RESULTS

5.1 Pharmacognostical studies

External features of the plant

C. retusa (Plate 1.1) was a shrub or much-branched small tree, 1-4 (-10)m tall; young branches hispid, with buds or short shoots producing clusters of leaves (Plate 1.3). Leaves alternate, clustered with axillary leaves, leaves usually 10-50mm long and 5–30 mm wide, sparsely scabrous along nerves below, base cuneate, margin entire, apex obtuse-truncate, bluntly 3-7 toothed, lateral nerves 5 pairs, arching near margin, impressed above, prominent below(Plate 2.1 – 2.2); Petiole 1-5mm long, stipules absent. Inflorescence in axil of leaves or on apex of short shoots, flowers in fascicles of 2-6 or in a cyme. Flowers (Plate 1.4 & 2.3) bisexual, (4-) 5-merous, axillary, solitary or 1 or 2 in the axile of leaves, small and white, bracteate; Pedicelled measures 5mm; Calyx 3-6mm long (Plate 2.4), with (4-)5 linear lobes, unequal, elliptic oblong, herbaceous, scabrous below in and out, decurrent, obtuse or apiculate; Corolla sub-rotate, white, 6-9mm in diameter (Plate 2.5), tube about 2 mm long, widening, lobes spreading, 2.5-4.5mm long, oblong elliptic, imbricate, 5mm, herbaceous obtuse; Stamens (4-)5 with filaments 2.5-3.5mm long and anthers oblong (Plate 2.6); Ovary superior, globose, about 1 mm in diameter, style deeply bifid, 4.5-6 mm long, free from base (Plate 2.7). Fruit drupe, globose (Plate 1.2), 5-6 mm in diameter, red or yellow, with 1-4 seeds, not breaking up into pyrenes. Seeds with a straight or slightly curved embryo, embedded in thin albumen. Seedling with epigeal germination; cotyledons leafy, green, hypocotyl elongated.

Microscopic features

Anatomy of the leaf:

In sectional view the leaf has less prominent midrib with small abaxial part, wide adaxial concavity and thick lamina (Plate 3.1). The midrib is 330µm thick and along the abaxial part 400µm wide. The epidermal layer of the midrib on the adaxial side consists of vertically oblong, wide, thick walled cells with thick cuticle. The adaxial epidermis is thick walled and abaxial epidermis thin walled. The ground tissue consists of angular compact, thick walled parenchyma cells.

The vascular strand is single, collateral and located in the middle part of the midrib. It consists of adaxial vertical lines of xylem elements and abaxial layer of phloem. There is a thick arc of sclerenchyma cells enclosing the vascular bundles on the abaxial part (Plate 3.2).

Lamina

The lamina is dorsi-ventral with distinct differentiation of adaxial and abaxial sides. The lamina is 250µm thick. The adaxial epidermal layer consists of vertically elongated rectangular, thin walled cells with thick cuticle (Plate 3.3). Some of the adaxial epidermal cells are highly dilated and raised above the surface and large calcium carbonate crystals are located in the dilated epidermal cell (Plate 4.1). These crystals are called calcium carbonate cystoliths. The abaxial epidermis has smaller, squarish, thick walled cells. The abaxial epidermis is stomatiferous. Some of the abaxial epidermal cells bear beak shaped thick walled epidermal trichome. The basal part of the trichome is very wide and the terminal part bent horizontally into conical structure. Within the lumen of the

trichome occurs small, cylindrical calcium carbonate crystal called cystoliths (Plate 4.2).

The mesophyll tissue of the lamina includes thick band of palisade cells which are 2 or 3 layered, narrow and cylindrical. The abaxial portion of the lamina includes spongy mesophyll tissue. The cells were spherical or lobed and loosely interconnected forming large air cavities (Plate 3.3).

Calcium oxalate druses were abundant in the palisade as well as spongy mesophyll tissue. The cells possessing the druses are wide and vertically oblong (Plate 3.3).

Leaf margin

The marginal part of the lamina bent down and the end is semicircular and broadly conical. The epidermal cells of the marginal part smaller with thick cuticle. The mesophyll tissues were normal in the marginal part. The leaf margin 120µm thick (Plate 5.2).

