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# Antioxidant activities and the chemical composition of the essential oil from *Eucalyptus pulverulenta* (Baby Blue Eucalyptus) grown in The Nilgiris

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**Abstract**
*Eucalyptus pulverulenta* (Baby Blue Eucalyptus) originated from Australia is one of the rare species of Eucalyptus family (Myrtaceae) and well adapted to the hilly regions of India. Apart from the cut foliage utility of the plant, its leaves have a pleasant aroma like most of the members of Myrtaceae. This study was designed to extract essential oil using hydro distillation method. Four different types of samples were used (*viz*. tender leaves, mature sticks, mature leaves along with sticks and mature leaves alone) among which matured leaves were found to have maximum amount of essential oil. GC-MS analysis of the essential oil was carried out to analyse the chemical composition. The major component of the essential oil was found to be 1, 8 cineole. The antioxidant ability of the essential oil to scavenge the free radicals was assessed using three antioxidant assays: DPPH assay, ABTS assay and FRAP assay.

**Keywords:** Hydro distillation, essential oil, Baby Blue Eucalyptus, antioxidant ability

## 1. Introduction
The genus *Eucalyptus* belonging to the family Myrtaceae, consisting of about 800 species shrubs and trees native to Australia, which are cultivated widely in various regions of the world (Coppen JW, 2002) [4]. Eucalyptus species have rich source of aromatic compounds in their aerial parts, which have been harnessed for the extraction of essential oil, using various methods, particularly the conventional hydro distillation. The Eucalyptus essential oil (EO), actually an aromatic volatile oil, is one of the world's most extracted, traded and consumed essential oils in terms of utility, volume and monetary value.
The study of essential oils has drawn the international attention for valuable properties like anti-microbial, antiseptic, and nematicidal potentials. Among all the Eucalyptus species, *Eucalyptus pulverulenta*, widely known as silver-leaved mountain gum, which is endemic to New South Wales, is a species of medium sized shrub to a small sized tree. The plants are identified by smooth bark (unlike most of the Myrtaceae members),slow growing nature, egg-shaped, heart-shaped (Sim type) or round (Baby Blue Sim type) leaves without pedicels exists in pairs of opposite arrangement, flower buds of three in a group, white flowers and cylinder shaped fruits.
This study aims to find out the chemical composition of the essential oil extracted from the leaves of Baby Blue Sim type of *Eucalyptus pulverulenta* through conventional hydro distillation, using gas chromatography coupled with mass spectrometry (GC-MS). The antioxidant potentiality of the essential oil was also established using three different antioxidant assays *viz.*, DPPH, ABTS and FRAP. The studies on the chemical composition of essential oil extracted from the Baby Blue Eucalyptus has been done at various parts of the world namely Australia (Joseph Brophy *et al.*, 1985) [8], Tuscany (Francesca Ieri *et al.*, 2019) [6], Morocco (Zrira S *et al.*, 2004) [15] and so on. This research is of first of its kind in analyzing the essential oil characteristics of Baby Blue Eucalyptus grown under the hilly regions of South Indian conditions.

## 2. Materials and Methods
### 2.1 Plant material collection and preparation
The herbage required for the hydro distillation of essential oil was collected at early morning from ten year old bushes of the *Eucalyptus pulverulenta* (Baby Blue Sim type) plants at the State Horticulture Farm, Thummanatty village, The Nilgiris. Harvested branches were tied into bunches and taken to the analytical lab at Tamil Nadu Agricultural University without

desiccation loss. The collected plant material was separated into tender leaves, mature sticks, mature leaves along with sticks and mature leaves alone. Finally, the sample materials were cut into a similar size of about 1 inch.

