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Exploring Wild Chaste Tree, Vitex agnus-castus L. of Oman

For Pharmaceutical Application

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Abstract:

Background: Plant-based medicinal molecules have utility in the treatment of several human ailments in either complementary or allopathic medicine or in combination. In this respect, several medicinal plants like the Chaste tree (*Vitex agnus castus* L.) are under use in both developing and developed countries. Oman is rich in plant biodiversity with about 1578 medicinal plant species, which also include *Vitex agnus castus* plants, distributed wildly across all geographical regions. The objectives of the study were to investigate on morphological characteristics, chlorophyll contents of leaves, essential oil, and chemical contents in Vitex agnus castus plants to explore the possibility of isolating its prime chemical compounds on a commercial scale.

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Methods: Leaf samples of ten randomly selected Vitex plants were collected from two diversified wadi sites. Edaphic features of sites of plant collection were recorded and chemical contents of soil, determined. Morphological traits were measured and chlorophyll contents, recorded. The essential oil was extracted and analyzed for chemical contents. The data were analyzed applying basic statistics.

Results: Al-Khoud plants showed higher expressivity in all the morphological traits and chlorophyll contents than those of Shafan plants. The EO yields were 0.12 % (v/w) and 0.10 % (v/w) for the plant samples of Al-Khoud and Shafan, respectively. The top ten chemical compounds of plants contributed over 80% of the total in both locations and were common with seven chemical compounds. Of the top ten chemical compounds, alpha-Pinene, L-Limonene, and beta- Eudesmol contributed the highest in the same range from 75.37% (Shafan) to 77.87% (Al-Khoud) of the area. These three compounds have the potentiality for use in pharmaceutical industries.

Conclusions: Wild Chaste tree plants can be explored with Good Agriculture Practices for their medicinal application.

KEYWORDS:

Morphology, Chlorophyll, Chemical compound, *Vitex agnus castus,* Lamiaceae

Introduction:

Several plants have been under record for their medicinal properties ever since the beginning of civilizations in the world. They have been reported to be used to treat many disease conditions under ethno medicine. Natural medicinal products from plants are now in vogue for their use as either alternative or complementary to allopathic medicine as a source of drugs in general for several ailments and in particular for cancer, diabetes, and blood pressure treatments in human beings[1-3]. In the past, traditional medicine was less preferred by the people as compared to modern medicine for use as the means of treatment for human diseases[4]. However, recently, there has been a sudden increase in the use of medicinal plants for health support and treatment of diseases in several countries, including developed ones[5-6]. As a result, several medicinal plant extracts are now prescribed in developed countries like the UK, Germany, China, and France[7-8]. In several countries like Africa, Asia, and Central and South America, rural communities still depend on ethnomedicine and rely on traditional plant-based medicines. However, in other countries, these plant-based medicines are integrated into mainstream health systems through purification and modification[9-10].

Oman has 1578 species that account for just 0.42% of the flowering plants in the world and 448 medicinal plant species from 283 genera and 95 families according to the "Socioeconomic Plants Conservation Strategy for the Sultanate of Oman" developed by the Oman Animal and Plant Genetic Resources Center (OAPGRC) in 2017[11]. Of these plant species, *Vitex agnus-castus*, which belongs to Lamiaceae, is known for its medicinal properties[12-13] and is wildly available in wadi habitats across all the governorates of Oman. It is native to the Mediterranean, European, and Asian countries and is grown as an ornamental plant[12]. Recently, many studies have indicated the plant-based medicinal product's effectiveness for treating not only PMS menstruation-related issues but also other diseases and disorders[13]. Given the above, the present research was conducted to compare and contrast the chemical contents of Vitex agnus castus plants from two wadi areas of Oman and discuss their importance and utility aspects in the medicinal world for serving humanity.

