# Volatile Components of Some Rutaceae Species

Antoaneta Ivanova[a], Ivanka Kostova[a,*], Hernan Rodriguez Navas[b], and Juan Villegas[c]

[a] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria. E-mail: kostiv1@yahoo.com
[b] Institute of Botany, National University, Heredia, Costa Rica
[c] Institute of Chemistry, National University, Heredia, Costa Rica

* Author for correspondence and reprint requests

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The volatile components of the aerial parts of *Ruta graveolens* and *Haplophyllum suaveolens*, as well as of leaves of *Zanthoxylum limoncello*, *Z. panamense* and *Z. setulosum* have been studied by GC/MS analysis.

*Key words*: Volatiles, *Ruta graveolens*, *Haplophyllum suaveolens*, *Zanthoxylum limoncello*, *panamense* and *setulosum*

## Introduction

The genera *Ruta* L., *Haplophyllum* Juss., and *Zanthoxylum* L. belong to the family Rutaceae. The plants of these genera find application in the traditional and contemporary medicine of many countries (Petit-Paly *et al.*, 1988; Charlton, 1998; Ahsan *et al.*, 2000; Shim *et al.*, 2001). The volatile oil from *R. graveolens* possesses phototoxical, bacteriostatical and anthelminthical activities (Petit-Paly *et al.*, 1988). The anti-inflammatory, anesthetic and hypotensive activities of the essential oil from the fruits of *Z. rhesta* (Ahsan *et al.*, 2000) and the repellent activity of the volatiles from *Z. piperitum* are also reported (Shim *et al.*, 2001).

The presence of coumarins, alkaloids, terpenes and flavonoids in *Ruta graveolens* has been reported (Kostova *et al.*, 1999). Data exist about the chemical composition of leaf, fruit and root oils from *R. graveolens* of German origin, as well as of the essential oil from the aerial parts of *R. graveolens* grown in Italy (Kubeczka, 1971, 1974; De Feo *et al.*, 2002). The total content of the essential oil in different Bulgarian populations of *R. graveolens* was established (Vitkova and Philipov, 1999). However, the composition of the essential oil of the Bulgarian *R. graveolens* has not been studied yet.

*Haplophyllum suaveolens* contains quinoline alkaloids, flavonoids, lignans and coumarins (Kostova *et al.*, 2000; Ivanova *et al.*, 2001b). Preliminary GC/MS investigations of the petroleum ether extract of the aerial parts of this plant species revealed the presence of aliphatic acids, phosphoric acid, glycerol and its esters, sesquiterpenes and sterols (Ivanova *et al.*, 2001a).

The genus *Zanthoxylum* is a rich source of alkaloids, coumarins, furocoumarins, terpenoids and other metabolites (Stermitz and Sharifi, 1977). The volatile components of *Z. limoncello, Z. panamense* and *Z. setulosum* have not been investigated.

The aim of the present study is to analyze the volatile components of the aerial parts of *R. graveolens* and *H. suaveolens* of Bulgarian origin, as well as of the leaves of *Z. limoncello, Z. panamense* and *Z. setulosum*, growing in Costa Rica.

## Experimental

### *Plant material*

Aerial parts of *Haplophyllum suaveolens* (DC.) G. Don fil. were collected in July 1998 in Ognyanovo (the region of Plovdiv, Bulgaria) and identified by Dr. A. Vitkova. A voucher specimen was deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

*Ruta graveolens* L. cultivated in the region of Gorna Orjahoviza, collected in 1996 and purchased from Bilkocoop Ltd. (Sofia, Bulgaria) was investigated.

The leaves of the three *Zanthoxylum* species were collected in May 2000 in the following regions of Costa Rica: *Zanthoxylum limoncello* (Planch & Oerst) – San Rafael, Heredia; *Zan-*

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*thoxylum panamense* (P. Wilson) – Sarapiqui, Heredia; *Zanthoxylum setulosum* (P. Wilson) – Cuidad Colon, San Jose. The collected *Zanthoxylum* species were authenticated by Dr. Hernan R. Navas and voucher specimens deposited at the Institute of Botany, National University, Heredia, Costa Rica.

