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The Open Natural Products Journal, 2014, *7*, 1-14

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Limnophila (Scrophulariaceae): Chemical and Pharmaceutical Aspects - An Update

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Abstract: The present resumé covers an up-to-date literature on *Limnophila* species. The botanical classification, ethno-pharmacology, and chemical constituents of *Limnophila* plants, as well as the biological activities and pharmacological applications of both distinct phytochemicals and medicinally active plant materials (formulations, extracts, etc.) are discussed in detail.

Keywords: Biological activities, Botany, Chemical constituents, Ethno-pharmacology, *Limnophila* species, Pharmacological applications, Taxonomical classification.

INTRODUCTION

Limnophila (family: Scrophulariaceae) [1-5] is originated from a *Latin* word that means pond-loving indicating its existence in aquatic environments. It is commonly known as 'Ambulia' (Asian marshweed). It is a perennial herb from Southeast Asia, tropical to subtropical Africa, Australia, and Pacific Islands; also finds adventive distribution in North America. *Limnophila* plants are widely distributed throughout India, and occupy a significant position in traditional systems of medicine. A number of plant species are in use as folk medicines in the treatment of various ailments. A number of works on chemical and pharmacological aspects of genus *Limnophila* have already been done. Here an attempt, for the first time as per our record, has been made to compile all these works that are in scattered in literatures. Although some works on the genus have been done, a major portion remains unexplored. This review is designed in such a fashion so that it would surely boost the ongoing research in this direction. That's why an all round and up-to-date resumé — covering its botany to ethnobotany, biological and pharmacological studies as well as phytochemicals as reported so far — on this important plant genus has been compiled.

BOTANICAL ASPECTS

Limnophila [6, 7] is an aquatic, or nearly aquatic, perennial herb found as submersed, emergent, and amphibious stem plant. Its natural habitats are rivers, lakes, ponds as well as marshy lands. The submerged stems are smooth and have feathery leaves with 30 mm long encircling about the stems. These differ from the emergent stems, which are covered with flat shiny hair and have leaves, generally lance-shaped, up to 3 cm long with toothed margins. Stems may be up to 12 feet long. The emergent stems are usually 2-15 cm above the surface of the water. Single white, pink, purple or blue to lavender flowers, sometimes with conspicuous spots, occasionally occur on the emerged portion of the stem. The flowers are stalkless and borne in the leaf axis, and are axillary and solitary or in axillary or terminal spikes or racemes, sessile or pedicellate. The lower portion (sepals) has five, green, hairy lobes, each 4-5 mm long. The upper portion is purple and composed of five fused petals forming a tube with two lips — adaxial lip (dorsal) is 2-lobed, while abaxial lip (ventral) is 3-lobed. The lips have distinct purple lines on the undersides. The fruit is capsule containing up to 150 seeds.

Limnophila reproduces through fragmentation of the stem or by seeds. In post-rainy session the fruits of *Limnophila* are mature, and the mats break loose from the hydrosoil — as the floating-mats move, they spread out the seeds throughout a wider area.

TAXONOMICAL BACKGROUND

The taxonomical classification [8, 9] of *Limnophila* plants are shown below:

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Scrophulariales
Family	Scrophulariaceae
Genus	*Limnophila* R. Brown

About 40 species [10] of the genus *Limnophila* are known; some common species are cited here:

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1874-8481/14

L. aromatica (Lamarck) Merrill. (Syn. *L. aromaticoides* Yang & Yen; *L. gratissima* Blum)
L. australis Wannan & Waterh.
L. balsamea (Benth.) Benth. (Syn. *L. thorelii* Bonati)
L. borealis Y. Z. Zhao & Maf.
L. brownii Wannan
L. chinensis (Osbeck) Merill (Syn. *L. chevalieri* Bonati; *L. hi*rsuta (Heyne ex Benth.) Benth.
L. connata (Buchanan-Hamilton ex D. Don)
Handel-Mazzetti
L. erecta Bentham
L. fragrans Seem
L. geoffrayi Bonati
L. hayatae Yamazaki
L. heterophylla (Linnaeus) Druce (Syn. *L. reflexa* Bentham)
L. indica (Linnaeus) Druce (Syn. *L. gratioloides* R. Brown; *L. racemosa* Bentham; *L. aquatica* Roxburgh)
L. laotica Bonati
L. laxa Bentham
L. micrantha (Benth.) Bentham
L. parviflora Yamazaki
L. poilanei Yamazaki
L. polyantha Kurz ex Hook.f. (Syn. *L. polyantha* Yamazaki)
L. repens (Bentham) Bentham (Syn. *L. conferta* Bentham; *L. dubia* Bonati; *L. sessilis* (Bentham) Fischer
L. rugosa (Roth) Merill (Syn. *L. roxburghii* G. Don)
L. sessiliflora (Vahl) Blume
L. siamensis Yamazaki
L. taoyuanensis Yang & Yen
L. verticillata Yamazaki
L. villifera Miq.
L. X ludoviciana Thieret
L. dasyantha Skan
L. glabra (Benj.) Kerr
L. hottonoides Druce
L. gigantean

TRADITIONAL USES

Limnophila plants are extensively used in the indigenous system of medicine, and are found to be useful and effective. Traditional uses of only a few of these significant plant species finding useful applications in the treatment of various ailments are mentioned here. The medicinal uses of these plant species are being cited on the basis of extensive literature survey.

(i) ***L. aromatica (Syn. L. gratissima)*:** 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. 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 an essential oil [11-13].

(ii) ***L. rugosa (Syn. L. roxburghii)*:** The plant shows numerous medicinal applications in the traditional system. The juice of the plant is rubbed over the body in pestilent fever. It is applied on elephantiasis with coconut oil. It is administered in diarrhoea, dysentery and dyspepsia. It is used as carminative and tonic. The essential oil is used as flavouring agent of food and perfuming of hair oils. The essential oil of this plant also exhibits significant anti-bacterial and anti-fungal activities. The plant had been accepted for "*Sugandhabala*" as it responded to Ayurvedic description of the drug. Infusion of leaves is used as diuretic and stomachic in the Philippine Islands and in India [12, 14, 15].

(iii) ***L. indica (Syn. L. gratioloides; L. racemosa; L. aquatica)*:** The plant has a refreshing and agreeable odour resembling to camphor or oil of lemon. *L. indica* is considered to be carminative and antiseptic. A liniment prepared from the plant is used in elephantiasis. The juice of the plant is rubbed over the body in pestilent fevers. It is given internally in dysentery combined with ginger, cumin and other aromatics [12-14].

