Pharmacognostic and Chemotaxonomic Studies on Indian Ratanjot

THESIS SUBMITTED
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
Botany
IN THE FACULTY OF LIFE SCIENCES
THE ALIGARH MUSLIM UNIVERSITY ALIGARH

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LUCKNOW (INDIA)
FEBRUARY, 1991
In the indigenous system of medicine, 'Ratanjot' is used for a variety of ailments, as an anthelmintic, in diseases of eye, in bronchitis, abdominal pains, itch, fever, wounds and burns etc. Leaves and flowers are used as stimulant and cardiac tonic. Ethanol extract of 'Ratanjot' showed bactericidal, fungicidal and anticancer activity when put through a wide screen of biological tests. The antibiotic activity was associated with the hexane soluble fractions of the root.

'Ratanjot' is popular not only in India but also in other Asian countries for imparting a pleasing red colour to food stuffs, oils, fats, medicinal preparations and also used for dying silk and wool.

The botanical identity of 'Ratanjot' sold in the crude drug market all over India in the form of isolated underground part with some remains of radical leaves, is a matter of concern for the botanists, as many plant species are being sold in the market under trade name 'Ratanjot'. The botanical authentication of market samples of 'Ratanjot' is difficult because of the absence of aerial parts.

Bole (1961, 1962) provided a convincing explanation that the material sold in the Indian markets under the vernacular name 'Ratanjot' is *Arnebia nobilis* which is imported into India from Afghanistan. From all available informations it becomes clear that this plant occurs only in Afghanistan and perhaps in some contiguous areas in West Pakistan, but not in India.
However, during the course of botanical survey and collection of plants from Himalayan region CDRI scientists observed that the roots resembling 'Ratanjot' in colour and texture were gathered by local collectors from the areas for commercial sale. This is the common practice of any trade to find out cheap, similar looking substances for adulteration or as substitute to the genuine item for easy monetary gain. It appears that this practice started in seventies after the much publicity of 'Ratanjot' for commercial utilization for colouring 'Vanaspati Ghee' and its extended permission to use it as colouring matter for use in and upon food stuffs under the prevention of Food Adulteration Rules.

Keeping this in view, a detailed Pharmacognostic and chemotaxonomic studies of six materials from five boraginaceous species viz. Arnebia benthamii, A. euchroma (Himachal Pradesh), A. euchroma (Laddakh), A. nobilis, Maharanga emodi and Onosma hispidum implicated as 'Ratanjot' and the market samples procured from important Indian markets viz. Amritsar, Bhopal, Bombay, Calcutta, Delhi, Hyderabad, Jammu and Lucknow were carried out. The detailed morphological (including SEM studies) and phytochemical parameters used as markers of respective botanical taxa to identify and authenticate the commercial samples.

The important salient features for the various species studied are:

Arnebia benthamii : (i) Roots 1-2.5 cm thick, (ii) number of vessels 210-230 per sq mm., (iii) vessels arrangement flame like,
(iv) xylem parenchyma apotracheal and confluent narrow, (v) parenchymatous cells wavy and suberized (also observed on maceration); (vi) hairs, unicellular, rugose with two tube like arms adjoining to broad bases on adaxial surface and ridged with multicellular bases comprised of irregular cells and a constricted joint on abaxial surface, (vii) epidermal cells polygonal, elongated, striated with raised partitions on adaxial surface and hexagonal or pentagonal finely striated, cell boundary raised on abaxial surface, (viii) stomata paracytic and (ix) presence of naphthazarins arnebin-3, -6 and 7, isomers of arnebin-1 and 4 with two additional compounds at hRf 18.7 and 30.0.

**Arnebia euchroma**: (i) Roots 3-3.5 cm in diameter, (ii) wood and bark readily exfoliated in the form of papery layers and only 2.5-5 mm broad woody portions discernible, (iii) presence of vessels in maceration and T.S. of peelings, (iv) wood parenchyma vasicentric apotracheal; (v) hairs unicellular, scanty on adaxial surface while dense on abaxial surface; (vi) presence of naphthazarins arnebin-1 to 7 and the stereo-isomers of arnebin-1 and -4 with some additional compounds at hRf 43.3, 46.7, 58.7 and 61.3.

The two materials of *A. euchroma* collected from Himachal Pradesh and Ladakh regions showed some differences in their morphological and chemical characters.

Himachal Pradesh material characterized by (i) number of vessels 260-305 in young and 410-475 in mature root per sq mm, (ii) presence
of suberized cells (on maceration), (iii) hairs scanty, 2-3 celled, hourglass shaped, finely folded with condyle shaped bases on adaxial surface while hairs dense, tape like, twisted, loosely folded forming interlocking arrangements with broad centrally depressed bases on abaxial surface, (iv) epidermal cells smooth, rectangular with depressed partition on adaxial side and slightly raised general surface on abaxial side, (v) stomata anomocytic; (vi) naphthaquinone 5.93%, (vii) percentages of successive extractives of alcohol and water 12.03 and 20.02 respectively and (viii) a naphthazarin at hRf 34.7.

However, the Laddakh material characterized by (i) number of vessels 370-400 in young and 455-530 in mature root per sq mm. (ii) hairs scanty, tape-like, short, finely folded surface on adaxial surface and elongated, strongly ridged on abaxial surface, (iii) epidermal cells elongated with tracheal shaped surface, (iv) stomata diacytic; (v) naphthaquinone 2.83% and (vi) percentages of successive extractives of alcohol and water 4.172 and 11.32 respectively.

Therefore, the material of Arnebia euchroma collected from Himachal Pradesh and Laddakh regions were considered as chemotype.

Arnebia nobilis : (i) Roots 1-5 cms in diameter, (ii) alternate rings of clusters of broad and narrow vessels, (iii) outermost zone comprised of groups of broad vessels in furrows while the inner most with groups of radially arranged narrow vessels interrupted by parenchymatous cells, (iv) number of vessels 500-700 per sq mm., (v) presence
of pith; (vi) hairs warty, dimorphic small and large; (a) small hairs with semilunar rings on bases, (b) broad hairs with warty blobs gradually disappearing and forming fine ridged surface towards the apex, having saucer shaped bases with 'U' shaped slight depression, (vii) epidermal cells hexagonal partitions raised on adaxial surface but rectangular with raised and swollen intercellular partitions on abaxial surface, (viii) stomata diacytic and paracytic and (ix) presence of naphthazarine arnebin-1 to 7 (except arnebin-2) along with the additional compound at hRf 36.7.

Although Shukla et al (1969) reported arnebin-2 from the roots of *A. nobilis* but it is not observed in our sample.

*Maharanga emodi*: (i) Roots 1-3 cm in diameter, sometimes branched, (ii) become very soft and mucilagenous when kept into the water for sometime, (iii) number of vessels 400-500 per sq mm. (iv) broad vessels mostly solitary or in a groups of 2-3 (v) central core comprised of a compact groups of narrow vessels, (vi) wood parenchyma paratracheal and aliform, confluent, (vii) most of the parenchymatous cells rectangular and filled with mucilage (also observed in maceration), (viii) hairs dimorphic, the smaller ones warty with digitally arranged multicellular bases, (ix) margins of epidermal cells indiscernible on adaxial surface but rectangular or pentagonal with convex surface and raised margins on abaxial surface, (x) stomata anomocytic and (xi) absence of the naphthazarins arnebin-1, 3, 7 and isomer of arnebin-4 and presence of additional compound at hRf 59.3.
Onosma hispidum: (i) Roots 1-7 cm in diameter, (ii) number of vessels 514-610 per sq mm. (iii) presence of fibres, (iv) hairs dimorphic (a) small, smooth with unicellular bases (b) large and broad, warty or smooth with cystolith like arranged multicellular bases on adaxial surface while in abaxial surface marginal hairs collapsed type and middle ones with branched root like bases, (v) epidermal cells elongated with raised partitions general surface folded on adaxial side and irregular, concave with raised partitions on abaxial side, (vi) stomata paracytic and flush, (vii) presence of all naphthazarins (except arnebin-6) and two other constituents at hRf 12.7 and 45.3.

Some other additional compounds are also observed at same hRf in more than one species. (Table - 1)

The significant variations in physico-chemical values (i.e. naphtho-quinones, phenolics, sugars, tannins, ash values and Soxhlet extractive percentage) from species to species were observed and represented by the Bar diagrams (Figs. 1-6).

On systematic comparison of the market samples with the authenticated materials it was revealed that all the market samples were the mixture of two or three botanical taxa except the Amritsar samples which showed very resemblance with Arnebia nobilis in its morphological and chemical parameters. Further, the market samples procured from Calcutta, Jammu
and Lucknow were the mixture of *Arnebia nobilis* and *Onosma hispidum* while Delhi sample consisted of *O. hispidum* and *Maharanga emodi*. The remaining samples from Bhopal, Bombay and Hyderabad comprised of *Arnebia nobilis* and or *Onosma hispidum* and some other boraginaceous taxa.
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* Any other plant species  CA = Cystolith like arrangement,  DA = Digitally arranged.
Phy. Chem. Constants of 'RATANJOT

GENUINE SAMPLES

NAPHTHAQUINONES

SUGARS

PHENOLICS

TANNINS

FIGURE - 1
Physico-Chemical Constant of "RATANJOT"

MABKET SAMPLES

FIGURE - 2
Ash Values of 'RATANJOT GENUINE SAMPLES

Figure 3.
Ash Values of "RATANJOT"

FIGURE 4
Soxhlet Extract % of "RATANJOT"

GENUINE SAMPLES

FIGURE -- 5
Soxhlet Extract, % of 'RATANJOT

FIGURE 6.
CERTIFICATE

This is to certify that the thesis entitled
"Pharmacognostic and Chemotaxonomic Studies on
Indian Ratanjot" embodies original and bonafide
work carried out under my supervision by Miss
Sayyada Khatoon and that no part of this thesis
has been submitted for any other degree or diploma.

(WAZAHAT HUSAIN)
Internal Supervisor
Prof. of Botany,
A.M.U. ALIGARH
This is to certify that thesis entitled "Pharmacognostic and Chemotaxonomic Studies on Indian Ratanjot" embodies original work carried out under my supervision by Miss Sayyada Khatoon and that no part of this work has been submitted for any other degree or diploma.

(External Supervisor)
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* * * *
ABBREVIATIONS

a - Crushed xylem
B - Bulbous cells
b - Bark
C. - Crystal of calcium oxalate
c - cortex
Ca - Cambium
cp - crushed and suberized xylem parenchyma
f - fibre
L - Lysigenous cavity
M - Medullary ray
m - mucilagenous cells
P - crushed parenchyma
p - xylem parenchyma
ph - phloem
pi - pith
R - radially arranged vessels
r - ray
S - sheath with coloured content
s - sheath without any content
Sc - suberized cells
t - trachied
V - group of vessels
v - vessel
x  -  xylem
xi  -  young wood
xii -  mature wood
xy  -  completely crushed xylem

-----------
AB  -  Arnebia benthamii
AEH -  Arnebia euchroma (Himachal Pradesh)
AEL -  Arnebia euchroma (Laddakh)
AN  -  Arnebia nobilis
ME  -  Maharanga emodi
OH  -  Onosma hispidum

-----------
Amt -  Amritsar
Bho -  Bhopal
Bom -  Bombay
Cal. -  Calcutta
Del -  Delhi
Hyd -  Hyderabad
Jam -  Jammu
Lko -  Lucknow
ACKNOWLEDGEMENTS

It is my privilege to record my deep sense of indebtedness to Dr. B.N. Mehrotra, Head of the Botany Division, Central Drug Research Institute, Lucknow for his indispensable guidance, everlasting encouragement and affectionate behavior throughout the course of present investigation.

It gives me immense pleasure to express my humble gratefulness to Prof. Wazahat Hussain, Botany Department, Aligarh Muslim University, Aligarh for his guidance, unflagging interest and invaluable help rendered during the tenure of this work.

My regards and honour are due to Dr. B.N. Dhawan, Director, Central Drug Research Institute, Lucknow and Dr. P.V. Sane, Director, National Botanical Research Institute, Lucknow for providing me laboratory facilities during the pursuit of this work.

My sincere thanks are also due to Dr. (Mrs.) Shanta Mehrotra, Scientist, National Botanical Research Institute, Lucknow whose unending encouragement, keen interest, valuable suggestions and fruitful discussions at all stages of my work helped in bringing my work to a successful completion.

I am deeply indebted and thankful to Dr. (Mrs.) Usha Shome, Head, Pharmacognosy Division, National Botanical Research Institute, Lucknow for her untiring help, encouragement and aesthetic suggestions.
Delighted, I feel to convey my sincere thanks to all the members of Botany Division, Central Drug Research Institute, Lucknow, especially Dr. B.S. Aswal, Dr. Ved Prakash and Dr. M.N. Srivastava for their valuable suggestions.

I am extremely grateful to Dr. V.K. Bajpai, Scientist in Electron Microscopy, Central Drug Research Institute, Lucknow for his valuable help in taking SEM photographs and P.Y. Guru, Scientist, Parasitology Division, CDRI for taking photographs of the present work.

I would like to thank Dr. Abu Shoeb and Dr. (Mrs.) Falak Anwar, Scientists, Medicinal Chemistry Division, Central Drug Research Institute, Lucknow for their untiring and continuous help and Mr. S.C. Sarkar for HPLC during the pursuit of this work.

I am thankful to Dr. J.S. Tondon, Scientist, Central Drug Research Institute, Lucknow for providing adequate samples of arnebins used in the present study.

It is unvanished truth that the treatise would never have materialized in the absence of my friends with whom I shared my joys and sorrows. In naming them I am likely to miss many of those who contributed in different ways. Nevertheless Simi, Ranu, Runjhun and Ghazala need be mentioned.

I would also like to thank Mr. Gajendra Singh and Mr. Shamim for making my work much easier with their expert typing and untiring patience.
My thanks are also due to Mr. Zameer Ahmad and Mr. Moeen Ahmad for statistical analysis and making computerized graphs for this work.

Financial support in the form of research fellowship from Indian Council of Medical Research, New Delhi, is gratefully acknowledged.

Words fail to come to express my sincere regards and appreciation to my all near and dear ones who were always a source of inspiration to me. I would like to pen down my heart-felt thanks to all the members of my family specially my beloved elder brother whose blessings, moral support, good wishes, love and affection had been one of the prime reasons for the successful completion of this work.

SAYYADA KHATOON
(SAYYADA KHATOON)
INTRODUCTION
Since disease, death and decay have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of the human intellect. From the earliest time, man and animals have had to distinguish between those substances which are poisonous and those which are not, and thus the knowledge of naturally occurring drugs has gradually developed. Although, there is no authentic record of medicine used by the primitive man, but the 'Rig Veda', which is the oldest record in the library of man, having been written between 4500 and 1600 BC., supplies many useful information on the subject (Kirtikar and Basu, 1933).

India, with its wide ecogeographic and climatic diversity, harbours a rich plant wealth, which has been used from time immemorial as herbal medicines for treatment of human and animal diseases. Of about 15000 species, of plants found in the Indian flora, more than 3000 species are considered to have medicinal properties of some description or the other.

whereas, most of the Western countries in the world exclusively depend on the modern system of medicine for their health care needs, the over-whelming majority of Asian countries still rely and depend on the indigenous system of medicine.
One of the most important problem faced by these systems is adulteration and substitution. Therefore, there is an imperative need for Pharmacognostical and Chemotaxonomical standardization and authentication of herbal drugs and their galenicals which are being used by the practitioners of these systems and also by Pharmaceutical firms manufacturing herbal medicaments.

Pharmacognosy, as is well known, is an applied science which deals with biologic, biochemical and economic features of natural drugs. In a broad sense it embraces a knowledge of the history, distribution, cultivation, collection, chemical and pharmacological evaluation, preservation and survey of the marketed drugs affecting the health of men and other animals.

Chemical characters can play a vital role in plant taxonomy where the biological criterias are ambiguous and conflicting in their distribution or in those cases where 'eye ball' taxonomy is not sufficiently available. Recently Harborne (1984) has emphasised that chemical data may be useful in solving the problem of plants particularly in case of medicinal plants where only certain organs eg. root, stem, leaf, seed and fruits are available.

In the present geological period more sophisticated analytical tools viz. HPLC, GLC, GC Mass, fingerprinting with the help of densitometer etc. have been developed to help taxonomists and pharmacognosists in solving out the problems of identification at generic and specific level.
'Ratanjot' is popular not only in India but also in other Asian countries for imparting a pleasing red-pink colour to food stuffs, oils, fats, medicinal preparation and also used for dying silk and wool (Anonymous, 1950).

In the indigenous system of medicine, it is used for a variety of ailments, as an anthelmintic, in diseases of eye, in bronchitis, abdominal pains, itch, fever, wounds and burns etc. (Kirtikar and Basu, 1933). In the bruised form it is used as an application to eruptions and have much value in Indian veterinary medicine. It is also applied with sweet oil to wounds and used as an antiseptic. Leaves and flowers of the plant are used as stimulant and cardiac tonic (Bhandari, 1949, Chopra et al., 1956, 1958). Ethanol extract of 'Ratanjot' showed bactericidal, fungicidal and anticancer activity when put through a wide screen of biological tests. The antibiotic activity was associated with the hexane soluble fractions of the root (Bhakuni et al., 1969).

Besides its medicinal uses the 'Ratanjot' is also used as a natural vegetable colouring matter. The isohexanyl naphthazarins, present on the outer surface of the roots of at least 150 species belonging to the family Boraginaceae, are responsible for the red colour. At least twelve European countries allow its use as colorant in food and wine (Papageorgiou, 1980).
The 'Ratanjot', on the basis of its morphological characters and therapeutic values, is of three types in Indian system of medicine and of four types in Unani system of medicine, where it is described under the name 'Shanjar' (Najmul-Ghani, 1920).

The values of Sanskrit and vernacular names of plants has been much questioned by botanists for purposes of identification because similar name is applied to two or more different plant species in the same region. The botanical identity of 'Ratanjot' sold in the crude drug market all over India in the form of isolated underground part with some remains of radical leaves, is a matter of concern for the botanists, as many plant species are being sold in the market under the trade name 'Ratanjot'. The botanical authentication of market sample of 'Ratanjot' is difficult because of nonavailability of aerial parts of concerned plant species.

Bole (1961, 1962) provided a convincing explanation that the material sold in the Indian markets under the vernacular name 'Ratanjot' is derived from the plant imported into India from Kabul (Afghanistan). The equivalent vernacular name in Afghanistan for 'Ratanjot' is 'Yarlang' or 'Yella rang'. The plant species is *Arnebia nobilis* Rech. f. From all available informations, it becomes clear that *Arnebia nobilis* occurs only in Afghanistan and perhaps in some contiguous areas in West Pakistan, but not in India.
However, during the course of botanical survey and collection of plant samples for biological screening programme of the Institute for the development of new drugs from plant sources in the Himalayan region, it was observed that the roots resembling 'Ratanjot' in colour and texture were gathered by local collectors from the areas (Personal observation of Dr. Mehrotra) for commercial sale. An appearance of root and root stocks available with the local collectors clearly indicates their origin to family Boraginaceae. On enquiry they explain that they forward these roots to market. It was not possible to authenticate their samples which are devoid of aerial portions required for botanical identity. This is the common practice of any trade to find out cheap, similar looking substances for adulteration or as substitute to the genuine item for easy monetary gain. It appears that this practice started in seventies after the much publicity of 'Ratanjot' for commercial utilization for colouring 'Vanaspati Ghee' and its extended permission to use it as colouring matter for use in and upon food stuffs under the prevention of Food Adulteration Rules.

Out of eight Boraginaceous species – namely Anchusa tinctoria Linn., Arnebia nobilis Rech.f., A. benthamii (Wall.ex G.Don) Johnston, A. euchroma (Royle) Johnston, A. hispidissima DC., Maharanga emodi (Wall.) DC., Onosma hispidum Wall. and O. hookeri Clarke described by Bole (1961) under Head III (See Review for detailed information), Anchusa tinctoria, the source of European 'Alkanet', is well known in the trade and its botanical identity is also well established.
Its roots differed considerably from the market samples of 'Ratanjot'. Similarly the roots of *Arnebia hispidissima*, though having almost similar colour, but are very thin, about 0.5 cm in diameter with entirely different morphology. Therefore, it does not merit further consideration as 'Ratanjot' of commerce or adulteration in it. Now remaining five species found in India with roots having closer resemblance to one another and sometimes with market samples, are the focus of our attention for study and comparison with *Arnebia nobilis*.

With all this in view, it has been decided to reinvestigate the market samples of 'Ratanjot' and to compare these with the botanically authenticated samples collected by taxonomists from Himalayan regions and with authentic sample of root of *Arnebia nobilis*. This will enable to establish the correct botanical identity of the market sample.
REVIEW OF LITERATURE
Realising the importance of 'Ratanjot' as dye and potential antimicrobial and anticancer activities, a good amount of phytochemical work has been carried out by various workers. However, only very little amount of work has been done on its standardization and authentication, particularly on morphological and microscopical aspects.

The research work so far carried out on different aspects has been grouped under the subheads viz; Pharmacognostical, Chemical, Biological and Economic importance.

A. PHARMACOGNOSTICAL:

Bisht et al. (1961) carried out pharmacognostic studies (macro and microscopic) on the root of Onosma echioides and found that the structure of the root was similar in many respects to that of the root of Alkanna tinctoria Tausch. One of the salient features of Onosma echioides was lysigenous cavities often present along the medullary rays and filled with a mucilagenous secretion.

Bole (1961) listed fifteen plant species mentioned in the literature under the vernacular name 'Ratanjot' belonging to four different families, viz. Apocynaceae, Boraginaceae, Geraniaceae and Rosaceae and indicated that this vernacular name is used in
a general sense to cover a range of red dye yielding materials rather than produce of a particular species.

**SPECIES REFERRED TO AS ‘RATANJOT’**

1. **Anemone obtusiloba** D.Don
2. **Clausena pentaphylla** (Roxb.) DC.
3. **Jatropha curcus** Linn.
4. **Lochnera rosea** (Linn.) Reichb. (= *Vinca rosea* Linn.)
5. **Viola serpens** Wall.
6. **Geranium nepalense** Sweet
7. **Potentilla nepalensis** Hook.
8. **Anchusa tinctoria** Linn. (= *Alkanna tinctoria* Tausch.)
10. **Arnebia benthamii** (Wall. ex G. Don) Johnson
    (= *Macrotomia benthamii* DC.)
11. **Arnebia euchroma** (Royle) Johnston
    (= *Macrotomia perennis* Boiss)
12. **Arnebia hispidissima** DC. (= *Lithospermum vestitum* Royle)
13. **Maharanga emodi** (Wall.) DC. (= *Onosma emodi* Wall.)
14. **Onosma hispidum** Wall.
    (= *Onosma echioides* sensu Clarke, non Linn.)
15. **Onosma hookeri** Clarke
All the above fifteen plants were classified under three groups, viz.;

(i) 5 plants (1 to 5), called 'Ratanjot', do not possess any coloured root.