Materials and Methods

1. Plant material and site features

The random leaf samples of Vitex agnus castus L. were collected during the Winter period of 2021, along with soil samples from the wadi habitat of Al-Khoud of Wilayat Al-Seeb of Muscat (58.110^{o} E; 23.558^{o} N) located at the altitude of 100 m. The leaf samples were also collected from the high mountain wadi site of Shafan of Wilayat Al-Khabora (56.669^{o} E; 23.767$_{o}$ N) of North Batinah, located at the altitude of 538 m (Figure 1; Plate1 (a-f); Table 1). At the same time, we also recorded relevant edaphic features of locations such as soil type, structure, important chemical contents, soil pH and salinity, and characters of 10 randomly selected plants. The voucher specimens were collected and submitted to the taxonomist at the Life Science Unit of Sultan Qaboos University. The identification of specimens had been confirmed by the taxonomist at the Sultan Qaboos University Herbarium (SPUH) and the voucher material was deposited in the herbarium collection under barcode number SQUH00006293 (https://herb.squ.edu.om/view_specimen/bFZ2RDVQY0k2UTd0SU5uWGVPaXZIUT09). The plant samples were dried under the shade prior to their use for the extraction of essential oil and chemical analyses.

a   b

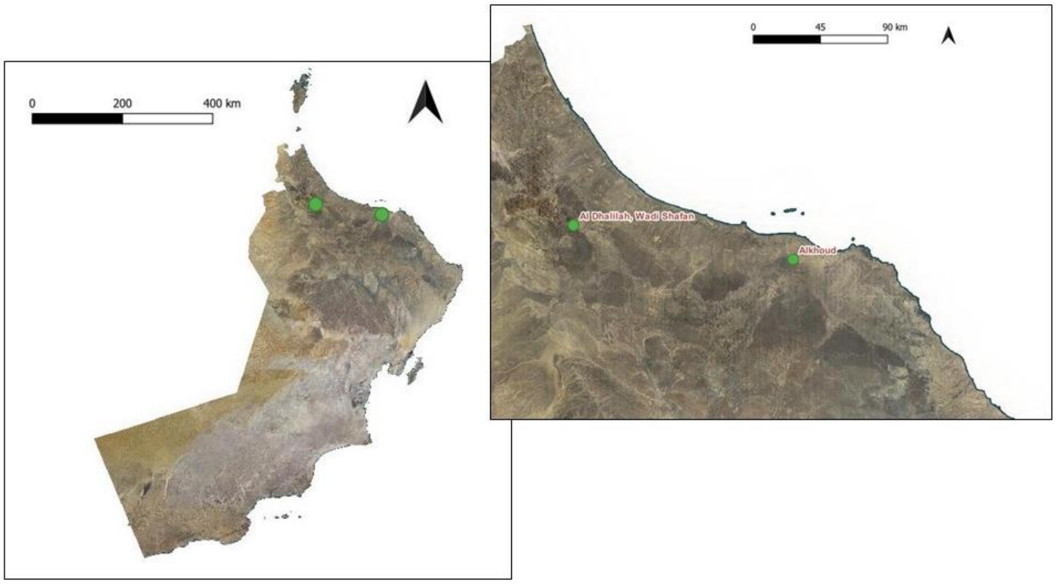

Figure 1. General map of Oman (a) and the zoomed two green dot locations (b) in Muscat and North Al-Batinah governorates

	
Plate 1a. General view of the plant in wadi habitat	Plate 1b. Twig Branches with leaves

Plate 1c. Closer view of leaf clusters

Plate 1d. Clusters of flowers (down) & buds (top)

Plate 1e. Inflorescences

Plate 1f. Inflorescence with clusters (10) of seeds

Plates 1 (a-f). Images of Vitex agnus castus plant found in the wadi habitats of Sultanate of Oman

The leaf samples of at least ten plants were randomly selected from each site. Plant characters such as plant height (m) (vertically from the base of the plant to the highest of tip the plant) and two plant widths (m), one from north to south direction (n-s) (W1) and the other from east to west (e-w) (W2) were measured by the ruler. The stem thickness (middle part of the plant) (mm) was measured by Vernier calipers. We also recorded the chlorophyll contents of leaf samples using an atLEAF CHL PLUS chlorophyll meter. This chlorophyll meter is now widely used for accurate, fast, and non-destructive chlorophyll estimates in several plant species like leaves of coffee[14], crop species[15], *Calamus diocus* and *Cleistostanthus*[16] and rubber plant *Hevea brasiliensis* Mull. Arg.[17]. The atLEAF CHL PLUS measures chlorophyll content in values ranging from 0 to 99.9, indicating the plant's health status; for example, the measurement value of 35 or above refers to grades of good health. Approximate plant area (m^2) was computed using formula of L x B by multiplying plant height (H) with the mean of two plant widths (W1 and W2) and approximate plant volume was calculated using the formula, HxLxB, by multiplying plant height (H), and the two plant widths (W1 and W2), referred above.

