

## Extraction and characterization of Eucalyptus leaves oil (Sudan)

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### Abstract

Eucalyptus is an evergreen tree known in Sudan as Caphour or Ban tree. The aim of this work was to extract and determine the constituents of Eucalyptus leaves essential oil. The plant leaves were collected from Shambat farms at Khartoum North and shed dried. The dry leaves essential oil was extracted by hydro-distillation and the oil yield was determined. GC/MS analysis was carried for the extracted oil. The oil yield was (4.29%), density (0.67g/cm<sup>3</sup>) and refractive index (1.473). The GC/MS identification showed the presence of seventy (70) compounds dominated by, alpha-phellandrene (26.36%), benzene 1-methyl-3-(1-methyl ethyl) (18.48%), 1,8-cineol (12.74%), beta.- phellandrene (8.29%), bicyclo [3.1.0]hex-2-ene, methyl-5- (1-me), 1H-Cycloprop [e]azulen-7-ol,decahydro-1 (2.12%) and globulol (1.98%).

**Keywords:** Eucalyptus microthecae, Oil yield, GC/MS, Antioxidant,  $\alpha$ - phellandrene

### Introduction

#### Plant origin

Eucalyptus is a naturally growing tree in Australia but, today it is distributed in many different tropical and subtropical climates, representing more than 900 species which are mainly cultivated for their essential oils (Singab *et al* 2011, Hanene Ghazghazi *et al.*, 2019, Ghareeb *et al.*, 2018, Havence, *et al.*, 2019) [6, 7, 24]. Eucalyptus was described as one of the important genera of *Myrtaceae* family as a large genus of evergreen trees and shrubs that have their native origin in Australia and Tasmania (Oyededeji 1999 [16], Batish *et al.*, 2008). According to Muhidin *et al.*, (2024) Eucalyptus is one of the most important genotype of the *myrtaceae* family which composes 140 genera and 3800 species distributed in tropical subtropical regions. In China, India, South Africa, Portugal, Brazil and Tasmania Eucalyptus is cultivated to produce essential oil on commercial scale (Shubhreet Kaur *et al.*, 2019) [23]. Eucalyptus essential oil is used in folk medicine and a folk cosmetic owing to it is antioxidant values (Sharma *et al.*, 2021) [21].

#### Plant names

Eucalyptus species names include rive red gum (Ghareeb *et al.*, 2018) [6], forest red gum (Shubhreet Kaur *et al.*, 2019) [23] and lemon scented gum (Imoisi and Okhale., 2024) [9].

#### Chemical properties

Eucalyptus leaves extract was reported to possess antioxidant, cytotoxic, antimicrobial, larvicidal, pesticidal and anti-dermatophytes activities and rich with bioactive molecules like gallic acid, toxifulin quercetin leuteolin and hesperidin (Ghareeb *et al.*, 2018) [6]. The ethanolic leaves extract was described as a good source of natural antioxidant and antimicrobial agents (Hanene Gh. *et al.*, 2019). Six phenolic compounds were identified in ethyl acetate extract which may be responsible for the reported bioactivities (Ghareeb *et al* 2018) [6]. Depending on Shubhreet Kaur *et al.*, (2019) [23] Eucalyptus leaves extract contains flavonoids as (35.88%), phenol (11.41%) in addition to saponin, quinone, carbohydrate, tannin, fat and antioxidant activity as (444.60). Eucalyptus fresh leaves oil

was reported to possess free radical scavenging potential, flavonoids, saponin, terpenoids and reducing sugars (mishra *et al*; 2010). N.Z. Immaroh *et al* (2021) [8, 13] reported the presence of 1,8-cineole, monoterpene, sesquiterpene, aldehyde and ketone as secondary metabolites in *Eucalyptus* leaves. E. leaves extract showed antioxidant, antiseptic, anti-inflammatory and antimicrobial activities, whereas the essential oil constituents showed herbicidal, insecticidal, antitumor and anti-leach activity (Kiryou 2005) [11].

#### Traditional and medicinal uses

In folk medicine Throughout the world Eucalyptus essential oil was used as anti-inflammatory, analgesic and antipyretic remedy to relieve rheumatism, muscular pain, cold and respiratory infections (Hanene *et al* 2019, Shubhreet Kaur *et al.*, 2019) [23].

