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Phytochemical Profiling, Cytotoxicity, and Antimicrobial Activities of Gum Extracts from the Stem Bark of *Eucalyptus globulus*

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ABSTRACT

Background: *Eucalyptus globulus* is well known for its medicinal applications. However, there are limited reports on the medicinal potential of the gum obtained from the stem bark of the plant. This study aims to evaluate the cytotoxicity, antimicrobial potential, and chemical constituents of the gum derived from the stem bark of *Eucalyptus globulus*.

Method: The gum was collected and extracted into methanol (EG-CE) and further fractionated into dichloromethane (EG-DF) and aqueous (EG-AR) fractions. These extracts were assessed for cytotoxic and antimicrobial activities. Phytochemical profiling was conducted using GC-MS to identify bioactive compounds.

Results: Bioassays revealed strong cytotoxicity of EG-CE (IC_{50} = 0.06 µg/mL) in the brine shrimp lethality test, surpassing EG-DF and EG-AR. EG-DF exhibited superior growth inhibitory effects on *Allium cepa* root (IC_{50} = 1.09 µg/mL) and *Sorghum bicolor* radicle (IC_{50} = 1.43 µg/mL), suggesting its potential as a natural herbicide. The antimicrobial assays demonstrated concentration-dependent activity, with EG-CE showing the highest efficacy against both clinical and standard strains of *S. aureus* (ATCC 25913) and *C. albicans* (ATCC 3147). EG-DF was most effective against *E. coli* (00726), highlighting the role of non-polar constituents in antimicrobial potency. GC-MS analysis identified bioactive compounds such as octadecenoic acid methyl ester, hexadecanoic acid, and cinnamic acid derivatives, known for antimicrobial and anti-inflammatory properties.

Conclusion: This study provides new insights into the bioactivity of *Eucalyptus globulus* gum, suggesting its potential contributions to natural product-based innovations.

1. Introduction

Infectious diseases are a major cause of public health concern and they account for about 17 million deaths annually (WHO, 2020) before the advent of COVID-19. To contain the devastation caused by infectious diseases, several antimicrobial agents have been identified and developed many of which have clinical efficacy against several strains of disease-causing pathogens. Following a prolonged and irrational use of commonly available antibiotics, there has arisen several resistant micro-organisms1.

The emergence of resistant strains of micro-organisms is the main drive and stimulation behind the ceaseless search for newer and more effective antimicrobial agents from all possible sources including medicinal plants and other products2. Medicinal plants are recognised as a prominent source of anti-infective agents that have been employed for the management of several types of infectious diseases

from time immemorial. From the few medicinal plants that have been explored, there has been identification of potent and novel anti-infective agents. However, most of the medicinal plants used in many traditional settings have not been scientifically evaluated 3 and in some cases, several morphological parts, tissues or naturally derived products of known plants are yet to be fully explored including the gum of *Eucalyptus globulus* stem bark.

Eucalyptus globulus Labill. (family: **Myrtaceae**) is famous for its extensive medicinal applications and is widely cultivated in many parts of the world for its economic value. Several parts of the plant have been used as analgesic, anti-inflammatory, and antipyretic remedies for the relief of symptoms arising from respiratory infections such as colds, flu, and sinus congestion 4. The plant has become famous for its essential oils which are highly demanded for its wide application in medicines and cosmetics 5. There are several reports in the literature on the medicinal uses of *E. globulus* leaves, essential oils and other parts. However, there are limited reports on the medicinal application of the gum obtained from the stem bark of the plant. Hence, this study is focused on evaluating cytotoxicity, antimicrobial potential and chemical constituents of the gum derived from the stem bark of *Eucalyptus globulus*.

2.0 Method

2.1 Plant Collection and Authentication: Dried gum exudates on the stem bark of the *Eucalyptus globulus* tree were collected in 2019 at Stadium Road, Ilorin, Kwara State, Nigeria (Latitude: 8.4803° N, Longitude: 4.5478° E). The plant was identified and authenticated by Mr. Bolu Ajayi of the Department of Plant Biology at the University of Ilorin Herbarium, where a voucher specimen was deposited with the number UILH/004/2020/1073.

2.2 Preparation of Extract: The gum was air dried to constant weight, pulverised and extracted into 70% methanol by cold maceration for 72 h. The extract obtained was concentrated *in vacuum* using a rotary evaporator and the crude extract obtained was labelled as **EG-CE**. A portion (50 g) of **EG-CE** was dissolved into aqueous methanol solution (1:3) and portioned into dichloromethane (non-polar) fraction and aqueous (polar) phase and concentrated. The crude extract of *Eucalyptus globulus* (EG-CE), dichloromethane fraction (EG-DF), and aqueous residues (EG-AR) were stored in the refrigerator at 4 °C until they were needed for bioassay.

2.3 Cytotoxic and Herbicidal Evaluation

2.3.1 Brine Shrimp Lethality (BSL) Assay: The cytotoxic potential of the gum extract and fractions were determined by their ability to kill the nauplii of *Artemia salina*. The nauplii of *A. salina* were obtained following a 48 h incubation of the *A. salina* eggs in natural seawater. The BSL assay was done using a method earlier used by Salawu and co-workers (2017)6.

