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Clerodendrum and Healthcare: An Overview - Part II Phytochemistry and Biotechnology

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ABSTRACT

The genus *Clerodendrum* is very widely distributed throughout the world and has more than five hundred species. Many species of this genus have been described in various indigenous systems of medicine and are used in preparation of folklore medicines for the treatment of various life-threatening diseases. From the genus few species are very well studied for their chemical constituents and biological activities, the latter having been covered in our previous review. This review mainly focuses on phytochemistry i.e. isolation, identification and characterization of chemical constituents and biotechnological prospects of the *Clerodendrum* genus. Some of the species described in the review are *Clerodendrum trichotomum*, *C. bungei*, *C. chinense*, *C. colebrookianum*, *C. inerme*, *C. phlomidis*, *C. petasites*, *C. grayi*, *C. indicum*, *C. serratum*, *C. campbellii*, *C. calamitosum* and *C. cyrtophyllum*. The major chemical constituents present in this genus were identified as phenolics, flavonoids, terpenes, steroids and oils. Biotechnological aspects have also been discussed in the review.

Keywords: flavonoids, *in vitro*, phenolics, steroids, terpenes

Abbreviations: BA, benzyl adenine; **CMV**, *Cucumber mosaic virus*; **GC**, gas chromatography; **HPLC**, high performance liquid chromatography; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **IR**, infrared; **MPLC**, medium pressure liquid chromatography; **MS**, Murashige and Skoog; **NAA**, α-naphthalene acetic acid; **NMR**, nuclear magnetic resonance; **PVY**, *Potato virus Y*; **TLC**, thin layer chromatography; **ToMV**, *Tomato mosaic tobamovirus*; **UV**, ultraviolet

CONTENTS

INTRODUCTION

Most of the earliest pharmaceuticals used were plant materials and they were used to treat diseases even before history was written (Houghton and Raman 1998). Documentation of the use of natural substances for medicinal purposes can be found as far back as 78 A.D., when Dioscorides wrote *De Materia Medica*, where he described thousands of medicinal plants. It included descriptions of many medicinal plants that remain important in modern medicine till today, not because they are continuously used for crude drug preparations, but because they serve as the source of important pure chemicals that have become mainstays of modern therapy (Ebadi 2007). The study and identification of chemical constituents present in plants is termed 'phytochemistry'. Before the 18th century progress in the field of phytochemistry was very slow and very few compounds such as starch, camphor, etc., were known. But the major thrust came in the 19th century, when 'nicotine', the first alkaloid, was isolated. In the 20th century with the isolation of many more compounds it gained more importance (Evans 2002). The main reason for interest in biologically active natural compounds was exemplified by changes that have occurred in the Western society with regard to pharmaceuticals during the last quarter of the 20th century (Ebadi 2007). So in the 20th century a major emphasis was given to the isolation, identification and elucidation of biosynthetic pathways of the isolated compounds. These studies were possible because of the use of various separation and identification techniques developed during this era (Harbone 1984; Mann *et al*. 1994; Kaufman *et al*. 1999).

All the chemical constituents found in plants are not biologically active molecules e.g. carbohydrates, protein, fats, etc. They are produced by plants for their own normal functioning and growth; these chemical constituents are termed primary metabolites. But there are certain compounds which are produced by plants mainly for their defence mechanism during adverse environmental conditions

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Invited Review

Figs. 1A and 1B: Flowering twigs of *C. inerme*.

Fig. 2 *C. phlomidis* in wild. Vegetative (**A**) and flowering (**B**) stages.

or pathogen attack, and these compounds are termed secondary metabolites and they have biological importance. These compounds are also termed biologically active compounds. These secondary metabolites contribute towards the therapeutic value of plants and when isolated from plants form not only valuable drugs but also valuable lead molecules. These lead molecules can be further modified chemically for designing synthetic molecules responsible for having better or similar biological activity as their natural counterparts.

The chemical constituents found in plants can broadly be grouped on the basis of their functional group. The major groups are phenolics, flavonoids, terpenoids, steroids, alkaloids, oils, etc. In this review the chemical constituents of the genus *Clerodendrum* are discussed in detail with reference to these groups. Biological activities of these chemical groups and individual compounds have been discussed in detail in our previous review on this genus (Shrivastava and Patel 2007). **Figs. 1** and **2** represent two species of the genus, *C. inerme* and *C. phlomidis* in the vegetative and reproductive stage.

PHYTOCHEMICAL INVESTIGATION OF *CLERODENDRUM* GENUS

Genus *Clerodendrum* [family: Lamiaceae (Verbenaceae)] was reported for the first time in 1753 (Hsiao and Lin 1995; Steane *et al*. 1999; Shrivastava and Patel 2007). This genus has more than 500 species and is very widely distributed throughout the world and comprises from herbs to small trees (Moldenke 1985; Rueda 1993). Few species of the genus like *C. indicum*, *C. phlomidis*, *C. serratum*, *C. trichotomum*, *C. chinense*, *C. petasites*, etc. are being extensively used as folk and traditional medicines in various parts of the world such as India, China, Korea, Japan, Thailand, Africa, etc. Various *Clerodendrum* species are reported to be used for remedial purpose in inflammatory disorders, diabetes, cancers, malaria, fever, etc. The traditional or ethnomedical claims of the species have also been evaluated. The biological activities of these species described in ancient literature have been reported to be associated with the chemical constituents present in the species (Shrivastava and Patel 2007). The major groups of chemical constituents present in the *Clerodendrum* genus are phenolics, flavonoids, terpenoids and steroids.

PHENOLICS

Phenolics constitute the largest group in plant secondary metabolites. In the *Clerodendrum* genus many phenolic compounds have been reported to be isolated from various species. The phenolic compounds in general and in the genus *Clerodendrum* are found in both free as well as bound to sugar moieties (Harbone 1984; Mann *et al*. 1994). On the basis of their structure phenolic compounds are further subgrouped into phenols, phenolic acids, phenyl propanoids, flavonoids, etc. As flavonoids represent a major constituent in this genus it will be dealt with separately. The various phenolic compounds isolated from the genus are listed in **Table 1**. All the major phenolic compounds which have been isolated from various species of *Clerodendrum* genus are given in **Fig. 3A-C**. Some of the phenolic compounds isolated were directly correlated with biologically activities such as antioxidant, antimicrobial, antiproliferative, antihypertensive and anticancer activities (Shrivastava and Patel 2007).

Table 1 Phenolic compounds isolated from genus *Clerodendrum*.

Species	Compound	Part	Reference
C. aculeatum	Cistanoside D, acteoside	Whole plant	Garnier *et al*. 1989
C. bungei	Anisic acid, vanillic acid, maltol, acteoside, leucosceptoside A, isoacteoside, jinoside	Whole plant	Zhou *et al*. 1982; Li *et al*. 2005
C. calmitosum	Pheophorbide related compounds	Whole plant	Cheng *et al*. 2001
C. cryptophyllum	Pheophorbide related compounds	Whole plant	Cheng *et al*. 2001
C. fragrans	Acteoside, leucosceptoside A, isoacteoside, methyl and ethyl esters of caffeic acid, jinoside	Whole plant	Gao *et al*. 2003
C. grayi	Lucumin, prunasin	Whole plant	Miller *et al*. 2006
C. inerme	(3-methoxy-4-hydroxyl phenyl) ethyl-*O*-2″, 3″-diacetyl-*α*-L-rhanopyranosyl-(1-3)-4-*O*-(E)-fernloyl-*β*-D-glucopyranoside, verbascoside, isoverbascoside, Neolignans (I-III)	Whole plant	Spencer and Flippen-Anderson 1981; Nan *et al*. 2005
C. indicum	Cleroindicin A-F	Aerial parts	Tain *et al*. 1997
C. infortunatum	Acteoside, fumaric acid, methyl and ethyl esters of caffeic acid	Whole plant, flower	Sinha *et al*. 1980, 1982
C. myricoides	Myricoide, acteoside	Root	Cooper *et al*. 1980
C. trichotomum	Kusagenin, indolizino[8,7-*β*] indole 5-carboxylic acids, acteoside, acteoside isomer, leucosceptoside A, martynoside, isomartynoside, isoacteoside, jinoside, trichotomoside	Whole plant	Tayoda *et al*. 1982; Sukurai and Kato 1983; Kim *et al*. 2001; Nagao *et al*. 2001; Kang *et al*. 2003; Chae *et al*. 2004, 2005, 2006; Lee *et al*. 2006

