



CHAPTER IV

CONCLUSION AND SUGGESTION FOR FUTURE WORKS

In this research, the leaves of *Piper betle* Linn. were investigated for their chemical constituents because of their utilization as described in the introduction. The results of the structural elucidation of the compounds from the leaves definitely increase data of the chemical constituents obtained of *Piper betle* Linn.

From research of the leaves of *Piper betle* Linn., eleven chemical constituents were obtained from chloroform extract which were separated by using column chromatography. Those compounds are as follows ::

- Mixture 1,** a mixture of 2,6-dimethyl cyclohexanol and 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone.
- Mixture 2,** a mixture of long chain alcohol (heptadecanol, nonadecanol, heneicosanol, docosanol, tetracosanol, pentacosanol, hexacosanol, triacontanol, hentriacontanol , dotricontanol and tritriacontanol)
- Mixture 3,** a mixture of long chain esters.
- Compound 4,** lupeol
- Mixture 5,** a mixture of long chain alcohol (heneicosanol and triacosanol)
- Compound 6,** a long chain ester (2-Propenyl hexanoate)
- Compound 7,** fridelan-3 β -ol
- Compound 8,** chavibetol
- Mixture 9,** a mixture of three steroids (β -sitosterol, stigmasterol and campesterol)
- Mixture 10,** a mixture of three steroids (β -sitosterol, stigmasterol and campesterol)
- Compound 11,** allylpyrocatechol

All substances isolated from chloroform crude extract of the leaves of *Piper betle* Linn. are summarized in table 58

Table 58 All substances isolated from chloroform crude extract of the leaves of *Piper betle* Linn.

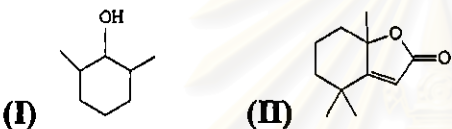
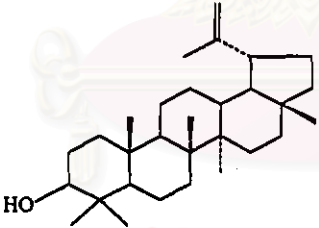
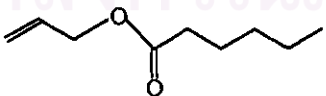
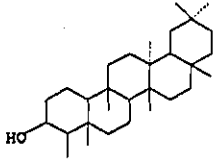
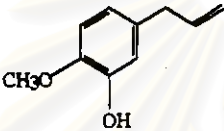
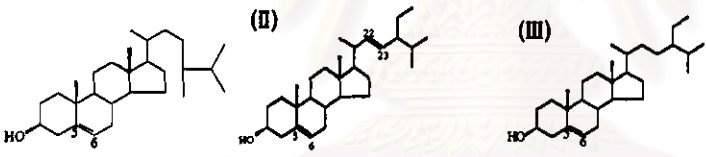
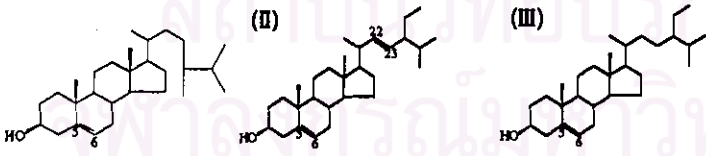
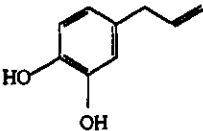
Substance	Weight (mg)	% wt. by wt. of dried leaves
a mixture of 2,6-dimethyl cyclohexanol (I) and 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone (II) (Mixture 1) 	30.0	6.38×10^{-3}
a mixture of long chain aliphatic primary alcohol (Mixture 2)	369.7	7.78×10^{-3}
a mixture of long chain esters (Mixture 3)	60.0	1.28×10^{-3}
Lupeol (Compound 4) 	159.5	3.39×10^{-3}
a mixture of long chain aliphatic primary alcohol (Mixture 5)	4.0	8.5×10^{-5}
2-Propenyl hexanoate (Compound 6) 	61.0	1.30×10^{-3}
Friedelan-3β-ol (Compound 7) 	70.0	1.49×10^{-3}

Table 58 (cont.) All substances isolated from chloroform crude extract of the leaves of *Piper betle* Linn.

