Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 https://dx.doi.org/10.4314/sa.v22i1.6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY AND ANTIBACTERIAL ACTIVITY OF STEM BARK OF *TERMINALIA GLAUCESCENS* ON SOME MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

Agu, G. C.[1]*, Onabanjo, A. M.[2], Efuntoye, O. M.[1], Banjo, A. O.[1] and Sossou, I. T.[3]
[1]Department of Microbiology, Faculty of Science, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Ogun State,
[2]Department of Biological Sciences, College of Science & Information Technology, Tai Solarin University of Education, Ijagun, PMB, 2118, Ogun State.
[3]Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, Redeemer's University, P.M.B. 230, Ede, Osun State, Nigeria.
*Corresponding author e-mail: georgiaagu@yahoo.com

Received: 18-01-2023
Accepted: 29-03-2023

ABSTRACT

Frequent antimicrobial resistance of bacterial pathogens has led to a search for plant origin to synthesize new drugs. Hence, the antibacterial potential of the stem bark part of Terminalia glaucescens plant needs to ascertain. *The 162 pure bacteria isolated from the patient's clinical samples were confirmed using standard procedures to be* Escherichia coli *(65),* Pseudomonas aeruginosa *(52), and* Klebsiella pneumonia *(45). The isolates were tested against seven commonly available antibiotics and the extracts. The identified bark of* T. glaucescens *was prepared for qualitative analysis using standard methods and also analyzed quantitatively using Gas Chromatography-Mass Spectroscopy (GC-MS). Alkaloid, saponin, flavonoid, steroids, tannin, terpenoid, and phenol were identified. The GC-MS analysis revealed 13 bioactive constituents with Neophytadiene, Squalene, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol and 9-eicosyne as active components. Antibiogram study revealed that most isolates, especially* E. coli *and* P. aeruginosa, *were 100% resistant to more than two antibiotics. The antibacterial activity of the stem bark of* T. glaucescens *was reflected by inhibition zones, ranging from 5.5 mm to 12.5 mm; Escherichia* coli *had the highest zone of inhibition (12.5 mm). The presence of phytochemicals and bioactive components in this stem bark suggests its effectiveness in treating infections and producing pharmaceutical products.*

Keywords: Antibacterial activity; GC-MS; Gram-negative bacteria; Multidrug-resistance; *T. glaucescens.*

INTRODUCTION

The emergence and increase in multidrug-resistant bacteria of the clinically important pathogen have led to the search for more active antibacterial agents of plant origin with the view of knowing potentially active components that would serve as a basis for the synthesis of new drugs. An analytical technique called Gas Chromatography-Mass Spectrometry (GC-MS) with a separating attribute of gas-liquid chromatography and mass spectrometry for identifying different substances present in the plant is been utilized to confirm the plant's active components (Chauhan *et al*., 2014).

Terminalia glaucescens, commonly found in the savannah region, is a green leafy plant of the family combretaceae; locally called Idi Odan in Yoruba, Baushe in Hausa, and Edo in Igbo dialect (Adebayo and Ishola, 2009). The plant is a deciduous, multipurpose perennial tree that grows above 15 m in height, with dark grey bark, deeply fissured; slash yellowish or reddish, rapidly turning darker (Saxena *et al*.,

2014). Researchers in different parts of the world have reported the ethnomedicinal usages of *Terminalia* species in treating numerous diseases. These include abdominal disorders, bacterial infections, colds, conjunctivitis, diarrhea, dysentery, fever, gastric ulcers, headaches, heart diseases, hookworm, hypertension, jaundice, leprosy, nosebleed, edema, pneumonia, skin diseases, and sore throats. Also, some researchers have extensively reported the activity of *T. glaucescens* against pathogens from various sources (Konan *et al*., 2014; Ukwueze and Ekpemogu, 2014; Adeeyo *et al*., 2018).

In Nigeria, *Terminalia glaucescens* is one of the medicinal plants widely used as chewing sticks for oral hygiene. Hence various types of research have been done on its antimicrobial activity against some oral pathogens, and these have been used as the basis for choosing it as chewing sticks (Ndukwe *et al*., 2005; Ogundiya *et al*., 2008; Mgbe and Mgbe, 2015). Reports on the antibacterial and bioactive components present in the ethanolic extracts of *T. glaucescens* (stem bark) against bacteria from urine, wound, and stool samples that might be responsible for infectious diseases have not been widely investigated. Furthermore, more information is needed in Nigeria on the GC-MS techniques of the ethanolic extracts of the stem bark of *T. glaucescens. In conclusion, t*his research was done to elucidate the chemical components in ethanolic extraction of the stem bark to authenticate the plant's antibacterial potential.