*Isolation of volatile compounds*

A part of the chloroform extracts of the studied plant species (260.0 mg from *R. graveolens*; 236.0 mg from *H. suaveolens*; 249.0 mg from *Z. limoncello*; 280.0 mg from *Z. panamense* and 230.0 mg from *Z. setulosum*) was subjected to a four-hour distillation-extraction in Lickness-Nickerson apparatus (Hendriks *et al.*, 1981). The volatile compounds were extracted from the distillate with diethyl ether. Yields: *R. graveolens* – 26.8 mg (10.3% of the lipophylic extract); *H. suaveolens* – 43.0 mg (18.2% of the lipophylic extract); *Z. limoncello* – 34.4 mg (13.8% of the lipophylic extract); *Z. panamense* – 33.0 mg (11.8% of the lipophylic extract) and *Z. setulosum* – 31.6 mg (13.7% of the lipophylic extract).

*Analysis and identification of volatile compounds by GC/MS*

The samples were investigated by analytical GC/MS on Hewlett-Packard Gas Chromatograph 6890 equipped with a Hewlett-Packard MS 5973 detector. HP5-MS capillary column (30 m × 0.25 $\mu$m film thickness, Agilent Technologies, Wilmington, Delaware, USA) was used. The temperature was programmed from 40° C to 280° C at a rate of 6° C · min$^{-1}$. Helium was used as a carrier gas at 9 ml · min$^{-1}$. The ion source was set at 250° C and the ionization voltage was 70 eV. The identification of components was accomplished by computer searches in the HP Mass Spectral Library NIST98 (Hewlett-Packard, Palo Alto, California, USA).

## Results and Discussion

The volatile compounds in *R. graveolens*, *H. suaveolens*, *Z. limoncello*, *Z. panamense* and *Z. setulosum* were analyzed by GC/MS. The results are summarized in Table I. Aliphatic acids, alcohols and ketones, hydrocarbons, mono- and sesquiterpenoides, and coumarins are the main groups of the compounds identified.

The presence of aliphatic acids is a characteristic feature of all samples under investigation. The biggest amount of aliphatic fatty acids is found in *Z. panamense* (17.2%), followed by *R. graveolens* (16.5%), *Z. limoncello* (14.7%), *H. suaveolens* (9.2%) and *Z. setulosum* (5.3%). Pentanoic, hexanoic, octanoic and nonanoic acids are common compounds in the volatiles of the five species. The branched fatty acids, 2-methylbutanoic and 3-methylbutanoic acid, are detected in trace amount only in *R. graveolens* and *H. suaveolens.* The $C_3$–$C_7$ fatty acids are in low amounts in all species investigated.

Aliphatic alcohols are present in *R. graveolens* and *Z. limoncello*, while aliphatic ketones are found only in *R. graveolens.*

Hydrocarbons are observed in the volatiles of *R. graveolens*, *Z. limoncello* and *Z. setulosum* – 1.8%, 3.1% and 6.3%, respectively.

Monoterpenoids are only detected in *H. suaveolens* ($\alpha$-terpineol, myrtenol), whereas sesquiterpenoids are observed in different quantitative and qualitative ratios in the volatile components of all species. The biggest amount of sesquiterpenoids is found in *Z. panamense* (21.1%), followed by *H. suaveolens* (19.0%) and *Z. setulosum* (16.0%).

The coumarin xanthotoxin is found in the volatiles of *R. graveolens*, while 4,4,5,7,8-pentamethyldihydrocoumarin is identified in *H. suaveolens* and *Z. setulosum*.

Our results show a high content of aliphatic acids, alcohols and ketones in *R. graveolens.* Pentanoic, hexanoic, octanoic and nonanoic acids, 2-undecanol and 2-undecanone are the main components. The identified 2-undecanol, 2-dodecanol and 2-tridecanol are biogenetic precursors of 2-undecanone, 2-dodecanone and 2-tridecanone. 2-Undecanone and 2-undecanol have been also found in the leaf and fruit oils of *R. graveolens* of German origin (Kubeczka, 1971), as well as in the root oil of *R. graveolens,* grown in Italy (De Feo *et al.*, 2002). No terpenes and only one sesquiterpenoid ($\beta$-eudesmol) have been detected in the volatiles of the aerial parts from the Bulgarian *R. graveolens.* The presence of terpenoids (elemol, myrcene, pregeijerene and geijerene) has also been reported in the essential oil of *R. graveolens* of German origin (Kubeczka, 1974).