**2.2 Essential oil extraction**

All the four types of sample materials were hydro distilled for essential oil extraction using a Clevenger type hydro distillation apparatus of 1 liter capacity. 125 g of the sample materials were weighed and kept in the round bottomed flask along with 500 ml of water and kept for an extraction time of 3 hours. The extracted essential oils were dehydrated using anhydrous ammonium sulphate and stored in amber bottles at 4˚C. The essential oil content in all the four types of samples was assessed. The sample with highest content of essential oil was used for bulk extraction, antioxidant assays and GC-MS analysis. The yield of essential oil (%) recovered from all the four samples were calculated using the formula:

Amount of oil extracted (g) = [Yield of essential oil (%) / Amount of herbage used for extraction (g)] × 100

**2.3 Determination of chemical composition by GC-MS analysis**

GC-MS analysis was carried out to identify the chemical components present in the essential oil. Sample for the GC-MS analysis was prepared by mixing 10 µL of essential oil with 5 ml of hexane. The essential oil sample was analysed by injecting into GC-MS (GC Clarus 500Perkin Elmer Analysis) using NIST (ver. 2005 MS data library). Initial temperature of the oven was set at 40°C, which was held for a minute.The temperature was then increased to 200 °C at 5°C per minute, which was again raised at 10 °C per minute upto 260 °C, and held for 6 minutes at that temperatuere. 1 µL of sample was injected at 260 °C, while the flow rate of carrier gas helium was 1.2 mL/ min. Mass spectrometer was operated with an electron ionization of 70eV. Tentative identification of the compounds was done by the comparision of mass spectra of each peak with the database (MS data library).

**2.4 Total antioxidant activity determination using FRAP analysis**

Total antioxidant activity of the extracted essential oil was determined using the ferric reducing antioxidant power analysis as described by Chu *et al.* (2000) [5]. 50 µLof the oil essential oil was mixed with methanol from which 1 mL of the methanolic extract was taken in a test tube. It was then mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%w/v). Followed by that the mixture was kept in boiling water bath at 50˚C for 25 minutes. 2.5 mL of tri chloro acetic acid (10% w/v) was added to all the test tubes from which 2.5 ml of working sample was pipetted out and added with 2.5 mL of distilled water. At last 500µL of ferric chloride solution (0.1% w/v) was added and kept for an incubation time of 30 minutes. The observance value of the prepared solution was read at 700 nm using UV-visible spectrophotometer. Higher the absorbance, higher will be the reducing activity of the antioxidant compounds present in the essential oil.

**2.5 DPPH assay of antioxidant activity determination**

1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) was used to study the antioxidant scavenging capacity of the essential oil and the standard using the procedure proposed by Blois (1958) [2] with slight modifications. Various concentrations of the essential oil and the pure antioxidant (ascorbic acid as a standard), *viz*., 10 µL, 20 µL, 30 µL, 40 µL, 50 µL diluted in 1.5 mL of methanol were prepared. Then, 1.5 mL of 0.2 mM methanolic DPPH solution was added to all the test tubes. The absorbance value of the essential oil samples were recorded as $A_T$ (sample), using a UV- visible spectrophotometer at 520 nm. All the test tubes were kept for an incubation period of 30 min at 25 °C before that, allowing the DPPH to be scavenged by the antioxidant compounds of the essential oil. The same procedures were followed for the standard ascorbic acid simultaneously and the absorbance values were recorded along with a blank experiment without the test material and the absorbance was recorded as $A_0$ (blank). The free radical scavenging ability of all the dilutions of the samples and standard were then calculated, which was represented as percent inhibition as per the following equation:

$$\% \text{ Inhibition} = 100 (A_0 - A_T) / A_0$$

Antioxidant capacity of the test compounds, i.e the essential oil sample was denoted as $IC_{50}$, which is the concentration of the sample material needed to cause a 50% decrease in the initial DPPH concentration.

