Chapter 2
Review of Literature

"Observation is a passive science, experimentation an active science"
-Claude Bernard

The review of literature on different species of *Clerodendrum* have been concentrated particularly on its medicinal and pharmacological aspects, with special emphasis on its ethno-medicinal use and evidence based scientific reports of its pharmacognostic activities. University of North Bengal library web portal (http://10.10.2.100/opac/opac.asp) and manual internet search were performed using various keywords related to the plant such as '*Clerodendrum* and traditional medicine, '*Clerodendrum* and therapeutic', '*Clerodendrum* and anti-cancer', '*Clerodendrum* and antioxidant' etc. In addition, reference and bibliographies of several published articles were searched for related keywords. Search for published research articles were separately performed in Medline, Scopus, Google Scholar and EBSCO.

2.1. Brief history of *Clerodendrum*

Clerodendrum is a flowering genus belongs to the family Lamiaceae; it is widely distributed in the tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and Asia. It is distributed in Northern Africa, Egypt and spreads through the remaining of Africa and Madagascar. The genus represents small trees, shrubs and herbs and is well known for its ornamental uses. Linnaeus first described the genus *Clerodendrum* in 1753, with the identification of *C. infortunatum*. Adanson changed the Latin name "*Clerodendrum*" to its Greek form "*Clerodendron*" in the year 1763 (Shrivastava and Patel, 2007) and after

a decade , Moldenke (1942)changed the Latinized name '*Clerodendrum*', which is currently used for the classification and description of the genus and species (Moldenke, 1985; Rueda, 1993; Hsiao and Lin, 1995; Steane *et al.*, 1999).

Clerodendrum is a very diverse and large genus with about 580 identified species are distributed throughout the world. Rajendran and Daniel (2002) recorded 23 species in India of which 16 were recorded from Arunachal Pradesh by Srivastava and Choudhary (2008).

2.2. Botanical Description

The different species of this genus are usually small trees, shrubs, herbs and climbers. Taxonomically the genus is characterized by simple opposite decussate leaves with most of them being petiolated. There is a wide variability in the sizes of the leaves. In *C. aculeatum*, the leaves range 0.9-4 cm in length and 0.3-1.4 cm in wide while *C. paniculatum* has leaves that are 6-35 cm long and 6-30 cm wide. The leaf shape is also variable with *C. walichii* having a lanceolate shape but *C. japanicum* being ovate shaped. The inflorescences of members of this genus are placed both axilliary and terminal position. The inflorescence ranges from 1-39 cm long and 1-25 cm wide. They may be cymes, panicles or solitary flowers which may be crowded or sparsely arranged. Almost all the species of *Clerodendrum* have foliaceous bracts and linear lanceolate bracteoles. The calyx is usually gamosepalous, commonly green but sometimes red or white. They are almost always campanulate, rarely elliptic, truncate, 5-lobed, glabrous or pubescent. The size of the corolla ranges from 0.6-4.0 cm long to 0.3-2.0 cm wide. The corolla is hypocrateriform and may be white, red, pink or purple. The corolla is glabrous, pubescent or glandular-puberulent. The fruits are mostly subglobose, drupaceous, ovoid or glabrous usually separating at maturity. The exocarp is fleshy and black when mature (Rueda, 1993).

2.3. Ethnomedicinal Uses of *Clerodendrum*

A number of species from this genus have been documented to be used by various tribes in Asia and Africa. Many species of the genus have been used in traditional systems of medicine in many countries like India, China, Korea, Thailand and Japan (Shrivastava and Patel, 2007). Various plant parts

