

Nutrition, Health Benefits and Applications of *Pluchea indica* (L.) Less Leaves

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Abstract

Pluchea indica (L.) Less (“Khlu” in Thai) is one of the most well-known indigenous medicinal plants of Southeast Asia and other warm climate countries. In Thailand, Khlu, traditionally used as folk medicine, is used for the treatment of kidney stones (stem), hemorrhoid (bark), inflammation, lumbago, and leucorrhoea (leaves). Consumption of Khlu leaves as a culinary herb offers significant health-promoting compounds, such as, dietary fiber, calcium, β -carotene, chlorogenic acid, caffeic acid and quercetin. Khlu tea has been commercially available in Thailand as a health-promoting drink. To date, several studies have revealed that both aqueous and alcohol extracts from Khlu leaves show high efficacy in the inhibition of lipid oxidation, scavenging of free radicals, and reduction of ferric ion. Furthermore, pharmacological activity of alcohol extracts of Khlu leaves have demonstrated anti-inflammatory, antinociceptive, and anti-tuberculosis properties. In addition, a recent study reported that the crude aqueous extract of Khlu leaves could potentially be used as a new anti-cancer agent due to its ability to inhibit the proliferation and migration of human brain malignant glioma cancer cells (GBM8401) and human cervical cancer cells (HeLa cells).

Keyword: *Pluchea indica* (L.) Less, Leaf, Khlu, Nutrition, Antioxidant activity, Anti-cancer agent

INTRODUCTION

Pluchea indica (Linn.) Less (“Khlu” in Thai) is taxonomically classified in the family Compositae (Asteraceae), and is also known by the names of Indian marsh fleabane or camphorweed, Kuo bao ju (Chinese), Munjhu rukha or Kukrakonda (Bengali), and Beluntas (Bahasa)¹. “Khlu” is a perennial shrub plant with small branches (0.5-2 m tall) widely found not only in the coastal line of Thailand but also in warm temperature regions of countries such as Malaysia, Indonesia, Australia, Taiwan, India, and Mexico². The Khlu leaves are described as simple, sessile, glabrous, obovate, serrated with an acute apex. Khlu leaves are 1-5 cm wide and 2-9 cm long^{3,4}. Flowering of Khlu occurs at terminal or axillary corymbs. Each rachilla is composed of violet/white flowers which

bear tiny fruits (cylindrical shape and about 0.7 mm long)².

Nutritive value of Khlu leaves

Khlu leaves possess a natural sweet taste and astringent flavor⁵ similar to other wild edible vegetables in Southeast Asia. Raw or blanched Khlu leaves are consumed as a side dish with Nam Prik (freshly made chili paste). In addition, Khlu leaves are often used as one of the ingredients in several local dishes such as yum (sour and spicy salad) and kang ped (spicy coconut milk soup)⁵⁻⁶.

The significant nutrients of Khlu leaves and two other native leafy vegetables normally used in several Thai dishes are given in Table 1. Indian mulberry leaves are clearly a good source of dietary fiber, calcium and β -carotene. The nutrient profiles

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of Khlu and Holy basil leaves are similar but Khlu leaves contain 7X more calcium and 2X more β -carotene. One hundred grams

of Khlu leaves have 251 mg of calcium, near the 297 mg Ca found in one serving (8 oz.) of 2% fat milk⁷.

Table 1. Nutritive values of Khlu leaves, Holy basil leaves and Indian mulberry leaves⁵

Nutrients	Khlu leaves (<i>Pluchea indica</i> (L.) Less)	Holy basil leaves (<i>Ocimum sanctum</i>)	Indian mulberry leaves (<i>Morinda citrifolia</i>)
Water (g/100 g)	87.53	86.83	76.56
Protein (g/100 g)	1.79	4.05	4.71
Fat (g/100 g)	0.49	0.48	1.42
Ash (g/100 g)	0.20	5.16	1.97
Insoluble dietary fiber (g/100 g)	0.89	0.86	3.15
Soluble dietary fiber (g/100 g)	0.45	0.42	1.19
Total dietary fiber (g/100 g)	1.34	1.28	4.34
Carbohydrate (g/100 g)	8.65	2.50	11.0
Calcium (mg/100 g)	251	32	470
β -Carotene (μ g/100g)	1,225	812	45,784
Vitamin C (μ g/100g)	30.17	27.94	3.48

Since Khlu can naturally grow in wet saline habitats, it may contain high amounts of sodium (Na) and chloride (Cl) due to osmotic effects and soil salinity⁸. Therefore, over consumption of Khlu leaves during long periods of time may present a health hazard, especially for individuals suffering from hypertension and cardiovascular disease.