The general procedure for isolation of phenolic compounds depends on the type of phenolic compound present i.e. whether it is present in glycosidic form or free form. For the extraction of phenolic moieties from its glycosides, the glycosides are first hydrolyzed; usually hydrolysis is carried out either with acid or alkali to break the glycosidic bond. The phenolic moieties are then extracted in non-polar solvents such as ethers. Extraction of free phenolic compounds is carried out by extracting the plant material with polar solvents. The extract obtained is then concentrated and the required compound is separated by various separation techniques such as preparative thin layer chromatography, column chromatography, HPLC and other techniques. Isolation of acteoside from flowers of *C. infortunatum* was carried out by extracting the material with alcohol after defatting it. The alcoholic extract was then successively extracted with various non-polar solvents like petroleum ether, *n*-hexane and diethyl ether and subjected to column chromatography which finally yielded acteoside (Sinha *et al*. 1982). Phenyl propanoid glycosides were isolated from stems of *C. trichotomum* by extracting the material with methanol and the methanolic fraction was further partitioned with solvents such as dichloromethane, ethyl acetate and *n*-butanol. From these ethyl acetate fraction was chromatographed which yielded acteoside, leucosceptoside A, martynoside, acteoside isomer, and isomartynoside (Kang *et al*. 2003; Chae *et al*. 2005). Another phenolic compound trichotomoside was also isolated from stems of *C. trichotomum* by extracting the material in methanol and was further partitioned with solvents like dichloromethane, hexane, butanol and ethyl acetate. The dichlormethane fraction was further subjected to column chromatography the fractions obtained were further separated and purified by MPLC which yielded trichotomoside (Chae *et al*. 2006). Cleroindicins from *C. indicum* were isolated by extracting the aerial parts in alcohol, which was further defatted with petroleum ether and residue obtained was chromatographed to obtain cleroindicins (Tain *et al*. 1997).

FLAVONOIDS

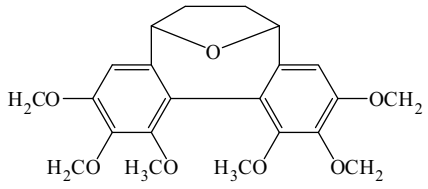
Flavonoids are one of the major groups present in *Clerodendrum* genus possessing promising biological activities. Flavonoids found in this genus are in both free and bound form. These flavonoids are further sub-grouped into catechins, leucoanthocyanidins, flavanones, flavanonols, flavones, anthocyanidins, flavonols, chalcones, aurones and isoflavones (Harbone 1984; Mann *et al*. 1994). Various flavonoids isolated from the *Clerodendrum* genus are mentioned in **Table 2**. These isolated flavonoids possess potent antioxidant, antimicrobial, antiasthmatic, antitumor and CNS-binding activities (Shrivastava and Patel 2007).

Isolation of flavonoids is carried out based on the polarity of the compounds. Less polar flavonoids are extracted with non-polar solvents such as chloroform, dichloromethane, diethyl ether or ethyl acetate, while polar flavonoids which are mainly glycosides are extracted with alcohols or mixture of alcohol and water e.g. the flavonoid hispidulin was extracted from alcoholic extract by partitioning with ethyl acetate/methanol/water. The organic phase obtained was again dissolved in ethanol and the insoluble fraction was fractionated with counter current chromatography in solvent system (chloroform/methanol/*n*-propanol/water) to obtain pure hispidulin (Hazekamp *et al*. 2001). Another flavonoid cleroflavone was isolated from leaves by extracting them with petroleum ether and then the extract was chromatographed which yielded pure cleroflavone (Ganapaty and Rao 1990). 7-hydroxyflavone, 7-hydroxyflavonone, naringin-4′-*O*-*α*-glucopyranoside and chalcone glucoside were isolated from flowers of *C. phlomidis* by extracting it in hexane and methanol, the hexane and methanolic extract were chormatographed which yielded these flavonoids (Anam 1997). Structures of isolated flavonoids are given in **Fig. 4**.

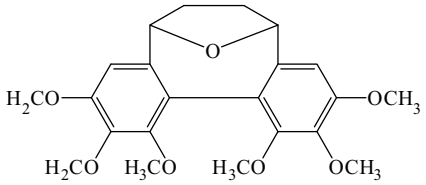
TERPENES

Many terpenoids have been reported from this genus. Broadly terpenes are grouped on the basis of isoprene units present into hemiterpenoid (C_5), monoterpenoid (C_{10}), sesquiterpenoid (C15), diterpenoid (C_{20}), sesterterpenoid (C_{25}), triterpenoid (C_{30}) and carotenoid (C_{40}). Terpenoids are generally found to be bound to sugar moieties by a glycoside linkage. Usually they are present as glycosides in their *β*-D-glucosidic form (Harbone 1984; Mann *et al*. 1994). Terpenes isolated and identified from *Clerodendron* genus are listed in **Table 3** and some of the terpenes had weak CNS activity, strong molluscicidal and fungitoxic activities (Shrivastava and Patel 2007). Structures of isolated terpenoids from the genus are shown in **Fig. 5A-C**.

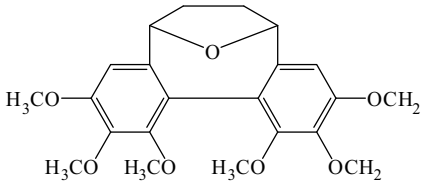
Isolation of terpenoids is generally carried out with non-polar and polar solvents. Triterpenoid Mi-saponin was isolated from roots of *C. wildii* by first extracting it with chloroform followed by methanolic, water and butanol extraction. The butanol extract obtained was then chromatographed to get pure Mi-saponin A. Inerminosides were isolated from leaves of *C. inerme* by extracting the leaves in methanol which was further partitioned with petroleum ether, diethyl ether and butanol. The butanol fraction was chromatographed which yielded inerminosides (Calis *et al*. 1994a). Megastigmane iridoid glycosides were also isolated by extracting the aerial parts of *C. inerme* with methanol



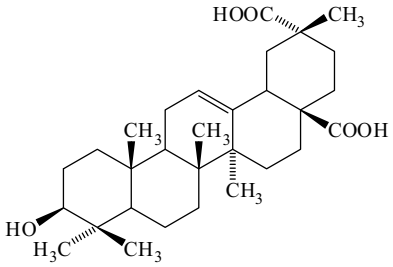
Neolignan I



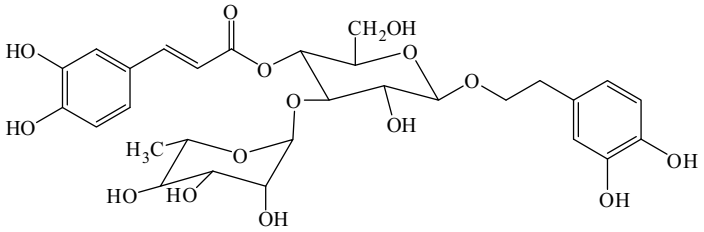
Neolignan II



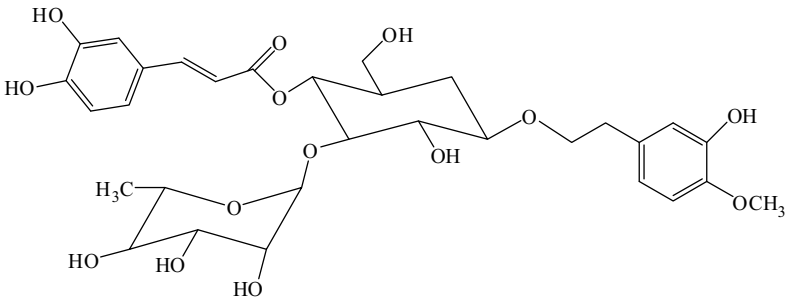
Neolignan III



Serratagenic acid



Acteoside



Jionoside D

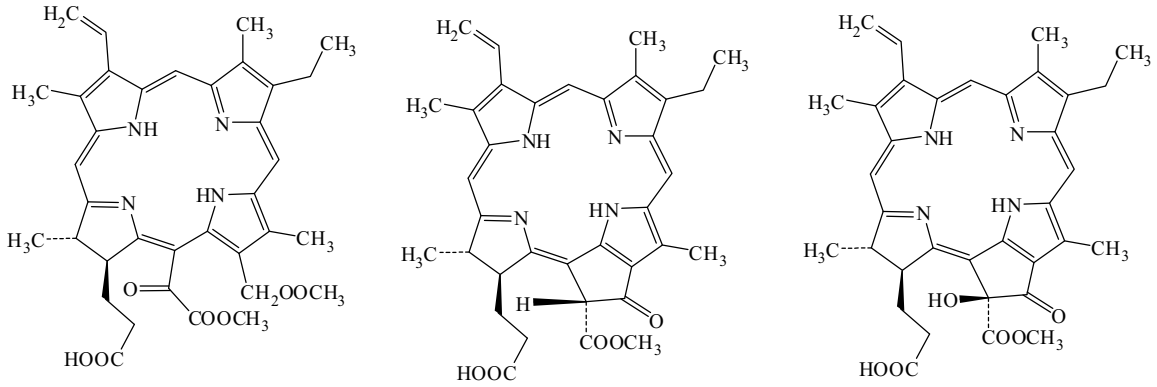
Fig. 3A Phenolic compounds of *Clerodendrum* genus.

and this methanolic extract was defatted with diethyl ether and the aqueous fraction was chromatographed which yielded iridoid glycosides (Kanchanapoom *et al*. 2001). Iridiod glucosides were also isolated from *C. incisum* by extracting the aerial parts with methanol and the methanolic extract was further chromatographed to get iridoid glucosides (Stenzel *et al*. 1986).

