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Review Article

Botany, uses, phytochemistry and pharmacology of selected *Etlingera* gingers: A review

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ABSTRACT: *Etlingera* species are large ginger plants which grow in clumps. Their rhizomes are subterranean, creeping and aromatic. Crushed leaves of each species emit a distinctive scent. Inflorescences are borne on erect stalks protruding from the ground (*Phaeomeria* group) or are found at the soil level (*Achasma* group). The colourful flowers and leaves of *Etlingera* make them very attractive plants. The World Checklist of Selected Plant Families has documented 100 accepted names of *Etlingera* species. In Peninsular Malaysia, 15 *Etlingera* species have been recorded. In Java, Indonesia, nine species are known, and Borneo has 42 species of which 16 were until recently unknown. Three species are reported in China. The various species of the genus are used as foods, spices, condiments, medicines and ornaments. *Etlingera elatior* (torch ginger) is the best known and most studied species. In this review, the current knowledge on the botany, uses, phytochemistry and pharmacology of leaves, inflorescences and rhizomes of five selected *Etlingera* species is reviewed. The species included *Etlingera elatior*, *Etlingera fulgens* and *Etlingera maingayi* of the *Phaeomeria* group, and *Etlingera littoralis* and *Etlingera rubrostriata* of the *Achasma* group.

KEYWORDS: Zingiberaceae, phenolic compounds, essential oils, bioactivities

INTRODUCTION

Etlingera Giseke of the tribe Alpinieae and family Zingiberaceae are tall forest plants, reaching 8 m in height and dominating gaps of disturbed forests.[1–3] Rhizomes are subterranean, creeping and aromatic. When crushed, the leaves emit a scent that is distinctive for each species. Inflorescences are borne on stalks protruding from the ground in the *Phaeomeria* group, or are found at the soil level in the *Achasma* group.[4,5] The recent inclusion of the *Achasma*, *Nicolaia* and *Geanthus* groups under *Etlingera* has been verified using phylogenetic analysis based on DNA sequencing.[6,7]

In the World Checklist of Selected Plant Families developed by the Royal Botanic Gardens at Kew, England, a total of 100 names of *Etlingera* species have been accepted.[8] In Peninsular Malaysia, 15 *Etlingera* species have been recorded.[5] In Java, Indonesia, nine species are known.[2] Borneo has 42 species of which 16 are new.[3] Three species (one endemic and one introduced) have been reported in China.[9]

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Botanists are continuing their work on the botanical systematics of *Etlingera* species. Three new species from northern Borneo have recently been described.[10] They are *Etlingera rubromarginata* (from Sabah, Sarawak and Brunei), *Etlingera belalongensis* (from the Temburong District of Brunei), and *Etlingera corrugata* (presently only known from Danum Valley, Sabah). From Sarawak, *Etlingera kenyalang*[11] and from Central Kalimantan, *Etlingera palangkensis*[12] have also been reported.

The most sensational story about the systematics of *Etlingera* is the description and publication of the same species on the same day. The species, found in both Peninsular Malaysia and Southern Thailand, was named *Etlingera terengganuensis* by C.K. Lim in Folia Malaysiana,[4] and *Etlingera corneri* by J. Mood & H. Ibrahim in Nordic Journal of Botany.[13] This evoked a publication to resolve the problem by K. Larsen who gave a brief background of the two papers and reasons why *E. corneri* is the future correct name.[14] The publication strongly recommended

Figure 1. Inflorescences (left) and plants (right) of *Etlingera elatior*.

that all botanists should work together for better understanding of the diverse Malesian flora. Such collaboration should be open, honest and with mutual respect for other researcher's work.

Plants of *Etlingera* have various traditional, local and commercial uses. In Sabah, Malaysia, the hearts of young shoots, flowers and fruits are consumed by indigenous communities as spice and condiment, eaten raw or cooked.[15] In Thailand, the fruits, young stems and flowers are eaten as salad and vegetable.[16] The colourful flowers and leaves of *Etlingera* make them very attractive plants. In recent years, they have gained popularity as ornamentals in parks and gardens.[4] There are very few reports on the use of rhizomes of *Etlingera* with the exception of *Etlingera punicea* rhizomes, which are used as spice ingredient for noodles and curries in the Chantaburi province of Thailand.[17]

One species in the genus, *Etlingera elatior* or torch ginger has commercial importance and is the best known and most studied. In this review, the current knowledge on the botany, uses, phytochemistry and pharmacology of leaves, inflorescences and rhizomes of five selected species of *Etlingera* is reviewed. The species included *E. elatior*, *E. fulgens* and *E. maingayi* of the *Phaeomeria* group, and *E. littoralis* and *E. rubrostriata* of the *Achasma* group.

BOTANY AND USES

Etlingera elatior

Synonyms: *Alpinia elatior*, *Elettaria speciosa*, *Nicolaia elatior*, *Nicolaia speciosa*, *Phaeomeria speciosa*.

