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Chemical Composition Similarity Relationships among the Various Organs of the *Ilex cornuta* Lindl. & Paxton Based on the Analysis of Hydrophilic Volatile Compounds

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Abstract

In performing molecular profiling of secondary metabolites, a lot of research has focused on biogenic volatile organic compounds with medium to low polarity. In this study, chemical composition similarity relationships among the various organs of the *Ilex cornuta* Lindl. & Paxton were assessed based on the analysis of hydrophilic volatile compounds. GC-MS analysis was conducted to characterize and classify the chemical compounds. A total of 36, 46, 42, 25, 64, 26 compounds have been respectively extracted from the root, stem, stem skin, leaf, flower and fruit. The six organs have 3 common compounds and large percentages of exclusive compounds ranging from 36.0% to 62.5% with a mean of 49.8%, indicating substantial component differences among the different organs. The percentage of overlapping compounds between each of the two organs ranges from 10.9% to 44.0%, which is relatively small, further demonstrating the strong organ specificity of the chemical composition. The overlapping index is used to reveal the similarity among the organs. The stem shares the maximum similarity while the fruit the minimum similarity with the other organs. Aside from fruit, the average overlapping indices between each of the other two organs correlate well to their physical proximity. In conclusion, hydrophilic volatile metabolites are a class of natural products that are rarely investigated but constitute a significant part of the plant chemical composition. Chemical profiling of these metabolites could provide a valuable tool for the plant taxonomy and help understand the chemically mediated biological phenomena.

***Keywords:** Chemical composition similarity, GC-MS, Hydrophilic volatile compounds, Ilex cornuta Lindl. & Paxton, Plant taxonomy*

Introduction

Plant taxonomy is traditionally conducted based on macroscopic and microscopic morphological characteristics. Growing evidence suggests that many biologically relevant entities could be missed in the studies that rely solely on morphological traits, particularly since speciation is not always accompanied by morphological change [1,2]. In recent years, plant chemical taxonomy has been developed to perform classification based on a wide array of biologically active secondary metabolites [3]. The expression of secondary metabolites could vary due to convergent evolution or differential gene expression [4], suggesting that the metabolite content of plants may reveal more information on the bioactive pattern of plants in comparison to morphology characterization [5].

In performing molecular profiling of secondary metabolites, a lot of research has focused on biogenic volatile organic compounds with medium to low polarity [6-9]. Volatile compounds are secreted and part of them are volatilized immediately after secretion [10,11]. The remaining part is stored in the special structure of the plant as in the case of essential oils [12-14]. Additionally, Berlinck and collaborators found that the vast majority of new compounds from natural sources reported in recent literature are compounds of medium to low polarity. Water-soluble, volatile, minor and photosensitive natural products are yet poorly known. One of the possible reasons for this trend could be that organic solvents of medium to low polarity used in isolation procedures require less time and less sophisticated instrumentation to be evaporated [15]. The author speculates that there is a class of hydrophilic volatile compounds in plants that are dispersed or dissolved in the water phase, evaporated with water vapor, and whose polarity and volatility are somewhere between essential oils and water-soluble compounds. To protect this type of ingredients from loss during extraction, water vapor distillation is used to collect volatile compounds that are dispersed or dissolved in the plant's water phase. The root, stem, stem skin, leaf, flower and fruit of the *Ilex cornuta* Lindl. & Paxton were analyzed as study samples. Volatile essential oils were removed by using Soxhlet extraction method. Hydrophilic volatile compounds obtained by water reflux extraction are characterized and classified by quantitative GC-MS. The study revealed the potential use of hydrophilic volatile metabolites in the plant taxonomy and understanding the chemically mediated biological phenomena.

Materials and Methods

Material

Ilex cornuta Lindl. & Paxton was collected in Nanjing, China. Its roots, stems, stem skins, leaves, flowers and fruits were washed, cut into pieces, dried at 30°C and stored at 2-8°C prior to use.

Chemicals and Reagents

Ethyl acetate was purchased from Xilong Chemical Co., Ltd (Shantou, China). Hexane was purchased from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Activated carbon was purchased from Shanghai Chemical Reagent Procurement Center (Shanghai, China). C7-C40 saturated alkanes standard was purchased from Anpel Laboratory Technologies Inc. (Shanghai, China).