such as leaf and root extracts of *C. phlomidis*, *C. serratum*, *C. indicum*, *C. chinense C. trichotomum* and *C. petasites* have been used for the treatment of asthma, rheumatism and other inflammatory diseases (Hazekamp *et al.*, 2001; Kang *et al.*, 2003; Panthong *et al.*, 2003; Choi *et al.*, 2004; Kanchanapoom *et al.*, 2005). Plant species such as *C. inerme* and *C. indicum* are used for the treatment of coughs, serofulous infection, buboes problem, venereal infections, skin diseases and as a vermifuge, febrifuge, snake and scorpion bites (Lobo *et al.*, 2006; Begum *et al.*, 1997; Kanchanapoom *et al.*, 2001). It was also reported that tribals use *C. inerme* as an antidote for poisoning of fish, crabs and toads (Begum *et al.*, 1997; Pandey *et al.*, 2003). *C. phlomidis*, *C. colebrookianum*, *C. calamitosum* and *C. trichotomum* have been used for antidiabetic, antihypertensive and sedative properties (Chaturvedi *et al.*, 1984; Khan and Singh, 1996; Cheng *et al.*, 2001; Kang *et al.*, 2003; Chae *et al.*, 2005; Choi *et al.*, 2004). *C. cyrtophyllum* and *C. chinense* are used for the treatment of fever, jaundice, typhoid and syphilis (Cheng *et al.*, 2001; Kanchanapoom *et al.*, 2005). Some species of *Clerodendrum* are cultivated as ornamental plants such as *C. inerme*, *C. thomosoniae*, *C. indicum* and *C. speciosum*.

2.4. Chemical constituent of *Clerodendrum*

Clerodendrum is an ethnomedicinally and traditionally important genus and used for the treatment of various diseases. Several researchers have been studied biologically active compounds from different species of *Clerodendrum* by isolation and identifying the compounds and other major constituents from these. Research report revealed that the several chemical compounds present in different species of *Clerodendrum,* are steroids, terpenoids and flavonoids (Praveen *et al.*, 2012).

2.4.1. Steroids

Steroids are a group of secondary metabolites which is present in the genus. Researchers have reported that *β* -sitosterol is present in the roots of *C. infortunatum*, roots also contain clerosterol, clerodone and clerodolone which is identified as 5, 25-sigmastadien_3β-ol, lup_20 (30)-en-3β -diol-12-one and 3β-hydroxylupan-12-one (Bhattacharjee *et al.*, 2011). *β*-sitosterol is also found in the roots of *C. phlomides* (Gokani *et al.*, 2011), *C.*

serratum (Jaya Babu *et al*., 1997), *C. paniculatum* (Joshi *et al.*, 1979), *C. fragrans* (Singh and Singhi, 1981) and stem of *C. indicum* (Prakash and Garg, 1981). *C. indicum* also contain clerodendrol (Tian and Sun, 1995). γ-sitosterol and clerosterol is present in the roots of *C. phlomides* (Gokani *et al.*, 2011). Clerodenoside-A is present in *C. japonicum* and it is identified as [β-3'- hydroxyl-4'-methoxyphenyl)-ethyl]-2",3"-di-o-acetyl-3-o-α-L-rhamnopyranosyl)-(4-o-feroloyl)-β-D-glucopyranoside (Tian and Sun, 1995). Clerosterol is also present in entire plant of *C. bungei* (Yang *et al.*, 2002) and leaves and stem of *C. inerme* (Akihisa *et al.*, 1989). Colebroside-A, colebrin A and B have also been isolated from the aerial parts of *C. colebrookianum* (Yang *et al.*, 2000). Stigmasterol, cholesterol, poriferasterol and 22-Dehydroclerosterol have been isolated from the aerial parts, leaves and stem of *C. fragrans* (Akihisa *et al.*, 1989). Bungein-A, cleroindicin-A, cleroindicin-C, cleroindicin-E and cleroindicin-F has been isolated from *C. bungei* (Yang *et al.*, 2002). List of steroids present in different species of *Clerodendrum* is depicted in Fig. 2.1.

2.4.2. Terpinoids

Terpenes are another class of secondary metabolites which include: monoterpenes, diterpenes, triterpenes, iridoids and sesquiterpenes. α-amyrin is isolated from the roots and stem of *C. fragans* (Singh and Singhi, 1981) and from the leaves and stem of *C. inerme* (Singh and Prakash, 1983). *β*-amyrin is isolated from the roots of *C. colebrookianum* (Joshi *et al.*, 1979)

