DISCUSSION

5. DISCUSSION

In the present investigation stated that the medicinal and nutritional properties of *Coldenia procumbens* has been discussed and reported. These are needed in human functioning system, health and development of immune system for the day to day life.

5.1 Proximate content of *Coldenia procumbens*

The high proximate content of *C.procumbens* was analyzed in *C.procumbens*. Igwenyi *et al*. (2014) reported that the proximate analysis showed higher moisture content (83.0%), crude fat (3.20%), ash content (3.80%), crude fiber (2.15%), protein (0.88%) and carbohydrate content (6.98%). The reductions of moisture content proximate the storage of the leaves and avoid the microbial growth and deterioration. The carbohydrate, lipid and protein content were very low and reduce the energy source, but large quantity of these compounds must be consumed to supply rich amount of nutrients to plants. The level of ash served as a medicinal properties and therapeutic function rather than dietary necessity.

In the present investigation that the proximate content of aerial parts of *Coldenia procumbens* were estimated. The proximate content of moisture, total ash, acid soluble ash, insoluble ash, dry matter, nitrogen free extract, crude fiber and crude fat results were significantly recorded respectively. The proximate content was responsible for biological activity of the test plant.

Similarly Hussain *et al*. (2009); Der Jiun *et al*. (2012) and Uusiku *et al.* (2010) studied that the whole plant of *P. pellucida* showed moisture content, protein, lipid, carbohydrate and caloric values. Hofman *et al*. (2002) revealed the ash content was

increased, those plant evaluation of functional properties (ie.) storage of food. In the present study the total ash content was 23.2±0.4 % were recorded in the aerial parts of *C.procumbens*.

Crude fibre is more important for food or plant is an indication of the level of non-digestible carbohydrate and lignin. In the present study the crude fiber content (5.2±0.3%) was esteemed in *C.procumbens* of aerial parts of plant. The low level of crude fibre enhanced the digestibility and high level can cause intestinal irritation, lower digestibility and decreased nutrient usage (Oladiji *et al.,* 2005).

5.2 Phytochemical properties of *C.procumbens*

Plants are the fundamental source of many phytochemicals and innumerable number of antimicrobial compounds like saponins, alkaloids, flavonoids, phenols, polyphenol, coumarin, anthroqunione, glycosides and terpenoids. These phytoconstituents have more therapeutic application against all pathogens (bacteria, fungi, virus etc.) (El-Astal *et al*., 2005; Marjorie, 1999). Alkaloids, carbohydrate, phenol, glycosides, terpenoids, flavonoids and saponins were reported from *C.fistula* plant (Pandya *et al*., 2012).

The estimated carbohydrate content in the leaves of *Ficus capensis* (73.77) was high and the main uses of carbohydrates are to produce required energy for the body but they are essential nutrients for adequate diet (Emebu and Anyika, 2011) and supplies energy to cells such as brain, muscle and blood (Ejelonu *et al*., 2011).

In the current investigation that the phytochemical active compounds of *Coldenia procumbens* L. were analyzed qualitatively and quantitatively analysed. According to Pamela *et al.* (2005), reported that the plant and animal proteins are

important in high nutritional value. Moreover, the higher levels of alkaloid, flavonoid, saponin and tannin contents were measured by Ezeabara and Nwafulugo (2015). The secondary metabolites are most commonly present in plants. The phytochemical compounds are depended upon the location, season, as well as method of extraction. Ezeabara and Egwuoba (2016) reported that the synthesis and accumulation of phytochemicals depend on species, age, season and climatic factors. Phytochemicals accumulate in plant organs such as fruits, flowers, leaves, stems, and roots.

Alkaloids (Zulak *et al*., 2006) and flavonoids (Crozier *et al*., 2006; Nagarajan and Sellamuthu, 2013) play a defensive role in the plant against herbivores and pathogens. Alkaloids have been linked with therapeutic consumptions for eras and one of their common biological properties is their cytotoxicity (Okwu, 2007), antispasmodic, antibacterial (Stray, 1998) and analgesic (Nobori *et al*., 1994). The capacity of flavonoids is owing to their ability to intricated with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). Flavonoids are also active antioxidant and show tough anticancer activities (Salah *et al*., 1995).

