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Full Length Article

Chemical Composition of Essential Oil from *Dalbergia odorifera* Flowers and HPLC Analysis of Tectorigenin in Its Leaves and Branches

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Abstract

Dalbergia odorifera T. Chen, as an important traditional Chinese medicinal plant, has been used in China over a long history. The chemical composition of volatile oil extracted from the *D. odorifera* flowers is described for the first time here. The volatile oil was extracted by hydro-distillation, and GC-MS was used for the chemical composition analysis. Tectorigenin, an isoflavonoid, was also isolated from the flowers. The structure of tectorigenin was established based on ^{1}H and ^{13}C NMR and HR-ESI-MS spectrometry. The main components of the volatile oil from the flowers were 4-hydroxy-4-methyl-2-pentanone (28.35%), phenethyl alcohol (12.17%), *cis*-5-ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol (8.71%), toluene (7.64%), *p*-xylene (5.93%), benzyl alcohol (5.72%) and ethylbenzene (5.35%). The tectorigenin contents in the xylem, phloem and leaves were determined by high-performance liquid chromatography (HPLC) as 75.44 μg/g, 104.26 μg/g and 393.11 μg/g, respectively, on a dry weight basis and 49.32 μg/g, 51.98 μg/g and 74.45 μg/g, respectively, on a fresh weight basis. The study provides an important theoretical basis for the further development and application of the *D. odorifera* flowers and tectorigenin.

Keywords: *Dalbergia odorifera*; Essential oils; GC-MS; Tectorigenin; HPLC analysis

Introduction

Dalbergia odorifera T. Chen (Family: Fabaceae) is a valuable tree indigenous to Hainan Province and has been gradually cultivated in South China (Sun *et al.* 2015; Zhao *et al.* 2020a). In view of its distinctive color, aromatic trunk, and high density, this valuable rosewood tree is particularly popular for manufacturing luxurious furniture, artifacts, and musical instruments (Tao and Wang 2010; Wariss *et al.* 2018). The heartwood of *D. odorifera* as an important traditional Chinese medicine in China has been used to treat cardio- and cerebrovascular diseases for a long time (Li *et al.* 2019; Zhao *et al.* 2020b). Due to its precious heartwood and medicinal and economic value, *D. odorifera* has been listed on the IUCN red list since 1998 because of the long-term overexploitation (Wariss *et al.* 2018; Liu *et al.* 2019).

As an increasingly popular traditional Chinese medicine, *D. odorifera* species have attracted much attention from phytochemists. Substantial research has been conducted on this species in recent years, and those studies mainly concerned with its chemical constituents and pharmacological activities of the heartwood (The SN 2017). Some previous studies have focused on the biological activities of the heartwood, such as its antimicrobial (Zhao *et al.* 2011; Wang *et al.* 2014), anti-inflammatory (Lee *et al.* 2014; Choi *et al.* 2017; Kim *et al.* 2018), antioxidant (Ma *et al.* 2013; Sun *et al.* 2015), anti-platelet aggregation (Tao and Wang 2010), angiogenic (Fan *et al.* 2017), antitumor (Park *et al.* 2016; Bastola *et al.* 2017; Meng *et al.* 2019) and vasodilatory (Sugiyama *et al.* 2002; Yang *et al.* 2013) effects. To date, 175 chemical constituents have been isolated from *D. odorifera* (Meng *et al.* 2019; Lu *et al.* 2019). Among them, flavonoids and volatile oils are the major secondary metabolites of *D. odorifera* (Zhao *et al.* 2000). Most studies on the chemical composition of this species have analyzed the heartwood and leaves and reported the isolation of flavonoids, neoflavonoids, isoflavonoids and other flavonoid derivatives (Barnes 2004; Coon *et al.* 2007). However, no reports on the secondary metabolites from the flowers have been published.

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In this study, we extracted the essential oils of *D. odorifera* flowers, and analyzed using GC-MS. Furthermore, tectorigenin, the main constituent, was isolated first time from the flowers of *D. odorifera* in this study. The antioxidant activities of the crude extracts as well as tectorigenin were also determined. As reported in previous studies, tectorigenin was also isolated from the heartwood and leaves, and the differences in the tectorigenin content among different organs were determined by using HPLC.

