



Short Communication

An appropriate solvent for the preparation of Prasaplai extract

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Abstract

Prasaplai is a Thai traditional formulation for relieving dysmenorrhea and adjusting the cycle of menstruation. Three fatty acid esters, (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl linoleate (**1**), (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl oleate (**2**) and (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl palmitate (**3**), were formed during storage by the interaction of components in the preparation. The recommended dose (1.0 g) of Prasaplai was separately extracted by exhaustive sonication with three different solvents; which are hexane, 40% ethanol and distilled water, and yielded 26.70 ± 0.11 mg (2.7% w/w), 33.96 ± 0.05 mg (3.40% w/w), and 49.83 ± 0.30 mg (4.98% w/w), respectively. The crude extracts were analyzed by HPLC for contents of the four major compounds i.e (*E*)-1-(3,4-dimethoxyphenyl)but-3-en-1-ol (compound D), (*E*)-1-(3,4-dimethoxyphenyl)butadiene (DMPBD), piperine, β -asarone, and three artifacts. The results showed that only the hexane extract contained the artifacts while the 40% ethanol extract contained the maximum amounts of the major active anti-inflammatory components, and water extract contained only compound D. These results suggest that the 40% ethanol extract should be the appropriate extract for the preparation of Prasaplai in modern dosage forms due to the high content of active anti-inflammatory agents in the extract.

Keywords: Prasaplai, artifact, fatty acid ester, HPLC, Thai traditional medicine

1. Introduction

Prasaplai is a drug preparation listed in the Thai traditional common household drug list for relieving dysmenorrhea and adjusting the cycle of menstruation (National list of essential drugs 1999). It is composed of ten crude plants and two pure compounds; which are the root of *Acorus calamus* L., the bulb of *Allium sativum* L., the pericarp of *Citrus hystrix* DC., the rhizome of *Curcuma zedoaria* Roscoe., the bulb of *Eleutherine palmifolia* (L.) Merr., the seed of *Nigella sativa* L., the fruits of *Piper retrofractum* Blume and *P. nigrum* L., the rhizomes of *Zingiber cassumunar* Roxb. and *Z. officinale* Roscoe, sodium chloride, and camphor (Poomchusri, 1973).

During the investigation in 2004 on Prasaplai formulation, three artifacts which are (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl linoleate (compound **1**), (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl oleate (compound **2**) and (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl palmitate (compound **3**) were originated during storage of the preparation (Nualkaew *et al.*, 2004) (Figure 1). These three compounds are formed by the interaction of components in Prasapai preparation on the first day after mixing all ingredients together. After an investigation of the origin of the artifacts by a systematic preparation of two components mixtures and after a subsequent HPLC analysis was conducted, it was found that the artifacts were formed from the mixture of the rhizomes of *Z. cassumunar* and the seeds of *N. sativa* (Nualkaew *et al.*, 2004). A possible explanation for this phenomenon is that the formation of artifacts may occur as a chemical reaction between alcohol [(*E*)-1-(3,4-dimethoxyphenyl)but-3-en-1-ol or compound D (**4**)] and carboxylic acids (fatty acids) under

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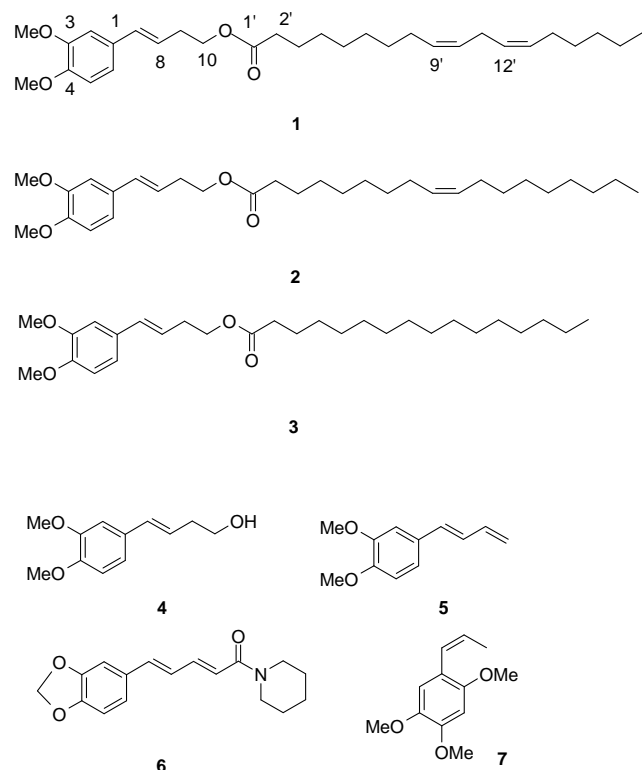


Figure 1. Chemical structures of compounds 1-7

special condition (Tangyuenyongwatana and Gritsanapan, 2008).

