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Phytochemical Study of Essential Oils of Six Medicinal Plants

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ABSTRACT

Introduction: *This work is an analytical study of Essential Oils (EO) extracted from six medicinal plants. This makes it possible to determine their physicochemical aspects, to calculate the yields of EO and to analyze their phytochemical compositions qualitatively and quantitatively in order to correlate them in new studies with antimicrobial activities.*

Materials and Methods: *Six medicinal plants were selected for our study: Artemisia vulgaris, Ocimum basilicum, Syzygium aromaticum, Thymus vulgaris, Origanum vulgare, and Rosmarinus officinalis. The extraction of the EO was carried out by hydrodistillation using a device of the clevenger type. The yield of EO is the ratio of the mass of the EO to the mass of the plant material used, expressed as a percentage. The physico-chemical characteristics were studied and the qualitative and quantitative analysis of the phytochemical composition was carried out using gas chromatography coupled with mass spectrometry.*

Results: *The yields obtained are very good, the physicochemical characteristics coincide with those described in the literature, the phytochemical composition is very divergent compared to those found by different authors, this is due to several factors such as the chemotype, Harvesting season, extraction technique, extraction equipment and technology, etc.*

Conclusion: *The essential oils studied are very rich in phytochemical substances belonging to different chemical families; this richness is promising of several biological activities, especially antimicrobial.*

Keywords: Phytochemical study, Essential oils, Six medicinal plants

INTRODUCTION

In view of the problems raised by microbial infections and parasitic infections over the last few years, the only reliable alternative to the use of antimicrobials and antiparasitic agents seems to be that of essential oils. Known empirically for centuries, their anti-infectious efficacy has been scientifically demonstrated *in vitro* and *in vivo* [1-4]. This work is an analytical study of EO extracted from six medicinal plants. This makes it possible to determine their physicochemical aspects, to calculate the yields of EO and to analyze their phytochemical compositions qualitatively and quantitatively in order to correlate them in new studies with antimicrobial activities.

MATERIALS AND METHODS

Plant material

The samples were collected in March in the Chtouka Ait Baha region of Sous-Massa-Draa (southern Morocco). The parts used are as follows:

Artemisia vulgaris	Leaf, buds and flowering tops
Ocimum basilicum	Flowered leaf and tops
Syzygium aromaticum	Flower buds
Thymus vulgaris	Flowering tops
Origanum vulgare	Flowering tops
Rosmarinus officinalis	Flowering tops

Extraction of the essential oils

The extraction of the EO was carried out by hydrodistillation using a device of the Clevenger type. Distillations by boiling 200 g of the plant material in 1 liter of distilled water for 2 h 30 min. The yield was calculated on the basis of dry matter. The hydrolate was treated with 10 g of NaCl to recover the EO of the aqueous part and the final EO was treated with anhydrous magnesium sulphate and stored in dark colored bottles at a temperature of 4°C. Then, it is diluted in methanol (1% v/v) before carrying out the GC and GC/MS analyzes according to the standard [5].

Yield

The yield of EO is the ratio of the mass of the EO to the mass of the plant material used, expressed as a percentage.

Physical and chemical properties

General characteristics

The appearance, the color, the odor, the solubility in various organic solvents and the boiling point are determined.

Density index

The relative density at 25°C can be measured with a Pycnometer (a vial used to determine the density of liquids or solubles). This mass is measured by a Pycnometer. The relative density at 25°C of an EO is the ratio of the mass of a volume of EO at 25°C to the equal mass of the volume of distilled water at 25°C [6].

Refractive index

The refractive index (change of direction of light when passing from one medium to another) of an EO is the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light beam of determined wavelength, passing from the air to the EO maintained at a constant temperature. This index can be measured by a Refractometer [6].

Chromatographic analysis

The qualitative and quantitative analysis of the EO components was determined by gas chromatography coupled with mass spectrometry. The sample changer is automatic, the volume injected is 1 µl, the carrier gas is helium and the column used is of the Elite-5ms type, whose length is 30 m and a diameter of 0.25 µm. The injection temperature is 250°C and that of the transfer line is 200°C. The ionization potential is 70 eV and the temperature of the source is 200°C.

