DISCUSSION

Pharmacognostical studies deal with the standardization, identification of controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, microscopical, preliminary phytochemical and physico-chemical analysis. Simpson (2010) has classified Boraginaceae into six types. Of these Ehretioideae is characterized by the four lobed ovary with two branched style and 2-4 chambered ovary. *C. retusa* fulfills all the features of the tribe Ehretioideae. Unlike taxonomic identification, pharmacognostic study includes parameters which help in identifying adulteration in raw drug form and also powder form (Sumitra Chandra, 2014), because it loses its morphological identity and easily prone to adulteration. The evaluation of standardization parameters described below through Transverse section (T.S.) or Longitudinal Section (L.S.) or Radial Longitudinal Section (R.L.S.) or Tangential Longitudinal Section (T.L.S.).

Microscopic evaluation (Kalyankar, 2014) also deals with determination of different types of trichomes, stomata, cell types, tissue organization and cell inclusions. The quantitative microscopical studies deals with stomatal number, stomatal index, palisade ratio, vein islet number and veinlet termination number are also used in identification and analysis of herbal drugs.

Crystals are made up of calcium carbonate and calcium oxalate. Calcium oxalate crystals which are usually located in the lumina of cells, are among the most widespread ergastic substances in various shapes, e.g. druses, prismatic crystals, raphides and styloides in angiosperms. Calcium carbonate material is usually deposited in cell walls but it is sometimes located within the lumina of

epidermal trichomes. In some families have both calcium carbonate and calcium oxalate crystals.

Calcium carbonate crystals are found only in a few families such as Moraceae, Urticaceae, Cucurbitaceae, Cannabinaceae, Acanthaceae and in some of the Combretaceae and Boraginaceae (Harisha *et. al.,* 2013).

Metcalfe and Chalk (1957) and Watson and Dallwitz (1991) explained the characteristic properties of the family Boraginaceae. One of the important characteristics features is the glandular and eglandular (covering) trichomes. The covering thick walled, harsh unicellular hairs have a characteristic rough feeling when handled. Bodies resembling cystoliths are frequent in basal part of these hairs and sometimes in the adjacent epidermal cells. Independent cystoliths also occur in *Cordia* and *Tounefortia.* Bider (1935) also found the nature of the cuticular striations at the bases of the hairs and elsewhere on the leaf epidermis to be unreliable for the diagnostic features. Cluster and other kinds of crystals are widely distributed.

Azizian *et. al.* (2000) reported that calcium carbonate crystals present in two forms in *Onosma* species, one deposited in cell wall of hairs and the other located in the base of large hairs. In certain members of Boraginaceae the lamina is said to be isobilateral. In the present study, *C. retusa,* the leaf is dorsi-ventral, the adaxial epidermal cells are highly dilated and raised above the surface and large calcium carbonate crystals (cystoliths) were located in the dilated epidermal cell. The abaxial epidermis is stomatiferous and cyclocytic type.

According to Metcalfe and Chalk (1957), the stomatal type in Boraginaceae is said to be ranunculaceous type (Anomocytyic type). However in the present

study the stomatal type of the lamina was found to be cyclocytic type with 3 to 6 subsidiary cells which are distinct from other surrounding epidermal cells in shape and size. The subsidiary cells are either in single circle or in two circles.

Akin and Binzet (2010) reported the presence of glandular and eglandular trichomes in the epidermis *Onosma angustissimum* Hausskn. & Bornm. and *O. cassium* Boiss. (Boraginaceae). The eglandular trichomes have ornamental cuticle and contain crystals in the bases. Palisade parenchyma cells are 3-layered on the upper surface and 1-2 layered on the lower surface.

Some of the abaxial epidermal cells bear beak shaped thick walled epidermal trichome with small calcium carbonate crystals in the lumen. The mesophyll consists of 2 or 3 layers of palisade cells. Calcium oxalate druses were abundant in the palisade as well as spongy mesophyll tissue. The adaxial epidermal cells are large polygonal with straight walls, stomata totally absent, numerous unicellular trichomes with spherical basal part. Within the spherical basal part small calcium carbonate cystoliths are present. The terminal part of the trichome stand straight on the epidermis and possess minute epicuticular calcium oxalate crystals which were clearly visible under polarized light. Solitary crystals and cluster crystals have been reported in certain members of Boraginaceae. Sand crystals are said to be abundant in most of the tissues of *Cordia* (Melcalfe and Chalk, 1957). In our study we are unable to locate sand crystals in the leaf.

