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Original Article

Bioactive Compounds in Aqueous Extract of Pilis, A Forehead Transdermal Herbal Medicine

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ABSTRACT

Introduction: Pilis is a traditional Javanese transdermal herbal formula applied on the forehead to cure dizziness, hazy vision, eyestrain, and fever. So far, there are no scientific report on the bioactive compounds of the aqueous extract of pilis. This study aimed to identify the main bioactive compounds in the aqueous extract of pilis, either fresh-made or commercial pilis. Then, the bioactivities of the identified bioactive compounds were described based on the relevant literature studies. **Materials and Methods:** LC-MS was used to analyze the aqueous extracts of various pilis. Relevant information on their bioactivity is available from the works of the literature. **Results:** The identified main bioactive compounds in the aqueous extract of pilis were: undulatoside A, viscumneoside II, kaempferol, quercetin, genistein, and 7-hydroxy-5,8-dimethoxyflavone. Most compounds were in glycosidic form. The fresh-made pilis was more abundant in the number of bioactive compounds than the commercial pilis. Their bioactivities include antioxidant, anti-inflammation, neuroprotective, anti-cancer, and anti-microbial activities. **Conclusion:** Aqueous extracts of pilis, particularly fresh-made pilis, are promising transdermal forehead formulas rich in particular bioactive compounds with healing capacities to cure encephalitis and dizziness.

Keywords: Dizziness, Encephalitis, Mother after birth, Overhead, Transdermal.

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INTRODUCTION

Pilis is a traditional Javanese paste formula composed of various herbal materials. It is known in several areas in Java to cure dizziness, headache, hazy vision, and eyestrain. Pilis traditionally treats childhood encephalitis (fever and headache, the local name-kejang demam), febrile seizure or convulsion, and maternal recovery following childbirth. Our previous studies were GC-MS analyses of the non-polar bioactive compounds from pilis. Moreover, many nonpolar bioactive compounds have anti-inflammatory activity. So far, there are no research reports on the aqueous extract containing polar constituents. This study was the first on the aqueous extract of pilis using LC-MS.[1,2]

Guaranteed commercially pilis is not yet available. The most crucial is its particlar bioactive compounds. Their minimum effective doses and effect on metabolic pathways must be verified to increase the pilis acceptability. The effect of pilis herbal ingredients on a metabolic pathway, their safety, and bioavailability is a priority for future scientific development.[3]

This study used LC-MS to identify the main compounds in the aqueous extract of pilis herbal formula. This study compared the composition of fresh-made and commercial pilis and collected information on the bioactivities of the identified bioactive compounds by searching relevant literature. This study's novelty identifies the polar bioactive compounds of the aqueous extract of pilis and compares the fresh-made pilis with the commercial pilis. This study is the first study on aqueous extracts using LC-MS.

MATERIALS AND METHODS

Pilis materials and extract preparation

Four different pilis were investigated: A local freshly produced pilis; and three other pilis were commercially available from traditional markets or medicine shops in different Indonesian cities. They are Kemilau, Ika, and Hadrasah pilis (Figure 1). Aqueous extracts were prepared by diluting 10 g of pilis with 100 mL of distilled water. The mixtures were then filtered and

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centrifuged. The obtained debris-free aqueous extracts were then subject to LC-MS analysis.

LC-MS analysis

LC-MS analysis was performed using Waters LCMS/MS-QTOF. The operation mode used Tof MSE, equipped with an Electrospray Ionization (ESI) source with positive and negative ion modes. The column type used was a C_{18} column (ACE HPLC Column, 25cm x 4.6mm). The mobile phases used were 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water, delivered at a total flow rate of 0.6 mL/min. A total of 0.5 g of the sample was dissolved in 10 mL of methanol and then homogenized in the ultrasonicator for 30 min. The suspension was filtered using a 0.22 m GHP/PTFE membrane filter, and then 10 µL of the sample was injected in to the LC-MS system. The screening process for active substances in samples using LCMS/MS-QTOF was carried out with UNIFI software, which has a mass spectrum library of natural active substances from the Waters database. UNIFI software can identify the mass spectrum of compounds in the sample, which matches the mass spectrum to compounds in the library. The compounds were identified when the mass error of analyte ≤ 5 ppm, isotope matches MZ RMS 6, analyte intensity 300, and there is one fraction with brake value < 4 in the fragment elucidation system. The measurement of phytochemical compounds in the aqueous extract of pilis was performed at least twice.