STEROIDS

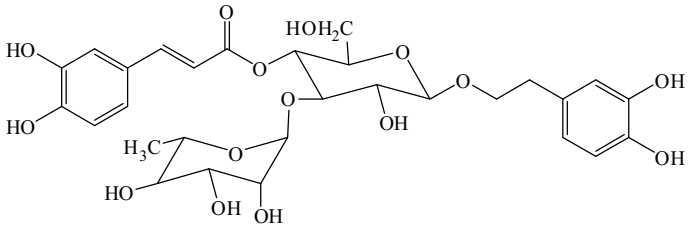
Steroids are terpenes based on the cyclopentane perhydroxy phenanthrene ring, but they are considered separately because of their chemical, biological and medicinal importance. Steroids are found in nature in free as well as in glycosidic form. There are many steroids reported from plants and they are termed phytosterols.

β-sitosterol was reported to be isolated from various species of *Clerodendrum* genus such as *C. inerme*, *C. fragrans*, *C. colebrookianum*, *C. paniculatum*, *C. tomentosum*, *C. bungei*, *C. phlomidis* and *C. infortunatum* (Chirva *et al*. 1980; Sinha *et al*. 1980; Singh and Singhi 1981; Hsu *et al*. 1983; Pinto and Nes 1985; Att-Ur-Rehman *et al*. 1997; Yang *et al*. 2002; Gao *et al*. 2003). (24*S*)-ethylcholestra-5, 22,25-triene-3*β*-ol was reported in *C. inerme*, *C. paniculatum* and *C. fragrans* (Singh and Singhi 1981; Singh and Prakash 1983; Hsu *et al*. 1983) and 24*β*-ethylcholesta-5,22*E*, 25(27)-trien-3*β*-ol was isolated from *C. splendens* (Pinto *et al*. 1985). Other steroids such as clerosterol and deucosterol were isolated from petroleum ether extracts of *C. fragrans* (Singh and Singhi 1981). *γ*-sitosterol was reported from *C.*

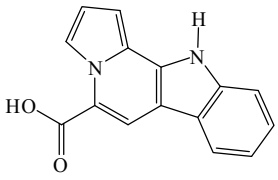


I II III

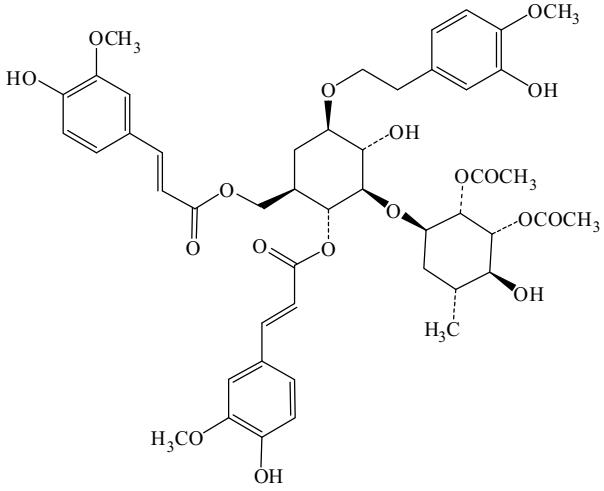
Pheophorbide related compounds



Verbascoside



Indolizino [8,7-b] indole 5-carboxlic acid



Trichotomoside

Fig. 3B: Phenolic compounds of *Clerodendrum* genus.

cyrtophyllum (Wu 1980) and *C. serratum* (Banerjee *et al*. 1969; Anynomous 2005), while 24*S*-stigmata-5,22,25-dine-3*β*-ol, 22*E*, 24*S*-stigmata 5,22,25-trine-3*β*-ol were isolated from hydroalcoholic extract of *C. mandarinorum* root bark, aerial parts of *C. inerme* and *C. campbellii* (Bolger *et al*. 1970a, 1970b; Zhu *et al*. 1996; Pandey *et al*. 2003). Also steroids such as clerosterol, taraxerol were reported from *C. colebrookianum*, *C. paniculatum*, *and C. tomentosum* species (Joshi *et al*. 1979; Chirva and Garg 1980; Goswami *et al*. 1996). New steroids colebrin A-E and colebroside were also isolated from aerial parts of *C. colebrookianum* (Yang *et al*. 2000a, 2000b). Cholestanol was also isolated from stem of *C. phlomidis* (Akihisa *et al*. 1989). Steroids such as taraxerol, glochidone, glochidonol, glochidiol were isolated from *C. bungei* (Gao *et al*. 2003). Some of the major steroids isolated have been shown in **Fig. 6A** and **6B**.

Steroids are extracted by various solvents, steroidal glycosides are extracted in polar solvents while free steroids are extracted with non-polar solvents. To obtain steroidal aglycan from steroidal glycoside, the glycoside is hydrolyzed and then extracted in non-polar solvents. *γ*-sitosterol was isolated from *C. serratum* roots by extracting it in petroleum ether which yielded a dark yellow residue which was further chromatographed to get *γ*-sitosterol. 4*α*-methyl sterols were also isolated from *C. inerme* by extracting the aerial parts with methanol. Then the methanolic fraction was concentrated and to it dimethyl ketone was added, the soluble fraction was further extracted with alkaline ethanol and then further extracted it with diethyl ether. The ether fraction was chromatographed which yielded 4*α*-methyl sterols (Akihisa *et al*. 1990). Other steroidal compounds like clerosterol, *β*-sitosterol were isolated from *C. colebrookianum* by extracting the leaves with hexane and subjecting the hexane fraction to column chromatography (Singh *et al*.

