Review Article

Chemical and Pharmacological Aspects of *Limnophila aromatica* (Scrophulariaceae): An Overview

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ABSTRACT

The present work offers a review addressing the chemistry and pharmacology of *Limnophila aromatica* (Lamk.) Merr. (Belonging to Scrophulariaceae family) regarded as one of the most significant plant species in traditional system of medicine and is established as a source of flavonoids, terpenoids etc. The isolated phytochemicals as well as different extracts exhibited significant biological activities such as antimicrobial, antioxidant, vascular protective activities. Exhaustive research regarding isolation of more phytochemicals and pharmacology study on this medicinal plant is still necessary so as to explore the plant regarding its medicinal importance. Therefore, the aim of this review is to boost up present day researchers in this direction to undertake further investigation of this plant for searching new drugs. The present review covers literature up to January 2014 and enlists 24 references.

Keywords: *Limnophila aromatica*, Scrophulariaceae, Chemical constituents, Biological activity.

INTRODUCTION

Limnophila aromatica (synonym: *Limnophila chinensis* var. *aromatica*; *Limnophila gratissima* Blume; known as kutna in Hindi and manganari in Malayalam; also called rice paddy herb) is a tropical and stout aromatic herb with 30-50 cm high found in South Bihar. Orissa, Sundarbans, Ala hills (Assam), Deccan and Western parts of South India, up to 600m in damp places, margins of ponds and backwaters belonging to the family of Scrophulariaceae. It is a much branched decumbent aromatic herb (odour of turpentine), copiously rooting at the lower nodes, leaves sessile, opposite, hear oblong or lanceolate, sharply serrate, flowers purplish in axillary and terminal racemes, pedicels long slender and

glandular, fruits small obovoid-oblong capsules covered by the striate calyx[1,2]. This plant is native to Southeast Asia, where it flourishes in hot temperatures and grows most often in watery environments, particularly in flooded rice fields[3,4]. It is used in Vietnam as an herb and aquarium plant. The plant was introduced to North America in the 1970s due to Vietnamese immigration following the Vietnam War. It is used in all traditional Cambodian soup dishes. It can grow in flooded rice paddies during wet season but it grows best on drained but still wet sandy soil of harvested rice paddies for a few months after the rainy season ended. The herb grows everywhere like wildfire after the rain stops at the end of monsoon season. It dies out soon after it flowers. Rural Cambodians often harvest them and put them on the roof of their houses to dry for later use[3,4].

The taxonomical classification of *Limnophila aromatica* is shown below:

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Asteridae
Order	Scrophulariales
Family	Scrophulariaceae
Genus	*Limnophila*
Species	*L. aromatica*
Binomial name	*Limnophila aromatica* (Lamk.) Merr.

Traditional use

The plant is used as a spinach, eaten raw or steamed. It is sour, slight bitter refrigerant emollient antiseptic, galactagogue, aperient, appetizer, digestive, carminative, anthelmintic, anti-inflammatory, diuretic and febrifuge[5-8]. It is useful in vitiated conditions of pitta, foul ulcers, agalactia, galactic impurities, anorexia, dyspepsia, helminthiasis, constipation, inflammations and strangury. The juice of the plant is used as a cooling medicine in fever and pharyngitis. It is given to nursing women, when the milk is sour. The plant emits turpentine-like odour and yields 0.13% of an essential oil[5-8]. It is used as a spice and medicinal herb in Southeast Asia[9-11].

MATERIALS AND METHODS

The chemical constituents isolated and identified from *Limnophila aromatica*, pharmacological activities exhibited by the isolated compounds as well as by the crude plant extracts were searched across the Medline (National Library of Medicine) and Science Direct databases. The data were updated in January 2014, using the search-terms Limnophila aromatica, Limnophila gratissima, chemical constituents, biological activities, pharmacological activities or properties of Limnophila heterophylla and Limnophila gratissima as keywords. In addition, the reference lists of all papers identified were reviewed.

RESULT AND DISCUSSION

Chemical constituents isolated from *L. aromatica*

Phytochemicals isolated so far from *L. aromatica* have been listed in **Table-1** and found that fifty-four phytochemicals have been reported; most of the isolated phyto-chemicals belongs to terpenoids, phenolics and flavonoids. Structures of these phytochemicals class of compounds **(1-54)** are included in **Figure-1**.

Biological activity exhibited by the plant and its phytoconstituents

A number of research group studied different biological activities of the crude plant extracts as well as its phytoconstituents and have been discussed in detail below:

Antimicrobial activity

Nanasombat and Teckchuen[20] investigated antibacterial efficacy of methanol extract of leaves against a number of bacteria **(Table-2)** using disc diffusion method and found significant antibacterial activity with MIC value ranging from 2.6 to 41.7 mg/mL. This extract exhibited greater efficacy against *B. cereus* and *S. aureus* (MIC value 2.6 mg/mL for both)[20].