MATERIALS AND METHODS

Procurement and preparation of *Terminalia glaucescens*

The bark of *T. glaucescens* was procured from farmland in Osun State and authenticated by a Botanist in Plant Science Department, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The bark of the *T. glaucescens was* rinsed in sterile water, spread on a sterile bench, and air-dried for three weeks. The dried stem bark was cut into pieces, ground using mortar and pestle, and then blended to a smooth powder using an electric grinder (Philips Bolmixer Melangeur HR 2846, Brazil). The powdered plant was packed in a sterile plastic bottle and stored in the refrigerator (4 °C) for further analysis. Using a slightly modified method of Ugwu *et al.* (2017), a hundred grams (100 g) of *T. glaucescens* were macerated using 70% ethanol (1000 ml) and filtered using Whatman filter paper (No 1). The filtrate collected was concentrated at 60 °C in a water bath (Grant Type SUB 14), while the dried crude extracts were weighed and stored at -20 °C for other analysis. The percentage yield was calculated as shown below.

$$\text{Percentage Yield} = \frac{\text{Weight of Extract X 100}}{\text{Weight of Crude Drug}}$$

Analysis of ethanolic extract using Gas Chromatography-Mass Spectroscopy (GC-MS)

The GC-MS analyses of the crude extract were carried out at the University of Lagos Central Research Laboratory. Qualitative analysis of the stem bark extract of *T. glaucescens* was carried out using the Gas Chromatography-Mass Spectroscopy (GC-MS) standard procedures of Harborne (Adeeyo *et al.* 2018). Ethanolic extract of stem-bark of *Terminalia glaucescens* was subjected to Gas Chromatography-Mass Spectroscopy (GS-MS) analysis using a Gas Chromatography (GC) System of Agilent Technologies (7890A) system coupled with a Mass Spectroscopy (MS) system of Agilent technologies (5975C) VLMSD. The extract was dissolved in High-Performance Liquid Chromatography (HPLC) grade methanol,

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 https://dx.doi.org/10.4314/sa.v22i1.6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

poured into anhydrous sulphate silica gel in a cotton wool ball, and filtered through with ethanol. Helium was used as the carrier gas. The stationary phase used is the model's column (Agilent technologies HP5MS) of lead 30 m with interval diameter (0.320 µml) and 0.25 µm thickness.

One (1) µml of crude extract was injected into GC with an auto-sampler that holds the extract, pushed by the mobile phase into the column. The interface between the MS and the GC is 250. The oven temperature used for the analysis was initially 80 °C which holds for 2 minutes and finally 240 °C for 6 minutes. With the aid of a computer-driven algorithm, significant constituents were identified using the Database of the National Institute of Standard and Technology (NIST), which has more than 62,000 patterns. A comparison was made between the spectrum of unknown components with the known component stored in the Database of NIST library, where the extract name, molecular weight, molecular formula, and chemical compound were ascertained (Adeeyo *et al.* 2018).

Preparation and identification of pure cultures

A total of 162 pure clinical cultures of *E. coli* (65), *K. pneumonia* (45), and *P. aeruginosa* (52) isolated from urine, wound, and stool samples were obtained from Ogun State General Hospital, Ijebu-Ode (Microbiology laboratory unit) Ogun State, Nigeria. The isolates were cultured on their appropriate selective medium (Eosine methylene blue and Centrimide agar, Oxoid UK) and subsequent incubation overnight at 37 °C to confirm their identity. The isolated bacteria were characterized and identified using biochemical tests and Gram's reaction (Cheesbrough, 2010).

Antimicrobial Susceptibility Test

The susceptibility profiles of the isolates to seven commonly available antibiotics: Amoxicillin (25 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Gentamicin (10 µg), Trimethoprim (5 µg), and Trimetoprim-sulphamethoxazole (1.25/23.75 µg) was determined using Kirby-Bauer method (Bauer *et al*., 1996). The stocked isolates before use were subcultured into nutrient agar, after which colonies were inoculated into 10 ml Mueller Hinton broth and compared with 0.5 McFarland turbidity standards. The agar plates were inoculated by swabbing using a sterile cotton swab per sample and allowed to stand for 5 minutes for absorption to take place. The Gram negative antibiotic discs were placed on the inoculated plates using sterile forceps and then incubated in an inverted position at 37 °C for 24 hours. The clear zones were measured using a ruler in millimeters (mm) and compared with the CLSI guidelines for bacteria (CLSI, 2015).

The antibacterial activity of the extract was done using the agar well diffusion method (Kurian, 2018). The test bacterial cultures (0.5 McFarland standards) were inoculated on Mueller Hinton agar duplicated plates using a sterile swab and allowed to stand for 10 minutes. Holes of 6 mm were bored into the inoculated media with a sterilized cork borer. Fifty (50 µg) of the plant extracts of concentration (100 mg/ml) were dispensed onto each well while ceftriaxone (30 µg) and ethanol (70%) served as positive and negative controls, respectively, and incubated at 37 °C. The inhibition zones were measured with a ruler to the nearest millimeter after 24 hours.

Data analysis

The collated results were analyzed using *Statistical Package for the Social Sciences* (Version 20). Simple means, percentages, and frequencies were computed.

RESULTS

The yield of the stem bark of *T. glaucescens* extract was 30.7%. The qualitative phytochemical analysis of some bioactive compounds of the extract also revealed the presence of alkaloids, saponins, flavonoids, steroids, tannins, terpenoids, and phenols. Anthraquinones and cardiac glycosides are absent (Table 1). The GC-MS analysis of stem bark extracts of *T. glaucescens* revealed the presence of 13 active compounds, with the majority belonging to fatty acids, steroids, and terpenoids (Fig 1-5: chromatogram and mass spectra). The retention time, the compound's name, peak area %, compound nature, molecular formula, and molecular weight for each chemical compound found in the extract are shown (Table 2).

