Part-B

Comparative Study of Colchicine Bearing Plants
1. INTRODUCTION TO MEDICINAL PLANTS

The importance of plants in human affairs is immense and has been so since the dawn of mankind. The main uses of plants have been a source of food, shelter, oxidizable material for fuel and medicinal. Since diseases death and decay have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of human intellect. Primitive man must have employed the most easily available things as therapeutic agents - The plants.

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants” (Wealth of India). Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties.

During the past seven or eight decades, there has been rapid extension of the allopathic system of medical treatment in India. It generated a commercial demand for pharmacopoeial drugs and products in the country, thus efforts were made to introduce many of these drug plants into Indian agriculture, and studies on the cultivation practices were undertaken for thos plants which were found suitable and remunerative for commercial cultivation (Shulz, 2001). In general, agronomic practices for growing poppy, isabgol, senna, cinchona, ipecac, belladonna, ergot and a few others have been developed and there is now localized cultivation of these medicinal plants commercially.
Plants have formed the basis for traditional medicine systems, which have been used for thousand or years in countries such as China (Chang and But, 1986) and India (Kapoor, 1990). The use of plants in traditional medicine systems of many cultures has been extensively documented. These plant-based systems continue to play an essential role in health care and the World Health Organisation estimates that 80% or the world’s inhabitants continue to rely mainly on traditional medicines systems for their health care. Plant products also play an important role in health care systems of the remaining 20% of the population, mainly residing in developed countries. Analysis of the data on prescriptions dispensed from community pharmacies in the US from 1959 to 1980, indicates that 25% contained plant extracts or active principles derived from higher plants and at least 119 chemical substances, derived from 90 plant species, can be considered as important drugs currently in use in one or more countries (Farnsworth et al., 1985). Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine. In addition, the use of so-called complementary or alternative herbal products has expanded in recent decades (Gurib-Fakim, A., 2006).

The isolation of the anti-malarial drug, quinine from the bark of Cinchona species (e.g. C. officinalis) was reported in 1820 by the French pharmacists, Caventou and Pelletier. The bark had long been used by indigenous groups in the Amazon region for the treatment of fevers, and was first introduced into Europe in the early 1600s for the treatment of malaria (Gurib-Fakim, A., 2006).
1.2 Secondary plant metabolites in drug discovery

Although natural products, particularly secondary metabolites, have formed the basis of medicines and the presence of these compounds in the biochemistry of the plant is very often difficult to justify. It has been suggested that these compounds may have been synthesized by the plant as part of the defense system of the plant, e.g. plants are known to produce phytoalexins as a response to attack by bacteria and fungi. The presence of highly toxic natural products has also been highlighted in some animals namely the Amazonian frogs so as to deter predation by other animals. Whatever the reasons for the presence of these compounds in nature, they provide an invaluable resource that has been used to find new drug molecules. fig. 1 gives an indication of the development of new drugs from leads coming from natural products (Gurib-Fakim, A., 2006).

![Figure 1](image-url)
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Botanical name</th>
<th>Indigenous use</th>
<th>Biologically active compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Adhatoda vasica</em></td>
<td>antispasmodic, antiseptic, cough</td>
<td>vasicin (lead molecule for Bromhexin and Ambroxol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>supressant</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Catharanthus roseus</em></td>
<td>diabetes, fever, cancer chemotherapy</td>
<td>vincristine, vinblastine</td>
</tr>
<tr>
<td>3</td>
<td><em>Condrodendron tomentosum</em></td>
<td>arrow poison, muscular relaxation</td>
<td>D-tubocurarine</td>
</tr>
<tr>
<td>4</td>
<td><em>Gingko biloba</em></td>
<td>asthma, anthelmintic</td>
<td>Ginkgolides</td>
</tr>
<tr>
<td>5</td>
<td><em>Podophyllum paltatum</em></td>
<td>laxative, skin infections, cancer</td>
<td>podophyllotoxin and lignans</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chemotherapy</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Prunus africana</em></td>
<td>Laxative, prostate hyperplasia</td>
<td>Sitosterol</td>
</tr>
</tbody>
</table>

**Table 1.** Botanical drugs used in traditional medicine and which have given useful modern drugs.

The Indian Pharmacopoeia (1966) recognizes eighty five drug plants whose ingredients are used in various pharmaceutical preparations. We shall however, confine our study to comparative study of colchicine bearing plants by using different extraction techniques.