Crude methanol extract of the plant exhibited weak antimicrobial activity against *Burkholderia pseudomallei*, causative agents of melioidosis-a disease found mostly in South-East Asia and Northern Australia. The extract at a concentration of 2.5 mg/disc (measured by disc diffusion method) showed inhibition zone 8mm both against *B. pseudomallei* strain A2 and G207 having minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) value greater than 128 mg/mL[21]. Ethanol extract of the plant was found to have antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pyrogens* and *Propionibacterium acnes*[22].

Antioxidant activity

Methanolic extract of *L. aromatica* was found to exhibit antioxidant activity against DPPH radical using α-tocopherol as standard[20]. The investigators noted EC_{50} value of 550.5 ± 12.2 µg extract/mg DPPH and accounted for this activity due to presence of phenolic compounds in the extract. They also determined phenolic content of the extract in terms of gallic acid equivalents as 42 µg GAE/mg dry extract[20].

L. aromatica (extracted by 80% ethanol) was found to have significant levels of antioxidant activities measured by DPPH scavenging (IC_{50} at 4.92±0.01mg/mL, FRAP antioxidant assays (IC_{50} at 74.38 ± 0.25 mmol of dry weight of vegetable, respectively) and also total phenolic constituentss (10.96 ± 0.02 mg gallic acid equivalence of dry weight of vegetable[22]).

The antioxidant properties of methanol extract, essential oil and authentic compounds relating to *L. aromatica* essential oil components were investigated employing various established *in vitro* systems, such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) radical scavenging and inhibition of lipid peroxidation[18]. The results indicated that methanol extract and essential oil of *L. aromatica* exhibited free radical scavenger, NO radical scavenger and antilipid peroxidation. The antioxidant activities of methanol extract were higher than essential oil. Eugenol **(54)** showed antioxidant activity against DPPH; Eugenol **(54)** and γ-terpinene **(25)** also exhibited anti-lipid peroxidation, however, the authentic compounds were found to have very weak activity against NO scavenging assay[18].

The aqueous extract of *L. aromatica* was found to exhibit *in vitro* antioxidant activity. The antioxidant activity was evaluated *in vitro* by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, the ferric reducing antioxidant power (FRAP) assay, the intracellular antioxidant activity in rat peritoneal macrophages by dihydro-fluorescein (DHF) assay, and the inhibition of nitric oxide (NO) production in RAW 264.7 macrophages[23]. The aqueous extract showed strong free radical scavenging activity as well as ability to scavenge intracellular oxygen radical in DHF assay when compared with ascorbic acid used as standard and significantly inhibited NO production **(Table-3)**. The total antioxidant capacity has been

measured in terms of ascorbic acid equivalence per mg of the extract[23].

In another investigation, water and various concentrations (50%, 75% and 100%) of methanol, ethanol and acetone in water, were used as solvent for extracting freeze dried *L. aromatica* and antioxidant activity, total phenolic content and total flavonoid content of the plant extracts were studied by using various *in vitro* assays[24]. The 100% ethanol extract showed the highest total antioxidant activity, reducing power and DPPH radial scavenging activity. The same extract also exhibited the highest phenolic content (40.5 mg GAE/gm extract) and the highest flavonoid content (31.11 mg QCE/gm extract) whereas 50% aqueous acetone as solvent gave the highest extraction yield. The investigator is in opinion that *L. aromatica* can be used in dietary applications having potential to reduce oxidative stress[24].

Vascular protective activity

The aqueous extract of *L. aromatica* exhibited *in vivo* vascular protective activity against male Sprague-Dawley rats. It was found that administration of aqueous extract (1 g/kg/d) to phenylhydrazine (PHZ) induced rats improved the hemodynamic status *i.e.* controls PHZ induced severe hemolysis and hemodynamic disturbances[23]. Vascular responsiveness to bradykinin, acetylcholine, and phenylephrine in PHZ-control rats was restored by the plant extract. The plant extract was also found to prevent loss of blood reduced glutathione and suppressed the formation of plasma malondialdehyde, plasma NO metabolites and blood superoxide anion. It was concluded that the plant extracts have potential roles in protection of vascular dysfunction owing to presence of antioxidants[23].

