Chapter V
Discussion

5.1. Phytochemical Investigations

Nature has provided a storehouse of remedies to cure all ailments of mankind (Kokate et al., 2002). Nature provides us drugs in the form of plants to cure the incurable diseases without any toxic effect (Trease et al., 1983). Plants synthesize some chemicals themselves which metabolize their physiological activities. These chemicals are often referred to as “secondary metabolites” which includes alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids. These phytochemicals are used to cure the disease in herbal and homeopathic medicine. Nowadays, most of the people depends on traditional methods to cure various ailments (Tyagi and Bohra, 2002; Okwu, 2004).

5.1.1. Preliminary Phytochemical Analysis

Plant based natural constituents can be obtained from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. (Gordon, 2001). Therefore, in the present study the different extracts (aqueous, petroleum ether, chloroform, ethanol, acetone) of whole plant of Caralluma geniculata was analyzed for preliminary phytochemicals and identified the presence of carbohydrates, flavonoid, phenolic compound, saponins, terpenoids, steroids, proteins, coumarins, quinones and phytosterols (Table 4.1). Similarly the availability of phytochemicals in different species of Caralluma was reported.
Alkaloids and glycosides were entirely lacking among the phytocomponents tested in the present study. Similarly, absence of alkaloids was reported in the ethanolic extract of *Caralluma dalzielii* and *Caralluma tuberculata* (Tanko et al., 2012; Rauf et al., 2013), whereas absence of glycosides in various solvent extracts (hexane, chloroform, ethyl acetate and methanol) of *Caralluma tuberculata* was reported by Rauf et al. (2013). Contrary to these observations, C-21 steroidal glycosides known as Carumbelloside – V with significant antinociceptive activity was isolated from *Caralluma umbellata* (Ray et al., 2012). Pregnane glycosides 12β,20-O-dibenzoyl-5α, 6-dihydrosarcostin β-oleandropyranosyl-(1→4)-β-cymaropyranosyl- (1→4) – β- digitoxypyranosyl- (1→4) – β- cymaropyranoside from the whole plant extract of *Caralluma adscendens* var. gracilis and pregnanes 12β -O-benzoyl -3β,11α,14β,20R- pentahydroxy-pregn-5-ene 11α-O-benzoyl-3β,12β,14β,20R-pentahydroxy - pregn–5–ene from *Caralluma pauciflora* (Reddy et al., 2011). Kishore et al. (2010) isolated two new pregnane compounds such as 3β–hydroxy–pregn–5–ene (CRUR I) and 3β, 14β – dihydroxy pregn–5–ene (CRUR II) from the roots of *Caralluma umbellata* and later, Ray and Nagaiah (2011) proved the antinociceptive and anti-inflammatory activity of pregnane glycoside Carumbelloside – II isolated from the whole plant of *Caralluma umbellata* and anti-inflammatory property of Carumbelloside-III obtained from the dried barks and flowers of *Carallum umbellata* (Ray et al., 2011). Presence of eleven novel pregnane glycosides was reported from the whole plants of *Caralluma adscendens* var. fimbriata by Kunert et al. (2008). Five pregnane glycosides were isolated from *Caralluma umbellata* (1-5) in addition to a known one (Russelioside E, 6) (Abdel-Sattar et al., 2008). Ahmed et al. (1988); Wadood et al. (1989); Abdel-Sattar (2013) stated that antihyperglycemic effect could be attributed to acetylated pregnane glycoside. Whole
plant of *Caralluma attenuata* possesses luteolin–4–O–neohesperidoside a flavonoid (flavones glycoside) (Ramesh *et al*., 1998). Megastigmane glycosides have been isolated from *Caralluma negevensis* (Jayakar *et al*., 2004). Twenty new pregnane glycosides were reported from the whole plant of *Caralluma negevensis* by Braca *et al.* (2002). Two new pregnane glycosides were isolated from the aerial part of *Caralluma wissmannii* (Dawidar *et al*., 2012).

Based on the nature of the soil in which the plant grow; the age of the plant at the time of collection, season and climate variation in the amount of chemical constituents may takes place (Jesudass *et al*., 2001).

Among the different phyto constituents identified carbohydrates serve as a primary energy source in the diet. It provides energy for working muscles, fuel for the central nervous system, permitting fat metabolism and preventing protein from being used as energy (food and nutrition board, 2002).

In nature, plants synthesize flavonoids to prevent damages caused by free radicals. The radical scavenging properties linked with the flavonoids which protect against oxidative stress, reducing the risk of heart diseases, preventing cancer, and slowing down the aging process in cells responsible for degenerative diseases (Hollman and Katan, 1997; Harvsteen, 2002), antibacterial (Cushnie and Lamb, 2011), anti-inflammatory (Martinez-Micaelo *et al*., 2012), antidiabetic and brain diseases (Bubols *et al*., 2013).

Phenolic compounds are the most abundant secondary metabolites of plants. Plant phenolics protect the plants against ultraviolet radiation or aggression by pathogens, parasites, predators and gives colours to it (Bravo, 1998; Archivio *et al*.,
Phenolic compounds reduces oxidative stress by inhibiting free radicals (Ames et al., 1993) simultaneously, reduces the chance of heart diseases (Jin and Mumper, 2010), cancer (Pieme et al., 2010), inflammation (Mohanlal et al., 2012) and diabetes (Scalbert et al., 2005). The availability of phenols changes in different seasons (Solar et al., 2006) and stress conditions (Diaz et al., 2001).

Saponins are secondary metabolites that remain in many plant species (Hostettmann and Marston, 1995). They are stored in plant cells as inactive precursors but can be readily converted into biologically active antibiotics with the help of plant enzymes in response to pathogen attack (Osbourn, 1996).

Terpenoids represent the largest class of secondary metabolites from the natural source. It serves as a protective agent against different pathogens such as insects, fungi and bacteria or growth regulatory molecules also it is a potential anticancer drugs (Kintzios, 2003). Terpenoids possess pharmacological activities like anti-viral, anti-malarial, anti-inflammatory and inhibitor of cholesterol synthesis (Mahato and Sen, 1977). Commercially, it is used as an agent of flavour and fragrance (Cragg, 1998; Dhingra et al., 2000). Terpenoids play a significant role in cellular function and maintenance. It represents a large component of volatile floral and fruit scents. It is the main constituent of essential oils (Dudareva and Pichersky, 2000; Closson and Monteleone, 2009; Reiss et al., 2009).

Steroids are an essential hormone. It includes anti-inflammatory (Jang et al., 2004), cytotoxic (Shao et al., 1997), antimutagenic (Tang and Gao, 2001) and antifungal activities (Shimoyamada et al., 1990).
Plant proteins are therapeutic agents. It has unique place in pharmaceutical biotechnology due to their significant role in cell biology particularly urolithosis (Aggarwal et al., 2014) and antifungal (Jamil et al., 2007). Soluble proteins play a role in resistance of plants to stress (Chkhubianishvili et al., 2011).