The edaphic characteristics of habitat and soil were described for the two wadi habitats (locations), and the range of chemical contents was determined following instructions using S1 Titan/Tracer 5/CTX equipment of Bruker developed based on energy dispersive X-ray fluorescence (EDXRF). This equipment is used to conduct soil mineral analysis, including soil chemical contents, in-situ or on prepared samples, powdered rock samples, "pulp", and others[18]. Only selected macro and micro elements relevant to plant nutrition are reported here.

2. Essential Oils Extraction

Extraction of essential oil of *Vitex* plants was done by using ETHOS X's advanced microwave extraction system[19] while the analyses of essential oils were accomplished by GC-MS analysis on a Shimadzu GC-2010 Plus Gas chromatograph. The chemical compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3) along with arithmetic retention indices on the capillary column and relative to a homologous series of n-alkane C7-C40. The results were confirmed by comparing the calculated retention indices with published literature.

3. Statistical Analysis

The standard errors (S.E. ±) were computed as a part of the basic statistics from the n observations wherever applicable in the study. The paired Student's *t*-test was used to compare the differences between the sample means of two locations for 37 chemical contents commonly present in the essential oils using the data analysis feature of the Excel 16 version. The test of significance was two-tailed at a p-value <0.025.

Results and Discussion

1. Soil Characters:

Both sites have typical wadi habitat features. The Al-Khoud site is at a low altitude (100 m) whereas the Shafan site is at a higher elevation (538 m) in the range of mountains. In both cases, the foothill plains are formed of sandy soil, particles much coarser, differential color from black, reddish to yellowish, and at some places pinkish colored granite-like materials. The rocks or boulders are partly exposed, more or less blunt-faced with little soil covered with vegetation of grasses, small creeping herbs, and medium to larger shrubs, which are mainly restricted to crevices or small depressions showing fine sediments. These are found to aggregate into pockets of smaller to bigger areas along with the length and width of wadis. Both the wadis are typically formed of soil that consists of more than 95% coarse sandy particles.

The soil material of both wadi sites has similar pH but varying E.C., indicating the influence of dryland salinity. Both the soils are formed of significant proportions of nitrate, silicon, and calcium and similar features in respect of other elements, but they differ in the contents of potassium and magnesium (Table 1).

Table 1. GPS data and main soil element contents (ppm) of two locations from where *Vitex agnus castus* leaf samples were collected

Particulars/ Description	Al-Khoud Wadi	Shafan Wadi
Longitude (E)	58.110	56.669
Latitude (N)	23.5584	23.76745
Altitude (m)	100	538
	ppm	ppm
Nitrate (NO3)	51250.00	74000.00
Phosphorus (P)	33.00	32.00
Potassium (K)	153.00	332.00
Magnesium (Mg)	4735.00	1773.00
Calcium (Ca)	5659.00	5251.00
Sulphur (S)	Traces	Traces
Iron (Fe)	5844.00	5183.00
Manganese (Mn)	68.00	82.00
Copper (Cu)	11.00	3.00
Molybdenum (Mo)		
Silicon (Si)	13462.00	14482.00
PH	7.830	7.730
EC(Electrical Conductivity) (dS $^{-1}$)	1.110	0.680

2. Plant Morphological Characteristics:

The plant characteristics of *Vitex agnus castus* were found different, with higher expressions of all the attributes at Al-Khoud wadi than at Shafan wadi (Table 2). Al-Khoud plants were taller (2.8 m) with an N-S canopy length of 1.4 m and E-W canopy length of 2.6 m compared to those of Shafan plants (1.2 m and 1.8 m, respectively). In addition, the plants of Al-Khoud wadi had higher middle stem thickness (13.4 mm) than those of Shafan plants (7.94 mm). Interestingly, when we approximated the canopy area and volume of each plant with simple formulae (LxB and LxBxH, respectively), we found in our study that each plant of *Vitex agnus castus* at wadi Al-Khoud occupies a higher space area, and plant volume of 3.64 m^2 and10.2 m^3 than that at wadi Shafan (2.16 m^2 and 3.46 m^3) (Table 2). These measurements assist researchers in fixing plant-to-plant spacing to maximize plant biomass yield under Good Agriculture Practice (GAP), as observed in *Neopierorhiza scrophulariiflora*[20], *Cynara cardunculus* L.[21] and medicinal and aromatic plants[22].

