*Acta Pharmaceutica Turcica*
*XXXX (1) 21-25 (1998)*

# STUDIES ON THE CONTACT DERMATITIC PROPERTIES OF INDIGENOUS PAKISTANI MEDICINAL PLANTS. DERMAL IRRITATING PROPERTIES OF EUCALYPTUS OIL CONTITUENTS

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*Irritant potential of eucalyptus oil (obtained by the distillation of Eucalyptus globulus leaves) and its purified compounds was investigated by open mouse ear assay, while their potencies were compared by $ID_{50}$ (Irritant dose in 50% individuals) after acute peak effects and by IU (Irritant units after chronic time). This effect was mainly due to the monoterpenes present in the oil, β Caryophyllene, 1,8 cineole-citronellal, crytone, ρ-cymene and α-terpinene were isolated and identified by chromatographic methods. Citronellal and cryptone were the most potent and persistent irritants. ρ-cymene and α-terpinene indicated an intermediate and less persistent irritant reaction, while 1,8-cineole and caryophyllene were the least irritating and least persistent compounds under the concentrations used.*

**Keywords** : *Eucalyptus Oil, Irritant potential, $ID_{50}$.*

## Introduction

Eucalyptus oil is used in perfume industry, for the manufacture of soaps, toothpastes, mouth washes and deodorants (1-4). Its use in various types of liniments, in inhalers for the relief of common cold, cough and bronchitis, in rheumatism, as an inhibitors for prostaglandin biosynthesis (5,6) and as mosquito repellent is well known (7-9). The terpenoid constituents of eucalyptus oil, obtained from leaves and other parts of various species of *Eucalyptus*, have been investigated for the presence of borneol, camphene, β-caryophyllene, 1,8-cineole, citral, citronellal, citronellol, citronellyl acetate, caryophyllene oxide, crytone, ρ-cymene, eudesmol, geraniol, isopropylhexane, isopulegol, limonene, linalool, linalyl acetate, myrcene, phellendrene, α-pinene, β-pinene, spathulenol, α-terpineol, γ-terpinene by many eminent workers (10-23). In the non-terpenoid area, a number of phenolic compounds have been isolated and characterized (24-26). Abe et al. determined proteins, lipids, sugars, fibers along with Na, K, Ca, Mg, P, Fe, Cu and Zn (27), while Pereira and Sardinha indicated high cellulose and low lignin content in the pulpwood of E. globulus (28).

In Pakistan eucalyptus oil has been used in cosmetics and in liniments, its harmful effects if any, particularly on the skin of human beings and animals have not been evaluated. The present communication describes the irritant effects of eucalyptus oil obtained from the leaves of locally occurring E. globulus on albino mice, followed by fractionation to isolate its active compounds, whose iritant potencies were evaluated by $ID_{50}$.

### Materials and Methods

*Plant Materials*

Leaves of *Eucalyptus globulus* (2 kg) were collected from the waste and uncultivated areas of Lahore, in July/August 1996. These were authenticated by the herbarium staff, Department of Botany, University of the Punjab, Lahore. The voucher specimen was deposited in the Herbarium of Pharmacognosy Section, Department of Pharmacy, University of the Punjab Lahore for Further reference. The plant material was air dried.

*Extraction*

The leaves of *E. globulus* were coarsely pulverized and subjected to steam distillation for 4 hours in a clevenger hydrodistillation apparatus. The oily layer was separated from the distillate and dried over anhydrous sodium sulphate. Physicochemical properties of the oil were determined according to the standard procedure (29) (Table 1).

*Column Chromatography*

2.6 g of the crude oil was absorbed on 4 g silica gels

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and fractionated into hydrocarbon and oxygenated part by silica gel 60 (70-230 mesh) column chromatography, eluting first with cyclohexane then with cyclohexane/ethyl acetate mixture. 20 ml fractions were collected and pooled, after monitoring with TLC and detecting the isolated compounds by iodine.

