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Litsea cubeba- A Deep Academic Report on the Global Distribution, Multifaceted Uses, Academic Research, and Essential Oil Chemistry Diversity of Cubeba (Lour.) Pers

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ABSTRACT: Litsea Litsea cubeba (Lour.) Pers., commonly known as Ma Gao, Mei Zhang, or Shan Ji Jiao, is an evergreen or deciduous, dioecious shrub or small tree belonging to the genus Litsea in the family Lauraceae [1, 2, 3, 4]. This species is rich in volatile essential oils with significant industrial and medicinal value and is considered a potential tree species for developing multifunctional industries such as pharmaceuticals, cosmetics, green protective agents, food flavorings, and low-cost bioenergy [5, 6, 7]. This report systematically organizes the global ecological distribution pattern and cross-regional ethnobotanical utilization characteristics of Litsea cubeba and delves into the active components of its essential oils and the multi-target pharmacological molecular mechanisms. Finally, it compares the regional and seasonal variations of the chemical composition of essential oils from multiple parts such as leaves, branches, and fruits, aiming to provide scientific empirical references for subsequent wild resource protection, genetic breeding, and deep processing.

Global ecological distribution and production area pattern

Litsea cubeba is widely distributed in tropical and subtropical climate zones, with a highly concentrated natural distribution in East Asia, South Asia and Southeast Asia [8, 9, 10]. Its main native habitats cover mainland China, Taiwan, Japan, India, Myanmar, Thailand, Vietnam, Laos, Cambodia, Malaysia, the Philippines and Indonesia [1, 7, 10]. In terms of spatial vertical distribution, this species grows in high-altitude mountainous areas such as the eastern Himalayas, Assam, Manipur and Arunachal Pradesh, with altitudes extending up to 2700 meters [10]. Studies show that Litsea cubeba is usually cultivated or grows wild in high-altitude environments above 1600 meters , and its growth altitude has a significant environmental selective pressure on the chemical accumulation and chemotype selection of essential oils [11].In mainland China, Litsea cubeba is commonly found in tropical and subtropical monsoon forests south of the Yangtze River, with Jiangxi Province being one of the core distribution areas, where wild Litsea cubeba forests cover more than 733.33 hectares [7, 12]. In Taiwan, Litsea cubeba (Maggao) is a well-known pioneer tree species in forests, with communities mostly formed in low- to mid-altitude forests, mountain landslide areas, newly reclaimed land, or logging sites at elevations of 300 to 2300 meters [13]. Litsea cubeba prefers cool and temperate environments and cannot adapt to extreme cold or high temperatures on planes. Therefore, its artificial introduction to plains

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cultivation often faces the bottleneck of not being able to flower or bear fruit successfully [13]. At the commercial trade level, mainland China is the world's largest producer and exporter of Litsea cubeba oil (LCEO), with an annual output of between 1,500 and 2,000 tons, or as high as 4.4 million pounds [10, 14]. Three-quarters of its annual exports are exported to Western countries such as the United Kingdom, the United States, France, Germany and the Netherlands [10]. Statistics show that from 2019 to 2023, mainland China's total exports of essential oil reached 1,682 tons [14], demonstrating the significant role this species plays in the international fragrance and pharmaceutical raw material market [5, 15].

Cross-regional ethnobotany and modern industrial utilization

Litsea cubeba has a long history of being used for food and medicine in different cultural regions of Asia. Its roots, stems, leaves, flowers, fruits and bark have all been used in unique ways.

Taiwanese Indigenous Culture and Folk Customs

In Taiwan, mountain pepper is called "Makao" by the Atayal indigenous people, symbolizing the continuation and endlessness of life [12, 13], while the Taroko people call it "Mqrip " [13]. The Qilan Mountain area is an important wild distribution area for mountain pepper in Taiwan [12]. Traditionally, the Atayal and other tribal residents use the dried mountain pepper fruit, which looks like black pepper, as a spice to remove fishy smells and enhance the aroma of meat and fish dishes [12, 16]. In terms of medical folklore, the indigenous people have long used the essential oil of mountain pepper leaves or fruit to treat headaches, tissue inflammation, hangovers, bronchitis and indigestion [17].