2.3.2 *Sorghum bicolor* Radical Growth Inhibitory Assay: Viable seeds of *Sorghum bicolor* were used to estimate the growth inhibitory potential of crude gumextract (**EG-CE**), dichloromethane fraction (**EG-DF**) and aqueous residue (**EG-AR**). Ten millilitres (10 mL) of 39.06, 156.25, 625, 2500 and 10000 µg/mL were prepared by four-fold dilution from an initial stock solution prepared from twenty milligrams (20 mg) of the CE, DF and AR dissolved in 20 mL of 5% DMSO (Sigma-Aldrich, Germany). Also, 10 mL of the same concentrations as above were prepared for cyclophosphamide (positive control). The different concentrations of the extract (10 mL) were poured into different petri-dish lined with cotton wool and filter (Whatman No.1). Ten viable seeds were spread on each of the petri-dishes and incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured after 96 hours incubation. The negative control seeds were treated with 10mL 5% DMSO in distilled water7. The experiment was repeated in three replicates for all concentrations and controls. The radical lengths were measured to the nearest millimetre. The percentage radical growth inhibition was calculated using the formula below;

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100\%$$

Where;
A = length of untreated radicle.
B = length of treated radicle with plant extracts/ cyclophosphamide

2.3.3 *Allium cepa* Root Growth Inhibitory Assay: The *A. cepa* root growth inhibitory assay was performed using a modified method described by Akinboro and Bakare8. Bulbs of *A. cepa* (50 ± 10 g) were washed with distilled water and grown in the dark over tap water at ambient temperature for 24-36 h until the roots had grown to approximately 2-3 cm in length. Twenty (20) mL of different concentrations of **EG-CE**, **EG-DF** and **EG-AR** (39.06, 1250, 2500, 5000 and 10000 µg/mL) were prepared

by dissolving extract in 20mL of 5 % DMSO, different concentrations were poured into different petri-dish and the base of each of three *A. cepa* bulbs were placed on a Petri-dishes containing each test sample (39.06 - 10000 µg/mL). The same concentrations as above were prepared for cyclophosphamide (positive control), while the negative control bulbs were treated with 20 mL of 5% DMSO in distilled water. The root lengths were measured at 0 and 96 hours for each concentration of extract and control was measured. The percentage root growth inhibition after treatment with extract/cyclophosphamide at 96 hours was determined. The percentage root growth inhibition after treatment with extract/cyclophosphamide at 96h was determined [9] using the formula below;

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100\%$$

Where;

A = length of untreated root.

B = length of treated root with plant extracts/ cyclophosphamide

2.4 Determination of Antimicrobial Activity

2.4.1 Test Organisms: Three clinical isolates and three type strains each of the bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Citrobacter freundii*), along with one clinical isolate and one type strain of the fungus (*Candida albicans*), were used as test organisms to determine the antimicrobial potential of the crude extract (EG-CE), dichloromethane fraction (EG-DF), and aqueous residue (EG-AR) of gum derived from *Eucalyptus globulus* stem bark extract.

Prior to the bioassay, the viability of all bacterial strains was confirmed by subculturing on nutrient agar and incubating at 37 °C for 24 hours. Morphologically consistent colonies were observed with no contamination. Gram staining and biochemical tests further confirmed their identity: *P. aeruginosa* (Gram-negative rods, oxidase-positive), *E. coli* (Gram-negative rods, lactose-fermenting), and *C. freundii* (Gram-negative rods, citrate-positive). The fungal strains were similarly revived on Sabouraud dextrose agar and confirmed microscopically.

Discrete colonies from overnight cultures were picked and emulsified in 5 mL of sterile normal saline, vortexed, and visually compared to a 0.5 McFarland turbidity standard to achieve approximate final concentrations of 1.5×10^8 CFU/mL for bacteria 10 and 2.0×10^6 CFU/mL for fungus 11. The turbidity standard was prepared by adding 9.6 mL of 1% aqueous barium chloride solution to 0.4 mL of 1% sulfuric acid, yielding a reference microbial density 12.

2.4.2 Antimicrobial Susceptibility Assay: The antimicrobial assay was performed using the disc diffusion technique earlier described by Ngamsurach&Praipipat (2022)13. A stock solution (50 mg/mL) of the test extract was prepared by dissolving 500 mg each of the extract/fraction in 10 mL of sterile distilled water. The bacterial test strains were spread over the nutrient agar plates by using separate sterile cotton buds. The fungal strain was spread over the potato dextrose agar plates. Four concentrations (50.00, 25.00, 12.50 and 6.25 mg/mL) of the extract/fraction were prepared by serial dilution. Thereafter, 5 mm diameter discs were impregnated with 100 µL of 50.00, 25.00, 12.50 and 6.25 mg/mL of extract/fraction to achieve a 5, 2.5, 1.25 and 0.63 mg/disc. The discs were mounted on inoculated agar and incubated. All bacterial plates were incubated at 37°C for 24 h and fungal plates at 25°C for 72h. Moreover, filter paper discs (5 mm diameter) containing standard antibiotics; gentamicin (0.04 mg/disc) and fluconazole (0.05 mg/disc) were used as positive controls. The zones of inhibition were recorded in millimetres (mm) as the diameter of growth-free zones around discs. The extract and standard antibiotics were independently tested in three replicates and the results were presented as mean±SEM. The extract is considered active against the organism upon which it showed a zone of inhibition greater than ≥10 mm14. An estimated zone of inhibition at a concentration of control (E-ZIC) was derived from a linear regression curve using GraphPad Prism. Activity index (AI) compares the antimicrobial activity of the sample/extract to the positive control using a mathematical model, the method was described in our earlier study 15. The formula below was used in calculating AI;