Table 1. Physicochemical characteristics of Eucalyptus oil.

| Characteristics | Values |
|---|---|
| Yield | 1.36% |
| Distillation time | 5.25 hours |
| Colour | Yellowish red |
| Specific gravity at 22°C | 1.62 |
| Refractive index at 25°C | 1.05 |
| Acid value | 6.61 |

The hyrdrocarbon part of the oil (1.26 g) was further chromatographed on silica gel 60 (140 g, 80-100 mesh) column, eluting first with petroleum ether (40-60°), then with petroleum ether/chloroform mixture. 10 ml fractions were collected and similar compounds were pooled.

The oxygenated part of the oil (86 mg) was subjected to third column, packed with 130 g of active neutral alumina (with activity I), eluted first with chloroform then with 2% methanol in chloroform. 10 ml fractions were also collected and similar compounds were bulked.

*Thin Layer Chromatography*

The silica gel ($PF_{254+360}$) thin layer analytical (25 mm) and preparative thin layer (75 mm) chromatographic plates were prepared according to the method of (del) Stahl (30). The solutions of following materials were prepared in chloroform (1%w/v) and applied to the chromatoplates using 5 µl Drummond microcaps. (i) Crude oil, (ii) Column fractions, (iii) Isolated compounds and the standard known compounds such as (iv) borneol, (v) camphene, (vi) 1,8-cineole, (vii) citronellal, (viii) citronellol (ix) cryptone, (x) caryophyllene, (xi) α-pinene, (xii) β-pinene and (xiii) α-terpineol. Solvent systems used for the development of TLC plates were cyclohexane/ethyl acetate (90:10 or 85:15) or petroleum ether/chloroform (90:10 or 80:20). Visualization of the chlromatogram was achieved by UV light or by using vanillin/sulphuric acid spraying reagent and heating the plates at 110°C for 5 minutes (30). The compounds from the pooled (smaller silica gel and alumina columns) fractions, that correspond with the standard compounds were further purified by preparative thin layers.

*Gas Chromatography*

The oil was also subjected to GC analysis on a Pye Unicam 204 model gas chromatographic apparatus, using CBP1 (non polar methyl silicone) and CBP20 (highly polar polyethylene glycol) capillary column with 20 m length and 0.25 internal diameter, along with the flame ionization detector (FIO). The retention times of various peaks were compared with the standard compounds. Other conditions of GC operation were (del) temperature programmed with initial column temperature at 90°C which was hold up for 10 min then raised at a rate of 5°C /min. Final column temperature was kept at 210°C for 20 min. The injection port temperature of 300°C was maintained. Nitrogen was used as a carrier gas under split system at a flow rate of 30 ml/min.

*Biological Assay*

*Animals*

Albino mice weighing 10 to 15 g were housed in cages on wood shavings in animal house under 20 3°C and relative humidity 46 3.6%. Palliated food and deionized water was available *ad libitum*.

*Procedure*

10 mg of the substance under test was dissolved in acetone to prepare a 10 mg/10 ml (w/v) solution. It was further diluted according to the method of Evans and Schmidt (31). Eight dilutions were prepared for the main assay. The pilot and main irritancy assay on mice ears were also adopted from Evans and Schmidt (31). For the main assay, group of 10 animals were used for each dilution. The results were tabulated as total number of red ears per dilution and $ID_{50}$ (Irritant dose in 50% individuals) were calculated by probit analysis (Table 2) (32).

Table 2. Chemical Constituents of Eucalyptus oil (as revealed by Gas Chromatogram)

| R. Time (min) | Compounds | Percentage |
|---|---|---|
| 8.69 | α -pinene | 0.25 |
| 9.10 | Camphene | 0.42 |
| 10.21 | β-Pinene | 0.36 |
| 13.05 | 1,8-Cineole | 25.52 |
| 19.10 | Borneol | 2.20 |
| 19.78 | α -Terpineol | 12.71 |
| 20.56 | Citronellal | 18.41 |
| 21.59 | Cryptone | 9.39 |
| 23.74 | Linalyl acetate | 1.12 |
| 26.64 | ρ-Cymene | 10.65 |
| 35.65 | β-Caryophyllene | 19.71 |

## Results and Discussion

Irritation due to eucalyptus oil was observed in the local people, who deals with the manufacture of cosmetic and liniment, where this oil was used as their main ingredient. The face, hands, arms and leg's skin was often involved. It produced inflammatory patches after prolong handling the oily preparation. It was thus worth while to look into the irritant

effects of eucalyptus oil obtained from the leaves of *E. globulus* on albino mice, followed by fractionation to isolate its active compounds. The physicochemical characteristics of this oil has been summarized in Table 1.