Diverse utilization of Southeast Asia and the Indonesian archipelago

In Thailand, ethnobotanical literature from 1996 to 2022 and field surveys from 2019 to 2021 show that local residents use 13 species of Litsea genus, 11 of which are mainly used for traditional herbal chemotherapies for colds, coughs and headaches [5, 18]. In northern Vietnam, residents traditionally pick Litsea flowers to make aromatic teas [2]; while in Java, Indonesia, the green fruit of Litsea is widely used as a vegetable accompaniment or as a substitute for Piper cubeba. Alternative seasonings for Lf.) [2]. In addition, the

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essential oils distilled from the leaves in western and central Java are named " Trawas oil" and " Krangean oil" respectively and are widely used in pharmaceutical preparations and soap fragrances [2]. The Dayak Kenyah people in eastern Kalimantan the Kenyah people mix the bark and fruit of the mountain pepper into internal or external medicines to reduce fever, relieve stomach pain and chest pain, and use it as a tonic and hangover cure [2].

Medical and specialty industries in India and mainland China

In the Assam region of India, mountain pepper is called " Mejankari " [10]. The local specialty "Muga silk " is made from Antheraea silk. The high-priced golden silk produced by the assama (a type of wild mulberry) from the leaves of the mountain lily is rare and has a natural creamy luster, and its market price is up to five times that of ordinary mulberry silk.[10] In addition, Indian residents also make salads, chutneys and pickles from the fresh green fruit.[10] In mainland China, the whole mountain lily plant is often used in the traditional Chinese medicine system to dispel cold, regulate qi, and relieve pain. It is mainly used to treat stomach cold and abdominal pain, cloudy urine, joint pain and traumatic injuries. [9, 10, 19]

In the modern industrial chain, in addition to essential oils serving as precursors for the synthesis of vitamins A, E, K and fragrance monomers (ionones) [2, 3, 5], mountain pepper is becoming a typical demonstration model of zero-waste biorefinery [6]. The fruit residue after steam distillation to extract volatile essential oils can be further pressed and refined to produce kernel oil rich in medium-chain fatty acids such as lauric acid, which can be used to convert into biodiesel or high-moisturizing cosmetic base oil [5, 6, 7]; the pressed cake and remaining waste can be converted into high-protein animal feed or green fertilizer [6, 7].

Active chemical components and multi-target pharmacological mechanisms

The bioactivity and pharmacological value of Litsea cubeba originate from its highly complex chemical composition, which mainly includes monoterpenes, sesquiterpenes, apophene alkaloids, and diterpenes (such as the unique cytotoxic diterpenoid cubelin) as secondary metabolites [10]. Among them, the volatile components of monoterpenes, mainly aldehydes (mainly geranialdehyde and neraldehyde, collectively known as citral), are the main active mediators [12, 19, 20].

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Broad-spectrum antimicrobial and physical blocking mechanisms

Litsea cubeba essential oil (LCEO) and its monomeric components (such as citral, limonene, etc.) exhibit significant bactericidal efficacy against Gram- positive bacteria, Gram-negative bacteria, filamentous fungi and yeasts [1, 8, 21].