Epidermal cells and stomata

The abaxial epidermal cells and stomata were studied from the surface view of the paradermal sections. The epidermal cells are small with polygonal walls. Stomata are densely distributed and are cyclocytic type. Each stoma is surrounded by 5 to 9 subsidiary cells. The subsidiary cells were wide and thick walled (Plate 6.2 & 6.3). The guard cells were broadly elliptical and measure 15 X 20µm. The stomatal pore is narrow and vertically elongated (Plate 6.1).

The adaxial epidermal cells are large polygonal cells with straight walls. Stomata are totally absent (Plate 6.4). Numerous unicellular covering trichomes with spherical basal part (Plate 6.5). Within the spherical basal part of the

trichomes occur. Small calcium carbonate cystoliths are also present (Plate 6.6). The terminal part of the trichome which stand straight on the epidermis, possess minute epicuticular calcium oxalate crystals which clearly seen under polarised light (Plate 6.7).

Venation pattern

Because of the thick and coriaceous nature of the lamina, the venation pattern is not clearly visible. The veins are fairly thick and vein lets form wide, angular islets. The vein terminations are seen arising from the vein islets which are either unbranched or branched (Plate 7.1 & 7.2).

Petiole

The petiole consists of middle biconvex portion and short, thick lateral wings (Plate 8.1). The middle part of the petiole consists of adaxial, wide small raised portion and abaxial thick semicircular midrib portion. The epidermal layer is a thin with small highly thick walled slightly papillate cells. The ground tissue includes circular, thick walled, less compact parenchyma cells. The vascular system includes median, large bowl shaped vascular strand and small, circular laterally placed accessory strands (Plate 8.2).

The proximal part of the petiole is plano-convex in sectional view without lateral wings. The petiole consists of smaller, median, vascular strand and two circular, smaller accessory strands. The proximal part of the petiole 650µm thick and 800µm wide (Plate 9.1 & 9.2).

The median vascular strand consists of several long parallel lines of xylem elements which are angular, narrow and thick walled. Along the lower part of the strand occurs a thick arc of phloem elements. A thin layer of sclerenchyma cells

occurs along the lower part of the vascular strand. The small accessory strands include a collateral vascular bundle with thick sclerenchymatous bundle cap.

Stem

The stem with well developed secondary growth was studied. It is 1.8mm in diameter. It consists of well defined periderm, wide cortex and thick, hallow cylinder of secondary xylem surrounded by thin layer of secondary phloem (Plate 10.1). The periderm is interior in origin. It is found located 2 or 3 layers inner to the cortical cells (Plate 10.1 & 10.2).

The periderm consists of 6 or 7 layers of tabular, thin walled, homogeneous, suberized cells (Plate 10.2 & 11.2). Inner to the periderm is wide, parenchymatous cortical zone where there are small, scattered mass of fibres (Plate 11.1).

Secondary phloem occurs in thin continuous layer along the outer surface of the xylem cylinder (Plate 10.2). Secondary xylem includes several thin radial lines of vessel elements and thick walled, lignified xylem fibres. There is a less distinct growth ring layer in the outer part of the xylem cylinder. The vessels are solitary and scattered in the outer part of the xylem cylinder and they occur in long radial multiples in the inner part (Plate 10.2 & 12.1). The vessels are circular or elliptical, thick walled with wide lumen. The vessels measure 20 to 50µm in diameter. The xylem fibres are heavily thick walled and lignified. They have wide lumen. The fibres along the growth ring border and more thick walled with narrow lumen (Plate 12.2).

Root

The root consists of very wide periderm and thick solid secondary xylem surrounded by secondary phloem (Plate 13.1). The periderm is replaced the epidermis and forms thick homogeneous tangentially elongated, tubular suberized cells. The periderm is 200µm thick (Plate 13.2 & 13.3, 14.1 & 14.2). The periderm includes entirely of phellem cells and phelloderm cells are not evident. Inner to the periderm is a narrow cylinder of cortex which 2 or 3 layered parenchymatous cells. Secondary phloem occurs in thick cylinder around the secondary xylem.

