# CHAPTER II

# LITERATURE REVIEW

## 2.1 DNA topoisomerases

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology (Osheroff, 1998). The first DNA topoisomerase was discovered by Dr. James Wang in 1971, from *Escherichia coli* (Wang, 1996). Topoisomerases control DNA structure by relaxing DNA supercoils and by resolving intertwined DNA strands as they arise during DNA transactions, particularly cell division (Wang, 1996). This is possible because DNA topoisomerases generate transient DNA strand breaks. There are two classes of topoisomerase, know as type I and type II enzyme. Type I topoisomerase acts by making a transient single-stranded break in DNA and allowing controlled rotation about the nick. In contrast, type II topoisomerase acts by making a transient double-stranded break in DNA and passing a separate double-stranded molecule through the break (Osheroff, 1998).

Every cell type so far examined contains DNA topoisomerases, and where a genetic test has been possible, at least one is essential for cell growth. Examples of the type of organisms in which topoisomerases have been studied include the bacteria *E. coli* and *Staphylococcus aureus*, yeast, the model plant *Arabidopsis*, *Drosophila*, and man. In addition, several viruses are known to encode a topoisomerase, for example, bacteriophage T4 and animal virus *vaccinia* (Bates and Maxwell, 2005). Examples of DNA topoisomerases are given in Table 2.1.

Table 2.1 Type of DNA topoisomerases (Bates and Maxwell, 2005).

| Enzyme | Type | Source | Subunit size (kDa) and composition | Remarks |
|---|---|---|---|---|
| Bacterial topoisomerase I (ω protein) | IA | Bacteria (e.g. *E. coli*) | 97 Monomer | Cannot relax positive supercoils |
| Eukaryotic topoisomerase I | IB | Eukaryotes (e.g. human ) | 97 Monomer | Can relax both positive and negative supercoils |
| *Vaccinia* virus topoisomerase I | IB | *Vaccinia* virus | 37 Monomer | ATP stimulates topoisomerase activity |
| Topoisomerase III[a] | IA | Bacteria (e.g. *E. coli*) | 73 Monomer | Potent decatenating activity |
| Reverse gyase | IA | Thermophilic Archaea (e.g. *Sulfolobus acidocaldarius*) | 143 Monomer | Can introduce positive supercoils into DNA (ATP-dependent) |
| DNA gyrase | IIA | Bacteria (e.g. *E. coli*) | 97 and 90 $A_2B_2$ | Can introduce negative supercoils into DNA (ATP-dependent) |
| T4 topoisomerase | IIA | Bacteriophage T4 | 58, 51, and 18 2 copies of each subunit | Can relax, but not supercoil, DNA (ATP-dependent) |
| Eukaryotic topoisomerase II | IIA | Eukaryotes (e.g. human topoisomerase IIα) | 174 Homodimer | Can relax, but not supercoil, DNA (ATP-dependent) |
| Topoisomerase IV[a] | IIA | Bacteria (e.g. *E. coli*) | 84 and 70 $C_2E_2$ | Can relax, but not supercoil, DNA, potent decatenase (ATP-dependent) |
| Topoisomerase VI | IIB | Archaea (e.g. *Sulfolobus acidocaldarius*) | 45 and 60 $A_2B_2$ | Can relax, but not supercoil, DNA (ATP-dependent) |

[a] Note that topoisomerase III and IV do not represent 'type' of topoisomerase mechanism.

## 2.2 Topoisomerase I

### 2.2.1 Structure of DNA topoisomerase I

Topoisomerase I (top1) enzymes share a common organization with four domains: the amino terminal, the core domain, the linker region, and the C-terminal domain (Pourquier and Pommier, 2001). (Figure 2.1) The N-terminal domain contains putative signals for the enzyme's nuclear localization. The core domain is essential for the relaxation of supercoiled DNA; it shows a high phylogenetic conservation, particularly in the residues closely interacting with the double helix. The C-terminal domain contains the active site enzyme tyrosine, which forms a transiently covalent phosphodiester bond between the enzyme and the DNA (González *et al.*, 2007).

