

## Original Article

# Major Bioactive Compounds of Pilis Plant Materials: A GC-MS Analysis

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### ABSTRACT

**Background:** As indigeneous herbal medicine (jamu) in Central Java, Indonesia, Pilis is applied topically to the forehead to cure dizziness, hazy vision, and eyestrain. For the future implementation, it is important to know the composition of plant materials used for Pilis and their major bioactive compounds. **Aims of the study:** This study aimed to determine the plant materials used for blending Pilis, and the major bioactive compounds of every plant materials. **Materials and Methods:** The blended plant materials for Pilis from homemade producers were collected and identified. For determination of bioactive compounds of every plant materials, extraction was done with hexane. The obtained crude extracts were analysed by GC-MS. **Results:** Eighteen plants materials of Pilis can be divided in two groups. The first group was the essential plant materials: *Acorus calamus* L., *Alyxia stellata* (J.R. Forst & G.Forst.) Roem & Schult., *Curcuma heyneana* Valetton & Zijp., *Foeniculum vulgare* Mill., *Kaempferia galanga* L., *Melalueca leucadendra* (L.) L., *Rosmarinus officinalis* L., *Syzygium aromaticum* (L.) Merr. & L.M. Perry, *Zingiber montanum* (J.Koenig) Link ex A.Dietr, *Zingiber officinale* Roscoe. The second group was the non-essential materials: *Amomum compactum* Sol. Ex Maton, *Cinnamomum verum* J.Fresl, *Cryptocarya massoy* (Oken) Kosterm, *Curcuma longa* L., *Piper cubeba* L.f., *Pluchea indica* (L.) Less., *Trigonella foenum-graecum* L., and *Usnea filipendula*. Several identified major bioactive compounds were amyrin, asarone, cinnamaldehyde, 1,8-cineole, dimethoxy phenyl butadiene (DMPBD), ethyl p-methoxycinnamate, eugenol, selinene, 4-terpineol, and zingerone. **Conclusion:** At least ten different plant materials are used for Pilis blending which has one or have limited number of dominant bioactive compounds.

**Key words:** Bioactive compound, GC-MS, Jamu, Java, Pilis, Topical herbal medicine.

### INTRODUCTION

Pilis is one of the Javanese unique herbal medicament. It is known as jamu (traditional medicine) for mainly after birth recovery of mother, but also for headache and fever.<sup>1</sup> As herbal medicine, Pilis is made from “ramuan”, a synergetic herbal blending, known through centuries of use. Pilis is known for the treatment of various types of headache and is prepared by blending the plant materials,

flattening and finally sticking on forehead and/or temple. The mixture of plant materials is generally crushed by stone mortar on stone pestle (“lumpang batu”), and then pounded with a stone pounder (“telenan batu”) in order to get fine grounding paste.

Every producer has their own combination and every batch from the producer is also not always the same. Therefore, it is necessary to study the usefulness and to improve the quality of Pilis. So far there is no quality standard available for the production of Pilis. This expresses one of the weakness of traditional herbal therapy. The plant material composition and quality are varied significantly, and therefore the quality of Pilis is not secured. Our previous study showed that Pilis contained several

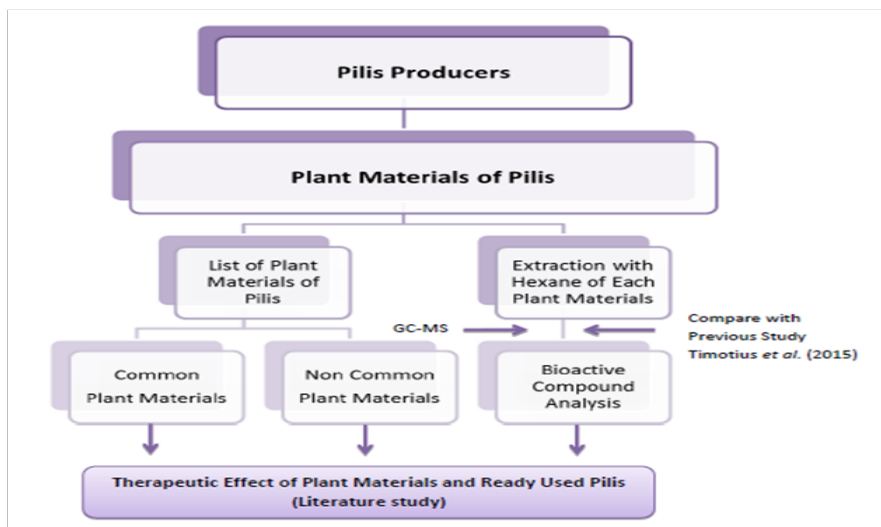
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Graphical Abstract

