INTRODUCTION
In the modern era of consumer conscious and consumer oriented market the quality of each and every item is gaining utmost importance, and quality assurance has become an integral part of all manufacturing practices. When it comes to pharmaceuticals, the quality becomes an indispensable factor for it has a direct bearing on efficacy and safety of the drug. Quality control of synthetic drugs is comparatively easy as these are single entities produced by well defined procedures, but it is not so in case of plant drugs which comprise a host of complex constituents elaborated by Nature's intricate reactions. It is for this reason that despite a flood of herbal drugs/formulations in the market their quality is not well defined, and consumers are totally on the mercy of manufacturers. With the increasing global interest in the revival of traditional systems of medicine, which mostly rely on herbal drugs, it has become obligatory on the part of all concerned to device ways and means to develop suitable standardization procedures with a view to produce quality herbal drugs.

About 80% of the world's population depends on traditional and indigenous medicines for their primary health care needs. The medicines used in various systems of traditional therapeutics viz., Chinese, Indian, and European are derived mainly from plants and plant products. A number of therapeutically active plants, plant products and their formulations have been incorporated in various Pharmacopoeias of the world, and many more are on their way to clinical trials. Plants and their formulations are usually complex in their composition, and are influenced by several factors such as age, geographical location and harvesting period of the medicinal plant involved.
Many a time medical research workers had a set back due to the use of unstandardized, controversial and unauthentic plant drugs, or plants collected in wrong season resulting in lack of potency of active constituents. It is important to use standardized plants, plant products and formulations for pharmacological studies and clinical trials.³

Many serious problems are confronted while developing standardization and quality control methods for herbal drugs, especially of multi-ingredient herbal formulations. The intrinsic value of the drug, i.e., the amount of therapeutically active ingredient(s), if known, must be specified. Pharmacognostic characters alone may not be adequate to ensure the quality of plant and plant products. Hence, standardization in terms of biological, chemical and physico-chemical standards is required. WHO Expert Committee on Specifications for Pharmaceutical Preparations observed the need to develop protocols for the quality control of plant material because of increasing demand and international trade in traditional medicines/natural products.⁴

1.1 Standardization of plant drugs

General protocols followed for the standardization of raw materials for herbs and herbal formulations include: authentication by detailed taxonomical studies, organoleptic evaluation, microscopical examination including quantitative microscopy, volatile matter, ash value, extractive value, chromatographic profile and quantitative estimation of marker component by various analytical methods. Analytical methods such as chromatographic (HPLC, TLC, GC etc.), spectroscopic (UV, IR, fluorescence etc.) and various chemical/physico-chemical (titrimetry, polarography etc.) are available for
standardization of plant drugs besides bioassay and pharmacognostical methods. Detection and determination of foreign matters such as soil, stones, insects, animal excreta and other non-drug plant materials, pesticide residue, heavy metals, microbial contamination and radioactive contamination are also included in the protocols.

1.2 Chromatographic methods

Certain analytical chromatographic techniques, especially HPLC\(^5\), TLC\(^5\) and GC\(^6\) are considered to be the most suitable for standardization and quality control of herbal drugs and extracts, and can be applied for both quantitative analysis of the bioactive ingredient(s) and qualitative analysis on the basis of chromatograms. These chromatographic techniques may be complementary to or substitute for one another. Each has its own merits and demerits with respect to accuracy, precision and reproducibility of assay. In general, chromatographic techniques are as selective as bioassays and immunoassays, and are quite sensitive and precise.

