Stability testing of a Plai extract

การศึกษาความคงตัวของสารสกัดไพล

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ABSTRACT

The stability of an extract of Plai, *Zingiber cassumunar* Roxb., one of the most popular herbs used as a topical anti-inflammatory and local soothing effect, was investigated. Rhizomes of Plai was collected, dried, pulverized and extracted by methanol. The effects of temperature Stability of Plai extract was conducted by monitoring of compound D, (*E*)-1-(3,4-dimethoxyphenyl)but-3-en-l-ol, one of the most active moieties of Plai. Factors affecting the stability of Plai extract investigated were pH, temperature and UV exposure. Plai extract was found to be fragile in aqueous solutions with a strong acidic pH at 3. At a temperature as high as 50°C, the major compound(s) of Plai extract decreased rapidly. However, UV exposure showed more reduction and potentially structural changes after 7 days. Thus, storage of Plai extract must be light-protected and kept cool at pH between 5 - 7.

บทคัดย่อ

ผงไพลสกัดด้วยเมทานอลและพัฒนาเป็นเจลสูตรเฉพาะ โดยใช้สำหรับต้านการอักเสบ เนื่องจากความคงตัวเป็นปัญหาสำคัญของพืชประเภทสมุนไพร ดังนั้นจึงทำการศึกษาความคงตัวสารประกอบสำคัญที่มีอยู่ในไพล (สารดี, ดีเอ็มพีบีดี (DMPBD, (*E*)-1-(3,4-dimethoxy phenyl) butadiene) ปัจจัยที่มีผลต่อการศึกษาความคงตัวของสารสกัดไพลมี 3 ปัจจัย คือ pH อุณหภูมิ และ แสงสว่าง ผลจากการศึกษาความคงตัวของสารสกัดไพลพบว่าทั้ง 3 ปัจจัยทำให้สารสกัดไพลมีปริมาณของสารดีลดลง จากปัจจัยของ pH พบว่า สารสกัดไพลมีความไม่คงตัวที่ pH 3 ส่วนปัจจัยของอุณหภูมิและแสงสว่างพบว่า เมื่อเก็บสารสกัดไพลที่อุณหภูมิสูง (50°C) ในที่ปลอดแสงสว่าง และที่อุณหภูมิห้อง (25°C) ในที่มีแสงสว่าง พบว่าปัจจัยทั้งสองมีผลทำให้ปริมาณสารดีลดลงอย่างรวดเร็วในเวลา 7 วัน แต่ปริมาณของสารสกัดไพลที่เก็บไว้ที่อุณหภูมิห้อง มีปริมาณสารดีลดลงมากกว่าสารสกัดไพลที่อุณหภูมิสูง ดังนั้นจึงควรเก็บสารสกัดไพลในที่ปลอดแสงสว่าง อุณหภูมิต่ำ และค่า pH 5-7

Key Words: Plai extraction, stability

คำสำคัญ: สารสกัดไพล ความคงตัว

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1.INTRODUCTION

Plai (*Zingiber cassumunar* Roxb. or *Zingiber purpureum* Roscoe., family Zingiberaceaeas) has been used as one source of herbs in Thai traditional remedies (Phongbunrod. 1965) and recently developed into topical dosage forms for anti-inflammation. It rhizome has been extracted for certain main ingredients which were identified to be *E*)-1-(3,4-dimethoxyphenyl)but-3-en-l-ol (compound D, Fig. 1), (*E*)-1-(3,4-dimethoxyphenyl)butadiene, compound D, cassumuin A, or cassumuin B Among these, compound D is the major ingredient which has shown to exert anti-inflammatory acticity (Jakchai. 2004). Various methods were used to extract the rhizomes of Plai (Ozaki et al. 1991). Most of the solvents used are hexane (Pongprayoon et al. 1997), chloroform (Amatayakul et al. 1979), and methanol. Methanol has been shown to give good yield with low irritability (Janpim et al. 2011)