Etlingera elatior (Jack) R.M. Sm. grows up to 5–6 m tall forming clumps.[1] Its aromatic rhizomes are stout (3–4 cm in diameter) and found just below the ground level. When crushed, the leaves emit a pleasant sour fragrance. The leaves are entirely green with a truncated base (Figure 1). Young leaves are sometimes flushed pink with petioles 2.5–3.5 cm in length. The species is native to Peninsular Malaysia, Southern Thailand and Indonesia but is cultivated throughout the tropics.[18] Inflorescences, which are shaped like a spear-head when young, are large and attractive with showy pink or red waxy bracts when in blossom. Fruiting heads are globular, greenish or red in colour, bearing many black seeds.

Young inflorescences of *E. elatior* are a common ingredient of sour curry dishes in Peninsular Malaysia.[18] It is also used in local rice dishes in the east coast. A decoction of the fruit is used traditionally to treat earache while leaves are applied for cleaning wounds.[19] Mixed with other aromatic herbs in water, the leaves are used by post-partum women for bathing to remove body odour. Farms in Australia and Hawaii are cultivating the species and selling its inflorescences as cut flowers on a commercial basis.

Etlingera fulgens

Synonyms: *Hornstedia fulgens*, *Phaeomeria fulgens*, *Nicolaia fulgens*.

Etlingera fulgens (Ridl.) C.K. Lim can be recognised by its shiny undulate dark green leaves.[1,4] The underside of young leaves is bright red, turning greenish on maturing (Figure 2). In older leaves, only the petiole and midrib are red.[1] The petioles are 1.5–2 cm in length. Rhizomes (up to 3 cm in diameter) occur just below the ground. The plant can grow up to 4–5 m tall. Crushed leaf sheaths emit a pleasant sour fragrance similar to that of *E. elatior*. Inflorescences are raised above the ground, blood red with incurved bracts.[4] Infructescences are globular with bulbous fruits. Distributed in Southern Thailand and Peninsular Malaysia, the species occurs in the lowlands and at higher altitudes.

Figure 2. Plants of *Etlingera fulgens* with bright red leaves at the underside.

Because of its attractive red colour of the young leaves and its blood red flowers, *E. fulgens* is gaining popularity as an ornamental in landscape gardens.[4] There are no reports of the traditional uses of its leaves or rhizomes.

Etlingera littoralis

Synonyms: *Achasma megalocheilos*, *Amomum littorale*, *Amomum megalocheilos*, *Hornstedtia megalocheilos*.

Etlingera littoralis (J. König) Giseke produces thick subterranean rhizomes of 3–3.5 cm in diameter.[1] The plant varies from 3–6 m tall (Figure 3). The crushed leaves do not have any scent. The leaves are bright green with young leaves sometimes flushed pink beneath. Petioles are 1.5–4.5 cm in length. Flowers of *E. littoralis* occur at the ground and can vary from entirely red to orange red with orange or yellow margins.[5] Lips of the flowers may be narrow and elongate or broad and spatulate. The species is common in lowland forests throughout Peninsular Malaysia and found growing together with other *Etlingera* species.[1,5] It also occurs in Southern Thailand and Indonesia.[16]

In Sabah, Malaysia, the hearts of young shoots, inflorescences and fruits of *E. littoralis* are consumed by the indigenous communities as condiment, eaten raw or cooked as vegetable.[15] In Thailand, the fruits are edible and the young stem, after removing the outer parts, yields an aromatic tender core that is eaten raw or cooked.[16] A decoction of rhizomes has been used to treat stomach ache, and as carminative and heart tonic.[20]

Etlingera maingayi

Synonyms: *Amomum maingayi*, *Hornstedtia maingayi*, *Phaeomeria maingayi*, *Nicolaia maingayi*.

Etlingera maingayi (Baker) R.M. Sm. is usually not more than 2 m in height, rising from running rhizomes.[4] Its leaves are usually lanceolate with red undulate fringes. When crushed, leaves emit an unpleasant oily smell. Young leaves are translucent with a reddish tinge (Figure 4). Inflorescences, borne on tall thin erect stalks, are recognisable by the white tomentose bracts with dark pink fringes. The flowers have bright pink lips with white streaks. The species is widely distributed in lowland and hill forests of Southern Thailand and Malaysia.[1,16] No traditional uses have been reported of the leaves and rhizomes except that the flowers are eaten as vegetables in Thailand.[16]

Etlingera rubrostriata

Synonym: *Etlingera metriocheilos* var. *rubrostriata*.

Etlingera rubrostriata (Holttum) C.K. Lim grows in clumps at edges of forests.[5] Leaves of this species can easily be recognised by their reddish-brown oblique bars at the upper surface and green lower surface (Figure 5). The leaf petiole is a distinct dark red colour and 1 cm in length. In Peninsular Malaysia, the species is quite widespread and occurs in higher altitudes. Wild populations can be observed in the forests of Ulu Gombak in Selangor and

Figure 3. Plants of *Etlingera littoralis* with bright green leaves.