non polar bioactive compounds.<sup>22</sup> In this study, the plant materials composition of Pilis and the major non polar bioactive compounds are reported.

Skin-topical herbal medicine or transdermal delivery is a non-invasive drug delivery, not as oral and nasal routes, and good for lipophilic drugs sometime with the use of skin penetration enhancers.<sup>3</sup> Since Pilis is delivered as topical medicine in forehead or temple, analysis of its non-polar fraction is basically needed to design a new formula for future application of Pilis. As a blending of various plant materials, it is necessary to identify and to document the composition of plant materials for Pilis production. It is importance to take the benefits from the indigenous wisdom of the practical use of Pilis, and then use them as new knowledge in developing Pilis as alternative medication in modern world.

The objectives of this study were to determine the plant materials blended for Pilis, and their major nonpolar bioactive compounds.

## MATERIALS AND METHODS

### Plant materials

Plant samples for this study were collected in 2014 from ten home-made producers before processing or blending into Pilis. Plant materials were stored at  $-20^{\circ}\text{C}$ . Plant samples were identified by Herbarium Bogoriense in Herbarium Bogoriense, Bogor, Indonesia. The voucher specimens were deposited at the Faculty's herbarium (FK No. P001 till P018).

### Extraction

The extractions were done with hexane as the previous study.<sup>2</sup> Prior to the extraction, the samples were crused and powdered or pounded till powders were obtained. 100 grams powder was extracted with hexane using Soxhlet for 8 hours. The obtained extracts were concentrated under pressure to provide a concentrated extract which was then injected to GC-MS analysis.

### GC MS analysis

Oil extraction and the chemical analysis with GC-MS was done as the previous study.<sup>2</sup> The the GC-MS analysis was done by using Agilent Gas Chromatography Model 6890 coupled to an Agilent 5973 Mass Selective Detector. Analytes were separated on an HP 5MS capillary column (60 meter x 0.25 mm x 0.25  $\mu\text{m}$ ). The following temperature program was pplied. Oven intitial temperature was  $100^{\circ}\text{C}$  (On) and maximum temperature was  $350^{\circ}\text{C}$ . Initial time was 0.00 min equilibrium time was 0.50 min. The initial front inlet (split) waw  $290^{\circ}\text{C}$ . Pressure was 20.90 psi, split ratio was 50:1, split flow was 49.8 ml/ml, total flow was 53 ml/min, and gas type was Helium. Compound identification was done by comparing with Willey 9 N11.L Mass Spectral library database.

## RESULTS

### *The essential plants materials for Pilis*

The plant materials used for Pilis is listed in (Table 1). From 18 recorded plant materials, ten were used by most Pilis home producers and therefore considered as essential plant materials for blending Pilis. The other eight materials were

