Int. J. Chem. Sci.: 9(2), 2011, 858-864
ISSN 0972-768X
www.sadgurupublications.com

PHYTOCHEMICAL STUDIES ON AERIAL PARTS OF *EUCALYPTUS GERANDIANA*

ANITA SHAKYA, NEETU ARYA, S. P. SHARMA and R. C. SAXENA*

Pest Control and Ayurvedic Drug Research Laboratory, S. S. L. Jain P.G. College,
VIDISHA – 464 001 (M.P.) INDIA

ABSTRACT

Eucalyptus gerandiana is commonly used in Indian traditional medicine for the treatment of variety of diseases. Over phytochemical studies on the *E. gerandiana* showed the presence of terpenoid in the decoction of the aerial part. However, the chemical constituents responsible for pharmacological activities remain to be investigated. Loss of weight on drying was 56.6% and the yield for ethanol and chloroform extracts were 3.06%, 2.02% respectively. Preliminary qualitative chemical analysis of extract was found positive for terpenoid. The Infra Red (IR) spectra of the crude extract revealed the presence of different functional group ranging from - C ≡ C stretching alkynes group (2170.4 cm^{-1}), C = C stretching for alkenes (1638.7 cm^{-1}), – OH stretching for hydroxyl group (1404.4 cm^{-1}), = C – H bonding for alkane group (771.7 cm^{-1}) and secondary alcohol stretching (1107.2 cm^{-1}). The main compound identified in *E. gerandiana* leaf was 2-hydroxy-1, 1′ 5, 9, 14, 20, 20′-heptamethyl-12-ene-triterpenoid.

Key words: *Eucalyptus gerandiana*, Phytochemical, Functional groups, Terpenoid.

INTRODUCTION

The genus Eucalyptus(family - Myrtaceae) comprises well-known plants of over 600 species[1]. Although most of the plants are native to Australia, numerous species have been introduced to other parts of world, including Iran, as economic and ornamental trees in forest[2], where the plants have become source of important fast-growing hardwood trees[3] and essential oils[4]. The Eucalyptus essential oils could be grouped into three types on the basis of their chemical constituents (medicinal, industrial and perfumer)[5-7].

E. gerandiana have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are know to contain substance that can be used for

*Author for correspondence; Ph.: 91 7592 237598;
E-mail: anitashakya4@yahoo.com; rcsvds@yahoo.com

therapeutic purposes or as precursors for the synthesis of useful drugs[8]. Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry[9]. *E. gerandiana* is an important ethnomedicinal plant belonging to the family, Myrtaceae. It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Eucalyptus oil is used as a counter irritant, an antiseptic and expectorant. It is used to relieve cough and chronic bronchitis in the inhalations. Solution of Eucalyptus oil is used as nasal drops. Eucalyptus oil has Eualyptol (1,8-cineole) as its active ingredient and this is responsible for its various pharmacological actions[10].

EXPERIMENTAL

Plant material

E. gerandiana leaves were collected around the Vidisha district and authenticated at department of the botany of the institute. A voucher specimen of the plant has been preserved in our herbarium record in pest control and ayurvedic drug research laboratory Vidisha for further reference.

Extraction method

The extraction of *E. gerandiana* shade dried powder material (40-60) mesh size was carried out separately by soxhlet apparatus in the laboratory using different solvents in increasing order of polarity. The extraction procedure adopted as given by Harborney (1948)[11]. Terpenoid are polar compound, so are extracted in 90% ethanol. Percentage yield of crude extract of *E. gerandiana* obtained 3.06 % with alcohol.

Isolation and purification of compound

There are various active compounds present in the plant extract so further isolation and purification was carried out, to find the pure active compound. The crude extract obtained from vacuum evaporator of plant was subjected to isolation, purification, chemical examination, spectral analysis and characterization of the compound.

Phytochemical screening of terpenoids

About 0.5 g of each extract in 2 mL of chloroform was taken and conc. H_2SO_4 carefully added to from a layer. A reddish brown colouration of the interface was formed to show positive sign for the terpenoid.

T. L.C. (Thin layer chromatography)

Separation was carried out on TLC plates for the presence of compound and their purity for the terpenoids and saponins. Different solvent system were used according to the method of Harborny[11] and measured with the cm scale for determining the R_f value defined by Brimley and Barrett (1953)[12].