Evidently, Ojokuku *et al*. (2010) explored that the flavonoids compounds are major functioning to control the following diseases of antibacterial, anti-thrombotic, antiallergic, anti-inflammatory, antimutagenic, antioxidant, antineoplastic and antiviral activities. Okwu and Josiah (2006) reported alkaloids are therapeutic agents for their analgesic, antispasmodic and bactericidal effects. The natural ascorbic acid plays a vital role for the body performance of human. The main role of phenolic compound indicated in antimicrobial properties against pathogens (Khoobchandani *et al*., 2010). Tannins also possess antiviral, antibacterial and antitumor activity and also

used as diuretic (Aiyelaagbe and Osamudiamen, 2009 and Dutta *et al*., 2013). Saponin has the property of precipitating and coagulating red blood cells (Okwu and Josiah, 2006).

Past year ago, Deshpande and Kadam (2013) reported that the natural antioxidants derived from plants to phenolic compounds of tocopherols, flavonoid, phenolic acids etc. Han *et al*. (2007) discussed that the biological properties of phenols are anti-carcinogen, anti-atherosclerosis, cardiovascular protection, anti-aging, anti-inflammation, improvement of endothelial function and apoptosis as well as cell proliferation activities and suppression of angiogenesis.

The main properties of saponins are coagulating and precipitating red blood cells, hemolytic activity, cholesterol binding properties, bitterness and formation of foams in aqueous solutions (Sodipo *et al*., 2000 and Just *et al*., 1998). Anthraquinones components support the management and therapeutic agents of cardiac disease, anti-tumor activities, antimalaria, antimicrobial, anti-viral and anti-oxidant activities.

Several reports of glycosides to agree in reduce the blood pressure (Nyarko and Addy, 1990). Bandyopadhyay *et al*. (2002) referred to glycosides as the main bioactive constituent that offers anti-ulcerative and anti-secretory effects. Plant glycosides, which are not normally lethal when ingested orally, are known to prevent chloride passage in the stomach. In the present study, the phytochemicals analysis support findings with Annapurna *et al*. (2003), who confirmed the presence of flavanoids, alkaloids, steroids, saponins and terpenes from the ether extract of *I. coccinea* and also presence of phenols, steroids and alkaloids were confirmed from the methanolic extract of *I. coccinea*.

5.3 Antimicrobial properties of *C.procumbens*

Antibiotics resistance are beginning study, using these agents for treating infections and it is a growing problem around the world (Sefton, 2000 and Cortes-Penfield *et al*., 2017). In the recent research studied that the effect of commercially antibiotics like streptomycin, tetracyclin, erythromycin, ampicillin and negative control was involved against selected bacteria. Among the antibiotics the streptomycin was more suitable against clinical bacteria exclusively *Bacillus cereus* because of multi drug antibiotics.

Omololu Aso *et al*. (2011) evaluated that the high resistance rates of *E. coli* was amoxycillin (100%), cotrimozazole (92.85%) and tetracycline (100%). These findings are comparable with the other studies (Bartoloni *et al*., 2006 and Sahaquillo Arce *et al.,* 2011). Cordoba (2017) reported that most of them bacteria are high resistant rate recovered in ampicillin antibiotics. The gram negative bacteria species can resistance to multiple antibiotics likes ciprofloxacin, ampicillin, tetracycline and fluconozole (Karam, 2016).

