

## GENETIC VARIABILITY TO ESSENTIAL OIL CONTENTS AND COMPOSITION IN FIVE SPECIES OF *EUCALYPTUS*

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### Abstract

Essential oils from the leaves of *Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Eucalyptus globulus*, *Eucalyptus crebra* and *Eucalyptus tereticornis* were extracted by hydro-distillation. Maximum oil (1.47 %) was found in *E. crebra* and minimum (0.58 %) in *E. tereticornis*. Specific gravity of the oils ranged between 0.86 to 0.91, refractive index 1.42 to 1.44 and optical rotation – 12.3 to 3.63 at 25 °C. Extracted oils were resolved and identified by GC/FID on Carbowex-20 M packed glass column. Maximum components (21) were detected in *E. crebra* and minimum (15) in *E. citriodora* and *E. globulus* oils, whereas in the oils of *E. camaldulensis* and *E. tereticornis* 16 and 19 components were resolved respectively. In *E. camaldulensis*, *E. globulus* and *E. tereticornis* oils five components ( $\alpha$ -pinene,  $\Delta^3$ -carene,  $\beta$ -phellandrene, 1,8-cineole and p-cymene), in *E. citriodora* oil four components ( $\alpha$ -pinene, 1,8-cineole, citronellal and citronellol) and in *E. crebra* oil three components ( $\alpha$ -pinene, limonene and 1,8-cineole) were identified. The 1,8-cineole, found in the oils of different species, was 72.17 % in *E. crebra*, 53.22 % in *E. camaldulensis*, 26.57 % in *E. globulus* and 50.51 % in *E. tereticornis*. Lemon scented compounds citronellal (74.65 %) and citronellol (6.13 %) were found in *E. citriodora* oil which were absent in the other *E.* species. Chemical composition of oils from all the species varied significantly which may be due to the differences in their genetic make up.

### Introduction

*Eucalyptus* is a tall evergreen tree native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in the Mediterranean and sub-tropic regions including Australia, China, India, Portugal, Spain, Egypt, Algeria, the southern United States, and South America (Leung & Foster 1996). It is known as *Mallee* in Australia and is used in traditional Australian Aboriginal medicines (Bown, 1995). Though native to Australia, its therapeutic uses have been introduced and integrated into traditional medicine systems, including Chinese, Indian Ayurvedic and Greeco-European. Its volatile oil is obtained by steam distillation from the fresh leaves or the fresh terminal branches. *Eucalyptus* oil contains mainly 1,8-cineole (eucalyptol), triterpenes (ursolic acid derivatives), monoterpenes ( $\alpha$ - and  $\beta$ -pinene, D-limonene, p-cymene), sesquiterpenes (aromadendrene, alloaromadendrene, globulol), aldehydes (myrtenal) and ketones (carvone) (Newall *et al.*, 1996).

*Eucalyptus* oil is used for industrial and medicinal purposes around the world for food flavouring, in confectionery, as detergent in aerosol, soap, chest rub, nasal and cough drops, inhalant, hand cleaner, perfume and as a solvent (Wood *et al.*, 1994). *Eucalyptus* oil is official in the Indian pharmacopoeia as a counter-irritant and mild expectorant (Anonymous, 1996), and also in the Chinese pharmacopoeia as a skin irritant used in neural pain (Tu, 1992). The present *Ayurvedic Pharmacopoeia* indicates its topical application for headache due to cold (Karnick, 1994). In both the United States

and Germany. eucalyptus oil is used extensively as an expectorant component of cough and cold compounds in various oral dosage forms, including lozenges and syrups, and as an inhalant in vapour baths. It is also used externally for percutaneous absorption in dosage forms, including the essential oils, liniments, and ointments (Leung & Foster, 1996; Duke, 1997). Both eucalyptus oil and eucalyptol have demonstrated strong antibacterial action against several strains of *Streptococcus*, as well as the expectorant activity (Leung & Foster, 1996). Oral ingestion of eucalyptus oil can be toxic unless diluted appropriately (Leung & Foster, 1996; Newall *et al.*, 1996). Eucalyptus oil is ingested orally to treat catarrh, used as an inhalant, and applied topically as a rubefacient (Newall *et al.*, 1996).

