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Physicochemical Properties and Antimicrobial activities of Essential oil extracted from *Eucalyptus globulus* leaves

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Abstract
Medicinal plants can serve as useful alternatives in antimicrobial therapy as well as sources of template molecules in new drug discovery. In this study, physicochemical properties and antimicrobial activity of *Eucalyptus globulus* leave oil were investigated to determine its efficacy against some bacterial pathogens. The leaves of the plant were collected, dried, pulverized and steam distillation was used to extract the oil. The results obtained were moisture content (1.4500 ± 0.1259%); ash content (3.7800 ± 0.3528%); % oil yield (3.1%); saponification value (266.4750mgKOH/g); iodine value (79.693gI_2/100g); acid value (1.2679mgKOH/g); free fatty acid value (0.6378%); specific gravity (0.7663g/ml); pH (4.4500); refractive index (1.4626); colour (Pale yellow); odour (mint); Solubility (insoluble in aqueous and soluble in organic solvents) respectively. The Minimum inhibitory concentration and minimum bactericidal concentration of the extract was obtained using the agar disc diffusion method and dilution broth method. The result of the sensitivity test revealed that the extract of the leaves demonstrated efficacy against *Escherichia coli* and *Staphylococcus aureus*. The MIC Indicates that *S. aureus* and *E. coli* were susceptible to the leave extract at low concentrations ranging 75mg/ml to 300mg/ml, while the MBC value was 300mg/ml for both pathogens. Thus, the antibacterial activity of the extract could probably be due to the bioactive metabolites such as 1, 8-cineole (Eucalyptol), o-cymene, α-pinene, γ-terpinene and α-terpineol. Hence, could be used as an alternative and complementary medicine in the treatment of ailments caused by these organisms.

Keywords: medicinal plants, essential oil, eucalyptus globulus leaves, escherichia coli and staphylococcus aureus

1. Introduction
Eucalyptus tree is a tall evergreen native in Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in the Mediterranean and sub-tropical region including Africa, China, India, Portugal, Spain, and southern United States (Pombal *et al*., 2014) [16]. Though native to Australia, its therapeutic uses have been introduced and integrated into traditional medicine systems in Greece, China, Africa and India. *Eucalyptus* is a diverse genus of flowering trees and shrubs in the myrtle family, myrtaceae (Hossain *et al*., 2008) [11].
The use and dependence on plants as medicine by man has been in existence for years and man continues to search for plants that can help in fighting diseases (Dawson *et al*., 2011) [7]. Medicinal plants have been used all over the world for the treatment and prevention of various ailments especially in developing Countries where diseases are more common and health care facilities and services are inadequate (Jose and Reddy, 2010) [13].
Oil is any neutral, nonpolar, chemical substance that is a viscous liquid at ambient temperatures and is both hydrophobic (immiscible with water) and lipophilic (miscible with other oils) (Wikipedia 2017). Oils may be animal, vegetable, or petrochemical in origin, and may be volatile or non-volatile. A vegetable oil is a triglyceride extracted from a plant. There are several types of plant oils, distinguished by the method used to extract the oil from the plant. Plant Oils extracted by Steam Distillation are called essential oils. An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compounds from plants. Studies have shown that certain essential oils may have the ability to prevent the transmission of some drug resistant strains of pathogen, specifically *staphylococcus*, *streptococcus* and *candida* (Newall, 2009) [15].
Eucalyptus oil is the generic name for oil from the leaf of eucalyptus, a genus of the plant family myrtaceae, native to Australia and cultivated worldwide. *Eucalyptus* oil has a history of wide applications such as pharmaceutical, antiseptic, repellent, flavoring, fragrance and industrial uses. *Eucalyptus* oil is obtained through a steam distillation process that removes the oil from the fresh, mature leaves. *Eucalyptus globulus* essential oils are made up of more than 100 different compounds. Its main chemical components are terpenes; α-pinene, O-cymene, Eucalyptol (1, 8-cineole), α-terpineol, γ-terpinene and Terpinen-4-ol (Edmond, 2013) [9]. The study was aimed at extracting, determining the physicochemical properties and antimicrobial activities of essential oils from *Eucalyptus globules* leaves.

2. Materials and Methods
Collection and identification of materials
The leaves of the plant were collected on 28th October, 2016 at University of Jos, Main campus, Bauchi road, Jos, Plateau state. The leaves were identified by an ethno botanist, Mr. J.J Azila with voucher number FHJ 096.