(iv) ***L. conferta:*** The plant has been employed to treat various types of skin diseases and conditions of inflammation in the indigenous system of medicine [14, 16].

CHEMICAL CONSTITUENTS OF *LIMNOPHILA*

The phytochemical investigation of the genus *Limnophila*, as carried out so far, has afforded a good number of compounds, previously known from other natural sources or isolated as new phytochemicals. These compounds are of varying structural skeletons and are classified into flavonoids (**1-23;** Fig. **1**) (Table **1**), terpenoids (**24-60;** Fig. **2**) (Table **2**), and miscellaneous (**61-87;** Fig. **3**) (Table **3**).

BIOLOGICAL/PHARMACOLOGICAL ACTIVITIES OF CRUDE PLANT MATERIALS AS WELL AS OF CHEMICAL CONSTITUENTS

A good number of biological/pharmacological works with different parts of *Limnophila* plants as crude extracts and also of pure chemical constituents isolated from these plant species have been reported so far. This section is an attempt to sum up all these findings.

Antimicrobial Activity

Limnophila plants are reported to exhibit significant antimicrobial activity. *L. racemosa* and *L. indica* extracts were found to inhibit the growth of *Xanthomonas campestris* and *X. malvacearum in vitro* [50]. Mishra *et al.* [51] also studied the antimicrobial activity of the same plant extracts against a number of bacterial species and obtained a convincing result (Table **4**) on the basis of which the workers pointed out that both the extracts of *L. racemosa* and *L. indica* bear certain antimicrobial components.

Antibacterial efficacy of the essential oil of *L. conferta* was also established by Reddy *et al.* [33] against the Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa***.** The significant antimicrobial activity of the essential oil of *L. conferta*, comparable with that of chloramphenicol used as standard and ethylene glycol as control solvent justifies the use of this plant in the indigenous system of medicine in controlling some infections. The oil was not found to be toxic at a dose level of 1.6 mL/Kg orally.

Reddy *et al.* [33] reported that the essential oil of *L. gratissima* (*Syn. L. aromatica*) shows a good antimicrobial activity (Table **5**) of the same order of that of the reference standards, streptomycin and chloramphenicol. Recently, antimicrobial efficacy of the plant extracts against number bacterial strains was found to be promising [52-54].

Further, Kapil *et al.* [55] reported that *Limnophila rugosa* essential oil and its constituents also show potent anti-bacterial activity against *Bacillus subtilis* and

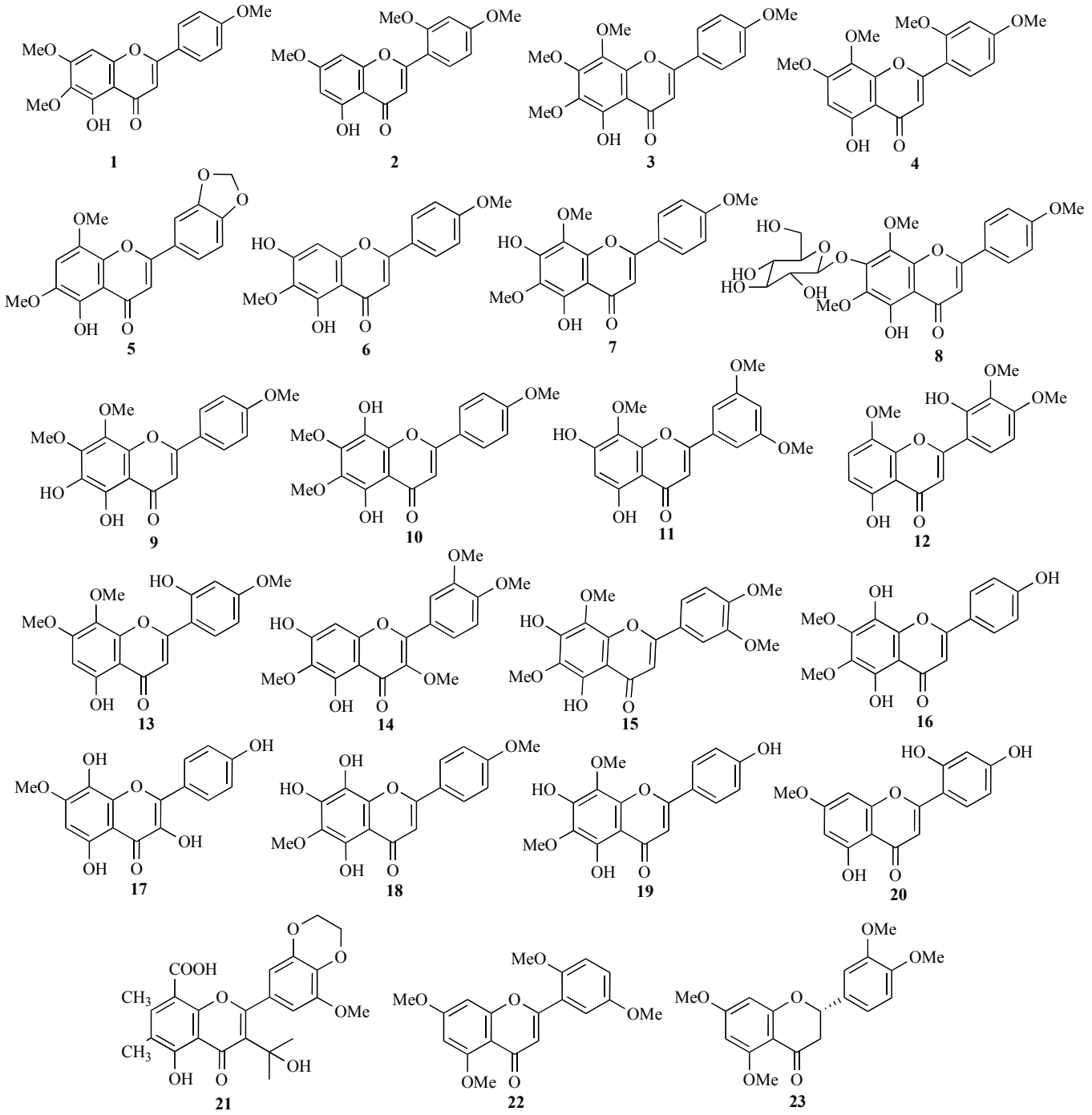

Fig. (1). Structures of flavonoids from *Limnophila.*

Salmonella typhi. Chloroform extract of the aerial parts of *L. geoffrayi* also found to possess antimycobacterial activities [24]. The essential oil of *L. rugosa* is reported to exhibit antifungal activity [13]. The essential oil of *L. conferta* is also a useful antifungal antidote. The antifungal activity of the oil at 1: 50 dilution in ethylene glycol was found to be of the same order as that of griseofulvin in chloroform used as standard (100 µg/0.1ml). In case of the dermatophytes viz. *Trichophyton mentagrophytes* and *Microsporum gypseum* the oil at a concentration of 100 µg/mL inhibited the growth of both the fungi, however less effective than the standard, miconazole (10 µg/mL) [33].