Colchicum extract was first described by Padanius Dioscorides, a Greek surgeon in the Roman Army in the first century BC as a treatment for gout in *De Materia Medica*. It’s an alkaloid first isolated in 1820 by the two French chemists P.S. Pelletier and J. Caventon (Pelletier and Caventon, 1820).
**SAR of colchicine**

![Chemical structures of colchicine and isocolchicine](image)

**Figure 2**

- Structure-activity studies reveal that the tri-methoxy benzene ring (A) and the methoxy tropane ring (C) of colchicine comprise the minimal structural features of the molecule needed for its high affinity binding to tubulin (Wallace *et al.*, 1991, Leighton *et al.*, 1991).

- Isocolchicine, a colchicine analog differing only in the relative position of the methoxy and carbonyl groups of ring C, is virtually inactive and is unable to inhibit tubulin assembly (Wallace *et al.*, 1991, Leighton *et al.*, 1991).

- The appropriate torsion angle (about 53 degrees) between rings A and C is required for tubulin binding ability (Quinn *et al.*, 1981).

- The acetamide on ring B can be replaced by other alkyl amides with retention of potency; however, the free amine has decreased antitubulin activity (Quinn *et al.*, 1981).

- On the C ring, demethylation of 10-methoxy to the 10-OH on ring C forms colchicine and destroys activity; however, replacement of the 10-methoxy with SCH₃ or NR₂ leads to increased potency (Quinn *et al.*, 1981).
1.3 Clinical uses of colchicine

**Gout** was also known as "the disease of kings" or "rich man's disease". It is represented as recurrent attacks of acute inflammatory arthritis, which involves the metatarsophalangeal joint of the great toe (podagra), in which there is a buildup of crystals from uric acid in joints of the body (Chen and Schumacher, 2008). Elevated levels of uric acid in urine can lead to uric-acid crystals precipitating in the kidney which may form kidney stones and lead to urate nephropathy (Tausche et al., 2009). The underlying cause of gout is hyperuricemia (Chen and Schumacher, 2008). Phagocytosis of urate crystals by human or rabbit neutrophils induced synthesis and release of a glycoprotein, CCF (crystal-induced chemotactic factor), which is chemotactically active both in vitro and in vivo. CCF has been proposed as a prime mediator of acute gouty attack. The anti-gout agent Colchicine has been shown to decrease the production and release of CCF in vitro (Spilberg et al., 1979). It, however, may cause stomach problems.
Acute pericarditis is a recurrent episode of pericardial inflammation (Carmichael et al., 1951; Connolly and Burchell, 1961). The accepted modalities for the disease include the non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressive agents, and pericardiectomy (Miller et al., 1982). Recently, results were reported from study on 51 patients who were treated with colchicine to prevent further relapses and who were followed up for more than 10 years (Adler et al., 1998).

Scleroderma- is a chronic autoimmune disease characterized by fibrosis, vascular alterations, and auto antibodies. There are no treatments available for scleroderma itself, rather individual organ system complications are treated (Gabrielli et al., 2009).

Anti-cancer- Anti-proliferative action of colchicine is the capacity of the tubulin-colchicine complex bound to the ends of microtubules to physically prevent the elongation of the microtubule polymer (Wallace et al., 1991; Niel and Scherrmann, 2006). Microtubules are main protein filaments that make up the cytoskeleton, which is crucial in regulation of many activities including cell migration, division, and polarization. Microtubule elongation stops, the mitotic spindle is disrupted, and cell division cannot proceed due to prevention of microtubule polymerisation. This effect makes colchicine effectively functions as a "mitotic poison" or spindle poison as tubulin availability is essential to mitosis. New thio-colchicine derivatives were designed as less toxic anticancer agent possessing the powerful anticancer activity of colchicine (Wallace et al., 1991).
1.4 Sources of Colchicine

The alkaloid colchicine among the Indian medicinal plants is contained in the corms of *Colchicum luteum* and the seeds of *Iphigenia*, to the extent of about 0.25% and 0.9% respectively (Kapadia et al., 1972). These plants are not available in sufficient quantities to warrant any commercial utilization. *Gloriosa superba* is another plant which also contains colchicine (Sarin et al., 1974). A mixture of alkaloids consisting mainly of colchicine has been isolated from dried tubers of *G. superba* (Clewer et al., 1915). Hence, *G. superba* acts as substitute plant of tropics to *Colchicum autumnale* for the alkaloid colchicine.