At the bacterial level, pepper oil exerts its antibacterial effect primarily through physical penetration and intracellular energy depletion. Using *Escherichia coli* and *Salmonella typhimurium * as models, when the essential oil concentration reaches the minimum inhibitory concentration (MIC, 0.125% v/v for *E. coli* and 0.4 mg/ mL for *Salmonella *), the essential oil, due to its high lipophilicity, penetrates the outer membrane, severely disrupting the cell membrane's phospholipid bilayer structure, leading to cell membrane perforation and disintegration [19, 22, 23]. At this point, large amounts of intracellular macromolecules such as nucleic acids and proteins leak out, causing irreversible cell depolarization [22, 23]. Simultaneously, the essential oil specifically binds to and inhibits key proton pumps on the cell membrane, causing intracellular... The essential oils completely inhibited the activity of Na^+-K^+-ATPase (79.9 % inhibition rate) and Ca^{2+}-Mg^{2+}-ATPase (54.6 % inhibition rate), thus blocking ATP energy production and interfering with the respiratory oxidation chain, ultimately leading to bacterial death [22, 23]. In addition, the essential oils significantly weakened the flagellar motility of bacteria and blocked the formation of pathogenic biofilms [22, 24]. At the fungal level, essential oil components have effects on dermatophytes (such as Trichophyton rubrum). rubrum, Microsporum gypsum Nannizzia Limonene in the essential oil exhibits excellent inhibitory effects against Candida albicans [1, 2, 8]. It mainly exerts its antifungal efficacy by disrupting the ultrastructure of fungal hyphae and organelle membranes [22]. Notably, limonene in the essential oil can exert a broad-spectrum antibacterial effect in the solid, liquid, and vapor phases. In particular, in the vapor diffusion method, Limonene essential oil shows an excellent bactericidal radius (LCEO inhibition zone reaches 90 mm, while pure limonene is 45 mm), confirming its high practical value in the field of vapor-phase preservation and active antibacterial packaging materials [8, 12].

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Anti-inflammatory and immune microenvironment regulation mechanisms

Litsea cubeba oil and citral play important roles in reducing tissue inflammation and protecting against endotoxin (LPS)-induced damage to the intestines, liver, and joints [1, 20]. Their molecular regulatory network mainly includes the following pathways:

1. **Direct targeting neutralization and blocking:** Molecular docking has confirmed that citral molecules (including α- and β -isomers) can form stable hydrogen bond interactions with specific amino acid residues of key pro-inflammatory cytokines (such as IL -1β*,* IL -6 and TNF- α), directly interfering with the downstream signal cascade amplification reaction of inflammatory factors [20].

2. **Transcription factors and inflammasome inhibition**: Citral significantly inhibits the activation of the nuclear factor NF - *κB* pathway, blocks its nuclear translocation, and thereby downregulates the transcriptional expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and prostaglandins [1, 20]. At the same time, it can inhibit the assembly and activation signaling of the NLRP3 inflammasome and reduce the release of mature IL-1β [1, 20].

3. PPAR- γ **pathway and antioxidant regulation**: Citral can act as a ligand activator of peroxisome proliferator-activated receptor γ (PPAR- *γ), and its anti-inflammatory properties can be reversed by the specific antagonist GW9662 [20].* In addition, it can upregulate the antioxidant response pathway of transcription factor Nrf2 and enhance the activity of superoxide dismutase and catalase in vivo [20, 23], thereby blocking ferroptosis in the inflammatory process [20].

4. **Intestinal barrier and microecological regulation**: Essential oils can effectively maintain the structure of colonic crypts, enhance the integrity of the mucosal epithelial barrier, and inhibit the proliferation of pathogenic bacteria by positively regulating the structure and diversity of the intestinal microecology, thereby reducing the systemic pro-inflammatory response triggered by LPS from the source [20].

Anti-tumor, antioxidant and cardiovascular regulatory mechanisms

In terms of anti-important activity, the fruit oil of Litsea cubeba exhibits significant organ-specific cytotoxicity. Studies have confirmed that the fruit oil can kill human oral cancer

