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# Volatile constituents of *Distichochlamys citrea* M. F. Newman and *Distichochlamys orlowii* K. Larsen & M. F. Newman (Zingiberaceae) from Vietnam

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**The characterization of chemical constituents of hydrodistilled essential oils from the rhizomes of *Distichochlamys citrea* M.F. Newman and *Distichochlamys orlowii* Larsen & M.F. Newman collected from Pù Mát National Park, Nghệ An Province, Vietnam, was performed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The main constituents of *D. citrea* oil were 1,8-cineole (23.0%), (*E*)-citral (18.9%) and (*Z*)-citral (15.0%). On the other hand, geranyl acetate (16.5%), β-elemene (9.2%), β-pinene (9.0%) and β-caryophyllene (7.9%) were the principal components of *D. orlowii*. The present paper is the first of its kind aimed at the characterization of the volatile compounds of *D. orlowii*.**

**Key words:** *Distichochlamys citrea, Distichochlamys orlowii,* essential oil composition, monoterpenes, sesquiterpenes.

## INTRODUCTION

The aim of the present study was to report the chemical compounds identified in the essential oil obtained from the rhizomes of *Distichochlamys citrea* M.F. Newman and *Distichochlamys orlowii* Larsen & M.F. Newman collected from Pù Mát National Park, Nghệ An Province, Vietnam. This is in continuation of an extensive research aimed at the characterization of the volatile compounds of poorly studied Vietnamese flora (Chau et al., 2015; Huong et al., 2016, 2017). *Distichochlamys* is a genus of plants in the ginger family. It has 4 known species, all endemic to Vietnam (Newman, 1995). The four species are: *D. benenica* Q.B. Nguyen & Skornick, *D. citrea* M.F. Newman, *D. orlowii* K. Larsen & M.F.Newman and *D. rubrostriata* W.J. Kress & Rehse (Newman, 1995; Rehse and Krees, 2003). *D. citrea* was discovered in Bach Ma National Park in Thua Thien Hue province earlier than the

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other species growing in Vietnam (Ty et al., 2015). This species has a distinct aroma and has been employed in traditional medicine in Vietnam as drugs and spices in foods to ameliorate internal disorders and inflammation related diseases (Ty et al., 2015). *Distichochlamys* species are distinguished from each other on the basis of leaf, inflorescence bract, lateral staminode and labellum characters (Rehse and Krees, 2003). They are small herbs forming dense tufts of few-leaved shoots. The inflorescence is terminal arising in the center of the radical leaves. The bracts are distichous, each subtending a few-flowered (Newman, 1995; Larsen and Newman, 2001; Rehse and Krees, 2003). The flowers are white and yellow. In *D. citrea,* the inflorescence bracts are spread and loosely imbricate while the labellum are divided with cleft extending less than half its length. However, in *D. orlowii,* the inflorescence bracts are densely imbricate while the labellum is yellow with purple veins, dark yellow medium band with two emarginated lobes (Larsen and Newman, 2001).

Till moment, scanty information are available in the literature on the chemical constituents of the volatile and non-volatile extracts and biological activity of *Distichochlamys* plants. The authors are aware of one reference describing the essential oil contents of *D. citrea* (Ty et al., 2015). High contents of 1,8-cineole (30.71% - 43.67%), β-citral (1.6% - 13.98%), α-citral (2.47% - 20.88%) and neryl acetate (4.14% - 11.11%) were present in the oils. Also, only one report in the literature describing the volatile and non-volatile compounds identified from rhizome of *D. rubrostriata* (Tuyet, 2012). The phytochemical investigation of the rhizomes of *D. rubrostriata* resulted in the isolation of 3,5-dihydroxy-4',7-dimethoxyflavon, sitosterol palmitate, 3',5-dihydroxy-4',7-dimethoxyflavonol-3-rutinoside and β-sitosterol (Tuyet, 2012). On the other hand, 1,8-cineole (13.20- 22.00%), (*Z*)-citral (14.15-22:26%), (*E*)-geraniol (12.47-12.75%), (*E*)-citral (18.49-22.13%) and geranyl acetate (6.61-14.92%) were the main constituents of the essential oil (Tuyet, 2012).

## MATERIALS AND METHODS

### Plant materials

Rhizomes of *D. citrea* and *D. orlowii* were collected from Pù Mát National Park, Nghệ An Province in August 2014. Botanical identification was performed by Dr. Dai DN and voucher specimens LTH 26 and LTH 441 respectively were deposited at the Botany Museum, Vinh University. Vietnam. Plant samples were air-dried for a week under room temperature prior to extraction.

