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 echoides 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 isohexenynaphthazarins, 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 naphthazarins, 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 (Pelleuir, 1832; Bolley, 1847; Betrabet and Chakravarti, 1933; Brockmann, 1935; Dusinsky' and Szokolay, 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, tetraacetyltetrabromoalkannin, dinitrotetraacetylalkannin and β-methylanthracene.

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 spectrometeric 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), Macrotomia 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 Macrotomia species (2.21%) and Onosma zerizaminum (1.59%). Pimenova and Tareeva (1980) observed the variation of shikonin content in subterranean parts of Macrotomia 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 hispdiissima (Jain and Mathur, 1967), Echium italicum, E. vulgare (Sherbanivs'kii, 1971), E. locopsis (Shcherbanovskii and Luks, 1974), Onosma polyphyllum and O. visianii (Sherbanivs'kii,1971). Shcherbanovs'kii (Loc. cit.) 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 euchroma (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), Macrotomia euchroma (Kyogoku et al., 1973), and Dehydroxyshikonin from the roots of Lithospermum enchromum (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 hispidesima* (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), *Macrotomia 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), *Macrotomia 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 Ghalib, 1986.

Kyogoku et al. (1973) identified and isolated α-methyl-n-butyrylshikonin from the roots of Lithospermum erythrorhizon, and the α-methyl-n-butyryl 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 β, B-dimethylacrylshikonin which was isolated from Lithospermum erythrorhizon (Ichiro et al., 1965; Krivoschekova 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 $\beta$, $\beta$-dimethylacrylic-shikonin 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. hirsutissima* (Afzal and Tofeeq, 1975).

Ichiro and Yoshimasa (1966), Hisamichi and Yoshizaki (1982) isolated $\beta$-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), $\beta$-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 as 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 isohexenynaphthazarin 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 monoterpenyl-benzoquinone (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). Poland et al. (1989) synthesized isoarnebifuranone via addition of tetrahydropyranyloxybutyne to cyclobuteredione and thermal ring enlargement of the adduct.
iii) **ANTHRACINONE**

Majumdar and Chakravarti (1940) isolated an anthracene derivative named as anchusin \( \text{C}_{30}\text{H}_{36}\text{O}_{9} \) 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 O\textsuperscript{7} -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 \( C_n H_{2n} O_2 \) \( (n=14, 16, 18, 20, 24 \text{ and } 26) \), (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 \( C_{18} \) nonconjugated tetraenoic acid (upto 17%) in addition to linolenic acid (0.3–50%) and other common \( C_{16} \) and \( C_{18} \) acids were present. Iodine values ranged from 88 to 225.

Hoerhammer et al. (1964) studied the constituents of the fruit of *L. officinale*. \( C_{18} \) 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.

Krolikowska 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, caffiec 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 β-amyrin, lupeol, β-sitosterol campesterol, stigmasterol, glycose, quercetin, rhamnose, bornesitol, syringin, rutin and sinapylaldehyde glucoside. Davis and Ross (1955) isolated β-sitostrolol 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. Caffiec 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 aerial 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, antiprotozoal, 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 naphthaquinones i.e. arnebin-1 and arnebin-3 were effective against rat walker – carcino-sarcoma-256 in-vivo, and each of the naphthaquinones inhibited the tumor cells in-vitro. Administration of arnebin-1 with mitemycin C or diphenyl sulphone-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, antifungal, 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. Weak 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 carcino-sarcoma (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 \textit{Alkanna tinctoria} to be located in its naphthoquinone pigments. Polymerization of naphthoquinones 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. \textit{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 \textit{Musca domestica} and \textit{Tribolium castaneum}. No activity was observed in the roots of \textit{Arnebia nobilis}. Prabhakar et al. (1981) observed that vitexin isolated from \textit{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 \( \beta, \beta \) -dimethyl-lacrylate is a strong antibacterial compound. Sankava (1983) noted
that \textit{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 \textit{A. euchroma} were not the inhibitors of prostaglandin biosynthesis. The inhibitory activity was found for shikonofuran B, C and de-O-methyllasiodiplodin. 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 \textit{Macrotomia euchroma} on plasma protein components. Further, Yamada et al. (1986) isolated an extraordinary patent anti-complementary substance from the roots of \textit{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 \textit{Alkanna tinctoria}.

Wessel et al. (1987) screened some selected plants for the isolation of pyrrolizidine alkaloids and antitumor activity. Extracts of \textit{Arnebia hispidissima} contained unsaturated alkaloids which were cytotoxic in-vitro to 'Ehrlich ascites carcinoma' cells. The major pyrrolizidine alkaloids - echimidine and monocrotaline isolated from \textit{A. hispidissima} were anticipated to be hepatotoxic to mammals on the basis of their molecular structure.
Hamdard et al. (1988) obtained alkannin monoacetate, alkannin α-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 callus 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 \( \beta \)-hydroxyisovalerylshikonin.

Kishimoto and Aota (1977) observed that the cosmetic preparation containing extracts of roots or stems of L. officinale with \( \beta \), \( \beta \)-dimethylacrylshikonin, acetylsahikonin, teracrylshikonin, shikonin, \( \beta \)-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 antiinflammatory 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 alongwith 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) DYE STUFFS:

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, Annotto, \( \beta \)-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.