The oil was fractionated into hydrocarbon and oxygenated terpenoid fractions through the column chromatogram on activated silica gel. The elusion with cyclohexane, isolated hydrocarbon, while the oxygenated component remained adsorbed on silica gel and was eluted later by ethyl acetate.

The hydrocarbon part of the oil was further resolved into various fractions after second column chromatography on silica gel, which were eluted with petroleum ether. These fractions after pooling and subjecting to the preparative thin layer analysis furnished 1,8-cineol, borneol and α-terpineol as predominant compounds.

The oxgenated part of the oil on the other hand, when further subjected to an active neutral alumina column, eluting with 2% methanol in chloroform, followed by preparative thin layers yielded β-caryophyllene, citronellal, cryptone, ρ-cymene and linalyl acetate as major compounds.

Purity of these compounds was confirmed by analytical TLC after using different solvent systems and also by GC. These compounds were identified by comparing their TLC and GC chromatogram with the standard samples.

Gas chromatographic analysis of *Eucalyptus globulus* oil also revealed a number of components, out of which 11 terpenes could be identified, after comparison with the standard samples (Table 2). Major constituents were 1,8-cineole (25.52%), β-caryophyllene (19.71 %), citronellal (18.41%), α -terpineol (12.71%), ρ-cymene (10.65), cryptone (9.39%), borneol (2.20%) and linalyl acetate (1.12%) along with many minor costituents.

Many mono- and sesquiterpenoid compounds including above mentioned compounds have previously been isolated and identified by many workers from eucalyptus leaf oil by TLC and GLC (10-23).

For comparing the irritant reactions due to the compounds present in eucalyptus oil,

Table 3. Irritant responses of the compounds isolated from eucalyptus oil on albino mice

| **Dose Levels (µg/5µl)** | | **COMPOUNDS** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1,8-Cineole** | **Citronellal** | **ρ-Cymene** | **Cryptone** | **α-Terpineol** | **β-Caryophyllene** |
| 20 | | ---- | ---- | ---- | ---- | ---- | 10/10 |
| 10 | | ---- | ---- | 10/10 | ---- | ---- | 9/10 |
| 5 | | 10/10 | ---- | 8/10 | 10/10 | 10/10 | 8/10 |
| 2.5 | | 8/10 | 10/10 | 7/10 | 9/10 | 8/10 | 5/10 |
| 1.25 | | 4/10 | 8/10 | 6/10 | 7/10 | 7/10 | 2/10 |
| 0.625 | | 3/10 | 7/10 | 5/10 | 5/10 | 4/10 | 1/10 |
| 0.3125 | | 2/10 | 4/10 | 3/10 | 4/10 | 3/10 | ---- |
| 0.15625 | | 1/10 | 3/10 | 1/10 | 2/10 | 1/10 | ---- |
| 0.078125 | | ---- | ---- | ---- | 1/10 | ---- | ---- |
| $ID_{50}$ | µg/5µl | 1.008 | 0.351 | 0.847 | 0.497 | 0.706 | 2.483 |
| | S.D. | 0.191 | 0.287 | 0.181 | 0.154 | 0.186 | 0.183 |
| | $x^2$ | 2.507 | 0.810 | 1.331 | 0.672 | 0.848 | 0.342 |
| | t | 4.50 | 1.50 | 3.25 | 3.00 | 4.00 | 5.50 |
| | U.C.L. | 1.654 | 0.567 | 1.467 | 0.811 | 1.128 | 3.778 |
| | L.C.L. | 0.616 | 0.163 | 0.443 | 0.293 | 0.419 | 1.569 |
| **IU(µg/ 5 µl) after** | **24 h** | 2.50 | 0.625 | 5.00 | 1.25 | 0.625 | >20 |
| | **48 h** | >20 | >10 | >5 | >10 | >10 | >20 |

Where....

