Chemical fingerprints and anti-inflammatory activity of polar fraction from *Cajanus cajan*

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**ABSTRACT**

*Cajanus cajan* (L.) Millsp., Leguminosae or pigeon pea is traditional edible pea crop widely distributed in the northern part of Thailand. Besides it is also served as food due to its high protein content, the seed has been used traditionally for the treatment of stomatitis, gingivitis and as energy stimulant. Previous studies have demonstrated its interesting biological activities including in vitro Anti-oxidation, suppression of inflammatory cytokine production in macrophage which was relevant to the presence of certain phenolic components. In this study, pigeon pea polar components i.e. n-butanol fraction from the aqueous ethanolic seed extract was investigated for chemical constituents and in vivo anti-inflammatory activity. HPLC-DAD-ELSD chromatographic analysis of the fraction was performed using X-Terra RP18 column with 0.1% trifluoroacetic acid and acetonitrile as mobile phase. Confirmation of components was done by comparision of R_t and absorbance spectral with reference standards. Four compounds were identified as genistin, genistein, soyasaponin I and soyasaponin II. Anti-inflammatory test was performed by carrageenan induced rat paw oedema model. The effect was observed at time 1, 2, 3 h after oedema induction. The polar fraction given orally to rats at 100 and 200 mg/kg, at time 3 h showed 35% and 61 % oedema inhibiting activity, respectively while that of diclofenac at 50 mg/kg was 68 %. The results indicate therapeutic potential of pigeon pea’s polar components which confirm its traditional uses as anti-inflammatory remedy.

**Keywords:** *Cajanus cajan*, anti-inflammation, Rat paw oedema, HPLC fingerprint
1. INTRODUCTION

Pigeon pea (Cajanus cajan) is a perennial plant widely cultivated in tropical and subtropical regions of many countries as well as in the north of Thailand. Besides serving as food due to its high protein content, the seeds have been used traditionally as a tea for the treatment of inflammation and blood disorders and as diuretic and energy stimulant [1, 2]. Previous studies have demonstrated its \textit{in vitro} antioxidation and suppression of inflammatory cytokine production in macrophage [3] and hypocholesterolemic effects [4] of which polar phenolic components take the responsibilities. In order to provide scientific evidences to confirm the traditional uses of pigeon pea, we investigated on its chemical components and anti-inflammatory activity in animal model focusing on the polar constituents.

2. MATERIALS AND METHODS

\textbf{Plant material:}

Mature seeds of pigeon peas collected from Kanchanaburi province in October 2011 were dried in hot air oven at 50°C for 6 h then pulverized into coarse powder and used for extraction.

\textbf{Extraction and fractionation:} Plant powder 1.5 kg was macerated with 15 L of 70% ethanol for 72 h with occasional stirring, then filtered, the method was repeated twice. The combined filtrate, after removal of ethanol under reduced pressure afforded 206.85 g crude extract (13.79\% yield). A 30 g portion of the extract was suspended in 200 mL of 10\% aqueous methanol and partitioned with 3 x 200 mL of \textit{n}-butanol. The combined \textit{n}-butanol solution was concentrated under reduced pressure to give 4.39 g of dried extract (Bu Fr).

\textbf{High performance liquid chromatography analysis}

BuFr was dissolved in methanol (1 mg/mL) and PTFE 0.45 μm filtered, then subjected to HPLC analysis. Confirmation of components was done by comparison of R\textsubscript{t} and absorbance spectral with reference standards i.e. soy isoflavones and soy saponins from Chromadex, USA.

\textbf{HPLC condition:} Waters Pump 600, thermostated column compartment and diode array detector (DAD), X-Terra RP18 column (150 mm x 3.9 mm i.d., particle size 4 μm) were used for the analysis. HPLC column was maintained at room temperature. HPLC analysis of the extract was carried out using the conditions applied from the methods for saponins analysis previously reported [5]. The gradient mode of mobile phases with water containing 0.1\% trifluoroacetic acid (solvent A) and acetonitrile (solvent B) : 100A for 3 min, 90A/ 10B for 17 min, 80A/ 20B for 10 min, 60A/ 40B for 20 min to 10A/ 90B isocratic for 10 min was used. The flow rate was 1.0 mL/min, the injection volume was 20 μL and the chromatograms were DAD detected at 205 nm. For evaporative light scattering detector (ELSD), a probe temperature 50°C, a gain of 6.0 and nebulizer nitrogen gas of 3.3 ± 0.1 bar were used.