However, further investigations on these artifacts are required for the development of a modern dosage form of Prasaplai. Normally, Prasaplai can be administered by two ways. First, it is recommended to take one tea spoonful of the powder of Prasaplai directly with water. The other way is to take it with spirit (40% ethanol). In Thai traditional medicine, the main recommendation for the administration of this drug with alcohol is to conduct it in the form of maceration. The preparation is macerated with 40% ethanol for a period of time and the decoction extract was taken (Ayurveda College, 2001). In order to develop the Prasaplai preparation in a modern pharmaceutical form, its extract is interesting to be used instead of the powdered form. Thus, the objective of this study was to find an appropriate solvent yielding the Prasaplai extract containing a high amount of active anti-inflammatory compounds. Ethanol (40% v/v) and water, which are normally taken with Prasaplai, were evaluated in this experiment. In addition, a non-polar solvent, hexane, which can extract the oil components in the preparation, was also evaluated.

2. Materials and Methods

2.1 Plant materials

All the plant components used for the Prasaplai pre-

paration were purchased from a traditional herbal drug store in Bangkok. They were identified by comparison with the specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (*A. calamus*: SMU021, *A. sativum*: SMU022, *C. hystrix*: SMU023, *C. zedoaria*: SMU024, *E. palmifolia*: SMU025, *N. sativa*: SMU 026, *P. retrofractum*: SMU027, *P. nigrum*: SMU028, *Z. cassumunar*: SMU 029, *Z. officinale*: SMU030) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

2.2 Chemical and standard compounds

Compounds **1-4**, and (*E*)-1-(3,4-dimethoxyphenyl)butadiene (DMPBD; **5**), were synthesized in our lab with purity higher than 95% (Tangyuenyongwatana and Gritsanapan, 2007, 2008). Piperine (**6**) was purchased from Aldrich (USA) and β -asarone (**7**) was isolated from rhizomes of *A. calamus*. The structure of β -asarone (**7**) was elucidated by comparing the ^1H and ^{13}C -NMR spectral data with a reference (Patra *et al.*, 1981).

2.3 HPLC system and condition

A HPLC system, a Knauer pump K-1001, and a Knauer Photometer K-2600 detector with detection at 254 nm were used for this experiment. The separation was performed on Kromasil 5 μm 100AC₁₈, 250 x 4 mm column, with a flow rate of 0.8 ml/min and the solvent system was a gradient elution of 1% acetic acid in water and CH_3CN starting from 85:15, 70:30, 55:45, 50:50, 30:70, 15:85, 0:100 and 0:100 at 0, 8, 25, 30, 55, 65, 80 and 100 min, respectively.

2.4 Content analysis

2.4.1 Preparation of Prasaplai extracts

For the hexane extract, the Prasaplai preparation (1.00 g), which is equal to one tea spoonful dose according to the medical indication, was transferred to the cotton bag and then the bag was sealed. The Prasaplai bag was placed in a 250 ml stopper flask and 50 ml hexane was added. Then the flask was sonicated for 30 min and the extract was decanted. The marc was added with the same amount of the solvent and re-sonicated in the same period of time. The extraction was continued until exhausted, monitored by TLC. The extracts were combined, filtered, and the filtrate were evaporated to dryness using a rotary evaporator. The weight of the crude extract was recorded. The extraction was done in triplicate.

For 40% ethanol and water extracts, the same procedure was carried out for each solvent except that freeze drying was used in the drying process of the water extract instead of using a rotary evaporator.

Table 1. Calibration equation, correlation coefficient, and limit of quantification of standard compounds.

Compound	Calibration equation	Correlation coefficient (r^2)	Limit of quantification LOQ ($\mu\text{g/ml}$)	Linear range ($\mu\text{g/ml}$)
1	$Y=105,593X + 1,772$	0.9992	1.10	54.0-272
2	$Y=102,038X + 700$	0.9996	0.90	37.0-188
3	$Y=536,401X + 1,332$	0.9992	1.60	1.80-32.0
4	$Y=775,997X + 4,257$	0.9997	1.50	54.0-272
5	$Y=2,108,049X - 31,833$	0.9994	1.46	20.0-40.0
6	$Y = 22,541X - 559.5$	0.9999	1.65	2.06-501.3
7	$Y = 35,325X + 110.8$	0.9998	1.19	3.21-780

2.4.2 HPLC analysis

Each crude extract was dissolved in ethanol, and adjusted to the volume in a 100 ml volumetric flask. The solution was filtered through a 0.45 μm membrane filter and 20 μL of the filtrate was injected into the HPLC system with the condition is described in chapter 2.3. The crude extract was injected in triplicate.