**S.D.** = Standard Deviation **$x^2$** = Chi square **t** = Time (hours) to peak reaction
**$ID_{50}$** = Irritant dose in 50% animals, Calculated by probit analysis
**U.C.L.** = Upper confidence limit. **L.C.L.** = Lower confidence limit
**IU** = Irritant units after 24 and 48 hours.

the number of mice indicating inflammatory reaction were counted at the time of peak irritancy, which differ from compound to-compound. The data was then analyzed by probit analysis (32), which enable us to compare the potencies by means of $ID_{50}$ (Irritant dose in 50% individuals). The chronic effects of eucalyptus oil compounds on the animals skin were also recorded after one and two days to ascertian the chronic inflammatory dose in a similar way as Hecker performed for croton oil (31,33). Our findings suggested that the irritant properties of eucalyptus oil was probably due to the compounds present in its monoterpenes part (Table 3).

Among the six isolated compounds, citronellal and cryptone appered to be the most potent and persistent irritants than all other compounds with least $ID_{50}$ (i.e.0.351 µg/5µl and 0.497 µg/5µl) after 1.5 and 3 hours respectvely. Their reactions were lasted for 24 hours, indicating IU = 0.625 and 1.25 µg/5µl after this time (Table 3). Moreover, the inflammatory reactions due to these compounds with at least ++ intensities emerged as red wheels that was scattered in 2.00 cm to 2.50 cm diameter areas of the animal's skin.

ρ-cymene and α-terpinene indicated an intermediate and less persistent irritant responses with $ID_{50}$ = 0.847 µg/µl and 0.706 µg/µl after 3.25 and 4.00 hours respectively. the inflammatory reactions due to these compounds with ++ intensities displayed in the form of an uneven red wheel that sweep from 1.20 cm to 1.75 cm diameter area of the animal's ears. The reactions of these compounds remained only up to 12 hours giving IU = 5.00 µg/5µl and 0.625 µg/5µl but did not continue more than 12 hours under the cocentrations used (Table 3).

1,8-cincole and caryophyllene on the other hand were the least irritant and least persistent compounds, with $ID_{50}$ = 1.008 µg/5µl and 2.483 µg/5µl after 4.50 and 5.50 hours respectively. The inflammatory reaction due to these compounds inidcated + intensity, scattered in the from of a weak red wheel that extent from 1.25 cm to 1.75 cm diameter area of the animal's ears. Here the reactions only persisted for less than 12 hours, under the concentrations used; giving IU=5.00 µg/µl and 0.625 µg/µl respectively (Table 3). It is likely that the potent and persistent inflammation induced by citronellal and cryptone was probably due to the result of some tissue damage in the animal skin; while the least persistent inflammation of 1,8-cineole and caryophyllene was possibly due to direct action at some skin receptor sites.

We concluded from our investigation that the eucalyptus oil from our local sources, contains closely related irritant monoterpenes and possibly be harmful to the animal and human's skin. Its use in cosmetics and liniments should be limited or it should be converted into some nonirritant material prior to its use.

Further work is obligatory to amplify this property through the preparation of their derivatives, that would possibility lead to the structure-activity relationship of such important irritant compounds.

## Acknowledgements

The authors are grateful to Mr. Abdus Sattar of PCSIR Lahore for providing the authentic samples of pure compounds and Mrs. Taseen Waseem of DTL, Lahore for arranging the albino mice for experiment.