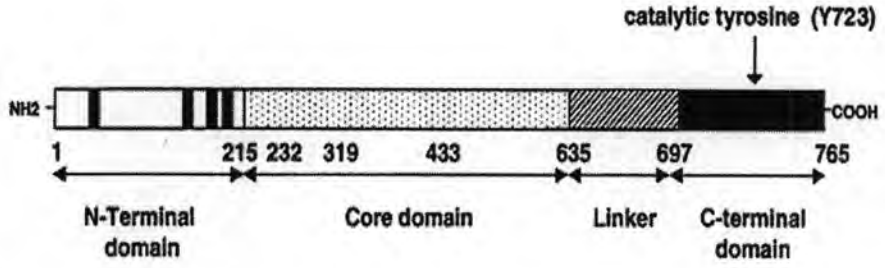



**Figure 2.1** Domain organization of human topoisomerase I (modify from Pourquier and Pommier, 2001).

### 2.2.2 Mechanism of action of topoisomerase I

Type I topoisomerases catalyze topological changes in duplex DNA by reversibly nicking one strand. Topoisomerase I conserve phosphodiester bond energy during catalysis by transiently forming a phosphotyrosine bond between the active-site tyrosine residue and the phosphate at one end of broken strand (Roca, 1995). Cleavage of one DNA strand with covalent attachment of top1 to the 5′ terminus of the nicked DNA leads to a top1 covalent complex (cleavage complex). Strand scission occurs through a trans-esterification in which a tyrosine hydroxyl group of top1 is linked to the 3′ phosphate of a phosphodiester bond, liberating the 5′ hydroxyl to generate a strand break (Pommier *et al.*, 1998) (Figure 1.1). This processive reaction dose not require ATP or divalent metal

binding, which is different from the case of topoisomerase II enzymes, which require both ATP hydrolysis and $Mg^{2+}$ (Pommier, 2009).

### 2.2.3 Inhibitors of topoisomerase I

Topoisomerase I inhibitors can be grouped into two main class; top1 poisons and top1 suppressors. Both inhibit catalytic activity (DNA relaxation), but top1 poisons trap cleavage complexes, while top1 suppressors inhibit the formation of cleavage complexes (Pommier *et al.*, 1998).

Camptothecin (CPT) (Figure 2.2) is a cytotoxic tryptophan-derived quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (top1). It was discovered in 1966 by M. E. Wall and M. C. Wani in systematic screening of natural products for anticancer drugs. It was isolated from the bark and stem of *Camptotheca acuminata* (Camptotheca, Happy tree), a tree native in China (Wall *et al.*, 1966). CPT and its derivatives are topoisomerase I-targeted drugs. They stabilized the cleavage complex. DNA replication forks collide with CPT stabilized enzyme-DNA intermediates producing DNA lesions that result in cell death (Wall and Wani, 1996). CPT induces top1-linked DNA breaks by preventing DNA re-ligation under physiological conditions, and it does not bind top1 alone (Lorence and Nessler, 2004). (Figure 2.3)