RESULTS

Physicochemical aspects, yield and composition of the essential oil of *Artemisia vulgaris*

Physicochemical aspect and yield

The EO obtained is fluid with a pale yellow color with a fresh herbaceous and camphorated odor, insoluble in water, having a density of 0.912 at 25°C and the refractive index is 1.480 to 25°C. The yield relative to the dry plant material is 0.5%.

Phytochemical composition

Chromatographic analysis revealed 66 compounds representing 95.15% of the HE (Table 1).

Physicochemical aspect, yield and composition of the essential oil of *Ocimum basilicum*

Physicochemical aspect and yield

The EO obtained is a clear, pale yellow liquid with a fresh, spicy and aniseed odor, insoluble in water, with a density of 0.962 at 25°C and the refractive index is 1.512 at 25°C. The yield relative to the dry plant material is 0.6%.

Phytochemical composition

Chromatographic analysis revealed 23 compounds representing 88.94% of the EO (Table 2).

Physicochemical aspects, yield and composition of the essential oil of *Syzygium aromaticum*

Physicochemical aspect and yield

The EO obtained is a more or less viscous liquid, of a light yellow color with a spicy odor, typical of eugenol, insoluble in water, with a density of 1.045 at 25°C and the refractive index is 1.530 at 25°C. The yield relative to the dry plant material is 12.5%.

Phytochemical composition

Chromatographic analysis revealed 13 compounds representing 98.71% of the EO (Table 3).

Physicochemical aspect, yield and composition of the essential oil of *Thymus vulgaris*

Physicochemical aspect and yield

The EO obtained is a more or less viscous liquid, of a light yellow color with a spicy odor, typical of eugenol, insoluble in water, with a density of 0.925 to 25°C and the refractive index is 1.502 at 25°C. The yield relative to the dry plant material was 1.9%.

Phytochemical composition

Chromatographic analysis revealed 25 compounds representing 98.83% of the HE (Table 4).

Table 1: Phytochemical composition of the EO of *Artemisia vulgaris*

Number	Component	Percentage (%)
1	Camphor	15.32
2	α-Thujone	10.92
3	Cis-Carveol	10.32
4	Camphene	6.82
5	Germacrene	6.52
6	α-Fenchene	4.91
7	β-Caryophyllene	4.36
8	Borneol	4.13
9	β-Thujone	3.65
10	1,8-Cineol	3.02
11	Sabinene	2.76
12	δ-Cadinene	1.32
13	Davanone	1.13
14	Lavandulyl acetate	0.96
15	Chrysanthenone	0.94
16	α-Humulene	0.92
17	Trans-Pinocarveol	0.91
18	α-Gurjunene	0.9
19	Myrtenal	0.86
20	Neoisothujanol	0.82
21	Isoborneol	0.79
22	β-Pinene	0.73
23	α-Pinene	0.72
24	4-Terpineol	0.72
25	α-Copaene	0.62
26	Bicyclogermacrene	0.62
27	Caryophyllene oxid	0.62
28	Cis-Sabinene hydrate	0.52
29	Thujanol	0.46
30	Tricyclene	0.45
31	β-Selinene	0.38
32	Trans-Sabinene hydrate	0.36
33	Allo-Aromadendrene	0.36
34	β-Cubebene	0.35
35	Y-Cadinene	0.35
36	Linalool	0.32
37	Verbenone	0.3
38	1.8-Dehydrocineol	0.28
39	p-Cymene	0.27
40	Perillaaldehyde	0.24
41	α-Murolene	0.23
42	Artemisia cetone	0.21
43	Cis-p-Menth-2-en-1-ol	0.21
44	Trans-Carveol	0.21
45	Silphiperfol-4,7(14)-diene	0.21
46	Y-Curcumene	0.21
47	T-Cadinol	0.2
48	Limonene	0.19
49	Trans-Muurola-4(14)5-diene	0.19
50	Oxyde d'humulene	0.18
51	β-Eudesmol	0.18
52	α-Eudesmol	0.16
53	Santolina triene	0.15
54	Carvone	0.15
55	α-Cadinene	0.15
56	(E)-β-Ocimene	0.14
57	Y-Terpinene	0.13
58	β-Bourbonene	0.13
59	Aromadendrene	0.13
60	Longiborneol	0.13
61	T-Murolol	0.13
62	α-Terpinene	0.12
63	Cuminaldehyde	0.12
64	β-Elemene	0.12
65	Thuj-3-en-10-al	0.11
66	Caryophylla-4(14),8(15)-dien-5-ol	0.11
Total		**95.15**