Introduction of *Eucalyptus* in Pakistan is not new, but the interest shown in the genus during the last two decades has been considerable and a lot of efforts towards its propagation have been made. Qadri (1966) recommended a large scale plantation of *Eucalyptus camaldulensis*, *E. citriodora*, *E. melanophloia*, *E. microtheca*, *E. robusta* and *E. tereticornis*. Worldwide production of *Eucalyptus* oil is about 3,000 tonnes a year. Major producers are China, Spain, Portugal, South Africa and Chile (Wood *et al.*, 1994). More than half of the world production of *Eucalyptus* oil comes from China (Hobart, 1995). Pakistan imported 8.95 and 8.16 tonnes of *Eucalyptus* oil in the years 1999-2000 and 2000-2001 by spending Rs. 1.6 and 1.97 millions, respectively (Anon., 2001). There is a vast introduction of *Eucalyptus* species in Pakistan and with a worldwide demand of *Eucalyptus* oil. Therefore, efforts were made to evaluate five different *Eucalyptus* species on the bases of their essential oil contents and compositions.

## Materials and Methods

**Collection of leaf samples:** Fresh leaves of *Eucalyptus camaldulensis*, *E. citriodora*, *E. globulus*, *E. crebra* and *E. tereticornis* were collected from Punjab Forestry Research Institute (PFRI), Faisalabad during the month of September 2002.

**Extraction of oil:** Extraction of oil from *Eucalyptus* leaves was carried out by hydro-distillation at atmospheric pressure. Fresh and clean leaves (about 700 g) from each species were taken in 3L round bottom flask. After adding 1.5 L water, the flask was connected with the distillation assembly and heated on a mental (electro thermal). After half an hour, boiling started; generated steam ruptured the cell walls of the leaves and released the oils they contained. Both steam and evolved oil were condensed and collected in the collector tube, attached with the condenser. Due to low density the condensed oil stayed at top of the condensed water, which was re-circulated in the distillation flask. This process was continued for three hours for maximum oil recovery. The oil was allowed to stand for sufficient time, to be clear, and then it was collected carefully after draining out condensed water.

**Recovery of absolute oil:** The oil extracted from all the *Eucalyptus* species under test was containing fraction of water. This was removed by adding small amount of anhydrous sodium sulphate and absolute oil obtained.

**Determination of physical constants of the extracted oils:** Extracted oil from each *Eucalyptus* species was subjected to the determination of specific gravity, refractive index and optical rotation by the methods described by Furnis *et al.* (1978).

**Table 1. Oil yield from each *Eucalyptus* species by hydro-distillation.**

| No. | Species                         | Oil % age |
|-----|---------------------------------|-----------|
| 1.  | <i>Eucalyptus camaldulensis</i> | 1.37      |
| 2.  | <i>Eucalyptus globulus</i>      | 1.10      |
| 3.  | <i>Eucalyptus crebra</i>        | 1.47      |
| 4.  | <i>Eucalyptus tereticornis</i>  | 0.58      |
| 5.  | <i>Eucalyptus citriodora</i>    | 1.17      |

**Table 2. Specific gravity of the oils obtained from each *Eucalyptus* species by hydro-distillation.**

| No. | Species                         | Specific gravity |
|-----|---------------------------------|------------------|
| 1.  | <i>Eucalyptus camaldulensis</i> | 0.90             |
| 2.  | <i>Eucalyptus globules</i>      | 0.88             |
| 3.  | <i>Eucalyptus crebra</i>        | 0.91             |
| 4.  | <i>Eucalyptus tereticornis</i>  | 0.89             |
| 5.  | <i>Eucalyptus citriodora</i>    | 0.86             |

Each determination in Tables 1-2 is mean of three replicates.

**Chemical composition of the extracted oils:** Determination of the chemical composition of the extracted *Eucalyptus* oil from each species was carried out by Perkin-Elmer gas chromatograph equipped with flame ionization detector (FID) and Shimadzu C-R4A chromatopac. The column used was 2 m x 2 mm i.d. glass, packed with 10 % carbowex 20 M on chromosorb WAW (80-100 mesh). Flow rates, nitrogen, 25 mL/min., hydrogen, 40 mL/min., air 500 mL/min. Injector temperature was 150 °C, detector temperature 200 °C. Column temperature programming: 80 °C for 1 min., 80-160 °C at 16 °C/min. and isothermal at 160 °C for 8 min.