(ii) Two plants (6 and 7), with red coloured roots belong to the family Geraniaceae and Rosaceae respectively.

(iii) Remaining eight plants (8 to 15) belong to the family Boraginaceae and have roots yielding a red dye.

He also clarified that the market samples are the roots of Boraginaceous species but not *Onosma echioides* Linn. as reported by several workers. The Linnean plant *O.echioides* is a native of Europe only and Johnston considered the Indian plant to be *O.hispidum* Wall.

Further, Bole (1962) concluded his investigations that the red dye yielding roots sold in Indian markets under the name 'Ratanjot' were derived from the plant *Arnebia nobilis* Rech.f. imported from Afghanistan.

B. **CHEMICAL**

1. **QUINONES**

The vast majority of naphthalene derivatives found in Nature are quinones, and the others are mainly related naphthols or naphthyl
ether. Increasing members of O-naphthaquinones (mainly of terpenoid origin) and binaphthaquinones have been isolated in recent years, and such compounds can no longer be considered rare.

(i) **Naphthaquinone**

The isohexenynaphthaquinins, commonly known as alkannins are lipophilic red pigments. They are found in the outer surface of the roots of at least 150 species, belonging to the genus *Lithospermum*, *Echium*, *Onosma*, *Anchusa*, *Alkanna*, *Arnebia*, *Macrotomia* of the family Boraginaceae. Their occurrence of *Jatropha glandulifera* (Euphorbiaceae), should be considered as an exception.

The structure of the alkannin, the first identified member of naphthaquinins, was elucidated by Brockman (1935) and oxidation reduction potential was determined by Moruzzi (1939). This compound was isolated from the roots of *Alkanna tinctoria*, the European alkanet (Pelleteir, 1832; Bolley, 1847; Betrabet and Chakravarti, 1933; Brockmann, 1935; Dusinsky' and Szokay, 1960; Papageorgiou, 1978), *Lithospermum arvense* (Krolkowska and Swiatek, 1966), *L. officinale* var. *erythrorhizon* (Sankawa et al., 1981), *Arnebia euchroma* (Liu, 1981), *A. hispidissima* (Khan et al., 1983), *A. nobilis* (Shukla et al., 1969 and named it as Arnebin-4). Rukuzin and Pekarskava (1976) observed the adsorption of alkannin from different solvents.
Previously, there was much controversy about the structure of alkannin. Liebermann and Romer (1887) said that this was a derivative of anthraquinone. Majima and Kuroda (1918) called it as shikonin, which was incorrect due to the position of one hydroxyl group and ultimately Brockmann (1935) established the correct identity of alkannin from the ultraviolet-visible absorption and found it a laevorotatory naphthazarin.

Betrabet and Chakravarti (1933) carried out chemical reactions with different compounds to form alkannin derivatives. They obtained a tetra acetate derivative of alkannin, dimethoxy-alkannin, tetrabenzoylalkannin, dimethoxydibenzoylalkannin, dicarboxyalkannin, hexabromoalkannin, tetrabromoalkannin, tetracetyltetrabromoalkannin, dinitrotetraacetylalkannin and β-methylantracene.

The difficulty involved in the isolation of suitable amount of pure alkannin from the extracts of Alkanet root lead Toribara and Underwood (1949) to recommend synthetic naphthazarin for being used as a reagent in the spectrometric determination of Berillium.

A quantitative determination of alkannin in whole root, root bark and lower leaves of Onosma echioides was carried out by Boldyrev (1940) and it was found 8.93%, 19.41% and 2.52% respectively, while in Arnebia euchroma, A. guttata and Lithospermum erythrorhizon, it was 1.11-4.98%, 0.83-4.90% and 1.57-2.38% respectively (Li et al.1986).
Shikonin, an antipode of alkannin, was isolated from the roots of Arnebia decumbens (Afzal and Ghalib, 1961), A. euchroma (Zhu et al., 1984), A. guttata (Lu et al., 1983; Zhu et al., Loc. cit.), A. tibetana (Romanova and Ban'kovskii, 1966), Echium rubrum (Romanova et al., 1967), Echium species (Shcherbanovskii, 1971), Lithospermum euchromum (Ichiro and Yoshimasa, 1966), L. erythrorhizon (Hisamichi and Yoshizaki, 1982; Krivoshchekova et al., 1976; Kuroda, 1918; Ichiro et al., 1965; Ichiro and Yoshimasa, Loc. cit.), L. officinale (Tareeva et al., 1966), Macrotronia echioides (Romanova et al., 1981), M. euchroma (Romanova et al., 1969), Onosma caucasicum (Romanova et al., Loc. cit.), O. polyphyllum (Shcherbanovskii, 1972), O. livanovii, O. sericeum, O. setosum (Romanova et al., Loc. cit.). Kagramanyan and Mnatsakanyan (1985) noted that the yield of shikonin in O. setosum was 0.07% and Moruzzi (1939) determined the oxidation reduction potential of shikonin. Kuroda and Wada (1939) carried out the synthesis of alkyl derivatives of naphthazarin, naphthopurpurin and their related compounds from the roots of Lithospermum erythrorhizon.

A qualitative and quantitative evaluation of naphthoquinones of Boraginaceae was carried out by Tareeva et al. (1970) and they observed that the largest amount of shikonin was detected in Arnebia tibetana (4.16%) followed by Macrotronia species (2.21%) and Onosma zerizaminum (1.59%). Pimenova and Tareeva (1980) observed the variation of shikonin content in subterranean parts of Macrotronia euchroma and found that it was significant within plants of different ages.
The maximum amount was detected during the reproductive phase of
the plants.

Brockmann (1935) showed that alkannin and shikonin could be
converted into an identical optically inactive monomethyl ether and,
in combination, yielded the racemic compound shikalkin, m.p. 148°.
Shikalkin was not found in the aerial parts. It was extracted from
the roots of *Arnebia hispidissima* (Jain and Mathur, 1967), *Echium
italicum*, *E. vulgare* (Sherbanivs'kii, 1971), *E. locopsis*
(Scherbanovskii and Luks, 1974), *Onosma polyphyllum* and *O. visianii*
(Sherbanivs'kii, 1971). Scherbanovskii also carried out
quantitative determination of this compound in both young and old
*O. visianii* and obtained 0.52% and 0.35% respectively. Nickel and
Carroll (1984) described a procedure to separate the shikonin and
alkannin naphthazarin pigments by reversed phase ion-pair high
performance liquid chromatography. Sankawa et al. (1977) isolated
some naphthazarin compounds from the benzene extract of the root
of *Macrotomia euchroma* carried out catalytical hydrogenation of
shikonin and isolated alkannane alongwith some other compounds.
Alkannane previously isolated from *Alkanna tinctoria* by Brockmann
(1935). The reduction in polar solvents resulted in the formation
of complex of mixtures of unidentified compounds along with
dihydroshikonin, alkannane, cycloshikonin and shikonin triacetate.

Later, Sankava et al. (1981) clarified that the naphthazarin
pigments obtained from 'Nan-Shikon' (*Macrotomia euchroma*) were the
derivatives of alkannin instead of shikonin on the basis of the report
of Tsukada et al. (1980). They obtained cycloalkannin, cycloalkannin
leucoacetate, alkannin triacetate, alkannin leucoacetate, alkannane
leucoacetate and cycloalkannin diacetate in the form of alkannin
derivatives.

Deoxyalkannin was isolated from the roots of Alkanna tinctoria
(Papageorgiou et al., 1980), Macrotomia cephalotes (Papageorgiou,
1979), Onosma heterophylla (Mellidis and Papageorgiou, 1987). Its
sterio-isomer deoxyshikonin or arnebin-7 was reported from Arnebia
cuchroma (Fu et al., 1984, Fu and Xiao, 1986, Zhu et al., 1984),
A. guttata (Lu et al., 1983; Zhu et al. loc.cit.), A. hispidissima
(Khan et al., 1983), A. nobilis (Shukla et al., 1973), Lithospermum
erythrorhizon (Krivoshche'kova et al., 1976; Hisamichi and Yoshizaki,
1982), L. euchromum (Komatsu et al., 1972), Macrotonia euchroma
(Kyogoku et al., 1973), and Dehydroxyshikonin from the roots of
Lithospermum euchromum (Komatsu et al., 1972).

The derivatives viz. diaacetate, dibenzoate, leucotetraacetate
and cycloarnebin-7 from arnebin-7 was formed by Shukla et al., (1973).
Anhydroalkannin was isolated from the roots of 'Ko-shikon' i.e.
Lithospermum erythrorhizon (Kyogoku et al., 1973).

Shukla et al. (1969) also isolated and identified the alkannin
acetate or Arnebin-3. Later this naphthazarin was also isolated
from Alkanna tinctoria (Papageorgiou et al., 1980), Arnebia hispidissima (Khan et al., 1983). From the roots of A. decumbens, shikonin acetate was isolated by Afzal and Ghalib (1986).

A pigment - monoacetyshikonin was isolated by Ichiro et al. (1965), from Lithospermum erythrorhizon; Majima and Kuroda (1972) also extracted this pigment from benzene fraction from the same plant. The acetyl alkannin was isolated from the roots of Arnebia euchroma (Fu and Xiao, 1986), Macrotemia cephalotes (Papageorgiou, 1979) and Lithospermum officinale (Kishimoto and Aota, 1974).

Ichiro and Yoshimasa (1966) separated out acetylshikonin by using TLC and this pigment was also found in Arnebia euchroma (Fu et al., 1984; Fu and Xiao, 1986; Zhu et al., 1984); Jatropha glandulifera (Ballantine, 1969), Lithospermum erythrorhizon (Hisamichi and Yoshizaki, 1982; Krivoshchekova et al., 1976) and Onosma setosum (Kagramanyan and Mnatsakanyan, 1985).

From the roots of Lithospermum erythrorhizon (Ichiro et al., 1965) and from L. officinale (Kishimoto and Aota, 1974) isobutylshikonin was also isolated, while isobutyrylshikonin was isolated only from the roots of L. erythrorhizon (Krivoshchekova et al., 1976).

Isovalerylalkannin was obtained from Alkanna tinctoria (Papageorgiou and Digenis, 1980), Macrotemia cephalotes (Papageorgiou,
1979) and Onosma heterophylla (Mellidis and Papageorgiou, 1987). Whereas the isovalerylshikonin was isolated from the roots of Lithospermum erythrorhizon by various workers viz. Kyogoku et al., 1973; Krivoshchekova et al., 1976; Hisamichi and Yoshizaki, 1982 and from Arnebia decumbens by Afzal and Ghali, 1986.

Kyogoku et al. (1973) identified and isolated α-methyl-n-butyl shikonin from the roots of Lithospermum erythrorhizon, and the α-methyl-n-butyl alkannin from Macrotomia cephalotes (Papageorgiou, 1979). Both the above naphthazarin compounds were found in the form of a mixture with isovaleryl derivative. Hisamichi and Yoshizaki (1982) isolated α-methyl-n-butyrylshikonin from the roots of Lithospermum erythrorhizon.

B, B-dimethyl acryl alkannin was isolated from the hexane extract of the roots of Arnebia nobilis and named as arnebin-1 (Shukla et al., 1969). The above mentioned naphthaquinone was also found in Alkanna tinctoria (Papageorgiou, 1978), Macrotomia cephalotes (Papageorgiou, 1979), Arnebia euchroma (Liu, 1981; Fu et al., 1984; Fu and Xiao, 1986), Onosma heterophylla (Mellidis and Papageorgiou, 1987).

The stereoisomer of arnebin-1 is B, B-dimethylacrylshikonin which was isolated from Lithospermum erythrorhizon (Ichiro et al., 1965; Krivoshchekova et al., 1976), Jatropha glandulifera (Ballantine,
1969), Alkanna hirsutissima (Afzal and Mohammad, 1983), Arnebia euchroma and A. guttata (Zhu et al., 1984). Sung et al. (1980) carried out a quantitative determination of β, β-dimethylacrylshikonin contents of Chinese medicinal plants, A. euchroma, A. guttata, Onosma hookeri, O. paniculata and Lithospermum erythrorhizon and it was found 2.4-3.6%, 1.21%, 0.27%, 0.75-0.92% and 0.132-1.0% respectively.

Teracrylshikonin was isolated from Lithospermum euchromum (Ichiro and Yoshimasa, 1966), from L. officinale (Kishimoto and Aota, 1974) and from Arnebia guttata, A. euchroma (Zhu et al., 1984).

Angelicalkannin was reported from the roots of Alkanna tinctoria (Papageorgiou and Digenis, 1980) and its isomer angelicshikonin was isolated from A. hispidissima (Afzal and Tofeeq (1975).

Ichiro and Yoshimasa (1966), Hisamichi and Yoshizaki (1982) isolated β-hydroxyisovalerylshikonin from the roots of Lithospermum erythrorhizon. The same compound was obtained from the roots of L. officinale. (Kishimoto and Aota, 1974), Arnebia guttata (Lu et al., 1983), A. euchroma and A. guttata (Zhu et al., 1984), β-hydroxyisovalerylalkannin was also isolated and identified from A. hispidissima (Khan et al., 1983) and from A. euchroma (Fu et al., 1984).
β-acetoxyisovaleryl alkannin was obtained from *Alkanna tinctoria* (Papageorgiou, 1977) and *Arnebia euchroma* (Pu et al., 1984; Pu and Xiao, 1986). A new naphthaquinone O-betaacetoxyisovalerylshikonin was isolated from *Macrotomia euchroma* by Cong 1984. He also described the mass spectrometric fragmentation and the characteristics of alkannin compounds.

Shukla et al. (1969, 1971) isolated arnebin-2 and identified it as β, β-dimethylacryl-hydroxyalkannin from the hexane soluble fraction acetyl hydroxyalkannin or arnebin-6 and hydroxyalkannan or arnebin-5 from the roots of *Arnebia nobilis*.

Pu and Xiao (1986) isolated a new compound from the roots of *A. euchroma* named 1-methoxyacetylshikonin. Afzal and Ghalib (1986) found that the roots of *A. decumbens* yielded 5,8-dihydroxy-2-(14-methylpent-13-enyl)-1,4-naphthoquinone.

Manabe et al. (1987) gave a procedure to extract the shikonin derivatives from the roots of *Lithospermum* species with supercritical carbon dioxide without using entrainer. The application of entrainer (ethanol or water) reduced efficiency with extraction.

Papageorgiou et al. (1985) carried out a quantitative determination of isohexenylnaphthazarin pigments by TLC. These pigments were isolated from the roots of *Alkanna tinctoria* and *Macrotomia*
cephalotes. The method was also used successfully for determination of alkannin isovalerate in blood.

The decrease of shikonin derivatives was examined in the roots of *Lithospermum erythrorhizon* during preservation at a sunny window side in glass bottles using HPLC. The calibration curves were linear at 0.5-8 μg for shikonin, 0.5-1.0 μg for β-hydroxyisovalerylshikonin, 1-26 μg for acetylshikonin, 0.5-1.8 μg for deoxyshikonin, 1-17 μg for α-methyl-n-butyrylshikonin and 1-17 μg for isovalerylshikonin. The recovery for these compounds was 92.7-104.8%. Decrease of the above six compounds after six months were 21.3%-7%, 26.4%, 27.8%, 23.9% and 24.5% respectively (Yoshizaki and Hisamichi, 1983).

Verma and Dass (1959) presented a report on a new acid-base indicator obtained by extracting 'Ratanjot' root i.e. *Onosma echioides* with ethyl ether. As used in alcoholic solution the indicator changes from a pinkish red in acid solution to a bluish violet in alkaline solution. The pH interval for the colour change is 7.5 to 8.8.

Nigam and Mitra (1964) extracted the colouring matter from *Arnebia hispidissima* and market 'Ratanjot' with 98 percent ethanol and found that the pigments of *A. hispidissima* were 78% soluble in fat while it was 93% soluble in case of market sample. The ultraviolet spectrum was approximately the same for both the plants (maximum near 280, 520 um).
Li et al. (1983) determined the total naphthaquinone pigments in Arnebia euchroma, A. guttata and Lithospermum erythrorhizon by using calorimetric method at 517 nm. The average contents were 2.47, 2.33 and 1.72 mg (as alkannin), respectively.

Tsukada et al. (1984) carried out 42 commercial samples of a crude drug 'Shikon', mostly from Japan, China, Hongkong and Korea for their red naphthaquinone pigment content. Morphologically these samples belong to the 'Ko-shikon' which is probably the roots of Lithospermum erythrorhizon or to 'Nan-shikon' which is the root of Arnebia euchroma. The total pigment content varied widely ranging from 0.1-7.7% in 'Nan-shikon' (23 samples) and from 0.5-3.2% in 'Ko-shikon' (19 samples). The circular dichroism (CD) measurements of the whole red pigments extracted from crude drugs with chloroform showed that some of the 23 samples of 'Nan-shikon' tested contained alkannin derivatives and minor amount of shikonin derivatives. In the case of whole pigments extracted from 'Ko-shikon' 12 samples contained a major quantity of shikonin derivatives (R-type) with a smaller amount of alkannin derivative (S-type) 5 samples contained a major amount of S-type with a minor quantity of R-type and 1 sample contained R-type and S-type in about the same quantity. Some of the 'Ko-shikon' samples containing alkannin derivatives might be the root of some other Boraginaceous species, such as A. guttata growing in inner Mangolia.
Five new furylhydroquinone derivatives named as shikonofurans A, B, C, D and E were isolated from the roots of Lithospermum erythrorhizon (Yoshizaki et al., 1982). Yao et al. (1983) obtained a mixture of shikonofuran B and C from the above plant.

Krivoshchekova et al., (1977) isolated an unknown quinone \( \text{C}_{12} \text{H}_{22} \text{O}_5 \) of orange yellow colour, m.p. 72-4° from the roots of L. erythrorhizon.

ii) **BENZOQUINONE** :

Some benzoquinone derivatives were also isolated. Denisenko et al., (1979) first of all, determined the structure of a new quinoid pigment identified as p-benzoquinone from L. erythrorhizon. Arnebinone, an orange coloured novel monoterpenylbenzoquinone (Yao et al., 1983 and Eisai Co. Ltd., 1984). Monoterpenyl benzohydroquinone (Sempuku, 1984 and Eisai Co. Ltd., 1984) were isolated from the root of Arnebia euchroma. The structure of arnebinol a new ansa-type monoterpenyl benzenoid from the same plant was also elucidated and isolated by Yao et al. (1983) and Sankawa (1983).

A new monoterpenylbenzoquinone named arnebifuranone was also isolated from A. euchroma (Yao et al., 1984). Foland et al. (1989) synthesized isoarnebifuranone via addition of tetrahydropyranloxybutyne to cyclobuteredione and thermal ring enlargement of the adduct.
iii) **ANTHRACINONE**

Majumdar and Chakravarti (1940) isolated an anthracene derivative named as anchusin \((C_{30}H_{36}O_9)\) from Alkanet roots. This anthraquinone formed \(\beta\)-methylanthracene with Zinc dust on distillation at reduced pressure. On acetylation it gave triacetyl anchusin and tetraacetylleucoanchusin, whereas tribenzoyl derivative of anchusin on benzoylation. A dimethoxy derivative was obtained on methylation and further acetylation gave dimethoxyacetyl derivative of anchusin.

(2) **TRITERPENE SAPONINS**:


(3) **ALKALOIDS**

Men'shikov and Petrova (1952) isolated makrotomine from the aerial parts of *Macrotomia echioides*. Amal and Ates (1971) isolated six alkaloids from *Echium italicum* var. *biebersteinii* and three from *E. diffusum*. Delorme et al. (1977) studied 31 Boraginaceae species for their alkaloids and polyphenolic compounds. Pyrrolizidinic alkaloids were identified in 23 species. The N-oxide alkaloid form was found in 12 species.
Broch-due and Aasen (1980) identified the pyrrolizidine alkaloid lycopsamine from *Anchusa officinalis*. Huizing and Malingre (1981) studied pyrrolizidine alkaloids and phenolic compounds in some members of the family Boraginaceae by means of TLC. Roeder et al. (1984) isolated three pyrrolizidine alkaloids from *Alkanna tinctoria* and found that O7-angelylretronecine was a monoester, whereas triangularine and dihydroxytriangularine were diesters. Wessel et al. (1987) investigated the major pyrrolizidine alkaloids of three Boraginaceous species. *Echium sericeum* produced symlandine, or its isomeric base symphytine, in addition to achimidine. *Arnebia hispidissima* yielded echimidine and monocrotaline.

(4) SUGARS:

Bourdu and Quillet (1953) found that the roots of *Anchusa sempervirens* containing two groups of sugars. One is based on fructosans containing fructose, sucrose and glucofructosans slightly polymerized while the other is based on glucose containing, glucose, sucrose, dextrans and starch. Again, Bourdu and Quillet (1954) observed that the amount of sugars was almost twice as much in roots during the dormant season. Fructose is the movable sugar which varies with the state of vegetative growth and the sucrose content while the starch is in storage form. Sosa et al. (1955) determined glucose, sucrose, and stachyose by paper chromatography.
Hikino (1985) isolated novel hypoglycemic polysaccharides, designated as Lithosperman A, B and C from the roots of *Lithospermum erythrorhizon*. Yamada et al. (1986) purified and characterized the acidic polysaccharide from the roots of *L. euchromum* and found that LR-polysaccharide II was composed of rhamnose, glucose, arabinose, xylose, mannose, galactose and glucose in molar ratios of 2: 2.5: 3.4: 2.8: 6.6: 9.6: 14.4. The polysaccharide also contain 15% galacturonic acid and 3.8% protein.

(5) **LIPIDS** :

Sosa (1958) isolated about 20 lipids from *L. officinale* and *L. purpuro-caeruleum*. The isolated compounds from the unsaponifiable fraction include: hydrocarbons, pigments, 3 sterols m.p. 117°, 139° and 144° respectively and an aliphatic alcohol m.p. 73°. The saponifiable fraction contains (1) normal and branched fatty acids of \( \text{C}_n\text{H}_{2n}\text{O}_2 \) \((n= 14, 16, 18, 20, 24 \text{ and } 26)\), (2) unsaturated fatty acids: hexadecanoic acid, m.p. 24.4°, Octadecatrienoic acid, m.p. 17-18°, (3) three hydroxy acids and a specific acid present in *L. purpurocaeruleum*.