The color, shape, and sizes of the isolates varied based on the selective media used. According to the different media used, the morphologies ranged from large mucoid purple growth, green metallic sheen to blue-green colonies (Table 3). The outcome of the Gram reaction and biochemical tests of the tested isolates are represented (Table 4). The antibiotic susceptibility revealed that most tested bacteria were resistant to two or more antibiotics. *Escherichia coli, P. aeruginosa, and K. pneumonia* had 100% resistance to four, three, and one antibiotic(s), respectively (Table 5). The antibacterial activity of the extract against the 162 bacterial isolates at 100 mg/ml concentrations gave varied activity. *Pseudomonas aeruginosa* isolates were the most sensitive to the effect of the extract, as 50 (96%) out of the 52 isolates were observed to susceptible to the extract. This was followed by *Klebsiella pneumonia*, in which 27 (60%) out of the 45 isolates had clear zones of inhibition. However, among the isolates tested, *E. coli* was the most resistant to the effect of the extract (41%) (Table 6).

Table 1: Phytochemicals present in the stem bark of *T. glaucescens*

Phytochemicals	***T. glaucascens***
Alkaloids	+
Saponins	+
Tannins	+
Anthraquinones	-
Flavonoids	+
Terpenoids	+
Steroids	+
Cardiac glycoside	-
Phenols	+

Key: (+) – Present, (-) – Absent.

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 *https://dx.doi.org/10.4314/sa.v22i1.6*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