Preparation of sample and extraction
The leaves were rinsed and air-dried at room temperature; the dried leaves were then pulverized. 450g of the pulverized sample was weighed and used for the extraction. The method of extraction used was steam distillation.

Physicochemical Properties of Oil
Physicochemical properties provide a base line for

suitability of oils (Barkatullah *et al*., 2012) [6]. Physicochemical properties such as moisture content, ash content % yield, density, specific gravity, pH, refractive index, acid value, free fatty acid, iodine value, saponification value, solubility (aqueous and organic), colour and odour were determined using Macaulay, 2015; AOAC with exception of colour and odour.

The color and odour of the *Eucalyptus globulus* essential oil was determined by visual and nostril observation by five volunteers.

Antibacterial activity of *Eucalyptus globulus* essential oil

The inhibitory effects of this essential oil were tested against *Escherichia coli* (*E. coli*) which is a gram negative bacteria, and *Staphylococcus aureus* (*S. aureus*) which is a gram positive bacteria by using agar disc diffusion method and dilution broth method. 5g of Meuller-Hinton (MH) agar was weighed using a weighing machine and added to 250ml of distilled water. The solution was then put in the autoclave for 45minutes for sterilization. The agar was poured into two petridishes, the first petridish containing 0.1ml of *E. coli* and the second petridish containing 0.1ml of *S. aureus.* After both cultures had solidified, 6 holes were then made on each using a cork borer. One hole in the centre (for the control) and five holes around for five different concentrations of the extract. The sample and the control were then inoculated and incubated for 24hours. The diameters of the inhibition zones were measured in millimeters (NCCLS, 2012) [14].

The broth micro-dilution method was used to determine MIC and MBC in accordance with NCCLS (2012) [14] and Yu *et al*., (2014). The MIC (Minimum Inhibitory Concentration) is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by turbidity. The MBC (Minimum Bacteriocidal Concentration) is defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed.

Stock solutions of the essential oil were prepared in Tween80 by mixing 10ml of tween80 with 3ml of the essential oil, followed by successive dilutions. The control was prepared by adding 3ml of Gentamicin to 10ml of tween80. After the MIC was determined, another agar media was prepared using 5g of Meuller-Hinton agar in 250ml of water. All the concentrations of the oil which hindered the growth of the bacteria during the MIC were then seeded by streaking the surface of the agar media using a hot wire lobe soaked in the respective concentrations of the oil. This was then incubated for 24hours, and from the results obtained the MBC was determined.

3. Results and Discussion

This study identifies the bases for suitability and utility of these oils in daily life. Physicochemical properties of oil like color, odor, density, specific gravity, refractive index, acid value, free fatty acid value, pH, iodine value, saponification value and solubility tells about the quality of these essential oils. Essential oils obtained from the leaves of *Eucalyptus globulus* were analyzed for physicochemical properties as shown in Table 1. The essential oil was pale yellow in appearance with mint odor. The percent yield of oil was 3.10%. The oil yield of the *Eucalyptus globulus* was less than the AFNOR (ISO) Standard, The deviation may be due to ecological factors.

The moisture content (1.450 ± 0.126%) was greater than the AFNOR ISO standard while the ash content (3.780±0.353%) was less than the standard, these may be as a result of environmental factors associated to where these trees were grown. Refractive index (1.463) gives information on the purity of the oil; the Result obtained for the refractive index of the essential oil was within the standard, which tells us that the oil is substantially pure. The value obtained for pH (4.450) is within range for Standard.

Specific gravity is the ratio of the density of water at 4^0C. Specific gravity values of oils are less than 1 for most of oil except few containing oxygenated aromatics compounds. In the present study, essential oil from *Eucalyptus globules* leaves has specific gravity and density of 0.782g/ml and 0.766g/ml respectively, these findings are well below the AFNOR (ISO) standard for essential oils. The acid value measures the amount of acids present in 1 gram of oil; oils with low free fatty acid value have more significant usage and edibility. The acid value and % free fatty acid value obtained are 1.268mgKOH/g and 0.638%.

Saponification value is the number of milligram of KOH which is required to saponify 1 gram of oil. The Saponification value (266.475mgKOH/g) was greater than AFNOR ISO standards but in accordance with Ejikeme *et al.*, (2010) [10] thus strengthening our findings.

