CHEMICAL COMPOUNDS, ANTICANCER AND ANTIOXIDANT ACTIVITIES OF VOLATILE OIL FROM *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms. AND *Polygonum odoratum* Lour.

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ABSTRACT

The volatile oils from fresh leaves of *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour. were isolated by hydrodistillation and analysed through a combination of gas chromatography-mass spectrometry (GC-MS). The essential oils were obtained in 0.0124, 0.1384 and 0.0567 % yield, respectively. All volatile oils exist as a yellow liquid. The major constituents of *Piper sarmentosum* Roxb. were β-caryophyllene (42.51%), α-selinene (11.27%), (+)-β-selinene (11.24%), β-elemene (10.85%)and α-humulene (6.99%). The identified constituents of *Polyscias fruticosa* Harms. include α-bergamotene (20.25%), γ-elemene (15.28%), and germacrene d (13.29%). Two peaks with relative peak areas of 25.19% and 14.53%, respectively remained unidentified. The major constituents of *Polygonum odoratum* Lour. were dodecanal (39.54%), decanal (21.14%), β-caryophyllene (10.10%), 1-tetradecanol (8.84%) and euparone (7.36%). In addition, the volatile oils from *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour. showed significant pharmacological effect (ED_{50} << 30 μg/ml) to anticancer activity, while *Piper sarmentosum* Roxb. oil exhibited only weak cytotoxic effect (ED_{50} > 30 μg/ml), from primary screening tests with P388 (mouse lymphocytic leukemia) cells. Moreover, none of the compounds exhibited antioxidant activity (IC_{50} values of >>100 μg/ml) in a DPPH radical scavenging assay.

KEYWORDS : GC-MS, Volatile oil, *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms., *Polygonum odoratum* Lour., Anticancer, Antioxidant

1. INTRODUCTION

Piper sarmentosum Roxb. (Piperaceae, Thai name: Chaplu), of which the leaves are used as food and traditional medicine in Thailand.[1] A study on the antioxidant activity of *Piper sarmentosum* Roxb. extract has been reported, [2] but none on anticancer activity. Another study also identified the chemical constituents of *Piper sarmentosum* Roxb. from an extract obtained through successive extraction with hexane and methanol using a Soxhlet apparatus. [3] *Polyscias fruticosa* Harms. (Araliaceae, Thai name: Lebkrut), of which the leaves are used as food and traditional medicine in Thailand. [4] A study on the leaves and roots of *Polyscias fruticosa* revealed eight new oleanolic acid saponins named polysciosides A to H and three known saponins. [5] From the literature review, there is no study on the antioxidant and anticancer activities of *Polyscias fruticosa* Harms. A *Polygonum odoratum* Lour. (Polygonaceae) has Thai name: Pakpaw. The leaves of pakpaw are used as food and traditional medicine in Thailand. [4] Several studies on the biological activities of plant extracts of family Polygonaceae have been reported. [6-8] However, there is no study on the chemical constituent of the volatile oils extracted from *Polygonum odoratum* Lour. The purpose of this work was to determine the antioxidant as well as anticancer activities of the volatile oils from *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour. leaves together with identifying the volatile components using gas chromatography-mass spectrometry (GC-MS)

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2. MATERIALS AND METHODS

Plant materials

Fresh leaves of *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour. were collected from Tah-guen Village, Sansai District, Chiang Mai, Thailand in September 2005. Fresh leaves were homogenized and hydrodistilled to obtain a volatile pale yellow oils in 0.0124 %, 0.1384 % and 0.0567 % yield respectively, based on the fresh weight of the sample. The sample was stored at low temperature prior to analysis.