Miura (1963) obtained fats from the roots of *L. officinale* var. *erythrorhizon*, from wild and as well as from cultivated forms and identified the fatty acid components of the fats by gas chromatography. Valeric and isovaleric acids were the source of characteristic odour.
Wagner and Koenig (1963) isolated octadeca-6,9,12,15-tetracenoic acid from the fruit of L. officinale. Kleiman et al. (1964) analysed the seed oils of 29 species of the family Boraginaceae and observed that the 6,9,12-octadecatrienoic acid (upto 27%) and C\textsubscript{18} nonconjugated tetraenoic acid (upto 17%) in addition to linolenic acid (0.3-50%) and other common C\textsubscript{16} and C\textsubscript{18} acids were present. Iodine values ranged from 88 to 225.

Hoerhammer et al. (1964) studied the constituents of the fruit of L. officinale; C\textsubscript{18} tetraenoic acid could be fractionated which by reason of ozonide breakdown was assigned the structures of 4,8,12,15- and 6,9,12,15-octadecatetraenoic acid.

Krolkowska and Swiatek (1966) carried out the phytochemical analysis of gromwell i.e. L. arvense. Analysis of acidic fraction yielded palmitic, oleic, linoleic, linolenic and two unidentified acids possibly cerotic acids. Some other compounds were also identified by neutral fraction and acetone soluble fraction.

Varvoglis (1972) isolated wax from Alkanna tinctoria and studied spectroscopically and found that this was a mixture of light esters. Siddiqui et al. (1983) studied seed oil of different families including the plant Arnebia hispidissima by chromatographic and spectroscopic techniques. All the oil contain palmitic, oleic, linoleic and linoic acids in varying amounts.
Mellidis and Papageorgiou (1987) extracted the lipids from the roots of *Onosma heterophylla* and observed that the wax fraction consists of esters of palmitic acids and its homologs with higher alcohols.

(6) **OTHER CONSTITUENTS**:

Gorman et al. (1956) isolated some constituents of *Lithospermum ruderale* and rutin was one of them. Johnston et al. (1963) said that the lithospermic acid was the principal polyphenol of the above plant. On alkaline fusion this polyphenol yielded catechol, caffic acid and protocatechuic acid.

Bandyukova et al. (1966) determined the chemical composition of plants in Northern Caucasus and separated narcissin from *Macrotomia euchromon*. Bech (1967) observed the presence of flavonoids in some *Lithospermum* species and found that *L. officinale* and *L. arvense* contained 0.54% and 0.59% rutin respectively. This was not found in *L. purpureo-coeruleum*.

Bandyukova et al. (1970) studied the flavonoid compounds. Quercetin glycosides and more rarely isorhamnetin and kaempferol were observed in Boraginaceae family. Sharma et al. (1972) studied the constituents of some plants including *Arnebia nobilis* from which
hexacosanol, heptacosanoic acid and sitosterol was isolated. Wagner et al. (1975) described the structure of lithospermic acid from Lithospermum officinale. Sosa et al. (1977) isolated a new glucoside from the roots of the above plant and L. purpureo-caeruleum and named as lithospermoside.

Annaev et al. (1983) isolated two new triterpene glycosides i.e. copterosides E and F from Arnebia hispidissima. Seoane et al. (1984) observed that the hexane and alcohol extractives of Lithospermum fruticosum leaves and stems yielded α-amyrin, lupeol, β-sitosterol campesterol, stigmasterol, glucose, quercetin, rhamnose, bornesitol, syringin, rutin and sinapylaldehyde glucoside. Davis and Ross (1955) isolated β-sitostrelol from L. officinale.

George (1985) isolated a new aliphatic ketone from the petroleum extractive of Onosma hispidum and its structure was elucidated as 5-noncosanone by chemical and spectroscopic means. Swiatek et al. (1987) examined the phenolic constituents from aerial and underground parts by using two dimensional TLC and PTLC. Caffeic acid was present in Lithospermum arvense while p-hydroxybenzoic, vanillic, syringic, p-coumaric and p-hydroxyphenylacetic acids in L. arvense. Phenolic acids were found more in aeril parts in the roots. Coumarins were found in both the parts of the plant. Hamdard et al. (1988) isolated vitexin from the flowers of Arnebia hispidissima.
C. BIOLOGICAL:

Rosen et al. (1955) observed the antigonadotropic activities of quinones and related compounds. 2-Methyl-1,4-naphthoquinone was found inactive and 2,6-dimethyl hydroquinone and hydroquinone affected reproductive process in-vivo tests upon injection, as measured by rat estrous cycle changes. All these quinones was inactive orally. After 7-12 days of injection, rats become refractory to these compounds and resumed normal cycle.

Dandiya and Arora (1957) observed that a dealcoholized and detannated extract of Onosma bracteatum lowers the blood pressure, depresses the heart and causes vasoconstriction. It also relaxes the small intestine and prevents the stimulating action of acetylcholine. Nikolov and Boyadzhier (1958) noted the effect of some medicinal plants and substances on Sarcinia lutea with a view to using them in cancer chemotherapy in which Alkanna tinctoria was one of them.

Carlos (1960) introduced a new flocculation test for liver diseases using colloidal Anchusa. Bhuvneswaran et al. (1963) studied the growth rates in rats fed with vanaspati (hydrogenated vegetable oil) coloured with 0.04% turmeric extract or 0.4% Onosma echioides. These were at a level of 10% in a poor rice diet, though slow, but almost normal. Female receiving O. echioides grew faster than females in the control or turmeric groups while the males slower. Liver
and serum cholesterol were lower in rats receiving turmeric than *O. echioides* or no colour. Histopathological examination revealed no abnormal fatty infiltration with any of diets.

Patel and Patel (1966) carried out three crude extracts of dried powders of 'Ratanjot' for antifungal and antibacterial activity. They observed that soxhlet extract possessed the greatest antimicrobial activity, particularly against Gram-positive bacteria.

Bhakuni et al. (1969) screened 300 plant materials for antibacterial, anticancer, antifertility, antifungal, antihelmintic, antiprotzoal, antiviral and pharmacological activities only antimicrobial and anticancer activities were confirmed in a 50% alcoholic extract of *Arnebia nobilis*.

Gupta and Mathur (1972) found that a 50% alcoholic extract of the roots of *A. nobilis* or either of the two naphthoquinones i.e. arnebin-1 and arnebin-3 were effective against rat walker - carcino-sarcoma-256 in-vivo, and each of the naphthoquinones inhibited the tumor cells in-vitro. Administration of arnebin-1 with mitomycin C or diphenylsulfone-4, 4'-diisothiocyanate was more effective against the tumor cell than were any of the drugs alone.
Dhar et al. (1972) carried out 287 plants for a wide biological screen including anticancer, chemotherapeutic and pharmacological activities in which A. hispidissima was showing antibacterial, antitumoral, antiprotozoal, antihelmintic, hypoglycemic and anticancer activity.

Hayashi (1977) carried out the pharmacological studies on crude drug and its preparation 'Shikon' Lithospermum erythrorhizon 'Shiunko' an ointment. The ether extract of 'Shikon' showed slight antipyretic action and decreased locomotor activity however the water extractive has positive inotropic and slightly positive chronotropic effects and no intestinal tract movement, and coagulation system. He also described the effect of shikonin and acetylshikonin on rodents. Week analgesic, moderate antipyretic and hypothermic action were also observed and in mice vasocontraction was noted in isolated rabbit ear vessel. The anti-inflammatory activity of shikonin and acetylshikonin was similar to that of phenyl butazone. 'Shiunko', a preparation of shikonin appeared effective for the treatment of cutaneous injuries. He compared the antiinflammatory action of 'Shiunko' with that of ether extract of 'Shikon' and observed that after topical application the Shikon extract inhibited the increase of vascular permeability induced by histamine, bromelain, bradykinin, anti-rat-rabbit serum, heating, and the increased local cutaneous body temperature induced by UV radiation and heating. These effects
of 'Shikon' were maximum at a concentration of 0.1 - 0.2%. Although there was no significant difference obtained between the action of 'Shikon' extract and those of 'Shiunko' ointment which has slightly stronger action. Thus 'Shiunko' ointment may be a good preparation for promoting healing of wounds which have inflammatory edema, redness and pyrexia.

Sankava et al. (1977) observed the antitumor activity of shikonin and its derivatives obtained from the roots of Lithospermum officinale var. erythrorhizon and Macrotomia euchroma. Shikonin showed highest activity against ascites cells of Sarcoma-180 in mice and completely inhibited tumor growth at a dose of 5-10 mg/kg/day while it was toxic at a higher dose i.e. more than 15 mg/kg/day and was inactive at a lower dose of 1 mg/kg/day. Four derivatives of shikonin i.e. alkannane, dihydroshikonin, cycloshikonin and shikonin triacetate and the fractions of the alcoholic plant extract were also showed similar antitumor activity.

Katti et al. (1979) observed that the arnebin derivatives showed anticancer activity. Arnebin-1 was active against walker carcinosarcoma (WM) in rats and P388 lymphoid leukemia (PS) in mice. Effective dose of arnebin-1 has been found to be 4 mg/kg and 3 mg/kg against WM and PS respectively. It was also been found to be a powerful inhibitor of reverse transcriptase and poliovirus replicase.
Papageorgiou (1978) noted that the alkannin esters of \( \beta \), \( \beta \)-dimethyl-acrylic acid, \( \beta \)-acetoxy-isovaleric acid, isovaleric acid and angelic acid were showed excellent wound healing properties in a clinical studies on 72 patients with ulcus cruris. In 1979 Papageorgiou et al. reported that the antimicrobial activity of root extract of Alkanna tinctoria to be located in its naphthoquinone pigments. Polymerization of naphthaquinones resulted in a complete loss of antimicrobial activity.

Lin et al. (1980) carried out studies on the antiinflammatory effect of chemical principle of 'Zi-Cao' i.e. Arnebia euchroma. Acetylshikonin inhibited histamine induced capillary permeability, rat paw edema, and cotton pellet granuloma and has antiinflammatory effects in adrenalectonized rats. Atal et al. (1979) screened the extractives of 644 plants for tannin estimation and insecticidal activity against Musca domestica and Tribolium castaneum. No activity was observed in the roots of Arnebia nobilis. Prabhakar et al. (1981) observed that vitexin isolated from A. hispidissima exhibited potent hypotensive, antiinflammatory and antispasmodic (non-specific) properties. Hypotensive effect of this compound was attributed to its ganglion-blocking properties and anti-inflammatory effects to its antihistaminic, anti-bradykinin and anti-serotonin properties.

Afzal and Mohammad (1983) found that shikonin \( \beta,\beta \)-dimethy lacrylate is a strong antibacterial compound. Sankava (1983) noted
that *A. euchroma* contained arnebinol derivative which showed inhibitory effect on prostaglandin biosynthesis. However, Yao et al. (1983) observed that the naphthoquinones, the major constituents of *A. euchroma* were not the inhibitors of prostaglandin biosynthesis. The inhibitory activity was found for shikonofuran B, C and de-O-methylasiodiplodin. Two other compounds arnebinol and arnebionone had less inhibitory activity. Sampuku (1984) isolated a monoterpenyl benzohydroquinone from the same plant and observed that, it also had prostaglandin biosynthesis inhibitory activity.

Yamada et al. (1985) noted the effect of oriental pharmaceutical polysaccharides from *Macrotomia euchroma* on plasma protein components. Further, Yamada et al. (1986) isolated an extraordinary patent anticomplementary substance from the roots of *Lithospermum euchromum*, which activated the human complement system in-vitro and the active principle was shown to be acidic polysaccharide. Seshadri et al. (1985) observed antifungal activity of *Alkanna tinctoria*.

Wessel et al. (1987) screened some selected plants for the isolation of pyrrolizidine alkaloids and antitumor activity. Extracts of *Arnebia hispidissima* contained unsaturated alkaloids which were cytotoxic in-vitro to 'Ehrlich ascites carcinoma' cells. The major pyrrolizidine alkaloids - echimidine and monocrotaline isolated from *A. hispidissima* were anticipated to be hepatotoxic to mammals on the basis of their molecular structure.
Hamdard et al. (1988) obtained alkannin monoacetate, alkannin \(\alpha\)-dimethylacrylate and (\(t\)) alkannin from the roots of *A. hispidissima* and observed that these naphthaquinones showing antimicrobial and anticancer activities.

D. **ECONOMIC IMPORTANCE**: 

The use of alkannins as dyes was known to ancient Greeks and Romans who employed the roots of *Anchusa tinctoria* for this purpose. The wound healing properties of these roots were described by Dioscarides.

The healing properties of the extracts of *Lithospermum* roots attracted the attention of the Chinese who used them as folk remedies. Even today the inhabitants of India and Pakistan employ these dyes. (Bole, 1961, Jain and Mathur, 1967).

Ironically the use as a food colorant survived and is still used as such in our days. Atleast twelve European countries allow its use as colorant in food and wine.

i. **MEDICINAL PREPARATIONS**: 

Papageorgiou (1978) described a pharmaceutical composition, especially salves, for treating ulcus cruris. It contain alkannin derivatives as active agents.
Inoue et al. (1986) prepared an oral bandage with good prolonged adhesion by using Lithospermum. Hasegawa et al. (1987) also prepared a transdermal tape suitable for intra oral application for a prolonged period. Tabata and Honda (1987) described a tropical bactericides containing deoxyshikonin as an active ingredient. Ointment and liquid formulation containing deoxyshikonin (pure or mixture) applied 2 or 3 times per day for 3 week provided almost complete control of athlete's foot. However there was no cure by a calamus extract of L. erythrorhizon containing 58% shikonin and its derivatives.

ii) **COSMETICS** :

Futagoishi (1973) and Matsui et al. (1977) used the Lithospermum extractives for cosmetics and pharmaceutical ingredients. The extractives contain isobutylshikonin, dimethylacryloyl shikonin and β-hydroxyisovalerylshikonin.

Kishimoto and Aota (1977) observed that the cosmetic preparation containing extracts of roots or stems of L. officinale with β, β-dimethylacrylshikonin, acetylshikonin, teracrylshikonin, shikonin, β-hydroxyisovalerylshikonin as an active ingredients are beneficial for skin and effective in preventing acne stains, freckles, sunburns etc.
Mutsui et al. (1978) described that the extractive obtained by a mixture of ethanol and propylene glycol (8:2) from the dried Lithospermum is useful in manufacturing liquid cosmetics.

Hatinguais and Belle (1980) described a natural hair coloring material which shows anti-inflammatory and antibacterial properties were obtained from Alkanna tinctoria or Onosma echioides.

A preparation of skin tonic cream was prepared by Lin (1986). This cream that softens, soothes and protect the skin, contains hypocrellin as the main ingredient along with root extract of Arnebia euchroma with other plant extract and/or vitamin E. Morimoto et al. (1986) noted that a cleanser contains a neutral or alkaline anionic surfactant as base and a powdered carrier coloured by shikonin or Shikon extract which change their colour when dissolved in water. Morimoto et al. (1987) described that the powdery bath preparations containing shikonin and various salts has therapeutic effects on skin disorders and haemorrhoids. It produces a blue colour in water when it contains alkaline salt, but the colour changes to red when an acidic salt is added.

iii) **Dyestuffs**:

Kozo et al. (1950) gave a preliminary report on the chemical identification of vegetable dyes used in ancient Japanese silk for Yellow, red and purple colour and found that shikonin
Mukerji et al. (1960) examined five colouring materials, namely Alkanet, Annatto, β-carotene, Turmeric and 'Ratanjot' to colour the vanaspati and found that only Ratanjot was fairly stable for practical purposes against physical and chemical treatments and it was considered as non-toxic or very little toxic.

Huttiagdi and Patel (1961) studied eleven market samples of 'Ratanjot' for its colouring properties. They used two alternative methods for colouration of vanaspati with 'Ratanjot'. Colour imparted to vanaspati was easily and completely removed by simple chemical methods, for example by shaking with alkali solution. More exposure to direct sunlight to 48 hours resulted in over 50% bleaching, and in one case the colour loss was complete. Other three physical methods i.e. heating, treatment with bleaching earth and bleaching carbon, resulted in colour loss ranging from 15 to 65%.

Minagawa et al. (1987) obtained natural mordant colour consisting of naphthaquinone derivatives and a variety of colours purple, reddish purple, purple black brown etc. could be developed on silk by using various mordants.

No detailed work on the field of pharmacognosy and chemotaxonomy of 'Ratanjot' has been on record and the present investigation includes the details of both these aspects of the genuine drug along with their substitutes and adulterants, and commercial samples procured from the different places of the country.
MATERIAL AND METHODS
A. **BOTANICALLY AUTHENTICATED SAMPLES**

Five root samples from following four species have been collected from various Himalayan regions and a small portion of root of *Arnebia nobilis* was taken from the specimen collected by Prof. Bole from Afghanistan for making comparison with the market samples. The voucher specimens of these species are preserved in Medicinal Plant Herbarium of the Institute.

1. *Arnebia benthamii* (Wall. ex G. Don)  
   **Collection Site** - Jaripani, Tehri Garhwal.  
   **Voucher Specimen No.** 11374

2. *Arnebia euchroma* (Royle) Johnston  
   **Collection Site** - Chotadhara, Lahul Valley, Himachal Pradesh.  
   **Voucher Specimen No.** - 6845

3. *Arnebia euchroma* (Royle) Johnston  
   **Collection Site** - Bodhikharbu, Leh, Jammu and Kashmir.  
   **Voucher Specimen No.** 16322

   **Collection Site** - Istalif Afghanistan. On barren hill slopes.  
   **Voucher Specimen No.** - AF 43

5. *Maharanga emodi* (Wall.) DC.  
   **Collection Site** - Chhangu, East Sikkim.  
   **Voucher Specimen No.** - 15807

   **Collection Site** - Gemeor, Lahul Valley, Himachal Pradesh  
   **Voucher Specimen No.** - 11487
B. MARKET SAMPLES

Eight market samples of 'Ratanjot' have been procured from various Indian markets.

Amritsar, Bhopal, Bombay, Calcutta, Delhi, Hyderabad, Jammu and Lucknow.

METHODOLOGY

There are several methods available for establishing the identity of herbal drugs. Some of these are Organoloptic, microscopic, physical, chemical and biological. In addition to this some diversified and specialized parameters such as tissue culture techniques, plant hormones, phytopharmacology, scanning electron microscopy and finger printing can play a meaningful role in solving out the problem of adulteration and substitution in the marketed crude drugs. Often more than one method have to be employed to detect adulteration and substitution.

The details of the method dealt, are given separately along with their respective chapters such as (i) Pharmacognosy (microscopic, SEM and powder studies) and (ii) Chemotaxonomy (Phytochemical Analysis and chemistry of Arnebia euchroma).
PART I

PHARMACOGNOSTIC STUDIES
CHAPTER I

BOTANICAL STUDIES
I.  **BOTANICAL DESCRIPTION**

1. *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnston

   **Echium benthamii** Wall.; **Macrotomia benthamii** (Wall.) A.DC.
   **Leptanthe macrostachya** Klotzsch; **Lithospermum benthamii** (Wall.ex G.Don)
   I.M.Johnston

   Erect perennial herb with thick root stock rich in purple dye. Stem solitary, fistulous unbranched, arising from the centre of the basal leaf-cluster, ca 90 cm long, densely covered with white, spreading trichomes, tuberculate at base. Basal leaves many, lanceolate, entire, acute, attenuated at both ends, with 3-5 prominent, longitudinally parallel ribs, 30 cm long and 3.5 cm broad, covered on both surfaces with silvery, thin, spreading trichomes. Middle and upper cauline leaves gradually reduced in size upward. Inflorescence a dense cylindrical thyrse, 35 cm long, 5-7 cm thick at maturity. Bracts linear-lanceolate, acuminate, densely hairy exceeding the calyx, which divided at the base, densely pubescent. Corolla pink or purple to maroon, usually shorter than the calyx and about 20-25 mm long, lobes ovate. Stamens borne at the middle of the corolla tube, 2-2.5 mm long. Style reaching the middle of the corolla tube, stigmas 2, broader than long, more or less separate. Nutlets broadest near the middle, 3-4 mm long with persistant calyx.

**Distribution**: Kashmir North and North Western India to Nepal and West Pakistan alt. 3000-4000 m.
2. *Arnebia euchroma* (Royle) I.M.Johnston

*Lithospermum euchromon* Royle; *Stenoselenium perenne* Schrenk ex Fisch & C.A.Mey; *Arnebia perennis* (Schrenk) Boiss.; *Macrotomia endochroma* Hook. f. & Thoms. ex Henderson & Hume; *M. perennis* (Schrenk) Boiss.; *M. onosmoides* Regel & Smir.; *M. euchroma* (Royle) Paulsen; *M. euchroma* var. *subacaulis* Lipsky; *M. oginoi* Kitamura; *Arnebia euchroma* subsp. *caespitosa* Rech. f. & Riedl.

Erect perennial, caespitose herb with thick root stock rich in purple dye. Stems many, arising from the axils of the leaves, which formed a sterile basal cluster of the preceding year, unbranched, pale to brownish purple, ca 50 cm long, covered sparsely or densely with white, thin or thick trichomes. Primary basal leaves lanceolate, entire, acute, attenuate towards the broad, base ciliate. Secondary basal leaves lanceolate, tapering towards the acute apex, winged, dilated, ciliate base with one strong prominent rib, 11 cm long and 15 cm broad, sparsely to densely covered on both surfaces with thick or thin trichomes. Lower middle cauline leaves sessile, lanceolate, broadest in the middle of the lower half, ca 8 cm long, 15 mm broad. Upper cauline leaves shorter and broader. Inflorescence terminal, subglobular, tending to become broader than long, not elongated in fruit, 5-6 cm in diameter, bracteate, bracts leaflike not exceeding the calyx. Pedicels erect, densely hairy. Calyx divided to the base, lobes hairy, linear - lanceolate, acute, ca 10 mm long, 1.5 mm broad.
in flower and enlarged in fruit. Corolla purplish-white, purple or brownish-purple, funnel shaped, 16-22 mm long, tube 10-15 mm long, lobes ascending, suborbicular to ovate. Anthers borne either at the middle of at the summit of the corolla tube. Style reaching the middle of the tube or slightly exerted, usually forked at the apex. Stigmas 2, compressed, rounded, broader than long. Nutlets grey, dusky, irregularly and coarsely tuberculate and more or less rugose.

Distribution: Kashmir 3000-4000 m, North West India to Nepal, West Pakistan.

3. Arnebia nobilis Rech.f.

Lithospermum nobile (Rech.f.) I.M.Johnst.