Table 2. Morphological characteristics of *Vitex agnus castus* at two locations of their samples' collection

Sl. No.	Characters	Wadi Al-Khoud	S.Em. (±)	Wadi Shafan	S.Em. (±)
1.	Plant Height (m)	2.8	0.012	1.6	0.021
2.	*Plant length (N-S) (m)	1.4	0.023	1.2	0.019
3.	*Plant length (E-W) (m)	2.6	0.009	1.8	0.001
4	Approximate plant area (m^2)	3.64	-	2.16	-
5	Approximate plant volume(m^3)	10.20	-	3.46	-
6	Stem thickness (middle) (mm)	13.4	0.001	7.94	0.011
7	Chlorophyll (atLEAF value)	56.9	0.029	51.00	0.032

N-S - North-South canopy length; E-W - East-West canopy length

We know that leaf color indicates the quantity and proportion of chlorophyll in leaves, which are closely associated with the status of nutrients in the plant. Hence, leaf color as a function of chlorophyll content could be applied as an index for diagnosing nutrient status[23-25]. The healthy growth of Al-Khoud plants was also manifested by their higher chlorophyll content in terms of atLEAF value (56.90) than those of Shafan plants (51.00). The health features of *Vitex* plants in these two locations suggested that Wadi Al-Khoud had a favorable environment in terms of water and climatic factors for the growth and development of *Vitex* plants.

Essential oil recovery:

The yield of Essential oil (EO) was 0.12% for the Al-Khoud Wadi sample and 0.10 % for the Shafan sample of *Vitex agnus castus.*Similar levels of extraction were obtained in the previous studies from 0.09%[26] to 0.35%[27-28]. However, higher levels of essential oil extraction have been also reported from the leaves (5.5%), flowers (6.2%) and fruits, (11.26%)[29].

Chemical Composition:

The analysis of essential oils of leaf samples of different parts of Vitex agnus-castus from two diverse locations of North Oman indicated different concentrations in the range of chemical constituents/ compounds (Tables 3 and 4). In the present study, of the total 84 volatile chemical compounds of *Vitex agnus castus* found, the highest as 61 chemical compounds were identified while 23 were not identified (UI), which in all, represent 100% of essential oil of Al-Khoud sample in the range from 0.007% (UI) to 39.573% (Alpha-Pinene) (Table 3). However, of the total 68 compounds separated for Shafan sample, only 48 chemical constituents were identified and 24, unidentified. These in total, represent 100 % of essential oil in the range from 0.054 UI) to 33.679% (Alpha Pinene) (Table 4).

Table 3. Chemical composition of *Vitex agnus castus* composite leaf sample from Al-Khoud Wadi site of Al-Seeb Wilayat, Muscat governorate, Oman[I]

Sl. No.	**Compound Name**	**R.T. (min)**	**%**	**KI**
1	alpha.-Pinene	7.933	39.573	945
2	L-Limonene	10.467	19.624	1038
3	.beta.-Eudesmol	25.595	6.721	1678
4	4(10)-Thujene	8.912	4.998	981
5	beta.-iso-Methyl ionone	31.16	3.632	1974
6	beta.-Myrcene	9.363	3.076	998
7	Germacrene D	21.938	2.896	1504
8	cis-3,14-Clerodadien-13-ol	31.854	1.991	2014
9	Germacrene B	23.619	1.086	1582
10	alpha.-Selinene	22.062	1.049	1510
11	alpha.-Phellandrene	9.737	1.043	1011
12	(-)-trans-Myrtanyl acatate	18.783	0.997	1367
13	Kolavelool	33.442	0.964	2108
14	beta.-Pinene	8.999	0.961	984
16	Linalool	12.329	0.780	1106
17	L-.alpha.-Terpineol	14.852	0.778	1210
18	o-Cymene	10.304	0.677	1032
19	U.I	31.243	0.567	1979
20	delta.-EIemene	18.516	0.547	1356
21	U.I	29.988	0.538	1908
22	trans-Carveol	15.716	0.485	1244
23	(-)-.beta.-Bourbonene	19.706	0.466	1406
24	Terpinolene	12.057	0.363	1095
25	Phytol	33.739	0.297	2127
26	alpha.-Bulnesene	22.245	0.278	1518

