Quinones are a large class of compounds endowed with rich and fascinating chemistry. 1,4- benzoquinone or p-benzoquinone is the basic structure of all quinoid compounds. They are widely distributed in the natural world, being found in bacteria, plants and arthropods and hence quinones are ubiquitous to living systems. Quinones play pivotal role in biological functions including oxidative phosphorylation and electron transfer. Their role as electron transfer agents in primary metabolic processes like photosynthesis and respiration is vital to human life. A large number of chemical derivatives with 1,4-benzoquinone as the basic subunit exhibit prominent pharmacological applications such as antibiotic, antitumor, antimalarial, antineoplastic, anticoagulant and herbicidal activity. Wide applications of quinones can also be found in the field of synthetic organic chemistry. Co-ordination chemistry of quinones is also quite rich from the perspective of designing magnetic materials and understanding photophysical properties. (Ignatious et al., 2011). In the present study two classes of quinones viz. Benzoquinone and Naphthoquinone are dealt with:

A) Two naturally occurring benzoquinones: Embelin and Thymoquinone  
B) Two naturally occurring naphthoquinones viz. Plumbagin and Shikalkin.

3.1 Extraction methods of Naphthoquinones and Benzoquinones

Extractions of quinonoids are generally carried out using organic solvents like hexane, chloroform, acetone, acetonitrile, ethyl acetate, methanol, etc. either at ambient temperature or at refluxing condition. However, in some cases, homogenization of the plant material in cold followed by sonication was performed to isolate sensitive compounds like shikonin or aloe-emodin. Ultrasonic bath was used to extract phylloquinone and naphthodianthrones. Samples of rhubarb, senna and herbal mixtures were also sonicated to extract the anthraquinonoid constituents. Recently, microwave-assisted extraction was applied for isolation of tanshinones from Salvia miltiorrhiza. Many anthraquinonoid metabolites exist along with their glycosides in roots of madder and rhubarb, and leaves and fruits of senna. Thus, samples were first extracted with aqueous alcohol, followed by hydrolysis to separate the aglycone part the two steps could be performed simultaneously also. Although quinonoids have been extracted by using various...
solvents, as per reports chloroform or dichloromethane would be the solvent of choice for getting the maximum yield of the lipophilic constituents; this has been established elegantly in the work of Liu et al. However, for an exception like lawsone, the tautomeric form helps it to be extracted preferentially with aqueous ethanol. Other recent developments in extraction technology as applied to quinonoids are the use of a non-CFC solvent like phytosol, and liquid carbon dioxide for SFE of Salvia and Hypericum sp. (Hazra et al., 2004). The methods for extraction and isolation are given under respective constituents.

3.2 Analytical techniques for quinones:
Most of the naturally occurring quinonoids could be separated by conventional methods like TLC, PTLC and CC, which have now largely been supplemented by HPLC, one of the most useful techniques for the analysis of natural products in complex biological matrices. Comparison of chromatograms, used as fingerprints, between authentic samples and unknowns, leads to the identification of drugs and/or search for adulterants. However, hydroxyquinonoids often tend to remain strongly adsorbed in the silica gel column. This problem has been efficiently countered by HSCCC, which is a support-free liquid–liquid partition chromatography, and eliminates the irreversible binding of the sample onto the solid support. HSCCC is applied for analytical as well as preparative separation of a few quinonoids of scientific and commercial importance. Again, droplet counter current chromatography was used to purify the quinonoids present in commercial ruberythric acid. Hyphenated methods such as HPLC coupled to PDA, MS and NMR in combination with biological screening have lately been developed for rapid survey of natural products. However, as of now, these coupled techniques are expensive, and do not allow a full on-line identification, except for some well-known compounds, such as phylloquinone. Other quinonoids reportedly isolated by such methods are 6-alkyl-substituted naphthoquinones from a Panamanian shrub Cordia linnei and a ‘quinone methide’ from Bobgunnia madagascariensis from Zimbabwe, none of which are used in Oriental medicines. Detectors are based mostly on UV, and in some cases fluorescence or electrochemistry provided good detection of some quinonoids. HPLC coupled with PDA detection has been used by many workers. Some of the LC–MS interfaces are ESI and APCI; such
hyphenated techniques were used to analyze sennosides, hypericin and alizarin analogues. More reports on quinonoids in the near future are highly warranted, going by the recent reviews on the applications of such nascent technologies for separation of many bioactive phenolic compounds and flavonoids. Recently, capillary electro-chromatography was applied for analysis of quinonoids, this method when coupled to MS allows a rapid characterization and quality control of quinoid components in natural products (Hazra et al., 2004). The different analytical techniques for analysis of Embelin, Thymoquinone, Plumbagin and Shikalkin are given below.

3.3 Color Reactions of Quinones:
The most useful diagnostic tests depend on the redox properties of quinones and the presence of hydroxyl groups. Leucomethylene blue is a useful spray for the detection of benzo- and naphthoquinones on paper or thin layer chromatography, the quinones appearing as blue spots on white background. Reduction to a colorless (or much less highly colored) product and easy restoration of the original color on oxidation is characteristic and distinguishes quinones from nearly all other natural compounds. Reoxidation can usually be affected simply by shaking the solution in air, but the leuco compounds of non hydroxylated benzo- and naphthoquinones do not oxidize so readily. Reduction is easily affected by neutral or alkaline sodium dithionate but many other reducing agents may be used. Catalytic hydrogenation may be employed quantitatively and sodium borohydride is convenient when reductions are to be followed spectrophotometrically. For hydroxyquinones, the color changes are more striking in alkaline solution and re-oxidation by air is more rapid.
Positive color reaction with magnesium acetate is a general test for a hydroxyquinone.

3.4 Pharmacological activity of quinones:
Pigments of various colors isolated from different sources have been identified as quinonoid compounds. Crude preparations of plants presently known to contain quinones as active ingredients were prescribed for more than 4000 years as purgatives or drugs. Throughout history several other medicinal benefits have been added to the list every year. The discoveries of antibiotic and antitumor properties of several naturally occurring
quinones have raised interest among scientists to explore their use as pharmaceuticals (Ignatious et al., 2011). The naphthoquinones are produced by higher plants, fungi, actinomycetes and exhibit a broad range of biological actions including fungicidal, antibacterial, insecticidal, phytotoxic, cytostatic, and anticarcinogenic. In plants they commonly occur in reduced and glycosidic forms e.g. 4β-glycoside of α-hydrojuglone, a constituent of walnut tree leaves. On extraction and workup or in soil they are oxidatively converted to coloured naphthoquinone. In some heart–woods, e.g. diospyros spp. Ebenaceae, naphthoquinones occur as monomers, complex dimmers and trimers (Pharmacognosy, 2000).