Erect perennial herb, stems simple, arising from the centre of the basal leaf cluster, 50 cm long, 8 mm thick at the base, covered with stout, white trichomes retrorsely appressed on the lower part of stem, spreading on the upper part 2-4 mm long. Basal leaves 20-25 cm long, 2-12 mm broad, linear, lanceolate, apex acute; lower cauline leaves similar to the basal ones with three strong longitudinal veins, covered on both surfaces with slender stiff, spreading to ascending trichomes; middle cauline leaves lanceolate 5-14 cm long, 5-8 mm broad near the base; upper cauline leaves gradually reduced upwards.
Inflorescence an elongate interrupted cylindrical thyrse, composed of small terminal or axillary cymes; cymes densely flowered. Bracts linear and shorter than the calyces, subtending leaves surpassing only the lower cymes, Calyx hispid 15-18 mm long in flower, lobes equalling to slightly surpassing the corolla lobes, broad near the base flowers heterostyloous. Corolla infundibuliformis 16-20 (-25) mm long; throat short and broad, limb about 5-7 (-10) mm in diameter, lobes ascending, apex rounded. Anthers attached at the summit of the corolla tube and partly excorted into the throat, 2-5 mm long. Style reaching to the middle of the corolla tube, bifid, stigmas -2, capitate. Nut about 7 mm long, apex subrostrate with keel on dorsal and ventral surfaces, general surface tuberculate marked with longitudinal striae sometimes irregularly wrinkled.

Distribution : Afghanistan, altitude 2400-3000m.

Maharanga emodi (Wall.) DC.
syn. Onosma emodi Wall; Onosma vestitum Wall.

Perennial from a strong tape root. Stems usually several, leafy, decumbent or ascending 20-50 cm long, 4-5 mm thick towards base, simple or producing loose floriferous branches above the middle. Cauline leaves acute, lanceolate -oblanceolate, elongate, mostly broadest at or above middle, 5-15 cm long, 7-20 mm broad, frequently
with some veins, upper surface hispid and minutely strigulose, hair bases not conspicuous, hairs more scanty on upper surface than on lower. Basal leaves oblanceolate, petiolate. Cymes terminal, usually forked, grouped to form a rounded terminal cluster usually 4-5 cm broad at anthesis. Calyx 6-10 mm long with short broad tube, lobes triangular or lance triangular, acute, 3-6 mm long, 2-3 mm broad at base. Carolla 9-13 mm long form a short tubular base 2-2.5 mm thick and 2 mm long abruptly expanding to 7-10 mm thick slightly below middle and then gradually contracting to a mouth, outside hispidulous or villulose, above the middle longitudinally plaited and hence more or less grooved below each lobe-sinus; below middle of corolla outside each plait replaced by a long sharply defined elliptic depression (hidden by calyx-lobe) with a deep pocket at upper end, because of the inflexure and depression below each lobe-sinus the intervening sectors of the corolla appear puffed out to from coarse convex longitudinal ribs especially prominent portion ended by an abrupt contraction, their most prominent part bulging out between the calyx lobes, chin like, resting on the bottom of the adjacent calyx sinus, corolla lobes small recurved 1.5-2 mm broad and 1-1.5 mm long, corolla inside below the middle bearing 5 thickish somewhat wedge shaped protuberances, coverage about the style, and bear the filament, on their upper inner extremity, protuberances somewhat hairy, containing a pocket like invagination originating on the outside of the corolla, base 2-3 mm long. Anthers 4-5.5 mm long, coheret at base only, included, sterile apex 0.5-1 mm long serrulate, filaments 1-2 mm long,
glabrous, born on the shoulder of a protuberance, flat, subulate-linear with evident midnervce, usually bent into a sigmoid curve, a pair of narrow wings along the crest of the protuberance; nectary a conspicuous collar, somewhat hairy. Style glabrous 10-12 mm long. Nutlets 2-3 mm long, dull coarsely tuberculate and also very abundently and minutely papillate.

**Distribution** : Eastern Himalayas, Garhwal to Western Bhutan. alt. 3300-4200 m.

*Onosma hispidum* Wall. ex D.Don  
Syn. *O. echioides* C.B. Clarke (non Linn.)

Perennial, bristly with spreading somewhat tawny hairs, minutely hispidulous, stem one to many, erect or ascending, simple or rarely branched, 10-50 or rarely 75 cm long, 3-6 mm thick towards the base. Leaves hispid and hispidulous on upper surface, the coarse hairs usually with discoid bases, veins in conspicuous, basal leaves usually persisting at flowering time, oblance-linear to narrowly oblanceolate, 10-40 cm long, 5-15 mm broad, apex obtuse, middle cauline leaves linear-oblong or lance-oblong, 4-8 cm long, 4-10 mm broad. Cyme terminal, forked, 3-4 cm broad at maturity, elongating, racemose, upto 15 cm long, bracts lanceolate. Calyx 12-15 mm long at anthesis on pedicels, lobes lanceolinar, tawny bristly, at maturity 15-25 mm long on pedicels with broad lobes. Corolla white, cream or pale yellow, 18-23 mm long,
from a base, 3-3.5 mm thick gradually expanding, 8-11 mm thick just below the sinus, outside commonly sparsely hispidulous on the lobes and elsewhere very minutely puberulent, inside completely glabrous. Anthers united basally and laterally to form a tube, 9-11 mm long, attached ± 2.5 mm above base, tip long, nectary a completely glabrous flange 0.3 mm high. Style 13-23 mm long, glabrous. Nutlets 6 mm long, smooth or obscurely roughened, somewhat lustrous.

**Distribution** : Western Himalayas, alt. 3000-4000 m.
II. MACROSCOPIC CHARACTERS

A. BOTANICALLY AUTHENTICATED MATERIALS

1. Arnebia benthamii (Fig. 1)

Dried root is thick sometimes 2.5 cm in diameter, tapering often twisted with a single headed crown which is covered by scales and the remains of the leaves bearing numerous dull whitish, bristly hairs. Externally the root is furrowed. The bark and sometimes the wood are more or less decomposed and separated into a number of dried papery layers. The layers are the exfoliate and are of reddish brown in colour. Fracture of the root is medium and the texture is loose. The smooth transverse surface shows central woody core with a surroundings of papery layers. The wood is semi-ring porous.

2. Arnebia euchroma : (Fig.2)

Dried root is thick 3-3.5 cm in diameter, tapering, fusiform with a single or several headed crown which is covered sparsely or densely with white, thin to thick trichomes, 1-2 mm long with minute to large tuberculate bases. The woody portion of the root is colourless and of 2.5 to 5.0 mm in diameter while the remaining part of the wood and the bark decomposed and readily exfoliate in the form of dark shining or dull purple brown coloured papery layers, when
handled, they stain the fingers red. Fracture of the root is short and the texture is loose. Smooth transverse surface shows distinct zonation and the wood is diffuse-porous.

3. *Arnebia nobilis*

Dried root is tapering 1-5 cm in diameter, fusiform, often twisted. Externally the root is often furrowed sometimes so deeply that the cylindrical form of the root is lost and irregular segments are formed. The layers of the bark and sometimes the wood readily exfoliate in the form of dried papery layers which are reddish purple in colour. The fracture of the root is short and the texture is medium coarse. The transverse surface showing semi-ring porous wood and distinct zonations surrounded by decomposed and separated bark layers; secondary phloem and secondary xylem, followed by internal reddish purple zone of decomposed xylem (Fig. 3).

4. *Maharanga emodi* : (Fig. 4)

The dried roots are thick, branched and measured upto 3 cm with a single headed crown of scales and remains of the basal leaves bearing numerous dull whitish, bristly hairs. Externally the root is often furrowed, but not so deep to form the irregular segments. In mature root the layers of bark are exfoliate and are of reddish
brown in colour. The wood is light brown or pale-yellow in colour and soft. Texture of the root is coarse. The transverse surface showing secondary phloem and secondary xylem which is pale yellow in colour with & surrounded by decomposed cortex and bark layers and the wood is semi-ringporous under the hand lense.

5. *Onosma hispidum* : (Fig. 5)

Dried root is reddish purple in colour, thick 1 to 7 cm in diameter and 6 to 25 cm in length, fusiform, often twisted with a single or several headed crown which is covered by scales and the remains of leaves bearing a number of dull whitish, bristly hairs. Externally the root is often furrowed sometimes so deeply that the cylindrical form is lost. The bark and often the wood are more or less decomposed and separated in to a number of (sometimes upto 30) dried papery layers. The layers of the bark readily exfoliate and are of reddish purple in colour. Fracture of the root is short and the texture is coarse. The smoothed transverse surface shows separate zonations of the wood surrounded by dark reddish brown colour decomposed bark and secondary phloem. The central core of each zone comprises of crushed parenchyma and decomposed vessels. The wood appears to be semi-ring porous.
8. **MARKET SAMPLES**: (Plates 3-5, Figs. 6-9)

All the market samples are almost similar in microscopic and macroscopic appearance (Figs. 6 & 7) and comprises of woody and papery pieces with some reddish brown coloured coarse powder. The woody portions are the roots as cleared by the presence of radical leaves and the absence of nodes and internodes. The woody pieces—the root, is 3-24 cm in length and 1-7 cm in diameter, fusiform, often twisted, tapering and sometimes branched as in Delhi sample. The root consist of a single headed crown as in Bhopal, Bombay and Delhi sample & several headed crown of radical leaves in all eight samples. Externally the roots are often furrowed, sometimes so deeply that the cylindrical form of the wood is lost. The layers of the bark and more often the wood are readily exfoliate in the form of dried papery layers which are reddish purple in colour and are major part of all the samples. The transverse surface showing semi-ring porous wood in all the samples and diffuse porous in few pieces of Bombay and Bhopal samples (Fig.8) Sone decomposed and separated zones of bark and xylem are also discernible in T.S. (Fig. 9). Few woody pieces in Delhi sample become very soft as compared to other samples and becomes mucilagenous when put into the water for sometime.
III. MICROSCOPIC STUDIES

METHODOLOGY:

Dried materials were employed for detailed histological studies. Wood pieces were macerated with hot 40% nitric acid to obtain full length measurements of tracheary elements. Hand and microtome sections were employed according to the nature of the material. The wood microtome sections were also cut 10 to 30 μ in thickness. These macerated tissues, transverse and longitudinal sections were stained in safranin as well as in safranin-fast green combination (Johnston, 1940). Permanent slides were prepared by the usual dehydration method. The sketches were made with Leitz Camera lucida.

The transverse sections of the wood and the peelings were treated with chlor-zinc-iodine, phloroglucinol and hydrochloric acid, concetrated Sulfuric acid and iodine for the identification of cellulose, hemicellulose, lignified, suberized or cutinized cell walls and with methylene blue or ruthenium red for the mucilagenous cell contents.

A. BOTANICALLY AUTHENTICATED MATERIALS

(i) ARNEBIA BENTHAMII: (Plate 5-8, Fig. 10-16)

Wood

In transverse section, the number of vessels varies from 210-230 per sq.mm and arranged somewhat flame like (Fig. 10). The broader
vessels are solitary or in a group of two or three surrounded by a cluster of narrower vessels which are often arranged in radial rows. Wood parenchyma is apotracheal and confluent-narrow (Fig. 11), Cambium is distinct, phloem made up of sieve tubes compound sieve plates, companion cells and thick walled suberized parenchyma which is arranged in radial rows and wavy in outline. Secondary cortex is very broad, about 25-30 layers of the cells and comprising of irregular shaped parenchyma (Fig. 12). All the parenchymatous cells i.e. wood parenchyma, ray parenchyma and cortical parenchyma are thick walled and made up of suberized wavy walls which become brown with Schultze's solution. The outer most margin is wavy with a thick cuticle. Fibres are absent.

In longitudinal sections, rays are distinct heterogenous and 3 to 8 cells broad. The secondary wall thickening of the vessels varies from scalariform to reticulate and intervascular pitting is alternate (Fig. 13).

In maceration the vessels are vary much in shape and size (Table 1) ranging from short and broad i.e. 123.2 μ long and 154.0 μ broad to elongated and narrow i.e. 200.2 μ long and 15.4 μ broad with tapering ends. They are exhibited a complete or incomplete simple perforation plate. The broader ones are drum or barrel shaped with terminal perforation plate while the elongated vessels are
primitive with lateral or sometimes terminal incomplete or complete perforation plate. Trachieds are very few and with bordered pits. Suberized elongated cells are present along with the xylem vessels (Fig. 14).

**PEELINGS**

T.S. of peelings show crushed, suberized parenchymatous cells. In maceration, two types of parenchymatous sheaths are present one with polygonal somewhat rounded cells with reddish brown content (Fig. 15) and another ones are elongated, tapering and without any coloured content in it (Fig. 16).

(ii) **ARNEBIA EUCROMA** : (Plate 9-12, Fig. 17-24)

**Wood**:

In T.S. of the young roots, (Fig. 18) the number of vessels varies from 260 to 305 per sq.mm in Himachal Pradesh sample and 370 to 400 per sq.mm in Laddakh sample while in mature root (Fig. 17) 410 to 475 in Himachal Pradesh sample and 455 to 530 in Laddakh sample. Vessels are arranged in tangential clusters of broad and narrow ones. Rosette crystals of calcium oxalate are present in the lumen of the few vessels. Xylem parenchyma is vasicentric and apotracheal type. The parenchymatous cells are of irregular in shape and become suberized at maturation and separated out in
the form of a continuous ring along with some vessels. Pith is absent. Cambium is distinct. The Phloem region is 10-15 cells broad and made up of sieve tubes, compound sieve plates, companion cells and parenchyma. Cortex is about 30-40 cells broad with rectangular or polygonal parenchymatous cells. Outer most layer of Cork cambium comprises of bulbous parenchymatous cells (Fig. 19). All the parenchymatous cells have cellulose cell walls except endodermis where the cell walls become blue with iodine and indicates that this is made up of hemicellulose.

In L.S. rays are few, distinct, heterogenous, short and only 2-3 cells broad. Secondary wall thickening of the vessels resemble to A. benthamii. Intervascular pitting is alternate. Fibres are absent (Fig. 20).

In maceration size of the vessels varies (Table 1) from broad and short i.e. 184.4 \( \mu \) and 200.2 \( \mu \) to elongated and narrow i.e. 231.0 \( \mu \) and 15.4 \( \mu \) (Fig. 21). Type of the vessels are same as in A. benthamii. Suberized parenchymatous cells are present in H.P. sample (Fig. 22).

**PEELINGS**

T.S. of peelings show few vessels with suberized crushed parenchymatous cells (Fig. 23). In maceration these are same as in A. benthamii except few additional vessels of narrow and broad type (Fig. 24).
(iii) **ARNEBIA NOBILIS**: (Plate 13-16, Fig.25-31)

**Wood**:

In T.S. number of vessels varies 500 to 700 per sq.mm. The outer most layer of xylem vessels comprises of the clusters of broad vessels and the inner most with narrow ones (Fig. 25). The middle portion of the wood having broad and narrow vessels in the form of a multiple clusters and sometimes in the form of alternate rings of both the type of vessels (Fig. 26). The narrow vessels are arranged in radial rows. xylem parenchyma is paratracheal and confluent narrow and at maturation they become suberized. Pith is present and made up of large, thin walled parenchymatous cells. Combium is distinct 3-4 cells broad any wavy in outline. Secondary phloem 15-20 cells broad and comprises of sieve tube, compound sieve plates, companion cells and parenchyma. The cortex is 25-30 cells broad with irregular shaped cellulosic parenchymatous cells (Fig. 27). Few layers of outer cortex become suberized and crushed showing brown colour with Schultze's solution. The outer most layer of cork cambium comprises of thick walled suberized bulbous parenchymatous cells. In L.S. the rays are very few, distinct, heterogenous and 4-6 cells broad. Secondary wall thickening of the vessels varies from annular to reticulate and intervascular pitting is alternate. Fibres are absent (Fig. 28).

In maceration the size of the vessels varies from 46.2 μ to 184.8 μ in length and 15.4 μ to 138.6 μ in breadth (Table 1).
They are of same type as in A. benthamii except the length. Trachieds are very few and with bordered pits (Fig. 29).

**PEELINGS:**

Transverse sections of the peelings are same as in A. benthamii, but the macerated peelings show three types of sheeth of parenchyma. The two types are almost similar to A. benthamii (Fig.30) and the third type with polygonal suberized cells without any granulated reddish brown contents in it (Fig. 31).

(iv) **MAHARANGA EMODI:** (Plate 16-19; Figs. 32-37)

**Wood:**

In T.S. number of vessels varies from 400 to 500 per sq.mm. Broad vessels are solitary or in a groups of 2 or 3 at some places with a surroundings of narrow vessels which are often arranged in radial rows (Fig. 32). Wood parenchyma surrounds the group of vessels, are paratracheal and aliform confluent and the cells are polygonal in shape. In young wood medullary rays are very broad about 7-14 cells broad and the parenchymatous cells of medullary rays are polygonal or somewhat rectangular in shape and arranged in radial rows (Fig. 33). Cambium and cork cambium are present. Phloem region is about 20 cells broad and comprises of sieve tubes with a compound sieve plate and few companion cells at some places. Phloem parenchyma
is somewhat rectangular in shape and having mucilage. Cortical region is very broad about 35-40 cells often arranged in radial rows and are polygonal or sometimes rectangular parenchymatous cells (Fig.34). Outermost 3-4 layers are suuberized and then 10-15 layers of parenchyma are in crushed form. The outer most margin is wavy with a thick cuticle. All the parenchymatous cells except few wood parenchyma contain mucilagenous substances which give brilliant pink colour with ruthenium red and blue with methylene blue. Pith is absent, the narrow vessels, are in a compact group in the central core.

In L.S. rays are distinct heterogenous 6-8 cells broad. The ray cells are thick walled and polygonal in shape and contain mucilage. Secondary wall thickening of the vessels also varies from spiral to reticulate. Intervascular pitting is alternate (Fig.35 & 36).

In macerated tissues the size of the vessels varies (Table 1) from short and broad i.e. 92.4 µ and 138.6 µ to elongated and narrow i.e. 184.8 µ and 15.4 µ. Thick walled parenchymatous cells with mucilagenous contents are present which are elongated and somewhat rectangular in maceration (Fig. 37).

PEELINGS

T.S. and macerations of the peelings show the similar characters as in A. benthamii.
OlOMOSWA HISPIDUM : (Plates 19-22; Figs. 38-43)

Wood

In T.S. the number of vessels varies from 514 to 610 per sq.mm. Xylem vessels are in a multiple group of broad and narrow ones. The broad vessels are arranged in tangential uniseriate clusters while the narrow ones are often arranged in radial rows (Fig. 38). The central core comprises a group of narrow vessels with a very few parenchymatous cells. At maturation vessels and parenchyma become crushed and a reddish brown content deposited in the crusted parenchymatous cells (Fig. 39). Wood parenchyma is apotracheal and confluent, sometimes it is paratracheal. Lysigenous cavities are present at some places in xylem parenchyma (Fig. 40). Medullary rays are radiating, broad and up to 35 cells in height and 12-15 cells in width. Lysigenous cavities with some mucilagenous substances are also present in medullary rays (Fig. 40). These cavities turn brilliant pink with ruthenium red and blue with methylene blue. The parenchymatous cells become suberized at maturation (Fig. 41). Cambium and cork cambium are distinct. Secondary phloem region is about 10-15 cells broad and comprises sieve tubes with compound sieve plates, companion cells and phloem parenchyma (Fig. 38). Fibres are present. Cortical region is very broad. The outermost 2-3 layers are suberized and somewhat bulging outwardly and are thick walled. The parenchymatous cells of cortex are somewhat rectangular and polygonal in shape and made up of cellulosic cell walls.
In L.S. rays are distinct, heterogenous 7-10 cells broad and composed of thin walled cellulosic parenchymatous cells which are at times slightly wavy in outline (Fig. 42).

On maceration the xylem vessels are vary much in shape and size (Table 1) ranging from broad and short i.e. 200.2 μ and 107.8 μ to elongated and narrow i.e. 154.0 μ and 30.8 μ. The types of vessels are same as in A. benthamii with simple terminal or lateral perforation plate. Fibres are present and trachieds are few with bordered pits (Fig. 43).

PEELINGS :

T.S. and macerated peelings are same as in Arnebia nobilis.
MARKET SAMPLES

(i) **AMRITSAR SAMPLE** : (Plates 22-25; Figs. 44-50)

**WOOD**

In T.S. the number of vessels varies from 410 to 600 per sq.mm. Vessels are often arranged in a multiple groups except a few which are solitary. Outermost layer of the xylem comprises tangential clusters of broad vessels and the inner most with narrow vessels which are arranged in radial rows of 10-15 vessels (Fig. 44). Wood parenchyma is paratracheal and confluent narrow and the cells are thin walled and irregular in shape. These become suberized at matura-
tion and in a mature wood piece these are with dark brown contents in globular form and crushed (Fig. 45). Medullary rays are short and at some places not very distinct. Cambium comprises of 2-4 layers of rectangular parenchymatous cells and wavy in outline, having a clusters of vessels in the furrow. Phloem consist of sieve tube, sieve plates, companion cells and radially arranged parenchyma. Pith is present in the centre of wood and comprises of thin walled polygonal or rounded, large parenchymatous cells while in mature wood pith is in crushed and decomposed form. Cork cambium is present and comprises of bulbous outline which is separated from the outer cortex and bark. Inner cortex 15-20 cells broad and made up of cellulosic irregular shaped parenchyma (Fig. 46).
In L.S. the rays are distinct heterogenous and 4-6 cells broad. The secondary wall thickening of the vessels also varies from spiral to reticulate. Intervascular pitting is alternate. Some trachieds are also present and fibres are absent (Fig. 47).

In maceration the size of the vessels varies (Table 1) from short and broad i.e. 107.8 μ and 138.6 μ to elongated and narrow 261.8 μ and 15.4 μ. Most primitive as well as most advanced vessels are present in a single piece of wood (Fig. 48).

PEELINGS

T.S. of peelings comprise of only crushed parenchymatous cells with reddish brown coloured contents. In maceration three types of parenchymatous sheeths are found one with polygonal or somewhat rounded cells with suberized cell walls and reddish brown content in it, the second one with elongated tapering colourless parenchymatous cells (Fig. 49), and the third type with polygonal suberized thick walled parenchyma without any coloured contents(Fig. 50).