Column chromatography

The small quantity of crude extract was followed by observation on silica gel column with ethyl acetate solution. Four fractions (E_1 to E_4) were collected by using solvent system $CHCl_3$: C_6H_6 : MeOH (43 : 43 : 13). All fractions were monitored by TLC until single spot was obtained.

Acid hydrolysis

Compound was heated in mixture of 2N-HCl (4 mL) and MeOH at 80°C for four hrs. on water bath. After removal of MeOH, the solution was extracted with EtOAc (4 mL x 3). The extraction was washed with distil water and then combined to give a dark brown powder. Purification of the product over silica gel and crystallization from MeOH offered a compound.

Methylation

Purified fraction were separately dissolved in MeOH. In this process compound was washed by MeOH until it got converted into crystals form. After methylation, solvents were evaporated and residues were controlled by TLC.

Table 1: Percentage yield of crude extract of *E. gerandiana* (leaf) by soxhelt apparatus in different solvent below 40°C

Solvent used (in mL)	Weight of powder (in gm)	Weight of extract (in gm)	Percentage of yield	Characteristics of extract
90 % ethanol	500	15.32	3.06	Brown
Chloroform	500	10.13	2.02	Yellowish Brown

Table 2: TLC of alcoholic crude extract of *E. gerandiana* (leaf) solvent system used $CHCl_3$: C_6H_6: MeOH (43 : 43 : 13)

Name of fractions	Colours of the fractions in			R_f value
	Visual light	Iodine chamber	U.V. light	
E_1	Brown	Dark Brown	Brown	0.90
E_2	Yellowish Brown	Brown Yellowish	Yellow Brown	0.69
E_3	Light Brown	Light Brown	Light Brown	0.36
E_4	Brown	Dark Brown	Brown	0.31

Table 3: Column Chromatography of the *E. gerandiana* crude extract

Solvent used	No. of fractions	Weight of fractions	Colours of fractions
$CHCl_3$: C_6H_6 : MeOH (43 : 43 : 13)	E_1	0.84	Cream
	E_2	1.32	Brownish cream
	E_3	0.90	Dark brown
	E_4	0.73	Light brown

RESULTS AND DISCUSSION

Quantitative phytochemical screening of the crude extracts of *E. gerandiana* demonstrated the presence of terpenoid. For further characterization and structure elucidation of active principles determined with the help of spectral analysis techniques viz. ^1H NMR and ^{13}C NMR, Mass spectroscopy and Infrared information, which shown following structure of the compound. The crude product was purified by column chromatography ($CHCl_3$: C_6H_6 : MeOH/43 : 43 : 13). The text compound was obtained yield for ethanol and chloroform extracts were (15.32g, 3.06%), (10.13 g, 2.02%), mp 190-230ºC; IR (KBr, Vmax in cm^{-1}) 3426 (≡ C-C), 2370 (-C ≡ C), 1638 (C = C), 1404 (C-O-OH), 771 (=C-H); [m^+] 391, m/z 43.05, 61.06, 81.0, 97.0, 99.10, 115.0, 136.0, 143.1, 153.1, 183.1, 235.1, 237.0, 279.1, 293.2, 337.0, 387.2 and 391.2 (Calcd; for $C_{30}H_{46}O$); ^1H NMR (300 MHz – $CDCl_3$) at δ 0.85 (3H, s CH_3) δ 0.87 (3H, s CH_3), δ 0.89 (3H, s CH_3), δ 0.97 (3H, s

CH_3), δ 1.00 (3H, s CH_3), δ 1.25 (3Hd, J_3 s – O Hz – HC_{20} – CH_3) and δ 1.57 (3H, s > C = C – CH_3).

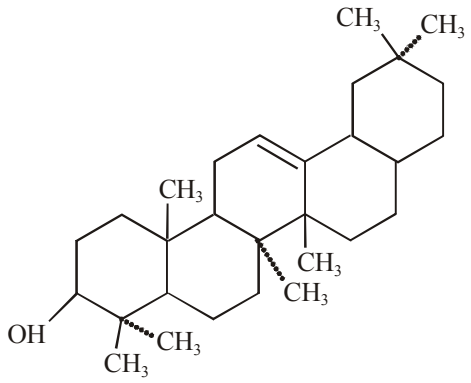

Fig. 1: (2-hydroxy-1, 1´ 5, 9, 14, 20, 20´ -heptamethyl-12-ene-triterpenoid)

