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DISTRIBUTION OF BIOACTIVE COMPOUNDS IN USNEOID LICHENS FROM WESTERN GHATS

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Abstract

Lichens form an important floral community, playing an important role in ecological succession. The Western Ghats harbour a number of plant resources including lichens, mosses and other lower plants. Among the lichen species, those belonging to *Usnea* have been used medicinally for ages. The present study is focusing on the chemical substances present in the Usneoid lichens from the Western Ghats. The study area mainly contains compositions of vegetations like evergreen, semi-evergreen, moist deciduous, dry deciduous and scrub forests. We have collected 38 species of *Usnea* lichens form Western Ghats. The collected *Usnea* lichens were found to be spread between 500- 3000m of altitude. All the collected lichens are subjected to colour test, Thin Layer Crystallography and microcrystallography. We have identified 13 different secondary metabolites from collected 37 species of lichens. Among them Usnic acid is common to all the species. Apart from that strictic acid and salazinic acid is present in majority of the species.

Key words *: Usnea*, Forest, Salazinic, Crystallography.

Introduction

Lichens are the combination of a mycobiont (fungus) and photobiont (algae). The organism consists of thalli made of fungal tissue in which the algal cells are situated. Hence it can grow photosynthetically. Lichens are the earliest colonizer of terrestrial habitats on the earth (Taylor *et al.,* 1995), and they are distributed in all form of environments from arctic to tropical regions and from plains to highest mountains. The fungal partner may contain characteristic secondary compound (Ahmadjian, 1993). These secondary metabolites are unique with respect to those of higher plants. Lichens produce diverse range of secondary metabolites; depsides, despidones, pulvinic acid. These compounds have attracted because of their antiviral, antibiotic, antioxidant, antitumor, allergenic and plant growth inhibitory activities (Muller, 2001; Boustie and Grube, 2005). Over 800 lichen metabolites have been identified so far (Huneck and Yoshimura, 1996). Of all thallophytes, lichens have been most extensively characterized with respect to their chemistry, and their metabolites are useful chemical characteristics in lichen taxonomy (Hegnauer, 1962). Some metabolites may also be produced by the fungus or the algae partner, while others are exclusively produced by synergistic action of both partners in lichens. The large concentrations of mainly phenolic compounds that are accumulated in the thallus are typical of lichens. These secondary metabolites have been produced to protect these organisms from herbivores (Lawrey, 1989). From ancient days lichens have used for various purposes, in particular as dyes, perfumes and in ethnomedicines. Lichens were used by various ethnic groups from the time of early civilization. The lichens were utilized for different purposes on account of their nutritive, medicinal, decorative, brewing, distilling, dyeing, cosmetic and perfumery properties. These different uses are substantiated by the complex lichen secondary metabolism, producing secondary compounds known as "Lichen substances".

Among the lichen, those belonging to *Usnea* are used in medicines from ages. Many species of *Usnea* are used as an ingredient of medicines by Ethnomedicinal practitioners in India and also in the world (Upreti and Chatterjee, 2007). *U. pictoides* shows inhibitory activity

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against clinical isolates of *Streptococci mutans,* (Kekuda *et al.,* 2013). *Usnea longissima* is known for its insecticidal activity (Yildirim *et al.,* 2012).

The presence of usnic acid in *Usnea pseudosinensis* was detected by the multi walled carbon nanotube modified pencil graphite electrode (MWCNT-PGE) ^{1}H and ^{13}C NMR confirms the structure (Kalachar, *et al.,* 2012). Four phenolic crystalline compounds are extracted from the *Parmotrema stuppeum*, the pure compounds showed moderate antioxidant activity (Jayaprakasha *et al.,* 2000). *Usnea ghattensis* showed the antioxidant component in methanol, acetone, ethanol and hexane extracts was derived from the secondary metabolites produced by the lichen mycobiont (Behera, 2005). The present study focusing at the different secondary metabolites present in the *Usnea* lichens from Western Ghats and their applications.

Materials and Methods

Usnea species are collected from the different parts of the Western Ghats. The Western Ghats forests are considered as the 18th mega biodiversity centre of the world, which stretches from Tapti valley in the north of Gujarat to Kanyakumari covering a distance of 1600 km with over 100km wide. The Western Ghats runs through different states of south-western India such as Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala, and covers various types of vegetations including evergreen, semi evergreen, deciduous, scrub and Montana forests.

