Sahel Journal of Life Sciences FUDMA (SAJOLS)
September 2024 Vol. 2(3): 98-103
ISSN: 3027-0456 (Print)
ISSN: 1595-5915(Online)
DOI: https://doi.org/10.33003/sajols-2024-0203-13

Research Article

Antibacterial Potentials and Phytochemical Screening of *Eucalyptus globulus* Leaves Extract against Selected Isolates

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ABSTRACT
In recent decades, medicinal plants have been of great interest as they have been the sources of natural products. As a result, there is a need to analyse the phytochemical and antibacterial properties of *Eucalyptus globulus* against *Escherichia coli, Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study was carried out to determine the percentage yield, phytochemical properties, antibacterial activity, minimum inhibitory and minimum bactericidal concentration (MIC/MBC) of the crude extracts using the reflux extraction, qualitative phytochemical, agar well diffusion and broth dilution method respectively; while aqueous, normal hexane and ethyl acetate were used as extraction solvents. Aqueous extract has the highest yield of 16.08%, ethyl acetate extract (8.76%) while n-hexane extract had 3.84%. Phytochemicals such as tannins, alkaloids, and phenols were present in all three solvent extracts; while terpenoids, glycosides and steroids were absent. Saponins and flavonoids were present in water extract, flavonoids were present only in the ethyl acetate extract. The most active with mean inhibition zone (MIZ) diameter of 13.33±0.58mm and 10.33±0.58mm against *Bacillus cereus* and *Staphylococcus aureus respectively* at 50mg/mL while the lowest activity of MIZ diameter of 8.00±2.00mm was obtained with same extract and concentration against *Escherichia coli*. N-hexane and ethyl acetate extracts show no activity against the test organisms. The lowest MIC of 15.6mg/mL and MBC of 31.3mg/mL were obtained against *Escherichia coli*. Based on these results, it can be concluded that *Eucalyptus globulus* leaf extracts possess antibacterial activity against some of the test organisms and can be considered for drug development.

Keywords: Antibacterial; *Eucalyptus globulus*; Leaves extracts; Phytochemical

Citation: Ewansiha, J.U., Fori, A., Aminu, D.U. & Ijabani, E. (2024). Antibacterial Potentials and Phytochemical Screening of *Eucalyptus globulus* Leaves Extract against Selected Isolates. *Sahel Journal of Life Sciences FUDMA,* 2(3): 98-103. DOI: https://doi.org/10.33003/sajols-2024-0203-13

INTRODUCTION

Eucalyptus*, Eucalyptus globulus,* is a flowering tree that belongs to Myrtle family (Myrtaceae). It has been used for thousands of years throughout human history. The genus eucalyptus contains more than 700 species and varieties and they have been successfully introduced worldwide. Eucalyptus is native to Australia and Tasmania and also in Africa and tropical to southern temperate America (Wilson *et al*, 2001). Variability is prevalent in morphology, growth habit, flower colour, leaves, stems and chemical composition.

Eucalyptus globulus is known by different names depending upon where you are in the world and its common name is "Australian Fever Tree", "Tasmania Blue Gum", "Southern Blue Gum" or "Blue Gum Tree" and "Stringy Bark". In Arabic language, it is known as "ban" or "kafur". In Burmese language it is known by "pyilon-chantha". The trade name of *Eucalyptus globulus* is "blue gum". In English language, it is

commonly known as "turpentine gas", "Tasmanian blue gum eucalypt", "Tasmanian blue gum", "southern blue gum", "fever tree", "blue gum eucalyptus" and "blue gum". In Japanese language, it is called "yukari-no-ki". In Spanish language, it is known as "eucalipto". In Swahili it is known as "mkaratusi", and in Hausa, it is called Zaiti, or Itacen Pol" (Orwa, *et al*., 2009).

Eucalyptus globulus is a complex species consisting of four further subspecies: Eucalyptus *bicostata, Eucalyptus pseudoglobulus, Eucalyptus globulus* and *Eucalyptus maidenii*. The only one variety of *Eucalyptus globulus* is *Eucalyptus globulus* var. compacta Labill-Dwarf blue gum (Chen *et al*., 2005). *Eucalyptus globulus* is a medium to large sized evergreen and broadleaf tree that can grow up to the height of 70 m and its diameter can be about 4 to 7 feet. Different parts of this plant are nutritionally very important and therapeutically highly valuable due to specific chemical composition as its essential oil contain esters, ethers, carboxylic acids, ketones, aldehydes, alcohols and hydrocarbons along with monoterpenes and sesquiterpenes. Phytochemical analysis of this plant has revealed that leaf oil contains 1,8-cineole, α-pinene, p-cymene, cryptone and spathulenol. In contrast, essential oil extracted from buds, branches and fruits constitutes α-thujene, 1,8-cineole and aromadendrene as major components. Due to these chemical compounds, *Eucalyptus glabrous* is found to be a potential anti-microbial, anti-fungal, anti-viral, anti-inflammatory, analgesic, anti-nociceptive and anti-oxidant agent of nature. Some recent scientific investigations have also revealed that essential oil of *Eucalyptus glabrous* also have anti-diabatic potentials that enhances its market value due to excessive usage in number of pharmaceutical products of traditional and advanced system of medicines (Hardel and Sahoo, 2011). Eucalyptus oil has numerous traditional uses especially in non-prescription pharmaceuticals but the market is small. Eucalyptus oil-based products have been used as a traditional non-ingestive treatment for coughs and colds.