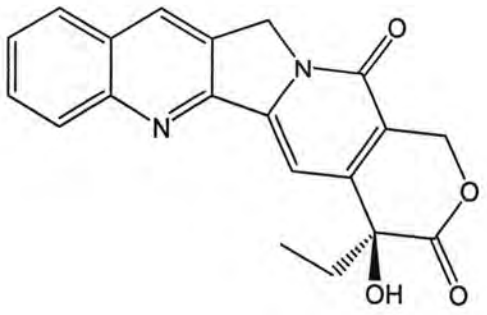



**Figure 2.2** Chemical structure of camptothecin.

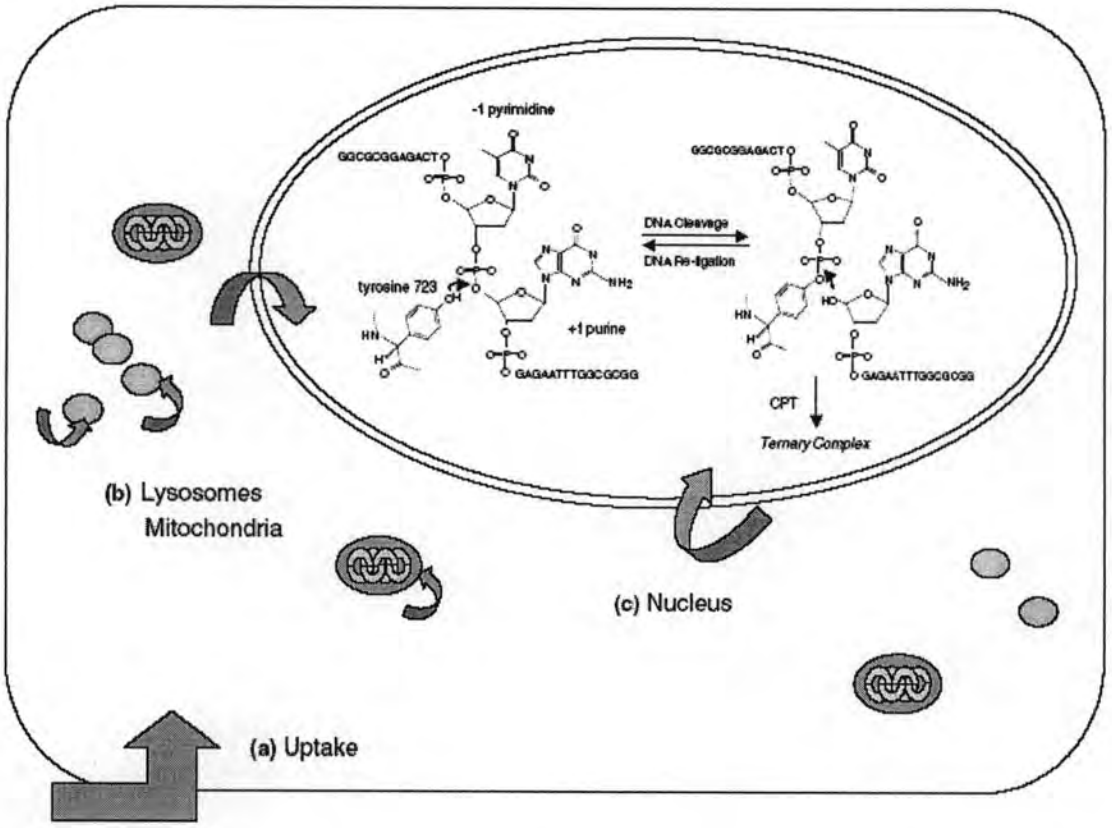



**Figure 2.3** Mechanism of action of CPT. (a) uptake, (b) lysosomal or mitochondrial sequestration, and (c) nuclear localization and stabilization of the cleavable complex (Lorence and Nessler, 2004).

### 2.2.4 Topoisomerase I as drug targeted for anticancer

Topoisomerase I is essential for cell growth. As a consequence, this enzyme is potential targets for anticancer drugs (Pommier *et al.*, 1998). Topoisomerase I-targeted drugs kill cancer cells in a unique and insidious fashion. Rather than robbing the cell essential enzyme functions, it increases the concentration of covalent topoisomerase I-cleaved DNA complex that is requisite, but fleeting intermediates in the catalytic cycle of this enzyme. As a result of this action, drug poison topoisomerase I and convert it to potent cellular toxin that generate breaks in the genetic material to treated cells (Osheroff, 1998).

## 2.3 Yeast cell-based assay

The budding yeast *Saccharomyces cerevisiae* has been a valuable model in establishing eukaryotic DNA topoisomerase I as the cellular target of specific antineoplastic agent including camptothecin, aclacinomycin A, and R-3, a rebeccamycin analogue (Osheroff and Bjornsti, 2001). This genetically tractable eukaryote has also been useful in study mechanisms of resistance to DNA topoisomerase I-targeted drugs and providing information about drug function, cell cycle specificity, mutations affecting drug sensitivity, and the cellular consequences of drug treatment (Nitiss and Wang, 1988; Bjornsti *et al.*, 1989; Reid, Benedetti, and Bjornsti, 1998).

### 2.3.1 Yeast: *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* is a species of budding yeast. *S. cerevisiae* cells are round to ovoid, 5–10 micrometres in diameter. It reproduces by a division process known as budding. It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model prokaryote (Wikipedia, 2009). Yeast cell can also be propagated as heterothallic a and $\alpha$ haploids or a/$\alpha$ diploids, produced by the fusion of haploid strains of opposite mating type. Under conditions of nutrient deprivation, diploids are induced to undergo meiosis, and the four spore products of a single meiosis can be recovered, grown out and analyzed (Reid *et al.*, 1998). The most remarkable aspect of yeast as a genetic system is that the sequence of the entire genome is known and this information is readily available in annotated DNA and protein database (http://genome-www.stanford.edu/Saccharomyces/).