The stem is well developed with secondary growth. It consists of well defined periderm, wide cortex and thick, hallow cylinder of secondary xylem surrounded by thin layer of secondary phloem. The cells of the periderm are strongly thick walled. The end wall perforations of the vessel elements are

simple. Reticulate perforations are not seen in the present study. But this type of perforations has been recorded in some species like *Cordia*.

The vessels in the stem are mostly solitary in the outer zone and short radial multiples in the inner zone. The xylem rays are thick walled and lignified. This observations seems to be common in other species of Boraginaceae.

The root consists of very wide periderm and thick solid secondary xylem surrounded by secondary phloem. Inner to the periderm was a narrow cylinder of cortex with 2 or 3 layered parenchyma cells. Secondary phloem occurs in thick cylinder around the secondary xylem. In many species of Boraginaceae a reddish substance has been recorded in the cells of the root. This red pigment is not present in any other part of the root tissue of *C. retusa*. It seems that the pigment formation might be a wound reaction.

Solereder (1908) has given a list of microscopical parameters of the members of Boraginaceae. The characters are isobilateral lamina, simple uniseriate hairs, ranunculaceous type of stomata present on both sides of the lamina. Petiole contains a median and a small lateral vascular bundle. Presence of cluster crystals in the mesophyll, sub-epidermal origin of periderm presence of isolated structure of pericyclic fibres, closed xylem cylinder with uniseriate rays and presence of septate fibres with simple pits. Afore of the above said characters only certain features such as isobilateral lamina and ranunculaceous type of stomata are not in accordance with our present observations. Other features mentioned by Solereder (1908) seem to reliable diagnostic character of protocol of Boraginaceae.

The root bark surface is smooth and even. The outermost part of the periderm undergoes exfoliation in the form of thick membrane. The bark consists

of two major zones namely outer periderm and inner secondary xylem. In between these two zones is a narrow cortex. The outer part of the periderm consists of thin walled, tabular, homogeneous, suberized phellem cells and towards the inner part the cells were wider and gradually become square shaped. The periderm becomes sharply delimited by a line of cortical parenchyma cells. The cortical zone gradually transits into wide secondary phloem zone. The secondary phloem consists of outer, wider zone of collapsed secondary phloem and inner narrow non collapsed secondary phloem. There are no distinct border separating the collapsed phloem and non collapsed phloem. Prismatic crystals of calcium oxalate are frequently seen both in the collapsed and non collapsed phloem. The crystals located in regular, radial lines and are located in the phloem parenchyma cells.

The tangential longitudinal section of the phloem shows the phloem rays non-storied. They are biseriate or less frequently uniseriate. The rays are hetero cellular possessing middle procumbent cells and marginal upright cells. The phloem viewed under polarized light showed large number of calcium oxalate crystals called styloids located in the parenchyma cells. The styloids are long, scale like parallel crystals with oblique ends. The styloids are upto 200µm long and about 10µm wide. In the list of plant possessing styloid type of crystals has been by Metcalfe and Chalk (1957) in their second volume of Anatomy of Dicotyledons, in which the family Boraginaceae was not given. Our finding of presence of abundant styloid crystals in *C. retusa* may be claimed to be a new finding.

The radial longitudinal section of the phloem shows phloem rays appear in horizontal ribbon like bands. The rays extend from inner phloem upto the inner

border of the periderm. The phloem parenchyma cells are in vertical rows. The periderm cells appear in compact radial files. The structural features of the phloem especially in the L.S. view, provides many diagnostic clues for the botanical diagnosis of the taxon. In *C. retusa* the phloem rays are non-storied and heterocellular and the cells possess calcium oxalate crystals. These features are truly dependable diagnostic features.

The powder microscopical studies revealed the following cell elements like xylem vessel abundant, long, narrow, cylindrical, with circular, wide, end wall perforation with bordered pits, some of them with long, narrow tails at one end or at both ends. Xylem fibres are abundant, very long, narrow and thick walled cells with tapering ends. Parenchyma cells are vertically elongated, parallel, rectangular, thin walled parenchyma cells mixed with other elements.