RESULTS

Main compounds of the aqueous extracts of pilis

LC-MS analyses revealed six main compounds: undulatoside A, viscumneoside II, kaempferol, quercetin, genistein-7,4'-di-O-β-D-glucoside, and 7-hydroxy-5,8-dimethoxyflavone, based on their relative abundance and chromatogram peak. Most bioactive compounds were in their glycoside forms. Not all pilis samples investigated contain all six of these compounds. The number of constituents in fresh-made pilis was higher than in commercial pilis (Table 1 and Figure 2).

The similarity of bioactive constituents among the investigated pilis was very low. The fresh-made pilis was richer in phytochemical constituents than the commercial pilis and possessed all six main bioactive compounds. Only two, undulatoside A and viccumneoside II were present in three pilis (fresh-made, Kemilau, and Ika pilis). Most of the six main compounds were present in their glycosidic forms Figure 3.

Bioactivities of the main bioactive compounds of pilis

Many works of literature described the bioactivities of the six main bioactive compounds in aqueous extracts of pilis (Table 2). Their bioactivities include antioxidant, anti-inflammation, antivirus, antimicrobial, anti-cancer, neuroprotection, and immunomodulation.[4-6]

DISCUSSION

This study showed evidence that pilis production's quality is not standardized nor available. The pilis quality varies depending on the kind of herbal materials and its end-drying process. The drying process applied for commercial pilis can eliminate many volatile compounds. Therefore, the composition of the bioactive compounds varies. The quality standard of pilis production should ensure its usage's efficacy.

The composition of the bioactive compounds of the polar extract of pilis differs from the non-polar extract. Nevertheless, many polar or non-polar compounds in pilis have anti-inflammatory and neuroprotective capacities.[1,2] These capacities are essential for the efficacy of pilis as an forehead paste.

The presence of undulatoside A and viscumneoside II (Table 1 and Figure 1) is unique in the aqueous extracts of pilis. Among these six compounds, undulatoside A and viscumneoside II may become molecular biomarkers for the quality of pilis. Undulatoside A inhibits nitric oxide production and has anti-inflammatory, anti-adipogenic, and antimicrobial activities.[7] Vicumneoside II possesses neuroprotective[8] and anti-cancer activities.[9]

Kaempferol and quercetin are antiinflammation compounds.[10] Kaempferol can weaken neuroinflammation and blood-brain barrier dysfunction to recover neurological deficits.[11] Quercetin is a flavonoid with neuroprotective activity associated with its antioxidant activity[12] and exhibits anti-inflammatory activity. The transdermal application using quercetin can attenuate inflammation.[13]

Genistein-7,4'-di-O-β-D-glucoside and 7-hydroxy-5,8-dime thoxyflavone-7-O-β-D-glucoside have anti-inflammatory activity. Genistein can be efficaciously administered by the transdermal route,[29] is found to pass through the blood-brain barrier, and possesses neuroprotective effects.[4,14-16] Genistein exhibits antioxidant, antiinflammation, and estrogenic properties and has a protective potential in neurodegenerative disorders. It plays a role in delaying the development of neurodegenerative disease.

7-Hydroxy-5,8-dimethoxyflavone is active against clinical isolates and displays antioxidant activity.[17] Flavonoids are very diversified and generally are well investigated. However, 7-hydroxy-5,8-dimethoxyflavone is less known and is relatively poorly studied.

Other compounds, such as isoschaftoside and betaine, are also important. Isoschaftoside can treat inflammatory-based ailments and vascular and nervous disorders. Isoschaftoside-containing extract attenuates $AlCl_3$-induced neurotoxicity in rats by inhibiting oxidative stress and inflammatory markers.[18] Betaine

Table 1: LC/MS-QToF analysis (relative abundance) for the phytochemicals screening of aqueous extract of Pilis.