1.4.1 *Colchicum autumnale:*

Colchicine is a toxic natural product and secondary metabolite extracted from plants of the genus *Colchicum* commonly known as *autumn crocus*, wild saffron and naked lady (Folpini and Furfori, 1995). It is also known as “Meadow saffron”. It is known as “naked lady” due to the fact that flowers emerge from the ground long after the leaves have died back. Colchicine is an alkaloid that is antimitotic and act by blocking mitosis by preventing tubulin polymerization to microtubules. It is present in most parts of the temperate areas of Europe, Asia and America (Hartung, 1953). It is an alkaloid prepared from dried corms and seeds of *Colchicum autumnale*. It has been used as an antiparasitic agent in ethnoveterinary use (Coassini and Poldini, 1988). In addition to its use in the treatment of gout, colchicine is also used in the treatment of Behcet’s syndrome and some forms of psoriasis (Miyachi et al., 1981; Wahba and Cohen, 1980; Zachariae et al., 1982).
The corm contains an alkaloid demecolcine (colchamine) in addition to colchicine that is best known as an orally-administered drug in the treatment of chronic myeloid leukaemia (Jelliffe and Maciver, 1956).

1.4.2 Colchicum luteum:

They contain the toxic alkaloid 'colchicine' which is used externally to relieve pain. The dried corms of *C. luteum* contain around 0.25% of colchicine and the seed contain about 0.4% of colchicine. The corms have aphrodisiac, carminative and laxative property. They are used in India to treat gout, rheumatism and also diseases of the liver and spleen (Wallis, 2005). When grown from seed its plant can take 4 - 5 years to flower. All parts of *C. luteum*, but especially the bulb, are poisonous causing vomiting, violent purging, serious inflammation of the stomach and bowels, and death (Castro, M., 1990).

1.4.3 Iphegenia indica

The systematic study on genus *Iphegenia* was carried out in search for new commercial source for colchicine. Studies on *Iphegenia indica* (L.) by A. Gray had revealed that its seeds contain as much as 0.51% colchicine. A number of *Iphegenia* species occur around Puna (Kaul *et al.*, 1964). Their seeds were collected in the month of August (1970) and after thorough drying at room temp. (-25°C) were analysed for colchicine content (Santavy, 1892). Since there is considerable confusion about the correct identity and taxonomic status of the various species of *Iphegenia* growing in India as presented in the literature, few important characteristics of 4 species were studied (Hooker, 1892).
1.4.4 *Gloriosa superba*

Colchicine levels in *Gloriosa superba* corms have been reported to the level of around 0.9% (DM) (Finnie and Staden, 1991). It grows in Africa, India and southeastern Asia [200]. Earlier studies revealed that colchicine levels are the highest during the initial growth of plant, and these levels decline during maturation in *Gloriosa superba* (Thakur *et al.*., 1975). Among the Indian medicinal plants, the corms of *Colchicum luteum* and the seeds of *Iphigenia* contain colchicine, to the extent of about 0.25% and 0.9% respectively (Kapadia *et al.*, 1972). A mixture of alkaloids consisting mainly of colchicine from dried tubers of *Gloriosa superba* has been isolated (Sarin *et al.*, 1974). *Gloriosa* is a genus of five or six species in the plant family colchicaceae, from tropical Africa, India and South-eastern Asia. The determination of colchicine in pharmaceutical preparations, in biological fluids and in plant extracts have been described by several analytical methods (Fayyad *et al.*, 2002; Ondra *et al.*, 1995)

Three different methods (Santavy, 1892; Alali *et al.*, 2004, Hayashi *et al.*, 1988) of extraction of colchicine have been studied and the concentration was quantified using high performance liquid chromatography (HPLC) in six different species of *Gloriosa*.

Out of three different methods studied for the extraction of colchicine, it was found that extraction with petroleum ether and dichloromethane was the best and most reliable method. The amount of colchicine in six different species of *Gloriosa*, viz., *Gloriosa superba*, *Gloriosa rothchildiana*, *Gloriosa planti*, *Gloriosa lutea*, *Gloriosa casuariana* and *Gloriosa vuchuria*, has been determined using HPLC method. Of the six different
species, *Gloriosa planti* exhibited the highest level of colchicines, followed by *Gloriosa lutea, Gloriosa casuariana* and *Gloriosa superba*. 