Table 2: Phytochemical composition of the EO of *Ocimum basilicum*

Number	Component	Percentage (%)
1	Linalol	44.07
2	Eugenol	13.65
3	Methyl eugenol	6.63
4	Fenchyl alcohol	3.24
5	Estragole	2.86
6	Caryophyllene	2.77
7	Isoeugenol	2.06
8	Methyl cinnamate	2.03
9	ɑ-Terpineo	2.02
10	1,8-Cineol	2.01
11	Geraniol	1.65
12	Citronellol	1.63
13	Terpinen-4-01	1.57
14	Cis-ocimene	1.02
15	ϒ-Terpinene	0.51
16	Limonene	0.31
17	8-Pinene	0.28
18	Fenchyl acetate	0.16
19	ɑ-Pinene	0.13
20	Camphor	0.12
21	p-Cymene	0.11
22	Camphene	0.07
23	ɑ-Terpinyl acetate	0.04
Total		**88.94**

Table 3: Phytochemical composition of the EO of *Syzygium aromaticum*

Number	Component	Percentage (%)
1	Eugenol	80.92
2	Eugenyl acetate	10.11
3	β-Elemene	5.32
4	β-Caryophyllene	2.36
5	ɑ-Thujene	Tr
6	β-Pineme	Tr
7	ϒ-Terpinene	Tr
8	Linalool	Tr
9	Terpinen-4-ol	Tr
10	ɑ-Terpineol	Tr
12	δ-Cadinene	Tr
13	myristic acid	Tr
14	Oleic acid	Tr
Total		**98.71**

Table 4: Phytochemical composition of the EO of *Thymus vulgaris*

Number	Component	Percentage (%)
1	Thymol	45.12
2	p-Cymene	18.02
3	Y-Terpinene	10.05
4	Linalol	3.72
5	Carvacrol	3.26
6	ɑ-Pinene	3.16
7	ɑ-Pinene	1.52

8	Myrcene	1.49
9	4-Terpineol	1.42
10	Caryophyllene	1.13
11	α-Thujene	1.09
12	Thymol methyl ether	1.09
13	α-Pinene	1.02
14	2-Isopropyl-4-methylanisole	0.85
15	2-Ethyl-2-hexen-1-ol	0.76
16	Isothymol	0.73
17	Borneol	0.69
18	Germacrene D	0.69
19	α-Phellandrene	0.62
20	p-Menth-2-en-1-ol	0.62
21	Camphor	0.61
22	Camphene	0.41
23	Sabinene	0.36
24	Terpinolene	0.25
25	Methyl 2-methylbutanoate	0.15
Total		**98.83**

Physicochemical aspect, yield and composition of the essential oil of *Origanum vulgare*

Physicochemical aspect and yield

The EO obtained is a clear liquid, pale yellow in color with a herbaceous and phenol odor, insoluble in water, with a density of 0.950 at 25°C and the refractive index is of 1.512 at 25°C. The yield relative to the dry plant material is 2.5%.

Phytochemical composition

Chromatographic analysis revealed 14 compounds representing 98.97% of the EO (Table 5).

Physicochemical aspect, yield and composition of the essential oil of *Rosmarinus officinalis*

Physicochemical aspect and yield

The EO obtained is a clear liquid, pale yellow in color with a fresh, rustic and more or less camphorous odor, insoluble in water, soluble in ethanol at 95° and in ether, with a density of 0.925 At 25°C, the refractive index is 1.475 at 25°C. The yield relative to the dry plant material is 2.9%.

Phytochemical composition

Chromatographic analysis revealed 31 compounds representing 99.94% of the HE (Table 6).

