5.0 SUMMARY AND CONCLUSION

The medicinal plants around the world contain many compounds with antibacterial activity. Researchers are much turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. The use of medicinal plants played a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antioxidant agents with significant activity against infective microorganisms.

The present research aims to report the pharmacological activities of leaf, root and stem of *Cissampelos pareira* and also reports the molecular characterization of the plant.

- The plant *C. pareira* L var. hirsuta for the proposed study was collected from different ecotypic regions such as Ambasamudram (AC1), Ooty (AC2), Peermade (AC3), Kumaracoil (AC4), Kozhikode (AC5), Yelahanka (AC6) and Ariyankuppam (AC7).

- The molecular characterization of seven samples of *C. pareira* performed by DNA barcoding using *matK* and *rbcL* gene sequencing. This method showed good amplification efficiency in species discrimination within the family of *Cissampelos* sp. ClustalW sequence alignment using BLAST search showed 842 nucleotides for *matK* and 697 for *rbcL* with 100% match except for AC6 *rbcL* which showed 1% variation.

- The phylogenetic analysis using maximum likelihood nearest neighbor interchange method revealed the intra and inter- species relationship that determines the detail evolutionary lineage of the family and the results clearly
revealed that \textit{matK} and \textit{rbcL} regions of the sample DNA, namely AC6 could distinguish among the plant species of the family. Thus phylogenetic study revealed the evolutionary history of \textit{C. pareira} among the genus and also with the closely related species.

- From the computational study on the translation expression profile of DNA barcoding sequences of seven samples of \textit{Cissampelos pareira} collected from different ecotypic locations reflects their unique molecular homology characters in their three letter codon region frequency except \textit{rbcL} DNA bar coding sequence of \textit{Cissampelos pareira} AC6 which showed slight variation of GAT, ATT, CAA when compared to the other bar DNA coding sequences. Thus the overall results of evolutionary divergence, phylogenetic and the molecular expression profile makes a solid conclusion on their common molecular functions, hence \textit{C. pareira} collected from Kumaracoil (AC4), Kanyakumari district, Tamilnadu was chosen for further studies.

- The phytochemical characterization of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of leaf, root and stem of \textit{C. pareira}, revealed that the various extracts of different parts of \textit{C. pareira} showed the presence of medicinally important bioactive compounds such as alkaloids, flavonoids, tannins, phenols, steroids, terpenoids, saponins, cardiac glycosides, aminoacids and volatile oil. Among the various extracts and different parts of \textit{C. pareira}, the ethyl acetate leaf extract showed the presence of more phytoconstituents.

- Thin layer chromatography was carried out in various extracts of different parts of \textit{C. pareira} such as leaf, root and stem with solvents such as hexane, chloroform, ethyl acetate, ethanol and aqueous using hexane : ethyl acetate : acetic acid (5 : 4 : 1) as mobile phase. TLC analysis of \textit{C. pareira} showed that the phytochemicals are present in varying quantities in different extracts which are evident from the number of spots with different R\textsubscript{f} value.

- The total phenolic and flavonoid content of different extracts of leaf of \textit{C. pareira}, calculated from the calibration curve (R\textsuperscript{2} = 0.997) ranged from 12.52 ±
1.09 to 47.76±1.09 mg GAE/g. and 36.19 ± 1.73 to 62.3±1.27 mg QE/g respectively.

The GC-MS analysis of ethyl acetate leaf extract of *C. pareira* revealed the presence of eight compounds. Two constituents namely 17-Pentatriacontene (C$_{35}$H$_{70}$), mw. 490 and 3,7,11,15-Tetramethyl 2 hexadecen-1-01 (phytol) C$_{20}$H$_{40}$O, mw.296 were found to be the major components at RT 52.65 and 31.95 with 28.30% and 24.90% peak area respectively. The other constituents identified are n-Hexadecanoic acid (RT 34.41), 1,2- Benzenedicarboxylic acid, disoocetyl ester (RT 44.33), Ergost-5-en-3-ol, acetate (RT 51.09), 1,30-Triacontanediol (RT51.47) and Stigmasta (RT 51.64).

In the present study, five different solvent extracts of leaf, root and stem of *C. pareira* were evaluated for exploration of their antibacterial activity against three pathogenic gram positive bacteria such as *E. faecalis*, *S. aureus*, *S. pyogenes* and three gram negative bacteria such as *V. cholerae*, *K. pneumoniae*, and *S. typhimurium* by agar-well diffusion assay. The ethyl acetate leaf extracts exhibited maximum growth inhibitory activity of 20.96±0.95 mm and 20.80± 0.72 mm against *K. pneumoniae* and *S. typhimurium*.

Further, the effectiveness of hexane, ethyl acetate and ethanol leaf extracts of *C. pareira* were determined by agar- disc diffusion method at three different concentrations such as 1.25, 2.5 and 5mg/disc against three pathogenic gram positive bacteria such as *E. faecalis*, *S. aureus*, *S. pyogenes* and three gram negative bacteria such as *V. cholerae*, *K. pneumoniae*, and *S. typhimurium*. The effectiveness of plant extracts were compared with standard antibiotic streptomycin. The results showed that at different concentrations, crude leaf extracts of *C. pareira* strongly inhibited the growth of all test bacterial strains. The ethyl acetate extract at a concentration of 5mg/disc exhibited maximum growth inhibitory activity of 15.50±0.50 mm and 15.47±0.21 mm against *S. pyogenes* and *S. typhimurium*.