Consequently, the findings of resistance to the following standard resistance rates Aztreonam (19.7%), augmentin (83.9%), ceftriaxone (34.5%), cefazoline (91.3%), piperacillin (51.8%), cefixime (38.2%), cefixime (62.9%), cotrimoxazole (23.4%), ciprofloxacin (25.9%), norfloxacin (17.2%), nalidixic acid (23.4%), gentamycin (11.1%), amikacin (11.1%), tobramycin (30.8%) and kanamycin (45.6%) against *Pesudomonas* sp. Based on cotrimoxazole resistance reported strongly recommended for empiric therapy of UTI (Le and Miller, 2001). According to the American

infectious disease society, bacterial resistance rates recommended empirical therapy not exceeding 10% (Gupta, 2011).

Medicinal plant extracts from many studies have shown that plants from similar genera with *C.procumbens* have antimicrobial activity. In our research deals with different concentration of 25, 50, 75 and 100µl and various solvents such as petroleum ether, aqueous, ethanol and methanol extracts of test plant was analysed against bacteria. The antibacterial potential of the *C.procumbens* was evaluated according to their maximum zone of inhibition against *B.cereus* and *E.coli* bacteria than followed by other bacteria recorded and methanolic solvent extracts of the test plant with 75µl concentration was more appropriate inhibition results recorded when compared with solvents of petroleum ether, aqueous and ethanol.

In our study correlated and reported that the ethanolic extract of *C.procumbens* is effective against gram positive as well as gram negative bacteria and fungi are *A.hydrophila, B. cereus, B. subtilis, E. aerogens, E. coli, K. pneumonia, P. vulgaris, P. aeruginosa, S. typhimurium, S. aureus, S. pyrogens, V. fischeri* and *C. albicans* (Shakila *et al*., 2017). Similar findings were reported in *Tamarindus indica* leaves (Asma *et al.*, 2015), and various species of *Ixora* have substantial antimicrobial activities (Annapurna *et al*., 2003; Latha *et al*., 2012), *I.parviflora* leaves (Saleha *et al.*, 2015) showed strong antimicrobial activity against *Salmonella typhi, Acinetobacter, Salmonella paratyphi* and *Bacillus subtilis.*

Previous report showed that the crude aqueous, hexane, benzene and ethyl acetate extracts from the leaf of *Tamarindus indica* were tested on five clinical isolates as *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, P.aeromonas*

and *Staphylococcus auerus* and maximum zone of inhibition observed in ethyl acetate extracts (Ushie *et al.,* 2013). *Tridax procumbens* extract of leaves and roots showed more significant antibacterial activity as compared to that flowers extract. The 100µl of acetone extracts of roots and a leaves showed superior antibacterial activity as compared to methanolic extract (Sowmya *et al*., 2015).

Khan *et al*. (2006) evaluated that the 72 fungal isolates including two quality control strains were determined by NCCLS/CLSI methods against fluconazole, itraconazole, voriconazole, amphotericin B and cancidas. Dermatophytes were also tested against terbinafine. The ethanolic extract of *Launaea procumbens* exhibited anticandidal activity against *C. tropicalis.* The inhibited results compared with standard antibiotics of chloramphenicol (30mcg/disc) and amphotericin B (100units/disc) (Jigna and Sumitra, 2006).

Iin the current investigation that the commercial antibiotics against the clinical fungi like *Aspergillus sydowii, A.raperi, A.terreus* and *Fusarium moniliforme* were tested with fluconozole, ketoconazole, clotrimazole and negative control were analysed. Among the antibiotics, the ketoconazole showed excellent performance against fungi particularly *Aspergillus raperi* than the other antibiotics of fluconozole, ketaconozole and clotrimazole and other fungi also with significant results performed.

In the present investigation the effect of antifungal properties of *C.procumbens* was performed against fungi. Similar approaches have been carried out by different researcher on antifungal activity of plant extract. *O. sanctum* extract showed antifungal activity (Singh *et al*., 1970 and Balta *et al*., 1971). Singh and Prasada (1993) found that, leaf extract of *O. sanctum* inhibited the growth of

Fusarium oxysporum. The leaf extract of *Mentha arvensis* showed maximum inhibition against *A. fumigatus* and minimum inhibition against *A. niger* at 30% concentration (Kaur *et al*., 2009).

