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Phytochemical analysis of *Stereocaulon pomiferum* and *Usnea baileyi* of Darjeeling hills by LCMS spectra method

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Abstract
Lichens have been central to human health and healing, not only as remedies in traditional medicine but as a reservoir of chemically active molecules known as phytochemicals. Theses naturally occurring secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides and polyphenols have made an invaluable starting point for drug discovery and development. LCMS analysis is one of the modern strategies used for exploration of phytochemicals. In this present study, two hill lichens namely *Sterecaulon pomiferum* and *Usnea baileyi* have been analysed. *S. pomiferum* confirms the presence of Terpenoids, Anthraquinone, Orcinol Tripepsides and Naphthaquinone. and from *U. baileyi* the presence of Stictic acid, Constictic acid, Methyl pseudosalazinate, Eumitrin A1, Usnic acid, 6-O-Methylaverantin and Bonnic acid could be observed from LCMS peaks obtained from methanolic extract of *U. baileyi* was observed.

Keywords: Lichens, Darjeeling Hill, *Stereocaulon Pomiferum*, *Usnea Baileyi*, phytochemicals, LCMS analysis

Introduction

The word lichen is derived from Greek word "Leprous" and refers to use of lichens in treating skin diseases due to peeling skin appearance. Lichens comprise a unique group that consists of two unrelated organisms, a fungus and an alga, growing together in a symbiosis. The medicinal uses to some extent been confirmed by studies which showed that many lichen metabolites such as depsides, depsidones and usnic acid are active against mycobacterium and gram-positive bacteria [1] (Vartia 1973).

Various biological activities of some lichens are known, such as: antimicrobial, antiviral, anti-tumor, anti-inflammatory, analgesic, antipyretic, antiproliferative and antiprotozoal [2, 3, 4]. The interest on the lichen secondary compound is increased because of ineffectiveness of some known previously reliable drugs [3].

India is a rich center of lichen diversity contributing of about 15% of the 13,500 species of lichens so far recorded in the world [5]. In India parmeloid lichens are extensively used in traditional medicine to treat several diseases and disorders e.g., headache, skin diseases, urinary trouble, boils, vomiting, diarrhoea, dysentery, heart trouble, cough, fever, leprosy and as blood purifier (Chandra and Singh 1971. [6]

The lichen members and the abundant distribution of lichens in the varied locations of Darjeeling Hills, the present study includes identification of bioactive compounds from lichens *Stereocaulon pomiferum* and *Usnea baileyi* from Darjeeling Hills. Their extracts were analyzed using LCMS (liquid chromatography-mass spectrometry) for the identification of bioactive compounds.

Materials and Method

Collection of lichen samples

Lichen samples were collected in paper polypacks from different sampling sites. Samples were collected from the barks of trees like *Alnus*, *Erythrina*, *Macaranga*, *Citrus*, *Betula*, *Prunus* as well as rocks and brought to the laboratory. Each specimen was preliminarily identified with the help of available literature, Key to Macrolichens [7]. The taxonomic identity of lichen samples was confirmed from the Lichenology Laboratory, National Botanical Research Institute, Lucknow, Uttar Pradesh, India and the voucher specimens were deposited in the Herbarium of the Postgraduate Department of Botany, Darjeeling Government College and Darjeeling, India.

Identification of active principle in lichen extract

Two lichen samples were air dried at room temperature (26˚C) for until complete drying and then it was ground to powder. Powdered lichen material (10g) was added to 100ml methanol, sonicated and shaken for 7 days in shaking incubator at room temperature. The extract was filtered through whatman filter paper no 42 and was concentrated using a rotary evaporator the obtained extracts were sent to SAIF, CDRI, Lucknow for LCMS analysis.

The mass spectrum as LCMS chromatogram of *U. baileyi* and *S. pomiferum* obtained from SAIF was studied following the literature - A catalogue of standardized chromatographic data of synthetic relationship for lichen substances [8] and lichen substances were determined.

