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Effects of Bioactive Compounds, Phytochemicals and Anti-inflammatory Inhibition of Pluchea indica Powder and Extracts

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ABSTRACT

This study investigated the bioactive components, phytochemical contents, anti-inflammatory activity, and enzyme inhibitory activities of dried Pluchea indica (L.) Less. powder and its hot water extract. The research objective was to evaluate their physical attributes, bioactive composition, phytochemical profile, anti-inflammatory potential, and contaminant levels in order to analyze their applicability for functional product development. The dried leaves were processed into powder and underwent hot water extraction. Physical parameters were analyzed, including moisture content, water activity, color, and pH. Bioactive substances were evaluated by assessing total phenolic content (TPC), total flavonoid content (TFC), anthocyanin concentrations, and DPPH radical scavenging activities. The extract displayed significantly reduced values, with certain compounds being undetectable, likely due to the thermal degradation. Phytochemical analysis revealed nine categories of chemicals in the dried powder: alkaloids, anthraquinones, carotenoids, flavonoids, glycosides, tannins, xanthones, triterpenes, and steroids. In contrast, the extract consisted of just alkaloids, xanthones, and steroids. Anti-inflammatory activity was assessed against diclofenac diethyl-ammonium. The desiccated powder exhibited notable inhibitory effect with an IC_{50} value of 0.41 ± 0.02 mg/mL, however the extract shown no quantifiable activity. The study of microbes and contaminants verified that both forms adhered to the Thai Community Product Standard TIS 480/2547. The findings indicate that dried P. indica powder may be utilized in antioxidant and anti-inflammatory functional meals and dietary supplements.

Keywords: *Pluchea indica, bioactive compounds, anti-inflammatory activity, phytochemicals, functional product*

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1. INTRODUCTION

Pluchea indica (L.) Less., known in Thailand as "Klu," is a coastal shrub in the Asteraceae family. It has been extensively used in culinary and traditional medical practices throughout Southeast Asia. P. indica has been used in folk medicine to manage many illnesses such as diabetes, inflammation, arthritis, hemorrhoids, and gastrointestinal issues. The leaves are traditionally infused into herbal tea for glycemic regulation; however, the roots and stems are used to reduce inflammation and promote wound healing (Hikmawanti et al., 2024). Traditional uses are increasingly supported by scientific research, which has confirmed the presence of a varied range of bioactive compounds. Prior studies have identified phytochemicals including thiophenes, sesquiterpenes, caffeoylquinic acids, flavonoids, phenolic compounds, alkaloids, and triterpenes. These compounds are recognized for their diverse biological activities, including antioxidant, anti-inflammatory, antidiabetic, antibacterial, and anticancer capabilities (Arsiningtyas et al., 2014; Buapool et al., 2013; Vongsak et al., 2018). This modest plant has received interest from both traditional knowledge systems and modern scientific research due to its extensive medicinal effects and nutritional worth.

In traditional Thai therapy, P. indica has been incorporated for nutritional and therapeutic purposes. The young leaves are used as fresh vegetables, cooked greens, or mixed into southern Thai curries, and valued for their slightly bitter and aromatic flavor. Dried leaves are used to produce herbal tea, with national guidelines advising a maximum intake of 5 grams per serving (Thai FDA Standard). The plant is recognized for its medicinal properties in treating diabetes, with tea being used to aid with glycemic regulation. Other traditional uses include remedies for hemorrhoids, gastric ulcers, urinary irritation, and fever.

The antioxidant components of P. indica have been thoroughly studied and are directly linked with the concentrations of total phenolic and total flavonoid content in various plant parts. Widyawati et al. (2014) evaluated significant antioxidant activity in leaf extracts, particularly when various polar solvents were applied, leading to diverse phytochemical extraction outcomes. Chiangnoon et al. (2022) discovered that stem extracts are notably rich in phenolics, with 4,5-O-dicaffeoylquinic acid recognized as a key contributor to free radical scavenging. In certain instances, P. indica extracts showed higher efficacy compared to synthetic antioxidants like BHT (butylated hydroxytoluene).

