CHAPTER EIGHT

SUMMARY
T. cuneifolia is a branched perennial shrub; grows in well-drained calcium rich soil in drying ponds or along the banks of small streams in the Marathwada region of Maharashtra. Plants start sprouting on the onset of monsoon and flower during August-September. The plants dry up by March-April & sprout again in rainy season. The associated plants are Phylanthus spp., Tephrosia purpurea (Fabaceae); Celosia argentea (Amaranthaceae); Tridax procumbens, Tricholepis radicans (Asteraceae); Lavandula bipinnata (Lamiaceae); Corchorus acutangulus (Tilliaceae); Lepidogathus cristata (Acanthaceae) and Trichodesma spp (Boraginaceae). Mature plants show tap roots system with many lateral roots.

The seeds show seed coat dormancy, which can be removed by mechanical or chemical scarification. Non-scarified seeds did not show germination for one month. Chemical treatment of concentrated H\textsubscript{2}SO\textsubscript{4} for 3 minutes and mechanical scarification using needle gave 100% germination within two days under petriplate test.

Roots are cylindrical and tapering; 27-64 cm long and 0.4-4 cm in diameter with brown traces of lateral roots. Cork is coarsely fibrous; inner surface where exposed white and fibrous; stolens absent. Transverse section of the root shows thin periderm; cortex made up of oval cells, which surrounds the phloem; vessels large; secondary xylem cells; medullary rays parenchymatus with pith at the center.

The ethanol (70%) extract of the roots of Taverniera cuneifolia exhibited twelve chromatophores, while that of Glycyrrhiza glabra yielded eight, corresponding to the Rf values 0.31 to 0.90 and 0.07 to 0.90 respectively. The standard Glycyrrhizin showed a peak at the Rf 0.31 and characteristic spectra at 200 nm. Both the plant extracts showed corresponding peak i.e. in Taverniera cuneifolia extract at the Rf 0.33 while Glycyrrhiza glabra extract at the Rf value 0.32, and showed quenching like Glycyrrhizin, under UV
254nm. On treatment with Anisaldehyde: Sulfuric acid reagent, standard Glycyrrhizin developed pink-violet spots at Rf 0.31 and similar violet-pink spot was observed at Rf 0.32 and 0.33 in G. glabra and T. cuneifolia lanes under visible light. Under UV 254 nm yellow green fluorescent zones were observed in standard Glycyrrhizin as well as in corresponding spots in G. glabra and T. cuneifolia lanes. Spectra of these compounds showed perfect matching with that of standard Glycyrrhizin. It was found that at least four compounds could be similar or closely related ones, as their spectra and absorption maxima matched. Glycyrrhiza glabra extract contained 15.88 % of Glycyrrhizin and 13.20% in Taverniera cuneifolia.

Alkaloid fractions on TLC resolved into thirteen chromatophores in the T. cuneifolia extract and nine in G. glabra extract. Visible matching of the spectra revealed that at least two compounds (Rf 0.26 and 0.55) could be identical/closely related ones to the G. glabra compounds. λ max of these compounds was also similar. The percentage of these compounds was found to be more in T. cuneifolia extract.

Saponin extract of T. cuneifolia and G. glabra separated, exhibited ten chromatophores in both the extracts. Plates showed seven brown spots at the Rf range 0.1 to 0.7 under UV 254 nm in G. glabra extract and three in T. cuneifolia extract at the Rf range 0.1-0.5. In T. cuneifolia, chromatophores of Rf 0.31, 0.38 and 0.97 could be identical or closely related to the G. glabra compounds of Rf. 0.33, 0.38 and 0.97 as it showed perfect matching of spectra and exhibited similar absorption maxima. The percentage of these compounds was more in T. cuneifolia.

Flavonoid extract of T. cuneifolia and G. glabra exhibited six chromatophores in T. cuneifolia extract and eleven in the G. glabra extract. Visual comparison of the spectra of T. cuneifolia
compounds of Rf 0.60 and 0.98 and *G. glabra* compounds of Rf 0.57 and 0.99 showed overlapping, indicating that these compounds could be closely related.

Cardiac glycoside extracts were resolved into eight and twelve chromatophores in *T. cuneifolia* and *G. glabra* extracts respectively. Plates when observed under UV 366 nm, showed blue, dark-blue fluorescent spots at the Rf range 0.5 - 0.9 in *T. cuneifolia* lane and four blue green and blue fluorescent spots at the Rf 0.7-0.9 in *G. glabra*. The *T. cuneifolia* chromatphores of Rf 0.01, 0.43, 0.71 and 0.81 could be closely related to the compounds of *G. glabra* of Rf 0.02, 0.43, 0.71 and 0.78, since the visual comparison of the spectra of these compounds showed overlapping. \( \lambda \) max was also found to be similar for these compounds.