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cells (OEC-M1), liver cancer cells (J5) and lung cancer cells (A549) in a dose-dependent manner [1], while the leaf oil did not show significant anti-cell proliferation efficacy in parallel tests, indicating that specific aldehyde components in the fruit oil are key to antitumor activity [1]. In addition, the free radical scavenging capacity (DPPH assay) of Litsea cubeba oil is significantly better than that of cinnamon oil, star anise oil and eucalyptus oil, and has excellent in vitro and in vivo antioxidant protection efficacy [25]. In cardiovascular regulation, the oil can regulate the ion flow of myocardial cells, normalize cardiac arrhythmia, and exert significant antihypertensive and vasodilatory effects by upregulating the cAMP level in smooth muscle and blocking PGF- *2α -induced smooth muscle spasm [2, 20, 26].*

Differences in essential oil composition of leaves, branches, and fruits and seasonal variations

The essential oil yield, chemical main components and percentage content of different organs and parts of Litsea cubeba exhibit extremely significant tissue specificity and are subject to dual regulation by geographical origin (chemotype variation) and harvesting period (seasonal fluctuations) [1, 3, 27].

Regional comparison of essential oil yield and chemical composition by part

A systematic comparison of research data from multiple typical producing areas reveals that, although the components of fruit essential oils are mainly monoterpenoid aldehydes in most producing areas, the chemical characteristics of leaf and branch essential oils exhibit a high degree of geographical diversity.

Table 1. The table below provides a detailed comparison of essential oil data from various parts of Litsea cubeba from Cameron Highlands, Malaysia; Baoshan, Yunnan, China; Jiayang, Taichung, Taiwan; and Balio Highlands, Sarawak [21, 27, 28, 29]:

Origin and Extraction Part	**Essential oil yield (production)**	**Main chemical components and their percentage content (%)**	**Characteristic chemical type classification**
Highlands, Malaysia [27]			
- Leaf blades (dried weight)	2.59% v/w	1,8- Cineole (48.8%), Sakunene (14.9 %), *α* -terpinene acetate (8.9%)	1,8- Cineole

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- Branches (dried weight)	0.02% v/w	1,8- Cineole (57.7%), α - terpinene acetate (11.4%), terpinene-4-ol (4.1%)	1,8- Cineole
- Fruit (dried weight)	0.27% v/w	Geraniol (44.7%), Neraldehyde (28.7%), Limonene (1.2%)	Citral
Chiayang, Taichung, Taiwan [29]			
- Blades (July, air-dried wood)	5.1 mL / 100g	1,8- Cineole (59.41%), β - terpinene (20.98%), terpineol (9.80%)	1,8- Cineole
- Fruit (September, dried fruit)	4.8 ~ 5.9 mL / 100g	Geraniol (47.01%), Neraldehyde (33.64%), Limonene (3.10%)	Citral
Baoshan, Yunnan Province, China [28]			
- Leaf (wet weight)	2.16% w/w	Linalool (67.37%), limonene (6.37%), β - bisabolene (6.03%)	Linalool type
- Fruit (wet weight)	5.36% w/w	Geranialdehyde (44.23%), Neraldehyde (36.51%), Citronellol (8.77%)	Citral
Bario Highlands, Sarawak [21]			
- Leaf blades (dried weight)	Approximately 8.1%	1,8- Cineole, α - Trinol	1,8- Cineole
- Fruit (dried weight)	medium yield	Citronellol (51.5%), limonene (10.4%), citronellol (8.9%)	Citronellol

Analyzing the data in the table above, the fruit of Litsea cubeba is usually the organ with the most efficient accumulation of volatile oils [5, 14]. In most typical producing areas, the fruit essential oil is dominated by the cis-trans isomers geranial (α - Citral) and neral (β - Citral) (totaling 70% to 85% or more) [5, 27, 29]. However, the Sarawak producing area presents an unusual **"citronellol/ citronellol type "** fruit essential oil (citronellol accounts for 51.5% , while citral accounts for only 2.6%), and its bark and root essential oils are also rich in high concentrations of citronellol and citronellol [21].Leaves of Litsea

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cubeba from Taiwan and Malaysia is a significant 1,8 -cineole typ **(content ranges from** 48.8% **to** 59.41% **) [27, 29]; while the essential oil of leaves from wild populations in Baoshan, Yunnan is a highly distinctive** linalool type, with a linalool content as high as 67.37% [28]. In addition, the content of 1,8- cineole (57.7%) in branch essential oil is often slightly higher than that in leaves of the same plant, but branches are rich in a higher proportion of terpene esters (such as α -terpineol acetate 11.4%), and their total oil yield (only 0.02%) is significantly lower than that of leaves and fruits, which makes the commercial value of extracting essential oil from branches alone low, and they are usually distilled together with leaves [27].

