THE COMPOSITION OF FRUIT ESSENTIAL OIL AND EXTRACT OF DRIED BUD OF *SOPHORA JAPONICA* L. CULTIVATED IN VIETNAM

THÀNH PHẦN TINH DẦU QUẢ VÀ PHẦN CHIẾT NỤ KHÔ CỦA *SOPHORA JAPONICA* L. TRỒNG Ở VIỆT NAM

Nguyen Thi Thu Huyen, Phan Dinh Chau, Pham Van Thiem
Hanoi University of Technology

ABSTRACT

In this study we reported identified compounds of the essential oil from the fresh bud and the flower of Sophora Japonica *L. cultivated in Vietnam analyzing by GC-MS. The major components were caryophyllene, α-humulene, (-)-caryophyllene oxide, 8-heptadecene, phytol and tricosane which were detected in both flower oil and bud oil. The results also showed that these oils were poor in monoterpenes. The major groups of the essential oil of bud and flower were found to be sesquiterpenes (11.45%, 56.69%), oxygenated sesquiterpenes (8.47%, 21.05%) and oxygenated diterpenes (15.62%, 5.72%), respectively. Moreover, the changing of the composition of flower oil and bud oil both from Hoabinh and Hungyen provinces was shown.*
Keyword: *Sophora Japonica L*., essential oil, flower, bud.

TÓM TẮT

Tinh dầu quả và phần chiết nụ khô của Sophora japonica L. ở Việt Nam được chiết và phân tích. Trong tinh dầu quả, 19 hợp chất chiếm 88,66% của dầu đã được tìm thấy. Các cấu tử chính của dầu quả là α-copaene (4,85%), phytol (33,35%), tricosane (9,85%), tetracosane (5,69%) và hexacosane (19,36%). Chúng tôi cũng đã xác định được 29 hợp chất trong hai phần chiết nụ khô. Các aldehyd được tìm thấy chủ yếu trong phần chiết n-hexan. Các monoterpene (chiếm 16.28% dịch chiết chloroform) cũng đã được tìm thấy trong thực nghiệm này.

I. INTRODUCTION

In the previous years, the bud, seed, bark, root, leaf, fruit and branch of *Sophora Japonica* L. were subjects for studies [1,2]. A large number of flavonoids were found in these materials and they are main source to be screened and to isolate drug. To explain the pharmacology of dried bud of *S. Japonica* L. in traditional remedies, we carried out extraction and defined composition of dried bud which was dried by heat. On the other hand, we also isolated and defined chemical composition of essential oil of fresh fruit of *S. Japonica* L. cultivated in Hungyen province. Many components which have not been detected in previous studies in the extract and essential oil were found in the present study [3].

II. EXPERIMENTAL

Plant material: The fresh fruit of *S. Japonica* L. was picked random in January 2009 from Hungyen province. The fresh bud of *S. Japonica* L. was collected from Thaibinh province in July 2008. The fresh bud was dried by heat after harvesting. The heat from the fire is transferred directly into the metal pan and the bud of *S. japonica* L. on the pan was turned frequently to dry.

Oil isolation: fresh fruit of *S. Japonica* L. was washed. 2.0 kilogram of the material were ground and distilled by Clevenger type apparatus for 8h. The corresponding white colored oils were recovered in yield of 0.05% (v/w fresh). The oils was dried over anhydrous sodium sulfate and stored in sealed brown vials kept at 4^0C for analysis afterward.

Extraction of volatile components: To avoid denaturing of chemical composition, solvelts were used to extract the dried bud. 5.00 gram of dried bud were ground and extracted in chloroform and n-hexane solvents by ultrasonic wave in two hours. The extracts were isolated on silicagel and florisil columns and analyzed by GC-MS after that.

Analysis: GC/MS analyses were carried out on a Shimadzu 2010 GC/MS system equipped with a DB-5 fused silica column (30m

x 0.25mm, film thickness 0.25um). The ion source and interface temperatures were set at 200^0C and 250^0C, respectively. Approximate 1mg (1µl) portion of each sample was dissolved in 1.5ml solvent and 0.5µl volume was injected into the column using a split ratio of 10:1 with injection temperature set to 250^0C.

- *Essential oil analysis:* The mass spectrometer was operated in 70eV with scan speed (1666) scanning from 40 to 800 m/z. The column temperature was initiated by a column temperature set at 70^0C for 2 min, increased to 230^0C at a rate of 7^0C/min and held for 15 min.

- *Extract analysis:* The mass spectrometer was operated in the 70eV mode with scan speed (666) scanning from 40 to 350 m/z. The column temperature was initiated by a column temperature which was set at 50^0C for 1 min, increased to 65^0C at a rate of 15^0C/min and held for 3min, increased to 210^0C at a rate of 10^0C/min and held for 6min, increased to 280^0C at a rate of 15^0C/min and held for 10min.

Identification of components: The compounds were identified by comparison their mass spectral fragmentation patterns with those stored in the spectrometer database using the National Institute of Standards and data Technology Mass Spectral database. Retention index on DB-5 column was found in references [4]. Percentage of the identified compounds was computed from peak areas. Total of oil or extract was set at 100%.