Sample Preparation

Each sample was sliced and dried at 30°C. After ground into powder, the samples were sieved through 80 mesh followed by 180 mesh. Approximately 6 g of the sample were subjected to Soxhlet extractor method with hexane for 24 hrs to remove essential oils and other lipophilic compounds. The remainder was then removed and dried at 30°C in the ventilation cabinet. Approximately 4 g of the dried powder was then added into a 6 x 7 cm nonwoven bag together with three glass balls of 4 cm diameter. At least 3 segments of thread were used to separate and tighten the bag into 3 parts, each containing a glass ball and even amount of the dried powder. The bag was then placed in a flask and 2100 mL of water was subsequently added to soak the powder for about 2 hrs. After reflux extraction for 6 hrs, 2 L of distilled water was collected. The same reflux extraction was repeated to collect another 1 L of distilled water for a total of 3 L. After cooling, activated carbon (4 g) was added to absorb the active ingredients from the 3 L of distilled water for about 8 hrs. The activated carbon containing the active ingredients was then filtered and dried at 30°C for 12 hrs. Ethyl acetate was subsequently added to isolate the active ingredients from the activated carbon using Soxhlet extractor method for 8 hrs. The resulting ethyl acetate extract was left in the ventilation cabinet to dry at 30°C. The dried active ingredients were finally re-dissolved using ethyl acetate, filtrated through 0.22 μm filter and analyzed using GC-MS.

GC-MS Analysis

Analysis of hydrophilic volatile compounds was performed using Shimadzu GCMS-QP2010 Single Quadrupole GC-MS (Kyoto, Japan). A Rxi-1 ms GC capillary column (30 cm length, 0.25 mm inner diameter and 0.25 μm thick film) from Shimadzu (Kyoto, Japan) was used for analysis.

One microliter of sample was injected in split mode with split ratio of 5 to 1. GC inlet temperature is set at 280°C. High purity nitrogen (≥99.999%) was used as carrier gas in constant flow mode at 1 mL/min. The initial temperature of the GC oven is set at 60°C and held for 1 min, then ramped at 4°C/min to 160°C and held for 3 mins, followed by 2°C/min to 280°C and held for 6 mins. Finally, the temperature is raised to 300°C at 4°C/min and held for 6 mins. The mass spectrometer was operated in positive electron ionization mode at 70 eV and all spectra were recorded in full scan with a mass range of 40-700 Da. The interface temperature is set at 280°C and ion source temperature is set at 250°C.

Data Processing and Compound Identification

The GC-MS data processing was done with Shimazdzu GCMS Solution software. Compound identification was performed by applying several assignments, e.g., reference standard analysis, retention index calculation, and by NIST08 Spectrum Library comparison. Only peaks with area greater than 3 million are analyzed. The overlapping percentage is calculated by the number of overlapping compounds divided by the total number of hydrophilic volatile compounds from each of the two organ and times 100. Overlapping index is calculated by the number of overlapping compounds squared and divided by the total number of hydrophilic volatile compounds from each of the two organs. In addition, hierarchical clustering analysis was performed with Python to assess the similarities between each of the two organs by analyzing the number of overlapping hydrophilic volatile compounds.

Results and Discussion

The root, stem, stem skin, leaf, flower and fruit of the *Ilex cornuta* Lindl. & Paxton contain compounds that are water soluble and can volatilize with water vapor. These hydrophilic compounds do not separate from the water phase and possess greater polarity than essential oils. The largest number (64) of hydrophilic volatile compounds are isolated from the flower and the smallest (25) from the leaf, indicating that the number of hydrophilic volatile compounds varies greatly from organ to organ. The hydrophilic volatile compounds include aromatics, fatty acids, furans, heterocycle, esters, alkanes, ketones, halogens and other types of small molecular compounds. This is a diverse group of molecules that could contribute to the expression of biological information about the plant. Tables 1-6 present the lists of hydrophilic volatile compounds identified from the root, stem, stem skin, leaf, flower and fruit, respectively. The bold and italic fonts in the table are used to refer to exclusive compounds that are only found in the specific organ and not contained in any other organ.

As shown in Table 7, the total number of hydrophilic volatile compounds isolated from the six organs ranges from 25 to 64. There are 3 common compounds in the six organs, *i.e.* Dodecanoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester and n-Hexadecanoic acid. This accounts for 12.0% of total hydrophilic volatile compounds for the leaf and 4.7% for the flower with an average of 8.4% for all the six organs, indicating the little commonality of the six organs. Each organ also has its exclusive compounds which are not found in any other organ. The percentage of exclusive compounds follows the order of flower > fruit > stem skin > root > stem > leaf. The flower has the largest number and percentage of the exclusive compounds, 40 and 62.5%, respectively. The leaf has the smallest number and percentage of the exclusive compounds, 9 and 36.0%, respectively. The stem and stem skin display medium numbers of exclusive compounds. The average percentage of the exclusive compounds in the six organs was 49.8%, nearly half, indicating strong organ specificity. These results provide evidence to support the