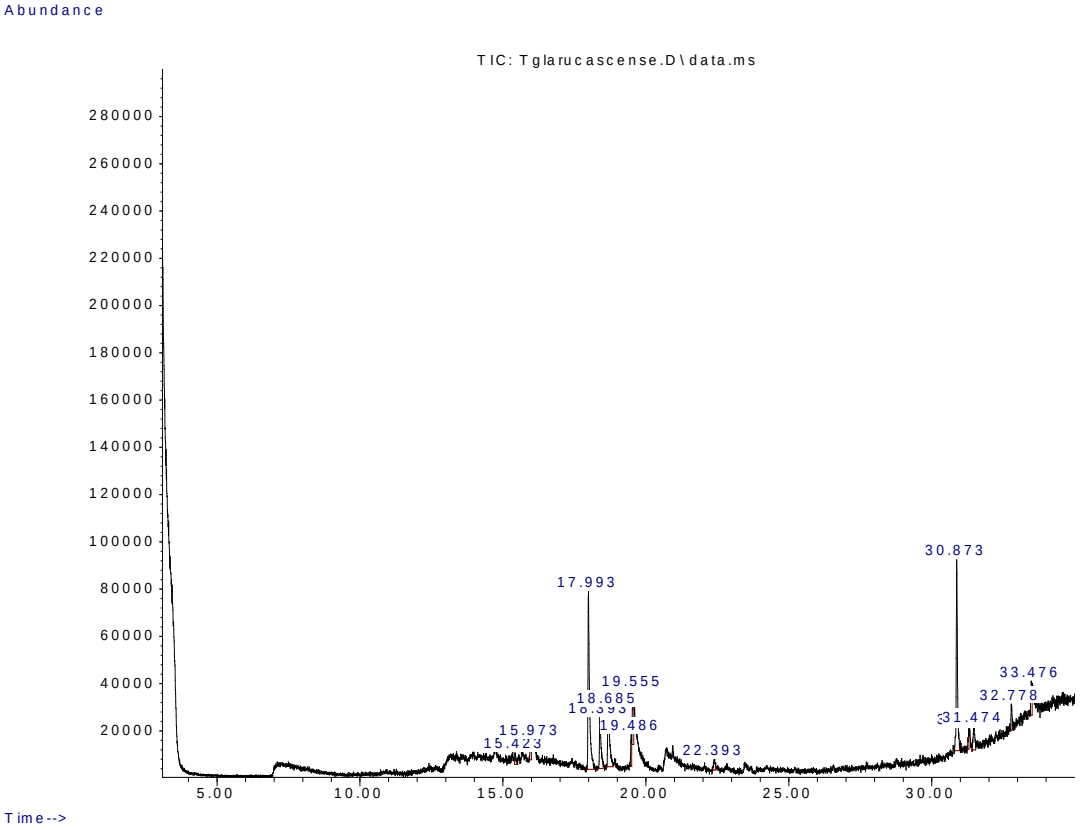

Figure 1: GC-MS of stem bark of *T. glaucascens*

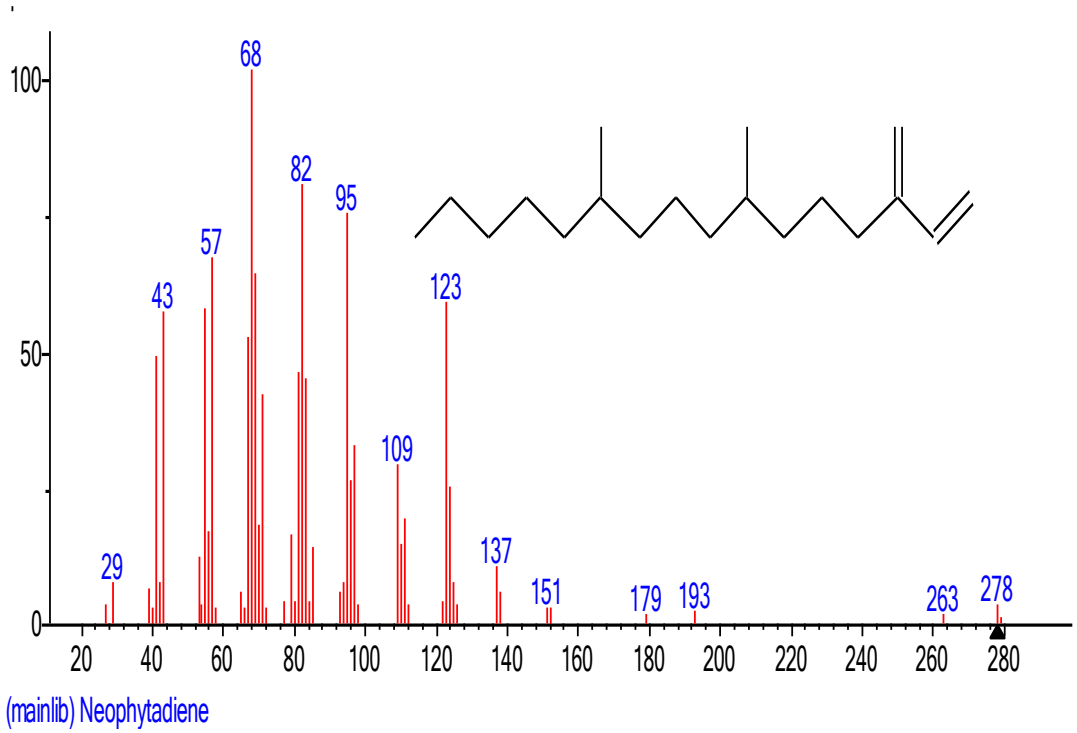

Figure 2: Mass spectra of Neophytadiene

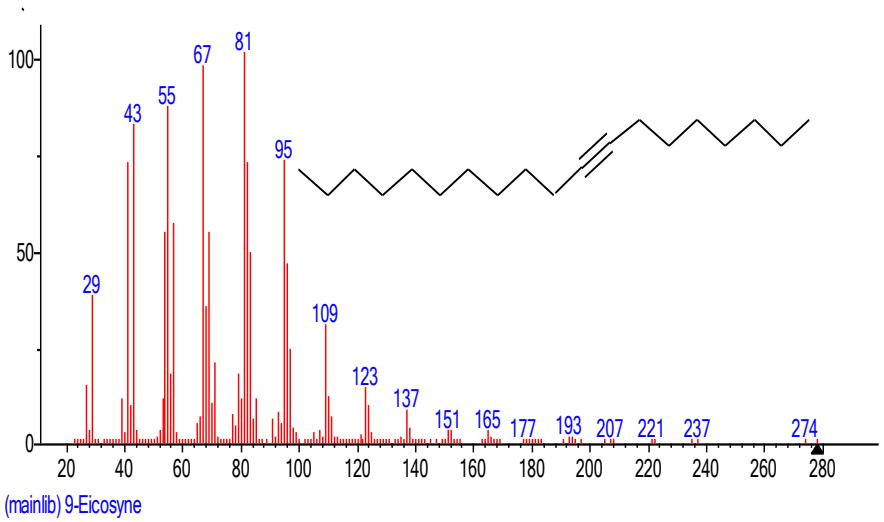

Figure 3: Mass spectra of 9- Eicosyne

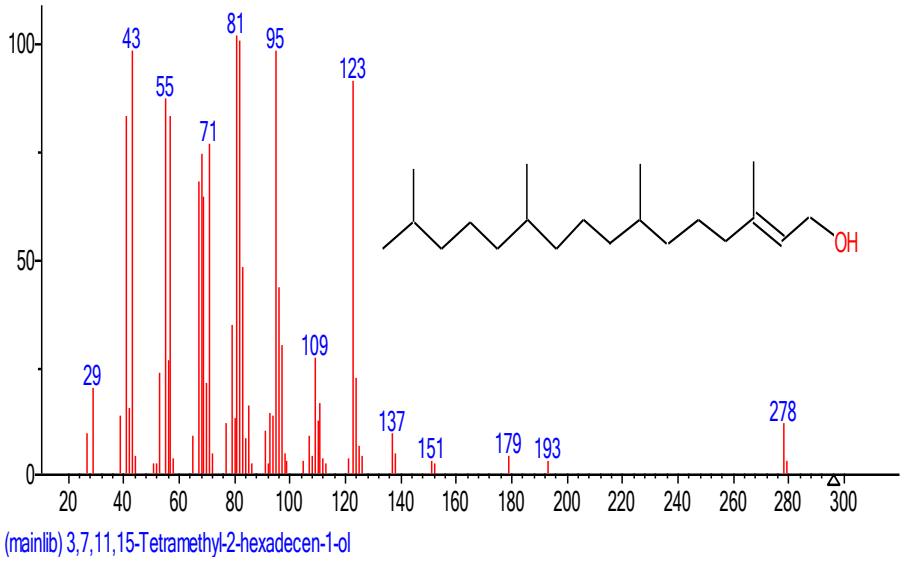

Figure 4: Mass spectra of 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol

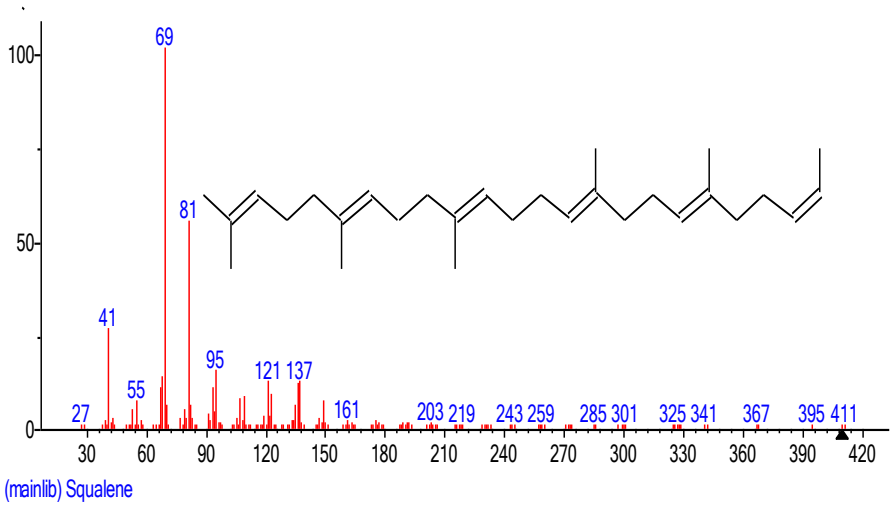

Figure 5: Mass spectra of Squalene

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 https://dx.doi.org/10.4314/sa.v22i1.6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Table 2: Detection of chemical compounds in ethanolic stem bark extract of *T. glaucescens*

Peak No	Retention time	Chemical Compounds	Peak%	Compound Nature	Molecular Formula	Molecular Weight
1	15.42	Paromomycin	1.36	Aminoglycoside	$C_{23}H_{45}N_5O_{14}$	615.6
2	15.97	1,3,6-Octatriene	2.41	Monoterpenoid	C_8H_{12}	108.18
3	17.99	Neophytadiene	28.0	Sequiterpenoid	$C_{20}H_{38}$	278
4	18.39	9-Eicosyne	8.34	Terminal alkyne	$C_{20}H_{38}O_2$	279
5	18.69	3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	12.1	Terpene alcohol	$C_{20}H_{40}O$	296
6	19.49	Hexadecanoic acid, methyl ester	2.71	Palmitic acid ester	$C_{17}H_{34}0_2$	270
7	19.56	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	6.97	Diterpenoid	$C_{30}H_{50}O$	426
8	22.39	Butanoic acid, 3 methyl-2-methylene-,methyl ester	1.68	Palmitic acid ester	$C_7H_{12}O_2$	128
9	30.87	Squalene	23.47	Triterpenoid	$C_{30}H_{50}$	410
10	31.29	5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	1.55			
11	31.47	Silicic acid, diethyl bis(trimethyl lsilyl) ester	4.13	Ester	$C_{10}H_{28}O_4Si_3$	296
12	32.78	2-Myristynoyl-glycinamide	2.35	Phenolic acid	$C_{16}H_{31}BrN_2O_2$	363
13	33.48	1-(Benzoxazol-2-ylthio)-propan 2-on	4.11		$C_{16}H_{28}N_2O_2$	280

Table 3: Cultural and morphological characteristics of the bacteria on various selective media

Growth on Eosine methylene blue agar	Growth on Centimide agar	Size of the colonies	Shapes of the colonies	Suspected organism
Green metallic sheen colonies	No growth	Medium	Flat	*E. coli*
Large mucoid purple colonies	No growth	Medium	Raised	*K. pneumonia*
Diffusible purple rough colonies	Blue-green diffusible colonies	Large	Circular	*P. aeruginosa*

Table 4: Gram reaction and biochemical characteristics of the bacterial isolates

Gram Staining	Tests									Triple Sugar Iron Test (TSI)							
Gram reaction	**Shapes**	**Motility**	**Indole**	**Urease**	**Citrate test**	**Catalase**	**Coagulase**	**Oxidase**	**Methyl red**	**Vogeus proskaeur test**	**Glucose**	**Lactose**	**Sucrose**	**Gas production**	**H2s production**	**Tsi reaction (slant)**	**Suspected organism**
_	Rods	+	+	_	_	+	_	_	+	_	+	+	+	+	_	Acidic	*E. coli*
_	Rods	+	_	_	+	+	_	+	_	_	_	_	_	_	_	Alkaline	*P. aeruginosa*
_	Rods	_	_	+	+	+	_	_	+	+	+	+	+	+	_	Acidic	*K. pneumoniae*

Key: + = positive, - = negative, MIU= Motility Indole and Urease test, H_2S= Hydrogen sulphide.