$$\mathbf{AI} = \frac{\mathbf{E - ZIC}}{\mathbf{ZOI}}$$

AI = Activity Index, E-ZIC= Estimated Zone of Inhibition at Concentration of Control, ZOI = Zone of Inhibition of Positive Control

2.5 Gas Chromatography-Mass Spectroscopy

The phytochemical investigation of the non-polar (dichloromethane fraction) of*E. globulus* extract was performed using GC-MS (Thermo Scientific Co.) equipment. The GC-MS instrument controlled parameters are as follows: Injection source (PAL Sampler), injection volume (2µL), column (Agilent technologies-HP-5MS),

column dimension (360 ˚C: 30 m x 250 µm x 0.25 µm), initial temperature (50˚C), program temperature (260 ˚C), initial pressure (9.05 psi), average velocity (38.724 cm/sec), holdup time (1.2912 min), run time (82.286 min). The sample dissolved in HPLC grade hexane was run full and compared by using Database- Spectrum MS-NW-1798. Suggestions with the highest confidence interval greater than 80 % were selected. Identification of the compounds was based on the comparison of retention indices and mass spectra of most of the compounds with data generated under identical experimental conditions by applying a two-dimensional search algorithm, considering the retention index, as well as mass spectral similarity. The relative percentage amount of each component was calculated by comparing its peak area to the total area of peaks in the chromatogram.

2.6 Data Analysis

Data obtained was analyzed by a GraphPad prism computer program. The concentration with 50% growth inhibition (IC_{50}) in *Sorghum bicolor* radical growth inhibitory assay and *Allium cepa* root growth inhibitory assay were estimated from a dose-response inhibition curve using a non-linear regression curve data analysis. The results are displayed as mean ± SEM of three replicates.

3. Results

3.1 Yield of Crude Extract and Fractions

Dark brown gum was collected from the stem bark of *E. globulus* and air-dried and pulverized. Hundred and fifty (150 g) grams of the gum was extracted into 70% methanol and yielded 83 g of EG-CE (55.3% $^{w}/_{w}$). Fifty grams (50 g) of the **EG-CE** was partitioned into dichloromethane and aqueous phase, the dichloromethane fraction led to about 9 g of dichloromethane soluble constituents yielding 18.0 % of EG-DF shown in Table 1. However, the aqueous residue accounted for 70% of the total yield of the fraction. The **EG-DF** was characterized by a strong aromatic smell while **EG-AR** was not characterised by an aromatic smell.

Table 1: Percentage Yield ($^{w}/_{w}$)of Crude Extract and Fractions of Gum Derived from *Eucalyptus globulus* Stem Bark

Sample	Weight (gram)	Percentage Yield (% $^{w}/_{w}$)
Gum Material	150.0	NA
EG-CE	83.0	55.3
EG-DF	9.0	18.0
EG-AR	35.0	70.0

Keys:
NA= not applicable
EG-CE = Crude extract of *E. globulus*, EG-DF = Dichloromethane Fraction, EG-AR = Aqueous Residues

3.2 Cytotoxic and Herbicidal Activity of the Extract and Fractions of *E. globulus* Gum.

The EG-CE, EG-DF and EG-AR demonstrated different levels of bioactivity in the brine shrimp lethality and growth inhibitory activities as shown in Table 2.

It was observed that EG-CE (IC_{50}=0.06±0.01 µg/mL), followed by EG-AR (IC_{50} = 0.09 µg/mL), and EG-DF (IC_{50} = 0.20 µg/mL). Compared to cyclophosphamide (IC_{50}=0.12±0.08 µg/mL) shown in Table 2 This indicates EG-CE's strong cytotoxic potential. However, EG-DF demonstrated the least cytotoxicity against the brine shrimp, interestingly EG-DF demonstrated the highest growth inhibitory activity in both ACRGI and SBRGI assays as shown in Table 2. The growth inhibitory activities of the EG-DF (IC_{50} of 1.09±0.06 and 1.43±0.13) were observed to be comparable to those afforded by the cyclophosphamide (IC_{50} of 1.07±0.19 and 1.61±0.84) in the two growth inhibitory model (ACRGI and SBRGI assays, respectively). The EG-AR also demonstrated better growth inhibitory activities than the EG-CE as shown in Table 2.