Table 1: List of the hydrophilic volatile compounds identified from the root of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Formula
1	*8.434*	*1041*	*2(3H)-Furanone, dihydro-4-hydroxy-*	$C_4H_6O_3$
2	*8.555*	*1044*	*2-Oxo-n-valeric acid*	$C_5H_8O_3$
3	*8.623*	*1047*	*2,3-Anhydro-d-galactosan*	$C_6H_8O_4$
4	*9.159*	*1064*	*Acetic acid, hexyl ester*	$C_8H_{16}O_2$
5	*9.767*	*1084*	*2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-*	$C_7H_{10}O_2$
6	12.753	1173	Octanoic Acid	$C_8H_{16}O_2$
7	13.662	1199	2-Furancarboxaldehyde,5-(hydroxymethyl)-	$C_6H_6O_3$
8	*16.053*	*1269*	*Nonanoic acid*	$C_9H_{18}O_2$
9	*19.301*	*1364*	*Benzaldehyde, 4-(methylthio)-*	C_8H_8OS
10	*23.459*	*1492*	*1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-*	$C_{10}H_{10}O_3$
11	*23.660*	*1498*	*3-Acetoxydodecane*	$C_{14}H_{28}O_2$
12	*25.161*	*1546*	*7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin*	$C_{14}H_{14}O_3$
13	25.496	1557	Dodecanoic acid	$C_{12}H_{24}O_2$
14	*25.696*	*1564*	*Estra-1,3,5(10)-trien-17. beta. - ol*	$C_{18}H_{24}O$
15	*26.109*	*1577*	*Butyric acid, 3-tridecyl ester*	$C_{17}H_{34}O_2$
16	26.829	1600	Hexadecane	$C_{16}H_{34}$
17	27.305	1613	Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-	$C_{14}H_{24}O_2$
18	28.020	1631	Thieno[3,2-c]pyridin-4(5H)-one	C_7H_5NOS
19	*28.671*	*1649*	*Dodecanoic acid, 3-hydroxy-*	$C_{12}H_{24}O_3$
20	30.636	1700	2-Bromotetradecane	$C_{14}H_{29}Br$
21	32.780	1750	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$
22	35.860	1821	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
23	37.894	1867	2a-isopropyl-9,10a-dimethyl-6-methylenedodecahydro-1H-cyclopenta[4',5']cycloocta[1',2':1,5] cyclopenta[1,2-b]oxiren-4-ol	$C_{20}H_{32}O_2$
24	*39.903*	*1912*	*1,2-Benzenedicarboxylic acid, butyl octyl ester*	$C_{20}H_{30}O_4$
25	41.687	1951	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
26	49.181	2119	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$
27	*50.098*	*2139*	*9-Octadecenamide, (Z)-*	$C_{18}H_{35}NO$
28	50.483	2148	Octadecanoic acid	$C_{18}H_{36}O_2$
29	59.601	2362	2-Methyloctadecan-7,8-diol	$C_{19}H_{40}O_2$
30	65.148	2499	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
31	73.761	2726	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$
32	*77.825*	*2840*	*3-Phenyl-2-ethoxypropylphthalimide*	$C_{19}H_{19}NO_3$
33	*83.874*	*3017*	*9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3.beta.,5Z,7E)-*	$C_{27}H_{44}O_3$
34	89.708	3197	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$
35	92.123	3265	Isophthalic acid, allyl pentadecyl ester	$C_{26}H_{40}O_4$
36	102.267	3561	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	$C_{35}H_{62}O_3$

Note: The bold and italic fonts are used to refer to exclusive compounds. RT: Retention time. RI: Reflex index.

Table 2: List of the hydrophilic volatile compounds identified from the stem of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Molecular
1	13.608	1198	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$
2	19.258	1363	4-Hydroxy-2-methoxybenaldehyde	$C_8H_8O_3$
3	*21.565*	*1433*	*Cyclopentanemethanol,.alpha.-(1-methylethyl)-2-nitro-, [1.alpha.(S*),2.alpha.]-*	$C_9H_{17}NO_3$
4	*23.85*	*1504*	*4,8-Decadienal, 5,9-dimethyl-*	$C_{12}H_{20}O$
5	*24.743*	*1533*	*Megastigmatrienone*	$C_{13}H_{18}O$