Table 5: Percentage antibiotic resistance pattern of the isolates

Organisms/Antibiotics	CIP	W	CRO	CN	SXT	AX	C
E. coli	65(100)	65(100)	11(16.9)	0(0)	65(100)	65(100)	33(50.8)
P. aeruginosa	1(1.92)	52(100)	36(69.2)	17(32.7)	52(100)	52(100)	41(78.8)
K. pneumonia	8(17.8)	24(53.3)	35(77.8)	9(20)	20(44.4)	45(100)	0(0)

CIP- Ciprofloxacin, CN- Gentamicin, SXT-Trimetoprim-sulphamethoxazole, AX- Amoxicillin, CN- Ceftriaxone, C- Chloramphenicol, W-Trimethoprim.

Table 6: Percentage antibacterial activity of stem bark of *T. glaucescens* against the isolates compared with CLSI

Isolates	S (%)	R (%)
Escherichia coli (65)	(38) 58.5	(27) 41.5
Pseudomonas aeruginosa (52)	(50) 96.2	(2) 3.8
Klebsiella pneumoniae (45)	(27) 60.0	(18) 40.0

Key: S= Sensitive, R=Resistance

DISCUSSION

Medicinal plants are of great value when it comes to the health of an individual and that of the communities. This study assessed the antibacterial activity of the stem bark of *T. glaucescens* and identified constituents responsible for the antibacterial activity using GC-MS analysis. The qualitative phytochemical analysis of the stem bark of *T. glaucescens* revealed alkaloids, saponin, terpenoids, tannin, flavonoids, steroids, and

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 https://dx.doi.org/10.4314/sa.v22i1.6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

phenol with the absence of glycosides and anthraquinone. This is similar to the study of Adeeyo *et al*. (2018), except for the presence of anthraquinone in their research. The result of the phytochemicals is also in line with the findings of Benjamin *et al*. (2018), but flavonoids were absent.

The GC-MS analysis of the stem bark of *T. glaucascens* revealed several compounds of pharmacological interest with most of the compounds belonging to fatty acids, steroids, and terpenoids. The key active components in the ethanolic stem bark extract of *T. glaucascens* are Neophytadiene, Squalene, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, and 9- eicosyne. These are responsible for pharmacological actions such as hepatoprotective, antioxidant, and antimicrobial activities. Neophytadiene, a terpenoid compound, has antipyretic, anti-inflammatory, antimicrobial, and antioxidant activity (Jayashree 2019). The 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol is a terpene alcohol and has antimicrobial and anti-inflammatory activities (Sudha *et al*., 2013).

Squalene is a triterpene that belongs to the phenolic compound and is a natural antioxidant found in most plants (Beltran, 2016; Rosale-Garcia *et al*., 2017b). Squalene acts as an anticancer agent (Gunes, 2013), an antibacterial agent (Popa *et al*., 2015), and an adjuvant for vaccines and drug carriers (Pasquale, 2015), amongst other pharmacological properties. The antibacterial activity of the plant used in this study could be attributed to the presence of the phytochemicals. According to Doss *et al*. (2009), tannins isolated from *Solanum trilobatum* leaves had the highest antibacterial activity against the tested bacteria. Also, Subhashini *et al*. (2010) reported phytochemicals' protective or disease-preventive properties, of which alkaloids, flavonoids, tannins, and phenolic were the most important compounds. Most of the isolates showed multiple drug resistance to the tested antibiotics. This could mean that these antibiotics have been abused; hence the possibility of the isolates to have acquired several resistant genes cannot be overlooked (Odumosu *et al*., 2015b; Olatunji *et al*., 2016; Lathamani and Subbannayya, 2018).

In this study, K. *pneumonia* demonstrates a great extent of resistance to β-lactam antibiotics and Sulphonamides, with no resistance to Chloramphenicol and little resistance to Gentamicin, Ciprofloxacin, which agrees with the report Aly *et al*. (2014). All isolates of *E. coli* showed 100% resistance to Amoxicillin, Trimethoprim, and Trimethoprim-sulphamethoxazole, which concurs with the report of Reuben and Owuna (2013). Resistance of 100% to Ciprofloxacin was also reported, which agrees with the findings of Omololu-Aso *et al*. (2017), who reported 92.86% resistance of *E. coli* to Ciprofloxacin.

All isolates of *P. aeruginosa* were 100% resistant to Amoxicillin, Trimethoprim, and Trimethoprim-sulphamethoxazole, with few isolates showing resistance to Ciprofloxacin, Gentamicin, Chloramphenicol, and Ceftriaxone. A similar result was reported in the sensitivity of *P. aeruginosa* to Ciprofloxacin, Gentamicin, and Ceftriaxone (Singh *et al*. 2017). The highest zone of inhibition was recorded in Ciprofloxacin. In contrast, the lowest clear zone of inhibition was observed in Ceftriaxone, which could be reasoned that Ciprofloxacin directly inhibits DNA synthesis, thereby being more efficient than Gentamicin and Ceftriaxone for the treatment of infections caused by *P. aeruginosa.*

The clear inhibition zones of the stem bark of *T. glaucescens* against the isolated bacteria ranged from 5.5 mm at 25 mg/ml to 12.5 mm at 100 mg/ml. This result is also in line with the study by Gbala and Anibijuwon (2018), who reported the antibacterial activity of *T. glaucescens* on *E. coli, K. pneumonia, Enterobacter aerogenes* and *Proteus mirabilis* isolated from patients suffering from dental caries. Bulana *et al.* (2014) also reported the antibacterial activity of *T. glaucescens* against *E. coli, S. aureus*, and *K. pneumoniae* in their study. The result of this study agrees with a recent work by Adeeyo *et al*. (2018), who reported antibacterial activities of *T. glaucescens* against *E. coli, K. pneumonia,* and *P. aeruginosa* (water microbial contaminants).