**Table 1: Pilis plant material**

Scientific Name	Family	Common Name	Local Name	Part Used	Dosage (mg/g.dw)
<b>Essential plant materials</b>					
<i>Acorus calamus</i> L.	Acoraceae	Sweet flag, Calamus	Dlingo	Rhizome	20-180
<i>Alyxia stellata</i> (J.R. Forst. & G. Forst.) Roem. & Schult.	Apocynaceae	Maile	Pulowaras, Pulosari, Palasan	Bark	30-40
<i>Curcuma heyneana</i> Valetton & Zijp	Zingiberaceae	Pale Turmeric	Temu Giring	Rhizome	50-310
<i>Foeniculum vulgare</i> Mill.	Apiaceae	Fennel	Adas	Seed	30-90
<i>Kaempferia galanga</i> L.	Zingiberaceae	Galangal, Aromatic Ginger, Sand Ginger	Kencur	Rhizome	70-340
<i>Melaleuca leucadendra</i> (L.) L.	Myrtaceae	Cajuputy	Merica Bolong	Fruit	20-140
<i>Rosmarinus officinalis</i> L.	Lamiaceae	Rosemary	Rabelam	Leaf	20-250
<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Myrtaceae	Clove	Cengkih	Fruit	1-25
<i>Zingiber montanum</i> (J.Koenig) Link ex A.Dietr	Zingiberaceae	Plai	Bengle	Rhizome	70-220
<i>Zingiber officinale</i> Roscoe.	Zingiberaceae	Ginger	Jahe	Rhizome	100-220
<b>Non essential plant materials</b>					
<i>Amomum compactum</i> Sol. Ex Maton	Zingiberaceae	Round cardamom	Kapulaga	Fruit	90-140
<i>Cinnamomum verum</i> J. Fresl.	Lauraceae	Cinnamon	Manis Jangan	Bark	40-140
<i>Cryptocarya massoy</i> (Oken) Kosterm.	Lauraceae	Massoia	Mesoyi	Bark	20-30
<i>Curcuma longa</i> L.	Zingiberaceae	Turmeric	Kunyit	Rhizome	250-300
<i>Piper cubeba</i> L.f.	Piperaceae	Cubeb	Kemukus	Fruit	20-40
<i>Pluchea indica</i> (L.) Less.	Compositae	-	Beluntas	Leaf	10-20
<i>Trigonella foenum-graecum</i> L.	Leguminosae	Fenugreek	Klabet	Seed	20-60
<i>Usnea filipendula</i>	Usneaceae	Usnea	Kayu angin	Fruiting Body	1-10

not commonly used, and therefore assumed as non essential plant materials, even they could be used as substitution if the essential was not available. None of the home made producers of Pilis weighted the plant materials before blending Pilis. Therefore the dosage was varied (Table 1).

### *The identified bioactive compounds of Pilis plant materials*

Major bioactive compounds of plant materials were identified as listed in Table 2 and 3. It is interesting to note that every Pilis plant materials have one or several dominant non polar compound(s). The eleven major bioactive compounds were amyrrin, asarone, caryophyllene, cinnamaldehyde, 1,8-cineole, dimethoxy phenyl butadiene (DMPBD), ethyl p-methoxycinnamate (EPMC), eugenol, selinene, 4-terpineol and zingerone.

## DISCUSSION

### *The plant materials of Pilis*

Ten plant materials are essential for Pilis. These materials were not monopoly of Pilis, but also often become component of other jamu (herbal medicines) and food spices. From the information available from scientific literatures of Pilis plant materials indicated the biological effects of these materials (Table 4). Most of them were known for their bioactivity in anti-inflammation, sedative or relaxant activities, and biostimulant effect (Table 4). The function of plant materials was overlapping. But there was indication of pairing of the materials, such as *Acorus calamus* and *Zingiber montanum*; and *Foeniculum vulgare* and *Alyxia stellata*, and *Kaempferia galanga* and *Syzygium aromaticum*. In Javanese tradition it was known to classify plant materials as “hot” and “cold”, probably similar with the concept yin and yang in Chinese traditional medicine.