The present study was carried out to evaluate the *in vitro* antifungal properties of *C.procumbens* with 75µl concentration has excellent antifungal activity against *Aspergillus raperi* than the other fungi and other concentration also because the methanolic extract of *C.procumbens* showed significant antifungal activity against fungi. The concentration of test plant extract is increase with the zone of inhibition also increased, but the bacterial growth is stationary phase to reduce the inhibition activity.

Similarly Luma *et al*. (2007) found that Eucalyptus extract showed inhibitory effect on pathogenic fungi. *C. citratus* showed maximum inhibition against *F.oxysporum* and minimum inhibition against *C. lunata* (Bansod and Rai, 2008). The result was an agreed with the finding of the leaf extract of *T. procumbens* showed maximum inhibition against *P. citrinum* and minimum inhibition against *C. lunata* at 30% concentration.

In recent times, Kakde *et al*. (2011) found that aqueous extract of *E. angophoroides* found to be fungitoxic for the growth of *Alternaria dianthicola*, *Curvularia pellescens, Fusarium oxysporum, Macrophomina phaseolina, Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum*. Mogle (2013) found that leaf extract of *P. hysterophorus, T. procumbens, A. mexicana, E. globulus* were inhibitory for the growth of *Aspergillus flavus, Aspergillus niger, Botrytis cinera, Chaetomium brasiliense, Penicillium digitatum, Rhizopus arrhizus* and *Rhizoctonia solani*. The antifungal

properties of *Ixora* leaves were studied (*Candida albicans* and *Saccharomyces cerevisiae*) by disc diffusion method (Annapurna *et al*., 2003). Jat and Agalave (2013) found that aqueous extract of *Eucalyptus angophoroides* and *Withania somnifera* was found to be antimycotic activity against fungi.

5.4 Anatomical study of *C.procumbens*

Plant anatomical is fundamental and plant development details about the plant and separate in plant belongs to the family (Patel *et al*., 2013). The anatomical studies of *C.procumbens* in vegetative organs of 4 different species classified as annual tropical light hygromesophyte according to its anatomical features. It mainly grows on frequently flooded river banks and on with red rice fields (Humphrey, 1932). The leaves are hypostomatic as also reported for several other *Piper* species. Two types of stomata, namely anomocytic and tetracytic were presented with the latter type being more abundant. (Bertocco *et al*., 2017; Silva *et al*., 2017; Machado *et al*., 2015; Santos *et al*., 2015; Gogosz *et al*., 2012; Raman *et al*., 2012).

In the present investigation of anatomy was studied in T.S section of leaf *C.procumbens* partly folded many times in longitudinal plane. Midrib and veins are axial part and deeply grooved along the folding of lamina. In the present study, crystal sand showing bipyramidal shapes were observed externally on the adaxial leaf surface.

Accordingly, the anatomical characteristics that can be used in differentiating *C. halicacabum, C. procumbens* and *C. pterocarpum,* i.e. epidermal cells size, palisade cells physical, parenchyma cells observation, type of crystal as well as epidermis micro-morphology characters *C. procumbens* and *C. pterocarpum* showed epidermal cells ratio for height:width of 2:1 and the main vascular tissue system is closed,

consists of continuous bicollateral vascular tissue which is O-shaped, from four to five strands, and the xylem is well developed (Norfaizal *et al*., 2017).

Nakamura *et al*. (2015) observed that the origin of the sub-epidermal layers in *Piper* leaves comes from the ground meristem and consequently should be considered a hypodermis. They suggested that the hypodermis would have the function of regulating the intensity of light reaching the chlorenchyma. Concerning the calcium oxalate crystals, they are by far the most prevalent and widely distributed mineral deposits in higher plants and typically develop within intravacuolar membrane chambers of specialized cells in any organ or tissue (Webb, 1999). These outgrowths play a role in plant defense especially with regard to phytophagous insects, avoiding insect feeding and oviposition responses, and the nutrition of larvae (Metcalfe and Chalk, 1988).

