PART A
CHEMICAL INVESTIGATIONS OF *PIPER* SPP. AND
*KOELPINIA LINEARIS* PALL.
INTRODUCTION

1. Natural Products in Therapeutics

   Natural Products as drugs play a significant role in the pharmaceutical care. The terrestrial plants have played a dominant therapeutic role in the treatment of human ailments from time immemorial. There is a romance and mystique surrounding traditional remedies which is lacking from the white tablets and sophisticated techniques of modern medicine. Various active compounds from barks, leaves and roots and other parts of the plants are found in a new guise in the existing treatments, or may be used as basis for design of novel medicinal agents.

   In the last 20 years there has been a renewal of interest in the area of natural products. Many pharmaceutical companies now have separate divisions dedicated to natural product research. The naturally occurring compounds themselves may be introduced as new drugs, or these may be used as leads for synthesis of active compounds or as starting material for some therapeutically active compounds.

   A number of natural products were on the list of the top-selling drugs for 1994 and 1995.1 Out of the 25 top earning drugs in 1995, 12 were derived from the natural products.2 Natural products continue to be important in terms of the compounds introduced in the 1990’s. A number of natural product based drugs were introduced from 1990-1998.3-11 The plants comprise 80% of the medicaments used in the primary health care in the developing world. About 79% of the world population is reliant on the traditional medicines to some extent.12 Plant drugs represent about 25% of the prescription market in the United States13,14 and in 1990, this equated to a retail value of approximately $15.5 billion.15
A number of drugs obtained from ethnopharmacological leads have been a significant milestone in Western medicine. The current opportunity to exploit ethnopharmacology may not last long as the world’s biodiversity and the existing knowledge will be lost, partly because of climate change, urban expansion and destruction of habitats in making plants extinct, and also breakdown in traditional societal structures for transmission of this knowledge. The threat is particularly acute in cultures where information is passed on by spoken words rather than written.2

The isolation of opium alkaloids such as morphine in early 19th century herald the start of reductionist approach, whereby plant extracts were replaced by pure active compounds isolated from the plant. Important drugs introduced in this way include digitoxin, digoxin, quinine, cocaine, atropine, hyoscine, reserpine, physostigmine, colchicine, tubocurarine, camptothecin, vinblastine, vincristine, podophyllotoxin and more recently, paclitaxel and artemisinin. Approximately 120 drugs are obtained from plants, a large number of therapeutic activities are mediated by these drugs and a host of drugs currently in use are still obtained from plants only.16

A lot of work has been done on the natural product pharmaceuticals in the last decade of the 20th century. Nature is a rich source of compounds, which regulate cellular proliferation by binding to specific proteins required for cell division. Anticancer and antiinfective agents from natural sources have been reviewed recently.12,17,18,19 A number of microbial products have been evaluated as immunosuppressants20 and antifungal agents.21

Since there are 250,000 species of flowering plants and vast number of other living organisms, and only very small fraction (about 10%) of the plants has been screened till now, a lot is yet to be done in the field of natural products chemistry.
Keeping in view the vast potential of the natural products, it was decided to investigate some traditionally used plants chemically. *Piper cubeb*, a widely used plant in Ayurvedic and other traditional systems of medicine and *Koelpinia linearis*, a folklore medicine from Ladakh region of Jammu and Kashmir were selected for the investigations.

2. Review of the work done on *Piper* species by other workers

*Piper* is a very large genus of shrubs, rarely herbs and trees, belonging to the Piperaceae family. There are over 700 species of *Piper* found throughout the tropical and subtropical regions of the world.\(^{22}\) About thirty species have been reported in India, of which *P. nigrum*, the black pepper and *P. betel*, the ‘pan’ or betel are cultivated widely. A large number of species of this genus have been recorded as occurring wild in various tropical and subtropical parts of India. Some of them are used as substitutes or adulterants for the cultivated species like *P. nigrum* and *P. betel*.\(^{23}\) The *Piper* species have high commercial, economical and medicinal importance. The plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic System of medicine for their medicinal properties\(^{24}\) and in folklore medicine of Latin America and West Indies.