CONCLUSION

The present article deals with an up-to-date review on the chemistry and pharmacology of *Limnophila aromatica*, a useful medicinal plant from Scrophulariaceae family finding applications in indigenous systems of medicine. The plant is used in different parts of the world for the treatment of several ailments and is the source of chemical constituents such as flavonoids, terpenoids etc. The isolated phytochemicals as well as different extracts exhibited significant biological activities such as antimicrobial, antioxidant, vascular protective activities. Exhaustive research regarding isolation of more phytochemicals and pharmacology study on this medicinal plant is essentially urgent so as to explore the plant regarding its medicinal importance. Therefore, the aim of this review is to boost up present day researchers in this direction to undertake further investigations of this plant and we do anticipate that this plant will be much effective in drug development programme in near future.

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Table 1. List of phytochemical constituents isolated from *L. aromatica*

Phytochemical constituents isolated from *L. aromatica*	Plant part	Reference
5,7-Dihydroxy-3,6,3',4'-tetramethoxyflavone (7-desmethyl artemetin,) **(1)**	Aerial parts and roots	12
5,7-Dihydroxy-6,8,4'-trimethoxyflavone (Nevadensin) **(2)**	Aerial parts and roots	13-14
5-Hydroxy-6,7,8,4'-tetramethoxyflavone (Gardenin B,)**(3)**	Aerial parts	13
Nevadensin-7-*O*-β-D-glucopyranoside **(4)**	Aerial parts	13
Isothymusin **(5)**	Aerial parts	13
Pilosin **(6)**	Aerial parts	13
8-Hydroxysalvigenin **(7)**	Aerial parts	13
Salvigenin **(8)**	----------	14
Pectolinaringenin **(9)**	Aerial parts	13
Triethyl carbinol **(10)**	Essential oil	15-16
Benzene **(11)**	Essential oil	15-16
2,4-Pentadione **(12)**	Essential oil	15-16
3-Hexen-2-one **(13)**	Essential oil	15-16
5-Methyl-5-nonenol **(14)**	Essential oil	15-16
α-Pinene **(15)**	Essential oil	15-17
β-Pinene **(16)**	Essential oil	17
Camphene **(17)**	Essential oil	15-16
1-Octen-3-ol **(18)**	Essential oil	15-17
Sabinene **(19)**	Essential oil	15-16
β-Myrcene **(20)**	Essential oil	15-16
2-Carene **(21)**	Essential oil	15-16
m-Cymene **(22)**	Essential oil	15-16
Z-Ocimene **(23)**	Essential oil	15-17
E-Ocimene **(24)**	Essential oil	17
γ-Terpinene **(25)**	Essential oil	15-16, 18
Terpenolene **(26)**	Essential oil	15-16
Acetic acid, tricyclo [4.4.0.0(3,8)] dec-9-en-4-yl ester **(27)**	Essential oil	15-16
Linalool **(28)**	Essential oil	15-17
α-Humulene/ α-Caryophyllene **(29)**	Essential oil	15-17
α-Terpineol **(30)**	Essential oil	17
Borneol www.iitrpr.ac.in	Essential oil	17
Trans- Shisool **(32)**	Essential oil	17
3-Cyclohexene-1-carboxaldehyde **(33)**	Essential oil	15-16
(-) Camphor **(34)**	Essential oil	15-16
p-Cymen-8-ol **(35)**	Essential oil	15-16
1,3-Cyclohexadiene-1-methanol, 4-(1-methylethyl)- **(36)**	Essential oil	15-16
Caryophyllene **(37)**	Essential oil	15-16
β-Farnesene **(38)**	Essential oil	15-17
Perillyl acetate **(39)**	Essential oil	17
Demethoxy-ageratochromene **(40)**	Essential oil	15-16

Caryophyllene oxide **(41)**	Essential oil	15-16
12-Oxabicyclo [9.1.0] dodeca-3,7-diene, 1,5,5,8-Tetramethyl **(42)**	Essential oil	15-16
2,6,9,9- Tetramethyl, 2,6,10-Cycloundecatriene-1-one **(43)**	Essential oil	15-16
Caffeic acid **(44)**	Aerial parts	14
Chlorogenic acid **(45)**	Aerial parts	14
Limonene **(46)**	Essential oil	15-18
Cis-Limonene oxide **(47)**	Essential oil	17
Trans-Limonene oxide **(48)**	Essential oil	17
Bornyl acetate **(49)**	Essential oil	17
Perillaldehyde **(50)**	Essential oil	17, 19
Cis-4-caranone **(51)**	Essential oil	19
Trans-4-caranone **(52)**	Essential oil	19
Caranyl acetate **(53)**	Essential oil	17
Eugenol **(54)**	Essential oil	18

Table 2. Antibacterial activity of crude methanol extract of *L. aromatica* leaves