Table 2: Brine Shrimp Lethality, *Allium cepa* Root Growth Inhibition and *Sorghum bicolor* Radicle Growth Inhibitory Effects of Gum Derived from *Eucalyptus globulus* Stem Bark

Bioassay	Concentration of Extract/fraction with 50% Lethality / Inhibition (IC_{50} µg/mL)			
	EG-CE	EG-DF	EG-AR	Positive Control
BSL	0.06±0.01	0.20±0.06	0.09±0.01	0.12±0.08
ACRGI	2.15±0.16	1.09±0.06	2.01±0.18	1.07±0.19
SBRGI	2.27±.013	1.43±0.13	1.94±0.09	1.61±0.84

Keys:
BSL = Brine Shrimp Lethality, ACRG = Allium cepa Root Growth Inhibition, SBRGI = Sorghum bicolour Radicle Growth Inhibition, EG-CE = Crude extract of *E. globulus*, EG-DF = Dichloromethane Fraction, EG-AR = Aqueous Residues

3.3 Antimicrobial activity of crude extract and fractions of *E. globulus* Gum

The extract and fractions of *E. globulus* gum and the standard antibiotics demonstrated concentration-dependent antimicrobial activity against clinical and typed strains of *S. aureus, E. coli, C. freundiii* and *C. albican* as shown in Table 3. The extracts at 25 mg/mL demonstrated a maximum zone of inhibition against the clinical isolate of *C. albican* (21±1.33 mm) and the typed strain of *C. freundiii* (25±0.29 mm). The crude extract appeared to be more active against typed strains of the test organism as shown in Table 3. The fractions obtained from the crude extract however displayed varied activity with EG-DF(non-polar) displayed the highest activity against *E. coil* with a zone of inhibition of 23±1.58 mm, while the polar fraction (EG-AR) appeared to display weak activity against the panel of micro-organisms compared to the EG-CE and EG-DF as shown in Table 3. A linear regression curve of the activity of the extracts and fractions was used to estimate the activity (zones of inhibition) of the extract and fractions at the concentration of the positive control (imipenem at 10µg /mL and fluconazole at 5µg/mL). The only EG-CE displayed a comparable activity to imipenem against *S. aureus* with an estimated 28 mm zone of inhibition. The activity of the extract/fractions against the positive control was measured as activity index (AI) and it was observed that the EG-CE was most active compared to the fractions (EG-DF & EG-AR), with EG-CE displaying the highest AI against clinical strains of *C. albican* (AI=0.84) and typed strain of *S. aureus* (AI=1). While EG-DF (AI=0.84) and EG-AR (AI=0.64) had the highest AI against *C. albican* and *E. coli*, respectively. The activity indices (AI) demonstrated that EG-CE consistently outperformed other fractions in antimicrobial potential.

Table 3. Antimicrobial Activity of Extract and Fractions of *Eucalyptus globulus* Gum

Sample	Conc. (mg/mL)	Clinical Isolate				Typed Strain (ATCC No)			
		S. aureus	*E. coli*	*C. freundiii*	*C. albican*	*S. aureus* (25913)	*E. coli* (00726)	*C. freundiii* (8090)	*C. albican* (3147)
EG-CE	**100**	26±1.33	30±3.14	25±2.38	30±2.42	28±2.47	30±3.33	36±1.17	27±1.46
	75	26±2.15	24±1.06	22±1.67	26±2.13	24±1.03	28±0.93	30±1.99	24±1.25
	50	24±1.36	22±3.33	20±1.56	25±1.33	20±1.81	27±1.28	28±1.57	20±0.39
	25	16±1.83	10±0.97	16±1.39	21±1.33	18±0.46	24±1.33	25±0.29	18±1.53
EG-DF	**100**	16±1.27	20±1.66	21±0.93	25±2.09	30±2.33	30±1.73	25±1.81	30±1.73
	75	14±1.48	18±1.47	17±1.24	25±1.88	24±1.80	28±1.19	25±1.25	25±1.28
	50	14±1.09	17±1.85	12±0.56	22±1.37	20±1.88	26±1.27	22±1.93	20±1.24
	25	12±0.33	13±1.31	7±1.03	20±1.73	15±0.25	23±1.58	20±1.56	18±1.73

EG-AR	**100**	20±1.89	22±1.28	25±1.89	20±1.33	18±1.27	32±1.39	26±1.88	18±1.47
	75	18±1.43	17±1.16	21±1.01	17±1.00	15±1.48	30±1.33	23±1.33	15±0.93
	50	16±1.44	16±1.40	17±1.39	15±0.56	12±1.21	25±1.25	21±1.77	12±1.46
	25	14±1.59	12±0.33	15±0.98	14±0.50	10±1.56	16±1.68	15±1.46	10±0.00
E-ZIC	**EG-CE**	15.01	6.00	13.50	18.50	14.00	22.50	21.00	14.50
	EG-DF	11.00	11.50	2.50	18.50	10.00	21.00	18.50	13.00
	EG-AR	12.00	9.00	11.00	11.50	7.00	12.50	12.50	7.00
Positive Control		28.0[a]	14.0[a]	18.0[a]	22.0[b]	14.0[a]	30.0[a]	40.0[a]	22.0[b]
Activity Index (AI)	**EG-CE**	0.54	0.43	0.75	0.84	1.00	0.75	0.53	0.66
	EG-DF	0.39	0.82	0.14	0.84	0.71	0.70	0.46	0.59
	EG-AR	0.43	0.64	0.61	0.52	0.50	0.42	0.31	0.32