5.5 Characterization studies of *C.procumbens*

UV absorbance is useful for estimating the dissolved aromatic carbon content for instance the phenolic hydroxyl groups in a sample (Tiainen *et al.*, 1999). In the current investigation studied that the identification of molecular reaction of methanolic extract of aerial part of *C.procumbens* were measured by UV spectrophotomerter and measurement also calculated. However, the intensity of light energy can be accumulated and oxidized in the form of particular reactions.

Evidently, the qualitative UV-VIS spectrum profile of ethanol extract of *Sarcostemma brevistigma* was selected at a wavelength from 200 to 1100 nm due to the sharpness of the peaks and proper baseline (Dhivya and Kalaichelvi, 2017). UV-VIS spectrum of *Micrococca mercurialis* plant extracts has absorption peaks at

214, 446 and 472 nm with the absorption of 0.599, 0.655 and 0.550 respectively (Kalaichelvi and Dhivya, 2017).

Fourier Transform Infrared Spectroscopy (FT-IR) is a high-resolution analytical technique to identify the functional compounds and elucidate the structural compounds (Hashimoto and Kameoka, 2008 and Hussain *et al*., 2007). FT-IR spectroscopy detects vibration frequencies and functional group of the components in the mixture of organic matters includes aromatic and aliphatic organic compounds (oxygen, nitrogen, sulfur functional groups).

In the present study in order to understand FT-IR results, it's helpful to know a little bit about the radiation and absorbance of infrared light at various peak wave length to adsorption caused by double bond for functional group identification of functional group like free O-H bond, polymeric association, chelate compounds, isocycanides, alkene, CH_2 nitro compounds and C-Cl bond were determined with respective retention time.

Previously Helm and Naumann (1995) suggested that the FT-IR spectroscopy is a valuable tool for detection of cellular components not present throughout the whole bacterial life cycle. The FT-IR might be used for the monitoring of dynamic processes such as accumulation and utilization of storage materials. It is also suggested that FT-IR can be used for the screening of speculation complementary to traditional staining methods. Moreover, FT-IR can be used for the detection of cell components which are simultaneously expressed. The feasibility of FT-IR for the analysis of biofilms has already been demonstrated.

The structure of the isolated compound 1 was elucidated on the basis of IR, $^{1}H/^{13}C/^{2}D$-NMR, and Mass spectra as Ehretioside A1. ^{1}H and ^{13}C NMR spectra indicated the presence of β-D-glucopyranose by signals at δ 5.07 (d, J-7.8 Hz, H1′) and δ104.04 (C1′) (Niranjan Kumar *et al*., 2013). The *Zehneria scabra* plant contains Gypenoside group component with evidence of other Cucurbitaceae member *Gynostemma pentaphyllum* (Rastogi and Mehrotra, 1998). Natarajan and Anton Smith, (2015) isolated antidiabetic compounds from *Leptadenia reticulate* and characterized in NMR studies.

Evidently, the methanolic extract of *C.procumbens* were analyzed by GCMS. Totally, 58 compounds including fractions with retention as well as molecular characterizations were predicting the compounds. Eventhough, the GC-MS analysis revealed the 27 chemical compounds in ethanolic leaf extract of *Waltheria indica* (Prabhanna and Jayaraj, 2018). The tetradecane chemical compound retrieved from *Hibiscus tiliaceus* (Nandagopalan *et al*., 2015), *Marsilea quadrifolia* (Karikalan and Udayakumar, 2014) and *Gymnema sylvestre* (Thirunavukkarasu *et al*., 2016). Similarly, phytol and squalene also showed the various biological activities as reported for *Coldenia procumbens* (Kesava and Usha, 2016).

A combination of GC-MS, FTIR, NMR and UV spectroscopic methods was used to characterize the various fractions of the phytochemical compounds of *C.procumbens*. Over the past years, many highly accurate and sensitive methods for the analysis of complex mixtures of compounds have been retrieved (Yalavarthi, 2016). However, GC-MS covers relatively larger classes of compounds.

