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A Phase I Study of Oral Phlai (*Zingiber cassumunar* Roxb.) Capsule in Healthy Adult Volunteers

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ABSTRACT

Zingiber cassumunar Roxb., called Phlai in Thai, is a traditional herb, has multiple bioactive properties including anti-inflammatory activity, smooth muscle relaxation, antihistamine activity, and mucin secretion lowering properties. As a result, Phlai may have the potential to treat asthma. Our phase I study aimed to study the safety profile of oral Phlai capsules in healthy adult volunteers during 12 weeks of consumption. Ten participants, aged 18-50, were prospectively enrolled. Blood samples were taken to measure complete blood count, blood urea nitrogen, creatinine, electrolyte, liver function and fasting blood sugar; urinary analyses for all, and urine pregnancy testing for female volunteers, were done. With normal lab results, the volunteers commenced taking two oral Phlai capsules once daily (200 mg/day) over 12 weeks. The same blood and urine tests were performed at 1, 2, 4, 12 and 14 weeks. The mean age (SD) of participants was 36.10 (7.26) years old. There were no laboratory abnormalities during the study and follow-up period except in one volunteer, a 25-year-old male with a slightly decreased white blood count of 3.6 x103/µl (neutrophil 39.8%) in the 1st week and 3.9 x10^3/µl (neutrophil 40.6%) in the 14th week. Four participants reported somnolence 4-5 hours after taking the drug during the first two weeks. In conclusion oral Phlai capsules (200 mg/day) for 12 weeks did not cause serious adverse events, including the laboratory abnormalities in our healthy volunteers. However, somnolence was found in 40% of the participants during the first 2 weeks and improved over time.

Keywords: Phlai; Safety profile; *Zingiber cassumunar*

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1. Introduction

Asthma is one of the most common chronic airway diseases worldwide. In the past 30 years, the prevalence of asthma has increased 2-3 times in both children and adults [1]. Asthma causes chronic inflammation and affects quality of life long-term; troublingly so, it can lead to unexpected death in acute exacerbated patients [2].

Zingiber cassumunar Roxb., known as "Phlai" in Thai, is a traditional herb in Southeast Asia, belonging to the Zingiberaceae family. The major bioactive component is compound D (Fig. 1), (*E*)-4-(3′, 4′-dimethoxyphenyl) but-3-en-1-ol, which is an anti-inflammatory, smooth muscle relaxant, antihistamine, and mucin-lowering secretion [3-6]. Preliminary studies using Phlai in child [7] and adult asthma patients [8] demonstrated positive outcomes without adverse effects. A study in rats on the pharmacokinetics of compound D revealed the absolute oral bioavailability was approximately 5%, yet the compound exerted excellent tissue distribution to several crucial organs, including the trachea and lungs, at 1 to 4 hours after dosage [9]. A safety evaluation of *Zingiber cassumunar* Roxb. extract granules showed no acute or chronic toxicity in rats [10]. Nonetheless, there is a lack of clinical toxicity information about this herbal medicine in humans.

The objective of this research was to study the safety profile of oral Phlai capsules in healthy adult volunteers after 12 weeks of regular consumption.

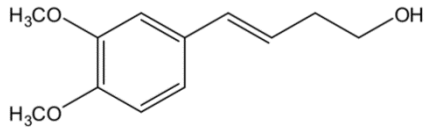

Fig. 1. The chemical structure of compound D.

2. Materials and Methods

This was a phase I study conducted in a single tertiary-care teaching hospital. Each Phlai capsule contained 100 mg of standardized Phlai extract, which is equivalent to 4 mg of compound D. The therapeutic dose of Phlai for asthma is 2 capsules (8 mg of compound D) [11]. The capsules were produced by the Quality Herbal Product Project, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Standard compound D was obtained from the Oral Biology Unit, Faculty of Dentistry, Thammasat University, Pathum Thani, Thailand. Thin-layer chromatography (TLC) was used to identify the presence of the bioactive component (compound D) in Phlai capsules, in accordance with the Thai Herbal Pharmacopoeia [12-13]. The chromatogram is shown in Fig 2. The quality, safety, and consistency of Phlai capsules were established in line with FDA standards.