Coumarin is a natural substance that has anti-tumour activity. It is used in cancer therapy (Lacy and Kennedy, 2004). Coumarins shows radical scavenging effect due to their antioxidant activities which prevent various human health problems such as diabetes, cardiovascular, brain diseases and inflammation (Bubols et al., 2013).

Quinones are important naturally occurring pigment which exhibit many biological activities such as antibacterial, anticancer and antiviral (Koyama, 2006; Ignacimuthu et al., 2009).

Phytosterols are a group of lipophilic steroid alcohols found in plants chemically they are similar to cholesterol. In plants it is found in the free form or esterified to fatty acids or as steryl glycosides (Ling and Jones, 1995). Phytosterols inhibit the uptake of both dietary and endogeneously produced (biliary) cholesterol from internal cells. This condition leads to decrease in serum total and LDL cholesterol levels (Law, 2000; Abumweis et al., 2008). Similarly, it helps to inhibit ovarian, breast, stomach and lung cancer (Woyengo et al., 2009), protects skin (Schwartz, 2011) and cardiovascular problems (Awad, 2000). Tanko et al. (2012) reported that hypoglycemic activity of Caralluma dalzielii may be due to the presence of secondary metabolites.

5.1.2. Fourier Transform Infrared Spectroscopy analysis

The FTIR spectrum is a widely used method to identify the functional group of the active components based on the peak value in the region of infrared radiation. The
FTIR spectrum of the ethanolic extract of *Caralluma geniculata* showed the presence of alkyne, methylene, isocyanate, cyanide ion thiocyanate ion and related ions, organic nitrates, aliphatic nitro compounds, methyl, ammonium ion, aromatic nitro compounds, amine, sulfonates, thioethers, disulfides, aryl disulfide [Table 4.2; Figure 4.1]. Among the different functional groups identified alkynes are bioactive as nematocides (Jørgen, 1988). Organic nitrates are highly useful in the treatment of cardiovascular disease (Gori *et al*., 2004). Ammonium ions play a crucial role in the metabolism of animals, plants and microorganisms. Ammonia is an important nutrient which is essential to the body to complete protein and amino acid synthesis (Beattie, 2014) also it has antimicrobial effect (Gupta *et al*., 1988). Aromatic nitro compounds are important intermediates in synthetic organic chemistry and chemical industry (Yan and Yang, 2013). The result of the present study proved the presence of different functional groups such as alkynes, organic nitrates, ammonium ions, aromatic nitro compounds etc. in ethanol extract of *Caralluma geniculata*. It shows the medicinal value of this plant hence this plant can be used to treat various diseases in future.

5.1.3. Gas Chromatography-Mass Spectrometry analysis

Gas Chromatography-Mass Spectrometry is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection and identification of unknown samples. It can identify trace elements in materials and quantify an amount of substance. The heights of the peak indicate the relative concentrations of the compound present in the plant extract. Priya *et al*. (2011) analyzed the ethanolic extracts of *Caralluma fimbriata* using GC-MS and identified 14 peaks among that n-Hexadecanoic acid (44.23%) showed maximum peak area, whereas
alcoholic extract of *Caralluma umbellata* revealed the presence of thirty two compounds and Lupeol (52.33%) was noticed with more peak area (Jeyakumar et al., 2013). Ethanolic extract of *Caralluma truncato-coronata* indicate the presence of 20 chemical constituents and showed a maximum peak area for thunbergol (68.05%) (Kalimuthu et al., 2013), where as Ravikumar (2014) reported 24 compounds in the same extract and maximum peak area was noticed in 1-Octadecene (11.91%). Similarly in the present study, Ethanolic extract of *Caralluma geniculata* showed the presence of 19 different phytoconstituents (Table 4.3; Figure 4.2). Among the different compounds identified Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-a-D-mannopyranoside showed maximum peak value (25.39%). The nature of the identified compounds were terpenoids and steroids (Table 4.4).

Sudha et al. (2013) proved the presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (peak value 20.45%), Hexadecanoic acid, ethyl ester (peak value 7.53%), Phytol (4.58%), Linoleic acid ethyl ester (peak value 0.63%) and Vitamin E (peak value 6.69%) in *Kirganelia reticulata*. Sathish et al. (2012) reported the presence of hexadecanoic acid, ethyl ester (peak value 1.69%) and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (peak value 3.71%) in *Vitex altissima*, whereas hexadecanoic acid, methyl ester (peak value 3.56%) and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (peak value 1.05%) and Linoleic acid ethyl ester (9,12-octadecadienoic acid (Z,Z)- (peak value 6.74%) were identified in methanolic extract of *Caralluma fimbriata* (Priya et al., 2011). These compounds were identified in ethanol extract of *Caralluma geniculata* also. Vitamin-E was reported in *Caralluma truncato-coronata* with peak value 9.45% (Kalimuthu et al., 2013) correspondingly, vitamin-E was identified in *Caralluma geniculata* with peak value 9.82%.
Among the various phytocompounds identified, borneol is a natural insect repellent. It is a component of many essential oils; also it is used in traditional Chinese medicine as moxa. It stimulates the production of gastric juices, improves blood circulation in heart, treats bronchitis, cough and cold, relieves pain caused by rheumatic diseases and sprains; reduces swelling and relives stress (Dukes, 2015).

3,7,11,15-Tetramethyl-2-hexadecen-1-ol exhibited antimicrobial and anti-inflammatory activity (Sathish et al., 2012).

Hexadecanoic acid, ethyl ester is an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic 5-alpha reductase inhibitor (Sathish et al., 2012; Sudha et al., 2013).

Linoleic acid ethyl ester is hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronal, insectifuge, antieczemic and antiacne (Sudha et al., 2013).

Astaxanthin is a naturally occurring carotenoid. It is an antioxidant (Pearson et al., 2006), anti-inflammatory and pain reliever (Lee et al., 2003). It shows a significant role in recovery of muscles, better endurance, enhanced strength, improved energy levels (Aoi et al., 2008) also, helps in diabetic retinopathy, eye strain (Yasunori et al., 2005; Sun et al., 2011) maintains skin moisture levels, smoothness, elasticity and fine wrinkles (Camera et al., 2009).

Docosanoic acid 1, 2, 3-propanetriyl ester is a skin conditioning agent, emulsifying agent and surfactant (Daffodil et al., 2012).
Vitamin-E possesses many biological functions the antioxidant activity being the most important and well known (Bell, 1987) also plays a role in neurological functions (Muller, 2010). It protects lipids and prevents the oxidation of polyunsaturated fatty acids (Whitney et al., 2011) and also attributed to hypoglycaemic activity (Sivan et al., 1996). Vitamin E is used as antiaging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic and anticoronary (Sudha et al., 2013).

The above mentioned compounds found in the ethanol extract of *Caralluma geniculata* can be used for the pharmacological work. Thus, this type of GC-MS analysis is the first step towards understanding the nature of bioactive compounds in the medicinal plant and also this kind of study will be useful for further detailed study. However, isolation of individual phytochemicals and analysing its bioactivity will give more beneficial results. Hence further studies are needed to confirm its bioactivity and safety.

