Contribution of HRC to the study on the chemistry of natural plants, chemotaxonomy, and biodiversity conservation

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Contribution of HRC to the Study on the Chemistry of Natural Plants, Chemotaxonomy, and Biodiversity Conservation

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1 Introduction

Study of the chemistry of natural products, chemotaxonomy, and biodiversity conservation is a relevant topic today and will play an important role in the future. Humankind's knowledge and understanding of these subjects is still very limited, and many plants and animals are steadily becoming extinct through the influence of humans and nature before they have been studied.

Vietnam is one of the countries well endowed with biodiversity. Its diverse climates and ecosystems, fertile river deltas, forests, and high mountains contribute to the natural richness of biodiversity. Vietnam has vast and very rich resources of medicinal and aromatic plants. This country is also ethnically diverse with a human population living in a variety of cultures. The interaction of this human diversity with nature is responsible for the development and maintenance of biodiversity.

HRC has become one of the most powerful techniques for the analysis of multicomponent mixtures from various origins. The separation of natural products by HRC, in combination with identification by mass spectrometry, is indispensable in establishing a systematic classification of the many plant species occurring in nature. We undertook a systematic investigation to determine the constituents present in the essential oils of medicinal and aromatic plants from Vietnam. In these studies HRGC and HRGC/MS are used for the characterization of the essential oils of various plant species from the families of *Lauraceae*, *Zingiberaceae*, *Compositae*, *Labiatae*, and *Rutaceae*. The analytical results are used for the classification of different chemotypes within morphologically similar plant species. Moreover, these results could possibly open up possibilities for industrial

processing and commercialization of plants with valuable constituents. Chemical data on some new Vietnamese plant oils are presented here.

2 Materials and Methods

2.1 Plant Material and Isolation of the Essential Oils

Plants were harvested in 1990–1994 during their flowering stage in the provinces of Lao Cai, Nghệ An, and Huế, and in Hồ Chí Minh City and Hanoi. Voucher specimens have been deposited in the herbarium of the Institute of Ecology and Natural Resources, Hanoi. The essential oils were prepared by steam distillation of fresh plant material in a glass apparatus during 3–6 h.

2.2 Essential Oil Analyses

The oils were investigated by capillary GC and GC/MS. GC analyses were carried out using Hewlett Packard 5710 and HP 5890 Series II gas chromatographs equipped with FID detectors. HP-1 fused silica columns (25 m × 0.32 mm i.d., 0.25 µm film thickness) were used. Samples were injected by split and splitless techniques. Injector and detector temperatures were maintained at 250 °C. The column ovens were programmed from 60 °C (after 2 min) to 220 °C at 4°/min and the final temperature was held for 20 min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components are based on the peak areas obtained, without FID response factor correction.

GC/MS analyses were carried out on Hewlett-Packard 5970A and HP 5971 mass selective detectors (MSD), directly coupled

to HP 5790A and HP 5890 gas chromatographs, respectively. 26 m × 0.22 mm i.d. columns, coated with 0.13 µm of CP-Sil 5CB were employed, using helium as carrier gas. The oven temperature program was 60 °C (3 min), then 5°/min to 250 °C (30 min). Other conditions were the same as described above.

The constituents of the oils were identified by matching their 70 eV EI mass spectra and (temperature programmed) retention indices with reference libraries and literature data [1–6]. Co-elution GC with reference compounds was used for confirmation of some of the compound identifications. Proton and ¹³C-NMR spectrometry were used for the structure elucidation of some major oil components if present in concentrations higher than 70%.

3 Results and Discussion

3.1 Labiatae Genera

Many species of *Labiatae* seem to be factories for the production of various compounds necessary for our life. During the past ten years the authors have thoroughly studied many plants of this family with the help of HRGC. The endeavor to investigate so many *Labiatae* oils is justified because of the widespread use of these valuable oils, and is spurred by the intricate chemical composition of various chemotypes, distributed in different phyto-geographical regions.