3.5 Biosynthesis of naturally occurring naphthoquinones and benzoquinones.

The biosynthesis of skeleton of naturally occurring quinone can originate from different carbon sources. Acetate and isoprene units are two major building blocks used in de novo synthesis of quinone skeletons. The term polyketide quinone and terpenoid quinone refer respectively to quinones possessing a skeleton originating from the acetate-malonate pathway or the isoprenoid pathway. Accordingly, quinones can be formed from phenolic systems and can be generated by either acetate or shikimate pathways, provided a catechol or quinol system has been elaborated, and many examples are found in nature (Medicinal Natural Products - A Biosynthetic Approach, 2000).
Plumbagin is biosynthesized by condensation of 6 acetate units which undergo condensation, cyclization and oxidation to form the same (Bringman et al., 1998). When the derivative of isoprene units are present in the side chain of quinone the term meroterpenoid is used e.g. Vit K and Co-enzyme Q. The examples of quinones biosynthesized from isoprene units include Thymoquinone (monoterpenoid), Mansonone B (sesquiterpenoid) and Royleanone (Diterpenoid). Naphthoquinones have been shown to be biosynthesized via variety of pathways including acetate and malonate (Plumbagin), shikimate/succinyl CoA combined pathway (lawsone) and shikimate /mevalonate combined pathway (alkannin). (Pharmacognosy, 2000)
3.6 Toxicity of Quinones:
The allergic potential of many benzoquinone and naphthoquinones is due to the fact that they act as Haptens: by combining themselves, through their nucleophilic centres, with amine and thiol function on macromolecules, they induce dermatitis by sensitization e.g. top primrose, The sensitization caused by Primula obconica (Top prime-rose) due to presence of 2-methoxy-6-pentylbenzoquinone, primin which causes localized pruriginous reactions and urticaria or erysipelatous-type rashes on eyelids, cheeks, chin, neck, fingers, hands and forearms. Similar problems also occur in wood industry. Workers exposed to sawdust may develop conjunctivitis and nasal reaction. Erythema and dermatitis with blisters are also frequent on exposed parts of body. The molecules incriminated are: lapachol, deoxylapachol and closely related compounds in teak (Tectona grandis) (Pharmacognosy and Phytochemistry - Medicinal Plants, 1999).

3.7 Benzoquinones:
3.7.1. Embelin
Embelin also known as Embellic acid is alkylated hydroxybenzoquinone derivative isolated from fruits of Embelia ribes (Myrsinaceae). Embelin is also present in fruits of Embelia robusta, leaves of Embelia aungustifolia, Embelia schimperi and fruits of Myrsine Africana, seeds of Rapanea laetevirens, fruits of Ardisia colorata, all belonging to family Myrsinaceae and Lysimachia punctata L. belonging to family Primulaceae (Bogh et al., 1995). Embelin was prepared synthetically by Fieser et al., 1948.

![Chemical structure of Embelin](image)

Figure 3.2. Chemical structure of Embelin
**Physical properties:**

Molecular formula : $\text{C}_{17}\text{H}_{26}\text{O}_{4}$  
Molecular weight : 294.39  
Melting point : 142-143ºC  
Solubility : Soluble in hot organic solvents chloroform, benzene, acetic acid and acetone. Practically insoluble in water. Soluble in alkali hydroxide solutions. Orange flakes obtained from methanol.  
Uses : Ammonium embelate is used as anthelmentic.

Embelin is a simple naturally occurring benzoquinone isolated from the species of Myrsinaceae, Lysimachia, Oxalidaceae families (Podolak and Strzalka, 2008; Podolak et al., 2005; Jiménez-Alonso et al., 2008; Feresin et al., 2003). It shows a number of diverse activities such as chemopreventive effect against DENA/PB-induced hepatocarcinogenesis in Wistar rats (Podolak et al., 2005), anti-fertility effects (Githui et al., 1991), wound healing (Kumara et al., 2007), Antibacterial (Chitra et al., 2003), free radical scavenging (Joshi et al., 2007) and *in vitro* cytotoxic activity against B16 and XC cell lines (Feresin et al., 2003). It has been shown that Embelin is a fairly potent, nonpeptidic, cell-permeable inhibitor of XIAP (X-linked inhibitor of apoptosis protein), and it represents a promising lead compound for designing an entirely new class of anticancer agents that target the BIR3 domain of XIAP (Mori et al., 2007; Chen et al., 2006). These antecedents justify the interest in evolving newer synthetic methods for the construction of Embelin derivatives.

**3.7.2. Embelin is isolated from dried berries of *E. ribes***

*Embelia ribes* fruits:

The plant *Embelia ribes* of the family Myrsinaceae is a small tree that grows throughout tropical and sub tropical regions of Old World. In Indian system of medicine ‘Ayurveda’, the plant is popularly known as *Vidanga* or *Bashmak* or *Krimigna* (Sanskrit); *Baberang* or *Wawrung* (Hindi) and it is used as one of the adjuvant in most of the drug preparations (Kumara et al., 2007). The dried fruits are globular, wrinkled or warty, varying in color
from dull red to nearly black; a short pedicel is usually present; pericarp is brittle and often encloses a single seed covered with a membrane; taste slightly aromatic and astringent. Fruits of *Embelia ribes* contain Embelin 2.5-3.1 %w/w on dried weight basis. Quercitol 1%, fatty ingredients 5.2%, an alkaloid christembine, a resinoid, tannins, and minute quantities of volatile oil (Wealth of India, 2006).

The methods reported for extraction and isolation of Embelin include Solvent extraction followed by column chromatography, isolation using hydrotropes and microwave assisted extraction (Chitra et al., 2003; Latha, 2006)

**Uses in traditional medicine.**

Dried fruits are used as anthelmintic, astringent, alterative, tonic, in ascariasis, in scorpion-sting and snake-bite. The decoction is useful in fevers and diseases of chest and skin. Infusion of roots is used for cough and diarrhea (Chitra et al., 2003). Some Ayurvedic formulations containing *E. ribes* fruit are *Sanjivani vati*, *Pippalyasavam*, *Dhanwantara ghritham*, *Kaisoraguggulu vatika* etc. (Ayurvedic Formulary of India, 1989)

**3. 7. 3 Extraction of Embelin**

**Soxhlet extraction:** Chitra et al., 2003 have reported isolation of Embelin from fruits of *E. ribes* using soxhlet extraction. Coarsely powdered berries of *E. ribes* (1.5 kg) were exhaustively extracted with *n*-hexane by cold extraction method (3 X 2L). After 72 h, the solvent was decanted and distilled off over boiling water-bath. The extract so obtained was concentrated in vacuo and subjected to column chromatography over silica gel (100-200 mesh). Elution of the column with benzene yielded an orange coloured powder which on crystallization with ether afforded orange plates of embelin (Yield: 4.5 g).