1.2.1 High performance liquid chromatography

HPLC has become very popular and well established technique for determining components for standardization and quality control of drugs in Pharmacopoeias of China, Japan, Britain and the United States of America.\(^7\) This technique is an alternative to bioassay for many drugs like oxytocin and insulin which can not be analysed by routine chemical methods,\(^8\) and a good correlation has been found between bioassay and HPLC methods.\(^9\) HPLC has been employed for the assay of some Chinese herbal preparations\(^10\) and for determining several undeclared drugs in herbal preparations.\(^11\) It has been used
extensively for qualitative as well as quantitative analysis of various phytoconstituents for quality control and standardization of plant drugs. Literature reveals that a large number of phytoconstituents have been analysed by HPLC in a variety of matrices like crude plant drugs, plant extracts, herbal formulations and various biological and clinical samples for the purpose of quality control, standardization, pharmacokinetics and toxicological studies. Some examples of analyses of phytoconstituents by HPLC include alkaloids in *Aconitum japonicum*, *Corydalis decumbens*, *Catharanthus roseus*, *Papaver somniferum*, tissue cultures and regenerates of *Cephaelis ipecacuanha*, lignans in *Phyllanthus amarus* and *Podophyllum* species, iridoids in *Gardenia jasminoides*, *Gentiana* species, *Swertia* species, *Valeriana* species, furanocoumarins in *Ammi visnaga* and *Peucedanum longifolia*; and glycosides in *Sapindus mukurosis*, *Anemone rivularis* and *Ginseng*.

1.2.2 Thin layer chromatography

TLC when compared with HPLC, has merits of being a relatively simple and inexpensive technique which can be applied for analysing even crude samples. It has been extensively used for identification and purity testing. Recent advances in instrumental TLC have enabled it to be used for quality control and standardization of natural products. TLC, supported by sophisticated instrumentation and computers, may be a substitute for HPLC in some applications. It has been used for standardization of *Rosmarinus officinalis* and its products and quality control of *Matricaria chamomilla* by qualitative identification of borneol and bornyl acetate. Five opium alkaloids have been analysed quantitatively on silica rods using the peak pyrolysis method.
method (TLC-FID)\textsuperscript{38} and qualitatively by routine TLC.\textsuperscript{39} TLC has been reported as one of the most important in-process quality control technique for developing methods for extraction, separation, quantification and standardization of constituents and their metabolites from various sources.\textsuperscript{40} Applications of TLC have been reviewed\textsuperscript{41-43} and TLC profiles of crude plant drugs have been evaluated, documented and compiled\textsuperscript{44,45}.

1.2.3 Gas chromatography

Both GC and HPLC have their own advantages, and one has to make selection of either technique based on physical and chemical properties of the material to be analysed. Volatile and low molecular weight samples are appropriately analysed by GC whereas polar and high molecular weight compounds by HPLC, though such compounds can also be analysed by pyrolysis-gas chromatography. Some examples of use of GC for plant drugs standardization include: determination of: securinine and allosecurinine in {	extit{Securinega}} species,\textsuperscript{46} essential oil components in {	extit{Ocimum basilicum}}\textsuperscript{47} and myristicin in {	extit{Daucus carota}}.\textsuperscript{48} Some plant species have been standardized on the basis of phenolic acid contents determined by GC.\textsuperscript{49}
Research envisaged

Standardization of drugs is indispensable for ensuring reproducibly uniform therapeutic efficacy. More than 2,500 plant species are in use as drugs in various traditional systems of medicine being practised over the globe. Unfortunately, standardized herbal drugs still remain a distant dream because of their complex and inconsistent composition attributable to a variety of factors viz. ontogenic, ecologic, harvesting time, drying method, etc. – a fact which limits their inclusion in the inventory of modern drugs, which because of scientific and sociopolitical backing, are the most accepted ones for use in clinical as well as veterinary practice. Till recently, very little inputs have been made in the direction of standardizing plant drugs both because of lack of proper facilities of handling complex mixtures of phytoconstituents and for want of information about the constituents responsible for therapeutic efficacy. With the advent of modern instrumentation and accumulating pharmacological data on the herbal drugs, it has now become possible to develop and devise methods of standardizing plant drugs in order to ensure uniformity of their efficacy, and make them acceptable to the drug control administration. Present investigations have been planned with this end in view, and attempt has been made to standardize selected herbal drugs with proven efficacy in traditional system of therapeutics. These drugs are: *Centella asiatica*, *Andrographis paniculata*, *Adhatoda vasica*, *Crataeva nurvala* and *Solanum surattense*. Methods have been established to standardize these plant drugs and their formulations on the basis of respective bioactive/major phytoconstituents employing modern analytical chromatographic techniques like HPLC and TLC densitometric scanning.
1.4 Literature review on the plants selected for the investigation