### 2.3.2 Yeast DNA topoisomerases

*S. cerevisiae* contains two types of DNA topoisomerases, type I DNA topoisomerases and type II DNA topoisomerase. As in human cells, type I enzymes transiently break a single strand of DNA duplex and type II enzymes cleave both strands of duplex DNA. Yeast contains two type I DNA topoisomerases, DNA topoisomerase I and DNA topoisomerase III. Yeast topoisomerase I is characteristic of other eukaryotic type I

enzymes in that it relaxes both positively and negatively supercoiled DNA (Reid *et al.*, 1998). However, in yeast the topoisomerase I gene is nonessential, presumably because in this single-celled organism, DNA topoisomerase II can compensate for the loss of DNA topoisomerase I (Bjornsti, Knab, and Benedetti, 1994). Topoisomerase III shares homology with the prokaryotic type I enzymes relaxing negatively but not positively supercoiled DNA (Reid *et al.*, 1998).

### 2.3.3 DNA topoisomerase I poisons in yeast

Topoisomerase I enzyme is the only topoisomerase that form a covalent link with the 3′-end of broken DNA while generating a 5′-hydroxyl end at the other end of break. In that respect, the eukaryotic top1 enzymes belong to the broader family of site-specific tyrosine recombinases of prokaryotes and yeast (Pommier, 2009). The purification and characterization of yeast topoisomerase I proteins resulted in the mapping of the active site tyrosine, residue 727. Yeast model clearly identified topoisomerase I as the cellular target of CPT and suggested that CPT causes cell death by converting topoisomerase I into a cellular poison, rather than simply inhibiting enzyme activity (Reid *et al.*, 1998).

### 2.3.4 Yeast strains and plasmids

Wild-type laboratory yeast strains are not sensitive to most DNA topoisomerase I-targeted drugs, possibly due to the relative impermeability of the cell wall/membrane or drug efflux (Reid *et al.*, 1998). This initial impediment to using yeast as a model system to establish the mechanism of drug action was overcome by developing drug-sensitive yeast strain, either by manipulating drug permeability or by in creasing the level of enzyme activity (Nitiss and Wang, 1988).

The yeast strain used in this study is *S. cerevisiae* strain RS190 (ATCC 208354, *MAT*a, *top1*$\Delta$), genotype a *top1-8 [top1::LEU2] ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*. It was purchased from American Type Culture Collection.

The yeast was integrating of the construct genes of *Arabidopsis thaliana topoisomerase I*. The gene of *A. thaliana topoisomerase I* was cloned by the Gateway

Cloning Technology (Invitrogen), and the integrity of the constructs were verified by DNA sequencing. The Gateway™ expression vector pYES-DEST52 was use for expression in *S. cerevisiae*. This plasmid also contains *URA3*, selectable markers for the selection and maintenance of plasmid bone sequences in auxotropihic yeast strain. Furthermore this plasmid has strong inducible promoter (*pGAL1*) enable the study of topoisomerase I enzyme function (Figure 2.4). The recombinant yeasts increasing the level of expression of *topoisomerase I* gene from the galactose-inducible *pGAL1* promoter. A high level of *topoisomerase I* expression from the *pGAL1* promoter on a high copy vector is toxic to repair-defective yeast strain. Expression of *GAL1* gene in *S. cerevisiae*, required for metabolism of the sugar galactose, is stringently regulated by the carbon source. Superimposed upon this well-characterized induction mechanism of *GAL1* gene expression is the global regulatory circuit of carbon catabolite repression, which represses expression of many genes involved in carbohydrate metabolism and respiration during growth on glucose (Flick and Johnston, 1990).

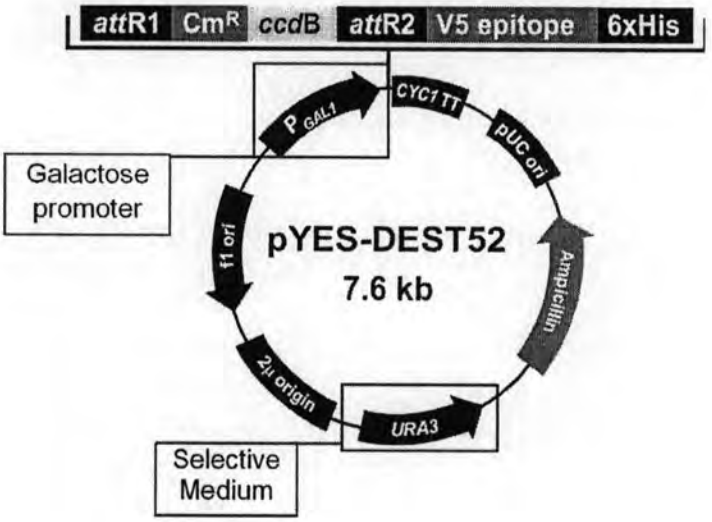



**Figure 2.4** pYES-DEST52 vector expression in *Saccharomyces cerevisiae* showing galactose promoter (p*GAL*1) and selectable marker gene (*URA*3).

