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Research Article

QUANTIFICATION OF LICHENS COMMERCIALLY USED IN TRADITIONAL PERFUMERY INDUSTRIES OF UTTAR PRADESH, INDIA

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ABSTRACT
Lichens are unique plants as they have peculiar morphology comprised of two microorganism's one alga and other fungus living in symbiotic association. Lichens produce unique secondary metabolites which are not known in other group of plants. Lichens are used by the mankind since ancient time for perfumery, spice, food, fodder and an incense material. In India lichens collected from Himalayas are exhaustively used for preparation of perfumes called 'Hina' and 'Attar' in different traditional perfumery industries of north India in Kannauj district of Uttar Pradesh. In the present paper an attempt has been made to inventories lichen species used by the perfumery industries in India based on the samples collected from different markets in the state of Uttar Pradesh. The members of lichen family Parmeliaceae including species of the genera *Bulbothrix, Cetrelia, Everniastrum, Flavoparmelia, Myelochroa, Nephromopsis, Parmelaria, Parmelinella, Parmotrema, Usnea* and together with the species of *Heterodermia, Leptogium, Ramalina* and *Lobaria* are exhaustively utilized in the preparations of perfume. The species of lichen genera Everniastrum and *Parmotrema* contributes the maximum in lichen material used in perfumery industry together with other 30 species.

Keywords: *Lichens, Parmeliaceae, North-India, Perfumery, Attar*

INTRODUCTION
India has a perfumery tradition that dates back to over 5000 years. Even during the days of Indus valley civilization in excavation at Harappa and Mohanjodaro, the proof of the existence of a perfumery industry in our country is confirmed by a water distillation still and receiver recovered from there.
The traditional Indian perfumery is the production of floral attars and otto and attar of Hina, Shamama, Rose, Kewra etc. It is also said that the discovery and development of process for the preparation of attar from Rose was noticed by Noorjahan, the Moghul queen. This was the beginning of the attar industries in India, which developed and progressed in and around Kannauj district (Uttar Pradesh), presently known as city of Attar.
Floral Attars may be defined as the distillates obtained by the hydro-distillation of flowers in Sandalwood Oil or other base materials like Dioctyl phthalate, Diethyl phthalate and liquid Paraffin.
The history of attars is very much associated with the history of Kannauj. Kannauj has been known for the natural attars from the Mughal period or even earlier when aroma bearing substances like Sandal, Musk, Camphor, Saffron were used as such (without isolation of odorous principles) and the range of such materials and essential oils were further enriched during the Mughal period, when new plants were brought by the Mughals from central India to this country.
The attars may be broadly categorized into three types on the basis of raw materials used. Floral Attars are manufactured from single species of flower such as *Rosa damascena* Mill.; *Pandanus odoratissimus* Jacq.*; Jasminum sambac* (L.)Sol.*; Lawsonia inermis* L.*; Jasminum grandiflorum* L. and *Anthocephalus cadamba* Miq. The herbal and spicy Attars are prepared by a number of herbs and spices such as Oakmoss (lichens), Sugandh mantra(*Homalomena aromatic* Schott), Laurel berry (Laurus nobillis), Juniper berry (*Juniperus communis* Thunb.), Cypriol (*Cyperus scariosus* R.Br.), Indian valerian (*Valeriana hardwickii* D.Don.), *Hedychium spicatum* Lodd., Daru Haldi (*Berberis aristata* DC.), Sugandh Bala (*Valeriana wallichii* DC.), Sugandha kokila (*Cinnamomum glaucescens* (Nees) Hand.- Mazz.),

Kulanjan (*Alpinia galangal* Willd.), Javitri/ Jaiphal (*Myristica fragrans* Houtt.), Cardmom (*Elettaria cardamomum* Maton), Clove (*Eugenia caryophyllata* Thunb.), Saffron (*Crocus sativus* Ten.) and Musk (*Abelmoschus moschatus* Medik.). The third category of attars are neither of floral nor herbal origin but produced by distillation of baked soil over base material.

The attars do have a good market potential in middle- east countries where it is used in the absence of alcohol. The attars are getting good market potential in international market because of their high class fragrances, cosmetics and even for aromatherapy. As for as national market concern, Indian attars are used in Tobacco (its extraordinary tenacity along with characteristic to withstand with tobacco note), Pan masala and Gutkha (Tobacco chewing material) as it is the area where consumption of attars are maximum due to its extraordinary stability along with taste in this product which comprised of betel nut, personal perfume, pharmaceuticals and Indian sweets.

In Indian traditional perfume preparation lichens are used in good quantity for preparation of Indian Attar particularly named as Hina and Shamama under the category of herbal and spicy attar. Lichens are known by different trade names such as 'Oakmoss', 'Stone Flowers', 'Pathar fool', 'Daggarphool', 'Chadila', 'Charrila', 'Chadilo', 'Iceland moss', 'Rock moss' (Anonymous, 1978). The quantities of lichens processed annually in the perfume industry in different region of the world are recorded by Moxham (1986). According to Moxham (1986) in India about 900-1000 tons of lichens are collected from the north-India are exploited for perfumes.