Traditionally, eucalyptus oil was strongly recommended as antimalaria, antiseptic, antispasmodic, antiviral, astringent, balsamic, decongestant, deodorant, depurative, diuretic, expectorant, febrifuge, hypoglycemic and rubefacient stimulant (Mirsha *et al.*, 2010). Eucalyptus species have potential usefulness as microbiostatic, antiseptic or as disinfectant agents and as effective ingredient in nasal decongestant to treat colds, flu and skin infections (Bashir and benali 2008, Imoisi and okhale, 2024) [3, 9]. Eucalyptus species extract and essential oils were used as food additives and employed in modern pharmaceutical and cosmetic industries (Hanene *et al*, 2019). Eucalyptus oil is employed as a major component in pharmaceutical preparations as anti-diabetic, anti-oxidative, antiviral, anti-bacterial, anti-fungal, anti-tumor, antihistaminic anti-cancer and to reduce the symptoms of colds and influenza (Raho and Benali M, 2012, sharma A. D. *et al* 2021) [17, 21]. Due to its antimicrobial, antifungal and insecticidal properties Eucalyptus citriara leaves oil found diverse application in traditional medicine (Imoisi and Okhale 2024) [9]. In Ethiopia eucalyptus *Camaldulensis* leaves are used to treat stomach ache, cough, swelling, wounds and eye infections, whereas it is essential oil was reported to possess many activities including antioxidant properties, antinociceptive activity ulcer healing-promoting activity and antibacterial activity (Muhidin *et al* 2024).

## Eucalyptus leaves oil

Eucalyptus essential oil is colorless liquid which contains volatile and aromatic compounds which may be obtained from plant seeds, flowers bark, stem and leaves (Immaroh *et al* 2021) [8]. Essential oils are complex mixture of hydrocarbons, aromatic derivatives, terpenes and oxygenated derivatives like mono-terpenes, sesqui-terpenoids, alcohols and esters (Sharma A. D. *et al* 2021) [21]. Eucalyptus essential oils possess various biological activities like antimicrobial, antiviral, fungicidal, insecticidal and herbicidal (Sharma AD *et al.*, 2021) [21]. E. leaves oil yield was reported by Hanene *et al* (2019) as (0.23- 0.25 %W/W) stating that, oil yield can be affected by maturity stage, extraction method, type of solvent, extraction conditions and environmental or genetic factors. Eucalyptus leaves oil yield was reported by Sharma A. D. (2021) [21] as (0.133%W/W). In a study of Muhammad A. A. *et al.*, (2025), *E. microtheca* oil exhibited high abundance of oxygenated sesquiterpenes (29.09%) and low abundance of sesquiterpenes (9.68%). Imosis and Okhale (2024) [9] reported hydrodistilled oil yield as (3.5%V/W). According to Hanene *et al* (2019) *E. marginata* leaves oil is dominated by 1,8-cineol (27.74%), propanal,2-methyle-3-phenyl (18.40%), cryptone (12.69%), (–)-spathulenol (7.22%), 2,5 diethyle-phenol (7.57%), 4-terpinol (5.25%) and p-cymene (5.17%), whereas *E. paniculata* leaves oil showed significantly different constituents including Globulol (54.86%), -Gurjunene (24.11%), 2-isopropyl-5-methyl-3-cyclohexen-1-one (8.58%), P-cymene (8.13%) and 1-Iodoctadan (1.18%). Essential oil constituents may differ due to genetic differences, isolation techniques, harvesting period, climatic conditions and soil characteristics (R.A. Ford *et al* 1988, P.Sartorelli *et al* 2007) [5, 20], Muhammad A. A. *et al.*, (2025) reported that, (1,8-cineole) is the dominant constituent in *E. microtheca* oil as (23.57%). Immaroh *et al* (2021) [8] described the major constituents of *E. globulus* leaves oil as 1,8 cineole (51.25%), camphor (9.58%), borneol (7.63%) and camphene (3.77%). Sharma *et al.*; (2021) [21] reported the major constituents of *E. leaves* oil as tannic acid (14%), vanillic acid (6.7), ascorbic acid (4.7%) and rutin (1.19%) by FPLC analysis, whereas GC-FID analysis indicated the presence of 42 compounds in *E. leaves* oil dominated by 1,8 cin (29%), cineol (29.00%), -pinene (16.9%), -pinene (16.00%), myrcene (7.22%), -pinene epoxide (10.73%) and eugenol (3.78%). According to S. Inouyea *et al.*, (2001) [10] *E. species* leaves oil constituents were dominated by 1,8-cineole (74.3%) but, the main contributor for bioactivity was alpha- terpineol (10.3%). Twenty nine constituents were identified by Imosis and Okhale (2024) [9] in

