

# Standardization of Prasaplai, a Thai traditional preparation for antidysmenorrhea

Prasan

Tangyuenyongwatana<sup>1</sup>

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**Abstract:** Prasaplai is a Thai traditional medicine preparation that has been used to treat dysmenorrhea for several decades. The preparation is composed of ten herbs and two compounds. Due to factors such as the inconsistency of active ingredients in raw materials, and the biological efficacy and reproducibility of therapeutic effects, traditional medicine preparations are required to be qualitatively and quantitatively standardized by modern methods so that they become more widely accepted. The prominent methods of choice are high-performance liquid chromatography and thin-layer densitometry. Each component of Prasaplai was collected from ten different locations in Thailand, and the amounts of active constituents in each of the component were analyzed using high-performance liquid chromatography. Prasaplai preparation also contains artifacts, which are formed during storage. These artifacts were evaluated periodically within 1 year of storage. Different extracting solvents for Prasaplai preparation were also analyzed to avoid the artifacts.

**Keywords:** Prasaplai, dysmenorrhea, standardization, HPLC, artifact

## Introduction

Prasaplai is a Thai traditional medicine preparation used to treat dysmenorrhea. The word “Prasaplai” is derived from “Prasa”, which means 50% in amount, and “Plai”, which is a Thai name for *Zingiber cassumunar* Roxb. Altogether, Prasaplai refers to the preparation that contains 50% of *Z. cassumunar*. It has been commonly used for a long time by Thai traditional practitioners as an antifatulent, to relieve dysmenorrhea, and to adjust the cycle of menstruation.<sup>1,2</sup> Prasaplai has been included as a Thai traditional common household drug since the first list was established in 1968. Additionally, it has also been included in the Thai traditional common household drug list and the national essential drug list AD 1999 of Thailand (list of herbal medicinal products) for relieving dysmenorrhea, especially in women who have less menstruation or an irregular cycle of menstruation.<sup>3</sup> Prasaplai formula consists of ten medicinal plants and two chemical compounds. It is a mixed powder of equal amounts of the pericarp of *Citrus hystrix* DC., the root of *Acorus calamus* L., the bulb of *Allium sativum* L., the bulb of *Eleutherine americana* (Aubl.) Merr. ex K. Heyne, the fruit of *Piper nigrum* L., the fruit of *Piper retrofractum* Vahl, the rhizomes of *Zingiber officinale* Roscoe and *Curcuma zedoaria* Roscoe, the seed of *Nigella sativa* L., and sodium chloride and camphor making a total of 50% of the formulation weight, and the remaining 50% comprises the rhizome powder of *Z. cassumunar* Roxb.<sup>2</sup>

Two clinical studies have investigated the efficiency and side effects of Prasaplai extract versus mefenamic acid in relieving primary dysmenorrhea.<sup>4,5</sup> The results

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showed that Prasapalai resulted in less pain and less side effects compared with mefenamic acid ( $P < 0.05$ ),<sup>4</sup> whereas Sriyakul et al reported no statistically significant difference between the effects of Prasapalai extract and mefenamic acid on the degree of pain relief. In addition, both Prasapalai extract and mefenamic acid showed no severe side effects.<sup>5</sup> Based on such results, this preparation became a well-known commercial product and has been widely used in most of the hospitals and health care units in Thailand. However, standardization of the preparation is lacking, which is necessary to ensure efficiency and safety. The quality assessment of each herbal component is also important to control the quantity of its major active ingredients and the quality of the whole formulation.

## Standardization of herbal components in Prasapalai preparation

Each herbal component of Prasapalai preparation was purchased during March–April 2007 from traditional drug stores in ten different locations in Thailand. The powder of each plant (100 mg) was sonicated three times in methanol (3 mL each) for 10 minutes. Each extract was decanted, filtered through a disk filter (0.45  $\mu\text{m}$ ), and adjusted to 10 mL in a volumetric flask to give a final concentration of 10 mg/mL. A 10  $\mu\text{L}$  volume of the sample solution was injected to high-performance liquid chromatography (HPLC) system (Table 1).<sup>6</sup>

## Validation method of HPLC for quantitative analysis of each plant component of Prasapalai preparation

### *A. calamus* L.

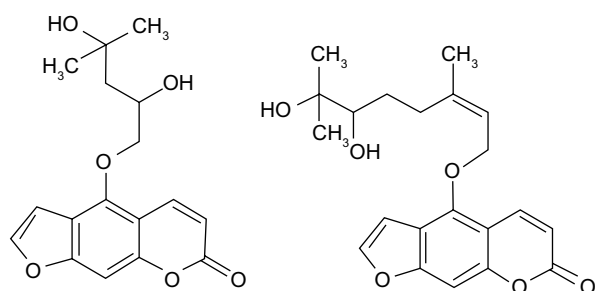
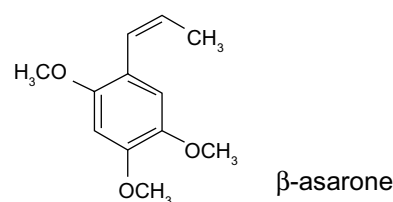
It is an herb of the Araceae family. The rhizome is traditionally used to soothe inflammation and treat skin infections. Calamus rhizome contains 2%–9% essential

oil, which comprises sesquiterpenes and phenylpropanoids. *cis*-Isoasarone ( $\beta$ -asarone) is the main component (up to 80%) of the essential oil (Figure 1). Other compounds include *cis*-methyl isoeugenol,  $\alpha$ - and  $\gamma$ -asarones, asarylaldehyde, acoroxide, acorin, calamine, linalool, calamol, calamenone, eugenol, methyl eugenol, azulene, pinene, cineole, camphor, and acorone, which is a sesquiterpene diketone with a bitter taste and spiral structure.<sup>7–9</sup>