**2.4 Bioassay-guided fractionation**

Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural sources, base on their use in traditional medicine (Itharat and Ooraikul, 2007). Many anticancer drugs were isolated from plants for example, camptothecin from *Camptotheca acuminata*, taxol from *Taxus brevifolia*, (Wall and Wani, 1996) and vinblastine and vincristine from *Catharanthus roseus* (Potier, 1980).

The study of bioactive compounds from plant has required the development of bioassay techniques, especially *in vitro* methods which allow a large number of plant extracts to be screened for activity, especially cytotoxicity, against yeast cell-based assay. A bioassay-guided fractionation was performed on the ethanolic extracts in order to isolate the bioactive compounds contributing to the to the topoisomerase I-targeted agents. After every fractionation process, the fractions were tested for topoisomerase I inhibitory activity against yeast cell-based assay.

**2.5 Botanical aspects and Chemical constituents of *Grangea maderaspatana* (L.) Poir.**

*Grangea maderaspatana* (L.) Poir. is known in various local names such as Phayaa-mutti พญามุตติ, or Yaa chaam luang หญ้าจามหลวง (Smitinand, 2001). It is prostrate or sometime erect annual herb in a family Compositae (Kasiwong, 1988) (Figure 2.5 a-d).

Rahman *et al.* (2008) studied on taxonomic of the family Asteraceae (Compositae). They reported taxonomic of *G. maderaspatana* as;

"Annual; stems many, prostrate, spreading from the centre, 10-30 cm. long, hair with soft white hairs. Leaves numerous, sessile, 2.3-6.6 cm. long, sinuately pinnatifid with 2-4 pairs of opposite or subopposite. Heads globose, 6.5-8.5 mm. diam.; solitary or 2-nate, on short leaf-opposed peduncles; flowers yellow. Achenes glandular, 2.5 cm. long including the pappus tube"