5.6 Antioxidant Activity

Plants have diverse groups of phenolic compounds such as simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. All these phenolic classes have gained extensive attention because of their physiological functions including free radical scavenging, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects (Manthey *et al*., 2001; Bandoniene and Murkovic, 2002).

Reducing antioxidant screening method is the simple and foremost assay method to assess the antioxidant potency of natural products in the process of drug discovery. They increase in the absorbance was due to reduction of Fe^{+3} to Fe^{+2} which indicated that the reducing potential of the extracts. The methanolic extract of *Annona cherimola* was found to have more reducing antioxidant power than hexane extract. Since phenolic compounds (flavonoids, tannins, hydroxycinnamate esters and lignin) are abundant in plant tissues and possess ideal structural chemistry for free radical scavenging activity.

A substance that inhibits oxidation, especially one used to counter act the deterioration of stored food products. A substance such as vitamin C, that removes potentially damaging oxidizing agents in a living organism. These antioxidants may be used for increasing shelf life of food products and improving the stability of lipids and lipid- containing foods by preventing loss of sensory and nutritional quality by preventing lipid peroxidation. These compounds may also find a place as food additives though studies are needed to evaluate their effectiveness within food matrices. Extracts exhibit antioxidant potential by reducing lipid peroxidation by *in vitro* method (Sudjaroen *et al.,* 2005). Raw and dry heated tamarind seed coats

exhibit good antioxidant activity against the linoleic acid emulsion system and the values were lower and higher than the synthetic antioxidant, butylated-hydroxy-anisole (BHA) and ascorbic acid respectively (Siddhuraju, 2007).

As per the antioxidant activity performed in different concentrations of 100, 200, 300, 400 and 500µl of *C.procumbens* methanolic extract studied in five methods as DPPH, Ferric reducing power assay, ABTS, superoxide scavenging assay and lipid peroxidation activity. The 400µl/ml concentration was excellent percentage activity observed in DPPH method when compared with standard (Gallic acid) and also IC_{50} were determined.

The $ABTS^{+}$ assay has been employed as an index that reveals the antioxidant activity of test samples (Wu *et al*., 2006). The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) (Benzie and Strain, 1996). Generally, the reducing properties are linked with the presence of compounds which exert their action by breaking free radical chain by donating a hydrogen atom (Duh *et al*., 1999).

5.7 Haemolytic activity

In antidenaturation assay conducted in denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo nephritis etc (Gell and Benacerraf, 1959). Heat denatured proteins are as effective as native proteins in provoking delayed hypersensitivity (Insel, 1990). Moreover it was already proved that Conventional NSAID's like phenylbutazone and indomethazine

does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins (Olga blokhina *et al*., 2003).

Mikami *et al.* (1983) reported in earlier, the ethanolic extract of *C. procumbens* extract behaves as an inhibitor of leukocyte migration and the formation of pleural exudates. Inflammation involves proliferation of macrophages, neutrophils and fibroflasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicated that the proliferative phase was effectively suppressed by the ethanolic extract of *C. procumbens* (Arul *et al*., 2005).

In the present study stated that the *in vitro* anti-inflammatory properties of *C.procumbens* were proved that the 400µl/ml was maximum percentage of activity by egg albumin concentration. The activity of methanolic extract of *C.procumbens* were statistically conformed with the 88.8% results at 400µl/ml concentration showed excellent activity observed when compared with diclofenac sodium as a standard and IC_{50} values also performed because of phytochemical constituents of tannins, saponins, alkaloids, anthroquinons and flavonoids content.

Similarly, Saleem *et al.* (2002) evaluated the *C. procumbens* has significant effect of anti-inflammatory activity which may be due to presence of chemical profile such as flavones, tri-terpenoids, flavonones and phenols were recorded.

