5 - Summary

This thesis comprises a review of literature which surveys information about the essential oils and non volatile antioxidant compounds of aromatic plants. It also gives a brief essay about different methods of extractions, and the factors effecting their chemical compositions. It comprises also some data about the reaction mechanism of antioxidants. Correlations between the essential oils and non volatile compounds and their antioxidant activities were reported.

The experimental part includes information about the materials utilised in the studies, the different experimental procedures and techniques adopted which include the extraction of essential oils of *E. camaldulensis var. brevirostris* leaves by HD and SFE. The non volatile antioxidant compounds of the leaves were extracted using Soxhlet extraction and Super critical fluid extraction (SFE) with and without modifier addition. The antioxidant activities were determined by the thiocyanate method. The essential oils and non volatile compounds were analysed by GLC-MS, HPLC, HPLC-MS, IR, UV, HNMR and MS.

The yield of essential oils of *E. camaldulensis var. brevirostris* leaves extracted by SFE (1.1 % fresh weight) is higher than that by HD (0.6 %). The HD and SFE extracts were subjected to GLC-MS analysis. According to GLC-MS analysis their chemical compositions differ qualitatively and quantitatively. Ninety different components were separated and most of them were identified by GLC-MS. The SFE extract showed 81 GLC signals, the HD oil showed only 66 signals. In both extracts (HD and SFE) the main constituents were found to be β-phellandrene (8.94 % and 4.09 %), p-cymene (24.01 % and 10.61 %), cryptone (12.71 % and 9.82 %) and spathulenol (14.43 % and 13.14 % respectively). The yield of monoterpene hydrocarbons in HD oil (0.288g/100g fresh leaves) was slightly higher compared to that in the SFE extract (0.242g/100g fresh leaves). The SFE extract possessed higher concentrations of

the sesquiterpene, light oxygenated and heavy oxygenated compounds. p-Cymen-7-ol was found in considerable amounts (HD: 0.58 %, SFE: 2.25 %) in the group of light oxygenated compounds. To our knowledge the occurrence of p-cymen-7-ol in E. species is not yet reported.

Both extracts show antioxidant activity which is attributed to their content of the phenolic compounds, mainly thymol and p-cymen-7-ol. p-Cymen-7-ol was found to exhibit an antioxidant activity very similar to that of BHA. The activity of the SFE is slightly higher than that of the HD extracts. The higher activity of the SFE extract corresponds to the fact that it contains higher concentration of p-cymen-7-ol (2.25 % versus 0.58 %).

The non volatile antioxidant compounds were extracted by different solvents (hexane, methylene chloride and ethanol) using different methods of extraction (solvent digestion, Soxhlet and CO_2-SFE modified with 10 % & 15 % ethanol). It was found that the yield in Soxhlet extraction is higher than that of the solvent (digestion) extract.

The antioxidant activities of all extracts were evaluated by the thiocyanate method. It was found that all the investigated extracts have antioxidant activities compared with BHA and BHT. The antioxidant activities of ethanol digestion extract and SFE extract (modified with 15 % ethanol) displayed a significant antioxidant activity. Therefore the ethanol (digestion) extract and SFE extract (modified with 15 % ethanol) of the leaves were separated by analytical HPLC. The main compounds were isolated by semi-preparative HPLC and identified by different methods (HPLC, HPLC-MS, UV, IR, HNMR and MS).

The main compounds in the ethanol (digestion) extract were identified as gallic acid and ellagic acid by their HPLC retention time as well as by their UV- and GC-MS spectra. The higher inhibitory effect upon linoleic acid after 12 days of the ethanol (digestion) extract (81.7 %) corresponds to the fact that it contains a higher concentration of ellagic acid (22.4 %) and gallic acid

(4.6 %). It was found that mixtures with the same proportion of gallic acid and ellagic acid have a good antioxidant activity (inhibitory effect 65.4 %), comparable with synthetic antioxidants.

The two main compounds in the SFE extract (modified with 15 % ethanol) were analysed by their IR, UV, ^{1}HNMR, HPLC-MS and high resolution MS spectra. The first was identified as 5-hydroxy-7,4′-dimethoxy flavone, the second identified as 5-hydroxy-7,4′-dimethoxy-8-methyl flavone. Both identifications have to be regarded as tentatively because of the impossibility to record ^{13}C-NMR spectra. The amount of the HPLC collected material was not enough. Uptill now the first compound was not reported to occur in any of the *Eucalyptus* species. But it is known that it occurs in other plant leaves, for example in rosemary. According to a computer search of the Chemical Abstract, 5-Hydroxy-7,4′-dimethoxy-8-methylflavone compound was not yet described as naturally occurring.

Both compounds have more or less the same antioxidant activity as BHA. The antioxidant activity of the two compounds is obviously due to the phenolic moiety of the structure.

The acute lethal (LD_{100}) and median lethal (LD_{50}) doses of the HD oil of *E. camaldulensis var. brevirostris* were respectively found to be 3.00 and 2.05 g / kg body weight in rats and accordingly the toxicity of the HD oil is low. It is comparable to that of the HD oil of *E. camaldulensis* leaves (LD_{50} 2-5 g / kg body weight, reported by the Research Institute for Fragrance Materials and regarded as safe)(UK).

p-Cymene [the major identified component in the HD oil (24.01 %)] was reported to be non toxic.

For evaluation of the toxicity of the lyophilised ethanol (digestion) extract the same test was performed. Mortality rate of rats fed with up to 9 g / kg

ndicates a very low lethality. That may be due to its high solubility in the biological fluids.