1.4.1 Centella asiatica

*Centella asiatica* Urban. (syn. *Hydrocotyl asiatica* Linn.; family Umbelliferae) is a prostrate perennial and faintly aromatic herb. The plant is popularly known as 'Mandukparni', however, in northern parts of India it is called Brahmi. The plant is common in India, Sri Lanka, Malaysia, Indonesia and other tropical and subtropical regions of the world. It grows in damp and shady places near banks of streams. Detailed pharmacognostic and histological studies of *C. asiatica* are well documented and reviewed.

1.4.1.1 Chemical constituents

The plant is rich in saponins, the most common and important being asiaticoside (1) and madecassoside (2). Asiaticoside was first isolated from the leaves of *C. asiatica* in 1941. Later, French workers isolated and hydrolysed it and obtained glucose, rhamnose and a triterpenoid aglycone, asiatic acid (3). Asiaticoside contains two molecules of glucose and one molecule of rhamnose attached to the carboxyl group by an unusual ester linkage. Chinese workers have also isolated asiaticoside from the plant and characterised it by mass and NMR spectroscopy. Asiatic acid has been reported as the main constituent of the plant collected from Madagaskar.

The structures of triterpenoidal trisaccharides, asiaticoside-A and asiaticoside-B (4) isolated from *C. asiatica* have been elucidated by spectroscopic analysis. Madecassic acid (5) has been isolated and characterized from Madagascarian variety of *C. asiatica*. Isolation and identification of asiaticoside and madecassoside from *C. asiatica* on the basis of NMR spectra,
physico-chemical properties and chemical reactions has also been done by Chinese workers. Madasiatic acid (6) (2α,3β,6β-trihydroxyurs-12-en-oic-acid) has also been reported in the plant from Madagascar.

Two saponins, brahminoside (7) and brahmoxide (8); two triterpenoid acids, brahmic acid and isobrahmic acid; betulic acid and stigmasterol have also been isolated from *C. asiatica*. The two saponins and the triterpenoid acids have been further examined, and the triterpenes reinvestigated. Brahmic acid and madecassic acid have been assigned similar chemical structure (5) (2α,3β,6β,23α-tetrahydroxyuro-12-en-28-oic acid) and designated as 6β-hydroxyasiatic acid. Similarly, madecassoside and asiaticoside-A have been assigned similar chemical structure (2) (O-α-L-rhamnopyranosyl(1→4)-O-β-D-glucopyranosyl(1→6)-O-β-D-glucopyranose ester of 2α,3β,6β,23α-tetrahydroxyurs-12-en-28-oic acid) and both may be named as 6β-hydroxyasiaticoside on the basis of chemical structure. Terminolic acid (9) is the aglycone of asiaticoside-B.

*C. asiatica* from Sri Lanka has been found to contain centic acid, centoic acid, centellic acid and centelloside. Indocentoic acid and indocentelloside have been reported from the Indian variety. Thankuniside with aglycone thankunic acid and isothankuniside with aglycone isothankunic acid have also been reported. The sugar components in both the cases were found to be glucose and rhamnose. Fourteen polyacetylenic compounds have been isolated from the underground parts of *C. asiatica*. A list of 21 terpenoids detected in the plant by gas chromatography has been reported. An alkaloid, hydrocotyline, two flavonoid glycosides, 3-glucosyl quercetin and
(1) R=H
(2) R=OH

(3) R=H
(5) R=OH

(6)

(7) R=Glc-Glc-Arb-Rha
(8) R=Rha-Glc-Arb
3-glucosyl kaempferol, and free amino acids have been reported from the plant. There is significant variation in glycosaponin contents of *C. asiatica* collected from different geographical locations within India and from other countries. Quantitative and qualitative analyses for asiaticoside and other saponins have been reported: quantitative TLC on glass powder and detection with anthrone reagent; chromatography coupled with colorimetric method; HPLC for asiatic acid in biological samples, and qualitative paper chromatography. A group of Chinese workers have used double developed TLC for quality control of the plant material on the basis of asiaticoside.