The secondary phloem elements occur in short radial lines, in the central part (Plate 13.3), while in the peripheral part the xylem elements are distributed in random circular zone (Plate 13.1 & 13.2). The xylem elements are vessels which are circular or elliptical, narrow and thick walled. They measure 10-50µm in diameter. The xylem fibres very thick walled with reduced lumen and lignified walls.

Root Bark

The surface of the bark is smooth and even. The outermost part of the periderm undergoes exfoliation in the form of thick membrane (Plate 15.1). The total thickness of the bark is 1.6mm. The bark consists of 2 major zones namely outer periderm and inner secondary xylem. In between these 2 zones is a narrow cortex (Plate 15.1).

The periderm 580 µm thick. The outer part of the periderm consists of thin walled, tabular, homogeneous, suberized phellem cells (Plate 15.2 & 16.1). The cell walls were uniformly thin. The radial walls are wavy and the tangential walls are straight (Plate 16.1).

Towards the inner part of the periderm the cell are wider and gradually becomes square shaped (Fig. 15.2). The periderm becomes sharply delimited by a line of cortical parenchyma tissue. The cortical cells are polyhedral and thick walled (Plate 15.2).

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 noncollapsed secondary phloem. There is no distinct border separating the collapsed phloem and noncollapsed phloem (Plate 17).

The noncollapsed phloem consists of radial rows of wide, angular, thick walled sieve elements and parenchyma cells (Plate 16.2). Calcium oxalate prismatic crystal are frequently seen both in the collapsed and noncollapsed phloem (Plate 18.2). The collapsed phloem exhibits thin, dark, tangential lines which represents the crushed sieve elements. The phloem parenchyma cells in this region are dilated. Calcium oxalate crystals are abundant in the phloem region. They are prominent prismatic type.

They are mostly cuboidal in shape. The crystals are located in regular, radial lines (Plate 18.1) and are located in the phloem parenchyma cells. The crystals are solitary in each cell and occupy the entire lumen on the parenchyma cells (Plate 18.3). The crystals are 12 x 12µm in size,

Tangential longitudinal section of the phloem

In TLS view the phloem rays appear non-storied. They are biseriate or less frequently uniseriate. The rays are heterocellular possessing middle procumbent cells and marginal upright cells. The rays are spindle shaped and thick walled. The uniseriate rays mostly possess upright cells. The rays are 110 to 200µm in height

and 30 to 40µm in thickness (Plate 19.1 & 19.2). The axile parenchyma cells are vertically elongated thick walled, arranged one below the other in vertical strands (Plate 19.1 & 19.2).

When TLS sections of the phloem viewed under polarized light large number of calcium oxalate crystal called styloids are seen located in the parenchyma cells. The styloids are long, scale like parallel crystals with oblique ends (Plate 20.1 & 20.2). The styloids are vertically oriented and occupy the entire length of the parenchyma cells. The styloids are upto 200 µm long and about 10µm wide (Plate 20.3).

Radial longitudinal section of the phloem

In RLS view the phloem rays appear in horizontal ribbon like bands (Plate 21.1 & 21.2). 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 phloem ray consists of two types of cells: those in the middle of the ray are square shaped or horizontally rectangular; these are called *procumbent cells*. The cells in the marginal part are vertically oblong. These cells are called *upright cells*. So, these rays are called *heterocellular rays*. The procumbent cells are 20 x 25µm in size. The upright cells are 20 x 30µm in size (Plate 21.1, 21.2 & 21.3).

Powder Microscopy

The powder preparation exhibits the following elements:

(i) Vessel elements:

The xylem vessel elements are abundant in the powder. They are long, narrow, cylindrical cells (Plate 22.2, 23.2, 24.1 & 24.2). The vessel elements have circular, wide, end wall perforation. Some of the vessel elements have long,

narrow tails at one end or at both ends. They are called tailed vessel elements (Plate 22.2). On the lateral walls are seen multiseriate, circular, dense bordered pits. The vessel elements are 350 – 450µm long and 30µm wide.

(ii) Fibres:

Xylem fibres are also equally abundant in the powder. They are very long, narrow, thick walled cells with tapering ends. They are upto 680µm long and 10µm thick in the middle. The fibres do not possess pits or perforations (Plate 22.1 & 22.2).