3.1.1 Essential Oils from *Elsholtzia* Species

The genus *Elsholtzia* (family *Labiatae*) is also a versatile genus, well-known for medicinal and aromatic properties. Most of the species of *Elsholtzia* grow wild throughout the tropical and subtropical regions of the world. Some species, like *E. ciliata* (Thunb.) and *E. cristata* Wild. are cultivated in various parts of the world. Many *Elsholtzia* species are used as spices and as common traditional medicine against influenza, headache, sore throat, measles, ostealgia, and furunculosis [7]. They are also haemostatic and are used to treat menorrhagia, metrorrhagia, haemoptysis, and bloody stools. The essential oils of this genus also have a broad spectrum of antimicrobial activities, a reason for exploring their potential therapeutic uses. The aim of our investigations of *Elsholtzia* species is, therefore, not only to reveal the chemical composition of the various essential oils, which may be helpful in their chemotaxonomy, but also to get to know the potential industrial interest in these species.

The volatile constituents of *E. blanda* Benth. have been analyzed previously [8–11]. The essential oils from the aerial parts of a Chinese variety have been reported to contain 1,8-cineole (28%) and α-phellandrene (9%) as major constituents [8,9]. Another Chinese chemotype gave oils containing linalool (33–56%), 1,8-cineole (6–19%), and acetophenone (2–10%) as main compounds [10]. An Indian chemotype yielded an oil containing mainly geranyl acetate (71%). In that particular oil another 28 compounds were identified [11], of which linalool (5%), geraniol (4%), and (*E*)-β-ocimene (3%) were the most important ones.

The volatile oil from the aerial parts of *E. blanda* Benth., grown in Vietnam, was investigated. The compounds identified in this oil are given in **Table 1** with their percentage composition in the second column of this table. Thirty-four components could be identified, of which 1,8-cineole was the major constituent. The

Table 1. Percentage composition of the essential oils from four Vietnamese *Elsholtzia blanda* chemotypes.

Compound ^{a)}	<i>E. blanda</i> Benth.	<i>E. blanda</i> (Benth.) var. spp.		
		specimen 1	specimen 2	specimen 3
α-thujene	0.5	tr	–	–
benzaldehyde	tr	–	–	–
α-pinene	4.3	2.7	1.5	tr
camphene	0.9	tr	2.2	tr
sabinene	2.2	0.8	0.6	tr
β-pinene	5.9	7.2	1.0	0.2
myrcene	1.4	1.3	0.4	–
α-phellandrene	tr	tr	–	tr
α-terpinene	tr	–	tr	–
<i>p</i> -cymene	0.1	tr	0.4	tr
1,8-cineole	62.0	52.7	13.4	tr
limonene	tr	0.5	2.9	–
(<i>Z</i>)-β-ocimene	1.5	1.7	0.2	–
acetophenone	0.1	0.3	0.1	–
(<i>E</i>)-β-ocimene	0.6	0.5	0.3	–
α-terpinene	2.4	tr	–	tr
<i>trans</i> -sabinene hydrate	0.6	0.2	–	–
<i>cis</i> -linalool oxide	–	0.1	–	–
terpinolene	0.2	–	–	tr
linalool	0.3	14.5	18.7	0.3
camphor	2.0	–	2.8	tr
borneol	0.1	–	1.0	tr
terpinen-4-ol	3.4	0.3	0.3	0.1
<i>trans</i> - <i>p</i> -menthen-2-ol	0.6	–	–	–
α-terpineol	5.0	2.9	0.5	tr
<i>trans</i> -piperitol	–	–	0.7	–
piperitone	0.1	–	2.8	–
geraniol	–	1.0	2.4	–
geranial	–	–	0.2	–
bornyl acetate	tr	–	1.9	–
geranyl acetate	–	0.1	31.3	–
α-copaene	0.2	–	–	–
<i>cis</i> -α-bergamotene	–	–	–	2.8
β-caryophyllene	2.4	2.3	4.1	25.3
<i>trans</i> -β-bergamotene	–	–	–	21.6
<i>trans</i> -α-bergamotene	0.4	–	–	–
α-humulene	1.5	0.3	0.7	13.5
γ-murolene	0.4	–	–	–
α-curcumene	–	–	–	0.2
germacrene D	–	2.6	0.8	–
(<i>E</i>)-β-farnesene	–	–	–	4.6
(<i>Z,E</i>)-α-farnesene	–	4.4	3.4	–
β-selinene	0.1	–	–	–
β-bisabolene	–	–	–	1.8
γ-cadinene	0.4	0.2	–	–
δ-cadinene	0.2	tr	–	–
β-sesquiphellandrene	–	–	–	5.3
caryophyllene oxide	0.1	0.2	0.9	7.0
β-asarone	–	–	–	7.5
α-asarone	–	–	–	6.6
trimethoxybenzaldehyde	–	–	–	1.9
parsley apiole	–	–	–	0.8
dill apiole	–	–	–	0.7
other compounds	tr	2.9	4.5	0.5