Analysis of volatile oils

The isolated oils were analysed by GC-MS. The GC-MS analysis was performed on Agilent 6890 gas chromatography coupled to electron impact (EI, 70 eV) with HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS) supplied by HP, USA (30.0 m × 250 μm, i.d. 0.25 μm film thickness). The analytical conditions were : helium as carrier gas (ca. 1.0 ml/min), injector temperature 260 °C, detector temperature 280 °C, oven temperature 40 °C (3 min hold) then at 10 °C/min to 108 °C, 3 °C/min to 188 °C and then at 4 °C/min to 280 °C (5 min hold). Programmed temperature Kováts retention indeces (RI) were obtained by GC-MS analysis of an aliquot of the volatile oil spiked with an *n*-alkanes mixture containing each homologue from *n*-C_8 to *n*-C_{30}. The GC-MS analysis conditions were the same as above.

Antioxidant Activity

The hydrodistillate was diluted in methanol to give a solution of concentration at 1000 ppm. This sample was further diluted to obtain five concentrations, using two-fold dilutions. Each concentration was tested in triplicate. A portion of sample solution (2 ml) was mixed with double volume of 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in absolute methanol and allowed to stand at room temperature for 30 min. The absorbance (A) was then measured at 514.5 nm (Hitachi U-2001 UV-Spectrophotometer). Vitamin E was tested in the same system as a positive control. The results are expressed as percentage inhibition. The percentage inhibition was calculated from the equation: % inhibition = $[1-(A_{sample}/A_{control})] \times 100$. IC_{50} value (inhibition concentration of sample required to scavenge DPPH radical by 50 %) was obtained by linear regression analysis of the dose response curve plot (% inhibition vs. concentration). [9]

Anticancer Activity

Anticancer primary screening tests were carried out at the National Cancer Institute, Department of Medical Services, Ministry of Public Health, Bangkok, Thailand. Mouse lymphocytic leukemia P388 cells were used in the cytotoxicity test. Cells were cultivated in RPMI-1640 medium supplemented with 10% newborn calf serum and incubateted in a 5% CO_2 incubator. For the experiment, a sample of the hydrodistillate was dissolved in dimethyl sulfoxide and finally diluted in culture medium. The solution (concentration of hydrodistillate 30 ppm) was added to the cell suspension with a cell density of 5×10^4 cells in 5 ml suspension. To determine the cell viability, trypan blue dye exclusion staining was used throughout the experiment. The percentage of cell growth and viability were carried out on day 4 after addition of the test material. Cytotoxic activity is expressed as the median effective dose, ED_{50}.

3. RESULTS AND DISCUSSION

Identification of the oil components were performed by comparision of the mass spectra with literature data (NIST and WILEY) and by a comparision of their retention indices (RI) [10,11] with those of authentic compounds or with those in the literature. [11,12] A typical gas chromatogram of volatile oil from *Piper sarmentosum* Roxb. leaves is presented in Figure 1. Table 1 shows the results of the GC-MS analysis for *Piper sarmentosum* Roxb. leaves. The most prominent component found were β-caryophyllene (42.51%), α-selinene (11.27%), (+)-β-selinene (11.24%), β-elemene (10.85%) and α-humulene (6.99%), with integrator peak areas expressed as a percentage of the total chromatographable components of the volatile oil. The extract also contained a homologous series on *n*-alkanes (C_{14}-C_{22}) present at trace level. A typical gas chromatogram of volatile oil from *Polyscias fruticosa* Harms. leaves is presented in Figure 2. The GC-MS data for *Polyscias fruticosa* Harms. leaves are summarized in Table 2. The major components that were identified include α-bergamotene (20.25%), γ-elemene (15.28%), and germacrene d (13.29%). Two peaks with relative peak areas of 25.19% and 14.53%,

respectively remained unidentified. Similarly, the extract also contained a homologous series on *n*-alkanes (C_{13}-C_{21}) present at trace level. A typical gas chromatogram of volatile oil from *Polygonum odoratum* Lour. leaves is shown in Figure 3. Table 3 presents the GC-MS data of the volatile oil from *Polygonum odoratum* Lour. leaves. The most prominent components found were dodecanal (39.54%), decanal (21.14%), β-caryophyllene (10.10%), 1-Tetradecanol (8.84%) and Euparone (7.36%). A trace amount of the homologous series on *n*-alkanes (C_{12}-C_{22}) was also determined in the extract. The chromatographable components of the volatile oil with integrator peak areas expressed as a percentage of the total peak area. These compounds accounted for approximately 90% of the total volatile oil components. Some components could not be identified from their mass spectra due to their relatively low abundance.

