Fourier Transform Infrared (FTIR) Characterization and Antimicrobial Activity of *Eucalyptus globulus* Leaves

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Abstract
Plants had been used for the healing of diseases ages ago before the use of recent clinical drugs. Such medicinal plants are also recognised to have therapeutic properties or as precursors for the synthesis of useful drugs. Eucalyptus globulus is an example of such plants known to possess therapeutic properties. Therefore, this research focuses on extraction, fractionation, Fourier Transform Infrared characterisation and antimicrobial analysis of Eucalyptus globulus leaves. The crude methanolic extract obtained from maceration was fractionated into n-hexane and ethyl acetate fractions. The results of the FTIR analysis of the fractions (n-hexane and ethyl acetate) showed presence of different functional groups such as carboxylic acids, aromatics and alkanes. The crude methanolic extract shows antimicrobial and antifungal activities against all tested organisms with zone of inhibition varying from 12 to 23 mm at Minimum Inhibitory Concentration of 200 mg/mL. However, the fractions (ethyl acetate and n-hexane) showed little sensitivity against tested organisms at the MIC of 150 mg/mL and 250 mg/mL respectively. These results showed that, the crude extract is rich in antimicrobial properties compared to the fractions, and can be used as antimicrobial phytomedicine.

Keywords: Extraction, Antimicrobial, Fractionation, *Eucalyptus globulus*, and FTIR

Introduction

Mankind has exploited plants such as grass, wild plants, herbs and fruit-yielding trees for their medicinal values. Nowadays, following the discovery of different type of medicinal plants and development of their therapeutic application, the practice of traditional medicine has well been acknowledged and established as a profession. Through a number of medical and clinical researches, researchers have been able to demonstrate the specific physiological activity of a particular medicinal plant by the extraction and isolation of its bioactive compounds. This has also helped in pharmacological studies and synthesis of drugs from medicinal plants with a reduced toxicity and side effect (Assareh *et al*., 2010).

E. globulus is a shrubby plant or a flowering tree belonging to the family *Myrtaceae*. Eucalyptus is basically native to Tunisia and Australia but has also been found in Africa and from tropical to southern temperate regions of America (Umer *et al*., 2015). *E. globulus* has a fresh mint-like smell and a spicy, cooling taste and has various concentrations of minerals.

Eucalyptus are highly valued for their wood and are also a good source of proteins, tannins, and dyes although eucalyptus oil being the most valuable product. The oil can be readily distilled from their leaves (Sartorelli *et al*., 2007). The essential oils from the *Myrtaceae* family exhibit diverse biological activities like bacteriostatic, fungistatic and anti-inflammatory effects (Ben *et al*., 2011). Eucalyptus extracts have been approved as food additives and are currently used in various cosmetics formulation. Saponins, tannins, steroids and flavonoids have been found in the leaf extract of eucalyptus. Alkaloids and flavonoids possess antimicrobial activity (Sartorelli *et al*., 2007). Traditionally, eucalyptus leaves have been used to heal wounds and fungal infections. Eucalyptus leaves show many activities such as antioxidant, antiseptic and anti-inflammatory (Ben *et al*., 2011). Besides antimicrobial activity, the essential oil and its constituents also show herbicidal (Setia *et al*., 2007), insecticidal and anthelmintics (Rudin, 2005; Park and Shin, 2005), anti-tumor and anti-leech activities (Kirton, 2005).

However, this research is focused on the extraction, fractionation, antimicrobial activity and FTIR analysis of the fractions and crude extract of *E. globulus* leaves.

Materials and Methods

Sample Collection

The *Eucalyptus globulus* leaves used were collected from Dekina Local Government Area of Kogi State, Nigeria, and was taken to Botany Department, Kogi state University Ayingba for proper identification. The specimen voucher number is PT-013

Sample Treatment

The collected plant samples were washed to remove sand particles and it was allowed to dry at room temperature for two weeks. After air drying, the Plant was pulverised to fine powder using mortar and pestle, and stored in an air tight container until required for analysis.

Extraction of Plant Sample

The powdered leaves (250 g) were extracted with 1000 mL of methanol using maceration for period of 72 hours. The percentage yield of the extract was calculated.

Fractionation of the Methanolic Extract

The methanolic extract (10 g) was soaked with 50 mL distilled water to make a suspension in a separatory funnel, and about equal volume of hexane was added. The mixture was shaken carefully, and allowed to stand until two clear layers are formed. The hexane layer (upper one) was separated to obtain the hexane fraction. The process was repeated at least three times. Afterwards, ethyl acetate was added to the aqueous layer and extracted in the same manner to get ethyl acetate fraction. The solvent from each fraction was evaporated, weighed and the yield was calculated.