6	25.469	1556	Dodecanoic acid	$C_{12}H_{24}O_2$
7	*25.681*	*1563*	*1-Cyclohexene-1-methanol, .alpha.,2,6,6-tetramethyl-*	$C_{11}H_{20}O$
8	26.105	1577	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	$C_{16}H_{30}O_4$
9	*26.245*	*1581*	*Phenol, 3,4,5-trimethoxy-*	$C_9H_{12}O_4$
10	*26.495*	*1589*	*2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)-but-2-en-1-ol*	$C_{14}H_{24}O$
11	27.127	1608	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$
12	27.37	1614	Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-	$C_{14}H_{24}O_2$
13	27.88	1628	Thieno[3,2-c]-pyridin-4(5H)-one	C_7H_5NOS
14	*28.27*	*1638*	*Spiro-[4.5]-decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-*	$C_{15}H_{24}O_2$
15	*28.685*	*1649*	*2-Bromo dodecane*	$C_{12}H_{25}Br$
16	*29.172*	*1662*	*Ethanol, 2-(octadecyloxy)-*	$C_{20}H_{42}O_2$
17	*29.971*	*1683*	*1-(2-Hydroxy-4,5-dimethoxy-phenyl)-ethanone*	$C_{10}H_{12}O_4$
18	*30.271*	*1691*	*2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-*	$C_{10}H_{10}O_3$
19	30.399	1694	Butanol, 1-[2,2,3,3-tetramethyl-1-(3-methyl-1-penynyl)-cyclopropyl]-	$C_{17}H_{30}O$
20	30.641	1700	Heptadecane	$C_{17}H_{36}$
21	31.037	1710	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$
22	*31.345*	*1717*	*4a-Dichloromethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one*	$C_{11}H_{14}Cl_2O$
23	31.75	1726	Adamantane, 1-thiocyanatomethyl-	$C_{12}H_{17}NS$
24	*32.052*	*1733*	*9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol*	$C_{15}H_{26}O_2$
25	32.512	1744	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$
26	*32.788*	*1750*	*Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-*	$C_{16}H_{30}O$
27	*33.683*	*1771*	*3-Isobutyryl-6-isopropyl-2,3-dihydropyran-2,4-dione*	$C_{12}H_{16}O_4$
28	*34.92*	*1800*	*Heneicosane*	$C_{21}H_{44}$
29	35.489	1813	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$
30	35.86	1821	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
31	37.316	1854	1-Hexadecanol	$C_{16}H_{34}O$
32	37.898	1867	2a-isopropyl-9,10a-dimethyl-6-methylenedodecahydro-1H-cyclopenta[4',5']-cycloocta[1',2':1,5]-cyclopenta-[1,2-b]oxiren-4-ol	$C_{20}H_{32}O_2$
33	*39.907*	*1912*	*1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester*	$C_{22}H_{34}O_4$
34	41.693	1951	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
35	*43.899*	*2000*	*Eicosane*	$C_{20}H_{42}$
36	*49.179*	*2119*	*12-Methyl-E,E-2,13-octadecadien-1-ol*	$C_{19}H_{36}O$
37	50.464	2148	Octadecanoic acid	$C_{18}H_{36}O_2$
38	59.603	2362	2-Methyloctadecan-7,8-diol	$C_{19}H_{40}O_2$
39	65.152	2499	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$
40	73.757	2726	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$
41	83.861	3016	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
42	89.711	3197	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$
43	92.16	3266	Isophthalic acid, allyl pentadecyl ester	$C_{26}H_{40}O_4$
44	*92.66*	*3280*	*17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one*	$C_{35}H_{52}O$
45	*94.571*	*3331*	*4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone*	$C_{29}H_{42}O_4$
46	102.263	3561	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	$C_{35}H_{62}O_3$

Note: The bold and italic fonts are used to refer to exclusive compounds. RT: Retention time. RI: Reflex index.