Materials and Methods

General experimental procedures

NMR spectra were recorded on a Bruker Avance-600 NMR spectrometer (Bruker, USA). HR-ESI-MS was carried out on Bruker maXis with an ESI interface and a Q-TOF mass spectrometer (Bruker, USA). The tectorigenin content was analyzed on a Prominence LC-16 HPLC system (Shimadzu, Japan). HPLC analysis of tectorigenin was performed at 40°C using a reversed-phase C_{18} column (Phenomenex, USA). Acetonitrile and water (30:70, v/v) with 0.01% TFA were used as the mobile phase and pumped at a flow rate of 1.0 mL/min for isocratic elution, with UV detection at 265 nm. Water was purified by an ultrapure water machine (Exceed-Cb-10, Aike, China). Acetonitrile was of HPLC grade.

Plant material and determination of the water content

Leaves, fresh flowers and current-growth branches of *D. odorifera* were manually collected in the campus of South China Agricultural University (SCAU) from 16-year-old artificially cultivated *D. odorifera* in May 2018, in Guangzhou, China. The taxonomical identification of the plant materials was performed by Dr. Mingxuan Zheng of College of Forestry and Landscape Architecture (SCAU, Guangzhou, China), where the voucher specimen (SCAULPMH-180518) of the plant was deposited.

The collected plant materials were first cleaned, and the dirt was removed. Then, the xylem and phloem of the current-growth branches were separated. The leaves, xylem and phloem were cut into small pieces and dried in an oven at 50°C. This process was repeated three times for each sample. The material was weighed before (wet weight) and after (dry weight) drying in the oven for several hours. The materials were dried until a constant weight was reached. The water content was calculated as:

$$[(Mass_{\text{wet weight}} - Mass_{\text{dry weight}})/Mass_{\text{wet weight}}] \times 100\%$$

Preparation, GC and GC-MS analysis of the flower essential oil

The volatile oil was extracted from the flowers (0.52 kg) of *D. odorifera* by hydro-distillation as our previous reported (Feng *et al.* 2017). GC and GC-MS were carried out by the same column and analysis conditions. The oven temperature was programmed as our previous reported (Feng *et al.* 2017).

Extraction of secondary metabolites from the flowers

Three different methods were used to extract secondary metabolites from the *D. odorifera* flowers. Fresh flowers from *D. odorifera* were extracted three times with ethyl acetate (EtOAc) at room temperature. The EtOAc extracts were dried under vacuum to obtain the crude extracts (sample 1). After extraction of the essential oils, the residue and water were separated by decreasing the temperature to room temperature. The flower residue was also extracted three times with EtOAc at room temperature. The EtOAc extracts were dried under vacuum to obtain sample 2. The water was first concentrated under vacuum and then extracted three times with EtOAc, and the EtOAc extracts were dried under vacuum to obtain sample 3.

Antioxidant activity analysis

Samples 1–3 were further evaluated the antioxidant activities. The radical-scavenging activity was determined with a spectrophotometric microplate method based on the reduction of a methanolic solution of DPPH according to our previous report (Shan *et al.* 2020).

Extraction and fractionation of tectorigenin

Sample 3 was a yellow solid and mainly contained one compound as identified on the basis of the HPLC analysis. Therefore, sample 3 was purified by recrystallization and repeated until the compound was purified. Finally, the recrystallized compound was chromatographed on a Sephadex LH-20 column with MeOH-$CHCl_3$ (1:1, V/V) to afford tectorigenin (14 mg).

Preparation of tectorigenin standard solutions

Tectorigenin (1 mg) was accurately weighed and placed in a 2 mL centrifuge tube, where it was dissolved in 1 mL of methanol and the mother solution was 1000 μg/mL. The mother solution was then diluted to 250 μg/mL～0.9765625 μg/mL with methanol using double and half dilution method, and stored at 4°C before use.

Preparation of sample solutions

D. odorifera xylem, phloem and leaves were extracted three times with EtOAc at room temperature to obtain crude extracts. Each sample solution was filtered (pore size, 0.22 μm) and then dissolved in methanol to attain a concentration of 25 mg/mL.

Method validation

The HPLC-UV method was validated according to the procedures described in ICH guidelines Q2 (R1) (ICH Harmonised Tripartite Guideline 2005) and our previous report (Shan *et al*. 2012) to validate the analytical methods.

Quantitative determination of tectorigenin in leaves and branches

The contents of tectorigenin in the samples, xylem, phloem and leaves of *D. odorifera* were determined by HPLC-UV. The crude extracts for the HPLC analysis were prepared as described above. The obtained peak area of tectorigenin was substituted into the regression equation to obtain the concentration and the RSD was calculated.