The medicinal chemistry of *E. gerandiana* has been carried out in detail, using various phytochemical techniques mentioned in the pharmacognocy of Kokate et al.[13] and phytochemical method by Harborney (1984).[11] A new triterpenoid glycosides was isolated by Yoshimistsu et al.[14] from thalicbrum plant extract. A sesquiterpenoid derivative from ferrule using ^{1}H NMR and GC/MS was reported by Koshama et al.[15] Fraga et al.[16] have also reported diterpenoid from a plant *Persea indica,* the MF of this substance $C_{20}H_{28}O_6$. They have used ^{13}C NMR, ^{1}H NMR and Mass spectrum along with IR and UV, which showed absorbance of an α-β unsaturated oxo groups. Isolation and characterization of monoterpenoid, diterpenoid, triterpenoid from the plants have been discussed in several papers of natural chemistry, such as Ahemed et al.[17] who have reported dT & MT conjugate from Salvia plant extract. Park et al.[18] have also reported the isolation of sesquiterpene lactones from Cichorium plant extract. Herbal compounds or plant based formulation are very much popular now a days for antibacterial, antifungal activities. From time *Immorial, neembakh* extract or cultis have been used against skin infection caused by bacteria and fungi, still it have the same validity and due to advancement of techniques of extraction, isolation & purification. These traditional medicines have been isolated and their formulation tested by the various pharmaceutical companies.

ACKNOWLEDGEMENT

Authors are thankful to SAIF, CDRI Lucknow for providing the spectral analysis

reports of the crude sample and one of as Neetu Arya acknowledge with thanks to UGC for Rajeev Gandhi Fellowship JRF.

REFERENCES

1. D. Boland, J. J. Brophy and A. P. N. House, Centre for International Agricultural Research, Inkata Press, Melbourne (1991) p. 25-155.
2. J. Hutchinson, The Families of Flowering Plants, Vol. II, University Press, London (1959) p. 303.
3. M. D. Dassanajake, A Revised Handbook to the Flora of Ceylon, Vol. II, Amerind Publishing Co., Washington (1981) p. 403-405.
4. M. B. Lawrence, Perfum. Flav., **13**, 39-40 (1987).
5. A. O. Oyedeji, O. N. Olawore, O. Ekundayo and W. A. Koenig, Flavour and Fragrance J., **14**, 241-244 (1999).
6. S. R. J. Robbins, Tropical Products Institute, 105-108 (1983).
7. A. R. Penfold and J. L. Willis, The Eucalyptus Botany, Cultivation, Chemistry and Utilization, Laonard Hill Ltd, London, Inter. Sci. Pub., (1992) p. 56.
8. I. Zh. Zhalolau, Tashkhad, B. Zhaeo, V. U. Khuhaeo and S. F. Aripova, Chem. of Nat. Compd., **38(1)**, 83-85 (2002).
9. T. K. Hayashi, M. Okamuka, Kawasaki and N. Morita, Phytochem., **35(2)**, 353-356 (1993).
10. D. Ghosh and K. S. Loddha, Pharmacognosy Magazine, **2(5)**, 15-26 (2006).
11. J. B. Harborne, Phytochemical Methods, Chapman and Hall, NY, **II** (1984) p. 288.
12. R. C. Brimley and F. C. Barrett, Practical Chromatography, Chapman and Hall Publication, London, 1-27 (1953).
13. P. K. Kokate, A. P. Purohit and S. B. Gokhle, Pharmacognosy, **III**, 235 (2005).
14. H. Yoshimitus, M. Nishidia, Zhi Zhong and T. Nohara, Chem. Pharm Bult., **48(6)**, 826-831 (2000).
15. K. Koshama, K. Isaka, P. Ondognil, O. Zevgeegiin, H. Mizukami and Y. Oinhar, Chem. Pharm Bult., **48(3)**, 353-356 (2000).
16. M. B. Fraga, D. Terrero, C. Gutierrez, A. Coloma, Phytochem., **56**, 15-320 (2001).

17. V. U. Ahemed, M. Zahid, M. S. Ali, A. R. Jassbi, S. Ahmad, Z. Ali and M. Z. Iqbal, Nat. Prod. Sci., **6(2)**, 66-69 (2000).

18. H. J. Park, S. H. Kwon, K. Yoo, W. T. Jung, K. Taelee, J. Llkim and N. H. Yong, Nat. Prod. Sci., **6(1)**, 86-90 (2000).

Revised : 14.02.2011 *Accepted : 16.02.2011*