Anti-inflammatory properties of P. indica have been validated using cellular and animal studies. Buapool et al. (2013) found that leaf extracts contained nitric oxide production and downregulated pro-inflammatory cytokines in macrophage cells through suppressing the NF-κB and MAPK pathways. Srisook et al. (2012) also noted the anti-inflammatory benefits in animal experiments by reducing edema and acute inflammation. These effects were linked to the inhibition of key inflammatory enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).

The plant's antidiabetic potential has received particular interest because of its ability to block α-glucosidase, an enzyme involved in carbohydrate digestion. Arsiningtyas et al. (2014) identified caffeoylquinic acid derivatives as principal inhibitors, noting enhanced activity in compounds that had numerous caffeate groups. A clinical trial conducted by Sirichaiwetchakoon et al. (2021) with 45 prediabetic participants showed that P. indica tea significantly enhanced postprandial blood glucose levels and contributed to decreases in cholesterol and triglycerides.

Recent pharmacological research confirms that P. indica consists of numerous medically relevant compounds. Caffeoylquinic acids are linked with antidiabetic activity, flavonoids are associated with antioxidant effects, and thiophenes are noted to have antibacterial qualities. P. indica possesses nutritional value, being rich in calcium, vitamin C, beta-carotene, dietary fiber, and iron, with its medical applications. These attributes position the plant as a suitable choice for the production of functional meals, nutraceuticals, cosmetics, and health beverages.

Modern initiatives in Thai agriculture have focused on promoting the processing of P. indica to enhance its economic value. This plant consists of a wide range of potential for application in high-value commercial products through suitable extraction and standardization methods.

Research Objectives:

1. To evaluate the physical properties of dried *Pluchea indica* before and after hot water extraction.
2. To analyze the bioactive compounds, phytochemical constituents, and anti-inflammatory activity of dried *P.india powder* and its hot water extract.
3. To assess the presence of contaminants in both the powder and extract forms.

2. METHODS

Sample Preparation

Fresh Pluchea indica plants were washed thoroughly with purified water and subsequently air-dried. The dried stems and leaves were then placed in a hot air oven at 50°C for a duration of 5 hours. The dried material was ground with a herbal grinder and filtered through a 100-mesh screen to acquire a fine powder of Pluchea indica.

For extract preparation, fresh Pluchea indica stems and leaves were washed, gently air-dried on mesh trays, and subjected to thermal extraction via a steam distillation unit at a 1:1 (v/w) ratio. The procedure was performed at 100°C for 1 hour and 30 minutes to acquire the aqueous extract.

Physical Quality Analysis

Physical quality was assessed using the community product standard TIS 480/2547. The evaluated parameters included:

- Color: Measured using the R.H.S. Color Chart (London, 2015 and 2019 editions)
- pH: Determined using a pH meter (SCHOTT Lab850 model)
- Moisture Content: Analyzed according to AOAC (2019) standard methods
- Water Activity (Aw): Measured using a water activity meter (EZ-200, Japan), also per AOAC (2019)

Phytochemicals Analysis according to the method of Fiadouse et al., 2011 and Sakunpak et al., 2014

Anti-inflammatory Analysis

Anti-inflammatory properties of *Pluchea indica* were examined utilizing Chandra's method and a protein denaturation assay final concentrations of **0.25**, **0.5**, **1**, **2**, and **4** mg/mL, the reaction mixture for each test included **0.2** mL of fresh hen's egg albumin, **2.8** mL of phosphate-buffered saline (pH **6.4)**, and **2** mL of various dosages of the tested extracts diluted in Tween-**20** and **2** mL of the **2** analyzed extracts were replaced with diclofenac diethyl-ammonium to serve as the study's positive control. The reaction mixtures were heated to **70** ⍰C for **5** min in an incubator. A UV–Vis spectrophotometer was used to measure the bioactivity at **660** nm after they had cooled to room temperature. The absorbance of tested samples compared to the control was used to calculate the percentage inhibition of protein denaturation. The strength of the anti-inflammatory activity of using the IC**50** value. By plotting the percentage inhibition against sample concentration, the sample concentration for **50 %** inhibition of albumin dena turation was calculated.