### 5.2. Anatomical Studies

Indian system of medicine such as Ayurveda and Siddha depends on crude drugs that are of plant origin. Therefore, the utilization of medicinal plants still play a vital role to fulfil the basic health needs in the developing countries like India (Ambasta, 1992). World Health Organization (WHO) states that the macroscopic and microscopic description of a medicinal plant is the first step towards establishing it’s identity and purity and should be carried out before any tests are undertaken (Anonymous, 2002).
5.2.1. Microscopic Characteristics of stem

The present study showed certain features of *Caralluma geniculata* similar to other Apocynaceae members. The four angled stem with thick and blunt wings and single layer of epidermis (Plate 4.1) was clearly identified in the present study. Similar condition was reported in *Caralluma sinaica* (Al-Massarani, 2011), whereas circular outline was reported in *Oxystelma esculentum* with single layer of epidermis (Poornima *et al.*, 2009). Semi-circular outline with two layers of epidermis was documented in *Vincetoxicum canescens* subsp. *canescens*, *Vincetoxicum canescens* subsp. *pedunculata* and *Vincetoxicum fuscatum* subsp. *boissieri* and also one layer of epidermis in *Vincetoxicum fuscatum* subsp. *fuscatum* and *Vincetoxicum parviflorum* (Ilcim *et al.*, 2010). The cortical zone is homogeneous consisting of angular thin walled parenchymatous cells (Plate 4.2), whereas it is heterogenous consisting of two layers of chlorenchyma followed by five to seven layers of parenchymatous cells in *Oxystelma esculentum* (Poornima *et al.*, 2009). In *Vincetoxicum canescens* subsp. *canescens*, *Vincetoxicum parviflorum* contain two layers of chlorenchyma followed by several layers of cells (Ilcim *et al.*, 2010) and 3-5 rows of collenchyma followed by several layers of parenchyma cells in *Caralluma sinaica* (Al-Massarani, 2011). Arrangement of several vascular strands in 4 angled outlines inside the cortex was observed in the studied *Caralluma geniculata* (Plate 4.3). Similarly, patches of phloem fibres present in the cortex in continuous ring was reported in *Oxystelma esculentum* (Poornima *et al.*, 2009), whereas *Caralluma sinaica* showed 15 to 20 free bundles arranged in a ring surrounding the central pith (Al-Massarani, 2011). Vascular strands of *Caralluma geniculata* stem include a small group of thick walled xylem elements and a cluster of phloem elements. Similarly, phloem clusters occur in some *Vincetoxicum* species (Ilcim *et al.*, 2010). Thin
walled parenchymatous cells are present in the central region of the *Caralluma geniculata* stem. Similar characters were reported in *Oxystelma esculentum* (Poornima *et al.*, 2009) and *Caralluma sinaica* (Al-Massarani, 2011).

### 5.2.2. Microscopic Characteristics of root

The root of *Caralluma geniculata* is thin, soft and delicate, whereas it is very hard in *Hemidesmus indicus* (Rajan *et al.*, 2011). The epidermal layer of *Caralluma geniculata* bearing several unicellular epidermal hairs (Plate 4.5) but it is an irregular cork, formed of 2-5 layers of tangentially elongated brownish cells in *Caralluma sinaica* (Al-Massarani, 2011). The cortex is made up of several layers of parenchymatous cells (Plate 4.6) as in *Caralluma sinaica* (Al-Massarani, 2011). In the present study vascular cylinder is thick, compact, circular cylinder of xylem elements surrounded by phloem elements (Plate 4.7 and 4.8) as reported in *Caralluma sinaica* (Al-Massarani, 2011). In *Caralluma geniculata* the three protoxylem points are slightly projecting outside the fiber cylinder. All the cells are thick walled lignified with narrow lumen. Anatomical features of this plant serve as a standard reference for identification and authenticity from its substitutes and adulterants.

There are few reports available in anatomical studies of the family Apocyanaceae whereas no authenticated reports were found in *Caralluma* species except *Caralluma sinaica*. So an attempt was made to study the anatomical features of *Caralluma geniculata*. In this study the plant was analyzed to find whether any special internal characters responsible for its endemism, reason for the availability of limited population of this plant or any special features which affect the normal flourish and growth of the plant in varied habitats irrespective of seasons. But the study revealed no
special feeding inhibitors such as waxes to avoid eating by herbivores or special types of defenses such as thorns or resin ducts or glandular trichomes.

5.3. *In vitro* antioxidant activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions produce free radicals that can start chain reactions in cell and simultaneously cause damage or death to the cell. Antioxidants are reducing agents, they oxidized themselves and terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions (Helmut, 1997). In the present study, ethanolic extract of *Caralluma geniculata* was tested for its scavenging activity against different free radicals.

5.3.1. Free radical scavenging activity on DPPH

DPPH (2, 2-diphenyl–1–picrylhydrazyl) assay is one of the most widely used methods for screening antioxidant activity of plant extracts (Nanjø *et al.*, 1996). DPPH is an organic chemical compound contain stable free-radical molecules also, a scavenger for other radicals. It has a deep violet colour in solution due to the strong absorption band centered at about 520nm and it becomes colourless or pale yellow when neutralized (Sharma and Bhat, 2009). This method depends on reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H during the reaction. This method helps to determine the anti-radical power of an antioxidant by measuring the decrease in the absorbance of DPPH spectrometrically (Mathew and Abraham, 2004). Ethanol extract of *Caralluma geniculata* was shown to scavenge directly the stable DPPH radical over a concentration range from 100µg/ml (7.84±2.43% inhibition) to 1000µg/ml (40.85±2.42% inhibition).
It scavenged the stable radical DPPH in a concentration dependent manner. The IC$_{50}$ value of *Caralluma geniculata* was calculated to be 413.6µg/ml, whereas, the standard Gallic acid was 4.42µg/ml. Similar findings were reported in *Caralluma edulis* (Ansari *et al*., 2005), *Caralluma flava* (Marwah *et al*., 2007), *Caralluma adscendens* (Tatiya *et al*., 2010), *Caralluma tuberculata* (Rauf *et al*., 2013) and *Caralluma umbellata* (Kalyani and Anuradha, 2013; Venkateswarlu *et al*., 2014).

### 5.3.2. Free radical scavenging activity on ABTS

ABTS (2, 2′- azino-bis 3-ethyl benzthiazoline- 6-sulphonic acid) is used by the food industry and agricultural researchers to measure the antioxidant properties of foods (Huarg *et al*., 2005). In this assay ABTS is converted to its radical cation by addition of potassium persulfate in the dark for 12-16 h at room temperature (Re *et al*., 1999). The ABTS radical cation is reactive towards many antioxidants such as phenolics, thiols and vitamin C (Walker *et al*., 2009). In this assay, total antioxidant activity reflects the tendency of hydrogen donating antioxidants to scavenge ABTS** comparable with that of Gallic acid. In the present study, ethanol extract of *Caralluma geniculata* exhibited 34.99±0.89% and 95.86±0.54% activity in the extract concentration ranging from 200-1000 µg/ml (Table 4.6). Similarly, antioxidant activity of *Caralluma adscendens* var. *attenuata*, *Caralluma stalagmifera* and *Boucerosia lasiantha* were reported by Madhuri *et al*.(2010).