In pharmaceutical industries the herbal plants are procured in powdered form. In order to find out the purity and genuineness of the powder, the drug must undergo microscopic analysis of the cell compounds in the powder.

The quantitative microscopical studies on *C. retusa* showed the mean value of stomatal number, stomatal index for adaxial epidermis, veinlet termination number, vein islet number and palisade ratio were found as 235, 15.11, 11.92, 6.88 and 50.8 respectively.

The determination of physico-chemical parameters such as foreign matter, ash values and extractive values will be useful in standardisation of the plant *C. retusa*. The plant material must be free from other parts of the same plant or other plants. They should be totally free from moulds or insects, along with excreta including contaminant likes sand, stones, poisonous and harmful foreign matter.

The determination of ash value is that the plant material burnt and residual ash is calculated as total ash content. Total ash is the assessment of the total amount of material left after burning and includes ash resulting from the part of the plant itself. The acid-insoluble ash was obtained after boiling of total ash with dilute hydrochloric acid and burning of the left over insoluble matter. The ash value of the plant *C. retusa* was found to be higher in leaf 12.73% and root and stem are comparatively same 2.64% and 2.63% respectively. The water soluble ash content of the drug indicated the presence of inorganic content found to be higher in leaf 7.48% followed by root 1.76% and stem 1.40%. The negligible amount of acid insoluble siliceous matter was detected higher in leaf 1.13% than root and stem. The moisture content was higher in leaf 10.19% followed by stem 8.47% and root 8.23%. The alcohol soluble extractive value indicated the presence of polar and non polar secondary metabolites. Alcohol soluble extractive value found to be higher in root 3.69 % and stem and leaf were found to be almost similar. Water soluble extractive value indicated the presence of sugar, acids and inorganic components, found to be higher in leaf 16.69 % than root 8.37 % and stem 6.17%.

The analysis of microbial load present in the plant materials showed that the total bacterial count (TBC) and total fungal count (TFC) for root was 3.5×10^3 and 1.7×10^3 and for stem it was 3.5×10^2 and 2×10^2 and for leaves 0.9×10^3 (TBC). The detection of the microbial load was under the permissible limits of WHO (1998). At the same time the bacteria *Enterobacteriaceae*, *Salmonella* spp, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* spp were found to be absent in the plant materials which indicated that the plant is not the carrier of

these microorganisms. The presence of potent contaminants was already been reported in herbal preparations (Czech *et. al*., 2001; Tassanaayakul, 2004).

Fungal contamination has been reported to affect the chemical composition of the raw materials and thereby decrease the medicinal potency of herbal drugs (Dubey *et. al.,* 2008). The most prominent fungal toxins reported are aflatoxins, zearalenone, ochratoxin and patulin, which are collectively known to cause hazards to the liver, nervous system, muscular system, respiratory organs as well as digestive and genital systems. The bacterial and fungal population are found in the locally available herbal medicines (Noor *et. al.,* 2013).

According to British Pharmacopoeia (2004) standards, the limits of microbial contamination are: total aerobic bacteria 10^5 cfu/g or ml, yeasts and moulds 10^4 cfu/g or ml, *Enterobacteriaceae* and other Gram negative organism 10^3 cfu/g and *E. coli* and *Salmonella* should be absent (Noor *et. al.,* 2013). Contaminants presenting serious health hazards have been reported to be *Salmonella*, *Escherichia coli, Staphylococcus aureus*, *Shigella* spp. and other Gram positive and Gram negative strains of bacteria (Noor *et. al.,* 2013).

Coliforms or members of the family Enterobacteriaceae are the most reliable indicators of faecal contamination which may indicate a possible presence of harmful, disease causing organisms (APHA, 1992; Pelczar *et. al.,* 1996; Jay, 1997). These bacteria make up approximately 10% of the intestinal microorganisms of humans and other animals. The significance of faecal coliforms is that if these specific bacteria are present, then other harmful microorganisms may also be present, such as *Salmonella, Shigella, E. coli, Pseudomonas* sp and *S. auereus* (Forest 2004; Hester 2004) and many more others.