Compounds	Mode	RT (min.)	Fresh Pilis*	Commercial pilis*		
				Kemilau pilis	Ika pilis	Hadasah pilis
Undulatoside A	Neg	5.2	42,265-44,384	7,815-7,847	10,822-11,018	nf
	Pos	5.1	26,580-26,705			
Viscumneoside II	Neg	9.2	37,389-37,666	4,124-4,468	nf	nf
	Pos	9.1	24,942-26,115			4,373-4,409
Quercetin-3-O-α-D-glucuronide	Neg	9.1	30,160-30,311	nf	nf	11,376-12,312
	Pos	9.0	9,370-9,945			6,121-6,271
Quercetin-3-O-(6”-O-acetyl)-β-D-glucopyranoside	Pos	6.9	2,969 -3,070	nf	nf	nf
Kaempferol-3-O-(2G-α-L-rhamnosyl)-rutinoside	Neg	8.4	77,006-77,382	nf	nf	nf
	Pos	8.3	8,517-9,299	nf	nf	nf
Genistein-7,4’-di-O-β-D-glucoside	Neg	9.5	52,092 -53,604	nf	nf	nf
7-Hydroxy-5,8-dimethoxy flavone-7-O-β-D-glucoside	Neg	13.9	44,638-44,911	nf	nf	nf
3-O-[β-D-Glucopyra-nosyl-(1→2)]-β-D-glucopyranosyl-7-O-α-L-glucopy ranosyl-kaempferol	Neg	7.8	3,996-4,349	nf	nf	nf
6-Hydroxykaempferol-3-O-glucoside	Neg	8.8	256,541,262,286	nf	nf	nf
3’,5-Dihydroxy-7,4’-dimethoxy flavone	Pos	13.9	15,631-16,751	nf	nf	nf
2,3-(S)-Hexahydroxydiphenoyl-D-glucose	Neg	1.6	431-489	nf	nf	nf
Trigonelline	Pos	0.5	3,908-4,278	nf	nf	nf
Trifolin	Neg	10.3	10,672 -11,616	nf	nf	nf
Myricetin	Neg	10.9	14,774-15,102	nf	nf	nf
Isoswertiajaponin	Neg	12.1	9,496-9,608	nf	nf	nf
Morin	Neg	12.9	11,298-11,469	nf	nf	nf
Acacetin-7-galactoside	Neg	13.2	13,222-13,660	nf	nf	nf
Isoschaftoside	Neg	7.7	nf	7,677 -8,659	14,059-14,489	nf
	Pos	7.6	nf	nf	5,726-6,835	
Betaine	Pos	0.6	nf	4,348-6,049	nf	5,750-8,758
4-O-β-D-Glucopyranosyl fagomine	Pos	0.8	nf	8,624-8,627	nf	nf
Armepavine	Pos	5.6	nf	7,671-7,929	nf	nf
Sinomenine	Pos	7.2	nf	5,176-5,234	nf	nf
Grosvenorine	Neg	8.4	nf	nf	12,520-13,510	nf
Neohesperidin	Neg	10.9	nf	nf	3,036-3,446	nf
Stachydrine	Pos	0.5	nf	nf	31,970-35,166	nf
Divaricatol	Neg	7.6	nf	nF	nF	1,077-1,178
Sappanol	Neg	6.5	nf	nf	nf	5,904-6,539
Apigenin-7-O-acetyl-β-D-glucoside	Neg	14.0	nf	nf	nf	4,260-4,540
2,3-Dihydroiriegnin	Neg	16.7	nf	nf	nf	6,354-6,675
Number of compounds			17	7	5	7

*: is relative abundance; nf: not detected.

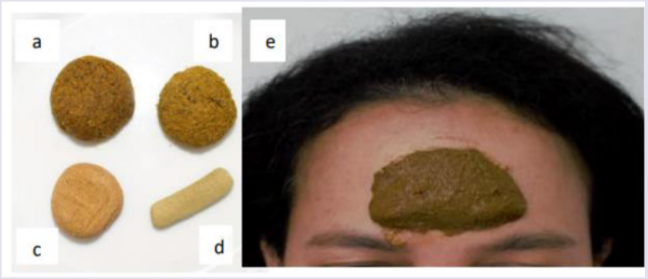

Figure 1: Photos of investigated pilis: (a) Fresh pilis; (b) Hadasah pilis, (c) Ika pilis, (d) Kemilau pilis, (e) pilis applied on forehead.