The high content of sesquiterpenoids and the presence of aliphatic acids, monoterpenoids, aro-

Table I. GC/MS analysis of the volatile components of *Ruta graveolens (R. g.), Haplophyllum suaveolens (H. s.), Zanthoxylum limoncello (Z. l.), Z. panamense (Z. p.)* and *Z. setulosum (Z. s.)* (in % of total ion current[a]).

| | *R. g.* | *H. s.* | *Z. l.* | *Z. p.* | *Z. s.* |
|---|---|---|---|---|---|
| *Aliphatic acids and esters* | *16.5* | *9.2* | *14.7* | *17.2* | *5.3* |
| Propanoic acid | – | – | – | – | tr[b] |
| Butanoic acid | – | – | tr | – | 0.6 |
| 2-Butenoic acid | tr | – | – | – | – |
| 2-Methylbutanoic acid | tr | tr | – | – | – |
| 3-Methylbutanoic acid | tr | tr | – | – | – |
| 3-Methyl-2-butenoic acid | – | tr | – | – | – |
| Pentanoic acid | tr | tr | tr | tr | tr |
| Hexanoic acid | tr | 0.4 | 1.2 | 0.9 | 1.3 |
| 2-Hexenoic acid | – | – | – | tr | – |
| 3-Hexenoic acid | – | – | – | tr | tr |
| 2-Ethylhexanoic acid | – | tr | – | – | – |
| Heptanoic acid | tr | tr | 1.2 | – | – |
| 3-Ethylheptanoic acid | – | tr | – | – | – |
| Octanoic acid | 0.5 | 1.1 | 1.1 | tr | 0.6 |
| 2-Octenoic acid | – | – | tr | – | – |
| Nonanoic acid | 0.8 | 0.7 | 2.3 | tr | 0.7 |
| 2-Nonenoic acid | – | – | 3.4 | – | – |
| Decanoic acid | 1.1 | 1.2 | 0.4 | – | 0.4 |
| 2-Dodecenoic acid | – | – | 0.8 | – | – |
| Dodecanoic acid | 1.8 | 2.2 | – | – | – |
| Tetradecanoic acid | 2.4 | 1.3 | – | – | – |
| Pentadecanoic acid | 0.3 | – | – | – | – |
| Hexadecanoic acid | 5.7 | 1.8 | – | 3.6 | – |
| Hexadecanoic acid, ethyl ester | – | – | 3.2 | 6.6 | 1.7 |
| Heptadecanoic acid | 0.5 | – | – | – | – |
| Octadecanoic acid, ethyl ester | – | – | – | 1.2 | – |
| 9,12-Octadecadienoic acid | 1.1 | – | – | – | – |
| 9,12-Octadecadienoic acid, ethyl ester | – | – | 0.9 | 2.1 | – |
| 9,12,15-Octadecatrienoic acid | 2.3 | – | – | 2.8 | – |
| 9,12,15-Octadecatrienoic acid, ethyl ester | – | – | – | – | – |
| Hexanoic acid, 2-ethylhexadecyl ester | – | 0.5 | 0.2 | – | – |
| *Aliphatic alcohols* | *3.0* | – | *1.3* | – | – |
| 2-Undecanol | 2.3 | – | – | – | – |
| 2-Dodecanol | 0.2 | – | – | – | – |
| 2-Tridecanol | 0.5 | – | – | – | – |
| 2-Nonen-1-ol | – | – | 0.3 | – | – |
| 1-Nonanol | – | – | 1.0 | – | – |
| *Aliphatic ketones* | *4.6* | – | – | – | – |
| 2-Undecanone | 1.3 | – | – | – | – |
| 2-Dodecanone | 0.2 | – | – | – | – |
| 2-Tridecanone | 0.5 | – | – | – | – |
| 6,10,14-Trimethyl-2-pentadecanone | 2.6 | – | – | – | – |
| *Hydrocarbons and derivatives* | *1.8* | – | *3.1* | – | *6.3* |
| Tetracosane | – | – | – | – | 0.6 |
| Pentacosane | 0.2 | – | 0.4 | – | 1.0 |
| Hexacosane | 0.2 | – | 0.6 | – | 1.3 |
| Heptacosane | 0.4 | – | 0.6 | – | 1.4 |
| Octacosane | – | – | 0.4 | – | 1.1 |
| Nonacosane | 0.2 | – | 0.3 | – | 0.9 |
| Hentriacontane | 0.3 | – | – | – | – |
| 2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene | – | – | 0.8 | – | – |
| 2-Acetoxytridecane | 0.2 | – | – | tr | – |
| 2-Propionyloxytridecane | 0.3 | – | – | – | – |

