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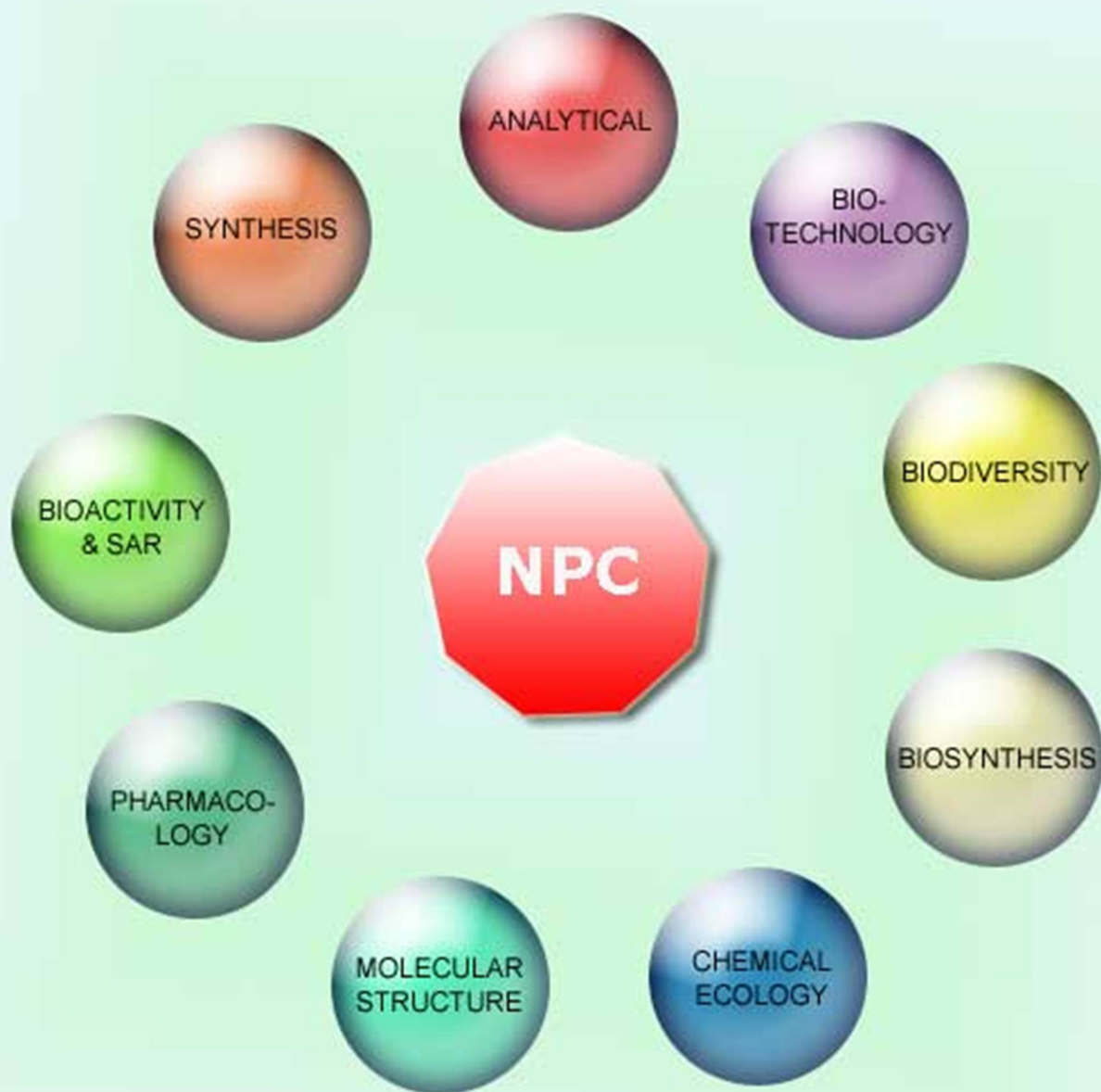
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## Volatile Composition of Six Horsetails: Prospects and Perspectives