A promising antifungal efficacy of *L. gratissima* (essential oil) was reported by Rao *et al.* [56]. At a dose of 0.1 mL, the essential oil of the plant showed inhibition zones of 20, 28 & 25 mm (diameter) respectively against *Aspergilus niger, Rhizopus oryzae* and *Candida albicans* while the reference standard, griseofulvin exhibited the respective inhibition zones of 18, 24 and 14 mm at a dose of 100 mg in $CHCl_3$. It appeared that the oil of *L. gratissima* is mostly active against *Rhizopus oryzae,* and the efficacy is greater than grisofulvin; thus the above findings are in support with the traditional uses of the plant oil as antiseptic [56].

Table 1. Flavonoid Constituents of *Limnophila*.

Compounds (Str. No.)	Source	Ref.
5-Hydroxy-6,7,4'-trimethoxyflavone (**1**) (Salvigenin)	*L. rugosa* (aerial parts and roots)	[17]
5-Hydroxy-7,2',4'-trimethoxyflavone (**2**)	*L. rugosa* (aerial parts and roots)	[18]
5-Hydroxy-6,7,8,4'-tetramethoxyflavone (Gardenin B) (**3**)	*L. aromatica* (aerial parts and roots)	[19]
5-Hydroxy-7,8,2',4'-tetramethoxyflavone (**4**)	*L. rugosa* (aerial parts and roots) *L. hetero-phylla* (aerial parts and roots)	[20] [21]
5-Hydroxy-6,8-di-methoxy-3',4'-methylene- dioxyflavone (**5**)	*L. indica* (aerial parts and roots)	[22]
5,7-Dihydroxy-6,4'-dimethoxyflavone (Pectolinaringenin) (**6**)	*L. aromatica* (aerial parts and roots)	[19]
5,7-Dihydroxy-6,8,4'-trimethoxyflavone (Nevadensin) (**7**)	*L. aromatica* (aerial parts and roots) *L. geoffrayi* (aerial parts) *L. heterophylla* (aerial parts and roots) *L. rugosa*	[19,23] [24] [25,26] [27]
Nevadensin-7-*O*-β-D-glucopyranoside (**8**)	*L. aromatica* (aerial parts and roots)	[19]
5,6-Dihydroxy-7,8,4'-trimethoxyflavone (**9**)	*L. indica* (aerial parts and roots)	[26]
5,8-Dihydroxy-6,7,4'-trimethoxyflavone (**10**)	*L. indica* (aerial parts and roots)	[28]
5,7-Dihydroxy-8,3',5'-trimethoxyflavone (**11**)	*L. rugosa* (aerial parts and roots)	[29]
5,2'-Dihydroxy-8,3',4'-trimethoxyflavone (**12**)	*L. indica* (aerial parts and roots)	[30]
5,2'-Dihydroxy-7,8,4'-trimethoxyflavone (**13**)	*L. heterophylla* (aerial parts and roots)	[31]
5,7-Dihydroxy-3,6,3',4'-tetramethoxyflavone (7-desmethyl artemetin, **14**)	*L. gratissima* (*Syn. L. aromatica*) (aerial parts and roots)	[32]
5,7-Dihydroxy-6,8,3',4'-tetramethoxyflavone (Hymenoxin, **15**)	*L. heterophylla*	[33]
5,8,4'-Trihydroxy-6,7-dimethoxyflavone (Isothymusin, **16**)	*L. geoffrayi* (aerial parts) *L. aromatica* (aerial parts and roots)	[23] [19]
3,5,8-Trihydroxy-7,4'-dimethoxyflavone (**17**)	*L. aromatica* (aerial parts and roots)	[19]
5,7,8-Trihydroxy-6,4'-dimethoxyflavone (Pilosin, **18**)	*L. aromatica* (aerial parts and roots)	[19]
5,7,4'-Trihydroxy-6,8-dimethoxyflavone (Demethoxysudachitin, **19**)	*L. rugosa*	[27]
5,2',4'-Trihydroxy-7-methoxyflavone (Artocarpetin, **20**)	*L. rugosa* (aerial parts and roots)	[34]
3',4'-Ethylenedioxy-5-hydroxy-3-(1-hydroxy-1-methylethyl)-6,7-dimethyl-5'-methoxy-flavone-8-carboxylic acid (**21**)	*L. indica* (aerial parts and roots)	[35]
5,7,2',5'-Tetramethoxyflavone (**22**)	*L. indica* (aerial parts and roots)	[36]
5,7,3',4'-Tetramethoxyflavanone (**23**)	*L. indica* (aerial parts and roots)	[36]