Figure 4. Leaves of *Etlingera maingayi*, translucent with a reddish tinge when young.

Figure 5. Leaves of *Etlingera rubrostriata* with attractive reddish-brown oblique bars.

Janda Baik in Pahang. The leaves have an aromatic fragrance when crushed. With striking oblique brown red bars at the upper surface of leaves, the species is attractive and has ornamental potentials. No traditional uses of its leaves and rhizomes have been reported.

PHYTOCHEMISTRY

Phenolic compounds

Phytochemical studies on *E. elatior* rhizomes led to the isolation of two new and six known compounds of diarylheptanoids, labdane diterpenoids and steroids.[21] The two new compounds were identified as 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone and 16-hydroxylabda-8(17),11,13-trien-15,16-olide. The known compounds included 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, demethoxycurcumin, stigmast-4-en-3-one, stigmast-4-en-6β-ol-3-one, stigmast-4-ene-3,6-dione and 5α,8α-epidioxyergosta-6,22-dien-3β-ol.

In a study screening 62 edible tropical plant species for flavonoid content, inflorescences of *E. elatior* were reported to have 286 mg/kg of kaempferol and 21 mg/kg of quercetin.[22] The phenolic content of *E. elatior* inflorescences was attributed to quercetin (1.2 mg/100 g).[23] Phytochemical screening of methanol extracts of inflorescences showed the presence of flavonoids, terpenoids, saponins, tannins and carbohydrates.[24] Phenolic, flavonoid, anthocyanin and tannin contents of inflorescences were 361 mg gallic acid equivalent (GAE)/100 g, 763 mg quercetin equivalent (QE)/100 g, 5.1 mg cyanidin 3-glucoside equivalent (CGE)/100 g and 468 mg catechin equivalent (CE)/100 g, respectively.[25] Nutritional contents of inflorescences showed high amounts of crude protein (12.6%), fat (18.2%) and fibre content (17.6%).[26] Major minerals were K (1589 mg/100 g), Ca (775 mg/100 g), Mg (327 mg/100 g), P (286 mg/100 g) and S (167 mg/100 g).

A study on the distribution of flavonoids in leaves of Zingiberaceae reported the occurrence of kaempferol 3-glucuronide, quercetin 3-glucuronide, quercetin 3-glucoside (isoquercitrin) and quercetin 3-rhamnoside (quercitrin) in *E. elatior*.[27] Six phenolic compounds were isolated from the methanol leaf extract of *E. elatior*.[28] They were caffeoylquinic acids of 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid (chlorogenic acid) and 5-*O*-caffeoylquinic acid methyl ester, and flavonoids of isoquercitrin, quercitrin and (+)-catechin. This was the first report of caffeoylquinic acids (CQA) including chlorogenic acid (CGA) in *Etlingera* and in Zingiberaceae.

CGA content in leaves of *E. elatior* (294 mg CGA/100 g) was significantly higher than flowers of *Lonicera japonica* or Japanese honeysuckle (173 mg CGA/100 g), the commercial source.[28] A protocol to produce a standardised extract of CGA with 40% w/w purity from leaves of *E. elatior* using column chromatography has been developed.[29,30] The protocol involved freeze-drying of leaves followed by extraction with 30% ethanol (repeated four times), fractionation of crude extract using Diaion HP-20 and fractionation of CGA extract using Sephadex LH-20. The entire fractionation process took only 6.5 h, using gravity flow. From 50 g of leaves, the final yield of CGA extract was 0.2 g (0.4%). CGA content of the standardised extract from leaves of *E. elatior* (40%) was 1.6 times that of commercial extracts from honeysuckle flowers (25%).

From the rhizomes of *E. littoralis*, six dihydrochalcones and two flavanones have been isolated.[31–33] Etlinglittoralin [1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylpropan-1-one] is a new dihydrochalcone reported in *Etlingera*. The others were known dihydrochalcones of 2',4',6'-trihydroxydihydrochalcone, 2',6',4-trihydroxy-4'-methoxydihydrochalcone, 2',6'-dihydroxy-4'-methoxydihydrochalcone, adunctin E and methyllinderatin, and known flavanones of pinocembrin and pinostrobin.