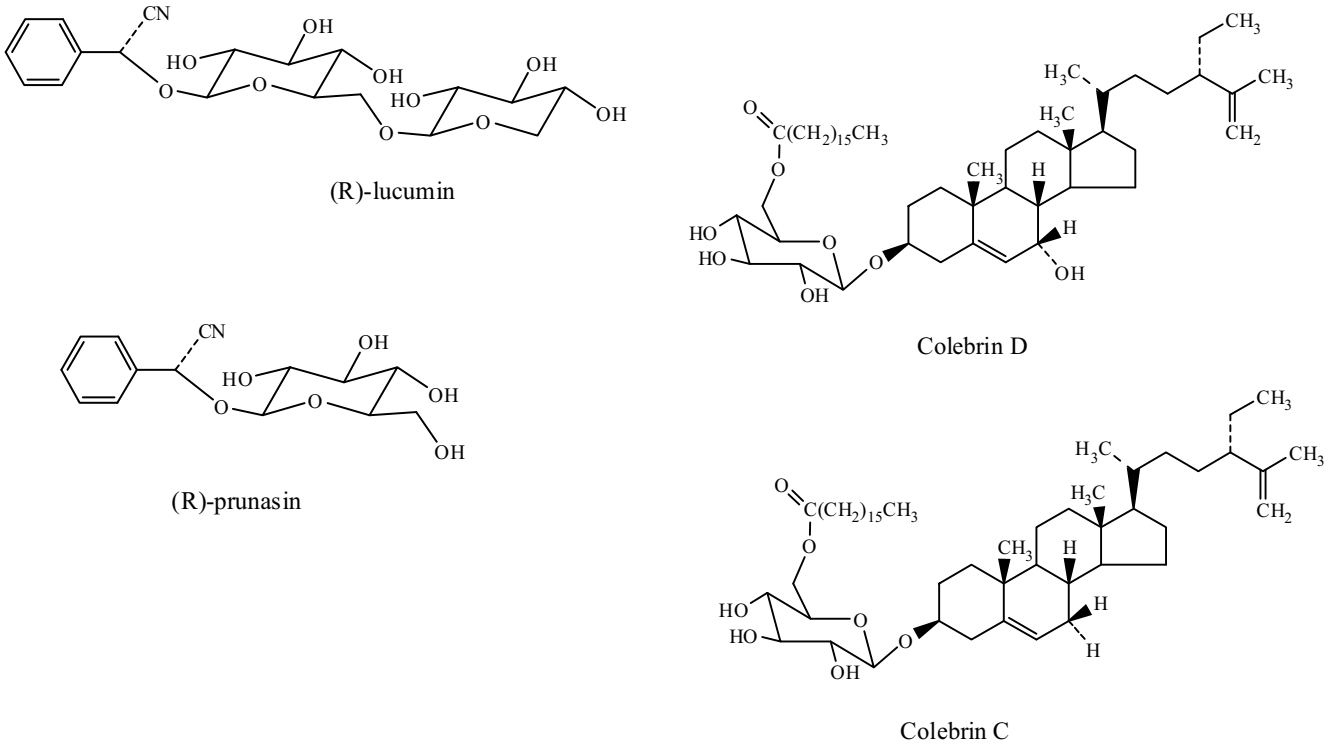


Fig. 3C: Phenolic compounds of *Clerodendrum* genus.

Table 2 Flavonoids isolated from the genus *Clerodendrum*.

Species	Compound	Part	Reference
C. fragrans	Kaempferol	Leaves	Gao *et al*. 2003
C. indicum	Hispidulin, scutellarein, scutellarein-7-*O*-*β*-D-glucuronide	Flowers	Sankara Subramanian and Ramachandran Nair 1973; Gunasegaran *et al*. 1993
C. inerme	Apigenin, acacetin, cosmosin, luteolin, cynaroside, salvigenin, 5-hydroxy-4′-7-dimethoxy-6-flavone, 5-hydroxy-4′-7-dimethoxy flavone, 4′-methylscutellarein	Aerial parts, stem, leaves	Vendatham *et al*. 1977; Achari *et al*. 1990; Raha *et al*. 1991; El-Shamy *et al*. 1996
C. infortunatum	Apigenin, acacetin and methyl esters of acacetin-7-*O*-glucuronide, cabruvin, quercetin, scutellarein, scutellarein-7-*O*-*β*-D-glucuronide, Hispidulin	Flowers, roots	Sankara Subramanian and Ramachandran Nair AG 1973; Sinha *et al*. 1981; Roy *et al*. 1996
C. mandarinorum	Cirsimartin, cirsimartin 4′-glucoside, quercetin-3-methyl ether	Roots	Zhu *et al*. 1996
C. nerrifolium	Cleroflavone	Leaves	Ganapaty and Rao 1990
C. petasites	Hispidulin	Flowers	Hazekamp *et al*. 2001
C. phlomidis	Apigenin, pectolinerngenin, chalconeglucoside, 2′-4-4′-trihydroxy-6′-methylchalcone, 7-hydroxyflavanone, an its *β*-D-glucoside, naringin-4′-*O*-*α*-glucopyranoside	Flowers, leaves, whole plant	Seth *et al*. 1982; Roy *et al*. 1994, 1995; Roy and Pandey 1995; Anam 1997, 1999
C. siphonenthus	Pectolinerngenin	Flowers	Pal *et al*. 1989
C. tomentosum	5-hydroxy-4′-7-dimethoxy flavone	Stems	Chirva and Garg 1980
C. trichotomum	Apigenin	Whole plant	Min *et al*. 2005

1995).

OTHER CHEMICAL CONSTITUENTS

Many other chemical constituents are also reported from the genus which include volatile constituents such as 5-*O*-ethylcleroindicin D, cleroindicin (A, C, E and F), linalool, benzyl acetate, benzyl benzoate, benzaldehyde and octen-3-ol which have been isolated from *C. bungei*, *C. canescens*, *C. cyrtophyllum*, *C. inerme* and *C. philippinum*, *C. buchholzii* (Yang *et al*. 2002; Yu 2004; Nyegue *et al*. 2005; Wong and Tan 2005). Inactive wax bungein A was also isolated from aerial parts of *C. bungei* (Yang *et al*. 2002). Amino acids such as lysine, arginine, serine, proline, threonine, glutamic acid; sugars like galactose, glucose and fructose and pentadecanoic acid-*β*-D-glucoside were also isolated from *C. inerme* (Desai and Baxi 1991; Pandey *et al*. 2006). Palmitic, oleic and linoleic acids were extracted from seeds of *C. infortunatum* (Siddiqui *et al*. 1973). 2-methyleicosa 2,9-diene,10,11,32-trimethyltetratriacontanol, pentatriacontane, palmitic acid were isolated from the leaves of *C. colebrookianum* (Singh *et al*. 1995). D-manitol was also isolated from *C. serratum* roots (Garg and Verma 2006). A cyclic hexapeptide cleromyrine I (Ala-Gly-Pro-Ile-Val-Phe) was isolated from *C. myricoides* by chiral chromatography (Bashwira *et al*. 1989) and two new spermidine alkaloids, myricoidine and dihydromyricoidine were also reported from *C. myricoides* (Bashwira and Hootele 1988); also other spermidine alkaloids buchnerine and *N′*-(*Z*)-*p*-methoxycinnamoylbuchnerine were isolated from leaves of *C. buchneri* (Lumba and Hootele 1993). Lectins and two pigments trichotomine and trichotomine G_1 were also isolated from fruits and leaves of *C. trichotomum* (Iwadare *et al*. 1974; Kitagaki-Ogawa *et al*. 1986). Glycoproteins CIP-29 and CIP-34 were isolated from *C. inerme* were reported to be responsible for inducing systemic resistance against tobacco mosaic virus in *Nicotina tabacum* (Prasad *et al*. 1995; Olivier *et al*. 1996), another protein identified as Crip-31 was also isolated from the same species and it was also showing systemic viral resistance against *Cucumber mosaic virus* (CMV), *Tomato mosaic tobamovirus* (ToMV) and *Potato virus Y* (PVY) in *Nicotiana tabacum* (Praveen *et al*. 2001) (**Fig. 7**).

5-*O*-ethylcleroindicin and bungein A were isolated by

Table 3 Terpenes isolated from the genus *Clerodendrum*.

Species	Compound	Part	Reference
C. chinense	Monomelittoside, melittoside, harpagide, 5-*O*-*β*-glucopyranosyl-harpagide, 8-*O*-acetylharpagide	Aerial parts	Kanchanapoom *et al*. 2005
C. colebrookianum	Triacatane, clerodin, clerodendrin A	Whole plant	Joshi *et al*. 1979
C. incisum	8-*O*-foliamenthoyleuphroside, 2'-*O*,8-*O*-difoliameuthoyleuphroside, plantarenaloside, euphroside	Whole plant	Stenzel *et al*. 1989
C. inerme	*α* and *β*-amyrin, royleanone dehydroroyleanone, caryoptin, 3epi-caryoptin, 14,15-dihydro-15*β*-methoxy-3-epicaryoptin, clerodermic acid, glutinol, gramisterol, Iridoids such as (inerminoside A-1, B, C, D), clerodendrins (A-H), clerodendrin B acetate, monomelittoside, inermes A, B, sammangaoside A-C, betulin, clerodermic acid	Leaves, aerial parts	Abdul-Alim 1971; Singh and Prakash 1983; Achari *et al*. 1990, 1992; Akihisa *et al*. 1990; Rao *et al*. 1993; Calis *et al*. 1994a, 1994b; Att-Ur-Rehman *et al*. 1997; Kanchanapoom *et al*. 2001; Kumari *et al*. 2003; Pandey *et al*. 2005
C. mandarinorum	Friedelanone, lupeol, betulinic acid	Roots	Zhu *et al*. 1996
C. neriifolium	(-)-Hardwickic acid	Leaves	Ganapaty and Rao 1990
C. paniculatum	Triacatane, clerodin, clerodendrin A, 3*β*-acetyloleanolic acid, 3*β*-acetyloleanolic aldehyde, glutinol	Leaves	Joshi *et al*. 1979; Hsu *et al*. 1983
C. phlomidis	Triacatane, clerodin, clerodendrin A	Whole plant	Joshi *et al*. 1979
C. serratum	Queretaroic acid, serratagenic acid	Whole plant	Rangaswami *et al*. 1969
C. siphonenthus	Unicinatone	Roots	Doraz *et al*. 2004
C. thomsonae	Monomelittoside, melittoside, harpagide, 5-*O*-*β*-glucopyranosyl-harpagide, 8-*O*-acetylharpagide, aucubin, reptoside, ajugoside, 8-*O*-acetylmioporoside	Aerial parts	Gabriele and Rimpler 1981
C. trichotomum	Clerodendrins (A-H)	Whole plant	Kawai *et al*. 1998
C. ugandenese	Ugandoside	Leaves	Gabriele and Rimpler 1983
C. uncinatum	Unicinatone	Roots	Doraz *et al*. 2004
C. wildii	Mi-saponin A	Roots	Toyota 1990

