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# Extraction and Antibacterial Activity of Essential Oils from *Eucalyptus globulus*

**Ahmed[1*], R. N., Sule[1], I. O., Zakariyah[1], R. F., Gambari – Ambali[1], R. O., Opoola[1], H. and Jimoh[2], F. A.**

[1]Department of Microbiology, University of Ilorin, Ilorin, Nigeria.
[2]Department of Biosciences and Biotechnology, Microbiology Unit, Kwara State University, Malete, Kwara State, Nigeria.

## Abstract

The essential oil of *Eucalyptus globulus* obtained by hydrodistillation (Fever tree) was investigated for activity against two bacteria (*Staphylococcus aureus* and *Klebsiella pneumoniae*). The oil was extracted using a Clevenger-type apparatus. Separation, identification and determination of percentage composition of compounds present in the oil were carried out by Gas Chromatography Mass Spectrometry. The antibacterial activity was investigated by agar diffusion technique using various concentrations of the essential oil: 100% v/v, 75% v/v, 50% v/v, 25% v/v, 15% v/v. Minimum inhibitory concentration was determined using turbidimetric assay by taking the absorbance with the aid of a spectrophotometer. Minimum bactericidal concentration was by plate assay. Gas Chromatography Mass Spectrometry revealed the presence of seven compounds, all amounting to 90.4% with globulol having the highest percentage composition of 30.1%. The evaluation of sensitivity of the oil revealed the highest activity at 100% v/v against both isolates. Minimum inhibitory concentration was obtained at 25% v/v when assayed against *K. pneumoniae* with no activity at all against *S. aureus* at the same concentration. Oil showed no bactericidal activity at all concentrations, suggesting only bacteristatic activity of the oil. Investigation from this work revealed that the essential oil of *E. globulus* exhibit activity against the tested isolates and can thus be employed in the treatment of infections caused by them.

**Keywords:** *Eucalyptus globulus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, hydrodistillation, Agar diffusion.

*Corresponding Author: Ahmed, R. N.
Email: anrisikat@gmail.com; anrisikat@unilorin.edu.ng

## 1. Introduction

Aromatherapy, a unique branch of herbal medicine employs the therapeutic properties of oils from various plants in the treatment of infections. Essential oils are the aromatic group of volatile liquids that come from plants. These oils come with characteristic pleasant aroma and have been established to possess pharmacological principles essential oils (Minter, 2005). The odor and taste of an essential oil is determined by the main chemical constituent of the mixture (Ikam, 1969).

The development of aromatherapy using plant oils was an offshoot of the fragrance industry. This term was coined in 1928 by a French perfume chemist, Rene Gattefossé, when he healed his burnt hand in a flagon of lavender oil (Minter, 2005). These oils are not found only in the flowers, but also frequently in the leaves, roots, rhizomes, seeds, rind, or bark of plants. Plants produce them mainly as a defense against insect attack, plant diseases, or extreme heat. The bioactivity of essential oils is the key to their potency as plant defenses and therapeutic properties. The use of essential oils, gums, and resins from plants dates back thousands of years and was particularly important in ancient Egypt and the civilizations of the Middle East with their incense trade routes.

In medieval times, perfume was believed to be important in the prevention of sickness. Before germ theory of disease was developed in the 19th century, foul and pestilential airs were believed to cause infection, hence the strewing of herbs on floors to release supposedly protective fragrances and the use of "tussie mussie" bouquets and pomanders as prophylactics. These may have been indirectly effective in repelling insect carriers of disease-causing organisms (Minter, 2005).

Researchers suggest that some species of *E. globulus* counteract influenza viruses, others are antimalarial or highly active against bacteria. The Eucalyptus (Myrtaceae) is used in control of several diseases derived from microbial infections (Ody, 1994 and Cowan, 1999). It has been reported to possess antifungal, antibacterial, antiviral, mosquito repellent and antioxidant properties (Burt, 2004 and Kordali *et al*., 2005).

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**Plate 1:** *Eucalyptus globulus* plant.