Biological Results

Preliminary testing showed that the volatile oils of *Piper sarmentosum* Roxb. leaves, *Polyscias fruticosa* Harms. leaves and *Polygonum odoratum* Lour. leaves have no significant antioxidant activity (IC_{50} values of >>100 ppm) when assayed for its ability to scavenge the DPPH radical (Table 4). The oil, however, had significant cytotoxic activity on mouse lymphocyte leukemia P388 cells with an ED_{50} << 30 ppm (Table 4)

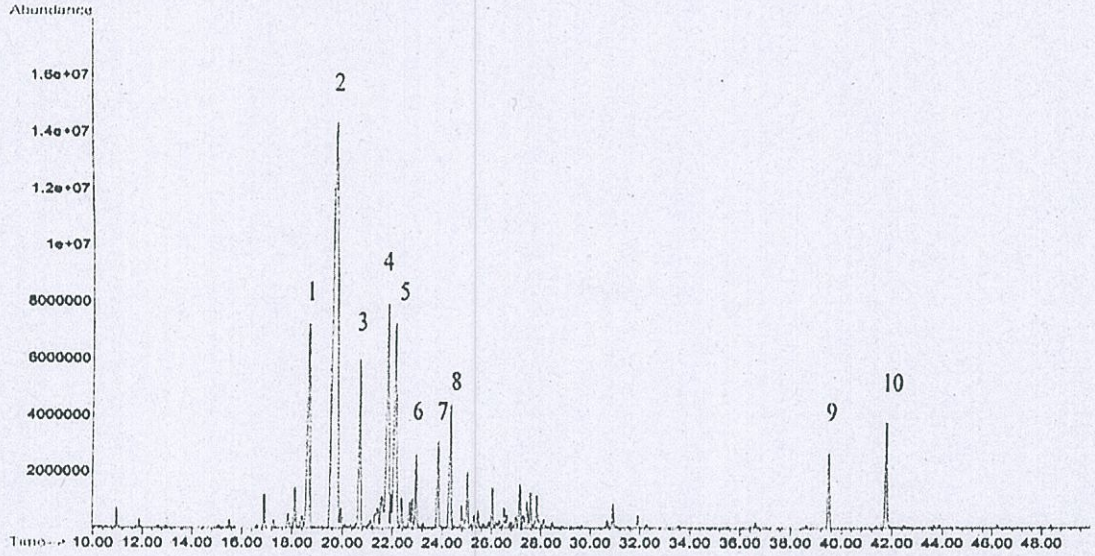

Figure 1. Gas chromatogram of the volatile oil of *Piper sarmentosum* Roxb. leaves.

Table 1. Volatile components in leaves of *Piper sarmentosum* Roxb.

Peak No.	RT	Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]
1	18.68	β-Elemene	10.85	1393	1391	204
2	19.78	β-Caryophyllene	42.51	1428	1418	204
3	20.71	α-Humulene	6.99	1457	1454	204
4	21.84	(+)-β-Selinene	11.24	1491	1485	204
5	22.15	α-Selinene	11.27	1504	1494	204
6	22.97	δ-Cadinene	2.05	1525	1524	204
7	23.87	Elemol	3.09	1552	1549	204
8	24.37	trans-Nerolidol	5.32	1566	1564	204
9	39.55	(E,E)-7,11,15-trimethyl-3-methylene-hexadeca-1,6, 11,14-tetraene	2.67	2028	-	272
10	41.84	Unidentified	4.02	2112	-	-

[a] RA, relative area (peak area relative to total peak area).
[b] RI, programmed temperature retention indices as determined on a HP-5MS column using a homologous series of n-alkanes.
[c] RI values from literature data.
[d] molecular weight from GC-MS (EI) data.