Results

Table 1: List of names, classes, mass spectrum and occurrence of lichen substances obtained from LCMS of methanolic extract of *Stereocaulon pomiferum* (SAIF 7904)

Sr. No.	Compound	Class	Mass spectrum (nm)	Also occurs in
1	Haemoventosin	Naphthaquinones	304, 302, 260	*Ophioparma ventosa*
2.	Phlebic acid B	Terpenoids	458, 440, 415, 387	*Peltigera aphthosa*
3	Methylpseudosalazinate	β-Orcinol Depsidones	402, 384, 369	*Pertusaria* sp.
4.	Stictic acid	â-Orcinol Depsidones	386, 368, 193, 191	*Xanthoparmelia conspersa*

5.	Loxodin (Methylnorlobariate)	Orcinol Depsidones	456, 424	*Xanthoparmelia flavescentireagens*
6.	Divaricatic acid	Orcinol Depsides	1,370, 193, 179	*Canoparmelia texana, Evernia divaricata*
7.	Fulgoicin	Orcinol â-Orcinol Depsidones	370, 368, 333, 325	*Fulgensia fulgida*
8.	Oxyskyrin	Anthraquinones	596	*Trypetheliopsis boninensis*
9.	Lobaric acid	Orcinol Depsidones	456, 438, 412, 235	*Protoparmelia badia*
10	Fragilin	Anthraquinones	318, 284, 277, 275	*Nephroma laevigatum*
11.	Xanthorin(1,5,8-trihydroxy-6-methoxy-3-methylanthraquinone)	Anthraquinones	300, 282, 272, 260	*Xanthoria elegans*
12.	Norsolorinic acid	Anthraquinones	370, 352, 327, 299	*Solorina crocea*
13.	2-*O*-Methylsekikaic acid	Orcinol Depsides	-1, 227, 224, 208	*Ramalina asahinae*
14.	4-*O*-Methylconhypoprotocetraric acid	β-Orcinol Depsidones	-1, 278, 223, 205	*Xanthoparmelia competita*
15.	Crustinic acid	Orcinol Tridepsides	-1, 301, 151	*Umbilicaria crustulosa*
16.	4-*O*-Methylolivetoric acid	Orcinol Depsides	1, 280, 262, 224	*Xanthoparmelia brattii*
17.	4-*O*-Demethylstenosporic acid	Orcinol Depsides	-1, 224, 206, 196	*Xanthoparmelia pokornyi*

The liquid chromatography-mass spectrometry analysis of methanolic extract of *Stereocaulon pomiferum* was found to contain 17 major compounds were confirmed based on their retention time, class and mass spectrum as shown in Table-1. The methanolic extract of *S. pomiferum* revealed the presence of mainly β-Orcinol Depsidones and Orcinol depsides classes of compounds some Terpenoids, Anthraquinones, Orcinol Tripepsides and Napthaquinones. The name of compounds identified are as follows Methylpseudosalazinate, Lobaric acid, Fulgoicin, Loxodin, 4-O-Methylconhypoprotocetraric acid, Constictic acid, Stictic acid, Eumitrin A1, Phlebic acid B, Haemoventosin, Oxyskyrin, Fragilin, Norsolorinic acid, Xanthorin, Norsolorinic acid, Divaricatic acid, 2-O-Methylsekikaic acid, 4-O-Methylolivetoric acid, 4-O-Demethylstenosporic acid, Crustinic acid and Haemoventosin.

Table 2: List of names, classes, mass spectrum and occurrence of lichen substances obtained from LCMS Chromatogram of methanolic extract of *Usnea baileyi* 7904(SAIF)

Sl No	Compound	Class	Mass spectrum (nm)	Also occurs in
1.	Bonnic acid	Orcinol Depsides	416, 236, 224, 207	*Ramalina boninensis*
2.	Methyl pseudosalazinate	â-Orcinol Depsidones	402, 384, 369	*Pertusaria* sp.
3.	6-*O*-Methylaverantin	Anthraquinones	368, 339, 325, 311	*Solorina crocea*
4.	Usnic acid	Usnic acid derivatives	344, 260, 233, 217	*Usnea* sp.
5.	Stictic acid	â-Orcinol Depsidones	386, 368, 193, 191	*Xanthoparmelia conspersa*
6.	Constictic acid	â-Orcinol Depsidones	402, 384, 356, 193	*Xanthoparmelia conspersa*
7.	Eumitrin A1	Ergochromes	680, 621, 561, 501	*Usnea baileyi*