(ii) BHOPAL SAMPLE : (Plates 26-28; Figs. 51-55)

WOOD

In T.S. the number of vessels varies from 410 to 470 per sq.mm. Vessels are in a multiple group of broad and narrow ones. The broad
vessels are mostly solitary or sometimes in a tangential group of 2-7.' Narrow vessels are mostly arranged in radial rows but they are solitary too at some places (Fig. 51) in some pieces, vessels are mostly solitary (Fig. 52). Wood parenchyma is paratracheal or apotracheal and confluent. Xylem parenchymatous cells are polygonal or somewhat rounded in shape and arranged in radial rows. Medullary rays are not distinct in most of the pieces of the wood while these are very broad, and radiating in other pieces of the wood and made up of flate, tangentially arranged parenchymatous cells with large lysigenous cavities. A lot of rosette crystals of calcium oxalate are present in medullary rays, mostly inside the lysigenous cavities (Fig. 53). In few pieces central core is dark brown in colour and wavy in outline. This zone comprises of decomposed vessels and parenchymatous cells. Cambium and cork cambium are distinct. Phloem made up of sieve tubes, compound sieve plates, companion cells and parenchyma. Cortex is only 4-5 cells broad. The outermost layer of cork cambium is bulbous and suberized. Bark comprises of 5-6 layers of crushed parenchyma (Fig. 54). Pith is absent.

In L.S. rays are long, 9-12 cells broad and heterogenous. Secondary wall thickening of the vessels also varies from spiral to reticulate. Intervascular pitting is alternate (Figs. 54 & 55). In some pieces of wood rosette crystals of calcium oxalate are also present inside the vessels (Fig. 55).

In maceration the primitive as well as advanced type of vessels are present (Table 1). The former one elongated and narrow i.e.
215.6 μ and 15.4 μ respectively and the later are drum or barrel shaped and measured up to 169.4 μ long 184.8 μ broad.

PEELINGS

T.S. and macerated peelings are same as in Amritsar market sample.

(iii) BOMBAY SAMPLE : (Plate 28-30; Figs. 56-60)

In T.S. number of vessels varies from 330 to 640 per sq.mm. Xylem vessels with large lumen are mostly solitary or sometimes in a groups of 2-3 (Fig. 56) while in few pieces broad vessels are mostly arranged in tangential clusters of 5-12 (Fig. 57). The narrow vessels are arranged in radial rows along with the broad vessels. Rosette crystals of calcium oxalate are present in some vessels. Xylem parenchyma is paratracheal and confluent broad. The parenchymatous cells are cellulosic, thin walled and polygonal in shape. In few pieces medullary rays are distinct and 5-12 cells broad and comprises of irregular shaped parenchyma with some lysigenous cavities at some places with rosette calcium oxalate crystals. Cortex is broad and showing bulbous suberized cells on the outer margin. Bark is about 5-8 cells broad with crushed suberized parenchyma. Cambium and cork cambium are distinct. The parenchymatous cells of cortical region are arranged in radial rows and irregular in shape (Fig. 58). Phloem comprises of sieve tubes with compound sieve plates, companion
cells and phloem parenchyma. Fibres are absent. In some wood pieces the central core comprises a distinct pith with suberized parenchymatous cells and a surrounding ring of decomposed xylem in which crushed parenchyma and vessels are observed (Fig. 9).

In L.S. rays are few, distinct, heterogenous, non-storied, 2 to 6 cells broad with polygonal parenchyma. Inter-vascular pitting is alternate. Secondary wall thickening of the vessels varies from spiral to reticulate depending on the width of the vessels (Fig. 59). In L.S. of few pieces rosette crystals of calcium oxalate are present in medullary rays and vessels (Fig. 60).

In maceration vessels trachieds and fibres are present. The size of the vessels varies (Table 1). Drum or barrel shaped vessels are 169.4 μ broad and 169.4 μ long while the tapering and elongated narrow ones are 277.0 μ long and 15.4 μ broad.

**PEELINGS:**

These are same as in Amritsar sample in T.S. and maceration both.

(iv) **CALCUTTA SAMPLE** : (Plates 31-32; Figs. 61-63)

In T.S. number of vessels varies from 300 to 560 per sq.mm. Broad vessels are solitary or sometimes in the form of tangential
clusters of 2-5 and in some 4-10 vessels, surrounded by a group of 2-8 radially arranged narrow vessels or trachieds (Fig. 61). Wood parenchyma apotracheal and confluent while in some other pieces it is paratracheal and the cells are cellulosic thin walled, polygonal or rectangular in shape. Medullary rays are distinct in some pieces with mucilagenous lysigenous cavities (Fig. 62) while it is not clear in few cases. Cambium and Cork cambium are distinct and the outer most layer of cork cambium become suberized and bulbous and comes out in the form of tiny outgrowths after the separation of crushed and decomposed outer cortex and bark. Cortical region is 15-20 cells broad and comprises of radially arranged irregular shaped parenchyma. Phloem made up of sieve tubes, compound sieve plates, companion cells and radially arranged phloem parenchyma. This region is also 12-20 cells broad. Pith is distinct and made up of crushed parenchymatous cells and a surrounding of old wylem with a few crushed vessels and suberized parenchyma. Rosette crystals of calcium oxalate are present in the lumen of the vessels.

In L.S. rays and heterogenous 3-5 and 4-9 cells broad. Secondary wall thickening also varies from spiral to reticulate. Intervascular pitting is alternate. Some trachieds and fibres are also present (Fig.63).

In maceration different types of vessels are present (Table 1) i.e. broad and short (123.2 μ 107.8 μ) with a terminal simple perforation plate and narrow and elongated (15.4 μ and 200.2 μ) with a simple complete or incomplete lateral or terminal perforation plate on both the ends.
PEELINGS:

T.S. and macerated peelings comprise of the similar structures as in Amritsar market sample.

(v) DELHI SAMPLE: (Plates 32-34; Figs. 64-67)

In T.S. the number of vessels varies from 500 to 600 per sq.mm. Xylem vessels are in a multiple group of broad and narrow vessels. In most of the pieces the broad vessels are arranged in tangential uniseriate clusters while in some these are solitary or in a group of 2-3 (Fig. 64). The narrow vessels are arranged in radial rows. Wood parenchyma paratracheal and confluent. Medullary rays are radiating and broad with some lysigenous cavities filled with mucilage. The ray parenchyma is mostly irregular or polygonal in shape while is some pieces it is rectangular also with mucilage (Fig. 65). Cambium and cork cambium are distinct. Phloem is made up of sieve tubes, compound sieve plates, companion cells and parenchyma which contains mucilage in some pieces of the wood (Fig. 64). Cortical region is very broad. The outermost layer of cork cambium bulging outwardly and are thick walled. Outermost 10-15 layers are suberized and in crushed form. Pith is not present but the central core comprises of a compact region of narrow vessels alongwith crushed suberized parenchyma.
In L.S. rays are distinct, broad, heterogenous. The secondary wall thickening of the vessels varies from spiral to reticulate. Intervascular pitting is alternate (Figs. 66 & 67).

In maceration the varying size of the vessels and few trachieds are present (Table 1). The broad and drum shaped vessels measures upto 138.6 μ wide and 107.8 μ long. While the elongated and narrow vessels are 215.6 μ long and 15.4 μ broad (Table 1). In few pieces mucilagenous cells are present.

**PEELINGS** :

T.S. and macerated peelings are same as in Amritsar market sample.

(vi) **HYDERABAD SAMPLE** : (Plates 34-36; Figs. 68-71)

In T.S. the number of vessels varies from 500 to 760 per sq.mm. In some pieces broad vessels are mostly solitary or in a group of 2-3 while in others they are in tangential clusters. The narrow vessels are radially arranged and surrounds the cluster of the broad vessels and form a group of radially arranged vessels towards the centre (Fig. 68). Rosette crystals of calcium oxalate are present in few vessels. Cambium is distinct and in some wavy in outline and comprises of clusters of broad vessels in its furrows (Fig. 69). In most of the pieces medullary rays
with lysigenous cavities are present. These cavities are also present in wood parenchyma and having mucilagenous contents. Phloem region is broad and comprises of sieve tubes with a compound sieve plate, companion cells and radially arranged phloem parenchyma. Inner cortex is 5-8 cells broad and the 8-10 layers of outer cortex are suberized and in decomposed form. Cork cambium is distinct and having bulbous thick walled parenchyma as its outermost layer. Bark is about 8-10 cells broad and readily separated down in the form of papery layers from the bulbous margin. Pith is present in some pieces while in the others central core comprises of consecutive rings of vessels and crushed parenchyma with an inner most zone of compact crushed vessels and parenchyma. This central core is dark brown in colour.

In L.S. rays are heterogenous, 3-5 and 8-10 cells broad (Fig.70). Secondary wall thickening of the vessels varies from annular to reticulate. Trachieds are vasicentric and few in numbers. Fibres are very few in some pieces.

In maceration the size and shape of the vessels varies (Table 1), measure upto 200.2 μ and 154.0 μ while the narrow and elongated upto 15.4 μ and 200.2 μ. Trachieds with bordered pits are observed the broad and short vessels (Fig. 71).

PEELINGS :

T.S. and macerated peelings are same as in Amritsar market sample.
In T.S. the number of vessels varies from 415 to 615 per sq.mm. The vessels are arranged in a multiple group of broad and narrow vessels. Mostly the broad vessels are in oblique tangential clusters and surrounded by radially arranged narrow vessels (Fig. 72). In few pieces the broad vessels are solitary. The xylem parenchyma is paratracheal and confluent comprises of irregular shaped, cellulosic thin walled cells. Medullary rays are distinct and 5-8 cells broad alongwith mucilage containing lysigenous cavities at some places Cambium and cork cambium are present. Phloem 20-30 cells broad and made up of sieve tubes, compound sieve plates, companion cells and phloem parenchyma. Secondary cortex is 15 -20 cells broad and the cellulosic thin walled, somewhat rectangular parenchymatous cells are arranged in radial rows. The cells of the outer most layer of cork cambium become bulbous and suberized at maturation and separated out from the bark which comprises of 8-10 layers of crushed parenchymatous cells. The central core of the wood is dark brown in colour and made up of consecutive rings of vessels and crushed parenchyma (Fig. 73). In few pieces pith is present.

In L.S. rays are heterogenous and 4-6 and 9-11 cells broad. Rosette crystals of calcium oxalate are present in the lumen of the vessels. Secondary wall thickening of the vessels varies from annular to reticulate. Inter vascular pitting is alternate. Few fibres and trachieds are also present (Fig. 74).
In maceration the vessels are of different types (Table 1). The broad and short are measured 138.6 μ and 107.8 μ long. While the narrow and elongated ones are 215.6 μ long and 15.4 μ broad.

PEELINGS:

T.S. and macerated peelings are same as in Amritsar market sample.

(viii) LUCKNOW SAMPLE: (Plate 38; Figs. 75-76)

In T.S. number of vessels varies from 515 to 570 per sq.mm. vessels are arranged in irregular clusters often in ulmiform tangential rows. In some pieces the broad vessels are mostly solitary or sometimes in a group of 2-4. Type of vessels varies narrow to broad. The narrow vessels are arranged in radial rows along with the broad ones (Fig. 73) in few pieces the narrow radially arranged vessels from groups towards the centre (Fig. 75). Rosette crystals of calcium oxalate are present in the vessels. Parenchyma apotracheal and in some paratracheal and confluent. Cambium and cork cambium are distinct. Cambium is wavy in outline and the vessel clusters are present in the furrows while the medullary rays with lysigenous cavities arise from the ridges. In some pieces medullary rays are not distinct and the outline of the cambium is slightly wavy. Secondary phloem is made up of sieve tube.
with compound sieve plates, companion cells and phloem parenchyma which is irregular in shape and arranged radially. Cortical region is not very broad. Inner cortex comprises of 8-10 layers of radially arranged cellulosic parenchyma and the outer cortex is in crushed form with suberized cells. At maturation the outer most layer of cork cambium become suberized and bulges out in the form of tiny outgrowths and bark is separated from these bulbous cells in the form of papery reddish brown layers. In some wood pieces pith is made up of large polygonal or somewhat rounded parenchymatous tissue (Fig. 75) which become suberized at maturation and present in decomposed form while in others the central core comprises of various zone of crushed wood parenchyma and vessels in the form of the dark brown coloured consecutive rings.

In L.S. rays are distinct heterogenous and 3-6 or 8-11 cells broad. Secondary wall thickening of the vessels varies from annular to reticulate. Trachieds are vasicentric and fibres are very few. Intervascular pitting is alternate (Fig. 76).

In maceration the size of the vessels differ (Table 1) from broad and short (169.4 μ and 107.8 μ) to narrow and elongated (15.4 μ and 300.8 μ) with tapering ends.

PEELINGS:

Macerated and T.S. peelings are same as in Amritsar market sample.
### TABLE 1: MEASUREMENTS OF TRACHEARY ELEMENTS OF 'RATANJOT'.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>LENGTH (in µ)</th>
<th>WIDTH (in µ)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. GENUINE</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Arnebia benthamii</em></td>
<td>77.0</td>
<td>215.6</td>
</tr>
<tr>
<td><em>Arnebia euchroma</em> (H.P.)</td>
<td>77.0</td>
<td>308.0</td>
</tr>
<tr>
<td><em>Arnebia euchroma</em> (L.)</td>
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<td>338.0</td>
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<td><em>Arnebia nobilis</em></td>
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<td>184.8</td>
</tr>
<tr>
<td><em>Mahaangha emodi</em></td>
<td>77.0</td>
<td>215.6</td>
</tr>
<tr>
<td><em>Onosma hispidum</em></td>
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<td>231.0</td>
</tr>
<tr>
<td><strong>B. MARKET</strong></td>
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<td></td>
</tr>
<tr>
<td>Amritsar</td>
<td>46.2</td>
<td>200.2</td>
</tr>
<tr>
<td>Bhopal</td>
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<tr>
<td>Lucknow</td>
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</tbody>
</table>

*Note: Hundred readings were taken for each sample.*
FIGURE 3: T.S. of Arnebia nobilis (under dissecting microscope).

FIGURE 4: Maharanga emodi
FIGURE - 9: T.S. of Lucknow Sample (under dissecting microscope).

FIGURE - 10: T.S. Root of Arnebia benthami.
FIGURE - 11: T.S. Root of *Arnebia benthamii* (crushed xylem).

FIGURE - 12: T.S. Root of *Arnebia benthamii* (cortical region with bark).

FIGURE - 14: Macerated woody portions of Arnebia benthamii.
FIGURE - 15: Peeling of Root of Arnebia benthamii.  
(Polygonal cells with coloured content).

FIGURE - 16: Peeling of Root of Arnebia benthamii.  
(Elongated cells without coloured content).
FIGURE - 17: T.S. mature Root of Arnebia euchroma.

FIGURE - 18: T.S. young Root of Arnebia euchroma.
FIGURE -19: T.S. Root of *Arnebia euchroma* (outer portion).

FIGURE - 20: L.S. Root of *Arnebia euchroma*. 
FIGURE - 21: Macerated Root of *Arnebia euchroma* (Laddakh sample).

FIGURE - 22: Macerated Root of *Arnebia euchroma* (H.P. Sample)
FIGURE 23: T.S. Peeling of Arnebia euchroma.

FIGURE 24: Peeling of Arnebia euchroma with vessels.
FIGURE 25: T.S. Root of *Arnebia nobilis* (inner portion).

FIGURE 26: T.S. Root of *Arnebia nobilis* (middle portion).

FIGURE - 28: L.S. Root of *Arnebia nobilis*. 
FIGURE - 29: Macerated wood of *Arnebia nobilis*.

FIGURE - 30: Macerated Peeling of *Arnebia nobilis*. 
FIGURE 31: Macerated Peeling of *Arnebia nobilis*.

FIGURE 32: T.S. Root of *Maharanga emodi*.
FIGURE - 33: T.S. Root of Maharanga emodi.

FIGURE - 34: T.S. Root of Maharanga emodi.
FIGURE - 35: L.S. Root of *Maharanga emodi*.

FIGURE - 36: L.S. Root of *Maharanga emodi*. 
FIG. 37: Macerated wood of *Maharanza emodi*.

FIG. 38: T.S. Root of *Onosma hispidum*.
FIGURE 39: T.S. Root of *Onosma hispidum*.

FIGURE 40: T.S. Root of *Onosma hispidum*. 
FIGURE 41: T.S. Root of Onosma hispidum.

FIGURE 42: L.S. Root of Onosma hispidum.
FIG. 43: Macerated Root of Onosma hispidum.

FIG. 44: T.S. wood of Amritsar sample.
FIGURE 45: T.S. wood of Amritsar sample.

FIGURE 46: T.S. Cortical region of Amritsar sample.
FIGURE - 47: L.S. wood of Amritsar sample.

FIGURE - 48: Macerated wood of Amritsar sample.
FIGURE - 49: Macerated Peeling of Amritsar sample (Types-1 & 2).

FIGURE - 50: Macerated Peeling of Amritsar sample (Type-3).
FIGURE 51: T.S. wood of Bhopal sample.

FIGURE 52: T.S. wood of Bhopal sample.
FIGURE - 53: T.S. wood of Bhopal sample.

FIGURE - 54: L.S. wood of Bhopal sample.
FIGURE 55: L.S. wood of Bhopal sample.

FIGURE 56: T.S. wood of Bombay sample.
FIGURE 57: T.S. wood of Bombay sample.

FIGURE 58: T.S. wood of Bombay sample.
FIGURE 59: L.S. wood of Bombay sample.

FIGURE 60: L.S. wood of Bombay sample.
FIGURE 63: T.S. wood of Calcutta sample.

FIGURE 64: T.S. wood of Delhi sample.
FIGURE 65: T.S. wood of Delhi sample.

FIGURE 66: L.S. wood of Delhi sample.
FIGURE 67: L.S. wood of Delhi sample.

FIGURE 68: T.S. wood of Hyderabad sample.
FIGURE 69: T.S. wood of Hyderabad sample.

FIGURE 70: L.S. wood of Hyderabad sample.
FIGURE - 71: Macerated wood of Hyderabad sample.

FIGURE 73: T.S. wood of Jammu sample.

FIGURE 74: L.S. wood of Jammu sample.
FIGURE 75: T.S. wood of Lucknow sample.

FIGURE 76: L.S. wood of Lucknow sample.
CHAPTER II

SEM STUDIES
PREPARATION OF THE MATERIAL

Dried radical leaves were slightly warmed with 1% aqueous sodium hydroxide solution for 15 minutes. The alkali solution was thoroughly washed with distilled water and the leaves remained as such in the distilled water for two hours and then dehydrated after passing through a series of different strength (30, 50, 70, 90 and 100%) of alcohol and finally with amyl acetate. These dehydrated samples were carried out for Critical Point drying by using Balzers Union (Type 11120 A) in presence of liquid carbon dioxide for half an hour. The leaves were cut into small pieces and hairs were removed from the pieces with the help of cellotape in order to get clear cuticular observations. Then pieces were coated with gold pelladium-twice, each for five minutes with SEM coating unit E 5000. Thereafter they were scanned under the Philips SEM 515 and photographs were taken.

OBSERVATIONS

BOTANICALLY AUTHENTICATED SAMPLES

1. ARNEBIA BENTHAMII (Plates 39-43; Figs. 77-85)

Adaxial surface

Hairy (Fig. 77); hairs unicellular, elongated with surface rugose, rugae irregular in shapes and sizes (Fig. 78), the hair bases broad, comprised of two tube like arms and forming somewhat triangular depression at the base (Fig. 80), apices acute (Fig. 79). Epidermal
cells polygonal and elongated, intercellular partitions raised and striated; stomata sparsed, paracytic type and the guard cells densely covered with wax (Fig. 81). Tertiary sculpture crystose.

**Abaxial surface**

Moderately hairy; hairs elongated, somewhat twisted, unicellular and ribbon shaped with acute apices (Fig. 82), hair surface ridged, ridges irregular in shape, mostly longitudinally elongated with smooth margins (Fig. 83); hair bases multicellular with a constricted joint, basal cells irregular in shape and size with raised intercellular partitions and depressed fine folded general surface (Fig. 84). Epidermal cells hexagonal or sometimes pentagonal with fine striations, surface deeply depressed and cell boundaries raised (Fig. 85). Tertiary sculpture crystose.

2. *ARNEBIA EUCHROMA* (Himachal Pradesh): (Plates 43-46; Figs. 86-92)

**Adaxial surface**

Hairs scanty (Fig. 86), cylindrical and two to three celled, mostly hook shaped with acute apices (Fig. 87), hair surface ridged, ridges slightly wavy, longitudinally elongated (Fig. 88), lower most cells of the hair comprised of a median suture started from the condyle shaped bases (Fig. 87). Epidermal cells smooth, elongated, rectangular, intercellular partitions depressed (Fig. 89), Stomata scanty and anomocytic. Tertiary sculpture crystose.
Abaxial surface

Surface hairy; hairs unicellular, twisted or hook shaped, varies in size with acute apices (Fig. 90), hair surface loosely folded forming interlocking arrangement (Fig. 91); hair bases broad centrally depressed with fine folds. Epidermal cells rectangular, surface slightly raised (Fig. 92). Tertiary sculpture crystose.

ARNEBIA EUCHROMA (Laddakh) (Plates 47-49; Figs.93-98)

Adaxial surface

Hairs scanty (Fig. 93); unicellular with fine folded surface (Fig. 94), and acute apices, hair bases unicellular broad with a deep depression in the middle (Fig. 93). Epidermal cells elongated and of various sizes, surface tracheal shape, intercellular partitions grooved (Fig. 95); stomata scanty and diacytic.

Abaxial surface

Densely hairy; hairs unicellular elongated, twisted, flattened and of various sizes with acute apices (Fig. 96), hair surface strongly ridged (Fig. 97); hair bases twisted and centrally depressed with folded margins and surface frilled (Fig. 98). Epidermal cells similar to adaxial surface.
4. **Arnebia nobilis** (Plates 50-55; Figs. 99-109)

**Adaxial surface**

Moderately hairy (Fig. 99); two type of hairs with acute apices; (i) hair surface warty, but general surface of the hairs covered with fine lines (Fig. 100), hair bases bulged with semilunar fine folds (Fig. 101). (ii) However, the basal portions of the hairs covered with smooth warty blobs (Fig. 102), but the warts are gradually disappearing forming smooth fine ridged surface (Fig. 103), hair bases saucer shaped with a 'U' shaped slight depression (Fig. 102).

Epidermal cells mostly hexagonal with raised intercellular partitions (Fig. 104); stomata diacytic or paracytic, (Fig. 105). Tertiary sculpture crystose type.