*E. citriodora* essential oil dominated by citronellal (46.87/isopulegal (7.68), citronellol (7.47%), 3-tetradecanal (4.98%) and citronellic acid (4.31%), suggesting that, the *E. species* essential oils may differ depending on geographics, environmental conditions and extraction methods. The major constituents of *E. leaves* oil can be classified into oxygenated mono-terpenes (28.57%), monoterpene hydrocarbons (21.42), oxygenated sesquiterpanes (21.42), monoterpene alcohol (0.071%) and other compounds (14.28%) (Imosis and Okhale, 2024) [9].

## Methodology

Eucalyptus leaf samples were collected from Shambat farm at Khartoum North (Sudan), dried at room temperature and one kilo gram of the dry leaves was extracted by hydrodistillation. The oil yield percentage, refractive index and density were determined. The constituents of the extracted oil were identified by GC/MS analysis.

## Results and discussion

In this study the essential oil yield of eucalyptus *microtheca* leaves was (4.29ml/100g). The extracted oil density was (0.67) and its refractive index was (1.474g/cm<sup>3</sup>). The relatively high oil yield (4.29% v/w) may indicate the suitability of the Sudanese eucalyptus *microtheca* leaves as a rich and cheap source for producing Eucalyptus essential oil in commercial scales. The ester number of eucalyptus oil was found to be (21.60), Saponification value (22.4). *E. microtheca* leaves oil applications may include, house hold products, perfumes, detergents, soaps, lotions, in addition to use as natural additive in foods, drinks, meat products, ice cream and confectionaries (Ali and Mohammed, 2020) [1]. The oil yield percentage in this study may nearly agree with that reported by Ali and Mohammed (2020) [1] as (5ml/100g) for *E. microtheca* leaves. Relatively lower range of *E. leaves* oil yield was described by N. Z. Immorah *et al.*, (2021) as (3.1 to 3.8%v/w).

Imosi and Okhale (2024) [9] reported oil yield as (3.5%v/w) for *E. Citriodora* leaves. Surbhi *et al.*, (2021) an oil yield range of (2.48 to 6.1%v.w) for eucalyptus *cinera* leaves. Muhaidin *et al.*, (2024) [14] reported leaves oil yield for two Eucalyptus species as (2.9 and 3.2% v/w). Significantly high eucalyptus leaves oil yield was reported by Durey and Sahu (2024) [4] as (8.48% v/w), Lemonene (5.44%) and globulol (3.05%).

Table (1) shows that the major components of eucalyptus oil were  $\alpha$ - phellandrene (26.35%), benzene, 1-methyl-3-(1-methylethyl) (18.48%), eucalyptol (12.74%), and bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methyl ethyl) (5.26%).

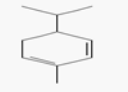
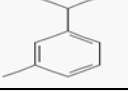

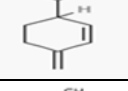

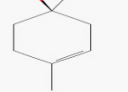
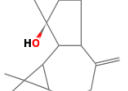
**Table 1:** The major constituents of *E. microtheca* leaves

Compound	Area%
$\alpha$ -phellandrene	26.35
(m-cymene) Benzene, 1- (methyl-3-(1-methylethyl)	18.48
Eucalyptol (1,8-cineole)	12.74
$\beta$ -phellandrene	08.29
( $\alpha$ -Thujene) bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1- methyl ethyl)	05.26
3-cyclohexen-1-ol,4-methyl-1-(1-methylet)-,R-	2.39
1H-Cycloprop [e]azulen-7-ol,decahydro-1,	2.12
Gamma- terpinene	1.23
Glbulol	1.98
Alpha-pinene	1.81
Alloaromadendrene	1.71
Gamma-Elemene	1.60
Beta Myrecene	1.44
Gamma terpinene	1.23
$\beta$ -Chamigrene Spiro [5.5]undec-2-ene,3,7,7-trimethyl-11-	1.05

