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Analysis of Essential Oil in *Sophora japonica* L. Flower Buds on Different Stages of Development by Gas Chromatography-Mass Spectrometry

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Abstract To investigate, for the first time, the chemical composition of essential oil of the buds species of *Sophora japonica* L., a native to eastern Asia and a popular species in almost all Europe, growing in Ukraine. A hydrodistillation apparatus was used for the extraction of volatile components of buds species and then it was analysed by gas chromatography equipped with a split-splitless injector (split ratio, 1:50) and flame ionization detector (FID). The oil was analyzed under linear temperature programming applied at 4℃/min from 50℃ - 340℃. Temperatures of the injector and FID detector were maintained at 280℃ and 300℃, respectively. The chemical analysis of the oil was carried out using gas chromatography coupled to mass spectrometry (GC-MS), to determine the chemical composition of the volatile fraction. The essential oil content in green bud, formed bud, and beginning of bud opening ranged from 0.00002 – 0.00193 g/100 g, 0.00002 – 0.00684 g/100 g and from 0.00003 – 0.00638 g/100 g, respectively. The qualitative and quantitative analysis led to the identification of 80 components that were identified in *Sophora japonica* L. flower buds. Out of these 71 components are from the green flower bud and the beginning of flower bud opening stages, and 67 components are from the formed flower bud stage. The major component found in green bud was 3-methoxypyridine (0.00193 g/100 g). In both formed bud and beginning of bud opening was dodecanoic acid with concentrations of 0.00684 g/100 g and 0.00638 g/100 g, respectively. Contents of *Sophora japonica* L. were significantly affected by the harvesting stage of bud flower. Harvesting at the formed bud stage yielded the highest essential oil content compared to buds harvested at other stages. Flowers harvested at the green bud and beginning of bud opening stages yielded a lower quality essential oil compared with flowers harvested at the formed bud stage.

Keywords: *Sophora japonica L., green bud, formed bud, bud opening, essential oil, GC/MS*

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1. Introduction

The popularity of Traditional Chinese medicines (TCMs) in the West is growing at a significant rate due to their high potential. On the other hand, several barriers still stand before TCM being accepted globally as an effective treatment for a variety of chronic and acute conditions. These challenges related to their safety and efficacy being markedly different from western scientific medicine [1].

Sophora japonica L., a genus of the Leguminosae family contains about 21 species, 14 varieties and 2 forms grown in China [2], is native to eastern Asia and a popular species in almost all Europe. It is a well-known TCM herb is widely cultivated in all parts of China. Buds and fruits have been used from early times as a hemostatic agent in TCM. Moreover, it is often planted as a street tree due to its beautiful deep green color foliage that is not attacked by insects and an advantage over Robinia trees in giving a denser shade and it is widely used in urban zones [3,4,5,6]. Additionally, it is officially listed in the Chinese Pharmacopoeia [7]. *Sophora japonica* L. can be used for the treatment of dizziness, headache, vascular hypertension, hemorrhoids and hematemesis [4,8]. It is also useful in skin-care and as a whitening agent [8]. Furthermore, pharmacologic studies as well as clinical

practice showed that it has anti-tumour, anti-fertility and anti-cancer activities [9].

Plant essential oil and their extracts have been used for many thousands of years, particularly in pharmaceuticals, natural therapies, and food preservation [10]. Furthermore, it has been long acknowledged that some plant essential oils exhibit antimicrobial properties, especially against bacterial pathogens and are therefore worthy of investigation.

Only a few methods have been reported in the literature for the analysis of *Sophora japonica* L. including analysis of rare earth elements using inductively coupled plasma mass spectrometry [4], and the isolation and purification of flavonoid and isoflavonoid compounds in *Sophora japonica* L. by adsorption chromatography [3]. Moreover, capillary electrophoresis coupled with electrochemical detector has been reported also for the analysis of rutin and quercetin in the flowers of *Sophora japonica* L [12].

Hence, an investigation of the volatile compounds present in *Sophora japonica* L. bud species can give a better understanding of the types of compounds present. Therefore, this paper reports, for the first time, the investigations of the chemical composition of essential oil in these buds at different stages of development in Ukraine. Additionally, increased knowledge on volatile components presents in *Sophora japonica* L. provide valuable data for understanding the effects of essential oils on the efficacy of herbal medicine.