**2.6 ABTS assay of antioxidant activity determination**

The radical scavenging ability of the extracted essential oil and standards were measured using ABTS free radical (2,2'-azinobis-3-ethylbenzothiazoline- 6-sulphonate) as reported by Re *et al.* (1999) [11]. Reactive ABTS solution was made by mixing 7 mM of ABTS at pH 7.4 (5 mM $NaH_2PO_4$, 5 mM $Na_2HPO_4$ and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration), followed by storing it for 16 hours at room temperature in the dark. The mixture was then diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using UV visible- spectrophotometer. For each sample (300 µL), the diluted methanol solution of essential oil was allowed to react with the ABTS solution (2700 µL) for 6 minutes after initial mixing, and then the absorbance was measured. As a control, ascorbic acid was employed. The $IC_{50}$ value, which represents the concentration necessary to scavenge 50% of ABTS radicals, was used to measure the capacity of free radical scavenging.

**3. Results and Discussion**

**3.1 Yield of *Eucalyptus pulverulenta* essential oil**

The percentage yields of the extracted essential oil from the four different plant parts were assessed and the results are displayed in the table 1. Among all the four samples, maximum oil content of 2.00% was recorded in the matured leaves, whereas the least recovery was recorded in the matured sticks (0.18%). Followed by the matured leaves, 1.86% of essential oil was recorded in the tender leaves on par with the matured leaves along with the sticks (1.71%) On comparison with the previous studies in *Eucalyptus pulverulenta*, the essential oil recovery percentage was found to be higher than that of the sample collected from Tuscany (1.1%), reported by Francesca Ieri *et al.* (2019) [6] and lesser than that of the sample collected from Australia (4.8%), using the steam volatilization method, reported by Joseph Brophy *et al.* (1985) [8].

**Table 1:** % of essential oil recovery from different parts of

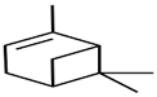
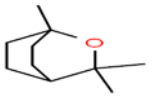
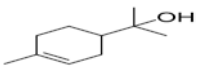
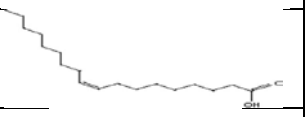
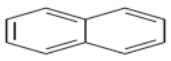
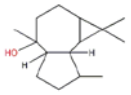
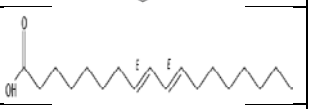
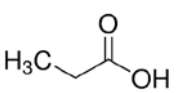
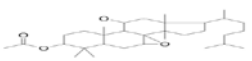
*Eucalyptus pulverulenta* (Baby Blue Sim type)

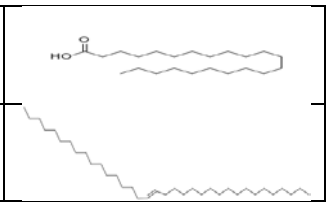
| Sample | % recovery (w/w) |
|---|---|
| Tender leaves | 1.86 |
| Matured leaves | 2.00 |
| Sticks alone | 0.18 |
| Matured leaves along with sticks | 1.71 |
| SEd | 0.0899 |
| CD (0.05) | 0.2073 |

### 3.2 Chemical composition of the essential oil

The GB-MS spectra of essential oil of *Eucalyptus pulverulenta* (Baby Blue Sim type) is given in figure 1. The major chemical composition of the essential oil determined by GC-MS is summarised in the table 2. Majority of the compounds were found to be monoterpene hydrocarbons and oxygenated monoterpenes with ketone group. 1,8 cineole (Eucalyptol) was found to be the major component in the current study which is in accordance with other Eucalyptus species also, *viz.*, *Eucalyptus parvula*, *E. cinerea*, and two types of E. *pulverulenta* itself (Francesca Ieri *et al.*, 2019) [6]. Similarly, higher amount of Eucalyptol (>80%) was reported in the essential oil of *E. pulverulenta* in Australia by Joseph *et al*. (1984) and *E. camuldulensis* by Siramon P and Ohtani Y (2006). Pentane 3-methyl, 1-Pentanol, a'-Pinene, Eucalyptol, ç-Terpinene, a'-Terpineol, Octadecanoic acid, Oleic acid, Naphthalene, Aromandendrene, Globulol, Methyl 8,10-octadecadiynoate, Propanoic acid, 7,8- epoxylanostan-11-ol, 3-acetoxy, Docosanoic acid and 17- pentatriacontene are other major components detected in the GC-MS analysis of the essential oil. Apart from Eucalyptol, a'-pinene, a'-terpinene, terpinolene, terpineol and globulol were some of the compounds found to be present in most of the related species of *E. pulverulenta.*