Table 2: Bioactivities of the main bioactive compounds of pilis.

Bioactivity	Bioactive compounds	References
Antioxidant	Quercetin	22
	Kaempferol	23
	Genistein	4,24
	7-Hydroxy-5,8-dimethoxyflavone	17
Antiinflammation	Undulatoside A	5
	Quercetin	13,25
	Kaempferol	10,23,25
	Genistein	4,14
Antivirus	Undulatoside A	26
	Kaempferol	26
	Quercetin	6
Antimicrobia	Undulatoside A	7
	Kaempferol	25
	Quercetin	25
	7-Hydroxy-5,8-dimethoxyflavone	17
Anticancer	Viscumneoside II	9
	Kaempferol	27,28
	Quercetin	6
	Genistein	15
	7-Hydroxy-5,8-dimethoxyflavone	29
Anti-adipogenesis	Undulatoside A	30
Neuroprotection	Viscumneoside II	8
	Quercetin	12
	Kaempferol	23,31,32
	Genistein	4,16
Immune-modulation	Kaempferol	28

might be helpful as a preventive agent against the activation of NF-κB induced during inflammation and aging.[19] Administration of betaine can significantly decrease the incidence of tumor formation with the downregulation of inflammation.[20]

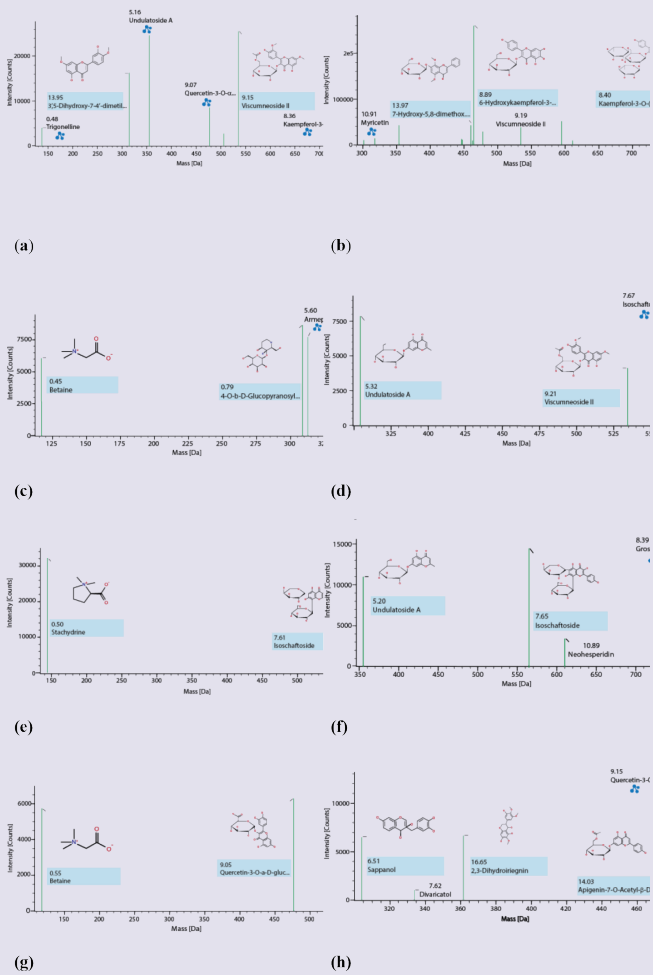

Figure 2: Chromatograms of investigated pilis: (a) fresh pilis (positive mode); (b) fresh pilis (negative mode); (c) commercial pilis-Kemilau (positive mode); (d) commercial pilis-Kemilau (negative mode); (e) commercial pilis-Ika (positive mode); (f) commercial pilis-Ika (negative mode); (g) commercial pilis- Hadasah (positive mode); (h) commercial pilis-Hadasah (negative mode).