Statistical analysis

All experiments were carried out in triplicate and the data obtained were compared among treatments using one-way analysis of variance (ANOVA SAS 9.4, SAS Institute, Cary, NC). Tukey's Honest Significant Differences (HSD) tests were conducted after each ANOVA for multiple comparisons. The significance level was determined at p =0.05 for all tests.

Results

Essential oil analysis

Fig. 1 shows the total ion chromatogram of the essential oil from the flowers of *D. odorifera*. The yield of volatile oil from the hydro-distillation of *D. odorifera* flowers was 0.082% (w/w, fresh weight). The GC-MS results are presented in Table 1. Sixteen components were identified in the essential oil from *D. odorifera* flowers and made up 96.00% of the volatile oil. Of them, the main components were 4-hydroxy-4-methyl-2-pentanone (28.35%), phenethyl alcohol (12.17%), *cis*-5-ethenyltetrahydro-*α*, *α*-5-trimethyl-2-furanmethanol (8.71%), toluene (7.64%), *p*-xylene (5.93%), benzyl alcohol (5.72%) and ethylbenzene (5.35%), and the relative contents of these compounds accounted for 73.87% of the total oil content. The relative percentages of the remaining components were below 5%. The essential oils from *D. odorifera* flowers were mainly consisted of alcohols, hydrocarbons, ketones and nitrogenous compounds on the basis of these data shown in Table 1.

Antioxidant activity

Samples 1–3 were submitted a DPPH free radical scavenging assay (Table 2). Sample 3 displayed the highest antioxidant activity with an IC_{50} of 0.35 μg/mL, which is higher than the IC_{50} of BHT, 7.14 μg/mL. Interestingly, both samples 1 and 2 displayed weak antioxidant activity,

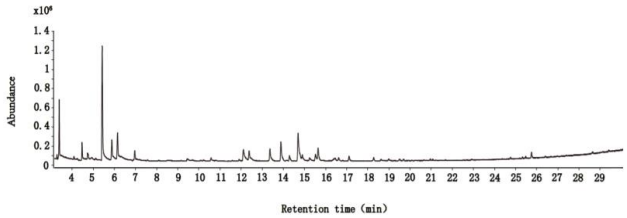

Fig. 1: Total ion chromatogram (TIC) of essential oil from the flowers of *D. odorifera*

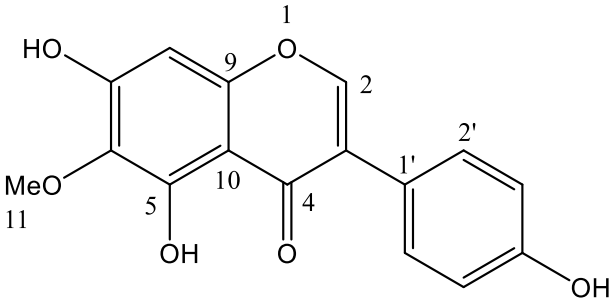

Fig. 2: The chemical structure of tectorigenin

with IC_{50} values greater than 200 μg/mL. Sample 3 was derived from the water remaining after extraction of the essential oil and contained compounds with high antioxidant activity, prompting further study.

Elucidation of the purified tectorigenin

As described above, sample 3 displayed the highest antioxidant activity and was therefore further purified by recrystallization and a Sephadex LH-20 column. The molecular formula of tectorigenin, $C_{16}H_{12}O_6$, was assigned by HR-ESI-MS (Fig. S1): m/z 301.0706 $[M+H]^+$ (calcd. 301.0707) and m/z 323.0526 $[M+Na]^+$ (calcd. 323.0526). After comparing the obtained ^1H and ^{13}C NMR (shown in Table 3) and HR-ESI-MS data with those reported in the literature (Lee *et al.* 2004), this compound was identified as tectorigenin, and its structure is shown in Fig. 2. Tectorigenin was obtained as a yellow powder (MeOH). However, tectorigenin did not show antioxidant activity at 200 μg/mL (results not shown).