### 5.3.3. Hydroxyl Radical Scavenging Activity

The hydroxyl radical is the highly reactive free radical produced in biological systems and responsible for the quick initiation of lipid peroxidation process (Hochenstein and Atallah, 1988). It is reported to cause oxidative damages to various biomolecules like
DNA, lipids and proteins (Spencer et al., 1994). In the present study ascorbic acid iron-EDTA was utilized to generate hydroxyl radical. The ethanol extract of Caralluma geniculata exhibited hydroxyl scavenging activity ranging between 19.90±2.08% and 55.88±3.62% (Table 4.7). From this observation it is clear that the ability of ethanol extract of Caralluma geniculata to scavenge hydroxyl radicals seems to be directly related to the prevention of propagation of lipid peroxidation, and simultaneously reducing the rate of chain reaction (Anusuya and Manian, 2013).

5.3.4. Nitric Oxide Radical Scavenging Activity

Nitric oxide is a bioregulatory molecule which shows many physiological activity such as regulation of blood pressure, neutral signal transduction, platelet function, antimicrobial and antitumor activity (Jagetia et al., 2004). In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs (Uمامaheswari and Chatterjee, 2008). Though, nitric oxide shows significant activity in different inflammatory process over production leads to various diseases. The toxicity of nitric oxide increases when it reacts with superoxide radical simultaneously produces highly reactive peroxynitrite anion (Huie and Padmaja, 1993).

Significant decrease in the nitric oxide radical due to the scavenging ability of extract and Gallic acid was recorded in Table 4.8. The ethanol extract of Caralluma geniculata showed maximum activity of 47.01% at 1000µg/ml; whereas, the standard Gallic acid produced 57.26% at very less concentration i.e., 50µg/ml. While Kalyani and Anuradha (2013) reported higher nitric oxide radical scavenging activity of Caralluma umbellata than the standard Ascorbic acid.
5.3.5. Superoxide Radical Scavenging Activity

Superoxide is biologically important because it decomposes to produce stronger oxidative species such as singlet oxygen and hydroxyl radicals (Dahl and Richardson, 1978). The ethanolic extract of \textit{Caralluma geniculata} has less ability to scavenge superoxide anions than the standard Gallic acid (Table 4.9). The superoxide scavenging activity may be attributed to pharmacologically active substances such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds (Patel \textit{et al.}, 2011).

5.3.6. Lipid peroxidation inhibiting activity

Peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury (Naik \textit{et al.}, 2011). The lipid peroxidation inhibitory activity of \textit{Caralluma geniculata} extract showed an IC$_{50}$ value of 30.28µg/ml, whereas, the standard Gallic acid exhibited 13.37µg/ml (Figure 4.4). Similarly, lipid peroxide scavenging activity was reported in the butanolic extract of \textit{Caralluma edulis} (Ansari \textit{et al.}, 2005) and \textit{Caralluma adscendens} (Tatiya \textit{et al.}, 2010).

Plants contain rich source of phenolic compounds. Phenolic compound is a group of phytonutrients which shows strong antioxidant activity (Ismail \textit{et al.}, 2004) by terminating the free radicals (Rani \textit{et al.}, 2011). Flavonoids and other plant phenolic compounds are common in the leaves, flowering tissues, and woody parts such as stems, bark and roots of plants. The antioxidant activity of phenolic compounds is mainly attributed to their redox properties, which lead them to act as reducing agents or hydrogen-atom donors. Natural antioxidants, function as free radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation (Pratt, 1992).
The present study confirmed the antioxidant property of *Caralluma geniculata* which inhibit the free radicals such as, hydroxyl and superoxide, lipid peroxide, nitric oxide, ABTS or 2,2’-azino-bis (3-ethyl benzo thiazoline-6-sulphonic acid) and 2,2, diphenyl picrylhydrazyl (DPPH) which is a stable free radical. The findings also suggest that, *Caralluma geniculata* could be a potential source of natural antioxidant that might have abundant importance as therapeutic agents in oxidative stress related degenerative diseases. The antioxidant activity of *Caralluma geniculata* may be due to the presence of coumarins.

5.4. *In vitro* Antidiabetic Activity

Diabetes is regarded as increased blood glucose levels and disturbances in the carbohydrate, fat and protein metabolism (Apparao et al., 2003). Diet, rich in carbohydrates lead to sharp rise in the blood glucose level as the complex carbohydrate in food is rapidly absorbed in the intestine aided by the α-amylase and α-glucosidase enzyme which break carbohydrate to simple absorbable sugars i.e., monosaccharides (Kwon et al., 2007). α-Amylase catalyzes the hydrolysis of starch and α-glucosidase catalyzes the final step in carbohydrate digestion which causes postprandial hyperglycemia. Inhibitors of α-amylase and α-glucosidase are helpful to reduce hyperglycemia by delaying carbohydrate digestion and also by reducing the rate of glucose absorption which simultaneously decreases the postprandial plasma glucose rise (Tarling et al., 2008). The antioxidant production decreases diabetes therefore it is clearly understood that an increased free radical level is the main reason for diabetes (Maritim et al., 2003). Many herbal extracts such as *Caralluma tuberculata* (Chopra 1956; Ali, 1986), *Caralluma edulis* (Wadood et al., 1989), *Wattakaka volabilis* (Ayyanar
et al., 2008), Gymnema sylvestre (Rao et al., 2010) and Catharanthus roseus (Ohadoma and Michael, 2011) have been reported for antidiabetic activities and are used in traditional medicine. Herbal extracts have been used directly or indirectly for the preparation of many modern medicines.

The in vitro α-glucosidase inhibitory results reveals that the ethanolic extract of Caralluma geniculata possess α-glucosidase inhibitory activities (Figure 4.5). The active components of the extract compete with the substrate for binding to the active site of the enzyme, also preventing or slowing down the breakdown of oligosaccharides to disaccharides (Shai et al., 2010). The IC₅₀ value (179.33µg/ml) shows that Caralluma geniculata has a potency and preference for α-glucosidase inhibition. A significant inhibitory activity (11.08 – 55.80%) was observed at different concentrations (100 – 500 µg/ml). This is agreed with previous reports that phytochemicals are strong inhibitors of α-glucosidase in Caralluma dalzielii (Tanko et al., 2012) and Caralluma fimbriata (Latha et al., 2014).

