

Composition of the Essential oil of *Artemisia absinthium* from Tajikistan

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Abstract: Three samples of *Artemisia absinthium* were collected from two different locations in the central-south of Tajikistan. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography - mass spectrometry. A total of 41 compounds were identified representing 72-94% of total oil compositions. The major components of *A. absinthium* oil were myrcene (8.6-22.7%), *cis*-chrysanthenyl acetate (7.7-17.9%), a dihydrochamazulene isomer (5.5-11.6%), germacrene D (2.4-8.0%), β -thujone (0.4-7.3%), linalool acetate (trace-7.0%), α -phellandrene (1.0-5.3%), and linalool (5.3-7.0%). The chemical compositions of *A. absinthium* from Tajikistan are markedly different from those from European, Middle Eastern, or other Asian locations and likely represent new chemotypes.

Keywords: *Artemisia absinthium*; essential oil composition; cluster analysis; myrcene; *cis*-chrysanthenyl acetate.

1. Introduction

Artemisia absinthium L. (Asteraceae), the major component of the notorious spirit drink absinthe [1,2], has been extensively studied. The plant has been used as an herbal medicine throughout Europe, the Middle East, North Africa, and Asia [3-17], and at least nine different chemotypes have been recognized based on essential oil compositions [18,19]. In this report, we present the chemical compositions of three different samples of *A. absinthium* collected from two different locations in the central-south of Tajikistan. To our knowledge, no previous work on *A. absinthium* from Tajikistan has been reported.

2. Materials and Methods

2.1. Plant Materials

Aerial parts of *A. absinthium* were collected from two regions of Tajikistan: Sample #1, the Khonaobod village, Muminobod region (38.107547 N, 69.966431 E, 1200 m above sea level), on 7 May 2010; Samples #2 and #3, the Chormaghzak village, Yovon region, (38.417502 N, 69.172175 E,

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1300 m above sea level), on 15 April and 25 July 2010. The plant was identified by F.S. Sharopov, and a voucher specimen (TJ2010-021) has been deposited in the herbarium of the Chemistry Institute of the Tajikistan Academy of Sciences. The air-dried samples were crushed and hydrodistilled for 3 h to give the yellow essential oils, 0.5-0.8% yield.

2.2 Gas Chromatographic – Mass Spectral Analysis

A gas chromatographic-mass spectral analysis was performed on the essential oils of *A. absinthium* using an Agilent 6890 GC with Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/s), and a fused silica capillary column (HP-5ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The carrier gas was helium with a flow rate of 1 mL/min, and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 minutes at 40°C, then ramp to 200°C at 3°C/min and finally to 220°C at 2°C/min. The interface temperature was 280°C. A 1% w/v solution of each sample in CH₂Cl₂ was prepared, and 1 μL was injected using a splitless injection technique. Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [20] and stored on the MS library [NIST database (G1036A revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical compositions of the *A. absinthium* oils are summarized in Table 1.

2.3 Numerical Cluster Analysis

A selection of 116 *Artemisia absinthium* essential oil compositions from the published literature [19,21-38] were treated as operational taxonomic units (OTUs). The percentage composition of 60 main essential oil components was used to determine the chemical relationship between the different *A. absinthium* essential oil samples by cluster analysis using the NTSYSpC software, version 2.2 [39]. Correlation was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition. The *A. absinthium* dendrogram is shown in Figure 1.