HPLC was performed using water and acetonitrile with gradient elution: 50:50, 43:57, 20:80, and 20:80 at 0, 10, 15, and 20 minutes, respectively. The linear regression equation is  $Y = 35.325X + 110.89$  with  $r^2 = 0.9998$  for  $\beta$ -asarone. The concentrations range between 3.21 and 780.19  $\mu\text{g/mL}$ . The marker for this plant is  $\beta$ -asarone, which is the major active compound in *A. calamus*. The content of  $\beta$ -asarone is in the range of  $2.05\% \pm 0.02\%$  to  $2.65\% \pm 0.01\%$  w/w. The average quantity of  $\beta$ -asarone in the dried powder is  $2.17\% \pm 0.02\%$ . Thus, *A. calamus* rhizome should contain  $\beta$ -asarone not  $< 2.0\%$  dry weight.<sup>6</sup>

### *C. hystrix* DC

It is a medium tree of the Rutaceae family. Fresh peels and dried fruits are used to relieve nausea and flatulence and control menstruation. The fruit peel of *C. hystrix* contains an essential oil of which the major compounds are terpenes, such as  $\beta$ -pinene, limonene,  $\beta$ -phellandrene, citronellol, and citronellal. The other constituents are linalool, borneol, camphor, sabinene, germacrene D, aviprin, umbelliferone, and  $\beta$ -sitosterol.<sup>7,10</sup> Coumarins are also isolated from the peel



**Oxypeucedanin hydrate      6',7'-dihydroxy bergamottin**

**Figure 1** Structure of marker compounds for *Acorus calamus* and *Citrus hystrix*.

**Table 1** HPLC system and common conditions

Pump	HP 1050, multisolvent system Millipore (Agilent Technologies, Santa Clara, USA)
Flow rate	1 mL/min
Column	Synergi Max-RP ( $C_{12}$ ), 150 $\times$ 4.6 mm, 4 $\mu\text{m}$ (Phenomenex, Torrance, CA, USA)
Guard cartridge	Security Guard (Phenomenex, USA)
Oven column	HP 1050 at 40°C (Agilent, USA)
Injector	HP 1050, Autosampler injection volume 10 $\mu\text{L}$ (Agilent, USA)
Detector	HP 1050, Photodiode array detector at 254 nm (Agilent, USA)

**Abbreviation:** HPLC, high-performance liquid chromatography.

of *C. hystrix*. They include bergamottin, 6',7'-dihydroxybergamottin (DHB), oxypeucedanin hydrate (OPH), psoralen, and auraptene.<sup>11</sup> Flavonoids, including myricetin, quercetin, kaempferol, luteolin, and apigenin, are also found in the leaf of *C. hystrix*.<sup>12</sup>

HPLC was performed using water and acetonitrile with gradient elution: 85:15, 70:30, 50:50, 20:80, and 20:80 at 0, 10, 20, 30, and 35 minutes, respectively. DHB and OPH are the two markers for this plant (Figure 1). The linear regression equations for DHB and OPH are  $Y = 24.036X + 22.851$  with  $r^2=0.9999$  and  $Y = 29.592X + 36.024$  with  $r^2=0.9999$ , respectively. The concentrations of DHB and OPH are 32.31–245.39  $\mu\text{g/mL}$  and 31.37–238.23  $\mu\text{g/mL}$ , respectively. The lower limits of dry weights of DHB and OPH are  $0.300\%\pm0.003\%$  and  $0.318\%\pm0.003\%$ , while the upper limits are  $0.709\%\pm0.002\%$  and  $0.543\%\pm0.002\%$ , respectively. The average amounts of DHB and OPH in the dried powder are  $0.491\%\pm0.124\%$  and  $0.446\%\pm0.076\%$  w/w, respectively. Thus, *C. hystrix* fruit peel should contain DHB and OPH not  $<0.40\%$  dry weight.<sup>6</sup>

