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# Chemical Composition and Bactericidal Activities of the Leaf Essential Oil of *Eucalyptus maculata* Hook

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## Abstract

This study examined the phytochemicals and medicinal potentials of the leaf essential oil of *E. maculata* from Nigeria. The essential oil was extracted by hydro-distillation technique and was analysed for various chemical composition using GC-MS. The essential oil was also screened for its antimicrobial activities against multi-drug resistant gram-positive and gram-negative bacteria using the agar well diffusion method. The GC-MS analysis revealed that *β*-citronellol (18.5%), *β*-pinene (9.4%), 2,6-dimethyl-2,6-octadiene (8.3%), *α*-pinene (7.1%), 2,6-dimethyl-1,3,5,7-octatetraene (6.7%), and citronellyl acetate (6.0%) were the major compounds in the leaf essential oil. The essential oil exhibited strong antibacterial activities against most of the tested bacteria, with diameters of inhibition zones ranging from 15.0-30.0 mm. The leaf essential oil of *E. maculata* is a possible alternative antibiotic drug for treatment of disease caused by multi-drug resistant bacteria.

**Keywords:** *Eucalyptus maculata;* Essential oil; Multi-drug resistant bacteria; Antibacterial

## Introduction

Medicinal plants are essential remedies for treatment of sicknesses and diseases from time immemorial. They contain pharmacologically active phytochemicals which are found in various parts of the plant and are very useful for mankind in many ways as drugs, flavours and preservatives [1-6]. *Eucalyptus maculata* Hook commonly called spotted gum; a member of *Myrtaceae* family is an attractive ornamental tree with significantly larger vascular tissue. It is adapted to water limited environments like drought and salinity. It is a fast growing and tall plant, usually about 35-45 m in height and average width of 1.5 m at breast height over bark. The immature leaves are glossy green and elliptic to ovate while the adult leaves are lanceolate and are 10-21 cm long and 1.5-3 cm wide. The flowers are small, white and clustered which develop to ovoid fruits, which are disc depressed; valves enclosed, nectar from the flowers, even the seed is sometimes eaten. Trunks are relatively long and usually clean and straight without branches for more than half their height. Bark is smooth to ground level and greenish cream when fresh. The wood is slightly greasy and gum veins are common [7,8]. *E. maculata* is commonly used locally for treatment of various diseases and preservatives in food and drugs [9-11]. Essential oils are volatile complex mixture of organic compounds found in plants as secondary metabolites and characterized by a strong odour [12,13]. They are normally formed in special cells in plants and commonly concentrated in some particular region such as leaves, bark, seed, fruit, root, flowers, twigs or stem [14]. They were thought to be the quintessence responsible for the odour and flavour of the plants [15]. They have been shown to not only possess broad-range antibacterial potentials, but also antioxidant, antiparasitic, pesticidal, antiviral, anticancer and anti-inflammatory properties [16,17].

Bacteria are developing thick resistance to synthetic antibiotics in recent time [18,19]. The resistance of bacteria to synthetic and commercial drugs has been a serious threat to human health and world economy [20,21]. Drug resistance by bacteria has also becoming a large and growing problem in infections that account for most of diseases in developing countries [21,22]. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections, therefore a serious need for alternative and cheap antibiotic from natural sources [23,24]. Moreover, because of side effects of using synthetic and commercial antibiotic there is need for natural antibiotics from plants [25,26]. It is necessary to take quick steps to save the entire globe from going into a post-antibiotic period, in which bacteria infections can become life threatening [20]. This study was designed to investigate the bactericidal activities of the leaf essential oil of *E. maculata* grown in Nigeria on some multi-drug resistant clinically isolated bacteria.

## Materials and Methods

### Plant material and extraction of the essential oil

Fresh and mature leaves of the plant were collected from Afforestation Research Station Kaduna, Nigeria and it was authenticated as *E. maculata* Hook. Air dried and pulverized leaves were subjected to hydro-distillation using all-glass Clevenger-type apparatus to extract the essential oil [27]. The obtained essential oil was then stored in vial in refrigerator to prevent evaporation.

### GC-MS analysis

The leaf essential oil was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m × 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temp at 60°C was held for 1.0 min, raised to 180°C for 3 min and then finally to 280°C held for 2 min); injection mode, Split ratio 41.6; injection temp, 250°C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 $mlmin^{-1}$;

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| Compound | Retention Index | Percentage Composition |
|---|---|---|
| *β*-pinene | 943 | 9.4 |
| *α*-pinene | 948 | 7.1 |
| 2,6-dimethyl-1,3,5,7-octatetraene | 966 | 6.7 |
| 2,6-dimethyl-2,6-octadiene | 985 | 8.3 |
| 1,8-cineole | 1059 | 2.3 |
| myecenol | 1064 | 1.8 |
| *exo*-fenchol | 1138 | 1.6 |
| *p*-menth-1-en-8-ol | 1143 | 1.8 |
| *β*-citronellol | 1179 | 18.5 |
| isopelegol | 1196 | 1.1 |
| 2-decyn-1-ol | 1275 | 2.6 |
| citronellyl acetate | 1336 | 6.0 |
| isoledene | 1373 | 2.0 |
| aromadendrene | 1386 | 1.5 |
| patchoulane | 1393 | 0.3 |
| caryophyllene | 1490 | 3.3 |
| ledol | 1530 | 1.9 |
| eudesm-4(4)-ene-11-ol | 1593 | 1.2 |
| guaiol | 1595 | 2.0 |
| cubenol | 1650 | 3.0 |
| 10,13-dioxotricyclo [6.3.3.0]tetradec-4-ene | 1833 | 0.3 |
| *trans*-geranylgeraniol | 2196 | 1.7 |
| erucylamide | 2625 | 0.7 |
| 1-heptacosanol | 3016 | 1.3 |
| **Percentage Total** | | **86.4** |