The presence of Stictic acid, Constictic acid, Methyl pseudosalazinate, Eumitrin A1, Usnic acid, 6-O-Methylaverantin and Bonnic acid could be observed from LCMS peaks obtained from methanolic extract of *U. baileyi.*

Discussion

The lichen substances comprise amino acid derivatives, sugar alcohols, aliphatic acids, macrocyclic lactones, mono-cyclic aromatic compounds, quinones, chromones, xanthones, dibenzofuranes, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl ethers [9]. Lichens and their metabolites have many biological activities: antiviral, antibiotic [10], antitumour, [11] antiherbivore [12], ecological roles [13] and enzyme inhibitory [14].

Lichen compounds occurring as phenolics with carbonyl as functional groups play an important role in withering of rocks due to complex metal ions which in turn leads to soil formation. The various biological activities of lichen compounds also help in colonization of terrestrial areas as these compounds have been used by man during ancient Chinese and Egyptian evolution. [15]

Secondary metabolites are products of polyketide pathway, mainly monocyclic and or bicyclic phenols joined by an ester bond (depsides), both ester and ether bonds (depsidones) and furan heterocycle (dibenzofurans). These metabolites are also reported to have defensive function and are known as photoprotecters with great antioxidant capacity. [16, 17] It can be used as preservatives in cosmetic products [18] Studied lichen *Stereocaulon pomiferum*, *Usnea baileyi* revealed the presence of salazinic acid, stictic acid and usnic acid respectively such compounds as reported by Paz *et al.* [19] protected human astrocytes from hydrogen peroxide induce damage. Such compound could also act as antioxidant agents in the neurodegenerative disorders associated with oxidative damage (e.g. Alzheimer's disease and Parkinson's disease). Usnic acid is also known to possess antiprotozoal, antiviral, antiproliferative, anti-inflammatory, analgesic, antipyretic and antitumour activities. [20, 21]

Anthraquinones such as Oxyskyrin, Fragilin, Xanthorin, Norsolorinic acid was obtained from lichen *Stereocaulon pomiferum*, are also studied earlier (Schnazi *et. al.*, 1990 and Sydiskis *et. al.*, 1991 [22, 23] anthraquinones possessed antiviral properties. Stictic acid isolated from *Lobaria pulmonaria* resulted moderate anticancer activity, and this compound could used as a lead compound for desgining of novel human colon adenocarcinoma drugs. [24]. The compound Eumitrin A1 with the formula structure C34H32O15, extracted from *U. baileyi* was examined its cytotoxic activity against Murine Leukemia P388 cells with IC504.5 µg/mL (very active. [25]

The chromatogram gives information on the relative concentrations of various compounds eluted as a function of retention time. The height of the peak indicates the relative concentrations of bioactive compounds. Mass Spectrometer analyses the structure of unknown compounds which are eluted at different time.

