Chromatographic evaluation and anthelmintic activity of Eucalyptus globulus oil

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Submitted: 27-03-2010 Revised: 28-03-2010 Published: 19-07-2010

ABSTRACT

In world Helminthes infections are the most widespread of all the infections in humans. The morbidity due to parasitic diseases has been increasing in our population. The gastrointestinal helminthes become resistant to the currently available anthelmintic drugs. Anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Due to this, there is a need to derive new chemical substances from natural sources, for helminthes control. In this study, volatile oil isolated from Eucalyptus globulus Labill was evaluated for its anthelmintic activity on adult Indian earthworms, Pheretima posthuma, which have anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. In concentrations of 0.05, 0.01 and 0.15 ml/ml, respectively, all the oil samples showed potent anthelmintic activity as compared to that of the standard drug albendazole at a concentration of 10 mg/ml.

Key words: Anthelmintic, Eucalyptus globulus, eucalyptus oil, Pheretima posthuma

INTRODUCTION

In world Helminthes infections are the most widespread of all the infections affecting humans. The morbidity due to parasitic diseases has been increasing in our population. The gastrointestinal helminthes become resistant to the currently available anthelmintic drugs. Therefore, treatment of helminthes diseases has become difficult. Anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. A new lead for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants which are generally considered to be very important sources of bioactive substances.

Eucalyptus globulus Labill (family Myrtaceae) is a lofty tree of about 90 m in height. Leaves are febrifuge, carminative, stimulant, expectorant, antiseptic, antimalarial and anthelmintic. Eucalyptus oil has powerful antiseptic and disinfectant properties. It increases the flow of saliva, gastric and intestinal juices and increases the digestion and appetite.

Bark of E. globulus contains ellagitannins, methyl and glycosyl derivatives of ellagic acid and free ellagic and gallic acids, gallotannins and catechin. Essential oil from this species has a therapeutic application in the treatment of pulmonary infections by inhalation and the monoterpene extracted from Eucalyptus citriodora, E. globulus and Eucalyptus tereticorni exhibits antibacterial activity. Besides antibacterial activity, the essential oil from eucalyptus shows analgesic and anti-inflammatory effects. Phytochemical analysis has established that the genus Eucalyptus contains monoterpenes. Two monoterpene glycosides, conjugated with gallic acid globulusin A and B, together with four known compounds, cypellocarpin A, eucaglobulin, cuniloside and (1S, 2S, 4R)-trans-2-hydroxy-1,8-cineole β-D-glucopyranoside, were isolated from hot water extracts of the leaves of E. globulus. The aim of this research was to study chemical composition and anthelmintic property of the volatile oil from the leaves of E. globulus.

MATERIAL AND METHODS

Plant material
The leaves were collected from Baramati region of Dist.
Pune and were authenticated by Prof. R. B. Deshmukh, Head, Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar.

**Isolation of Essential Oil**

The essential oil was isolated from the fresh leaves by hydrodistillation in a clevenger-type apparatus, yielding essential oil 0.53% w/v.

**Evaluation of Anthelmintic Activity**

Indian adult earthworms (*Pheretima posthuma*) of 3–5 cm length and 0.1–0.2 cm width were used for anthelmintic activity because of their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.\[16,17\] The earthworms were divided into four groups containing five earthworms in each group. Oil was dissolved in water using tween 80. All the test samples and the standard drug were freshly prepared before starting the experiment. The solution of oils and standard solution were poured in petridishes. All the earthworms were washed in normal saline solution before they were released into petridish containing 10 ml solution of *E. globulus* oil in concentrations of 0.05, 0.1 and 0.15 ml/ml, respectively, and albendazole (10 mg/ml). The time taken for the worms to get paralyzed and killed was noted. All readings were expressed as mean and standard error of mean (SEM) of three animals in each group.\[18\]

**Evaluation of Volatile Oil**

Volatile oil was evaluated for optical rotation and refractive index as per the standard procedure.\[19\]

**Thin layer chromatography**

Thin layer chromatography was performed using silica gel G as the stationary phase, toluene-ethylacetate (97:3) as the mobile phase and vanillin-sulfuric acid as the spraying reagent.\[20\]

**RESULTS**

Essential oil from *E. globulus* contains 1,8-cineole as the major component\[21,22\] and is used in the treatment of pulmonary infections\[23\] and also exhibits antibacterial activity.\[12\] The result of the present study reveals that *E. globulus* oil in concentrations of 0.05, 0.1 and 0.15 ml/ml showed significant anthelmintic activity as compared to the standard drug albendazole at a concentration of 10 mg/ml, as shown in Figure 1. *E. globulus* oil showed anthelmintic activity in a concentration-dependent manner. Evaluation of *E. globulus* oil showed an optical rotation of +0.8° and a refractive index of 1.4554. Chromatographic study confirmed the presence of borneol, linalool, cineol, geranyl acetate, anethol, saffrol as the phytoconstituents of eucalyptus oil, as shown in Table 1.

**DISCUSSION**

An Indian adult earthworm (*P. posthuma*) has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.\[16,17\] Result indicates that time taken for albendazole for causing paralysis and death of *P. posthuma* was 5.82 ± 0.466 and 6.54 ± 0.429, respectively, whereas *eucalyptus* oil at a concentration of 0.15 ml/ml causes paralysis and death at 4.598 ± 1.151 and 6.57 ± 1.374, respectively. So, the present investigation concludes that *E. globulus* oil has anthelmintic potential due to the presence of borneol, linalool, cineol, geranyl acetate, anethol, saffrol as phytoconstituents.