III. RESULTS AND DISCUSSION

3.1 Composition of essential oil from fresh fruit of *S. Japonica* L.

The composition is reported in Table 1. A chromatogram of essential oil of *S. Japonica* L. fruit is also shown in Figure 1.

19 components which account approximately 88.66% of the oil were detected. While the essential oil is composed from monoterpenes which account for approximately more than 50%, the essential oil of fruit of *S. Japonica* L. was absent in monoterpenes in this study [5,6]. The major groups in fruit oil were found to be sesquiterpenes (11.36%), oxygenated sesquiterpenes (0.82%) and oxygenated diterpenes (33.35%). The main components of the essential oil were α-copaene (4.85%), phytol (33.35%), tricosane (9.85%), tetracosane (5.69%) and hexacosane (19.36%). The less predominant constituents are composed of 2-pentanone, α-cubebene, β-bourbonene, caryophyllene, α – humulene, alloaromadendrene, germacrene-D, δ-cadinene, (-)-caryophyllene oxide, tetradecanal, 8-heptadecene, hexadecanoic acid methyl ester, eicosane and docosane.

3.2 Composition of extracts from dried bud of *S. Japonica* L.

The composition of extract from the dried bud which was dried by heat is shown in the Table 2. A chromatogram of the n-hexane extract from the dried flower bud in Thaibinh province was also shown in Figure 2.

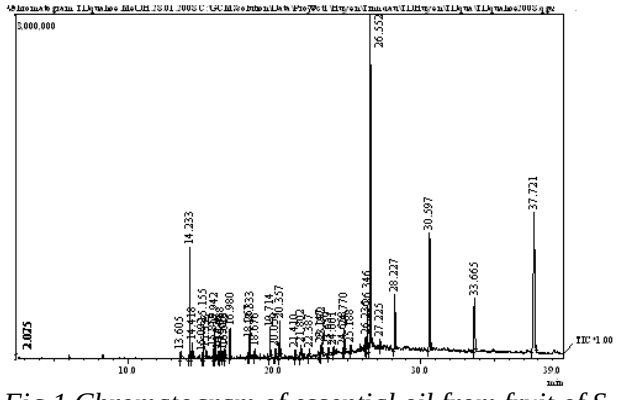

Fig.1 Chromatogram of essential oil from fruit of S. Japonica L. from Hungyen province

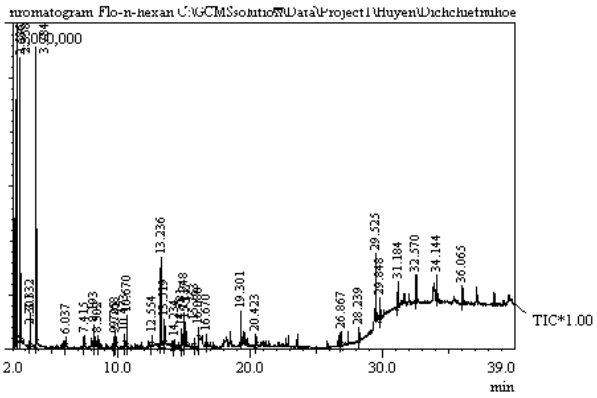

Fig.2 Chromatogram of the n-hexane extract from the dried bud of S. Japonica L. from Thaibinh province

Table 1. The essential oil of fruit of S. Japonica L from Hungyen province

N[0]	Similarity	RI[a]	Component[b]	Content[c] (%)
				Fresh fruit
1	89	688	2-pentanone	0.25
2	96	1355	α-cubebene	0.25
3	95	1379	α-copaene	4.85
4	96	1386	β-bourbonene	0.97
5	97	1438	caryophyllene	1.52
6	96	1455	α - humulene	0.39
7	97	1461	alloaromadendrene	1.36
8	94	1479	germacrene-D	0.76
9	95	1520	δ-cadinene	1.26
10	92	1573	(-)-caryophyllene oxide	0.82
12	96	1615	tetradecanal	1.77
11	96	1672	8-heptadecene	1.29
13	93	1901	hexadecanoic acid, methyl ester	0.31
14	97	2000	eicosane	1.00
15	97	2128	phytol	33.35
16	97	2200	docosane	3.61
17	97	2301	tricosane	9.85
18	97	2400	tetracosane	5.69
19	97	2598	hexacosane	19.36
Sesquiterpene hydrocarbons				11.36
Oxygenated sesquiterpenes				0.82
Oxygenated diterpenes				33.35
Nonterpenes				43.13
Unknown				11.34
Total identified				88.66

[a]RI retention index measured relative to *n*-alkane on DB-5 column and referenced in [5] references

[b] The compounds listed in order of elution from DB-5 column

[c]: the content base on the percentage of peak area

Table 2. The volatile compounds of dried bud of S. Japonica L. from Thaibinh province