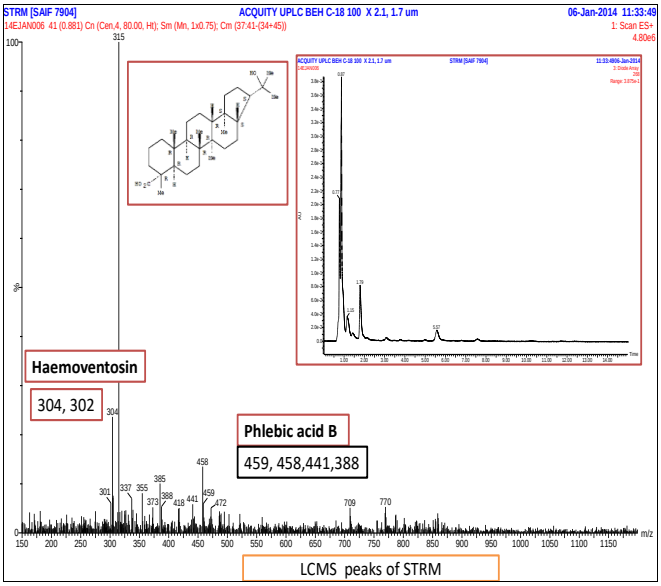
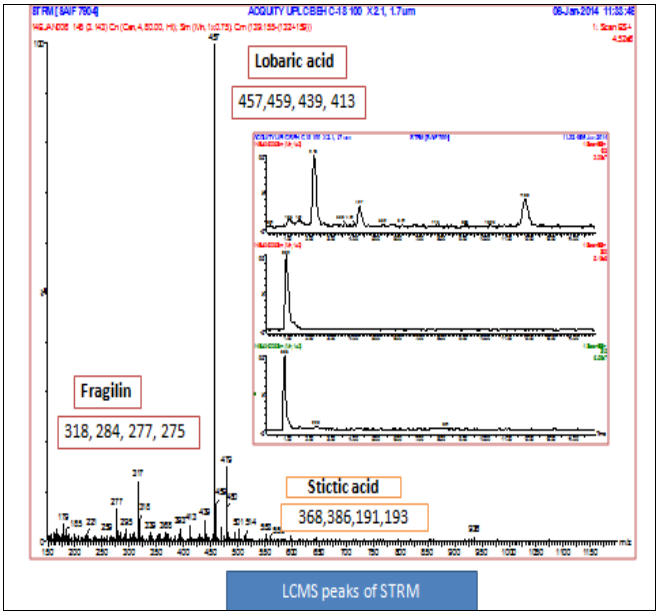


Fig 1: a



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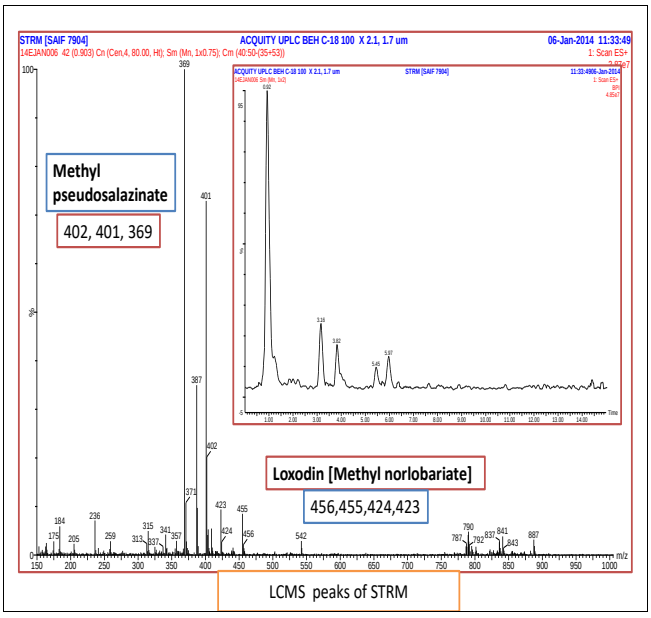

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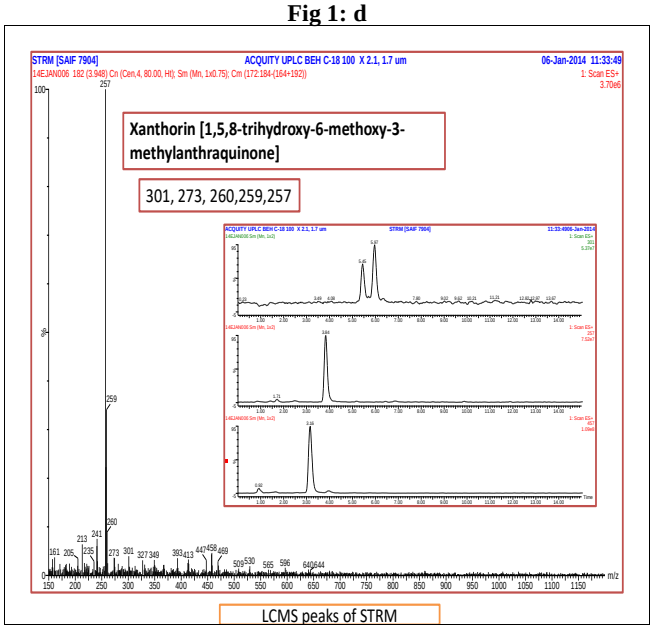


