

accession number AY962268. Liu *et al.* [3] showed that Cypriot Sicilian-like virus strains isolated in 1985 differed from the Sicilian Sabin strain by 11.5% in the M segment. Sequence analysis of all three segments of the strain isolated during the present study is currently in progress and will enable analysis of variation among Cypriot strains isolated during different periods.

The infected soldiers were staying in the Athalassa National Forest Park, situated *c.* 5 km south-east of the capital Nicosia, at an altitude of *c.* 130–190 m. The area has a typical semi-dry Mediterranean climate, with maximum temperatures in July and August of 35°C. The main local vegetation consists of shrubs and herbaceous species, mainly *Tamarix* spp., *Thymus capitatus*, *Zizyphus lotus* and salt cedars. Intensive forestation of the region began in 1904 with the planting of various tree species, mainly pines, eucalyptuses, cypresses, acacias and casuarinas. Geologically, the area is formed from biocalcarenes, sandstones, and sandy marls, so the subsoil has extremely poor moisture-retaining capacity; however, there is a gorge with two streams flowing through the area, with two dams and extensive hygrophytic vegetation nearby. The area seems to be an ideal environment for the sandfly vectors of the virus, which prefer a cool, shaded, slightly damp environment. Only the female sandflies bite, as a blood meal is necessary for ovulation. The flies rest during the day and feed at night. All of the infected patients recalled numerous insect bites.

In conclusion, sandfly fever is endemic in Cyprus, where cases occur sporadically or in outbreaks. Clinicians should consider sandfly fever in the differential diagnosis of patients with symptoms similar to those described above, particularly if the patient has recently visited an endemic region. Measures for the prevention of the disease (e.g., protective clothing and use of insect repellent) should be taken in endemic areas, especially during the summer, when sandflies are active. Further studies are in progress to determine the vector of this virus strain.

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RESEARCH NOTE

Antibacterial effects of *Eucalyptus globulus* leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders

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ABSTRACT

The antibacterial activity of *Eucalyptus globulus* leaf extract was determined for 56 isolates of *Staphylococcus aureus*, 25 isolates of *Streptococcus pyogenes*, 12 isolates of *Streptococcus pneumoniae* and seven isolates of *Haemophilus influenzae* obtained from 200 clinical specimens of patients with respiratory tract disorders. MIC₅₀s for these species were 64, 32, 16 and 16 mg/L, respectively; MIC₉₀s were 128, 64, 32 and 32 mg/L, respectively; and MBCs were 512, 128, 64 and 64 mg/L, respectively. These results suggest that further studies to clarify the possible therapeutic role of *E. globulus* leaf extract in the treatment of respiratory tract infection are warranted.

Keywords Antibacterial activity, *Eucalyptus globulus*, leaf extract, respiratory tract infection, therapy

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Eucalyptus spp. (family Myrtaceae) originated in Australia, but these plants now grow in almost all tropical and sub-tropical areas, and are cultivated in many other climates. Much research has been conducted on the medicinal properties of *Eucalyptus* spp. The leaf extract or essential oil from the leaves of *Eucalyptus* spp. has been reported to possess antifungal, antibacterial, mosquito repellent and antioxidant properties [1–3]. Against this background, the present study investigated the antibacterial effects of *Eucalyptus globulus* leaf extract against pathogenic bacteria isolated from patients with respiratory tract infection.

Clinical specimens from 200 patients (112 male, 88 female; aged 15–62 years) with pharyngitis and other respiratory tract infections who had been referred to the Imam Khomani and Shariati hospitals (Tehran, Iran) were collected between September 2003 and September 2004. The specimens were cultured on blood agar and cefsulodin chocolate agar (Difco, Detroit, MI, USA) containing horse blood 7% v/v, and were incubated at 37°C for 24 h. Identification of bacterial isolates was based on standard biochemical tests [4–7]. The pathogenic bacteria isolated comprised 56 *Staphylococcus aureus*, 25 *Streptococcus pyogenes*, 12 *Streptococcus pneumoniae* and seven *Haemophilus*

influenzae; no growth was observed for 100 (50%) specimens.

E. globulus leaf extract was prepared by the School of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran) by extraction with methanol (80°C) using the percolation method [8,9], dissolved in sterile Mueller–Hinton broth (Difco), with or without horse blood 5% v/v, and then filtered using 0.5-µm filters (Millipore, Billerica, MA, USA). Different concentrations of *E. globulus* leaf extract were used to prepare a series of dilution tubes. For the broth dilution susceptibility tests, a standard inoculum of each microorganism (1×10^6 CFU/mL, equivalent to a 1:500 dilution of a suspension equal to a McFarland standard of 1.0) was added to an equal volume of each concentration of *E. globulus* leaf extract, and to a tube of the growth medium without added leaf extract to serve as a growth control. An uninoculated tube of medium was incubated to serve as a negative control. After incubation for 18–24 h at 37°C, the lowest concentration of the *E. globulus* leaf extract that inhibited growth of the organism was designated as the MIC. Following determination of the MIC, 0.1 mL from each of the tubes without visible turbidity was subcultured on to blood agar and cefsulodin chocolate agar, and then incubated at 37°C for a further 24–48 h. Following incubation, the number of colonies obtained was compared with the number of CFU/mL in the original inoculum. The lowest concentration of leaf extract that allowed <0.1% of the original inoculum to survive was defined as the MBC [5,10]. The results obtained are summarised in Table 1. MIC₅₀, MIC₉₀ and MBC values ranged from 16 to 64 mg/L, from 32 to 128 mg/L, and from 64 to 512 mg/L, respectively, depending on the species.

Staph. aureus, *Strep. pyogenes*, *Strep. pneumoniae* and *H. influenzae* are the most important causes of bacterial infection of the human respiratory tract. The emergence of resistance to conventional

Table 1. The sensitivity of pathogenic bacteria isolated from patients with respiratory tract disorders to *Eucalyptus globulus* leaf extract

Pathogenic bacteria	<i>Eucalyptus globulus</i> leaf extract (mg/L)		
	MIC ₅₀	MIC ₉₀	MBC
<i>Staphylococcus aureus</i> (n = 56)	64	128	512
<i>Streptococcus pyogenes</i> (n = 25)	32	64	128
<i>Streptococcus pneumoniae</i> (n = 12)	16	32	64
<i>Haemophilus influenzae</i> (n = 7)	16	32	64

antimicrobial agents poses a serious problem for physicians, and necessitates ongoing development of new antimicrobial agents that can inhibit the growth of or kill resistant organisms. Eucalyptal (1,8-cineole) is the active ingredient of eucalyptus oil that is responsible for its various pharmacological activities. Pharmacopoeial-grade dried eucalyptus leaf must contain a minimum of 2.0% v/w volatile oil, composed mainly of 1,8-cineole. Oil of eucalyptus has been used traditionally as an antiseptic and in the treatment of respiratory tract infections. However, scientific and toxicological data regarding its antibacterial action are lacking, and its current applications are focused on topical use as an antiseptic [11,12]. The results of the present study with bacteria isolated from patients with respiratory tract infections suggest that further studies to clarify the possible role of *E. globulus* leaf extract in the treatment of respiratory tract infections are warranted.

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