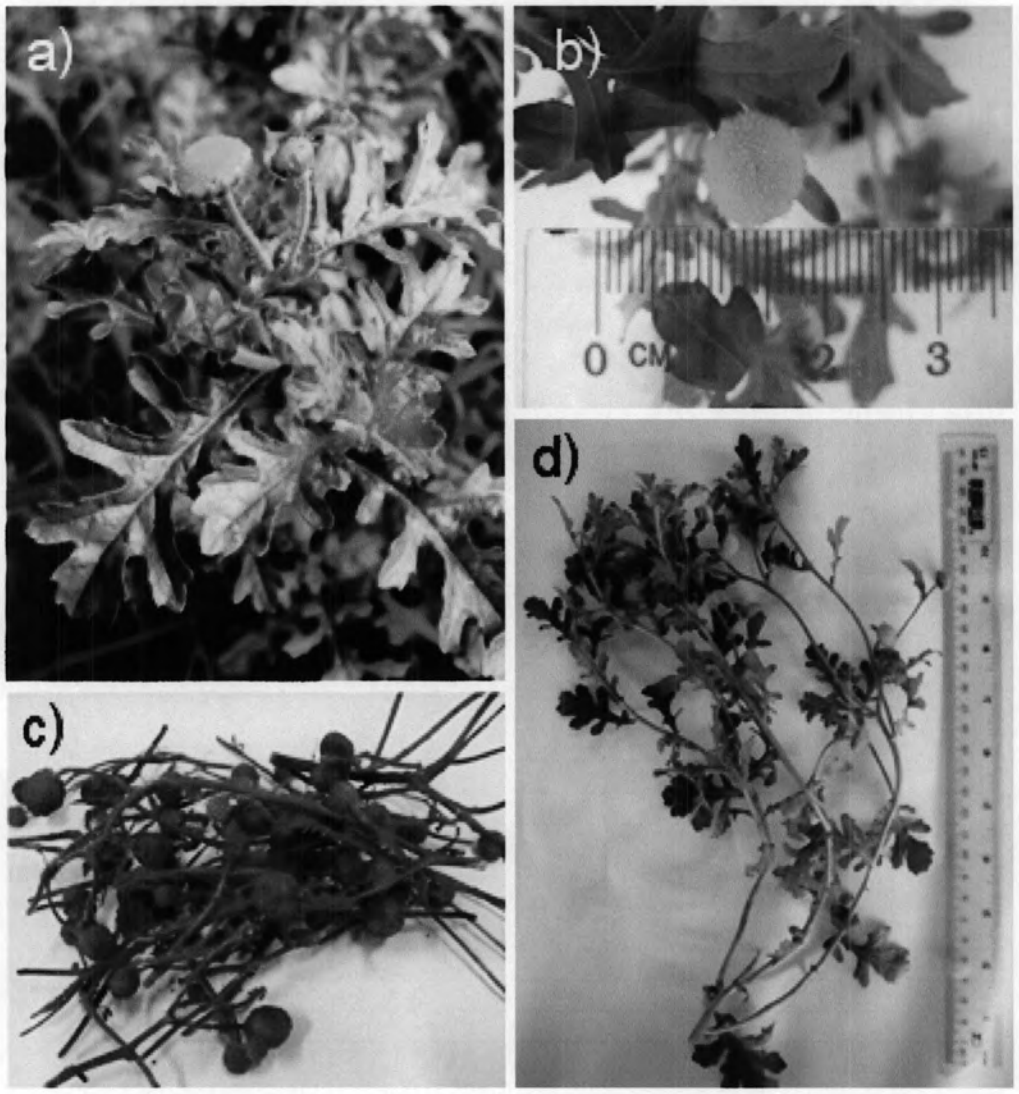



**Figure 2.5** *Grangea maderaspatana* (L.) Poir. a) habitat, b) flower, c) dried plants, and d) whole plant.

*G. maderaspatana* has been used in traditional medicine. The leaves are used as stomachic, a sedative, a carminative, an emmenagogue, an anti-flatulent, and an analgesic (Kasiwong, 1988; Ahmed *et al.*, 2001). A crude $CHCl_3$ extract of *G. maderaspatana* exhibits strong cytotoxic activity (Ruangrungsi *et al.*, 1989). The chemical substances isolated from these plants are summerized in Table 2.2

**Table 2.2** Chemical constituents and structures found in *Grangea maderaspatana*.

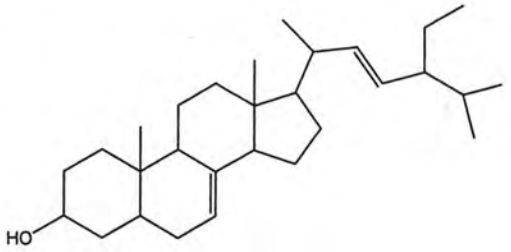
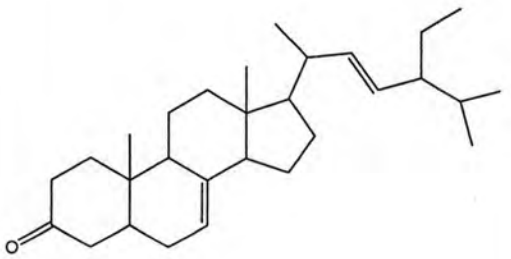
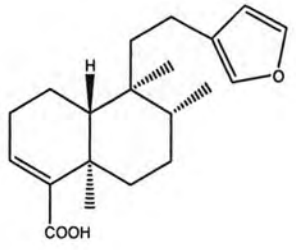
| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| Chondrillasterol |  | Aerial part | Iyer and Iyer, 1978 |
| Chondrillasterone |  | Aerial part | Iyer and Iyer, 1978 |
| Hardwickiic acid |  | Aerial part | Pandey *et al.*,1984; and Singh, Jain, and Jakupovic, 1988 |

Table 2.2 (continued)

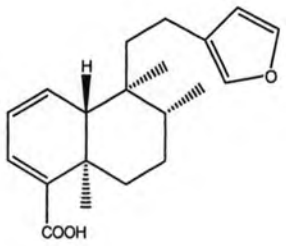
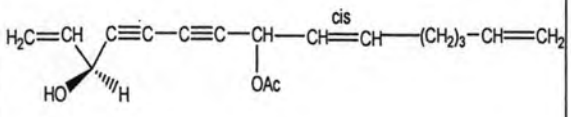
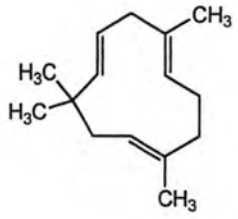
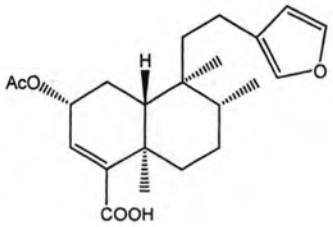
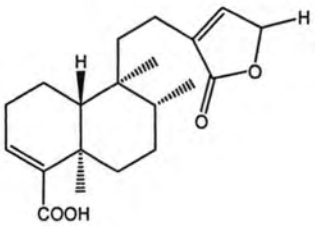
| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| *ent*-15,16-Epoxy-1,3,13(16),14-clerodatetraen-18-oic acid |  | Aerial part | Pandey *et al.*,1984 |
| 3-Hydroxy-8-acetoxypentadeca-1,9,14-trien-4,6-diyne |  | Aerial part | Pandey *et al.*,1984 |
| α-Humulene |  | Aerial part | Singh *et al.*,1988 |
| 2α-Acetoxy hardwickiic acid |  | Aerial part | Singh *et al.*,1988 |
| 16-Oxo-15,16H-hardwickiic acid |  | Aerial part | Singh *et al.*,1988 |