3. Results and Discussion

The three essential oils from Tajikistan, sample #1 from the Muminobod region collected at the budding (pre-flowering) stage, and two samples from the Yovon region, collected during the budding (pre-flowering) period and the full flowering period, #2 and #3, respectively, showed qualitative similarities, but some notable quantitative differences. The major components in *A. absinthium* essential oil #1 were *cis*-chrysanthenyl acetate (15.5%), myrcene (8.6%), germacrene D (8.0%), β-thujone (7.3%), linalool acetate (7.0%), linalool (5.6%), and dihydrochamazulene 1 (5.5%). By comparison, *A. absinthium* essential oil #2 was dominated by myrcene (22.7%) and dihydrochamazulene 1 (10.5%), with lesser amounts of *cis*-chrysanthenyl acetate (7.7%), dihydrochamazulene 4 (6.2%), linalool (5.3%), and α-phellandrene (5.3%). Interestingly, *A. absinthium* essential oil #3 (Yovon region, flowering stage) was more similar in chemical composition to sample #1 (Muminobod region, pre-flowering) than to sample #2 (Yovon region, pre-flowering stage), with *cis*-chrysanthenyl acetate (17.9%), myrcene (9.2%), and linalool (7.0%) as major components, but also high concentrations of dihydrochamazulene 1 (11.6%) and dihydrochamazulene 3 (5.7%).

Table 1. Chemical compositions of *Artemisia absinthium* essential oils from Tajikistan.

| RI ^a | Compound | Percent Composition | | |
|-----------------|---|---------------------|-----------------|-----------------|
| | | #1 ^b | #2 ^c | #3 ^d |
| 941 | α -Pinene | 1.3 | 0.9 | t |
| 992 | Myrcene | 8.6 | 22.7 | 9.2 |
| 1004 | α -Phellandrene | 2.4 | 5.3 | 1.0 |
| 1024 | <i>p</i> -Cymene | 2.0 | 2.8 | 0.7 |
| 1030 | 1,8-Cineole | 1.1 | t ^e | t |
| 1036 | Santolina alcohol | 1.9 | 1.0 | t |
| 1058 | γ -Terpinene | 0.8 | 0.9 | t |
| 1100 | Linalool | 5.6 | 5.3 | 7.0 |
| 1105 | α -Thujone | 2.9 | 0.9 | 0.5 |
| 1115 | β -Thujone | 7.3 | 2.2 | 0.4 |
| 1153 | Menthone | 1.1 | t | t |
| 1161 | <i>cis</i> -Chrysanthenol | --- | --- | 0.7 |
| 1189 | α -Terpineol | 1.1 | t | t |
| 1217 | γ -Isogeraniol | --- | --- | 0.4 |
| 1225 | Unidentified ^f | --- | --- | 0.9 |
| 1235 | Pulegone | 1.2 | t | 0.4 |
| 1240 | Carvone | 2.4 | t | t |
| 1253 | <i>cis</i> -Piperitone epoxide | 3.2 | 1.5 | t |
| 1256 | Linalool acetate | 7.0 | 1.6 | t |
| 1261 | <i>cis</i> -Chrysanthenyl acetate | 15.5 | 7.7 | 17.9 |
| 1283 | 1-Phenyl-2,4-pentadiyne | --- | --- | 0.3 |
| 1291 | Lavandulyl acetate + Thymol | 3.2 | 3.0 | 1.8 |
| 1300 | Carvacrol | 1.5 | t | 0.9 |
| 1365 | Neryl acetate | --- | --- | 0.4 |
| 1365 | Piperitenone oxide | 2.7 | 0.9 | --- |
| 1391 | β -Elemene | --- | --- | 0.4 |
| 1418 | (<i>E</i>)-Caryophyllene | 3.0 | 3.1 | 2.6 |
| 1425 | Lavandulyl isobutanoate | tr | 0.9 | 0.7 |
| 1481 | Germacrene D | 8.0 | 4.8 | 2.4 |
| 1492 | Neryl isobutanoate | 1.5 | 1.7 | 2.0 |
| 1496 | Bicyclogermacrene | --- | --- | 0.5 |
| 1511 | Lavandulyl 2-methylbutyrate | --- | --- | 3.3 |
| 1514 | Dihydrochamazulene 1 ^g | 5.5 | 10.5 | 11.6 |
| 1518 | Dihydrochamazulene 2 ^h | --- | 1.1 | 1.6 |
| 1575 | Neryl 2-methylbutanoate | 2.2 | 2.8 | 4.9 |
| 1582 | Neryl isovalerate | 2.7 | 2.7 | 4.4 |
| 1600 | Geranyl 2-methylbutanoate | --- | --- | 1.1 |
| 1606 | Geranyl isovalerate | --- | --- | 0.6 |
| 1613 | Dihydrochamazulene 3 ⁱ | --- | 3.1 | 5.7 |
| 1627 | Dihydrochamazulene 4 ^j | --- | 6.2 | 2.7 |
| 1638 | (2 <i>S</i> ,5 <i>E</i>)-Caryophyll-5-en-12-al | --- | --- | 0.8 |
| 1651 | Dihydrochamazulene 5 ^k | --- | 3.4 | 2.5 |
| 1655 | Pogostol | --- | --- | 0.4 |
| 1709 | Unidentified ^l | --- | --- | 1.9 |