Bioactivity Analysis

DPPH Radical Scavenging Activity

The antioxidant activity was evaluated utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as outlined by Jirasatid & Nopharatana (2018). A 1 mL aliquot of the sample was combined with 3 mL of a 95% ethanol-dissolved DPPH solution and left to react in the dark at room temperature for 30 minutes. The absorbance was then measured at 515 nm utilizing a UV-Visible Spectrophotometer (Shimadzu UV-1601, Japan). The % inhibition was calculated using the following equation:

$$\%DPPH\ inhibition\ =\ ((A_0\ -\ A_1)\ /\ A_0)\ \times\ 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Total Phenolic Content

The Folin-Ciocalteu technique was used to quantify phenolic content (Srisuk et al., 2021). A 1.5 mL aliquot of 10% Folin-Ciocalteu reagent was combined with 0.30 mL of the sample and 3 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark for 30 minutes. Absorbance was measured at 765 nm. Total phenolic content was quantified as gallic acid equivalents (GAE) in mg/mL.

Total Flavonoid Content

The flavonoid concentration was determined using the pH differential method as described in AOAC (2019) and Mongkontanawat & Lertnimkongkol (2015). The extract was diluted tenfold and mixed with two separate buffer solutions: 0.2 M potassium chloride at pH 1.0 and 0.4 M sodium acetate at pH 4.5. The samples were incubated for 30 minutes, and absorbance was measured at 520 and 700 nm. The flavonoid concentration was calculated using the following equation

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$$

$$\text{Total Flavonoid (mg/L)} = \frac{A \times 449.2 \times \text{dilution factor} \times 103}{26,900}$$

Values were expressed as cyanidin-3-glucoside equivalents (mg/L).

Phytochemical Constituents

Phytochemical Constituents of Pluchea indica Powder and Steam-Distilled Extract Analyzed according to High performance liquid chromatography (HPLC) (AOAC, 2019)

3. RESULTS AND DISCUSSION

The physical quality of dried Pluchea indica before thermal extraction was evaluated in accordance with Thailand's Community Product Standard (TIS 480/2547) set by the Ministry of Industry. This evaluation included moisture content, water activity, pH, color, odor, and the detection of foreign particles. Results are shown in Table 1.

Table 1. Physical quality of *Pluchea indica* powder prior to extraction, compared with the TIS 480/2547 community product standard.

Property	Standard Requirement	Measured Result
Moisture content according to the Community Product Standard (TIS 480/2547), Ministry of Industry, Thailand	Not more than 12 percent by weight	3.76±0.95

Aw	-	0.21±0.02
Color According to the Community Product Standard (CPS) No. 480/2004 by the Ministry of Industry	Must have a good color that reflects the natural appearance of the dried herb.	Light green, consistent with the natural color of Pluchea indica powder.
L* value (Lightness)	-	55.28±0.06
a	-	12.19±0.05
b	-	19.28±0.12
pH	-	6.23±0.02
General Characteristics According to the Community Product Standard TCPS 480/2547 issued by the Ministry of Industry, Thailand.	Must be dry, not clumped, and free from visible mold.	It is a dry powder, not clumped, and no visible mold is present.
Odor According to the Community Product Standard, TIS 480/2547, Ministry of Industry, Thailand.	Must have a pleasant odor characteristic of dried herbal products.	Has a pleasant odor characteristic of Pluchea indica.
Foreign Matter According to the Community Product Standard (TCPS 480/2547), Ministry of Industry, Thailand.	Must not contain any foreign matter that is not part of the herb, such as hair, soil, sand, or gravel.	No foreign matter was found in the Pluchea indica powder.

Physical Quality of Dried *Pluchea indica* Powder

The evaluation of the physical quality of dried Pluchea indica powder indicated that the material is suitable for use in many industrial sectors and conforms with the Thai Community Product Standard (TCPS 480/2547) established by the Ministry of Industry. The moisture level was found to be 3.76 ± 0.95%, which is within the optimal range for the storage of dry products. This aligns with the findings of Ibrahim et al. (2022), who noted that low-moisture Pluchea indica products effectively inhibit microbial proliferation. Chiangnoon et al. (2022) also found that dried Pluchea indica leaves, containing 10.00 ± 0.04% moisture, preserved their medicinal compounds over prolonged durations without degradation.