Keys:
EG-CE = Crude extract of *E. globulus*, EG-DF = Dichloromethane Fraction, EG-AR = Aqueous Residues, E-ZIC= Estimated Zone of Inhibition at Concentration of Control,
a = Zone of Inhibition of imipenem at 10 µg /mL, **b** = Zone of Inhibition of fluconazole at 5 µg/mL, EG-CE = Crude extract of *E. globulus*, EG-DF = Dichloromethane Fraction, EG-AR = Aqueous Residues

3.4 GC-MS Analysis of Non-polar fraction of *E. glabrous gum* extract

The compounds identified in the non-polar fraction of *E. glabrous* gum extract by GC-MS analysis are listed in the order of their column elution time (Table 4). In the EC-DM fraction, 96 compounds were detected. The most dominant of all the identified compounds were octadecenoic acid, methyl ester (4.04%), Pyrrolidine, 1-[3α,7α,12α-tris(trimethylsiloxy)-5β-cholan-24-oyl]- (3.9%), eicosene (3.75%), Heneicosane (3.75%) and Cinnamic acid, p-hydroxy-, methyl ester (3.22%). The other constituents present in appreciable amounts were Methylparaben (0.04%), Behenic alcohol (0.99%), Sakuranin (0.47%), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (0.92%), Diosmetin 7-O-glucoside (0.59%) and Methyl p-coumarate (0.03%). Most of the compounds had been reported to possess antimicrobial activities as shown in Table 4.

Table 4: Relative percentage of compounds detected in dichloromethane fraction of *E. globulus* by GC-MS

Peak Number	Retention Time	Identified Compounds	Area Sum %
1	17.93	Succinic acid, methylene-, dimethyl ester	0.08
2	18.51	α-Isophoron	0.06
3	19.36	Paraethylphenol	0.98
4	20.31	Coumaran	0.25
5	21.03	Pelargonic acid	0.01
6	22.14	1-(Ethylene-1'-oxy-2'-thio)-2,5-dimethylcyclopentane	0.02
7	22.57	Hydroxyphenyl)glyoxal	0.04
8	22.98	Methyl cinnamate	0.46
9	23.55	Cinnamic acid	0.12
10	23.65	Cinnamic acid	0.55
11	23.72	Piceol	0.91
12	23.86	Methylparaben	0.04
13	24.08	cinnamic vinyl ester	0.06
14	24.63	Phenol, 3,5-bis(1,1-dimethylethyl)-	0.04
15	25.02	Nonanedioic acid, dimethyl ester	0.05
16	25.6	Methyl 4-hydroxyhydrocinnamate	0.04
17	25.76	Methyl 3-hydroxy-3-phenylpropanoate	0.25

18	26.66	Methyl p-coumarate	0.03
19	27.73	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	0.04
20	27.83	Cyclobuta[a]dibenzo[c,f]cycloheptadiene, 7-oxo-	0.12
21	28.00	Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0]pentane), 1',4',2,6,6-pentamethyl-	0.05
22	28.18	Heptanone, 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclopropen-1-yl]-	0.07
23	28.51	Cinnamic acid, p-hydroxy-, methyl ester	3.22
24	28.87	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-	0.61
25	29.22	Dodecanol, 3,7,11-trimethyl-	0.17
26	29.38	Octadecane	1.57
27	30.16	Hydroxycinnamic acid, ethyl ester	0.24
28	31.33	Dibutyl phthalate	0.14
29	31.69	Hexadecanoic acid, methyl ester	0.06
30	31.80	Methyl-6-(1-methyl-1-phenylethyl) phenol	0.05
31	32.00	Tetradecane, 2,6,10-trimethyl-	0.09
32	32.22	Hexadecenoic acid, methyl ester, (Z)-	0.11
33	32.87	Hexadecanoic acid, methyl ester	2.9
34	33.70	Hexadecane, 5-butyl-	0.26
35	34.19	Hexadecanoic acid	2.74
36	34.39	Propyl 14-methyl-pentadecanoate	0.21
37	35.35	Benzenedicarboxylic acid, butyl 8-methylnonyl ester	0.67
38	35.67	Eicosene	3.75
39	36.33	Acetic acid n-octadecyl ester	0.49
40	39.69	Heneicosylformate	0.31
41	40.41	Octadecadienoic acid (Z,Z)-, methyl ester	1.74
42	40.82	Octadecenoic acid, methyl ester, (E)-	4.04
43	42.31	Octadecanoic acid, methyl ester	1.72
44	42.74	Octadecadienoic acid (Z,Z)-	0.29
45	43.15	13-Octadecenoic acid	0.98
46	43.89	Eicosane, 2,4-dimethyl-	0.4
47	44.69	Octadecanoic acid	0.64
48	46.00	Hexadecanoic acid, butyl ester	0.48
49	46.39	Docosene	0.65
50	46.74	Heneicosane	3.64
51	47.46	Behenic alcohol	0.99
52	48.18	Hexadecanoic acid, 2-hydroxyethyl ester	0.58
53	49.05	Butyl 2-ethylhexyl phthalate	0.76
54	50.18	Heptadecane, 2,6,10,15-tetramethyl-	0.52
55	50.97	Eicosanoic acid, methyl ester	0.39
56	51.54	Methyl-6-heneicosen-11-one	0.39
57	52.05	Docosane, 11-decyl-	0.19
58	52.39	Octadecanoic acid, butyl ester	0.37
59	52.55	Tetracosanol-1	0.51
60	52.7	Triacontane	2.77
61	53.05	6-Octadecenoic acid, (Z)-	0.12
62	53.17	n-Tetracosanol-1	0.94
63	53.57	Octadecanoic acid, 2-hydroxyethyl ester	0.3
64	53.73	Alpha-phenyl-alpha-tropylacetaldehydetosylhydrazone	0.6
65	54.63	Hexacosane	0.54
66	55.19	20-methyl-heneicosanoate	0.68
67	55.85	3-Phenylamino-5-phenyl-1,1,2,2-cyclopentanetetracarbonitrile	0.56