Pilis has been used for centuries in traditional medicine to treat various neurological diseases, including nervousness, dizziness, and headaches. Pilis influences the central nervous system activity, including antiepileptic, sedative, antipsychotic, anxiolytic, sedative, and antidepressant, and has antinociceptive effects. Additionally, the therapeutic effect of pilis might result from the synergistic interactions of various secondary metabolites. Further studies of the central nervous system activity of pilis are required. The mechanisms of action, target sites, pharmacokinetics, metabolic mechanisms, adverse effects, and interactions of bioactive compounds of pilis must also be investigated.[8]

The pilis, either from the aspect of its composition or the method of delivery, may help in treating brain-related disorders, such as encephalitis, dizziness, and depression. However, there is a need for tailored-made pilis in order to have a highly effective treatment. It is necessary to develop existing pilis into user-friendly dosage

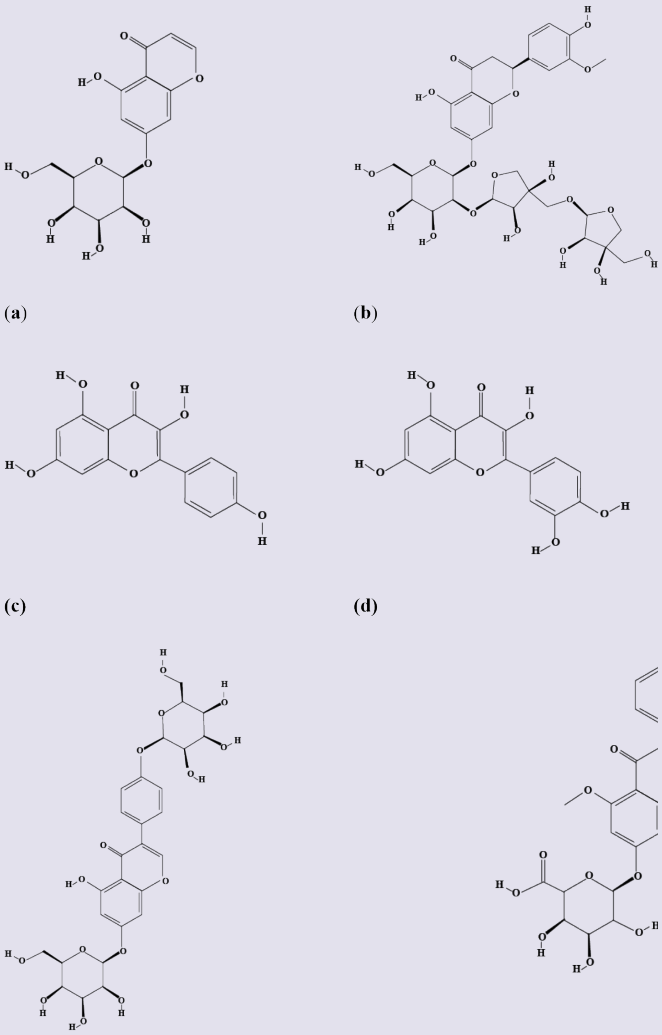

Figure 3: Chemical structure of the main bioactive compounds from aqueous extracts of pilis: (a) undulatoside A, (b) viscumneoside II, (c) kaempferol, (d) quercetin, (e) genistein-7,4′-di-O-β-D-glucoside, (f) 7-hydroxy-5,8-dimethoxyflavone-7-O-β-D-glucoside.

forms with several advantages for major brain disorder states. They require sustained drug release, reduced drug dosing frequency, improved tolerance and adherence, suitability for use in diverse populations and different treatment scenarios, and fewer side effects. The transdermal forehead route is a non-invasive drug delivery route that can provide the above mentioned benefits. The pilis patch possible be available in the future.[21]

CONCLUSION

This study's findings suggest that pilis is an excellent herbal medicine for protection against brain inflammation. Further research is needed regarding drug availability via transdermal administration. The application of modern delivery methods, such as nanoparticles (pilisome) and patches, are necessary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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