Bioactive compounds of Khlu leaves

Bioactive compounds are defined as secondary metabolites of plants which typically occur in small quantities in foods⁹ but are highly beneficial to health and are physiologically active when consumed¹⁰. Bioactive compounds are also known as

“Phytochemicals” (“phyto” meaning plant). Examples of typical phytochemicals found in fruits and vegetables are terpenoids, phenolic compounds, glucosinolates and chlorophylls, etc.

Phytochemical fractionation studies have revealed that plant extracts obtained from the genus *pluchea* are comprised of phenolic acids, flavonoids, tannins, monoterpenes, triterpenoids, eudesmane-type sesquiterpenoids, chalcones, phenylpropanoids, benzenoids, ligand glycosides and steroids^{4,11}. The concentration of phenolic acids, flavonoids, anthocyanins and carotenoids found in Khlu and similar culinary leaves are shown in Table 2.

Table 2. Bioactive compounds of Khlu leaves, Hoary basil leaves and Indian mulberry leaves¹²

Bioactive compounds	Concentration (mg/ 100 g fresh weight)		
	Khlu leaves (<i>Pluchea indica</i> Less)	Hoary basil leaves (<i>Ocimum americanum</i> L.)	Indian mulberry leaves (<i>Morinda citrifolia</i>)
Total phenolic acids	28.48±0.67	2.23±0.06	3.24±0.09
Chlorogenic acid	20.00±0.24	0.32±0.01	2.31±0.05
Caffeic acid	8.65±0.46	2.03±0.06	nd
Ferulic acid	nd	0.16±0.00	0.76±0.04
Total flavonoids	6.39	7.22	33.42
Quercetin	5.21±0.26	1.89±0.10	23.67±1.62
Kaempferol	0.28±0.02	2.47±0.18	9.75±0.69
Myricetin	0.09±0.03	nd	nd
Luteolin	nd	2.12±0.05	nd
Apigenin	nd	0.74±0.04	nd
Total anthocyanins	0.27±0.01	0.11±0.01	1.12±0.04
β-carotene*	1.70±0.05	1.56±0.20	0.33±0.00
Total carotenoids	8.74±0.34	7.35±0.45	3.28±0.21

nd = not detected above limit of detection;

*Concentration of β-carotene is expressed in term of mg βCE/ 100 g fw; βCE=β-carotene equivalents.

In general, chlorogenic acid, caffeic acid and quercetin are found in fruits, vegetables and grains. Khlu leaves also contain significant amounts of chlorogenic acid, caffeic acid and carotenoids. Major flavonoids in Khlu leaves are quercetin and kaempferol, which are found in much higher concentration in Indian mulberry leaves. As expected, all three green leafy herbs contain low amounts of anthocyanins.

Phenolic compounds have been proven to be protective or potentially protective agents against many fatal illnesses and chronic diseases, especially cancers. For instance, it was reported that chlorogenic acid could reduce methylazoxymethanol acetate-induced colon and liver carcinogenesis in hamsters¹³. Caffeic acid has anti-proliferative effects on cervical cancer cell lines¹⁴. The preclinical model studies have shown that quercetin and kaempferol can inhibit prostate cancer¹⁵ and ovarian cancer¹⁶, respectively, by acting as a chemo-preventive agent. It has also been demonstrated that carotenoids, β-carotene,

and, anthocyanins can prevent cancers, promote immune systems, and help maintain good health of vital organs such as heart, blood vessels, and liver⁹⁻¹⁰.

Antioxidant activity of Khlu leaves

Antioxidant activity of plant extracts are of great interest for medical science since oxidative stress is now considered as a critical factor in the pathogenesis of various diseases, including cancer and cardiovascular disease⁹⁻¹⁰. Reactive oxygen species (ROSs) are free radicals and that can readily cause severe damage on DNA, proteins and lipids in human cells. This action probably initiates and accelerates the progression of carcinogenesis and abnormal vascular cell proliferation⁹. Dietary phytochemicals can eliminate ROSs via several mechanisms, including stimulating the immune system, chelating transition metal, modulating gene expression and hormone metabolism¹⁰.