### 1.4.1.2 Pharmacology and clinical studies

*C. asiatica* enjoys considerable reputation as a medicinal herb. Its medicinal uses and therapeutic efficacy have been well reviewed. It has been used in various countries in their traditional systems of medicine. The plant is an official drug in the Dutch, French, Mexican, Spanish, Venezuelan pharmacopoeias and also in the Extra Pharmacopoeia.
The first clinical study made as early as 1904 has shown \textit{C. asiatica} to be useful in ameliorating the symptoms, and producing general improvement of leprotics.\cite{88} Asiaticoside\cite{89} and centelloside\cite{90} are reported as antileprotic. Asiaticoside and oxyasiaticoside inhibited \textit{in vitro} the growth of \textit{Mycobacterium tuberculosis}.\cite{91} Alcoholic extract of the plant has shown tranquilizing and sedative action.\cite{92,93} In anaesthetised dogs, it produced slight respiratory stimulation, hypotension and bradycardia.\cite{94} Antidepressant\cite{95} and antianxiety\cite{96} effects of \textit{C. asiatica} have also been noted in experimental animals. The herb has been found to produce significant intellectual improvement in mentally retarded children.\cite{97,98} The effect of the aqueous extract of \textit{C. asiatica} leaves on learning and memory was studied in albino rats and results were found significant.\cite{99} Improvement with total triterpenoid fraction of \textit{C. asiatica} in venous hypertensive patients has been observed.\cite{100-102} Post-phlebitic syndrome and venous insufficiency have also been treated successfully with the triterpenoid fraction of \textit{C. asiatica}.\cite{103,104}

In the Extra Pharmacopoeia, \textit{C. asiatica} has been mentioned under dermatological agents. Intramuscular injection or implants of asiaticoside in animals provoked local leucocytes and increased vascularization of tissue.\cite{105,106} Medicaments with cicatrisive and dermatropic properties based on a derivative of asiaticoside have been reported.\cite{107} Glycol extract of \textit{C. asiatica} along with some other ingredients has been incorporated in skin ointment.\cite{108} Asiaticoside as 1\% ointment or 2\% powder, or intramuscular injection of 25 mg hastened the healing of wounds without any significant side effect.\cite{109} Madecassol (a proprietary formulation containing asiaticoside, madecassoside and asiatic
acid) when applied locally on wounds in rats, promoted the proliferation of granulation and healing but had no therapeutic effect when administered orally. Contact dermatitis due to Madecassol has been observed. Complete recovery of trophic ulcer with extract of *C. asiatica* has been reported. It also inhibited gastric ulceration induced by cold and restraint stress in rats. Asiatic acid, madecassic acid and asiaticoside have been found to stimulate human collagen-I synthesis *in vitro*. A comparison of asiaticoside and asiatic acid shows that the sugar moiety of the molecule does not seem to be necessary for this biological activity. Treatment with *C. asiatica* provided relief to some common ailments of aged persons. The drug significantly increased serum IgM and IgG (immunoglobulins) indicating immunity enhancing properties. Alcoholic extract of the whole plant has exhibited antiprotozoal activity against *Entamoeba histolytica*. Narcotic analgesic activity of the plant has also been reported.

1.4.2 *Andrographis paniculata*

*Andrographis paniculata* Nees (family Acanthaceae) indigenously known as Kalmegh is an erect annual herb growing wild throughout India and neighbouring countries. The morphological and histological characters of *A. paniculata* have been described in detail.