The lowest MIC/MBC of the plant extracts in different concentrations of the active plant extract were 15.6mg/mL and 31.3mg/mL against *Escherichia coli;* MIC/MBC was 62.5mg/mL and 125mg/mL against *Pseudomonas aeruginosa*. 125gm/mL and 250mg/mL were obtained as MIC/ MBC against *Bacillus cereus* and *Staphylococcus aureus* respectively.

MATERIALS AND METHODS

Collection and Processing of Plant Materials

The plant species of *Eucalyptus globulus* leaves were harvested from Adamawa State College of Nursing and Midwifery, Yola Premises. The taxonomic identification and authentication was done at the Plant Science Department of Modibbo Adama University, Yola, Nigeria, where the plants were certified. The leaves of *Eucalyptus globulus,* was examined and screened. The plant materials were dried at room temperature, with free airflow for quick drying and to minimize direct sunlight after collection. The plant materials were crushed and grounded using pestle and mortar and then filtered to obtain a fine texture with a reduced surface area. The powder was stored in tightly closed glass containers in the dark at room temperature for further analysis.

Extraction of Crude Extract of *Eucalyptus globulus*

The extraction was done using reflux extraction method as described by (Ewansiha *et al*., 2020). The reflux extraction method was used to obtain the crude extract. The solvents used were normal hexane, ethyl acetate, and aqueous. One hundred grams (100 g) of the well-blended dried plant materials were dissolved in 400 ml of the extracting solvents. After refluxing, the mixtures were filtered with filter paper to obtain a clear filtrate, then concentrated to a semi-solid substance with a Rotary Evaporator and dried using water bath to yield the crude extract.

Percentage yield of the extract was calculated from each weight of the extracts using the formula below:

$$\text{Percentage Yield} = \frac{Dry\ Weight\ of\ extract\ x\ 100}{Dry\ weight\ of\ whole\ sample\ extracted}$$

Preparation of Extract Stock Concentration for Antibacterial Screening

Two hundred, and two hundred and fifty milligrams (200 mg and 250 mg) of the n-hexane, ethyl acetate, and aqueous crude extract were weighed in 5 mL each of 20% Dimethyl sulfoxide (DMSO) to give 40 mg/mL and 50mg/mL concentrations (Ewansiha *et al*., 2016).

Qualitative Phytochemical Analysis

The crude extracts were subjected to phytochemical analysis to screen for the presence of secondary metabolites: steroids, alkaloids, tannins, saponins, flavonoids, terpenoids and phenols. The phytochemical screening was carried out using standard procedure as reported by Evans (2002).

Source of Organisms

Samples from inanimate objects (Floor, Air, Reception Bench, Beddings and Locker, laboratory coat) were collected from the environment of Specialist Hospital, Yola, for isolation and identification of potential Nosocomial bacteria Pathogens.

Confirmation of Organism Identity

The samples were cultured on freshly prepared nutrient agar and were incubated at 37^{o}C for 24 hours. After incubation, the colonial morphology of the isolates was observed and documented.

Identification of Isolates

The developed colonies were identified using Grams staining techniques and appropriate biochemical tests according to Chesbrough. (2006). Organisms isolated and identified include *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus,* and *Staphylococcus aureus.*

Antibacterial Screening

Their susceptibility pattern to common antibiotics was determined and found to be susceptible to the commonly used antibiotics. The test organisms obtained were confirmed using the methods of Chesbrough (2006), by observing their cultural growth characteristics each. Biochemical confirmatory tests were also performed to further confirm the identity of each of the test organisms. The organisms were checked for any form of impurity and then stored at 4 ºC in slants of nutrient agar till use.