Table 5: Phytochemical composition of the EO *Origanum vulgare*

Number	Component	Percentage (%)
1	Carvacrol	62.23
2	Y-Terpinene	11.02
3	p-Cymene	9.06
4	Thymol	3.26
5	Linalol	2.43
6	Myrcene	2.32
7	Carvacrol methyl-ether	1.82
8	α-Terpinene	1.62
9	α-Thujene	1.23
10	α-Pinene	1.12
11	Cis-Sabinene hydrate	1.01
12	Limonene	0.72
13	β-Bisabolene	0.62
14	β-Caryophyllene	0.51
	Total	**98.97**

Table 6: Phytochemical composition of the EO of *Rosmarinus officinalis*

Nimber	Component	Percentage (%)
1	1,8-Cineol	41.04
2	Camphor	14.35
3	α-Pinene	12.89
4	β-Pinene	8.36
5	Camphene	4.32
6	Limonene	2.92
7	β-Trans-terpineol	2.13
8	(E)-Caryophyllene	1.95
9	Myrcene	1.73
10	α-Terpinene	1.53
11	α-Terpineol	1.27
12	Terpin-4-ol	0.92
13	α-Terpinen-7-al	0.91
14	Linalool	0.82
15	E-β-ocimene	0.69
16	α-Humulene	0.46
17	Caryophyllene oxide	0.43
18	1,4-Cineol	0.41
19	Verbenone	0.39
20	Terpinolene	0.36
21	ϒ-Terpinene	0.31
22	3-Octanne	0.24
23	α-Thujene	0.23
24	α-Phellandrene	0.23
25	Tricyclene	0.21
26	δ-Cadinene	0.21
27	2-β-Ocymene	0.16
28	α-Campholenol	0.15
29	Thuja-2,4(10)-diene	0.12
30	Sabinene	0.11
31	δ-3-Carene	0.09
Total		**99.94**

DISCUSSION

Essential oil of *A. vulgaris*

The yield obtained (0.5%) is very high compared to that found by Mucciarelli *et al.* (Yield not significant), Ghanmi *et al.,* demonstrated that the yield of essential oil relative to the dry plant material depends on the harvest date [7]. The phytochemical analysis made it possible to deduce that it is a camphor chemotypes (15.32%), the ketones (Camphor, α and β thujone and others) represent more than 30% as described by Govindaraj *et al.* [8].

The sesquiterpenes (Humulene, cadinene, caryophyllene) with more than 25%, which are a class of terpenes formed of three isoprene units and of molecular formula $C_{15}H_{24}$. The monoterpenes then come (Limonene, p-cymene, Sabinene, Cis-sabinene hydrate, etc.) with more than 20%, monoterpenols (Thujanol, neoisothujanol, 4-terpineol, borneol, etc.) with more than 10%.

Mucciarelli and al. brought back the following majority composition: camphor 48%, camphene 9%, verbenone 9%, trans-verbenol 7% and β-caryophyllene 4%. The essential oil comparison of *A. vulgaris* with the other species shows a great variability of the components, the majority compounds of *A. capilaris* are α-thujone (0-40%), borneol (0-15%), ε-cadinene (0-46%), those of *A. rehan* is the davanone (44%), trans-ethylcinnamate (3%), bornyl acetate (2%), for the species petrosa one finds the 1,8-ceneole, β-pinene and borneol, essential oil of *A. annua* is made up of camphor (7-44%), artemisia-ketone (6-26%), germacrene-D (14-24%), β-caryophyllene (5-15%), that of *A. arbuscula* is made up of artemiseol (29%), santolinate of methyl (15%), 1,8-cineole (15%) and camphor (7%) [9]. The essential oil of Artemisia bleached on grass-alba is made up mainly d'α-thujone (43.85%), trans-acetate of sabinyle (17.46%) and β-thujone (10.10%), accompanied by small quantity of 1,8-cineole (3.30%), chrysanthenone (2.32%) and chrysanthenyl acetate (3.93) [9].

Essential oil of *O. basilicum*

The yield obtained (0.6%) is close to that reported by Akgül and al. which found a yield of 0.5% [10]. The yield and the chemical composition of essential oils of *O. canum Sims* according to Yayi-Ladekan and al. vary depending on time and the sunning. Abundant the morning at 7 a.m.

(1.71 ± 0.01%), the yield decrease gradually with the increase in the solar rays, until its minimum at 1 p.m. (1.35 ± 0.01%) when the sun is with the zenith, before growing again with its highest value (1.78 ± 0.02% to 7 p.m.) with the sunset [11].