The Western Ghats harbour almost 45% of the total lichens in India, the highest for any region in the country. Out of these, 253 species are endemic to Western Ghats (Nayaka and Upreti, 2006). We collected 38 different species of *Usnea* from different parts of the Western Ghats.

Survey was done regularly in different habitats of Western Ghats. The representative lichen specimens were collected along with their substratum irrespective of their growth form. The three major substrates such as wood, rock and soil were considered as the microhabitats, the woody substratum includes tree trunks, branches, twigs, wood logs and stumps. The corticolous lichens growing on tree trunks at reachable height usually collected and canopy lichens found fallen on the ground was collected. The data on locality, altitude, vegetation type, and microhabitat were recorded. The specimens were identified with the help of morphological, anatomical and chemical character. The identification of collected lichens is done by using standard manual (Awasthi, 2007). The secondary metabolites are identified by performing colour test, TLC and microcrystallogrphic methods on the *Usnea*. The colour tests are performed with the usual reagents, *i.e.*, K test (5% Potassium hydroxide), C (aqueous solution of Calcium hypochlorite), I (Iodine) and

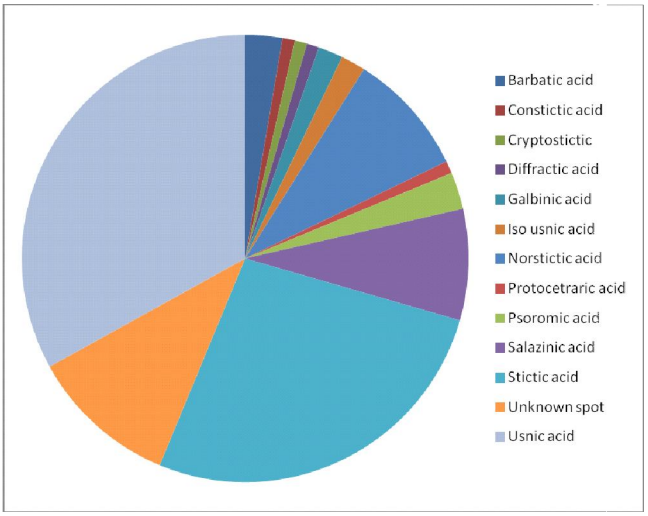


Fig. 1: Pi-chart distribution of secondary metabolites in *Usnea* of Western Ghats.

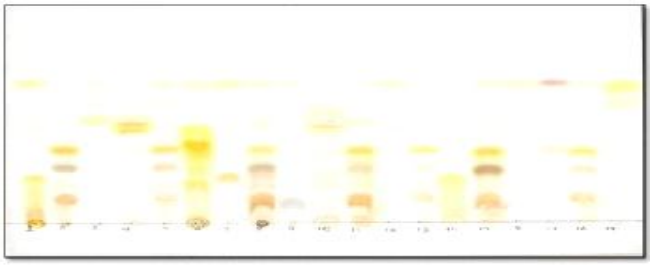


PLATE-1

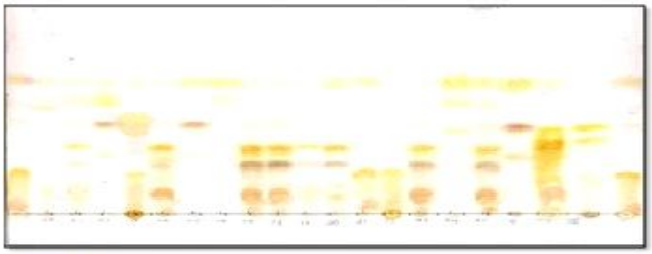


PLATE-2

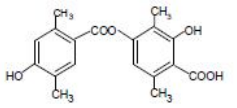
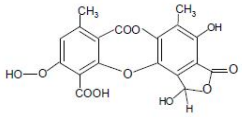
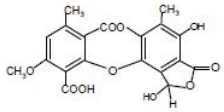
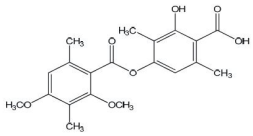
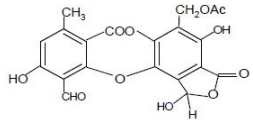
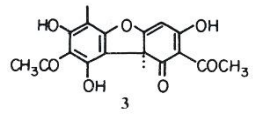
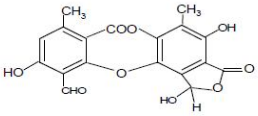
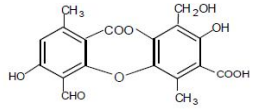
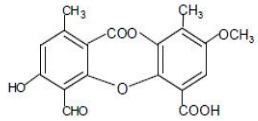
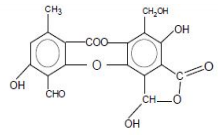
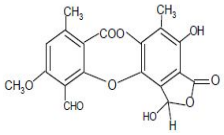
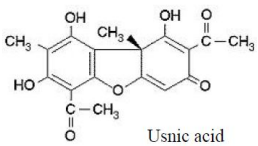
Plate 1:

1- *Parmelinella wallichiana,* 2- *Usnea aciculifera,* 3- *U. austro-indica,* 4- *U. baileyi,* 5- *U. bismolliscula,* 6- *U. complanata,* 7- *U. coralina,* 8- *U. compressa,* 9- *U. dasaea,* 10- *U. fragalis,* 11- *U.eumitriodes,* 12- *U. ghattensis,* 13- *U. gigas,* 14- *U. himalayana,* 15- *U. himantodes,* 16- *U. inermis,* 17- *U. lucea,* 18- *U. leucospilodea,* 19- *U. luridorufa.*

Plate 2:

C- *Parmelinella wallichiana,* 20- *U. maculate,* 21- *U. nilgirica,* 22- *orientalis,* 23- *U. pangiana,* 24- *U. picta,* 25- *U. pictoides,* 26- *U. pseudosinensis,* 27- *U. rigidula,* 28- *U. rubicund,* 29- *U. sinensis.* 30- *U. spinosula,* 31- *U. splendens,* 32- *U. stigmata,* 33- *U. stigmatoides,* 34- *U. subflorida,* 35- *U. subfloridana,* 36- *thomsonii,* 37- *U. undulata,* 38- *U. vulneraria.*

Table 1: Table showing the secondary metabolite, its structure, bioactivity and number of species in which it is present.

Sl.No.	Secondary Metabolite	No. of Species	Structure	Bioactivity
1.	Barbatic acid	3		Antimicrobial activity, antiproliferative activity (Kumar and Müller, 1999)
2.	Constictic acid	1		Anticancerous activity (Shrestha *et al.*, 2014)
3.	Cryptostictic	1		Antimicrobial activity (Cobanoglu *et al.*, 2006)
4.	Diffractic acid	1		Antifungal agent, antiviral, antitumor, analgesic and antipyretic; inhibition of leukotriene B4 biosynthesis (LTB) in leukocytes (Kumar and Müller, 1999)
5.	Galbinic acid	2		Antibacterial activity (Sultana and Afolayanb, 2011)
6.	Iso usnic acid	2		Antibacterial activity
7.	Norstictic acid	10		Antibacterial activity (Sultana and Afolayanb, 2011)
8.	Protocetraric acid	1		Antimicrobial activity (Rankovic *et al.*, 2008)
9.	Psoromic acid	3		ioxidant and cardiovascular protective activities hera *et al.*, 2009)
10.	Salazinic acid	9		Antibacterial activity, antioxidant activity (Paz *et al.*, 2010)
11.	Stictic acid	30		Antimicrobial, anticancer activity and antioxidant activity (Paz *et al.*, 2010; Pejin *et al.*, 2013)
12.	Usnic acid	37	 Usnic acid	Antitumor, antimutagenic, antioxidant, analgesic, antipyretic, enzyme inhibitory, hepato-protective and antimicrobial activities (Mayer *et al.*, 2005; Rankovic *et al.*, 2008;Behera, 2009)

PD (Paraphenylenediamine). Also Thin Layer Chromatography (TLC) in solvent A (180ml tolune: 60ml dioxine: 8ml Acetic acid) using standard technique (Culberson, 1972, Walker and James, 1980) was performed.

Results and discussion

Among all the collected *Usnea* we identified 13 different secondary metabolites (Fig. 1). Usnic acid is common secondary metabolite found in almost all species. Apart from the Usnic acid, Salazinic and stictic acid is present in most of the species. These 13 chemical compounds belong to different chemical classes such as dibenzofurane, depsidone and didepside. Most of the secondary metabolites belong to depsidones followed by didepside and only Usnic acid belongs to the class dibezofurane. Except *U. picta, U. pangiana* and *U. himantodes* all the collected *Usnea* species contain the