^{a)} Listed in the order of elution from a HP-1 column; tr = trace = < 0.1%.

oxygenated compounds (>70%) play an important role in the odor of this oil. Another variety of this plant, *E. blanda* (Benth.) var. spp., was found in the Lào Cai area. This plant gave a high yield of essential oil (1.64% on the basis of dried material), which is a light yellow liquid with acceptable odor. The composition of this oil is also listed in Table 1 (third column). The oil of this species also contains mainly 1,8-cineole, but the linalool content is appreciably higher. Other compounds such as (*Z*)- β -ocimene, *trans*-sabinene hydrate, *cis*-linalool oxide, terpinen-4-ol, α -terpineol, geraniol, and geranyl acetate contribute to the aromatic odor of this oil.

Recently we have found another *E. blanda* (Benth.) var. spp. species in the Sapa area (Lào Cai province). The oil of this species contained geranyl acetate (31.3%), linalool (18.7%), and 1,8-cineole (13.4%) as main constituents (see Table 1, fourth column). This oil has a special odor, like rose oil.

A fourth variety of *E. blanda* (Benth.) var. spp., distributed in the Sapa area, has been found and analyzed. Table 1, column five, shows the percentages of the components identified in the oil of this species. The main characteristic components were sesquiterpenes, a promising value for fixative purposes.

According to their main oil constituents, the investigated specimens of *Elsholtzia blanda* Benth. from Vietnam can thus be classified into four chemotypes: I: 1,8-cineole (62%), II: 1,8-cineole (53%) and linalool (15%), III: geranyl acetate (31%), linalool (19%), and 1,8-cineole (13%), and IV: the sesquiterpene (99%) chemotype.

Table 2. Percentage composition of the essential oil from Vietnamese *Elsholtzia communis* (L.).

Compound ^{a)}	area %	Compound	area %
3-methylbutanal	tr	α -copaene	0.1
3-methylbutanoic acid	tr	β -bourbonene	0.9
α -pinene	0.4	β -caryophyllene	4.5
camphene	0.2	<i>trans</i> - α -bergamotene	0.2
sabinene	tr	α -humulene	1.4
β -pinene	tr	germacrene D	1.3
α -terpinene	tr	germacrene B	0.3
1,8-cineole	0.1	γ -cadinene	0.1
limonene	0.1	δ -cadinene	0.5
acetophenone	tr	spathulenol	0.3
γ -terpinene	tr	caryophyllene oxide	0.5
linalool	0.2	β -asarone	0.4
camphor	tr	α -cadinol	0.1
α -terpineol	tr	α -asarone	0.4
elsholtzia ketone	82.3	apiole	tr
piperitone	tr	dill apiole	tr
		other compounds	4.9

^{a)} Listed in the order of elution from a HP-1 column; tr = trace = < 0.1%.

Table 3. Percentage composition of the essential oil from Vietnamese *Perilla* species.

Compound ^{a)}	"purple" <i>Perilla</i>	"green" <i>Perilla</i>	<i>P. frutescens</i> (L.) Britt.
2-methylpropanal	—	—	tr
1-buten-3-one	—	—	tr
3-methylbutanal	—	—	tr
furfural	—	—	tr
2-ethylbutanal	—	—	tr
α -thujene	—	—	tr
benzaldehyde	tr	tr	tr
α -pinene	tr	tr	—
1-octen-3-ol	tr	tr	2.8
β -pinene	0.7	0.2	—
3-octanol	tr	tr	—
myrcene	tr	tr	—
α -terpinene	—	—	0.2
1,8-cineole	0.3	0.7	tr
limonene	3.4	6.8	2.2
(<i>Z</i>)- β -ocimene	—	—	tr
(<i>E</i>)- β -ocimene	—	—	tr
γ -terpinene	—	—	tr
terpinolene	—	—	0.1
linalool	0.7	0.7	3.2
methyl salicylate	tr	tr	—
α -terpineol	0.2	0.2	tr
perilla ketone	—	—	77.4
isopiperitenone	—	—	tr
perillal	6.7	16.1	1.0
safrole	—	—	tr
perilla alcohol	0.1	tr	—
(<i>Z</i>)-methyl geranate	—	—	tr
eugenol	tr	tr	tr
damascenone	tr	0.6	—
methyleugenol	tr	tr	—
α -copaene	tr	tr	0.1
β -bourbonene	tr	tr	tr
β -elemene	0.3	0.3	0.1
β -caryophyllene	20.4	13.6	4.1
α -humulene	2.1	1.3	0.5
germacrene D	1.8	1.8	0.6
myristicin	7.5	7.2	—
(<i>E,E</i>)- α -farnesene	tr	tr	tr
δ -cadinene	tr	tr	tr
elemicin	50.0	40.7	—
(<i>Z</i>)-nerolidol	0.1	0.2	0.2
caryophyllene oxide	tr	0.2	3.2
δ -cadinol	—	—	0.1
α -cadinol	—	—	0.1
other compounds	4.9	8.6	2.9