**Column chromatography:** Podolok et al.; 2005, isolated embelin by chloroform extraction of the drug powder of *Lysimachia punctata* L. of the family Primulaceae followed by Silica-gel column chromatography using hexane and ethyl acetate; yield 1.3% w/w.

**Microwave assisted extraction:** Latha C, 2007, developed a rapid and efficient microwave-assisted extraction (MAE) process for the selective extraction of embelin from
Embelia ribes was developed. Solvent selection, microwave energy input and solid loading were optimized. The rate of extraction and purity of embelin depends upon the solvent used and exposure time to microwaves. Maximum MAE was achieved in acetone with total yield of 92% (w/w) embelin with 90% (w/w) purity with 1% (w/v) raw material loading at 150 W power level in 80 s. Non-polar solvents, such as hexane and dichloromethane, were not effective for the selective extraction of embelin.

Hydrotropes: Latha in 2006 devised an alternate strategy of the extraction of embelin (2,5-dihydroxy-3-undecyl-p-benzoquinone) from fruits of Embelia ribes was carried out by Latha 2006 by using Hydrotropes. The aromatic hydrotropes such as sodium n-butyl benzene sulfonate (NaNBBS), and sodium cumene sulfonate (NaCS) were found to be effective for the selective extraction of Embelin, with a recovery of 95% embelin from the aqueous solution of hydrotropes. In this method drug powder was soaked in a hydrotrope solution for 3 hours at 50º C. After extraction the solution was filtered, the filtrate diluted with acidified water so that the Embelin precipitates out, this is then filtered and recovered. Hydrotropic extraction shows a tremendous potential for commercial production of hydrophobic naturally occurring compounds in future, as the process of extraction is economically feasible. A single step process of extraction gives pure embelin with a recovery of 92% as compared to conventional multiple extraction and purification process. After the recovery of the compounds the hydrotropic solutions may be reused since there is no chemical reaction between the hydrotrope moiety and the extracted products. The simple recovery step along with no contamination of the product by the hydrotrope and a potential reuse of the hydrotrope solution make the technique economically attractive for the extraction of embelin.

3.7.4. Methods of Analysis of Embelin:

Pal et al.; 1995 developed a UV-Vis spectrophotometric method for analysis of Embelin from fruits of E. ribes based on its colour reaction with pyridine.

containing 0.1% v/v o- phosphoric acid 90:10. The detection was carried out at 288 nm and the Rt of Embelin was found to be 7 min.

Latha, C.2007, carried out analysis of Embelin by HPTLC using 20 x 20 cm silica gel 60 F254 plates and developed with ethyl acetate/formic acid/acetic acid/water (94:1:1:2). After development, plates were scanned at 280 nm.

The Indian Herbal Pharmacopoeia describes a HPTLC method for analysis of E. ribes fruit using mobile phase comprising of Butanol: Propanol: Ammonia (4N) (7:1:2). After development plates were scanned at 280 nm. The Rf of Embelin was found to be 0.31.

Shela et al., 2009 have developed a RP-HPLC method with photodiode array detection was established for the determination of major constituent, Embelin in Embelia ribes samples. The Embelin was separated by using isocratic mode consisting of 0.1 % trifluoroacetic acid in water and methanol (in proportion of 88:12) at a flow rate of 1.0 mL/min. Under these conditions, a plot of integrated peak area versus concentration of Embelin was found to be linear over the range of 5.0-75.0 μg/mL, with a relative standard deviation of 0.61-0.96 %. The limit of detection was 20 ng on column and the limit of quantitation was 50 ng on column. The determination of the Embelin content in various solvent extracts exhibited a mean content of 0.44-33.0 % w / w. Recovery experiments led to a mean recovery rate of 96.49 ± 2.42 %.

Podolok and Strzalka, 2008, developed a RP-HPLC method for qualitative and quantitative determination of the pharmacologically active benzoquinones, embelin and rapanone, in different organs of eight Lysimachia species by reversed-phase high-performance liquid chromatography. An analytical Hypersil BDS C-18 column and a mobile phase of water containing 0.1% v/v H₃PO₄ and acetonitrile (10:90) at a flow rate of 1.0 mL min⁻¹ were used. UV detection was carried at 286 nm. The recovery of the method was 81.5% for embelin and 80.5% for rapanone. Good linearity (r > 0.999) was obtained for both compounds. The leaves of L. ephemerum had the highest amount of rapanone (1.69%) while the roots of L. punctata had the highest amount of embelin (1.28%).

3. 7. 5 Pharmacological activities of Embelin:

<table>
<thead>
<tr>
<th>Pharmacological Activity</th>
<th>Reference</th>
</tr>
</thead>
</table>

© 2014, Galvina M. Ferreira, Institute of Chemical Technology
<table>
<thead>
<tr>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective and hypolipidemic effect in chemically induced hepatocarcinogenesis in wistar rats</td>
<td>Jagadeesh et al.; 2009</td>
</tr>
<tr>
<td>Hepatoprotective against paracetamol induced acute hepatocellular damage in mice</td>
<td>Tabassum and Agrawal; 2003</td>
</tr>
<tr>
<td>Inhibitor of X Chromosome-Linked Inhibitor-of-Apoptosis Protein, Blocks Nuclear Factor-κB (NF-κB) Signaling Pathway Leading to Suppression of NF-κB-Regulated Antiapoptotic and Metastatic Gene Products</td>
<td>Ahn et al.; 2007</td>
</tr>
<tr>
<td>Anti-tumor, anti-inflammatory and analgesic</td>
<td>Chitra et al.; 1994</td>
</tr>
<tr>
<td>Induce apoptosis in human myeloid HL-60 cells</td>
<td>Xu et al.; 2005</td>
</tr>
<tr>
<td>induce cleavage of receptor interacting protein (RIP) through activation of caspases during pancreatitis</td>
<td>Mareninova et al.; 2006</td>
</tr>
<tr>
<td>A cell-permeable, small molecular weight inhibitor of the X-chromosome-linked inhibitor-of-apoptosis protein (XIAP), an anti-apoptotic protein, through structure-based computational screening of a traditional herbal medicine three-dimensional structure database of 8221 individual traditional herbal products</td>
<td>Nikolovska-Coleska et al., 2004</td>
</tr>
<tr>
<td>Wound healing</td>
<td>Kumara et al.; 2007</td>
</tr>
<tr>
<td>Antibacterial activity against against <em>Staphylococcus aureus</em>, <em>Streptococcus pyogenes</em>, <em>Shigella flexneri</em>, <em>S. sonnei</em> and <em>Pseudomonas aeruginosa</em>; moderate against <em>Salmonella typhi</em>, <em>S. boydii</em> and <em>Proteus mirabilis</em></td>
<td>Chitra et al.; 2003</td>
</tr>
<tr>
<td>Free radicals scavenging activity</td>
<td>Joshi et al.; 2007</td>
</tr>
</tbody>
</table>
3.8.1. Thymoquinone:

Thymoquinone is an isoprenoid quinone obtained from the seeds of *Nigella sativa* Linnaeus variety hispidula (brachyloba) that belongs to the botanical family *Ranunculaceae*.