The *Piper* species have been widely investigated both phytochemically and biologically. These investigations have led to the isolation of a variety of biologically active compounds *viz.* alkaloids, amides, propylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, chalcones, dihydrochalcones, flavones and flavanones. The genus has been reviewed comprehensively for the phytoconstituents recently by Parmar *et al.*\(^{22}\), and by others.\(^{25,26}\)
2',6'-Dihydroxy-4'-methoxychalcone (1) isolated from *P. aduncum* has been reported to show *in vitro* activity against promastigotes and intracellular amastigotes of *Leishmania amazonensis*. The leaf extract of the plant also shows antifungal and antibacterial activity, and moderate wound healing activity. The leaf and seed extracts of *P. guineense* possess a depolarising neuromuscular blocking action. The extract of this plant on repeated treatment enhances spontaneous uterine muscle contraction in rats, justifying the usage of this plant in Nigerian traditional system of medicine for its oestrogenic and oxytocic properties.

The kavapyrones isolated from *P. methysticum*, commonly known as kava, are reported to have anticonvulsant, analgesic and centrally acting muscle relaxant properties. The constituent (+)-kavain (2) is reported to cause reduction of both Ca$^{2+}$ and Na$^+$ currents through voltage activated Na$^+$ and Ca$^{2+}$ channels. Gleitz et al. have reported COX inhibition by (+)-kavain resulting in suppression of TXA2 which...
induces aggregation of platelets and exocytosis of ATP by its binding on TXA2 receptor. The most popular application of kava is as natural anxiolytic, comparable to a number of prescription medications, including benzodiazepines. It also potentiates other medicines like barbiturates.34,35

The methanolic extract of *P. sarmosum* leaves shows a marked neuromuscular blocking activity36 and hypoglycemic activity.37 The ethanolic extract of *P. longum*, a commonly used ingredient of many Ayurvedic preparations, causes a significant increase in weight of lungs, spleen and reproductive organs. It also induces a significant increase in sperm motility and sperm count.38 The essential oil of *P. angustifolium* exhibits bacteriostatic and fungistatic activities against *Trichophyton mentagrophytes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus* and *Escherichia coli*.39

2.1 Review of work done on *Piper cubeb*

*Piper cubeb*, a liana-like climber, is native of Indonesia. It is cultivated in India, but fruits are mostly imported. The leaves are glabrous, ovate oblong with cordate or rounded base. The fruits are sub-globose, 6-8 mm in diameter, somewhat apiculate and stalked, hence are also known as tailed pepper. The pericarp is dusky red to slightly brown, rarely greyish in colour. This is a very variable species cultivated mostly in Java and Sumatra. It is also cultivated in India in Mysore and in Western ghats in Kerela. The fruits commonly called as cubebs possess a spicy odour and are somewhat bitter and acrid in taste. Fruits obtained from *P. clussi* DC and *P. guineense* DC are also used as substitutes; they are often called false cubebs and are obtained from Africa.
The most characteristic constituent of cubeb is the essential oil (oil of cubeb), the proportion of which varies from 5-20%. In addition, the fruits contain resinous matter (6.4-8.5%), gum, colouring matter, fixed oil, starch and nitrogenous substances. The oils distilled from true fruits are characterized by a pronounced laevo rotation and a high specific gravity, whereas most oils from spurious cubebs exhibit dextro rotation and a comparatively low specific gravity.

Cubebs are used as drugs and as spice. The powdered fruits are employed in the treatment of dysentery, catarrh and as an aromatic stimulant, local irritant, antiasthmatic, diuretic, carminative, sedative and in rheumatism. They have been used as an internal antiseptic in gonorrheal uretheritis and also as an expectorant and
stimulant to the bronchial mucus membrane. The most commonly used preparation is in form of cubeb oleoresin, which is prepared by extracting cubebs with alcohol or ether. Oil of cubebs is used for flavouring of bitters and cigarettes and as condiment.