Essential oils

From the essential oil from young inflorescences of *E. elatior*, 49 aromatic compounds were identified.[34] The

major classes consisted of aliphatic alcohols and aldehydes, while the terpenoids constituted 18%. A similar study with inflorescences of *E. elatior* in the Amazon revealed that the main alcohol, aldehyde and terpenoid compounds were dodecanol, dodecanal and α-pinene, respectively.[35]

The composition of essential oils varied between the different parts of *E. elatior*.[36] Major components of leaves, stems, flowers and rhizomes were (*E*)-*β*-farnesene, *β*–pinene, 1,1-dodecanediol diacetate, cyclododecane and (*E*)-5-dodecane. Examination of the essential oils from *E. elatior* inflorescences revealed that the main compounds were dodecanol, dodecanal and α-pinene.[34,35] The major constituents of oils from *E. elatior* leaves were *β*-pinene (24.9%) and 1-dodecene (24.3%),[37] and sesquiterpenes of (*E*)-farnesene (13.6%) and (*E*)-caryophyllene (8.6%).[38]

A comparative study on the essential oils of leaves, rhizomes and roots of *E. elatior* reported the dominance of mono- and sesquiterpenoids.[39] The major components detected were myrcene (13.5%), α-humulene (11.8%) and *β*-caryophyllene (10.7%) in the leaf oil, camphene (18.0%) in the rhizome oil, and *β*-pinene (16.9%) in the root oil. A white-flowered variety of *E. elatior* known as 'Thai Queen' studied had similar oil profiles except for a higher level of α-pinene and a lower concentration of myrcene in the leaf oil. There was a marked increase in dodecanol and dodecanal in the leaves (18.9% and 15.9%, respectively) and in the rhizomes and roots (12.9% and 10.6%, respectively). This would imply that the leaves, rhizomes and roots of *E. elatior* and its variety 'Thai Queen' contain similar essential oils but differ in content.

Essential oils from leaves of four *Etlingera* species (*E. elatior*, *E. fulgens*, *E. maingayi* and *E. rubrostriata*) in Peninsular Malaysia were analysed using GC and GC-MS.[38] The oils differed in composition and content. Sesquiterpenes, comprising mainly of (*E*)-farnesene (13.6%) and (*E*)-caryophyllene (8.6%) were the major constituents of oil of *E. elatior* leaves. Oil of *E. fulgens* leaves consisted mainly of dodecyl acetate (21.6%), pentadecanol (14.1%) and hexadecanol (3.6%).[38] These compounds were also present in *E. elatior* but in much smaller amounts. Dodecanal, detected in leaves of *E. elatior* (3.1%) and *E. fulgens* (8.1%), was previously reported in inflorescences of *E. elatior*.[34] Oils of *E. elatior* and *E. fulgens* were different in composition despite having a similar aroma.[38] Oil of *E. rubrostriata* leaves was the most diverse, with 23 compounds identified. Leaves of *E. maingayi* had the highest yield of oil (1317 mg/100 g) with only four compounds, comprising mainly of dodecanoic acid (44.6%) and decanoic acid (42.6%).

A study on the essential oils of leaves, rhizomes and roots of *E. littoralis* reported high levels of phenylpropanoids.[39] (*E*)-methyl isoeugenol was the major component of the leaves (37.7%), and of the rhizomes and roots (58.1%). An analysis of the essential oils of *E. fulgens* showed the dominance of non-terpenes (65.8–85.8%) over terpenes (8.5–24.5%).[40] Some of the major constituents were (*Z*)-9-hexadecen-1-ol (15.0% in leaves; 7.2% in stems), n-dodecyl acetate (16.6% in stems; 16.3% in rhizomes), and cyclodecane (12.2% in stems; 11.3% in rhizomes).

Among the various components of essential oils of *Etlingera*, fatty acids of dodecanoic (lauric) acid and decanoic (capric) acid, aldehydes of dodecanal, monoterpenes of α-pinene and *β*-pinene, and sesquiterpene of (*E*)-caryophyllene have been reported to possess antibacterial, antifungal and antiviral properties.[41,42] *β*-Caryophyllene and α-pinene display anti-inflammatory activity against the carrageenin-induced hindpaw oedema in rats,[43] and cytotoxic activity against HeLa, A-549 and HT-29 human cancer cells.[44] The anti-inflammatory mechanism of terpenoids involves the inhibition of nuclear factor-κB (NF-κB) signalling pathways, which are major regulators in the pathogenesis of inflammatory diseases and cancer.[45,46]

PHARMACOLOGY

Antioxidant activity

Past studies examining the antioxidant activity of ginger species were confined to rhizomes.[47–51] Ginger rhizomes such as those of *Zingiber officinale* (ginger) and *Curcuma longa* (tumeric) have been widely used as spice, condiment and traditional medicine. The rhizomes have been reported to contain antioxidants with lipid peroxidation inhibition properties comparable to α-tocopherol. Although leaves of some ginger species have been used for food flavouring and traditional medicine, little research on their antioxidant properties has been undertaken until recent years.