## Results and Discussion

**Percentage oil yield:** The percentage oil obtained from the leaf samples of *Eucalyptus camaldulensis* was 1.37 %, *E. globulus* 1.10 %, *E. crebra* 1.47 %, *E. tereticornis* 0.58 % and *E. citriodora* was 1.17 % (Table 1). Maximum oil was obtained from *E. crebra* (1.47 %) and minimum from *E. tereticornis* (0.58 %). For *E. camaldulensis* our results are a little higher than those of Ernest (1954), who determined 1.14 % oil in the same species. In case of *E. globulus* our findings resembled with those of Li & Madden (1995) and Peter (2000), they reported it 1.25 and 0.75-1.25 % respectively. Peter (2000) obtained 0.5-2 % oil from *E. citriodora*, our recovery of oil from the same species (1.17 %) lies in between their limits. The percentage data for remaining species was not available in the literature to be compared with.

**Specific gravity:** The values of specific gravity for different *Eucalyptus* oil samples (Table 2) were 0.90, 0.88, 0.91, 0.89 and 0.86 for *E. camaldulensis*, *E. globulus*, *E. crebra*, *E. tereticornis* and *E. citriodora* respectively. For the oil from *E. camaldulensis* our results of specific gravity were close to those of Nicolle (1981) who reported 0.91 at 30 °C. For *E. citriodora* oil, Anonymous (2002) reported 0.86 specific gravity which resembled exactly with our findings. In case of *E. globulus* oil the sp. gr. reported by

Perry (1992) was 0.91, which was slightly higher than our results in the same species. For the remaining two varieties sp. gr. data were not available in the literature for comparison.

**Refractive index:** The values of refractive index (Table 3) of the *Eucalyptus* oil samples measured with the help of Abb's refractometer at 25 °C were 1.44, 1.44, 1.43, 1.44 and 1.42 for *E. camaldulensis*, *E. globulus*, *E. crebra*, *E. tereticornis* and *E. citriodora* respectively. Our refractive index results of *E. camaldulensis* oil differed with the findings of Semblat *et al.* (1979) and Nicolle (1981), they reported it 1.58, 1.48-1.49 and 1.349 respectively. This variation may be due to climatic and/or soil differences. For both the *E. globulus* and *E. citriodora* oils Anonymous (2002) reported refractive index 1.45, which nearly resembled with our findings. Refractive index for the oils of remaining two species was not found in the literature.

**Optical rotation:** The optical rotation values of the *Eucalyptus* oils extracted from different species were observed by Lippich polarimeter at 25 °C. The results were found -2.3°, -5.1°, +3.63°, -10.64° and +2.3° for *E. camaldulensis*, *E. globulus*, *E. crebra*, *E. tereticornis* and *E. citriodora* respectively. Our results for optical rotation of the oil from *E. camaldulensis* were close to the findings of Semblat *et al.* (1979) who reported it as -12.1. The optical rotation reported by Anonymous (2002) for *E. citriodora* oil ranged between -5.0 - +2.0 and that by Perry (1992) for *E. globulus* oil ranged between -10.0 - +10.0. Our findings lie within the mentioned limits for the oils from both the species. Whereas the literature was found silent for the optical rotation of the oils of *E. crebra* and *E. tereticornis*.

**Chemical composition of *Eucalyptus camaldulensis* oil:** Gas chromatographic analysis (Fig. 1) of *E. camaldulensis* oil indicated the presence of 16 components, of which only 5 were identified by matching the retention time of the available standards (Tables 5 & 6). The components found present may be  $\alpha$ -pinene (6.0 %),  $^3\Delta$  carene (1.8 %),  $\beta$ -phellandrene (7.94 %), 1,8-cineole (53.22 %) and p-cymene (21.59 %) at retention times 0.65, 0.94, 1.14, 1.47 and 1.76 minutes respectively. For  $\alpha$ -pinene (peak No.3, fig. 1) and  $^3\Delta$  carene (peak No.4, fig. 1) our findings were much higher than reported by Li & Madden (1995) and Bignell *et al.* (1997), they reported  $\alpha$ -pinene 0.6 % and  $^3\Delta$  carene 0.25 % respectively in the oil of *E. camaldulensis*. But in case of  $\beta$ -phellandrene (peak No.5, fig. 1) our findings were close to those of Fedel *et al.* (1999), who found it 8.94 %. 1,8- cineole (peak No.6, fig. 1) was the major compound found present in its oil, its concentration was 53.22 %. Our findings of 1,8-cineole lie between the range (34.07-68.25 %) found by Shieh (1996) in the oil of same species. The compound detected at retention time 1.76 min. (Table 6) may be p-cymene (peak No.7, Fig. 1) as its retention time resembled with the retention time of p-cymene standard (Table 5). The concentration of p-cymene (21.59 %) found was much higher than the findings of Li *et al.* (1994) and Bignell *et al.* (1997), they reported it 0.13 % and 0.1 % respectively. Whereas it was close to the findings of Fedel *et al.* (1999), who reported the presence of p-cymene 24.01 % in the oil of same species.