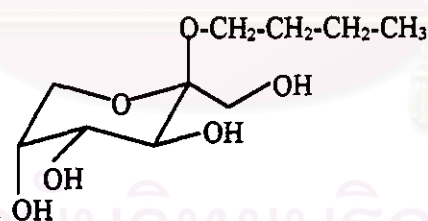
Substance	Weight (mg)	% wt. by wt. of dried leaves
<p>Chavibetol (Compound 8)</p> 	956.2	0.02
<p>A mixture of three steroids (campesterol (I), stigmasterol (II) and β-sitosterol (III))</p>  <p>(Mixture 9)</p>	450.0	9.57×10^{-3}
<p>A mixture of three steroids (campesterol (I), stigmasterol (II) and β-sitosterol (III))</p>  <p>(Mixture 10)</p>	612.0	13.02×10^{-3}
<p>Allylpyrocatechol (Compound 11)</p> 	3.11	0.07

This work is the first report on the occurrence of 2,6-dimethyl cyclohexanol, 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone, lupeol, 2-propenyl hexanoate and fridelan-3 β -ol in this particular species.

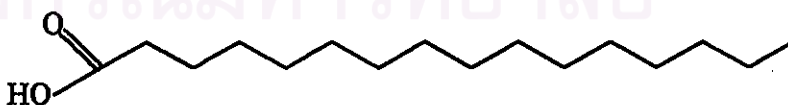
In this research, chemical constituents of the leaves of *Piper betle* Linn. was obtained from *n*-butanol extracts, which were separated by column chromatography. The structures of those substances have been proposed but were not confirmed yet :

- PBBu 1,** 2-O-*n*-butyl- β -fructopyranose (I)
PBBu 2, The major component might be hexadecanoic acid (II)
PBBu 3, A mixture of 3 unidentified compound
PBBu 4, A mixture of 2 unidentified compound
PBBu 5, The major component might be a mixture of diethoxydimethyl silane (III), 2(3H)-benzothiazolone (IV) and unidentified compound
PBBu 6, The major component unidentified

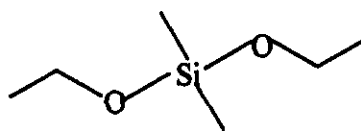
The proposed structure of substance which were found in *n*-butanol extract are shown below.



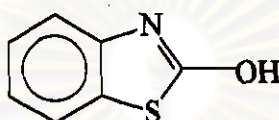
(I) 2-O-*n*-butyl- β -fructopyranose



(II) Hexadecanoic acid



(III) Diethoxydimethyl silane



(IV) 2-(3H)-benzothiazolone

For bioassay results, the chloroform crude extract of the leaves of *Piper betle* Linn. showed inhibitory effects for *Human Leukemia Carcinoma* (HL-60), *Human Hepatocellular Carcinoma* (Bel-7402) and exhibited cytotoxicity activity against brine shrimp (*Artemia salina* Linnaeus) with LC_{50} value of 8.36 $\mu\text{g} / \text{ml}$ (high activity).

The results of brine shrimp (*Artemia salina* Linnaeus.) cytotoxic lethality test of isolated compound.

From the results of brine shrimp cytotoxic lethality test, it was found that chavibetol (**Compound 8**) and allylpyrocatechol (**Compound 11**) revealed much higher toxicity than those of the others.

Suggestion for future works

From this work, the preliminary work on cardiac glycoside testing on *n*-butanol crude extract was performed. The results clearly confirmed the presence of cardiac glycoside and other compounds in this fraction. However, attempt to separate to get pure compounds were not successful. The aid of modern techniques of separation such as MPLC or preparative HPLC may need to be applied for the better resolution of this separation.

However, the preliminary screening test against various cell lines and brine shrimp cytotoxicity test is still needed to be confirmed before being certain of these results.

Compounds related to the isolated chavibetol and allylpyrocatechol should be synthesized and studied for structure activity relationship. The outcome from this examination would lead to the finding of new and environmental friendly biological activity.

Moreover, the chemical constituents and biological activity study of other parts of *Piper betle* Linn. should be investigated. Another aspect that would make this research fulfill is the chemotaxonomy study of *Piper betle* Linn.