The measured water activity (Aw) was 0.21 ± 0.02, a value significantly lower than the microbial growth threshold. The majority of bacteria cannot grow at Aw levels beneath 0.60, while molds generally are not able to survive at Aw levels below 0.70. The low Aw found in this study indicates significant potential for microbiological stability and prolongation of shelf life. Moreover, these conditions aid in maintaining moisture-sensitive bioactive components, such as flavonoids and phenolic constituents, by minimizing the likelihood of hydrolysis and oxidation.

Color analysis indicated values of L* 55.28 ± 0.06, a* 12.19 ± 0.05, and b* 19.28 ± 0.12. The results demonstrate a natural pale green tint characteristic of the unrefined herb. The physical characteristics, encompassing texture and scent, conformed to TCPS standards, appearing as a dry, non-clumping powder devoid of visible mold and exhibiting a distinctive aroma of Pluchea indica.

The physical characteristics of the dried Pluchea indica powder were deemed appropriate for subsequent commercial advancement. The reduced moisture and water activity levels enhance prolonged storage stability, while the pH and overall physical characteristics conform to national product standards. These findings endorse the utilization of Pluchea indica powder as a prospective raw material for enhanced health goods and supplements.

Table 2. Bioactive Properties of Dried *Pluchea indica* Powder and Extract

Characteristic	Dried Pluchea indica Powder	Steam-Distilled Extract of Pluchea indica
Total phenolic content (mg GAE/g)	210.30a ± 1.12 mg/GAE	59.76b ± 0.59mg/GAE

DPPH %	21 a ±0.02 mg/ml	0.02 b±0.00 mg/ml
Total flavonoid content (mg QE/g)	187±1.34 mgQE/g	NS
Anthocyanin (mg/l)	212.10±1.25	NS

Values are means ± SD. Values with different small letters in a column are significantly different at p≤0.05.

Table 2 presents the comparison of bioactive compounds between dried *Pluchea indica* powder and heat-extracted *Pluchea indica*. The dried powder exhibited significantly higher total phenolic content (210.30 ± 1.12 mg GAE/g) than the extract (59.76 ± 0.59 mg GAE/g), a difference of approximately 3.5-fold. This finding is in line with Chiangnoon et al. (2022), who reported that powder forms of *Pluchea indica* tend to retain higher concentrations of phenolic compounds compared to extracts, likely due to the thermal degradation of certain complex or high-molecular-weight phenolics during the extraction process.

A similar trend was observed in antioxidant activity measured by DPPH free radical scavenging. The dried powder showed substantial inhibition at 21 ± 0.02 mg/ml, while the extract had a negligible value of 0.02 ± 0.00 mg/ml. The prolonged heat exposure during extraction (100°C for 1 hour and 30 minutes) may have contributed to the degradation of heat-sensitive antioxidant components. These results support the direct relationship between antioxidant activity and phenolic content in the samples.

In terms of flavonoid content, the dried powder demonstrated a significant amount of 187 ± 1.34 mg QE/g, whereas the extract showed no detectable flavonoids (NS). This may suggest that flavonoids exhibit greater thermal stability in powder form or are poorly water-soluble and thus not retained during heat extraction. Ibrahim et al. (2022) highlighted the importance of flavonoids as bioactive agents with antioxidant, anti-inflammatory, and anticancer properties. The presence of flavonoids in powdered form reinforces its value for functional food applications.

Anthocyanin content was also detected only in the dried powder (212.10 ± 1.25 mg/L) and not in the extract. Anthocyanins are natural pigments with potent antioxidant capacity but are known to be sensitive to both chemical and thermal degradation. Their absence in the extract suggests that the extraction conditions may have led to their breakdown.