The lipid peroxidation inhibition (LPI) activity of methanol and dichloromethane (DCM) extracts of rhizomes of four *Etlingera* species was analysed using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) assays.[49] Methanol and DCM extracts of *E. elatior* showed higher LPI activity than α-tocopherol for both assays. Results of the FTC assay showed that methanol and DCM extracts of *E. littoralis* rhizomes had higher inhibition activity than α-tocopherol. A comparable amount of α-tocopherol was used. The higher LPI activity of plant extracts may be attributed to their multiple components that are able

to inhibit the formation of lipid peroxides. Similar findings have been reported in a number of other plant species. However, the results may only apply to LPI activity but not to other antioxidant activities such as free radical scavenging, reducing ability and ion chelation.

Using the FTC assay, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, demethoxycurcumin and 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one isolated from the rhizomes of *E. elatior* showed stronger LPI activity than α-tocopherol.[21] Stigmast-4-en-3-one and stigmast-4-en-6*β*-ol-3-one displayed high anti-tumour activity using the inhibition of Epstein-Barr virus (EBV) activation assay.[52] Ethyl acetate extracts showed strong cytotoxic activity against CEM-SS and MCF-7 cancer cells using the methylthiazole tetrazolium (MTT) assay. Inhibition concentration (IC_{50}) of rhizome extracts was 4.0 and 6.3 mg/ml, compared to tamoxifen with IC_{50} of 30 and 15 μM, respectively.

The total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of leaves and rhizomes of five wild and six cultivated ginger species of *Alpinia*, *Boesenbergia*, *Curcuma*, *Elettariopsis*, *Etlingera*, *Scaphochlamys* and *Zingiber* were compared.[53] In general, leaves of wild and cultivated *Etlingera* species contain the most antioxidants by having the highest TPC and AEAC. Respective values were 1110 mg GAE/100 g and 963 mg AA/100 g for *E. maingayi*, and 2390 mg GAE/100 g and 2280 mg AA/100 g for *E. elatior*. The outstanding leaf TPC and AEAC of both *E. maingayi* and *E. elatior* were 7–8 times higher than those of rhizomes.

Antioxidant properties of leaves of 26 ginger species belonging to nine genera and three tribes were screened.[54] *Etlingera* had the highest values followed by *Alpinia* and *Hedychium* (Table 1). Values of *Boesenbergia*, *Curcuma*, *Elettariopsis*, *Kaempferia*, *Scaphochlamys* and *Zingiber* were significantly lower. The outstanding antioxidant properties of *Etlingera* species were attributed to their size and growth habitat. Plants of *Etlingera* are the largest among the gingers and can grow up to 8 m in height. They grow in gaps of disturbed forest and are continually exposed to direct sunlight. The other genera are small- to medium-sized herbs. It has been postulated that larger ginger plants growing in exposed forest sites have stronger antioxidant properties than smaller plants growing in shaded sites.

Leaves of five *Etlingera* species were assessed for total phenolic content (TPC), and antioxidant activities based on ascorbic acid equivalent antioxidant capacity (AEAC), ferric reducing power (FRP), ferrous ion chelating (FIC) ability and LPI activity.[55] Highest TPC, AEAC and FRP values were found in leaves of *E. elatior* followed by *E. rubrostriata* (Table 1). The relative order was *E. elatior* > *E. rubrostriata* > *E. littoralis* > *E. fulgens* > *E. maingayi*. It was reported that leaves of *Etlingera* species with high TPC, AEAC and FRAP have low FIC ability and *vice versa*. This would mean that phenolic compounds in the extracts responsible for antioxidant activities of scavenging free radicals and reducing ferric ions might not be directly involved in ferrous ion chelation. FIC ability of *E. maingayi* and *E. fulgens* was much higher than that of young tea leaves (*Camellia sinensis*) which is well known for its superior antioxidant properties. All *Etlingera* species, particularly *E. maingayi*, had higher LPI activity than young leaves of *C. sinensis* (Figure 6).

Table 1: Phenolic content and antioxidant activity of leaves of 26 ginger species[54,56]

Genus	Species	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Alpinia	*A. zerumbet*	1990 ± 62	2180 ± 42
	A. purpurata	1190 ± 174	1100 ± 113
	A. zerumbet	1150 ± 41	1250 ± 184
	'variegata'	744 ± 61	800 ± 62
	A. malaccensis	392 ± 50	90 ± 36
	A. galanga		
Boesenbergia	*B. rotunda*	260 ± 8	157 ± 2
Curcuma	*C. zanthorrhiza*	503 ± 57	287 ± 39
	C. aeruginosa	282 ± 78	140 ± 47
	C. mangga	275 ± 36	118 ± 11
	C. longa	230 ± 19	113 ± 18
Elettariopsis	*E. latiflora*	423 ± 26	395 ± 27
	E. slahmong	346 ± 45	269 ± 67
	E. smithiae	303 ± 18	147 ± 21
Etlingera	*E. elatior*	2390 ± 329	2280 ± 778
	E. rubrostriata	2250 ± 113	2290 ± 118
	E. littoralis	2150 ± 94	1990 ± 87
	E. fulgens	1280 ± 144	845 ± 158
	E. maingayi	1110 ± 93	963 ± 169
Hedychium	*H. coronarium*	820 ± 55	814 ± 116
Kaempferia	*K. galanga*	146 ± 9	77 ± 7
	K. rotunda	140 ± 48	46 ± 15
	K. pulchra	112 ± 9	30 ± 3
Scaphochlamys	*S. kunstleri*	203 ± 21	171 ± 33
Zingiber	*Z. officinale*	291 ± 18	96 ± 7
	Z. spectabile	242 ± 7	121 ± 24
	Z. ottensii	162 ± 13	52 ± 6

Abbreviations: TPC = total phenolic content, AEAC = ascorbic acid equivalent antioxidant capacity, GAE = gallic acid equivalent, AA = ascorbic acid.