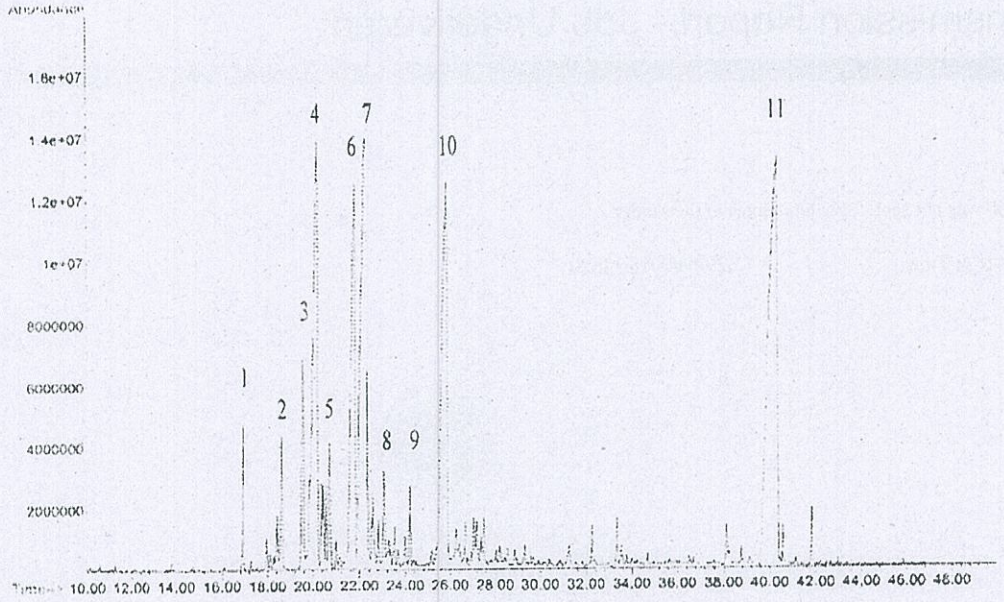

Figure 2. Gas chromatogram of the volatile oil of *Polyscias fruticosa* Harms. leaves.

Table 2. Volatile components in leaves of *Polyscias fruticosa* Harms.

Peak No.	RT	Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]
1	16.92	δ-Elemene	1.37	1341	1339	204
2	18.62	β-Elemene	1.61	1395	1391	204
3	19.54	Bicyclo[4.4.0]dec-1-en,2-isopropyl-5-methyl -9-methylene	3.33	1423	-	204
4	20.12	γ-Elemene	15.28	1440	1433	204
5	20.67	α-Humulene	2.28	1456	1454	204
6	21.70	Germacrene D	13.29	1485	1480	204
7	22.16	α-Bergamotene	20.25	1498	1436	204
8	23.01	δ-Cadinene	1.88	1526	1524	204
9	24.15	Germacrene B	0.98	1562	1556	204
10	25.76	Unidentified	14.53	1610	-	-
11	40.17	Unidentified	25.19	2051	-	-

[a] RA, relative area (peak area relative to total peak area).
[b] RI, programmed temperature retention indices as determined on a HP-5MS column using a homologous series of n-alkanes.
[c] RI values from literature data.
[d] molecular weight from GC-MS (EI) data.