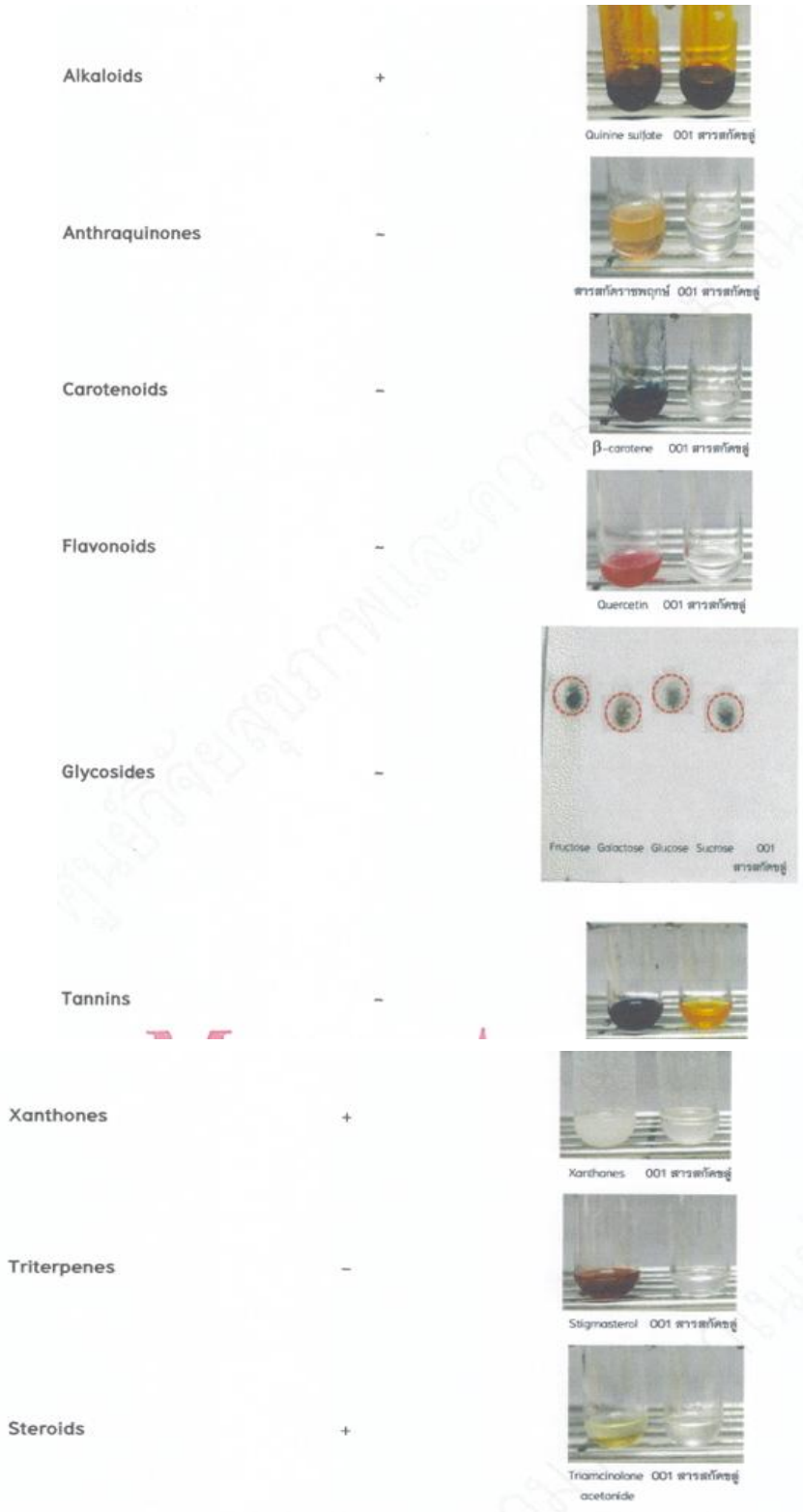
In conclusion, the bioactive compound profiles of dried *Pluchea indica* powder and its extract differ significantly. These differences may result from multiple factors: (1) degradation of heat-sensitive compounds during the extraction process, particularly due to prolonged exposure to temperature, light, and oxygen, and (2) variations in concentration due to differences in the sample state (powder vs. extract). These findings suggest that dried *Pluchea indica* powder offers higher potential as a source of bioactive compounds and could serve as a promising ingredient in the development of functional foods and nutraceutical products.