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Six horsetails were investigated for volatile organic compounds (VOC) by GC-MS using organic solvent extraction. Seventy-five VOC biosynthesized from the shikimic, lipidic and terpenic pathways including isoprenoid derivatives were detected from these putative natural resources. *E. palustre* var. *americana* contained mainly lipidic derivatives, i.e., 1-octen-3-ol (mushroom-like odor), (*E*)-2-hexenoic acid (fruity odor) and (*E*)-2-hexenal (green odor). Many isoprenoid flavour precursors, i.e., 3-oxo- $\alpha$ -ionol (spicy odor) and (*E,E*)-pseudoionone (balsamic odor), as well as odorous benzenic derivatives, i.e., phenylethanal (hyacinth, lilac note) and 2-phenylethanol (rose odor) contributed to the odor of *E. arvense*. The volatile pattern of *E. telmateia* is dominated by high amounts of isoprenoids and benzenic derivatives. The complex volatile profiles of *E. hyemale* and *E. ramosissimum* are based on ferulic acid isomers, along with either (*E*)-2-heptenal (green vegetable-like odor) or 4-vinylguaiaicol (spicy clove smoky odor) for *E. hyemale* and *E. ramosissimum*, respectively. The broad spectrum of *E. scirpioides* shows the lowest VOC content with high amount of isoprenoids (46.9%), mainly ionone derivatives. *Equisetum* resources are of great interest as bioactive litter and new potential functional feed ingredients.

**Keywords:** Horsetails, *Equisetum*, Volatile Organic Compounds, (*E*)-2-Heptenal, 4-Vinylguaiaicol, Ferulic acid isomers, (*E,E*)-Pseudoionone, 3-Oxo- $\alpha$ -ionol.

Horsetails have a subcosmopolitan distribution. The fifteen well-known *Equisetum* species always grow in wet sites and form large long-lived populations via highly developed rhizomes [1a,1b]. In the new phylogenetic classification, they belong to Monilophytes, where they are closer to ferns than in the previous classifications [1c,1d]. *Equisetum* species biosynthesize a great number of various phytochemicals, which may possess health-promoting properties. Horsetails have been frequently used in medicine since Greek antiquity to treat wounds and inflammations of the skin and to cure fractures, thanks to the action of silicic acid and various identified minerals [2a]. Recently *E. arvense*, commonly used as a traditional Polish medicine, was investigated for silicon from whole plant and tea bags [2b]. Nowadays several species of horsetails are still used in traditional medicine around the world, i.e., *E. debile* against jaundice and hepatitis, as well as for treatment of urinary tract problems, including kidney stones [2c,2d], *E. giganteum* as a diuretic [2e], and *E. telmateia* as an antiseptic [2f]. *E. ramosissimum*, *E. arvense* and *E. telmateia* were investigated for antioxidant activity, mainly due to polyphenols [2g-2k], and *E. hyemale* for both antioxidant and anti-inflammatory properties [2l]. Recently, anti-cancer activity of *E. hyemale* extract was demonstrated on murine leukemia L1210 cells [2m]. *E. arvense* and *E. palustre* were recently utilized in cosmetics to regenerate the skin and stabilize the connective tissue due to flavonoid and saponin derivatives [3a,3b]. Nowadays *Equisetaceae* extracts are patented for uses in skin treatments and capillary preparations for hair problems [3c,3d].

However, very few data have been reported on volatile organic compounds (VOC) of horsetails and their biological activities. Two

research teams published on wild *Equisetum* essential oils from China and Serbia, respectively [4a-4d]. Within the framework of our investigations for natural volatiles in mushrooms and plants [4e-4j], especially in ferns in the Monilophytes group, six wild horsetails were investigated for VOC.