Table I. (continued)

| | *R. g.* | *H. s.* | *Z. l.* | *Z. p.* | *Z. s.* |
|---|---|---|---|---|---|
| *Monoterpenoides* | – | *0.3* | – | – | – |
| α-Terpineol | – | 0.3 | – | – | – |
| Myrtenol | – | tr | – | – | – |
| *Sesquiterpenoides* | *0.7* | *19.0* | *4.1* | *21.1* | *16.0* |
| β-Bourbonene | – | 0.3 | – | – | – |
| Eremophylene | – | 1.9 | 0.3 | – | – |
| Copaene | – | – | 0.3 | tr | – |
| Copaene-8-ol | – | 3.3 | – | – | – |
| Oplopanone | – | 1.1 | – | – | – |
| Bergamotene | – | – | 0.5 | 0.8 | – |
| β-Bergamotene | – | – | tr | – | – |
| Caryophyllene | – | – | 0.5 | 0.8 | – |
| Caryophyllene oxide | – | – | – | 5.0 | 3.4 |
| β-Eudesmol | 0.7 | – | – | – | 1.5 |
| Spathulenol | – | 12.4 | – | 3.9 | 4.4 |
| δ-Cadinene | – | – | 0.6 | 2.2 | – |
| γ-Cadinene | – | – | 0.6 | 3.4 | – |
| Sesquiphellandrene | – | – | 0.7 | – | – |
| Ledene | – | – | 0.3 | – | – |
| Isoledene | – | – | – | tr | – |
| β-Elemene | – | – | – | 0.3 | – |
| β-Selinene | – | – | – | 2.1 | – |
| Selina-4(14),7(11)-diene | – | – | – | 2.6 | – |
| Farnesol | – | – | 0.3 | – | – |
| Nerolidol | – | – | – | – | 6.7 |
| *Diterpenes* | | | | | |
| Phytol | 0.6 | 0.6 | 2.0 | 6.1 | 5.1 |
| *Aromatic alcohols* | – | *0.5* | – | – | – |
| Phenylethyl alcohol | – | 0.5 | – | tr | – |
| Benzyl alcohol | – | tr | – | – | – |
| *Coumarins* | *0.6* | *3.2* | – | – | *1.8* |
| 4,4,5,7,8-Pentamethyl-dihydrocoumarin | – | 3.2 | – | – | 1.8 |
| Xanthotoxin | 0.6 | – | – | – | – |
| *Others* | *1.1* | *2.6* | *2.6* | *5.1* | *5.6* |
| Dihydroactinidiolide | 0.9 | 1.6 | 0.4 | 1.8 | 4.8 |
| 2-Propenoic acid, 3-(4-methoxyphenyl), 2-ethylhexyl ester | 0.2 | 1.0 | 0.7 | 0.5 | 0.8 |
| Isopropylpalmitate | – | – | 1.5 | 2.8 | – |

[a] The ion current generated depends on the characteristics of the compounds and is not true quantitation.
[b] tr – trace.

matic alcohols and coumarins are specific for *H. suaveolens.* Spathulenol (12.4%) is the main volatile component. The hexanoic, octanoic, dodecanoic, tetradecanoic and hexadecanoic acids and the sesquiterpenes spathulenol and β-bourbonene have already been found in the petroleum ether extract of the same plant (Ivanova *et al.*, 2001a).

The presence of aliphatic acids, hydrocarbons, sesquiterpenoids, diterpenes and coumarins is established for *Zanthoxylum* species. The content of these structural classes varies for the studied plants. Hexadecanoic acid and its ethyl ester are the main components of *Z. panamense* (3.6% and 6.6%, respectively), while 2-nonenoic (3.4%) and nonanoic (2.3%) acid and the ethyl ester of hexadecanoic acid (3.2%) are those of *Z. limoncello.* The sesquiterpenoids caryophyllene oxide (5.0%), spathulenol (3.9%), γ-cadinene (3.4%) and selina-4,7-diene (2.6%) are major compounds in *Z. pana-*

*mense*; nerolidol (6.7%), spathulenol (4.4%) and caryophyllene oxide (3.4%) are in big amount in *Z. setulosum.*

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