2. Experimental

2.1. Plant Material

Sophora japonica L. buds growing in Ukraine were collected from healthy trees, harvested during every stage of development, namely; green bud, formed bud and beginning of bud opening. Bud species were air-dried, ground, and sifted through 0.5 mm mesh screen to obtain a uniform particle size. After collection, the samples were immediately separated and hydrodistilled.

2.2. Hydrodistillation

The essential oil content of *Sophora japonica* L. buds at different stages of development was determined by hydrodistillation as described elsewhere in the European Pharmacopoeia [15]. Plant samples (0.5 – 5 g) with the aid of 10 mL distilled water were hydrodistilled in a Clevenger- type apparatus. The essential oil had been distilled with water for 2 h, collected, dried under anhydrous sodium sulphate and stored at 4 ℃ until used for further experiments. Adsorbed compounds were washed down into a dry vial of 10 mL with a slow addition of 3 mL of pure pentane. The washed sample was concentrated by using of a stream of nitrogen to a final volume of 2 µL. Tridecane was used as an internal standard at a concentration of 50 µg/g (wt/wt). Hydrodistillation method was performed in triplicate for each sample and the mean values of the extraction yields were reported.

2.3. GC/MS Analysis

Quantitative analysis was carried out using gas chromatography system (Agilent Technologies, model 6890, Waldbronn, Germany). The unit was equipped with a split-splitless injector (split ratio, 1:50) and flame ionization detector (FID). OPTIMA-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used. The oil was analyzed under linear temperature programming applied at 4℃/min from 50 – 340℃. Temperatures of the injector and detector (FID) were maintained at 280℃ and 300℃, respectively. Concentrations (g/100 g) of the essential oil components were calculated, obtained by FID, assuming a unity response by all components determined by GC/MS analysis. The chemical analysis of the essential oil was carried out using gas chromatography-mass spectrometry (GC/MS) coupled to a Saturn 2000 mass spectrometry detector (Varian-Chrompack, model 3800, gas chromatography-mass spectrometry (GC/MS) (Varian chrompack, model 3800, GC/MS/MS-200 (Saturn, Netherlands)). The chromatographic conditions were as follow: column oven program, 60℃ (1 min, isothermal) to 246℃ (3 min, isothermal) at 3℃/min; the injector and detector temperatures were 250 and 300℃, respectively. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses) was utilized. The actual temperatures in MS source reached approximately 180℃. The ionization voltage was 70 eV. A hydrocarbon mixture of *n*- alkanes (C8 - C20) was analyzed separately by GC/MS under same chromatographic conditions using the same HP-5 column.

2.4. Identification of Compounds

Essential oil components were identified by computer search using their mass spectra either with known components [16], comparison of the mass spectra of reference compounds and a mass-spectrum library (Wiley/NIST database) [7].

3. Results and Discussion

Essential oils from the buds at different stages of developments were obtained from hydrodistillation, and their chemical components were determined by GC/MS. Hydrodistillation method is described elsewhere in the European Pharmacopoeia [15]. Simply, in this method, the plant material is boiled in water using a heat source. Then the volatile material is carried by steam through tubes and then is cooled. The volatile essential oil is removed from the top of the hydrosol. Although hydrodistillation using a clevenger type apparatus is a common method, but the results are still better than the ones obtained using other methods reported [18].

The chemical components of the three different essential oils are given in Table 1. The results show that the number of components in the green bud and in the beginning of bud opening were similar at about 61 regardless the components type. The formed buds had 67 chemical components.

Table 1. Chemical composition of volatile compounds of *Sophora japonica* L. buds on different stages of their development. Data are given as mean (n = 3).