Table 3: List of the hydrophilic volatile component identified from the stem skin of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Molecular
1	12.765	1173	Octanoic Acid	$C_8H_{16}O_2$
2	13.818	1204	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$
3	19.283	1364	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$
4	*21.526*	*1432*	*2H-Pyran-2-one, 5,6-dihydro-6-pentyl-*	$C_{10}H_{16}O_2$
5	*23.372*	*1489*	*4,6-di-tert-Butyl-m-cresol*	$C_{15}H_{24}O$
6	*23.599*	*1496*	*12-Oxa-[tetracyclo[5.2.1.1(2,6).1(8,11)]]dodecan-10-ol, 3-acetoxy-*	$C_{13}H_{18}O_4$
7	*23.856*	*1504*	*2,6-Dimethoxybenzoquinone*	$C_8H_8O_4$
8	*25.171*	*1547*	*1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-*	$C_{15}H_{26}O$
9	*25.317*	*1551*	*2(5H)-Furanone, 4-methyl-5,5-bis(2-methyl-2-propenyl)-*	$C_{13}H_{18}O_2$
10	25.462	1556	Dodecanoic acid	$C_{12}H_{24}O_2$
11	*25.694*	*1564*	*2-Oxabicyclo[3.3.0]oct-7-en-3-one, 7-(1-hydroxypentyl)-*	$C_{12}H_{18}O_3$
12	*25.922*	*1571*	*Dodecane, 2,6,10-trimethyl-*	$C_{15}H_{32}$
13	26.114	1577	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	$C_{16}H_{30}O_4$
14	*26.335*	*1584*	*3-Butyl-4-nitro-pent-4-enoic acid, methyl ester*	$C_{10}H_{17}NO_4$
15	26.514	1590	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$
16	26.838	1600	Heptadecane	$C_{17}H_{36}$
17	27.227	1611	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$
18	*27.929*	*1629*	*2,6,10,10-Tetramethyl-1-oxaspiro-[4.5]decan-6-ol*	$C_{13}H_{24}O_2$
19	*28.288*	*1638*	*4-Isobenzofuranol, octahydro-3a,7a-dimethyl-, (3a.alpha.,4.beta.,7a.alpha.)-(.+-.)-*	$C_{10}H_{18}O_2$
20	*29.187*	*1662*	*Ethanol, 2-(hexadecyloxy)-*	$C_{18}H_{38}O_2$
21	29.827	1679	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl-	$C_{13}H_{22}O_2$
22	*29.956*	*1682*	*Cyclopentanone, 2-(1-adamantyl)-*	$C_{15}H_{22}O$
23	*30.308*	*1692*	*alpha. Isomethyl ionone*	$C_{14}H_{22}O$
24	30.649	1700	2-Bromotetradecane	$C_{14}H_{29}Br$
25	31.047	1710	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$
26	31.774	1727	Adamantane, 1-thiocyanatomethyl-	$C_{12}H_{17}NS$
27	*32.083*	*1734*	*E,E-6,8-Tridecadien-2-ol, acetate*	$C_{15}H_{26}O_2$
28	32.522	1744	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$
29	32.801	1751	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$
30	*33.682*	*1771*	*7-Bromo-3a,6,6-trimethyl-hexahydro-benzofuran-2(3H)-one*	$C_{11}H_{17}BrO_2$
31	35.475	1813	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$
32	35.876	1822	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
33	*37.903*	*1867*	*Dodecane, 1,2-dibromo-*	$C_{12}H_{24}Br_2$
34	*39.916*	*1912*	*Dibutyl phthalate*	$C_{16}H_{22}O_4$
35	41.653	1951	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
36	*65.178*	*2500*	*1,2-Benzenedicarboxylic acid, diisooctyl ester*	$C_{24}H_{38}O_4$
37	73.760	2726	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$
38	93.411	3301	1,2-Benzenedicarboxylic acid, diundecyl ester	$C_{30}H_{50}O_4$
39	93.650	3307	Isophthalic acid, allyl pentadecyl ester	$C_{26}H_{40}O_4$
40	*100.522*	*3505*	*9-Octadecenoic acid (Z)-, phenylmethyl ester*	$C_{25}H_{40}O_2$
41	*101.150*	*3525*	*2,6-Lutidine 3,5-dichloro-4-dodecylthio-*	$C_{19}H_{31}Cl_2NS$
42	102.301	3562	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	$C_{35}H_{62}O_3$

Note: The bold and italic fonts are used to refer to exclusive compounds. RT: Retention time. RI: Reflex index.

Table 4: List of the hydrophilic volatile component of the leaf of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Molecular
1	13.543	1196	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$
2	*14.110*	*1212*	*2-Furancarboxaldehyde, 6-(hydroxymethyl)-*	$C_6H_6O_4$
3	*14.318*	*1218*	*2-Furancarboxaldehyde, 7-(hydroxymethyl)-*	$C_6H_6O_5$
4	*25.089*	*1544*	*Bicyclo[3.2.0]heptan-6-one, 2-acetyl-3,3-dimethyl-7-(1-methylethyl)-*	$C_{14}H_{22}O_2$
5	25.453	1556	Dodecanoic acid	$C_{12}H_{24}O_2$
6	25.692	1564	trans-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}O$
7	*26.117*	*1577*	*4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol*	$C_{13}H_{20}O_2$
8	*26.493*	*1589*	*7-Heptadecene, 1-chloro-*	$C_{17}H_{33}Cl$
9	26.831	1600	Hexadecane	$C_{16}H_{34}$
10	28.088	1633	3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo-, ethyl ester	$C_9H_{11}NO_4$
11	30.644	1700	Heptadecane	$C_{17}H_{36}$
12	*31.038*	*1710*	*Hexadecane, 2,6,11,15-tetramethyl-*	$C_{20}H_{42}$
13	*32.440*	*1742*	*2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-*	$C_{13}H_{18}O_3$
14	32.801	1751	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$
15	34.479	1790	Pentadecyl trifluoroacetate	$C_{17}H_{31}F_3O_2$
16	34.925	1800	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$
17	35.476	1813	Nonadecane	$C_{19}H_{40}$
18	35.872	1822	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
19	41.601	1949	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
20	43.458	1991	1-Nonadecene	$C_{19}H_{38}$
21	59.608	2362	2-Methyloctadecan-7,8-diol	$C_{19}H_{40}O_2$
22	73.760	2726	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$
23	92.239	3268	Isophthalic acid, allyl pentadecyl ester	$C_{26}H_{40}O_4$
24	*93.235*	*3296*	*1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ester*	$C_{18}H_{26}O_5$
25	*94.006*	*3317*	*Phthalic acid, propyl octadecyl ester*	$C_{29}H_{48}O_4$