| RI ^a | Compound | Percent Composition | | |
|-----------------|------------------------------------|---------------------|-----------------|-----------------|
| | | #1 ^b | #2 ^c | #3 ^d |
| 1730 | Chamazulene | --- | --- | 0.8 |
| 1946 | 9-Geranyl- <i>p</i> -cymene | 2.8 | 1.3 | 1.5 |
| 1997 | (<i>Z</i>)-Nuciferol isobutyrate | --- | --- | 1.0 |
| 2004 | (<i>E</i>)-Nuciferol isobutyrate | 1.3 | 1.7 | 3.3 |
| 2496 | Unidentified ^m | --- | --- | 0.9 |

^a RI = "Retention Index" with respect to a series of normal alkanes on a HP-5ms column.

^b Sample #1 from Muminobod region, flowering stage.

^c Sample #2 from Yovon region, flowering stage.

^d Sample #3 from Yovon region, fruiting stage.

^e t = "trace" (< 0.05%).

^f Monoterpenoid; EIMS: 154(7%), 136(77%), 121(58%), 107(29%), 93(100%), 79(59%), 69(94%), 55(35%).

^g Isomer not determined; EIMS: 186(38%), 171(25%), 157(100%), 142(56%), 128(23%), 115(19%).

^h Isomer not determined; EIMS: 186(43%), 171(29%), 157(100%), 142(59%), 128(26%), 115(21%).

ⁱ Isomer not determined; EIMS: 186(83%), 171(47%), 157(100%), 142(63%), 128(50%), 115(34%).

^j Isomer not determined; EIMS: 186(90%), 171(100%), 157(53%), 143(65%), 128(55%), 115(38%).

^k Isomer not determined; EIMS: 186(99%), 171(100%), 157(53%), 143(66%), 128(51%), 115(31%).

^l EIMS: 184(66%), 169(77%), 157(76%), 143(100%), 128(77%), 115(49%).

^m EIMS: 322(14%), 251(13%), 186(100%), 157(34%).

A. absinthium from Tajikistan represents a new chemotype, a myrcene/*cis*-chrysanthenyl acetate chemotype. Chialva and co-workers [18] had noted six different chemotypes of European *A. absinthium*, three "pure" chemotypes: (a) (*Z*)-6,7-epoxyocimene, (b) sabinyl acetate, and (c) β -thujone, and three "mixed" chemotypes: (d) β -thujone/(*Z*)-6,7-epoxyocimene, (e) β -thujone/sabinyl acetate, and (f) (*Z*)-6,7-epoxyocimene/chrysanthenyl acetate/sabinyl acetate. A subsequent study by Orav and co-workers [19] revealed three additional chemotypes of *A. absinthium*: (g) sabinene/myrcene, (h) neryl butanoate, and (i) 1,8-cineole (not represented in the cluster analysis, Figure 1, of the present study). The results of this present cluster analysis shows that there are numerous additional "mixed" chemotypes of *A. absinthium*, in particular, a (*Z*)-6,7-epoxyocimene/chrysanthenyl acetate (devoid of sabinyl acetate) cluster (j) and numerous small clusters (two samples) or individual representatives of "mixed" chemotypes. Additionally, samples from outside Europe (e.g., Middle East, central Asia, Cuba) differ markedly in their chemical compositions from those of European origin (see Figure 1 for examples). There are individual samples from Europe, also, that show different chemical characteristics than the previously defined chemotypes (a-j).