**Table 2: Major bioactive compounds of essential Pilis plant materials**

Scientific Name	Oil Recovery (mg/g.dw)	GC MS Analysis	
		Major Compound	Relative Amount (%)
<i>Acorus calamus</i> L.	25.00	Asarone(Beta)	46.36
<i>Alyxia stellata</i> (J.R. Forst. & G. Forst.) Roem. &Schult.	26.90	Amyrin (Beta) Amyrin acetate (alpha,beta)	74.59
<i>Curcuma heyneana</i> Valetton & Zijp	17.20	n.i.*	-
<i>Foeniculum vulgare</i> Mill.	22.40	1,8-Cineole	50.53
<i>Kaempferia galanga</i> L.	26.00	Ethyl p-methoxycinnamate (EPMC)	55.58
<i>Melaleuca leucadendra</i> (L.) L.	36.00	Selinene (Alpha, Beta Delta) Caryophyllene	25.63 13.45
<i>Rosmarinus officinalis</i> L.	11.00	Asarone (Beta)	33.76
<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	46.00	Eugenol (Acetyl)	83.94
<i>Zingiber montanum</i> (J.Koenig) Link ex A.Dietr	11.60	Dimethoxy Phenyl Butadiene (DMPBD)	39.40 31.66
<i>Zingiber officinale</i> Roscoe.	19.00	Zingerone	30.72

Note: \* : no identified major compound(s)

**Table 3: Major compounds of non essential Pilis plant materials**

Scientific Name	GC MS Analysis	
	Major Compound	Relative Amount
<i>Amomum compactum</i> Roem. & Schult.	1,8-Cineole	53.96
<i>Cinnamomum verum</i> , J. Fresl, syn. <i>C. Zeylanicum</i>	Cinnamaldehyde	72.15
<i>Cryptocarya massoia</i> (Oken) Kosterm.	Massoilactone	69.97
<i>Curcuma longa</i> Linn. Syn. <i>Curcuma domestica</i> Val.	n.i.*	-
<i>Piper cubeba</i> L.	Cadinene (Delta)	42.47
<i>Pluchea indica</i> L.	n.i.*	-
<i>Trigonella foenum-graecum</i> L.	n.i.*	-
<i>Usnea filipendula</i>	1,8-Cineole	67.86

Note: \* n.i. : no identified major compound

### Major bioactive compounds of Pilis plant materials

Eleven major bioactives compounds were detected in this study (Pilis plant materials) and in line with the result of the previous study (ready to use Pilis).<sup>2</sup> This means that the bioactive compounds from the Pilis plant materials accumulated in the ready to use Pilis. Bioactive compounds of Pilis plant materials, no matter how diverse, appeared to have similar components with the ready-to-use Pilis. During the blending process of Pilis, the bioactive compounds were probably not transformed into other compounds.

In stead of using plant materials, Pilis can be composed directly from the extracts of Pilis plant materials, or mixture of pure Pilis bioactive compounds. And then a new formula of Pilis can be designed by using the available extracts in the market, such as clove oil, cajuput oil, rosemary oil, ginger oil etc. Eventhough, the balanced composition and amount should be recognized for the efficacy and biosafety of Pilis. The new delivery approach of Pilis, such as lotion or patch, can be investigated via *in vitro*, pre clinic and/or clinical study. Literature studies of the Pilis bioactive compounds gave evidency that they could be important for anti inflammation, sedation and biostimulation (Table 5).