### *C. zedoaria* Roscoe

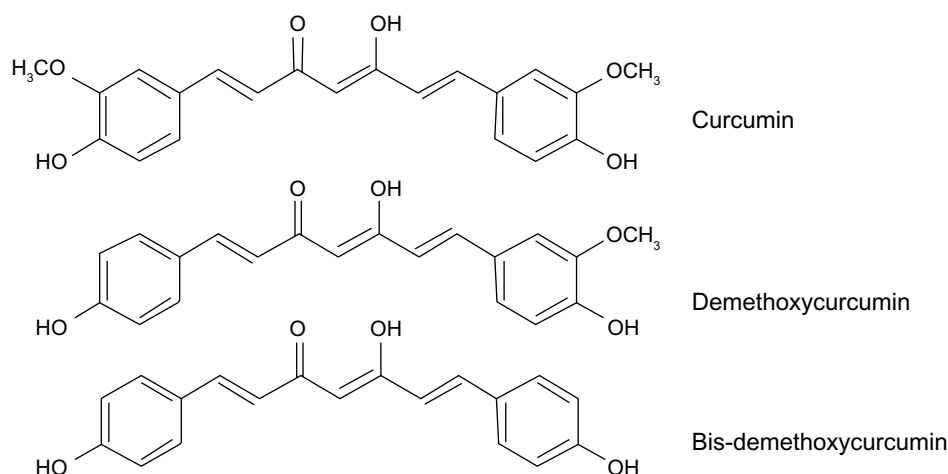
It is an herb in the Zingiberaceae family. The rhizome is traditionally used as a decoction for flatulent colic. The essential oil from the rhizomes contains  $\alpha$ -pinene, D-camphor, D-camphene, and cineole,<sup>13</sup> while a great number of sesquiterpenes were also isolated. They are derived from germacrane-, elemene-, cadinane-, eudesmane-, and guainane-type skeletons. For example, curdione and germacrone-7,8-epoxide are germacrane type; zedoarone and curzerene are elemene type; pyrocurzerenone and curzeone are cadinane type; curcolone is eudesmane type; and procurcumenol and zedoarol are guainane type.<sup>14</sup> In addition, curcumin (CCM) and its

related compounds bis-(4-hydroxycinnamoyl)-methane and 4-hydroxycinnamoyl feruloyl methane are also detected in the rhizome of *C. zedoaria*.<sup>15</sup>

HPLC was performed using water and acetonitrile with gradient elution: 50:50, 50:50, 60:40, 60:40, 50:50, and 50:50 at 0, 8, 10, 15, 16, and 20 minutes, respectively. The linear regression equations and concentration ranges for three makers (Figure 2), namely, CCM, demethoxycurcumin (DMC), and bis-demethoxycurcumin (BMC), are shown in Table 2. The lower limits of CCM, DMC, and BMC are  $0.320\%\pm0.002\%$ ,  $0.228\%\pm0.002\%$ , and  $0.120\%\pm0.001\%$  w/w, respectively. The upper limits of CCM, DMC, and BMC are  $0.732\%\pm0.003\%$ ,  $1.427\%\pm0.055\%$ , and  $0.436\%\pm0.021\%$  w/w, respectively. The average quantities of CCM, DMC, and BMC in the dried powder are  $0.616\%\pm0.125\%$ ,  $0.912\%\pm0.352\%$ , and  $0.252\%\pm0.104\%$  w/w, respectively. Thus, *C. zedoaria* rhizome should contain CCM, DMC, and BMC not  $<0.5\%$ ,  $0.6\%$ , and  $0.15\%$  dry weight, respectively.

### *E. americana* (Aubl.) Merr. ex K. Heyne

It is an herb of the Iridaceae family. The bulb of this plant is traditionally used as a carminative, and together with galangal, it can treat a cold in children. The ethanol extract from the bulb of *E. americana* contains three naphthalene derivatives, which are eleuterol, eleutherin, and isoeleutherin.<sup>16</sup> Upon further investigation, hongconin, a new naphthalene derivative, was isolated. Its structure was elucidated based on chemical and spectroscopic methods.<sup>17</sup> Anthraquinone and methyl ester of 3,4,8-trihydroxy-1-methyl-anthra-9,10-quinone-2-carboxylic acid were also found in the rhizome of *E. americana*.<sup>18</sup> Recently, Paramapojn et al<sup>19</sup> isolated a new compound, eleutherioside A, from the bulb of this



**Figure 2** Structures of markers for *Curcuma zedoaria*.

**Table 2** Linear regression equation and concentration ranges of markers for *Curcuma zedoaria*

Compound	Regression equation	$r^2$	Range
Curcumin	$Y = 42.596X - 119.78$	0.9995	3.95–197.60
Demethoxycurcumin	$Y = 47.673X - 30.152$	0.9999	4.82–96.30
Bis-demethoxycurcumin	$Y = 37.402X - 150.74$	0.9994	4.67–93.40

**Note:** Reproduced from Paramapojn S. Standardization and Biological Activities of Prasapalai, a Thai Traditional Medicine [Ph.D. thesis]. Salaya: Mahidol University; 2008.<sup>6</sup>

plant (Figure 3). Its structure was also elucidated based on spectroscopic methods.