Of the five *Etlingera* species studied, leaves of *E. elatior* had the highest TPC and AEAC.[55] Values were significantly higher than those of inflorescences and rhizomes (Table 2). The relative order was leaves > inflorescences > rhizomes. Leaves which are exposed to sunlight are known to have greater concentrations of flavonoids than unexposed parts below the soil surface such as roots and

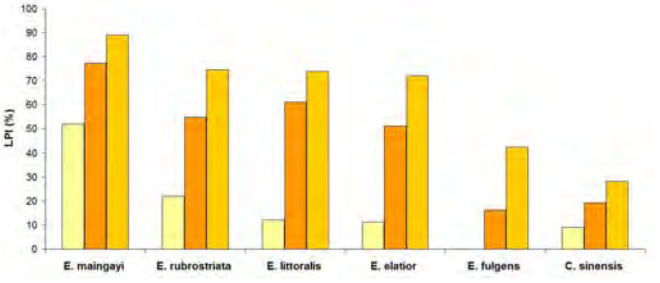

Figure 6. Lipid peroxidation inhibition (LPI) activity (%) of leaves of five *Etlingera* species in comparison with young leaves of *Camellia sinensis* (fresh weight).[55] For each species, LPI was tested at 0.2, 1.0 and 2.0 mg in 3 ml (left, middle and right bars).

Table 2: Phenolic content and antioxidant activity of different parts of *Etlingera elatior* (fresh weight)[55]

Plant part	TPC (mg GAE/ 100 g)	AEAC (mg AA/ 100 g)	FRP (mg GAE/ 100 g)
Leaves	3550 ± 304[a]	3750 ± 555[a]	2000 ± 210[a]
Inflorescences	295 ± 24[b]	268 ± 45[b]	150 ± 20[b]
Rhizomes	187 ± 46[c]	185 ± 59[c]	90 ± 20[c]

Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC) and ferric reducing power (FRP) values are means ± SD (n = 3). For each column, values followed by the same letter (a–c) are not statistically different at $P < 0.05$, as measured by the Tukey HSD test. Abbreviations: GAE = gallic acid equivalent, AA = ascorbic acid, HSD = honestly significant difference.

rhizomes. TPC and AEAC of *E. elatior* leaves were eight times higher than those of rhizomes.[54]

Leaves of highland populations of *Etlingera* species were found to have higher TPC and AEAC than lowland counterparts.[55,56] Leaves of *E. elatior*, *E. rubrostriata* and *E. fulgens* had significantly higher values with greater altitude, while leaves of *E. littoralis* were marginally higher (Table 3). Higher altitudes seem to trigger an adaptive response in leaves of *Etlingera* species. The stronger antioxidant properties of highland populations might be due to environmental factors such as higher UV-B radiation and lower air temperature. Studies have shown that enhanced UV-B radiation induces production of phenolic compounds in plants,[57] and low temperatures enhance the synthesis of phenylalanine ammonia lyase in plants, leading to increased production of flavonoids and other phenolics.[58]

Antioxidant properties of methanol extracts, non-polymeric (NP) fractions and polymeric tannin (PT) fractions of leaves and inflorescences of *E. elatior* have been investigated.[59] Highest phenolic content and antioxidant activity were observed in the PT fractions of leaves (575 ± 79 mg GAE/g extract and 616 ± 13 mg AA/g extract) and inflorescences (191 ± 14 mg GAE/g extract and

Table 3: Phenolic content and antioxidant activity of leaves of four *Etlingera* species sampled from highland and lowland locations (fresh weight)[55]

Etlingera species	Altitude (m)	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
E. elatior	400	3550 ± 304[a]	3750 ± 555[a]
	100	2390 ± 329[b]	2280 ± 778[b]
E. rubrostriata	300	3480 ± 390[a]	3540 ± 401[a]
	100	2430 ± 316[b]	2640 ± 508[a]
E. littoralis	800	2810 ± 243[a]	2930 ± 220[a]
	100	2340 ± 386[a]	2220 ± 913[a]
E. fulgens	400	2270 ± 31[a]	2030 ± 126[a]
	100	1280 ± 143[b]	845 ± 158[b]

Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) values are means ± SD (n = 3). For columns of each species, values followed by the same letter (a–b) are not significantly different at $P < 0.05$ measured by the Tukey HSD test. ANOVA does not apply between species. Abbreviations: GAE = gallic acid equivalent, AA = ascorbic acid, HSD = honestly significant difference.