(iii) Parenchyma cells:

Vertically elongated, parallel, rectangular, thin walled parenchyma cells are seen mixed with other elements (Plate 22.1, 23.1 & 23.2, 24.1). The parenchyma cells have thin walls and wide lumen. Some of the parenchyma cells possess dark spherical bodies (Plate 24.1). The parenchyma cells are 200 – 400µm long and 25µm wide.

Quantitative Microscopy

Quantitative microscopy such as stomatal number, stomatal index, vein islet number, veinlet termination number and palisade number were studied and presented (Table 1). The mean value of stomatal number (abaxial epidermis), stomatal index (abaxial epidermis), veinlet termination number, vein islet number and palisade ratio were found as 235 ± 17.32, 15.11 ± 1.35, 11.92 ±2.11, 6.88 ± 1.73 and 50.8± 9.23 respectively showing the characteristic features of the plant.

5.2 Organoleptic and Physico-chemical parameters

The oraganoleptic parameters of the plants are

Root - Root earthy brown in colour, bark very thick, bark very difficult to peel, very strong, odour distinct and taste bitter.

Stem - Stem - Cylindrical, light brown with thin bark, bark peelable, odourless, taste characteristic of its own.

Leaves - Leaves obovate, spathulate, upto 3cm long and 1-5cm broad; obovate, spathulat thick, leaf tip bluntly 3 to 7 toothed,

Analytical / Physico-chemical parameters

The physico-chemical standards for root, stem and leaves powders of the plant (80mesh) are given in (Table 2). The plant contains Loss on drying at 105ºC at 8.23%, 8.47% and 10.19%; ash value for 2.64%, 2.63% and 12.73%. for root, stem and leaves respectively. The water soluble ash for root 1.76%, stem 1.4% and leaves 7.48%. The acid insoluble ash values obtained for root was 0.76%; stem 0.079% and for leaves 1.13%). The alcohol soluble extractive for root 3.69%, stem 2.35% and foe leaves 2.35% shows the extraction of polar chemical constituents. The water soluble extractive values for root 8.37%, stem 6.17% and for leaves 16.69% indicates the presence of inorganic chemical constituents.

5.3 Quality Control Parameters:

Analysis of Microbial Load:

The microbial load present in the plant materials were given in Table 3. The results of the experiment revealed that the total bacterial count and total fungal count for root was 3.5×10^3 and 1.7×10^3 and for stem was 3.5×10^2 and 2×10^2 and for leaves it was 0.9×10^3. The detection of the microbial load was found below 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.

Analysis of Heavy Metal:

The results of the heavy metals found in the plant material were given in Table 4. The heavy metal contents viz. of lead, cadmium, mercury and arsenic as per Ayurvedic Pharmacopoeia of India (2008) showing that the results were found to be within the permissible limits viz. 10, 0.3, 1 and 3 ppm respectively. The plant is hence considered non-pollutant in the environment and the plant cannot cause any ailments due to the heavy metals.

Analysis of Aflatoxins:

The results of the analysis of various aflatoxins found in the plant material were given in the (Table 5). 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.

Analysis of Pesticide Residues:

The various pesticidal residues HCH (all isomers), DDT (all isomers), DDE (all isomers), Endosulphan (all isomers) etc., were tested in the plant. All the mentioned pesticides were not detected. The results were shown in (Table 6).

5.4 Phytochemical Analysis:

Preliminary phytochemical studies

Phytochemical screening of *C. retusa* of alcohol extract of root, stem and leaf were analysed and results were tabulated (Table 7). The results revealed that the presence of alkaloids, phenol, flavanoid, triterpenoid, steroid, coumarin, glycosides/sugars, tannins and quinine were found in the alcohol extracts of root, stem & 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.

5.5 TLC / HPTLC finger print analysis:

TLC profile of petroleum ether extracts of root, stem and leaf

TLC profile of petroleum ether extracts of the root, stem and leaf were developed using Toluene : Ethyl acetate (9 : 1) as mobile phase. TLC profile at UV-254 nm, UV-366 nm and after derivatized with vanillin - sulphuric acid was shown in Fig. 1. At 254 nm 8, 2 and 4 spots were observed for root, stem and leaf respectively (Table 8). The R_f value of 0.56 was commonly observed for all the three the extract. At 366nm 8, 4 and 10 spots for root, stem and leaf were seen (Table 9). The plate after derivatization with vanillin-sulphuric acid showed 7, 5 and 10 spots were observed for root, stem and leaf respectively (Table 10). The R_f value of 0.82, 0.51 and 0.36 were commonly observed for all the extract.