68	56.04	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	0.47
69	56.19	Hexacosene	0.2
70	56.28	Hexacosane	1.36
71	56.61	Hexacosene	0.08
72	56.70	1-Hexacosanol	0.58
73	57.16	Docosanoic acid, 2-hydroxy-, methyl ester	0.25
74	57.26	1-Heptacosanol	1.34
75	57.39	α-N-Normethadol	0.19
76	57.67	Heptacosane	0.5
77	58.10	Tetracosanoic acid, methyl ester	0.47
78	58.31	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	0.92
79	58.80	Sakuranin	0.47
80	58.88	Propen-1-one, 1-(2,4-dihydroxy-6-methoxyphenyl)-3-(2-hydroxyphenyl)-	1.6
81	58.95	Heptacosane	0.68
82	59.11	Decanedioic acid, bis(2-ethylhexyl) ester	1.37
83	59.40	1-Octacosanol	0.79
84	59.84	Methyl 2-hydroxy-tetracosanoate	0.25
85	59.98	Androstan-9-thiocyanato-3,11,17-trione	1.25
86	60.25	Octacosane	0.35
87	60.72	Hexacosanoic acid, methyl ester	0.39
88	61.68	Tetratriacontane	0.69
89	62.95	Diosmetin 7-O-glucoside	0.59
90	63.27	Tetratriacontane	0.26
91	64.61	1,37-Octatriacontadiene	0.51
92	67.25	Stigmastanol	0.26
93	68.66	Sitosterol	0.53
94	69.8	Nonacosane	0.32
95	77.23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	0.79
96	77.70	Pyrrolidine, 1-[3α,7α,12α-tris(trimethylsiloxy)-5β-cholan-24-oyl]-	3.9

4. Discussion

This study investigated the cytotoxicity and antimicrobial potential of the methanol extract and its fractions derived from the gum of *Eucalyptus globulus*. Eucalyptus species, particularly *E. globulus*, have long been used for treating infectious diseases 16. However, there are limited reports on the ethnomedicinal applications of *E. globulus* gums. The gum, brownish in colour, was found to contain approximately 55% methanol-soluble constituents, with 18% being soluble in dichloromethane. This study marks the first evaluation of the cytotoxic and antimicrobial potential of*E. globulus*gum.

The results demonstrated that *Eucalyptus globulus* stem bark is a valuable source of biologically active compounds, consistent with prior studies on Eucalyptus species' medicinal properties 17. The aqueous residues (EG-AR) had the highest yield (70%), indicating the abundance of water-soluble bioactive compounds such as polysaccharides and phenolic acids 18.

The methanol extract and its fractions exhibited notable cytotoxicity and growth-inhibitory activity. The crude extract (EG-CE) showed stronger cytotoxic effects compared to the fractions, yet all extracts were classified as highly cytotoxic based on the brine shrimp lethality assay 19. The dichloromethane fraction (EG-DF) displayed the most significant inhibitory effects on *Allium cepa* root and *Sorghum bicolor* radicle growth. These findings are in line with prior studies on the herbicidal properties of diterpenoids in plant gums 20. Similarly, essential oil from *E. globulus* has been reported to exhibit brine shrimp cytotoxicity, with an LC_{50} of 9.59 µL/mL21.