\textbf{Anti-inflammatory test}

\textbf{Animals:}

Male Wistar rats purchased from Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand were housed in the animal facility of Thailand Institute of Scientific and Technological Research (TISTR) under standard conditions (25 ± 2°C), 50-60\% of humidity and 12 h/12 h light/dark cycles. Food and water were allowed \textit{ad libitum}. The animals were kept under laboratory conditions for one week prior to the start of the experiment. The Animal Ethics Committee of TISTR approved all experimental protocols.

\textbf{Carrageenan induced paw oedema [6]}

Rats weighing 80-100 g were divided in groups of six: vehicle control (distilled water), positive control (diclofenac 50 mg/kg, test sample I (BuFr 100 mg/kg) and test sample II (BuFr 200 mg/kg). At the beginning of the experiment, initial paw volumes were determined using a water plethysmometer (Ugo Basil, Italy). Then, individual animal group was orally received sample accordingly. One hour after sample administration, paw oedema was induced by injection of 0.1 mL of carrageenan (\textit{$\lambda$}-carrageenan, type IV, Sigma) diluted in saline in the right hind foot pad. Paw volumes were determined at time 1, 2, and 3 h after oedema induction. The percentage of oedema was calculated as follows:

\[
\text{% Oedema} = \left( \frac{T_t - T_0}{T_0} \right) \times 100
\]

where \( T_0 \) = initial paw volume and \( T_t \) = paw volume after sample application and induced oedema. The percentage of oedema inhibition was calculated with reference to vehicle control group.
Analysis of Data

The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey’s test. *P* values less than 0.05 (*P* < 0.05) is considered significant.

3. RESULTS

HPLC analysis

HPLC chromatogram of BuFr detected under DAD at 205 nm (Fig. 1D) showed the presence of isoflavones genistin and genistein at R_t of 25.90 and 36.74 respectively as confirmed by R_t of standard isoflavones (Fig. 1A). Peaks corresponding to soyasaponin I and soyasaponin II were also observed at R_t 38.94 and 39.55 as confirmed by ELSD (Fig. 1B, Fig. 1C). Structurally, there have been no UV active chromophores in saponin molecule. Thus, DAD detector at 205 nm gave a low intensity peak of soyasaponin I and soyasaponin II while the saponin selective ELSD detector gave higher intensity peaks (Fig. 1C, Fig. 1D).

Anti-inflammatory activity

BuFr at 100 and 200 mg/kg orally given to rats exhibited significant oedema inhibitory effect at time 2 and 3 h similar to that of diclofenac 50 mg/kg (Table 1). Maximum inhibitory effects of 54% (100 mg/kg) and 64% (200 mg/kg) were observed at 2 h after oedema induction while that of diclofenac appeared to be more than 64% at time not less than 3 h. The oedema inhibitory effect of BuFr was in a dose dependent manner. The result is in accordance with its chemical profile obtained from this study as well as a previously reported on *in vitro* anti-inflammatory activity [3].

![HPLC Chromatograms](https://example.com/hplc_chromatograms.png)

Figure 1. HPLC Chromatograms of polar fraction from pigeon peas (BuFr) and reference standards.

(A) standard isoflavones, DAD 205 nm, (B) soyasaponins, ELSD, (C) BuFr, ELSD, (D) BuFr, DAD 205 nm
Table 1. Oedema inhibitory effect of polar fraction, BuFr from pigeon pea tested by carrageenan induced paw oedema in rat.

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Oedema</th>
<th>Oedema inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Control</td>
<td>31.06 ± 2.27</td>
<td>63.40 ± 7.84</td>
</tr>
<tr>
<td>Diclofenac 50 mg/kg</td>
<td>23.44 ± 1.93</td>
<td>20.92 ± 3.60*</td>
</tr>
<tr>
<td>BuFr 100 mg/kg</td>
<td>29.36 ± 3.18</td>
<td>28.59 ± 3.51*</td>
</tr>
<tr>
<td>BuFr 200 mg/kg</td>
<td>22.44 ± 3.41</td>
<td>22.45 ± 5.24*</td>
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* p < 0.05, compared with control using Tukey’s test

4. CONCLUSIONS

n-Butanol fraction from the 70% aqueous ethanolic extract of pigeon peas contained both isoflavones and saponins detected by HPLC-DAD-ELSD. Four components could be characterized from HPLC-DAD profile at 205 nm including genistin, genistein, soyasaponin I and soyasaponin II. The fraction given orally at 100 and 200 mg/kg significantly showed in vivo anti-inflammatory effect tested by carrageenan induced rat paw oedema. Our study demonstrates both chemical and pharmacological evidences to support the traditional use of pigeon peas as anti-inflammation remedies.

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REFERENCES