

CHAPTER IV

CONCLUSION

In our course of research, the chemical constituents from the roots of *Cladogynos orientalis* Zipp. ex Span. have been investigated these plant in family Euphorbiaece. The roots of *C. orientalis* were extracted with organic solvents. Hexane, dichloromethane and ethyl acetate crude extracts showed high activity against brine shrimp, so these three crude extracts were separated by chromatography techniques to obtain two mixtures and eight compounds as follows.

1. Mixture **1**, a mixture of long chain aliphatic ester
2. Compound **2**, β -sitosterol
3. Mixture **3**, a mixture of steroids (stigmasterol and β -sitosterol)
4. Compound **4**, chettaphanin II
5. Compound **5**, chettaphanin III
6. Compound **6**, cyperenoic acid
7. Compound **7**, (-)-spathulenol
8. Compound **8**, scopoletin
9. Compound **9**, chettaphanin I
10. Compound **10**, taraxerol

From the literature surveys^(4,5) chettaphanin I and II were a novel furanoditerpenes which were separated from *C. orientalis* in 1970 and 1971 respectively. They have not been reported about biological activity of their isolated compounds. So, this study also provided additional spectroscopic information (1D-NMR, 2D-NMR, MS and IR) of chettaphanin II, which was not previously reported. A computerized literature search (STN International) indicated that this was the first report of compound **5**, chettaphanin III. However, this plant was reported to contain 9 known substances as shown in Table 26 and the biological activity of some pure compounds also shown in Table 27.

Table 26 All isolated substances from the roots of *C. orientalis*

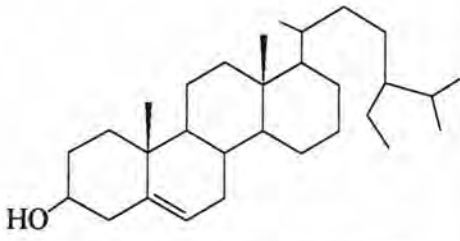
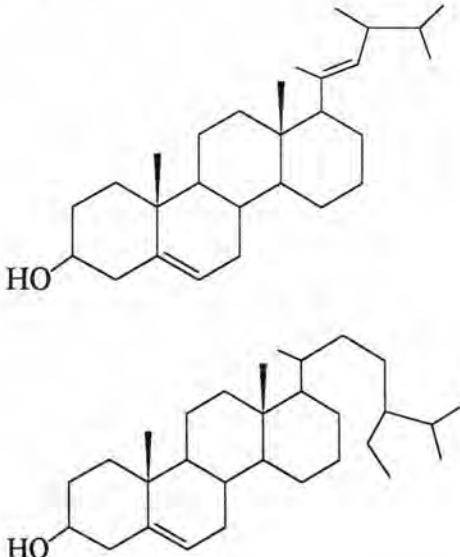
Isolated substances	Weight (mg)	% wt by wt of the roots
1. A mixture of long chain aliphatic esters (Mixture 1)	7	0.07×10^{-3}
2. β -sitosterol (Compound 2) 	17	0.17×10^{-3}
3. A mixture of steroid (stigmasterol and β -sitosterol) (Mixture 3) 	23	0.23×10^{-3}

Table 26 (cont.)

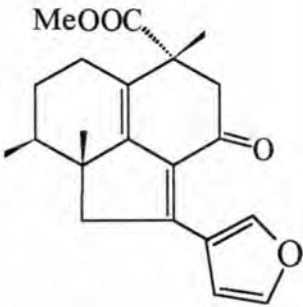
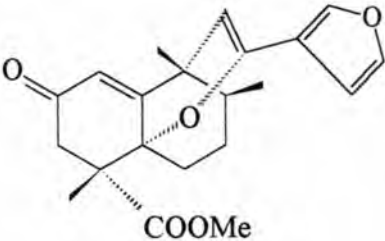
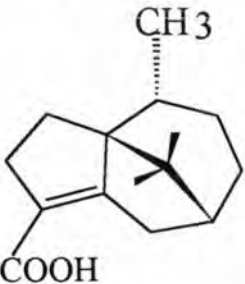
Isolated substances	Weight (mg)	% wt by wt of the roots
<p>4. Chettaphanin II (Compound 4)</p> 	27.9	0.28×10^{-3}
<p>5. Chettaphanin III (Compound 5)</p> 	40.2	$0.40 \times 10^{-3}\%$
<p>6. Cyperenoic acid (Compound 6)</p> 	75.2	$0.75 \times 10^{-3}\%$

Table 26 (cont.)