### Hydrodistillation of the essential oils

About 500 g each of air-dried and pulverized rhizomes (using grinding mill) of each plant were subjected separately to hydrodistillation in an all glass Clevenger apparatus for 4 h at normal pressure, according to an established procedure (Vietnamese Pharmacopoeia, 1997). Briefly, 500 g of the pulverized sample were carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out in an all glass Clevenger-type distillation unit designed according to the specification. The volatile oils distilled over water and were collected in the receiver arm of the apparatus into a separate clean and previously weighed sample bottles. The processes were done in triplicate. The oil was kept under refrigeration (4°C) until the moment of analysis.

### Gas chromatography (GC) analysis of the oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with flame ionization detector (FID) and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm). Temperature parameters: column oven- 40°C, injection port-250°C, detector-260°C. Time programming: 40°C for 2 min, temperature raised to 220°C (10 min hold) at 4°C/min. Carrier gas used was $H_2$ (1 mL/min), split ratio 10:1, volume injected: 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. Retention indices (RI) value of each component was determined relative to the retention times of a homologous *n*-alkane series ($C_4$-$C_{32}$) with linear interpolation on the HP-5MS column. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

### Gas chromatography-mass spectrometry (GC-MS) analysis of the oils

GC/MS was performed on HP 5973 MSD mass spectrometer with HP 6890N Plus GC system fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm). The conditions were the same as described above for GC with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu.

### Identification of the constituents

Peaks were identified by comparison of relative GC retention indices with standards from literature, retention indices on HP-5 MS column, peak enrichment on co-injection with authentic standard wherever possible and comparison of mass spectra with literature data (National Institute of Science and Technology, NIST, 2001).

## RESULTS

The yield of the essential oils were 0.25% (v/w, *D. citrea*), and 0.35% (v/w, *D. orlowii*), calculated on a dry weight basis. Oil samples were light yellow in colouration. Table 1 indicates the chemical constituents present in the oil, their percentages as well as retention indices on HP-5MS column. The classes of compounds obtained in *D. citrea* rhizome oil were mainly the oxygenated monoterpenes (79.4%). The monoterpene hydrocarbons (4.4%), sesquiterpene hydrocarbons (2.3%) and oxygenated sesquiterpenes (5.8%) occurred in much lower amounts. The main constituents of *D. citrea* oil were 1,8-cineole (23.0%), (*E*)-citral (18.9%) and (*Z*)-citral (15.0%). There are significant amounts of geraniol (9.3%), α-cedrol

**Table 1.** Volatile compounds of *D. citrea* and *D. Orlowii.*

| Compounds [a] | Class | RI (Cal.) | RI (Lit.) | Percentage composition (%) | |
|---|---|---|---|---|---|
| | | | | *D. citrea*[b] | *D. orlowii* [b] |
| α-Thujene | mh | 920 | 921 | 0.2 | 0.1 |
| Tricyclene | mh | 926 | 926 | - | 0.1 |
| α-Pinene | mh | 939 | 932 | 1.4 | 2.2 |
| Camphene | mh | 953 | 946 | 1.1 | 2.8 |
| β-Pinene | mh | 970 | 976 | 1.9 | 9.0 |
| 6-Methyl-5-hepten-2-one [c] | nt | 988 | 987 | 1.9 | - |
| β-Myrcene | mh | 990 | 988 | - | 0.8 |
| α-Phellandrene | mh | 1006 | 1004 | - | 0.1 |
| δ-3-Carene | mh | 1011 | 1008 | - | 0.2 |
| α-Terpinene | mh | 1017 | 1014 | 0.1 | 0.1 |
| *o*-Cymene [c] | mh | 1024 | 1021 | - | 0.2 |
| Limonene | mh | 1032 | 1030 | - | 3.1 |
| 1,8-Cineole | mo | 1034 | 1032 | 23.0 | - |
| (*Z*)-β-Ocimene | mh | 1043 | 1037 | - | 0.1 |
| (*E*)-β-Ocimene | mh | 1052 | 1044 | - | 0.2 |
| γ-Terpinene | mh | 1061 | 1056 | 0.3 | 0.3 |
| α-Terpinolene | mh | 1080 | 1082 | - | 0.3 |
| Isoterpinolene | mh | 1088 | 1088 | - | 2.5 |
| Fenchone | mo | 1089 | 1089 | 0.2 | - |
| Linalool | mo | 1100 | 1095 | 1.2 | 3.1 |
| *trans*-Pinocarveol | mo | 1139 | 1140 | 0.3 | - |
| Camphor | mo | 1145 | 1141 | - | 0.3 |
| *allo*-neo-Ocimene | mh | 1147 | 1147 | - | 0.8 |
| Citronellal | mo | 1153 | 1158 | 0.1 | - |
| Borneol | mo | 1167 | 1167 | 1.8 | 0.3 |
| Terpinen-4-ol | mo | 1177 | 1177 | 4.1 | - |
| α-Terpineol | mo | 1189 | 1187 | 4.6 | 0.1 |
| α-Thujenal [c] | mo | 1189 | 1189 | 0.4 | - |
| Myrtenal | mo | 1209 | 1194 | - | 0.1 |
| Fenchyl acetate | mo | 1228 | 1226 | 0.2 | 0.1 |
| Nerol | mo | 1234 | 1239 | 0.3 | - |
| (*Z*)-Citral (= Neral) [c] | mo | 1251 | 1249 | 15.0 | 4.6 |
| Geraniol | mo | 1253 | 1249 | 9.3 | 0.9 |
| (*E*)-Citral (= Geranial) [c] | mo | 1270 | 1273 | 18.9 | - |
| Bornyl acetate | mo | 1289 | 1287 | - | 2.1 |
| Myrtenyl acetate | mo | 1326 | 1330 | - | 1.0 |
| Bicycloelemene | sh | 1327 | 1337 | 0.1 | - |
| Citronellyl acetate | mo | 1360 | 1357 | - | 0.2 |
| Neryl acetate | mo | 1362 | 1365 | - | 0.1 |
| α-Copaene | sh | 1377 | 1374 | - | 0.2 |
| Geranyl acetate | mo | 1381 | 1378 | - | 16.5 |
| β-Elemene | sh | 1391 | 1387 | - | 9.2 |
| α-Cedrene | sh | 1412 | 1409 | - | 0.2 |
| β-Caryophyllene | sh | 1419 | 1417 | - | 7.9 |
| γ-Elemene | sh | 1437 | 1434 | - | 0.3 |
| Aromadendrene | sh | 1441 | 1439 | 0.4 | - |
| α-Humulene | sh | 1454 | 1452 | - | 4.9 |
| γ-Gurjunene | sh | 1477 | 1479 | 0.2 | 3.4 |
| α-Amorphene | sh | 1485 | 1484 | - | 0,2 |
| β-Selinene | sh | 1486 | 1486 | 0.8 | 0.4 |