Table 2.2 (continued)

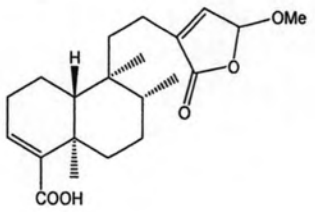
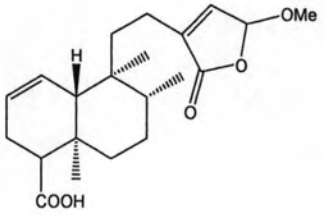
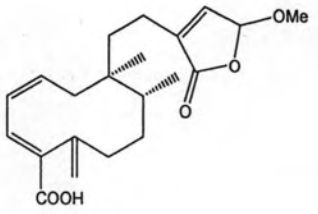
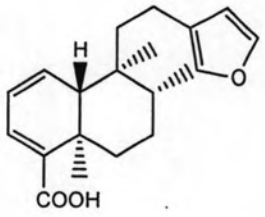
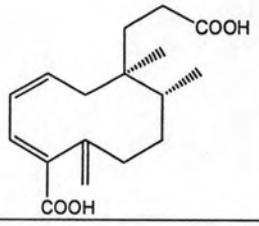
| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| 15-Methoxy-16-oxo-15,16H-hardwickiic acid |  | Aerial part | Singh *et al.*,1988; and Krishna and Singh, 1999 |
| 15-Methoxy-16-oxo-mdoresedic acid |  | Aerial part | Singh *et al.*,1988 |
| 15-Methoxy-16-oxo-15,16H-stricic acid |  | Aerial part | Singh *et al.*,1988 |
| Strictic acid |  | Aerial part | Singh *et al.*,1988 |
| nor-Strictic acid |  | Aerial part | Singh *et al.*,1988 |

Table 2.2 (continued)

