1. Introduction

Eucalyptus oils are obtained by distillation of the leaves of *Eucalyptus* and have aromas characteristic of the particular species used. The oils are classified in the trade into three broad types according to their composition and main end-use: medicinal, perfumery and industrial. Of these, the most important in terms of volume of production and trade is the medicinal type, characterised by a high cineole content in the oil. This, and the perfumery type, are discussed below. The medicinal type of oil may be sold as such, neat, in pharmacies and other retail outlets or in the form of sprays, lozenges, cough sweets and ointments or in formulation with other oils. It is used as an inhalant or chest rub to ease breathing difficulties, as a mouthwash in water to refresh or ease the throat, and as a skin rub to provide relief from aches and pains. Anti-plaque solutions in dental hygiene are a recent application. Although employed for medicinal purposes, the pleasant flavour and fragrance properties of cineole-rich eucalyptus oils play an important role in their acceptance and utilization on such a large scale.

2. Chemical components of Eucalyptus Oil

*Chemical variability, antifungal and antioxidant activity of Eucalyptus camaldulensis essential oil from Sardinia*
Essential oil (EO) from aerial parts of Eucalyptus camaldulensis Dehnh., growing wild in different localities of Sardinia (Italy), was extracted by steam distillation and analyzed by gas chromatography (GC) FID and GC-ion trap mass spectrometry (ITMS). The yields of EO (v/dry wt) ranged between 0.2-0.5%. Samples were harvested between April and December to study the seasonal chemical variability of the EO. The chemical composition varied depending on the different origins and showed strong fluctuation during the vegetative stage. Thirty-seven compounds, accounting for at least 97.7% of the total EOs were identified, the major components being p-cymene (27.8-42.7%), 1,8-cineole (4.1-39.5%), beta-phellandrene (3.9-23.8%), spathulenol (2.1-15.5%) and cryptone (3.2-10.2%). The oils possessed moderate amounts (1.4-4.7%) of two uncommon aldehydes, cuminal and phellandral. The essential oils were screened for their antifungal activities against common phytopathogenic fungi using the paper disk diffusion method and they showed activity at low doses against the fungi tested. The antioxidant activity, assessed by DPPH-test and expressed as Trolox equivalent antioxidant capacity, showed values ranging between 0.5 and 5.8 mmol/L.

**Extraction and determination of volatile constituents in leaves of Eucalyptus citriodora**

The volatile constituents in leaves of Eucalyptus citriodora, including oil fraction and water-soluble fraction, were extracted and determined. Oil fraction of volatile components was obtained through steam distillation. Ether was used as the solvent to extract the water-soluble fraction of volatile compounds from the liquid left after steam distillation in order to know the quantity and constituents of volatile compounds dissolved in the water phase. The oil yield in the oil fraction was 1.36%, and the oil yield in the water-soluble fraction was 0.48% (both on fresh weight basis). Both oil fraction and water-soluble fraction were analyzed by gas chromatography-mass spectrometry (GC-MS) method. The results showed that 37 compounds (97.36%) in the oil fraction and 10 compounds (82.05%) in the water-soluble fraction were identified. There were 12 hydrocarbon compounds and 25 oxygenated compounds identified in oil fraction. The major constituents in oil fraction were citronellal (57.00%), followed by citronellol (15.89%) and citronellyl acetate (15.33%). Alcohols dominated the compounds in water-soluble fraction. cis-p-Menthane-3, 8-diol (53.43%) and trans-p-menthane-3, 8-diol (16. 48%) were found to be the major compounds, which have
the activity to repel insects. It is concluded that the comprehensive utilization value of leaves of Eucalyptus citriodora was enhanced owing to the extraction of water-soluble volatile components.