![Chemical Structure of Thymoquinone](image)

**Figure 3.3. Chemical Structure of Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone)**

**Physical properties:**
- Molecular formula: $C_{10}H_{12}O_2$
- Molecular weight: 164
- Melting point: 44-46°C
- Solubility: Soluble in hot organic solvents chloroform, benzene, light petroleum ether and acetone. Practically insoluble in water. Yellow prisms obtained from light petroleum ether.

**Uses:** Antiepileptic, anti-inflammatory and hepatoprotective

The interest in isolated Thymoquinone has been growing during the last years due to its diverse pharmacological activity such as hepatoprotective (Al-Gharably *et al.*, 1997; Nagi *et al.*, 1999), anti-convulsant (Hossein *et al.*, 2004), anti-inflammatory (Canonica *et al.*, 1963), anti-mycobacterial (Naito *et al.*, 1952) agent. The hepatoprotective effects of...
Thymoquinone have been well documented and have been found to be related to its strong antioxidant potential.

**Thymoquinone is isolated from seeds of *Nigella sativa***

3. 8.2. *Nigella sativa* seeds

*Nigella sativa* Linnaeus plant is an erect profusely branched herb that can attain heights of 40 and up to 70 cm. It bears alternate leaves, terminal white flowers and capsule like fruits. The latter are filled with black ovoid or pyramidal seeds attaining lengths and widths ranging from 2.5 to 3.5 mm and widths from 1.5 to 2 mm. respectively. The principle constituents of the seed are oil 31-35%, protein 16-19%, carbohydrate 33-34%, fibre 4.5-6.5%, moisture 5-7% etc. The major component is the fixed oil whereas the volatile oil constitutes 04-0.7% of the seeds’ weight. The volatile oil comprises of Thymoquinone upto 27.8%, Carvacrol 5.8-11.6%, p-cymene 15-31%, p-terpineol 4.6-6%, longifoline, t-anethole etc. (El Tahir and Bakeet., 2006)

**Uses in traditional medicine.**

The seeds of *Nigella sativa* L., commonly known as black seed, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases (Muhtasib *et al*., 2006).

3. 8.3. **Methods of Analysis of Thymoquinone:**

Pereira *et al.*; 2011 developed a validated HPTLC method for determination of Thymoquinone in *Nigella sativa* extracts, commercially available marketed oils, polyherbal formulations and in lipid-based oral and parenteral formulations prepared in-house. Analysis of thymoquinone was performed on TLC aluminium plates pre-coated with silica gel 60F-254. Linear ascending development was carried out in twin trough glass chamber, saturated with mobile phase consisting of toluene–cyclohexane (8 : 2, v/v) at ambient temperature. Camag TLC scanner III was used for the spectrodensitometric scanning and analysis in absorbance mode at 254 nm. The method was found to give compact spots for Thymoquinone with *R* value of 0.28.
Ghosheh et al.; 1999 developed a RP-HPLC method for quantifying the putative pharmacologically active constituents: thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ), and thymol (THY), in the oil of Nigella sativa seed. Extraction of the constituents from the oil was carried out using C18 PrepSep mini columns followed by quantification of the recovered constituents by HPLC on a reversed-phase muBondapak C18 analytical column, using an isocratic mobile phase of water:methanol:2-propanol (50:45:5% v/v) at a flow rate of 2 ml min\(^{-1}\). UV detection was at 254 nm for TQ, DTQ, and THY, and at 294 nm for THQ. The above four compounds were separated with good resolution, reproducibility, and sensitivity under these conditions.

3.8.4. Pharmacological actions of Thymoquinone:

<table>
<thead>
<tr>
<th>Action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity in carcinomas such as Ehrlich ascites cells, Dalton’s Lymphoma ascites cells and sarcoma</td>
<td>Aqel.; 1993</td>
</tr>
<tr>
<td>Anthelmintic effect against some cestodes such as Ascaris lumbricoidis, Taenia saginata or Hymenolepsis nana</td>
<td>Akhtar and Riffat.; 1991</td>
</tr>
<tr>
<td>Hepatoprotective effect against CCl(_4)-induced liver fibrosis and cirrhosis</td>
<td>Turkdogan et al.; 2000</td>
</tr>
<tr>
<td>spasmolytic and a bronchodilatory actions, respectively that involved a calcium channel blocking mechanism</td>
<td>Gilani et al.; 2001</td>
</tr>
<tr>
<td>lipid lowering and insulin sensitizing effect</td>
<td>Benhaddou et al.; 2004</td>
</tr>
<tr>
<td>Galactogogue effect</td>
<td>Agrawal et al.; 1971</td>
</tr>
<tr>
<td>Cytotoxicity against HEP-2 cancer cells</td>
<td>Rooney and Ryan.; 2005</td>
</tr>
</tbody>
</table>

Naphthoquinones:

Naphthoquinones are yellow or orange pigments essentially from plants and are characteristic of some Angiosperm families including Ebenaceae, Droseraceae and
Bignoniaceae. They are almost always 1,4- naphthoquinones and they are in very rare cases, 1,2-naphthoquinones. The most common substituent are hydroxyl and methyl groups, at C-2 on aromatic ring or both, they are sometimes prenylated (Pharmacognosy and Phytochemistry - Medicinal Plants, 1999).