Fresh aerial parts of six *Equisetum* species were investigated for their volatile profiles using GC-MS: *E. arvense* L., *E. palustre* var. *americana* Vict. and *E. telmateia* Ehrh. from the *Equisetum* subgenus; *E. hyemale* L., *E. ramosissimum* Desf. and *E. scirpioides* Michx. from the *Hippochaete* subgenus. The main volatile components of *Equisetum* sp. were identified by comparison with the National Institute of Standards and Technology Mass Spectral Library [5a], retention indices [5b] as reported in the literature for the eleven French ferns previously studied [4i,4j].

Seventy-five VOC biosynthesized from the shikimic, lipidic and terpenic pathways were detected and sixty-five identified from the concentrated diethyl ether extracts of the six horsetails (Table 1).

Twenty-one VOC participated in the general odor of *E. arvense*. This plant contained the highest biodiversity of isoprenoid flavor precursors (75.8%), i.e., 3-hydroxy-7,8-epoxy- $\beta$ -ionol (17.5%), (*E,E*)-pseudoionone (13.1%) and 3-oxo- $\alpha$ -ionol (9.8%; Table 1) [6a,6b], as well as odorous benzenic derivatives, i.e., phenylethanal (3.8%; hyacinth, lilac note), 2-phenylethanol (3.8%; rose odor), benzaldehyde (2.2%; bitter almond odor) and homovanillic acid (3.8%). The last compound is well-known as one of the quercetin metabolites from *E. arvense* extract when administered to humans [6c].