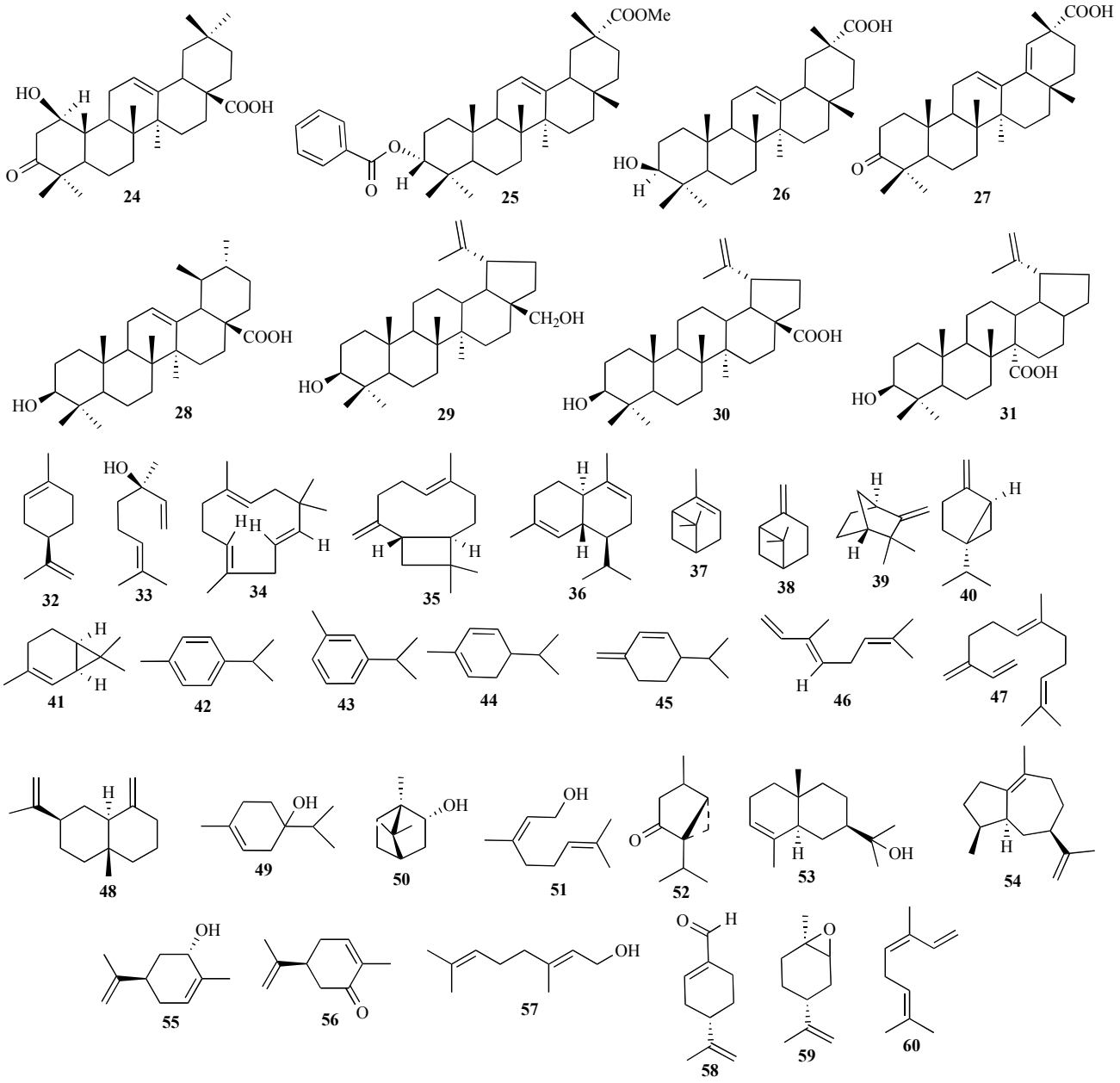

Fig. (2). Structures of terpenoids from *Limnophila.*

Methanolic extract of the whole plants of *L. indica* was found to possess potent antidiarrheal and anti-dysentery efficacy with mild antacid activity. Subhadra *et al.* [57] performed both *in vitro* and *in vivo* studies to evaluate the activities. *In vitro* antibacterial studies with the methanolic extract revealed that at 100 mg/ml it showed more potent activity than gentamycin at 1 mg/ml against *S. aureus* and *E. coli*, while exhibiting similar antibacterial potency as that of gentamycin against the bacterial strains *P. aeruginosa* and *B. substilis*. The study further unearthed an excellent activity of *L. indica* against three *Shigella* species such as *S. flexineri*, *S. dysentery* and *S. boydii*. It is worthy to mention that the plant extract was found to have greater efficacy against *S. flexineri* than the standard drug ceftazidime. The zones of inhibition (in mm scale) for the drug (at 1 mg/ml) and the extract (at 100 mg/ml) were measured as 15.0 ± 0.0 & 18.33 ± 0.58 against *S. flexineri*, 28.0 ± 1.0 & 18.67 ± 0.58 against *S. dysentery* and 20.0 ± 0.0 & 19.0 ± 0.0 against *S. boydii*, respectively [57]. Compared with the normal control group, both the metahnolic extract and ceftazidime were found to be more active against the *Shigella* species ($P<0.01$). The minimum inhibitory concentrations (MICs) for the methanolic plant extract to inhibit the growth of *S. flexineri*, *S. dysentery* and *S. boydii* were measured as 230, 233 and 235 µg/ml, respectively. The authors further performed *in vivo* experiments to disclose antidiarrheal activity of the *L. indica* plant extract; the methanolic plant extract at a lower dose of 100 mg/kg showed more potent antidiarrheal activity than that of loperamide (at 3 mg/kg), a standard drug having several side-effects. Hence, this plant can be a potent substitute for the synthetic antidiarrheal and anti-dysentery drugs [57].

Table 2. Terpenoid Constituents of *Limnophila.*

Compounds (Str. No.)	**Source**	**Ref.**
1β-Hydroxy-3-keto-olean-12-en-28-oic acid (**24**)	*L. rugosa* (aerial parts and roots)	[37]
Methyl-olean-12-ene-3α-benzoyloxy-29-carboxylate (**25**)	*L. heterophylla* (aerial parts and roots)	[38]
3α-Hydroxyolean-12-ene-29-oic acid (Katonic acid) (**26**)	*L. heterophylla* (aerial parts and roots)	[39]
3-Oxo-olean-12(13),18(19)-dien-29α-carboxylic acid (**27**)	*L. indica* (aerial parts and roots)	[40]
Ursolic acid (**28**)	*L. heterophylla* (aerial parts and roots) *L. rugosa* (aerial parts and roots)	[21] [41]
Betulin (**29**)	*L. rugosa*	[42]
Betulinic acid (**30**)	*L. rugosa*	[42]
3β-Hydroxy-lup-20(29)-en-27-oic acid (**31**)	*L. rugosa*	[27]
R-(+)-Limonene (**32**)	*L. heterophylla* (Essential oil) *L. geoffrayi* (Essential oil)	[43] [44]
R-Linalool (**33**)	*L. rugosa* (Essential oil) *L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[43] [45-47] [44]
α-Humulene (**34**)	*L. rugosa* (Essential oil)	[39]
β-Caryophyllene (**35**)	*L. rugosa* (Essential oil) *L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[39] [45-47] [44]
α-(+)-Cadinene (**36**)	*L. heterophylla* (Essential oil)	[39]
α-Pinene (**37**)	*L. heterophylla* (Essential oil) *L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[39] [45-47] [44]
β-Pinene (**38**)	*L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[47] [44]
Camphene (**39**)	*L. aromatica* (Essential oil)	[45,46]
(+)-Sabinene (**40**)	*L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[45,46] [44]
2-Carene (**41**)	*L. aromatica* (Essential oil)	[44-46]
p-Cymene (**42**)	*L. heterophylla* (Essential oil) *L. geoffrayi* (Essential oil)	[43] [44]
m-Cymene (**43**)	*L. aromatica* (Essential oil)	[45,46]
α-Phellandrene (**44**)	*L. conferta* (Essential oil)	[33]
β-Phellandrene (**45**)	*L. conferta* (Essential oil) *L. geoffrayi* (Essential oil)	[33] [44]
β-Ocimene (**46**)	*L. conferta* (Essential oil) *L. geoffrayi* (Essential oil)	[33] [44]
trans-β-farnesene (**47**)	*L. conferta* (Essential oil)	[33]