Both the crude extract and fractions inhibited the growth of clinical and standard strains of *S. aureus*, *E. coli*, *C. freundii*, and *C. albicans*. EG-CE exhibited the highest antimicrobial activity, particularly against *S. aureus* (ATCC 25913) and *C. albicans*, while EG-DF displayed superior activity against *E. coli* and *C. albicans* compared to the aqueous residue (EG-AR). These results are in agreement

with prior reports of antimicrobial activity in Eucalyptus essential oils, which have been suggested as alternatives to synthetic germicides 22. The broad-spectrum activity of EG-CE may be attributed to the synergistic action of phenolic acids and fatty acid esters, as corroborated by Oliveira et al. (2023)23.

The GC-MS analysis of EG-DF revealed an array of pharmacologically significant compounds, including octadecenoic acid methyl ester**,** hexadecanoic acid**,** eicosene**,** heneicosane**,** andcinnamic acid derivatives. Several constituents, such asmethylparaben**,** sakuranin, andmethyl p-coumarate, have documented antimicrobial properties. Octadecenoic acid methyl ester and hexadecanoic acid, comprising 4.04% and 2.74% of the fraction, respectively, are known for their antimicrobial and anti-inflammatory effects –24. Additionally, stigmastanol and sitosterol, identified in the GC-MS analysis, are associated with anticancer and cholesterol-lowering activities, further highlighting the therapeutic potential of the dichloromethane fraction'25.

Conclusion

This study demonstrates the significant bioactive potential of *Eucalyptus globulus* stem bark extracts and fractions. The findings reveal notable cytotoxic and antimicrobial, with the crude methanol extract (EG-CE) exhibiting the strongest effects. The GC-MS analysis identified various bioactive compounds, including hexadecanoic acid, cinnamic acid derivatives, and octadecenoic acid methyl ester, which contribute to the observed biological activities. These results support the potential application of *Eucalyptus globulus* gum extracts as eco-friendly cytotoxic agents and natural antimicrobials. Further research should focus on isolating and characterizing individual bioactive compounds, exploring their synergistic interactions, and conducting in vivo studies to validate their efficacy.

Conflict of Interest: All authors declared no conflict of interest

Authors' Contributions: KMS conceptualized the study, designed the experimental approach, and contributed to the analysis of data. Supervised the research process and provided critical inputs to the manuscript. KMS and SA Conducted the plant collection, preparation of extracts, and bioassays. Contributed to data interpretation and manuscript writing. KMS and SNN Performed antimicrobial susceptibility testing and GC-MS analysis. Assisted with data analysis and contributed to the manuscript revision. KMS and OS Assisted in plant identification and contributed to the manuscript revision. All authors read, edited, and approved the final version of the manuscript.

REFERENCES

1. Fatima Z, Purkait D, Rehman S, Rai S, Hameed S (2023) Biological and environmental hazards, risks, and disasters. In: Fatima Z, Purkait D, Rehman S, Rai S, Hameed S (eds) Biological and Environmental Hazards, Risks, and Disasters. Elsevier, pp. 197–220.
2. Agarwal H, Bajpai S, Mishra A, Kohli I, Varma A, Fouillaud M, Dufossé L, Joshi NC (2023) Bacterial pigments and their multifaceted roles in contemporary biotechnology and pharmacological applications. *Microorganisms,* 11(3):614.https://doi.org/10.3390/microorganisms11030614
3. Innocent E, Marealle AI, Imming P, Moeller L (2022) An annotated inventory of Tanzanian medicinal plants traditionally used for the treatment of respiratory bacterial infections. *Plants*, 11(7):931. https://doi.org/10.3390/plants11070931
4. Silva J, Abebe W, Sousa SM, Duarte VG, Machado IL, Matos FJA (2003) Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *Journal of Ethnopharmacology,* 89(2-3):277–283. https://doi.org/10.1016/j.jep.2003.09.007PubMed
5. Bachir RG, Benali M (2012) Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine,* 2(9):739–742. https://doi.org/10.1016/S2221-1691(12)60220-2
6. Salawu KM, Ogbole OO, Abiodun OO, Ajaiyeoba EO (2017) Antioxidant, brine shrimp lethality, and antiproliferative properties of gel and leaf extracts of *Aloe schweinfurthii* and *Aloe vera*. *Journal of Herbs, Spices & Medicinal Plants*, 23(3):263–271. https://doi.org/10.1080/10496475.2017.1318328 Taylor & Francis Online
7. Salawu KM, Ogbole OO, Abiodun OO, Ajaiyeoba EO (2021) Ethnobotanical survey, phytochemical screening, growth inhibitory effects and

cytotoxicity evaluation of medicinal plants used for cancer management in Ilorin metropolis, Nigeria. *Archives of Basic and Applied Medicine,* 9:168–175.