**Table 1:** Percentage of volatile organic compounds<sup>a</sup> in fresh aerial part of horsetails.

| Compounds                              | RI <sup>b</sup> | <i>Equisetum arvense</i> | <i>Equisetum palustre</i> var. <i>americana</i> | <i>Equisetum telmateia</i> | <i>Equisetum hyemale</i> | <i>Equisetum ramosissimum</i> | <i>Equisetum scirpioides</i> |
|--|-----------------|--------------------------|---|----------------------------|--------------------------|-------------------------------|------------------------------|
| <b>Aromatic compounds</b>              |                 | <b>18.0</b>              | <b>15.3</b>                                     | <b>26.3</b>                | <b>25.5</b>              | <b>76.6</b>                   | <b>34.8</b>                  |
| Benzaldehyde                           | 962             | 2.2                      | 1.2   |                            |                          |                               | 0.5                          |
| Benzylalcohol                          | 1044            |                          |   | 4.4                        |                          |                               |                              |
| Phenylethanal                          | 1046            | 3.8                      | 1.0   | 1.4                        |                          | 1.9                           | 6.5                          |
| Acetophenone                           | 1068            |                          | 3.5   |                            |                          |                               |                              |
| 2-Phenylethanol                        | 1117            | 3.8                      | 2.0   |                            |                          | 6.5                           | 1.8                          |
| Benzoic acid                           | 1187            |                          | 1.2   |                            |                          |                               | 2.9                          |
| Benzothiazole                          | 1228            |                          | 0.5   |                            |                          |                               | 0.8                          |
| Phenylacetic acid                      | 1266            |                          | 1.5   |                            |                          | 1.9                           | 1.2                          |
| 4-Vinylguaiaicol                       | 1316            | 2.2                      |   | 18.1                       | 5.9                      | 19.6                          | 2.3                          |
| Isovanillin                            | 1404            | 2.2                      | 1.0   | 2.4                        | 0.7                      |                               |                              |
| (Z)-3-Hexenyl benzoate                 | 1552            |                          | 0.3   |                            |                          |                               | 3.5                          |
| Homovanillic acid                      | 1561            | 3.8                      |   |                            |                          |                               | 15.2                         |
| (Z)-Ferulic acid                       | 1777            |                          |   |                            | 8.6                      | 18.7                          |                              |
| (E)-Ferulic acid                       | 1889            |                          |   |                            | 10.3                     | 28.0                          |                              |
| Esculetine                             | 1890            |                          | 3.3   |                            |                          |                               |                              |
| <b>Lipid derivatives</b>               |                 | <b>6.2</b>               | <b>58.8</b>                                     | <b>3.7</b>                 | <b>69.7</b>              | <b>20.2</b>                   | <b>18.3</b>                  |
| (E)-2-Hexenal                          | 851             |                          | 6.6   |                            |                          |                               |                              |
| (Z)-3-Hexenol                          | 855             |                          | 2.5   |                            |                          |                               |                              |
| (E)-2-Hexenol                          | 866             |                          | 2.0   |                            |                          |                               |                              |
| Hexanol                                | 870             |                          | 1.0   |                            |                          |                               |                              |
| (E)-2-Heptenal                         | 956             |                          | 2.1   |                            | 22.4                     | 2.3                           | 4.1                          |
| 1-Octen-3-one                          | 977             |                          | 0.8   |                            |                          |                               |                              |
| 1-Octen-3-ol                           | 984             |                          | 12.4  |                            |                          |                               |                              |
| 2,3-Octanedione                        | 985             | 1.6                      | 0.8   | 1.4                        |                          |                               | 0.3                          |
| 3-Octanol                              | 1000            |                          | 1.3   |                            |                          |                               |                              |
| (E,Z)-2,4-Heptadienal                  | 1001            |                          |   |                            | 1.7                      |                               | 3.8                          |
| Hexanoic acid                          | 1009            | 2.2                      |   |                            | 0.7                      | 0.9                           | 1.2                          |
| (E,E)-2,4-Heptadienal                  | 1013            |                          |   |                            | 1.0                      |                               | 0.9                          |
| (Z)-3-Hexenoic acid                    | 1017            | 1.1                      | 9.9   | 2.3                        |                          |                               |                              |
| (E)-2-Hexenoic acid                    | 1037            | 1.3                      | 10.7  |                            |                          |                               |                              |
| NI <sup>c</sup>                        | 1041            |                          |   |                            | 4.5                      |                               |                              |
| NI <sup>c</sup>                        | 1064            |                          |   |                            | 3.8                      |                               |                              |
| (E)-2-Octenol                          | 1072            |                          | 1.3   |                            |                          |                               |                              |
| Allyl 3-oxo-butanoate                  | 1075            |                          |   |                            | 2.1                      |                               |                              |
| Heptanoic acid                         | 1096            |                          | 0.3   |                            | 3.1                      | 1.9                           |                              |
| 6-Hydroxy-6-methylheptan-2-one         | 1109            |                          |   |                            | 6.2                      |                               |                              |
| Allylheptanoate                        | 1123            |                          |   |                            | 2.8                      |                               |                              |