27	Bornyl acetate	17.253	0.268	1304
28	Nerolidol	23.526	0.249	1578
29	Methyl geraniate	18.115	0.246	1340
30	U.I	19.033	0.235	1378
31	U.I	34.036	0.227	2145
32	n-Amyl isovalerate	12.467	0.219	1112
33	Cadinol T	25.329	0.205	1665
34	Isospathulenol	25.085	0.191	1653
35	Camphene	8.236	0.183	956
36	ent-Germacra-4(15),5,10(14)-trien-1.beta.-ol	26.271	0.170	1711
37	U.I	20.83	0.166	1455
38	(-)-Terpinen-4-ol	14.464	0.164	1194
39	Viridiflorol	24.936	0.158	1645
40	(Z)-.beta.-Caryophyllene	20.503	0.147	1441
41	L-trans-Pinocarveol	13.451	0.143	1152
42	U.I	25.433	0.127	1670
43	gamma.-Terpinene	11.24	0.126	1066
44	cis-Sesquisabinene hydrate	23.132	0.122	1559
45	Pinocarvone	14.103	0.116	1179
46	Epimanool	31.938	0.103	2019
47	U.I	25.281	0.100	1662
48	.beta.-copaene	20.736	0.100	1451
49	U.I	33.532	0.099	2114
50	Nerolidol	23.368	0.096	1570
51	Caryophyllene oxide	24.187	0.089	1609
52	.beta.-Elemene, (-)-	19.826	0.085	1411
53	gamma.-Elemene	20.782	0.083	1453
54	trans-Verbenol	13.6	0.083	1159
55	U.I	25.726	0.078	1684
56	alpha.-Terpinene	10.073	0.073	1024
57	Estragole	15.21	0.073	1224
58	U.I	23.789	0.071	1590
59	U.I	25.225	0.071	1659
60	Myrtenyl acetate	17.593	0.069	1318
61	U.I	14.182	0.068	1183
62	alpha.-Thujene	7.651	0.063	935
63	U.I	34.925	0.060	2200
64	U.I	19.765	0.051	1409
65	delta.-EIemene	18.455	0.050	1354
66	trans-Ocimene	10.643	0.049	1044
67	2,4(10)-Thujadiene	8.384	0.046	962
68	Alloaromadendrene	21.089	0.045	1467
69	U.I	20.952	0.045	1461
70	U.I	13.869	0.040	1170
72	U.I	16.36	0.039	1269
73	U.I	22.811	0.037	1545
74	3-Methyl-2-butenoic acid, 3-methylbutyl ester	14.55	0.035	1198
75	Isopentyl hexanoate	16.25	0.035	1264
76	α-Compholene aldehyde	13.098	0.030	1138
77	U.I	7.43	0.026	926
78	U.I	14.654	0.026	1202
79	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	12.927	0.018	1131
80	p-Mentha-1(7),8-dien-2-ol	14.726	0.013	1205
81	(-)-Carvone	16.207	0.012	1263
82	U.I	15.006	0.009	1216
83	U.I	13.316	0.007	1147
84	U.I	15.537	0.007	1237
Essential oil recovered	**0.12 % v/w**			

[1]RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified (not detected)

Table 4. Chemical composition of *Vitex agnus castus* composite leaf sample from Shafan wadi site of Al-Khabora Wilayat, North Al-Batinah Governorate, Oman