Iodine value is the measure of unsaturation in the oil; the iodine value (79.693mgI_2/100g) obtained for this study was within standard implying the low level of unsaturation in the oil. The oil sample was insoluble in aqueous but soluble in organic solvent.

Table 1: Physicochemical properties of *Eucalyptus globulus* essential oil

Parameter	Result	AFNOR (ISO) 2015
Moisture content (%)	1.450±0.126	0.115 ± 0.006
Ash content (%)	3.780±0.353	4.600 ± 0.003
Color	Pale Yellow	Colorless – Golden
Refractive index (25º)	1.463	1.462 – 1.464
Odor (Smell)	Minty	Minty
Specific gravity (25ºC)	0.782g/ml	0.820g/ml
Density (g/ml)	0.766	0.813
Sap value (mgKOH/g)	266.475	251.000
Acid value (mgKOH/g)	1.268	1.195
Free fatty acid (%)	0.638	0.608
Iodine value (mgI_2/100g)	79.693	20.000 – 100.000
pH	4.450	4.000 – 8.000
Oil yield (%)	3.100	4.300
Solubility	Soluble in ether and chloroform	Soluble in organic solvent

Table 2: Zones of inhibition (in mm) of *E. coli* and *S. aureus* against *Eucalyptus globulus* essential oil. Zones of inhibition (mm)

Test organism	Concentration (mg/ml)					Control (Gentamicin)
	300	150	75	37.5	18.75	
E. coli	15	11	9	5	nil	13
S. aureus	29	23	19	13	5	17

Table 3: Minimum Inhibitory Concentration (MIC) of *Eucalyptus globulus* essential oil

Test organism	Concentration (mg/ml)					MIC
	300	150	75	37.5	18.75	
E. coli	-	-	+	+	+	150
S. aureus	-	-	-	+	+	75

Key; + = Growth, - = No growth

Table 4: Minimum Bacteriocidal Concentration (MBC) of *Eucalyptus globulus* essential oil

Test organism	Concentration (mg/ml)					MBC
	300	150	75	37.5	18.75	
E. coli	-	+	nill	nill	nill	300
S. aureus	-	+	+	nill	nill	300

Key; + = Growth, - = No growth

In the antibacterial result by agar well diffusion (Table 2), the oil extract was found to be effective against the tested organism; *Escherichia coli* and *Staphylococcus aureus*. The addition of *Eucalyptus globulus* essential oil in broth cultures inoculated with *S. aureus* and *E. coli* inhibited the growth of these organisms. The rate of inhibition was greater on the Gram positive *S. aureus* than that observed on the Gram negative *E. coli.* When assessed individually, the essential oil demonstrated antibacterial activity at peak values (300mg/l) against *E. coli* (15mm) and *S. aureus* (29mm) respectively. This is similar to results reported by Bachir & Benali, 2012 [5].

Table 3 shows MIC values of (18.75 – 300mg/l) for essential oil demonstrated inhibitory capabilities on the tested organism with values ranging from (75 - 300mg/l) while the MBC value was 300mg/ml for both pathogens as shown in Table 4.

The bactericidal activity of the essential oil highlights the usefulness of the essential oil in the treatment of typhoid fever, anemia, bloody diarrhea and urinary tract infections (Bachir & Benali, 2008) [4].

The antibacterial activity of *Eucalyptus globulus* essential oil has been found to be as a result of the components 1, 8-cineole (Eucalyptol), o-cymene, α-pinene, γ-terpinene and α-terpineol (Edmond, 2013) [9].

4. Conclusion

The physicochemical properties of the extracted *Eucalyptus globulus* essential oil were in agreement with the AFNOR ISO 2015 standards with slight deviation, indicating that the extracted oil is of relatively good quality.

The results of the antibacterial activity reported show that essential oil of *Eucalyptus globulus* has antibacterial activity against Gram positive bacteria (*S. aureus*) and Gram negative bacteria (*E. coli*). Hence, the efficacy of *Eucalyptus globulus* essential oil against these microorganisms can provide a scientific ground for the application of the oil in the prevention and treatment of bacterial infections caused by *Staphylococcus aureus* and *Escherichia coli*, which have developed resistance to some antibiotics. The results also show that the herb is a good means to explore new alternative antibacterial agents to combat pathogenic microorganisms.

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