The WHO permissible limits for the total aerobic microbial count ($1x10^5$), total fungal count ($1x10^3$) and Enterobacteriaceae ($1x10^1$) and the absence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp, and *Pseudomonas* spp. The European Pharmacopoeia also interprets that *E. coli* and *Salmonella* species, should not be present in herbal preparations (Kalyankar *et. al.,* 2014). The presence of *E. coli* and *Staphylococcus aureus* indicates the possible presence of enterotoxins. Moisture content increase the possibility of fungal toxic products especially aflatoxins.

The analysis of heavy metals i.e., infectivity by toxic metals can either be unintentional or intentional, infectivity by heavy metals likes lead, cadmium, mercury and arsenic in herbal preparation can be recognized to many causes, together with environmental pollution and can be dangerous for the health of the consumer. The possible intake of the toxic metal can be predicted based on the level of its occurrence in the product and the suggested or estimated dosage of the product. Instrumental analyses are used when the metals are present in very small quantities or when the analysis is quantitative. The most important methods usually used are Atomic Absorption Spectrophotometer (AAS), Neutron Activation Analysis (NAA) and Inductively Coupled Plasma (ICP). The analysis of aflatoxin in the plant *C. retusa* was carried out by AAS showed the presence of lead 0.1521ppm, 0.0751ppm and 0.2531ppm within the permissible limit (API, 2008) in root, stem and leaves respectively. The permissible limits viz. 10, 0.3, 1 and 3 ppm for Lead, Cadmium, Mercury and Arsenic respectively. The plant was hence considered non-pollutant in the environment and the plant cannot cause any ailments due to the heavy metals.

The causative toxins known that aflatoxins (B1, B2, G1, G2) are food-borne secondary toxic metabolites produced during the growth of *Aspergillus flavus* and *A. parasiticus* group of fungi. These are highly substituted coumarin derivatives containing a fused dihydrofurofuran moiety. Aflatoxin B1 and AFB2 are named because of their strong blue fluorescence under UV light, whereas AFG1 and AFG2 fluoresced greenish yellow (Verma, 2004).

Aflatoxins are responsible for various toxicological effects such as hepatic, gastrointestinal and carcinogenic diseases and have the capacity to cross the placental barrier and cause genetic defects at foetal stages (Maxwell *et. al*., 1998). Aflatoxins in plant and herbal products can be hazardous to health though they are absorbed in minute quantity. The analysis of aflatoxins in *C. retusa* using HPLC showed that the aflatoxins B_1, B_2, G_1 and G_2 found below the detecting limit and hence the present study shows the non-toxic effect of the plant.

Pesticides are mainly used to eliminate or control pests (Margni *et. al*., 2002; Waxman, 1998). There are three main groups of pesticides are insecticides, herbicides and fungicides. There are also rodenticides, nematicides and molluscides (Waxman, 1998). Herbicides are the most commonly used pesticide, followed by insecticides and fungicides (Ahmed, 2001). Pesticide exposure can cause a variety of adverse effects ranging from simple irritation of skin and eyes to more sever effects such as affecting the nervous systems mimicking hormones causing reproductive problems and also causing cancer. The presence of pesticides has been reported in medicinal plants by Ahmed (2001) and Sarkhail (2012). The analysis of various pesticidal residues like HCH (all isomers), DDT (all isomers),

DDE (all isomers), Endosulphan (all isomers) etc., were tested in the plant *C. retusa*. All the above mentioned pesticides were not detected.

Aarthi *et. al.* (2014) reported the preliminary phytochemical analysis of successive extracts like hexane, chloroform, ethyl acetate and methanol were carried out. The steroids and triterpene were present in hexane extract, whereas phenols, flavanoid, tannin, acids, alkaloids, sugars and saponins were present in methanol extract. Chandarappa *et. al.* (2012) reported the phytochemical screening of leaves extract of various solvents like petroleum ether, methanol and chloroform was also reported. The methanol extract showed the presence of alkaloid, flavanoid, saponins, phenols, tannins and cardiac glycosides.

In the present study the phytochemical screening of alcohol extract of root, stem and leaves showed the presence of alkaloids, phenol, flavanoid, triterpenoid, steroid, coumarin, glycosides/sugars, tannins and quinine were found to be alcohol extracts of root, stem and leaf. Saponin was fond to be only in leaf extract of the plant. Amino acids, carboxylic acids and furanoid were absent in all parts of the plant.