Coumarin extracts of *T. cuneifolia* and *G. glabra* were separated to twelve chromatophores in both the plant extracts. Plates when exposed to UV 366 nm, showed two blue and a blue green fluorescent spots in *T. cuneifolia* lane at 0.37-0.6 Rf range, while five slightly fluorescent spots were present in *G. glabra* lane at 0.25-0.9 Rf range. *T. cuneifolia* compounds of Rf 0.30, 0.35 and 0.71 could be closely related to the *G. glabra* compounds of Rf 0.29, 0.37 and 0.68 as their spectra and \( \lambda \) max showed similarity.

*G. glabra* root powder contains 6.9 % sugar while *T. cuneifolia* root powder has 2.2%.

Our results showed that *Tavemiera cuneifolia* exhibits concentration dependent, *in-vivo* Anti-inflammatory activity. Ethanol and chloroform extracts showed considerable activity, which was comparable to that of the standard Anti-inflammatory agent Na-Diclofenac (1.8 mg/200 gram body weight). Ethanol fraction was the most potent one while other fractions failed to inhibit carrageenan induced paw edema in rats.
Cytotoxicity of the crude extract of *Tavemiera cuneifolia* was studied using TTMT (Testing of Traditional Medicine for Toxicity & Anti-HIV activity) assay method. Different concentrations of extracts were tested i.e. double dilutions ranging from 500 µg/ml to 7.8-µg/ml etc. and subtoxic concentration of the extract was determined. It was found that the crude extract causes severe toxicity at the concentration more than 250 µg/ml, while it inhibited cell growth considerably at 62.5 µg/ml concentration. About 50 % growth inhibition of the cells was recorded at this concentration while 31.25 µg/ml concentration was found to be nontoxic to the cells, showing no effect on cell growth. Thus the concentration is called as subtoxic concentration.

Subtoxic concentration was used for the Anti-HIV activity testing. Results of the Anti-HIV activity of crude extracts tested by cell associated method is expressed as the CPE (Cytopathic Effect) and the presence of p24 antigen. Respective controls were also kept simultaneously and AZT (a known anti-retroviral drug) is used as a positive control. It was found that, at subtoxic concentration, extract doesn't have any effect on virus replication. At 62.5 µg/ml concentration it has shown p24 antigen level comparable to that of virus control. The cytopathic effect was also observed at this concentration. At the lower concentrations i.e. 15.06-µg/ml, cytopathic effect was found to be comparable to that of virus control, while level of p24 antigen was found to be more than virus control.