(Table 2) contd….

Compounds (Str. No.)	**Source**	**Ref.**
(+)-β-Selinene (**48**)	*L. conferta* (Essential oil)	[33]
Terpinen-4-ol (**49**)	*L. conferta* (Essential oil)	[33]
Borneol (**50**)	*L. conferta* (Essential oil)*L. geoffrayi* (Essential oil)	[33] [44]
Nerol (**51**)	*L. conferta* (Essential oil)	[33]
Dihydroumbellulone (**52**)	*L. conferta* (Essential oil)	[33]
α-Eudesmol (**53**)	*L. heterophylla* (Essential oil)	[43]
α-Bulnesene (**54**)	*L. rugosa* (Essential oil)	[43]
trans-Carveol (**55**)	*L. geoffrayi* (Essential oil)	[44]
S-Carvone (**56**)	*L. geoffrayi* (Essential oil)	[44]
trans-Geraniol (**57**)	*L. geoffrayi* (Essential oil)	[44]
S-(-)-Perillaldehyde (**58**)	*L. geoffrayi* (Essential oil)	[44]
(+)-*cis*-Limonene oxide (**59**)	*L. geoffrayi* (Essential oil)	[44]
cis-Ocimene (**60**)	*L. geoffrayi* (Essential oil)	[44]

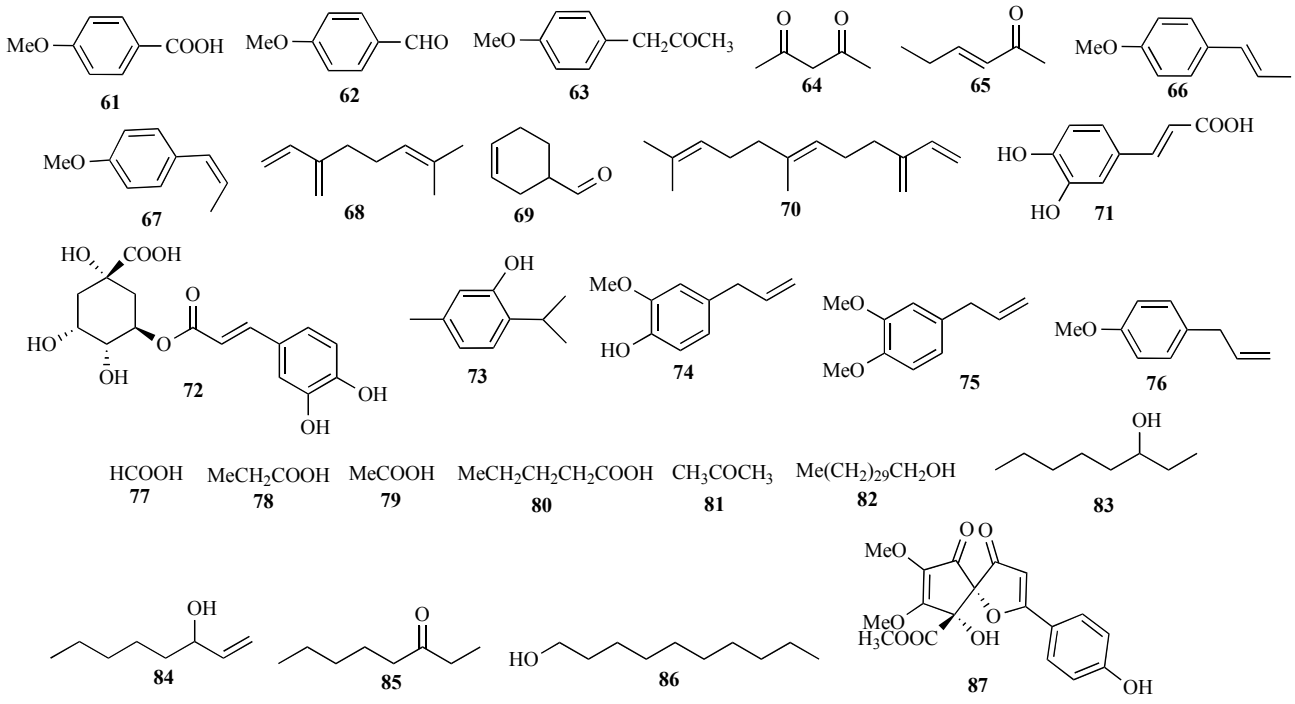

Fig. (3). Structures of miscellaneous compounds from *Limnophila.*

Do *et al.* [58] studied the comparative effect of different solvents (methanol, ethanol and acetone in water) in extracts of *L. aromatica* and evaluated their antioxidant activity, total phenolic content, and total flavonoid content of the freeze-dried *L. aromatica* extracts. The extract obtained by 100% ethanol was found to exhibit the highest total antioxidant activity, reducing power and DPPH radical scavenging activity. The same extract also exhibited the highest phenolic content (40.5 mg gallic acid equivalent/g of defatted *L. aromatica*) and the highest flavonoid content (31.11 mg quercetin equivalent/g of defatted *L. aromatica*); thus, *L. aromatica* can be useful in dietary applications with a potential to reduce oxidative stress [58].