236 ± 28 mg AA/g extract), suggesting that tannins are important antioxidants. Tannins are polymeric phenolic compounds of intermediate to high molecular weight.[60,61] Due to their higher molecular weight and greater degree of hydroxylation of aromatic rings, tannins exhibit strong antioxidant activities such as free radical scavenging, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation. Other bioactivities of tannins include antibacterial, antiviral, anti-carcinogenic and anti-inflammatory effects.

The effects of different drying methods on the antioxidant properties of leaves of *E. elatior* have been reported.[62] All methods of thermal drying (microwave-, oven- and sun-drying) of leaves resulted in drastic declines in antioxidant activity (Table 4). Many studies have reported losses in antioxidant activity of plant samples following

Table 4: Percentage gain (+) or loss (–) in total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric reducing power (FRP) of leaves of *Etlingera elatior* following drying compared to fresh leaves (fresh weight)[62]

Drying treatment	Water loss (%)	Gain/loss (%) compared to fresh leaves		
		TPC (mg GAE/ 100 g)	AEAC (mg AA/ 100 g)	FRP (mg GAE/ 100 g)
Microwave-drying	75	−40	−59	−44
Oven-drying	68	−42	−58	−43
Freeze-drying	76	+26	+45	+36

TPC, AEAC and FRP are means ± standard deviation (n = 3). Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid. Drying protocols: fresh leaves (1 g) were microwave-dried for 4 min, oven-dried at 50 °C for 5 h, and freeze-dried overnight. Extraction: leaves (1 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of methanol.

thermal treatments. Loss in antioxidant activity of heat-treated samples was attributed to thermal degradation of phenolic compounds and to the inhibition of antioxidant enzyme activities. Non-thermal drying (air- and freeze-drying) of *E. elatior* leaves had contrasting effects. Significant losses were observed in air-dried leaves. However, there was significant gain in antioxidant activity of freeze-dried leaves compared to fresh leaves. There is no thermal and/or enzyme degradation in freeze-drying. Freeze-drying is known to have high extraction efficiency because ice crystals formed within the plant matrix can rupture cell structure, which allows exit of cellular components and access of solvent, and consequently better extraction. The HPLC chromatogram of freeze-dried *E. elatior* leaves showed greater amounts of minor compounds than fresh leaves.[63] Similarly, significantly higher phenolic content and antioxidant activity of freeze-dried inflorescences of *E. elatior* have been reported.

Antibacterial activity

Leaf extracts of five *Etlingera* species were assessed for antibacterial activity using the disc-diffusion method.[55] All species inhibited Gram-positive bacteria of *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus* but not Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis*. Leaves of *E. elatior*, *E. fulgens* and *E. maingayi* exhibited moderate inhibition against the three Gram-positive bacteria. Moderate inhibition was shown by the leaves of *E. rubrostriata* on *B. cereus* and *S. aureus*, and by the leaves of *E. littoralis* on *S. aureus*.

The antibacterial activity of inflorescences of *E. elatior* has been studied. Ethanol extracts displayed antibacterial activity with minimum inhibitory concentration (MIC) of 200 µg/ml against *P. aeruginosa*, 400 µg/ml against *Bacillus megaterium* and 800 µg/ml against *E. coli*.[64] Methanol extracts inhibited *S. aureus*, *E. coli*, *Bacillus thuringiensis*, *Bacillus subtilis* and *Proteus mirabilis* with MIC ranging from 1.6–25 mg/ml.[24]

Antibacterial properties of methanol extracts, non-polymeric (NP) fractions and polymeric tannin (PT) fractions of leaves and inflorescences of *E. elatior* showed that methanol extracts and NP fractions of *E. elatior* leaves and inflorescences displayed antibacterial activity with minimum inhibitory dose (MID) ranging from 1.0–2.0 mg/disc.[59] PT fractions displayed antibacterial activity only after the addition of ethylenediamine tetraacetic acid (EDTA). EDTA has been reported to permeabilise the outer membrane of bacteria, making them more susceptible to antibiotics and certain antiseptic agents.

Using the wet disc diffusion method, essential oils extracted from leaves of four *Etlingera* species inhibited Gram-positive bacteria of *B. cereus*, *M. luteus* and *S. aureus* with no activity against Gram-negative bacteria of *E. coli*, *P. aeruginosa* and *S. choleraesuis*.[38] Leaf oil of *E. maingayi* had the strongest activity with MIC of 6.3 mg/ml against *B. cereus* and *M. luteus* and 12.5 mg/ml against *S. aureus*. Based on MIC, ranking was *E. maingayi* > *E. rubrostriata* > *E. elatior* > *E. fulgens*. Variation in antibacterial activity of the leaf oils can be attributed to qualitative and quantitative differences in the constituents of individual oils.