Sl. No.	Compound Name	R.T. (min)	%	KI
1	alpha.-Pinene	7.659	33.679	935
2	L-Limonene	10.208	14.379	1028
3	.beta.-Eudesmol	25.333	12.410	1665
4	beta.-iso-Methyl ionone	30.885	4.395	1959
5	cis-3,14-Clerodadien-13-ol	31.595	3.961	1999
6	4(10)-Thujene	8.687	3.381	973
7	Pinocarvone	13.874	2.453	1171
8	L-trans-Pinocarveol	13.225	2.073	1144
9	Kolavelool	33.175	1.961	2092
10	beta.-Myrcene	9.144	1.525	990
11	o-Cymene	10.076	1.323	1024
12	Germacrene D	21.676	1.311	1492
13	(-)-trans-Myrtanyl acetate	18.55	1.257	1358
14	Estragole	14.776	1.053	1192
16	U.I	32.092	0.986	2028
17	beta.-Eudesmene	21.805	0.882	1498
18	beta.-Pinene	8.775	0.764	976
19	Linalool	12.106	0.699	1097
20	Bornyl acetate	17.021	0.613	1295
21	trans-Verbenol	13.371	0.548	1150
22	U.I	30.97	0.521	1963
23	U.I	23.356	0.454	1570
24	(-)-Terpinen-4-ol	14.236	0.424	1185
25	α-Compholene aldehyde	12.866	0.395	1130
26	(-)-.beta.-Bourbonene	19.46	0.384	1395
27	U.I	31.198	0.341	1976
28	U.I	33.781	0.339	2129
29	Methyl geraniate	17.885	0.338	1330
30	U.I	27.197	0.324	1757
31	U.I	29.723	0.318	1889
32	alpha.-Phellandrene	9.514	0.317	1003
33	(Z)-β-Caryophyllene	20.279	0.312	1431
34	L-.alpha.-Terpineol	14.631	0.283	1201
35	U.I	11.829	0.283	1087
36	Cineole	10.272	0.276	1031
37	trans-Carveol	15.494	0.262	1235
38	U.I	31.322	0.259	1983
39	Guaia-1(10),11-diene	21.994	0.250	1507
40	U.I	14.581	0.242	1199
41	U.I	18.804	0.229	1368
42	p-Mentha-1(7),8-dien-2-ol	14.496	0.225	1196
43	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	12.697	0.208	1123
44	gamma.-Terpinene	11.019	0.205	1058
45	U.I	33.295	0.204	2099
46	Estragole	14.986	0.201	1215
47	U.I	31.483	0.194	1992
48	U.I	13.951	0.189	1174
49	U.I	13.653	0.186	1162
50	U.I	34.769	0.185	2190
51	U.I	25.48	0.185	1672
52	Camphene	8.024	0.172	948
53	delta.-EIemene	18.277	0.167	1346
54	(-)-Carvone	15.97	0.145	1253
55	U.I	15.311	0.126	1228
56	Nonanal	12.245	0.122	1105
57	alpha.-Terpinene	9.852	0.107	1016
58	U.I	14.429	0.099	1193
59	U.I	13.092	0.092	1139
60	Citronellol	15.889	0.088	1250
61	U.I	7.227	0.083	919
62	U.I	12.162	0.081	1102

63	.beta.-Elemene, (-)-	19.586	0.075	1401
64	3-Methyl-2-butenoic acid, 3-methylbutyl ester	14.327	0.074	1189
65	Rose oxide	12.432	0.072	1113
66	alpha.-Thujene	7.442	0.068	927
67	2,4(10)-Thujadiene	8.178	0.067	954
68	U.I	11.704	0.054	1082
Essential oil recovered	**0.10 % v/w**			

ƚ RT –Retention Time (minutes); KI- Distribution Constant; UI-Un-identified (not detected)

Of the chemical contents of the plant samples of two locations, only 37 chemical contents were common and typical (Table 5). These chemical contents were found to be statistically similar based on the results of paired t-test performed ($p<0.05$) (Table 6). The figures indicated the stability of these chemical contents retained in the plant samples irrespective of geographical differences.

Table 5. Chemical contents of *Vitex agnus castus*, which are common in the plant samples of Wadi Al-Khoud site of Al-Seeb Wilayat, Muscat governorate, and Wadi Shafan site of Al-Khabora Wilayat, North Batinah governorate in Oman