**Table 3. Refractive indices of the oils obtained from each *Eucalyptus* species by hydro-distillation.**

| No. | Species                         | Refractive index |
|-----|---------------------------------|------------------|
| 1.  | <i>Eucalyptus camaldulensis</i> | 1.44             |
| 2.  | <i>Eucalyptus globules</i>      | 1.44             |
| 3.  | <i>Eucalyptus crebra</i>        | 1.43             |
| 4.  | <i>Eucalyptus tereticornis</i>  | 1.44             |
| 5.  | <i>Eucalyptus citriodora</i>    | 1.42             |

**Table 4. Optical rotation of the oils obtained from each *Eucalyptus* species by hydro-distillation.**

| No. | Species                         | Optical rotation |
|-----|---------------------------------|------------------|
| 1.  | <i>Eucalyptus camaldulensis</i> | -12.3            |
| 2.  | <i>Eucalyptus globulus</i>      | - 5.1            |
| 3.  | <i>Eucalyptus crebra</i>        | + 3.63           |
| 4.  | <i>Eucalyptus tereticornis</i>  | -10.64           |
| 5.  | <i>Eucalyptus citriodora</i>    | + 2.3            |

**Table 5. Retention time of individual standards on 10 % carbowex 20 M column.**

| No. | Standard compound             | Retention time (min.) |
|-----|-------------------------------|-----------------------|
| 1.  | $\alpha$ -pinene              | 0.65                  |
| 2.  | <sup>3</sup> $\Delta$ -carene | 0.94                  |
| 3.  | $\beta$ -phellandrene         | 1.14                  |
| 4.  | Limonene                      | 1.34                  |
| 5.  | 1,8-cineole                   | 1.48                  |
| 6.  | p-cymene                      | 1.76                  |
| 7.  | citronellal                   | 4.02                  |
| 8.  | citronellol                   | 5.98                  |