| (Z)-3-Nonenol                          | 1158            |                          | 0.7   |                            |                          |                               |                              |
| (E)-2-Nonenal                          | 1162            |                          | 0.8   |                            |                          |                               |                              |
| Ethylmethylmaleimide                   | 1242            |                          | 0.7   |                            | 1.0                      |                               | 1.2                          |
| NI <sup>c</sup>                        | 1256            |                          |   |                            | 3.1                      |                               |                              |
| (E)-2-Decenal                          | 1263            |                          |   |                            |                          | 7.1                           | 1.1                          |
| Methylvinylmaleimide                   | 1273            |                          | 0.3   |                            | 0.3                      |                               | 0.6                          |
| Nonanoic acid                          | 1283            |                          | 1.3   |                            |                          | 1.9                           |                              |
| (E,Z)-2,4-Decadienal                   | 1296            |                          |   |                            |                          | 0.9                           | 1.1                          |
| 2,4,4-Trimethyl-2-butenolide           | 1298            |                          |   |                            | 2.4                      |                               |                              |
| NI <sup>c</sup>                        | 1301            |                          |   |                            | 3.8                      |                               |                              |
| NI <sup>c</sup>                        | 1316            |                          |   |                            | 6.2                      |                               |                              |
| (E,E)-2,4-Decadienal                   | 1321            |                          |   |                            |                          | 2.3                           | 4.1                          |
| NI <sup>c</sup>                        | 1348            |                          |   |                            | 2.2                      |                               |                              |
| 2,4-Decanedione                        | 1373            |                          |   |                            | 2.4                      |                               |                              |
| Lauric acid                            | 1570            |                          | 1.3   |                            |                          | 2.8                           |                              |
| Myristic acid                          | 1773            |                          | 2.0   |                            |                          |                               |                              |
| <b>Terpenes</b>                        |                 | <b>0.0</b>               | <b>0.7</b>                                      | <b>2.9</b>                 | <b>0.0</b>               | <b>0.0</b>                    | <b>0.0</b>                   |
| Linalool                               | 1102            |                          | 0.7   |                            |                          |                               |                              |
| $\beta$ -Caryophyllene                 | 1422            |                          |   |                            |                          |                               |                              |
| <b>Isoprenoid derivatives</b>          |                 | <b>75.8</b>              | <b>25.2</b>                                     | <b>70.6</b>                | <b>2.4</b>               | <b>3.3</b>                    | <b>46.9</b>                  |
| 1,2-Dimethylcyclohexanol               | 1113            |                          | 0.7   |                            |                          |                               |                              |
| 1-Acetyl-2,2-dimethylcyclohexane       | 1257            |                          |   |                            | 2.4                      |                               |                              |
| $\alpha$ -Ionone                       | 1433            |                          | 0.2   | 1.4                        |                          |                               |                              |
| Dihydroactinidiolide                   | 1539            | 4.4                      |   |                            |                          |                               |                              |
| (E,Z)-Pseudoionone                     | 1596            | 4.9                      | 2.1   |                            |                          |                               |                              |
| NI <sup>c</sup>                        | 1603            |                          | 1.2   |                            |                          |                               |                              |
| (E,E)-Pseudoionone                     | 1611            | 13.1                     | 1.8   | 2.3                        |                          |                               |                              |
| NI <sup>c</sup>                        | 1617            | 0.6                      | 1.3   |                            |                          |                               |                              |
| 3-Oxo-7,8-dihydro- $\alpha$ -ionone    | 1628            |                          | 1.2   | 5.6                        |                          |                               |                              |
| 3-Hydroxy- $\beta$ -ionol              | 1640            | 8.2                      | 1.5   |                            |                          |                               |                              |
| 3-Hydroxy- $\alpha$ -ionone            | 1645            |                          | 1.8   | 10.3                       |                          |                               | 5.0                          |
| NI <sup>c</sup>                        | 1648            |                          | 3.3   | 15.0                       |                          | 2.3                           | 7.0                          |
| 4-Hydroxy- $\beta$ -ionol <sup>d</sup> | 1653            | 2.2                      | 1.3   |                            |                          |                               |                              |
| 3-Oxo- $\alpha$ -ionol                 | 1658            | 9.8                      |   | 6.8                        |                          |                               |                              |
| 3-Hydroxy-7,8-dihydro- $\beta$ -ionol  | 1659            | 4.4                      |   |                            |                          |                               |                              |
| 3-Hydroxy- $\beta$ -ionone             | 1668            |                          |   |                            |                          |                               | 13.5                         |
| 3-Hydroxy-7,8-epoxy- $\beta$ -ionol    | 1678            | 17.5                     | 4.3   | 25.4                       |                          | 0.9                           | 15.5                         |
| 4-Oxo- $\beta$ -ionone                 | 1680            | 4.4                      |   |                            |                          |                               |                              |
| 4-Hydroxy-7,8-dihydro- $\beta$ -ionol  | 1694            | 6.5                      | 4.6   | 3.8                        |                          |                               |                              |
| 3-Hydroxy-7,8-epoxy- $\alpha$ -ionone  | 1755            |                          |   |                            |                          |                               | 2.9                          |
| NI <sup>c</sup>                        | 1790            |                          |   |                            |                          |                               | 2.9                          |