3.9.1. Plumbagin

Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone, Fig. 3.4) is found in the roots of *Plumbago zeylanica* and several *Drosera* species. Plumbagin is found to occur in *Limonium carolinianum* (sea lavender), *Plumbago* (leadwort), *Plumbago capensis* (cape leadwort) or *P. auriculata, P. coerulea P. europaea, P. rosea, P. pulchella, P. scandens, P. zeylanica* (Ceylon leadwort), *Statice limonium* (English sea lavender). Plumbagin is also contained in members of the *Droseraceae* (Sundew) family. This family of insectivorous plants comprises some 105 species in four genera. *Drosera* L., the largest genus, is usually found in acid bogs. The following *Drosera* species have been reported to contain plumbagin: *D. anglica* Hudson, *D. auriculata* Backh., *D. binata* Labill., *D. capensis* L., *D. cistiflora* L., *D. indica* L. *D. intermedia* Hayne *D. longifolia* L.

![Figure 3.4. Chemical structure of Plumbagin (5-hydroxy-2-methylplumbagin)](image)

**Physical properties:**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C(<em>{11})H(</em>{8})O(_{3})</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>188.17</td>
</tr>
<tr>
<td>Melting point</td>
<td>78 -80°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>Freely soluble in chloroform, toluene, methanol and acetone, slightly soluble in hot water. Needle shaped crystals obtained from light petroleum ether.</td>
</tr>
</tbody>
</table>
Plumbagin is a yellow naphthoquinone pigment, which occurs in plant roots as a colorless combined form that can be processed to plumbagin by acid treatment. Plumbagin is currently of interest because of its activities such as anticancer, leshmanicidal, antimicrobial, anti-fungal, anti-feedant, anti-fertility, anti-mutagenic and insecticidal (Krishnaswamy and Purushothaman, 1980; Parimala and Sachdanandam, 1993; Kayser et al., 2000; Didry et al., 1994; Shin et al.; 2007; Tokunaga et al., 2004; Bhargava, 1984; Kubo et al., 1983). Vyas and Lal have found plumbagin to give fairly good results in early cases of leucoderma and baldness of head (Indian Materia Medica, 1954) Plumbagin is reported to be isolated by solvent extraction . Bothiraja et al. 2011.

3. 9. 2. Plumbagin is isolated from roots of Plumbago zeylanica.

Plumbago zeylanica L. roots

Plumbago zeylanica L. is a semi-climbing, perennial and sub-scandent shrub with semi woody stem and numerous branches that grows in tropical and subtropical regions of Asia, Australia and Africa. The dried roots as sold in market occur as cylindrical pieces of varying length and are less than 1.25 cm in thickness, reddish brown in color with fairly thick shriveled, smooth or irregularly fissured brittle bark marked with small projections representing scars of rootlets. Roots have a short fracture, an acrid and biting taste and disagreeable odor (Wealth of India, 2006; Ayurvedic Pharmacopoeia of India). The principle compounds present in the roots include Plumbagin (1), 3,3'-biplumbagin(2) plumbagic acid, (3) and its two glucosides viz. 3'-O-β-glucopyranosyl plumbagic acid(4) and 3'-O-β-glucopyranosyl plumbagic acid methyl ester (Lin et al., 2003; Dinda et al., 1998) 3,8-dihydroxy-6-methoxy-2-isopropyl-1,4-naphthoquinone and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone (Gupta et al., 1999). The dimers of plumbagin reported include chitranone(5) (Sankaram et al., 1976), maritinone (6) (Tezuka et al., 1973), elliptinone (7) and zeylanone (8) and isozeylanone (9)(Sankaram et al., 1979), isoshinanolone (10) (Gunaherath et al., 1983),methylen-3,3'-diplumbagin (Gunaherath and Gunatilaka, 1988) and 1,2(3)-tetrahydro-3,3'-biplumbagin (11) (Gunaherath et al., 1983) and its trimer plumbazeylanone (12), (Fig. 3.4)which is probably 5b,11a,12,12a-tetrahydro-1,7-dihydroxy-5b-(8-hydroxy-3-methyl-1,4-naphthoquinon-2-yl)-5a,12a-
dimethyl-5aH-dibenzo[b,h]fluorene-5,13:6,11-dquinone (Kamal et al., 1984). Chlorplumbagin though reported to be present in roots by Gunaherath et al., 1983 has conflicting reports about its presence as such or whether the compound reported was an artifact as presence of chloro compounds in plants are unlikely. A new class of furano-naphthoquinone has also been isolated from the roots of P. zeylanica which includes 2-isopropenyl-9-methoxy-1,8-di-oxa-dicyclopenta[b,g]naphthalene-4,10-dione, 9-hydroxy-2-isopropenyl-1,8-dioxo-dicyclopenta[b,g]naphthalene-4,10-dione, 2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxo-dicyclopenta[b,g]naphthalene-4,10-dione and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone which were characterized using chemical and spectroscopic techniques (Kishore et al., 2010). A new flavonoid 2-(2,4-dihydroxy-phenyl)-3,6,8-trihydroxy-chromen-4-one is also reported to be isolated from roots (Nile and Khobragade, 2010). Two new triterpenoids, 1-keto-3β,19α-dihydroxyurs-12-ene-24,28-dioicacid-di-methylester and its 3-O-β-D-arabinopyranosyl derivative were isolated from P. zeylanica (Gupta et al., 1998). Five coumarins viz. seselin (14), 5-methoxyseselin (14), suberosin (15), xanthyletin (16) and xanthoxyletin (17) are found to be present in roots of P. zeylanica. 1,2-benzendicarboxylicaciddiisooctyl ester (diisooctyl phthalate) is also reported to be isolated from roots (Gunaherath et al., 1983; Rahman and Anwar, 2006).
Figure 3.5. Some bioactive compounds from *Plumbago zeylanica*
Uses in Traditional medicine:

In Taiwan, the whole plant of *P. zeylanica* and its roots have been used as a folklore medicine for the treatment of rheumatic pain, dysmenorrhea, carbuncles, contusion of the extremities, ulcers and elimination of intestinal parasites (The Illustrated Medicinal Plants of Taiwan, 2003). In Africa, it is used in southwestern Nigerian folk medicine for parasitic diseases, scabies and ulcers (De Paiva *et al*., 2003). According to the Indian system of medicine the plant is used in sprue, malabsorption syndrome, piles and inflammatory diseases of anorectum (Ayurvedic Pharmacopoeia of India, 1999). Root is used as intestinal flora normalizer, it stimulates digestive processes and is also used for dyspepsia. Root paste is applied in order to open abscesses. Paste of roots prepared with milk, vinegar or salt and water, is used externally in treatment of leprosy and other obstinate skin diseases. A cold infusion is used for influenza and black water fever. In Siddha medicine, the plant is an ingredient of number of drug formulations for treating cancers of the uterus, breast, lungs and oral cavity, in addition to haemorrhoids. The tincture of the root bark is employed as antiperiodic. The root powder of *P. zeylanica* is an important ingredient of number of ayurvedic formulations used in treatment of flatulence, dyspepsia, piles, intermittent fevers chronic and muscular rheumatism. It is also used in treatment painful affections of the joints, epilepsy, hysteria, mania and other mental disorders. Root is used generally as a stimulant adjunct to other preparations in the form called ‘Trimada’ consisting of Plumbago root, Baberang seeds and tubers of *Cyperus rotundus*. Hakims use it in rheumatism and enlargement of the spleen. It acts as a powerful sodophoric (Encyclopedia of Indian Medicinal Plants, 2004). In Ethiopia, it is traditionally used for the treatment of wound, eczema, scabies, leishmania, leprosy and rheumatoid pain (Medicinal Plants and Enigmatic Health Practices of Northern Ethiopia, 1993). However, many of these tribal therapies are not supported by systematic ethnobotanical and ethnopharmacological research.