**Abaxial surface**

Densely hairy; hairs irregular in shapes and sizes with acute apices (Fig. 106); hair surface smooth and covered with flakes of tertiary sculpture (Fig. 107); hair bases twisted, flat and narrow with deep depression (Fig. 108). Epidermal cells elongated, rectangular with raised swollen intercellular partitions, general surface depressed or sometimes slit like (Fig. 109). Tertiary sculpture varies.
5. **MAHARANGA EMODI** (Plates 55-58; Figs. 110-116)

**Adaxial surface**

Sparsely hairy, two types of hairs (Fig. 110) with acute apices;

i) **Small**:

Hair surface warty (Fig. 111) warts smooth somewhat oval shaped and of various sizes, remaining surface smooth (Fig. 112), hair bases broad, cup shaped with lateral depression (Fig. 111).

ii) **Large and Broad**

Hair surface smooth, hair bases broad, multicelled (Fig. 113), arranged digitally (Fig. 110). Margins of epidermal cells not cleared, at some places margins sinuous (Fig. 110). Stomata anomocytic and scanty.

**Abaxial surface**

Sparsely hairy, unicellular hairs with acute apices (Fig. 114), surface covered with oblique, long and fine lines (Fig. 115), hair bases saucer shaped surrounded by a ring of epidermal cells (Fig. 114). Epidermal cells mostly rectangular or pentagonal with convex surfaces and raised cell margins (Fig. 116). Tertiary sculpture granular.
6. **Onosma hispidum** (Plates 59-63; Figs. 117-125)

**Adaxial surface**

Moderately hairy, two types of hairs with acute apices:

(i) **Small**

Hair surface smooth, hair bases unicellular and broad (Fig. 117).

(ii) **Large and Broad**

Hair varies in sizes, surface warty and remaining surface covered with fine lines (fig. 118) or smooth (Fig. 119 & 120), hair bases multicellular and the basal cells arranged in cystolith like fashion (Fig. 121).

Epidermal cells elongated and of various sizes, intercellular partitions raised and the general surface folded (Fig. 122), stomata paracytic, flush and scanty. Tertiary sculpture crystose.

**Abaxial surface**

Sparsely hairy; marginal hairs collapsed type (Fig. 123), hairs on the middle surface ridged, ridges longitudinal and irregular in shape (Fig. 124), hair bases branched root like with a fine central minute (Fig. 125). Epidermal cells irregular in shapes and sizes, intercellular partitions raised and surface concaved (Fig. 125). Tertiary sculpture crystose.
B. MARKET SAMPLES

1. AMRITSAR SAMPLE (Plates 50-55; Figs. 99-109)

Moderately hairy (Fig. 99), two types of hairs, with acute apices: (i) Hair surface covered with fine lines along with some warts, hair bases flat (Fig. 100). (ii) Smooth warts on the basal portion and converted rugulose type towards the apices, hair bases saucer shaped (Fig. 102).

Epidermal cells elongated, somewhat hexagonal with raised intercellular partitions (Fig. 104). Stomata raised, diacytic or paracytic. Densely covered with crystose tertiary sculpture (Fig. 105).

**Abaxial surface**

Densely hairy, hairs irregular in shapes and sizes with acute apices (Fig. 106), hair surface smooth or sometimes covered with fine lines and flakes of tertiary sculpture (Fig. 107), hair bases twisted with a deep depression (Fig. 108). Epidermal cells elongated, intercellular partitions raised and swollen, general surface depressed or sometimes only a slit like and covered with fine ridges (Fig. 109).

2. BHOPAL SAMPLE

Two types of leaves:

(a) Moderately hairy, two types of hairs: (i) short, smooth with unicellular hair bases (Fig. 117) and (ii) elongated… broad,
wartgy (Fig. 118) or smooth (Fig. 119) with multicellular hair bases (Fig. 121). Epidermal cells elongated and of various sizes, intercellular partitions raised, general surface folded (Fig. 122). Stomata paracytic flush and scanty. Tertiary sculpture crystose.

(b) Moderately hairy, two types of hairs: (i) Small, hook shaped, warty with unicellular hair bases and (ii) large, elongated, warty, remaining surface covered with fine folds, hair bases broad and three armed (Fig. 126). Epidermal cells thick walled, mostly short, pentagonal or sometimes elongated with depressed intercellular partitions, general surface centrally depressed with fine cracks. Stomata anomocytic, flush and scanty (Fig. 127).

Abaxial surface

(a) Densely hairy, marginal hairs collapsed type (Fig. 123), hairs on middle surface ridged, ridges irregular in shape (Fig. 124), hair bases broad branched root like with a central suture (Fig. 125). Epidermal cells irregular in shapes and sizes, intercellular partitions raised and general surface concaved (Fig. 125). Tertiary sculpture crystose.

(b) Densely hairy (Fig. 128), hairs unicellular, smooth with fine oblique depressions (Fig. 129), irregular in shape, hair bases bend with a triangular deep depression (Fig. 130). Epidermal cells irregular with ridged surface (Fig. 131). Tertiary sculpture granular.
3. **BOMBAY SAMPLE** : Three types of leaf surfaces:

**Adaxial surface**

(a) Moderately hairy (Fig. 99), two types of hairs (i) warty, warts smooth, remaining hair surface ridged (Fig. 100), hair bases bulged with semilunar fine folds (Fig. 101), acute apices (ii) warty blobs present only on the basal portion of the hairs and warts gradually disappearing and forming rugulate type towards the acute apices (Fig. 103), hair bases saucer shaped with 'U' shaped slight depression. Epidermal cells mostly hexagonal with raised intercellular partitions (Fig. 104), stomata diacytic or paracytic, densely covered with crystose tertiary sculpture (Fig. 105).

(b) Moderately hairy, two types of hairs: (i) smooth, short with unicellular hair bases (Fig. 117) and (ii) warty (Fig. 110) or smooth (Fig. 119), broad with cystolith like arranged multicellular hair bases. Epidermal cells elongated, irregular with raised intercellular partitions and folded general surface. Stomata flush and scanty.

(c) Sparsely hairy, two types of hairs: (i) small hook shaped with unicellular hair bases and (ii) large, elongated broad with three armed bases. Hair surface warty with fine folds (Fig. 126). Epidermal cells pentagonal, thick walled with depressed intercellular partitions and shrink general surface. Stomata anomocytic, flush and scanty (Fig. 127).
Abaxial surface

Densely or moderately hairy, hairs smooth and in some leaves finely ridged, unicellular, cylindrical, irregular in sizes (Fig.106), tertiary sculpture crystose or in the form of flakes (Fig. 107), hair bases twisted, flat, narrow, with a deep depression (Fig. 108) or in some leaves branched hair bases with a suture at the joint (Fig.125), while in others triangular or somewhat spoon-like (Fig. 130). Epidermal cells irregular in shapes and sizes, intercellular partitions raised and general surface concaved or depressed, sometimes only a slit like. Tertiary sculpture varies.

4. CALCUTTA, JAMMU AND LUCKNOW SAMPLE

Two types of leaves in all the three samples:

Adaxial surface

(a) Sparsely hairy (Fig. 99), hairs warty, remaining hair surface covered with finelines (Fig. 100), hair bases flat with few semilunar folds (Fig. 101) or saucer shaped with a 'U' shaped slight depression (Fig. 102). Epidermal cells hexagonal, with raised intercellular partitions (Fig. 104); stomata diacytic, densely covered with crystose tertiary sculpture (Fig. 105).

(b) Moderately hairy, two types of hairs: (i) Smaller ones smooth with unicellular hair bases (Fig. 117) and (ii) Board hairs
warty (Fig. 118) or smooth (Fig. 119) with multicellular cystolith-like arranged hair bases (Fig. 121). Epidermal cells elongated, intercellular partitions raised and the general surface folded, stomata flush and scanty (Fig. 122). Tertiary sculpture crystose.

**Abaxial surface**

(a) Densely hairy, hairs irregular in shape and sizes with acute apices (Fig. 106), hair surface smooth and covered with flakes of tertiary sculpture (Fig. 107), hair bases twisted, flat, narrow with a deep depression (Fig. 108). Epidermal cells elongated rectangular with raised intercellular partitions, general surface depressed or sometimes only a slit like (Fig. 109).

(b) Sparsely hairy, marginal hairs collapsed type (Fig. 123), middle hairs ridged, hair bases branched root like with a fine central suture. Epidermal cells irregular and covered with crystose tertiary sculpture (Fig. 125); stomata paracytic, flush and scanty.

5. **DELHI SAMPLE**: Two types of leaves:

**Adaxial surface**

(a) Sparsely hairy, two types of hairs (Fig. 110); (i) small hairs warty, warts smooth somewhat oval shaped, hair bases broad, cup shaped and laterally depressed (Fig. 111) and (ii) Broad hairs
smooth with digitally arranged, multicellular hair bases (Fig.110). Margins of epidermal cells not cleared; stomata anomocytic.

(b) Moderately hairy, two types of hairs: (i) small hairs with smooth surface and unicellular hair bases (Fig. 117) and (ii) broad and large hairs with warty surface (Fig. 118), hair bases broad and multicellular with cystolith like arrangements (Fig.121). Epidermal cells elongated, intercellular partitions raised and the general surface folded; stomata paracytic flush and scanty. Tertiary sculpture crystose (Fig. 122).

**Abaxial surface**

(a) Hairs scanty, hair surface covered with oblique long and fine lines, hair bases saucer shaped with a surrounding ring of epidermal cells. Epidermal cells mostly pentagonal with convex surfaces. Tertiary sculpture granular (Fig. 114).

(b) Sparsely hairy, hair surface ridged (Fig. 124), hair bases branched root like with a suture (Fig. 125). Epidermal cells irregular in shapes and sizes, intercellular partitions raised and general surface concaved. Tertiary sculpture crystose.

6. **HYDERABAD SAMPLE** : Two types of leaves:

**Adaxial surface**

(a) Sparsely hairy (Fig. 99), two types of hairs with acute
apices: (i) Warty, general surface covered with fine lines (Fig. 100), hair bases flat with few semilunar fine folds (Fig. 101) and (ii) warty blobs present just above the bases, warts disappearing and forming rugulose type towards the acute apices, hair bases saucer shaped (Fig. 102). Epidermal cells mostly hexagonal, with raised intercellular partitions, stomata diacytic or paracytic, densely covered with crystose tertiary sculpture (Fig. 104 & 105).

(b) Sparsely hairy, hair surface ridged (Fig. 132), hair bases flat. Epidermal cells irregular in shape (Fig. 133). Stomata paracytic, guard cells comprised of concentric rings of striae and also extended wings on lateral sides (Fig. 134).

**Abaxial surface**

(a) Densely hairy (Fig. 106), hair surface smooth and covered with flakes of tertiary sculpture (Fig. 107), hair bases twisted, flat, narrow with a deep depression (Fig. 108). Epidermal cells rectangular with raised swollen intercellular partitions (Fig. 109). Tertiary sculpture varies.

(b) Moderately hairy, hair surface ridged, ridges fine and short (Fig. 135), hair bases flat with a triangular depression towards the hair surface. Epidermal cells thick walled irregular in shape, intercellular partitions raised (Fig. 136). Tertiary sculpture crystose.
### TABLE 2: COMPARATIVE CHARACTERS OF HAIRS (REDICLE LEAVES) OF 'RATANJOT' 

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* Any other plant species  
CA = Cystolith like arrangement,  
DA = Digitally arranged.
FIGURE - 77: Adaxial surface of leaf of *Arnebia benthamii*.

FIGURE - 78: Hair surface of Adaxial surface (*Arnebia benthamii*).
**FIGURE - 79**: Acute apex of hair (*Arnebia benthamii*).

**FIGURE - 80**: Hair base of Adaxial surface (*Arnebia benthamii*).
FIGURE 81: Epidermal cells with stomata of Adaxial surface (Arnebia benthamii).

FIGURE 82: Abaxial surface of leaf of Arnebia benthamii.
FIGURE - 83: Abaxial surface - Hair surface (Arnebia benthamii).
FIGURE - 84: Abaxial surface - Hair base (Arnebia benthamii).
FIGURE 85: Abaxial surface - Epidermal cells (Arnebia benthamii).

FIGURE 86: Adaxial surface of leaf of Arnebia euchroma (H.P.).
FIGURE 87: Adaxial surface with hair type of *Arnebia euchroma* (H.P.)

FIGURE 88: Adaxial surface - Hair surface (*Arnebia euchroma*) (H.P.)
FIGURE - 89: Adaxial surface-Epidermal cells (*Arnebia euchroma*) (H.P.).

FIGURE - 90: Abaxial surface of leaf of *Arnebia euchroma* (H.P.).
FIGURE 91: Abaxial surface - Hair surface (*Arnebia euchroma*) (H.P.)

FIGURE 92: Abaxial surface - Hair base (*Arnebia euchroma*) (H.P.)
FIGURE 93: Adaxial surface of leaf of *Arnebia euchroma* (L.).

FIGURE 94: Adaxial surface-Hair surface (*Arnebia euchroma*) (L.).
FIGURE 95: Adaxial surface-Epidermal cells (Arnebia euchroma) (L.).

FIGURE 96: Abaxial surface of leaf of Arnebia euchroma (L.).
FIGURE 97: Abaxial surface-Hair surface *Arnebia euchroma* (L.).

FIGURE 98: Abaxial surface-Hair bases *Arnebia euchroma* (L.).
FIGURE 99: Adaxial surface of leaf of *Arnebia nobilis*.

FIGURE 100: Adaxial surface-Hair surface (*Arnebia nobilis*).
Figure 101: Adaxial surface-Hair base (*Arnebia nobilis*).

Figure 102: Adaxial surface-Hair base (*Arnebia nobilis*).
FIGURE 103: Adaxial surface-Hair surface (Arnebia nobilis).

FIGURE 104: Adaxial surface-Epidermal cells (Arnebia nobilis).
FIGURE 105: Epidermal cells with stomata (*Arnebia nobilis*).

FIGURE 106: Abaxial surface of leaf of *Arnebia nobilis*. 
FIGURE 107: Abaxial surface-Hair surface (*Arnebia nobilis*).

FIGURE 108: Abaxial surface-Hair bases (*Arnebia nobilis*).
FIGURE - 109: Abaxial surface - Epidermal cells (Arnebia nobilis).

FIGURE - 110: Abaxial surface of leaf of Maharanga emodi.
FIGURE 111: Adaxial surface-Hair surface (*Maharanga emodi*).

FIGURE 112: Warts on the hair surface (*Maharanga emodi*).
FIGURE 113: Adaxial surface—Hair bases (Maharanca emodi).

FIGURE 114: Abaxial surface of leaf of Maharanca emodi.
FIGURE 115: Abaxial surface-Hair surface *Maharanga emodi*.

FIGURE 116: Abaxial surface-Epidermal cells *Maharanga emodi*.
FIG. 117: Adaxial surface-Hairs dimorphic (Onosma hispidum).

FIG. 118: Adaxial surface-Hair surface (Onosma hispidum).
FIGURE 119: Adaxial leaf surface of *Onosma hispidum*.

FIGURE 120: Adaxial surface; hair surface (*Onosma hispidum*).
FIGURE 121: Adaxial surface-hair base (Onosma hispidum).

FIGURE 122: Adaxial surface-Epidermal cells (Onosma hispidum).
FIGURE 123: Abaxial surface—Marginal hairs (Onosma hispidum).

FIGURE 124: Abaxial surface—Hair surface (Onosma hispidum).
FIGURE - 125: Abaxial surface of leaf of *Onosma hispidum*.

FIGURE - 126: Adaxial surface-Hair dimorphic (Bhopal Sample).
FIGURE - 127: Adaxial surface-Epidermal & Cells & Stomata (Bhopal Sample).

FIGURE - 128: Abaxial surface-Hair surface (Bhopal Sample).
FIGURE - 129: Abaxial surface-Hair surface (Bhopal Sample).

FIGURE - 130: Abaxial surface-Hair base (Bhopal Sample).
FIGURE 131: Abaxial surface-Epidermal cells (bhopal Sample).

FIGURE 132: Adaxial surface-Hair surface (Hyderabad Sample).
FIGURE 133: Adaxial surface—Epidermal cells (Hyderabad Sample).

FIGURE 134: Aăxial surface—Stomata (Hyderabad Sample).
FIGURE 135: Abaxial surface-Hair surface (Hyderabad Sample).

FIGURE 136: Abaxial surface-Epidermal cells (Hyderabad Sample).
CHAPTER III

POWDER STUDIES
Quantitative analysis

Physico-chemical characters such as ash values, tannins, sugars, phenolics, napthaquinones and percentage extractive (I.P. method) were determined from air dried material according to the following methods, and results are recorded in Table 3 & 4.

1. Determination of Percentage Extractives:

For determination of percentage extractives of water and alcohol, about 2 gm of the air dried powdered material was macerated with 100 ml of desired solvents of specified strength in a closed flask for about 24 hours. The mixture was vigorously shaken at intervals. After 24 hours the solution was rapidly filtered without any loss of solvent. Then from the filterate about 20 ml of the solution was evaporated in a flat bottomed shallow disc at 100°C till it is completely dried and weighed. The percentage of each extractive was calculated with reference to the air dried material. (Indian Pharmacopoeia, 1966, 948 A, B)

ASH VALUES

TOTAL ASH

About 2 gm of accurately weighed powdered material was taken in a silica crucible previously ignited and weighed. The ground material was scattered in fine, even layer on the bottom of the
The crucible was placed in a muffle furnace at a temperature of 600°C for about 4 hours, then cooled and weighed. The percentage of the total carbon free ash was calculated with reference to the air dried material.

**WATER SOLUBLE ASH**

Carbon free ash was boiled with distilled water in a crucible for 5 minutes, filtered through the ashless filter paper, ignited and weighed. The percentage of water soluble ash was calculated with reference to the air dried material.

**ACID INSOLUBLE ASH**

Carbon free ash was boiled with 25 ml of dilute hydrochloric acid (10%) in a crucible for 5 minutes, filtered through ashless filter paper, washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried material (Indian Pharmacopoeia, 1966, 947).

**ESTIMATIONS OF TANNINS SUGARS AND TOTAL PHENOLICS**

2 gm of the powdered material was extracted with 100 ml of distilled water. After filtration, saturated lead acetate solution (prepared in 50% alcohol) was added to the filtrate, to precipitate the tannin in the form of lead tannate and then sodium carbonate or potassium oxalate was added to the filtrate to remove excess lead acetate and filtered. The filtrate was carried out for the estimation of sugars.
**TOTAL PHENOLICS**

The precipitate of lead tannate was washed with water thoroughly, dried, powdered and suspended in the alcohol. It was decomposed by passing hydrogen sulphide gas for half an hour. The excess of gas was removed by boiling the solution and filtered. The clear solution was concentrated under reduced pressure. The residue was weighed accurately in the form of total phenolics. The percentage of total phenolics was calculated with reference to the powdered material.

**TANNINS**

The above residue was dissolved in 50 ml of water and precipitated with 25 ml of 5% copper acetate solution. The precipitate was washed thoroughly, dried, incinerated and weighed as copper oxide. The tannin content was calculated by multiplying the weight of copper oxide obtained with 1.305.

**SUGARS** (Anonymous, 1965)

**Shaffer-Somogyi Micro Method**

a. **Shaffer-Somogyi Carbonate 50 Reagent, 5 gm Potassium Iodide**

25 gm of each of anhydrous sodium carbonate and potassium sodium tarterate (Rochelle salt) were dissolved in about 500 ml of
water in a two litre beaker and added through funnel with tip under surface, with stirring, 75 ml solution of 100 gm copper sulphate/litre. 20 gm sodium bicarbonate and 5 gm potassium iodide was added and dissolved. This solution was transferred to one litre volumetric flask and then added 250 ml of 0.001 N potassium iodate (3.567 gm. dissolved and diluted to 1 litre). Diluted it up to mark and stored overnight before use.

b. **Iodide-Oxalate Solution**

2.5 gm potassium iodide and 2.5 gm potassium oxalate were dissolved in water and diluted to 100 ml. This solution was prepared fresh weekly.

c. **Thiosulphate Standard Solution 0.005 M**

Prepared daily from standardized stock 0.1 N solution. (25 gm sodium thiosulphate was dissolved in 1 litre of water. Boiled gently for 5 minutes and transferred while hot to storage bottle, previously cleaned with hot sulphuric acid-potassium dichromate solution and rinsed with warm boiled water. This solution was stored in dark, cool place and it is notable that the unused portion is never return to stock bottle).
Standardization of Thiosulphate Solution

Accurately weighed 0.2-0.23 gm potassium dichromate (AR) (Sample dried at 100°C for two hours) and placed in g-S-I flask (or g-S flask) and mixed 2 gm potassium iodide which was dissolved in 80 ml of chlorine free water and added with swirling, 20 ml of 1.0 N-HCL and immediately placed in dark for 10 minutes and titrated with sodium thiosulphate solution, adding starch solution after most of iodine has been consumed.

\[
\text{Normalith} = \frac{\text{gm K}_2\text{Cr}_2\text{O}_7 \times 1000}{\text{ml Na}_2\text{S}_2\text{O}_3 \times 49.032}
\]

d. Starch Solution

About 2.5 gm soluble starch was rubbed with mercuric iodide in little water and dissolved in about 500 ml of boiling water.

DETERMINATION

5 ml reagent/a/ was added to the 5 ml solution containing 0.5 to 2.5 mg glucose into 25x200 mm test tube and mixed by swirling and also prepared a control by using 5 ml water and 5 ml reagent/a/. These tubes capped with bulb or funnel and placed in boiling water bath for 15 minutes and then the tubes were removed carefully without agitation and kept in running water for cooling for five minutes. The caps were removed and 2 ml of iodide oxalate solution and then 3 ml of 2N-sulphuric acid (56 ml/L) was added from the side of each
tube. Then mixed thoroughly to ensure that all cupric oxide is dissolved and kept stand in cold water bath for 5 minutes. This solution was titrated with 0.005 N-sodium thiosulphate using starch as an indicator d. After subtracting the titration of test solution from that of control and determined amount glucose in 5 ml solution from the table. Control determinations were made with known amounts of glucose and applied correction for any deviation from tabulated equivalents.

Shaffer Somogyi Dextrose-thiosulphate Equivalent:

\[
\text{mg glucose} = (0.1099) \left( \text{ml } 0.005 \text{ N - Na}_2\text{S}_2\text{O}_3 \right) + 0.048
\]

**ESTIMATION OF TOTAL NAPHTHAQUINONES**

**CALORIMETERIC METHOD**

A standard solution of Arnebin-1 (a naphthoquinone) was prepared by dissolving 5 mg compound in 25 ml of alcohol. Different dilution were made from this standard solution and noted optical density (O.D.) of each dilution at a wavelength, \( \lambda = 515 \text{ nm} \).