**Table 2:** Chemical composition of the essential oil extracted from *Eucalyptus pulverulenta*

| Peak | R time | Compound | Chemical formula | Structure |
|---|---|---|---|---|
| 1 | 2.04 | Pentane 3-methyl- | $C_6H_{14}$ |  |
| 2 | 4.66 | 1-Pentanol | $C_5H_{12}O$ |  |
| 3 | 8.19 | a'-Pinene | $C_{10}H_{16}$ |  |
| 4 | 11.06 | Eucalyptol | $C_{10}H_{18}O$ |  |
| 5 | 15.67 | a'-Terpineol | $C_{10}H_{18}O$ |  |
| 6 | 17.13 | Octadecanoic acid | $C_{18}H_{36}O_2$ |  |
| 7 | 18.50 | Oleic acid | $C_{18}H_{34}O_2$ |  |
| 8 | 22.36 | Naphthalene | $C_{10}H_8$ |  |
| 9 | 22.91 | Aromandendrene | $C_{15}H_{24}$ |  |
| 10 | 25.91 | Globulol | $C_{15}H_{26}O$ |  |
| 11 | 30.54 | Methyl 8,10- octadecadiynoate | $C_{19}H_{30}O_2$ |  |
| 12 | 35.21 | Propanoic acid | $C_3H_6O_2$ |  |
| 13 | 37.35 | 7,8- epoxylanostan-11-ol, 3-acetoxy | $C_{32}H_{54}O_4$ |  |

| | | | | |
|---|---|---|---|---|
| 14 | 41.16 | Docosanoic acid | $C_{22}H_{44}O_2$ |  |
| 15 | 45.50 | 17- pentatriacontene | $C_{35}H_{70}$ |  |

The major compound Eucalyptol is used as a constituent in cosmetic products, cough controlling drugs, dentistry and aromatherapy (Neumann W *et al*., 2015) [10]. It is also proposed to possess anti-inflammatory activity and have potential to treat respiratory problems like bronchitis and asthma (Juergens UR *et al*., 1998) [9]. Eucalyptol inhalation for a prolonged time increases the blood flow to cerebral area of the brain, due to the Eucalyptol concentration in the blood, thereby exhibiting cardiovascular effects also (Soares MCMS *et al*., 2005) [14]

There is a correlation between the chemical constituents and the antioxidant activity of the essential oil and the main constituents of the essential oil like 1,8 cineole and terpinene compounds were reported to produce lesser antioxidant activities, on comparison with potential antioxidant constituents like thymol (Siramon P and Ohtani Y, 2006).

As per the previous reports, the chemical constituents of the essential oil may significantly differ based on the species, chemo types, season of sample collection, extraction methods, geographical factors and so on, which in turn makes a considerable change in the antioxidant, antimicrobial, and other desirables properties of the essential oil (Coppen JW, 2002) [4].