In cell free assay method, 62.5 µg/ml, 31.25 µg/ml & 15.6 µg/ml concentrations of crude *T.cuneifolia* root extracts were tested. It was found that at these concentrations, extract doesn't have any effect on virus growth as well as p24 antigen production. At 62.5 µg/ml concentration, it showed less cytopathic effect but comparable level of p24 antigen after 5 days incubation.
Ten-gram root powders of *Tavemiera cuneifolia* and *Glycyrrhiza glabra* extracted in seventy-percentage ethanol yielded 2.0 g and 2.12 g crude extracts respectively. Two hundred-gram root powder of *Tavemiera cuneifolia* extracted sequentially with different solvents yielded 1.391g in Petroleum Ether; 1.122g in Toluene; 0.6780g in Chloroform; 9.106 g in Ethanol and 4.882g in Distilled Water. The extractive values of these extracts was found to be 8, 7, 4, 53, and 28 percentage for Petroleum Ether, Toluene, Chloroform, Ethanol and Distilled Water respectively. Crude extracts of both *Tavemiera cuneifolia* and *Glycyrrhiza glabra* showed comparable activity against majority of the tested bacteria including gram positive and gram negative ones, except for few. In the case of *S.aureus* B, a gram-positive bacteria and *E. coli* CSH57, *E. coli* X239, *C. glutamicum*, the gram-negative members, activity of *Glycyrrhiza glabra* extract was found to be more. It caused 92, 68, 26 and 27 percentage of growth inhibition respectively at 8-mg/disc concentration. *Tavemiera cuneifolia* crude extract showed 61, 56, 26 and 0-percentage growth inhibition respectively at same concentration. In the case of *Glycyrrhiza glabra* extract *S. aureus* B was found to be most sensitive. The percentage of growth inhibition of various strains by *Glycyrrhiza glabra* crude extract was as follows: *S.aureus* 'A' (64%); *B. megaterium* (31%), *B. subtilis* (69%), *E. coli* CSH57 (68%), *E. coli* (X239) (26%), *P. aeruginosa* (0%) and *C. glutamicum* (27%). In the case of *Tavemiera cuneifolia* crude extract, *B. subtilis* was the most sensitive (69% inhibition) while *E. coli* (w), *E.coli* KL 16, *C. glutamicum* and *K. planticola* were not inhibited. The percentage of growth inhibition of various bacteria caused by *Tavemiera cuneifolia* extract was as follows. *B. megaterium* (35%), *S. aureus* A (64%), *S. aureus* 'B' (61%), *E.coli* CSH 57 (56%), *E. Coli* X239 (26%), *P. aeruginosa* 2488 (61%), *P. aeruginosa* (w) (61%) and *P. putida* (56%).
Differential antibacterial activity was observed in different extracts of *Taverniera cuneifolia*. Petroleum ether extract caused 25% growth inhibition at 8mg/disc concentration in the case of streptomycin resistant strain of *E.coli* CSH 57. Toluene extract was found effective against all the gram-positive bacteria tested. *B.subtilis* was the most sensitive one and showed 36% growth inhibition at 8 mg/disc concentration, while *S. aureus* ‘B’ strain was least sensitive showing only seven percent growth inhibition. In the case of *S.aureus A*, and *B.megaterium* only 9% growth inhibition was observed. Among the gram-negative bacteria, *E.coli* (w), *E.coli* (X239), *P. aeruginosa* (w) and *C. glutamicum* were found sensitive i.e. 8-mg/disc concentration caused 23, 13, 11 and 11% growth inhibition respectively.

Chloroform extract displayed inhibitory effect against all the bacterial strains except *E.coli* (X239) and *P. putida*. *B.subtilis* and *B. megaterium* were found to be the most sensitive ones showing 36% growth inhibition at 8 mg/disc concentration, while *S. aureus* ‘A,’ *S. aureus* ‘B’ were the least sensitive ones among the gram positive group showing 13% growth inhibition. Among the gram negative bacteria, *E. coli* (w) was found to be the most sensitive (46% inhibition) while *P. aeruginosa* (w) strain was least sensitive showing only 18% growth inhibition at 8 mg/disc concentration.

Ethanol extract exhibited activity against *B. subtilis* a gram positive member and *C. glutamicum* and *P. aeruginosa* (w) two gram negative member i.e. it caused 36 and 38% growth inhibition respectively, at 8 mg/disc concentration. *S.aureus A*, a gram positive member showed no effect up to 8 mg/disc concentration while other members of gram positive bacteria showed slight growth inhibition i.e. *S.aureus A*, (7%) and *B.megaterium* (5%).
In the case of Distilled water extract, 26% growth inhibition was observed in *S. aureus* A, the only member of the gram positive group, while 53% growth inhibition was observed in *E. coli* (w) strain, the only member of the gram negative group at 4 mg/disc concentration.

*Aspergilli* were found to exhibit varied sensitivity to ethanol extracts of both the plants. *G. glabra* extract showed a maximum of 60% growth inhibition of *A. oryzae*. The sensitivity of *Aspergillus* species was in the following order: *A. oryzae* (60%), *A. flavus* (51%), *A. niger* (46%), *A. parasiticus* (AP 456) (36%) & *A. parasiticus* (32%). Antifungal activity exhibited by *T. cuneifolia* extract was less compared to that of *G. glabra*. It caused only 30% inhibition of the growth of *A. oryzae*.

Methanol extract of *G. glabra* caused a maximum of 46% growth inhibition of *A. niger*. The sensitivity of other *Aspergillus* species was as per the following order: *A. oryzae* (39%), *A. flavus* (34%), *A. parasiticus* (AP 456) (26%) & *A. parasiticus* (26%). *T. cuneifolia* extract caused 40% growth inhibition of *A. flavus*, which was higher, compared to *G. glabra* (34%).