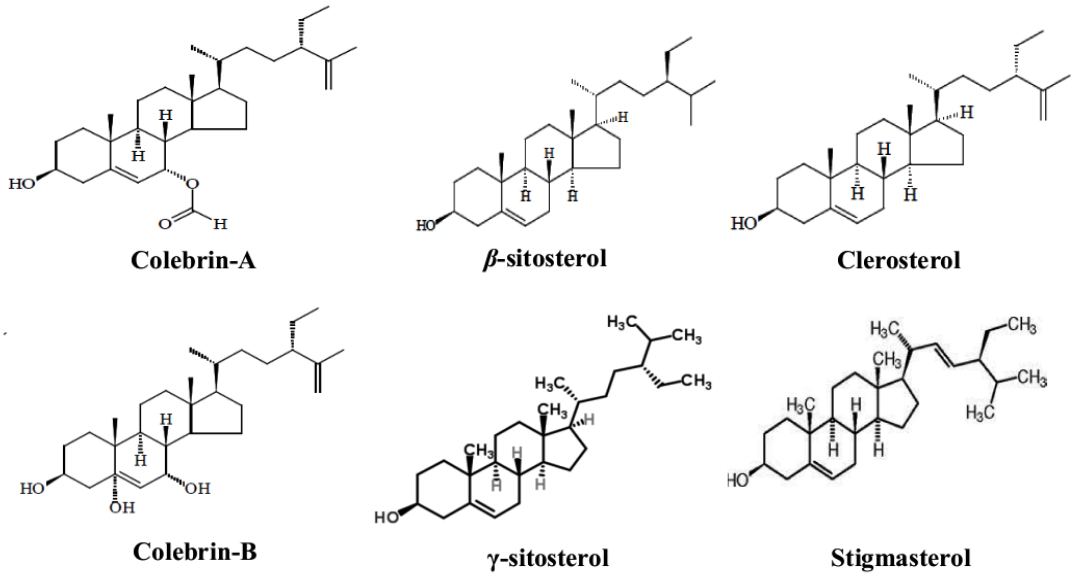

Fig. 2.1. Selected steroid phytocompounds present in different *Clerodendrum* species.

and from the leaves and stem of *C. inerme* (Parveen *et al.*, 2010) and from the roots of *C. paniculatum* (Joshi *et al.*, 1979). Luperol has been isolated from *C. viscosum* (Bhattacharjee *et al.*, 2011). Clerodendrin A is a diterpene which has been isolated from the roots of *C. phlomoides* (Gokani *et al.*, 2011) and Clerodendrin B, C and friedelin have been isolated from the leaves of *C. inerme* (Rao *et al.*, 1993). Clerodin which is a diterpene has been isolated from the flowers of *C. infortunatum*, *C. phlomides* and the leaves of *C. brachyanthum* (Bhattacharjee *et al.*, 2011; Gokani *et al.*, 2011; Lin *et al.*, 1989). Diterpenes like clerodinin A, B and C have been isolated from the leaves of *C. brachyanthum* (Lin *et al.*, 1989). Melittoside, monomelittoside and harpagide are isolated from *C. fragans* (Singh and Singhi, 1981). Ajugoside has been isolated from the leaves of *C. thomsoniae* (Lammel and Rimpler, 1981). Oleanolic acid has been isolated from the leaves and stem of *C. colebrookianum* (Yang *et al.*, 2000). Clerodinin A and oleanolic acid have been isolated from *C. infortunatum* (Sannigrahi *et al.*, 2012). List of terpinoids present in different species of *Clerodendrum* is depicted in Fig. 2.2.

2.4.3. Flavonoids

Flavonoids are another class of compounds that have been isolated from different species of *Clerodendrum*. Scutellarein has been isolated from the aerial parts of *C.*