**Table 1.** Cont'd.

| | | | | | |
|---|---|---|---|---|---|
| Eudesma-4,11-diene | sh | 1490 | 1494 | 0.2 | - |
| β-Himachalene | sh | 1495 | 1499 | - | 0.9 |
| Bicyclogermacrene | sh | 1500 | 1500 | - | 3.6 |
| β-Bisabolene | sh | 1506 | 1502 | 0.3 | - |
| (*E, E*)-α-Farnesene | sh | 1508 | 1505 | - | 0.4 |
| δ-Cadinene | sh | 1525 | 1522 | - | 1.3 |
| γ-Selinene [c] | sh | 1529 | 1532 | - | 0.5 |
| β-Sesquiphellandrene | sh | 1543 | 1545 | 0.2 | - |
| Elemol | so | 1550 | 1548 | - | 0.2 |
| (*E*)-Nerolidol | so | 1563 | 1561 | - | 0.2 |
| Ledol | so | 1565 | 1561 | - | 0.2 |
| Spathulenol | so | 1578 | 1577 | - | 1.9 |
| Caryophyllene oxide | so | 1583 | 1581 | 0.4 | 2.7 |
| Viridiflorol | so | 1593 | 1591 | 0.1 | 0.8 |
| Guaiol | so | 1601 | 1600 | 0.1 | 0.4 |
| α-Cedrol | so | 1601 | 1602 | 5.2 | - |
| τ-Muurolol [c] | so | 1646 | 1644 | - | 2.9 |
| α-Cadinol | so | 1654 | 1652 | - | 0.5 |
| Lepidozene | so | 1676 | 1676 | 0.1 | - |
| Valerenol | so | 1715 | 1711 | - | 0.3 |
| (*E,E*)- α-Franesol [c] | so | 1718 | 1722 | - | 1.1 |
| Mint sulfide [c] | sh | 1741 | 1743 | - | 0.3 |
| Phytol | dt | 2125 | 2119 | - | 0.3 |
| Total | | | | 93.8 | 98.5 |
| Monoterpene hydrocarbons | | | | 4.4 | 23.9 |
| Oxygenated monoterpenes | | | | 79.4 | 29.4 |
| Sesquiterpene hydrocarbons | | | | 2.3 | 33.7 |
| Oxygenated sesquiterpenes | | | | 5.8 | 11.2 |
| Diterpenes | | | | - | 0.3 |
| Non-terpenes | | | | 1.9 | - |

[a] Elution order on HP-5MS column; (RI Cal.) Retention indices on HP-5MS column; (RI lit.) Literature retention indices; [b] Standard deviation (SD ±) were insignificant and excluded from the Table to avoid congestion; - Not identified; [c] Mode of identification, retention indices, mass spectrum and co-injection; mh, monoterpene hydrocarbons; mo, oxygenated monoterpenes; sh, sesquiterpene hydrocarbons; so, oxygenated sesquiterpenes; dt, diterpenes; nt, non-terpenes

(5.2%) α-terpineol (4.6%) and terpinen-4-ol (4.1%).