CONCLUSION

The outcome of this study revealed the potential of the stem bark of *T. glaucescens*, which can be utilized as an alternative therapy in combating and curtailing the infections caused by these multidrug-resistant bacteria and may also be useful in the production of a useful pharmaceutical product. However, there is a need for further studies to establish the mechanism of action of the studied plant and its toxicity using an animal model.

Declaration of Competing of Interest: None

REFERENCES

Adebayo, E.A. and Ishola, O.R. (2009). Phytochemical and antimicrobial Screening of crude extracts from the root, stem bark and leaves of *Terminalia glaucescens. African Journal of Pharmacology* 3(5): 217-221.

Adeeyo, A.O., Odiyo, J. and Odelade, K. (2018). Chemical profiling and antimicrobial properties of phyto-active extracts from *Terminalia glaucescens* stem against water microbial contaminants. *The Open Biotechnology Journal* 12: 1-15.

Aly, M.M., Khalil, S. and Metwaly, A. (2014). Isolation and Molecular identification of *Klebsiella* microbe isolated from chicks. *Alexandria Journal of Veterinary Sciences* 43: 97-103.

Anokwuru, C.P., Tankeu. S., Van-Vuuren, S., Viljoen, A., Ramaite, I.D., Taglialatela-Scafati, O. and Combrinck, S. (2020). Unravelling the Antibacterial Activity of *Terminalia sericea* Root Bark through a Metabolomi Approach. *Molecules* 25: 3683. https://doi.org/10.3390/molecules25163683

Ayelaagbe, O., Olaoluwa, O., Oladosu, I. and Simon, G. (2014). A New Triterpenoid from *Terminalia glaucescens* (Planch. ex Benth). *Record of Natural Products* 8(1): 7-11. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standard single disc method. *American Journal of Clinical Pathology* 45(4): 493-496.

Beltran, G., Bucheli, M.E., Aguilera, M.P., Belaj, A. and Jimenezi, A. (2016). Squalene in Virgin Olive oil: screening of variability in olive cultivars. *European Journal of Lipid Science and Technology* 118: 1250-1253.

Benjamin, T., Fodouop, S.P.C., Fokam, M.T., Zigla, G., Jules-Roger, K. and Léopold, N.T. (2018). Antibacterial activities of some medicinal plants used for treatment of infectious diseases in the Vina and Mayo-Louti divisions of Cameroon. *European Journal of Medicinal Plants* 22(3): 1-13.

Bharti, S. (2020). Agro-climatic zone-based identification of elite *Terminalia arjuna* accessions concerning to arjunolic acid production. *SN Applied Sciences* 3: 236. https://doi.org/10.1007/s42452-021-04207-1.

Scientia Africana, Vol. 22 (No. 1), April, 2023. Pp 55-66 https://dx.doi.org/10.4314/sa.v22i1.6
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Bulama, J.S., Dangoggo, S.M., Bwala, Y.A., and Abah, J.O. (2014). Phytochemicals and Antibacterial Evaluation of Root Bark Extract of *Terminalia glaucescens. Journal of Applied & Pharmaceutical Sciences* 4 (02): 129-132.

Chauhan, A., Goya,l M.K., and Chauhan, P. (2014). GC-MS Technique and its Analytical Applications in Science and Technology. *J Anal Bioanal Tech* 5:222.

Cheesbrough, M. (2010). District Laboratory Practice in Tropical Countries. *Cambridge University Press.* Pp 434.

Clinical and Laboratory Standards Institute (CLSI) (2015). Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement *CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.*

Doss, A., Mohammed, M.H. and Dhanabalan, R. (2009). Antibacterial activity of tannins from the leaves of *Solanum trilobatum Lim. Indian Journal of Science and Technology* 2(2): 41-43.

Gbala, I.D. and Anibijuwon, I.I. (2018). Antibacterial activity of *Terminalia glaucescens, Mangifera indica* and *Mitracarpus villosus* on carbapenem-resistant Enterobacteriaceae. *African Journal of Clnical & Experimental Microbiology* 19 (4): 251-259.

Gunes, F.E. (2013). Medicinal use of Squalene as a natural antioxidant. *Clinical Experimental Health Sciences*. 3:220-228.

Jayashree, I.W, Geetha, D.H. and Rajeswari, M. (2015). GC-MS Analysis of Bioactive Constituents of *Glochidio nellipticum*. *International Journal of Pharmaceutical Sciences and Research* 6(6): 2546-2550.

Konan, F.K., Guessennd, N.K., Oussou, K.R., Bahi, C., Coulibaly, A., Djaman, A.J. and Dosso, M. (2014). Effect antibactérien de l'extraitaqueux de l'écorce de *Terminalia glaucescens* Planch ex Benth (Combretaceae) surla croissance *in-vitro* des entérobactériesproductrices de bêta-lactamases à spectreélargi (EBLSE). *International Journal of Biology and Chemical Sciences*. 8: 1192-1201.

