Chapter 7

Chapter – 7. Summary & Conclusion and Recommendations.

The aims of the study were to evaluate the leaves of *Pimenta dioica* (Family: Myrtaceae) pharmacognostically, screen the leaves phytochemically, extract essential oil from leaves collected in two prominent seasons of South Kanara, Karnataka state, Summer and monsoons, evaluate chemical composition of various essential oils of *Pimenta dioica* (Allspice) leaves and to identify compounds which may be responsible for the biological activities. In addition, the effect of seasons on the chemical composition of essential oil was studied as well as the influence of the essential oils on the biological activities. The aims of the study were achieved and the major findings are listed below.

Pharmacognostical evaluation through macroscopy and microscopical studies revealed absence of trichomes of any type on the epidermis. Preliminary Phytochemical screening of leaves of Allspice showed a prominence in phenol along with some tannins. The total phenol content of Allspice leaves was in the range of 712.848±10.32 to 848.42±10.324 mg of GAE/g. A maximum value was however seen in samples of leaves collected during March and July respectively.

There was a distinct seasonal variation in yield of essential oils distilled from leaves of Allspice during two prominent seasons of South Kanara District, Karnataka State, India, Summer and Monsoon in a range of 1.2 – 2.5 % w/v. Oil distilled from leaves collected in
March and April showed percentage yields of 2.5 and 2.0 respectively, while that from month of June showed 2.0% w/v.

Preliminary chromatographic profiling of various essential oil samples of Allspice leaves by TLC and HPTLC revealed the presence of a phenol, Eugenol which was prominent than other compounds. We can therefore corroborate our earlier finding of total phenolic content in allspice leaves.

GC-MS studies of various essential oil samples showed that there was a very distinct seasonal variation in composition of the oils. A maximum of 30 compounds were identified in the volatile oil from leaves collected in May while a maximum of 24 compounds were identified in June sample. Amongst the more prominent compounds identified in various samples were Eugenol, Chavicol and β-Pinene, while the other compounds were in trace amounts. While almost all the oil samples showed prominence of Eugenol and Chavicol, only one sample (March month) showed a combination of Eugenol and β-Pinene. However the content of Eugenol was maximum in April sample (53.5%) while those from July and August exhibited 64.32 % and 59.36 % respectively. A conclusion can be drawn from above findings that the best season to distil essential oil of Pimenta dioica leaves collected during months of April, July or August from South Kanara region of Karnataka state, India for isolation of eugenol.

The various essential oil samples showed variation in biological activities depending upon the month of collection of Allspice leaves as evident from scavenging of following free radicals: DPPH, •O₂, •OH,
NO• and ABTS•⁺ when compared to the positive control, eugenol. A
distinct activity was seen in oil samples of February, April, August,
September and October months.

All the essential oil samples of Allspice leaves exhibited
significant antimicrobial activity against various test micro-organisms
at very low concentrations of 20 or 40 µl/ml in comparison with the
standard drug, Ofloxacin (1 mg/ml). The presence of eugenol in
greater amounts than other constituents substantiates our results.

As the leaves of Pimenta dioica are indicated by traditional
healers as an anti-inflammatory agent in folk medicine, the oils were
evaluated for the same using croton oil induced ear edema.
Application of croton oil topically has been used to screen
antiinflammatory steroids and nonsteroidal agents and it also
promotes events of inflammatory processes such as cell infiltration
and proliferation, oedema, production of arachidonic acid and its
metabolites, cytokines and other pro-inflammatory mediators. The
essential oil samples extracted from leaves collected in different
seasons exhibited significant antiinflammatory activity as shown by
our results which are evident in summer sample of February and
monsoon sample of August. The essential oils also showed a decrease
in vascular permeability induced by croton oil.

Anthelmintic activity of essential oils of allspice leaves collected
in summer and monsoon were evaluated separately using Pheritima
posthuma (Earth worm), in comparison with a standard drug
Albendazole. The summer samples of February and April months and
monsoon samples of July and August exhibited significant activity against the earthworms.

All the above results on biological activity lead to a deduction that the main component Eugenol along with its other constituents may have contributed synergistically. Though eugenol formed the main component, the concentration differed in essential oils extracted from leaves collected during various months of two main seasons, summer and monsoon, prevailing in South Kanara District of Karnataka State. This leads to the conclusion that seasonal variation exists for the biological activities of various essential samples of *Pimenta dioica* leaf oil.

One possibility for future studies is to isolate the various components responsible for the above activities one by one and show the mechanism of action as to whether eugenol alone is responsible or it acts along with other components in synergy.