With many different chemotypes of *A. absinthium* from diverse geographical locations, it is interesting to speculate that traditional medicinal uses may vary depending on geographical location (and possibly chemical characteristics of the plant). A summary of the ethnopharmacological uses of *A. absinthium* is presented in Table 2, along with the characteristic chemotype(s). A perusal of Table 2 shows some common medicinal uses such as digestive, antihypertensive, and antipyretic, regardless of geographical origin or chemotype. Although *Artemisia absinthium* has been extensively studied, it is apparent from this current work that there are numerous essential oil chemotypes depending on geographical location, and much additional work is necessary in order to help sort out the factors responsible for the very different chemical profiles of this interesting and economically important medicinal plant.

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *A. absinthium* samples, based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA).

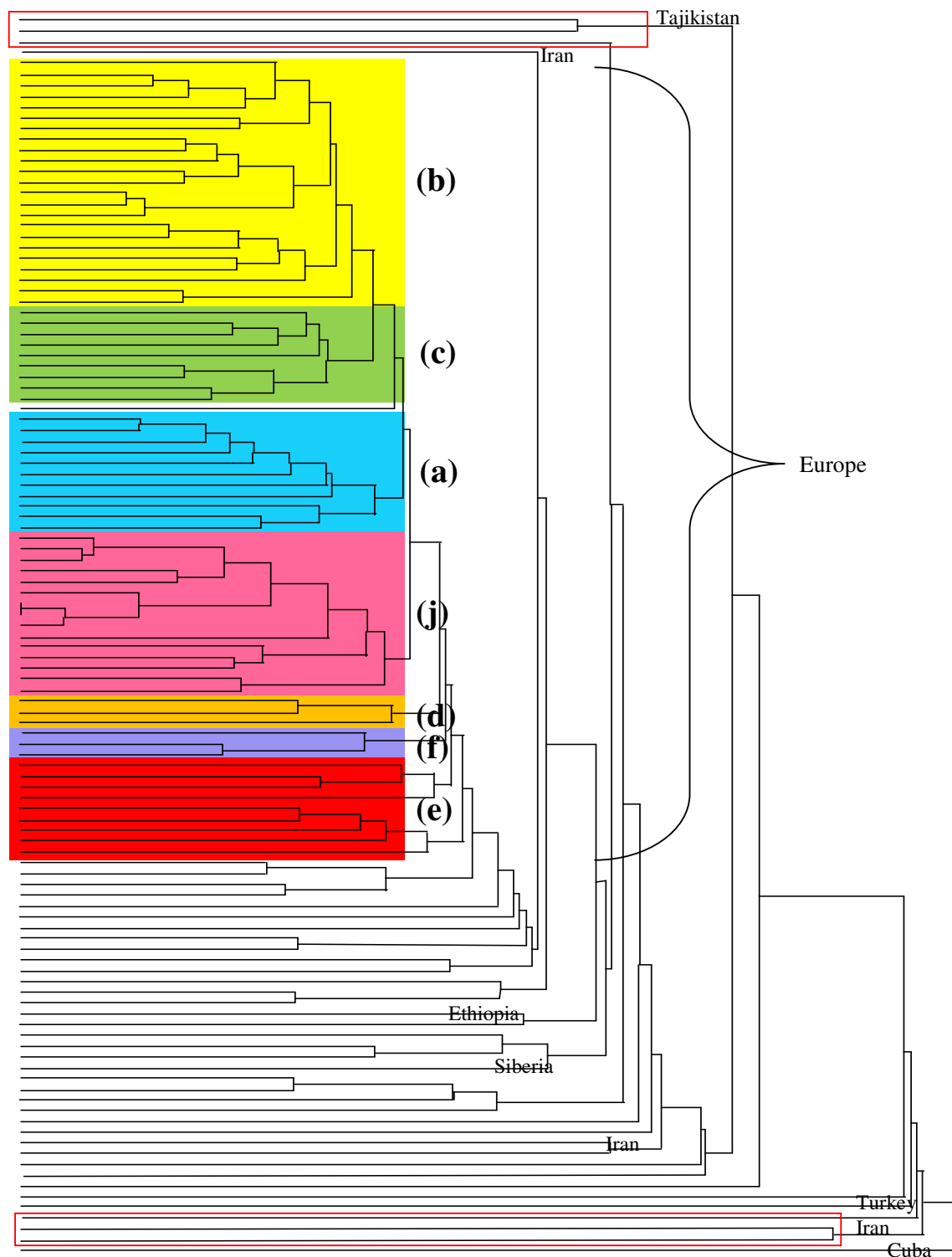


Table 2. Ethnopharmacology of *Artemisia absinthium*.

| Geographical location | Traditional medicinal use | Ref. | Chemotype(s) | Ref. |
|---------------------------------|--|------|---|-----------------|
| Bosnia and Herzegovina | Infusion used for gastrointestinal ailments, stomachache; decoction used for stomachache. | [15] | β -Thujone Epoxyocimene/ β -thujone Sabinyl acetate/ β -thujone/Z- β -ocimene Fenchene/linalyl 3-methylbutanoate | [32] |
| China (Urumqi) | Used to treat hepatic disorders. | [16] | --- | --- |
| Croatia (northern Istria) | Infusion used as a digestive. | [14] | β -Thujone | [27] |