1.4.2.1 Chemical constituents

Andrographolide (10), a bitter diterpenoid lactone, is the major and important constituent of *A. paniculata*. Nineteen fold increase in the content of andrographolide has been achieved by tissue culture techniques. Other diterpenoids reported in literature include: neoandrographolide (11);
14-deoxy-11-oxoandrographolide (12), 14-deoxy-11,12-didehydroandrographolide (13) and 14-deoxyandrographolide (14),\textsuperscript{124} 3,14-dideoxyandrographolide (15), 14-deoxyandrographiside (16) and andrographiside (17),\textsuperscript{125,127} andrographanan (18) and 14-deoxy-12-methoxyandrographolide (19).\textsuperscript{128} Bisaboloenoid lactones, paniculides A (20), B (21) and C (22) have been reported from tissue cultures of \textit{A. paniculata}.\textsuperscript{129} Seven other unidentified bitter constituents have also been isolated from the herb.\textsuperscript{130}

A flavanone glucoside, andrographidine A (23) and five flavone glucosides, andrographidine B (24), C (25), D (26), E (27) and F (28) have been isolated from the roots of \textit{A. paniculata}.\textsuperscript{131} Flavonoids isolated from the roots include andrographin (29), panicolin (30), 5-hydroxy-7,8,2',3'-tetramethoxyflavone (31), apigenin 4',7-dimethyl ether (32), 5-hydroxy-7,8-dimethoxyflavone (33),\textsuperscript{132,133} 5-hydroxy-7,8-dimethoxyflavanone (34) and 5-hydroxy-3,7,8,2'-tetramethoxyflavanone (35).\textsuperscript{134} Caffeic acid, chlorogenic
(13) $R_1 = R_2 = \text{OH}$
(14) $R_1 = R_2 = \text{OH}$
(15) $R_1 = \text{H}, R_2 = \text{OH}$
(16) $R_1 = \text{OH}, R_2 = \text{O-Glc}$

(17) $R_1 = R_2 = \text{H}$
(18) $R_1 = R_2 = \text{H}$
(19) $R_1 = \text{OH}, R_2 = \text{OMe}$
(20) $R_1 = \alpha-\text{H}, R_2 = \beta-\text{OH}, R_3 = \text{H}$
(21) $R_1 = \alpha-\text{H}, R_2 = \beta-\text{OH}, R_3 = \text{OH}$
(22) $R_1, R_2 = \text{O}, R_3 = \text{OH}$
(23) R1=R4=H, R2=O-Glc, R3=OH
(24) R1=R4=H, R2=O-Glc, R3=OH
(25) R1=R2=R3=H, R4=Glc
(26) R1=H, R2=R3=OMe, R4=Glc
(27) R1=R2=H, R3=OMe, R4=Glc
(28) R1=OH, R2=R3=OMe, R4=Glc
(29) R1=R2=H, R3=R4=OMe
(30) R1=R2=H, R3=OH, R4=OMe
(31) R1=H, R2=H, R3=OMe
(32) R1=OMe, R2=H, R3=R4=H
(33) R1=R2=R3=H, R4=OMe
(34) R1=R2=H
(35) R1=R2=OMe
acid, 135 carvacrol and eugenol 136 have also been reported from the herb. An iridoid glucoside, procumbide, has been isolated along with other earlier reported constituents from A. paniculata. 137

Recently, six new diterpenoids, 14-epi-andrographolide (36), isoandrographolide (37), 14-deoxy-12-methoxyandrographolide (38), 12-epi-14-deoxy-12-methoxyandrographolide (39), 14-deoxy-12-hydroxyandrographolide (40), 14-deoxy-11-hydroxyandrographolide (41) as well as two new diterpenoid glucosides, 14-deoxy-11,12-didehydroandrographiside (42) and 6'-acetylneoandrographolide (43), and four new diterpenoid dimers, bisandrographolides A (44), B (45), C (46) and D (47) have been isolated from the plant during investigation of cell differentiation inducers. 138 Various methods reported for the estimation of andrographolide in the plant materials include gravimetric, 139 titrimetric, 140,141 spectrophotometric, 142,143 TLC 144-146 and HPLC. 147