## 2. Materials and methods

### Collection and identification of plant materials

The leaves of *Eucalyptus globulus* were collected in the early hours of the morning from the University of Ilorin, Nigeria. The plant material was authenticated at the herbarium unit of the Department of Plant Biology, of the same University.

### Extraction of essential oil

Fresh leaves of *Eucalyptus globulus* were pulverized with the aid of iron mortar and pestle and 500g was introduced into a round bottom flask of a Clevenger apparatus. Distilled water measuring two to three times the weight of the leaves was added to the flask. This was placed on a thermo-stating heating mantel for 3 hours according to the British pharmacopoeia specification (British Pharmacopoeia, 1993). Condensed water and oil from the heated crude plant material was collected and trapped in the Clevenger, at regular intervals the denser water was drained off. At the end of the extraction the resulting less dense oil in the Clevenger was collected into a glass sample bottle sealed and preserved by refrigeration (4°C) until required for analysis.

### Gas chromatography (G.C) analysis

Gas chromatography was performed on an orion micromat 4/2 double focusing gas chromatography system. Qualitative data were obtained by electronic integration of FID percent without the use of correction factors.

**Gas chromatography – mass spectrometer (GC/MS) analysis**

GC could not identify the individual compounds contained in the essential oil, hence, MS was also carried out. The percentage composition of the oils was computed in each case from GC peak areas. Identification of components was based on comparison of retention indices, determined relative to the retention times of series of n-alkanes and mass spectra with those of authentic samples and with data from literature (Adams, 2007).

**Collection and maintenance of test organisms**

*Klebsiella pneumoniae* and *Staphylococcus aureus* were collected from the culture collection unit of the Department of Microbiology, University of Ilorin. They were maintained on appropriate agar slants at $4^0$c and routinely sub-cultured for the purpose of purity.

**Antibacterial sensitivity assay**

**Agar well diffusion technique**

Nutrient agar was poured into sterile petri dishes and allowed to solidify. Swab stick was used to streak test culture on solidified agar. Wells of approximately 5mm in diameter were made on the surface of the medium with the aid of a sterile cork borer. Each well was filled with 0.5ml of different concentrations of the oil:100%, 75%, 50%, 25% and 15% v/v. The plates were incubated at 37$^o$C for 24 hours. Sensitivity of the organisms to the essential oil was recorded by reading zones of inhibition around the wells (Jahangirian *et al*., 2013).

**Determination of minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) of essential oil was determined using turbidimetric method, by incorporating a known concentration of the oil in tubes containing broth and test isolate. Control experiments were set up without the oil. Contents of all test tubes were checked for turbidity at 0hours and 24hours using Spectrophotometer (Black, 1996).

**Determination of Minimum Bactericidal Concentration**

One milliliter of contents of test tubes that showed no increase in turbidity in the MIC assay were inoculated onto nutrient agar and incubated at 37$^o$C and observed for growth. The

lowest concentration that showed no growth on the recovery plate was regarded as minimum bactericidal concentration**.**

## 3. Results

### Recovery of oil by hydrodistillation

Hydrodistillation of leaves of *E. globulus* yielded 1.7% (8.5g) of essential oil (w/w based on fresh weight of mature leaves) with a spicy aromatic odour and an amber colour which was highly volatile at room temperature and pressure.

### Isolation and characterization of chemical constituents of oil

The total percentage composition of compounds found in the oil was 90.4% with globulol having a retention index of 1576 and being the most abundant (30%). Table 1 shows individual compounds, their retention indexes and percentage compositions as observed by Gas Chromatography-Mass Spectrometry.

### Effect of different concentrations of essential oil of *Eucalyptus globulus* on test isolates.

The effect of different concentrations of essential oil of *Eucalyptus globulus* leaves on *Staphylococcus aureus* and *Klebsiella pneumoniae* are shown in Figure 1. The highest concentration of the oil (100%) demonstrated highest activity against *S. aureus and K. pneumoniae* with inhibition zones of 35mm and 18mm respectively. The sensitivity plates showing susceptibility of test organisms to the essential oil of *E. globulus* as reflected by zones of inhibition are presented in plates 2-5.