Table 5: List of the hydrophilic volatile component identified from the flower of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Molecular
1	*9.359*	*1070*	*2,2-Dimethyl-3-vinyl-bicyclo[2.2.1]heptane*	$C_{11}H_{18}$
2	*9.987*	*1091*	*Cyclohex-3-enecarboxaldehyde, 2,4,6-trimethyl-, oxime*	$C_{10}H_{17}NO$
3	*12.197*	*1156*	*Phenol, 3-ethyl-*	$C_8H_{10}O$
4	12.649	1170	Benzoic acid	$C_7H_6O_2$
5	*12.797*	*1174*	*Glucosamine, N-acetyl-N-benzoyl-*	$C_{15}H_{19}NO_7$
6	*13.333*	*1190*	*Benzothiazole*	C_7H_5NS
7	*15.613*	*1256*	*Phenol, 2,3,5-trimethyl-*	$C_9H_{12}O$
8	*16.214*	*1273*	*5H-Inden-5-one, 1,2,3,6,7,7a-hexahydro-*	$C_9H_{12}O$
9	*16.640*	*1286*	*Hydroquinone*	$C_6H_6O_2$
10	*17.145*	*1300*	*Cyclohexanol, 1-methyl-4-(1-methylethylidene)-*	$C_{10}H_{18}O$
11	*17.280*	*1304*	*Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)-*	$C_{10}H_{18}O$
12	*17.772*	*1319*	*2,7-Octadiene-1,6-diol, 2,6-dimethyl-*	$C_{10}H_{18}O_2$
13	18.160	1330	trans-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}O$
14	*18.430*	*1338*	*(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene*	$C_{10}H_{18}O_2$
15	19.298	1364	4-Hydroxy-2-methoxybenaldehyde	$C_8H_8O_3$
16	19.508	1370	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	$C_9H_{12}O_2$
17	*21.040*	*1417*	*Phenol, 2-pentyl-*	$C_{11}H_{16}O$
18	*21.311*	*1425*	*2-Propen-1-ol, 2-methyl-3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (E)-*	$C_{13}H_{22}O$
19	*21.602*	*1434*	*3-(2-Hydroxy-cyclopentylidene)-2-methyl-propionic acid*	$C_9H_{14}O_3$

20	21.838	1442	*5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dimethyl-α-methylene-2-oxo-, methyl ester*	$C_{16}H_{20}O_4$
21	23.259	1486	*8-Methylenecyclooctene-3,4-diol*	$C_9H_{14}O_2$
22	23.514	1494	*1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone*	$C_{13}H_{18}O_2$
23	24.011	1509	*1-Acetamido-1,2-dihydro-2-oxopyridine*	$C_7H_8N_2O_2$
24	24.675	1531	*cis-Z-.alpha.-Bisabolene epoxide*	$C_{15}H_{24}O$
25	24.767	1534	*Cyclopentan-1-al, 4-isopropylidene-2-methyl-*	$C_{10}H_{16}O$
26	25.085	1544	*Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-*	$C_{13}H_{18}O_3$
27	25.514	1558	Dodecanoic acid	$C_{12}H_{24}O_2$
28	25.685	1563	*Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-*	$C_{12}H_{19}NO_3$
29	25.899	1570	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl-	$C_{13}H_{22}O_2$
30	26.127	1578	*Ledol*	$C_{15}H_{26}O$
31	26.498	1590	1-Hexadecanol	$C_{16}H_{34}O$
32	26.840	1600	Hexadecane	$C_{16}H_{34}$
33	27.155	1609	*Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta.,17.beta.)-*	$C_{22}H_{32}O_2$
34	27.486	1617	*Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-*	$C_{10}H_{18}O_2$
35	28.099	1634	3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo-, ethyl ester	$C_9H_{11}NO_4$
36	28.615	1647	*Bromoacetic acid, dodecyl ester*	$C_{14}H_{27}BrO_2$
37	28.684	1649	*Chloroacetic acid, 4-tetradecyl ester*	$C_{16}H_{31}ClO_2$
38	29.178	1662	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$
39	29.777	1678	*2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin*	$C_{13}H_{22}O_3$
40	29.951	1682	*1-Cyclopropene-1-pentanol, .alpha.,.epsilon.,.epsilon.,2-tetramethyl-3-(1-methylethenyl)-*	$C_{15}H_{26}O$
41	30.651	1701	2-Bromotetradecane	$C_{14}H_{29}Br$
42	31.045	1710	*Tetradecane, 1-chloro-*	$C_{14}H_{29}Cl$
43	31.355	1717	*5.beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol*	$C_{15}H_{26}O$
44	31.582	1722	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$
45	31.771	1727	*Pentane-2,4-dione, 3-(1-adamantyl)-*	$C_{15}H_{22}O_2$
46	32.092	1734	Butanol, 1-[2,2,3,3-tetramethyl-1-(3-methyl-1-penynyl)-cyclopropyl]-	$C_{17}H_{30}O$
47	32.468	1743	*Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-*	$C_{11}H_{18}N_2O_2$
48	32.769	1750	Tetradecanoic acid	$C_{14}H_{28}O_2$
49	33.645	1771	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$
50	34.485	1790	Pentadecyl trifluoroacetate	$C_{17}H_{31}F_3O_2$
51	34.932	1801	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$
52	35.479	1813	*1-Octanol, 2-butyl-*	$C_{12}H_{26}O$
53	35.873	1822	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
54	36.928	1845	*5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1',2'-d]pyrazine*	$C_{14}H_{22}N_2O_2$
55	37.910	1867	*2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-*	$C_{20}H_{40}$
56	39.389	1900	Nonadecane	$C_{19}H_{40}$
57	41.675	1951	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
58	43.480	1991	1-Nonadecene	$C_{19}H_{38}$
59	46.996	2069	*3-Chloropropionic acid, heptadecyl ester*	$C_{20}H_{39}ClO_2$
60	48.984	2114	*9,12-Octadecadienoic acid (Z,Z)-*	$C_{18}H_{32}O_2$
61	49.228	2120	9-Octadecenal, (Z)-	$C_{18}H_{34}O$
62	50.660	2152	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
63	52.394	2192	*9-Tricosene, (Z)-*	$C_{23}H_{46}$
64	73.770	2727	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$