Cytotoxic activity

Ethanol extracts from inflorescences of *E. elatior* were reported to have cytotoxic activity against HeLa cells.[64] The cytotoxic dose (CD_{50}) values were 10–30 µg/ml. In a different study, methanol leaf extracts of *E. elatior* did not exhibit cytotoxic effect on normal WRL-68 (human liver) and Vero (African green monkey kidney) cells.[28]

Screening of 38 edible plants in Thailand for inhibition of Epstein-Barr virus (EBV) activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) showed that leaf and stem extracts of *E. elatior* were moderately active whilst the rhizome extracts were only weakly active.[65] Using the same assay, DCM rhizome extracts at 20 µg/ml of *Etlingera* species showed that *E. littoralis* had an excellent inhibition of 100% while *E. elatior* exhibited 71.2% inhibition.[49] The inhibitory assay for EBV activation is an effective *in vitro* method for assessing the anti-tumour promoting properties of plant samples and for predicting chemopreventive potential *in vivo*.[63] The inhibitory effect of plant extracts is classified as strongly active (>70%), moderately active (50–70%), weakly active (30–50%) and inactive (<30%).

Anti-tyrosinase activity

Leaves of five *Etlingera* species were assessed for anti-tyrosinase activity using the modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate.[54] Leaves of *E. elatior* displayed significantly stronger activity than those of *Hibiscus tiliaceus* which were used as a positive control. Activity of *E. fulgens* leaves was comparable to *H. tiliaceus*. The overall ranking was *E. elatior* > *E. fulgens* > *E. maingayi* > *E. rubrostriata* > *E. littoralis*.

Hepatoprotective activity

Inflorescences of *E. elatior* have been reported to possess hepatoprotective properties against lead-induced hepatotoxicity in male Sprague-Dawley rats.[66] Treatment with 300 mg of extracts per kg body weight significantly reduced hepatic lipid hydroperoxide (LPO) and protein carbonyl (PC) content in the serum, increased antioxidant

enzyme levels in the liver, and reduced lead levels in the blood after 21 days. The study concluded that the hepatoprotective effect against lead toxicity in rats may be attributed to the powerful lead chelating ability of the *E. elatior* extract. In a related study, the effect of *E. elatior* extracts of inflorescences on the bone marrow of male Sprague-Dawley rats exposed to lead acetate toxicity was examined.[67] The study similarly concluded that the species has a powerful antioxidant effect which protects bone marrow oxidative damage induced by lead acetate.

Another follow-up study evaluated the antioxidant effects of *E. elatior* inflorescence extract against lead-induced changes in serum free radical scavenging enzymes and lipid hydroperoxides in rats.[68] Lead acetate in drinking water elicited a significant increase in LPO and PC contents. There was also a significant decrease in total antioxidants, superoxide dismutase, glutathione peroxidase and glutathione S-transferase levels with lead acetate treatment. Supplementation of *E. elatior* extract resulted in reduced serum LPO and PC contents, and significant increase in total antioxidants and antioxidant enzyme levels.

CONCLUSION

While botanists are working on the systematics of *Etlingera* gingers as new species are identified, chemists are following up with their research findings on the phytochemistry of various plant parts of selected species. Some comparative work has been done on their antioxidant, antibacterial, cytotoxic and anti-tyrosinase properties. Of the species in this genus, *E. elatior* is the best known and most studied in view of its commercial potential. Research on the composition and some bioactivities of essential oils of leaves, rhizomes and inflorescences of *E. elatior* has been conducted. Two new and six known compounds of diarylheptanoids, labdane diterpenoids and steroids have been isolated from the rhizomes. Five flavonoids and three caffeoylquinic acids including chorogenic acid (CGA) have been isolated from the leaves. The CGA content in leaves of *E. elatior* is significantly higher than flowers of *L. japonica* (Japanese honeysuckle), the commercial source. A protocol to produce a standardised extract of CGA with 40% w/w purity from leaves of *E. elatior* has been developed. Antioxidant properties of leaves, rhizomes and inflorescences have been compared, with the effects of different drying methods on the antioxidant properties of its leaves assessed. The antioxidant activity of highland populations of *Etlingera* species is higher than lowland counterparts. Extracts and fractions from leaves and inflorescences show antibacterial and cytotoxic activities. Inflorescence extracts have potent antioxidant activities against lead-induced oxidative stress in liver, bone marrow and blood serum of rats. With more in-depth research being conducted on *E. elatior*, there are enormous prospects for studies on the phytochemistry and pharmacology of *Etlingera* gingers, particularly the lesser-known species. The potential of *E. elatior* as a candidate species for development of functional food and other pharmaceutical products, and as an alternative source of CGA, warrants further investigation.

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