FT-IR Analysis

Fourier transform infrared (FTIR) spectroscopy was used to reveal all the possible functional groups present in the fractions (hexane

and ethyl acetate). IR absorption spectra were recorded in the 4000-650 cm^{-1} range on a Perkin-Elmer FT-IR spectrometer model 2000 using KBr pellets. The fractions (hexane and ethyl acetate) were placed in contact with KBr disc and FT-IR spectra were collected at the frequency of 4000-650 cm^{-1} by coadding 32 scans at a resolution of 8 cm^{-1}. The analysis was carried out by Agilent Technologies, Katsina, Nigeria.

Antimicrobial Assay

Collection and Identification of Test Organisms

The organisms tested with the methanolic extract for antimicrobial activity were pure clinical isolates of *Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Aspergillus niger and Candida albican*. The bacterial isolates were obtained from the Department of Microbiology, Kogi State University, Anyigba.

Antimicrobial Screening

The antimicrobial activity of the methanolic plant extract was determined using agar well diffusion method which has been recommended as a standard protocol for conducting antimicrobial screening (Irobi *et al*., 1994). The microbes were cultured with nutrient broth and incubated at a temperature of 37°C for a period of 24 h. The cultured microbes were sub-cultured again for another 24 h to obtain purer microbial colonies. Mueller-Hinton agar powder (14 g) was prepared in 500 mL distilled water and sterilized in an autoclave for 15 min at a temperature of 120°C in order to get rid of any possible contamination of the agar. The petri dish together with the cork borer, wire loop and other materials needed for the work were sterilized to eliminate unwanted contaminations. 1 mL colony forming units (cfu) each of the microbes were respectively transferred into the sterilized petri dish using syringes. A 100 mL of the prepared Mueller-Hinton agar were poured into the petri dish containing the various microbes and allowed to solidify, and this was repeated for the controls (negative control (water) and positive control (streptomycin)). A 6 mm cork borer was used to bore wells on the media. Clear labels were made on each of the petri dishes based on the concentration and type of

extract. Crude extract (0.15 µg to 0.20 µg) of methanol was dissolved in 10 mL of distilled sterile water. A 100 µL of the various concentrations of each extract were inoculated into each well and allowed for the extract to diffuse into the Muller-Hinton agar and subsequently placed in the incubator at 37°C for 24 h. The antimicrobial activity of the extract was monitored by observing the various zones of inhibition after the incubation by measuring the diameter of the zones of inhibition using vernier caliper. Streptomycin was used as a positive control and distilled sterile water was used as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

The estimation of MIC for the crude methanolic extract was carried out using the method of Akinpelu and Kolawole (2004). 0.5 mL of varying concentrations of the extract (100, 150, 200 mg/mL) were dispensed into each test tubes containing nutrient broth, inoculated with loopful of each test organism. 0.5 Mcfarland turbidity standard was adopted. A tube containing nutrient broth with test organism, but no extract serve as control. The MIC was taken from the tube with least concentration, showing no visible turbidity after 24 hours incubation at 37 °C. Therefore, growth was examined by observing turbidity.

Results

The results of FTIR for the fractions (hexane and ethyl acetate) and the antimicrobial analysis of the crude methanolic extract and fractions are presented in the Tables 1-8 and Figures 1-2.

Fourier transform Infrared (FTIR) spectroscopy was used to reveal all the possible functional groups present in the crude methanolic extract fractions (Tables 1-2; Figures 1-2). IR absorption spectra were recorded in the 4000-650 cm^{-1} range. Tables 3-8 shows the antimicrobial activity and minimum inhibition concentration (MIC) of the crude methanolic extract and fractions (ethyl acetate and n-hexane) on *Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Aspergillus niger and Candida albican*.