The validation of the assay for linearity was determined by the analysis of five different concentrations of standard solutions. The linear ranges of the compounds **1-4** and **6-7** were shown in Table 1. The intraday precision of injection results demonstrated the relative standard deviation (RSD) of precision of less than 3% and the interday precision of less than 4%. The recovery studies were preceded by spiking the standard compounds in three different concentrations to a control Prasapalai extract. After the HPLC analysis, the mean recovery of the compounds **1-7** were 101.3 ± 2.42 , 98.27 ± 0.80 , 101.3 ± 2.3 , 101.5 ± 2.6 , 101.0 ± 1.45 , 99.12 ± 1.72 , and 100.4 ± 1.92 %, respectively.

3. Results and Discussion

For each Prasapalai extract, four major standard compounds, which are compound D, DMPBD, piperine, β -asarone (**4-7**), and the three artifacts were used to prepare the calibration curves for the HPLC analysis. Linearity of each compound was determined by using five concentrations in the certain ranges. Linear regression equations, correlation coefficients (r^2), and limit of quantification (LOQ) were shown in Table 1. The results of regression analysis revealed that the calibration curve of each compound had a correlation coefficient > 0.9991 .

The hexane extract was prepared by sonication of Prasapalai preparation with hexane in an ultrasonic bath. The exhaustive extraction was monitored by TLC and completed when using 250 ml (50 ml x 5) of hexane. After evaporation to dryness, 26.70 ± 0.11 mg (2.7% w/w) of crude hexane extract was obtained as a brown color residue. The crude extract was then dissolved in ethanol and analyzed with the HPLC in a gradient elution mode. From the HPLC chromatograms (Figure 2 (A)), the hexane crude extract comprised

many peaks including the peaks of active anti-inflammatory ingredients, which are compound D (**4**) (Panthong *et al.*, 1990) and DMPBD (**5**) (Jeenapongsa *et al.*, 2003) from *Z. cassumunar*. The other active compounds, which were piperine (**6**) from *P. nigrum* and *P. retrofractum*, β -asarone (**7**) from *A. calamus*, and compounds **1-3** were also selected for quantitative analysis. The amount of each compound is shown in Table 2.

The 40% ethanol extraction of Prasapalai preparation was carried out in the same procedure as the previous experiment. The exhaustive extraction was completed using 250 ml (50 ml x 5) of 40% ethanol, monitored by TLC. After evaporation to dryness, 33.96 ± 0.05 mg (3.40% w/w) of crude 40% ethanol extract was obtained as a brown color residue. The crude extract was then analyzed by HPLC in the same procedure as the previous assay. From the HPLC chromatograms (Figure 2 (B)), 40% ethanol crude extract comprised peaks lesser than the hexane extract and lacked of the three artifacts peaks. Compounds **4-7** were still apparent in the crude ethanol extract. The amount of each marker compound in the extract is shown in Table 2.

For the water extraction of Prasapalai preparation, the exhaustive extraction was completed using 250 ml (50 ml x 5) of water, monitored by TLC. After freeze drying, 49.83 ± 0.30 (4.98% w/w) of crude water extract was obtained as a yellow color residue. From the HPLC chromatograms (Figure 2 (C)), the water crude extract comprised mainly a peak of **4** and lacked the three artifacts peaks. The amount of **4** is shown in Table 2.

Among all Prasapalai extracts, 40% ethanolic extract seems to be the appropriate extract for the further developing of a modern dosage form of Prasapalai, because this extract contains the maximum amount of the major anti-inflammatory agents, **4** and **5**, and also the other active agents. In addition, the 40% ethanol extract did not have the artifacts, which still play an uncertain role in the preparation. The role of these artifacts is under further investigation.