HPTLC finger print profile of petroleum ether extract of root

HPTLC finger print profile of petroleum ether extract of root was scanned at 254nm showed 10 peaks (Fig. 2) of which 3 were major peaks at R_f value of 0.09, 0.38 and 0.64 whereas others were moderately smaller peaks.

HPTLC finger print profile of petroleum ether extract of stem

HPTLC finger print profile of petroleum ether extract of stem was scanned at 254nm showed 10 peaks (Fig. 3) of which 2 were major peaks at R_f value of 0.64 and 0.71 whereas others were moderately smaller peaks.

HPTLC finger print profile of petroleum ether extract of leaf

HPTLC finger print profile of petroleum ether extract of leaf was scanned at 254nm showed 9 peaks (Fig. 4) of which one was major peak at R_f value of 0.65 whereas others were moderately smaller peaks.

Densitometric chromatogram of petroleum ether extracts of root, stem and leaf.

The desnsitometric chromatogram was used for comparison of root, stem and leaves extracts (Fig. 5). At R_f value of 0.64 peak was present in all parts of the extracts, of root, stem and leaves.

TLC profile of chloroform extracts of root, stem and leaf

TLC profile of chloroform extracts of the root, stem and leaf were developed using Toluene: Ethyl acetate (9: 1) as mobile phase. TLC profile at UV-254nm, UV-366nm and after derivatized with vanillin - sulphuric acid was shown in Fig.6. At 254nm 3, 4 and 4 spots were observed for root, stem and leaf respectively (Table – 11). At 366nm 1, 8 and 8 spots for root, stem and leaf were observed (Table – 12). The plate after derivatization with vanillin-sulphuric acid showed 2, 6 and 7 spots for root, stem and leaf respectively (Table – 13). The R_f value of 0.76 was commonly observed for all the extract.

HPTLC finger print profile of chloroform extract of root

HPTLC finger print profile of chloroform extract of root was scanned at 254nm showed 3 peaks (Fig. 7) and all are moderately smaller peaks.

HPTLC finger print profile of chloroform extract of stem

HPTLC finger print profile of chloroform extract of stem was scanned at 254nm showed 13 peaks (Fig. 8) of which 3 were major peaks at R_f value of 0.06, 0.37 and 0.63 whereas others were moderately smaller peaks.

HPTLC finger print profile of chloroform extract of leaf

HPTLC finger print profile of chloroform extract of leaf was scanned at 254nm showed 6 peaks (Fig. 9) and all the peaks are moderately smaller peaks.

Densitometric chromatogram of chloroform extracts of root, stem and leaf.

The desnsitometric chromatogram was used for comparison of root, stem and leaves extracts (Fig. 9). At R_f value of 0.88 peak was present in all parts of the extracts, whereas at 0.62 showed both root and stem.

TLC profile of alcohol extracts of root, stem and leaf

TLC profile of alcohol extracts of the root, stem and leaf were developed using Chloroform : Methanol (8: 2) as mobile phase. TLC profile at UV-254 nm, UV-366 nm and after derivatized with vanillin - sulphuric acid was shown in Fig. 11. At 254nm 7, 3 and 3 spots were observed for root, stem and leaf respectively (Table 14). The R_f value of 0.69 and 0.13 were commonly observed for all the three part of the extract. At 366nm showed 6, 5 and 3 spots for root, stem and leaf respectively (Table 15). The R_f value 0.57 and 0.49 were commonly observed all the three part of the extract. The plate after derivatization with vanillin-sulphuric acid showed 7, 6 and 5 spots were observed for root, stem and leaf respectively (Table 16). The R_f value of 0.77, 0.71, 0.49 and 0.37 were commonly observed all the three part of the extract.

HPTLC finger print profile of alcohol extract of root

HPTLC finger print profile of alcohol extract of root was scanned at 254nm showed 11 peaks (Fig. 12) of which 4 were major peaks at R_f value of 0.13, 0.42, 0.52 and 0.56 whereas others were moderately smaller peaks.