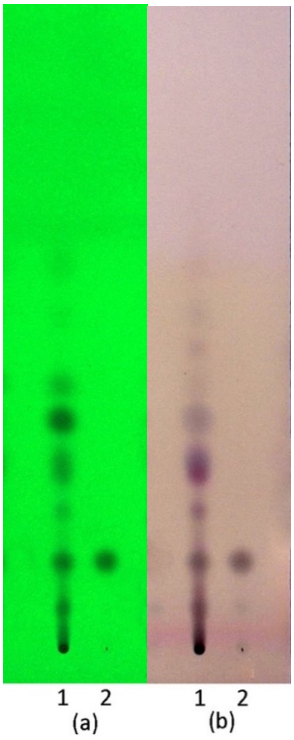

Fig. 2. Thin-layer chromatogram of Phlai capsule. Plate (a) was photographed under UV 254 nm light. Plate (b) was stained with anisaldehyde-sulfuric acid reagent and heated at 110 degrees Celsius for 10 minutes. Lanes 1-2 correspond to ethanolic extract of Phlai capsules and compound D, respectively.

Ten healthy adult volunteers were prospectively enrolled: 5 men and 5 women. The inclusion criteria were 18-50 years of age, no underlying diseases, no current medication use, body mass index less than 30 kg/m2, no prior history of smoking, and no current alcohol consumption. Exclusion criteria were pregnancy or plans to become pregnant in the next 3 months, breast feeding, abnormal initial laboratory results, and refusal to be enrolled.

The volunteers were informed about the study details, including the possible adverse effects of oral Phlai, and consent forms were signed by each volunteer. The principal investigators took the medical history and performed physical examinations (vital signs, cardiovascular, respiratory, and gastrointestinal) of each volunteer.

Ten milliliters of blood were drawn for complete blood count (CBC), blood urea nitrogen (BUN), creatinine (Cr), electrolyte, liver function test (LFT), and fasting blood sugar (FBS). Urinary analysis (UA) for all, and urine pregnancy test for female volunteers, was done. If laboratory results were all normal, volunteers commenced taking two oral Phlai capsules once daily (200 mg/day), which is the therapeutic dose for asthma as previously mentioned, for 12 weeks. During the weekdays, the volunteers were directly observed consuming the two capsules by the investigators; on the weekends, participants were relied upon to take the capsules of their own accord. Blood and urine samples were taken for laboratory tests as stated before at 1, 2, 4, 12, and 14 weeks after starting Phlai intake. We divided safety profiles into 1) hematotoxicity; shown by CBC, 2) nephrotoxicity; shown by BUN, Cr, electrolyte and UA, 3) hepatotoxicity; shown by LFT and 4) Metabolic abnormality; shown by FBS.

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medicine, Thammasat University, Thailand (IRB No. MTU-EC-IM-1-172/58) and was conducted in accordance with the Declaration of Helsinki. Informed consent from all volunteers was obtained before starting.

2.1 Statistical analysis

Data are expressed as mean ± standard deviation (SD), median (interquartile ranges), and percentage when appropriate. Continuous data differences between groups were analyzed using paired t-test. Linear mixed model analysis was used to compare laboratory results.

3. Results and Discussion

3.1 Results

The mean age SD of participants was 36.10 (±7.26) years old. The height and weight (SD) were 1.63 (±0.09) m and 62.38 (±11.22) kg, respectively. The other baseline characteristics are given in Table 1. There were no laboratory abnormalities during drug intake or the follow-up period (Table 2), except for one volunteer, a 25-year-old male, who had a slight decrease in white blood count (WBC) to 3.6 $x10^3/\mu l$ (neutrophil 39.8%, lymphocyte 44.7%) in the 1st week and 3.9 $x10^3/\mu l$ (neutrophil 40.6%, lymphocyte 42.9%) in the 14th week. Fig 3, a line graph for liver enzyme levels during the study, shows non-clinically significant increases in alkaline phosphatase (ALP) and aspartate aminotransferase (AST) at the 1st and 2nd weeks, after taking the Phlai capsules. Four (40%) participants reported somnolence 4-5 hours after taking the capsules during the first two weeks. There was one report of dry mouth 5 minutes after consumption during the first two weeks. No other adverse events were reported. All of the urine pregnancy tests of female volunteers during this study were negative.

