## Chapter-2. Literature Survey

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2.1 ESSENTIAL OILS

There are numerous factors which affect the composition and yield of essential oil. In some scenarios it becomes difficult to separate the factors from each other since majority of the factors are interdependant\(^1\). The factors that play a major role are seasonal variation, genotype and allelopathy, geographical location, plant part used or processing methods\(^{23,38-39}\).

Seasonal variation among plants is intricately interlinked with maturity variation, since the growth stage is largely affected by the season. Literature based upon seasonal change in phytochemicals is well documented\(^{40-42}\). Intensity of sunlight available during cultivation has also been reported as an important factor which controls the type of phytoconstituent available, month to month variation has also been reported for essential oil of *Santolina rosmarinifolia*\(^{43,44}\).

In *Artemisia afra*, oil yield was found to be more during the reproductive period\(^45\). Variation has also been reported in chemical profile of Mentha and Tagetes species collected from different geographical locations\(^{46,47}\). The differences in such chemical constituents may be due to the variability in the texture of soil available\(^2\). In some cases, climatic factors also have been reported to affect chemical profile of the essential oils\(^{48,49}\).
Investigations have also been carried out on variation in yield of essential oil based upon the difference in collection of the plant part in basil; it was found that oil obtained from shorter flower spikes yielded less amount of oil when compared to oil obtained from long flower spikes\textsuperscript{50}.

Studies carried out on Peppermint revealed that the monoterpenoids content of the oil varied when the herb was cut and dried partially\textsuperscript{51}. In Bay leaves however, no significant difference was found in the yield when the oil was isolated from fresh and dry sample respectively, but oil composition was found to vary\textsuperscript{52}.

Hydrodistillation is considered to be the ideal method for extraction of oil since damage to the constituents is minimal\textsuperscript{53}. Comparative analyses was done in \textit{Ocimum} species between hydro and steam distillation and it was revealed that both the methods produced the same phytoconstituents although the yield was found to be more in case of steam distillation\textsuperscript{54}. In studies done on Tea tree oil no significant difference was observed when oil was isolated employing both the methods\textsuperscript{55}.

Another factor found to play a major role in affecting the yield and composition of essential oils is the duration of the distillation process\textsuperscript{56,60}. The process may occur due to the penetrative power of boiling water which enters the oil gland and releases the oil. The oil components once released are vaporized immediately; oxygenated
compounds which are polar in nature are distilled first since they are water soluble than non oxygenated compounds\textsuperscript{56-60}.

Plenty of work has been carried out on the characterization of essential oils. Methods such as gas chromatography coupled with mass spectra, gas chromatography with flame ionization detection are the ideal methods of choice\textsuperscript{61-70}.

Essential oils are very versatile in their biological applications which range from activities such as anticancer, antioxidant, antimicrobial or antiinflammatory\textsuperscript{65}.

2.2 ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS

The essential oils of leaves and berries of \textit{Juniperus phoenicea} (leaves and berries), \textit{Lauris nobilis}, \textit{Thymus capitatus}, \textit{Eucalyptus gracilis} and \textit{Melaleuca armillaris} have been reported to show good antioxidant activity by the DPPH and ABTS assay. Furthermore the compounds responsible for antioxidant activity were reported to be p-cymene, β-elemene and β-myrcene\textsuperscript{71}.

Good antioxidant activity was shown via the DPPH, ABTS and FRAP assay methods by the essential oil from the leaves of Brazilian cherry tree (\textit{Eugenia uniflora} L.)\textsuperscript{72}.

Essential oils from stem, adult leaves, immature flower and fruits of \textit{Eucalyptus oleosa} were screened for free radical scavenging activity via the DPPH and ABTS assay, the mature adult leaves were found to show better free radical scavenging potential\textsuperscript{73}.
Four Eucalyptus species namely, salubris, salmonophloia, oleosa and gracilis growing in Southern Tunisia were screened for antioxidant activity by DPPH and ABTS assay methods. *E. oleosa* was reported to show better activity\(^7^4\).

The volatile oil collected by hydrodistillation from the aerial parts of *Eucalyptus camaldulensis* growing in different areas of Sardinia were found to have good antioxidant activity when assayed by DPPH method\(^7^5\).

Comparative study of essential oils from the decaying as well as fresh leaves of *Eucalyptus tereticornis* was done by scavenging studies of DPPH, OH radical and superoxide anion. Both the oils were found to have good antioxidant activity\(^7^6\).

### 2.3 ANTIMICROBIAL ACTIVITY

A large number of methods are available for evaluating the antimicrobial activity of essential oils\(^6^9,7^7,7^8\). Methods such as Kirby Bauer disc diffusion method, Agar well diffusion method, determination of MIC are the most preferred method for the measurement of antimicrobial activity of phytoconstituents\(^7^9-8^9\).

Essential oil from two chemotypes of *Pimenta pseudocaryophyllus* from Brazil were studied for their antimicrobial activity by broth dilution method. Essential oil from citral chemotype showed good activity against gram positive bacteria\(^9^0\).