Identification of tectorigenin by HPLC-UV

The chromatograms of the tectorigenin standard, the real sample solution and the mixed standard solution are shown in Fig. 3. Tectorigenin could be distinguished based on its retention times. Fig. 3 (A) shows the HPLC-UV profile of the tectorigenin standard. The retention time of tectorigenin was 18.50 min when isocratically eluted with MeCN-H_2O (30:70) containing 0.01% TFA, with detection at 265 nm. Fig. 3 (B)-(D) shows the HPLC-UV profiles of the EtOAc

Table 1: Chemical composition of the essential oil from the flowers of *D. odorifera*

No.	Retention time	Compound	Molecular formula	Molecular weight	RA (%)[a]
1	3.389	Toluene	C_7H_8	92	7.64
2	4.470	Acetic acid, butyl ester	$C_6H_{12}O_2$	116	3.04
3	4.731	1-Cyclopropyl-1-methyl-ethylamine	$C_6H_{13}N$	99	1.58
4	5.426	4-Hydroxy-4-methyl-2-pentanone	$C_6H_{12}O_2$	116	28.35
5	5.879	Ethylbenzene	C_8H_{10}	106	5.35
6	6.148	*p*-Xylene	C_8H_{10}	106	5.93
7	6.965	*o*-Xylene	C_8H_{10}	106	1.95
8	12.108	Benzyl alcohol	C_7H_8O	108	5.72
9	12.380	Benzeneacetaldehyde	C_8H_8O	120	4.01
10	13.887	*cis*-5-Ethenyltetrahydro-α,α-5-trimethyl-2-furanmethanol	$C_{10}H_{18}O_2$	170	8.71
11	14.291	Linalool	$C_{10}H_{18}O$	154	1.58
12	14.696	Phenethyl alcohol	$C_8H_{10}O$	122	12.17
13	14.899	Isophorone	$C_9H_{14}O$	138	2.31
14	15.520	Benzyl nitrile	C_8H_7N	117	2.19
15	15.645	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	152	4.01
16	25.753	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	1.46

a: RA indicates the relative amount (individual peak area relative to the total peak area)

Table 2: Antioxidant activities

Samples	IC_{50} (μg/mL)
Sample 1	246.78 ± 0.99b
Sample 2	316.98 ± 2.52a
Sample 3	0.35 ± 0.01d
BHT	7.14 ± 0.08c

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

Table 3: ^{13}C-NMR and ^{1}H-NMR data for tectorigenin (in DMSO-d_6)

Position	1(δ in ppm, J in Hz)	
	δ_C	δ_H
2	154.0	8.33 (1H, s)
3	121.8	-
4	180.5	-
5	152.7	-
5-OH	-	13.05 (1H, s)
6	131.4	-
7	153.2	-
7-OH	-	9.59 (1H, s)
8	93.8	6.50 (1H, s)
9	157.5	-
10	104.8	-
11	59.9	3.75 (3H, s)
1'	121.2	-
2', 6'	130.1	7.37 (2H, d, J = 8.5 Hz)
3', 5'	115.0	6.82 (2H, d, J = 8.5 Hz)
4'	157.4	-

extracts of the xylem, phloem and leaves, respectively. The tectorigenin peak eluted at 18.50 min for all three samples. A tectorigenin standard was added to crude extracts and analyzed to confirm the peak at 18.50 min. Fig. 3 (E)-(G) shows the HPLC profiles of the crude xylem, phloem and leaf extracts containing the tectorigenin standard. The peak at 18.50 min in Fig. 3 (E)-(G) was significantly higher than that in Fig. 3 (B)-(D). Therefore, the peak at 18.50 min in Fig. 3 (B)-(D) corresponded to tectorigenin.

Calibration curves

Based on the above results, tectorigenin was further selected for quantitative analysis by HPLC-UV. The linear equation was Y = 3398998.33X - 2834.37 (R = 0.9994), where X represent the quantity (μg) of the sample injected for one time, Y represent the peak area, and R is the correlation coefficient. The R value showed a good linearity over a range of 0.009765625 ~ 2.5 μg of injected sample.

Precision

The mean RSDs in the tectorigenin content for intra- and interday detection at three different levels (Table 4) were less than 1.09% and 1.43%, respectively. Because these values are below 2.0%, the method has good reproducibility.

Accuracy

The accuracy was calculated as the means of a standard addition experiment and the result was shown in Table 5. The mean recovery (n = 9) of tectorigenin in xylem, phloem and leaves were 95.99%, 96.87% and 100.50%, respectively, and the mean RSDs were 0.79%, 0.72% and 0.80%, respectively. These satisfactory recoveries and low RSDs confirmed the suitability of this method for the analysis of tectorigenin.

LOD and LOQ

Under the validated HPLC conditions, the LOD and LOQ were determined at an S/N values of approximately 3 and 10, respectively, from injections of 0.9342 and 1.3023 ng, respectively.