**Table 6. Possible compounds determined in the oil of *Eucalyptus camaldulensis* extracted by hydro-distillation.**

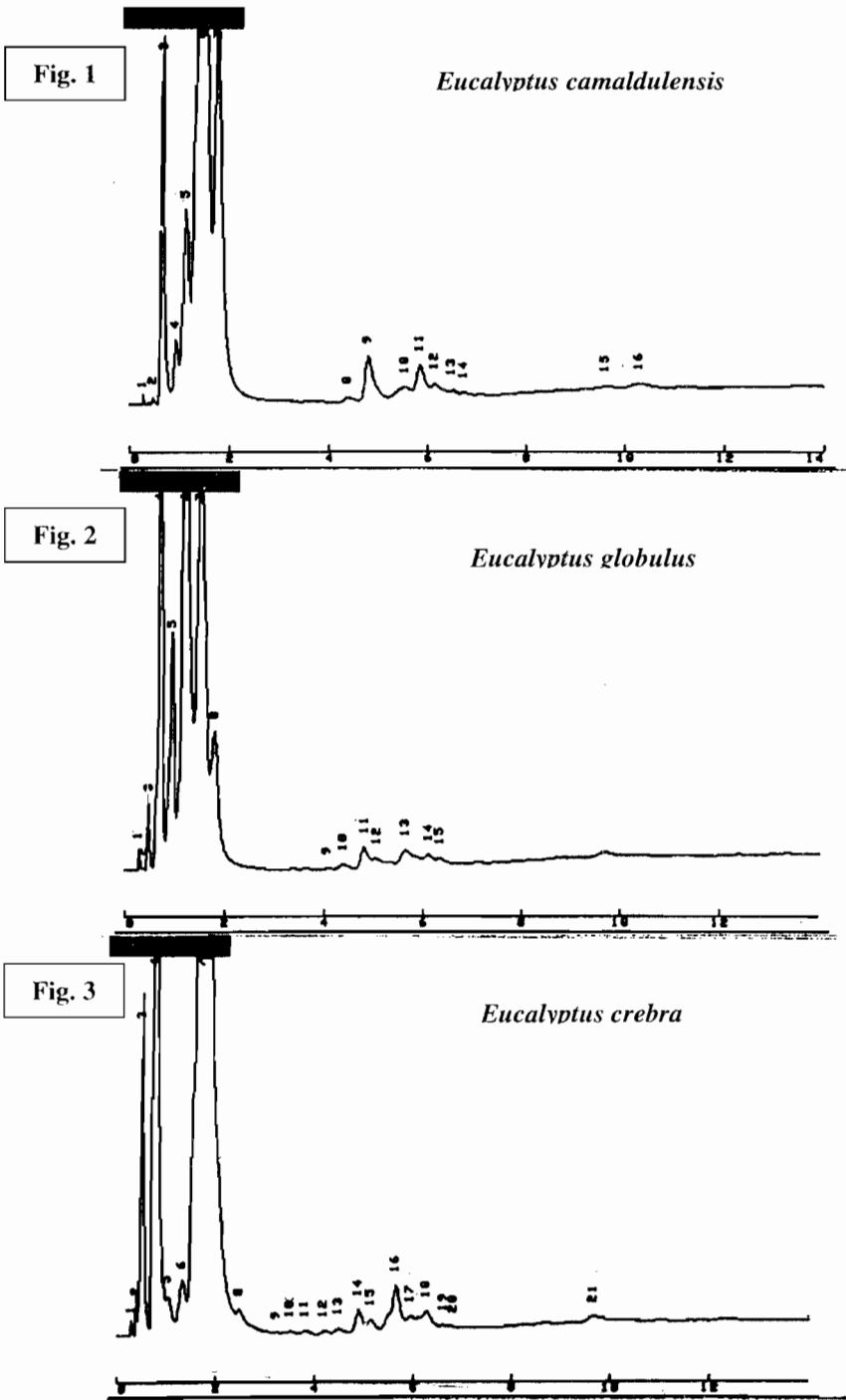
| Peak No. | Retention time (min.) | Concentration (% age) | Possible compound             |
|----------|-----------------------|-----------------------|-------------------------------|
| 3.       | 0.65                  | 6.0                   | $\alpha$ - pinene             |
| 4.       | 0.94                  | 1.8                   | <sup>3</sup> $\Delta$ -carene |
| 5.       | 1.14                  | 7.94                  | $\beta$ -phellandrene         |
| 6.       | 1.47                  | 53.22                 | 1,8-cineole                   |
| 7.       | 1.76                  | 21.59                 | p-cymene                      |

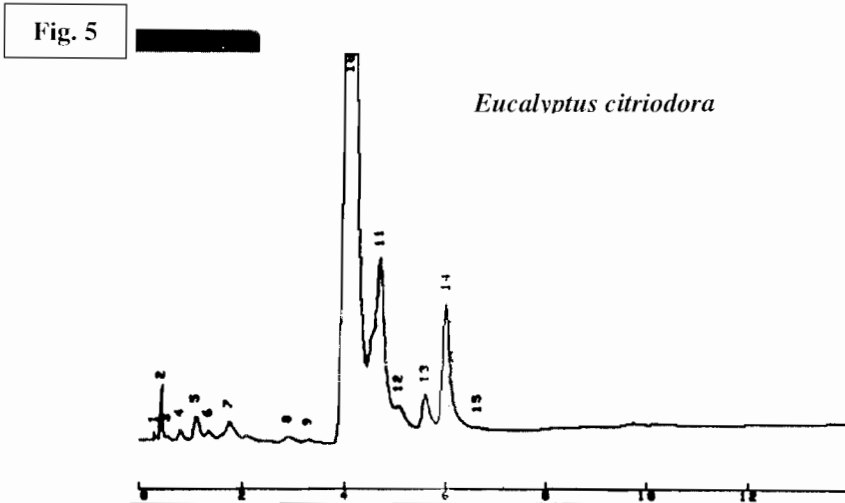
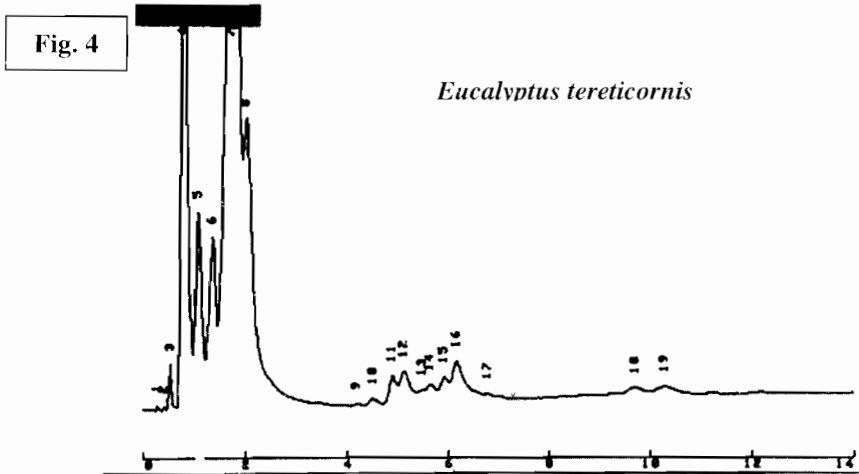
Each determination in Tables 3-6 is mean of three replicates.