The alcoholic extract of cubebs shows antibacterial activity against *Micrococcus pyogenes* var. *aureus*, while acetone extract was found to be effective as mosquito larvicide. Cubeb oil is reported to be effective against influenza virus and *Bacillus typhosus*. When taken by mouth it is found to exert antiseptic effect in urine. The Arabian and Persian physicians in the 11th century used this plant as internal antiseptic in gonorrhoeal infections. It is also used in the treatment of cataract.

The constituents of *P. cubeb* can be divided into amides, lignans, neolignans, terpenes and miscellaneous compounds. The Table 1 enlists various constituents isolated from *P. cubeb*.

**Table 1: Constituents of *Piper cubeb***

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Structure</th>
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<tr>
<td><strong>Amides</strong></td>
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<tr>
<td>Aschantin</td>
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<td>Compound</td>
<td>Structure</td>
<td>Page</td>
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<tr>
<td>(-)-Clusin</td>
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<td>(-)-Cubebin</td>
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<td>(-)-Cubebinin</td>
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<td>(β)-O-Ethylcubebin</td>
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<td>Compound</td>
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<tr>
<td>Heterotropane</td>
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<td>Sesamin</td>
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<td>(-)-Thujaplicatin trimethylene</td>
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<td>(-)-Yatein</td>
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<td>Neolignans</td>
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<table>
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<td>Bicyclosesquiphellandrene</td>
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<td>(+)-4-Carene</td>
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<td>1,8-Cineol</td>
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<tr>
<td>Compound</td>
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<td>Cubebol</td>
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<tr>
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<tr>
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<td>α-Phellandrene</td>
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<td>Sabinene</td>
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<td>α-Terpinene</td>
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<td>(+)-Crotepoxide</td>
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<td>Piperenol A</td>
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<td>Piperenol A-triacetate</td>
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<tr>
<td>Piperenol B</td>
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3. Review of the work done on *Koelpinia linearis* Pall. by other workers

*Koelpinia linearis* Pall. (Family Asteraceae, nom. alt. Compositae) (var. *vodar*) is a toxic alpine xerophyte growing in Ladakh region of Jammu and Kashmir in the Western Himalayan range at the height of 14,000 ft. It is an erect or decumbent, simple or branched annual herb. The plant is 15-30 cms tall. The leaves are mostly

![Figure 2: Koelpinia linearis Pall. plant](image-url)
radical, linear, entire, gradually narrowed at both ends. Flowers are yellow coloured, peduncled, bracts 5-7, narrow, subequal and dorsally spinescent. The plant flowers and fruits between June to August. The plant is distributed in Central Western Asia and North Africa. It is found in Kashmir, Ladakh, Lahul Spiti, Western Tibet, Rawalpindi and Peshawar area of Indian subcontinent on open sandy slopes.56-60

The plant is used ethnomedically in the Ladakh region of Kashmir for rheumatism. The major constituents of this plant are coumarins, cichrin, esculin and esculitin.61 Razdan et al.62 have reported that the plant is a rich source of triterpenoids and coumarins. Shah et al.63 isolated a sterol, 5β,24α-stigmasta-8,22-dien-3β-ol (3) from the methanolic extract of plant. They also reported the isolation of β-sitosterol and stigmasterol from the methanolic extract.
Some, unusual long chain alkanoic acid esters of lupeol (4), some of which carry odd carbon acid moiety, have been reported from methanolic extract of aerial parts of the plant.\textsuperscript{64, 65}

\[ \text{R}=\text{OCOC}_n\text{H}_{2n+1}; \; n = 13-17 \]

