

Identification and Quality Control Assessment of Two Herbal Medicines by Volatile Oil Analysis

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ABSTRAK

Analisis komponen meruwap secara ko-kromatografi dengan sampel tulen di atas dua turus berbeza kekutuban, KG-SJ dan indeks penahanan linear digunakan pada dua ubatan herba Air Mancur® (Jamu Tujuh Angin dan Jamu Nafsu Makan) sebagai satu kaedah pantas untuk tujuan pengenalpastian dan penilaian kualiti. Tidak terdapat perbezaan komposisi dan terdapat variasi kecil pada tahap setiap komponen di antara minyak daripada sediaan serupa yang diperolehi daripada tiga lokasi yang berlainan. Minyak-minyak yang diperolehi daripada dua ubatan herba didapati mempunyai beberapa persamaan komposisi dan ini menunjukkan penggunaan ramuan beraroma utama yang serupa dalam sediaan. Kedua-dua minyak kaya dengan seskuiterpina di mana ar-kurkumena (19.6 – 27.1%), β -kurkumena (8.4 – 13.2%) dan xantorizol (7.0 – 9.9%) adalah komponen yang utama. Kehadiran jumlah yang ketara mentol (7.4 – 10.6%) dan anetol (4.1 – 5.9%) dalam Jamu Tujuh Angin dan kepekatan timol (9.6 – 11.4%) yang lebih tinggi dalam Jamu Nafsu Makan boleh digunakan untuk membezakan minyak-minyak tersebut.

Kata kunci: Ubatan herba, komposisi minyak, ar-kurkumena, β -kurkumena, xantorizol.

ABSTRACT

Analysis of volatile components by co-chromatography with authentic samples on two columns of different polarity, GC-MS and linear retention indices were applied to two herbal medicines of Air Mancur® (Jamu Tujuh Angin and Jamu Nafsu Makan) as a rapid method for identification and quality assessment purposes. There was no compositional differences and little variation in the levels of each component between the oils of similar preparation obtained from three different locations. The oils obtained from the two herbal medicines were found to possess some compositional similarities, suggesting the use of mainly similar aromatic ingredients in

the preparations. Both oils were rich in sesquiterpenoids where α -curcumene (19.6 – 27.1%), β -curcumene (8.4 – 13.2%) and xanthorrhizol (7.0 – 9.9%) were the major components. The presence of appreciable amounts of menthol (7.4 – 10.6%) and anethol (4.1 – 5.9%) in Jamu Tujuh Angin and a higher concentration of thymol (9.6 – 11.4%) in Jamu Nafsu Makan may be used to differentiate the oils.

Key words: Herbal medicines, oil composition, α -curcumene, β -curcumene, xanthorrhizol.

INTRODUCTION

A herbal preparation is prone to adulteration during collection of plant materials and manufacturing. Misidentification of plant species and admixture of the desired plant part with other plants and fraudulent activities such as substitution of genuine plant part with inferior varieties, exhausted drugs or superficially similar but cheaper materials may result in products of poor quality. The concentration of the phytochemicals and the ratio between different constituents vary continually i.e. in a dynamic state as they are synthesized and metabolized continuously in the living tissues. Plant materials obtained from identical botanical species may also show considerable qualitative and quantitative differences in the content of the ingredients due to genetic and ecological factors. Improper handling of the plant materials during collection, preparation, drying and storage may cause deterioration in the activity of the plant materials and hence the quality of the herbal preparations (Phillipson 1993).

The therapeutic value of a crude drug depend on the amount of active ingredients present in the preparation. Thus, it is important that the plant materials should be in uniform quality, both as regards to origin and cleanliness and also with respect to the content of the active constituents (Phillipson 1993). The crude drugs should conform to the standards set by pharmacopoeias to qualify to be used clinically. The properties of the crude drugs considered for evaluation are the structural form (to establish identity and purity), the analytical standard (to determine the amount of active constituents) and the physical standards (to establish physical characters) (Ibrahim 1998).

The present paper reports on the qualitative and quantitative chemical analyses of the volatile components of two herbal preparations as a rapid method for identification and quality assessment.