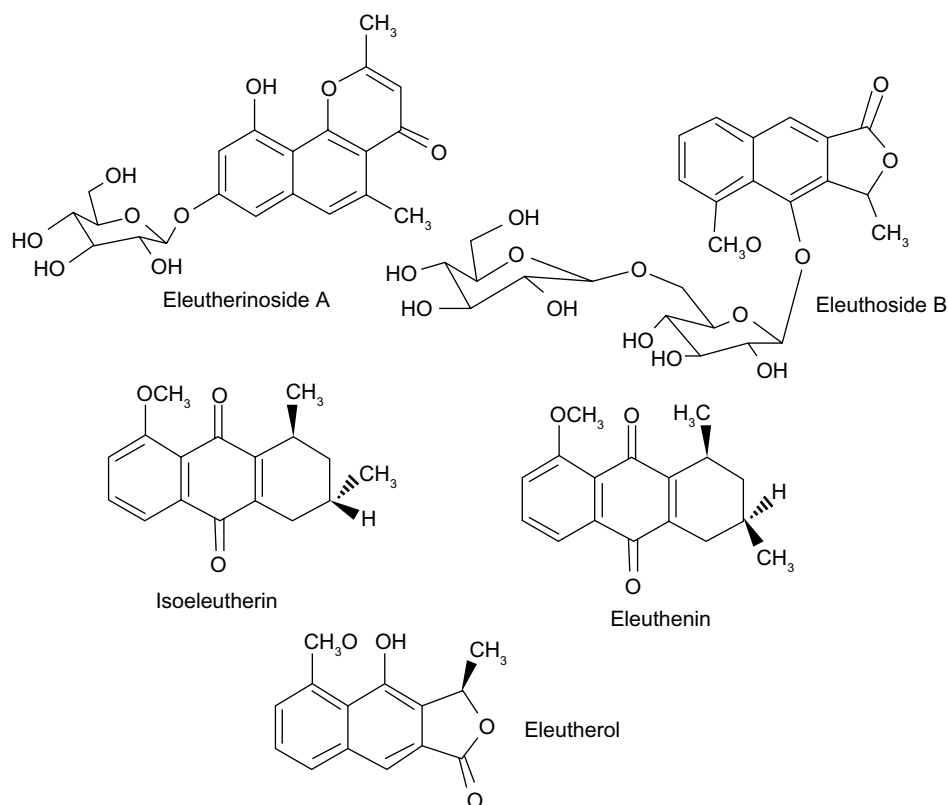
HPLC was performed using water and acetonitrile with gradient elution: 85:15, 70:30, 55:45, 50:50, 40:60, 20:80, and 20:80 at 0, 10, 15, 20, 23, 25, and 30 minutes, respectively. The linear regression equations and concentration ranges for five makers, namely eleutherinoside A (A), eleuthoside B (B), isoeleutherin (C), eleutherin (D), and eleutherol (E), are shown in Table 3. The amounts of A, B, C, D, and E in the dried powder of *E. americana* bulbs collected from ten locations are found in the ranges of  $0.011\% \pm 0.001\%$  to  $0.158\% \pm 0.001\%$ ,  $0.001\% \pm 0.001\%$  to  $0.260\% \pm 0.006\%$ ,  $0.089\% \pm 0.001\%$  to  $0.161\% \pm 0.002\%$ ,  $0.138\% \pm 0.003\%$  to  $0.267\% \pm 0.004\%$ , and  $0.099\% \pm 0.002\%$

to  $0.196\% \pm 0.003\%$  w/w, respectively. The average contents of A, B, C, D, and E in the dried powder are  $0.091\% \pm 0.045\%$ ,  $0.169\% \pm 0.083\%$ ,  $0.112\% \pm 0.021\%$ ,  $0.210\% \pm 0.039\%$ , and  $0.135\% \pm 0.028\%$  w/w, respectively. Therefore, *E. americana* bulb should contain A, B, C, D, and E not  $<0.05\%$ ,  $0.1\%$ ,  $0.1\%$ ,  $0.2\%$ , and  $0.1\%$  dry weight, respectively.<sup>6</sup>

### *N. sativa* L.

It is an herb of the Ranunculaceae family. The seed is used as a carminative, diuretic, and anthelmintic. The essential seed oil contains thymoquinone, nigellone, and 2-methyl-4-isopropyl-*p*-quinone.<sup>20</sup> Seeds contain fatty acids, including palmitic, myristic, oleic, linoleic, and linolenic acids.  $\beta$ -Sitosterol and flavonol triglycosides are also found in the seeds of *N. sativa*.<sup>21</sup>

HPLC was performed using water and acetonitrile with gradient elution: 85:15, 80:20, 60:40, 50:50, 20:80, and 20:80 at 0, 5, 10, 15, 20, and 25 minutes, respectively. The linear regression equation is  $Y = 95.279X + 81.601$  with  $r^2 = 0.9998$  for thymoquinone (Figure 4). The concentrations range between 1.47 and  $357.06 \mu\text{g/mL}$ . The content of thymoquinone in the dried powder of *N. sativa* seeds from ten locations is found in the range of  $0.112\% \pm 0.004\%$  to  $0.369\% \pm 0.004\%$  w/w. The average amount of thymoquinone

**Figure 3** Structures of markers for *Eleutherine americana*.

**Table 3** Linear regression equation and concentration ranges of makers for *Eleutherine americana*

Compound	Regression equation	$r^2$	Range
Eleutherinoside A	$Y = 26.758X + 3.989$	1.000	6.28–201.0
Eleutherinoside B	$Y = 32.556X + 53.882$	0.9999	7.09–227.0
Isoeleutherin	$Y = 26.972X + 76.761$	0.9998	6.34–203.0
Eleutherin	$Y = 27.711X - 10.931$	1.000	6.69–214.0
Eleutherol	$Y = 57.651X + 121.17$	0.9996	7.28–233.0

**Note:** Reproduced from Paramapojn S. Standardization and Biological Activities of Prasaplai, a Thai Traditional Medicine [Ph.D. thesis]. Salaya: Mahidol University; 2008.<sup>6</sup>

is 0.220%±0.106% w/w. Thus, *N. sativa* seeds should contain thymoquinone not <0.1% dry weight.<sup>6</sup>