KI*	Name of constituents	g/100g**

		Green bud	Formed bud	Beginning of bud opening
		Saturated hydrocarbons		
1000	Decane	0.00010	0.00051	0.00025
1100	Undecane	0.00005	0.00021	0.00010
1400	Tetradecane	0.00023	0.00107	0.00054
1452	2,6,10- trimethyldodecane	0.00028	0.00088	0.00068
1500	Pentadecane	0.00020	0.00135	0.00074
1600	Hexadecane	0.00013	0.00104	0.00032
1700	Heptadecane	0.00008	0.00034	0.00053
1800	Octadecane	0.00009	0.00055	0.00026
1900	Nonadecane	-	-	0.00018
1971	Eicosane	0.00006	0.00027	0.00015
2100	Heneicosane	0.00009	0.00063	0.00033
2300	Tricosane	0.00023	0.00173	0.00115
2400	Tetracosane	0.00011	0.00064	0.00039
2500	Pentacosane	0.00035	0.00199	0.00134
2700	Heptacosane	0.00011	0.00057	0.00054
2740	Nonacosane	0.00009	0.00060	0.00043
		Aldehydes, ketones, alcohols		
962	Dihydro -2- methyl furanone -3	-	0.00005	-
979	Trans-2-hexenal	0.00018	0.00016	0.00035
982	2,5- furandione	-	0.00007	-
986	Furfural	0.00011	0.00062	0.00034
1048	1,3- cyclopentanedione	-	0.00009	-
1069	Trans -2-heptenal	0.00010	-	0.00017
1077	2,3-octanedione	0.00002	-	-
1088	6-methyl -5-hepten -2-ol	0.00002	-	-
1123	Benzaldehyde	0.00009	0.00052	0.00012
1147	5- methylfurfural	0.00012	0.00062	0.00019
1158	Cis-2,4-heptadienal	0.00003	-	0.00008
1232	3,5-octadiene-2-ol	-	0.00002	0.00008
1290	Trans-2-octenal	0.00003	-	0.00005
1313	Cis-3,5-octadiene -2-one	0.00012	0.00048	0.00013
1328	Trans-3,5-octadiene -2-one	0.00009	0.00017	0.00014
1330	Dimethylcyclohexanol	0.00012	0.00043	-
1336	6- methyl-3,5-heptadien-2-one	0.00006	0.00018	0.00016
1351	Trans-2-nonenal	0.00008	0.00236	0.00013
1362	Decanal	-	0.00005	-
1394	3,5,7- nonatriene -2-one	0.00003	-	0.00008
1418	2,4-decadienal	0.00005	0.00014	0.00009
1624	β-ionone	0.00019	0.00095	0.00030
1635	β-ionone-5,6-epoxide	0.00018	0.00089	0.00030
1913	Hexahydrofarnesylacetone	0.00069	0.00405	0.00246
		Heterocyclic compounds		
1036	2,6-dimethylpyrazine	0.00003	0.00016	0.00006
1046	2,3-dimethylpyrazine	0.00005	0.00010	0.00009
1054	Acetylfuran	-	0.00002	-
1123	2,3,5-trimethylpyrazine	0.00003	0.00011	0.00003
1248	3-methoxypyridine	0.00193	0.00546	0.00065
1320	5-ethyl -2,3- dimethylpyrazine	0.00003	-	-
1321	2,3,5,6-tetramethylpyrazine	0.00005	0.00010	-
1414	3-pentylfuran -2(5*H*)-one	0.00004	0.00028	0.00016
1537	5-pentyl-furanone -2	0.00016	0.00044	0.00024
1557	Dihydro -5-pentyl-furanone-2	0.00013	0.00064	0.00020
		Fatty acids and their ethers		
1086	Hexanoic acid	-	0.00089	-
1274	Heptanoic acid	-	0.00051	-
1391	Nonanoic acid	-	0.00261	0.00072
1466	Decanoic acid	-	0.00186	0.00066
1655	Dodecanoic acid	0.00008	0.00684	0.00638
1858	Tetradecanoic acid	0.00023	0.00290	0.00382
1942	Pentadecanoic acid	-	0.00058	0.00041
1974	Methyl palmitate	0.00007	0.00091	0.00029
2190	Methyl oleate	-	0.00052	0.00059
2199	Methyl linoleate	0.00005	0.00096	-
2224	Methyl linolenate	0.00006	0.00071	-
2258	Oleic acid	0.00008	-	-
2272	Linoleic acid	0.00033	0.00213	0.00119
2349	Iso-linoleic acid	-	-	0.00022
		Terpenoids		
1301	Trans-linalool oxide	-	0.00010	-
1304	Linalool	0.00068	0.00381	0.00066
1353	Camphor	0.00017	0.00042	0.00026
1355	Terpinen-4-ol	0.00008	0.00047	0.00010
1388	Verbenone	-	0.00027	-
1431	α-copaene	-	-	0.00005
1493	Piperitone	-	-	0.00027
1568	Geranylacetone	0.00008	0.00046	0.00019
1574	Germacrene d	-	-	0.00013

Aromatic compounds				
1333	2-methoxyphenol	0.00003	0.00014	-
1483	2-methoxy-4-vinylphenol	0.00035	0.00194	0.00037
1517	Eugenol	0.00104	0.00619	0.00122
1880	Benzophenone	0.00011	0.00052	0.00028
Unsaturated hydrocarbons				
1227	Trans-2,4-heptadien	0.00006	0.00008	0.00015
1697	Heptadecene -8	0.00065	0.00572	0.00306
2834	Squalene	0.00011	0.00068	-