**Table 1**: List of compounds isolated from essential oil of *Eucalyptus globulus*

| **Compounds** | **Retention index** | **Percentage composition** |
|---|---|---|
| Thujene | 931 | 8.3 |
| α-phellandrene | 1005 | 1.4 |
| Piperitone | 1250 | 1.9 |
| β-Elenene | 1375 | 27.1 |
| α-Gurjuene | 1409 | 2.9 |
| Aromodendrene | 1439 | 18.7 |
| Globulol | 1576 | 30.1 |
| **Total** | | 90.4 |

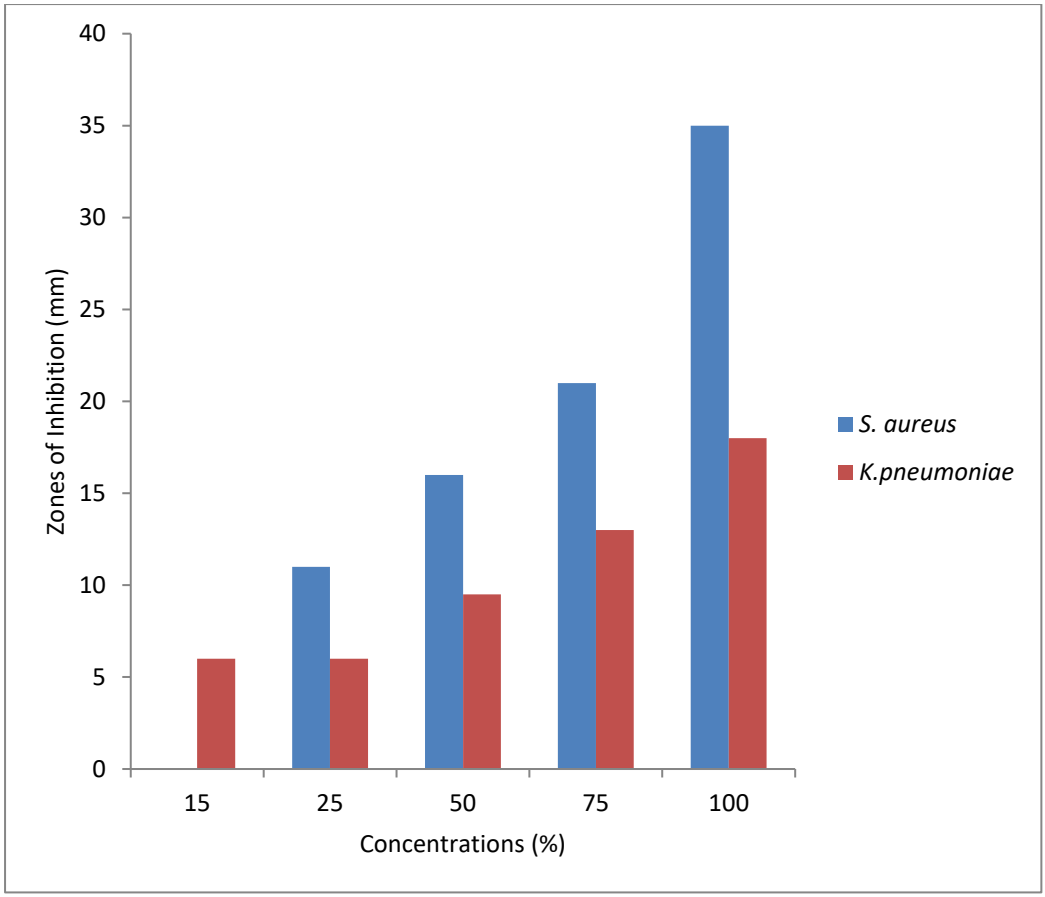


**Figure 1:** Sensitivity of test organisms to different concentrations of essential oil of leaves of *E. globulus*.

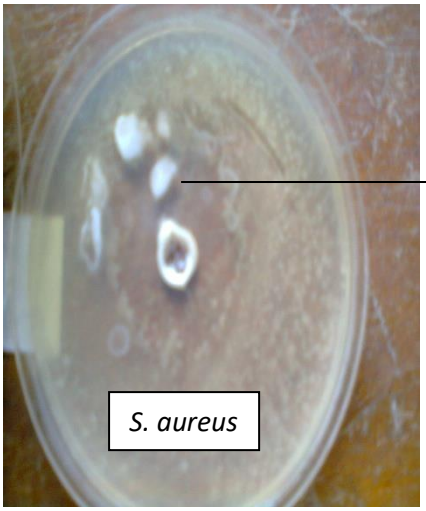