The composition of essential oil differs according to the chemotype, Guenther, Zola and the National Academy of the Plants with Perfumes, Medicinal, Aromatic and Industrial indexed four chemotypes: The first chemotype rich in linalol (40%) and methyl chavicol (25%), it is the European type. The second chemotype is that of the Island of Réunion with a renor raised out of methyl chavicol (more than 85%) and linalol traces. The third chemotype is that of Bulgaria, Sicily, Egypt, India and Haiti, it is poor (traces) in (E)-methyl cinnamate, out of methyl cinnamate, linalol and methyl chavicol. The fourth chemotype is that with eugenol, met in Morocco and the Seychelles islands. It is rich in linalol and eugenol [12].

Rodrigues and al. found a composition of 60.96% in estragole, followed by 27.27% from linalol and 4.07% of 1,8-cinéol [13]. The principal compounds found by Gradinariu and al. are linalol 31%, camphor, β-elemene, α-bergamotene and bornyl-acetate, estragole 15.57%, eugenol 2.64% and the 1,8-cinéole 3.29% [14].

Essential oil of *S. aromaticum*

The yield obtained (12.5%) is lower than that found by De Mello *et al.* (15%) for nails [15], Razafimamonjison *et al.*, showed that the leaf essential oil yield in the four developmental stages was: Young leaves (5.1%), expanded leaves 1 (4.5%), expanded leaves 2 (4.1%) and mature leaves (3.8%) [16]. Lee *et al.* reported a low yield of 3% [17]. The chemical composition shows a high content of eugenol (80.92%), 10.11% of eugenyl acetate, and 5.32% of β-Elemene. Lee *et al.* found a total of nine chemical compounds, with 49.0% of eugenol and 7.5% of caryophyllene as major compounds [17].

Razafimamonjison *et al.* have demonstrated that when eugenol reaches its highest percentages of foamed leaves 2 (84.00-90.48%) and mature leaf stage (88.32-90.22%), eugenyl acetate reaches the lowest levels (0.96-7.16% and 0.36-1.64%, respectively), but when eugenyl acetate reaches a maximum percentage at the young leaf stage with (61.44-65%, 52%), eugenol reaches a minimum of 25.43-30.38% [16]. Many reports have stated that eugenol is the main compound of the essential oil of *S. aromaticum*. For example, Bauer *et al.* have reported that the essential oil of S. aromaticum is composed of 75% to 85% of eugenol [18]. Farag *et al.* also reported that about 85% of eugenol was found in the essential oil of *S. aromaticum* [19]. Another study by Kong *et al.* claimed that the essential oil of S. aromaticum had 68% of eugenol [20].

Essential oil of *T. vulgaris*

The yield obtained (1.9%) is very high compared to that found by El-Akhal *et al.* (1%) [21]. The same yield (1%) was found by Imelouane *et al.* [22]. Cheurfa *et al.* have found an essential oil yield of about 2% [23], El Ajjouri et al. have reported a yield that ranges between 1.75 and 2.05% [24], that found by Dob et al. is *0.9%.* The yield obtained by Moldão-Martins for *T. zygis* ranges from 2.3-3.6%. The yield of essential oil reached its peak during the flowering phase (0.9-1.4%) and its minimum during the dormant period (0.15%) [25]. The essential oil of *T. vulgaris* studied is a thymol chemotype (45.12%), which is the main constituent of the oxygenated fraction. This chemotype is low in carvacrol (3.26%), unlike the carvacrol chemotype which contains a high proportion of carvacrol as obtained by Boukhatem (83.8%) [26,27].

A carvacrol chemotype described by Cheurfa *et al.* revealing a content of 34.62% in carvacrol and 27.43% in thymol [23]. Deletre reported 30.5% of thymol, 23.7% of p-cymene and 13.6 of carvacrol for a thymol chemotype [27,28]. El-Akhal *et al.* found an essential oil composed of 41.39% in thymol and 2.06% in carvacrol [21], François *et al.* [29] Roman *et al.* [30], Pino *et al.* [31], and Naguib *et al.* [32] found a thymol content of 40.1%, 60.3%, 34.6%, 36.6% and 44.77% respectively.

