# Phytochemical Analysis and Antimicrobial Properties of Eucalyptus torelliana Oils

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**Abstract**- Normal oil and Essential oil from fresh leaves of *Eucalyptus torelliana* (F. Muell) were extracted using solvent (Soxhlet) and steam distillation extraction methods respectively. The leaves were screened for the presence of secondary metabolites and the extracted oils for their antimicrobial potentials. The phytochemical screening revealed the presence of Phytates (27.81 mg/g) Phytic acids (7.833mg/g) Oxalates (3.061mg/g) Tannins (2.457mg/g) Phenols (30.00%) Flavonoids (2.948%) Saponins (3.166%) and Alkaloids (2.84%). In addition to this the leaves were found to contain anthraquinone. The two oils extracted from the leaves of *Eucalyptus torelliana* were studied for their antimicrobial activity against the following isolated microorganisms: *Xanthomonas axonopodis, Shigella dysenterine, Pseudomonas solanecearium, Streptococcus faecales, Staphylococcus aureus, Salmonella enterica, Erwinia carotovora, Salmonella typhi.* The oils were found to inhibit at the microorganism isolates. The zone of inhibition exhibited by the extracts on the tested microorganism was between 22-6mm. The oil extracted from *Eucalyptus torelliana* has antibacterial activities against enteric pathogens and the oil may be potential source of new antimicrobial sagainst enteric organisms.

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Keywords- Enteric microorganisms, essential oil, Eucalyptus torelliana, secondary metabolites, solvent extraction

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## **1** INTRODUCTION

The need to study medicinal plants cannot be overemphasized for many reasons including widespread use of plants in folk medicine, rescuing traditional medicinal plants and knowledge about them from imminent loss as well as the need of health for all. Since the first earth summit in Rio de Janeiro, there has been a sustained global awareness of the importance of the plethora of biodiversity and natural resources from tropical forests for several purposes. This stems not only from their ecotourism potentials, the forest products derivable there from, but also from the ethnobotanical and ethnomedicinal uses attached to the plant genetic resources obtained from these forests (Obute, 2005).

A large proportion of the world's population depends on traditional medicine because of the scarcity, high costs of orthodox medicine and unpleasant side effects (Tagboto, and Townson, 2001). Medicinal plants have provided the modern medicine with numerous plants derived therapeutic agents (Sanda et al., 2011). Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Oladunmoye et al., 2009). Orthodox medicines and cosmetics with high expensive nature and other unwanted side effects have led many to looking for a replaceable chemical entity of high quality, lower side effects and high effectiveness in contemporary and alternative medicines which come along with lower price rate. Phytochemicals are exogenous substances with diverse biochemical and biomolecular activities which is beneficial to human beings as a medicine or cosmetics.

The essential oils of the leaves have been used in the treatment of lung diseases and were stated to have antitubercular effect (Oyedeji *et al.*, 1999). Hot water extracts of dried leaves of *E. torelliana* are reportedly used traditionally as analgesic, anti-inflammatory and remedies for cancer-related symptoms and intestinal disorders (Silifat *et al.*, 2005). Eucalyptus has been used for many therapeutic effects thus the objective of this study is to extract the oil in the plant and evaluate the antimicrobial properties of the oil.

### **2 MATERIALS AND METHODS**

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### 2.1 PLANT COLLECTION AND IDENTIFICATION

The leaf samples of *Eucalyptus torelliana* was collected and identified at the Forestry Research Institute of Nigeria (FRIN) in Ibadan, Oyo State.

### **2.2 PREPARATION OF PLANT MATERIALS**

Fresh leaf samples of *Eucalyptus torelliana* were rinsed using sterile water to remove debris on it. The leaves were air-dried at room temperature for 21days then pulverized into a fine powder using a blender. The pulverized sample was stored in an air tight container at room temperature.

### **2.3 EXTRACTION OF OIL**

The normal oil was extracted by Soxhlet method of extraction, using N-hexane as the extracting solvent. The oil was extracted from dried leaves by immersing 100g of the pulverized sample in 350ml of N-hexane in a round bottom flask. The essential oil on the other hand was extracted by steam distillation. The pulverized leaves were boiled in 1 litre of water in a round bottom flask fitted with Clavenger apparatus. The setup was placed inside heating mantle and boil for 90 minutes. As the contents of the flask boils, the essential oil vaporized and condensed after passing through a condenser. The condensed essential oil was collected from the tap on the Clavenger apparatus.

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The plant secondary metabolites were screened according to Trease and Evans (1989); Sofowora (1996) and Guilei (1982).

# 2.4 EVALUATION OF ANTIMICROBIAL ACTIVITIES OF EUCALYPTUS OIL

All glassware were sterilized at 15psi (121°C) for 15 min. Thirty-eight grams of Muellar-Hinton Agar was dissolved in 100ml of distilled water. The mixture was gently heated to dissolve the media completely. It was then sterilized by autoclaving at 121°C for 15 min. The Agar media were allowed to cool to 45°C - 50°C. The microorganisms used were cultured aerobically 37°C for 24 h on peptone water and antibacterial test was carried out on the Muellar-Hinton agar plate. Agar well diffusion method of Martina et al. (2013) was employed. Pure isolates of each microorganism previously cultured on peptone water were seeded on plate. Sterile cork borer of 10mm diameter were used to make wells on the solidifying agar into which 0.5ml volume of the oil was aseptically introduced while the control has 0.10ml Ampiclox. The plates were incubated at 37°C for 24 hours and the zones of inhibition around the wells were measured with a digital Vernier calliper. Results were quoted as the radii (mm) of the zone of inhibition around the well (subtracting the radius diameter of the cup borer). The microorganisms used in this study are: Staphylococcus aureus, Erwinia carotovora, Xanthomonas axonopodis, Pseudomonas solacerium, Salmonella enterica, Shigella dysenterine, Salmonella typhi, Streptococcus faecalis.