Table 2: Different chemical classes present in *Usnea.*

Sl. No.	Species	Tannin	Alkaloids	Phenols	Flavinoids	Didepside	Tetradepside	Xanthone	Depsidone	Dibenzofurane	Chromome	Anthraquinone
1	*Usnea aciculifera*	-	-	-	-	-	-	-	+	+	-	-
2	*Usnea austro indica*	+	-	-	-	-	+	-	-	+	-	-
3	*Usnea baileyi*	-	-	-	-	-	-	-	+	+	-	-
4	*Usnea bismolliscula*	+	-	+	-	-	-	-	+	+	-	-
5	*Usnea complanata*	-	-	-	-	-	-	-	+	+	-	-
6	*Usnea compressa*	-	-	-	-	-	-	-	+	+	-	-
7	*Usnea corallina*	-	-	-	-	-	-	-	+	+	-	-
8	*Usnea dasaea*	+	-	-	-	-	-	-	+	+	-	-
9	*Usnea eumitriodes*	-	-	-	-	-	-	-	+	+	-	-
10	*Usnea fragailis*	-	-	-	-	-	+	-	-	+	-	-
11	*Usnea ghattensis*	-	-	-	+	-	-	-	-	+	-	-
12	*Usnea gigas*	+	-	-	-	-	-	-	+	+	-	-
13	*Usnea himalayana*	-	-	-	-	-	-	-	+	+	-	-
14	*Usnea himantodes*	-	-	-	-	-	-	-	+	-	-	-
15	*Usnea cf. inermis*	+	-	-	-	-	-	-	+	+	-	-
16	*Usnea leucospilodea*	+	-	-	-	-	-	-	+	+	-	-
17	*Usnea lucea*	-	-	-	-	-	-	-	+	+	-	-
18	*Usnea luridorufa*	-	-	-	-	-	-	-	+	+	-	-
19	*Usnea maculata*	-	-	-	-	-	-	-	+	+	-	-
20	*Usnea nilgirica*	-	-	-	-	-	+	-	-	+	-	-
21	*Usnea orientalis*	-	-	-	-	-	+	-	-	+	-	-
22	*Usnea pangiana*	+	-	-	-	-	+	-	+	-	-	-
23	*Usnea picta*	+	-	-	-	-	-	-	+	-	-	-
24	*Usnea pictoides*	-	-	-	-	-	-	-	+	+	-	-
25	*Usnea pseudosinensis*	-	-	-	-	-	+	-	+	+	-	-
26	*Usnea rigidula*	+	-	-	-	-	-	-	+	+	-	-
27	*Usnea rubicunda*	+	-	-	-	-	-	-	+	+	-	-
28	*Usnea sinensis*	+	-	-	-	-	-	-	-	+	-	-
29	*Usnea spinosula*	-	-	-	-	-	-	-	+	+	-	-
30	*Usnea splendens*	-	-	-	-	-	-	-	+	+	-	-
31	*Usnea stigmata*	-	-	-	-	-	-	-	+	+	-	-
32	*Usnea stigmatoides*	-	-	-	-	-	-	-	+	+	-	-
33	*Usnea subflorida*	-	-	-	-	-	+	-	+	+	-	-
34	*Usnea subfloridana*	-	-	-	-	-	-	-	+	+	-	-
35	*Usnea thomsonii*	+	-	-	-	-	-	-	-	+	-	-
36	*Usnea undulata*	+	-	-	-	-	-	-	+	+	-	-
37	*Usnea vulneraria*	-	-	-	-	-	-	-	+	+	-	-

chemical class dibenzofurane. Next to the dibenzofurane, depsidone is the chemical class which is found in 30 species of *Usnea*. Tannins and tetradepsides are rarely found chemical classes. Phenols and flavinoids are found only in one species, that is in *Usnea bismolluscula* and *U.ghattensis* respectively. Alkoloids, didepside, xanthone, chromome and anthraquin are not found in any of the collected *Usnea* species table 2.

These secondary metabolites show different bioactivities. Usnic acid and stictic acids are most common secondary metabolites found in most of the *Usnea*. Usnic acid is found in all the lichen species collected, which has antitumor, antimutagenic, antioxidant and many other properties. Stictic acid is present in 30 species of *Usnea* and shows antimicrobial, anticancer and antioxidant activity. Norstictic acid shows antibacterial activity and is found in 10 species of *Usnea* collected. Cardiovascular activity was known to found in psoromic acid, which is found in 3 species of *Usnea* table 1. Most of the secondary metabolites present in *Usnea* show antimicrobial activity. Among all the secondary metabolites Usnic acid shows highest medicinal value. It shows Antitumor, anti mutagenic, antioxidant, analgesic, antipyretic, enzyme inhibitory, hepato-protective and antimicrobial activities table 1.

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