1.4.2.2 Pharmacology and clinical studies

A. paniculata has a very bitter taste, and has been used traditionally for the relief of general debility, dysentery, dyspepsia and liver disorders. The plant is an ingredient of 25 out of 40 commercial polyherbal formulations available in the Indian market for treatment of liver ailments. 148 Alcoholic extract of leaves of A. paniculata and andrographolide possess antihepatotoxic activity against carbon tetrachloride and alcohol. 149,150 Hepatoprotective activity of andrographolide has also been reported against galactosamine and paracetamol. 151 Similar experiments have further corroborated the hepatoprotective potential of andrographolide. 152-154 The aqueous extract of the
(36) R = α or β-OMe
(37) R = β or α-OMe
(38) R = α or β-OH
(39) R = β or α-OMe
(40) R = α or β-OH
herb was found to increase biliary flow and liver weight in rats and decrease the duration of hexabarbitone induced sleep in mice. Clinical studies using an aqueous decoction of *A. paniculata* has revealed its hepatoprotective activity. During screening, *A. paniculata* has been found to be the most active among various plants frequently used in herbal formulations for liver ailments.

The plant has also been reported to possess antifertility, antidiarrhoeal, antipyretic, antihepatitis B virus surface antigen, anti-inflammatory, antiplatelet aggregation and moderate anticomplementary activity. Recently, some of the compounds isolated from the plant have shown potent cell differentiation-inducing activity on mouse myeloid leukemia (M1) cells.

### Adhatoda vasica

*Adhatoda vasica* Nees (syn. *Adhatoda zeylanica* Medic.; family Acanthaceae), popularly known as Vasaka, is a small evergreen, subherbacious bush growing all over the plains and sub-Himalayan region of India and neighbouring countries. Microscopic and macroscopic characters of leaves and stem of the plant have been described in detail. An adulterant of Vasaka, *Ailanthus excelsa* Roxb, has been distinguished from the drug on the basis of comparative microscopical studies.

#### Chemical constituents

A pyrroloquinazoline alkaloid, vasicine (48) was isolated and characterized from *A. vasica*. Chemistry and pharmacology of vasicine
Other alkaloids isolated from the plant include vasicol (49),\textsuperscript{173} adhatonine (50),\textsuperscript{174} vasicinone (51),\textsuperscript{175,176} vasicinol (52),\textsuperscript{177,178} vasicinolone (53)\textsuperscript{179} and 1,2,3,9-tetrahydroxy-5-methoxypyrrolo-(2,1-b)-quinazoline-3-ol (5-methoxyvasicine) (54)\textsuperscript{180}. Two aliphatic hydroxyketones, 37-hydroxyhexatetracont-1-en-15-one and 37-hydroxyhentetracontan-19-one have also been isolated from \textit{A. vasica}.\textsuperscript{181}
Seasonal variation in various constituents, total alkaloids and vasicine has been reported. Total alkaloidal content is maximum (~2%) during the months of August-October and minimum (~0.5%) during February-March. Comparatively higher concentration of glycosides and N-oxides of vasicine occur during February-March. Variations in pharmacognostic characters and alkaloidal content in two types (BLV — tall and bushy, and SLV — comparatively shorter) of *A. vasica* have been discussed. Gibberelic acid and chloramphenicol have been found to increase total alkaloidal content in the plant. Vasicine has been estimated in plant material by gravimetric, titrimetric, and spectrophotometric methods.