### *P. retrofractum* Vahl

This plant is a vine of the Piperaceae family. The fruit is traditionally used as a carminative and for preparing a decoction for the treatment of diarrhea. The major active constituents of the fruit are alkaloid piperine (Figure 4) and volatile oil. The other compounds are phenolic amides, such as guineensine, pellitorine, and *N*-isobutyl-2*E*,4*E*,8*Z*-eicosatrienamide. The neutral compounds are methyl piperate and  $\beta$ -sitosterol, while the acidic compound is piperic acid.<sup>22</sup> Techadamrongsin et al reported many alkaloids in the fruits, including 2,4-decadienoylpiperidine, (2*E*,4*E*)-*N*-eicosadienoylpiperidine, (2*E*,14*E*)-*N*-eicosadienoylpiperidine, (2*E*,4*E*,14*E*)-*N*-isobutyleicosatrienamide, 1-(2*E*,4*E*)-octadecadienoylpiperidine, 1-(2*E*,4*E*)-octadecatrienoylpiperidine, piperamine, (2*E*,4*E*,14*E*)-*N*-isobutyloctadecatrienamide, pipartine, piperlonguminine, piperundecalidine, piperonaline, sylvetin, retrofractamide A, C, D, piperoctadecalidine, dihydropiperlonguminine, pipericide, octadecadienamide, eicosatrienamide, and *n*-isobutyldeca-*trans*-4-dienamide.<sup>23</sup>

HPLC was carried out using water and acetonitrile with gradient elution: 60:40, 55:45, 40:60, 30:70, 10:90, and 10:90 at 0, 10, 15, 25, 30, and 35 minutes, respectively. The linear

regression equation is  $Y=22.541X-55.953$  with  $r^2=1.000$  for piperine. The concentrations range between 2.06 and 501.29  $\mu\text{g/mL}$ . The amount of piperine in the dried powder of *P. retrofractum* fruits from ten locations are found in the range of 2.84%±0.03% to 3.61%±0.03% w/w. The average content of piperine in the dried fruit powder is 3.24%±0.26% w/w. Therefore, *P. retrofractum* fruits should contain piperine not <3% dry weight.<sup>6</sup>

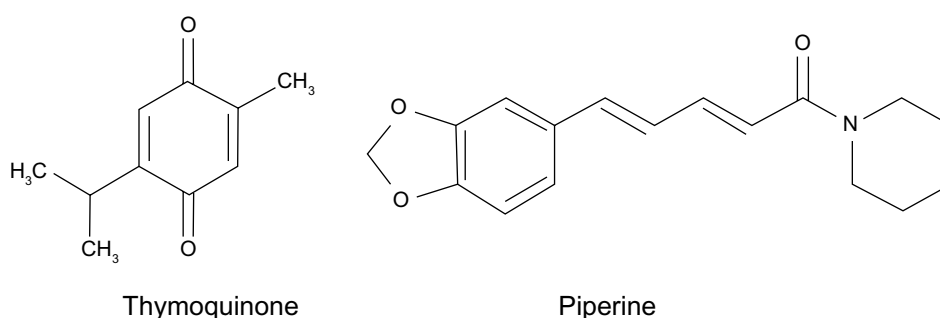
### *P. nigrum* L.

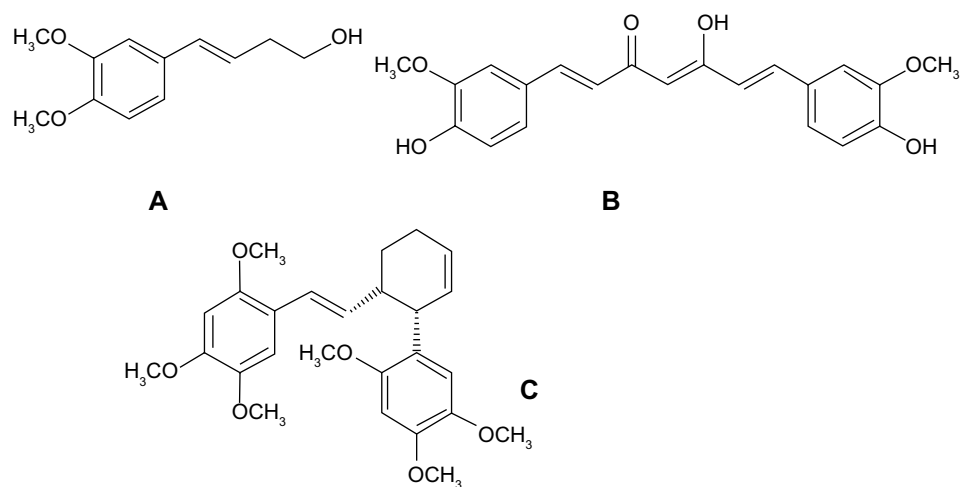
It is a vine of the family Piperaceae. The fruit is traditionally used for cough, rhinitis, and anemia. The fruit consists of piperine, piperetine, amide-peperylin, piperoleins A and B, *N*-isobutyl-cicosa-*trans*-2-*trans*-4-diamide, guineensine, and sarmentine. Other alkaloids include chavicine, piperidine, piperettine, methyl caffeic acid, piperidide,  $\beta$ -methyl pyrrolidine, and a series of vinyl homologs of piperine and their stereoisomers. Essential oil from the fruit contains mainly sabinene (15%–25%), caryophyllene,  $\alpha$ - and  $\beta$ -pinene, citronellol,  $\alpha$ -thujene, myrcene, limonene, terpinene, *p*-humulene, selinene, camphene, linalool, terpineol, and nerolidol in varying amounts.<sup>24–27</sup>