	Chloroform extraction		n-Hexane extraction	
N[0]	Comp.[a]	Cont.[b] (%)	Comp.[a]	Cont.[b] (%)
1	propanoic acid, 2-methyl-, methyl ester	0.58	hexanal	10.58
2	butanoic acid, methyl ester	0.31	heptanal	0.31
3	butanoic acid, ethyl ester	0.98	2-heptanal	0.30
4	α-pinene	0.41	2-pentylfuran	0.71
5	(-)-β-pinene	0.37	n-octanal	0.32
6	β-myrcene	0.46	2-octenal	0.39
7	p-cymene	0.49	n-nonanal	0.92
8	l-limonene	13.44	decanal	0.31
9	1,8-cineole	0.25	trans-2-decenal	1.22
10	γ-terpinene	0.35	n-undecanal	0.22
11	dl-menthone	0.37	2-undecenal	0.80
12	l-menthol	1.01	dodecanol	0.32
13	anethole	0.39	1-heptadecene	0.96
14	8-heptadecene	0.34	γ-stearolactone	0.50
16	hexadecanoic acid, methyl ester	0.44		

[a] The compounds listed in order of elution from DB-5 column.

[b]: The content base on the percentage of peak area

Table 2 showed that aldehydes were major components in the n-hexane extract. Other chemical compounds were found including 2-undecenal, dodecanol (fatty alcohol), γ-stearolactone (sugar). Hexanal was the most abundant constituent (about 10.58%) in the extract. In the chloroform extract, 16 components were detected consisted mainly of monoterpenes which account 16.28% of the extract. 7 monoterpenes which have not been detected in flower oil and bud oil in our previously study were α-pinene (0.41%), (-)-β-pinene (0.37%), β-myrcene (0.46%), p-cymene (0.49%), γ-terpinene (0.35%), dl-menthone (0.37%), anethole (0.39%) [7]. The monoterpene which contains 13.14% of the extract was l-limonene.

A total of 29 compounds were identified in two extracts of the *S. Japonica* L. bud and the composition differ from that shown in our previous results with the same extraction method [3]. The major chemical changes can be explained by the thermal degradation and oxidation of main constituents in dried process. In the experiment, we dried the bud of *S. Japonica* L. by heating. The artificial drying does carry a risk of drying the bud too rapidly, in which case some component will be broken, leading to a highly change of composition. In Vietnam, it is possible to dry the bud of *S. Japonica* L. just by spreading them out in the sun when the weather is dry enough. If the weather is bad, the bud must be control heat because if the *S. japonica* L. bud is not adequately dried, it is likely to go mouldy. The uncontrolled temperature in the drying process causes changing composition of the material. Some hydrocarbon chains were broken and formed aldehydes, alcohols, ketones, *etc*. and these components contributed to the more special natural scent of dried bud of *S. Japonica* L..

Although the heat-treated bud was used effectively in traditional remedies in centuries, the pharmacology explanation has not been reported clearly before. The effective treating diseases of heat-dried bud can depend on the increase or decrease of rutin and quercetin content in drying process. Our experiments show the difference of the volatile composition between heat-dried bud and air-dried bud. Thus, we can conclude the change of the volatile composition in drying process is one of causes making the heat-treated bud more effective in treating some diseases.

IV. CONCLUSIONS

In the experiment, 19 compounds were shown by GC-MS which account approximately 88.66% of the fruit oil. The major constituents of the oil were α-copaene (4.85%), phytol (33.35%), tricosane (9.85%), tetracosane (5.69%), hexacosane (19.36%). In addition, n-hexane and chloroform extracts of heat-dried bud were studied and we found 36 compounds. In the extracts, aldehydes were a major group found in n-hexane extract and monoterpenes were a major group found in chloroform extract. The results opened a new trend in study on the dependence of pharmacology of heat-dried bud and drying process.

REFERENCES

1. *Huang, Hsu-shan* (Taipei. TW); United States Patent 20050181080 (2005).
2. *Hao-yun Feng*; Studies on the cosmetic bioactivity for *Sophora japonica* L. and *Lycium chinense* Mill., Master's Thesis, Chinese (2006).
3. *Nguyen Thi Thu Huyen, Pham Xuan Thang, Phan Dinh Chau, Pham Van Thiem*; Journal of science and technology in Vietnam, Vol. 45 (1B), 497-502 (2007).
4. http://www.kovats.org/
5. *Abbas Aflatuni*; The yield and essential oil content of mint (*Mentha SP*.) in northern ostrobothnia, Oulu University press (2005).
6. Antonios Michaelakis, Spiridon A. Theotokatos, Georgios Koliopoulos, Nikos G. Chorianopoulos; Molecules 12, (2007).
7. *Nguyen Thi Thu Huyen, Pham Van Thiem, Phan Dinh Chau*; in submitted to Journal of Science and Technology (of Technological Universities).

Contact: Nguyen Thi Thu Huyen - Tel: (+84)912.411.769; E-mail: thuhuyen_hut06@yahoo.com
Center for Education and Development of Chromatography, Hanoi University of Technology