Standardization of Inoculum

Using a wire loop, the test organisms were taken from the agar slant and then subcultured into test tubes containing nutrient broth which were incubated for 24 hours at 37ºC. The organisms' suspensions in the broth were then standardized by the addition of normal saline to obtain a turbidity and population density, equivalent to a 0.5 McFarland standard. Exactly 99.5ml of 1% $BaCl_2$ was added to 0.5ml of 1% H_2SO4 to obtain 100ml of BaSO4 which is equivalent to 0.5 McFarland's turbidity standard equivalent to 1.0 X 10^8 cfu/ml population for bacterial isolates (Ewansiha *et al*., 2017)

Antibacterial Susceptibility Test of the Crude Extract

A 6mm diameter sterile cork borer was used to make wells in the medium. One hundred microliter (100µL) 0.1mL of 40mg/mL and 50mg/mL concentrations of the extracts; positive control, 30 µg/mL doxycycline and negative/solvent control, dimethyl sulfoxide (DMSO), were transferred into the wells, respectively, and the plates were allowed to stand on the bench for about 30min to allow proper diffusion of the extracts through the culture medium and all plates were incubated at 37^{o}C for 18 to 24 hours. After incubation, the culture plates were observed for the formation of a clear zone around the wells which corresponds to the antibacterial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. The tests were carried out in triplicate (Ewansiha, 2020).

Determination of activity index of the extracts

The activity index of the crude plant extract was determined using the following relation (Ewansiha *et al*., 2017); Activity index (A.I.) = Mean of zone of inhibition of the extract**/**Zone of inhibition obtained for standard antibiotic drug

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum Inhibitory Concentration (MIC) was carried out using the broth dilution method. The n-hexane extracts of *Eucalyptus globulus* which showed activity to the isolates were weighed at 1000mg, i.e.,1g and dissolved in 2mL of 20% DMSO as stock extract. The stock extract was dispensed wholly into 2mL nutrient broth, given 4mL in the first broth tube. 2mL from the first broth tube was dispensed serially into other tubes, to the 8th tube, given 250mg/mL, 125mg/mL, 62.5mg/mL, 31.3mg/mL, 15.6mg/mL, 7.81mg/mL, 3.91mg/mL and 1.95mg/mL. The tubes were inoculated with 0.1 mL standard inoculum of 1.0x10^8 (CFU/mL) prepared from 18-24h old culture, equivalent to McFarland Turbidity Standard of 0.5. The tubes were incubated at 37^{o}C for 24h, and observed for growth in the form of turbidity, compared with the control broth tube mixed with extract, the other broth tube inoculated with test organism for Minimum Inhibitory Concentration (MIC). The test tubes with the lowest dilution with no detectable turbidity by visual inspection were considered the MIC. The MBC values were determined by subculturing a loop full from the MIC tubes and other tubes without turbidity on sterile nutrient agar plates and incubated at 37^{o}C for 24h. After incubation, the concentration at which no visible growth was seen was recorded as the Minimum Bactericidal Concentration (MBC).

RESULTS

The highest percentage yield of the extract was observed in the aqueous extract which was 16.08% of the total sample extracted, 8.76% ethyl acetate extract and the least, 3.84% was obtained for n-hexane extract, as shown below in **Table (1)**. Phytochemicals identified from the n-hexane, ethyl acetate and aqueous extracts of *Eucalyptus globulus* leaves include the following secondary metabolites: tannins, flavonoids, alkaloids and phenols as shown in **Table (2)**. Terpenoid glycosides and steroids were absent, saponins and flavonoids were present in water extract, and flavonoids were present only in ethyl acetate extract

Antibacterial activity of *Eucalyptus globulus* against clinical isolates

Table 3 shows the antibacterial activities of the extracts and that of standard antibiotic (doxycycline) at different concentrations of 50mg/mL, 40mg/mL, and 30µg/mL of the doxycycline against the test organisms. The aqueous extracts showed considerable activity compared to n-hexane and ethyl acetate extracts.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 4 showed the MIC/MBC of *Eucalyptus globulus* extract of 250mg/mL, 125mg/mL, 62.5mg/mL, 31.3mg/mL, 15.6mg/mL, 7.81mg/mL, 3.91mg/mL and 1.95mg/mL against the test organism.

Table 1. Percentage yield of N-Hexane, Ethyl Acetate and Aqueous extracts of *Eucalyptus globulus* leaves

Plant	WWPP (g)	NHE		EAE		AQE	
		(g)	(%)	(g)	(%)	(g)	(%)
Eucalyptus globulus	100	3.84	3.84	8.76	8.76	16.08	16.08

Key: WWPP = Weight of whole plant part, NHE = n-hexane extract, EAE = ethyl acetate extract, AQE = aqueous extract

Table 2. Qualitative Phytochemical constituents of *Eucalyptus globulus* leaves

Extracts	Saponins	Tannins	Terpenoids	Flavonoids	Alkaloids	Glycosides	Steroids	Phenols
NHE	-	+	-	-	+	-	-	+
EAE	-	+	-	+	+	-	-	+
AQE	+	+	-	+	+	-	-	+