In 2010, the antioxidant activities of Khlu leaves and other ten leafy vegetables,

grown in Indonesia, were determined by four principal methods: DPPH[•] scavenging, ABTS[•] Trolox equivalent antioxidant capacity (TEAC), ferric reducing power, and inhibition of lipid oxidation¹⁷. ROSs are highly reactive so that the synthetic free radicals such as DPPH[•] and ABTS[•] are commonly used to determine the ability of an antioxidant to scavenge these free radicals. Additionally, ABTS[•] is more suitable for measuring activity of hydrophilic and lipophilic antioxidants while DPPH[•] is suggested for use in hydrophilic antioxidants¹⁸. In many antioxidant activity assays, Trolox, a water soluble derivative of vitamin E, is used as a standard antioxidant, generally expressed as micromole Trolox equivalent ($\mu\text{mol TE}$) per weight of sample. Therefore, a sample with a higher TEAC value has a greater antioxidant activity. Ferric reducing power is an indirect measurement of antioxidant activity; a compound with a high reducing power possesses good anti-oxidation capacity. Nonetheless, the measurement of antioxidant activity by determination to its ability to

inhibit lipid peroxidation is more related to human health, since the lipid peroxidation is the main effect of oxidative stress and the cause of oxidative damage in living cells¹⁹. Generally, the ability of an antioxidant to inhibit lipid oxidation is often expressed in terms of inhibition of TBARs formation in the control (without addition of antioxidant). The term "TBARs" stands for thiobarbituric acid reactive substances. The TBARs value indicates the occurrence of products of lipid peroxidation which are subsequently reacted with thiobarbituric acid. Antioxidant activity, total phenol and total flavonoids contents of Khlu leaves, hoary basil leaves and sweet leaves are shown in Table 3. Interestingly, Khlu leaves exhibited the highest efficacy on inhibition of linoleic acid oxidation, DPPH[•] scavenging, and ferric reducing power regardless of amount of phenols and flavonoids. This finding simply indicates that there are other phytochemical compounds, also present in Khlu leaves, which possess good antioxidant activity.

Table 3. Total phenols, total flavonoids and antioxidant activity of Khlu leaves, Hoary basil leaves and sweet leaves¹⁷

Antioxidant activity	Khlu leaves (<i>Pluchea indica</i> Less)	Hoary basil leaves (<i>Ocimum americanum</i> L.)	Sweet leaves (<i>Sauropus anrogynus</i> (L) Merr.)
Total phenols (mg GAE/ g fw)	0.831±0.129	0.812±0.119	1.490±0.15
Total flavonoids (mg / g fw)	6.39±0.27	7.22±0.36	143±6
DPPH ($\mu\text{mole TE/ g fw}$)	96.4±15.2	23.8±1.3	7.72±0.88
ABTS ($\mu\text{mole TE/ g fw}$)	3.75±0.16	1.94±0.05	1.81±0.08
Ferric reducing ($\mu\text{mole TE/ g fw}$)	81.1±0.6	47.9±0.7	70.6±1.0
% Inhibition of lipid peroxidation*	98.5±0.4	97.0±0.2	84.7±0.2

GAE = Gallic acid equivalent; TE = Trolox equivalent; Trolox is the water soluble derivatives of vitamin E.

* Lipid peroxidation is determined in linoleic acid emulsion.

Application of Khlu leaves as a medicinal herb

In Asia, Khlu is commonly used for local food and folk medicine. Khlu is used as

a natural medicine in three forms: decoction, poultice and infusion. Table 4 shows some typical traditional medicinal uses of Khlu in Thailand, India and Indonesia.

Table 4. Summary of traditional uses of “Khlu” in Thailand, India and Indonesia

Country	Medical part (form)	Reported medicinal or traditional uses
Thailand	Stem (decoction)	Treatment of kidney stones (diuretic agent) ^{3-4,11}
	Bark (decoction)	Treatment of hemorrhoid ^{4,11}
	Fresh leaf (poultice)	Treatment of gangrenous ulcer ^{3,11}
	Leaf (infusion/decoction)	a nerve tonic ^{3, 11} Treatment of inflammation, lumbago and leucorrhoea ²⁰
India	Root (decoction)	astringent and antipyretic ²¹
Indonesia	Leaf (infusion/decoction)	appetite stimulant, anti-diaphoretic, antipyretic, digestive aid, deodorant, antibacterial, anti-diarrhea, antitussive, emollient. ^{12,17}