Kurian, S., Joseph, L. and Josekumar, V.S. (2018). Phytochemical evaluation, GC-MS analysis and antimicrobial activity of the leaves petiole of *Artocarpus heterophyllus-Lam. Asian Journal of Pharmacy and Pharmacology*. 4(3): 280-287.

Lathamani, K. and Subbannayya, K. (2018). Virulence determinant and extended spectrum beta-lactamase production in *Klebsiella pneumonia* isolated from a tertiary care hospital, South India. *Journal of Laboratory Physicians* 10(2): 155-161.

Mgbe, C.G. and Mgbe, E.K. (2015). The antimicrobial properties of *Terminalia Glaucescens* and *Zanthoxylum Zanthoxyloides* extracts and the spectrum of their activity. *South American Journal of Public Heath* 3(4): 1-11.

Ndukwe, K.C., Okeke, I.N., Lamikanra, A., Adesina, S.K. and Aboderin, O. (2005). Antibacterial activity of aqueus extracts of selected chewing sticks. *Journal of Contemporary Dental Practice.* 6(3): 086-094.

Odumosu, B.T., Adeniyi, B.A. and Chandra, R. (2015b). Occurrence of amino modifying enzymes genes (aac(6')- I and ant(2")-I) in clinical isolates of *Pseudomonas aeruginosa* from Southwest Nigeria. *African Health Science* 15: 1277-1281.

Olatunji, M.K., Albert, O. and Fausat, J. (2016). Distribution profile of Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli* isolates from as a river (Nigeria). *Journal of Applied*

Science & Environmental Management 20(4): 973-977.

Ogundiya, M.O., Kolapo, A.L., Okunade, M.B. and Adejumobi, J. (2008). Evaluation of phytochemical composition and antimicrobial activity of *Terminalia glaucescens* against some oral pathogens. *Advances in Natural and Applied Sciences* 2(2):89-93.

Omololu-Aso, J., Omololu-Aso, O.O., Adekanye, A., Owolabi, T.A. and Shesha, A. (2017). Antimicrobial susceptibility pattern of *Escherichia coli* isolates from clinical sources at tertiary health care setting, Ile-Ife, South Western Nigeria. *European Experimental Biology* 7:5.

Pasquale, A.D., Preisi, S., Silva, F.T.D. and Garcon, N. (2015). Vaccine adjuvants: from 1920-2015 and beyond. *Vaccines* 3: 320-343.

Reuben, R.C. and Owuna, G. (2013). Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. *International Journal of Pharmaceutical Science Invention* 2: 38-44.

Rosale-Garcia, T., Jimenez-Martinez, C., Cardador-Martinez, A., Martin-del, S.T., Garlicia-Luna, L.A., Tellez-Medina, D.I. and Gloria, D. (2017b). Squalene extraction by supercritical fluids from traditionally puffed *Amaranthus hypochondriacus* seeds. *Journal of Food Quality* 2017(687912): 1-8.

Saleha, A., Yasmina, S. and Daniel, C. (2012). High throughput screening to determine the antibacterial activity of *Terminalia ferdinandiana* (kakadu plum): A proof of concept. *Journal of Microbiological Methods* 182: 106169. https://doi.org/ 10.1016/j.mimet,2021.106169

Saxena, V.G., Mishra, G., Saxena, A. and Vishwakarma, K.K. (2014). A comparative study on quantitative estimation of tannins in *Terminalia chebula, Terminalia belerica, Terminalia arjuna* and *Saraca indica* using spectrophotometer. *Asian Journal of Pharmacy and Clinical Research* 6: 148-9.

Singh, S., Dutta, U., Bhat, A.K., Gupta, S., Gupta, V. and Jamwal, S. (2017). Morpho-cultural and biochemical identification of *Pseudomonas* sp. isolated from the rhizosphere of different vegetable crops and study its efficacy on *Solanum melongena* (Brinjal). *Journal of Pharmacognosy and Phytochemistry* 6(2): 22-28.

Subhashini, R., MahadevaRao, U.S., Sumathi, P. and Gayathri, G. (2010). A comparative phytochemical analysis of cocoa and green tea. *Indian Journal of Science and Technology* 3(2): 188-192.

Sudha, T., Chidambarampillai, S. and Mohan, V.R. (2013). GC-MS analysis of bioactive components of aerial parts of *Fluggeale ucopyrus* Willd (Euphorbiaceae). *Journal of Applied Pharmaceutical Science.* 3(05): 126-130.

Ugwu, C. C., Ezeonu, I. M., Mbah-Omeje. K. N., Agu, C. C. and Onuorah, S. C. (2017). Evaluation of the Antimicrobial Effects of *Syzygium aromaticum* (Clove) and *Garcinia kola* (Bitter kola) extracts singly and in Combination, on Some Bacteria. *World Journal of Pharmacy and Pharmaceutical Sciences.* 6(12): 1-13.

Ukwueze, S.E. and Ekpemogu, D.U. (2014). Comparative Study on the Phytochemical, Phenolic and Antioxidant Profiles of the Leaf, Root, and Stem barks of *Terminalia glaucescens (planch.exbenth). Indo American Journal of Pharmaceutical Research.* 4(12):5801-5807.