Trace level of ( $\alpha$ -phellandrene was reported as 0.44%) by Hanene *et al.*, (2019) in *E. Pauciflora* leaves oil compared with the percentage of 1,8-cineole in *E. marginata* leaves oil compared with the percentage of 1,8-cineole in *E. marginata* leaves oil (27.27%) and globulol availability was (1.98%). Muhidin *et al.*, (2024) didn't reports phellandrene in two eucalyptus species leaves oil where 1, 8-cineole was the major constituent as (62.86 and 93.9%) followed by  $\alpha$ -pinene and p-cymene. Phellandrene was not detected also by Sharma *et al.*, (2021) [21] who described the isomers  $\alpha$ -pinene (16.9%) and  $\beta$ -pinene (16.01%) as the major constituents in *E. leaves* oil representing (32.91%) of the total, whereas 1,8-cineole was (28.94%),  $\alpha$  pinene epoxide (10.73%),  $\beta$ -myrcene (7.22%) and eugenol (3.78%).

N. Z. Immorah *et al.*, (2021) reported 1,8-cineole in *E. globulus* leaves oil as (51.25%) followed by camphor (9.58%),  $\alpha$ -pinene (9.22%) borneol (7.63%) and camphene (3.77%). Thujene (bicyclo [3.1.0] hex-2-ene, 2-methyl-5-(1-methyl ethyl)) is a natural flavoring obtained from eucalyptus, it is a natural organic compound classified as a monoterpene. It forms a major part of the constituents in essential oils of a variety of plants, and contributes a strong spicy flavor of certain herbs (Rofida Abd Allah Mohammed, 2018) [18]. Sonia Ben Rabeh, *et al.*, (2025) [25], studied different species of *Eucalyptus* and concluded that, the main bioactive compounds including, 1,8-cineole,  $\alpha$ -pinene, spathulenol, and  $\beta$ -eudesmol.

**Table 2:** shows the structures of some constituents of *Eucalyptus microtheca* leaves extracted oil.

Name	Structure
$\alpha$ -phellandrene	
Benzene, 1-(methyl-3-(1-methylethyl)-	
Eucalyptol	
$\beta$ -phellandrene	
Bicycle [3.1.0]hex-2-ene Methyl-5-(1-met)	
3cyclohexen-1-ol, 4methyl-1-(1-methyle)	
1H-Cycloprop [e] azulene-7-ol,decahydro-	

Significant qualitative and quantitative differences in *E. microtheca* leaves oil composition were described by Ali and Mohammed (2020) [1] where the highest available constituents were camphene (20.60%), 4-carene (18.53%), 1,8-cineole (11.96%), terpin-4-ol (8.7%), p-cymene (8.39%),

$\beta$ -pinene (4.18%),  $\alpha$ -pinene (3.69%) and  $\alpha$ -copaene (3.41%). In this study 1, 8-cineole was (12.74%) and  $\alpha$ -pinene (1.81%). Imoisi and Okhale reported citronella as the major constituent of *E. citriodora* leaves oil (46.87%) in addition to isopulgol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%), citronellic acid (4.31%) and p-methane-3,8-diol (3.83%). Maghsoodlou M. T. *et al.*, (2015) [12] reported that, the major constituents of *E. microtheca* as,  $\alpha$ -phellandrene (16.487%), aromadendrene (12.773%),  $\alpha$ -pinene (6.752%), globulol (5.997%), ledene (5.665%), P-cymen (5.251%), and  $\beta$ -pinene (5.006%). The chemical composition of eucalyptus leaves essential oils may strongly reflect the genetic and environmental differences between *Eucalyptus* species.

## Conclusion

- In this study phellandrene was the highest available constituent in *E. microtheca* leaves oil whereas, 1,8-cineole was the third one.
- From oil yield percentage sight of view the Sudanese eucalyptus *microtheca* leaves may be considered as a cheap and rich potential source of essential oil which can be produced in economically feasible amounts for medicinal, pharmaceutical, cosmetics, perfumery, and food additives purposes.
- Further research efforts may be required in the field of *E. microtheca* tree cultivation and its leaves oil application.

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