Table 1: FTIR bands of Absorption for n-Hexane Fraction

Wave number (cm^{-1})	Assignment
3368	O-H_{str} (Carboxylic acid)
2854; 2925	C-H_{str} (Aliphatic)
1689	C=O_{str} (Carboxylic acid)
1622; 1514	C=C_{str} (Aromatic)
1033; 1461	C-H_{bend} (Aliphatic)
1272; 1179	C-O_{str} (Acyl)
717; 769; 832; 918	C-H_{bend} (Aromatic)

Table 2: FTIR bands of Absorption for Ethyl Acetate Fraction

Wave number (cm^{-1})	Assignment
3394	O-H_{str} (Carboxylic acid)
2851; 2921	C-H_{str} (Aliphatic)
1689	C=O_{str} (Carboxylic acid)
1514; 1629	C=C_{str} (Aromatic)
1041; 1093; 1458	C-H_{bend} (Aliphatic)
1179; 1275	C-O_{str} (Acyl)
665; 683; 724; 773	C-H_{bend} (Aromatic)

Table 3: Antimicrobial Activity of the Methanolic Extract on Test Organisms

	Concentration (mg/mL)/Zone of inhibition (mm)				
Test organisms	**100 mg/mL**	**150 mg/mL**	**200 mg/mL**	**Positive control**	**Negative control**
Bacteria					
Staphylococcus aureus	15 mm	20 mm	23 mm	33 mm	0 mm
Salmonella typhi	12 mm	13 mm	15 mm	0 mm	0 mm

Bacillus Subtillis	12 mm	15 mm	17 mm	31 mm	0 mm
Fungi					
Candida albican	17 mm	18 mm	19 mm	0 mm	0 mm
Aspergillus niger	12 mm	14 mm	16 mm	0 mm	0 mm

Table 4: Antimicrobial Activity of the Ethyl Acetate Fraction on Test Organisms

	Concentration (mg/mL)/Zone of inhibition (mm)					
Test organisms	**100 mg/mL**	**150 mg/mL**	**200 mg/mL**	**250 mg/mL**	**Positive control**	**Negative control**
Bacteria						
Staphylococcus aureus	10 mm	15 mm	19 mm	25 mm	20 mm	0 mm
Salmonella typhi	8 mm	10 mm	12 mm	14 mm	16 mm	0 mm
Bacillus Subtillis	12 mm	15 mm	18 mm	20 mm	28 mm	0 mm
Fungi						
Candida albican	16 mm	9 mm	12 mm	14 mm	18 mm	0 mm
Aspergillus niger	15 mm	18 mm	20 mm	24 mm	16 mm	0 mm

Table 5: Antimicrobial Activity of the n-Hexane Fraction on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)					
	100 mg/mL	**150 mg/mL**	**200 mg/mL**	**250 mg/mL**	**Positive control**	**Negative control**
Bacteria						
Staphylococcus aureus	6 mm	6 mm	6 mm	6 mm	7 mm	0 mm
Salmonella typhi	0 mm	0 mm	0 mm	0 mm	10 mm	0 mm
Bacillus Subtillis	0 mm	0 mm	18 mm	18 mm	16 mm	0 mm
Fungi						
Candida albican	6 mm	6 mm	9 mm	9 mm	18 mm	0 mm
Aspergillus niger	6 mm	6 mm	10 mm	10 mm	17 mm	0 mm

Table 6: Minimum Inhibitory Concentration (MIC) of the Methanolic Extract on Test Organisms

	Concentration (mg/mL)/Zone of inhibition (mm)				
Test organisms	**100 mg/mL**	**150 mg/mL**	**200 mg/mL**	**Positive control**	**Negative control**
Bacteria					
Staphylococcus aureus	NG	NG	NG	NG	G
Salmonella typhi	G	G	NG	G	G
Bacillus Subtillis	G	NG	NG	NG	G
Fungi					
Candida albican	NG	NG	NG	G	G

Aspergillus niger	G	NG	NG	G	G

Key: NG = No growth, G = Growth

Table 7: Minimum Inhibitory Concentration (MIC) of the Ethyl Acetate Fraction on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)					
	100 mg/mL	150 mg/mL	200 mg/mL	250 mg/mL	Positive control	Negative control
Bacteria						
Staphylococcus aureus	G	G	NG	NG	NG	G
Salmonella typhi	G	G	G	G	G	G
Bacillus Subtillis	G	G	G	NG	NG	G
Fungi						
Candida albican	G	G	G	G	G	G
Aspergillus niger	G	NG	G	NG	G	G

Key: NG = No growth, G = Growth

Table 8: Minimum Inhibitory Concentration (MIC) of the n-Hexane Fraction on Test Organisms

Test organisms	Concentration (mg/mL)/Zone of inhibition (mm)					
	100 mg/mL	150 mg/mL	200 mg/mL	250 mg/mL	Positive control	Negative control
Bacteria						
Staphylococcus aureus	G	G	G	G	G	G
Salmonella typhi	G	G	G	G	G	G
Bacillus Subtillis	G	G	G	NG	G	G
Fungi						
Candida albican	G	G	G	NG	NG	G
Aspergillus niger	G	G	G	G	NG	G