Note: The bold and italic fonts are used to refer to exclusive compounds. RT: Retention time. RI: Reflex index.

Table 6: List of the hydrophilic volatile component identified from the fruit of the *Ilex cornuta Lindl. & Paxton.*

No	RT	RI	Compound	Molecular
1	*9.133*	*1063*	*Mequinol*	$C_7H_8O_2$
2	*9.303*	*1069*	*Phenol, 4-methyl-*	C_7H_8O
3	*9.430*	*1073*	*Hexane, 3-bromo-*	$C_6H_{13}Br$
4	*9.923*	*1089*	*Phenylethyl Alcohol*	$C_8H_{10}O$
5	*10.510*	*1107*	*4-Acetylbutyric acid*	$C_6H_{10}O_3$
6	12.643	1169	Benzoic acid	$C_7H_6O_2$
7	13.559	1196	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$
8	*15.378*	*1249*	*1,5-Cyclooctadien-4-one*	$C_8H_{10}O$
9	*17.652*	*1315*	*Phenol, 2,6-dimethoxy-*	$C_8H_{10}O_3$
10	19.180	1361	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$
11	*21.852*	*1442*	*2-Ethoxyphenylacetonitrile*	$C_{10}H_{11}NO$
12	*22.103*	*1450*	*Benzeneacetonitrile, 4-hydroxy-*	C_8H_7NO
13	*22.466*	*1461*	*Coumarin, 8-methyl-*	$C_{10}H_8O_2$
14	*25.187*	*1547*	*1,4-Benzenediol, 2-(1,1-dimethylethyl)-*	$C_{10}H_{14}O_2$
15	25.508	1558	Dodecanoic acid	$C_{12}H_{24}O_2$
16	*25.876*	*1569*	*3,5-Octadienoic acid, 7-hydroxy-2-methyl-, [R*,R*-(E,E)]-*	$C_9H_{14}O_3$
17	25.938	1571	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	$C_9H_{12}O_2$
18	*26.125*	*1577*	*1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one*	$C_{13}H_{20}O_2$
19	*26.492*	*1589*	*4-Chloro-3-n-hexyltetrahydropyran*	$C_{11}H_{21}ClO$
20	27.323	1613	Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]-	$C_{14}H_{24}O_2$
21	30.643	1700	Heptadecane	$C_{17}H_{36}$
22	32.760	1750	Tetradecanoic acid	$C_{14}H_{28}O_2$
23	35.878	1822	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
24	41.694	1952	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
25	*48.863*	*2111*	*9,12-Octadecadienoic acid, methyl ester*	$C_{19}H_{34}O_2$
26	49.244	2120	9-Octadecenal, (Z)-	$C_{18}H_{34}O$

Note: The bold and italic fonts are used to refer to exclusive compounds. RT: Retention time. RI: Reflex index.

Table 7: The number and percentage of the common and exclusive hydrophilic volatile compounds identified from the six organs.

Organ	Root	Stem	Stem Skin	Leaf	Flower	Fruit
Total Compounds	36	46	42	25	64	26
Common Compounds	3					
Percentage of Common Compounds	8.3%	6.5%	7.1%	12.0%	4.7%	11.5%
Exclusive Compounds	17	21	21	9	40	15
Percentage of Exclusive Compounds	47.2%	45.7%	50.0%	36.0%	62.5%	57.7%

practice of the traditional herbal medicine to treat the diseases using either the whole plant or part of the plants depending on which part contains the substances that can be used for therapeutic purposes.