Seasonal fluctuations in essential oil content of leaves and fruits of Taiwan mountain pepper (Ma Gao)

in central Taiwan by the Taiwan Forestry Research Institute, the cumulative yield and chemical structure of essential oils from leaves and fruits have significant seasonal variation characteristics. This ebb and flow pattern is crucial for precision agriculture harvesting [29].

Annual fluctuations in leaf essential oils (Table 2)

Table 2. The oil yield of Litsea cubeba leaf extract is highest during the hot summer and autumn seasons [29]. The table below shows the yield and main component variation of leaf extracts harvested in different months of the year (unit: air-dried weight ml/100g and relative percentage %) [29]:

Harvest month	**Leaf essential oil production**	**1,8- Cineole content**	**β - Pine Oil Content**	**terpineol content**	**Lemon oil content**
January (JAN)	2.0 ml/100g	66.62% **(highest)**	14.12%	3.23%	4.01%
March (MAR)	1.5 ml/100g	62.75%	19.03%	2.43%	5.46%
May (MAY)	3.1 ml/100g	59.38%	20.04%	5.12%	2.58%
July (JUL)	5.1 ml/100g **(maximum)**	59.41%	20.98%	9.80% **(highest)**	3.12%
September (SEP)	4.7 ml/100g	59.35%	22.49% **(highest)**	7.15%	2.80%
November (NOV)	3.6 ml/100g	59.18%	16.37%	4.18%	3.50%

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The analysis of the table above shows that the yield of essential oil from the leaves is... **The annual peak value was reached in July (** 5.1 ml/100g **)** , while it dropped to the lowest value in January and March (1.5–2.0 ml /100g) [29]. Although the purity of 1,8-cineole in the essential oil was higher in winter and spring (62.75% –66.62 %), the study suggests that commercial collection of leaves should be strictly limited to **July to September each year,** considering the overall oil yield and the abundance of forest biomass (July to September is the period of most vigorous growth of branches and leaves) [29].

Changes in chemical maturity during fruit essential oil development (Table 4)

From fruit set in May to maturity in September, the essential oil content and composition of Litsea cubeba undergo dramatic secondary metabolic changes [7, 29].

Table 3. The table below shows the dynamic changes in essential oil yield and main components throughout the fruit's development [29]:

Harvest month	**Fruit essential oil yield range**	**Geranialdehyde content**	**Nerol content**	**Lemon oil content**	**1,8- Cineole content**
May (MAY)	2.6 ~ 3.5 ml/100g	38.98%	27.09%	11.82% **(highest)**	4.09%
June	3.3 ~ 4.6 ml/100g	42.42%	26.89%	8.70%	4.74%
July (JUL)	4.5 ~ 5.6 ml/100g	46.98%	32.23%	3.44%	1.86%
August (AUG)	4.7 ~ 5.8 ml/100g	47.11% **(highest)**	33.59%	2.81% **(lowest)**	3.18%
September (SEP)	4.8 ~ 5.9 ml/100g **(maximum)**	47.01%	33.64% **(highest)**	3.10%	2.03%

The above developmental patterns indicate that:

1. **Citral is positively correlated with maturity:** in the early stage of development in May, the content of geranialdehyde and nerol is the lowest, while as the fruit gradually develops to the ripening period in August and September, the combined proportion of the two climbs to a historical high of **over** 80%, giving the essential oil an excellent lemon aroma [29].