*Analysis of essential oil from the leaves and fruits of Eucalyptus tereticornis in Guangxi province by GC-MS*

The objective of the study was to analyze the chemical composition and their relative contents of essential oil from the leaves and fruits of Eucalyptus tereticornis in Guangxi province. The column temperature was controlled by a program with a capillary column HP-5 MS, and the MS analysis was performed with EI and quadrupole mass analyzer. The chemical compositions were identified by NIST98 searching and mass spectra comparing, and their relative contents were determined by using normalization method of chromatographic peak areas. Thirty nine compounds constituting 96.69% of the oil of leaves were identified. The major components were eucalyptol (27.93%), 1R-alpha-pinene (22.60%), isopinocarveol (8.71%); 36 compounds constituting 93.50% of the oil of fruits were identified. The major components are 1R-alpha-pinene (32.88%), eucalyptol (13.64%), D-limonene (8.31%).

*Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus*

The leaf-essential oil from Eucalyptus robusta and E. saligna, the latter in two phenologic stages, were analysed by gas chromatography coupled to mass spectrometry (GC-MS). The major constituent in E. robusta oil was the monoterpane alpha-pinene (73.0% of the total amount). The oil composition of the E. saligna was dependent of the phenologic stage. In the vegetative phase, the major constituents were p-cymene (54.2%) and gamma-terpinene (43.8%), while during the blossoming alpha-pinene became the major constituent followed by p-cymene (22.5%). Additionally, the antimicrobial activity for all three oils was evaluated against Staphylococcus aureus, Escherichia coli and Candida albicans. The E. robusta oil presented the highest growth inhibition for all the microorganisms tested.
3. Clinical studies

Antioxidant activity

Characterization and antioxidant activity of essential oils from fresh and decaying leaves of Eucalyptus tereticornis

The composition of essential oils hydrodistilled from fresh and decaying leaves of Eucalyptus tereticornis was analyzed by means of gas chromatography and mass spectrometry, and a total of 68 constituents were identified. The essential oils were assayed for antioxidant activity in terms of scavenging of 2,2-diphenyl-1-picrylhydrazil (DPPH) and hydroxyl (OH(*)) radical, and superoxide anion (O2(-*)). The major constituents of the fresh leaf oil were alpha-pinene (28.53%) and 1,8-cineole (19.48%), whereas in the decaying leaf oil, beta-citronellal (14.15%), (-)-isopulegol (13.35%), and (+)-beta-citronellol (10.73%) were the major components. Both essential oils exhibited a strong radical scavenging activity against DPPH radical with IC50 values of 110 and 139.8 microg/mL for fresh and decaying leaf oil, respectively (IC50 of BHT = 164.2 microg/mL). Further, the essential oils (at 400 microg/mL) also exhibited OH(*) (56-62%) and O2(-*) (65-69%) scavenging activity parallel to the commercial antioxidant BHT/ascorbic acid. However, unlike the essential oils, the major monoterpenic constituents exhibited significantly less scavenging activity (<35% DPPH or OH(*); at 400 microg/mL). The study concluded that fresh and decaying leaves of E. tereticornis are a source of monoterpenoid rich oil exhibiting antioxidant activity.

Immunomodulatory, anti-inflammatory and analgesic activities
Antioxidant properties of essential oils. Possible explanations for their anti-inflammatory effects

Pathogenesis and symptoms of inflammatory processes are accompanied and/or initiated by the production of reactive oxygen species (ROS). The effects of essential oils on these processes have been studied with the aid of biochemical model reactions simulating these pathological events. It can be shown that Myrtol Standardized and Eucalyptus oil ameliorate inflammatory processes by interacting with aggressive oxygen radicals of the OH.-type and interfere with leukocyte activation. These activities partially allow attenuation of oxidative attack and damage introduced by infections or environmental impacts.

Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices

Eucalyptus oil (EO) and its major component, 1,8-cineole, have antimicrobial effects against many bacteria, including Mycobacterium tuberculosis and methicillin-resistant Staphylococcus aureus (MRSA), viruses, and fungi (including Candida). Surprisingly for an antimicrobial substance, there are also immune-stimulatory, anti-inflammatory, antioxidant, analgesic, and spasmolytic effects. Of the white blood cells, monocytes and macrophages are most affected, especially with increased phagocytic activity. Application by either vapor inhalation or oral route provides benefit for both purulent and non-purulent respiratory problems, such as bronchitis, asthma, and chronic obstructive pulmonary disease (COPD). There is a long history of folk usage with a good safety record. More recently, the biochemical details behind these effects have been clarified. Although other plant oils may be more microbiologically active, the safety of moderate doses of EO and its broad-spectrum antimicrobial action make it an attractive alternative to pharmaceuticals. EO has also been shown to offset the myelotoxicity of one chemotherapy agent. Whether this is a general attribute that does not decrease the benefit of chemotherapy remains to be determined. This article also provides instruction on how to assemble inexpensive devices for vapor inhalation.
Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial

Airway hypersecretion is mediated by increased release of inflammatory mediators and can be improved by inhibition of mediator production. It was recently reported that 1.8-cineol (eucalyptol), which is known as the major monoterpen of eucalyptus oil, suppressed arachidonic acid metabolism and cytokine production in human monocytes. Therefore, the aim of this study was to evaluate the anti-inflammatory efficacy of 1.8-cineol by determining its prednisolone equivalent potency in patients with severe asthma. Thirty-two patients with steroid-dependent bronchial asthma were enrolled in a double-blind, placebo-controlled trial. After determining the effective oral steroid dosage during a 2 month run-in phase, subjects were randomly allocated to receive either 200 mg 1.8-cineol t. i.d. or placebo in small gut soluble capsules for 12 weeks. Oral glucocorticosteroids were reduced by 2.5 mg increments every 3 weeks. The primary end point of this investigation was to establish the oral glucocorticosteroid-sparing capacity of 1.8-cineol in severe asthma. Reductions in daily prednisolone dosage of 36% with active treatment (range 2.5-10 mg, mean: 3.75 mg) vs. a decrease of only 7% (2.5-5 mg, mean: 0.91 mg) in the placebo group (P = 0.006) were tolerated. Twelve of 16 cineol vs. four out of 16 placebo patients achieved a reduction of oral steroids (P = 0.012). Long-term systemic therapy with 1.8-cineol has a significant steroid-saving effect in steroid-depending asthma. This is the first evidence suggesting an anti-inflammatory activity of the monoterpen 1.8-cineol in asthma and a new rational for its use as mucolytic agent in upper and lower airway diseases.

Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes in vitro

Cineole (eucalyptol) is the isolated active agent of eucalyptus oil. Traditionally, it is recommended for treating the symptoms of airway diseases exacerbated by infection. The inhibitory effect of 1.8-cineole on LPS-and IL1beta-stimulated mediator production by human monocytes in vitro was examined. For the first time, it is reported on a dose-dependent and highly significant inhibition of production of tumor necrosis factor-alpha, interleukin-1beta, leukotriene B4 and thromboxane B2 by 1.8-cineole. In summary, this is
the first report on a new mechanism of action of monoterpines suggesting 1.8-cineole as a strong inhibitor of cytokines that might be suitable for longterm treatment of airway inflammation in bronchial asthma and other steroid-sensitive disorders.

**Antinociceptive properties of 1,8-Cineole and beta-pinene, from the essential oil of Eucalyptus camaldulensis leaves, in rodents**

1,8-cineole (cineole) and beta-pinene, two monoterpines isolated from the essential oil obtained from Eucalyptus camaldulensis Dehn leaves were tested for antinociceptive properties. Tail-flick and hot-plate methods, reflecting the spinal and supraspinal levels, respectively, were used in mice and/or rats using morphine and naloxone for comparison. Cineole exhibited an antinociceptive activity comparable to that of morphine, in both algesic stimuli. A significant synergism between cineole and morphine was observed, but naloxone failed to antagonize the effect of cineole. Beta-pinene exerted supraspinal antinociceptive actions in rats only and it reversed the antinociceptive effect of morphine in a degree equivalent to naloxone, probably acting as a partial agonist through the mu opioid receptors. From structure-activity relationships of the pairs morphine+cineole and naloxone+beta-pinene, it was shown that similarities exist in the stereochemistry and in the respective atomic charges of these molecules. Further studies are in progress in order to elucidate the mechanism of action of the two terpenoids.