References

Ayurveda College. 2001. The textbook of Thai traditional medicine, 3rd ed, Samjarearnpanich, Bangkok, Thai-

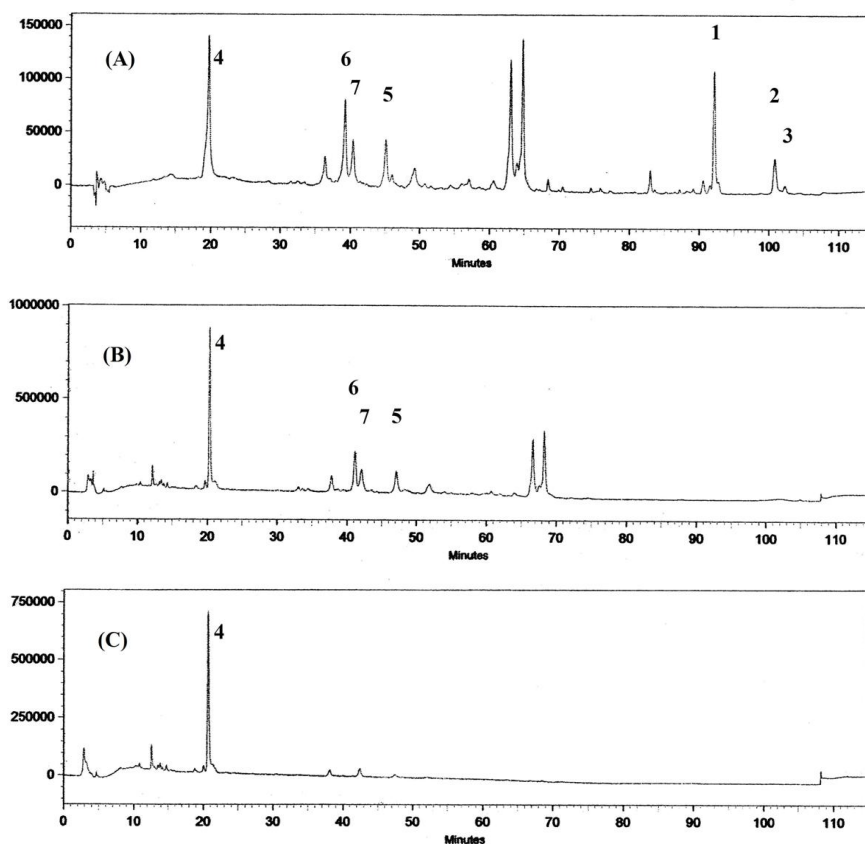


Figure 2. HPLC chromatograms of Prasapalai in hexane (A), 40% ethanol (B) and Water (C) extracts; 1: Compound 1, 2: Compound 2, 3: Compound 3, 4: Compound D, 5: DMPBD, 6: Piperine, and 7: β -asarone.

Table 2. Amounts of marker compounds in Prasapalai extracts.

Compound	Amount(% w/w)		
	Hexane	40% Ethanol extract	Water
1	0.906 \pm 0.005	-	-
2	0.340 \pm 0.003	-	-
3	0.103 \pm 0.009	-	-
4	0.187 \pm 0.002	0.687 \pm 0.083	0.595 \pm 0.040
5	0.097 \pm 0.013	0.118 \pm 0.001	-
6	0.144 \pm 0.012	0.408 \pm 0.010	-
7	0.119 \pm 0.003	0.237 \pm 0.007	-

land. pp. 260-261.

Jeenapongsa, R., Yoorathaworn, K., Pongprayoon, U. and Sriwatanakul, K. 2003. Anti-inflammatory activity of (*E*)-4-(3,4-dimethoxyphenyl)butadiene from *Zingiber cassumuar* Roxb. *Journal of Ethnopharmacology*. 87, 143-148.

Panthong, A., Kanjanapothi, D., Niwatananun, V., Tuntiwachwuttikul, P., Reutrakul, V. 1990. Anti-inflammatory activity of compounds isolated from *Zingiber cassumuar*. *Planta Medica*. 56, 655.

National list of essential drugs 1999. National essential drug list committee. p 2.

Nualkaew, S., Gritsanapan, W., Petereit, F. and Nahrstedt, A. 2004. New fatty acid esters originate during storage by the interaction of components in Prasapalai, a Thai traditional medicine. *Planta Medica*. 70, 1243-1246.

Patra, A. and Mitra, A.K. 1981. Constituents of *Acorus calamus*: structure of acoramone. carbon-13 NMR spectra of *cis*- and *trans*-asarone. *Journal of Natural Products*. 44, 668-669.

- Poomchusri, N.T. 1973. Ayurvedic study. 2nd ed, Promjakkapimp, Bangkok, Thailand. p.194
- Tangyuenyongwatana, P. and Gritsanapan, W. 2007. Biological evaluation of fatty acid esters originated during storage of Prasapalai, a Thai traditional medicine. *Natural Product Research* 21, 990-997
- Tangyuenyongwatana, P. and Gritsanapan, W. 2008. A study on artifacts formation in the Thai traditional medicine Prasapalai. *Planta Medica*. 74, 1403-1405.