HPTLC finger print profile of alcohol extract of stem

HPTLC finger print profile of alcohol extract of stem was scanned at 254nm showed 11 peaks (Fig. 13) of which one was major peak at R_f value of 0.13 whereas others were moderately smaller peaks.

HPTLC finger print profile of alcohol extract of leaf

HPTLC finger print profile of alcohol extract of leaf was scanned at 254nm showed 10 peaks (Fig. 14) of which 3 were major peaks at R_f value of 0.15, 0.70 and 0.79 whereas others were moderately smaller peaks.

Densitometric chromatogram of alcohol extracts of root, stem and leaf.

The desnsitometric chromatogram was used for comparison of root, stem and leaves extracts (Fig. 15). At R_f value of 0.13 and 0.42 peaks were present in root and stem extract, whereas at 0.54 showed both stem and leaves.

5.6 Cytotoxicity Studies

The cell lines when observed under inverted microscope for any significant morphological changes such as shrinking of the cells, membrane blebbing, ballooning, chromatin condensation, formation of apoptotic bodies were observed in predicting the apoptotic mechanism for cell death. Meanwhile, vacuolations of the cytoplasm and formation of double membrane vesicle containing organelles were also assessed for autophagic cell death. Hence, it is concluded from this preliminary cytotoxic study, the plant extracts of root, stem and leaves was not toxic to vero cell lines (30µg/ml) and this cytotoxicity assay was considered as one of the basic parameter (Plate 25).

5.7 Pharmacological studies

Acute toxicity

The animals received the drug upto the dose from 50mg/kg body weight to 2000mg/kg body weight. The continuous observation of the animals for behavioural, neurological and autonomic response viz. awareness, irritability, spontaneous activity, convulsion, righting reflex, corneal reflex, urinary, salvation

and pilo-excitation showed the normal behaviour. No mortality in animals was observed in the entire drug treated group indicating that the plant C. *retusa* was safe up to 2000mg/kg body weight. The observation revealed non-toxicity of the plant.

Analgesic activity

The analgesic effect of *C. retusa* on thermal stimulus by hot plate method was summarized in Table 17. All the doses of extracts of 50, 100 and 200mg/kg induced significant analgesic activity and the observations are compared to standard in respect to intensity of heat and duration. The results showed (Table 17) that the extracts of various parts of root, stem and leaves showed the maximum response as 59.11%: 76.91%; 77.9% for root, 52.26%: 58.35%; 66.33% for stem and 71.87%: 63.56%; 90.87% for leaves at 30 min and 60 min respectively.

Among the various extracts the maximum activity was observed in leaf extract at a dose of 200mg/kg followed by root and stem extract at a dose of 200mg/kg. The root extract the maximum response of 59.11% and 76.91% was observed at 60min for 50mg/kg and 100mg/kg whereas 77.9% for 30min at 200mg/kg. The stem extract the maximum response of 66.33%, 58.35% and 52.26% was observed at 30min for 200mg/kg, 100mg/kg and 50mg/kg respectively. The leaf extract the maximum response of 90.87%, 71.87% and 63.56% was observed at 60min for 200mg/kg, 50mg/kg and 100mg/kg..

The analgesic activity was slowly decreasing from 60min to 180min irrespective of all the doses of various extracts.

Since p values were greater than 0.05 there was no significant difference among groups in initial time. But in 30min, 60min, 90min, 120min and 180min there was significant difference among groups at 1% level since p value which was less than 0.01 with regard to analgesic response. Based on DMRT the control was significantly differs with standard and test groups at 5% level also. The standard was significantly differs with test groups in 30min duration except Group-IV and V. The standard has similar response to Group - IV and V at 90min.

Anti-inflammatory activity

The alcoholic extracts of various parts of *C. retusa* showed significant anti-inflammatory activity and the observations are compared to standard (Table 18). The 100mg/kg and 200mg/kg of various extracts of root, stem and leaves showed inhibition of paw edema percentage of 54.61%, 50.14% and 45.67% at 100mg/kg respectively at the end of 6hr compared to the standard with 67.6%.