Essential oil of *O. vulgare*

The yield obtained is average relative to that found by Kanias *et al.* (8.8%), 19 of the 31 samples had an essential oil yield of 8% to 16.6%. This range can be considered as an extremely high level. Data from the literature did not show a yield of more than 8.2% in essential oil of the genus *Origanum*. Some typical examples of the yield of essential oil of the genus *Origanum* are: *O. syriacum* (3.7%), *O. vulgate* (1.3%-6.5%). *O. minutijolium* (1.1%-2.5%) and O. *sipyleum* (0.36%-1.68%) [33-35]. The essential oil obtained is composed of 62.23% of carvacrol, 11.02% of γ-terpinene, 9.06 of p-cymene, 3.26% of thymol and 2.43 of linalool [36]. Chatzifragkou et al. found slightly similar proportions in carvacrol 56.3% and in thymol 16.4% [37]. Veres et al. [38] and Raina et al. [39] found a composition rich in thymol 33.92% and relatively low in carvacrol 6.90%.

Veres et al. studied two subspecies *O. vulgare ssp. Hirtum* and *O. vulgare ssp. Vulgate.* Essential oil of *O. vulgare ssp. Hirtum* contained carvacrol (76.4%), 7-terpinene (6.6%), and p-cymene (4.7%) as main constituents, while the main compounds of the essential oil of *O .vulgare ssp.* (7.2%), 7-terpinene (5.1%), and spathulenol (4%, 8%), carvacrol was not found in this subspecies. The differences between the minor components of the two subspecies were also found; it means that they differ not only in their oil content, but in their chemical character as well as the main constituents of two subspecies are aromatic compounds of carvacrol and p-cymene, respectively [36].

Essential oil of *R. officinalis*

The yield obtained (2.9%) is very high compared to those found by Khia *et al.* which demonstrated regionally varied values, Rchida samples yielded a better yield of essential oil (2.21%) compared to Berkine and Aknoul, respectively, with grades of 1.87% and 1.29%. Fechtal *et al.* indicated that yields of essential oil from two oriental provenances (El Ayat and Debdou) ranged from 0.5-2.9%. Yields of essential oil from the three provenances in Tunisia are of the order of (1.25%, 1.27% and 1.35%) [38].

Soliman *et al.* found yields of 0.14 and 0.4% for two samples from St. Catherine, Sinai and Giza respectively. Our sample is composed of 1,8-cineol (41,04%), camphor (14,35%), α-pinene (12,89%) and camphene (4.32%). Khia *et al.* found a 1.8-cineole content of 50.80%, 44.75% and 42.73% for the three samples respectively from Berkine, Rchida and Aknoul. The AFNOR and NF ISO 4730 standards have a content ranging between 38 and 55% [39].

A camphor content of 7.36%, 11.66% and 11.94% for the three regions, values of 5 and 15% according to the AFNOR and NF ISO 4730 standards. An α-pinene content of 6.72%, 9.58% and 13.83% for the three regions, values of 9 and 14% according to the AFNOR and NF ISO 4730 standards. A camphene content of 3.13%, 3.89% and 5.57% for the three regions, values of 2.5 and 6% according to the AFNOR and NF ISO 4730 standards, these values are reported according to Khia *et al.* [40].

According to Soliman *et al.* the composition for the two samples from St Catherine, Sinai and Giza was verbenone (12.3%), camphor (11.3%), bornyl acetate (7.6%) and limonene (7.1%), respectively were the main constituents for the first sample. The main constituents for the second

sample were camphor (14.9%), α-pinene (9.3%) and 1.8-cineole (9.00%) [41-43].

Touafek *et al.* Found a content of 29.5% in 1,8-cineole, 12% in 2-ethyl-4,5-dimethylphenol, and 11.5% in camphor [43]. Two methods (hot and cold) have been studied for HD-HSME and compared with hydrodistillation as the reference method. The cold process was judged to be superior to the hot method. 32 compounds were identified, including α-pinene (48.7%), camphene (13.7%), 1,8-cineole (13.7%), myrcene (4.5%) and camphor (2.7%) were found to be the main constituents. The results were in good correlation with those obtained by the hydrodistillation process [44].

CONCLUSION

The phytochemical analysis of the essential oils studied showed a great biodiversity and a richness of phytochemical substances, these substances obviously have different activity according to the content and the combination with other substances. The comparison of the qualitative and quantitative composition as well as the physicochemical characteristics of our essential oils with the results of other authors was not always unanimous, due to several factors such as chemotype, harvest season, extraction equipment and technique, etc.

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