MATERIALS AND METHODS

PLANT MATERIALS

The herbal preparations, in powdered form and each packed in a plastic packet, were the products of Air Mancur® (Wonogiri, Solo, Indonesia), namely Jamu Tujuh Angin (JTA) (registration no. MAL 19985483T) and Jamu Nafsu Makan (JNM) (registration no. MAL 19988714T). Each preparation was obtained from three different locations in Malaysia i.e. Kuala Lumpur, Penang and Malacca, to ensure that they were samples from different manufacturing batches. According to the label each JTA packet contained 7 g of the following 7 major ingredients: *Alpinia galanga* (rhizome) (10%), *Zingiber* sp. (rhizome) (20%), *Z. purpurei* (rhizome) (10%), *Curcuma* sp. (rhizome) (10%), *Amomum* sp. (fruit) (8%), *Parkia* sp. (seed) (20%), *Foeniculum vulgare* (fruit) (10%). JNM was mainly 7g of a mixture of *Alpinia galanga* (rhizome) (15%), *Zingiber officinale* (rhizome) (10%), *Zingiber* sp. (rhizome) (15%), *Curcuma* sp. (rhizome) (30%) and *Coriandrum sativum* (fruit) (10%).

Each powdered sample was weighed by an electronic analytical balance (4 decimal points) to determine the uniformity of weight of the different batches. The weights were averaged from at least three different samples.

MOISTURE DETERMINATION

The moisture content of the herbal preparations was determined by azeotropic method. Each sample was soaked in toluene and distilled in a Dean-Stark apparatus for a few hours (3 – 4 hrs) until there was no further increase in the amount of water collected. The yields were averaged over three experiments.

OIL ISOLATION

The herbal materials were subjected to water distillation in Clevenger-type apparatus for 8 hrs. The oily layers obtained were separated and dried over anhydrous magnesium sulfate. The yields were averaged over three experiments and calculated based on dry weight of the plant materials.

ANALYSIS OF THE OILS

The oils were analyzed on a Shimadzu GC 14A chromatograph equipped with a FID detector using a DB-5 capillary column (25 m x 0.25 mm, 0.25 mm film thickness). The operation parameters were: nitrogen as carrier gas at 50 cm/s, injector and detector temperatures were maintained at 250°C. The column was programmed initially at 75°C for 10 min, then 3°C/min to

210°C and held for 1 min. The oils were also examined using a DB-1 stationary phase column (25 m x 0.25 mm, 0.25 µm film thickness) programmed from 60°C for 10 min, then 3°C/min to 180°C and held for 10 min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components are based on peak areas obtained, without FID response factor correction. Temperature program linear retention indices of the compounds were also determined relative to n-alkanes (van den Dool & Kratz 1963).

The oils were also analysed by GC-MS with Hewlett-Packard GC-MSD 5890 series 2 mass spectrometer (70eV direct inlet) on a SE-30 column (25 m x 0.25 mm) initially at 60°C for 10 min, then 3°C/min to 180°C for 10 min with helium as carrier gas. The constituents were identified by comparison of their retention indices with literature values and their mass spectral data with those from the Wiley mass spectral database, and in some cases by co-chromatography on the different columns with authentic samples (Adams 1989; McLafferty & Stauffer 1989; Davies 1990).

RESULTS AND DISCUSSION

The weight of powdered material in each packet for both *Jamu Tujuh Angin* (JTA) and *Jamu Nafsu Makan* (JNM) showed little variation, i.e. 7 ± 0.0142 g, indicating uniformity and accuracy in the amount of materials used in different batches of preparation. The moisture contents of the preparations varied in a narrow range of 7.1 – 7.8% which is within the range recommended by most pharmacopoeias (10%). Water distillation of the samples gave the following oil yields: JTA (Kuala Lumpur) (0.5%), JTA (Penang) (0.6%), JTA (Malacca) (0.6%), JNM (Kuala Lumpur) (0.3%), JNM (Penang) (0.4%), JNM (Malacca) (0.4%).

The list of constituents in the essential oils of JTA and JNM is shown in Table 1. The results show that there was no compositional differences and little variation in the levels of each component between the oils of similar preparation obtained from the different locations. The gas chromatogram of JTA oils revealed the presence of at least 58 components. Fifty-one compounds had been identified in the oils representing about 89% in the Kuala Lumpur sample, 88% in the Penang sample and 87% in the Malacca sample. The unidentified compounds showed mass fragmentation patterns indicative of sesquiterpene hydrocarbons and their oxygenated derivatives. The oils were made up mainly of sesquiterpenoids, constituting 77.4%, 77.8% and 79.3%, respectively. The major components which contributed to the spicy-herbaceous odor of the oils were ar-curcumene, menthol, xanthorrhizol, β -curcumene, anethol, α -eudesmol, α -zingiberene and β -eudesmol.