Accordingly, Arul *et al.* (2005) explained that the aerial parts of the plant *C. procumbens* possess significant anti-inflammatory activity in rats. Further studies involving the purification of the chemical constituents of the plant and the biochemical pathways may result in the development of a potent anti-inflammatory

agent with a low toxicity and better therapeutic index. From the results of present study it is concluded that the methanolic extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

5.8 Cytotoxicity assay

In the present study stated that the cytotoxicity of methanolic extract of *C.procumbens* on the liver cancer line (MCF7 cell line) was performed. The various concentration of the plant extract of 100, 200, 300, 400 and 500µg/ml treated. Among the concentration of plant extract the 500µl/ml concentration showed excellent inhibition activity of hepatoprotective activity and percentage of growth of inhibition also calculated accordingly. The cytotoxicty effect of methanolic extract of *C.procumbens* would be useful to obtain hepatoprotective potentially represented.

Recently worked, as cell line study reported in griseofulvin is an orally active nontoxic antifungal drug and treatment of skin infections in both humans and animals. If the mechanism of action is believed to occur to inhibit the fungal cell at the time of mitosis cell division observed the strong cytotoxic effect in the damage to liver cells (Otang *et al*., 2014). While there exists a number of reports on the antiproliferatory activity of Griseofulvin against various cancer cell lines, these antiproliferatory effects occur at concentrations well above that required to inhibit fungal mitosis. One of the reasons for the relative safety of Griseofulvin at clinically useful doses may be the fact that it accumulates to high levels in the keratin layers of the skin, where it acts on the growth of dermatophytes. Such an effect is completely lost in the *in vitro* Chang liver model. The IC_{50} value of 9.07µg/ml (approximately 25µM) obtained for the Chang liver cells is similar to that reported for HeLa (20µM) (Panda *et al*., 2005) and K562 cells (44µM) (Zhong *et al*., 2010).

According to Gertsch (2005) *in vitro* bioassays of biological extracts that possess IC_{50} values greater that 200µg/ml are not considered meaningful as it is unlikely that such concentrations will be reached *in vivo* and due to the probability that high concentrations of plant extracts will significantly change the physiochemical environment in terms of ionic strength, pH and osmolarity which will impact on the cell viability independent of the extract itself leading to artificial toxicity. Depending on the dosage and duration of treatment, the cytotoxic effects of Gasteria bicolor and *Pittosporum viridiflorum* may be considered relatively weak (but not entirely absent) and less of a toxicity risk.

5.9 Hepatoprotective activity by *in vivo* study

In the recent investigation suggested that the methanolic extract of *C.procumbens* extract with different models of albino rat was CCl_4 given orally treated and analysis of hepatotoxicity activity. As it is evident from biochemical, haematological and histopathological parameters of the rats were determined. The haematological parameters of RBS, PCV, HB, platelet, total WBC and percentage of differential counts increased I-IV group of albino rat when compared with other treatment respectively.

Many reports indicated that overdose CCl_4 can produce centrizonal hemorrhogie hepaticnecrosis in human and experimental animals (Hinson, 1981). Based on the result, the acute oral toxicity studies for methanolic extracts of both the plants of *S.amaranthoids* and *O.umbellate* would be rewarded as safe and no mortality found out during experimental periods of the animals. CCl_4 induced hepatic injury is commonly used as an experimental method for the hepatoprotective effects of medicinal plants extracts. The extent of hepatic damage is assessed by histological evolution level of various biochemical parameters in circulation (Somnath *et al*., 2017).

Some workers are reported, the effect of *C.longa* on liver function as well as the immune functional activities of peritoneal macrophages in CCl_4 on the liver intoxicated Swiss albino mice. Evidence for the adverse effect of CCl_4 on the liver function when enzymes such as SGOT and SGPT were found elevated in the serum. Since the estimation of serum enzymes such as SGOT and SGPT was a good indicator for the pathological manifestation of jaundice, there was no doubt the CCl_4 induces liver damages in these experimental animals. However, *in vivo* administration of *C.longa* aqueous extract causes hepatoprotective activities in CCl_4 toxicity. The enzyme level goes back to the normal level as in the case of control group (Mahuya *et al.*, 2011).