The Thin layer chromatography is one of the simplest chromatographic method based on principle of either partition or adsorption of sample in between mobile and stationary phase. Generally stationary phase comprises of silica gel, alumina, cellulose, keislgur, starch, etc. And mobile phase includes use of number of polar and nonpolar solvent depending upon nature of the sample. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved.

The WHO accepts fingerprint chromatography as an identification and quality evaluation technique for medicinal herbs and it is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. Fingerprints can be a unique identification utility for herbs and their different species (CHMP, 2005 and Welsh *et. al*., 1996). Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies (Sampathkumar and Ramakrishnan, 2011).

In the present study chromatogram of root, stem and leaves were carried out and the number of peaks at 254nm, 366nm and VS reagent were studied. Similar studies are also reported by Sampathkumar and Ramakrishnan (2011) on ethanolic extract of stem, bark and leaf of *Naringi crenulata* by HPTLC methods.

Aarthi *et. al.* (2014) reported the TLC photo documentation and HPTLC finger print of chloroform extract of aerial parts. The HPTLC finger printing showed 16 peaks at 254 nm with major peaks at 0.98, 0.91 and 0.41. The TLC photo documentation showed four visible spots under 254 nm, 8 spots under 366 nm and 4 spots after derivatization for aerial parts.

The HPTLC profile of petroleum ether extracts of various parts of the were developed using Toluene : Ethyl acetate (9 : 1) as mobile phase. At 254 nm R_f 0.56 was commonly observed all the three part of the extract. At 366 nm showed 8, 4 and 10 spots for root, stem and leaf respectively. The plate on derivatization with vanillin-sulphuric acid showed 7, 5 and 10 spots were observed for root, stem and leaf respectively and the R_f value was 0.82, 0.51 and 0.36 and commonly

observed for all the three extracts. The desnsitometric chromatogram was used for comparison of root, stem and leaves extracts the peak at R_f was 0.64 and was similar for all the extracts of root, stem and leaves.

The HP TLC profile of chloroform extracts of various parts of the plant were developed using Toluene: Ethyl acetate (9: 1) as mobile phase. At 254 nm 3, 4 and 4 spots were observed for root, stem and leaf, at 366 nm showed 1, 8 and 8 spots for root, stem and leaf and the plate was after derivatization with vanillin-sulphuric acid, it showed 2, 6 and 7 spots were observed for root, stem and leaf respectively. The R_f 0.76 was commonly observed all the three part of the extract. The desnsitometric chromatogram was used for comparison of root, stem and leaves extracts. At R_f 0.88 peak was present in all parts of the extracts, whereas at 0.62 showed both root and stem.

The HPTLC profile of alcohol extracts of various parts of the plant were developed using Chloroform : Methanol (8: 2) as mobile phase. At 254 nm 7, 3 and 3 spots were observed for root, stem and leaf, at 366 nm showed 6, 5and 3 spots for root, stem and leaf and the plate was after derivatization with vanillin-sulphuric acid, it showed 7, 6 and 5 spots were observed for root, stem and leaf respectively. The R_f 0.77, 0.71, 0.49 and 0.37 were commonly observed all the three part of the extract. The desnsitometric chromatogram was used for comparison of root, stem and leaves. At R_f 0.13 and 0.42 peaks were present in root and stem extract, whereas at 0.54 showed both stem and leaves.

Toxicity studies are important because they show that there are risks with an inappropriate use of some of these extracts as therapeutics for any ailments. Cytotoxicity is cell damage but the definitions vary depending on the nature of the

study whether cells are killed or their metabolism altered. This intern will facilitate in the identifications of toxicants at an early stage of drug discovery and development from plant sources. Among the various studied models for toxicity study, cytotoxicity assays are one among the indispensable tools to predict toxicity. Cytotoxicity is the cell-killing property of a chemical compound. In contrast to necrosis and apoptosis, the term cytotoxicity does not indicate a specific cellular death mechanism. There are many observable morphological and biomedical differences between necrosis and apoptosis (Nabil Ben Salem Abid *et. al*., 1994).

Botanicals such as herbal products and nutraceuticals are often regarded as low risk since they have been used by human throughout history. However some of them may reveal a very strong and even toxic activity in humans, which especially refers to extracts, concentrate or pure compounds obtained from plants. For this reason it is very important to conduct screening test to assess both the beneficial effects and the toxicity of the plant materials (Sieniawska *et. al*., 2013).