^{a)} Listed in the order of elution from a HP-1 column; tr = trace = < 0.1%.

Two more species of the genus *Elsholtzia* from Vietnam have been investigated. **Table 2** lists the chemical composition of the essential oil from *E. communis* (L.). No reports on the volatile constituents of this plant could be found in the literature. The analytical results show that *E. communis* (L.) from Vietnam belongs to the *Elsholtzia* ketone chemotype. Its volatile oil is a valuable source of *elsholtzia* ketone (82.3%).

The composition of the volatile oils from *E. ciliata* grown in two different areas in Vietnam has been compared recently [12]. These oils contained geranial (20–27%), neral (15–21%), limonene (11–14%), and (*Z*)- β -farnesene (11–12%).

3.1.2 Essential Oils from *Perilla frutescens*

Perilla frutescens (Labiatae) is a medium sized genus of strongly scenting perennial herbs distributed in many places of Vietnam. *Perilla* species are used in traditional medicine and are also well known as a source of valuable compounds, which are either used as such in perfumery and allied industries, or as starting material for the synthesis of other products.

On steam distillation, the plants yield about 0.25% of essential oils. The chemical composition of the oils from “purple” and “green” *Perilla* is presented in **Table 3**. Elemicin (50.0 and 40.7%), β -caryophyllene (20.4 and 13.6%), and perillal (6.7 and 16.1%) are the main constituents of these respective oils. Another local variety, *P. frutescens* (L.) Britt., however, was found to yield an oil with perilla ketone (77.4%) as major constituent (see **Table 3**, last column).

Two chemotypes of *P. frutescens* had already been found previously in Vietnam [13,14]. From these and the present results it may be concluded that there exist at least four chemotypes of *Perilla frutescens* in Vietnam. The first chemotype has perillal as major constituent [13]. The second type contains mainly limonene, piperitone, and β -caryophyllene [14]. The third chemotype is rich in elemicin, perillal, and β -caryophyllene, while the fourth type is characterized by perilla ketone (*cf.* **Table 3**). The third chemotype is reported here for the first time and is a promising source of elemicin.

3.2 Compositae Genera

Compositae is one of the largest, diverse, and most important families in the flora of Vietnam. Several species of this family have been used as sources of natural material for food, for the production of herbicides and growth regulators, and as drugs in traditional and modern medicine. In recent years we have studied various genera of the family Compositae such as several species of *Artemisia*, *Ageratum*, *Blumea*, *Eupatorium*, *etc.*

In the course of these investigations we found some typical chemotypes in Vietnam, differing substantially from chemotypes in other parts of the world. For example, the essential oil from *Blumea lanceolaria* (Roxb.) Druce growing in India contained 99.5% of *p*-cymene [15], while the oils from the Vietnamese variety contained 95.0% of methylthymol [16].

Another example is *Ageratum conyzoides* L. The oils from the Indian chemotypes contained mainly precocene II (*syn.* *agerato-*

chromene) [17,18]. Plant oils from Nigeria have been reported to contain both precocene I and II as major constituents [19]. The Vietnamese chemotype also contains precocene I (29%) and precocene II (31%), but in addition a substantial amount of β -caryophyllene (17%) [20].