Table 8 presents the number of overlapping compounds, overlapping percentage and overlapping index. The stem and stem skin share the largest number (15) of overlapping compounds. The overlapping percentage is calculated to be 32.6% for the stem and 35.7% for the stem skin. The smallest number (5) of overlapping compounds are found between root and fruit, leaf and fruit. The percentage of overlapping compounds between each of the two organs ranges from 10.9% to 44.0%, which is relatively small, further demonstrating substantial component differences among the different organs. The overlapping index is used to reveal the similarity among the organs. Two organs share the same number of overlapping compounds, but the overlapping index could be different if the total number of the hydrophilic volatile compounds differs. The more total number of the hydrophilic volatile compounds, the less the percentage of the overlapping compounds and smaller the overlapping index. That is why the average overlapping indices between the two organs is introduced to normalize the difference. In addition, total average overlapping indices is derived to calculate the mean of the average overlapping indices between each organ and the other five organs. Based on Table 8, the total average overlapping indices for each organ follows the order of stem > stem skin > root > leaf > flower > fruit. The total average overlapping indices for the stem is the greatest at 3.056, indicating the stem share the maximum similarity with the plant. The total average overlapping indices for the fruit was the smallest at 1.090, indicating that the fruit share the minimum similarity with the plant. And there is not much difference in the average overlapping indices between fruit and the other five organs. Except fruit, the average overlapping indices between each of the two organs correlate well to their physical proximity. The root, stem and stem skin are the organs that the plant survive and grow, and their total average overlapping indices are greater than 2.5. The overlapping index differences among these three organs are small, and they share the most in common. As an evergreen plant, the leaf is symbiotically related to the plant

Table 8: The number of overlapping compounds, overlapping percentage and overlapping index.

Organ 1	Organ 2	Number of overlapping compounds	Overlapping percentage	Overlapping index for Organ 1	Overlapping index for Organ 2	Average overlapping indices between organ 1 and 2	Total average overlapping Indices
Root	Stem	14	38.9%	5.444	4.261	4.853	2.522
	Stem skin	11	30.6%	3.361	2.881	3.121	
	Leaf	9	25.0%	2.250	3.240	2.745	
	Flower	7	19.4%	1.361	0.766	1.064	
	Fruit	5	13.9%	0.694	0.962	0.828	
Stem	Root	14	30.4%	4.261	5.444	4.853	3.056
	Stem skin	15	32.6%	4.891	5.357	5.124	
	Leaf	9	19.6%	1.761	3.240	2.501	
	Flower	10	21.7%	2.174	1.266	1.720	
	Fruit	6	13.0%	0.783	1.385	1.084	
Stem skin	Root	11	26.2%	2.881	3.361	3.121	2.710
	Stem	15	35.7%	5.357	4.891	5.124	
	Leaf	9	21.4%	1.929	3.240	2.585	
	Flower	10	21.4%	1.929	1.266	1.598	
	Fruit	6	14.3%	0.857	1.385	1.121	
Leaf	Root	9	36.0%	3.240	2.250	2.745	2.435
	Stem	9	36.0%	3.240	1.761	2.501	
	Stem skin	9	36.0%	3.240	1.929	2.585	
	Flower	11	44.0%	4.840	1.891	3.366	
	Fruit	5	20.0%	1.000	0.962	0.981	
Flower	Root	7	10.9%	0.766	1.361	1.064	1.844
	Stem	10	15.6%	1.563	2.174	1.859	
	Stem skin	10	14.1%	1.266	1.929	1.598	
	Leaf	11	17.2%	1.891	4.840	3.366	
	Fruit	7	10.9%	0.766	1.885	1.326	
Fruit	Root	5	19.2%	0.962	0.694	0.828	1.090
	Stem	6	23.1%	1.385	1.000	1.193	
	Stem skin	6	23.1%	1.385	0.857	1.121	
	Leaf	5	19.2%	0.962	1.000	0.981	
	Flower	7	26.9%	1.885	0.766	1.326	

although the relationship between each leaf and the plant is cyclical, so the leaf is secondarily related to the plant. The flower and fruit are also cyclically related to the plant and have the most distant relationship. The leaf, flower and fruit are necessary but not survival organs for the growth of the plant. The relationship between the organs and the plant generated from the analysis of the hydrophilic volatile compounds is consistent with their biological function.

Conclusion

The root, stem, stem skin, leaf, flower and fruit of the *Ilex cornuta* Lindl. & Paxton contain hydrophilic volatile compounds that are evenly distributed in the water phase of the various organs of the plant and can volatilize with water vapor. The number and type of hydrophilic volatile compounds vary from organ to organ. There is only a small number of common compounds among the six organs and the number of overlapping compounds between each of the two organs is also relatively small. In addition, there are large number of exclusive compounds from each organ. Therefore, it is possible to identify the plant through the assessment of the hydrophilic volatile compounds isolated from each individual organ.

In conclusion, we found that hydrophilic volatile metabolites are a class of natural products that are rarely investigated but constitute a significant part of the plant chemical composition. Chemical profiling of these secondary metabolites could provide a valuable tool for identification and authentication of the plant samples, as well as resolving taxonomic problems and understanding the chemically mediated biological phenomena.

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