Quantitative determination of tectorigenin in leaves and branches

The water contents are shown in Table 6. The water content in leaves was the highest, followed by that in phloem and then that in xylem, with values of 81.05, 49.52 and 34.63%, respectively. The content of tectorigenin was calculated in leaves and branches on the basis of the water content, as

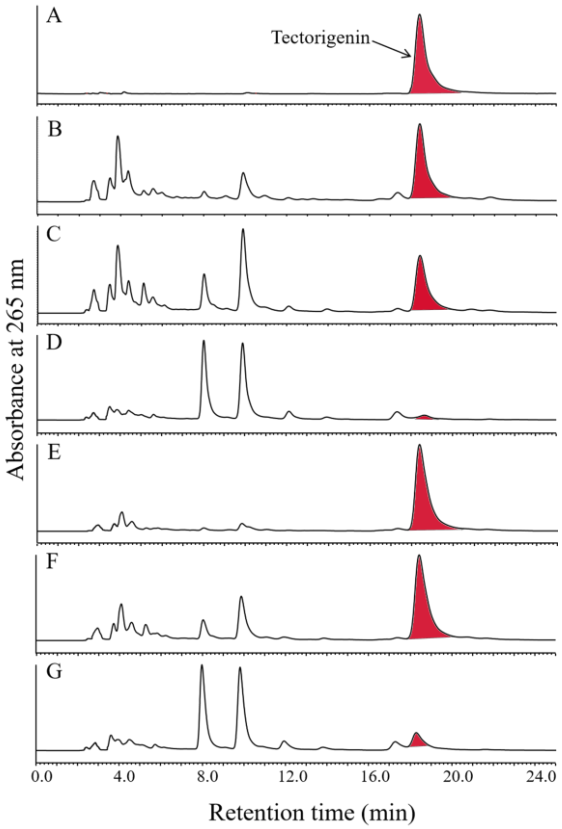

Fig. 3: HPLC-UV analysis of tectorigenin in xylem, phloem and leaves of *D. odorifera*
(A) HPLC profile of the tectorigenin standard; (B)-(D) HPLC profiles of the crude xylem, phloem and leaf extracts, respectively (E)-(G) HPLC profiles of the crude xylem, phloem and leaf extracts containing the tectorigenin standard, respectively

shown in Table 7. The content of tectorigenin in the xylem, phloem and leaves was 75.44, 104.26 and 393.11 μg/g, respectively, on a dry weight basis and 49.32, 51.98 and 74.45 μg/g, respectively, on a fresh weight basis.

Discussion

Volatile oil is an important component of *D. odorifera*. To the best of our knowledge, the heartwoods are a valuable source of essential oils that can be used as a precious perfume fixative. In previous studies, the volatile oils obtained from *D. odorifera* leaves, heartwood and seeds were analyzed and reported. 2-methoxy-4-vinylphenol and *n*-hexadecanoic acid with the relative percentages 21.73 and 13.97%, respectively, were the major compounds in volatile oil from leaves (Bi *et al.* 2004). Liu (2009) reported the major components of the volatile oil obtained from heartwood via steam distillation were nerolidol and caryophyllene oxide, and the relative percentages were 57.36 and 22.22%, respectively. Guo *et al.* (2011) analyzed the chemical composition of the seeds essential oil and the main components were *p*, *p*, *p*-triphenyl phosphine imide and bis(1-methylethyl) peroxide with the relative

Table 4: Precision and RSDs of the determination of tectorigenin by HPLC

Compound	Intraday (n = 5)		Interday (n = 9)	
	Concentration (μg/mL)	RSD (%)	Concentration (μg/mL)	RSD (%)
Tectorigenin	3.91	1.09	3.91	1.17
	31.25	0.50	31.25	1.43
	250.00	0.38	250.00	1.12

Table 5: Recoveries and RSDs of tectorigenin in leaves and branches

Sample	Added concentration (μg/mL)	Recovery (%)	RSD (%)
Xylem	0.5 X	95.21	0.44
	1.0 X	98.42	1.45
	2.0 X	94.33	0.49
Phloem	0.5 X	97.41	1.02
	1.0 X	96.38	0.70
	2.0 X	96.83	0.45
Leaf	0.5 X	101.10	1.09
	1.0 X	100.34	0.81
	2.0 X	100.05	0.49