**ACKNOWLEDGMENTS**

The authors are thankful to the Management and Prof. R. N. Patil, Principal, of S.V.P.M's College of Pharmacy, Malegaon (Bk), Baramati, for providing necessary facilities and also to Prof. R. B. Deshmukh, Head, Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, for the authentication of the plant.

| Spots from bottom | R<sub>g</sub> values | Phytochemical constituents |
|-------------------|---------------------|---------------------------|
| 1                 | 0.266               | Borneol                   |
| 2                 | 0.312               | Linalool                  |
| 3                 | 0.4666              | Cineol                    |
| 4                 | 0.632               | Geranyl acetate           |
| 5                 | 0.785               | Anethol                   |
| 6                 | 0.814               | Saffrol                   |
REFERENCES

1. Tagboto S, Townson S. Antiparasitic properties of medicinal and other naturally occurring products. Adv Parasitol 2001;50:199-205.
2. Coles GC. Nematode control practices and anthelmintic resistance on British sheep farms. Vet Rec 1997;141:91-3.
3. Turnipseed SB, Roybal JE, Rupp HS, Gonzales SA, Pfenning AP, Hurbut JA. Confirmation of avermectin residues in food matrices with negative-ion atmospheric pressure chemical ionization liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom 1999;13:493-9.
4. Hammonds JA, Fielding D, Bishop SC. Prospects for plant anthelmintics in tropical veterinary medicine. Vet Res Commun 1997;21:213-28.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed, Vol 1. Dehradun: International book Distributor; 1985. p. 1044-5.
6. Nadkarni AK. Dr. K.M. Nadkarni’s Indian Materia Medica. 3rd ed, Vol 1. Bombay: Popular Prakashan; 1992. p. 512-6.
7. Yazaki Y, Hillis WE. Polyphenol of Eucalyptus globulus, E. regnans and E. deglupta. Phytochemistry 1976;15:1180-1.
8. Fechtal PM, Riedl B. Analyse de extraits tannants des 'ecores des eucalyptus apr es hydrolyse acide par la chromatographie en phase gazeuse couplée avec la spectrometrie de masse (GC–MS). Holzforschung 1991;45:269-73.
9. Conde E, Cadahia E, Diez-Barra R, Garcia-Vallejo MC. Polyphenolic composition of bark extracts from Eucalyptus camaldulensis, E. globulus and E. rudis. Holz Roh Werkstoff 1996;54:175-81.
10. Cadahia E, Conde E, Fernandez de Simon B, Garcia-Vallejo MC. Tannin composition of Eucalyptus camaldulensis, E. globulus and E. rudis. Part II. Bark Holzforschung 1997;51:125-9.
11. Low D, Rawal BD, Griffin WJ. Antibacterial action of the essential oils of some Australian Myrtaceae with special references to the activity of chromatographic fractions of oil of Eucalyptus citriodora. Planta Med 1974;26:184-9.
12. Ramezani H, Singh HP, Batish DR, Kohli RK. Antifungal activity of the volatile oil of Eucalyptus citriodora. Fitoterapia 2002;73:261-2.
13. Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol 2003;89:277-83.
14. Foudill-Cherif Y, Meklati BY, Verzera A, Mondello L, Dugo G. Chemical examination of essential oils from the leaves of nine Eucalyptus species growing in Algeria. J Essent Oil Res 2000;12:186-91.
15. Hasegawa T, Takano F, Takata T, Niiyama M, Ohta T. Bioactive monoterpenic glycosides conjugated with gallic acid from the leaves of Eucalyptus globules. Phytochemistry 2008;69:747-53.
16. Thorn GW, Adams R, Braunwald E, Isselbacher K, Petersdorf R. Harrison’s Principle of Internal Medicine. New York: Mcgraw Hill Co; 1977. p. 1088.
17. Vigar Z. Atlas of Medical Parasitology. 2nd ed. Singapore: P.G. Publishing House; 1984. p. 216.
18. Tambe V, Nirmal S, Jadhav R, Ghogare P, Bhalke R, Girme A, Bhambar R. Anthelmintic activity of Wedelia trilobata leaves. Indian J Nat Prod 2006;22:27-9.
19. Anonymous, Indian Pharmacopoeia. Vol 2, New Delhi: The Controller of Publications; 1996. p. A-137.
20. Wagner H, Bladt S. Plant drug analyses: A thin layer chromatography atlas. 2nd ed, New Delhi, India: Springer; 1996. p. 149.
21. Silvestre AJ, Cavaleiro JA, Delmonb D, Filliatre C, Bourgeois G. Analysis of the variation of the essential oil composition of Eucalyptus globules Labill. from Portugal using multivariate statistical analysis. Ind Crops Prod 1997;6:27-33.
22. Vituro Cl, Molina AC, Heit Cl. Volatile components of Eucalyptus globules Labill. ssp. bicostata from Jujuy, Argentina. J Essent Oil Res 2003;15:206-8.

Source of Support: Nil, Conflict of Interest: None declared.