3.2 Discussion

Two oral Phlai capsules given once daily (200 mg/day) for 12 weeks, appeared to be well-tolerated in the healthy adult volunteers: no drug interruption occurred due to adverse events; in fact, no adverse events

were reported. We used the 12-week period to examine the short-term side effects of Phlai capsules, which would help to support another study on the "efficacy of Phlai capsules in allergic rhinitis patients". The investigators plan to do a long-term study of Phlai capsules in the future. The most common reported side effect was somnolence, which improved after 2 weeks of consumption. The somnolence and aforementioned dry mouth may have arisen from potential antihistamine effects of *Zingiber cassumunar*, as was reported in guinea pig tests [3-4]

Table 1. Baseline characteristics and initial laboratory results.

Characteristic / Laboratory results (n=10)	**Number**
Gender (%)	Male (50%)
Age (mean ± SD), years	36.10 ± 7.26
Height (mean ± SD), meters	1.63 ± 0.09
Weight (mean ± SD), kilograms	62.38 ± 11.22
Body mass index (mean ± SD), kilograms/meters^2	23.17 ± 2.00
Systolic blood pressure (mean ± SD), mmHg	118.50 ± 12.91
Diastolic blood pressure (mean ± SD), mmHg	72.20 ± 8.23
Heart rate (mean ± SD), /min	82.10 ± 7.64
Respiratory rate (mean ± SD), /min	19.80 ± 0.63
Body temperature (mean ± SD), Celsius	36.54 ± 0.56

Table 2. Laboratory results after commencing Phlai capsules compares to baseline.

Week	**0**	**1**	**2**	**4**	**12**	**14**	**p-value**
Hematotoxicity							
Haemoglobin (mean ± SD), g/dL	13.87 ±1.44	13.91 ±1.43	14.03 ±1.38	13.97 ±1.29	14.26 ±1.61	14.17 ±1.20	0.016
White blood cells (mean ± SD), x10^3/µl	6.50 ±0.95	6.17 ±1.53	6.07 ±0.98	7.18 ±1.81	7.05 ±1.04	6.85 ±1.70	0.040
Neutrophils (mean ± SD), %	56.59 ±9.73	54.77 ±9.21	53.53 ±7.36	58.50 ±6.76	57.83 ±8.20	55.58 ±9.31	N/A
Lymphocytes (mean ± SD), %	32.43 ±6.61	32.57 ±7.85	33.46 ±5.67	29.89 ±6.78	30.09 ±7.23	32.18 ±7.95	N/A
Monocytes (mean ± SD), %	6.94 ±2.00	7.83 ±1.36	7.58 ±1.65	7.23 ±1.61	7.67 ±1.46	7.85 ±1.40	0.283
Eosinophils (mean ± SD), %	3.44 ±2.54	4.21 ±2.00	4.75 ±2.77	3.82 ±1.86	3.72 ±2.40	3.81 ±2.41	N/A
Basophils (mean ± SD), %	0.50 ±0.32	0.62 ±0.23	0.68 ±0.25	0.56 ±0.19	0.69 ±0.55	0.58 ±0.12	0.482
Platelets (mean ± SD), x10^3/µl	242.90 ±50.21	237.90 ±43.80	241.50 ±45.85	239.00 ±50.00	237.90 ±54.97	248.60 ±52.45	N/A
Nephrotoxicity							
Blood urea nitrogen (mean ± SD), mg/dL	11.76 ±2.01	12.34 ±1.83	13.01 ±3.13	12.06 ±2.57	11.75 ±1.99	11.20 ±1.88	N/A
Creatinine (mean ± SD), mg/dL	0.87 ±0.17	0.85 ±0.20	0.87 ±0.20	0.86 ±0.18	0.85 ±0.18	0.88 ±0.20	0.767
Sodium (mean ± SD), mEq/L	139.00 ±1.33	138.50 ±1.84	137.70 ±1.49	137.70 ±2.45	137.00 ±0.67	138.90 ±2.64	N/A
Potassium (mean ± SD), mEq/L	4.21 ±0.26	4.06 ±0.28	4.10 ±0.21	4.03 ±0.24	4.23 ±0.29	4.17 ±0.28	0.262
Chloride (mean ± SD), mEq/L	103.00 ±1.76	102.40 ±3.20	103.80 ±2.57	102.30 ±3.23	102.30 ±1.64	103.20 ±2.98	N/A
Bicarbonate (mean ± SD), mEq/L	28.44 ±1.88	28.36 ±2.49	28.56 ±2.32	28.45 ±2.13	29.04 ±2.53	28.62 ±2.23	N/A
Hepatotoxicity							