Fig 1: e

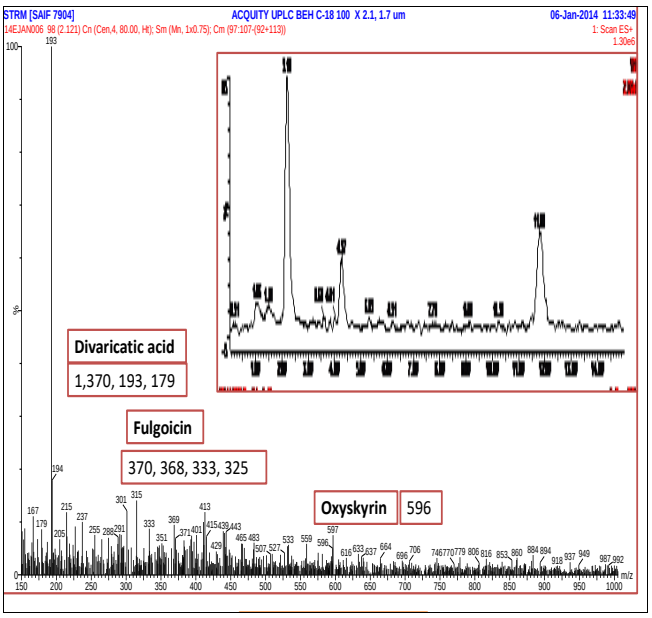


Fig 1: c

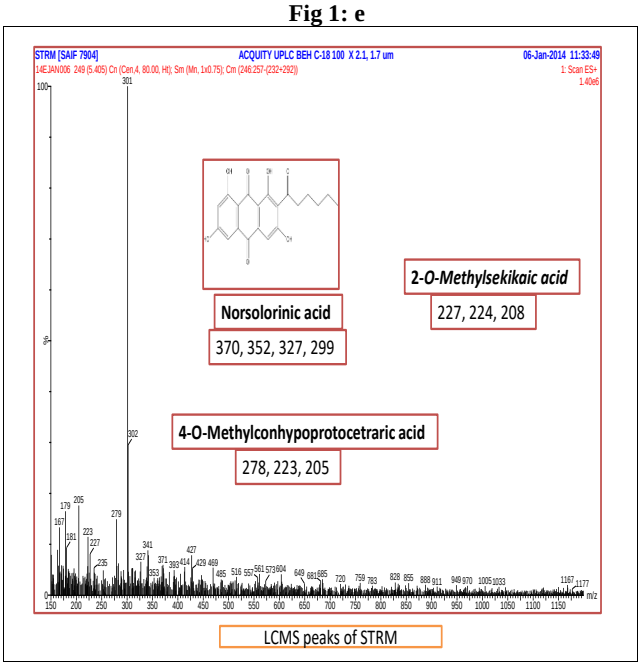
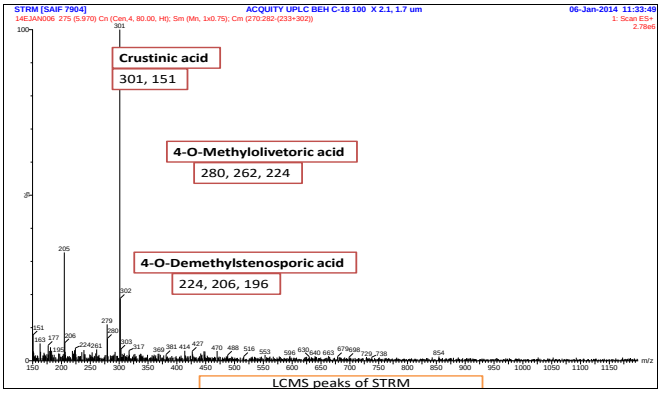