However, significant quantity of monoterpene hydrocarbons (23.9%), oxygenated monoterpenes (29.4%), sesquiterpene hydrocarbons (33.7%) and oxygenated sesquiterpenes (11.2%) were identified in the rhizome oil of *D. orlowii*. The oil contained a trace quantity of diterpenes (0.3%). It was observed that geranyl acetate (16.5%), β-elemene (9.2%), β-pinene (9.0%) and β-caryophyllene (7.9%) were the principal components of *D. orlowii*. Other compounds of qualitative importance include α-humulene (4.9%), (*Z*)-citral (4.6%), bicyclogermacrene (3.6%), γ-gurjunene (3.4%), linalool (3.1%) and limonene (3.1%).

## DISCUSSION

Of the total of 77 compounds identified in the oil samples, only seventeen of them are common to both oils. Although terpene compounds predominates in the essential oils, it should be noted that each oil sample has its own compositional different from another. For example, high contents of oxygehated monoterpene were observed in *D. citrea*, whereas *D. orlowii* consist of diversed terpene compounds. A noteworthy observation was that 1,8-cineole, (*E*)-citral and α-cedrol, some principal cmpounds of *D. citrea* were not identified in *D. orlowii*. In addition, the content of geraniol (9.3%) in *D. citrea* is much higher than that of *D. orlowii* (0.9%). Also, several compounds such as geranyl acetate, β-elemene, β-caryophyllene, α-humulene which are present in *D. orlowii* were conspicuously absent in *D. citrea*.

The authors are aware of one literature citation on the essential oil of *D. citrea* (Ty et al., 2015) in which the main compounds were identified to be 1,8-cineole (30.71

- 43.67%), β-citral (1.6 - 13.98%), α-citral (2.47 - 20.88%) and neryl acetate (4.14 - 11.11%). Except neryl acetate, all the other compounds mentioned above were also of identified in significant quantity in the present investigated oil sample. The quantitaive and qualitative compositions of 1,8-cineole, (*Z*)-citral and (*E*)-citral in present and previously studied oil samples, confers similarity between *D. citrea* (Ty et al., 2015) and *D. rubrostriata* (Tuyet, 2012).

The biological activity of an essential oil may be due to the main constituents or a synergy between the main constituents and some minor compounds. Literature information has shown that the chemical compounds identified in the essential oils of the studied *Distichochlamys* species possessed some biological potential. For example, 1,8-cineole was known to exhibited several biological activities such a anti-inflammatory (Juergens, 2014) and allelopathic (Nishida et al., 2005). The antitumor activities of β-elemene (Zhan et al., 2012), β-caryophyllene (Legault and Pichette, 2007) and 1,8-cineole (Juergens et al., 2004) against human cell cancer lines have been reported. Geranly acetate ha possessed antinociceptive (Quintans-Júnior et al., 2013), antifungal and anti-inflammatory (Gonçalves et al., 2012) effects. Essential oil with high contents of citral (mixture of neral and geranial) was found to displayed cytotoxic activity on human tumor cell lines, antioxidant activity and the free radical scavenging capacity (Maggi et al., 2013). Thus, a combination of phytochemicals with reported bioactivity in the essential oils of the studied *D. citrea* and *D. orlowii* growing in Vietnam may contribute to their biological activities.

Although little is known about the volatile components of genus *Distichochlamys*, the chemical constituents of essential oils from several species of other genus in the family Zingiberaceae have been widely reported as new species are being discovered. Recently, the leaf volatile components of a newly discovered species, *Zingiber nitens* M.F. Newan, was found to contained δ-elemene (17.0%), β-pinene (12.8%) and β-elemene (8.8%) while the stem comprised mainly δ-elemene (20.1%), germacrene D (8.6%) and bicyclogermacrene (8.1%) with β-pinene (21.0%), δ-elemene (12.8%) and bornyl acetate (11.8%) making up the root (Hung et al., 2017). *Stahlianthus campanulatus* O. Kuzt (Dai et al., 2017) another newly analysed species in Zingiberaceae has its major constituents as stahlianthusone (27.6%), α-copaene (16.7%) and camphor (14.7%). The essential oil compositions of several other plants in the family were newly described in our laboratory (Chau et al., 2015; Huong et al., 2017). It is well known the chemical compositions of an essential depends on several factors such as intra- and inter-specific variations, age of the plants, climatic and environmental conditions, chemotype, handling and processing conditions etc. These factors may have been responsible for the variations in the chemical constituents of essential oils within the family Zingiberaceae.

The present paper provides new information on the chemical constituents of essential oil of *D. orlowii*. In addition, relative differences were observed between the present and previously investigated oil samples of *D. citrea*. Moreover, it was well established that different species of plant may contained different phytochemicals.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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