Table 3. Phytochemical Constituents of Pluchea indica Powder and Steam-Distilled Extract

Phytochemical Class	Pluchea indica Powder	Steam-Distilled Extract
Alkaloids	+	+
Anthraquinoes	+	-
carotenoids	+	-
Flavonoids	+	-
Glycosides	+	-
Tannins	+	-
Xanthones	+	+

Triterpenes	+	-
Steroids	+	-

Note: "+" indicates presence; "–" indicates absence of the compound



Phytochemical Constituents of Steam-Distilled Extract

Table 3 showcases the comparative findings of phytochemical screening between Pluchea indica powder and its steam-distilled extract. The powdered form showed a wider range of bioactive components, confirming the presence of nine phytochemical categories: alkaloids, anthraquinones, carotenoids, flavonoids, glycosides, tannins, xanthones, triterpenes, and steroids. Conversely, the steam-distilled extract exclusively exhibited alkaloids, xanthones, and steroids. This notable discrepancy where the powder maintains threefold the phytochemical variety relative to the extract likely arises from component loss during the extraction procedure.

Alkaloids, found in both forms, are recognized for their pharmacological applications, including analgesic, antihypertensive, and antitussive effects. Their stability in both forms validates their resilient chemical structure. Ibrahim et al. (2022) indicated that the alkaloids in P. indica demonstrate anti-inflammatory, antibacterial, and neurological properties, justifying their persistence after extraction. Anthraquinones were identified just in the powder and are acknowledged for their antibacterial and anticancer attributes. Ruan et al. (2018) discovered that anthraquinones in P. indica significantly suppressed bacterial and fungal proliferation. Their lack in the extract may be attributed to low water solubility and thermal deterioration during extended distillation.

Carotenoids, present exclusively in the powder, are natural pigments possessing potent antioxidant and anticancer characteristics. Buapool et al. (2013) documented elevated β-carotene levels in P. indica, consistent with the previously noted b* value (19.28±0.12) from colorimetric study. Carotenoids exhibit significant sensitivity to heat, light, and oxygen, elucidating their lack in the extract. Flavonoids were solely contained in the powder. These chemicals are crucial in antioxidant, anti-inflammatory, and anticancer processes. Vongsak et al. (2018) found several flavonoids in P. indica, including quercetin, kaempferol, and luteolin. The employed extraction method may have been inadequate for isolating these thermolabile chemicals.

Glycosides, present solely in the powder, are recognized for their ability to augment cardiac output and regulate heart rate. Arsiningtyas et al. (2014) validated the existence of caffeoylquinic acid derivatives in P. indica glycosides that block α-glucosidase. These chemicals are unstable at elevated temperatures and fluctuating pH levels, which accounts for their absence in the extract. Tannins, present exclusively in the powder, demonstrate antioxidant and anti-inflammatory properties. Ibrahim et al. (2022) highlighted their function in neutralizing free radicals. The protein-binding activity of tannins and their propensity for co-precipitation during extraction may explain their absence in the extract.

Xanthones, present in both the powder and extract, exhibited excellent thermal and aqueous stability. These chemicals have been documented to suppress nitric oxide synthesis in macrophage cells (Ruan et al., 2018), confirming their involvement in antioxidant and anti-inflammatory mechanisms. Triterpenes were solely present in the powder and are linked to immune regulation and anti-inflammatory properties. Hikmawanti et al. (2024) identified many triterpenoid chemicals in P. indica, which exhibit low solubility in water and are likely to be lost during aqueous extractions. Steroids found in both forms, indicating significant thermal and aqueous stability. Phytosterols in P. indica are recognized for their ability to lower cholesterol and reduce inflammation, positioning them as being significant for functional food innovation.

The greater variety of bioactive components preserved in P. indica powder, as opposed to the steam-distilled extract, underscores the advantages of utilizing the entire dried plant in nutraceutical applications. The powdered version retained a greater quantity of heat-sensitive and water-insoluble chemicals, rendering it a more advantageous option for forthcoming product development in functional foods and herbal therapies.

