Essential Oil from the Fruits of *Fissistigma bracteolatum* and *Fissistigma maclurei*

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Authors NVH, THT and DND collected the plant samples and performed the hydrodistillation of the oil, while authors DND and TDT designed the study and performed the instrumental analysis on the oil samples. Author IAO managed the literature searches, wrote the first and final draft of the manuscript. All authors read and approved the final manuscript.

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**ABSTRACT**

**Aims:** The aim of this study was to isolate essential oils from the fruits of *Fissistigma bracteolatum* and *Fissistigma maclurei* (Annonaceae) and investigate the volatile constituents present they contained.

**Study Design:** The study involves the hydrodistillation of essential oils from the air-dried fruit samples of *F. bracteolatum* and *F. maclurei* and analysis of their chemical compositions by GC and GC-MS.
Place and Duration of Study: Fruits of *F. bracteolatum* and *F. maclurei* were collected from Pù Mát National Park, Nghệ An Province, Vietnam, in May 2014. Analysis of the oil samples was performed between June and August, 2014.

Methodology: About 500 g of air-dried fruit samples was shredded and their oils were obtained by separate hydrodistillation for 4 h at normal pressure, according to the Vietnamese Pharmacopoeia specifications. The chemical constituents of the distilled oils were analyzed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS).

Results: The main constituents in the fruit of *F. bracteolatum* are α-pinene (15.5%) and δ-cadinene (11.0%), with significant amounts of β-caryophyllene (8.0%) and germacrene D (7.0%) while spathulenol (21.2%) and β-cubebene (10.6%) are the quantitatively significant compounds of *F. maclurei* fruit.

Conclusion: The present oil compositions of fruit of *F. bracteolatum* and *F. maclurei* were reported for the first time for these species and the results were found to differ from previous studies on other parts of the plants.

Keywords: *Fissistigma bracteolatum*; *Fissistigma maclurei*; monoterpenes; sesquiterpenes.

1. INTRODUCTION

In recent times, the analyses of chemical constituent of essential oils from Vietnamese plants have received attention [1-3]. This paper therefore present the chemical compounds identified in the fruit essential oils of two *Fissistigma* species. *Fissistigma bracteolatum* Chatt. is a scandent evergreen shrub which grows up to 10m long. The leaves are ovate-oblong in shape. Flowering takes place from March to June while fruiting occurs between August and November. This herb is used for the treatment of contusions and strains [4]. Phytochemical studies have revealed that *F. bracteolatum* contains chalcones and dihydrochalcones with potent inhibitory effects against superoxide anion production [5-8], phenanthrene [7], flavonoids [9] and alkaloids [10]. The essential oil from the leaf was reported to contained *epi*-α-muurolol (17.8%), caryophyllene oxide (13.1%), bicycloelemene (10.5%), while β-bourbonene (13.6%), *(E)*-β-ocimene (12.6%) and *(E,E)*-α-farnesene (11.2%) were the main volatiles of the stem bark [11]. The monoterpene myrcene (83%) occurred as the most abundant compound in another result [12]. However, β-myrcene (17.0%), β-caryophyllene (7.4%), δ-cadinene (6.9%), and bicyclogermacrene (6.2%) in the leaf, as well as β-caryophyllene (20.0%), δ-cadinene (9.9%), α-humulene (6.4%), bicyclogermacrene (6.3%) and linalool (6.2%) in the stem were the principal components of other investigated sample [2].

*Fissistigma maclurei* Merr. (syn. *Meiogyne maclurei* Sinclair) are climbers that grow up to 6 m tall. Little is known about the pharmacological potential and chemistry of this plant. The major compounds of the leaf oil of *F. maclurei* [13] were germacrene D (26.1%), spathulenol (10.0%) and α-terpinene (8.2%).

Till moment there are no reports on the chemical constituents of essential oils from the fruits of the studied plant species. This paper reports for the first time the components of fruit volatile of both *F. bracteolatum* and *F. maclurei* grown in Vietnam.

2. MATERIALS AND METHODS

2.1 Plant Material

Fruits of *F. bracteolatum* and *F. maclurei* were collected from Pù Mát National Park, Nghệ An Province, Vietnam, in May 2014. Botanical identification was carried out by Dr. Dai. Voucher specimens LVH 425, LVH 426 respectively have been deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Hydrodistillation of Essential Oil

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 [14]. The volatile oils distilled over water and were collected separately in the receiver arm of the apparatus into a clean and previously weighed sample bottles. The oils were kept under refrigeration until the moment of analyses.
2.3 Analysis of Essential Oil

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H₂ (1 mL/min), injector temperature (PTV) 250ºC, detector temperature 260ºC, column temperature programmed from 60ºC (2 min hold) to 220ºC (10 min hold) at 4ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST [15] and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values [16].