TABLE 1. Percentage composition of the volatile components of Jamu Tujuh Angin (JTA) and Jamu Nafsu Makan (JNM)

Compound	Jamu KL	Tujuh PP	Angin M	Jamu KL	Nafsu PP	Makan M	RI	Method of identification
α -Thujene	t	t	t	t	0.1	t	929	a,b
α -Pinene	t	0.6	0.4	0.5	t	0.1	937	a,b,c
Sabinene	t	0.3	0.2	t	0.3	0.1	970	a,b,c
β -Pinene	t	t	t	0.1	t	0.1	979	a,b,c
Myrcene	t	t	0.1	0.1	t	0.	990	a,b,c
α -Terpinene	t	t	t	t	t	t	1014	a,b,c
p-Cymene	0.1	0.3	0.2	0.5	0.5	0.3	1024	a,b,c
Limonene	t	0.7	0.3	-	-	-	1027	a,b,c
1,8-Cineole	-	-	-	0.1	0.2	0.1	1030	a,b,c
γ -Terpinene	0.2	0.3	0.2	0.3	0.6	0.4	1055	a,b,c
Terpinolene	t	t	t	0.1	0.1	0.1	1089	a,b,c
Fenchone	0.1	0.4	0.3	t	0.1	0.1	1095	a,b,c
Linalool	t	t	t	2.5	1.8	2.0	1098	a,b,c
Camphor	0.3	0.6	0.3	0.4	0.8	0.6	1146	a,b,c
p-Menthone	1.1	1.7	1.5	-	-	-	1155	a,b,c
Borneol	1.6	1.5	1.5	0.1	0.1	0.1	1166	a,b,c
Menthol	10.6	7.4	8.2	-	-	-	1173	a,b,c
Terpinen-4-ol	-	-	-	0.2	0.3	0.2	1178	a,b,c
α -Terpineol	1.0	1.6	1.0	1.3	2.4	2.2	1188	a,b,c
cis-Piperitol	t	t	t	-	-	-	1198	a,b
trans-Piperitol	0.4	0.9	0.4	-	-	-	1206	a,b
Nerol	0.1	0.1	t	-	-	-	1232	a,b,c
Neral	t	t	t	-	-	-	1241	a,b,c
Geraniol	t	0.1	t	-	-	-	1260	a,b,c
Geranial	t	t	t	-	-	-	1273	a,b,c
Anethol	5.9	4.1	4.9	1.5	1.8	1.3	1290	a,b,c
Thymol	0.2	0.6	0.3	11.4	9.6	10.0	1295	a,b,c
Eugenol	0.1	0.2	0.1	0.1	0.1	0.1	1359	a,b,c
Geranyl acetate	0.2	0.4	0.4	0.6	0.6	0.4	1390	a,b,c
β -Elemene	0.1	0.7	0.5	0.1	0.2	0.2	1407	a,b
β -Caryophyllene	0.6	0.7	0.5	0.3	0.	0.2	1419	a,b,c
γ -Elemene	0.1	2.4	2.0	1.0	0.2	0.1	1439	a,b
α -Humulene	0.4	0.1	0.2	0.3	0.9	0.6	1451	a,b,c
(Z)- β -Farnesene	1.4	1.9	1.3	0.6	2.0	1.3	1468	a,b
Ar-curcumene	23.5	19.6	20.8	27.1	25.4	26.8	1482	a,b
α -Zingiberene	3.2	5.2	3.8	2.8	2.5	2.8	1494	a,b,c
α -Bisabolene	0.2	0.5	0.3	0.1	0.1	0.1	1505	a,b
β -Curcumene	10.3	8.4	12.6	13.2	12.3	11.9	1523	a,b,c
β -Sesquiphellandrene	3.6	4.1	4.3	2.9	2.1	2.5	1526	a,b
α -Cadinene	0.4	0.5	0.2	-	-	-	1541	a,b,c
(E)-Nerolidol	0.1	0.2	0.1	0.2	0.3	0.1	1560	a,b,c
Caryophyllene oxide	1.0	1.5	1.4	0.3	0.5	0.2	1578	a,b,c
β -Elemenone	3.2	3.3	3.0	2.6	2.3	2.4	1605	a,b
Curzerenone	0.3	0.2	0.1	0.2	0.1	0.1	1616	a,b