ALT and AST are non plasma specific enzymes which hydrolyses aliphatic, aromatic or heterocyclic phosphate compounds requiring pyridoxal phosphate as coenzyme for deamination of aspartic acid and alanine respectively mostly in the liver (Khan and Sultana, 2009). Very high values of ALT are seen in acute hepatitis, either toxic or viral in origin. Both ALT and AST levels are increased in liver diseases with ALT > AST (Muriel *et al*., 2007). Very high activity of serum ALP than normal may be noticed in extra hepatic obstruction or cholestasis. ALP is also produced by epithelial cells of billiary canaliculi and obstruction of bile with consequent irritation of epithelial cells leads to secretion of ALP into serum (Manonmani *et al*., 2015).

The enzyme analysis also significantly improved in the level of liver enzymes like ALT, AST, ALP and bilirubin content in the IV group of animal treated with *C.procumbens* plant extract than the other groups of the animal. According to the biochemical analysis, the IV group of animal was excellent increased in albino rats than the other groups. In the biochemical like total protein, albumin, globulin, glucose, bilirubin and cholesterol regained to the normal position because of the test

plant phytocompounds which responsible for hepatoprotective activity can improved accordingly.

Reports of Arun and Balasubramanian (2011) indicated that the animals treated with *Phyllanthus niruri* for a period of 21 days significantly decreased the levels of these lipoperoxidative products in plasma and tissues of alcohol induced rats.

Serum albumin is quantitatively the most important protein synthesized by the liver and reflects the extent of functioning liver cell mass. Since albumin has a fairly long half life of 20 days, it is not a good indicator of acute liver diseases (Shahjahan *et al*., 2004). The liver sections of CCl_4 intoxicated mice showed massive fatty changes, necrosis, degenerated nuclei and wall of bile capillaries, ballooning degeneration and broad infiltration of the lymphocytes and Kupffer cells around the central vein and the loss of cellular boundaries. The normal architecture of the liver was lost and extensive damage was detected in the sections from CCl_4 treated group of rats. The intralobular vein was badly damaged with wide spaces at some sinusoids.

In treated group, there was a mild inflammatory change and greater area of regeneration which is a clear indication of the improvement of functional status of hepatic cells and almost equal to the normal control group. The reduction in body weight was minimal and liver enlargement was also less compared to the animals in toxic control group. All these lesions in the Plant extract + CCl_4 groups were significantly attenuated. Free radicals induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations (Unander *et al*., 1995 and Wolf, 1999).

Reduction in the levels of SGOT, SGPT, ALP and TB towards the normal value is an indication of regeneration process. The histological examination of the liver sections revealed that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with extracted and intoxicated with CCl_4, the normal cellular architecture was compared to silmyrin thereby confirming the protective effect of both the extracts. The methanolic extract of *S.amaranthoids* and *O.umbellate* have shown very significant hepatoprotection against CCL_4 induced hepatotoxicity in albino rats in reducing SGOT, SGPT, ALP and TB levels. Liver sction of *S.amaranthoids* and *O.umbellate* treated animal groups clearly showed normal hepatic cells thereby confirming hepatoprotective activity (Somnath *et al*., 2017).

Previous study reported that increased serum transaminase levels (AST, ALT), indicating acute hepatotoxicity recovered to normal levels after ~2 weeks. No increase in the transaminase levels was observed (Oguz *et al*., 2002).

The quantitatively serum albumin was improved and most important protein synthesized by the liver with reflects the extent of function liver cell mass and supportive evidence for biochemical, histopathological and enzymes were improved with no apparent damage was found in the liver section showed normal parenchymal architecture and well developed cytoplasm respectively. However, the results of the present study the methanolic extract of *C.procumbens* has potent hepatopotective activity observed from the experiments.