The ethyl acetate extract has also shown very low MIC valueof 15.6mg/ml against *S. aureus*, *S. pyogenes* and *K. pneumoniae*. This showed that the extract has good
antibacterial activity. The further antimicrobial activity of plant extracts was strengthened by low MBC values obtained in the plant extracts against bacterial strains. However, the low MBC value was obtained in ethyl acetate extract of \textit{C. pareira} against \textit{K. pneumoniae} and \textit{S. aureus}. The MIC and MBC values were compared with the standard broad spectrum antibiotic streptomycin.

The antifungal activity of various solvent extracts of leaf, root and stem of \textit{C. pareira} have been studied on three fungal pathogens such as \textit{A. niger}, \textit{A. fumigates} and \textit{P. chrysogenum}. The antibiotic fluconazole (10µg/disc) was used as standard. The ethyl acetate leaf extract showed maximum inhibitory activity of 11.63±1.55 mm against \textit{A.fumigates} and 11.43±1.30 mm against \textit{A. niger} while the standard antibiotic fluconazole displayed pronounced antagonistic activity of 22.83±1.66 mm against \textit{A. fumigates}.

The ethyl acetate leaf extract of \textit{C. pareira} displayed fungistatic (MIC) and fungicidal (MFC) activity against pathogenic fungal strains was considerably higher. The MIC of ethyl acetate leaf extract of \textit{C. pareira} was 31.25 µg/ml against \textit{A. fumigates} indicating strong antifungal potential, while fluconazole, the standard antifungal drug showed activity against all the fungal strains tested. The lowest MFC (62.5 µg/ml) was observed for ethyl acetate leaf extract of \textit{C. pareira} against \textit{A. fumigates} which proved to possess the highest fungicidal activity.

In the current research, the \textit{in vitro} antioxidant activities of hexane, ethyl acetate and ethanol leaf extracts of \textit{C. pareira} at varying concentrations (20-100 µg/ml) using different assays such as DPPH (1, 1 - Diphenyl-2- picry l- hydrazil) free radical scavenging, hydrogen peroxide radical scavenging, nitric oxide scavenging, superoxide radical scavenging and reducing power assay and. The IC\textsubscript{50} values of the extracts and ascorbic acid were obtained using the linear regression equation. The lower value of IC\textsubscript{50} indicates a higher antioxidant activity. The ethyl acetate extract was found to be potent antioxidant activity for all the assays tested except superoxide radical scavenging assay. All the other extracts showed moderate activity when compared with the respective standard compound.
The total phenol contents are closely related with antioxidant activity. In the present study, the results showed significant good linear positive correlation ($R^2=1.000^{**}$) between the total phenol content and $H_2O_2$ free radical scavenging assay at $P< 0.05$ level and $P< 0.01$ level in a two tailed Pearson’s correlation. However, there was no significant correlation between total flavonoid content and antioxidant activity.

Based on the antimicrobial and the antioxidant activity of various leaf extracts of *C. pareira*, it was observed that the ethyl acetate extract exhibited significant antimicrobial and antioxidant activity. Hence ethyl acetate leaf extract was focused on for the isolation and characterization of bioactive compounds.

Bioactive compound was isolated using column chromatography and subjected to various spectroscopic analysis viz. NMR, FT-IR and mass spectrometry to elucidate the structure. On the basis of the spectroscopic data the isolated compound could be attributed to salicylic acid.

The *in vitro* anticancer and cytotoxicity assay of the isolated compound (salicylic acid) was carried out against human epithelial ovarian cancer cell lines. PA-1 and OWA-42 and on L929 fibroblast cells was used as normal cell line (control). The result inferred that the isolated compound strongly inhibited the growth of PA-1 and OWA-42 cancer cell lines in dose dependent manner and displayed strong cytotoxic activity with lower $IC_{50}$ value 10.22 µg/ ml on PA-1 cancer cells, when compared to the standard drug doxorubicin with $IC_{50}$ value 5.87µg/ ml and with lower $IC_{50}$ value 11.83µg/ ml on OWA-42 cells when compared to the standard drug doxorubicin with $IC_{50}$ value 5.32µg/ ml. The normal cells (L929 fibroblast) treated with isolated compound recorded moderately higher $IC_{50}$ value 35.29 µg/ ml, when compared to the standard drug doxorubicin with higher $IC_{50}$ value 61.55 µg/ ml. From the study, it was evident that a maximum concentration of isolated compound had showed 84.55±0.46% growth inhibition, thus indicated the non-toxic nature of the isolated compound towards L929 fibroblast cells.

In conclusion, the results of the present study unveiled that, the ethyl acetate leaf extract of *C. pareira* possess potent antimicrobial activity against the tested
pathogenic bacterial and fungal strains. Antioxidant assay results implied the dose dependent increase in free radical scavenging property. The studies on purification and structural elucidation of bioactive compound provide a benchmark data to utilize this plant for the development of new therapeutic drugs. The in vitro MTT cytotoxic assay results indicated that the compound salicylic acid isolated from the ethylacetate leaf extract showed promising anticancer activity against human ovarian cancer cell lines. On the basis of the results in the present study, it is concluded that the selected plant *C. pareira* is endowed with potential pharmacological activities such as antimicrobial, antioxidant and anticancer and the results of the present study scientifically justifies their use in the folklore remedies since ancient times. This scientific study also revealed the efficacy of the selected plant and it would definitely have wide scope in future. Hence, the leaf can be recommended therapeutically for the investigation of medicinal claims. These observations will stimulate further research in the field of phytochemistry and also in the clinical applications of phytochemical constituents of *C. pareira*. 