In the present study the cytotoxic studies on the alcohol extract of various parts were carried out and the cell lines were observed under inverted microscope does not show any significant morphological changes. Hence it is concluded from this preliminary cytotoxic study, the plant extracts of root, stem and leaves is not toxic to vero cell lines (30µg/ml) and this cytotoxicity assay was considered as one of the basic parameter. The American National Cancer Institute (NCI) guidelines set the limit of activity for crude extracts at 50% inhibition (IC50) of proliferation of less than 30µg/ml after then exposure time of 72hrs (Abdel-Hameed *et. al*., 2012). However the crude extract with IC50 less than 20µg/ml is considered highly toxic (Mahavorasirikul *et. al*., 2010; Vijayarathna and Sasidharan, 2012).

This *in-vitro* cytotoxic assays offer quick, simple and cost efficient way of testing the toxicity and forms an important tool for screening of plant extracts.

In literature studies Jowi *et. al.,* (2008) reported the plant *C. retusa* have a high potential infighting the growth and multiplication of cancer cells.

Chandrappa *et. al.,* (2014) reported the flavonoid quercetin from stem of *C. retusa* was analyzed for its anticancer activity on HepG2 cell lines by MTT assay, Hoechst 33342 staining and Caspase-3 colorimetric assay shows significant and concentration-dependent anticancer activity at 100 μg/ml and 80 μg/ml doses after 24 and 48 h of treatment on HepG2 cell line in MTT assay, significant cell apoptosis have shown at 53μg/ml.

However in the present study the plant extract is not toxic to veo cell line, since the cytotoxic assay is considered as one of the basic parameters to be studied before any drug designing and development. However the root extract possess anti-snake venom properties so it is very much necessary to test the LD_{50} for the root extract. The root extract may possess a good anticancer properties also

The impetus for pharmacology came from the need to improve the outcome of therapeutic intervention by doctors, who were at that time skilled at clinical observation and diagnosis. One of the basic trends of pharmacology is that the drug molecules must exert some chemical influence on one or more constituents of cells in order to produce a pharmacological response (Rang *et. al*., 2003). Before screening the biological activity of the plants, the toxicity is an important factor for the therapeutic values. Toxicity is a complex term with many influencing factors such as dosage and xenobiotics. Toxic effects are generally categorised according to the site of the toxic effects. Acute toxicity occurs almost immediately

(hours/day) after an exposure to the drug. An acute exposure is usually is a single dose or a series of doses received within 24 hours period. Sub-chronic toxicity results from repeated exposure for several weeks or months and is a common human exposure pattern for some pharmaceuticals and environmental agents. It represents cumulative damage to specific organ systems and takes many months or years to become a recognizable clinical disease.

The hot plate test is a well-known method for assessing acute heat pain sensitivity in mice. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The heat stimulation sensitizes peripherally nerve endings and the impulses generated propagate to the brain via the spinal cord. Hence this test is primarily used to evaluate the capability of a substance to inhibit pain of central origin.

In the present study, the activity was more in the leaf extract compared with the control standard and decreased activity in the root extract then stem extract.

The analgesic activity of the sample was comparable with that of the standard diclofenac. The results obtained in the present study suggest that *Carmona retusa* leaf extract possess maore analgesic activity. The leaves of the plant is used as tea, tonic in Philippines.

The reaction of living tissue to injury which comprises a series of changes of the terminal vascular blood and connective tissues, which tend to eliminate the injurious agents to repair the damaged tissues, is called as inflammation. Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is defensive mechanism of the organism to remove the injurious stimuli as well as to initiate the healing

process for the tissue. In absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism (Coussens and Werb, 2002). The formation of edema in paw results a synergism between various inflammation mediators which increases the vascular permeability and the blood flow. Carrageenan induced paw edema is used widely for determining the acute phase of inflammation.

Chandrappa *et. al*. (2013) reported the *in-vitro* anti-inflammatory activity of ethanol extract of stem of *Carmona retusa* and was investigated by human red blood cell membrane stabilization method, heat induced hemolysis and proteinase inhibitory activity. The activity was found maximum in human red blood cell membrane stabilization method, heat induced hemolysis and proteinase inhibitory activity as 55.72%, 56.37% and 61.75% respectively at 400µg/ml concentration. Minimum anti-inflammatory activity has shown at 50µg/ml concentration as 28.92µg/ml, 15.43µg/ml and 11.33µg/ml in the methods used.