Microorganisms	Diameter of inhibition zone (mm)	MIC value (mg/mL)
Bacillus cereus	21.0 ± 5.2	2.6
Listeria monocytogenes	12.2 ± 3.4	20.8
Pseudomonas fluorescens	9.7 ± 4.2	20.8
Salmonella typhimurium	8.7 ± 0.6	10.4
Staphylococcus aureus	12.5 ± 2.5	2.6
Yersinia enterocolitica	11.2 ± 3.9	41.7

Table 3. Antioxidant activity and total antioxidant capacity (FRAP assay) of *L. aromatica* aqueous extract

IC_{50} value against DPPH assay (µg/mL)	IC_{50} value against DHF assay (µg/mL)	IC_{50} value to inhibit NO formation (µg/mL)	Ascorbic acid equivalence (µg/mg extract)
10.78 ± 0.31	17.52 ± 2.57	553 ± 51	188 ± 7

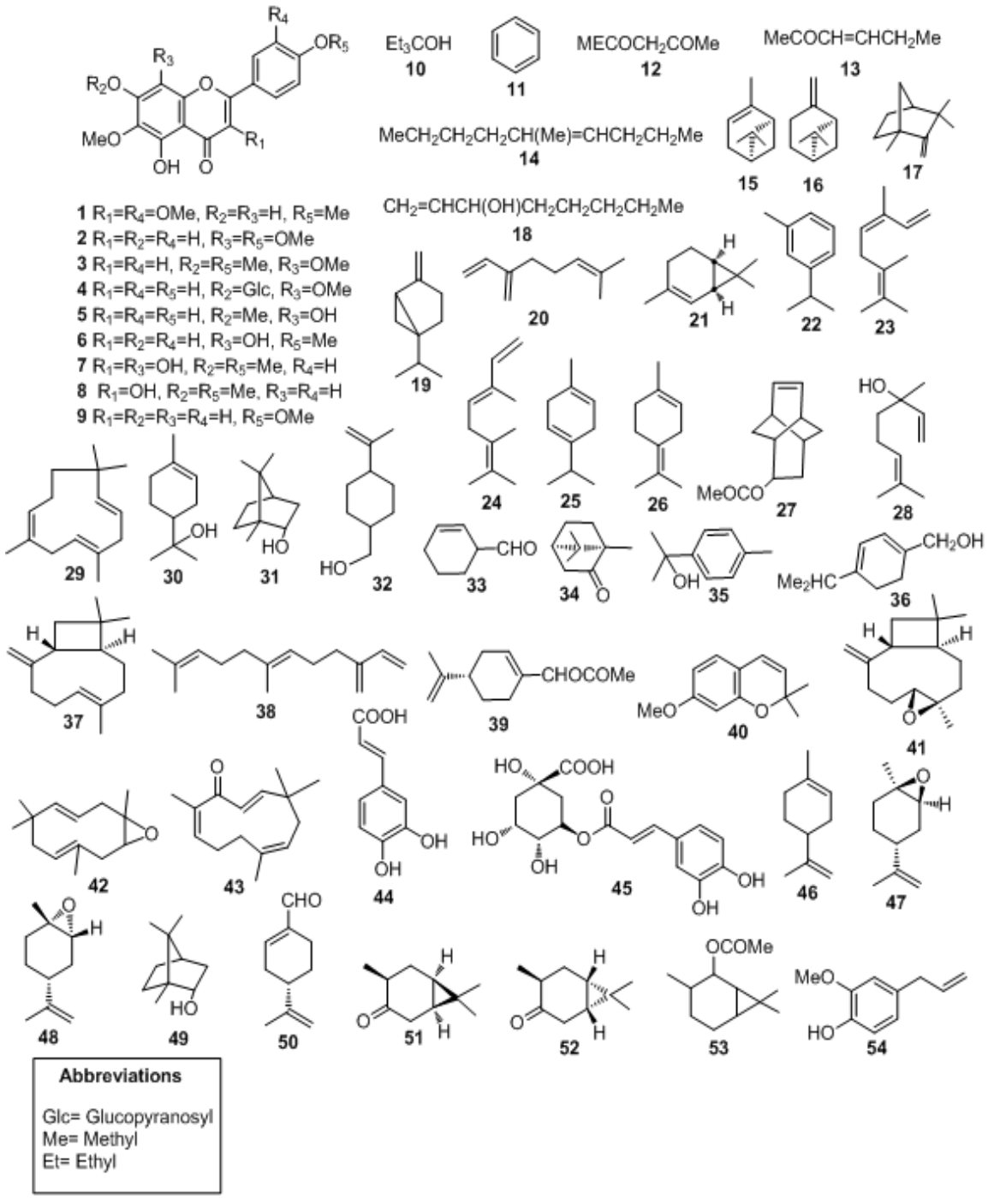

Figure 1. Structure of chemical constituents isolated from *L. aromatica*

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Figure 2. ***Limnophila aromatica***