1.4.3.2 Pharmacology and clinical studies

The extract and juice of *A. vasica* leaves have been used traditionally for the treatment of bronchitis, asthma, bleeding piles, pyorrhoea and tuberculosis. The bronchodilatory and expectorant properties of the leaves of *A. vasica* have been attributed to vasicine. Inhalation of fumes of *A. vasica* leaves has shown encouraging results in the treatment of bronchial asthma. Expectorant effect of bromhexine and its derivatives from *A. vasica* have been investigated, and the findings suggest that these compounds stimulate secretory activities of cells. Wintry, a proprietary formulation containing vasicine and vasicinone from leaves of the plant has shown promising results in double blind clinical trials. Abortifacient activity of *A. vasica* has been demonstrated clinically and in experimental animals. Significant antimicrobial activity of the plant against gingival inflammation and pyorrhoea has also been reported. The plant has been evaluated as wound healing agent for veterinary use.
1.4.4 *Crataeva nurvala*

*Crataeva nurvala* Buch. Ham. (syn. *Crataeva religiosa* Hook. f. & Thomas. non Forst. f.; family Capparaceae), indigenously known as Varun, is a moderate sized deciduous evergreen tree growing widely in all parts of India and cultivated in the vicinity of temples. The macroscopic and microscopic characteristics of leaves, roots and stem bark of the tree have been reviewed. The salient diagnostic microscopic characters and chemical tests of authentic and commercial samples of the bark have been illustrated. Traditional use, chemical constituents, pharmacology and reports on clinical studies for antiurolithiatic activity of *C. nurvala* have been reviewed.

1.4.4.1 Chemical constituents

Stem bark of *C. nurvala* contains lupeol (55) as the major constituent along with other minor constituents like alkaloids cadabacine (56) and cadabacine diacetate (57), flavonoids (−)-catechin (58), (−)-epiafzelechin-5-glucoside (59) and (−)-epiafzelechin (60), diosgenin (61), ceryl and cetyl alcohols, glucocapparin (isothiocyanate glucoside). Maximum (2.2%) and minimum (0.7%) concentration of glucocapparin has been noticed in the seed and root samples respectively. Lupeol, lupeol acetate, α-spinasterol acetate, ψ-taraxasterol (63), 3-epilupeol (64), β-sitosterol as the major constituents and lupenone, β-sitosterol acetate as the minor constituents along with sugars, aminoacids, rutin and quercetin have been isolated from root bark of the plant.
1.4.2 Pharmacology and clinical studies

The stem bark of *C. nurvala* has been used in the indigenous system of medicine in India for various urinary disorders. Clinical studies on aqueous decoction of the stem bark of *C. nurvala* have shown it to be effective in the management of urolithiasis.\(^{217}\) It has also been found to be effective in experimentally induced urolithiasis\(^{218}\) but has not produced any effect in isolated duodenum of experimental albino rats.\(^{219}\) Treatment with *C. nurvala* bark decoction has lowered the levels of Na\(^+\), K\(^+\) and ATPases of small intestine of experimental rats fed on calculi-producing diet.\(^{220}\) Ethanol extract of the bark has been reported to possess significant antilithiatic activity in experimentally induced calculi in rats,\(^{221,222}\) and antifertility\(^{223}\) and antiinflammatory\(^{224}\) activities. Lithiatriptic property of the stem bark has been attributed to lupeol.\(^{225}\) Dried aqueous decoction of the stem bark of *C. nurvala* has been put to clinical trials by the Indian Council of Medical Research (ICMR) with a view to assess its antiurolithiatic activity.

1.4.5 Solanum surattense

*Solanum surattense* Burm. F. (syn. *Solanum xanthocarpum* Schrad. & Wendl.; family Solanaceae), a perennial herb, is distributed throughout India, Pakistan, Sri Lanka and other neighbouring countries. Pharmacognostic characteristics of the herb have been reported.\(^{226}\)

1.4.5.1 Chemical constituents

Like other non-tuberous *Solanum* species, *S. surattense* contains glycoalkaloids and is a well known source for steroidal alkaloid solasodine (65).\(^{227-229}\) From the fruits of *S. surattense*, cycloartanol (66),
cycloartenol (67), sitosterol (68), stigmasterol (69), campesterol (70), cholesterol (71), sitosteryl glucoside (72), stigmasteryl glucoside (73), solamargine (74), β-solamargine (75) and 4α-methyl-24-ethylcholest-7-en-3β-ol have been isolated and characterized besides solasonine (76) and solasodine as reported earlier.\textsuperscript{230}