Distilled water extract of *G. glabra* showed 34% growth inhibition of *A. niger*. Sensitivity of other *Aspergillus* species to *G. glabra* extract was in the following order: *A. flavus* (29%), *A. oryzae* (19%), *A. parasiticus* (12%) & *A. parasiticus* (AP 456) (8%). Antifungal activity of distilled water extract of *T. cuneifolia* was near about the same as compared to that exhibited by *G. glabra*. It showed 32% inhibition of growth of *A. niger*. *Trichophyton* species showed no effect at 0.12, 0.16 and 0.20 mg/ml concentrations of the extracts of both the plants (data not shown) and thus higher concentration of ethanol extract (0.5, 1.0 and 2.0 mg/ml) was tested. Ethanol extract of *G. glabra* showed excellent activity and caused 72% growth inhibition at 2 mg/ml concentration while at the same concentration *T. cuneifolia* extract showed 60% growth inhibition. Interestingly the clinical isolates of *Candida albicans*...
tested were not inhibited at any of the concentrations tested. The species of Aspergillus used in this study exhibited differential sensitivity to the standard antifungal drug Amphotericin B.

Extracts of both the plants tested, exhibited concentration dependent antifungal activity against majority of the plant pathogenic fungi. All the plant pathogens tested were found sensitive to ethanol extracts of both the plants. G. glabra extract showed maximum antifungal activity at (0.20 mg/ml) concentration, against A. brassicicola, where it caused 47% growth inhibition, F.moniliformae and F. oxysporum f sp. vasinfectum were the least sensitive (32% growth inhibition). T. cuneifolia extract showed 32% growth inhibition of F. oxysporum f sp vasinfectum while F. oxysporum f sp vasinfectum (NCIM 1072) was least sensitive (4% growth inhibition).

Methanol extract of G.glabra caused 41 % growth inhibition of Fusarium oxysporum f sp vasinfectum, while F. moniliformae was least sensitive (16 %). The sensitivity of other organisms were as follows: A. brassicicola (30 %), M.phaseolina (30 %) and F. oxysporum f sp vasinfectum (NCIM 1072) (18 %). T.cuneifolia methanol extract was found to be more effective against M.phaseolina (43 % growth inhibition) but least sensitive against Fusarium oxysporum f sp vasinfectum (NCIM 1072) (8 %).

Distilled Water extract of G.glabra showed a maximum of 20 % growth inhibition of A. brassicicola while it had no effect on the growth of F.moniliformae (0%). T. cuneifolia extract caused 22% growth inhibition of A. brassicicola and Macrophomina phaseolina while on F.moniliformae it showed no effect (0% growth inhibition).

The plant pathogenic fungi tested were found to be sensitive to the extracts of both the plants. The efficacies of extracts were varied. Ethanol extract of G.glabra was most
effective while in the case of *T. cuneifolia* methanol extract was the best. On the other hand distilled water extract of both the plants were the least effective. Among the fungi tested, *A. brassicicola* and *M. phaseolina* were found to be more sensitive organisms than others.

Incubation of potato discs with *A. tumefaciens* for twelve days induced profuse number of galls (tumors) on potato discs. In the case of discs treated with plant extracts, it was found that the numbers of galls were reduced considerably. In the case of *T. cuneifolia* extract treated discs, average number of gall were found to be eleven while that of *G. glabra* extract treated discs were nine tumors. Both the plant extracts exhibited considerable anti tumor activity i.e. *T. cuneifolia* crude extract showed 50% inhibition of tumor formation which is comparable to that of *G. glabra* extract (59%) at 0.250 mg/ml concentration. Extracts of both the plants exhibited no effect on the *in vitro* growth of *A. tumefaciens* up to the 30mg/plate concentration.

Protective effects of both the plant extracts were studied using *Salmonella typhimurium* as a model. It was found that both the plant extracts exhibit concentration dependent protective effect against EMS induced toxicity. *G. glabra* extract caused about 97% survival of *Salmonella typhimurium* at the concentration of 8 mg/plate. 6-mg/plate concentration of *T. cuneifolia* extract supported 75% survival of *Salmonella typhimurium*.

In the present study, we have tested the efficacy of crude extracts of *G. glabra* and *T. cuneifolia* on serum induced germ tube induction in *Candida albicans*. It was found that *T. cuneifolia* extract inhibited serum induced germ tube formation in *C. albicans* considerably i.e. 85 %, while *G. glabra* extracts showed only 3% inhibition. Effect of both the plant extracts on the growth of *C. albicans* tested separately, showed that both of the plant extracts does not affect the *C. albicans* growth upto 10 mg/ml concentration.
It was found that both the plant extracts inhibited browning of apple juice considerably. Crude extracts of *G. glabra* and *T. cuneifolia* were effective. The chloroform and ethanol fraction of *T. cuneifolia* also inhibited browning of apple juice. In addition to this coumarins, 7-hydroxy coumarin and 7-methoxy coumarin present in *G. glabra* was also found effective.