Table 4. Anti-inflammatory Activity of *Pluchea indica* Powder and Steam-Distilled Extract

Anti-inflammatory Activity	**Diclofenac diethylammonium (IC50, mg/mL)**	**Dried Pluchea Powder (IC50, mg/mL)**	**Pluchea Extract (IC50, mg/mL)**
Anti-inflammatory Activity (IC50, mg/mL) Compared with Diclofenac Diethylammonium	0.08b±0.02	0.41a±0.02	**NS**

Anti-inflammatory Activity

The study into anti-inflammatory activity revealed a notable difference between dried Pluchea indica powder and its extract form. The dried powder demonstrate significant anti-inflammatory potential, with an IC_{50} value of 0.41 ± 0.02 mg/mL. This aligns its traditional use in topical treatments for wound care or inflammation reduction. The extract showed no anti-inflammatory activity (NS). In comparison to the typical anti-inflammatory medication diclofenac diethylammonium

($IC_{50} = 0.08 \pm 0.02$ mg/mL), the dried powder demonstrated approximately 5.1 times reduced potency, while the result remains statistically significant.

Comparison with Standard Anti-inflammatory Drug

Diclofenac, a non-steroidal anti-inflammatory medication (NSAID), functions by blocking cyclooxygenase (COX) enzymes essential for the synthesis of prostaglandins implicated in inflammation. The IC_{50} value of 0.08 ± 0.02 mg/mL underscores its powerful efficacy, while NSAIDs are associated with potential gastrointestinal and renal adverse effects. The difference between the dried powder and the extract may be due to several factors: the powder may possess a more concentrated form of active chemicals, or the synergistic interaction among various compounds in the powder may augment efficacy. Heat-sensitive chemicals may have deteriorated or been eliminated during extraction.

The data indicate that Pluchea indica powder may serve as a promising natural anti-inflammatory medication, particularly for chronic inflammatory disorders such arthritis, asthma, and inflammatory bowel disease. Nonetheless, additional research is necessary to ascertain long-term safety, optimal dosage, and any drug interactions. (References: Ibrahim et al., 2022; Hikmawanti et al., 2024; Ruan et al., 2018)

Table 6. Heavy Metal and Microbial Contamination Test Results of Dried *Pluchea indica* Powder and Extract According to Community Product Standard (TIS 480/2547), Ministry of Industry, Thailand

Contaminants	Standard Criteria According to Community Product Standard TIS 480/2547	Dried Pluchea Powder (IC50, mg/mL)	Pluchea Extract (IC50, mg/mL)
Lead	Must Not Be Detected	NS	NS
Mercury	Must Not Be Detected	NS	NS
Cadmium	Must Not Be Detected	NS	NS
Yeast and Mold	Yeast and mold: Not more than 100 colonies per 1 gram sample	NS	NS
Total Microorganisms	Total microorganisms: Not more than 5×10^5	2.3×10^1	1.6×10^1

The outcomes of heavy metal and microbiological analysis demonstrate that both dried Pluchea indica powder and its extract possess suitable safety quality. No harmful heavy metals, including lead, mercury, or cadmium, were identified, in accordance with the Thai Community Product Standard (TCPS 480/2547).

The total microbial count in the dry powder was 2.3×10^1 CFU/g, while in the extract it was 1.6×10^1 CFU/g, both remaining under the permitted limit of 5×10^5 CFU/g. No yeast or mold was identified. This indicates that the production and storage processes were suitable and complied with the standards of TCPS 480/2547.

4. CONCLUSION AND RECOMMENDATIONS

The study suggests that dried Pluchea indica powder possesses significant potential for application in functional meals, cosmeceuticals, and health supplements. It encompasses a variety of bioactive components, demonstrates antioxidant and anti-inflammatory effects, and offers superior phytochemical characteristics compared to the extract form. Currently, Thailand's food and drug rules authorize only hot water extracts from Pluchea leaves in specific cosmeceutical products.

Despite exposure to elevated temperatures and prolonged extraction durations, these hot water extracts continue to preserve essential bioactive components, including alkaloids, xanthones, and steroids. Furthermore, both the powder and extract were determined to be devoid of heavy metal and microbiological contamination, thereby complying with the safety standards of the Thai Community Product Standard (TCPS 480/2547).

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