**Table 4: List of bioactivities of Pilis plant materials (literature review)**

Scientific Name	Bioactivity
<b>Essential Pilis Plant Materials</b>	
<i>Acorus calamus</i> L.	Nervous disorder, sedative Halocinogenic <sup>4,5</sup>
<i>Alyxia stellata</i> (J.R. Forst. & G. Forst.) Roem. &Schult.	Stimulant (Increase resistance to disease, reduce muscular fatigue) <sup>6</sup>
<i>Curcuma heyneana</i> Valetton & Zijp	Antidepressant effects (curcumin) <sup>7</sup>
<i>Foeniculum vulgare</i> Mill.	Myorelaxant <sup>8</sup>
<i>Kaempferia galanga</i> L.	Sedative activity <sup>9,10</sup>
<i>Melaleuca leucadendra</i> (L.) L.	Sedative, stimulant <sup>6</sup>
<i>Rosmarinus officinalis</i> L.	Antidepressant-like effect <sup>11</sup>
<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Relaxant <sup>12</sup>
<i>Zingiber montanum</i> (J.Koenig) Link ex A.Dietr	Smooth-muscle relaxant <sup>13</sup>
<i>Zingiber officinale</i> Roscoe.	Post-operative nausea and vomiting prevention in general anaesthesia patients <sup>14</sup>
<b>Non Essential Pilis Plant Materials</b>	
<i>Amomum compactum</i> Sol. Ex Maton	Anti-inflammatory <sup>15</sup>
<i>Cinnamomum verum</i> , J. Fresl.	Anti-inflammatory <sup>16</sup> Memory enhancer <sup>17</sup>
<i>Cryptocarya massoy</i> (Oken) Kosterm.	Stimulate recovery after childbirth and restore vitality <sup>6</sup>
<i>Curcuma longa</i> L.	Anti-inflammatory <sup>18</sup>
<i>Piper cubeba</i> L.f.	Anti-inflammatory <sup>19</sup>
<i>Pluchea indica</i> (L.) Less.	Anti-inflammatory <sup>20</sup>
<i>Trigonella foenum-graecum</i> L.	Anti-inflammatory <sup>21</sup>
<i>Usnea filipendula</i>	Anti-inflammatory <sup>22</sup>

**Table 5: The list of major bioactive compounds in Pilis plant materials as well as in Pilis (literature review)**

Name of the compound	Bioactivity
Amyrin (Alpha, Beta)	Anti-inflammatory <sup>23</sup> , Psychedelic/ psychoactive activity, tranquilizer effect <sup>24</sup>
Asarone (beta)	Stimulant action (4), psychoactive substance <sup>26</sup>
Caryophyllene	Anti-inflammatory <sup>27</sup>
1,8-Cineole	Anti-inflammatory <sup>28,29,30</sup> Natural enhancer <sup>3</sup>
Cinnamaldehyde	Anti-inflammatory <sup>7,31,32</sup>
Dimethoxy Phenyl Butadiene (DMPBD)	Anti-inflammatory <sup>33</sup> , analgesic <sup>34</sup>
Ethyl methoxycinnamate (Ethyl p-)	Treatment of dyspepsia and headache, pain (analgesic), promoting vital energy <sup>35</sup>
Eugenol	Relaxant <sup>36</sup> Potent depressant of peripheral nervous activity <sup>37</sup>
Selinene (Alpha, Delta)	Anti-inflammatory <sup>38</sup>
4-Terpineol	Induction of vascular smooth muscle relaxation <sup>3</sup>
Zingerone	Anti-inflammatory <sup>39</sup>

## CONCLUSION

In conclusion, Pilis is composed from at least ten different plant materials. Every plant material is known for their specific major bioactive content. Eleven major bioactive compounds were detected.

### Highlights of Paper

- Pilis is a mixture of blended herbal materials used traditionally in Java for recovery of afterbirth mothers by putting it on the forehead.
- It was familiar long time ago, and can be improved to become a modern transdermal drug in the future, such as universal oil, patch, or ointment/cream.
- After GC MS analysis of Pilis, several bioactive compounds were identified.

### Author Profile

- **Prof. Dr. Kris Herawan Timotius:** presently working in the Department of Herbal Medicine, KridaWacana Christian University (UKRIDA). His main interests are to find novel bioactive compounds from herbal materials, and transdermal drug delivery system of the herbal bioactive compounds.
- **Ita:** presently following postgraduate program at Department BioMedical Science of Soonchunhyang University, Korea. Her main interest are molecular aspects of bioactive compounds.
- **Adit:** presently working in the Department of Herbal Medicine as junior researcher. His main experts are in elucidating bioactive compounds, HPLC, and using PAGE for screening of bioactive compounds.

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