Decoction

Decoction and infusion are used to extract water soluble components from hard and soft plant materials, respectively. In general, infusion contains more aromatic flavors of volatile ingredients but is lower in total soluble solids content than decoction. For decoction, fresh herbs (stem, bark, and root) should be simmered for 30 min or more²². Some key considerations for preparation, quantities and times from Khlu are as follows,

1. Fresh Khlu should be sliced (not ground) prior to decoction²². According to Thai crude medicine, the suggested amount of water for decoction is about 3-5 fold of total weight of herbs²³. Nonetheless, for easy preparation at home, one small handful of fresh Khlu leaves should be simmered with 1-1.5 cups of water.
2. The simmering step of decoction is considered to be adequate for medical purposes when the color change of decoction by heating time is not noticeable²². For Khlu decoction, heating for 30-45 min is normally required²³. This should be followed by filtration over 3

layers of cheese cloth while it is hot. Any sediment found in the decoction after cooling can be mixed back into the liquid portion by gently shaking it several times prior to be used as a remedy²².

3. The common prescription for a treatment with medicinal decoctions is to drink only half a cup (ca. 75 mL) three times a day, preferably one half hour before each meal²²⁻²³, especially in case of an acute health problem²³. When the symptoms of the health problem disappear or if no change is seen after three days, the decoction should be discontinued²².

Infusion²²

Traditionally, there are two kinds of infusions based on preparation temperature: hot and cold. The hot infusion is made by pouring warm or hot water over dried leaves and steeping the infusion in a covered container for 15-30 min, or longer if the infusion is to be used cold. A good example of hot infusion is placing a tea bag or tea ball in a cup of hot water and allowing it to steep a couple of minutes before drinking.

The cold infusion is used when any active compound of herb is heat sensitive. It is simply prepared by soaking the herb in cold water or milk for several hours.

Khlu “Tea”

Around the world, Khlu leaves and numerous leafy herbs, such as rosemary, peppermint, mulberry, lotus, mate, persimmon, bamboo, lemongrass, are commercially available as “teas” and commonly consumed as an infusion²⁴. It is well established that consumption of certain herbal teas especially green tea in adequate quantity and with suitable frequency and proper preparation helps promote human health, by boosting immune system, and lower the risk of certain chronic diseases such as allergy, anxiety, arteriosclerosis, depression, dyslipidemia, headache, hypertension, hypoglycemia, intestinal disorders, insomnia, muscle cramps, sinus, etc^{22,24-25}. Nonetheless, there has been some concern regarding the misuse of Khlu as one of the main ingredients of commercial herbal teas for weight reduction, due to its diuretic property²⁶. This effect is only temporary, because the hypothalamus will soon initiate the feeling of thirst. The retail price of commercial Khlu tea in Thailand ranges between US \$ 2-6/100 grams.

Guidelines for Khlu tea production²⁷

1. Remove all blemished leaves from the freshly plucked leaves, then thoroughly clean and air dry at ambient temperature.
2. Use a sharp knife to cut leaves into a narrow band, 0.5 cm in width. (optional step)
3. Blanch for 3-5 min in almost boiling water to fix green color, and subsequently dip in cold water to avoid overheating.
4. Remove excess water by using a low speed centrifuge (such as a salad spinner) then place on a perforated stainless tray or plastic bucket for 10-15 minutes.

5. Roast on a heated aluminum pan for 30 minutes, constantly turning the leaves upside down by hand. This is a very important step for the manufacture of a high quality Khlu tea. Basically, proper heat treatment contributes four essential benefits to the quality of Khlu tea as follows;
 - i Inactivation of heat resistant deteriorative enzymes such as oxidative and hydrolytic enzymes,
 - ii Reduction of microbial load,
 - iii Formation of desirable thermally generated aroma and flavor compounds, and
 - iv Enhancement of phytochemical compounds extractability by disruption of cell membranes and cell walls²⁸.
6. Dry in a hot air oven (or a solar dryer). In order to inhibit growth of spoilage micro-organisms in “tea” the final moisture content should not be higher than 10%.
7. Pack in a food grade container (~5 grams/tea bag) and properly seal.