**Plate2:** Sensitivity plate of *S. aureus* to 100% v/v essential Oil of E. globulus

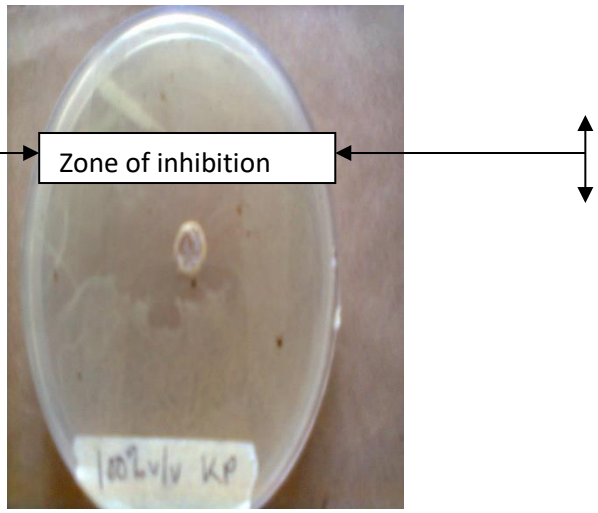


**Plate 3:** Sensitivity plate of K. *pneumoniae* to 100% v/v essential oil of *E. globulus*

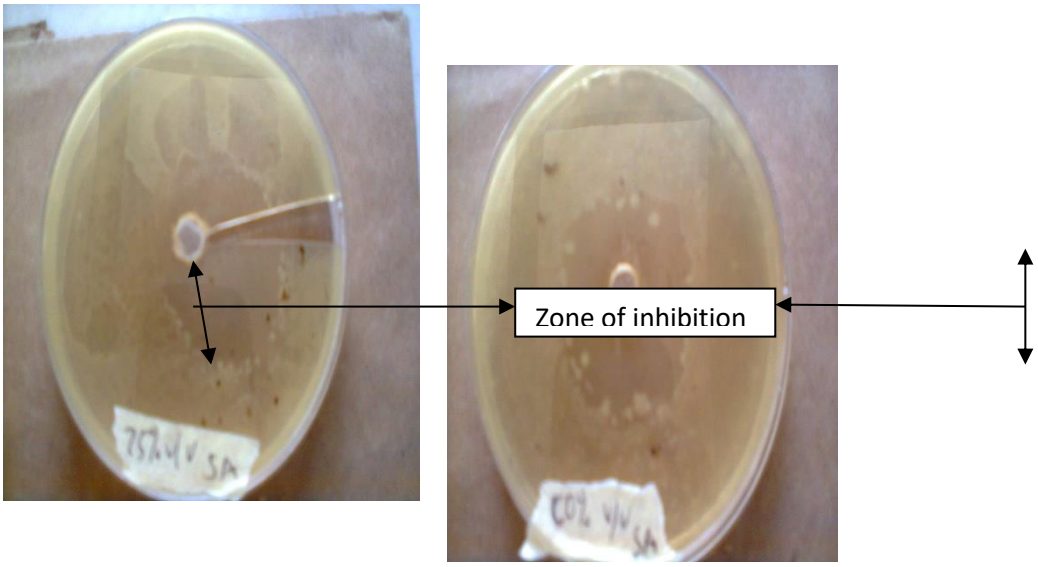


**Plate 4:** Sensitivity plate of S. *aureus* to 75% essential oil of *E. globulus*

**Plate 5:** Sensitivity plate of S. *aureus* to 50% v/v essential oil of *E. globulus*

**Evaluation of minimum inhibitory concentration and minimum bactericidal concentrations of essential oil of *E. globulus***