Sl.No.	Compound name	% (Shafan)	% (Al Khoudh)
1	alpha.-Pinene	33.679	39.573
2	L-Limonene	14.379	19.624
3	.beta.-Eudesmol	12.410	6.721
4	beta.-iso-Methyl ionone	4.395	3.632
5	cis-3,14-Clerodadien-13-ol	3.961	1.991
6	4(10)-Thujene	3.381	4.998
7	Pinocarvone	2.453	0.116
8	L-trans-Pinocarveol	2.073	0.143
9	Kolavelool	1.961	0.964
10	beta.-Myrcene	1.525	3.076
11	o-Cymene	1.323	0.677
12	Germacrene D	1.311	2.896
13	(-)-trans-Myrtanyl acatate	1.257	0.997
14	beta.-Pinene	0.764	0.961
15	Linalool	0.699	0.780
16	Bornyl acetate	0.613	0.268
17	trans-Verbenol	0.548	0.083
18	(-)-Terpinen-4-ol	0.424	0.164
19	α-Compholene aldehyde	0.395	0.030
20	(-)-.beta.-Bourbonene	0.384	0.466
21	Methyl geraniate	0.338	0.246

22	alpha.-Phellandrene	0.317	1.043
23	(Z)-.beta.-Caryophyllene	0.312	0.147
24	L-.alpha.-Terpineol	0.283	0.778
25	trans-Carveol	0.262	0.485
26	p-Mentha-1(7),8-dien-2-ol	0.225	0.013
27	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans-	0.208	0.018
28	gamma.-Terpinene	0.205	0.126
29	Estragole	0.201	0.073
30	Camphene	0.172	0.183
31	delta.-EIemene	0.167	0.547
32	(-)-Carvone	0.145	0.012
33	alpha.-Terpinene	0.107	0.073
34	.beta.-Elemene, (-)-	0.075	0.085
35	3-Methyl-2-butenoic acid, 3-methylbutyl ester	0.074	0.035
36	alpha.-Thujene	0.068	0.063
37	2,4(10)-Thujadiene	0.067	0.046
	Total	91.161	92.133

Table 6. Results of paired t-test performed for the 37 common chemical contents of essential oils of the plant samples of *Vitex agnus castus* from two geographically diverse locations in Oman

Location	Al-Khoud	Shafan
Mean	2.463858382	2.490001439
Variance	37.26104386	50.93064102
Observations	37	37
Pearson Correlation	0.974304794	
Hypothesized Mean Difference	0	
df	36	
t Stat	-0.0874	
P(T<=t) one-tail	0.465	
t Critical one-tail	1.688	
P(T<=t) two-tail	0.931	
t Critical two-tail	2.028	

Interestingly, the top ten higher contents of the Al-Khoud sample accounted for 84.65 % of its essential oil recovered (Table 7), whereas those of Shafan sample accounted for 80.22 % of its essential oil (Table 8).

Of the top ten higher contents of the Al-Khoud sample, alpha-Pinene had the highest of 39.573%, followed by L-Limonene (19.624 %), beta Eudesmol (6.721%), and 4(10) Thujene (4.998%) (Tables 7). The remaining six compounds, namely beta iso methyl ionone, beta-Myrcene, Germacrene D, cis-3,14 Clerodadien 13 ol, Germacrene B, and alfa Seinene, were with a lower degree of concentration (<5 %; Table 7).

Table 7. Top 10 chemical compounds of *Vitex agnus castus* composite leaf sample from Al-Khoud Wadi site of Al-Seeb Wilayat, Muscat governorate, Oman[Ɨ]

Sl.No.	Compound Name	R.T. (min)	%	KI
1	alpha.-Pinene	7.933	39.573	945
2	L-Limonene	10.467	19.624	1038
3	.beta.-Eudesmol	25.595	6.721	1678
4	4(10)-Thujene	8.912	4.998	981
5	beta.-iso-Methyl ionone	31.16	3.632	1974
6	beta.-Myrcene	9.363	3.076	998
7	Germacrene D	21.938	2.896	1504
8	cis-3,14-Clerodadien-13-ol	31.854	1.991	2014
9	Germacrene B	23.619	1.086	1582
10	alpha.-Selinene	22.062	1.049	1510
	Total		84.65	

Ɨ RT –Retention Time (minutes); KI- Distribution Constant

Table 8. Top 10 chemical compounds of *Vitex agnus castus* composite leaf sample from Shafan wadi site of Al-Khabora Wilayat, North Al-Batinah Governorate, Oman[Ɨ]