Lupeol has been isolated from tissue cultures of \textit{S. surattense}.\textsuperscript{231} Norcarpesterol\textsuperscript{232} and five new steroidal compounds\textsuperscript{233}: 4α-methyl-24ξ-ethyl-5α-cholest-7-en-3β,22ξ-diol, 3β,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one, 3β-benzoxy-14β,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one, 3β-benzoxy-14α,22ξ-dihydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one and 3β-(p-hydroxy)-benzoxy-22ξ-hydroxy-4α-methyl-24ξ-ethyl-5α-cholest-7-en-6-one have been isolated and characterized from the plant. Coumarins have also been reported from the plant.\textsuperscript{234} Dried plant gives 10.8% ash consisting mainly of potassium nitrate, carbonate and sulphate.\textsuperscript{226} Suspension tissue cultures of \textit{S. surattense} yielded diosgenin and solasodine\textsuperscript{235} and showed many fold increase in solasodine content when grown in RT media with cholesterol.\textsuperscript{236} \textsuperscript{13}C-NMR spectroscopy of solasodine glycosides and other glycoalkaloids has been reported.\textsuperscript{237,238} Seeds of \textit{S. surattense} are rich in essential amino acids.\textsuperscript{239,240} Various methods for the estimation of solasodine content in different \textit{Solanum} species include: gravimetric,\textsuperscript{241,242} titrimetric,\textsuperscript{243} spectrophotometric,\textsuperscript{244,245} TLC,\textsuperscript{246} GC,\textsuperscript{247} HPLC\textsuperscript{248,250} and enzymatic.\textsuperscript{251}
(65) R=H
(66) R=Me
(67) \( \Delta 22 \)
(68) R=H
(69) R=H, \( \Delta 22 \)
(70) R=Glc
(71) R'=Glc, \( \Delta 22 \)
(72) R=a-L-Rha-(1-2-Glc)-a-L-Rha-(1-4-Glc)-p-D-Glc
(73) R=a-L-Rha-(1-2-Gal)-(1-D-Glc-(1-3-Gal)-\( \beta \)-D-Gal
(74) R=a-L-Rha-(1-2-Glc)-a-L-Rha-(1-4-Glc)-\( \beta \)-D-Glc
(75) R=a-L-Rha-(1-2-Gal)-\( \beta \)-D-Glc-(1-3-Gal)-\( \beta \)-D-Gal
(76) R=a-L-Rha-\( \beta \)-D-Glc
1.4.5.2 Pharmacology and clinical studies

*S. surattense* is popular in the traditional therapeutics as an antiasthmatic, expectorant, antiphlogistic and diuretic.\(^{252,253}\) Clinical efficacy of the dried aqueous decoction prepared from whole plant is under clinical investigation by ICMR for the treatment of bronchial asthma. Preliminary clinical trials of *S. surattense* have shown significant improvement in some respiratory diseases.\(^{254}\) Both glycoalkaloid and fatty acid fractions from the extract of the plant caused liberation of histamine from chopped lung tissue. Beneficial effect of the drug on bronchial asthma may be attributed to the depletion of histamine level.\(^{226}\) Fatty oil isolated from the seeds of *S. surattense* showed significant antifungal activity.\(^{255}\) Alcoholic extract of *S. surattense* seeds has shown spermicidal activity in experimental rats.\(^{256}\) Solasodine has been reported to possess antispermatogenic activity.\(^{257-259}\) Inhibition of proliferation of murine spleen cell cultures points to immunomodulatory potential of solasodine.\(^{260}\) Hypocholesterolaemic and antiatherosclerotic effects of solasodine in cholesterol fed rabbits have been observed.\(^{261}\) The plant has also shown antipyretic activity.\(^{160}\)