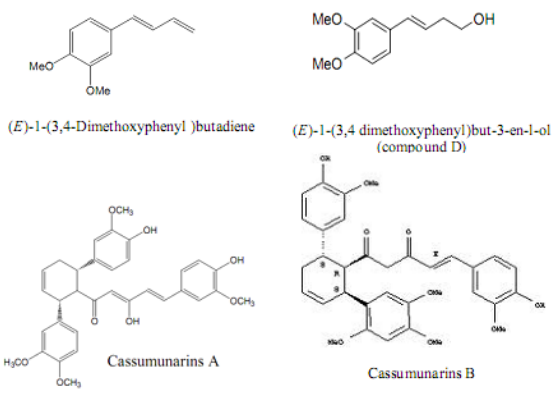


Fig. 1 Structure of components found in Plai extract with the major being (*E*)-1-(3,4 dimethoxyphenyl)but-3-en-l-ol (compound D) (Tangyuenyongwatana et al. 2009).

Anti-inflammatory activity of the methanolic extract of the rhizomes of Plai was demonstrated by several models, i.e. carragenan-induced edema rats, acetic acid-induced vascular permeability and writhing symptoms in mice (Ozaki et al. 1991). For sustainable product development of the Plai extract, it is essential to understand the effect of potential conditions which may degrade the main ingredient(s). Usually, degradation of a compound occurs depending on various factors such as temperature and pH due to reactions such as hydrolysis, oxidation, photolysis, and recemization (Nimmannit. 1991.). Herbal extracts are generally instable under various conditions. Curcumin was reported to be hydrolyzed at pH 7.2 in phosphate buffer (Ansari et al. 2005).

Since water is the most important solvent in biological system as well as in a production process, thus, the effect of water on the major compound of a Plai extract is vital for further product development for safe use. Also, light, particularly UV radiation, is the source of energy which accelerates oxidation of a compound which results in degradation. The objective of this study was to compare the factors involved in degradation of a Plai extract.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

Methanol (VWR International S.A.S, VWR, France), carbopol 940 (Merck, USA), poly(vinyl alcohol) (PVA, Sigma-Aldrich, Germany), citric acid (Analar, VWR International Ltd, England) and disodium hydrogen phosphate (Carlo Erba, Carlo

Erba Reagenti) were purchased and used as received. Compound D was extracted and separated by column chromatography as previously described (Nualkaew. 2004).

2.2 Extraction

The extraction of Plai rhizomes was conducted as previously reported (Jakchai. 2004.) Plai rhizomes were locally collected, cleaned, pulverized and dried. The dried powder was soaked in 70% ethanol for 7 days and lyophilized to 'Plai powder'.

2.3 Plai solution

A 20 mg/ml of the Plai powder in methanol was freshly prepared and diluted with deionized water which was adjusted to pH 3, 5, 7 and 9. A final concentration of about 0.4 mg/ml of the Plai powder in the aqueous solution was obtained.

2.4 Storage conditions of the Plai solution

The aqueous solutions of Plai were kept in tightly-closed amber containers at a controlled temperature of 50±2 °C in a hot air oven (ED series 7200 Tuttlingen, Germany). UV exposure was performed at a controlled wavelength range of 320 – 360 nm using a UV lamp.

2.5 UV Spectrophotometry and Validation

The Plai solutions were firstly scanned for its full range of spectra using UV spectrophotometer (Shimadzu Corporation, Japan) to identify the maximum peak which was 269 nm and used throughout the study. Within-day and between-day analysis of 6 replicates gave reliable and reproducible in a linear relationship at the concentration range of 0.09 – 0.53 mg/ml with the limit of detection at pH 3 of 0.01 mg/ml and limit of quantification at 0.16 mg/ml. The Plai solutions used for UV spectrophotometer savings and environmental pollution more than high performance liquid chromatography (HPLC) analysis.

2.6 High performance liquid chromatography (HPLC) analysis

A gradient HPLC system for determination of compound D (Jakchai. 2004) was used. In brief, the HPLC (Shimadzu Corporation, Japan) consisted in a C18 column (5 µm, 250 × 4 mm, Phenomenex, Japan) with a mobile phase (a gradient between acetonitrile and 0.2% acetic acid in water) and UV detection at 254 nm. Gradient flow of the mobile phase started from 30% acetonitrile and increased to 35, 65, 50 and 100% at 5, 10, 20 and 30 min with a constant flow rate of 0.8 ml/min.