100 mg of powdered material was dissolved in 100 ml of alcohol and made three dilutions of each sample. Optical density of each dilution was taken at the same wavelength and total naphthoquinones were estimated with the help of standard curve plotted for Arnebin-1 (Concentration against optical density) (Figure 137).
TABLE 3: PHYSICO-CHEMICAL CONSTANTS OF 'RATANJOT' (All values: Mean ± SD)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Naphthaquinones (%)</th>
<th>Phenolics %</th>
<th>Tannins %</th>
<th>Sugars %</th>
<th>Total Ash %</th>
<th>Acid Insoluble Ash %</th>
<th>Water Soluble Ash %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GENUINE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arnebia benthamii</td>
<td>0.35±0.008</td>
<td>2.36±0.048</td>
<td>0.804±0.008</td>
<td>2.33±0.021</td>
<td>11.66±0.06</td>
<td>6.45±0.10</td>
<td>4.72±0.07</td>
</tr>
<tr>
<td>Arnebia euchroma (H.P.)</td>
<td>5.93±0.029</td>
<td>2.21±0.022</td>
<td>0.684±0.006</td>
<td>1.97±0.113</td>
<td>6.20±0.055</td>
<td>0.79±0.06</td>
<td>0.92±0.04</td>
</tr>
<tr>
<td>Arnebia euchroma (L.)</td>
<td>2.83±0.04</td>
<td>2.15±0.12</td>
<td>0.615±0.004</td>
<td>1.56±0.06</td>
<td>5.90±0.10</td>
<td>0.64±0.038</td>
<td>0.63±0.025</td>
</tr>
<tr>
<td>Arnebia nobilis</td>
<td>10.5±0.02</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Maharange emodi</td>
<td>0.25±0.01</td>
<td>1.98±0.05</td>
<td>0.476±0.009</td>
<td>2.36±0.03</td>
<td>9.045±0.10</td>
<td>1.785±0.038</td>
<td>3.995±0.10</td>
</tr>
<tr>
<td>Onosma hispidum</td>
<td>7.75±0.03</td>
<td>1.40±0.54</td>
<td>0.819±0.008</td>
<td>0.09±0.11</td>
<td>12.77±0.06</td>
<td>2.77±0.08</td>
<td>3.85±0.095</td>
</tr>
<tr>
<td><strong>MARKET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amritsar</td>
<td>12.8±0.231</td>
<td>1.35±0.024</td>
<td>1.81±0.245</td>
<td>0.115±0.005</td>
<td>10.746±0.021</td>
<td>0.703±0.013</td>
<td>0.241±0.016</td>
</tr>
<tr>
<td>Bhopal</td>
<td>7.4±0.23</td>
<td>1.68±0.023</td>
<td>1.675±0.011</td>
<td>0.114±0.003</td>
<td>10.086±0.015</td>
<td>1.865±0.017</td>
<td>2.346±0.024</td>
</tr>
<tr>
<td>Bombay</td>
<td>6.6±0.046</td>
<td>4.19±0.022</td>
<td>0.819±0.002</td>
<td>0.202±0.004</td>
<td>18.42±0.012</td>
<td>1.490±0.011</td>
<td>9.10±0.076</td>
</tr>
<tr>
<td>Calcutta</td>
<td>11.25±0.057</td>
<td>2.54±0.034</td>
<td>0.918±0.003</td>
<td>0.202±0.004</td>
<td>11.30±0.01</td>
<td>0.96±0.03</td>
<td>0.85±0.013</td>
</tr>
<tr>
<td>Delhi</td>
<td>6.4±0.058</td>
<td>1.80±0.011</td>
<td>0.892±0.007</td>
<td>1.98±0.034</td>
<td>10.80±0.03</td>
<td>2.56±0.019</td>
<td>3.42±0.02</td>
</tr>
<tr>
<td>Hyderabad</td>
<td>7.0±0.115</td>
<td>1.31±0.031</td>
<td>0.571±0.006</td>
<td>0.245±0.007</td>
<td>14.35±0.024</td>
<td>6.59±0.017</td>
<td>3.08±0.014</td>
</tr>
<tr>
<td>Jammu</td>
<td>13.9±0.057</td>
<td>2.38±0.022</td>
<td>0.951±0.007</td>
<td>0.292±0.007</td>
<td>11.004±0.016</td>
<td>0.926±0.013</td>
<td>7.785±0.02</td>
</tr>
</tbody>
</table>
| Lucknow             | 15.9±0.115          | 2.305±0.013 | 0.333±0.008 | 0.256±0.003 | 11.15±0.021  | 0.99±0.007          | .01
### TABLE 4:

PERCENTAGE OF ALCOHOL AND WATER EXTRACT (IP METHOD) OF 'RATANJOT'.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alcohol Extractive</th>
<th>Water Extractive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>GENUINE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arnebia benthamii</td>
<td>14.0</td>
<td>28.1</td>
</tr>
<tr>
<td></td>
<td>(13.8-14.6)</td>
<td>(27.9-28.3)</td>
</tr>
<tr>
<td>Arnebia euchroma (H.P.)</td>
<td>28.0</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>(27.2-29.0)</td>
<td>(21.8-22.3)</td>
</tr>
<tr>
<td>Arnebia euchroma (L.)</td>
<td>22.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>(21.8-22.2)</td>
<td>(19.5-21.0)</td>
</tr>
<tr>
<td>Maharanga emodi</td>
<td>6.0</td>
<td>38.2</td>
</tr>
<tr>
<td></td>
<td>(5.8 - 6.4)</td>
<td>(38.0-38.6)</td>
</tr>
<tr>
<td>Onosma hispidum</td>
<td>14.0</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>(13.7-14.3)</td>
<td>(12.0-12.3)</td>
</tr>
<tr>
<td><strong>MARKET</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amritsar</td>
<td>15.0</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>(14.5-15.6)</td>
<td>(7.8-8.5)</td>
</tr>
<tr>
<td>Bhopal</td>
<td>8.0</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>(7.9 - 8.1)</td>
<td>(9.8 - 10.3)</td>
</tr>
<tr>
<td>Bombay</td>
<td>22.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>(21.7-22.5)</td>
<td>(11.9-12.1)</td>
</tr>
<tr>
<td>Calcutta</td>
<td>13.2</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>(13.0-13.6)</td>
<td>(9.5-10.0)</td>
</tr>
<tr>
<td>Delhi</td>
<td>14.4</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>(14.2-14.5)</td>
<td>(22.3-22.5)</td>
</tr>
<tr>
<td>Hyderabad</td>
<td>16.1</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>(16.0-16.2)</td>
<td>(11.5-11.7)</td>
</tr>
<tr>
<td>Jammu</td>
<td>10.6</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>(10.5-10.8)</td>
<td>(9.7-10.1)</td>
</tr>
<tr>
<td>Lucknow</td>
<td>13.2</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>(13.0-13.3)</td>
<td>(10.1-10.3)</td>
</tr>
</tbody>
</table>
B. Qualitative Analysis

The study of powder was performed by the following methods:

The colour, odour and taste of powder were noted. A small quantity of the powder was treated with chloral hydrate and mounted in glycerine and slides were observed under the microscope.

Behaviour of the powdered drugs with different chemical reagents was studied and observations are recorded in tables 5-7. Following tests were performed for qualitative analysis.

TEST FOR SUGARS

1. Resorcinol Test for Ketoses

This is known as Selivonoff's test. A crystal of resorcinol was added to the extract with an equal volume of concentrated hydrochloric acid. A rose colour is produced if ketoses are present.

2. Modified Selivonoff's Test for Aldoses

For this test dilute hydrochloric acid was used instead of concentrated hydrochloric acid red colour indicated the presence of aldoses in the extract.
3. **TEST FOR PENTOSES**

Small quantity of extract was taken in a test tube and added 2-4 drops of concentrated hydrochloric acid and a little of phloroglucinol (1%). Formation of red colour on heating indicated the presence of pentoses.

4. **KELLER-KILIOMI TEST FOR DE-OXYSUGARS**

The extract was mixed with acetic acid containing a trace of ferric chloride and transferred to the surface of concentrated sulphuric acid at the junction of two liquid a reddish brown colour is produced which gradually become blue, shows the presence of de-oxysugars.

5. **TEST FOR CARBOHYDRATES**

In the extract of the powder, equal volume of Molisch's reagent was added. the presence of blue-violet colour indicated the presence of carbohydrates.

**TEST FOR PROTEINS**

i. Extract treated with Millon's reagent. White precipitate was obtained, it turned red on heating and indicated the presence of proteins.
ii. Extract treated with saturated picric acid. The brown or orange colour indicated the presence of proteins and peptides.

iii. **XANTHOPROTEIN TEST**

Extract was treated with concentrated nitric acid followed by an excess of ammonia. The presence of dark orange colour indicated the xanthoprotein.

iv. **BIURET TEST FOR PEPTIDES AND PROTEINS**

To the extract 1 ml of 10% sodium hydroxide followed by a drop of copper sulphate solution was added. A violet or pink colour is produced if peptides and proteins are present but negative with amino acids.

v. **TEST FOR CYSTEIN**

Extract treated with 40% sodium hydroxide and a drop or 2 of 10% lead acetate, development of black or brown colour indicated the presence of cystein.

vi. **TEST FOR TRYPTOPHANE**

The extract treated with acetic acid and concentrated sulphuric acid, a violet colour indicated the presence of tryptophane.
vii. **TEST FOR CARDENOLIDES**

10% sodium hydroxide solution and 0.3% sodium nitroprusside solution was added to the extract. Appearance of transient pinkish red colouration indicated the presence of cardenolides (Dan et al. 1978).

**TEST FOR STARCH**

The term starch represents a group of polysaccharides giving characteristic stains with aqueous iodine ranging from brown to blue (Peach and Tracey, Vol. II, 1955).

**TEST FOR OIL**

In the extract added sudan III and 50% alcohol. The presence of brown precipitate indicated the presence of oil.

**TEST FOR TANNINS**

The extract was treated with 5% alcoholic ferric chloride solution. Green colour indicated the presence of gallo-tannins while the brown colour for the tannins.

**TEST FOR ALKALOIDS**

A drop of extract is placed on the filter paper and then specific alkaloidal reagents (Wagner's, Mayer's, Dragendorff's reagent)
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Powder + Reagents</th>
<th>Arnebia benthamii (Himachal Pradesh)</th>
<th>Arnebia euchroma (Laddakh)</th>
<th>Arnebia nobilis</th>
<th>Maharanga emadi</th>
<th>Onosma hispidum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1N-NaOH in Methenol</td>
<td>Yellow</td>
<td>Blue</td>
<td>Purple</td>
<td>Bluish purple</td>
<td>Yellow</td>
</tr>
<tr>
<td>2.</td>
<td>1N-NaOH in Water</td>
<td>Yellow</td>
<td>Yellow with Blue at some places</td>
<td>Greenish yellow</td>
<td>Blue</td>
<td>Yellow</td>
</tr>
<tr>
<td>3.</td>
<td>50% KOH</td>
<td>Greenish yellow</td>
<td>Very light Green</td>
<td>Green with Yellow tinge</td>
<td>Yellowish Brown</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>4.</td>
<td>1N-HCl</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>5.</td>
<td>50% H₂SO₄</td>
<td>Light brown</td>
<td>Purple</td>
<td>Dark Brown</td>
<td>Reddish Brown</td>
<td>Light Brown</td>
</tr>
<tr>
<td>6.</td>
<td>50%HNO₃</td>
<td>Light Brown with Yellow tinge</td>
<td>Dark Brown with Yellow tinge</td>
<td>Brown with Orange tinge</td>
<td>Dark Brown</td>
<td>Brown with Yellow tinge</td>
</tr>
<tr>
<td>9.</td>
<td>Acetic Acid</td>
<td>No change</td>
<td>Dark pink</td>
<td>Pinkish Brown</td>
<td>Bright Red</td>
<td>No change</td>
</tr>
<tr>
<td>10.</td>
<td>Iodine water</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
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<tr>
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<td>Amritsar</td>
<td>Bhopal</td>
<td>Bombay</td>
<td>Calcutta</td>
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<tr>
<td>2. 1N-NaOH in water</td>
<td>Blue</td>
<td>Yellow with blue at some places</td>
<td>Blue &amp; Yellow</td>
<td>Blue &amp; Yellow</td>
<td>Yellowish Green</td>
<td>Blue &amp; Yellow</td>
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<tr>
<td>3. 50% KOH</td>
<td>Green with Yellow tinge</td>
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<td>Light yellow with Green tinge</td>
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TABLE -7.: COMPARATIVE, QUALITATIVE ANALYSIS OF ALL THE SAMPLES OF 'RATANJOT'.

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</tr>
</tbody>
</table>

I20
were added. The presence of white to creamy precipitate indicated the presence of alkaloids.

**STUDY OF THE POWDER**

The powder is reddish purple in colour, odourless and tasteless. Under the microscope it is characterized by the presence of numerous fragments of lignified xylem vessels, isolated or in groups with spiral to reticulate secondary wall thickenings. Trachieds with bordered pits are also present at some places. Patches of suberized cells are present, which are somewhat polygonal or elongated in surface view. The polygonal or somewhat rounded parenchymatous cells contain dark red colour substance. Portions of unlignified hairs with walls sometimes striated are observed. Bulbous hair bases surrounding parenchymatous cells are also present. (Fig. 138).
Parenchymatous cells

Crystals

Hairs A.

Tracheary Elements

D.

FIGURE-138
PART II

CHEMOTAXONOMIC STUDIES
PHOTOCHEMICAL EVALUATION

This study divided into two parts: (1) deals with the preliminary phytochemical studies (qualitative and quantitative evaluation) and (2) the detailed chemical studies of Arnebia euchroma (collected from Laddakh) to isolate the active constituents especially the naphthazarins for the comparison of these constituents with the market samples of 'Ratanjot' and with other botanical taxa which are reported to be used as 'Ratanjot'.

1. PRELIMINARY PHYTOCHEMICAL STUDIES

Methods used

10 gms of powdered material was extracted in a Soxhlet apparatus with hexane, benzene, chloroform, alcohol and water successively. Solvents from the respective extractives were evaporated and percentage of each solid extractive was calculated with reference to the material used for extraction. These extractives were further screened for steroids and triterpenoids (L B test), flavonoids (Shinoda's test), saponins, tannins (ferric chloride test), reducing sugars (Benedict's test and Fehling test), glycosides, alkaloids (Mayer's reagent, Wagner's reagent, Marme's reagent and Dragendroff's reagent), resins (turbidity test) by the help of following methods (Peach & Tracy, 1955).
(i) **Test for steroids and Triterpenoids**

A portion of the extract was taken in a dry test tube and dissolved in chloroform or benzene. To this was added 1 ml of acetic anhydride and then concentrated sulphuric acid was poured from the side of the test tube. The appearance of green or blue green colour at the junction of two liquid showed the presence of steroids and of violet to pink colour showed the presence of triterpenoids.

(ii) **Test for Saponins**

A portion of residue obtained after evaporating the respective solvents was dissolved in distilled water and shaken vigorously. A honey comb froth, persisting for 15 minutes indicated the presence of saponins.

(iii) **Test for Flavonoids**

A few drops of concentrated hydrochloric acid and magnesium turnings were added to 1 ml of extract dissolved in alcohol. The presence of flavonoids was indicated by the development of pink or magenta colour.

(iv) **Test for Tannins**

Equal quantity of ferric chloride (5% solution W/V) was added to alcoholic and aqueous extractives. The presence of tannins was recorded by the development of blue or green or violet colour.
(v) **Test for Reducing Sugars**

The extractives were dissolved in distilled water and made alkaline by adding 10% solution of sodium hydroxide and then added 5 ml of Benedict's reagent or Fehling solution B and A. The tubes were kept in boiling water for 15 minutes. A red or bright brick red precipitate indicated the presence of the reducing sugars.

(vi) **Test for Glycosides**

(a) After complete precipitation of reducing sugars the filtrate was hydrolysed with dilute hydrochloric acid and then the same test of reducing sugars was repeated, red or brick red precipitate indicated the presence of glycosides or polysaccharides.

(b) The alcoholic, chloroform and water extractives were treated with acetic acid, ferric chloride and 2-4 drops of concentrated sulphuric acid. Formation of blue colour indicated the presence of glycosides.

(vii) **Test for Resins**

Extractives were dissolved in acetone and this solution was transferred to distilled water, turbidity indicated the presence of resins.
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<th>SAMPLE</th>
<th>EXTRACTIVE PERCENTAGE</th>
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<td>Hexane</td>
</tr>
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<td>GENUINE</td>
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</tr>
<tr>
<td>Arnebia benthamii</td>
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</tr>
<tr>
<td>Arnebia euchroma (H.P.)</td>
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<td>Arnebia euchroma (L.)</td>
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<td>Maharanga emodi</td>
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<td>Onosma hispidum</td>
<td>1.928 ± 0.08</td>
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Each value - Average ±SD
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<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaloids</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Resins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Resins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Xanthoproteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>Tannins</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Saponins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Am. - Amritsar; Bh. - Bhopal; Bo - Bombay; Ca - Calcutta; De - Delhi; Hy. - Hyderabad; Ja - Jammu; Lu
(viii) **Test for Alkaloids**

Benzene, chloroform, alcoholic and water extractives were treated with some of the specific alkaloidal reagents mentioned above. The formation of white or creamy precipitate or the development of turbidity clearly indicate the presence of alkaloids.

**OBSERVATIONS**

Results of quantitative and qualitative evaluation of 'Ratanjot' in all the samples are recorded in Tables 8 to 10.

2. **DETAILED CHEMICAL STUDIES OF ARNEBIA EUCHROMA (LADDAKH SAMPLE)**

(i) **EXTRACTION OF THE PLANT MATERIAL**

The air dried 1 Kg powdered root of *Arnebia euchroma* (Laddakh sample) was extracted (6 times) with hexane and the combined extracts concentrated to dryness under reduced pressure at a temperature not exceeding 50°C. The dark red viscous residue (39.8g.) thus obtained was chromatographed over silica gel.

(ii) **CHROMATOGRAPHY OF THE HEXANE EXTRACTIVES**

The column was eluted in the sequence: hexane, hexane-benzene (3:1), hexane - benzene (1:1), hexane - benzene (1:3), benzene,
benzene - chloroform (3:1), benzene - chloroform (1:1), Chloroform and the fractions collected were each 250 ml. The elution schedule is shown in the table.

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Eluant</th>
<th>TLC</th>
<th>Substance isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15</td>
<td>Hexane</td>
<td>Two spots</td>
<td>Wax</td>
</tr>
<tr>
<td>16-20</td>
<td>Hexane-Benzene (3:1)</td>
<td>One spot</td>
<td>Arnebin-7</td>
</tr>
<tr>
<td>21-30</td>
<td>&quot;</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>31-55</td>
<td>Hexane-Benzene</td>
<td>One spot</td>
<td>A mixture of Arnebin-1 and its stereoisomer.</td>
</tr>
<tr>
<td>56-65</td>
<td>Hexane-Benzene (1:1)</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>66-72</td>
<td>Hexane-Benzene (1:3)</td>
<td>One spot</td>
<td>Arnebin-3</td>
</tr>
<tr>
<td>73-80</td>
<td>Benzene</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>81-90</td>
<td>&quot;</td>
<td>One spot</td>
<td>Arnebin-4</td>
</tr>
<tr>
<td>91-100</td>
<td>Benzene-Chloroform(3:1)</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>101-115</td>
<td>&quot;</td>
<td>One spot</td>
<td>B-sitosterol</td>
</tr>
<tr>
<td>116-126</td>
<td>&quot;</td>
<td>One spot</td>
<td>Arnebin-2</td>
</tr>
<tr>
<td>127-138</td>
<td>&quot;</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>139-143</td>
<td>&quot;</td>
<td>One spot</td>
<td>Arnebin-5</td>
</tr>
<tr>
<td>144-150</td>
<td>Benzene-Chloroform (1:1)</td>
<td>Two spots</td>
<td>Mixture</td>
</tr>
<tr>
<td>151-158</td>
<td>Chloroform</td>
<td>One spot</td>
<td>Arnebin-6</td>
</tr>
</tbody>
</table>

**Fraction 31-35 (AEH-2)**

Although the red viscous residue obtained from the above fractions could not be separated out by column chromatography and PTLC but on further separation with the help of HPLC according to the method adopted by Nickel and Carroll (1984), it was separated
out into two sterio-isomers of arnebin-1 viz. B, B-dimethylacryl alkannin and B, B-dimethylacryl shikonin.

**Fraction 116-126 (Arnebin-2)**

The residue from these fractions, on recrystallisation from hexane-benzene yielded dark red flowery needless m.p. 92-94°C, molecular formula $C_{21}H_{24}O_7$ identified as B, B-dimethylacryl-hydroxy-alkannin.

**Fraction 66-72 (Arnebin-3)**

The residue from these fractions, on crystallisation from hexane, afforded dark red needles m.p. 104-5°C, molecular formula $C_{18}H_{18}O_6$ named acetylshikonin.

**Fraction 81-90 (AEH-5)**

The residue obtained from these fractions was a red viscous substance and was difficult to purify the substance by using PTLC and column chromatography. Two separate peaks were obtained from the fractions when using reserved-phase ion-pair (HPLC) described by Nickel and Carroll (1984). These two peaks indicate the sterio-isomers viz. alkannin and shikonin.
Fraction 139-143 (Arnebin-5)

The residue from these fractions was crystallised as dark red needles from hexane-benzene m.p. 111-112°C, molecular formula $C_{16}H_{18}O_5$, identified as hydroxy alkannan.

Fraction 151-158 (Arnebin-6)

These 8 fractions, gave a single spot on TLC and identified as acetyl hydroxy-alkannin having molecular formula $C_{18}H_{20}O_7$, are amorphous in nature.

Fraction 16-20 (Arnebin-7)

The residue from these fractions, on crystallization from hexane, m.p. 95°C, molecular formula $C_{16}H_{16}O_4$, identified as deoxyshikonin.

Fraction 101-115 (β-Sitosterol)

The residue from these fractions, on crystallisation from methanol, afforded white crystals, m.p. 135°C

All the above compounds were identified with the help of comparative TLC and finger printing of authentic samples of arnebins.
III. COMPARATIVE THIN LAYER CHROMATOGRAPHY

TLC of hexane extractives and isolated naphthaquinones was performed in methanol-benzene on precoated silica gel 60-254 plates. After eluting the plates were carried out for finger printings by using Hitachi 650 Fluorescence spectrophotometer (Figs.139 to 152).