<sup>a</sup> Relative percentage of the VOC based on the GC-MS chromatographic area; <sup>b</sup> RI = Retention Indices on SLB<sup>TM</sup>-5MS column (Supelco); <sup>c</sup> NI = not identified; <sup>d</sup> = tentatively identified.

The volatile profile of *E. palustre* var. *americana* showed forty-four VOC with mostly lipidic derivatives (58.8%), i.e., 1-octen-3-ol (12.4%; mushroom-like odor), (*E*)-2-hexenoic acid (10.7%; fruity odor), as well as odorous compounds such as (*E*)-2-hexenal (6.6%; green odor), (*Z*)-3-hexenol (2.5%; grass, fresh odor) [6d], hexanol (1%; balsamic odor), and (*E*)-2-nonenal (0.8%; fatty, green odor) [6e]. Only one terpenic derivative was identified, i.e., linalool (0.7%; floral, orange odor). The 1-octen-3-ol responsible for the mushroom-like odor has also been reported from many mushrooms [6f,6g], ferns [4i,4j] and Angiosperms [6h]. (*E*)-2-Hexenoic acid (10.7%), with a fruity, herbal odor, has also been found in both ferns [4i,4j] and mushrooms [4h].

The volatile pattern of *E. telmateia* based on fifteen VOC was dominated by a large number of isoprenoid derivatives (70.6%), i.e., 3-hydroxy-7,8-epoxy- $\beta$ -ionol (25.4%) and 3-hydroxy- $\alpha$ -ionone (10.3%), as well as one benzenic derivative, i.e., 3-methoxy-4-hydroxystyrene also called 4-vinylguaiaicol (18.1%; spicy clove smoky odor).

Ionol and ionone derivatives were identified from quince leaves [7a] and ferns [4i,4j];  $\alpha$ -ionone shows a more floral *smell* than  $\beta$ -ionone. Isoprenoids, widely produced by animals, microbes and plants, are of great interest in the industrial production of pharmaceuticals, colorants and flavors. Research has focused on their transformation into functional isoprenoids [7b].

The major VOC from *E. hyemale* derived from the lipidic pathway (69.7%), such as (*E*)-2-heptenal (22.4%; green vegetable-like odor with fatty undertone), and the shikimic pathway (25.5%), i.e., (*E*)- and (*Z*)-ferulic acid isomers (10.3% and 8.6%, respectively), 4-vinylguaiaicol (5.9%) and isovanillin (0.7%, vanilla odor). Ferulic acid derivatives are converted into 4-vinylguaiaicol by decarboxylation, then oxidized to vanillin [7c].

Cinnamic acid derivatives have received much attention in medicinal research, more recently as synthetic antitumor agents [7d,7e].

*E. ramosissimum* showed the highest amount of shikimic derivatives (76.6%), i.e., (*E*)-ferulic acid (28.0%), 4-vinylguaiaicol (19.6%), (*Z*)-ferulic acid (18.7%), and 2-phenylethanol (6.5%; rose odor). Lipid derivatives, such as (*E*)-2-decenal (7.1%), also contributed to the complex odor of *E. ramosissimum*.

The volatile profiles of *E. ramosissimum* and *E. hyemale*, both belonging to the *Hippochaete* subgenus, showed similarities, with both producing the same main shikimic compounds (ferulic acid isomers and 4-vinylguaiaicol). 2-Phenylethanol and (*E*)-2-decenal were previously detected in several ferns (*Adiantum capillus-veneris*, *Blechnum spicant* and *Asplenium trichomanes*) [4i,4j]. In addition, (*E*)-2-decenal, a natural plant and mushroom VOC with a plastic oxidized mutton fatty odor was an abundant key component of the strong "stink bug" odor with (*E,Z*)-2,4-decadienal and (*E,E*)-2,4-decadienal [7f]; these three VOC were also found in *A. capillus-veneris*, *E. ramosissimum* and *E. scirpioides*.