The occurrence of uneven carbon acids as esters in nature is not normal; fatty acids with even carbons are elaborated. The possible explanation for presence of such compounds in the plant is that biogenetic routes direct themselves in a different path under different ecological conditions. The dry and high altitude conditions, as in case of \textit{K. linearis}, and exposure to UV radiations can bring about certain photocatalysed reactions. The mono and sesquiterpenoids undergo drastic variation in sunlight and
most potent hydroxylated monoterpenoids which originate through same acetate mevalonate pathway, as fatty acids, are elaborated better during the dark hours while esterification dominates during the day light.\textsuperscript{66}

Three new seco-triterpenoids have been reported recently from the methanolic extract of the plant. Two of these compounds, 8,14-secours-8(26),12-dien-3\(\beta\)-ol (5)
and 8,14-secours-8(26),13-dien-3β-ol (6), have two carbon-carbon double bonds at different positions in the secoursane ring. A dihydroxy compound 8,14-seco-27-norurs-12-en-3β,20α-diol (7) has also been isolated.

Keeping in view the potential of the plant we decided to work further on it.

4. Research Envisaged

In view of changing scenario towards utilization of medicinal plants coupled with newer techniques that are available for characterization and evaluation of bioactive molecules, a lot of emphasis is being given currently to the studies of herbal drugs and folklore medicines. For the present studies *Piper cubeb* and *Koelpinia linearis* Pall. interested us.

*Piper cubeb*

The genus *Piper* belongs to the family *Piperaceae*, which includes four genera and about two thousand species. These are found throughout the tropical and subtropical regions of the world. The fruits of *Piper cubeb*, commonly know as “cubebs”, are used in indigenous systems of medicine. Arabian and Persian physicians in the eleventh century also used this plant as an internal antiseptic in gonorrhoeal infections. It is also used in the treatment of cataract, dysentery, and as an aromatic stimulant, local irritant, antiasthmatic, diuretic, carminative, sedative, and antirheumatic. Alcoholic extract of the fruits shows antibacterial activity against *Micrococcus pyogens* var. *aureus.* Amides isolated from *Piper* species are well known for their antibacterial, insecticidal, CNS, hepatoprotactive, antileishmanic and antiinflammatory activities. Recent studies have shown that piperine and allied compounds are excellent “bioavailability enhancers,” when given in admixture with some antitubercular drugs. Enhancement of blood levels of the drugs such as
rifampicin, phenytoin, pentobarbitone, theophylline, propranolol and dapsone have been recorded when these drugs were co-administered with piperine.

Lignans have been reported as potent anticancer compounds and their biogenetic precursors. A number of such compounds have already been found in *Piper cubeb* and other related species which bear a typical bisbenzyl butyrolactim system for example (-)-cubebin, (-)-hinkonin, (-)-clusin, and (-)-dihydrocubebin. Some of the lignans isolated from *Piper* species have been reported to be platelet activating factor antagonists. Cyclohexane epoxides such as pipoxide, isotepoxide, (+)-piperenol A, (+)-piperenol B, (+)-crotepoxide, and (+)-zeylenol, have been reported from different *Piper* species. These interesting molecules have analgesic and antitumour activity. Bicyclosesquiphellandrene and epibicyclosesquiphellandrene have also been isolated from *Piper cubeb*.

**Koelpinia linearis** Pall.

*Koelpinia linearis* Pall. is a toxic alpine xerophyte from Ladakh region of Jammu and Kashmir (India), which is rich a rich source of triterpenoids. It is used as folklore medicine in Ladakh in a number of ailments. So far very little work has been done on the plant is still unexplored.

The literature survey done on the above plants, namely, *Piper cubeb* and *Koelpinia linearis* Pall., reveals that the plants offer considerable scope for further studies on chemical constituents and exploring the biological potentials. It was envisaged to investigate further *Piper cubeb* for newer amides, lignans, and bicyclosesquiphellandrene and epibicyclosesquiphellandrene derivatives, and undertake the studies on their biological potentials. *Koelpinia linearis* is scarcely explored plant chemically, but offers a considerable biological potential. It was,
therefore, desired to carry out systematic investigation on *K. linearis* and test the constituents for biological activity.