In the present study an attempt has been made to identify and quantify the various species of lichens used in the preparation of various Indian attars at Kannauj district, Kannauj a town of north India in the state of Uttar Pradesh. Together with the lichens the waste materials consist of parts of twigs, humus soil is also estimated which are also distillated along with the lichens used for preparation of attars.

MATERIALS AND METHODS

More than one kilogram of lichen material was procured from four different perfumery industries of Kannauj city of Uttar Pradesh. Three replicates each of 100 gm were segregated from the samples and the species in each 100 gm were recorded after their authentic identification. Weight of individual species was estimated in all the replicates.

The morphological characters were studied using a Leica $_{EZ4}$ stereomicroscope while the anatomical characters were studied in Leica^{TM} DM 500 optical microscope. The colour spot tests were carried out on cortex and medulla with usual chemical reagents such as aqueous Potassium Hydroxide (K),Steiner's stable Paraphenylene diamine (PD) and aqueous Calcium Hypochlorite (C).The chemical substances were identified with the help of thin-layer chromatography (Orange *et al.,* 2001; Elix *et al.,* 1993). For species identification relevant key and monographs on Indian lichens were used (Awasthi *et al.,* 2007; Upreti *et al.,* 2005).

RESULTS AND DISCUSSION

The complete identification of lichen material procured from perfumery industries revealed the presence of 31 species belonging to 15 genera and 5 families, of which the member of family Parmeliaceae exhibit their maximum representation by 9 genera and 23 species.

Different lichen species encountered in the lichen material used for perfumery exhibit the great variation in their chemical substances. Most lichen substances are derivatives of depsidones and depsides.

It is well evident from the Table 1, that *Everniastrum cirrhatum* contributes the maximum amount of 10.24-14.28 gm dry weight in the raw material followed by *E. nepalense, Parmotrema nilgherrense* and *P. reticulatum* which ranges from 8.26-9.28, 1.53-3.95 and 0.13-4.06 gm dry weight respectively. Most of the species which showed higher contribution in the material have wide distribution in Himalayas in temperate and subalpine regions found growing on different phorophytes and other available substrates. Owing to bigger thallus lobes and covering larger area, the species are easily recognized and collected frequently, thus contributes maximum in the raw material. *E. cirrhatum* is one of the most frequently used lichen species in Ayurveda and Unani medicinal system under the name 'Charrila' as carminative and

aphrodisiac and considered useful in dyspesia, spermatorrhoea, amenorrhoea, calculi, diseases of blood and heart, stomach disorders, enlarged spleen, bronchitis, bleeding piles, scabies, leprosy, excessive salivation, sourness of throat, toothache and general pain (Chandra and Singh, 1971).

Table 1: Quantification of Lichen species in raw materials used by the traditional perfumery industries in Uttar Pradesh

S.N.	Species name	Quantity of Lichen samples (gm)			
		Site -1	Site -2	Site -3	Site -4
1	*Bulbothrix meizospora* (Nyl.) Hale	NA	0.27±0.03	0.60±0.07	NA
2	*Bulbothrix setschwanensis*(Zahlbr.) Hale	0.53±0.08	NA	NA	NA
3	*Cetrelia braunsiana* (Müll. Arg.) W.L. Culb. & C.F. Culb.	0.29±0.03	NA	NA	NA
4	*Cetrelia cetrarioides*(Delise) W.L. Culb. & C.F. Culb.	0.52±0.06	0.08±0.01	0.24±0.01	0.63±0.01
5	*Everniastrum cirrhatum* (Fr.) Hale ex Sipman	**12.26±0.75**	**10.24±0.86**	**14.28±0.98**	**11.70±0.39**
6	*Everniastrum nepalense* (Taylor) Hale ex Sipman	**8.26±0.29**	**8.63±0.11**	**9.28±0.83**	NA
7	*Flavoparmelia caperata*(L.) Hale	NA	NA	0.13±0.01	9.59±0.88
8	*Heterodermia boryi*(Fée) Hale	0.26±0.04	NA	0.24±0.06	0.57±0.09
9	*Heterodermia diademata* (Taylor) D.D. Awasthi	0.76±0.13	1.98±0.17	0.62±0.08	NA
10	*Leptogium askotense* D.D. Awasthi	0.02±0.02	0.02±0.01	NA	NA
11	*Leptogium pedicellatum* P.M. Jørg.	0.04±0.01	NA	0.04±0.02	NA
12	*Lobaria retigera*(Bory) Trevis.	NA	0.05±0.01	0.37±0.01	NA
13	*Myelochroa denegans*(Nyl.) Elix& Hale	0.56±0.07	NA	NA	NA
14	*Myelochroa subaurulenta* (Nyl.) Elix& Hale	NA	NA	0.08±0.01	NA
15	*Nephromopsis laii* (A. Thell&Randlane) Saag& A. Thell	NA	NA	0.04±0.01	NA
16	*Nephromopsis stracheyi* (C. Bab.) Müll. Arg.	0.79±0.14	NA	NA	NA
17	*Parmelaria subthomsonii* D.D. Awasthi	0.45±0.11	NA	NA	NA
18	*Parmelaria thomsonii*(Stirt.) D.D. Awasthi	0.85±0.11	0.50±0.01	2.75±0.05	9.28±0.33
19	*Parmelinella wallichiana* (Taylor) Elix& Hale	0.53±0.11	NA	NA	NA
20	*Parmotrema austrosinense* (Zahlbr.) Hale	NA	NA	0.13±0.05	0.56±0.05
21	*Parmotrema nilgherrense* (Nyl.) Hale	**3.49±0.76**	**3.95±0.19**	**3.71±0.24**	1.53±0.16
22	*Parmotrema reticulatum* (Taylor) M. Choisy	**3.55±0.31**	0.13±0.01	**4.06±0.10**	**2.21±0.01**
23	*Pyxine meissneriana*Nyl.	NA	0.71±0.02	NA	NA
24	*Ramalina conduplicans*Vain.	0.40±0.10	0.39±0.01	0.67±0.02	0.64±0.04
25	*Ramalina sinensis*Jatta	0.23±0.04	0.24±0.02	NA	NA
26	*Usnea longissima* Ach.	0.08±0.01	NA	0.09±0.01	NA
27	*Usnea orientalis*Motyka	1.42±0.21	1.93±0.06	2.65±0.01	2.25±0.05
28	*Usnea perplexans*Stirt.	0.46±0.09	NA	0.73±0.03	NA
29	*Usnea pseudosinensis*Asahina.	0.06±0.013	NA	0.08±0.01	NA
30	*Usnea subfloridana*Stirt.	0.06±0.01	NA	NA	NA
31	*Usnea diffracta*Vain.	0.05±0.01	0.03±0.01	NA	0.04±0.01
	Waste materials	64.15±2.92	70.85±2.51	59.21±1.98	61±2.08