Special notes for Khlu tea

1. In Thailand, according to the Notification of Ministry of Public Health No. 280 (B.E.2547), Khlu is not included in a group of allowed herbs (18 kinds) to be used as raw materials in herbal tea production²⁹. Therefore, it is strongly recommended to use a product name such as “Khlu tea” instead of “Khlu herbal tea”.
2. It is wise to purchase Khlu tea from reliable manufactures such as local community herbal product producers subsidized by BEDO, Thailand Government Organization, for the highest quality and safety due to their tea productions compliance with cGMPs (current good manufacturing practices).

3. Even though the estimated shelf-life of tea is about one year, the degradation of bioactive compounds progresses with storage time. It is suggested to buy enough to consume within 4 months.
4. A common good storage practice is to keep Khlu tea in an air-tight and opaque container to avoid gaining moisture from atmosphere and to retard auto-oxidation. Additionally, storage in a cold and dry place will greatly help preserve bioactive compounds of Khlu tea.
5. A visual indicator of the deterioration of Khlu tea is the change to a yellowish color. The fading of green color is due to the loss of chlorophyll pigments by oxidation, so the yellow color of carotenoid pigments within Khlu tea are more noticeable.
6. Brewing temperature is definitely one of the significant parameters which not only provides good sensory attributes but also insures high efficacy of extraction/preservation of bioactive compounds from tea or herbal tea. Traditionally, indigenous people living in the coastal line of Thailand brew Khlu leaves (whole; fresh or dried) in boiling in water for 30 minutes²³.
7. Drinking Khlu tea in large amounts will increase the feeling to urinate, since Khlu leaves contain diuretic agents.

Recent update on pharmacological activities of extracts from Khlu leaves

A recent search on ScienceDirect database by using selected keywords (pluchea

+indica+antioxidant) in the month of September 2014 returned 8 research papers related to antioxidant and pharmacological activity of *Pluchea indica* (L.) Less. Only two scientific research papers were related to pharmacological studies on aqueous extracts of leaves. The pharmacological studies of the aqueous extracts of Khlu leaves are of great interest since nowadays, as mentioned before, the consumption of Khlu tea is widespread in Thailand as a health promoting drink. Thus, only a brief commentary of these research papers is provided for this review as following.

Srisook and others (2012) prepared a hot water extract from Khlu herbal tea (HWEP) by boiling commercial Khlu herbal tea powder in distilled water at a ratio of 1:5 for 30 minutes. The extract was subsequently concentrated and spray dried prior to analysis. It was reported that HWEP exhibited antioxidant and anti-inflammatory activities³⁰. Based on DPPH scavenging assay, HWEP showed greater antioxidant activity than positive control BHT but slightly less than ascorbic acid. Furthermore, HWEP (25-400 µg/mL) showed potent inhibitory effects against lipopolysaccharide-induced nitric oxide (NO) and prostaglandin E₂ (PGE₂) production in RAW 264.7 macrophages.

Cho and coworkers (2013) studied the anti-cancer effect of crude aqueous extracts of Khlu dry powder (leaves or root). They used distilled water (75-80 °C) as an extraction media in the ratio of 1:8 by wt. The obtained crude extracts were filtered and subsequently freeze-dried. Based on cell proliferation and viability assays, crude aqueous extraction of Khlu leaves (100-300 mg/mL) showed good suppression on proliferation and viability of GBM8401 and Hela cancer cells³¹.

Table 5. Pharmacological activities of non-aqueous extracts from Khlu leaves

Pharmacological activities	Extraction method	Type of study
Anti-inflammatory ³² , Antinociceptive ³²	Cold maceration in 70% ethanol, 2 days	animal models
Anti-tuberculosis ³³	Maceration in 80% Methanol, 7-14 days, 25-30°C	<i>In vitro</i> : Tetrazolium bromide microplate assay (TEMA)
Anti-inflammation ³⁴	Ethyl acetate fraction of ethanol extract	Macrophages RAW264.7 and animal models

CONCLUSIONS

There is considerable evidence that Khlu leaves contain several significant nutrient constituents and bioactive compounds. However, over consumption of Khlu leaves as a culinary herb or tea could pose a health hazard to some individuals due to their diuretic properties and high sodium content. Nonetheless, recent pharmacological studies have demonstrated that aqueous and/or alcohol extracts from Khlu leaves possess important pharmacological activities such as anti-inflammatory, antioxidant, antinociceptive, anti-tuberculosis and anti-cancer activities.

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