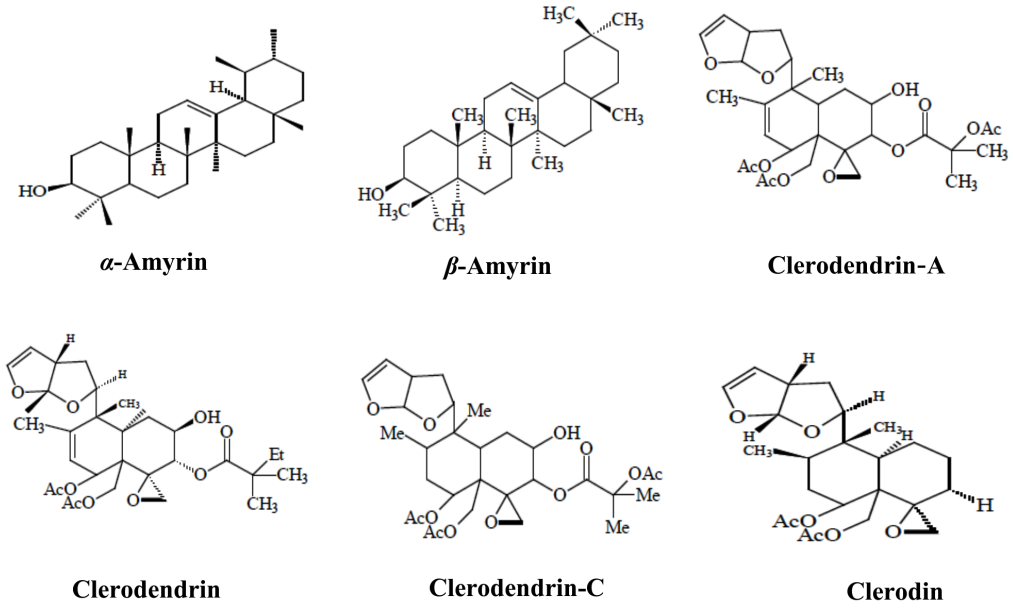

Fig. 2.2. Selected terpinoids present in different *Clerodendrum* species.

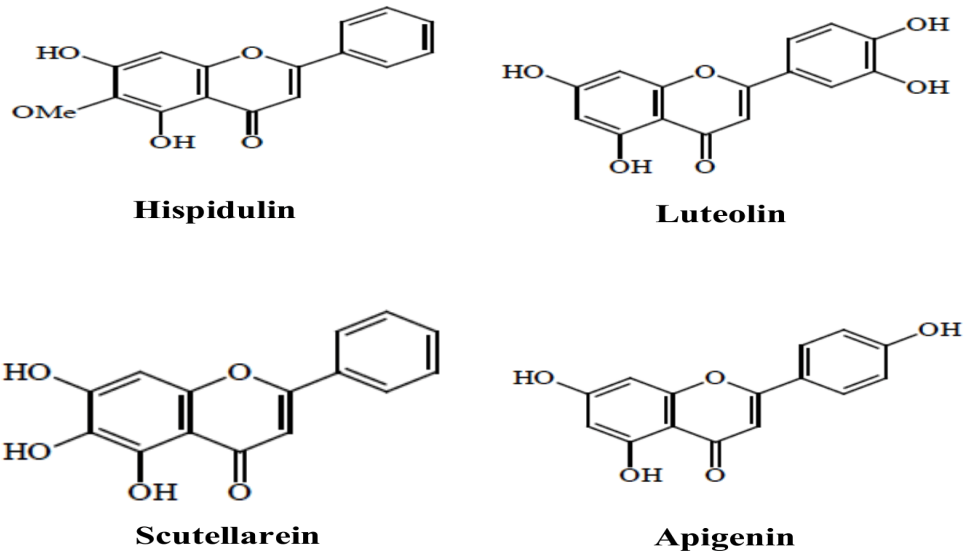

Fig. 2.3. Selected flavonoids present in different *Clerodendrum* species.

indicum (Tian and Sun, 1995). Apigenin has been isolated from the leaves and stem of *C. inerme* (Pandey *et al.*, 2006). Hispidulin has been isolated from the flowers of *C. phlomoides* (Gokani *et al.*, 2011) and from the aerial parts of *C. indicum* which is also contain hispidulin 7-0-glucuronide (Tian and Sun, 1995). Luteolin is another major flavonoid that has been isolated from the flowers of *C. phlomoides* (Gokani *et al.*, 2011). List of terpinoids present in different species of *Clerodendrum* is depicted in Fig. 2.3.