The present study, an in vitro inhibitory effect of ethanol extract of Caralluma geniculata on α-amylase was evaluated (Figure 4.6). A significant inhibitory activity (25–66.36%) was observed against α-amylase at different concentrations (100 – 500 µg/ml) with IC₅₀ value 101.87µg/ml. Antidiabetic activity of medicinal plant may be attributed to the secondary metabolites such as glycosides, flavonoids, tannins, etc. (Suba et al., 2004), coumarins (Bubols et al., 2013). The phytochemical analysis of ethanol extracts of Caralluma geniculata revealed the presence of coumarins. Therefore, the phytoconstituents may be responsible for the antidiabetic activity. Correspondingly, antidiabetic effects were reported in Caralluma tuberculata (Ahmed and Shaikh, 1989;
Abdel – Sattar et al., 2013), Caralluma arabica (Radhakrishnan et al., 1999), Caralluma attenuata (Venkatesh et al., 2003; Jeyahar et al., 2004; Mayur 2010), Caralluma sinaica (Habibuddin et al., 2008), Caralluma adscendens (Mali et al., 2009; Tatiya et al., 2010; Bhuvaneswari and Manivannan, 2014), Caralluma dalzielii (Tanko et al., 2012), Picralima nitida (Kazeem et al., 2013), Caralluma fimbriata (Latha et al., 2014) and Caralluma umballesta (Venkateswarlu et al., 2014). The extract of Caralluma geniculata inhibits α-glucosidase and α-amylase, so this plant could be effective in the treatment of diabetes.

5.5. In vivo Antiobesity Activity

Obesity is a medical condition in which excess body fat has accumulated to the extent it may have an adverse effect on health (Haslam and James, 2005). It resulted from an imbalance between food intake and energy expenditure, terminating in excessive accumulation of fat in adipose tissue, liver, muscle, pancreatic islets and other organs involved in metabolism (Hassan and Roy, 2007; Ogden et al., 2007). Further, it has been found to be associated with various disorders such as coronary heart disease, dyslipidemia, glucose intolerance, diabetics, respiratory complications, hypertension obstructive sleep apnea, osteoarthritis of large and small joints, hyperlipidemia, gall bladder disorders, cerebrovascular accidents, restrictive pulmonary disease and certain cancers (Haslam and James, 2005; Flegal et al., 2007; Eckel, 2008; Hu et al., 2008). Factors comprising lack of exercise, sedentary lifestyles and consumption of energy rich diets are responsible to the etiology of obesity (Ekanem et al., 2007). The gain in body weight is due to increased fat mass as a result of preadipocyte proliferation and differentiation and accumulation of lipids in the liver (Roca et al., 1999; Llado et al., 2000). Antiobesity agents are able to suppress appetite and induce satiety (Calixto, 2000).
which will help individuals to control their appetite. At last these different functions of antiobesity medicinal plants will cause a reduction of food and energy intake (Haaz et al., 2006).

Anti-obesity activity of several plant extracts without any adverse effects have been proved earlier. It includes *Caralluma fimbriata* (Kamalakkannan et al., 2010), *Caralluma adscendens* var. *attenuata*, *Caralluma stalagmifera* and *Boucerosia laisantha* (Madhuri et al., 2010). HFD induced obesity has been considered as the most popular model due to its high similarity with the usual route of obesity effect in human (Buettner et al., 2007). Chemical compounds such as saponins, flavonoids and some triterpenoids are attributed for the antiobesity property in certain plants (Yun, 2010). The phytochemical investigation of the present study also reveals that *Caralluma geniculata* contains these chemical constituents.

Orlistat (40 mg/kg bwt) also has been included in the study as a standard, inorder to compare the level of antiobesity activity of *Caralluma geniculata* to that of a standard drug. It is an intestinal lipase inhibitor. It is not systematically absorbed, but acts within the gut lumen to block the digestion of approximately 30% of ingested dietary fat; for a person on a typical western diet containing 40% energy from fat, this will result in an energy loss of about 200 kcal per day (Sjostrom et al., 1998).

5.5.1. Body Weight, Body, Mass Index, Organ Weight and Mesenteric Fat Pad Weight

The Indian tribes have consumed *Caralluma* for centuries as a vegetable. People used it during famines to reduce appetite and as a thirst quencher. *Caralluma adscendens* var. *fimbriata* was used by Indian tribes to suppress hunger and enhance endurance. The
Indian Health ministry has incorporated *Caralluma adscendens* var. *fimbriata* on a list of medicinal plants as vegetable eaten raw or used as pickles and curries. There is no adverse side effect or toxicity (Karuppusamy *et al.*, 2013).

Elevated feeding of foodstuffs rich in calories such as high fat diet is associated with low physical activity leads to increased body weight. Correspondingly, effect of *Caralluma fimbriata* extract in overweight individuals of fifty men and women (25-60 years) with a Body Mass Index (BMI) greater than 25 kg/M² were experimented. Waist circumference and hunger levels produced a significant decline in the experimental group over the observation period (Kuriyan *et al.*, 2007). Likewise, the effect of *Caralluma fimbriata* extract on appetite and lipid profile in rats fed with HFD was examined and identified significant decline of food intake and body weight (Ambadasu *et al.*, 2013). Human studies have revealed that increased fat intake is associated with body weight gain which can lead to obesity. This study also proved that rats exposed to high fat diet for 30 days cause a significant increase of animal’s body weight, consequently verifying the obese status (Neyrinck *et al.*, 2009). However, there was no significant difference observed in the normal rats. Treatment of HFD rats with aqueous and ethanol extract of *Caralluma geniculata* at 300 mg/kg bwt caused a remarkable reduction of body weights when compared to HFD induced obese rats (Table 4.10). Thus, the result suggests that supplementation of *Caralluma geniculata* at 300 mg/kg bwt are capable of preventing body weight gain. This result was in agreement with the appetite suppressant and antiobesogenic effects of *Caralluma fimbriata* extract on rats fed with cafeteria diet (Kamalakkannan *et al.*, 2010).
Body Mass Index (BMI) is a useful measure of overweight and obesity. It is calculated from height and weight of an organism. BMI is an estimate of body fat and a factor to determine the risk for diseases that can occur with more body fat. The higher BMI denotes the higher risk for certain diseases such as heart diseases, high blood pressure, type 2 diabetes, gall stones, breathing problems and certain cancers. In the present study administration of aqueous and ethanol extract of *Caralluma geniculata* altered the level of BMI to normal (Table 4.10) in experimental group when compared to HFD induced obese group.

High Fat Diet resulting in increased organ weight (Barr and Craken 1984). The result of the present study also showed a significant increase in organ weight in HFD induced obese group (Table 4.11). Organs such as liver, kidney and heart weights were examined in order to determine whether these modulated diets have undesirable side effects on the body. In general, enlargement of the organs might be an indicator for pathogenic development. Administration of *Caralluma geniculata* extract at 300mg/kg bwt reduced the organ weights significantly in experimental group.

Construction of foods rich in calories associated with low physical activity leading to increased fat deposition in tissues. This accumulation of fat in mesenteric region is considered as a factor of obesity. In the present study administration of *Caralluma geniculata* extract reduced the mesenteric fat pad weights significantly in a dose dependent manner (Table 4.11).