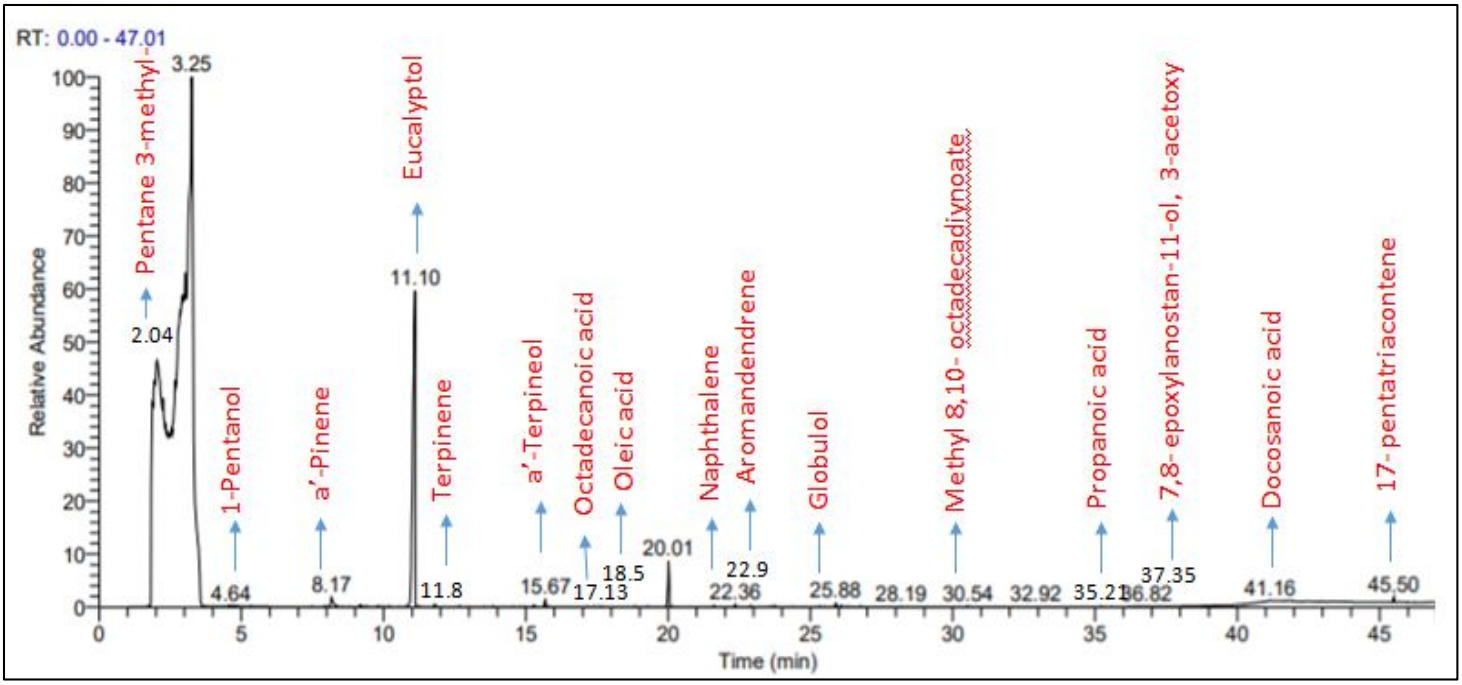



**Fig 1:** Chromatogram of the essential oil extracted from *Eucalyptus pulverulenta*

### 3.3 Antioxidant ability of the essential oil

The antioxidant potential of the *Eucalyptus pulverulenta* essential oil was expressed in $IC_{50}$values in the ABTS and DPPH assays of antioxidant determination, which represents the concentration of the essential oil or a standard antioxidant required to scavenge the 50% of the stable ABTS and DPPH radicals during the incubation period for free radical scavenging. The $IC_{50}$ values derived in those two assays are displayed in the form of table 3.

**Table 3:** $IC_{50}$ values obtained in ABTS assay and DPPH assay

| | **$IC_{50}$ in ABTS (µL/mL)** | **$IC_{50}$ in DPPH (µL/mL)** |
|---|---|---|
| Sample (essential oil) | 62.96 | 117.3 |
| Standard (ascorbic acid) | 54.23 | 81.26 |

The higher $R^2$ values derived for the ABTS (0.982) and DPPH (0.983) assays, through the linear regression equation framed by plotting the percentage inhibition values against the concentration of the essential oil proves that the $IC_{50}$ values of both ABTS and DPPH assays are significant (Fig. 2)