continued

TABLE 1. *continued*

Compound	Jamu KL	Tujuh PP	Angin M	Jamu KL	Nafsu PP	Makan M	RI	Method of identification
β -Eudesmol	0.1	1.0	0.3	1.2	1.8	1.6	1634	a,b
α -Eudesmol	4.9	4.6	4.4	2.3	1.1	2.0	1651	a,b
Ar-Tumerone	0.1	0.1	0.1	0.3	0.2	0.1	1677	a,b
α -Tumerone	0.7	0.8	0.5	0.2	0.1	0.1	1689	a,b
Germacrone	1.8	2.1	1.6	1.5	1.7	1.3	1707	a,b
(Z,Z)-Farnesol	0.2	0.3	0.2	0.2	0.2	0.1	1713	a,b
β -Tumerone	0.1	0.1	0.1	0.1	0.1	0.1	1723	a,b
Zerumbone	0.2	0.2	0.1	1.1	1.5	1.5	1733	a,b
Xanthorrhizol	9.9	7.2	8.2	7.5	7.0	8.0	1766	a,b,c
Tetradecanoic acid	0.6	0.4	0.4	0.3	1.3	1.0	1769	a,b
Other sesquiterpenoids	11.1	12.1	12.7	13.8	16.4	16.6		

KL = Kuala Lumpur; PP = Pulau Pinang; M = Malacca; Percentages were obtained by peak-area normalization on column DB-5, all relative response factors being taken as 1; RI = retention index; t = trace; tentative identification for all compounds, except for c; a = mass fragmentation; b = retention index; c = co-chromatography with authentic sample.

Forty-four compounds were identified in the chromatogram of JNM oils where sesquiterpenoids were the major group, constituting 80.0% in the Kuala Lumpur sample, 79.3% in the Penang sample and 80.7% in the Malacca sample. Ar-curcumene, β -curcumene and xanthorrhizol were the major representatives of sesquiterpenoids while the most abundant non terpene present in the oils was thymol. Other compounds present at appreciable amounts (greater than 1% concentration) in the oils were β -sesquiphellandrene, α -zingiberene, β -elemenone, linalool, α -terpineol, anethol, β -eudesmol, α -eudesmol, germacrone and zerumbone.

The oils of JTA and JNM were found to possess some compositional similarities, suggesting the use of mainly similar aromatic ingredients in the preparations. Both oils were rich in sesquiterpenoids where ar-curcumene (19.6 – 27.1%), β -curcumene (8.4 – 13.2%), xanthorrhizol (7.0 – 9.9%), α -zingiberene (2.5 – 5.2%), β -sesquiphellandrene (2.1 – 4.1%), α -eudesmol (1.1 – 4.9%), β -elemenone (2.3 – 3.3%) and germacrone (1.3 – 2.1%) were present as major components. However, the oils could be distinguished from each other by the levels of menthol, anethol, thymol, linalool, borneol and p-menthone. JTA oils were characterized by the presence of appreciable amounts of menthol (7.4 – 10.6%), anethol (4.1 – 5.9%), p-menthone (1.1 – 1.7%) and borneol (1.5 – 1.6%). In contrast, JNM oils contained a higher concentration of thymol (9.6 – 11.4%) and linalool (1.8 – 2.5%), but conspicuously smaller proportion of anethol (1.3 – 1.8%) and absence of menthol and p-menthone.

The labels of the herbal medicines gave incomplete information on the contents as the names of some ingredients were merely provided at the genera level or were excluded. It would be useful for quality control purposes that the composition of the products were concisely presented using botanical names up to the species level. The presence of certain compounds in the oils could be used as markers for the plant materials used in the herbal medicines. The high concentration of xanthorrhizol in both herbal medicines might indicate the presence of rhizome of *Curcuma xanthorrhiza* in the preparations (Ibrahim et al. 1999). The use of fruit of *Foeniculum vulgare* in JTA could be confirmed by the presence of significant amount of anethol in the oils (Tiserand & Balacs 1995). The relatively high levels of linalool in JNM might be contributed by the fruit of *Coriandrum sativum* (Hethelyi & Nyaradi-Szabady 1990).

Literature studies indicated that none of the plant samples used in the products have been reported to contain significant amounts of menthol or thymol. The presence of significant amounts of menthol in JTA and thymol in JNM may suggest that these compounds could possibly be added to the herbal medicines as adjuvants. Analysis of volatile components could be employed as a helpful method for rapid identification and quality control of aromatic herbal medicines.

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