2.4.4. Miscellaneous

Several other chemical compounds have been found in different species of *Clerodendrum.* Benzoic acid, cabsurin and quercetin have been isolated from aerial parts and roots of *C. infortunatum* respectively (Bhattacharjee *et al.*, 2011). Friedelin, betulinic acid, syringic acid, ρ-methoxy benzoin acid have been isolated from *C. inerme*. Martinoside, monoacetyl martinoside have been isolated from *C. japonicum* and *C. bungei* (Tian and Sun, 1995; Yang *et al.*, 2002). Glyceryl -1-docosoicate, acetoside, maslinic acid have been isolated from *C. colebrookianum* (Yang *et al.*, 2000). Racemic dihydro rengyolone, rengyoxide, rengyoside B, cornoside and racemic rengyolone,dihydro cornoside have been isolated from *C. fragrans* (Kanchanapoom *et al.*, 2005). Betulinic acid and octadecanoic acid have been isolated from *C. bungei* (Yang *et al.*, 2002).

2.5. Biological activities of *Clerodendrum*

Clerodendrum is a traditionally and ethnomedicinally important genus which is used to treat various healthcare systems. Many researchers have been carried out a number of *in-vivo* and *in-vitro* assays to prove certain chemicals present in this plant and the validity of these traditional claims. These studies showed that the different species of the genus possess potent antimicrobial, anti-inflammatory, anti-malarial, anti-diabetic, anti-cancer, analgesic and anti -oxidant activities (Shrivastava and Patel, 2007). Some major activities are described below.

2.5.1. Anti-microbial activity

Many species from the genus *Clerodendrum* were documented in ancient literature and have showed potent anti-microbial activity. Ethanol extract of leaves and stems of *Clerodendrum infortunatum* showed potent anti-microbial activity against some gram positive, gram negative bacteria and fungal strains including *E. coli, Bacillus subtilis, Staphylococcus aureus and Aspergillus niger, Aspergillus flavus Candida albicans* (Rajurkar, 2011). Ethanol and aqueous extracts of leaf, stem and root of *Clerodendrum viscosum* showed potent antibacterial activity against some gram positive, gram negative bacterial and fungal strains viz., *Staphylococcus aureus*, *Sercinia lutea, Bacillus subtilis, B. megaterium, B. cereus* and *Streptococcus haemolyticus, Salmonellae typhi, Shigella dysenteriae*, *Escherichia coli, S. shiga, S. boydii*, *S. sonnei*, *Proteus* sp., *Klebsiella pneumonia, Pseudomonus aeruginosa, Agrobacterium tumefaciens*, *Erwinia chrysanthemi* and *Xanthomonas phaseoli* (Tamta *et al.*, 2012; Oly *et al.*, 2011). Petrol, benzene, ethanol, methanol, ethly acetate and aqueous extracts of leaves and roots of *Clerodendrum inerme* exhibited anti-microbial activity against some human pathogenic bacteria (Khan and Khan, 2005; Chahal *et al.*, 2010). Leaves and stems of *Clerodendrum phlomidis* and *Clerodendrum inerme* exhibited anti-fungal activity against some fungal pathogen (Anitha and Kannan, 2006). Roots extract of *Clerodendrum serratum* showed potent anti-microbial activity against some gram positive and gram negative bacteria (Singh *et al.*, 2012). Various extracts of leaves of *Clerodendrum paniculatum* showed

potent anti-microbial activity against some gram negative bacteria (Joseph *et al.*, 2011). Ethanolic extracts of leaves of *C.philippinum* exhibited anti-microbial activity against *E. coli, S. aureus, Bacillus* and *Klebsiella* (Venkatanarasimman *et al.*, 2012). The flower chloroform extracts of *C. chinense* and *C. splendens* were active against *Plasmodium falciparum* with an IC_{50} value of $<10 \mu g mL^{-1}$. Chloroformic extracts of the stem and flowers of *C. chinense* were active against *Trypanosoma cruzi* with an $IC_{50}=1.21$ and 1.12 $\mu g mL^{-1}$ respectively. Chloroformic extracts of the leaves of *C. chinense* and *C. splendens* showed promising activities against *T. cruzi* with an $IC_{50}=3.39$ and 1.98 $\mu g mL^{-1}$ respectively (AbouZid *et al.*, 2013).