*Kováts retention index (KI) relative to C8–C20 n-alkanes on the HP-5MS column [24].
**Mean ± SD (n=3).
«–»: substance's absence.
I: Retention indices
OM: Oxygenated monoterpenes
SH: Sesquiterpene hydrocarbons
OS: Oxygenated sesquiterpenes
IP: Isoprenoids
TT: Triterpene
DT: Diterpenes hydrocarbons
AL: Aldehyde

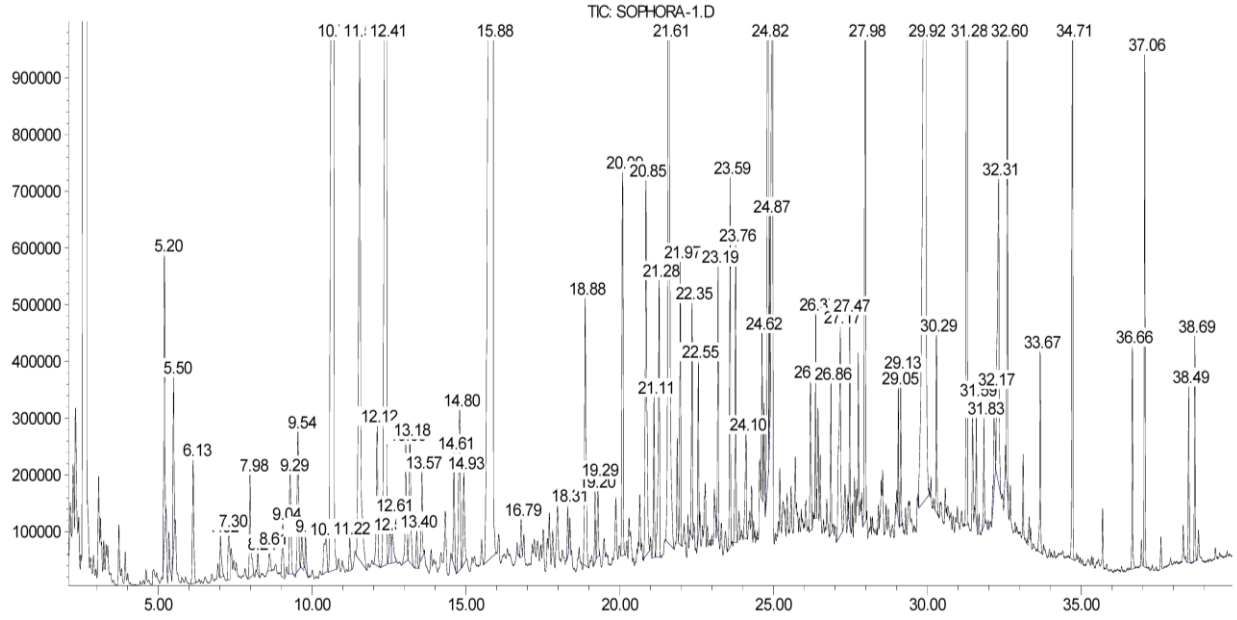

Figure 1. A typical chromatogram of *Sophora japonica* L. bud (green bud) volatile compounds. Please refer to text for GC conditions