# **3 RESULTS**

Qualitative phytochemical screening of the leaves showed that *E. torelliana* had tannins, saponins, phytic acid, phytate, flavonoids, anthraquinone, oxalates and phenols while the quantitative screening revealed that it contains 7.833mg/g phytic acid, 27.81mg/g phytate, 2.948mg/g flavonoid, 2.457mg/g tannins, 3.166mg/g saponins and 3.061mg/g oxalate.

# 3.1 ANTIBACTERIAL ACTIVITIES OF THE E. TORELLIANA OIL

The results of the antimicrobial activity of the oils against the test organisms revealed that the two oils inhibited the growth of all the test organisms. The ampiclox fractions also showed good activity on the isolates. The normal oil was not effective on S.typhi alone but the essential oil and ampiclox are effective on it. The zone of inhibition of normal and essential Eucalyptus oils ranged between 22-6mm with the normal oil having maximum effect on X. axonopodis, S. dysenterine, P. solacerium, S. faecales, and S. aureus. It has minimum effect on S. enterica and E. carotovora and it has no effect on S. typhi. The essential oil has maximum effect on P. solacerium, S. faecales, S. aureus, S. enterica and S. typhi. It has a minimum effect on X. axonopodis, S. dysenterine and E. carotovora while the control has a maximum effect on all the tested bacteria (Table 1).

Table 1. Antimicrobial properties of Eucalyptus torelliana oil

	Zones of inhibition		
Test organisms	Normal oil (mm)	Essential oil (mm)	Ampiclox (mm)
Xanthomonas axonopodis	22	9	25
Shigella dysenterine	20	8	20
Pseudomonas solanecearium	19	15	21
Streptococcus faecales	17	17	18
Staphylococcus aureaus	16	14	20
Salmonella enterica	13	13	25
Erwinia carotovora	6	9	18
Salmonella typhi	-	14	16

### **3.2 DISCUSSION**

Results of the phytochemical analysis of *Eucalyptus torelliana* oil revealed the presence of tannins, saponins, flavonoids, phytates, phytic acid and anthraquinones. Ahmad *et al.* (1998) and Shaliff (2001) have independently reported the presence of these components in members of the family Myrtaceae to which the plants used in this study belong. Babayi *et al.* (2004) had reported that the phytochemical analysis of the crude extracts of Eucalyptus species revealed the presence of saponin, phytate, flavonoids, oxalate, alkaloid, tannins, cardiac glycoside and phenols, thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components.

Phytochemical and antibacterial activity of *Eucalyptus globulus* extract showed that it is mainly due to the presence of phytochemical compounds such like tannins, saponins, steroids, reducing sugar, phenols, glycosides,

triterpenes and tripenoid but anthraquinone was absent (Sasikala & Kalaimathi, 2014). *Eucalyptus camaldulensis* also has tanins, saponins, and cardiac glycosides but anthraquinone and alkaloids are absent (Ayepola & Adeniyi, 2008). The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus, this plant could be utilized as an alternative source of useful antimicrobial drugs.

One of the molecular actions of tannins is to form complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation resulting in the inhibition of cell protein synthesis (Stern *et al.*, 1996). The presence of tannins therefore plays a significant role in the antimicrobial activity of the extracts. The antimicrobial activity of *Eucalyptus torelliana* could partly be explained by the presence of anthraquinones. The bacteriostatic and bactericidal activities of anthraquinone from Cassia italica have been established (Kazmi et al., 1994).

This study showed that Eucalyptus torelliana is an effective inhibitor of microbial growth as the oils extracted from it showed varying degrees of activity against the test organisms. There were little differences in the activity of ampiclox and this shows that both extracts can be exploited as antimicrobial agents. The normal oil showed higher activities against Xanthomonas axonopodis compared with the essential oil that has high effectiveness on Streptococcus faecales.

It was observed that the activity of the normal eucalyptus oil and ampiclox were very similar as the measurements of the zones of inhibition are very close. This is in support of the antimicrobial activity of Eucalyptus citriodora against Salmonella typhii as reported by Akin-Osanaiye et al., 2007. This relates that the two oils are very potent against Xanthomonas axonopodis, Shigella dysenterine, Pseudomonas solanecearium, Streptococcus faecales, and Staphylococcus aureus but higher doses of the antimicrobial agents will be required in infections caused by Salmonella typhii and Erwinia carotovora. The essential oil and the normal eucalyptus oil have antimicrobial activities and thus confirmed the historical use of eucalyptus oil as an antibacterial agent (Kumar, 1988).

# **4** CONCLUSION

The results of this study therefore form a good basis for selection of E. torelliana for further phytochemical and pharmacological investigation for its use as possible antimicrobial agents in the treatment of infections.

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