Recently, Brahmachari and his group [26] reported on the evaluation of antimicrobial potential of two bioflavonoids, *i.e.*, 5,7-dihydroxy-6,8,4′-trimethoxyflavone (nevadensin; **7**)

Table 3. Miscellaneous Compounds of *Limnophila.*

Compounds (Str. No.)	Source	Ref.
p-Methoxybenzoic acid (**61**)	*L. rugosa* (Essential oil)	[48]
Anisaldehyde (**62**)	*L. rugosa* (Essential oil)	[48]
Anisylacetone (**63**)	*L. rugosa* (Essential oil)	[43]
2,4-Pentanedione (64)	*L. aromatica* (Essential oil)	[45,46]
3-Hexen-2-one (65)	*L. aromatica* (Essential oil)	[45,46]
trans-Anethole (**66**)	*L. rugosa* (Essential oil)	[43]
cis-Anethole (**67**)	*L. rugosa* (Essential oil)	[43]
β-Myrcene (**68**)	*L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[45,46] [44]
3-Cyclohexene-1-carboxaldehyde (**69**)	*L. aromatica* (Essential oil)	[45,46]
β-Farnesene (**70**)	*L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[45,46] [44]
Caffeic acid (**71**)	*L. aromatica* (Essential oil)	[23]
Chlorogenic acid (**72**)	*L. aromatica* (Essential oil)	[23]
Thymol (**73**)	Essential oil of *L. conferta*	[33]
Eugenol (**74**)	*L. aromatica* (Essential oil)	[23]
Methyleugenol (**75**)	*L. geoffrayi* (Essential oil)	[44]
Methylchavicol (**76**)	*L. rugosa* (Essential oil)	[48]
Formic acid (**77**)	*L. rugosa* (Essential oil)	[48]
Propionic acid (**78**)	*L. rugosa* (Essential oil)	[48]
Acetic acid (**79**)	*L. rugosa* (Essential oil)	[48]
Valeric acid (**80**)	*L. rugosa* (Essential oil)	[48]
Acetone (**81**)	*L. rugosa* (Essential oil)	[48]
Hentriacontanol (**82**)	*L. rugosa* (Essential oil)	[41]
3-Octanol (**83**)	*L. geoffrayi* (Essential oil)	[44]
1-Octen-3-ol (**84**)	*L. aromatica* (Essential oil) *L. geoffrayi* (Essential oil)	[45-47] [44]
3-Octanone (**85**)	*L. geoffrayi* (Essential oil)	[44]
1-Decanol (**86**)	*L. geoffrayi* (Essential oil)	[44]
Limnophila-spiroketone (**87**)	*L. geoffrayi* (aerial parts)	[49]

and 5,6-dihydroxy-7,8,4′-trimethoxyflavone (**9**), isolated from *L. heterophylla* and *L. indica*, respectively, against the microbial strains *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Alternaria solani*, and *Candida albicans*. Compounds **7** and **9** exhibited moderate but broad antimicrobial activities against both Gram-positive and Gram-negative bacteria and also against the fungal pathogens. Compound **7** showed a bactericidal effect against *E. coli* and *S. aureus* (MICs of 200 and 250 mg/ml, respectively), while compound **9** was found to effectively kill *B. subtilis* by cell lysis. The detailed experimental results are presented in Table **6**. The scanning electron microscopic pictures (Fig. **4**) clearly delineate morphological deformities of the microbial cells observed by the effect of compounds **7** and **9** [26].

The same laboratory of Brahmachari [40] also reported on the isolation of a potent antimicrobial pentacyclic triterpenoid constituent, characterized as 3-oxo-olean-12(13),18(19)-dien-29α-carboxylic acid (**27**) on the basis of detailed spectral studies, from the aerial parts and roots of *L. indica*. The antibacterial effect of the compound **27** was evaluated against three *Gram*-positive (*Bacillus subtilis*

Table 4. Antimicrobial activity of *L. racemosa* and *L. indica* [51].

Plant extracts	Diameter of the inhibition zone including diameter of well (10 mm) in mm.								
	Species* 1	2	3	4	5	6	7	8	9
L. racemosa (whole plant)	29	30	28	32	25	24	22	19	20
L. indica (whole plant)	18	28	25	30	26	25	26	18	17
Control	28	24	20	20	26	22	18	16	16

*1. *Bacillus anthracis* 4. *Bacillus subtilis* 7. *Staphylococcus albus*
2. *Bacillus mycoides* 5. *Pseudomonas* sp. 8. *Xanthomonas campestris*
3. *Bacillus pumilus* 6. *Salmonella paratyphii* 9. *Xanthomonas malvacearum*

Table 5. Antimicrobial activity of *L. gratissima* [33].

Bacteria	Diameter of inhibition zone (mm)		
	Essential oil *L. gratissima* (0.1mL)	Chloramphenicol (positive control) 25µg	Streptomycin (positive control) 50µg
Bacillus subtilis	18	18	21
Staphylococcus aureus	16	15	21
Escherichia coli	14	18	23
Pseudomonas aeruginosa	15	17	20

Table 6. Anti-microbial study by microdilution method for compounds 7 & 9, and thymol (positive control) against the bacteria *E. coli*, *S. typhimurium*, *S. aureus*, *B. subtilis*, and fungi *C. albicans* and *A. solani*, assessed by the Disk Diffusion and the Broth Microdilution Methods. Values are arithmetic means with ranges in parentheses (n = 3).

Microorganism	Incubation period (h)	Inhibition zone diameter (mm)[a]						Minimum inhibitory concentration (MIC) values (µg/ml)					
		7		9		Thymol		7		9		Thymol	
		Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range	Arithmetic mean	Range
E. coli	24	20	15-25	–[b]	–	21	19-23	200	150-250	–	–	200	150-200
S. typhimurium	24	14	12-17	12	9-14	18	14-21	300	250-350	500	450-550	225	200-260
S. aureus	24	18	16-20	–	–	16	14-18	250	200-375	–	–	300	275-325
B. subtilis	24	–	–	18	15-21	19	16-21	–	–	300	200-350	350	300-400
C. albicans	24	–	–	19	17-21	20	17-22	–	–	225	200-300	400	350-450
A. solani	24	19	18-21	–	–	20	18-21	200	150-300	–	–	350	300-400

[a]Inhibition zone diameters were assessed at corresponding MIC concentrations obtained by microdilution method (200–500 µg/disk). [b] –: No microbial growth inhibition. [Source: Brahmachari *et al.*, *Chem. Biodiv*., **2011**, *8*, 1039-1151; reproduced with permission]