2.7 Statistical analysis

The data are presented as the mean ± SD. Comparisons between groups were conducted by using one-way analysis of variance (ANOVA) and any significant difference was determined at the level of 0.05.

3. RESULTS

Results obtained from an *In Vitro* stability of Plai solution showed the % remaining of Plai extract concentration when compared to initials of Plai dilutions at various pH after 7 d storage at 50°C. At this accelerated temperature, the effect on the % remaining was significantly observed at pH 3 ($p < 0.001$) but not pH 5 – 9 (all $p > 0.05$).

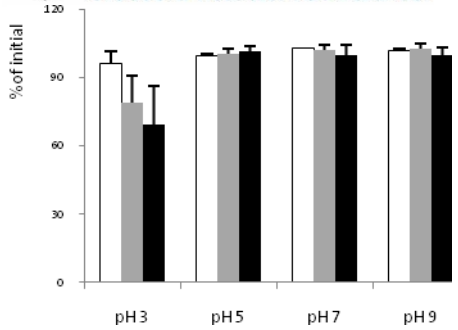


Fig. 2 Effect of storage at 50°C of Plai powder dissolved in aqueous solutions at pH 3 – 9 shown as a percentage of initial. (n = 6) White column represents 1 day after exposure, while grey columns 3 days and black columns 7 days.

UV exposure between 320 – 360 nm at an ambient temperature caused the spectrum shift of the Plai extract as shown in Table 1. At the pH ranges between 3 – 9, λ_{max} at 269 nm was all shown to be a hyperchromic shift suggesting some structural changes as a result of UV light exposure. It is noted that this effect was observed within 1 day. A further study was conducted to compare between % of initials of aqueous solutions of the Plai powder at pH 3 upon storage in 2 accelerated conditions, i.e. 25°C with UV exposure and 50°C with light-protection. The results shown in Fig. 3 suggest that both conditions have been hostile to the major compound(s) in Plai powder. Secondly, the stability of the major compounds of Plai powder was greatly affected by UV exposure than higher temperature. There was only about 10% remaining after UV exposure for 7 days. Thus, storage of Plai extract must be light-protected.

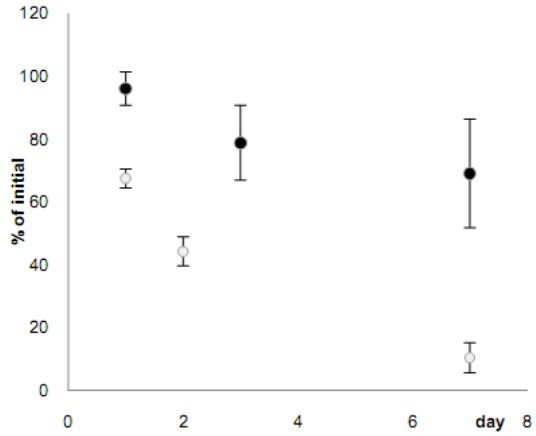
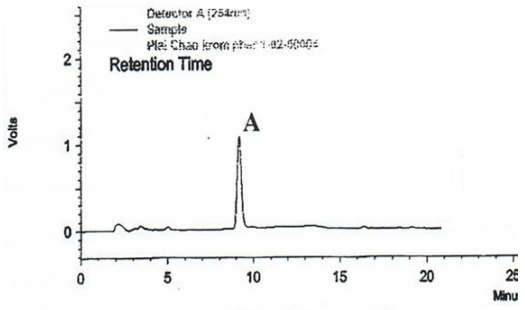


Fig. 3 Effect of storage conditions on Plai powder in aqueous solutions at pH 3; light circles kept at 25°C + UV exposure and dark circles at 50°C + light-protected; shown as percentage of initial. (n = 6)

Table 1 Effect of UV exposure (320-360 nm) on λ_{max} shift from 269 nm of Plai aqueous solutions stored in tightly-closed translucent glass containers at 25 ± 1°C. (n=6)