**Effect of Eucalyptus globulus oil on lipopolysaccharide-induced chronic bronchitis and mucin hypersecretion in rats**

The aim of this study is to see the effect of Eucalyptus globulus oil on bronchiolitis and mucin hypersecretion in chronic bronchitis induced by lipopolysaccharide in rats. Rat model was established by intratracheal instillation of lipopolysaccharide 0.2 mg. Pathological changes, alteration in bronchoalveolar lavage fluid (BALF) and immunohistochemistry characters were examined after 3 weeks and the effect of E. globulus oil was observed. Characters of pathological manifestations of chronic bronchitis were found after instillation of LPS. Inflammatory cell infiltration and bronchiolitis severity were significantly reduced
after administration of E. globulus oil. Especially in 300 mg x kg(-1) treated rats, there were significant decreases of mucin content in BALF and MUC5ac expression in trachea and bronchiole epithelium. Optical density and mucins area% detected by image analysis system were apparently lower than those in model group. As concluded by the results, E. globulus oil has the anti-inflammatory effect on chronic bronchitis induced by lipopolysaccharide in rats and the inhibitio effect on hypersecretion of airway mucins.

**Analgesic and anti-inflammatory effects of essential oils of Eucalyptus**

Many species of the genus Eucalyptus from the Myrtaceae family are used in Brazilian folk medicine for the treatment of various medical conditions such as cold, flue, fever, and bronchial infections. In the current investigation, we evaluated the analgesic and anti-inflammatory effects of essential oil extracts from three species of Eucalyptus employing various standard experimental test models. Using acetic acid-induced writhes in mice and hot plate thermal stimulation in rats, it was shown that the essential oils of Eucalyptus citriodora (EC), Eucalyptus tereticornis (ET), and Eucalyptus globulus (EG) induced analgesic effects in both models, suggesting peripheral and central actions. In addition, essential oil extracts from the three Eucalyptus species produced anti-inflammatory effects, as demonstrated by inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan, and vascular permeability induced by carrageenan and histamine. However, no consistent results were observed for some of the parameters evaluated, both in terms of activities and dose-response relationships, reflecting the complex nature of the oil extracts and/or the assay systems used. Taken together, the data suggest that essential oil extracts of EC, ET, and EG possess central and peripheral analgesic effects as well as neutrophil-dependent and independent anti-inflammatory activities. These initial observations provide support for the reported use of the eucalyptus plant in Brazilian folk medicine. Further investigation is warranted for possible development of new classes of analgesic and anti-inflammatory drugs from components of the essential oils of the Eucalyptus species.
Antibacterial, antifungal, antiviral and anticancer activities

Eucalyptus species leaves have been traditionally used to heal wounds and fungal infections. Essential oils and extracts of some Eucalyptus species possess antimicrobial and antitumor properties. It was sought to determine antimicrobial and cytotoxic activities of oils and extracts of leaves, stems, and flowers of Eucalyptus sideroxylon and Eucalyptus torquata grown in Egypt. An agar diffusion method was used to analyze antimicrobial activities of essential oils and extracts of Eucalyptus against medically important gram-positive and gram-negative bacteria. A sulphorhodamine B assay was used to analyze the in vitro cytotoxic activities of oils and extracts against Human hepatocellular carcinoma cell line (HEPG2), and Human breast adenocarcinoma cell line (MCF7). Gram-positive bacteria were highly susceptible to oils and extracts of both Eucalyptus species. With the exception of Escherichia coli, gram-negative bacteria were resistant to extracts, but susceptible to the oil obtained from at least one organ of E sideroxylon and E torquata. Although Aspergillus flavus and Aspergillus niger were resistant to the extracts, essential oils of E sideroxylon and E torquata generally exhibited moderate to high antifungal activities against Candida albicans, A flavus and A niger. Oils of E torquata stems exhibited cytotoxic activities on MCF7 cells followed by oils of E torquata leaves and E sideroxylon leaves. However, oils from both species failed to exert cytotoxic effects on HEPG2 cells. This was the first report of antimicrobial and antitumor properties of E sideroxylon and E torquata. Results suggest a wider use of Eucalyptus species products in pharmaceutical, cosmetic, and food preparations.