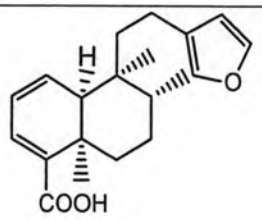
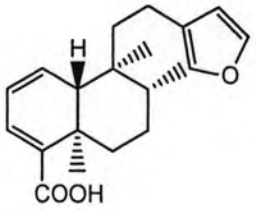
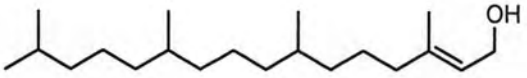
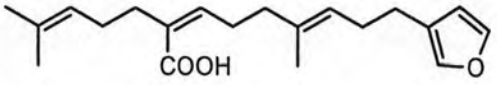
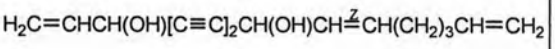
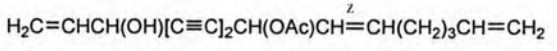
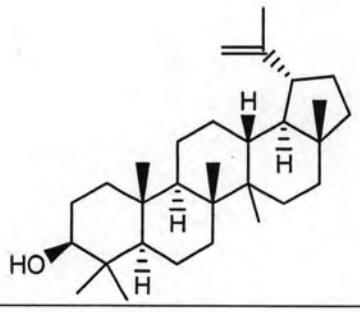
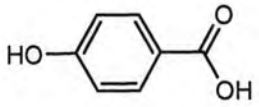
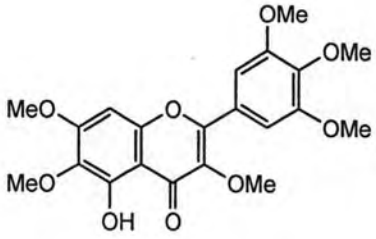
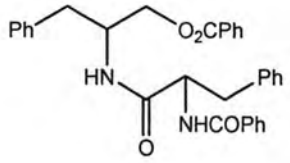
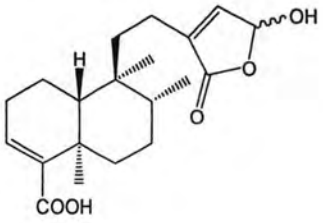
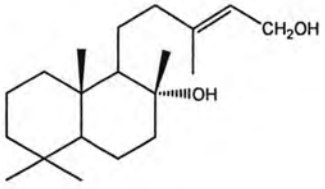
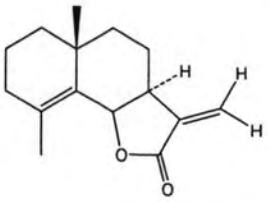
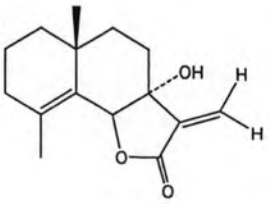
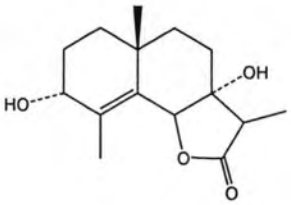
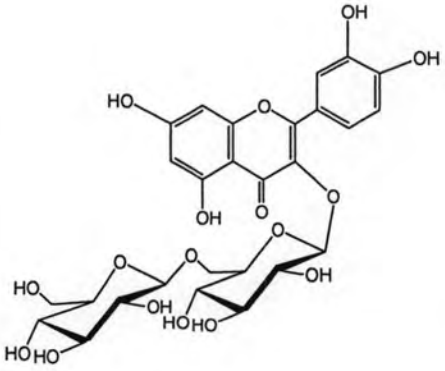
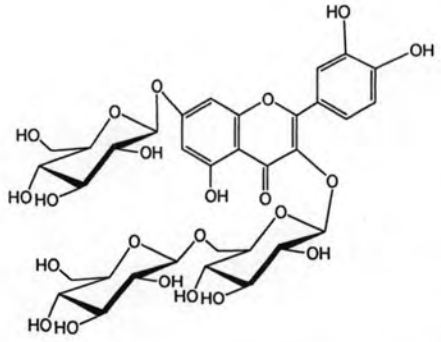
| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| 10-epi-Nidoresedic acid |  | Aerial part | Singh *et al.*,1988 |
| 10β-Nidoresedic acid |  | Aerial part | Singh *et al.*,1988 |
| Phytol |  | Aerial part | Singh *et al.*,1988 |
| Centipedic acid |  | Aerial part | Singh *et al.*,1988 |
| Dihydroxypentadeca-1,9,14-trien-4,6-diyne |  | Aerial part | Singh *et al.*,1988 |
| Hydroxy-8-acetoxy pentadeca-1,9,14-trien-4,6-diyne |  | Aerial part | Singh *et al.*,1988 |
| Lupeol |  | Aerial part | Singh *et al.*,1988 |

Table 2.2 (continued)

| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| p-Hydroxybenzoic acid |  | Aerial part | Singh *et al.*,1988 |
| 5-Hydroxy-3,6,7,3′,4′, 5′-hexamethoxy flavone |  | Aerial part | Singh *et al.*,1988 |
| Phenyl alanine derivative |  | Aerial part | Singh *et al.*,1988 |
| 15-Hydroxy-16-oxo-15,16H-hardwickiic acid |  | Aerial part | Krishna and Singh, 1999 |
| 8-Hydroxy-13E-labdane-15yl-acetate |  | Aerial part | Rojatkar, Chiplunkar, and Nagasam pagi, 1994 |

| Chemical substance | Chemical structure | Plant part | Reference |
|---|---|---|---|
| (-)-Frullanolide |  | Aerial part | Ruangrungsi *et al.*, 1989 |
| (-)-7α-Hydroxy-frullanolide |  | Aerial part | Ruangrungsi *et al.*, 1989 |
| (+)-11α,13-Dihydro-3α,7α-dihydroxy-frullanolide |  | Aerial part | Ruangrungsi *et al.*, 1989 |
| Quercetin 3-gentiobioside |  | Aerial part | El-Karemy *et al.*, 1987 |
| Quercetin 3-gentiobioside-7-glucoside |  | Aerial part | El-Karemy *et al.*, 1987 |