Storage time	pH 3	pH 5	pH 7	pH 9
1 d	+2.8	+3.8	+5	+3
2 d	+13.2	+13.2	+5.8	+10
7 d	+13.2	+17.2	+17	+17.6

(a)



(b)

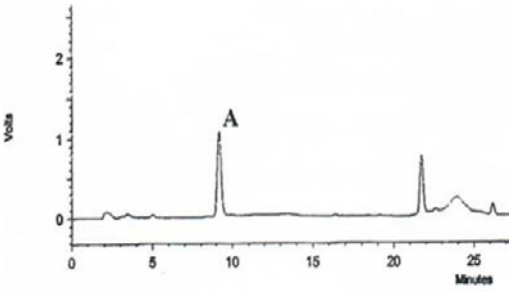


Fig. 4 HPLC chromatograms of (a) compound D and (b) Plai extract (column Phenomenex 250×4 mm, 254 nm, acetonitrile and 0.2%acetic acid in water as the mobile phase, gradient from acetonitrile 30 to 100% within 30 min).

Fig. 4 (a) shows the HPLC chromatogram of compound D at 0.8 mg/ml and compares to that of the Plai extract in (b). The retention times of the peaks of compound D was about 9 min with a peak area of about 1.5×10^{8} while that of the Plai extract showed the same retention time at 9 min and a peak area of about 1.2×10^{7} when using the same concentration for analysis. This indicates that our extraction method from the Plai rhizomes used in this study gave compound D as the major component and can be the marker for analysis.

4. Discussion Conclusions

Factors affecting the stability of Plai extract investigated were pH, temperature and UV exposure. Plai extract was found to be fragile in aqueous solutions with a strong acidic pH at 3. At a temperature as high as 50°C, the major compound(s) of Plai extract decreased rapidly. However, UV exposure showed more reduction and potentially structural changes after 7 days. Thus, storage of Plai extract must be light-protected and kept cool at pH between 5 - 7.

Acknowledgements

Thai Research Fund – Master Research Grants (TRF-MAG; MRG-WI 535S026) is thanked for the funding of the research. T.C. Pharmachem Company is thanked for scholarship through TRF-MAG and other supports.

References

Phongbunrod, S., 1965 *Maithet Muang Thai*, Kasaem Bannakit, Bangkok, pp. 373.

Pongprayoon, U., Tuchinda, P., Claeson, P., et al. 1997. Topical anti-inflammatory activity of the major lipophilic constituent of the rhizome of *Zingiber cassumunar*, Part2, Hexane extractives: *Phytomedicine*,*3(4)*: 323-26

Amatayakul, T., Cannon, J., Dampawan, P., Dechatiwongse, T., Gils, RF., Huntrakul, C., Kusamran, K., Mokkhasamit, M., Raston, C., Reutrakul, V., and White, A. 1979. Chemistry and crystal structures of some constituents of Zingiber cassumunar. *Australian Journa of Chemistry,32(1)*: 71-88

Ozaki, Y., Kawahara, N., and Harada, M. 1991. Anti-inflammatory effect of *Zingiber cassumunar.*

Janpim, K., Sakkumduang, W., Nualkaew, S., Priprem, A. 2011. Development of a Plai gel. **;** Loas Journal of Science 2: 604-607.

Nimmannit, U., 1991. *Stability test*, Chulalongkorn University, Bangkok, pp. 47.

M.J. Ansari, S., Ahmad, K., Kohli, J., Ali, Khar. 2005. R.K. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* 39: 132–138

Nualkaew S, Gritsanapan W, Tiangda C. Acute toxicity of a thai traditional preparation : Prasaplai. Mahidol University Annual Research Abstract 2004; 1: 213.

Jakchai, K. 2004. Formulation and satisfaction assessment of Prasaplai in Primary Dysmenorial Patient at Primary care center:Mahasarakham University. *A Special Project Submitted In Partial Fulfilment Of The Requirement For The Bachelor Degree Of Science In Pharmacy Faculty Of Pharmacy Mahasarakham University.*