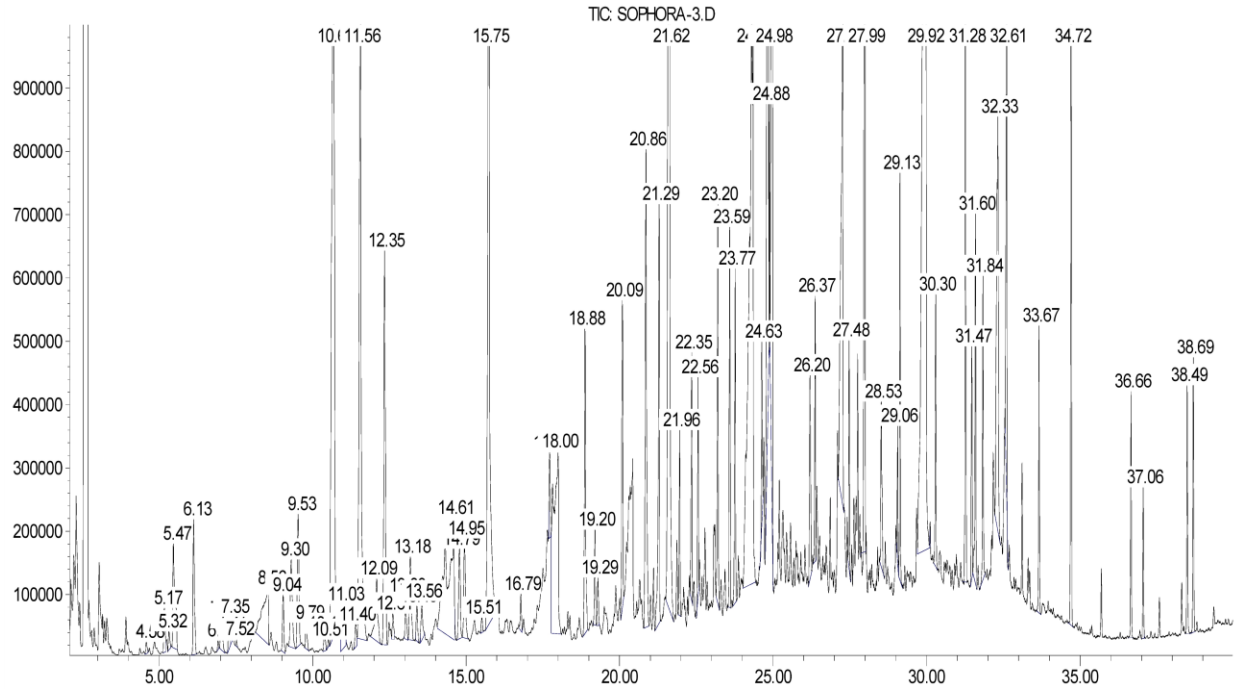

Figure 2. A typical chromatogram of *Sophora japonica* L. bud (formed bud) volatile compounds. Please refer to text for GC conditions

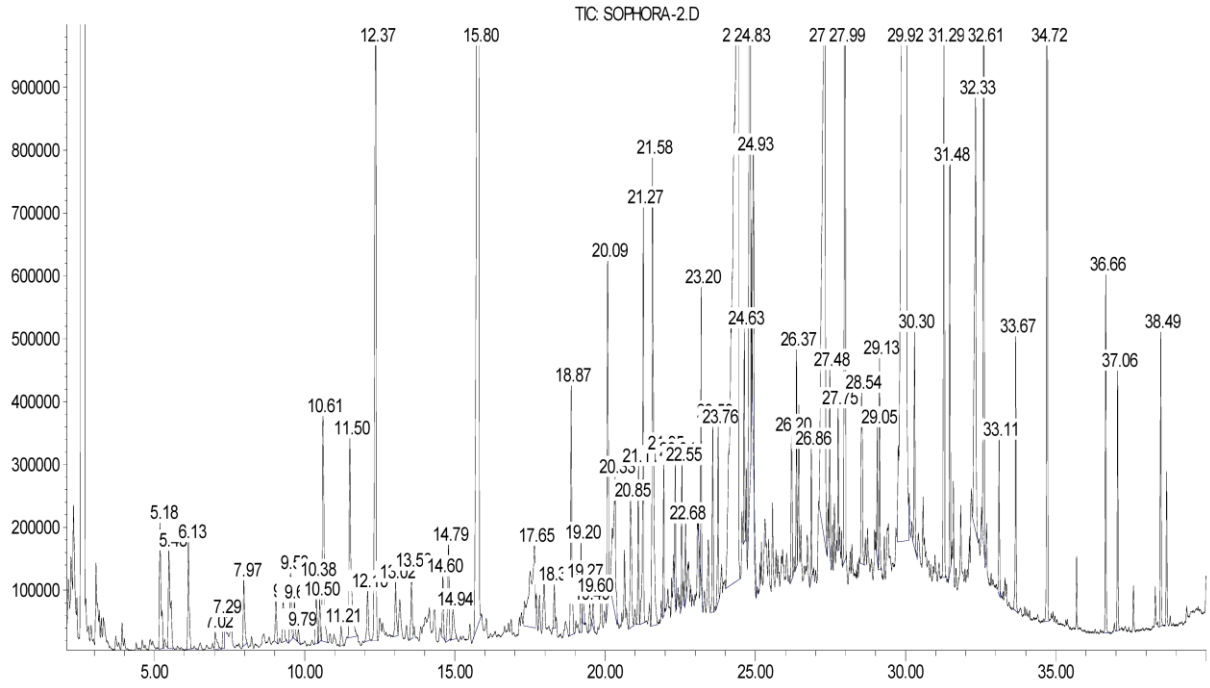

Figure 3. A typical chromatogram of *Sophora japonica* L. bud (beginning of bud opening) volatile compounds. Please refer to text for GC conditions

As results revealed, 80 components were identified in all buds types. Out of which 71 components were from the green flower bud and beginning of flower bud opening stages, and 67 from formed flower bud stage. Components found include saturated hydrocarbons, aldehydes, ketones, alcohols, heterocyclic, fatty acids and their ethers, terpenoids, aromatic and unsaturated hydrocarbons (Table 1).