extracting the aerial parts of *C. bungei* in methanol and further defatting it with petroleum ether. The residue obtained was partitioned with ethyl acetate and *n*-butanol successsively. The ethyl acetate fraction was chromatographed which yielded the two compounds, 5-*O*-ethylcleroindicin and bungein A (Yang *et al*. 2002). Isolation of spermidine alkaloids from *C. buchneri* leaves was carried out by extracting the leaves with methanol. Methanolic fraction was filtered and the filtrate obtained was acidified with dilute acid and then neutralized, this neutralized fraction was further extracted with chloroform. The chloroform extract was concentrated and the residue was distributed between chloroform and aqueous citric acid. It was further basified with alkali and then extracted with chloroform which yielded crude alkaloidal fraction. This fraction upon chromatography yielded two spermidine alkaloids (Lumbu and Hootele 1993). For isolation of sugars and amino acids, first the material was defatted and then the remaining residue was extracted with hydro-alcoholic mixture. The filtrate thus obtained was concentrated and acidified with dilute acid and then extracted with non-polar solvents like ether. The aqueous acidic fraction remained after separation was further extracted with ethyl acetate for removal of flavonoids. The aqueous fraction then obtained was neutralized and subjected to column chromatography which yielded sugars and amino acids. Volatile constituents from fresh plant material were reported to be extracted by steam distillation (Houghton and Raman 1998).

GENERAL ISOLATION AND EXTRACTION METHOD

Ideally, the plant material to be used is collected fresh at the right stage of growth and then dried under the shade or in oven at 40-45°C, the dried plant material is used for the extraction. Drying of plant material should be carried out under control conditions to prevent the changes occurring in its constituents due to drying. In the case of volatile constituents, fresh plant material is used because drying leads to degradation/loss of volatile constituents from the material. Another criterion is that plant material should be free from any type of contamination before it is used for isolation studies because contamination can also lead to loss or degradation of chemical constituents present. Prior to extraction the plant material should be authenticated. To investigate/isolate chemical constituents the method mainly employed is based on fractionation by solvents of varying polarity. The fractions obtained after extraction with various solvents are then subjected to different separation techniques like precipitation techniques, TLC, paper chromatography, column chromatography, HPLC, GC, etc. to yield pure compounds (**Fig. 8**). The procedure of isolation can be modified depending on the substance that is under investigation (Harbone 1984; Mann *et al*. 1994; Houghton and Raman 1998).

IDENTIFICATION OF COMPOUNDS

Once the compound is isolated, it is necessary to identify it. The compound identification is done by determining the properties of the compound such as melting point, boiling point, purity, solubility and R_f value of the compounds. For characterization of the compounds various analytical techniques such as ultra violet (UV) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and X-ray crystallography are used. In the case of ultraviolet spectroscopy the underline principle is the amount of light absorbed by the compound. A spectrum of the compound is recorded against solvent blank in which it is dissolved. The compound will absorb maximum amount of light at a specific wavelength which is termed as absorbance maxima. Such spectral measurements help in checking the purity of the isolated compound. While in infrared spectroscopy the type of chemical group present in the compound can be identified on the basis of bending and stretching vibration property of the compound. IR spectroscopy helps in structural elucidation and identification of the compound. In NMR spectroscopy the principle is based on the spinning property of the active nucleus so it will also have a magnetic moment and an angular momentum. The ratio of these two properties (magnetic moment and angular momentum) is utilized as a characteristic property of a compound for its identification. Molecular weight of the compound is determined by mass spectroscopy where it determines the molecular weight based on the mass to charge ratio of the particles present in the compound. So by using these techniques compound are identified and its structures are elucidated. The data obtained then can be compared with the authentic standards materials and confirmed. In case where authentic samples are not available the above data are exploited to identify and cha-

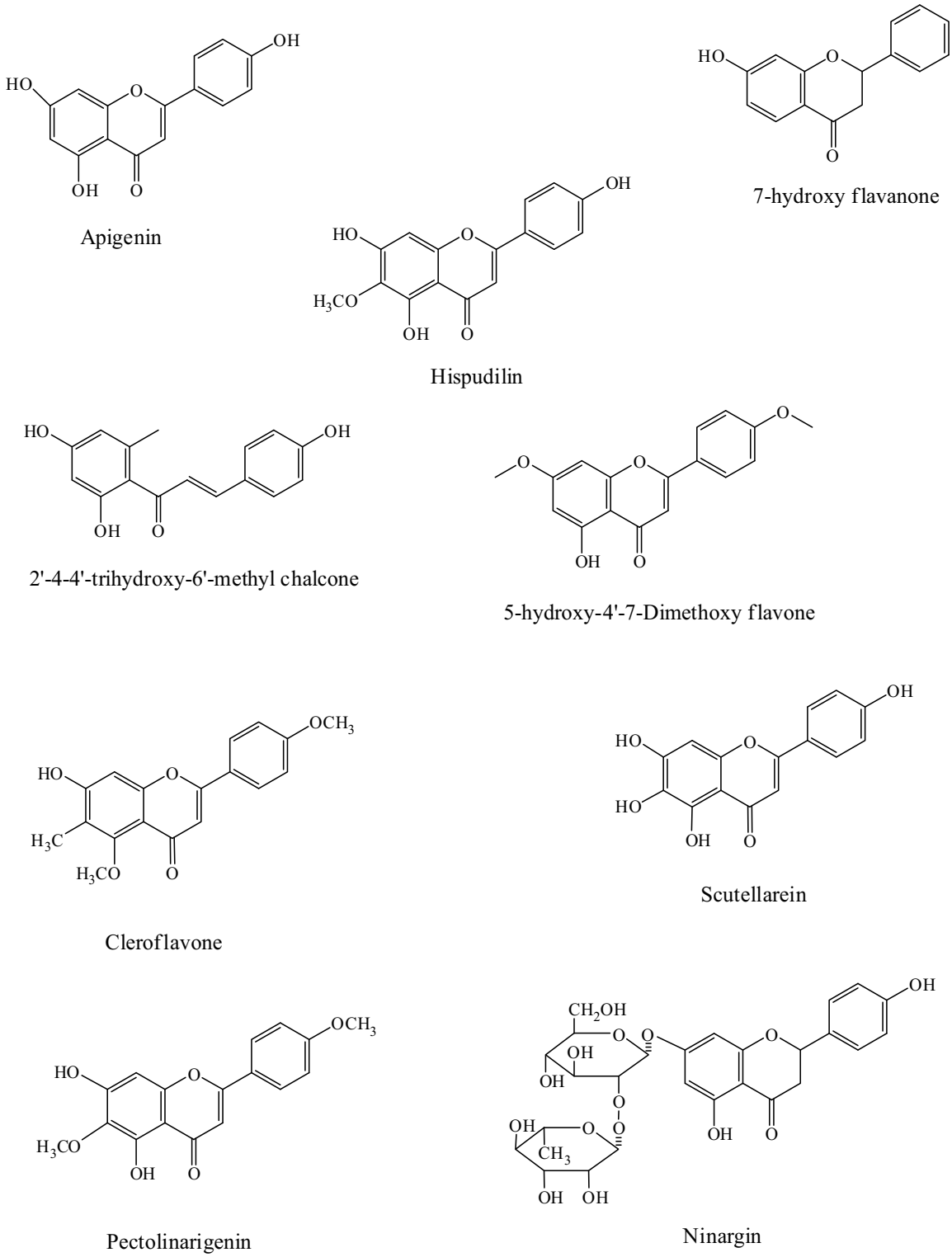


Fig. 4 Flavonoids of *Clerodendrum* genus.

racterize the isolated compounds (Harbone 1984; Houghton and Raman 1998; Anonymous 1 2004).