4 Conclusions

Cataloguing plants by their morphological character is not sufficient for classification. Knowledge of the composition of the essential oils from (still) numerous species provides a better means for systematic classification, based on chemotaxonomy. In this report several examples of different chemotypes of otherwise morphologically similar plants have been described. HRC may thus help to increase our knowledge of the great biodiversity and, indirectly, to the conservation of numerous valuable plants.

References

- [1] Y. Masada, Analysis of essential oils by gas chromatography and mass spectrometry, Wiley, New York, NY (1967).
- [2] P. Sandra and C. Bicch, Capillary gas chromatography in essential oil analysis, Hüthig, Heidelberg, Germany (1987).
- [3] L.M. Libbey, A Paradox Data Base for GC/MS Data on Components of Essential Oils and Other Volatiles, *J. Essent. Oil Res.* 3 (1991) 193.
- [4] D. Henneberg, B. Weimann, and W. Joppck, Library Search System MASS-LIB, version 5.3, Max-Planck-Institut für Kohlenforschung, Mülheim a.d. Ruhr, Germany (1989). Using this software the following data bases were searched: a) F.W. McLafferty, Wiley/NBS registry of mass spectral data, Wiley, New York, NY, 4th ed. (1988); b) D. Henneberg, B. Weimann, and W. Joppck, MPI library of mass spectral data, Max-Planck-Institut für Kohlenforschung, Mülheim a.d. Ruhr, Germany (1989); c) M.C. ten Noever de Brauw, J. Bouwman, A.C. Tas, and G.F. La Vos, Compilation of mass spectra of volatile compounds in food, TNO-CIVO Institutes, Zeist, The Netherlands (1988).
- [5] F.W. McLafferty and D.B. Stauffer, Mass spectrometry library search system BenchTop/PBM, version 3.0, Palisade Co., Newfield, NY, (1993). Using BenchTop/PBM the following database was searched: F.W. McLafferty and D.B. Stauffer, The Wiley/NBS registry of mass spectral data, 5th ed., Wiley and Sons, New York, NY (1991).
- [6] National Institute of Standards and Technology, PC Version of the NIST/EPA/NIH mass spectral database, version 4.5, U.S. Department of Commerce, Gaithersburg, MD, USA (1994).
- [7] Đỗ Tất Lợi, Medicinal Plants and Prescriptions from Vietnam, Science and Technology Publisher, Hanoi, Vietnam (1988).
- [8] H. Fang, Y. Xu., and J. Lin, Proceedings 11th International Congress on Essential Oils, Fragrances and Flavours, Nov. 1989, New Delhi, India, Vol. 4, p. 49.
- [9] H. Fang, H. Duan, Y. Xu, T. Zhou, and J. Lin, *Seppu* 11 (1993) 69.
- [10] B. Cheng, X. ma, X. Yu, and J. Ding, *Yunnan Zhiwu Yanjiu* 11 (1989) 91.
- [11] H.J. Bestmann, J. Rauscher, O. Vostrowsky, A.K. Pant, V. Dev, R. Parihar, and C.S. Mathela, *J. Essent. Oil Res.* 4 (1992) 121.
- [12] N.X. Dung, L.V. Hac, L.H. Hải, and P.A. Leclercq, *J. Essent. Oil Res.* 7 (1995), in press.
- [13] H.V. Phiet and H.T. Huong, *Tập San Hóa Học (J. Chem., Vietnam)* 16(2) (1978) 24.
- [14] N.X. Dung, L.D. Cu, L.D. Mõi, and P.A. Leclercq, *J. Essent. Oil Res.* 7 (1995), in press.
- [15] S.C. Dutta, B.N. Saha, M.G. Pathak, and R.K. Mathur, *Indian Perfum.* 33 (1989) 38.
- [16] N.X. Dung, D.T. Lôi, D.T. Hùng, and P.A. Leclercq, *J. Essent. Oil Res.* 3 (1991) 285.
- [17] E. von Rudloff and V.K. Sood, *Perfum. Essential Oil Rec.* 60 (1969) 303.
- [18] V.K. Sood, *Flavour Ind.* 4 (1973) 77.
- [19] O. Ekundayo, I. Laakso, and R. Hiltunen, *Planta Med.* (1988) 55.
- [20] N.X. Dung, P.T.T. Tho, N.V. Dan, and P.A. Leclercq, *J. Essent. Oil Res.* 1 (1989) 135.

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