2.5.2. Anti-inflammatory activity

Inflammation is a complex biological reaction of vascular tissues against destructive agents such as pathogens, damaged cells or irritants. Many species of the genus have showed potent anti-inflammatory activity. In 1988 Surendrakumar, showed that *C. phlomoidis* significantly reduced paw edema induced by carrageenan in rats at a dose of 1 g/kg. The anti-inflammatory activity of methanol extract of *Clerodendrum inerme* exhibited sub-chronic (cotton pellet-induced granuloma) models significantly (Yankanchi and Koli, 2010). The ethanolic root extract of *C. serratum* showed considerable anti-inflammatory activity in carrageenan-induced edema in rats and also in the cotton pellet model in experimental mice, rats and rabbits (Singh *et al.*, 2012). *C. petasites* was reported to show moderate anti-inflammatory activity in the acute phase of inflammation in rats. The ED_{50} values of the experiment were reported to be 2.34 mg/ear and 420.41 mg/kg in rats (Panthong *et al.*, 2003). The methanolic extract of leaves of *C. trichotomum* showed significant anti-inflammatory activity in experimental rat, mice and Raw 264.7 macrophage cells (Choi *et al.*, 2004). The methanol leaves extract of *C. infortunatum* displayed vital anti-inflammatory activity against the carrageenan induced rat paw edema (Das *et al.*, 2010). The aqueous extract of root bark of *Clerodendrum phlomidis* showed anti-inflammatory activity against carrageenan induced rat paw edema and acetic acid induced peritonitis in mice (Parekar *et al.*, 2012).

Examples of Free Radicals

Name	Formula	Comment
Hydrogen atom	H'	The simplest free radical.
Trichloromethyl	$CCl_3^{\cdot}$	A carbon-centered radical (i.e., the unpaired electron resides on carbon). $CCI_3^{\cdot}$ is formed during metabolism of CCl_4 in the liver and contributes to the toxic effects of this solvent. Carbon-centered radicals usually react fast with O_2, to make peroxyl radicals, e.g., $CCI_3^{\cdot} + O_2 \rightarrow CCI_3O_2^{\cdot}$
Superoxide	$O_2^{\cdot -}$	An oxygen-centered radical. Selectively reactive.
Hydroxyl	$OH^{\cdot}$	A highly reactive oxygen-centered radical. Attacks all molecules in the human body.
Thiyl	$RS^{\cdot}$	A group of radicals with an unpaired electron residing on sulfur.
Peroxyl, alkoxyl	$RO_2^{\cdot}$, $RO^{\cdot}$	Oxygen-centered radicals formed (among other routes) during the breakdown of organic peroxides.
Oxides of nitrogen	$NO^{\cdot}$, $NO_2^{\cdot}$	Nitric oxide is formed in vivo from the amino acid L-arginine. Nitrogen dioxide is made when $NO^{\cdot}$ reacts with O_2 and is found in pol- luted air and smoke from burning organic materials, *e.g.,* cigarette smoke.

2.5.3. Anti-malarial activity

Malaria is one of the most important parasitic infections of humans and every year 863,000 deaths occur throughout the world (Zailani *et al.*, 2009; Deressa *et al.*, 2010). Many species of the genus *Clerodendrum* have been reported for its antimalarial activities because of the presence of a bitter principle. The methanol extract of the leaves of *C. myricoides* significantly ($p<0.05$) inhibited parasitaemia of *P. berghei* (Deressa *et al.*, 2010). The ethanolic extract of leaves of *C. violaceum* showed antimalarial activity against *Plasmodium berghei* (Zailani *et al.*, 2009). The alcoholic extract of *C. phlomidis* showed antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 48 µg/ml (Simonsen *et al.*, 2001). The petroleum ether extract of *C. inerme* also inhibited the growth of larvae of *Culex quinquefasciatus, Culex pipiens*, and *Ades aegypti* (Gayar and Shazli, 1968; Kalyanasundaram and Das, 1985).