BIOTECHNOLOGY AND ITS FUTURE PROSPECTS

In the recent past, there has been a resurgence of interest in herbal medicines, which has disturbed the equation of demand and supply. To deal with these demands search of a potential alternative method for supply of good quality raw material has become a prime importance. In the last few decades biotechnological methods, specifically the plant tissue culture system, has emerged as a potential alternative source of high quality plant material. However, very little work has been reported on tissue culture aspects of *Clerodendrum* genus. Direct shoot regeneration from leaf explants of *C. inerme* was reported by Baburaj *et al*. (2000) on MS medium supplemented with BA alone at 4 mg/l. The regenerated shoots were rooted in MS medium supplemented with IAA (2 mg/l). In our laboratory we have reported micropropagation of *C. inerme* using axillary buds. Axillary buds were multiplied using BA at 16 µM with 3% sucrose. Rooting of the regenerated shoots was observed in basal MS medium without plant growth regulators. The phytochemical profile of *in vitro* plants was found to be similar to that of *in vivo* plants (Kothari *et al*. 2006). Adventitious shoot regeneration in MS medium supplemented with BA (5 mg/l), NAA and IBA (0.5 mg/l of each), was reported in *C. aculeatum*. The shoots were rooted in MS medium con-

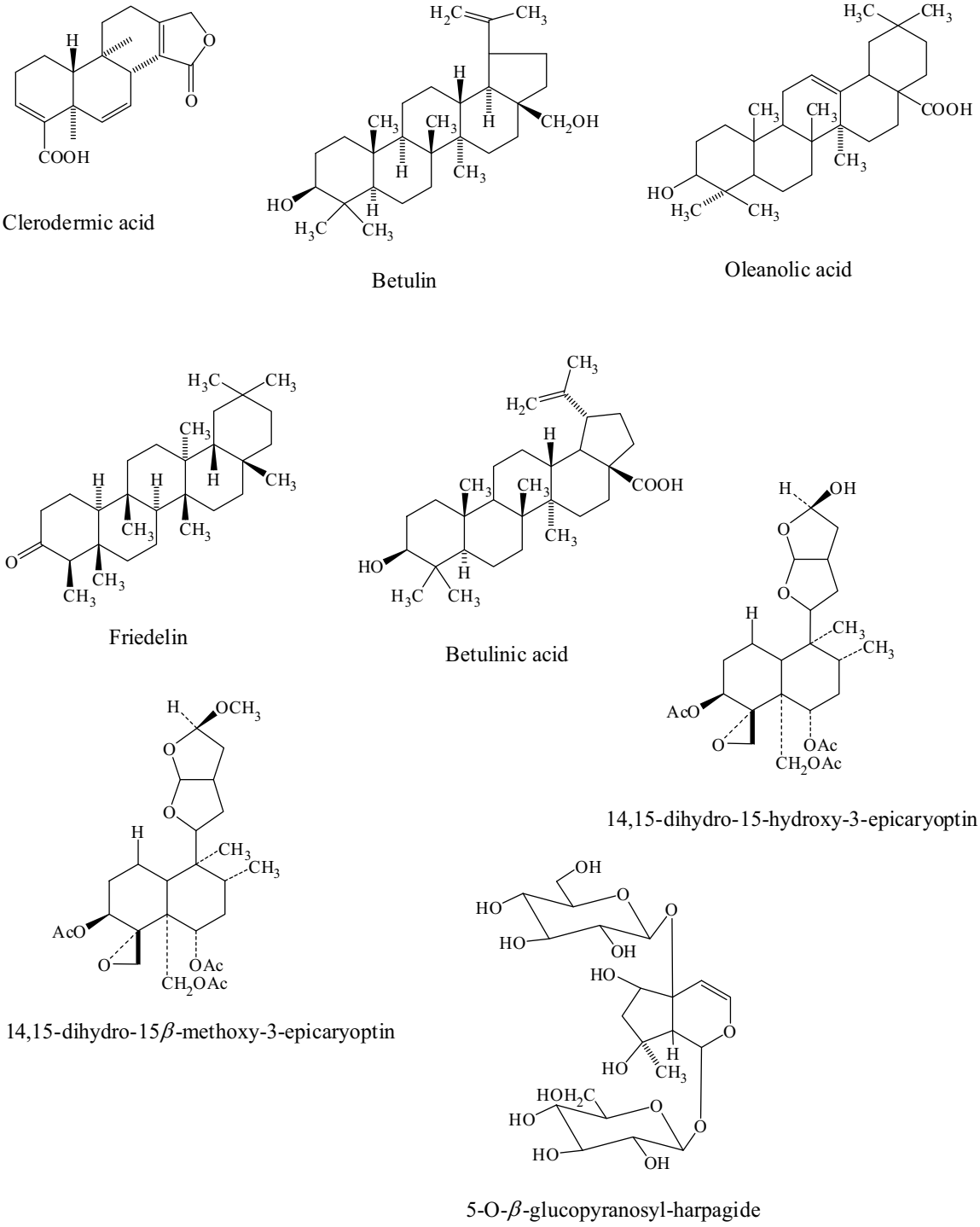


Fig. 5A: Terpenes of *Clerodendrum* genus.

taining 0.5 mg/l of NAA and IBA (Srivastava *et al*. 2004). Multiple shoot regeneration was observed in *C. colebrookianum* with six different cytokinins. Optimum shoot induction was observed with the medium containing BA (Mao *et al*. 1995).

From the above reports, it is clear that only certain aspects of biotechnology have been worked out in a few species of the genus. Extensive research has to be done in this field of biotechnology, especially in the area of molecular taxonomy because the genus shows much diversity and a clear pedigree of the genus is not yet known.

SUMMARY

Few species of *Clerodendrum* genus have been an important source of medicine for thousands of years and have been extensively investigated for their chemical constituents. Still the genus has tremendous potential which has not yet unfolded. The need of the hour is to explore the potential of various species of this widely distributed and available genus to fight against many diseases. New transgenic varieties could be created as efficient green production lines for pharmaceuticals by using genetic engineering and tissue culture for multiplying and conserving the species, which are difficult to regenerate by conventional methods and to save them from extinction.

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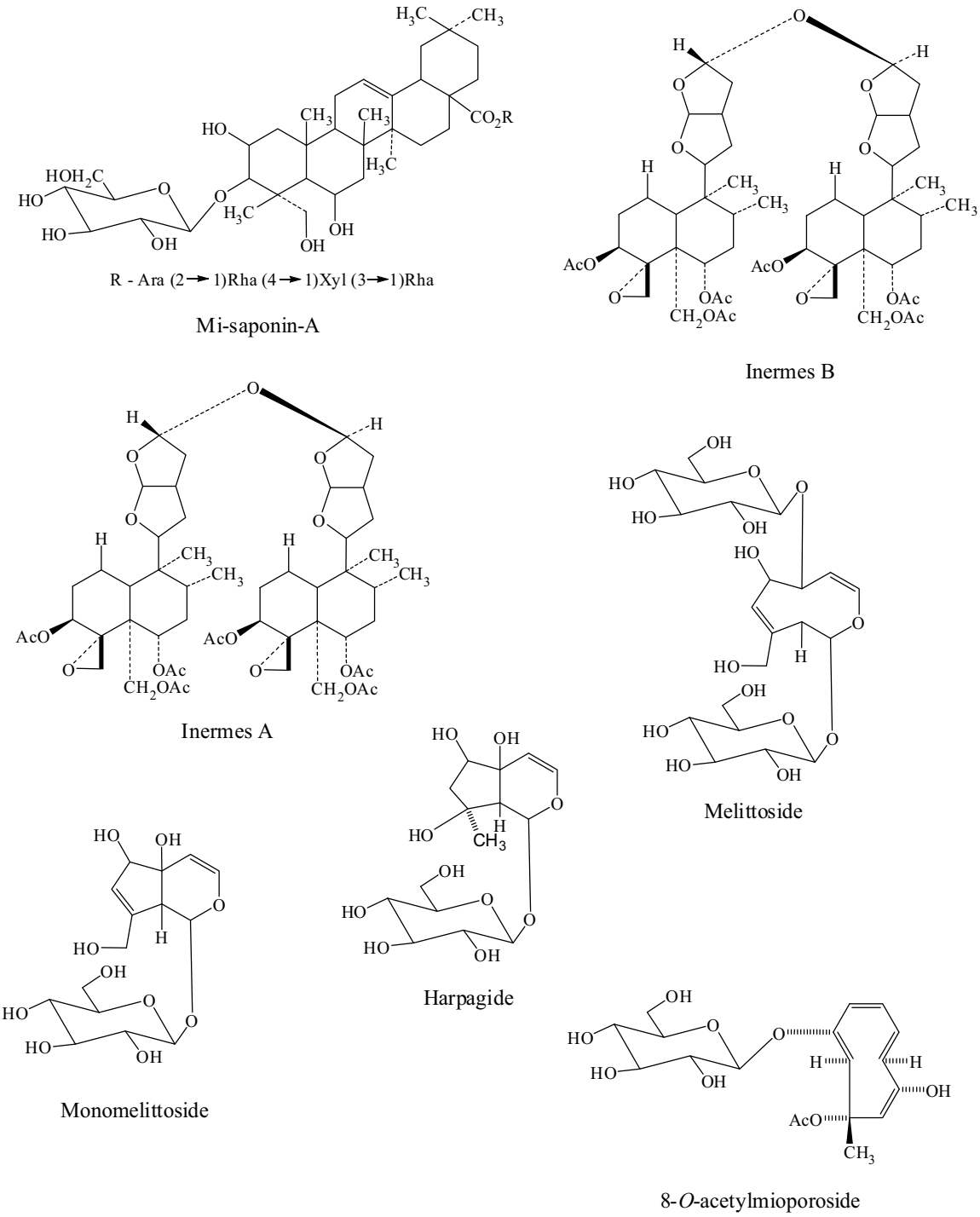