The antimicrobial activity of the essential oils from the leaves of *Eugenia uniflora* and *Plinia trunciflora* were studied against yeast and
bacteria, where good antibacterial activity was observed against *Streptococcus equi* and *Staphylococcus epidermidis*, and also against yeast such as *Candida* species and *Cryptococcus*\textsuperscript{91}.

The *in vitro* antifungal activity of *Eucalyptus citriodora* was investigated and found to show effective inhibition against *Aspergillus* (flavus, fumigates, nidulans and terreus species) by agar well diffusion methods\textsuperscript{92}.

The essential oils of *Cinnamomum verum*, clove, lemongrass (citratus and martini species) along with their major phytoconstituents cinnamaldehyde, eugenol, citral and geraniol were evaluated for their antifungal activity against *Aspergillus fumigatus* and *Trichophyton rubrum*. Cinnamaldehyde was found to have the maximum antifungal activity; the least activity was exhibited by *Cymbopogon martini*\textsuperscript{93}.

Seasonal variation of essential oil from three species of *Leptospermum* (flavescens, petersonii and madidum) was studied and antibacterial activity was evaluated. The activity was found to vary depending upon the season of collection and oils were found to be more active against Gram positive bacteria\textsuperscript{94}.

Using four methods (agar well, filter paper disc, broth dilution by turbidimetric and rezazurin reduction assay) the antibacterial activity of essential oils of *Lavandulae aetherleum*, *Limonis aetherleum* and *Melaleuca aetherleum* was determined against *S.aureus* and *E.coli*. The results portrayed that broth dilution method showed better activity than
agar diffusion method. The presence of resazurin enhanced the activity further\textsuperscript{95}.

The antibacterial activity of essential oils of 	extit{Eugenia} species (\textit{brasiliensis}, \textit{beaurepaireana} and \textit{umbelliflora}) was studied against \textit{S.aureus}, \textit{P.aeruginosa} and \textit{E.coli}. Exceptional activity was portrayed by \textit{Eugenia umbelliflora} and \textit{E. brasiliensis} against \textit{S.aureus}\textsuperscript{96}.

Antifungal activity of tea tree oil was determined against Candida, \textit{Debaryomyces hansenii} and \textit{Schizosaccharomyces pombe} as well as dermatophytes Microsporum and Tricophyton species by broth dilution test and contact test\textsuperscript{96}.

\textbf{2.4 ANTHELMINTIC ACTIVITY}

The WHO has suggested usage of traditional medicines against parasitic diseases in its Tropical diseases control programme\textsuperscript{97}. One of the major uses of anthelmintic drugs is in veterinary medicine\textsuperscript{98}.

Presently anthelmintic studies have been conducted on the earth worm \textit{Pheritima posthuma}, using plant extract or essential oils\textsuperscript{97,98}. In all the studies comparison was done using synthetic anthelmintic agents.

The oil of \textit{Piper betel} (Piperaceae) has shown good anthelmintic activity \textit{in vitro} when used against \textit{Pheritima posthuma} as the test worm\textsuperscript{100}.

Anthelmintic studies of the essential oils of \textit{Cymbopogon nardus} (Graminaceae), \textit{Zanthoxylum alatum} (Rutaceae) and \textit{C. citratus}
(Graminaceae) have revealed that the oil of *C. nardus* has very good effect against earthworms, while the oils of *Z. alatum* and *C. citratus* have moderate activity. Studies done on Cymbopogon species reveal that the oil obtained from nardus species shows good activity\(^{101}\).

In a study carried out on the rhizome oil from *Hedychium coronarium* and *H. spicatum* (Zingiberaceae) better activity was seen when compared to piperazine phosphate against earthworm and tapeworm as the test worms\(^{101}\).

Similarly, better anthelmintic activity was also exhibited by essential oil obtained from *Boswellia serrata* (Burseraceae) and *Cinnamomum tamala* (Lauraceae) when tested *in vitro* against tapeworm using piperazine citrate as the standard\(^{100}\).

Essential oil obtained from the seed of *Inula racemosa* (Compositae), *Gardenia lucida* (Rubiaceae), *Randia dumetorum* (Rubiaceae), *Psitacia integrrima* (Anacardiaceae), *Litsea chinensis* (Lauraceae) and *Cyperus rotendus* (Cyperaceae), have shown good anthelmintic properties against earthworm and tapeworm\(^{101}\).

Good *in vitro* anthelmintic activity against *H. contortus* was shown by eugenol and essential oil of *Ocimum gratissimum*\(^{101}\).

### 2.5 ANTI-INFLAMMATORY ACTIVITY

The data reported by some authors suggest that essential oil extracts of three species of *Eucalyptus*, viz., *Eucalyptus citriodora,*
*E. tereticornis* and *E. globulus* belonging to family Myrtaceae, possess both central and peripheral analgesic effects along with neutrophil-dependent as well as independent anti-inflammatory activities\(^\text{102}\).