The HPLC conditions, linearity, and range of concentration are the same as those for *P. retrofractum*. The content of piperine in the dried powder of *P. nigrum* fruits from ten locations is in the range of 2.54%±0.01% to 3.97%±0.01% w/w. The average amount of piperine in the dried powder is 3.37%±0.48% w/w. Thus, *P. nigrum* fruits should contain piperine not <3% dry weight.<sup>6</sup>

### *Z. cassumunar* Roxb.

It is an herb of the family Zingiberaceae. The rhizome is used as a carminative and antispasmodic and for treatment of digestive problems. Amatayakul et al isolated six aromatic compounds from the rhizome of *Z. cassumunar*, which are *cis*-3-(2',4',5'-trimethoxyphenyl)-4-[(*E*)-2''',4''',5'''-trimethoxystyryl]

**Figure 4** Structures of markers for *Nigella sativa* and *Piper retrofractum*.



**Figure 5** Structures of markers for *Zingiber cassumunar*.

**Notes:** (A) *E*-4-(3,4-dimethoxyphenyl)but-3-en-1-ol, (B) curcumin, and (C) *cis*-3-(2',4',5'-trimethoxyphenyl)-4-[(*E*)-2''',4''',5'''-trimethoxystyryl]cyclohex-1-ene.

cyclohex-1-ene; *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-3''',4'''-dimethoxystyryl]cyclohex-1-ene; *cis*-3-(3',4'-dimethoxyphenyl)-4-[(*E*)-2''',4''',5'''-trimethoxystyryl]cyclohex-1-ene; (*E*)-4-(3',4'-dimethoxyphenyl)but-3-en-1-ol (compound D); (*E*)-4-(3',4'-dimethoxyphenyl)but-3-en-1-yl acetate; and 8-(3,4-dimethoxyphenyl)-2-methoxynaphtho-1,4-quinone.<sup>28</sup> Kuronayaki et al reported three compounds with the same core structure.<sup>29</sup> In 1981, Tuntiwachwutikul et al reported phenylbutanoids from the rhizome of *Z. cassumunar*, which are (*E*)-4-(3',4'-dimethoxyphenyl)but-1,3-diene; (*E*)-4-(3',4'-dimethoxyphenyl)but-3-ene; (*E*)-4-(2',4',5'-trimethoxyphenyl)but-1,3-diene; (*E*)-4-(2',4',5'-trimethoxyphenyl)but-3-ene; (*E*)-4-(3,4-dimethoxyphenyl)but-3-en-1-yl palmitate; 3,4-dimethoxybenzaldehyde; and 2,4,5-trimethoxybenzaldehyde.<sup>30</sup>

The HPLC conditions comprise BDS Hypersil C<sub>18</sub> column as a stationary phase. The mobile phase comprises water and acetonitrile with gradient elution: 30:70, 45:65, 60:40, 45:55, 45:55, 70:30, 70:30, 55:45, and 30:70 at 0, 8, 10, 11.5, 30, 35, 40, 45, and 50 minutes, respectively. The linear regression equation and concentration ranges for the three markers (Figure 5), namely *E*-4-(3,4-dimethoxyphenyl)but-3-en-1-ol (A); CCM (B); and *cis*-3-(2',4',5'-trimethoxyphenyl)-4-[(*E*)-2''',4''',5'''-trimethoxystyryl]cyclohex-1-ene (C), are

**Table 4** Linear regression equation and concentration ranges of makers for *Zingiber cassumunar*

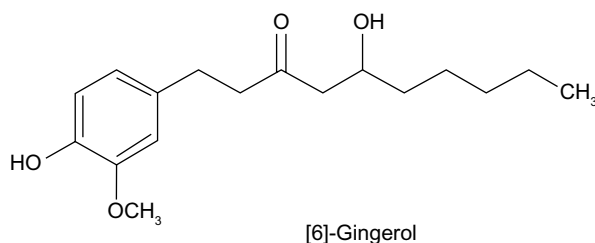
Compound	Regression equation	r <sup>2</sup>	Range
A	Y = 170.45X – 32.391	0.9998	6.31–202.0
B	Y = 51.603X – 59.176	0.9995	3.22–103.0
C	Y = 82.033X – 177.72	0.9992	3.16–101.0

**Note:** Reproduced from Paramapojn S. Standardization and Biological Activities of Prasaplai, a Thai Traditional Medicine [Ph.D. thesis]. Salaya: Mahidol University; 2008.<sup>6</sup>

shown in Table 4. The dried rhizomes of *Z. cassumunar* were collected from 14 locations. The contents of A, B, and C in the powdered rhizomes are in the range of 0.400%±0.001% to 1.642%±0.005%, 0.066%±0.001% to 0.265%±0.002%, and 0.027%±0.001% to 0.287%±0.004% w/w, respectively. The average contents of A, B, and C are 0.895%±0.374%, 0.131%±0.073%, and 0.126%±0.076% w/w, respectively. Therefore, *Z. cassumunar* rhizomes should contain A, B, and C not <0.8%, 0.13%, and 0.12% dry weight, respectively.<sup>6</sup>