Fig. 5B: Terpenes of *Clerodendrum* genus.

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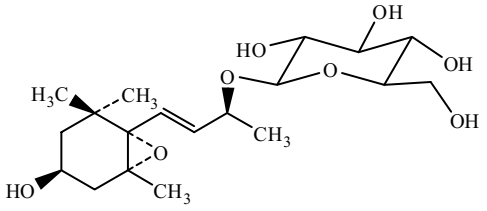
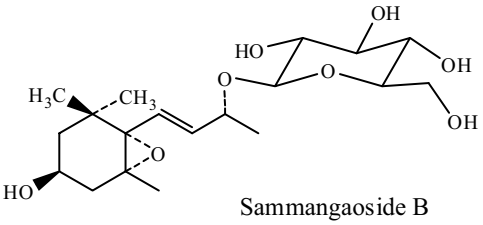
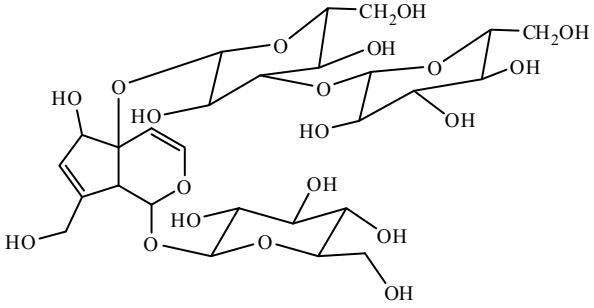


Sammangaoside A



Sammangaoside B



Sammangaoside C

Fig. 5C: Terpenes of *Clerodendrum* genus.

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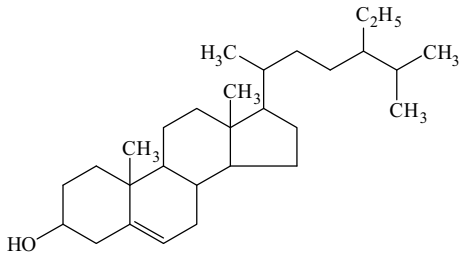
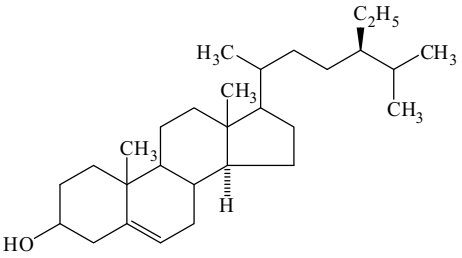
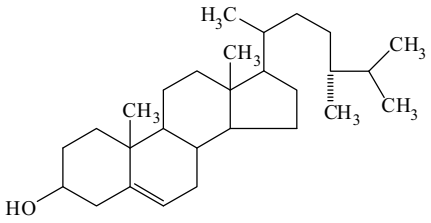


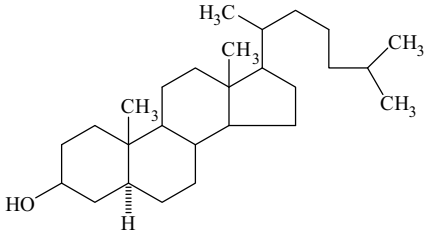
β-Sitosterol



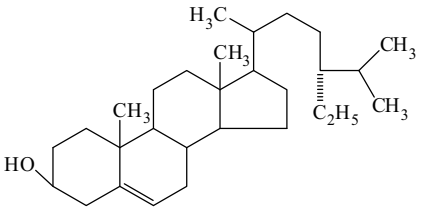
γ-Sitosterol



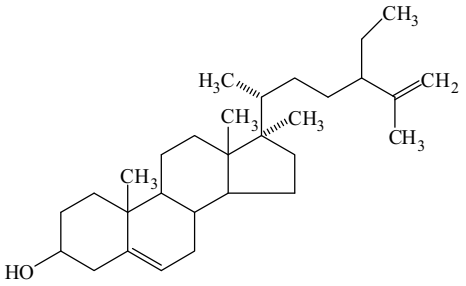
Campesterol



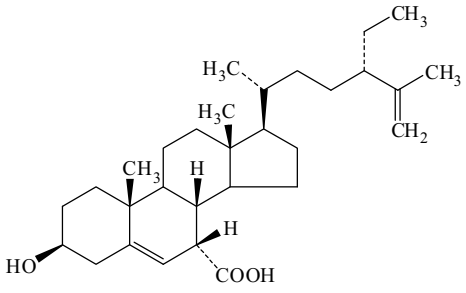
Cholestanol



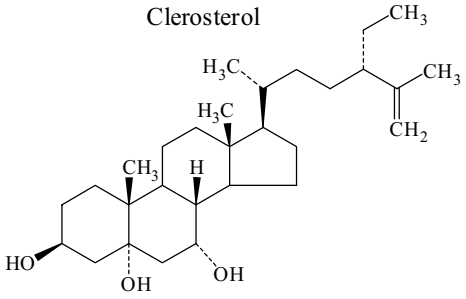
24-ethyl cholesterol



Clerosterol



Colebrin A



Colebrin B

Fig. 6A: Steroids of *Clerodendrum* genus.

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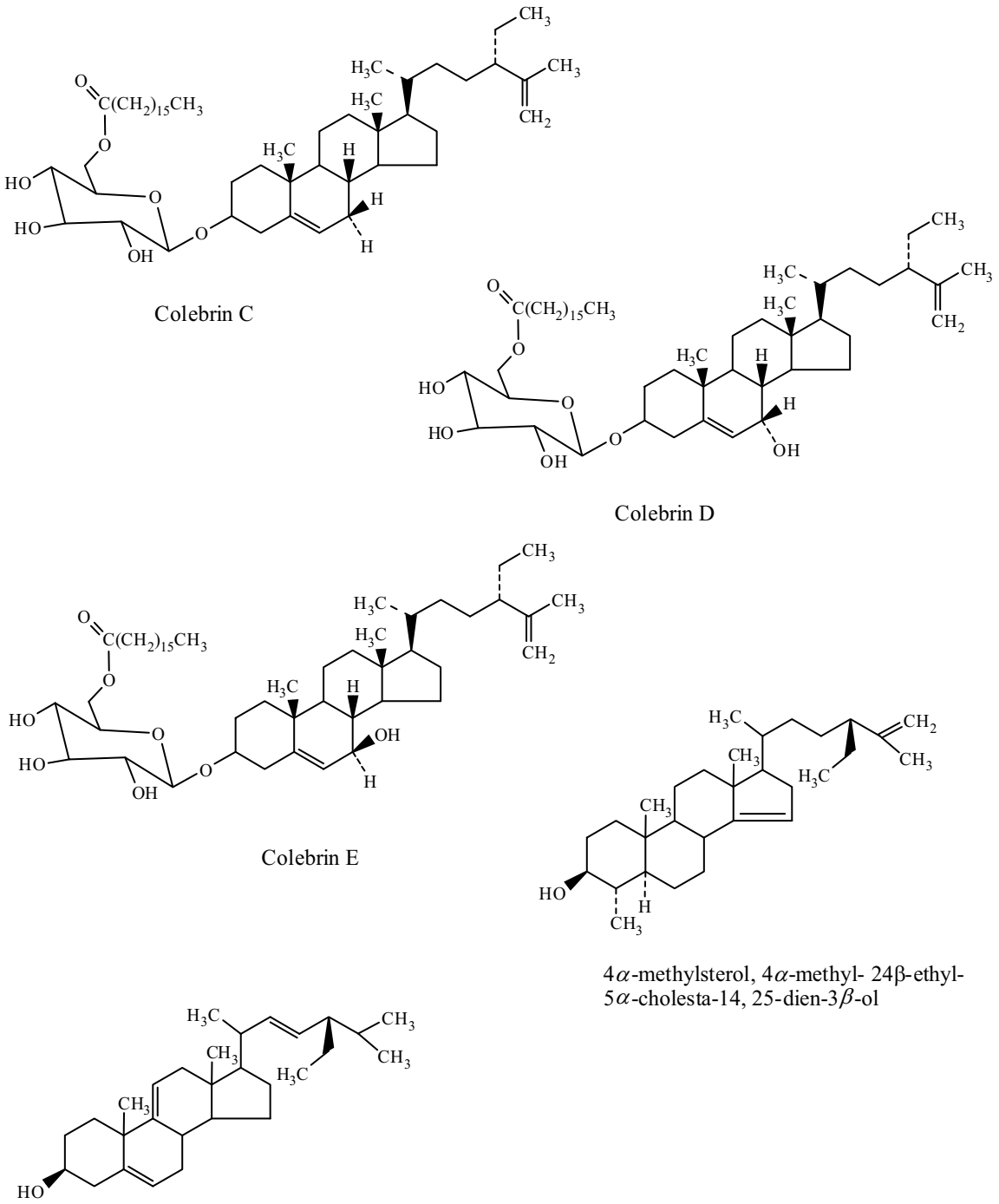