3.9.3 Extraction and Isolation of Plumbagin

**Solvent Extraction:** Bothiraja *et al*. 2011, have reported a method for extraction of Plumbagin from roots of *P. zeylanica*. In this method roots of *P. zeylanica* were cold
maceration with a mixture of chloroform/dichloromethane (1:1) and the extract was successively washed with water, saturated sodium bicarbonate and water. The concentrated washed extract was dissolved in n-hexane and recrystallized to get plumbagin. The cold maceration yielded 1.2% (w/w) fine crystalline orange needles of plumbagin.

3. 9. 4. Methods of Analysis of Plumbagin:

Babula et al., 2006, optimized the simultaneous analysis of the most commonly occurring naphthoquinones (1,4-naphthoquinone, lawsone, juglone and plumbagin) by high performance liquid chromatography coupled with diode array detector (HPLC-DAD). The most suitable chromatographic conditions were as follows: mobile phase: 0.1 mol l⁻¹ acetic acid: methanol in ratio of 33:67 (%, v/v), flow rate: 0.75 ml min⁻¹ and temperature: 42°C. The wavelength used for simultaneous determination of the naphthoquinones of interest was 260 nm. Naphthoquinones were separated on a reversed-phase Zorbax C18-AAA chromatographic column (150 mm×4.6 mm, 3.5 µm particle size, Agilent Technologies, USA) in an isocratic mode. Auto sampler injection was 5 µl. The Rt of plumbagin was 7.49 min.

Hsieh et al., 2005, developed an LC-MS method for analysis of Plumbagin. The crushed roots of *P. zeylanica* L. were ground from lumps to powder and boiled with H₂O, 50% EtOH, or 95% EtOH. Chromatographic separation of plumbagin from the herb was carried out using a ZORBAX Extend-C18 column (150×4.6 mm I.D.; 5µm) that was eluted with the mobile phase of water–methanol (10:90, v/v). Multiple reaction monitoring (MRM) was used to monitor the transition of the deprotonated molecule *m/z* 187 [M–H]⁻ to the product ion *m/z* 159 [M–H–CO]⁻ for plumbagin analysis. The limit of quantification was determined to and accuracy of 1 ng/ml.

Wang and Huang, 2005 developed a HPLC method for analysis of Plumbagin in *P. zeylanica* roots. The HPLC system consisted of a Hewlett-Packard Model 1100 system (Darmstadt, Germany), equipped with a multisolvent delivery system and an ultraviolet (UV) detector. The column was a LiChrospher®100RP18e, 5 µm, 4.0 mm internal diameter (i.d.) × 250mm (Merck, Darmstadt, Germany). The mobile phase was composed of water-methanol with gradient elution as follows: 0 min, 2:98; 10 min, 50:50; 30 min, 100:0; 40 min, 100:0. The mobile phase was filtered under vacuum through a 0.45µm
membrane filter before use. The flow rate was 1 ml/min with UV absorbance detection at 254 nm. The Rt of Plumbagin was 24 min.

Unikrishnan et al, 2008 have developed a HPTLC and HPLC method for estimation of Plumbagin in P. zeylanica and P. indica. Plates were developed in a TLC chamber previously saturated with the solvent system hexane: ethyl acetate (8:2). Development distance was 90 mm. Detection and quantification was performed with a Camag TLC Scanner 3 at 265 nm. The Rf of Plumbagin was found to be 0.65. The HPLC method was performed using a Shimadzu HPLC system (Kyoto, Japan) consisting of LC-10ATVP pump, a rheodyne injector, SPD M10AVP photodiode array detector and Class-VP 6.12 SP5 integration software was used for the analysis. The stationary phase was Phenomenex Luna C 18 (2) (250×4.6mm) column with 5 μm particle size with a C18 guard column (Phenomenex, 4×2.0 mm ID). The mobile phase consisting of methanol (HPLC grade, Merck) and sodium dihydrogen phosphate (5 mM) in the proportion (9:1 v/v) was used. The mobile phase was degassed by sonication before use. The column was equilibrated with the mobile phase for an hour and then pumped at the rate of 0.8 ml/min. Detection was done at 265 nm.

3. 9. 5. Pharmacological actions of Plumbagin:

<p>| Exhibits antifungal activity against all plant pathogenic fungi tested including Alternaria alternata, Aspergillus niger, Bipolaris oryzae, Fusarium oxysporum, Phytophthora capsici, Rhizoctonia solani, Rhizopus stolonifer var. stolonifer and Sclerotinia sclerotiorum. | Shin et al.; 2007 |
| anti-(H.) pylori activity, with 0.02–0.16 mg/ml as minimum inhibitory concentrations | Wang and Huang.;2005 |
| suppress the activation of NF-κB in tumor cells | Checker et al.; 2009 |
| suppresses STAT-3 activation through induction of protein tyrosine phosphate | Sandur et al.; 2010 |
| selectively induces apoptosis in prostrate cancer cells but not in immortalized non-tumorogenic prostate | Aziz et al.; 2008 |</p>
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<tr>
<th>Epithelial RWPE-1 cells</th>
<th>Inhibits invasion and migration of liver cancer HepG2 cells by decreasing production of matrix metalloproteinase-2 and urokinase-plasminogen activator</th>
<th>Shih et al.; 2009</th>
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<td>Radiosensitizing effects i.e. plumbagin in combination with 2 Gy of radiation was very effective in inducing apoptosis, when compared to a higher radiation dose of 10 Gy alone.</td>
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<td>Nair et al.; 2008</td>
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<td>Antihyperlipidimic activity</td>
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<td>Sharma et al.; 1991</td>
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<td>Anti-feedant activity against insectual pests (herbivores) as well as fungal pathogens</td>
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<td>Babula et al.; 2009</td>
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<td>Anti coagulant activity</td>
<td></td>
<td>Santhakumari, and Rathinam, 1978</td>
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<td>Antiprogestational activity in rats.</td>
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<td>Dhar and Rao, 1995</td>
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<td>Plumbagin is also shown to have a glucogenic effect.</td>
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<td>Olagunju et al.; 1999</td>
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### 3.10.1. Shikalkin