Sl.No.	Compound Name	R.T. (min)	%	KI
1	alpha.-Pinene	7.659	33.679	935
2	L-Limonene	10.208	14.379	1028
3	.beta.-Eudesmol	25.333	12.410	1665
4	beta.-iso-Methyl ionone	30.885	4.395	1959
5	cis-3,14-Clerodadien-13-ol	31.595	3.961	1999
6	4(10)-Thujene	8.687	3.381	973
7	Pinocarvone	13.874	2.453	1171
8	L-trans-Pinocarveol	13.225	2.073	1144
9	Kolavelool	33.175	1.961	2092
10	beta.-Myrcene	9.144	1.525	990
	Total		80.22	

Ɨ RT –Retention Time (minutes); KI- Distribution Constant

Comparatively, of the top ten higher contents of the sample of another location (Shafan, Wilayat AlKhabora), alpha-Pinene had the highest of 33.679% concentration, followed by L-Limonene (14.379 %) and beta Eudesmol (12.410 %) (Table 8). In contrast, the remaining seven compounds, namely beta iso methyl ionone, cis 3,14-Clerodadien-13-ol, 4(10), Thujene, Pinocarvone, L-trans-Pinacarveol, Kolavelool, and beta-Myrcene were with a lower degree of concentration (<5 %; Table 8). Interestingly, the top six constituents are similar in both locations, irrespective of the degree of their concentrations. Our results are comparable with earlier results in respect of higher contents of alpha-pinene (14.83%) and Limonene (10.29%) among other 29 constituents of their leaf samples in Iran[30], Turkey[31] and Morocco[27]. Alpha-Pinene levels were almost two-fold (Al-Khoud sample-39.573% and 33.679%) whereas Limonene contents were 28-48% more in our studies as compared to earlier results[12, 26-27, 29-30, 32].

Regarding our samples, we found them rich in three chemical compounds, Alpha-Pinene, Limonene, and Eudesmol, irrespective of the quantity in both diversified locations. However, these three together contributed the highest of 77.87% of the top ten compounds in the Alkhoud sample in comparison with that (75.37%) in the Shafan sample. Alpha-Pinene is a monoterpene found in various plant species like cannabis, eucalyptus, conifers, citrus, and oils of the most aromatic plants such as rosemary, *Vitex agnus castus,* Salvia species, etc. It is also known to have antibiotic, anti-inflammatory, antimicrobial, apoptotic and anti-metastatic properties[33-35]. Limonene is the most common terpene found in medicinal and aromatic plants besides plants like citrus and cannabis. It has anti-inflammatory, antioxidant, anti-stress, and possibly diseases like cancer-preventing properties[36-37] whereas β-Eudesmol has several pharmacological benefits[38]. It is one of the active ingredients present in most essential oils and has antioxidant and antimicrobial activities. It is reported to suppress tumor cell proliferation, growth, and migration of human tumor cells[38]. Besides, it is known to have several protective effects on the nervous system[38-40]. Given the merits of these three compounds available in *Vitex agnus castus*, as stated above, it is recommended that this medicinal plant species be explored for isolation of these plant-based molecules for their safe application towards the welfare of humanity. The isolation of molecules is commercially feasible on a large scale by producing large biomass of the plants by growing *Vitex agnus castus* in the field with good agriculture practices (GAP) formulated using specific plant characteristics like approximated plant area (m^2) and plant volume (m^3) for optimum plant density with irrigation scheduling and fertilizer management. The low levels of essential oil extraction observed in the present study can be enhanced through elicitation treatments by using elicitors such as Salicylic acid as reported recently in *Ocimum gratissimum* L[41].

CONCLUSIONS

Vitex agnus castus plants were found to be different in morphological features depending on the edaphic features of locations but stable in the contents of most of the compounds. There exists the possibility of adapting Good Agriculture Practice (GAP) to produce the highest yield of herbage yield of vegetative parts of Vitex plants based on morphological features under wild conditions to extract these three compounds at a commercial scale.

Ethical approval: Ethical approval is not required for our research.

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Authorship contributions

SKN planned the investigation, statistically analyzed the data and written the original draft and modified/revised based on inputs of all other authors. JNA and AHA devised and helped in the chemical analysis and collection and preservation of plant samples, respectively. ARA and AAA collected seed and plant samples. FAA, HKA and ASA helped in extraction of essential oil and its chemical analyses. AF confirmed the specimens as that of Vitex agnus castus L. and NAS arranged for funds and supervised the investigation.

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