Fig 1: f



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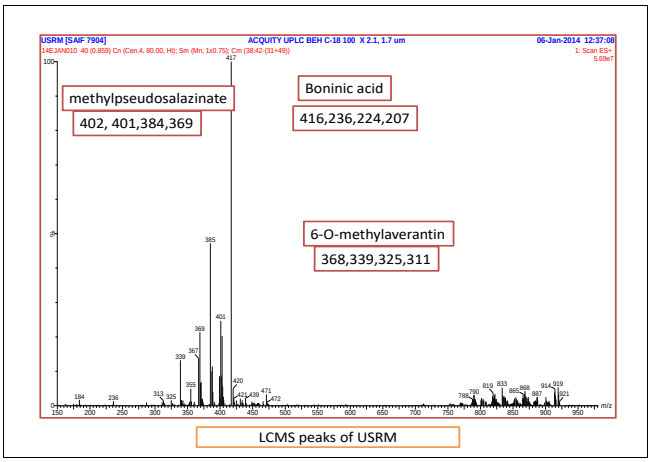

Fig 1: h

Fig 1.a LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig 1.b LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig 1.c LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig.1d LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig.1e LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig.1.f LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig.1g LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*
Fig.1h LCMS spectral peaks and respective compounds of methanolic extract *Stereocaulon pomiferum*