5.5.2. Lipid Profiles

High fat diet significantly increased the total cholesterol level and serum triglyceride level in HFD induces obese rats. Supplementation of aqueous and ethanol
extract of *Caralluma geniculata* at 300mg/kg bwt significantly lowered the total cholesterol and triglyceride level when compared to the obese control group (Table 4.12 and 4.13). The decline in triglycerides level is owing to an increase in activity of endothelium bound lipoprotein lipase which hydrolyzes the triglyceride into fatty acids or may be attributable to an inhibition of peripheral lipolysis so that fatty acids are not released and get converted into triglyceride (Tenpe *et al*., 2007).

Plasma nonesterified fatty acids are also known as free fatty acids, which has the tendency of insulin resistance. It carries triacylglycerol stored in adipose tissue to its sites of utilization. Elevated level of free fatty acids in obesity arises from an increased adipose tissue mass. The process of fatty acid development from adipose tissue is usually inhibited by insulin, meanwhile once if the plasma free fatty acid levels are elevated, it will inhibit insulin, anti-lipolytic action and simultaneously increases the rate of free fatty acids release in to the circulation (Eaton *et al*., 1969; Jensen *et al*., 1989; Frayn and Coppack, 1992; Frayn *et al*., 1996). Accumulation of fat in and around the pancreas is associated with impaired β-cell function (Tushuizen *et al*., 2007; Heni *et al*., 2010) and increase the chance of diabetes (Lee *et al*., 1994; Boden, 1997; Mook *et al*., 2004; Nolan *et al*., 2006). The elevated free fatty acids concentration were associated with many abnormalities of carbohydrate metabolism such as endocrine and nutritional disorders (Eckel *et al*., 2005). Over supply of free fatty acid to liver may cause dyslipidemia, including hypertriglyceridemia and the antherogenic lipoprotein profile (Ginsberg *et al*., 2005).

Human adipogenesis is mainly due to the activity of phospholipids (Fei *et al*., 2008). Impaired phospholipid-mediated signaling systems lead to cardiac hypertrophy,
diabetic cardiomyopathy and heart failure (Tappia and Singal, 2008). These symptoms are connected with accumulation of too much of cardiac lipid. In the present study reduction in the level of tissue free fatty acids, tissue and serum phospholipids was noticed in experimental group after the administration of *Caralluma geniculata* extract (Table 4.14).

Very low density lipoprotein cholesterol (VLDL) serve as a means for the liver to transfer excess energy in the form of triglycerides therefore, VLDL assemblage is based on the availability of triglyceride (Choi and Ginsberg, 2011). The reduction in VLDL levels in experimented groups could be directly connected to a decline in triglyceride levels as it is well established that VLDL particles are the main transporters of triglycerides in plasma (Howell *et al*., 1998). This could be attributed to saponins, steroids and flavonoids present in the experimental plant *Caralluma geniculata*. Several studies showed that plant saponins and steroids are responsible for both hypolipidemic and antihyperlipidemic activities (Ghule *et al*., 2009). Flavonoids and other polyphenolic compounds also have been recognised for their hypocholesterolemic and hypolipidemic activity (Chan *et al*., 1999). Similarly hypolipidemic activity of *Caralluma adscendens* on triton and methimazole induced hyperlipidemic rats was proved by Sakore *et al*. (2012). Hypolipidemic effects of the methanolic extract of *Caralluma tuberculata* was investigated by Abdel-Sattar *et al*. (2011) in streptozotocin induced diabetic rats. The hypolipidemic action of the extract was evident by the significant decrease in the levels of total cholesterol, triglycerides and LDL cholesterol compared to diabetic rat values. At the same time the extract increased the cardio-protective lipid HDL-cholesterol 1.40.90 percent as compared to diabetic rat value. The data suggest that methanolic extract of *Caralluma tuberculata* has hypolipidemic effect in STZ-induced diabetic rats.
Total cholesterol/HDL cholesterol and LDL: HDL cholesterol ratios are also predictors of cardiovascular risks as per National Cholesterol Education Program Expert Panel. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries, a rise in LDL may cause deposition of cholesterol in the arteries and aorta and hence it is bad for health and a direct risk factor for coronary heart diseases (Ramakrishnan, 1994). HDL is responsible for the transportation of cholesterol from peripheral tissues to the liver for metabolism (Kumar et al., 2010) where it gets excreted as bile salts (Tan et al., 2004). Obesity severely affects serum HDL cholesterol level (Wang and Peng, 2011). Epidemiologic studies proved that higher plasma HDL cholesterol concentration reduces cardiovascular disease (Gordon et al., 1977; Castelli et al., 1986). One of the significant features of obesity is dyslipidemia branded by high levels of triglycerides in very-low-density lipoproteins and low levels of high-density lipoprotein cholesterol (Poirier et al., 2006). Caralluma fimbriata showed significant reduction in serum lipid profile (total cholesterol, triglycerides and VLDL) levels also, increase in the HDL cholesterol levels (Ambadasu et al., 2013).

In the present study, reduction in LDL and VLDL and increase in HDL level was observed in experimental group after the administration of Caralluma geniculata extract (Table 4.15). It shows that aqueous and ethanol extract of Caralluma geniculata has the ability to reduce the incidence of obesity and suggesting its cardioprotective nature. Similarly, hypolipidemic effect of Caralluma adscendens was proved by Tatiya et al. (2010) using reductive ability method. The treatment produced significant decline in total cholesterol, LDL, triglyceride and an increase in HDL in the tested diabetic animal group.
Antherogenic index is regarded as a marker of obesity recently. It has been reported that the higher the value of antherogenic index, the higher the risk of developing coronary heart diseases and vice versa (Treasure et al., 1995; Tan et al., 2004). The present study exhibited reduction in antherogenic index in a dose dependent manner and thus provides cardioprotection (Table 4.15).

5.5.3. Liver Enzyme Markers

Phosphatases are important and critical enzymes in biological processes. They are responsible for detoxification, metabolism and biosynthesis of energetic molecules for different functions (Bengt and Kent 1975). Biochemical impairment and lesions of the tissue and cellular function takes place if any changes occur in these enzymes (Khan et al., 1995). Hepatoprotective effect of Caralluma geniculata extract reported in this study was evident from the significant decrease of the elevated serum levels of liver enzymes (AST, ALT, ALP and GGT) in obese rats (Table 4.16). This reduction may be due to the consequence of liver damage by the antioxidant efficacy of the plant extract (Zamora et al., 1991; Nakano et al., 1999).

5.5.4. Lipid peroxide (LPO) reduced glutathione and glutathione peroxidase (GPx)

In general, obesity is associated with oxidative stress which results from an imbalance between the production of free radicals and an effective antioxidant system. Lipid peroxidation is involved in the oxidative modifications of low density lipoproteins and leads to the formation of atherosclerotic lesions (Yesilbursa et al., 2005). One of the most frequently used biomarkers providing an indication of lipid peroxidation level is the plasma concentration of malondialdehyde (MDA) a by-product of lipid peroxidation process (Nielsen et al., 1997). Recent studies also revealed that obesity is associated with
enhanced lipid peroxidation (Davi et al., 2002; Olusi et al., 2002). The present study also showed high LPO levels in obese rats than in normal rats. Administration of Caralluma geniculata extract altered the modified LPO levels in experimental groups (Table 4.17).