Data represent mean (±SD) of three separate measurements. NA denotes not available.

Everniastrum nepalense, Flavoparmelia caperata and *Parmelaria thomsonii*, another most common species in the Himalayas exhibit their higher contribution in the raw material within the ranges of 8.26-9.28, 0.13-9.59 and 0.5-9.28 gm dry weight respectively. *Parmelaria subthomsonii* and *Parmelinella wallichiana* species also have bigger thallus in size but fund growing in few localities ranges from 0.08 to 1.98 per 100gm. Though, *Leptogium askotense, L. pedicellatum, Lobaria retigera, Myelochroa subaurulenta, Nephromopsis laii, Usnea longissima, U .pseudosinensis, U. subfloridana* and *U. diffracta* have a bigger thallus but shows lower quantity ranges from 0.02-0.09 gm, as most of these species do not grow luxuriantly in the Himalayas. *Bulbothrix setschwanesis, Cetrelia braunsiana, Myelochroa denegans, M. subaurulenta, Nephromopsis stracheyi, Parmelaria subthomsonii, Parmelinella wallichiana, Pyxine meissnerina and Usnea subfloridana* showed their poor representation in few samples with lower ranges. Apart from the traditional perfumery industry the lichensare also known for their medicinal values. Rankovic *et al.,* (2007 and 2008) demonstrated a significant antimicrobial activity of Flavoparmelia *caperata* and *Bulbothrix setschwanesis* exhibited inhibition of tyrosinase and xanthine oxidase activity (Behera and Makhija, 2002). The extract of *Parmotrema austrosinense* showed beta-glucosidase inhibitor activity (Lee and Kim, 2000). Natural product Longissimone A isolated from *U. longissima* found to possess anti-inflammatory activity in a cell based contempory assay (Choudhary *et al.,* 2005). The medicinal uses of lichens by different ethnic groups in India are available. *Heterodermia diademata* is used by Nepalese of Chaunje Basti in Sikkim for application on cuts and wound as plaster to protect from water infection (Sakalani and Upreti, 1992). *Lobaria retigera* is one of the constituent of Chinese medicine (Hu *et al.,* 1980); the Lepchas of Sikkim also use the smoke of this lichen to relieve eye pain (Sinha and Singh, 2005). Lichen species contributed only 30-50% of the total raw material used by attar industries in India, while larger amount (50-70%) of the raw material is a waste comprised of mosses, small twigs, humus, soil and dust accumulated on the lower side of the lichen thallus trapped within the network of root like structures (Rhizinae). The present account of lichen species recorded in the raw material will help to understand the quality of lichens used for preparation of traditional perfumes in India. The aromatic esters in the lichens are the components that contribute to the characteristic "green scent" and act as fixative in the perfume. Since harvesting lichens in large quantity is not sustainable due to their slow growth, improved culture techniques would enable the production of larger biomass of lichens or enhance the quantity of secondary metabolites. *In vitro* culture of mycobiont is the alternative method of producing the metabolites.

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