RESULTS

The Rf values were calculated from the finger prints of all the samples and compared them with the markers of naphthaquinones.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Isolated Naphthaquinone</th>
<th>Peaks</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AEH-2</td>
<td>Two</td>
<td>0.78; 0.753</td>
</tr>
<tr>
<td>2.</td>
<td>Arnebin-2</td>
<td>One</td>
<td>0.267</td>
</tr>
<tr>
<td>3.</td>
<td>Arnebin-3</td>
<td>One</td>
<td>0.64</td>
</tr>
<tr>
<td>4.</td>
<td>AEH-5</td>
<td>Two</td>
<td>0.56; 0.533</td>
</tr>
<tr>
<td>5.</td>
<td>Arnebin-5</td>
<td>One</td>
<td>0.213</td>
</tr>
<tr>
<td>6.</td>
<td>Arnebin-6</td>
<td>One</td>
<td>0.167</td>
</tr>
<tr>
<td>7.</td>
<td>Arnebin-7</td>
<td>One</td>
<td>0.827</td>
</tr>
</tbody>
</table>

The finger prints of hexane extractive taken with the help of Hitachi Fluorescence Spectrophotometer at excitation and emission
305 and 614 nm respectively are compared with the isolated naphthazarins and following results are obtained:

Arnebin-1 is present in almost all the samples studied viz. Arnebia euchroma (both the samples). A. nobilis Onosma hispidum and the market samples of Amritsar, Bombay, Calcutta, Delhi, Hyderabad, Jammu and Lucknow except in A. benthamii, Maharange emodi and Bhopal sample where it is absent. Similarly, its sterio-isomer - β,β-dimethyl-acrylshikonin is also observed in all the samples except A. nobilis, Amritsar and Hyderabad market samples. However, arnebin-2 is present in a very low concentration at Rf 0.267 in four genuine sample viz. A. euchroma (both the samples), M. emodi and O. hispidum and all the market samples expect the Amritsar sample where it is absent. Remaining naphthoquinones arnebin-3 to arnebin-7 are present in all the samples studied except in M. emodi where arnebin-3 and arnebin-7 are absent. Similarly in A. benthamii arnebin-4 and arnebin-5 are not found. Presence of shikonin- the sterio-isomer of arnebin-4 is observed in all the samples except in A. nobilis, M. emodi and the commercial sample procured from Amritsar market.
Arnebia benthamii

FIGURE-139

Arnebia euchroma (H.P.)

FIGURE-140
Arnebia euchroma (L.)

Figure 141

Arnebia nobilis

Figure 142
Maharanga emodi

FIGURE-143

Onosma hispidum

FIGURE-144
Amritsar sample

FIGURE-145

Bhopal sample

FIGURE-146
Delhi sample

FIGURE-149

Hyderabad sample

FIGURE-150
DISCUSSION AND CONCLUSION
'Ratanjot' have long been used as antipyretic, anthelmintic, antiseptic, bactericidal, fungicidal and in ailments of eye, lungs, abdomen and skin etc. In addition to its medicinal uses it is also employed for imparting a pleasing red colour to food stuffs, oils, fats and some galenicals. As there is lot of confusion with regards to the correct botanical identity of 'Ratanjot' and various botanical taxa belonging to four families, Apocynaceae, Boraginaceae, Geraniaceae and Rosaceae, are being reported to be 'Ratanjot'. Bole (1961, 1962) after thorough survey and screening of the literature described in detail the various species used as 'Ratanjot' and concluded that the material sold in Indian markets under this vernacular name is *Arnebia nobilis* Rech. f. which is imported from Afghanistan. However, CDRI scientists during their survey of Himalayan region observed that all the roots gathered by local collectors from this region and forwarded to whole sale dealers for commercial sale were resembling those of 'Ratanjot' in colour and texture these belonged to the family Boraginaceae.

Keeping this in view, a detailed study of five boraginaceous species viz. *Arnebia benthamii*, *A. euchroma* (two samples: one from Himachal Pradesh and another from Laddakh), *A. nobilis*, *Maharanga emodi* and *Onosma hispidum* implicated as 'Ratanjot' and the market samples procured from various important drug markets viz. Amritsar, Bhopal, Bombay Calcutta, Delhi, Hyderabad, Jammu and Lucknow was carried out.
On systematic comparison of the market samples with the authenticated materials it was revealed that the commercial samples procured from Amritsar showed very close resemblance with *Arnebia nobilis* in its morphological and chemical parameters viz. (i) alternate rings of clusters of broad and narrow vessels, (ii) outermost zone comprised of groups of broad vessels in furrows while the innermost with groups of radially arranged narrow vessels interrupted by parenchymatous cells, (iii) presence of pith; (iv) dimorphic hairs, (v) epidermal cells-hexagonal on adaxial surface while rectangular with raised intercellular partitions on abaxial surface, (vi) stomata diacytic and paracytic; and (vii) presence of naphtazarins: arnebin -1 to 7 (except arnebin -2) and other compounds at hRF 32.0, 36.7, 48.7, 85.3 and 92.0 and therefore it was identified as *A. nobilis*.

Similarly, on comparison the market samples of Calcutta, Jammu and Lucknow were found to be the mixture of *A. nobilis* and *Onosma hispidum* as certain pieces from these samples possess morphological characters of *A. nobilis* described earlier while some pieces showed very close affinities with *Onosma hispidum* in (i) presence of fibres; (ii) dimorphic hairs - small and large, larger ones having cystolith like multicellular bases on adaxial surface, (iii) two types of hairs (a) collapsible generally on margins and (b) tape like with branched bases on abaxial surface, (iv) epidermal cells folded on adaxial surface and concave on abaxial surface and (v) stomata paracytic and flush.
Further, the chemical studies also indicated that all the compounds present in these market samples were either components of *Arnebia nobilis* or *Onosma hispidum* and in this way all the naphthazarins arnebin-1 to 7 and isomers of arnebin -1 and 4 were present along with the additional compounds at hRf 12.7, 15.3, 32.0, 34.7, 36.7, 45.3, 48.7, 70.0, 85.3, 88.0, 92.0, 95.3 and 97.1 except in Calcutta sample where a compound at hRf 34.7 was not observed (figs. 153 & 154).

Likewise some of the pieces from Delhi market resemble *Maharanga emodi* in several characters viz. (i) branched roots becoming very soft and mucilagenous when soaked in water, (ii) broad vessels mostly solitary or in a group of 2-3, (iii) central core comprised of a compact group of narrow vessels, (iv) parenchymatous cells mostly rectangular and filled with mucilage; (v) hairs dimorphic - smaller warty; larger ones broad, smooth with digital multicellular bases, (vi) margins of epidermal cells indiscernible on adaxial side but rectangular or pentagonal with convex surfaces and raised margins on abaxial surface and (vii) stomata anomocytic. On the other hand some of the pieces of this sample are similar to *Onosma hispidum*, therefore, the Delhi sample seems to be the mixture of *Onosma hispidum* and *Maharanga emodi*.

These findings are also supported by the phytochemical results obtained during the course of present study viz. presence of all the naphthazarins, sterioisomers of arnebin-1 and 4 and other compounds at
Diagramatic Representation of \( \text{hRf} \) values of compounds of Genuine 'Ratanjot'

\( (100 \equiv 360) \)

- \text{Arnebia benthamii}
- \text{Arnebia euchroma (H.P.)}
- \text{Arnebia euchroma (L.)}
- \text{Arnebia nobilis}
- \text{Maharanga emodi}
- \text{Onosma hispidum}

FIGURE-153

[In \text{Arnebia benthamii—}compounds present at \( \text{hRf} \) 16.7, 18.7, 24.0, 30.0, 32.0, 40.7, 56.0, 64.0, 70.0, 75.3, 82.7 and 95.3]
Diagramatic Representation of hRf values of compounds of Market Samples.

(100 = 360)

- Amritsar
- Bhopal
- Bombay
- Calcutta
- Delhi
- Hyderabad
- Jammu
- Lucknow

FIGURE - 154
Furthermore, Bhopal and Bombay samples in general showed very close resemblance with each other in having (i) diffuse porous wood, (ii) broad vessels mostly solitary, (iii) clusters of rosette crystals of calcium oxalate in medullary rays and vessels; (iv) dimorphic hairs (a) smaller ones hook shaped (b) larger ones broad with three armed bases on adaxial surface while on abaxial surface spoon shaped bases, (v) epidermal cells pentagonal thick walled with fine cracks on general surface and (vi) stomata paracytic and flush. However the aforesaid characters did not occur in any of the boraginaceous species presently studied. On the contrary some of the pieces of these samples resembled greatly with Onosma hispidum also. In addition to this Bombay sample also showed similarity with Arnebia nobilis in almost all the characters described earlier. It was therefore, concluded that the Bhopal sample is the mixture of Onosma hispidum and some other boraginaceous taxa (which could not be identified) while the Bombay sample consists of O. hispidum, Arnebia nobilis and the unidentified plant material also present in Bhopal sample.

The above findings were also confirmed by the chemical data of aforesaid market samples. The compounds at hRf 12.7, 21.3, 24.0, 29.3, 34.7, 36.7, 38.0, 45.3, 53.3, 56.6, 64.0, 70.0, 75.3, 82.7, 85.3, 88.0, 95.3 and 97.1 present in both the market samples are the components
of Onosma hispidum with three additional compounds. The Bombay sample comprised of few other compounds at hRf 16.7, 48.7, 78.0 and 92.0 which are the components of Arnebia nobilis.

The remaining market sample from Hyderabad was found to be the mixture of two plant materials i.e. A. nobilis and altogether different boraginaceous taxa (not found in any of the market samples studied till now). The characters of this taxa are (i) monomorphic hairs with flat bases on adaxial surface, finely ridged with triangularly depressed flat bases on abaxial side, (ii) epidermal cells irregular, thick walled on lower surface, (iii) stomata paracytic, guard cells comprised of concentric rings of striae extended laterally too and (iv) presence of naphthazarins- arnebin-1 to 7, isomer of arnebin-4 and with some other compounds at hRf 15.3, 32.0, 34.7, 36.7, 43.3, 48.7, 52.0, 70.0, 85.3, 88.0, 92.0, 95.3 and 97.1.

Besides, composit profile of the samples on the basis of their physico-chemical constants is presented in the form of bardagrams (Figs 55-60) in order to study the comparative proportion of different plant materials present in the commercial samples. The average of physico-chemical constants of Amritsar sample (identified as Arnebia nobilis) and Onosma hispidum were compared with those of Calcutta, Jammu and Lucknow samples and very interesting results were obtained. All the three market samples were almost alike and had more quantity of A. nobilis as compared to
to *O. hispidum* whereas the Delhi sample on comparison with the average values of these constants of *O. hispidum* and *Maharanga emodi* showed that in this sample the percentage of *O. hispidum* was higher.

It is significant to note that none of the market samples resemble the two other species of *Arnebia* i.e. *A. benthamii* and *A. euchroma* also considered to be 'Ratanjot'. However, during the course of this study noteworthy observations were made. Although the genuine material of *A. euchroma* collected from Himachal Pradesh (H.P.) and Laddakh regions resembled closely in their gross morphology except the presence of few suberized cells in H.P. material (on maceration) but much variation was observed in their physico-chemical values and TLC finger prints such as: (i) the naphthaquinone percentage almost double in H.P. material (5.93%) as compared to the Laddakh material (2.83%), (ii) successive Soxhlet extractive percentage in alcohol and water: 12.03 and 20.02 in the former and 4.172 and 11.321 in the latter respectively, (iii) a major peak of naphthazarin at hRf 34.7 on excitation 305nm and emission 614nm in H.P. material only.

The above differences were also confirmed by the ultramorphological characters of radical leaves of both the materials as revealed under scanning electron microscope. Some of the significant variations are: (i) hook shaped, 2-3 celled hairs with smooth surface in H.P. material and ribbon like, unicellular with fine folded surface in Laddakh material on adaxial surface, (ii) hairs loosely folded in H.P. material but strongly
ridged in Laddakh material on abaxial surface, (iii) epidermal cells smooth, rectangular in H.P. material while elongated, tracheal shape in Laddakh material and (iv) stomata anomocytic in H.P. material but diacytic in Laddakh material.

From the above study it was concluded that A. euchroma collected from Himachal Pradesh and Laddakh regions are considered as chemotype.

From the foregoing discussion it is very clear that all the market samples except the Amritsar identified as Arnebia nobilis, are the mixture of two or three botanical species in varying proportions viz. Calcutta, Jammu and Lucknow samples are the mixture of Arnebia nobilis and Onosma hispidum, while the Delhi sample has Onosma hispidum and Maharanga emodi and the remaining samples from Bhopal, Bombay and Hyderabad comprise of Onosma hispidum and/or Arnebia nobilis and some other boraginaceous taxa. Another striking feature of this study is the presence of naphthazarins, specially the arnebins and their isomers responsible for the red colour of 'Ratanjot' in all the botanical species and the market samples studied. This incidently proved very useful in identification of market samples as it forms a characteristic feature of the family Boraginaceae.
Phy. Chem. Constants of "RATANJOT"

Ash Values of "RATANJOT"

FIGURE - 155

FIGURE - 156.
Soxhlet Extract. % of "RATANJOT"

Comp. with Cal, Jam, Lko.

FIGURE - 157
Physico-Chem. Constants of "RATANJOT".
GENUINE SAMPLE COMPARED WITH DELHI

FIGURE - 158
Ash Values of "RATANJOT".

Soxhlet Extractive % of "RATANJOT".

FIGURE - 159

FIGURE - 160.
SUMMARY
In the indigenous system of medicine, 'Ratanjot' is used for a variety of ailments, as an anthelmintic, in diseases of eye, in bronchitis, abdominal pains, itch, fever, wounds and burns etc. Leaves and flowers are used as stimulant and cardiac tonic. Ethanol extract of 'Ratanjot' showed bactericidal, fungicidal and anticancer activity when put through a wide screen of biological tests. The antibiotic activity was associated with the hexane soluble fractions of the root.

'Ratanjot' is popular not only in India but also in other Asian countries for imparting a pleasing red colour to food stuffs, oils, fats, medicinal preparations and also used for dying silk and wool.

The botanical identity of 'Ratanjot' sold in the crude drug market all over India in the form of isolated underground part with some remains of radical leaves, is a matter of concern for the botanists, as many plant species are being sold in the market under trade name 'Ratanjot'. The botanical authentication of market samples of 'Ratanjot' is difficult because of the absence of aerial parts.

Bole (1961, 1962) provided a convincing explanation that the material sold in the Indian markets under the vernacular name 'Ratanjot' is Arnebia nobilis which is imported into India from Afghanistan. From all available informations it becomes clear that this plant occurs only in Afghanistan and perhaps in some contiguous areas in West Pakistan, but not in India.
However, during the course of botanical survey and collection of plants from Himalayan region CDRI scientists observed that the roots resembling 'Ratanjot' in colour and texture were gathered by local collectors from the areas for commercial sale. This is the common practice of any trade to find out cheap, similar looking substances for adulteration or as substitute to the genuine item for easy monetary gain. It appears that this practice started in seventies after the much publicity of 'Ratanjot' for commercial utilization for colouring 'Vanaspati Ghee' and its extended permission to use it as colouring matter for use in and upon food stuffs under the prevention of Food Adulteration Rules.

Keeping this in view, a detailed Pharmacognostic and chemotaxonomic studies of six materials from five boraginaceous species viz. *Arnebia benthamii*, *A. euchroma* (Himachal Pradesh), *A. euchroma* (Laddakh), *A. nobilis*, *Maharanga emodi* and *Onosma hispidum* implicated as 'Ratanjot' and the market samples procured from important Indian markets viz. Amritsar, Bhopal, Bombay, Calcutta, Delhi, Hyderabad, Jammu and Lucknow were carried out. The detailed morphological (including SEM studies) and phytochemical parameters used as markers of respective botanical taxa to identify and authenticate the commercial samples.

The important salient features for the various species studied are:

*Arnebia benthamii*: (i) Roots 1-2.5 cm thick, (ii) number of vessels 210-230 per sq mm., (iii) vessels arrangement flame like,
(iv) xylem parenchyma apotracheal and confluent narrow, (v) parenchymatous cells wavy and suberized (also observed on maceration); (vi) hairs, unicellular, rugose with two tube like arms adjoining to broad bases on adaxial surface and ridged with multicellular bases comprised of irregular cells and a constricted joint on abaxial surface, (vii) epidermal cells polygonal, elongated, striated with raised partitions on adaxial surface and hexagonal or pentagonal finely striated, cell boundaries raised on abaxial surface, (viii) stomata paracytic and (ix) presence of naphthazarins arnebin-3, -6 and 7, isomers of arnebin-1 and 4 with two additional compounds at hRf 18.7 and 30.0.

_Arnebia euchroma_: (i) Roots 3-3.5 cm in diameter, (ii) wood and bark readily exfoliated in the form of papery layers and only 2.5-5 mm broad woody portions discernible,(iii) presence of vessels in maceration and T.S. of peelings, (iv) wood parenchyma vasicentric apotracheal; (v) hairs unicellular, scanty on adaxial surface while dense on abaxial surface; (vi) presence of naphthazarins arnebin-1 to 7 and the stereo-isomers of arnebin-1 and 4 with some additional compounds at hRf 43.3, 46.7, 58.7 and 61.3.

The two materials of _A. euchroma_ collected from Himachal Pradesh and Laddakh regions showed some differences in their morphological and chemical characters.

Himachal Pradesh material characterized by (i) number of vessels 160-305 in young and 410-475 in mature root per sq mm, (ii) presence
of suberized cells (on maceration), (iii) hairs scanty, 2-3 celled, hook shaped, finely folded with condyle shaped bases on adaxial surface while hairs dense, tape like, twisted, loosely folded forming interlocking arrangements with broad centrally depressed bases on abaxial surface, (iv) epidermal cells smooth, rectangular with depressed partitions on adaxial side and slightly raised general surface on abaxial side, (v) stomata anomocytic; (vi) naphthaquinone 5.93%, (vii) percentages of successive extractives of alcohol and water 12.03 and 20.02 respectively and (viii) a naphthazarin at \text{hRf} 34.7.

However, the Laddakh material characterized by (i) number of vessels 370-400 in young and 455-530 in mature root per sq mm. (ii) hairs scanty, tape-like, short, finely folded surface on adaxial surface and elongated, strongly ridged on abaxial surface, (iii) epidermal cells elongated with tracheal shaped surface, (iv) stomata diacytic; (v) naphthaquinone 2.83% and (vi) percentages of successive extractives of alcohol and water 4.172 and 11.32 respectively.

Therefore, the material of \textit{Arnebia euchroma} collected from Himachal Pradesh and Laddakh regions were considered as chemotype.

\textit{Arnebia nobilis} : (i) Roots 1-5 cms in diameter, (ii) alternate rings of clusters of broad and narrow vessels, (iii) outermost zone comprised of groups of broad vessels in furrows while the inner most with groups of radially arranged narrow vessels interrupted by parenchymatous cells, (iv) number of vessels 500-700 per sq mm., (v) presence
of pith; (vi) hairs warty, dimorphic small and large; (a) small hairs with semilunar rings on bases, (b) broad hairs with warty blobs gradually disappearing and forming fine ridged surface towards the apex, having saucer shaped bases with 'U' shaped slight depression, (vii) epidermal cells hexagonal partitions raised on adaxial surface but rectangular with raised and swollen intercellular partitions on abaxial surface, (viii) stomata diacytic and paracytic and (ix) presence of naphthazarine arnebin-1 to 7 (except arnebin-2) alongwith the additional compound at hRf 36.7.

Although Shukla et al (1969) reported arnebin-2 from the roots of *A. nobilis* but it is not observed in our sample.

**Maharanga emodi**: (i) Roots 1-3 cm in diameter, sometimes branched, (ii) become very soft and mucilagenous when kept into the water for sometime, (iii) number of vessels 400-500 per sq mm. (iv) broad vessels mostly solitary or in a groups of 2-3 (v) central core comprised of a compact groups of narrow vessels, (vi) wood parenchyma paratracheal and aliform, confluent, (vii) most of the parenchymatous cells rectangular and filled with mucilage (also observed in maceration), (viii) hairs dimorphic, the smaller ones warty with digitally arranged multicellular bases, (ix) margins of epidermal cells indiscernible on adaxial surface but rectangular or pentagonal with convex surface and raised margins on abaxial surface, (x) stomata anomocytic and (xi) absence of the naphthazarins arnebin-1, 3, 7 and isomer of arnebin-4 and presence of additional compound at hRf 59.3.
Onosma hispidum: (i) Roots 1-7 cm in diameter, (ii) number of vessels 514-610 per sq mm. (iii) presence of fibres, (iv) hairs dimorphic (a) small, smooth with unicellular bases (b) large and broad, warty or smooth with cystolith like arranged multicellular bases on adaxial surface while in abaxial surface marginal hairs collapsed type and middle ones with branched root like bases, (v) epidermal cells elongated with raised partitions general surface folded on adaxial side and irregular, concave with raised partitions on abaxial side, (vi) stomata paracytic and flush, (vii) presence of all naphthazarins (except arnebin-6) and two other constituents at hRf 12.7 and 45.3.

Some other additional compounds are also observed at same hRf in more than one species. (Table - 13)

The significant variations in physico-chemical values (i.e. naphthaquinones, phenolics, sugars, tannins, ash values and Soxhlet extractive percentage) from species to species were observed and represented by the Bar diagrams (Figs. 161-166).

On systematic comparison of the market samples with the authenticated materials it was revealed that all the market samples were the mixture of two or three botanical taxa except the Amritsar samples which showed very resemblance with Arnebia nobilis in its morphological and chemical parameters. Further, the market samples procured from Calcutta, Jammu
and Lucknow were the mixture of *A. nobilis* and *Onosma hispidum* while Delhi sample consisted of *O. hispidum* and *Maharanga emodi*. The remaining samples from Bhopal, Bombay and Hyderabad comprised of *Arnebia nobilis* and or *Onosma hispidum* and some other boraginaceous taxa.
## TABLE 13: COMPARATIVE CHEMICAL STUDIES OF 'RATANJOT'.

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Phy. Chem. Constants of 'RATANJOT GENUINE SAMPLES

NAPHTHAQUINONES

PHENOLICS

SUGARS

TANNINS

FIGURE - 161.
Physico-Chemical Constant of "RATANJOT"

M A R K E T  S A M P L E S

FIGURE - 162
Ash Values of 'RATANJOT GENUME SAMPLES

FIGURE - 163.
Soxhlet Extract % of "RATANJOT"

GENUINE SAMPLES

H \\
E X A N E  B E N Z E N E  C H L O R O P O R M

A L C O H O L  W A T E R

FIGURE - 165
Soxhlet Extract. % of 'RATANJOT

FIGURE - 166.
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Original not seen.