The major component in the green bud was 3-methoxypyridine (0.00193 g/100 g). In both formed bud and beginning of bud opening was dodecanoic acid with concentrations of (0.00684 g/100 g) and (0.00638 g/100 g), respectively. Other components present in significant contents as being described in Table 1. The retention data, chemical composition and chromatographic traces of green bud, formed bud and beginning of bud opening essential oils are shown in Figure 1, Figure 2, Figure 3 and Table 1.

As seen, great variability in the chemical compositions of the three essential oils from the buds types was observed. In both formed bud and beginning of bud opening essential oils of *Sophora japonica* L., dodecanoic acid was the major component. In the green bud and formed bud essential oils this component is followed by eugenol with concentrations of 0.00104 g/100 g and 0.00619 g/100 g, respectively, while in the beginning of bud opening essential oil, the second major component was tetradecanoic acid with a concentration of 0.00382 g/100 g.

Most substances are extracted at the stage of formed bud. On the other hand, it should be admitted that at this stage substances with potential pharmacological properties are in the highest proportion based on the following components found, namely: dodecanoic acid (0.00684 g/100 g), eugenol (0.00619 g/100 g), heptadecene-8 (0.00572 g/100 g), 3-methoxypyridine (0.00546 g/100 g), hexahydrofarnesylacetone (0.00405 g/100 g), linalool (0.00381 g/100 g), tetradecanoic acid (0.00290 g/100 g), nonanoic acid (0.00261 g/100 g), trans-2-nonenal (0.00236 g/100 g), and linoleic acid (0.00213 g/100 g).

Linalool has antibacterial, wound-healing and anti-inflammatory properties [19,20], eugenol, a phenolic compound has antiseptic, anaesthetic and analgesic effects, antimicrobial, anti-oxidant, anti-inflammatory, anticonvulsant, anticarcinogenic, antimutagenic, repellant and antifumigant activities [21,22] and linoleic acid plays an important role in arachidonic acid and prostaglandin synthesis and maintains cellular membrane structure that is necessary for visual apparatus and nervous system functioning [23].

Additionally, at stages of green bud and formed bud, squalene with concentration of 0.00011 g/100 g and 0.00068 g/100 g, respectively was also identified. Squalene is an unsaturated fluidal triterpene hydrocarbon which lowers blood cholesterol level and also has anti-inflammatory and antioxidative properties.

4. Conclusions

The essential oils, obtained from flower buds in different stages of development, namely; green bud, formed bud and beginning of bud opening from *Sophora japonica* L. by hydrodistillation, were analysed by gas chromatography-flame ionization detection (GC/FID) and gas chromatography-mass spectrometry (GC/MS). Essential oil profile of *Sophora japonica* L. flower buds was produced; it showed significant differences between buds species. A total of 80 identified volatile components were found in the extract of *Sophora japonica* L. flower buds. 71 components were found in the green flower bud and the beginning of flower bud opening stages, and 67 components in the formed flower bud stage (Table 1). Moreover, the results obtained showed that GC/MS is a powerful tool that offers a simple and highly sensitive way to evaluate the quality of *Sophora japonica* L. species, which may be of significant value to industrial as well as

regulatory bodies. To our knowledge, this is the first in-depth screening and comparative investigation of the essential oils present in buds flower species of *Sophora japonica* L.. Contents of *Sophora japonica* L. were significantly affected by the harvesting stage of bud flower. Harvesting at the formed bud stage yielded the highest essential oil content compared to buds harvested at other stages. Flowers harvested at the green bud and beginning of bud opening stages yielded a lower quality essential oil compared with flowers harvested at the formed bud stage. These results showed also that the composition of essential oils in the plant at different growth stages changed and the production of a specific component of these essential oils depended on which stage of growth the plant was at when flowers were harvested.

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Statement of Competing Interests

The authors have no competing interests.

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