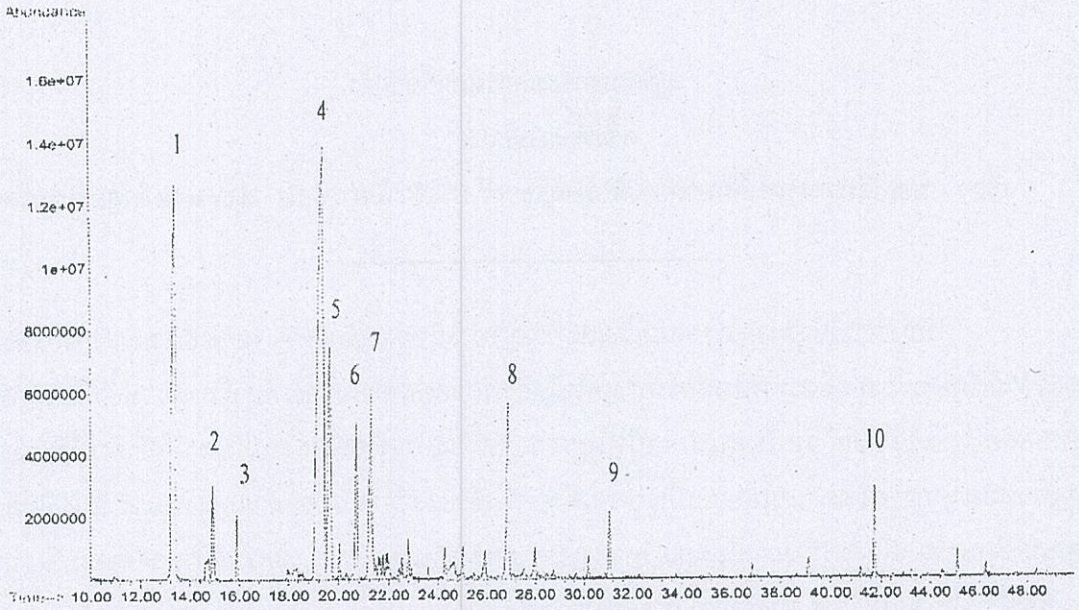

Figure 3. Gas chromatogram of the volatile oil of *Polygonum odoratum* Lour. leaves.

Table 3. Volatile components in leaves of *Polygonum odoratum* Lour.

Peak No.	RT	Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c](Lit.)	MW[d]
1	13.36	n-Decanal	21.14	1212	1204	156
2	14.93	1-Decanol	3.23	1272	1272	158
3	15.91	Undecanal	1.13	1307	1306	170
4	19.31	Dodecanal	39.54	1417	1407	184
5	19.62	β-Caryophyllene	10.10	1427	1418	204
6	20.68	α-Humulene	4.47	1460	1453	204
7	21.25	1-Tetradecanol	8.84	1476	-	204
8	26.86	Euparone	7.36	1640	-	218
9	31.03	Drimenol	1.93	1761	1759	222
10	41.75	Neophytadiene	2.25	2111	1949	278

[a] RA, relative area (peak area relative to total peak area).
[b] RI, programmed temperature retention indices as determined on a HP-5MS column using a homologous series of n-alkanes.
[c] RI values from literature data.
[d] molecular weight from GC-MS (EI) data.

Table 4. Antioxidant and cytotoxic activity of *Piper sarmentosum* Roxb. leaves, *Polyscias fruticosa* Harms. leaves and *Polygonum odoratum* Lour. leaves volatile oils.

volatile oils sample	Antioxidant (IC_{50} ppm)		Cytotoxicity (ED_{30} ppm)	
Piper sarmentosum Roxb.	>>100	Inactive	>30	Slightly active
Polyscias fruticosa Harms.	>>100	Inactive	<<30	Very active
Polygonum odoratum Lour.	>>100	Inactive	<<30	Very active

4. CONCLUSIONS

The components of the volatile oil from fresh leaves of *Piper sarmentosum* Roxb., *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour. have been analyzed by GC-MS. The three volatile oils contained terpenoids compounds as the major components which some of them can be clearly identified, however some of them can not be done. The volatile oil showed no significant antioxidant activity but showed interestingly significant cytotoxicity on P388 leukemia cells.

5. ACKNOWLEDGEMENTS

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