Reduced glutathione constitutes the first line of defence against free radicals in the liver and it is also responsible for the maintenance of protein thiols and acts as a substrate for Glutathione peroxidase (Moonkyu et al., 2004). The result of the present study indicates that reduced glutathione contents decreased in HFD induced obese group were restored after the treatment with Caralluma geniculata extract (Table 4.17).

Glutathione peroxidase (GPx) plays a primary role in minimizing oxidative damage. Reduction of this enzyme may result in deleterious oxidative changes due to the accumulation of toxic products. As enzymatic antioxidants are saturated by excessive levels of free radicals, the presence of non-enzymatic antioxidants is essential for the removal of free radicals (Allen, 1991). The present study also confirmed the effect of Caralluma geniculata extract to increase the level of GPx gradually in a dose dependent manner (Table 4.17).

5.5.5. Superoxide Dismutase and Catalase

Superoxide Dismutase and Catalase are two important enzymes that execute against toxic oxygen derived free radicals such as superoxide (O2-) and hydroxyl ions (.OH) in the biological system. They are associated in the direct elimination of reactive oxygen metabolites. It is one of the most effective defenses of the living body against diseases (Nagao et al., 1986; Ray et al., 2000). In obese rats the activity of antioxidant enzymes (SOD and CAT) is less in renal tissues as compared to normal group. Treatment
with extract of *Caralluma geniculata* significantly elevated the activity of antioxidant enzymes such as SOD and CAT (Table 4.18).

### 5.5.6. Blood Glucose, Serum protein and Creatine kinase

Obesity is highly associated with imbalance in glucose and insulin homeostasis (Hotamisligil *et al.*, 1993). High Fat Diet induced obesity can result in insulin resistance. Obesity is accompanying with defective insulin synthesis and decreased insulin efficiency to regulate glucose metabolism in the peripheral tissue (Boden, 1997; Kahn and Flier, 2000). High blood glucose may simultaneously lead to heart diseases, stroke, diabetic retinopathy, kidney failure and amputations (Fasanmade *et al.*, 2008). In the present study, significant increase in blood glucose level in obese control group was noticed (Table 4.19). Treatment with aqueous and ethanol extract of *Caralluma geniculata* almost normalized the glucose level in dose dependent manner. The drug might have enriched the secretion of insulin from the β-cells of the islets of pancreas or improved the productivity of insulin which enables the distribution of glucose from blood to target tissues.

Serum proteins or blood proteins are proteins present in blood plasma. They play a role in different functions including transport of lipids, hormones, vitamins and metals in the circulatory system. It regulates acellular activity and immune system (Adkins *et al.*, 2002; Jacob *et al.*, 2005). Administration of aqueous and ethanol extract of *Caralluma geniculata* almost normalized the serum protein level in a dose dependent manner (Table 4.19). It shows that the extract of *Caralluma geniculata* is potent to regulate serum protein.
Creatine Kinase (CK), also known as phosphocreatine kinase, is an enzyme that catalyzes the transfer of one phosphate group from ATP to creatine generating phosphocreatine, an important energy reservoir in muscle and brain tissue. CK is a dimeric protein made up of B (brain) and M (muscle) subunits. CK-MB is an isoenzyme. It is an important indicator of renal health. It is an easily measurable byproduct of muscle metabolism that is emitted without any change by the kidneys (Wells, 2000). Creatine and phosphocreatine are catalyzed by creatine kinase and produces creatinine. Creatinine is filtered from the blood and removed by kidney by the action of glomerular filtration, also by proximal tubular secretion. Little or no tubular reabsorption of creatinine happens. If the kidney fails to remove creatinine properly the level of creatinine rises in the blood (Shemesh et al., 1985; Allen, 2012). Elevated level of creatine kinase in brain increases the chance of Alzheimer’s disease, in heart increases the risk of hypertension and in the blood indicate the chance of heart disease, muscular dystrophy, nerve damage to thyroid disorders and kidney malfunction. In the present study the level of creatine kinase increased significantly in HFD induced obese rat (Table 4.19). Administration of plant extract reduced the level of creatine kinase in a dose dependent manner and simultaneously avoids renal dysfunction.

Among the two different extract (aqueous and ethanol) of Caralluma geniculata experimented for antiobesity activity, both extracts showed its efficacy against obesity but aqueous extract of Caralluma geniculata produced comparatively better result in many parameters than ethanol extract. The reason is the antiobesity components of Caralluma geniculata are more soluble in water than the ethanol. Water is more effective in extracting the solute compared to ethanol because it has higher polarity and shorter chain (Pin et al., 2009). In aqueous extract the chance of chemical reaction is reduced.
very much than the ethanol extract and simultaneously reduces the chance of side effect. The biological value of plants depends on their bioactive constituents (Veermuthu et al., 2006). Chemical compounds such as saponins, flavonoids and some triterpenoids are attributed for the antiobesity property in certain plants (Yun, 2010). The phytochemical investigation of the present study also reveals that *Caralluma geniculata* contains these chemical constituents.

5.6. **In vitro propagation**

Biotechnological techniques, particularly plant cell, tissue and organ culture provides new means for clonal multiplication and *in vitro* conservation of valuable, endangered indigenous germplasm rapidly (George and Sherrington, 1984; Arora and Bhojwani, 1989; Bhojwani et al., 1989; Constable, 1990; Chandel and Pandey, 1991; Sharma and Chandel, 1992). Micropropagation allows rapid production of high quality, disease-free and uniform planting material. The plants can be multiplied under a controlled environment irrespective of the season and weather on a year-round basis. Micropropagation has been accepted as a suitable technology in the development and conservation of several medicinal plants. However, the cost of production must be reduced (Brink *et al.*, 1998).

5.6.1. **Shoot multiplication from apical meristem explant**

Any part of the plant (meristem, node, internode, root, seed, and embryo) can be cultured to propagate a new plant. Apical meristem perhaps the most popular source of explants to initiate tissue culture. The apical meristem maintains itself, differentiated into new tissues and organs, and communicates signals to the rest of the plant (Medford, 1992). As meristem tips are free from viruses, generation of pathogen free plants are
possible through apical meristem culture (Jha and Ghosh, 2005) also, an easy source for rapid plant propagation as reported in a number of plants. A protocol for \textit{in vitro} plant regeneration via apical meristem regeneration was established in \textit{Caralluma adscendens} var. \textit{attenuata} (Aruna \textit{et al.}, 2012), \textit{Caralluma pauciflora} (Kiranmai \textit{et al.}, 2012) and \textit{Caralluma lasiantha} (Aruna \textit{et al.}, 2012).

\textit{In vitro} growth of plant is highly determined by the proportion of the culture medium. The main components of culture media are mineral salts and sugar as carbon source and water. Other components may include organic supplements, growth regulators, a gelling agent (Gamborg \textit{et al.}, 1968, Gamborg and Phillips, 1995). The basic MS (Murashige and Skoog, 1962) is the most widely used media. The present study also carried out using MS media with slight modification.