### *Z. officinale* Roscoe (ginger)

It is an herb of the Zingiberaceae family. The rhizome is used as stomachic, antiemetic, and anti-inflammatory agent and as an expectorant. Ginger rhizome oleoresin contains mainly pungent principles such as homologous gingerol, shogaol, and zingerone. Zingerone and shogaol are found in small amounts in fresh ginger but in large amounts in stored products. The series of gingerol and related compounds are composed of [3]-, [4]-, [5]-, [6]-, [8]-, [10]-, and [12]-gingerols; [3]-, [4]-, [5]-, [6]-, [8]-, and [10]-shogaols; [4]-, [6]-, [8]-, and [10]-gingerdiols; [6]-methylgingerdiol; and [4]- and [6]-gingerdiacetate.<sup>31,32</sup>



**Figure 6** Structure of a marker for *Zingiber officinale*.

HPLC was performed using water and acetonitrile with gradient elution: 50:50, 50:50, 30:70, 20:80, 10:90, and 10:90 at 0, 7, 10, 15, 20, and 30 minutes, respectively. The linear regression equation is  $Y=10.938X+17.08$  with  $r^2=1.000$  for [6]-gingerol (Figure 6). The concentrations range between 1.61 and 390.63  $\mu\text{g/mL}$ . The quantity of [6]-gingerol in the dried powder of *Z. officinale* rhizomes collected from ten locations is found in the range of  $0.263\%\pm 0.018\%$  to  $1.120\%\pm 0.054\%$  w/w. The average amount of [6]-gingerol in the dried powder is  $0.641\%\pm 0.245\%$  w/w. Thus, *Z. officinale* rhizomes should contain [6]-gingerol not  $<0.4\%$  dry weight.<sup>6</sup>

## Formation of artifacts in Prasaplai preparation

During the investigation on Prasaplai formulation, three artifacts (*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**) are originated during storage of the preparation<sup>2</sup> (Figure 7). These three compounds are formed by the interaction of components in Prasaplai preparation on the first day after mixing all ingredients together. After investigating the origin of the artifacts by a systematic preparation of two-component mixtures and analysis with HPLC, it is found that the artifacts are formed from the mixture of the rhizomes of *Z. cassumunar* and the seeds of *N. sativa*. A possible explanation for this phenomenon is that the formation of artifacts may occur from an esterification reaction between alcohol ((*E*)-1-

(3,4-dimethoxyphenyl)but-3-en-1-ol or compound D) and carboxylic acids (fatty acids) in a special condition.<sup>33</sup> These artifacts continue to be formed and saturated after 73 days of storage and are stable for 1 year.<sup>34</sup>

## Standardization of artifacts in Prasaplai preparation

According to the Thai traditional way, Prasaplai can be administered by two approaches. First, it is recommended to take one teaspoon of the Prasaplai powder directly with warm water. The other method is to take it with spirit (40% ethanol). The preparation is macerated with 40% ethanol for a certain period of time and the decoction extract is taken.<sup>1</sup> In order to produce Prasaplai preparation in a more modern pharmaceutical form, its extract is used instead of the powdered drug.

## Preparation of Prasaplai extract

Tangyuengwatana and Gritsanapan studied the extraction method of traditional preparation of Prasaplai.<sup>35</sup> Hexane, 40% ethanol, and distilled water were used as extracting solvents. The target markers used in this study were compound D ((*E*)-1-(3',4'-dimethoxyphenyl)but-3-en-1-ol), (*E*)-4-(3',4'-dimethoxyphenyl)butadiene (DMPBD),<sup>36</sup> and compounds **1**, **2**, and **3**. All of these compounds were synthesized with purity  $>95\%$ .

For hexane extract, 1 g of Prasaplai preparation, which is equal to one teaspoon dose according to the medical indication, was transferred into a cotton bag and then the