**Synonym**

Alkannin, shikonin

Alkannin/ Shikonin are isohexenylnaphthazarin pigments isolated from the roots of plants belonging to family Boraginaceae. Alkannin/Shikonin is present in roots of *Alkanna tinctoris*, *Arnebia nobilis* Rech. f., *Arnebia hispidissima*, *Arnebia densiflora*, *Alkanna frigida*, *Alkanna orientalis* and *Lithospermum erythroxylon* belonging to the family Boraginaceae (Papageorgiou et al., 2006, Singh et al., 2003; Yazdinezad et al., 2009, Bozan et al., 1997, Tawfik et al., 2007). Shikalkin has also been prepared synthetically by Lu et al., 2008, Pulley and Czako, 2004 and Couladouros et al., 1997.
Figure 3.6. Chemical structure of Alkannin and Shikonin

(±)-5,8-dihydroxy-2-(1-hydroxy-4-methyl-3-pentenyl)-1,4-naphthoquinone

**Physical properties:**

- **Molecular formula**: $\text{C}_{16}\text{H}_{16}\text{O}_{5}$
- **Molecular weight**: 288.10
- **Melting point**: 148°C
- **Solubility**: Soluble in chloroform, benzene, acetic acid and acetone. Soluble in alkali hydroxides. Practically insoluble in water and hydrochloric acid. Needle shape crystals obtain from hexane.

**Uses**: Colourant.

As important naturally occurring pigments that are widely distributed in nature which plays vital roles in the biochemistry of living cells and exert relevant biological activities, naphthoquinones exhibit cytostatic and antimicrobial activities. This may be due to their ability to act as potent inhibitors of electron transport, uncouplers of oxidative phosphorylation, intercalating agents in the DNA double helix, bioreductive alkylating agents of biomolecules, and producers of reactive oxygen radicals (Wu et al., 2005). Alkannin and shikonin (A/S) are potent pharmaceutical substances with a wide spectrum of biological properties and comprise the active ingredients of several pharmaceutical and cosmetic preparations, besides being used as food colorants (Assimopoulou et al., 2004). They are reported to possess anti-inflammatory, wound healing, antimicrobial,
immunostimulant activities, and anti-tumor activity (Papageorgiou et al., 1999, Papageorgiou et al., 2008).

3.10.2. Arnebia nobilis Rech. f. roots

Arnebia nobilis is herbaceous perennial, 20-50 cm in height, found in the western Himalayas from Kashmir to Kumaon at altitudes of 3000-4200 m. Leaves are oblong 12.5 cm x 0.8 cm in dimensions and flowers are purplish white to purple or brownish, present in compound cymes. It is dried roots appear as purple brown pieces of roots, rootstocks, 5-10 cm long and 3-6 cm in diameter, covered with several layers of thin scaly bark of same color. The root contains up to 0.1% alkannins, which are lipophilic isohexenylnaphthazarin red pigments; tannins and wax. An air dried market sample yielded 2.5% of a very crude viscous red dye which on chromatographic analysis gave seven naphthaquinones designated as arnebins and identified as: Arnebin-1 (alkannin β,β-dimethylacrylate; yield, 0.375%), arnebin-2 (β,β-dimethylacrylic ester of hydroxyalkannin; yield, 0.047%), arnebin-3 (alkannin acetate; yield, 0.075%), arnebin-4 (alkannin), arnebin-5 (C₁₆H₁₈O₅, mp 111-112°C), arnebin-6 (C₁₈H₂₀O₇, mp 88-90°C) and arnebin-7 [5,8-dihydroxy-2(4'-methylpent-3'-enyl)-1,4-naphthaquinone] (Khatoon et al., 2003).

Traditional uses of Arnebia nobilis roots.
Ratanjot is attributed to 8 species of Boraginaceae belonging to genera Alkanna, Arnebia, Maharanga and Onosma. The root and the rootstock which form the actual drug are considered to be anthelmintic, antiseptic and antipyretic. They are claimed to be useful in burns eczema, wounds and eruptions and are also used for treating diseases of eyes, bronchitis, abdominal pains, itch etc (Khatoon et al., 2003).

3. 10. 3. Extraction and Isoltion of Alkannin and Shikonin

Supercritical Fluid extraction: Akgun et al., 2011, have reported a method for extraction of Alkannin from Alkanna tinctoria using supercritical carbondioxide. A two step process was used; extraction of alkannin derivatives with supercritical CO₂ followed by alkaline hydrolysis of alkannin derivatives. A Box-Behnken experimental design was used to evaluate the effect of three variables, pressure (50–350 bar), temperature (30–80°C) and CO₂ flow (5–20 g min⁻¹) at 1:30 ratio of alkanna root:CO₂ amount. Optimum conditions were determined as 80 °C, 175 bar, 5 g min⁻¹ CO₂ flow yielding the highest total alkannins
(1.47%) which was higher than conventional hexane extraction (1.24%) providing a solvent-free alternative for industrial production.

**Rapid Solid Liquid Dynamic Extraction:** Sagratini *et al.*; 2008 have devised a method for extraction of naphthazarin pigments using Rapid solid liquid dynamic extractor. The process takes place in an extraction vessel specifically designed for the purpose. In detail, the extraction takes place during the passing of the system from a static phase, in which solvent and solid are under a pressure of 7 atm, to a dynamic phase, at atmospheric pressure, in which the rapid release of extracting liquid from the inside of a solid matrix transports mechanically the extractable compounds towards the outside. An extractive cycle includes a static phase (7 atm of pressure using nitrogen, without stirring) and a dynamic phase (atmospheric pressure, with mechanical stirring). RLSDE was performed at room temperature with 15 g of dried and pulverized roots of *O. echioides* (Ofena) and 500 mL of each extracting solvent. Each cycle was set by alternating static phases (120 s the first, then 30 sx 4) and dynamic phases (30 s x 5), for 6 h and 4 min as total time and a total number of 56 cycles. After the extraction process, the mixture was filtered on paper, evaporated under reduced pressure at 40ºC.