Key: NG = No growth, G = Growth

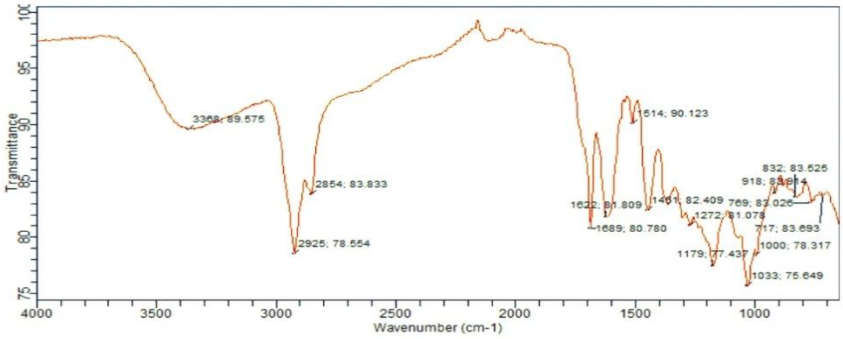

Figure 1: FTIR Spectra of the Hexane Fraction

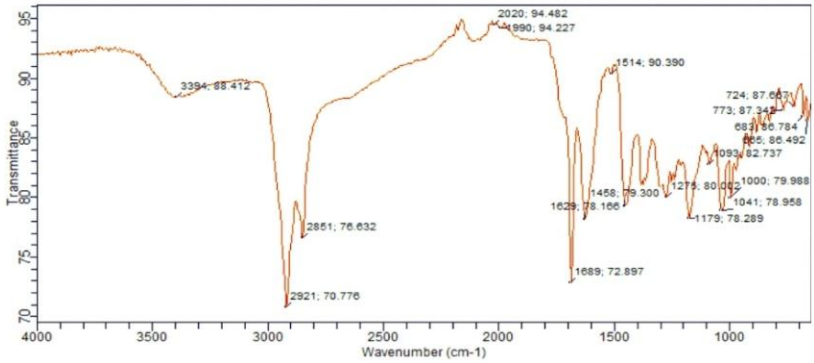

Figure 2: FTIR Spectra of the Ethyl Acetate Fraction

Discussion

Tables 1-2 and Figures 1-2 shows the interpretation and FTIR spectra of the fractions (hexane and ethyl acetate) of the crude methanolic extract of *Eucalyptus globulus* leaves.

n-Hexane Fraction: The characteristic absorption band were exhibited at 3368 cm^{-1} (O-H stretching for carboxylic acid), 2854 and 2925 cm^{-1} (C-H stretching for aliphatic), 1689 cm^{-1} (C=O stretching for carboxylic acid), 1514 and 1622 cm^{-1} (C=C stretching for aromatic), 1033 and 1461 cm^{-1} (C-H bending for aliphatic), 1179 and 1272 (C-O stretching for acyl), and the peaks at 717, 769, 832 and 918 reveals C-H bending for aromatic.

Ethyl acetate Fraction: The characteristic absorption band were exhibited at 3394 cm^{-1} (O-H stretching for carboxylic acid); 2851 and 2921 cm^{-1} (C-H stretching for aliphatic); 1689 cm^{-1} (C=O stretching for carboxylic acid), 1514 and 1629 cm^{-1} (C=C stretching for aromatic); 1041, 1093 and 1458 cm^{-1} (C-H bending for aliphatic); 1179 and 1275 (C-O stretching for acyl); and the peaks at 665, 683, 724 and 773 reveals C-H bending for aromatic.

Tables 3-5 show the antimicrobial activity of the crude methanolic extract and fractions (ethyl acetate and n-hexane) of *Eucalyptus globulus* leaves on the test organisms (*Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Aspergillus niger and Candida albican*).

The zone of inhibition expressed by various concentrations of the crude methanolic extract (Table 3) on the test organisms range from 12 – 23 mm. *Candida albican* and *Staphylococcus aureus* were sensitive in all the varying concentration whereas, all the test organisms are sensitive at the highest concentration (200 mg/mL) only.

