strong activity against *S. aureu*s with MIC 2.23 mg/100mL and moderate effect against *C. albicans* (MIC= 4.36 mg/100mL), table 3.

Table 3. Antimicrobial activity of hydro-distilled constituents of *C. tubulosa*

Type of strain	**Strain**	**Minimum inhibitory concentration (MIC) mg/100 mL**		
		EO	**Ciprofloxacin**	**Fluconazole**
Gram-positive	*Staphylococcus aureus*	2.23±0.52	0.185±0.09	NT
	Enterococcus faecalis	12.47±0.48	0.096±0.02	NT
	Bacillus cereus	15.68±0.43	0.182±0.011	NT
	Listeria monocytogenes	6.06±0.54	0.093±0.07	NT
Gram-negative	*Escherichia coli*	18.35±0.39	0.184±0.05	NT
	Salmonella typhimurium	26.71±0.41	0.095±0.07	NT
	Pseudomonas aeruginosa	23.53±0.32	0.096±0.03	NT
	Klebsiella pneumonia	31.61±0.49	0.188±0.06	NT
Fungi	*Candida albicans*	4.36±0.38	NT	0.191±0.01

NT: not tested

4. Conclusion

The volatile components of *C. tubulosa* or the commonly known as Desert Ginseng are composed mainly of hexanal (15.98%), *trans*-sabinyl acetate (12.22%), allo-aromadendrene (9.30%), nonanoic acid (6.66%), 3*Z*-hexeny-2-methyl butanoate (6.09%), valeranone (5.25%), (*E*, *E*)-α-Farnesene (3.18%), α-pinene (3.06%), linalool isovalerate (3.03%) and α-humulene (1.8%), which were characterized by their retention times and the fragmentation pattern for each, in the GC-MS chromatogram, as well as comparison to the literature. These components showed promising antioxidant effect at concentration of 80 µg/mL and comparatively similar results upon using three methods of assay. It also exhibited strong antimicrobial and antifungal activities against *S. aureus L. monocytogenes and C. albicans* compared to ciprofloxacin and fluconazole.

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Conflict of Interest

The authors declare no conflicts of interest.

ORCID
Arafa Musa: 0000-0003-0979-556X
Khaled F. El-Massry: 0000-0001-9029-2243
Ahmed H. El-Ghorab: 0000-0002-5500-3124
Amr Farouk: 0000-0002-5261-2397
Hazim M. Ali: 0000-0003-0781-0850
Mohamed A Abdelgawad: 0000-0001-9035-5638
Ibrahim A. Naguib: 0000-0002-5923-1466
Ehab M. Mostafa: 0000-0001-8841-9786

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