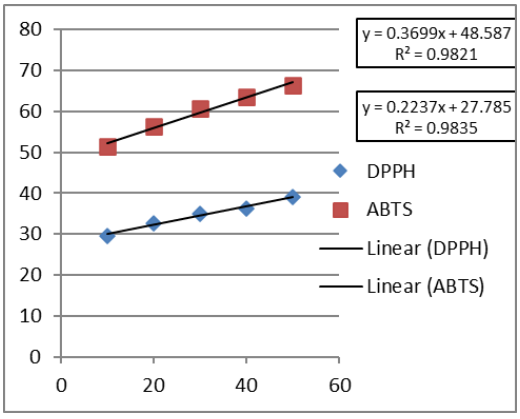



**Fig 2:** % I values (y axis) of different concentrations (x axis) of essential oil in ABTS and DPPH assays

From the table 4, it is evident that concentration dependent antioxidant activity of the essential oil, as the %I value increase from 51.48 to 66.34 in the ABTS assay and from 79.18 to 86.50% in case of DPPH assay. When compared with the % I values of ascorbic acid (93.25 to 94.79% in ABTS assay and 79.18 to 86.50% in DPPH assay), the % I values of the essential oil was lesser, significantly. From the table 4, the $IC_{50}$values of the essential oil obtained in ABTS and DPPH assays are 62.96 µL/mL and 117.3µL/mL, respectively. It means lesser the $IC_{50}$value, lesser will be the concentration of essential oil required to produce 50% inhibition of the free radical. Higher $IC_{50}$ value in DPPH assay and lower $IC_{50}$ value in ABTS assays obtained in this study has been found to be similar to the findings of Celeste de Jesus Pereira Franco *et al.* (2021) [3] and Hajer Nacer Ben Marzoug *et al.* (2011) [7].

**Table 4:** % I values of the essential oil obtained from ABTS assay and DPPH assay

| | % I in ABTS assay | | % I in DPPH assay | |
|---|---|---|---|---|
| **Concentrations** | **Sample** | **AA** | **Sample** | **AA** |
| 10 µL | 51.48 | 93.25 | 29.55 | 79.18 |
| 20µL | 56.28 | 93.77 | 32.78 | 81.15 |
| 30µL | 60.77 | 94.18 | 34.88 | 82.84 |
| 40µL | 63.55 | 94.38 | 36.29 | 84.52 |
| 50µL | 66.34 | 94.79 | 38.98 | 86.50 |

(%I – Percentage Inhibition value, AA- Ascorbic acid standard, sample- essential oil)

The strong antioxidant ability of the essential oil has been attributed to their phenolic constituent like 1,8 cineole, whereas terpinene compounds were also reported to have high antioxidant activity (Ruberto G and Baratta MT, 2000) [12].
The assessment of total antioxidant activity of the essential oil was carried out using the FRAP assay and the result showed that the essential oil has a ferric reducing antioxidant power with the value of 1.83 mg GAE/g. Similar studies on FRAP assay using *Eucalyptus camelduleńsis* (15.02 mg GAE/g) reported by Aisha Ashraf *et al.* (2015) [1] reveals that the value for ferric reducing antioxidant power of the Baby Blue Eucalyptus essential oil is lesser.

## 4. Conclusion

The extraction of essential oil from *Eucalyptus pulverulenta* (Baby Blue Sim) type was done by Clevenger method. The chemical composition and antioxidant studies were carried out in the extracted essential oil. The current study has established the correlation between the chemical composition and the antioxidant ability of the essential oil. This property is due to the presence of phenols, terpenes, hydrocarbon compounds, etc., in the essential oil. The steam distilled essential of *Eucalyptus pulverulenta* can be recommended as an antioxidant additive in cosmetics, food, and beverage industries. Further studies may be carried out in exploring the seasonal variation in the composition and the antioxidant activity of the essential oil.

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