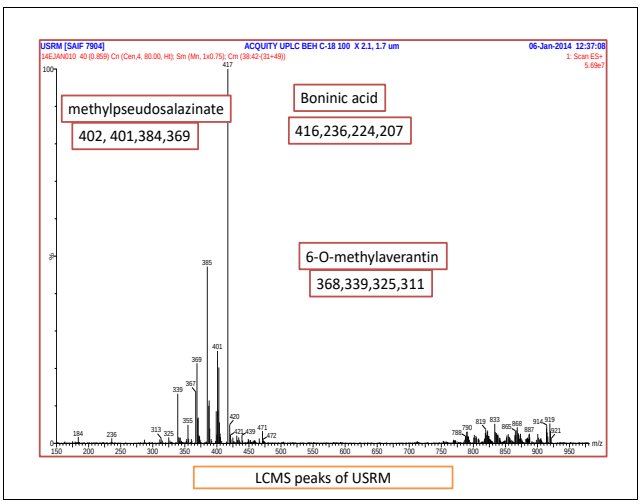

Fig 2: a

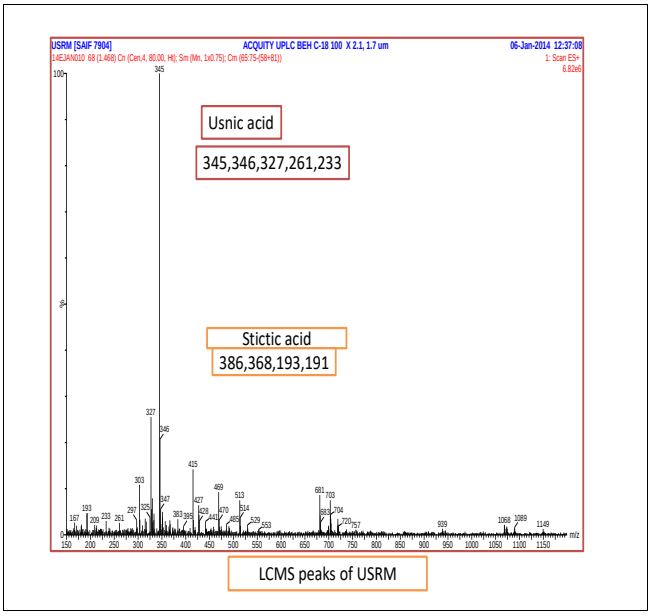

Fig 2: b

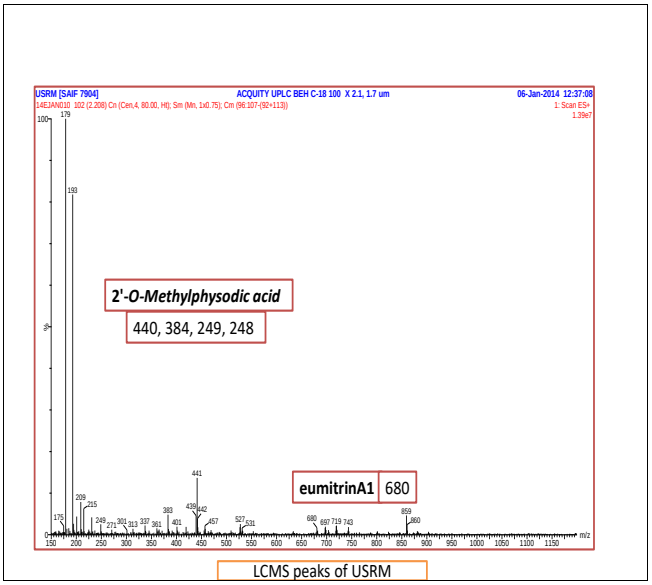

Fig 2: c

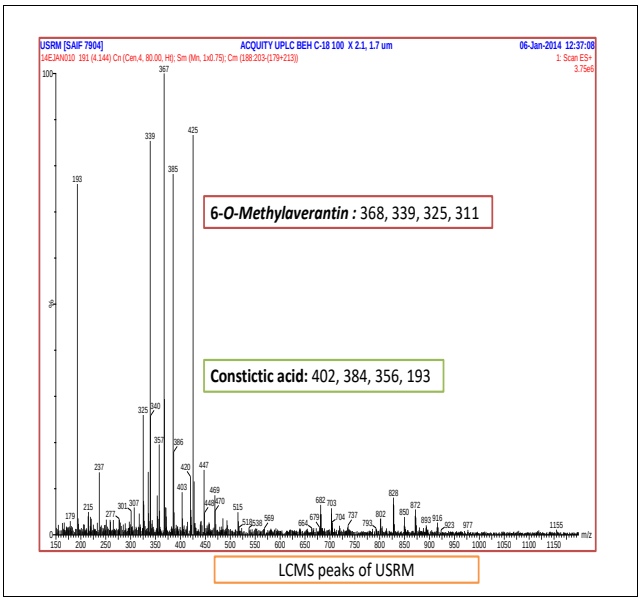

Fig 2: d

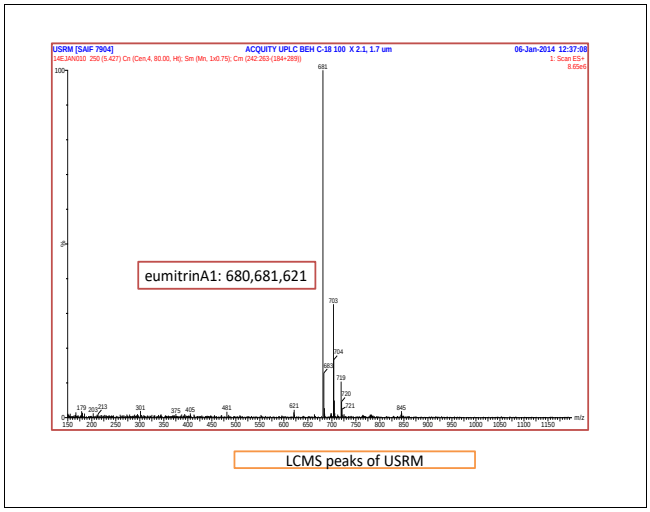

Fig 2: e

Fig 2.a LCMS spectral peaks and respective compounds of methanolic extract *Usnea baileyi*
Fig 2.b LCMS spectral peaks and respective compounds of methanolic extract *Usnea baileyi*
Fig.2c LCMS spectral peaks and respective compounds of methanolic extract *Usnea baileyi*
Fig.2d LCMS spectral peaks and respective compounds of methanolic extract *Usnea baileyi*
Fig.2e LCMS spectral peaks and respective compounds of methanolic extract *Usnea baileyi*

Conclusion

The Methanolic extract of *Stereocaulon pomiferum* and *Usnea baileyi* revealed the presence of therapeutically important bioactive compounds like flavonoids, glycosides, alkaloids, coumarins, terpenoids, saponins using LCMS (liquid chromatography-mass spectrometry) analysis. These bioactive components possess important pharmacological activities and could be useful for treating various human ailments. Further occurrence of different group of phytochemicals *S. pomiferum* and *U. baileyi* samples may be exploited for the development of widely acceptable agents to combat disorders without any side effects. They may offer a promising reservoir of various therapeutic agents.

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