Broad spectrum herbal therapy against superficial fungal infections
Skin disease associated with keratinized tissues in animal and human beings has been investigated. The essential oil of Eucalyptus pauciflora in vitro showed strong antifungal activity at 1.0 microl/ml against human pathogenic fungi, viz. Epidermophyton floccosum, Microsporum audouinii, M. canis, M. gypseum, M. nanum, Trichophyton mentagrophytes, T. rubrum, T. tonsurans and T. violaceum. The oil has heavy doses of inoculum potential at 1.0 microl/ml. Moreover, it did not exhibit any adverse effects on mammalian skin up to 5% concentrations. Further, the oil was formulated in the form of ointment 'BSHT' (broad spectrum herbal therapy) (1% v/v) and subjected it to topical testing on patients attending the outpatient department of M.L.N. Medical College, Allahabad. Fifty patients were selected on the basis of KOH-positive results and diagnosed as either tinea pedis, tinea corporis or tinea cruris. After the second week of treatment, all patients were KOH-negative. At the end of medication, 60% of patients recovered completely and 40% showed significant improvement from the disease. No KOH-negative cases of relapse were observed when patients were re-examined after 2 months following the end of treatment. Thus, the ointment can be exploited commercially after undergoing successful multicenter clinical trials, which are in progress.

Antibacterial essential oils in malodorous cancer patients: clinical observations in 30 patients

Malodorous necrotic ulcers in cancer patients are of major concern as it leads to social isolation and poor quality of life. Current medications and topical therapies have proven inadequate in their ability to reduce foul smell to acceptable levels. The positive experience in using antibacterial essential oils in patients with incurable head and neck cancer and associated malodorous necrotic ulcers is being reported. All patients received a standard course of therapy with oral or systemic antibiosis. In addition, the ulcers were rinsed with an antibacterial essential oil mix (mainly based on Eucalyptus oil) twice a day. All patients experienced complete resolution of the foul smell by only the third or fourth day of therapy. As a secondary effect it was seen that besides smell reduction the oils had anti-inflammatory effects on neoplastic ulcers. In some patients ulcers started to heal and achieved complete re-epithelialization. The patients experienced great personal relief upon
resolution of their malodorous conditions. Quality of life improved significantly with the resulting reintroduction of social contact with friends and relatives.

Effect of eucalyptus essential oil on respiratory bacteria and viruses

The activity of Eucalyptus globulus essential oil was determined for 120 isolates of Streptococcus pyogenes, 20 isolates of S. pneumoniae, 40 isolates of S. agalactiae, 20 isolates of Staphylococcus aureus, 40 isolates of Haemophilus influenzae, 30 isolates of H. parainfluenzae, 10 isolates of Klebsiella pneumoniae, 10 isolates of Stenotrophomonas maltophilia and two viruses, a strain of adenovirus and a strain of mumps virus, all obtained from clinical specimens of patients with respiratory tract infections. The cytotoxicity was evaluated on VERO cells by the MTT test. The antibacterial activity was evaluated by the Kirby Bauer paper method, minimum inhibitory concentration, and minimum bactericidal concentration. H. influenzae, parainfluenzae, and S. maltophilia were the most susceptible, followed by S. pneumoniae. The antiviral activity, assessed by means of virus yield experiments titered by the end-point dilution method for adenovirus, and by plaque reduction assay for mumps virus, disclosed only a mild activity on mumps virus.

Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures

Effective disinfection and antisepsis is pivotal in preventing infections within the healthcare setting. Chlorhexidine digluconate (CHG) is a widely used disinfectant/antiseptic possessing broad-spectrum antimicrobial activity; however, its penetration into bacterial biofilms and human skin is poor. The aim of this study was to investigate the antimicrobial efficacy of crude eucalyptus oil (EO) and its main component 1,8-cineole (a recognized permeation enhancer), alone and in combination with CHG, against a panel of clinically relevant microorganisms grown in planktonic and biofilm cultures. MICs and minimum bactericidal/fungicidal concentrations were determined for each microorganism grown in suspension and biofilm using microbroth dilution and ATP bioluminescence, respectively.
Chequerboard assays were used to determine synergistic, indifferent or antagonistic interactions between CHG and EO or 1,8-cineole. Antimicrobial activity was demonstrated by CHG, EO and 1,8-cineole; however, CHG was significantly more active against microorganisms in both planktonic and biofilm modes of growth (P < 0.05). Crude EO was significantly more efficacious against microorganisms grown in suspension compared with 1,8-cineole (P < 0.05). Synergistic activity was demonstrated between CHG and both EO and 1,8-cineole against suspensions of Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), Escherichia coli and Candida albicans, and biofilm cultures of MRSA and Pseudomonas aeruginosa. In conclusion, CHG may be combined with either crude EO or its major component 1,8-cineole for enhanced, synergistic antimicrobial activity against a wide range of microorganisms in planktonic and biofilm modes of growth; however, the superior antimicrobial efficacy associated with crude EO alone, compared with 1,8-cineole, favours its combination with CHG.

Comparative study on the antiviral activity of selected monoterpenes derived from essential oils

Essential oils are complex natural mixtures, their main constituents, e.g. terpenes and phenylpropanoids, being responsible for their biological properties. Essential oils from eucalyptus, tea tree and thyme and their major monoterpane compounds alpha-terpinene, gamma-terpinene, alpha-pinene, p-cymene, terpinen-4-ol, alpha-terpineol, thymol, citral and 1,8-cineole were examined for their antiviral activity against herpes simplex virus type 1 (HSV-1) in vitro. These essential oils were able to reduce viral infectivity by >96%, the monoterpenes inhibited HSV by about >80%. The mode of antiviral action has been determined, only moderate antiviral effects were revealed by essential oils and monoterpenes when these drugs were added to host cells prior to infection or after entry of HSV into cells. However, both essential oils and monoterpenes exhibited high anti-HSV-1 activity by direct inactivation of free virus particles. All tested drugs interacted in a dose-dependent manner with herpesvirus particles thereby inactivating viral infection. Among the analysed compounds, monoterpenic hydrocarbons were slightly superior to monoterpane alcohols in their antiviral activity, alpha-pinene and alpha-terpineol revealed the highest selectivity index. However, mixtures of different monoterpenes present in
natural tea tree essential oil revealed a ten-fold higher selectivity index and a lower toxicity than its isolated single monoterpenes.

**Myorelaxant effect**

*Relaxant effects of the essential oil of Eucalyptus tereticornis and its main constituent 1,8-cineole on guinea-pig tracheal smooth muscle*

The effects of the essential oil of Eucalyptus tereticornis Sm. (EOET) on guinea-pig tracheal smooth muscle were investigated. EOET (10 - 1000 microg/mL) relaxed the tracheal basal tonus with an EC (50) value of 125.3 [52.2 - 300.9] microg/mL. Its maximal relaxation (40 +/- 6 %) was significantly lower than that evoked by aminophylline (209 +/- 34 %). The K (+)-(60 mM)-induced contractions were significantly reduced by both EOET (200 - 1000 microg/mL) and its main constituent 1,8-cineole (600 - 1000 microg/mL). Acetylcholine (1 microgM)-induced contractions were significantly enhanced by 1,8-cineole (10 - 1000 microg/mL). However, they were significantly enhanced and reduced by lower (200 - 400 microg/mL) and higher (800 - 1000 microg/mL) concentrations of EOET, respectively. Electrical field stimulation-induced contractions were significantly increased by EOET (100 - 600 microg/mL). In conclusion, EOET produces myorelaxant effects on guinea-pig isolated trachea, an effect that seems to result from a complex interaction between its monoterpenoid constituents.

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