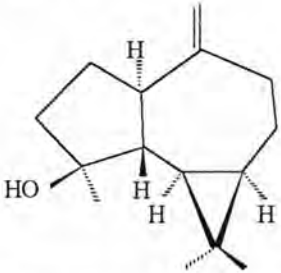
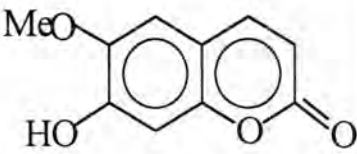
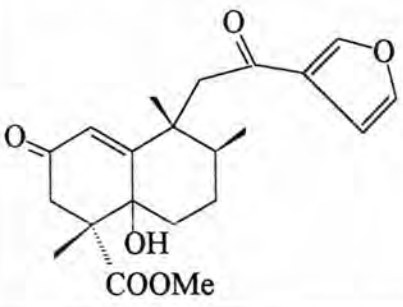
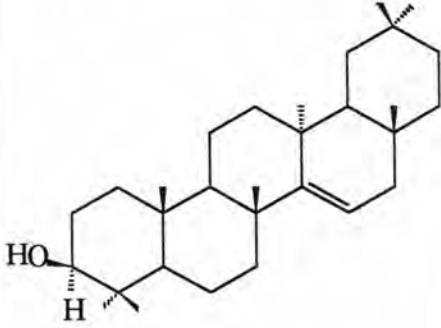
Isolated substances	Weight (mg)	% wt by wt of the roots
<p>7. (-)-Spathulenol (Compound 7)</p> 	194.6	$1.95 \times 10^{-3}\%$
<p>8. Scopoletin (Compound 8)</p> 	7.1	$0.07 \times 10^{-3}\%$
<p>9. Chettaphanin I (Compound 9)</p> 	2400	0.02%
<p>10. Taraxerol (Compound 10)</p> 	52	$0.52 \times 10^{-3}\%$

Table 27 The biological activity of isolated compounds from the roots of *C. orientalis*

Compound \ Activity	6	7	9	10
Brine shrimp	32.58	72.64	>1000	>1000
<i>C. cucumerinum</i> ⁽¹⁾	x	n	x	x
<i>C. albicans</i> ⁽¹⁾	√	n	x	x
DPPH ⁽²⁾	x	n	x	x
β-carotene ⁽²⁾	x	n	x	x
<i>A. aegypti</i> ⁽³⁾	x	x	x	x
<i>E. coli</i> ⁽⁴⁾	+	-	-	-
<i>B. cereus</i> ⁽⁴⁾	+	-	-	-
<i>S. aureus</i> ⁽⁴⁾	+	-	-	-
<i>S. derby</i> ⁽⁴⁾	+	+	-	-
K562 ⁽⁵⁾	n	n	+	n

(1) = antifungal

+ = weak inhibited

(2) = antioxidant

- = not inhibited

(3) = Lavicidal

x = no activity observed

(4) = antibacterial

√ = activity observed

(5) = anticell lines

n = no test

Chettaphanin II(4), cyperenoic acid(6), (-)-spathulenol(7) and chettaphanin III(5) and scopoletin(8) showed cytotoxicity to brine shrimp with LC₅₀ 19.95, 32.58, 72.64, 79.54 and 125µg/ml, respectively. In addition, compound 6 also showed significant antibacterial activity against *E. coli*, *B. cereus*, *S. aureus*, *S. derby*, and antifungal activity against *Cladosporium albicans*. Compound 9 was weakly on insect antifeedant (*G. mellonella*).

Proposal for the Future Work

Plants of the family Euphorbiaceae are known to produce a variety of diterpenoids. The naturally occurring diterpenoids always play an important role because of their biological activity as insect antifeedants, antifungal, antitumor and antimicrobial agents^(53,54). More importantly, chettaphanin II and chettaphanin III (new furanoditerpene) isolated from the roots of *C. orientalis* showed cytotoxicity to brine shrimp at LC_{50} 19.95 and 79.54 $\mu\text{g/ml}$. These two compounds provided many possibilities to carry on further investigation for biological activity (anticell lines, insecticide and antimalarial). Chettaphanin II also can be derived from chettaphanin I⁽⁵⁾ which is isolated from *C. classifolius* and *C. orientalis* as a major component. So chettaphanin II and chettaphanin III might be proposed as bioactive compounds in medicine extracted from this plant. Mechanism for the conversion of chettaphanin I to chettaphanin III should be investigated both biologically and chemically.

The investigation from the roots of *C. orientalis* leads to isolate one new furanoditerpene which has activity to brine shrimp. Another aspect that would provide more fulfillment to this research is the chemotaxonomic study on chemical constituents from the other parts of *C. orientalis*.