Abstract

The large, crustose lichen families such as Thelotremataceae and Graphidaceae are important components of tropical diversity, with many undescribed species. In these families generic concepts may need to be reconsidered in the light of tropical material. The lichen family Graphidaceae is one of the largest and widely distributed groups of lichens in the lichen flora of India are still inadequately studied and the taxa reported till now are mostly in need of revision.

The present dissertation is based on the research work done by me during the last two and a half years on the lichen family Graphidaceae in three chapters of the thesis.

The first chapter of the thesis deals with the comprehensive taxonomic studies of the lichen genus *Graphis* Adans. ex Müll. Arg. which is the largest cosmopolitan genus in the family Graphidaceae claiming over 300 species at world level and previously known by 74 species in India.

The morphological, anatomical, and chemical studies of 1500+ specimens including type/authentic material have resulted into the recognition of as many as 137 species of *Graphis* from India, which includes 11 new records, 8 varieties and as many as 48 new species and 7 unnamed species for India.

The morphotaxonomic account of the 137 species of the lichen genus *Graphis* from India has been prepared and presented in the thesis along with a key for the identification of all species from India. The detail taxonomic descriptions of the species together with the chemistry followed by a short discussion on their geographical distribution in world, their relationship and illustrations have been given.

The second chapter of the thesis deals with the screening of bioactivities in natural thalli of some species (77 species) belonging to the lichen genera namely *Graphina*, *Graphis*, *Phaeographina*, *Phaeographis* and *Phaeographopsis* (Family Graphidaceae).

Lichens have long been recognized as containing bioactive compounds, but little attempts have been made to screen them.

For the present studies 77 species of the lichen family Graphidaceae have been searched for tyrosinase-inhibitory activity, xanthine oxidase inhibitory activity and superoxide scavenging activity (SSA). Tyrosinase-inhibiting material extracted with MeOH, Acetone, Ethanol, Dimethyl sulphoxide in water and water only. Methanol has been found to be suitable for extracting good amounts of tyrosinase-
inhibiting component from the natural thallus. Inhibitory activity of xanthine oxidase was carried out by using enzymatic method with cytochrome c and non enzymatic method with nitro blue tetrazolium (NBT).


Half-inhibiting concentration (IC$_{50}$, µg/ml) has been found to be much lower than the standard tyrosinase-inhibitors like Ascorbic acid, Azealic acid, Curcumin, Kojic acid, Mimosine and Tropolone, and thus can compete with the other commercially available tyrosinase inhibitors. The extracts of these species have been found to be stable at 4°C.

The potential of the extracts for scavenging of superoxide and inhibition of xanthine oxidase under various physiological conditions have been evaluated. Out of 77 species, 30 species gave extract yields in the range of 7-77.5 µg and were found to show both superoxide scavenging activity (SSA) and inhibition of xanthine oxidase (IXO) activities. The methanolic extracts of the species have showed inhibition of xanthine oxidase remained stable at 4°C and inhibitory properties of xanthine oxidase is found to be IC$_{50}$ = 2.0 to 5.26 µg/ml and superoxide scavenging capacity as IC$_{50}$ = 3.63 to 13.88 µg/ml.

The third chapter deals with the standardization of a method of lichen tissue culture *in vitro* for their ex-situ conservation.

Lichens are very slow growing organisms. Research in the field of cultivating lichens and their symbionts will open a door to the mass production of lichen substances and their pharmaceutical and technical applications.

With the declining in the area of natural habitats, ex-situ preservation can be used as supplemental tool for maintaining lichen germplasm, offering insurance against the possibility of loss of organisms from the wild.

With this view, in the present studies successful attempt has been made to develop methods for the ex-situ conservation and the long term preservation of the tissue obtained from the fragments of the natural thallus of the three species namely *Graphis guimarana* Vainio, *Graphis nakanishiana* Patw. & Kulk., and *Graphis schizograpta* Müll. Arg. This was one of the first attempts in India to culture crustose lichens *in vitro*.
Graphis subchrysocarpoides

Graphis contortuplicata

Graphis collateralis

Graphis afzelii
Studies in the family Graphidaceae
(Lichenized Ascomycetes)

Introduction

Lichens are nature's most remarkable alliances and the first recognized symbionts, combining the growth of fungi and algae or cyanobacteria in an intimate biological union. Such an association, besides conferring several advantages on lichens, has enabled them to thrive in all sorts of diverse environments in different parts of the world. An arsenal of over eight hundred chemicals called lichen substances unique to lichens protect them by bacteria, and other fungi. These pigments, toxins and antibiotics have made lichens very useful to people in diverse cultures especially as a source of dyes, medicines, agrochemicals and other exploitable compounds.