MTCC121, *Staphylococcus aureus* MTCC96, *Listeria monocytogenes* MTCC657), *Lactococcus lactis* subsp. *lactis* LABW4 and four *Gram*-negative (*Salmonella typhimurium* MTCC98, *Escherichia coli* MTCC1667, *Pseudomonas aeruginosa* MTCC741, *Pantoea ananatis* MTCC2307) bacteria. The compound **27** showed its activity against *Gram*-positive bacteria at very low concentrations within a range of 25-30 µg/ml, whereas it showed a variable sensitivity against *Gram*-negative bacteria. It could kill *E. coli* and *P. aeruginosa* at lower concentrations (40 and 30 µg/ml, respectively), but a comparatively higher amounts (75 and 100 µg/ml, respectively) of the compound were required to kill *P. ananatis* and *S. typhimurium*. The overall results are shown in Table **7**. Interestingly, the triterpenoid **27** was found not to

Fig. 4a)

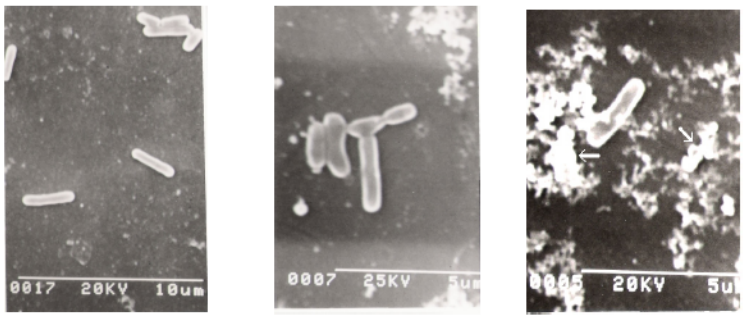

Fig. 4b)

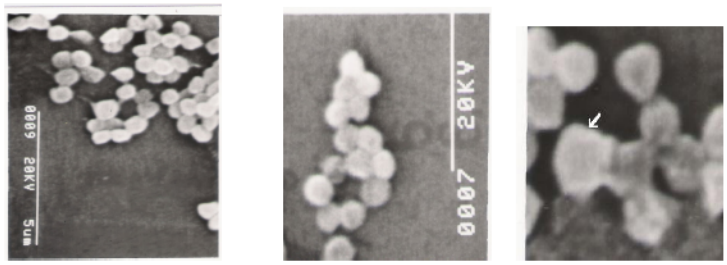

Fig. 4c)

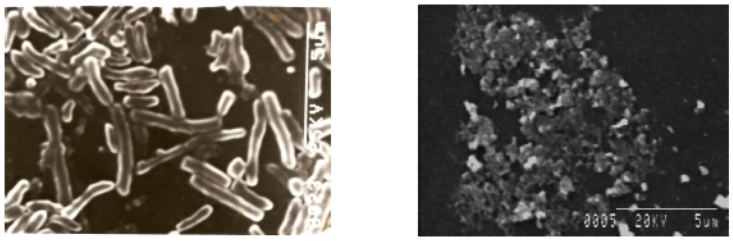

Fig. (4). Scanning electron microscopic pictures: **a**) effect of compound **7** on the morphology of *Escherichia coli*, **b**) effect of compound **7** on the morphology *Staphylococcus aureus*, and c) effect of compound **9** on the morphology of *Bacillus substilis*. Arrows indicate morphological deformities of the microbial cells. [Source: Brahmachari *et al.*, *Chem. Biodiv*., **2011**, *8*, 1039-1151; reproduced with permission].

Table 7. Antibacterial activity of compound 27 and the positive control oxytetracyclin against the Bacteria *E. coli, S. typhimurium, S. aureus, B. subtilis*, *P. aeruginosa*, *P. ananatis* and *L. monocytogenes* assessed by the disk diffusion and the broth microdilution methods. Values are arithmetic means with ranges in parentheses (n=3).

Microorganisms	Incubation Period [h]	Inhibition zone diameter [mm][a]		Minimum inhibitory concentration (MIC) [µg/ml]	
		Compound 27	Oxytetra-cyclin	Compound 27	Oxytetra-cyclin
E. coli	24	22 (20-24)	25(23-27)	40	10
S. typhimurium	24	15 (14-17)	28(27-29)	100	10
S. aureus	24	25 (23-27)	21(19-22)	30	20
B. subtilis	24	28 (26-29)	19(18-20)	25	20
P. aeruginosa	24	25 (23-26)	31(29-32)	30	10
P. ananatis	24	15 (14-17)	—[b]	75	R[c]
L. monocytogenes	24	25 (24-27)	19(20-24)	30	20
L. lactis subsp. *lactis* LAB W4	24	—[b]	28 (27-29)	—[b]	12

[a] Inhibition zone diameters were assessed at corresponding MIC concentrations obtained by microdilution method (200–500 mg/disk). [b] –: No microbial growth inhibition. [c] Resistance. [Source: Brahmachari *et al.*, *Fitoterapia*, **2013**, *90*, 104-111; reproduced with permission]

inhibit a probiotic lactic acid bacterium *Lactococcus lactis* subsp. *lactis* LABW4 under *in vitro* condition and to possess no toxicity in Swiss albino mice [40]. Detailed studies on the mode of action were also reported by this group [40].

Anti-Inflammatory Activity

Reddy *et al.* [33] studied the anti-inflammatory activity of the essential oil and crude extract of *L. conferta* and also of nevadensin (a chemical constituent), isolated from the plant, in acute and chronic inflammatory model employing the method of Winter *et al.* [59]. Carrageenan-induced rat paw edema was compared at '0' and '3' hours with that of control (4% gum acacia mucilage). In tests for acute inflammatory activity, nevadensin (5,7-dihydroxy-6,8,4'-trimethoxy- flavone) showed significant inhibition ($P< 0.001$, dose 75 mg/Kg oral, % inhibition 45.28) but neither the volatile oil nor the crude extract, showed any significant activity compared to the control. However, in chronic inflammation model, the crude extract of *L. conferta* reduced ($P< 0.001$, dose 500 mg/Kg/day oral) the weight of dry granuloma (22.1±1.4 mg % of body weight) compared to the control value (36± 1.86 mg % of body weight). Nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone) has recently been reported to have *in vitro* weak inhibitory activity against cyclooxygenase-1 and 2 (COX-1 and COX-2) as studied in COX catalyzed prostaglandin biosynthesis assay [60].