3. RESULTS AND DISCUSSION

The yield of essential oils were 0.18% (v/w, F. bracteolatum) and 0.20% (v/w, F. maclurei), calculated on a dry weight basis. Oil samples were leaf light yellow in colouration. Table 1 indicates the chemical constituents present in the oils, their percentages as well as retention indices on HP-5 column.

| Compounds          | RI (Cal.) | RI (Lit.) | Percent composition of essential oils of F. bracteolatum and F. maclurei |
|--------------------|-----------|-----------|---------------------------------------------------------------------|
| α-Pinene           | 939       | 932       | F. br 15.5, F. ma 0.7                                               |
| Camphene           | 953       | 946       | -                                                                   |
| β-Pinene           | 980       | 976       | -                                                                   |
| β-Myrcene          | 990       | 988       | F. br 1.4, F. ma 0.1                                               |
| α-Phellandrene     | 1006      | 1004      | -                                                                   |
| p-Cymene           | 1026      | 1024      | -                                                                   |
| Limonene           | 1032      | 1030      | F. br 2.0, F. ma 0.5                                               |
| (E)-β-Ocimene      | 1052      | 1044      | F. br 0.7, F. ma 0.1                                               |
| γ-Terpinene        | 1061      | 1056      | F. br 2.3, F. ma 0.1                                               |
| α-Terpinolene      | 1090      | 1089      | F. br 2.0, F. ma 0.1                                               |
| n-Nonanal          | 1106      | 1100      | -                                                                   |
| β-Citronellol      | 1228      | 1232      | -                                                                   |
| (Z)-Citral         | 1250      | 1249      | -                                                                   |
| Bornyl acetate     | 1289      | 1287      | -                                                                   |
| 2-Undecanone       | 1291      | 1294      | -                                                                   |
| Bicycloelemene     | 1327      | 1337      | -                                                                   |
| α-Cubebeene        | 1351      | 1345      | -                                                                   |
| α-Copaene          | 1377      | 1374      | F. br 1.1, F. ma 1.5                                               |
| β-Bourbonene       | 1385      | 1381      | -                                                                   |
| β-Cubebeene        | 1388      | 1387      | F. br 5.4, F. ma 10.6                                             |
| α-Gurjunene        | 1412      | 1409      | -                                                                   |
| β-Caryophyllene    | 1419      | 1417      | F. br 8.0, F. ma 6.9                                              |
| α-Guaiene          | 1440      | 1433      | F. br 3.7, F. ma -                                                  |
| Aromadendrene      | 1441      | 1439      | F. br 1.2, F. ma 1.0                                               |
Thirty compounds representing 97.5% of the total oil contents were identified in the fruit of *F. bracteolatum*. The main classes of compounds present in the oil were monoterpene hydrocarbons (23.9%), sesquiterpene hydrocarbons (44.1%) and oxygenated sesquiterpenes (28.6%). Oxygen-containing monoterpene compounds are conspicuously absent in the oil. The main constituents of the oil were α-pinene (15.5%), δ-cadinene (11.0%), β-caryophyllene (8.0%), germacrene D (7.0%) and β-cubebene (5.4%). It was well noted that some compounds such as epi-α-muurolol, bicyclemene, β-bourbonene, (E)-β-ocimene, (E,E)-α-farnesene-humulene, bicyclogermacrene and linalool [2,11] that are characteristics of previous reports were not identified in the present oil sample. In addition, the contents of myrcene (1.4 vs.83%) and caryophyllene oxide (4.0 vs.13.1%) are low when compared with previous analysis [12].
Table 2. Main chemical constituents of previously studied *Fissistigma* oil samples