*E. scirpioides* showed the lowest VOC content with a broad spectrum based on twenty-five VOC. High amounts of isoprenoids (46.9%), mainly ionone derivatives, were identified as 3-hydroxy- $\beta$ -ionone (13.5%) or 3-hydroxy-7,8-epoxy- $\beta$ -ionol (15.5%). Lower amounts of aromatics (34.8%), i.e., homovanillic acid (15.2%) and phenylethanol (6.5%; hyacinth, lilac note) [4f,4h], already found in *Gymnocarpium dryopteris* and *Phegopteris connectilis* [4j], as well

as lipid derivatives, i.e., (*E*)-2-heptenal (4.1%), (*E,E*)-2,4-decadienal (4.1%; orange-like odor) and (*E,Z*)-2,4-heptadienal (3.8%) complete the volatile pattern of *E. scirpioides*.

Among the six *Equisetum* species investigated for volatiles belonging either to *Hippochaete* or *Equisetum* subgenera, the quantitatively major volatiles were aromatic compounds, i.e., 4-vinylguaiaicol, homovanillic acid, and (*Z*)- and (*E*)-ferulic acid isomers, which were particularly present in the *Hippochaete* subgenus. Ferulic acid, a precursor of vanillin with antioxidant activity, may be effective in preventing cancer [7g]. 3-Hydroxy-7,8-epoxy- $\beta$ -ionol and 3-hydroxy- $\beta$ -ionone were the isoprenoid derivatives detected in the highest amount. Another megastigmane glucoside, i.e., (6*R*,9*S*)-3-oxo- $\alpha$ -ionol 9-*O*- $\beta$ -D-glucopyranoside, was previously reported in *E. debile* [7h]. On the other hand, lipids (C<sub>6</sub>-C<sub>10</sub>) constituted the most diversified VOC content in horsetails with more than 20% of (*E*)-2-heptenal in *E. hyemale*.

## Conclusion

*Equisetum* resources have great potential as new bioactive litter and potential functional feed ingredients due to the importance of their biologically active constituents, for example silicon, phenolic derivatives and volatile organic compounds [2a-2b,4b,8a,8b].

In addition, each investigated *Equisetum* species synthesizes numerous volatiles known to exhibit insecticidal, pesticidal and/or arthropod repellent properties. These plants may be useful sources of chemicals for the control of micro-organisms of medical, veterinary and agricultural importance. Except *E. palustre*, which is a well-known livestock poison, containing the alkaloid palustrine, *Equisetum* natural resources are of major importance as new potential functional feed ingredients and bioactive litter.

## Experimental

**Plant material:** Fresh aerial parts of *Equisetum arvense* L., *E. palustre* var. *americana* Vict., *E. ramosissimum* Desf., and *E. scirpioides* Michx. were collected on 25<sup>th</sup> November, 2009 from the Botanical Garden of Limoges. *E. telmateia* Ehrh and *E. hyemale* L. were obtained on 31<sup>st</sup> August, 2010, from the Botanical Garden of Strasbourg. Voucher specimens are deposited at the Laboratory of Botany (Faculty of Pharmacy, Limoges, France).

**Plant part and GC-MS analyses:** Fresh aerial parts of *Equisetum* were cubed and extracted with diethyl ether (Carlo Erba, 6 ppm BHT). After one-week of maceration at room temperature, the concentrated organic extracts were used for Gas Chromatography-Mass Spectrometry (GC-MS) analyses, as reported in the literature [4i,4j]. The analyses were performed at the Platform for Chemical Analyses in Ecology of the "SFR 119 Montpellier Environnement Biodiversité".

VOC were identified by comparison with National Institute of Standards and Technology Mass Spectral Library [5a], retention indices listed in the literature [5b], and our own data bank. Internal standards (*n*-alkanes) were used as reference points in the calculation of relative retention indices.

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