Tables 4-5 show the zones of inhibition expressed by various concentrations of the fractions; n-hexane fraction (0 – 18 mm) and ethyl acetate (8 – 25 mm). *Staphylococcus aureus*, *Bacillus subtilis and Aspergillus niger* were sensitive to the ethyl acetate fraction at the highest concentration (250 mg/mL) whereas, *Bacillus subtilis* and *Candida albican* were sensitive to the n-hexane extract at the highest concentration (250 mg/mL).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (antibiotic, bacteriostatic or antifungal) drug that will inhibit the visible growth of a microorganism after overnight incubation (Tripathi, 2013). Table 6 shows that the crude extract inhibits growth at 100 mg/mL (0.10 µg/mL) and, didn't show any sign of turbidity or growth for *Staphylococcus aureus and Candida albican.* Therefore, the value 100 mg/mL (0.10 µg/mL) was taken as the minimum inhibitory concentration. However, turbidity at the highest concentration was not observed for all the test organisms. Table 7 shows that the ethyl acetate fraction inhibits growth at 150 mg/mL (0.15 µg/mL) and, didn't show any sign of turbidity or growth for *Aspergillus niger*. So, 150 mg/mL was taken as the MIC. However, at the highest concentration, turbidity was not observed for *Staphylococcus aureus, Bacillus subtilis and Aspergillus niger*.

Table 8 shows that the n-hexane fraction inhibits growth at the highest concentration (250 mg/mL) and so, this value is taken

as the MIC. However, at this concentration, the n-hexane fraction was sensitive against *Bacillus subtilis* and *Candida albican*.

Conclusion

Plants are found in nearly all regions of the world. The prevailing climatic, soil and environmental conditions often play a vital role in determining the type of plant species that could be found in such region. Due to the challenges associated with drug resistance, which have made scientists to search for effective and sustainable means of managing the problem. Plants have emerged as an alternative to synthetic antibiotics which is prone to reoccurring drug resistance. The result of antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of *Eucalyptus globulus* against bacterial (*S. aureus, S. typhi* and *B. subtilis*) and fungi (*Aspergillus niger* and *Candida albican*) isolates used in this study. The MIC value of different organisms are verified, and thus, MIC are assays capable of verifying that the compound has antimicrobial activities, and that it gives reliable indication of the concentration of medicine required to inhibit the growth of microorganisms.

References

Akinpelu, D. A. and Kolawole, D. O. (2004). Phytochemistry and Antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum). *Science Focus*. 7: 64 -70.

Assareh, M. H., Sedaghati, M., Kiarostami, K. and Zare, A. G. (2010). Seasonal changes of essential oil composition of *Eucalyptus maculate Hook. Iranian Journal of Medicinal and Aromatic Plants*. 25: 580-588.

Ben, M. H. N., Romdhane, M., Lebrihi, A., Mathieu, F. and Couderc. F. (2011). *Eucalyptus oleosa* essential oils: chemical composition and antimicrobial and antioxidant activities of the oils from different plant parts (stems, leaves, flowers and fruits). *Molecules*. 16(2): 1695-1709.

Irobi, O. N., Moo-Young, M. and Anderson, W. A. (1996). Antimicrobial Activity of Annatto (*Bixa orellana*) extract. *International Journal of Pharmacognosy*. 34(2): 87 – 90.

Kirton, L. G. (2005). Laboratory and field test of the effectiveness of the lemon-eucalyptus extract, Citriodiol, as a repellent against land leeches of the genus *Haemadipsidae*. *Annals of Tropical Medicine & Parasitology*. 99:695-714.

Park, I. K. and Shin, S. C. (2005). Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitemes speratus Kolbe*). *Journal of Agricultural and Food Chemistry*. 153:4388- 4392.

Rudin, W. (2005). Protection against insects. *Therapeutische Umschau*. 62:713-718.

Sartorelli, P., Marquioreto, A. D., Amaral-Baroli, A., Lima, M. E. L. and Moreno, P. R. H. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of eucalyptus. *Phytotherapy Research*. 21:231-233.

Setia, N., Batish, D. R., Singh, H. P. and Kohli, R. K. (2007). Phytotoxicity volatile oil from Eucalyptus citriodora against some weedy species. *Journal of Environmental Biology*. 28:63-66.

Tripathi, K. D. (2013). Essentials of Medical Pharmacology (7th ed.). New Delhi, India: Jaypee Brother Medical Publishers. Pp. 696-697.

Umer, H., Muhammad, I. J., Rafia, R. and Farwa, N. (2015). A Review on *Eucalyptus globulus*: A New Perspective in Therapeutics. *International Journal of Chemical and Biochemical Sciences*. 8: 85-91.