In another study, the fruit essential oil of *Cinnamomum insularimontanum* was shown to decrease the expression of IKK, iNOS, and nuclear NF-κB along with an increase in IκBα in a dose dependent manner\(^\text{102}\).

Anti-inflammatory activity of volatile oil of *Myrtus communis* was determined by croton oil induced ear oedema, myeloperoxidase activity in mice. Significant reduction was seen. The oil also showed inhibition in cotton pellet granuloma and serum TNF-α and IL-6\(^\text{103}\).

*In vitro* and *In vivo* anti-inflammatory activity of *Myriciaria tenella* and *Calycorectes sellowianus* revealed significant reduction in neutrophil chemotaxis. When evaluated by carrageenan induced paw edema, *Myrciaria tenella* oil showed results similar to indomethacin\(^\text{102}\).

### 2.6 Plant Description

The plant in question not being of Indian origin, has not been mentioned in the standard texts like Kirtikar and Basu, Nadkarni, Dymock and Watt. Only the Wealth of India is the oldest book available, which has literature about *Pimenta* or Allspice.
Common or Other Local, Multi-lingual Names

Allspice, Jamaica Pepper, Pimento, Malagueta, Piment Jamaique, Pimenta, Pimienta Gorda, Dulce, English Spice, Tabasca, Toda Especial, Toute Epice103.

Synonym

Synonyms for *Pimenta dioica* (L.) Merrill are *Eugenia pimenta* DC.; *Myrtus dioica* L.; *Myrtus pimenta* L.; *Pimenta officinalis* Lindley, Allspice tree, Jamaican pepper tree, Pimento tree104,105.

Botanical Description

Botanical name of allspice is *Pimenta dioica* (L.) Merr., and belongs to Myrtaceae. It possesses an aromatic taste and flavor resembling a mixture of cinnamon, cloves and nutmeg, hence the name allspice106,107.

Distribution/Ecology

*Pimenta dioica* is native to the Caribbean region, especially Jamaica and Cuba; and trees grow naturally at a mean average temperature of 18°C - 24°C106.

Parts Used/Key Uses

The essential oils of *P. dioica* leaves and fruits are utilized in food industry – mainly meat and tanning industries– as well as in perfumery compositions and cosmetic products. The therapeutic properties of the essential allspice oils are anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, muscle relaxant, rubefacient, acaricidal, carminative, stimulant and tonic108.
**Chemistry**

Allspice berries owe their characteristic odour to the presence of an essential oil (3.3-4.5%), concentrated mainly in the pericarp. Quercitannic acid (over 8%) responsible for their astringency, a soft resin with a burning taste, fixed oil (5.8%), proteins (5.8%), crude starch (20%) and traces of an alkaloid. The oil contains eugenol (65-85%) as the principal constituent, together with eugenol methyl ether (9.6%), phellandrene, cineole, caryophyllene and a terpene alcohol.

The dried leaves on steam distillation yield 0.7-2.9 % of an essential oil, which like berry oil contains, eugenol as its main component, but with inferior odour and flavour. The leaves also contain tannin\textsuperscript{109}.

According to Trease and Evans, Pimento fruits yield about 3-4.5% of volatile oil which shows a phenol content of 65-80%. The oil is also shown to contain cineole, (-)-phellandrene and caryopyllene amongst some 44 compounds identified\textsuperscript{109}.

Two oils produced by steam distillation of the leaves of *P.dioica* of Jamaican origin were examined by GC/MS, by Tucker and Maciarello, which were found to be rich in eugenol (66.38-79.24%)*\textsuperscript{110}.

The leaf oil of *P.dioica* of Cuban origin has been analysed by a combination of capillary gas chromatography and GC/MS and main constituent was found to be eugenol - 54.26%\textsuperscript{111}.
Vincenzi M De, et Al., has shown that methyl eugenol is a natural constituent of a number of aromatic plants and their essential oil fractions. In Pimento (*Pimenta officinalis*), the content of methyl eugenol was found to be about 5.0 - 8.8 %\(^{112}\).

**Antimicrobial activity**

Ground spices were examined *in vitro*, by Bava and Vanetti, for antibacterial effects on the growth of *Yersinia enterocolitica*. An agar-diffusion technique indicated that among the spices evaluated, the eugenol-containing cloves and allspice (*P.dioica*) had the strongest inhibitory activity\(^ {113}\).

**Chemical Constituents**

**Phenylpropanoids**

A phenylpropanoid, *threo*-3-chloro-1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol, has been isolated from the berries of *Pimenta dioica* together with five known compounds, 4-hydroxy-3-methoxycinnamaldehyde, eugenol, 3,4-dimethoxycinnamaldehyde, vanillin and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol\(^ {112}\).

**Tannins**

Two C-glycosidic tannins namely vascalaginone and grandininol were also identified from Pimento berries\(^ {110}\).