In the present study, the activity was more in the root extract (200mg/kg) 54.61% compared with the control standard 67.6% followed by decreased in activity of 100mg/kg or root extract then, 200 and 100mg/kg of stem and 200mg/kg and 100mg/kg of leaves extract.

The anti-inflammatory activity of the sample was comparable with that of the standard diclofenac. The results obtained in the present study suggest that *Carmona retusa* can be a potential source of anti-inflammatory agents

Many plants have been reported for anti-inflammatory activities. *Salacia oblonga* Wall. root bark powder, *Azima tetracantha* Lam. leaf powder and *Gmelina asiatica* Linn. root powder are screened for anti-inflammatory activities.

Bergenin - a glucoxanthose isolated from the pods *Peltophorum pterocarpum* is equivalent to phenylbutazone in curing rats against carrageenan induced hind paw oedema (Menon *et. al*., 1982). Kumar *et. al*. (2013) reported that nearly 67 medicinal plants are anti-inflammatory agents. Some of the plants are *Bacopa monnieri*, *Adhatoda vasica, Mangifera indica*, *Garcinia mangostana*, *Lantana camara, Emblica officinalis* etc.,

As infection is a major cause of morbidity and mortality in wound patients, these herbal extracts may be useful in preventing infection that leads to high risk of sepsis. Results demonstrate that the alcoholic extract ointment of various parts of the plant *C. retusa* would be capable of promoting wound healing activity.

The topical application of drugs is an efficient therapy method of destroying microbial populations because the availability of the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of microorganisms, amount of inoculums, and host immune response are important factors that can cause massive damage during infection. Normally, common wound pathogens are S. aureus, C. albicans and P. aeruginosa.

A number of secondary metabolites/active compounds isolated from plants have been demonstrated in animal models (in vivo) as active principles responsible for facilitating healing of wounds. Some of the most important ones included tannins from *Terminalia arjuna*, oleanolic acid from *Anredra diffusa*; polysaccharides from *Opuntia ficus-indica*; gentiopicroside, sweroside and swertiamarine from *Gentiana lutea*; shikoninderivatives (deoxyshikonin, acetyl shikonin, 3-hydroxy-isovaleryl shikonin and 5,8-Odimethyl acetyl shikonin) from *Onosma argentatum*; asiaticoside, asiatic acid, and madecassic acid from *Centalla*

asiatica; quercetin, isorhamnetin and kaempferol from Hippophae rhamnoides; curcumin from *Curcuma longa* (Karodi *et. al*., 2009).

The results of the antimicrobial activity prove the efficacy of the various parts of the plant against the microorganisms. Plant produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens.

Chandrappa *et. al*. (2012) also reported that the stem extract showed antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella flexnari* and *Bacillus subtilis.*

Penecilla and Magno (2011) reported that the extract of aerial part of *C retusa* showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* where as there is no activity against *Escherichia coli.*

In the present study only the alcoholic extract of the root of *C. retusa* showed a maximum activity towards *Escherichia coli* where as it is susceptible to stem and leaf extracts.

Among the 10 microorganisms tested *Bacillus subtilis* was found to be the most sensitive organisms followed by *Bacillus cereus and Candida albicans*, *Pseudomonas putida* and *Staphylococcus aureus* and *Escherichia coli* and *Klebsiella pneumonia.*

The two microorganism *Enterobacter aerogenes* and *Pseudomonas aeruginosa* are resistant to all the extracts even at the dose of 100µg/ml.

The infectious diseases are the leading causes of death worldwide, accounting, 13.3 billion deaths, which constitutes about 25% of all deaths. The reasons for the increases in the incidence of infectious diseases are not fully understood and may be due to emergence of multi drug resistant bacteria such as *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Enterococcus* species. Antibiotic resistance achieved by inactivation of drugs, modification of drug targets and reduction in the concentration of drug that reaches the target1. To overcome this problem there is an urgent need for promising antimicrobial compounds to fight against the emerging and re-emerging infectious diseases.