**Table 1:** Chemical Chemical composition of the leaf essential oil of *Eucalyptus maculata*

| | Leaf Essential Oil | | | Synthetic Antibiotic | |
|---|---|---|---|---|---|
| | | | | GEN | CXC |
| Conc. (µgml$^{-1}$) / Organisms | 1000 | 100 | 10 | 10 µg | 5 µg |
| ***E. coli*** | 30 | 30 | 30 | 22 | - |
| ***K. pneumoniae*** | 15 | 15 | 15 | 21 | - |
| ***P. aeruginosa*** | 18 | 18 | 18 | 20 | - |
| ***P. mirabilis*** | 18 | 18 | 18 | | |
| ***S. agalactiae*** | 20 | 20 | 20 | - | - |
| ***S. aureus*** | 30 | 30 | 30 | - | - |
| ***S. typhimurium*** | 18 | 18 | 18 | 21 | - |

**Key note:** - = Resistant, not sensitive (<8 mm), sensitive (9-14 mm), very sensitive (15-19 mm) and ultrasensitive (>20 mm)

**Table 2:** Zones of Inhibition (mm) showing the Antimicrobial activities of the Leaf Essential oil of *E. maculata*.

pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 mlmin$^{-1}$; column flow rate, 0.99 mlmin$^{-1}$; ion source temp, 200°C; interface temp, 250°C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature [28].

### Screening for antimicrobial activities

The antibacterial potentials of the leaf essential oil solutions were determined by agar-well diffusion method against multi-drug resistance gram-positive (*Staphylococcus aureus* and *Streptococcus agalactiae*), and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacteria. Mueller Hinton agar plates were prepared by the manufacturer's instructions. Pure isolates of bacteria was sub-cultured in the recommended specific medium at 37°C for 24 h. All the bacteria cultures were adjusted to 0.5 McFarland standards, inoculum of each test organism was swabbed onto the specific media plates and kept for 15 min for adsorption, and then 6 mm diameter wells were bored with a sterile borer in the inoculated agar plates. Different concentrations of the essential oil solutions were pipetted directly into the wells of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for 30 min for diffusion of the essential oil solutions to take place and incubated at 37°C for 24 h. The antibacterial activities of the essential oil were compared with synthetic antibiotics i.e., gentamicin (GEN) and cloxicillin (CXC). The bactericidal activities of the essential oil solutions indicated by the formation of zones of inhibition around the wells were measured in millimetre using a transparent ruler [29].

## Results and Discussion

### Chemical composition of the leaf essential oil

The GC-MS analysis of the leaf essential oil of *E. maculata* revealed that the leaf essential oil has 24 organic compounds representing 86.4% of the total composition. The results showed that the major identified compounds in the leaf essential oil were *β*-citronellol (18.5%), *β*-pinene (9.4%), 2,6-dimethyl-2,6-octadiene (8.3%), *α*-pinene (7.1%), 2,6-dimethyl-1,3,5,7-octatetraene (6.7%) and citronellyl acetate (6.0%). 1,8-cineole which is a principal component of most *Eucalyptus* essential oils was of very low percentage in in the leaf essential oil of *E. maculata* grown in Nigeria (Table 1). This result is similar to what was obtained from the leaf essential oil of the plant from Brazil, Iran and Tunisia [30-32]. Most of the secondary metabolites present in this essential oil were reported to have good pharmacological properties [33-36].

### Antimicrobial activities

All the tested bacteria showed sensitivity to the leaf essential oil of *E. maculata* compared to synthetic antibiotic (Table 2). Zones of inhibition values of the essential oil against the tested gram-positive and gram-negative bacteria ranged between 15.0-30.0 mm. It is noteworthy that the zone of inhibition remained unchanged despite the increase in concentration. The tested bacteria were found to be resistant to cloxicillin (CXC) but some were sensitive to gentamicin (GEN) synthetic antibiotics. The antibacterial potentials of the leaf essential oil from Nigeria were more active than that of leaf essential oil of *E. maculata* from Tunisia which gave lower zones of inhibition between 7.0–9.0 mm against *E. faecalis*, *S. aureus*, *E. coli* and *P. aeruginosa* [32]. The investigated essential oil showed strong activities against multi-drug resistant bacteria due to the phytochemicals in the essential oil as well as the possible synergistic interaction between phytochemicals to penetrate the cell membrane of the organisms, inhibit their growth and proliferation; also induced a toxic effects to the membrane structures [37-44]. The investigated essential oil as a natural antibiotic substance is locally available, easily accessible, easy to extract, inexpensive, environmentally safe and friendly [45,46].

## Conclusions

In this study it was shown that the leaf essential oil of *E. maculata* grown in Nigeria contains medicinally active phytochemicals. The results indicated that the essential oil exhibited strong bactericidal activities against tested multi-drug resistant gram-positive and gram-negative bacteria. The main compounds that responsible for the activities of the essential oil should be isolated. Moreover, clinical trials may be needed for further tests for the potentials of this essential oil as

a natural antibiotic agent.

## Conflict of Interest

We have no conflict of interest.

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