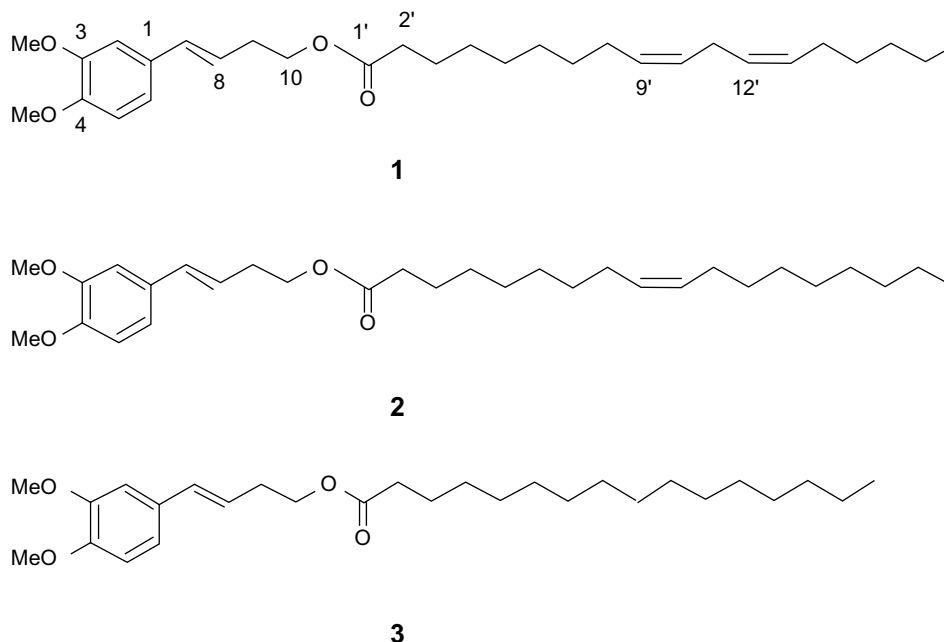


Figure 7 Structures of artifacts 1–3.

bag was sealed. The Prasapalai bag was placed in a 250 mL stopper flask and hexane (50 mL) was added. Then, the flask was sonicated for 30 minutes and the extract was decanted. The process was continued until exhausted as monitored by thin layer chromatography. The extracts were combined, filtered, and evaporated to dryness using a rotary evaporator. The weight of the crude extract was recorded.

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

## Determination of appropriate solvent for Prasapalai extraction

For each Prasapalai extract, four major standard compounds, which are compound D, DMPBD, and the three artifacts, were used to prepare the calibration curves for HPLC analysis.<sup>35</sup> The 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  $\mu$ m 100A C<sub>18</sub>, 250×4 mm column, flow rate was 0.8 mL/min, and the solvent system was a gradient elution of 1% acetic acid in water and CH<sub>3</sub>CN 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 minutes, respectively.

Linearity of each compound was determined by using five concentrations in certain ranges. Linear regression equations, correlation coefficients ( $r^2$ ), and limit of quantification are shown in Table 5. The results of regression analysis of each compound had a correlation coefficient >0.9992.

The hexane extract was prepared by sonicating Prasapalai preparation with hexane in an ultrasonic bath. After evaporation to dryness, 26.70±0.11 mg (2.7% w/w) of crude hexane extract was obtained as a brown residue. HPLC chromato-

gram showed that the hexane crude extract comprised the peaks of active anti-inflammatory ingredients, compound D, DMPBD, and three artifacts, which are compounds 1–3.

For the 40% ethanol extraction of Prasapalai preparation, the same procedure was carried out as mentioned in the previous experiment. After evaporation to dryness, 33.96±0.05 mg (3.40% w/w) of brown crude 40% ethanol extract was obtained. HPLC chromatogram showed that the 40% ethanol crude comprised peaks less than those of the hexane extract and lacked the three artifact peaks. Compound D and DMPBD still appeared in the crude 40% ethanol extract.

For the water extraction of Prasapalai preparation, after freeze drying, 49.83±0.30 (4.98% w/w) of crude water extract was obtained as a yellow residue. HPLC chromatogram showed that the water crude extract comprised mainly compound D peak and lacked the three artifact peaks.

Among the Prasapalai extracts, 40% ethanol extract seemed to be the appropriate extract for the development of modern dosage forms of Prasapalai because it contained the highest amount of major anti-inflammatory agents, compound D and DMPBD.

## Conclusion

In modern times, standardization of herbal preparation is essential for the quality of drugs because of the variation in the concentration of their active principles. Quality assessment of an herbal formulation is important to justify their acceptability in modern medicine system. Prasapalai, a popular Thai traditional medicine preparation that has been used to treat dysmenorrhea, is composed of ten herbs that need to be standardized to avoid the inconsistency of active ingredients in the raw materials. Prasapalai preparation is standardized by a modern method using HPLC. The amounts of active compounds in each raw material collected from ten different places in Thailand were evaluated and the information was recorded as a guidance. Another interesting aspect in this preparation is the artifacts, which are originated during storage of the preparation. These artifacts can be avoided by using 40% ethanol extract of Prasapalai powder, which thus changes the way of administration of Prasapalai. The standardization of Prasapalai preparation is essential for assessing the quality of the product and promoting the interest of people who suffer from dysmenorrhea.

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**Table 5** Calibration equation, correlation coefficient, and limit of quantification of standard compounds for standardization of Prasapalai preparation

Compound	Regression equation	( $r^2$ )	LOQ ( $\mu$ g/mL)	Range ( $\mu$ g/mL)
Compound D	$Y = 775997X + 4257$	0.9997	1.50	54.0–272
DMPBD	$Y = 2108049X - 31833$	0.9994	1.46	20.0–40.0
Compound 1	$Y = 105593X + 1772$	0.9992	1.10	54.0–272
Compound 2	$Y = 102038X + 700$	0.9996	0.90	37.0–188
Compound 3	$Y = 536401X + 1332$	0.9992	1.60	1.80–32.0

**Note:** Reproduced from Tangyuenyongwatana P, Gritsanapan W. Standardization and Biological Activities of Prasapalai, a Thai Traditional Medicine. *Songklanakarin J Sci Technol*. 2009;31(5):527–531.<sup>35</sup>

**Abbreviations:** DMPBD, (E)-1-(3,4-dimethoxyphenyl) butadiene; LOQ, limit of quantification.

## Disclosure

The authors declare no conflicts of interest in this work.

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