### References

1. Thilivahali, N.T., Von Wandruszka, R.M.A.: S. Afr. J. Chem. 39, 95 (1986)
2. Ahmadouch, A., Bellakdar, J., Berrada, M., Denier, C., Pinel, R.: Fitotherap. 56, 209, (1985)
3. Boelens, M. H.: Perfum. Flav. 9, 1, Dec./Jan. (1985)
4. Montejo Loret De Mola, L., Magranar Hernandez, A., Rosado Perez, A., Baluja, R.R.: Rev. Cub. Farm. 14, 177 (1985)
5. United States Food And Drug Administration (Rock ville, MD20857 USA) fed. Redist. 53, (156), 305-22-64 12 Aug. (1988)
6. Wagner, H., Wierer, M., Bauer, R.: Planta Medica (3), 184, (1986)
7. Collin, D.A., Brady, J.N., Curtis, C.F.: Phytother. Res. 7, 17, (1993)
8. Nishimura, H.: Taijo Enaugi 14, 10, (1988)
9. Nishimura, H.: Kagahu to Scibutsa 27, 486, (1989)
10. Canigureral, S., Vita, R., Iglesia, J., Bellahhdar, J., Idrissi, A.K.: J. Essential Oil. Res. 4, 543, (1992)

11. Chaudri, D.C., Suri, R.K.: Indian Perfum. 35, 30, (1991)
12. Gibson, A., Doran, J.C., Bogsanyi, D.: Flavour Fragrance J. 6, 129, (1991)
13. Cheng, Z., Wu, H., Zang, G.: Fenxi Ceshi Tongbao 8, 1, (1989)
14. Holeman, M., Rombourg, M., Fechtal, M., Gorrichon, J.P., Lassaigne, G.: Plant Med. Phytother. 21, 311 (1987)
15. Manzoor-i-Khuda, M., Rehman, M., Yusaf, M., Chowdhu, J.U., Wahab, M.A.: Bangladesh J. Sci. Ind. Res. 22, 152, (1987)
16. Prabhu, V. V., Theagarajan, K.S.: Curr. Sci. 56, 522, (1987)
17. Brophy, J. J., Lassak, E. V., Toia, R. : Planta Med. 2, 170, (1985)
18. Boelens, M.H.: Perfum. Flavor. 9, 1, 3, (1984) (Pub. 1985)
19. Miranda, M., Zayas, J.P.: Cuba. Rev. Cub. Farm. 19, 121 (1985)
20. Weston, R.J.: Phytochem. 23, 1943, (1984)
21. Rao, B.R.R., Singh, S.P., Rao, E. V. S. P., Chandrasekhara, G., Ramesh, S.: Indian Perfum 28, 153, (1984)
22. Chen, Y. D., Yang, L., S-X. Li., Jiang, Z.R.: Chem. Indust. Forest. Prod. 3, 14, (1983)
23. Miranda, M., Perez Z. J., Rosado, A.: Rev. Cienc. Quim. 14, 211, (1983)
24. Salama, A.M., Ismail, M.K., Ali, M.I.A., Ouf, S. A.: Egypt J. Physiol. Sci. 14, 75, (1990)
25. Borphy, J., Lassak, E. V. :Flavour Fragrance J. 6, 265, (1991)
26. Wagner, H., Wierer, M., Bauer, R. : Planta Med. 3, 184, (1986)
27. Abe, M. Tamura, Y., Kato, H., Sakabe, Y.: Nagoya-shi Eisei Kenkyushoho 34, 91, (1988)
28. Pereira, H., Sardinha, R. Appita 37, 661, (1984)
29. Ernest, G.: The Essential Oils, D. Van Nostrand Co. Inc. New York Vol-1, P.112, (1955)
30. Stahl, E. "Thin layer chromatography" Springer- Verlag, Berlin, Heidelberg, New York pp. 52, (1969)
31. Evans, F.J., Schmidt, R.J.: Inflammation 3, 215 (1979)
32. Finney, D.J.: Probit Analysis. 3rd ed. Cambridge University Press (1971)
33. Hecker, E. "Methods of Cancer Research". H. Bush (ed.) Academic Press, London (1977)

Accepted: 14.04.1997