Since p values less than 0.01 there is significant difference among groups at 1% level with regard to inhibition of paw edema at 1hr, 2hr, 3hr, 4hr and 6hr. Based on DMRT the control was significantly differs from standard group and test groups at 5% level also. The standard was significantly differs from all the test groups.

Wound healing activity

The alcoholic extracts of various parts of *C. retusa* showed significant wound healing activity and the observations were compared to standard in respect of number of days required to cure (Table 19). The 5% and 10% ointments were prepared from various extracts using petroleum jelly as base. The 10% and 5%

extract of root, stem and leaves showed the percentage of wound healing activity in the order of 99%, 95%, 94%, 90%, 85% and 82% on 13th day when compared to the standard nitrofurazone ointment the wound heals in the end of 11th day itself.

Since p values less than 0.01 there is significant difference among groups at 1% level with regard to wound healing activity at 3, 5, 7, 9, 11 and 13 day. Based on DMRT the control is significantly differ from standard group and test groups at 5% level also. The standard was significantly differ from all the test groups

Wound enclosure

The topical application of various extract ointments at different concentrations (5% and 10%) elicited a significant reduction in the wound area (Table 20). There was no significant differences among the groups upto 3rd day, but from day 5 there were significant differences among all groups, On day 7th, the wound contraction of standard and test were found to be significant in compared to control group. On day 11, wound was completely healed for standard group while for test group almost at complete healing stage. On day 13th, test group healed 99.10% and control group showed 61.57% healing. The time required for complete epitheliazation of the excision wound was an important parameter to assess the wound healing process. When compared with the control, the wound healing activity of extract ointments were found to be highly significant.

5.8 Antimicrobial studies:

The results of the antimicrobial activity of alcohol extract (Plate 26 – 28) of *C. retusa* were observed and tabulated (Table 21). The standard drug ampicillin

was used for comparison. A significant growth inhibition was shown by most of the organisms tested indicating the profound potency of the drug.

Among the 10 microorganisms were tested the alcoholic extract of the root showed maximum activity towards the organisms *Bacillus subtilis* (25mm), *Bacillus cereus* and *Escherichia coli* (22mm), *Staphylococcus aureus* and *Candida albicans* (20mm), *Klebsiella pneumonia* (16mm), *Salmonella typhimurium* (15mm) and *Proteus vulgaris* (14mm) for 100mg/ml concentration. The alcoholic extract of stem also showed significant activity against the microorganisms *Bacillus subtilis* (22mm), *Staphylococcus aureus* and *Candida albicans* (20mm), *Bacillus cereus* (18mm), *Klebsiella pneumonia* (13mm) and *Salmonella typhimurium* (10mm) at 100mg/ml concentrations.

The leaf extracts showed activity only against the microorganisms *Proteus vulgaris* (15mm), *Klebsiella pneumonia* and *Bacillus subtilis* (14mm), *Salmonella typhimurium* and *Enterobacter aerogenes* (12mm) and *Bacillus cereus* (8mm) at 10mg/ml concentrations.

No activity was observed against the microorganism *Pseudomonas aeroginosa* for all the alcoholic extracts such as root, stem and leaves. The organism is susceptible to all the extracts. The root extract does not show any activity towards the organism *Enterobacter aerogenes.* The stem extract does not show any activity towards the organism *Proteus vulgaris*, *Enterobacter aerogenes* and *Escherichia coli.* Whereas the leaf extract does not show activity against *Escherichia coli*, *Pseudomonas aergenes, Staphylococcus aureus* and *Candida albicans* for even the highest concentration (100mg/ml).

Minimum Inhibitory Concentration:

The result observed for the minimal inhibitory concentration is observed and tabulated in (Table 22). The MIC study revealed that the lowest concentration i.e., the MIC value for the alcoholic extract of root and stem extract showed 6.25mg/ml for all the organisms like *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Candida albicans*, *Proteus vulgaris* and *Escherichia coli* tested. The MIC value for leaf extract also showed 6.25mg/ml for the *Proteus vulgaris*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Salmonella typhimurium, Bacillus subtilis* and *Bacillus cereus* tested except *Bacillus cereus* which showed the MIC 25mg/ml under the experiment.