**Chemical composition of *Eucalyptus globulus* oil:** Gas chromatograms (Fig. 2) of *E. globulus* oil showed 15 peaks of different compounds from which only 5 compounds were identified by matching their retention time with the standards (Table 5 and 7). The identified compounds were  $\alpha$ -pinene,  $^3\Delta$ -carene,  $\beta$ -phellandrene, 1,8-cineole and p-cymene. Concentration of  $\alpha$ -pinene (peak No. 4, fig. 2) found in the oil of *E. globulus* was 16.78 % which lie within the range (1-18 %), reported by Chalchat *et al.* (1995) for the oil of the same species. We found  $^3\Delta$ -carene (peak No.5, Fig. 2), 8.05 % and  $\beta$ -phellandrene (peak No. 6, Fig. 2), 32.12 % in the extracted oil. In case of  $\beta$ -phellandrene our results resembled with the findings of Li & Madden (1995). They found *E. globulus* oil enriched with  $\beta$ -phellandrene but did not mention its concentration. In our findings 1,8 cineole (peak No. 7, Fig. 2) was found 26.57 % but most of the literature indicates its percentage in the oil of same species more than 70 %, however, some studies had shown the concentration of 1,8 cineole as little as 4 % (Chalchat *et al.*, 1995). In our findings 1,8 cineole found in the extracted oil was toward lower range. Peak No. 8, Fig. 2 may be of p-cymene, its concentration was found 8.91 %, which is within the range (1-27 %) as determined by Chalchat *et al.* (1995).

**Chemical composition of *Eucalyptus crebra* oil:** The gas chromatograms (Fig. 3) of extracted *E. crebra* oil showed 21 peaks of different compounds from which only 3 compounds were identified (Tables 5 & 8). The identified compounds were  $\alpha$ -pinene, limonene and 1,8-cineole having concentrations 19.82, 0.53 and 72.17 % respectively (Table 8). The  $\alpha$ -pinene (peak No. 4, fig. 3) was found 19.82 %. Our findings for  $\alpha$ -pinene were within the range (0.1–28.3 %) reported by Bignell *et al.* (1997) for the oil of *E. crebra*. Concentration of limonene, 0.53 % (peak No. 6, Fig. 3) was also within the range of the findings of Bignell *et al.* (1997), as they found it 0–24.15 %. Bignell *et al.* (1997) reported the concentration of 1,8 cineol in *E. crebra* in a range of 0.4–63.2 %. Our findings of 1,8 cineol (peak No. 7, Fig. 3) was 72.17 which is higher than the findings of Bignell *et al.* (1997). This higher concentration of 1,8 cineol in the oil of *E. crebra* may be due to soil and/or climatic changes.

**Chemical composition of *Eucalyptus tereticornis* oil:** Gas chromatogram (Fig. 4) of *E. tereticornis* oil indicated the presence of 19 components, of which 5 components were identified (Tables 5 & 9). The identified compounds were  $\alpha$ -pinene,  $^3\Delta$ -carene,  $\beta$ -phellandrene, 1,8-cineole and p-cymene. Concentration of  $\alpha$ -pinene (peak No. 4, Fig. 4) found at retention time 0.65 min was 19.75 % (Table 9). Peak No 5 (Fig. 4) at retention time 0.95 min (Table 10) resembled with the retention time of standard  $^3\Delta$ -carene, its amount was 4.9 %. Peak No 6 (Fig. 4) at retention time 1.20 min. showing concentration 4.62 %, may be of  $\beta$ -phellandrene as its retention time resembled with its standard (1.14 min., Table 5). A prominent peak No 7 (Fig. 4) of 1,8 cineole showing concentration 50.51 % appeared at 1.50 min. Its concentration was found nearly close to *E. camaldulensis* oil (53.22) included in our study. Another peak (peak No. 8, fig. 4) at retention time 1.80 min (Table 10) may be of p-cymene, its concentration was found 12.31 %. Chemical composition of the oil of *E. tereticornis* was not available in the literature. Therefore, it was not possible to compare our findings with the findings of other researchers on this species.