Wolseley and Laouwhoff (International Lichenological Newsletter 34(2): 45-46, Dec. 2002) while emphasizing the importance of studies of biodiversity in tropical regions of the world, stated that, "tropical regions in the world cover a vast area between the Tropics of Cancer and Capricorn, ranging from extensive continental masses dominated by tropical forests in S. America, Africa and SE Asia to oceanic islands of the Pacific. Aptroot and Sipman (1997) suggest that the tropics contain from one third to one half of the total lichen diversity of our planet, yet despite early exploration and collecting in tropical areas our knowledge of the lichens of this region is still very patchy. These are also areas where rapid loss of biodiversity is taking place due to increased pressures from logging, mining, and tourism etc., so that there is urgency for tackling disappearing tropical diversity".

They further stated that large, crustose families such as Thelotremataceae and Graphidaceae are important components of tropical diversity, with many undescribed species. In these families generic concepts may need to be reconsidered in the light of tropical material.
In view of the vast area, it's broad range of altitudinal zones and its considerable climatic variation; the lichen flora of India is very diverse. In contrast to macrolichens, considerable number of the taxa of microlichens, which are ecologically significant as indicators of primary forests from India are yet insufficiently known, revised or even investigated (Awasthi 1991).

The Graphidaceae is a large family comprising 16 genera and 918 species at world level (Kirk et al. 2001) and is characterised by lirelliform ascomata with branched or unbranched lirellae; excipulum tissues usually carbonaceous; unitunicate asci with apical pore apparatus; ascospores usually thick walled and many septate and photobiont usually filamentous green algae.

Like many other microlichens (crustose) the family Graphidaceae, which is one of the largest and widely distributed groups of lichens in India and represented mainly by 4 genera namely Graphina (60 species), Graphis (74 species), Phaeographina (25 species), and Phaeographis (32 species) (Awasthi 2000) is still inadequately studied and the taxa reported till now are mostly in need of revision. Although, several taxa of family Graphidaceae in India have been recorded in many scattered publications, through the preliminary work of Indian lichenologists, there has never been a comprehensive treatment of the members of the family Graphidaceae in India.

The apparent structural simplicity and short taxonomic characters of this family present major taxonomical obstacles in its perusal. Generic concept in crustose lichens is rapidly changing and the generic and family arrangement of the Graphidaceae is still very incompletely settled.

Also, in view of extinction of several lichen species through man-made atmospheric and terrestrial pollution and destruction of primary forests in India where the conservation resources are scarcest, it is urgently and highly desirable to develop methods for their ex-situ conservation in vitro. Such ex-situ preservation of rare lichen germplasm can provide a resource for research as well as for re-introduction if a species is lost in the wild.
In this background and as a part of our comprehensive program at Agharkar Research Institute, the studies in the lichen family Graphidaceae from India was undertaken by me for my dissertation for the award of Ph.D.

The present investigation has been carried out with the aims (i) to investigate the species diversity in the lichen genus *Graphis* (Family Graphidaceae) in India, and to understand the delimitation and circumscription of the genus and species, evaluation of characters for speciation and correct nomenclature in the light of modern trends in lichen taxonomy. The survey of *Graphis* is a first step towards producing the comprehensive taxonomic account of the family Graphidaceae in India, (ii) to screen natural thalli for bioactivities in this group of lichens and (iii) to standardize methods for lichen tissue culture *in vitro* for the ex-situ conservation and their long-term preservation of some species of *Graphis*.

The present thesis is based on the research work done by me, during the last two and a half years (2000-2003) on the lichen family Graphidaceae and has been presented in the following three chapters.

**Chapter I.** The comprehensive taxonomic account of the lichen genus *Graphis* (Family Graphidaceae) from India.

**Chapter II.** Screening of bioactivities in natural thalli of some taxa of lichen family Graphidaceae.

**Chapter III.** Standardization of method of lichen tissue culture *in vitro* for their ex-situ conservation.