Cytokinins favour \textit{in vitro} shoot proliferation (Thorpe, 1993), differentiation (Schuller \textit{et al.}, 2000) and seem to play a key role in cell cycle synchronization (Dobrev \textit{et al.}, 2002). The response of explants to different cytokinins (BAP and KN) was more or less similar when used individually (Table 4.20 and 4.21) but when added with low concentration of auxin produced better results in the present study. MS medium fortified with BAP and NAA together produced multiple shoots within 4-6 days of inoculation from apical meristem. The data in respect of average number of days required shoot induction frequency, average number and length of shoot per explant and different concentrations of each hormone on shoot tip explant were summarized in Table 4.22.

\textbf{5.6.2. Shoot multiplication from nodal explant}

Nodal explants found to be the second most effective explant in inducing good quality multiple shoots for large scale propagation and conservation. When compared to
apical meristem the number of shoots developed from nodal explants was a little lesser. There are several reports in which nodal explants are more effective in Asclepiadaceae, these include *Caralluma edulis* (Gurdeep et al., 1992), *Tylophora indica* (Sharma and Chandel, 1992; Kaushik et al., 2010), *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Caralluma candelabrum* (Beena et al., 2003), *Leptadenia reticulata* (Arya et al., 2003), *Cryptolepis buchanani* (Prasad et al., 2004), *Decalepis arayalpathra* (Ganga prasad et al., 2005; Sudha et al., 2005), *Wattakaka volubilis* (Chakaradhar and Pullaiah, 2006; Kumar et al., 2011), *Caralluma adscendens* (Arana et al., 2009), *Ceropegia intermedia* (Karuppusamy et al., 2009), *Marsdenia brunoniana* (Ugraiah et al., 2010), *Caralluma edulis* (Rathore et al., 2010), *Caralluma bhupenderiana* (Ugraiah et al., 2011), *Caralluma adscendens* var. *attenuata* (Aruna et al., 2012), *Caralluma lasiantha* (Aruna et al., 2012), *Boucerosia diffusa* (Ramadevi et al., 2012), *Gymnema sylvestre* (Manonmani and Francisca, 2012), *Ceropegia thwaitesii* (Muthukrishnan et al., 2012), *Asclepias curassavica* (Reddy et al., 2012), *Caralluma diffusa* (Prabu et al., 2013), *Caralluma stalagmifera* var. *longipetala* (Naik et al., 2014) and *Caralluma diffusa* (Kalimuthu, 2014).

Though several plant growth regulators available for shoot induction BAP and KN were widely used. In the present study nodal explants with axillary bud were also cultured on MS basal medium supplemented with different cytokinins (BAP, KN and PBA) and auxins (IAA, IBA and NAA) either individually or together.

The efficacy of BAP on shoot proliferation from axillary buds was explained in different species of Asclepiadaceae (Sharma and Chandel, 1992; Rani and Rana, 2010; Manonmani and Francisca, 2012; Ugraiah et al., 2010; Ramadevi et al., 2012; Ugraiah...
et al., 2011; Prabu et al., 2013; Aruna et al., 2012; 2012a; Karuppusamy et al., 2009; Kaushik et al., 2010; Murthy et al., 2010), however, in the present study MS medium containing KN is more effective than BAP and PBA for induction and proliferation of axillary buds on *Caralluma geniculata* (Table 4.24-4.26).

Significant effect of BAP in addition to an auxin has been proved in many medicinal plants of Asclepiadaceae such as, *Gymnema sylvestre* (Reddy et al., 1998), *Holostemma annulare* (Sudha et al., 1998), *Hemidesmus indicus* (Sreekumar et al., 2000), *Holostemma ada-kodien* (Martin, 2002), *Leptadenia reticulata* (Arya et al., 2003) and *Ceropegia candelabrum* (Beena et al., 2003). Similarly thin cell layers obtained from nodes of *Ceropegia spiralis* were cultured on the medium provided with BAP and NAA produced shoot with extensive growth (Murthy et al., 2010). High concentration of BAP with low concentration of NAA produced significant multiple shoots (Table 4.27) from nodal explants. Similarly, Kumar et al. (2012) reported shoot induction from the leaf segments of *Pergularia daemia* on MS medium containing BAP (0.5mg/l) in combination with NAA (0.1mg/l). Similarly in the present study also BAP in combination with low concentration of NAA produced multiple shoots from nodal explants within a short period (Table 4.27).

5.6.3. *In vitro* rooting

Shoots produced from apical meristem and nodal explants were excised and transferred on MS basal medium. After 5 days of incubation, rooting was observed (Plate 4.9f). Of the three auxins (IAA, IBA and NAA) tested independently NAA produced 100% root sprouting frequency (Table 4.29-4.32) though number of root induction is very less. Similarly in many studies, auxins such as IAA, IBA and NAA
were used to induce rooting. Higher frequency of rooting was reported when MS medium augmented with NAA on *Caralluma sarkariae* (Sreelatha et al., 2009), *Ceropegia intermedia* (Karuppusamy et al., 2009), *Marsdenia brunoniana* (Ugraiah et al., 2010), *Ceropegia spiralis* (Murthy et al., 2010), *Caralluma bhupenderiana* (Ugraiah et al., 2011), *Gymnema sylvestre* (Manonmani and Francisca, 2012), *Boucerosia diffusa* (Ramadevi et al., 2012) and *Caralluma diffusa* (Kalimuthu et al., 2014). In the present study Maximum number of rooting resulted on BAP 2mg/l with NAA 6mg/l (Table 4.30; Plate 4.9h). Survival of plantlets required strong, healthy, long roots, which were obtained from BAP 2mg/l with NAA 6mg/l with mean number of 10.6 roots per explant. It denotes that the higher concentration of NAA increased the number of root initiation in a dose dependent manner.

In this study the combined effect of three different plant growth hormones such as BAP, KN and NAA was experimented using different concentration. But the plant did not show any significant growth.

5.6.4. Hardening and acclimatization

The well rooted plantlets were removed from the culture tubes with care and were washed thoroughly with distilled water to remove the culture medium. Then they were transferred to the paper cup containing a mixture of sterile farmyard manure, red soil and sand in the ratio of 1:2:1 (Plate 4.11 a & b). The well established plants were transferred to the pots for acclimatization (Plate 4.11c).

Vermicompost, soilrite mix, vermiculite, etc. is generally used for transplantation as it ensured high frequency survival of plantlets prior to outdoor transfer (Agretious
et al., 1996). However, mixture of sterilized farmyard manure, red soil and sand was used in this study as it is easily available and reduced the cost of transplantation.

The *in vitro* propagated plants showed higher survival, subsequent growth and establishment. The plants produced numerous axillary flowers (Plate 4.11d) but showed very poor seed set. It showed that the chance of multiplication of *Caralluma geniculata* by seed germination is very less. In such case tissue culture is the only way for mass multiplication of this valuable medicinal plant. So the derived standard protocol can be used for mass multiplication of *Caralluma geniculata*. 