Table 6: Water content of xylem, phloem and leaves

Samples	Water content (%)
Xylem	34.63 ± 0.55c
Phloem	49.52 ± 3.02b
Leaf	81.05 ± 0.78a

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

Table 7: Contents and RSDs of tectorigenin in leaves and branches

Samples	Average (μg/g dry weight)	Average (μg/g fresh weight)	RSD (%)
Xylem	75.44 ± 0.63c	49.32 ± 0.41c	0.83
Phloem	104.26 ± 1.38b	51.98 ± 0.69b	1.32
Leaf	393.11 ± 2.57a	74.45 ± 0.49a	0.65

Mean ± standard deviation. Values with same letter differ non-significantly (P>0.05)

percentages 35.3 and 16.4%, respectively. As can be seen from the report above, the major constituents in *D. odorifera* leaves differ from those in the heartwood and seed oil. However, the chemical composition of volatile oil from *D. odorifera* flowers has not been reported. In this study, the volatile oil of *D. odorifera* flowers, extracted by steam distillation and analyzed by GC-MS, revealed the following main components: 4-hydroxy-4-methyl-2-pentanone, phenethyl alcohol, *cis*-5-ethenyltetrahydro-*α*, *α*-5-trimethyl-2-furanmethanol, toluene, *p*-xylene, benzyl alcohol and ethylbenzene. The present analysis also showed that the types and relative contents of the volatile oil components varied greatly in different parts of plant tissues. In addition, the chemical composition also varies according to the extraction method.

Flavonoids are considered the important active principle components in many herbs because of the extensive pharmacological activities (Zhao *et al.* 2020a). As previously reported, flavonoids are the main secondary metabolites and about 99 flavonoids have been identified from *D. odorifera* (Zhao *et al.* 2020b). In previous reports, pharmacological studies revealed flavonoids to be antioxidant components

and parts of flavonoids displayed stronger antioxidant activity than those of BHT (Wang *et al.* 2000; Hou *et al.* 2011). In this study, the extract of *D. odorifera* flowers was found to contain tectorigenin, an isoflavone that did not show any antioxidant activity in the DPPH assay. At present, no reports have described the antioxidant activity of tectorigenin. The above results from other studies are consistent with our results in this study. Sample 3 from the flowers of *D. odorifera* in this study displayed significant in *vitro* antioxidant activity, but the active components remain unknown. Therefore, the active antioxidant constituents of *D. odorifera* flowers should be elucidated in the future.

This investigation has shed light on utilization of the flowers from *D. odorifera*, a Chinese medicinal plant of greater economic and ecological importance, for the study of essential oils and secondary metabolites. This study is the first of its kind in this plant species which involves the use of flowers for biochemical analysis, unlike previous studies which were primarily focused on heartwood. The evidence also suggests that tectorigenin as the major plant metabolite in flower tissues which needs further functional studies and can prove to be very useful.

Conclusion

The is the first study to clarify chemical composition of volatile oil from flowers of *D. odorifera* and to identify tectorigenin as the key constituent in the flowers secondary metabolites. The main components of the volatile oil from *D. odorifera* flowers were 4-hydroxy-4-methyl-2-pentanone, phenethyl alcohol, *cis*-5-ethenyltetrahydro-α, α-5-trimethyl-2-furanmethanol, toluene, *p*-xylene, benzyl alcohol and ethylbenzene. The distribution of tectorigenin in branches of *D. odorifera* was also analyzed. The tectorigenin contents in the xylem, phloem and leaves were determined by HPLC to be 75.44 μg/g, 104.26 μg/g and 393.11 μg/g, respectively, on a dry weight basis and 49.32 μg/g, 51.98 μg/g and 74.45 μg/g, respectively, on a fresh weight basis. This information will pave the way for further functional analysis, experimentation, development and application of *D. odorifera* flowers with keen focus on tectorigenin as an important antioxidant agent.

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Author Contributions

ZM, YW and TS performed the research and recorded the spectra. ML, TC, JL, HH, TS and DW prepared the flower essential oils and performed the GC-MS analysis. ZM, YW and TS isolated and structurally characterized tectorigenin. HS and JW performed the antioxidant assay. TS, TC, JL and HS performed the HPLC analysis of tectorigenin. TS, YW and CT contributed to the data collection process. TS and DW contributed by preparing figures. TS, DW and ZM designed the research. TS, YW, DW and ZM completed the draft of the manuscript. All the authors contributed to the writing, editing and revising of the manuscript.

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