Antitubercular Activity

Nevadensin and isothymusin (6,7-dimethoxy-5,8,4'-trihydroxyflavone), isolated from the chloroform extract of the aerial parts of *L. geoffrayi*, were reported to exhibit growth-inhibitory activity against *mycobacterium tuberculosis* H 37Ra with equal MIC value of 200 µg/mL [15]; however the efficacy is relatively lower than those of the standard drugs (used during the experiment) rifampicin (MIC 0.003-0.0047 µg/mL), isoniazid (MIC 0.025-0.05µg/mL) and kanamycin sulphate (MIC 1.25-2.5µg/mL). But the flavone, nevadensin was found to be more effective (MIC values: 100 µg/mL for nevadensin; 10µg/mL for streptomycin used as standard) against the H 37Rv strain of *M. tuberculosis* as reported by Reddy *et al.* [33]. The investigators suggested that the compound shows no toxicity up to 600 µg/Kg orally in acute toxicity studies.

Wound Healing Activity

The crude alcoholic extract of *Limnophila conferta* was reported to possess wound-healing property [33]. The effect was studied in three different experimental wound models. Animals were wounded under pentobarbitone [40 mg/Kg/intraperitoneal(IP)] anesthesia (supplemented with ether) to bear either incision/ or excision/ dead space wound. The crude extract was given in the dose of 500 mg/Kg/orally, (once daily) up to 10 days (incision and dead space wound) or until complete healing (excision wound) and the tensile strength was measured on the 10^{th} day.

In the excision wound model, the crude extract showed significant ($P<0.001$) reduction on the epithelisation period (17.22±0.46 days) compared to that of the control (unreacted wounded animals); (21±0.1 days) and significant inhibition in the rate of wound contraction on the 4^{th}, 8^{th}, 10^{th} and 12^{th} days. The 16^{th} day onwards significant enhancement ($P<0.001$) in wound contraction (97.59±0.64%) was shown by crude extract compared to that of control (93.2±1.48). Effects of crude extract on other wound models were insignificant.

Antioxidant Activity

Suksamrarn *et al.* [24] reported significant antioxidant activity of chloroform extract of aerial part of *Limnophila geoffrayi*. Bioassay-guided fractionation of the extract led to the isolation of two pentaoxygenated flavones — one is nevadensin (7) and the other is isothymusin (**16**), of which only the latter exhibited antioxidant activity against the radical scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the IC_{50} value of 7.7µg/mL. The efficacy is almost comparative with the standard antioxidant compound 2,6-di-(*tert*-butyl)-4-methylphenol (BHT, IC_{50} =5.7 µg/mL).

It is interesting to note that isothymusin (**16**) while shows strong antioxidant property, nevadensin can't — this contrasting difference in the behaviour may be explained on the basis of structure/activity relationship. The free 4'-hydroxy group in isothymusin molecule exerts delocalization with the 4-keto group after the 4'-hydrogen being abstracted. The *p*-hydroquinone nature of the A-ring possibly also contributes to the relatively high antioxidant activity of the compound. It should also be noted that the free 7-hydroxyl group of nevadensin does not exert any radical scavenging activity by similar mechanism to that of the free 4'-hydroxyl group as observed in case of isothymusin; one possible cause may be the steric hindrance developed due to the two adjacent methoxyls, although such effect is not observed in case of BHT. The antioxidant efficacy of isothymusin, isolated from other sources was also established by Wang *et al.* [61] and also by Kelm *et al.* [62].

Recently, methanolic extract of *L. aromatica* and its essential oil as well was found to possess significant *in vitro* antioxidant properties assessed in DPPH and nitric oxide (NO) radical scavenging, lipid peroxidation, and ferric reducing antioxidant power (FRAP) assays [52,54,63-66]. The results are promising, and are in accordance to the traditional uses of the plant.

Cytotoxic Activity

The dihydroxytrimethoxyflavone, nevadensin isolated from the plant *L. conferta* was also reported to display moderate cytotoxic activity [33]; the test compound showed 100% cytotoxicity at a concentration of 75 µg/mL both in Dalton's lymphoma ascites tumour and Ehrlich ascites tumour (using Swiss albino mice model). The compound was found to be more effective than wogonin that showed only 24.1% cytotoxicity in both the tumours at the same concentration [33]. This findings support the view of Dong *et al.* that the methoxylated flavones possess moderate cytotoxic activity [66].

Anthelmintic Activity

From the studies of Reddy group [33] with the essential oil of *L. conferta* on a variety of worms, it appears that the oil might be used as a potent and effective antidote against

Table 8. Essential oil of *L. conferta* against earth worm model [33].

Test material/Positive controls	Worms	Dose (mg/mL)	Time required for Paralysis (min)	Time required for death (min)
Oil of *L. conferta*	Earth worm	1.7	125	142
Piperazine citrate		1.7	188	242
Mebendazole		4.0	180	238

Table 9. Essential oil of *L. conferta* against round worm model [33].

Test material/Positive controls	Worms	Dose (mg/mL)	Time required for death (min)
Oil of *L. conferta*	Round worm	2.0	240
Thymol		2.0	378
Mebendazole		2.0	380
Piperazine citrate		2.0	323

Table 10. Essential oil of *L. conferta* against tape worm model [33].

Test material/Positive control	Worms	Dose (mg/mL)	Time required for paralysis & death (min)
Oil of *L. conferta*	tape worm	1.7	55
Piperazine citrate		1.7	165

such parasites. The oil exhibited dose-dependent anthelmintic activity against the test organisms, and in each case the oil was found to be more effective than the standards used. The experimental results are tabulated in Tables (**8-10**).

CONCLUDING REMARKS

Limnophila plants are widely distributed world-wide, and find immense applications in traditional systems of medicine in many countries. Although some works on the chemical and pharmacological aspects of these plants have already been done, a major portion remains unexplored. This present resume is an attempt to compile an all round and up-to-date literature covering its botany to ethnobotany, biological and pharmacological studies as well as phytochemicals as reported so far, with a goal to boost the ongoing research in the field of dynamic bioactive natural products directed toward the searches for 'promising leads' in modern drug development processes.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The author is thankful to the UGC, New Delhi (FIST Programme) for financial support.

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Received: October 30, 2014 Revised: December 07, 2014 Accepted: December 08, 2014