CHEMICAL CONSTITUENTS OF ESSENTIAL OILS FROM THE RUTACEAE FAMILY

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In the framework of our study on the family Rutaceae for cosmetic product development, various plant parts from selected species were investigated by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and linear retention indices. Species that were collected and extracted for essential oils were as follows; *Citrus halimii, C. aurantifolia*, *C. hystrix* (hybrid) papeda hybrid, *C. medica* var. *enthrog, C. medica* var. *2, C. medica* var. *sarcodactylis, Zanthoxyllum acanthopodium, Tetractomia tetranda* and *Murraya koenigii.* Identification of chemical components was confirmed by comparison of retention times and calculated retention indices with literature values and comparison with authentic standards. In general most of the essential oils were complex mixtures of terpenoid hydrocarbons; the monoterpenoid and sesquiterpenoid. The major chemical components observed in all of the oils were reported in this paper.

Introduction

Rutaceae is a large family comprising 160 genera and 1650 species largely distributed in the tropical and subtropical parts of the world (Jones 1995). The family included the genus *Citrus, Zanthoxyllum acanthopodium, Tetractomia tetranda and Murraya koenigii.* Some of these species were often used in cooking and medicinal preparation. The essential oils obtained from the leaves or the fruit peels were also used in perfumery. Initial studies conducted at FRIM have shown that many of the Malaysian essential oils possessed many useful chemical components with bioactivity properties that could be beneficial to the herbal and cosmetic industries (Nor Azah *et al.* 2001, Zaridah *et al.* 2003). Some of these oils, the limau purut (*C. hystrix*) has been found to be suitable as fragrance ingredient in toiletries products (Nor Azah *et al.* 2005). Therefore as part of our chemical and biological profiling of Rutaceae plants for cosmetic product development, the essential oils of the lesser known species from the Rutaceae family were reported in this paper.

Materials and methods

Extraction of the essential oil

Plant samples were collected from various locations (Cameron Highlands, Kuala Krau, Pahang, Sabak Bernam, Jelebu, Negeri Sembilan) in Peninsular Malaysia. Voucher specimens were deposited at the Herbarium of the Forest Research Institute Malaysia, Kepong. The leaf, stem bark or rhizome materials were subjected to Clavenger type water distillation apparatus for 8 hours. The oily layers obtained were separated and dried over anhydrous sodium sulphate.

Analysis of essential oils

Quantitative GC analysis was carried out using Shimadzu GC 2010, Shimadzu GC 14A and Hewlett–Packard GCMSD 5890 series II /5971A apparatus using, respectively, fused silica capillary columns CBP1 (25 m x 0.25 mm, 0.25 mm film thickness) and ZB1 (30 m x 0.25 mm, 0.25 mm film thickness) for GC and DB1 (30 m x 0.25 mm, 0.25 mm film thickness) for GC-MS. The gas chromatograph was equipped with FID using split mode injection technique and the operating parameters were helium as carrier gas at a flow rate of 1ml/min, injector temperature 250 ^{0}C, and detector temperature 250 ^{0}C. With the CBP1 column, the gas chromatograph was programmed, initially at 60 ^{0}C for 10 minutes, then to 230 ^{0}C at 3 ^{0}C/min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components were based on peak areas obtained. For GC-MS analysis, the temperature program adopted for DB1 was 60 ^{0}C with an increase of 3 ^{0}C/min till 230 ^{0}C, with helium as carrier gas, ion source temperature 250 ^{0}C and electron energy 70 eV. The identification of chemical components was confirmed by comparison of retention times and calculated retention indices with literature values (Jennings & Shibamoto 1980) and comparison with authentic standards.

Results and discussion

The chemical composition of the essential oils studied were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Majority of the essential oils contained mixtures of monoterpenes and sesquiterpenes. The identification of chemical components was confirmed by comparison of retention times and calculated retention indices with literature values and comparison with authentic standards. All of the *Citrus* fruit peel essential oils were made up of mixtures of monoterpenoids and sesquiterpenoids with limonene being the principal component. The fruit peel oil of *C. aurantifolia* was made up of limonene (28.9%), -terpineol (14.3%)

and -pinene (10.6%) as the major components. Other components present in lesser amounts were terpinen-4-ol (3.1%), terpinolene (2.1%) and (*E)*-citral (1.7 %). *Citrus halimii* fruit peel oil was entirely made up of limonene (85.9%) while the fruit peel oil of *C. hystrix* (hybrid) papeda hybrid constituted of limonene (57.5%), γ-terpinene (13.0%), -pinene (9.7%) and linalool oxide (3.1%). Limonene and γ-terpinene were also the major components found in the peel oils of *C. medica* var. *enthrog* (72.7% and 13.1%), *C. medica* var. 2 (70.6% and 15.6%) and *C. medica* var. sarcodactylis (48.0% and 26.3%). The peel oils of *C. medica* var.1 however, was made up entirely of limonene (84.5%) and α-terpineol (4.2%) (Mohd. Ali *et al.* 2005).

The leaf oil of *Zanthoxyllum acanthopodium* was characterized by the presence of 1, 8-cineole (34.6%) and limonene (30.8%). The other main constituents were -terpineol (6.2%), -pinene (5.3%) and citronellal (3.9%). The fruit oil was totally made up of monoterpenoids amongst which geranyl acetate (30.0%), citronellal (23.3%), limonene (15.1%) and geraniol (11.9%) were most predominant. The chemical composition of the twig and stem oils was similarly characterized by the presence of α-pinene (41.0%, 35.8% respectively), terpinen-4-ol (9.41, 18.5% respectively) and β-pinene (16.0%, 12.6% respectively).

Limonene (58.5%) was the most abundant compound in the leaf oil of *Tetractomia tetranda*. Other chemical components present in the leaf oil were -pinene (5.8%), -caryophyllene (3.9%), terpinolene (3.3%) and safrole (2.5%). Unlike all of the other essential oils, majority of the chemical constituents detected in the leaf oil of *Murraya koenigii* were mainly sesquiterpene hydrocarbons. The major component of the leaf oil was β-caryophyllene (28.1%), α-humulene (6.2%), δ-guiane (9.5%), β-selinene (5.0%). Major monoterpenes seen in this oil was identified as β- phelanderene (7.2%) and *trans*-beta ocimene (4.1%).

Conclusion

Malaysia is blessed with a large variety of useful phytochemicals such as essential oils and flavanoids from the Rutaceae family. Results obtained from this study will be useful in the future selection of plant materials for domestication, cultivation and product development.

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