Key: - = Absent, + = Present; NHE = N-Hexane Extract**,** EAE = Ethyl Acetate Extract**,** AQE = Aqueous Extract

Table 3. **Mean Diameter of Zones of Inhibition of *Eucalyptus globulus* crude Extract against the Test Organisms**

Organism	Plant Extracts (mg/mL)						Control	
	N-Hexane		Ethyl Acetate		Aqueous		DOX (µg/mL)	D (mL)
	(40)	(50)	(40)	(50)	(40)	(50)	(30)	(0.1)
E. coli	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	8.00±2.00^{b}	14.00±1.00^{c}	0.00^{a}
B. cereus	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	12.33±0.58^{b}	13.33±0.58^{c}	17.00±0.00^{d}	0.00^{a}
P. aeruginosa	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00a	14.66±0.57^{b}	0.00^{a}
S. aureus	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	10.00±0.00^{b}	10.33±0.58^{b}	15.50±0.57^{c}	0.00^{a}

Key: DOX – Doxycycline, D – Dimethyl Sulfoxide. (CLSI, 2021). Values on the same row with different superscripts are significantly different ($p \leq 0.05$)

Table 4. Minimum inhibitory and Bactericidal Concentration of *Eucalyptus globulus* Aqueous

Isolate	Extract Concentration in mg/mL									
	250	125	62.5	31.3	15.6	7.81	3.91	1.95	MIC	MBC
E. coli	-	-	-	-	-	+	+	+	15.6	31.3
B. cereus	-	-	+	+	+	+	+	+	125	250
P. aeruginosa	-	-	-	+	+	+	+	+	62.5	125
S. aureus	-	-	+	+	+	+	+	+	125	250

Key: + = Growth and - =No growth, MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

DISCUSSION

The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed (Obeidat, 2011). Modern-day pharmacopoeia however contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated from plants. Involvement in medicinal plants as a re-budding health assistance has been fueled with the rising charges of prescription drugs in the safeguarding of personalized health and well-being and the bio prospecting of new plant derived drugs (Lucy and Edgar, 1999). An increasing dependence on the use of these medicinal plants in the industrialized organizations has been traced towards the extraction and development of drugs and chemotherapeutics from these plants as well as from conventionally used herbal remedies (Obeidat, 2011). The therapeutic properties of plants could be based on their anti-oxidant, anti-microbial, antipyretic

effects of the phytochemical's constituents in them (Adesokan *et al*., 2008). According to World Health Organization, medicinal plants would be the greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness (Nascimento *et al*., 2000).

From the result obtained, water can be said to be the best solvent (used in the study) for extraction of these leaves extract of *Eucalyptus globulus,* as it gives higher yield of the extract of 16.08%, ethyl acetate 8.76% and n-hexane 3.84%. The result did not conform with the reported by Abdulmumin *et al*. (2021), on though, *Eucalyptus camaldulensis*, aqueous leaves extract 7.30%, ethyl acetate extract 9.29% and n-hexane 1.40%. Phytochemical screening of aqueous extracts discovered that the leaves of *Eucalyptus globulus* contain tannins, alkaloids and phenols, which does not conform to the report by Gerard *et al*. (2011), whose flavonoids, glycosides, terpenoids and saponins were present, but alkaloids absent, with only tannins which made the reports similar. Based on the antibacterial testing conducted, aqueous extracts of *Eucalyptus globulus* showed activity against the test organisms at all concentrations of 40mg/mL and 50mg/mL, slightly lower than the standard antibiotic doxycycline, and considered sensitive, as reported by Abalaka (2011). The lowest MIC/MBC recorded in this study was 15.6mg/mL and 31.3mg/mL against *Escherichia coli* the lower the MIC the more potent is the drug for use in treatment as reported by Mouton and Vinks (2007).

Limitations of the study

1. Only a few groups of bacteria were tested, so the results cannot be used to conclude the plant's potential effects on other organisms.
2. The inactivity of the extract according to the antibacterial study using n-hexane, ethyl acetate or aqueous as the solvent do not disqualify *Eucalyptus globulus* as a potential product of antibacterial study since other solvents like methanol and acetone may extract another secondary metabolite.

CONCLUSION

Based on the result obtained from this study, it can be concluded that:

There is the presence of phytochemical constituents such as tannins, alkaloids and phenols in medicinal plants which gives them the potentials to exert their antibacterial activity.

Aqueous extract was the most active is indicative of the fact that the polar constituents were more potent than the non-polar constituents.

Eucalyptus globulus leaves aqueous extract can be considered for drug development production, because it possesses potent antibacterial activity against the test organisms.

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