Figs. 1-5. Gas chromatograms of the oils extracted from different *Eucalyptus* species on 10 % Carbowex 20 M column.

**Chemical composition of *Eucalyptus citriodora* oil:** The chromatogram (Fig. 5) of *E. citriodora* oil showed 15 peaks of different compounds from which only 4 compounds were identified. The identified compounds were  $\alpha$ -pinene, 1,8 cineole, citronellal and citronellol by comparing their retention times with the standards (Tables 5 & 10). Peak no. 4 may be of  $\alpha$ -pinene as its retention time (0.67 min) resembled with standard  $\alpha$ -pinene (0.65 min, Table 5). Its concentration was found 0.2 %, nearly similar to the amount (0.1 %) determined by Bignell *et al.* (1997). The retention time 1.48 min of peak no. 7 resembled with the retention time of standard compound 1,8 cineole. Its concentration was 0.68 %, whereas Bignell *et al.* (1997), found it 0.1 %. Peak No. 10 at retention time 4.02 min was the major compound and its concentration was 74.65 %, which resembled with the determinations made by Betts & Thomas (2000), they found it



72.9-80.5 %. The peak at retention time 5.98 min. resembled with the retention time of standard compound citronellol (Table 5). Its concentration was found to be 6.13 %, which was close to the findings of Sukhmal *et al.* (1998) and Betts & Thomas (2000), they found it 5.4 %.

**Table 7. Possible compounds determined in the oil of *Eucalyptus globulus* extracted by hydro-distillation.**

| Peak No. | Retention time (min.) | Concentration (% age) | Possible compound             |
|----------|-----------------------|-----------------------|-------------------------------|
| 3.       | 0.66                  | 16.78                 | $\alpha$ - pinene             |
| 4.       | 0.91                  | 8.05                  | <sup>3</sup> $\Delta$ -carene |
| 5.       | 1.15                  | 32.12                 | $\beta$ -phellandrene         |
| 6.       | 1.47                  | 26.57                 | 1,8-cineole                   |
| 7.       | 1.76                  | 8.91                  | p-cymene                      |

**Table 8. Possible compounds determined in the oil of *Eucalyptus crebra* extracted by hydro-distillation.**

| Peak No. | Retention time | Concentration | Possible compounds |
|----------|----------------|---------------|--------------------|
| 4.       | 0.68           | 19.82         | $\alpha$ - pinene  |
| 6.       | 1.34           | 0.53          | Limonene           |
| 7.       | 1.50           | 72.17         | 1,8 cineole        |

**Table 9. Possible compounds determined in the oil of *Eucalyptus tereticornis* extracted by hydro-distillation.**

| Peak No. | Retention time (min.) | Concentration (% age) | Possible compound             |
|----------|-----------------------|-----------------------|-------------------------------|
| 4.       | 0.69                  | 19.75                 | $\alpha$ -pinene              |
| 5.       | 0.95                  | 4.9                   | <sup>3</sup> $\Delta$ -carene |
| 6.       | 1.20                  | 4.62                  | $\beta$ -phellandrene         |
| 7.       | 1.50                  | 50.51                 | 1,8-cineole                   |
| 8.       | 1.80                  | 12.31                 | p-cymene                      |

**Table 10. Possible compounds determined in the oil of *Eucalyptus citriodora* extracted by hydro-distillation.**

| Peak No. | Retention time (min) | Concentration (% age) | Possible compounds |
|----------|----------------------|-----------------------|--------------------|
| 4        | 0.67                 | 0.2                   | $\alpha$ - pinene  |
| 7        | 1.48                 | 0.68                  | 1,8 cineole        |
| 10       | 4.02                 | 74.65                 | Citronellal        |
| 14       | 5.98                 | 6.13                  | Citronellol        |

Each determination in Tables 7-10 is means of three replicates.

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