2.5.4. Anti-diabetic activity

Diabetes Mellitus (Type 2) is

characterized by hyperglycemia due to insulin resistance and insulin deficiency resulting from β-cell dysfunction. According to World Health Organization, 200 million cases of diabetes were reported worldwide in the year 2000 and the number is estimated to rise to 300 million by 2030. In India, it is becoming a killer disease next to coronary heart disease. The causes could be attributed to sedentary lifestyle, lack of physical exercise and obesity. A decoction of the entire *C. phlomidis* plant has been reported to have antidiabetic activity. A dose of 1g/kg showed antidiabetic effect in alloxan-induced hyperglycemia in rats. It further showed antihyperglycemic activity in human adults at a dose of 15-30 g/day (Chaturvedi *et al.*, 1984). The methanolic extract of leaves of *Clerodendron inerme* showed anti-diabetic activity. A dose of 200 mg/kg showed a very significant and progressive reduction in glucose level induced by streptozotocin in rats (Rajeev *et al.*, 2012).

2.5.5. Anti-cancer activity

Cancer is the second leading cause of death in many of the developed countries and every year it is increase exponentially. The aqueous extract of leaves of *Clerodendron inerme* showed anti-cancer activity. A dose of 500 mg/ kg body weight significantly prevented the tumor formation (oral carcinogenesis) induced by 7, 12-dimethylbenz anthracene (DMBA) in rats (Manoharan *et al.*, 2008). The leaf extract of *Clerodendrum serratum* showed anti-cancer activity against skin carcinogenesis induced by 7, 12-dimethylbenz anthracene (DMBA) in mice (Chinchali *et al.*, 2011). The ethanolic extract of roots of *Clerodendrum paniculatum* showed anticancer activity but to a lesser extent (John *et al.*, 2008).

2.5.6. Analgesic activity

Analgesic is a very common term which includes pain, inflammation, fever etc. and creates many complications in day to day life of human beings. Many species of *Clerodendrum* have been reported for analgesic activity. Petroleum ether, Ethyl acetate and Methanolic extract of aerial parts of *Clerodendrum phlomidis* showed analgesic activity in mice (Vijayamirtharaj *et al.*, 2011). *Clerodendrum inerme* displayed significant analgesic activity in acetic acid induced writhing in rats (Yankanchi and Koli, 2010). The ethanolic extract of leaves of

Clerodendrum viscosum showed analgesic activity induced by pethidine in rats (Das *et al.*, 2011). The Ethanolic Extract of the leaves of *Clerodendrum viscosum* at a dose of 200mg/kg ($p<0.01$) exhibited significant peripheral analgesic activity in mice (Chandrashekar and Rao, 2013).

2.5.7. Anti-oxidant activity

Reactive Oxygen Species (ROS) and many of the free radicals are natural by -products which are constantly generated *in-vivo* for specific metabolic purposes. ROS is responsible for many of the human diseases like cancer, viral infections, diabetes, cardiovascular diseases and inflammations and it also damage the biological molecules like DNA, lipids and proteins (Halliwell, 2006). The antioxidants present in the medicinal plants minimize the formation of reactive oxygen species (ROS). The ethanolic extract of arial parts of *Clerodendrum serratum* showed good antioxidant properties against DPPH and Nitric oxide radical whereas *Clerodendrum serratum* root extract showed good antioxidant properties against DPPH (Ismail and Leelavathi, 2011). The ethanolic extract of the leaves of *Clerodendrum infortunatum* Linn showed significant antioxidant activity against DPPH free radical scavenging activity, reducing power assay and scavenging of hydrogen peroxide (Modi *et al.*, 2010). The methanolic extract of leaves of *Clerodendrum inerme* showed highest free radical and antioxidant activity (Gurudeeban *et al.*, 2010). The ethanolic extract of roots of *Clerodendrum viscosum* showed maximum scavenging of the radical cation, nitric oxide radical, ferric ion radical and 1,1- diphenyl, 2-picryl hydrazyl (DPPH). The aqueous extract showed only moderate activity (Pankaj *et al.*, 2007). The ethanolic extract of roots of *Clerodendrum phlomidis* showed best free radical scavenging activity than that of other three extracts petroleum ether, chloroform, ethyl acetate (Sathish *et al.*, 2011).

2.5.8. Other biological activity

The leaf juice *C. phlomidis* showed significant anthelmintic activity against *Ascaris lumbricoides*, *Phreitima posthuma* and *Taenia solium* (Garg and Siddiqui, 1992). *Clerodendrum phlomidis* showed potent antidiarrhoeal activity against castor oil induced diarrhea and PGE_2 induced enteropooling in rats (Rani *et al.*, 1999).