| Plant            | Part     | Main constituents                                                                 | References |
|------------------|----------|-----------------------------------------------------------------------------------|------------|
| *F. chloroneurum*| leaf     | (E)-α-bergamotene (52.0%) and (E)-β-caryophyllene (9.0%)                           | [17]       |
| *F. cupreonitens*| leaf     | (E)-β-caryophyllene (27.7%) and bicyclogermacrene (14.7%)                         | [17]       |
| *F. pallens*     | leaf     | germacrene D (30.2%), bicyclogermacrene (26.4%) and bicycloelemene (18.3%)        | [17]       |
| *F. bicolor*     | leaf     | (E)-β-caryophyllene (13.3%), (E)-α-bergamotene (13.1%), (Z)-β-farnesene (12.3%) and p-cymene (8.9%) | [17]       |
| *F. shangtzeense*| leaf     | (E)-β-caryophyllene (28.1%), bicyclogermacrene (10.3%) and α-muurolene (8.8%)     | [17]       |
| *F. petelotii*   | leaf     | α-pinene (15.4%), bicyclogermacrene (13.9%), bicycloelemene (10.8%) and (E)-β-caryophyllene (9.9%) | [17]       |
| *F. maclurei*    | leaf     | germacrene D (26.1%), spathulenol (10.0%), α-terpinene (8.2%) and bicyclogermacrene (6.6%),     | [13]       |
| *F. rufinerve*   | leaf     | α-santalene (14.3%), beta-caryophyllene (6.3%) and terpinen-4-ol (6.3%)            | [13]       |
| *F. villosum*    | leaf     | bicyclogermacrene (13.0%), bicyclogermacrene (11.5%), spathulenol (11.4%) and germacrene D (10.6%) | [11]       |
| *F. villosum*    | stem     | elema-1,3,11(13)-triene-12-ol (17.0%), germacrene D (10.3%), globulol (8.2%) and heneicosane (8.2%), | [11]       |
| *F. latifolium*  | leaf     | γ-elemene (14.9%), dihydro carveol (14.0%), eugenol (9.2%) and viridiflor (9.1%)     | [11]       |
| *F. latifolium*  | stem     | γ-elemene (19.0%), valerenol (16.9%), eugenol (11.6%) and viridiflor (8.5%)          | [11]       |
| *F. glaucesens*  | leaf     | β-bourbonene (16.4%), eicosane (9.1%) and and β-caryophyllene (6.1%) docosane (5.8%) and heptadecane (5.1%) | [11]       |
| *F. glaucesens*  | stem     | β-caryophyllene (9.7%), α-bisabolol (9.5%), γ-terpinene (7.6%), bicyclogermacrene (5.8%), bicyclogermacrene (5.1%) and longifolene (5.0%). | [11]       |
| *F. scandens*    | leaf     | (E)-β-ocimene (14.8%) and limonene (11.0%)                                        | [1]        |
| *F. scandens*    | stem     | campheronone (13.0%) and (E)-β-ocimene (8.8%)                                     | [1]        |
| *F. poilanei*    | leaf     | β-elemene (33.2%), germacrene D (10.9%), bicyclogermacrene (8.7%) and bicyclogermacrene (8.5%) | [1]        |
| *F. poilanei*    | stem     | β-elemene (36.9%), germacrene D (12.9%) and benzyl benzoate (10.0%)               | [1]        |
| *F. acuminatissimum* | leaf  | β-bisabolene (14.5%), β-elemene (12.2%) and caryophyllene oxide (11.0%)           | [1]        |
| *F. acuminatissimum* | stem  | caryophyllene oxide (15.6%), β-bisabolene (12.1%), and spathulenol (9.8%)          | [1]        |
| *F. thorelli*    | leaf     | γ-terpinene (22.0%), β-phellandrene (7.3%), and bicyclogermacrene (7.2%)          | [12]       |
| *F. oldhamii*    | leaf     | (E)-β-ocimene (10.2%), β-caryophyllene (23.7%) and γ-cadinene (27.2%)             | [12]       |
| *F. polyanthoides* | leaf  | β-phellandrene (14.3%), (E)-β ocimene (43.4%) and α-phellandrene (8.3%)           | [12]       |
| *F. rubigirosa*  | leaf     | (E)-β-ocimene (21.4%) and δ-cadinene (13.3%)                                   | [18]       |

On the other hand, forty-five compounds amounting to 95.8% of the total oil contents were identified in *F. maclurei* fruit oil. The classes of compounds found in the oil were mainly sesquiterpenes with 44.9% of the hydrocarbon contents and 47.2% of the oxygenated
derivatives. Monoterpenes are less common (3.3%). The constituents occurring in higher proportions were spathulenol (21.2%) and β-cubebene (10.6%), along with bicyclogermacrene (7.0%), β-caryophyllene (6.9%) and δ-cadinene (6.9%). A previous report on the leaf oil of F. maclurei [13] identified germacrene D (26.1%), spathulenol (10.0%) and α-terpinene (8.2%). It could be seen that spathulenol content of the fruit oil competes favourably with that of the leaf. However, germacrene D and α-terpinene were not identified in the fruit oil.

There is dirt of literature on the volatile compositions of both fruit oils and as a result the present study may represent the first of its kind. The observed compositional variations between the present oil samples and previous studies may be attributed to the different plant parts being analysed. It is well known that different parts of the same plant accumulate different phytochemicals. For example F. scandens leaf oil comprised mainly of (E)-β-ocimene and limonene while campherenone and (E)-β-ocimene were identified in higher proportions in the stem oil.

Literature information revealed that the chemical constituents of some Vietnamese grown Fissistigma have been studied and reported (Table 2). Although ubiquitous monoterpane and sesquiterpene were the main compounds identified in this study, it is evident that each sample has its own compositional pattern different from each other.

4. CONCLUSION

In the present investigation of chemical constituents of essential oils from the fruits of F. bracteolatum and F. maclurei were found to be different from previous investigated oil samples from other parts of the plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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