8. Akinboro A, Bakare AA (2007) Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *Journal of Ethnopharmacology,* 112(3):470–475. https://doi.org/10.1016/j.jep.2007.04.014PubMed
9. Salawu KM, Ogbole OO, Abiodun OO, Ajaiyeoba EO (2021) Phytochemical, nutritional composition and heavy metals content of *Allium cepa* (onion) and *Allium sativum* (garlic) from Wudil central market, Kano state, Nigeria. *Biokemistri,* 33(3):311–317.
10. Simões LA, de Souza AC, Schwan RF, Dias DR (2021) Using wild yeasts to modulate the aroma profile of low-alcoholic meads. Springer.
11. Prakit B, Chaiyod R, Khongkool K, Chanasit W, Lertworapreecha M (2025) Multifunctional probiotic and safety attributes Heyndrickxia coagulans isolated from stingless bee honey. *Annals of Microbiology*, 75(3):3. https://doi.org/10.1186/s13213-025-01791-0ResearchGate
12. Karthiga Devi K, Natarajan K (2015) Isolation and characterization of a bioflocculant from Bacillus megaterium for turbidity and arsenic removal. Mining. *Metallurgy & Exploration,* 32(2):222–229. https://doi.org/10.1007/BF03402479SpringerLink
13. Ngamsurach P, Praipipat P (2022) Antibacterial activities against Staphylococcus aureus and Escherichia coli of extracted Piper betle leaf materials by disc diffusion assay and batch experiments. *RSC Advances,* 12(43):26435–26454. https://doi.org/10.1039/D2RA04611C
14. Usman H, Abdulrahman F, Ladan A (2007) Phytochemical and antimicrobial evaluation of *Tribulus terrestris* L. (Zygophylaceae). Growing in Nigeria. *Research Journal of Biological Sciences,* 2(3):244–247.
15. Salawu K, Oyerinde A, Bello R (2021) Antiproliferative and antimicrobial activities of *Citrus limon* (L.) Burm. f. stem bark extract. *Nigerian Journal of Basic and Applied Sciences,* 29(1):49–54. https://doi.org/10.4314/njbas.v29i1.7
16. Bachir RG, Benali M (2012) Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Medicine,* 2(9):739–742. https://doi.org/10.1016/S2221-1691(12)60220-2
17. Ebrahimi Y, Abdalkareem Jasim S, Mohammed BA, Salman NA, Jabbar AM, Hameed NM, Goudarzi MA, Parsaei P (2024) Determination of antioxidant properties of *Mentha longifolia*, *Pistacia khinjuk*, and *Eucalyptus globulus*. *Caspian Journal of Environmental Sciences,* 22(3):601–606. https://doi.org/10.22124/cjes.2022.6065
18. Zhu J, Zhang Z, Wang R, Zhong K, Zhang K, Zhang N, Liu W, Feng F, Qu W (2022) Review of natural phytochemical-based self-assembled nanostructures for applications in medicine. *ACS Applied Nano Materials,* 5(3):3146–3169. https://doi.org/10.1021/acsanm.1c04242
19. Padmaja R, Arun PC, Prashanth D, Deepak M, Amit A, Anjana M (2002) Brine shrimp lethality bioassay of selected Indian medicinal plants. *Fitoterapia,* 73(6):508–510. https://doi.org/10.1016/S0367-326X(02)00171-3
20. Gu C-Z, Xia X-M, Lv J, Tan J-W, Baerson SR, Pan Z-Q, Song Y-Y, Zeng R-S (2019) Diterpenoids with herbicidal and antifungal activities from hulls of rice (*Oryza sativa*). *Fitoterapia*, 136:104183. https://doi.org/10.1016/j.fitote.2019.104183ReferenceCitationAnalysis
21. Akolade JO, Olajide OO, Afolayan MO, Akande SA, Idowu DI, Orishadipe AT (2012) Chemical composition, antioxidant and cytotoxic effects of *Eucalyptus globulus* grown in north-central Nigeria. *Journal of Natural Products and Plant Resources,* 2(1):1–8.Scholars Research Library+2Academia+2Academia+2
22. Čmiková N, Galovičová L, Schwarzová M, Vukic MD, Vukovic NL, Kowalczewski PŁ, Bakay L, Kluz MI, Puchalski C, Kačániová M (2023) Chemical composition and biological activities of Eucalyptus globulus essential oil. *Plants,* 12(5):1076.https://doi.org/10.3390/plants12051076MDPI+1PMC+1
23. Oliveira CSD, Moreira P, Cruz MT, Pereira CMF,

Silva AMS, Santos SAO, Silvestre AJD (2023) Exploiting the integrated valorization of *Eucalyptus globulus* leaves: Chemical composition and biological potential of the lipophilic fraction before and after hydrodistillation. *International Journal of Molecular Sciences*, 24(7):6226. https://doi.org/10.3390/ijms24076226

24. Allijn IE, Brinkhuis RP, Storm G, Schiffelers RM (2019) Anti-inflammatory properties of plant derived natural products–a systematic review. *Current Medicinal Chemistry*, 26(24):4506–4536. https://doi.org/10.2174/0929867325666190523357PMC+2PubMed+2ResearchGate+2

25. Annuur RM, Triana D, Ernawati T, Murai Y, Aswad M, Hashimoto M, Tachrim ZP (2024) A review of cinnamic acid's skeleton modification: Features for antibacterial-agent-guided derivatives. Molecules, 29(16):3929. https://doi.org/10.3390/molecules29163929MDPI+1PubMed+1