| Croatia (Žejane) | Decoction of aerial parts drunk as a digestive. | [8] | Epoxyocimene/ β -thujone | [23] |
| Cuba | Antimalarial | [40] | Bornyl acetate | [27] |
| France | Appetite stimulant, anthelmintic, antibacterial, antipyretic, emmenagogue. | [4] | Epoxyocimene/ <i>cis</i> -chrysanthyl acetate | [31] |
| Iran (Elburz Mountains) | Anthelmintic, antifungal, antimicrobial, choleric, digestive, diuretic. | [17] | α -Thujone β -Thujone β -Pinene | [29] [26,33] |
| Italy (Marche, Abruzzo, Latium) | Leaves used as an anthelmintic; infusion of leaves used as a digestive (treat lack of appetite); decoction used as antiemetic; poultice of aerial parts applied to relieve tendonitis. | [9] | Epoxyocimene Epoxyocimene/ β -thujone Linalool/ β -thujone | [18] |
| Italy (Piedmont Alps) | Antiparasitic (veterinary). Infusion used as antihypertensive, digestive, anthelmintic (children). | [14] | | |
| Italy (Tuscany) | Used as an antihypertensive. | [5] | | |
| Pakistan (Gilgit District) | The whole herb is used to treat fevers, especially malaria. It is also used as an anthelmintic for children. | [10] | --- | --- |
| Tunisia | Used to treat malaria. | [13] | --- | --- |
| Turkey (Kirkclareli Province) | Aerial parts (infusion) used as an abortive, to treat stomach ache, as an appetizer, and a blood depurative. Decoction used as an appetizer, to treat stomach ache, diabetes, tuberculosis, antihypertensive. Young shoots eaten to treat malaria. Leaves chewed as an antihypertensive. Leaves applied to wounds. Leaf decoction used to treat diabetes and as an antihypertensive. | [11] | Chamazulene | [30] |
| Turkey (K. Maras) | Used as an antipyretic, to heal wounds, and to treat stomach problems. | [7] | | |

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