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2. **The biosynthetic dynamics of monoterpenes and monoterpenoids:** Limonene reached its peak (11.82%) during the young fruit stage (May) and then dropped sharply to about 3% as the fruit matured [29]. This indicates that the biosynthetic pathway of monoterpenes in plants is strongly inhibited in the later stages of fruit maturity, while the biosynthetic pathway of monoterpenoids (citral) is dominant [5].

3. **Trade-offs in harvesting decisions: Although** the essential oil yield peaks in September (up to 5.9 ml/100g) [29], the fruit is fully ripe and blackened at this time and is very easy to fall off, which makes manual harvesting extremely difficult due to wild bird feeding [7, 29]. Therefore, in practice, the best harvesting period is **from July to August each year (when the fruit is green and firm), when** the oil yield is very close to the peak and efficient harvesting is easy to carry out [7, 29].

Modern Processing Separation Technology and Industrialization Prospects

To further enhance the added value and specific pharmacological activity of Litsea cubeba oil, modern separation engineering has introduced physical refining techniques such as vacuum fractional distillation (VFD) and molecular distillation (MD) [14, 24]. These techniques can precisely cut heat-sensitive terpene components based on boiling point differences under low pressure and short heating time, avoiding the thermal polymerization or oxidation of citral and other compounds [11, 24]. Fractions (such as VFD3 and MDH2) prepared using vacuum fractionation and molecular distillation techniques are effective against food poisoning bacteria (Escherichia coli, Staphylococcus aureus) and crop pathogens (Agrobacterium rhizogenes). The antibacterial efficacy of rhizogenes is significantly better than that of the raw essential oil extracted directly [24]. Refined fractions can significantly improve their application precision in green agricultural protection and targeted pharmaceutical formulations by precisely downregulating the biofilm synthesis-related metabolic pathways of bacteria and more efficiently disrupting the cell wall barrier [24]. However, the large-scale development of the mountain pepper industry still faces significant challenges. As this species is dioecious and its seeds have a very deep physiological dormancy, its natural germination rate in the wild is usually only about 10% [3, 13]. This has led to the industry's past reliance on wild collection, and has even caused ecological damage in some forest areas by cutting down female plants in order

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to "rush to harvest" [13]. At present, agricultural research institutions have successfully developed a standardized artificial germination and seedling raising technology, which has significantly increased the germination rate of pepper seeds from 10% to nearly 70% [13]. This technological breakthrough has laid the scientific foundation for the field planting and standardized cultivation management of high-yield artificial forests, and is expected to achieve a win-win development of environmental conservation and high-value forest chemical industry in the future [6, 13].

Conclusion

Litsea As an important economic and ecological pioneer tree species in subtropical and tropical high-altitude mountainous areas, *Cubeba* has demonstrated unique cross-regional utilization value and chemical diversity [7, 11, 13]. Pharmacologically, its rich monoterpenoid components, such as citral, exhibit excellent antibacterial, antifungal, anti-inflammatory, and antihypertensive potential by physically disrupting pathogen cell membranes, blocking respiratory chain ATPase activity, and inhibiting NF-κB inflammatory *signals*, suppressing NLRP3 inflammasomes, and binding free inflammatory cytokines [1, 20, 22].

Comparative analysis of essential oil components confirms that the essential oil from Litsea cubeba fruit is predominantly citral in most parts of the world, while the leaves and branches exhibit highly diverse geochemical characteristics, mainly divided into 1,8-cineole and linalool types [27, 28, 29]. In production practice, July to September is the optimal extraction period for leaf essential oil, while July to August is the golden window for commercial harvesting of fruit [29]. In the future, by combining efficient artificial seedling cultivation and germination technology, vacuum molecular distillation refining technology, and a comprehensive development model of "waste-free biorefining," Litsea cubeba will exert a more profound industrial influence in diverse fields such as natural medicine, cosmetics, green pesticides, and bioenergy [6, 13, 24].

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