The result of minimum inhibitory concentration of essential oil of *Eucalyptus globulus* against test organisms is shown in Table 2. The lowest concentration of the oil that showed activity for both test organisms was 25%. All concentrations of essential oil of *E. globulus* used showed no bactericidal activity against *Staphylococcus aureus* and *Klebsiella pneumoniae.*

**Table 2:** Determination of Minimum Inhibitory Concentration

| **Concentrations (v/v)** | ***S. aureus*** | | ***K. pneumonia*** | | **MIC** |
|---|---|---|---|---|---|
| | **0 HOURS** | **24HOURS** | **0 HOURS** | **24 HOURS** | |
| 100 | 0.804 | 0.590 | 0.539 | 0.306 | + |
| 75 | 1.940 | 1.802 | 2.903 | 2.709 | + |
| 50 | 2.729 | 2.580 | 0.544 | 0.395 | + |
| 25 | 0.423 | 0.227 | 0.551 | 0.408 | + |
| 15 | 0.473 | 0.373 | 0.541 | 0.469 | + |
| 0 (CONTROL) | 0.483 | 0.747 | 0.321 | 0.543 | _ |

**MIC =25% v/v Key:** + = inhibition present, - = inhibition absent.

## 4. Conclusion

With the growing interest in the use of essential oils in both food and pharmaceutical industries, examination of various plant parts for therapeutic purposes is very key. The analysis of Gas Chromatograpy/Mass Spectrometry (Table 1) varied with the results of other researchers (Damjanović-Vratnica *et al*., 2011). This could be as a result of differences in species or factors such as geographical location, climatic conditions, soil type among others. Eucalyptus species produce numerous volatile compounds in large amounts, especially isoprenoids, which are accumulated in glands abundantly distributed throughout the leaf parenchyma and bark (Moleyar and Narasimham, 1986; Rakotonirainy and Lavédrine, 2005).

In the evaluation of the effect of varying concentrations of oil on the bacteria (Figure 1) it was observed that activity was maximum at 100% and minimum at 15% when assayed against both organisms. This observation shows clearly that effectiveness of the oil is dependent on concentrations, suggesting that at lower concentrations, when the oil is diluted the strength is weakened by such diluents thus leading to a reduction in efficacy. Also the presence of diluents in the lower concentrations may have been responsible for a reduction in the rate of diffusion of the oil during the agar well assay. Thus, reducing movement of the vapour from the oil in the surrounding agar medium and subsequently limiting its activity. It has already been shown that the antimicrobial activity of volatile compounds results from the combined effect of direct vapor absorption on microorganisms and indirect effect through the medium that absorbed the vapor (Trivedi and Hotchandani, 2004). This is in line with researches conducted by Inouye *et al.* (2001), Trivedi *et al*. (2004) and Ghalem *et al*. (2008), where higher concentrations resulted in increased activity against test organisms.

The result obtained for the MIC indicating the lowest concentration of oil that demonstrated activity revealed that the oil was very potent since at 25% a remarkable decrease in absorbance was still observed. However, the bactericidal assay showed that none of the varied concentrations had cidal effect on the test isolates. This may be due to the volatile nature of the oil which may have been weakened upon exposure in subsequent assays such as MBC.

From the results emanating from this work, it is evident that essential oil of *E. globulus* extracted by hydrodistillation was effective against *S. aureua* and *K. pneumoniae* producing remarkable zones of inhibition even at low concentrations. The exploitation of the oil resulting from this plant would be a cost effective approach in indigenous health care

management as an alternative antibacterial agent for the management of infections resulting from *S. aureus* and *K. pneumoniae.* This is particularly important since the dilemma of resistance has become a scourge to the human race and natural product chemists are combing the earth for alternative source of antimicrobials.

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