Fig. 6B: Steroids of *Clerodendrum* genus.

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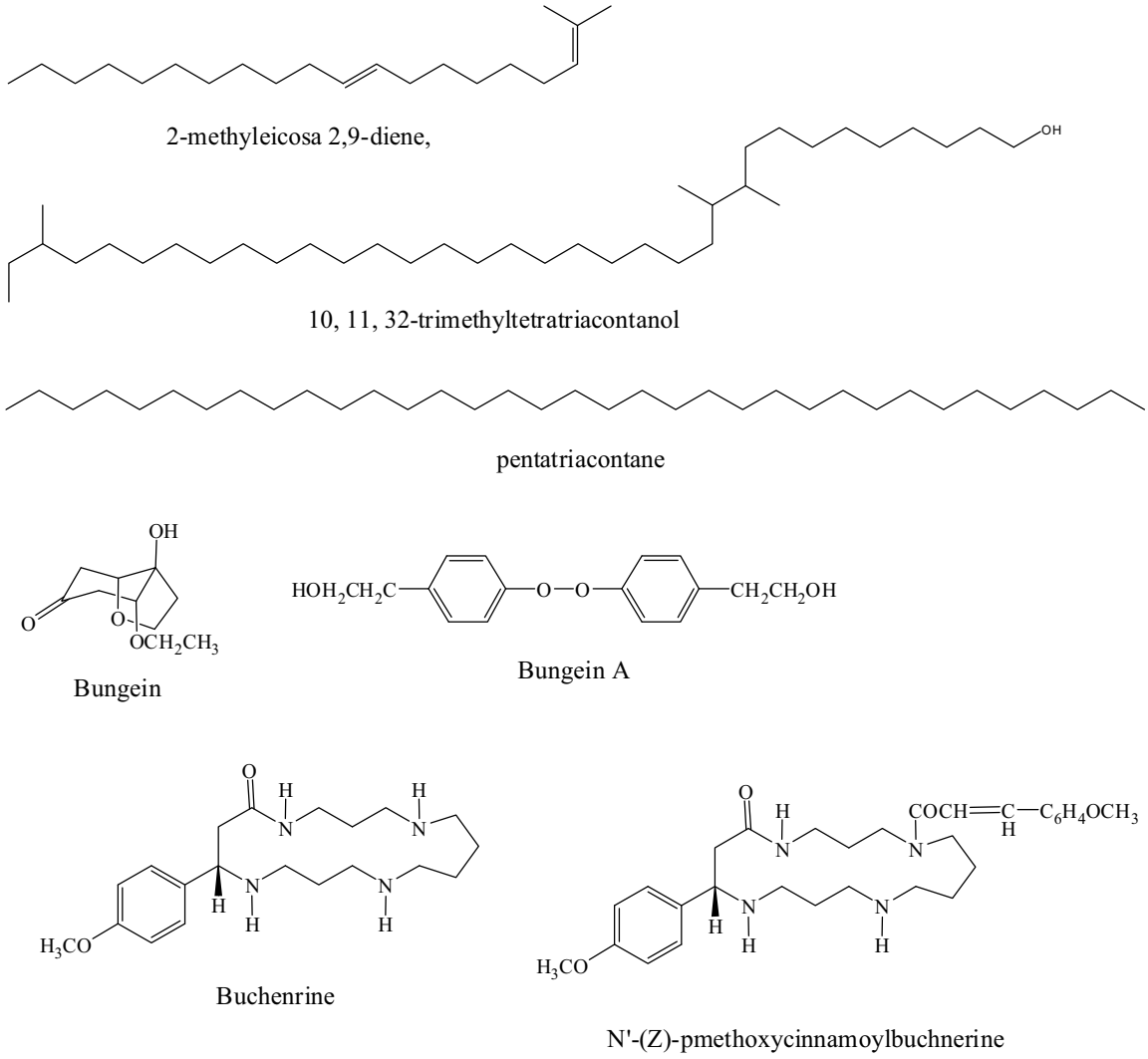

Fig. 7 Other chemical constituents of *Clerodendrum* genus.

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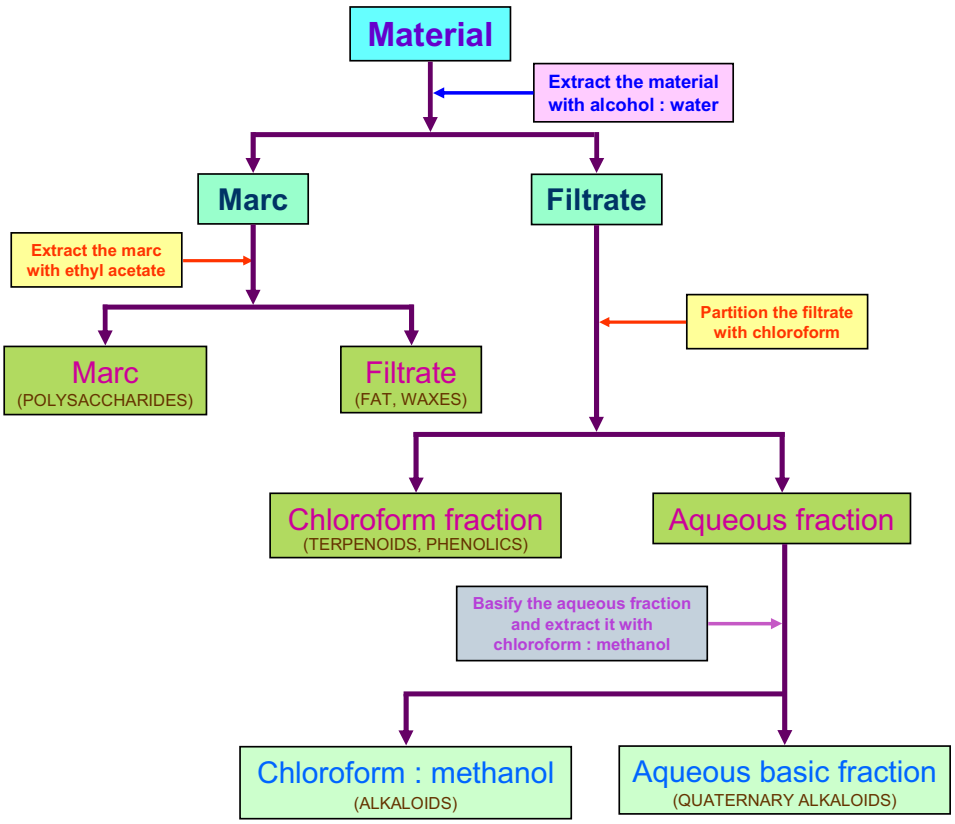

Fig. 8 General schematic diagram for isolation of chemical constituents. Based on Harbone 1984.