Albumin (mean ± SD), g/dL	3.95 ±0.35	4.05 ±0.26	4.07 ±0.38	3.92 ±0.27	3.91 ±0.36	3.94 ±0.32	0.138
Globulin (mean ± SD), g/dL	3.48 ±0.32	3.34 ±0.22	3.52 ±0.27	3.47 ±0.18	3.57 ±0.19	3.55 ±0.23	N/A
Total bilirubin (mean ± SD), mg/dL	0.50 ±0.19	0.59 ±0.26	0.63 ±0.18	0.71 ±0.33	0.54 ±0.17	0.58 ±0.26	0.732
Aspartate aminotransferase (mean ± SD), U/L	18.30 ±6.50	21.20 ±4.57	21.80 ±7.05	18.10 ±5.38	18.40 ±4.53	18.20 ±5.22	0.153
Alanine aminotransferase (mean ± SD), U/L	29.60 ±11.28	29.60 ±12.18	29.70 ±10.68	27.90 ±11.36	27.30 ±11.51	28.40 ±13.53	N/A
Alkaline phosphatase (mean ± SD), U/L	62.90 ±16.73	66.10 ±16.52	65.10 ±13.41	58.50 ±12.11	57.40 ±12.28	58.10 ±11.26	N/A
Metabolic abnormality							
Fasting blood sugar (mean ± SD), mg/dL	96.13 ±4.82	95.09 ±7.56	92.88 ±5.69	94.88 ±5.55	94.10 ±5.08	93.54 ±6.44	0.366

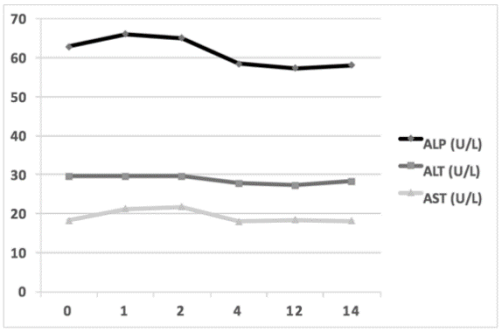

Fig. 3. The levels of mean liver enzymes during the study shows non-significant increases in ALP, and AST at the 1st and 2nd week after taking the Phlai capsules.
ALP; alkaline phosphatase, ALT; alanine transaminase, AST; aspartate aminotransferase

ALP and AST, but not alanine transaminase (ALT), levels did not significantly change during the 1st and 2nd week, yet all returned to almost baseline, or even below, at the 4th and 12th week during administration of the Phlai capsules. All levels subsequently remained normal at the 2nd week after stopping drug administration. A previous toxicity assessment in rats and monkeys had revealed that the plant might be hepatotoxic; although, this may have been due to dose irregularity within the capsules themselves [11]. However, a recent study demonstrated no liver toxicity measured in both an acute and chronic (270 days) toxicity assessment [10]. This study also suggests that a dose of 200 mg/day of oral Phlai capsules does not present any danger to healthy human livers.

The slight decrease in WBC, apparent in one volunteer, might have been non-specific, possibly from viral infection, or less likely, from the drug itself. However, we consider it most likely to be non-specific and within the range of normal variation. This assessment is based on the fact that this participant's initial WBC before taking the drug was 4.5 $x10^3$/µl (neutrophil 45.2%, lymphocyte 39.7%); his highest WBC was in the 12th week, at 6.2 $x10^3$/µl (neutrophil 60.8%, lymphocyte 21.4%). Nevertheless, more data is required to investigate this possible phenomenon.

As previously stated, the minor adverse effects, somnolence and dry mouth, may be owed to the antihistamine effect of the Phlai itself, gradually decreasing to non-appearance over time.

A limitation of this study was the small size of the study population, making it difficult to demonstrate statistical significance in laboratory deviations, as such, the study was designed to rely on clinical significance instead. Moreover, as a phase I study, it is reasonable to limit the number of participants being exposed to new drugs.

4. Conclusion

Oral Phlai capsules once daily (200 mg/day) over 12 weeks did not cause serious adverse events, including laboratory abnormalities in healthy volunteers, i.e., no aberrations in complete blood count, kidney function, liver function, and fasting blood sugar. The somnolence found in 40% of the participants during the first 2 weeks improved over time. Phlai appears to be safe for human consumption in controlled doses; however, more research is needed on potential side effects, such as lowered WBC and the previously reported hepatoxicity, on asthmatic patients with other pre-existing conditions.

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