**High Speed Counter current Chromatography:** Assimopoulou *et al.*; 2009 developed a High-speed counter-current chromatographic method (HSCCC) for preparative isolation and purification of A/S and their esters from extracts of *Alkanna tinctoria* roots, as well as commercial samples. HSCCC experiments were carried out using a model CCC-1000 multilayer coil high-speed counter-current chromatograph (Pharma-Tech Research Corp., Maryland, USA) equipped with a multilayer coil (total capacity 305 mL). The solvent was pumped into the assembly and the speed of the apparatus was regulated at 1000 rpm. The coil rotates around its axes as it simultaneously revolves around a central axis, producing an efficient mixing of the two phases while retaining a sufficient amount of the stationary phase in the column. A manual injection valve with a low-pressure four-way valve (Rheodyne Inc., California, USA) and a 10 mL sample loop was used to introduce the sample into column for separation.
Complexation and Base hydrolysis: Assimopoulou et al.; 2009 have also developed a method for isolation of Alkannin and Shikonin. In this method the roots of *Alkanna tinctora* were extracted in n-hexane which was then evaporated and fractionated in methanol to remove wax. The naphthazarin pigments were chelated using copper acetate and filtered to wash of other impurities. The pigments were regenerated using dilute hydrochloric acid and extracted in hexane. The hexane extract was dried and resuspended in aqueous sodium hydroxide solution for generation of monomeric alkannin which was acidified and extracted to obtain pure alkannin.

3. 10. 4. Methods of Analysis for Shikalkin:

Bozan et al.; 1997 developed a HPLC procedure for quantitative determination of the alkannin derivative naphthaquinones, ILI3-dimethylacrylalkannin(I), teracrylalkannin (II) and isovalerylalkannin (III)+et-methyl-n-butylalkannin (IV), contained in *Arnebia densiflora* roots and extracts was introduced. This procedure, as the mainstay of the study, enabled separation of the compounds within only 12 min on a reversed-phase column. HPLC experiments were conducted using Ultracarb ODS C-20 (5 µm particle size, 25 cmX4.6 mm I.D., Phenomenex, USA) column with a flow-rate of 1 ml/min at ambient temperature. The mobile phase was methanol-water-formic acid (95:5:0.1). A Shimadzu SPD6 AV UV-Vis detector, set at 520 nm, was used. These alkannin derivatives were well separated with a Ultracarb ODS-20 column and eluted within 12 min. The peaks with retention times of about 5.7 min, 6.5 min and 9.0 min correspond to 13,13-dimethylacrylalkannin (I), teracrylalkannin (II) and isovaleryl alkannin (III)+et-methyl-n-butylalkannin (IV), respectively.

Bozan et al.; 1997 devised an improved HPLC procedure for quantitative determination of the alkannin derivative naphthaquinones, ILI3-dimethylacrylalkannin(I), teracrylalkannin (II) and isovalerylalkannin (III)+et-methyl-n-butylalkannin (IV), contained in *Arnebia densiflora* roots and extracts was introduced. Experiments were conducted using a Ultracarb ODS C-20 (5 µm particle size, 25 cm x 4.6 mm I.D., Phenomenex, USA) column with a flow-rate of 1 ml/min at ambient temperature. The mobile phase was methanol-water-formic acid (95:5:0.1). A Shimadzu SPD6 AV UV-Vis detector, set at 520 nm, was used.
Bozan et al.; 1999, developed a HPTLC method for estimation of ββ-dimethylacrylalkannin, teracrylalkannin and isovalerylalkannin α-methyl-n-butylalkannin in *Arnebia densiflora* roots. Silica gel 60 F254 20 x 20 cm, 0.2-mm thick, Merck plates were used. The mobile phase was n-hexane: Ethylacetate: Acetic acid:: 100:15:1. The mobile phase was allowed to run a distance of 100 mm in the saturated tank. Detection was carried out at 520 nm.

Papageorgeo et al.; 2002 developed a method utilizing size-exclusion chromatography (SEC) has been used for the first time for qualitative and quantitative analysis of monomeric and polymeric hydroxynaphthoquinone alkannin and shikonin derivatives. detector wavelength was set at 520 nm. The column used was a GPC 300 mm x 8 mm i. d. MZ-Gel SD plus column containing 10µm particles with 100 Å pores (MZ Analysentechnik, Mainz, Germany); separations were performed at ambient temperature. The mobile phase, THF (Merck, LiChrospher, HPLC grade) was degassed before use; mobile-phase flow rate was 0.5 mLmin⁻¹.

Assimopoulou and Papageorgiou, 2004 developed a chiral HPLC for qualitative and quantitative determination of the enantiomeric pair of A/S. Measurements were performed by means of a liquid chromatograph, equipped with a UV–Vis detector (Fasma 500), and a Marathon III HPLC pump (all from Rigas Labs, Thessaloniki, Greece), and software for process control an data handling (Chrom & Spec for Windows, Ampersand Ltd, Multi-Channel Chromatography Data Station, version 1.44a).The detector wavelength was set at 520 nm. The column used was Kromasil HPLC KR100-5CHI-TBB no. E6177 [packing CHI-TBB = o,o’-bis(4-tertbutylbenzoyl)-N,N'-diallyl- l-tartardiamide], obtained from EKA Chemicals (Bohus, Sweden), 250 x 4.6 mm; separations were performed at ambient temperature. The mobile phase hexane:2-propanol 90:10 (HPLC grade), was degassed before use; the mobile phase flow rate was adjusted to 0.4 mL/min, in order to obtain sufficient resolution of alkannin and shikonin peaks. The retention times of alkannin and shikonin under the experimental conditions applied were, respectively, 14.1 and 14.5 min.

Assimopoulou et al.; 2006, also carried out TLC analysis was performed for each of the hexane extracts of *Alkanna* species for preliminary identification of the constituents (TLC plates Kieselgel 60 F254). The elution solvent system was benzene: chloroform: acetone (50:50:1 v/v/v).The Rf of Alkannin was found to be 0.21.
Assimopoulou et al.; 2006, have developed a high performance liquid chromatography/photodiode array/mass spectrometry (HPLC/PDA/MS) method to establish the identity of monomeric hydroxynaphthoquinones of the alkannin series and other metabolites from Boraginaceous root extracts. Chromatographic separation was carried out on an XTerra C18 5 µm 3.0 × 250 mm HPLC column (Waters Co., USA) thermostatted at 40°C, by a gradient elution program, of Ammonium formate buffer: Acetonitrile: Water: 10:40:50 to 10: 90:0 in 45 min.

3.10.5. Pharmacological actions of Shikalkin

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<td><strong>Anti-oxidant and Free radical Scavenging</strong></td>
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<td><strong>active against W256 in rats and P388 lymphoid leukemia in mice</strong></td>
<td>Gupta and Mathur, 1972</td>
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