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Prospects of isolating new antimicrobial compounds from plants: The case of *Azadirachta indica* bark extract

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ABSTRACT

Objectives: Essential oils and extracts from medicinal plants have been shown to have antimicrobial properties in several investigations carried out in regions with diverse floras. This study intends to evaluate the antimicrobial activity of *Azadirachta indica* (Neem plant) bark extract on microbial isolates.

Materials and Methods: The plant's bark was cut out of the tree, dried, and pulverized using a mechanical grinder. The crushed barks were split in half, one half macerated in ethanol and the other put through the Soxhlet apparatus. The ethanol extract of plant bark was used to analyze microbial isolates (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*). The active components in the extracts were analyzed using gas chromatography-mass spectrometry.

Results: According to the inhibition zone width, mean inhibition concentration, and lowest bactericidal concentration, all organisms were shown to be sensitive to the antibacterial activities of *A. indica* at varied doses of the extracts utilized. For every isolate examined, the minimum inhibitory concentration (MIC) of the extract was 12.5 mg/mL; however, *B. subtilis* had a concentration of 25 mg/mL. The extract had bactericidal activity on all the isolates except *Bacillus* sp. The minimum bactericidal concentration (MBC) for the isolates was 12.5 mg/mL for *P. aeruginosa*, *S. aureus*, and *C. albicans*, and 100 mg/mL for *E. coli*. Among the principal compounds discovered are pentadecanoic acid, 14-methyl-methyl ester, stigmasterol, 9-octadecanoic acid (z)-methyl ester, methyl stearate, n-hexadecanoic acid, linoelaidic acid, and Vitamin E.

Conclusion: Our research showed that the ethanol extract from *A. indica* bark contains several bioactive compounds with antimicrobial properties.

Keywords: Antimicrobial susceptibility testing, *Azadirachta indica*, Neem plant, antimicrobial agents, Bioactive compounds

INTRODUCTION

Bacteria are the primary causative agent in most infectious diseases.[1] Identification of microbial susceptibilities to various antibiotics has become essential with the availability of laboratory culture techniques for cultivating bacteria. This allows medical professionals to promptly start patients on the right therapeutic regimens.[2]

To combat microbial resistance, there has been a rise in interest in recent years in the discovery and synthesis of new antimicrobial chemicals sourced from various sources. Antimicrobial activity screening techniques have thus drawn more interest.

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Plants might be a possible answer to the shortage of novel antibacterial agents and the fight against antibiotic resistance.[3,4] Numerous complex and structurally diverse compounds may be found in plants and other natural sources. As potential antibacterial agents, many research work have been focused on examining plant and microbial extracts, essential oils, pure secondary metabolites, and freshly synthesized chemicals.[5-7]

Historically, medicinal plants are utilized in many regions of the globe, where access to modern healthcare is restricted[8] and it has been posited that traditional medicines are used by about 80% of the world's population.[9]

Azadirachta indica, commonly known as the neem plant, is mostly grown on the Indian subcontinent. Before the emergence of written history, humans utilized neem widely to cure a variety of diseases due to the numerous beneficial effects of their many constituents.

Annual antimicrobial resistance globally has continued to rise to about 750,000,[10] with a predicted increase to 10 million by 2050.[11] In tropical countries, infectious illnesses account for almost half of all fatalities. Even with breakthroughs in microbiology understanding and management, drug-resistant microbial outbreaks and the emergence of previously recognized disease-causing bacteria continue to pose serious public health risks in affluent nations.[12] This study aims to determine if the *A. indica* bark has an antimicrobial effect on the microbial isolates and also identify some of the organic compounds present in the extracts.

MATERIALS AND METHODS

Plant identification and collection

A. indica plant material was taken from the Department of Pharmacognosy Medicinal Garden at Madonna University Elele Campus in Rivers State, Nigeria. The plant material was authenticated at the medical plants herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, and assigned a Voucher number: FPI 2475.

Extraction of plant's active ingredients

The extraction of the plants was done as previously reported.[13,14] *A. indica* fresh bark was washed with purified water and air-dried for 14 days. A mechanical blender was used to pulverize the bark, and the particle size was further decreased using an electric grinding mill. 200 g of the powdered material was placed into a Winchester container containing 900 mL ethanol. The mouth of the bottle was closed with a lid to prevent solvent evaporation and then the powder was allowed to macerate for 72 h and then carefully filtered to separate the marc from the extract using a filter paper (Whatman Cat No 1001 150). The filtrate was then filtered again using filter paper transferred to a beaker and later concentrated in a 45°C-water bath for 20 min. For additional analysis, the extract was weighed and computed. The ethanol extract weighed 22.5 g giving a percentage yield of 11.25%.

To get the requisite fixed oils, the Soxhlet extraction method[13] was applied. 200 g of the powdered material was utilized. The solvent utilized was N-hexane (400 mL), a non-polar solvent with a boiling point of 68°C. The extraction procedure involves adding the grounded neem bar (200 g) into the extractor which is connected to a round bottom flask attached to the heat source. Four hundred milliliters of the solvent (n-Hexane) were poured into the connected round bottom flask, and the heat source was switched on and regulated to 68°C for evaporation of the solvent. The solvent on heating evaporates, passing through the condenser condenses into the extractor carrying the plant extract, and solubilizes the desired extract. The solvent continues to condense into the extractor until it reaches above the siphon bend on the extractor and the extract in a mixture with the solvent flows back to the connected round bottom flask through the siphon tube. The cycle continues for 48 h. After the extraction process, the mixture of the solvent and extract was allowed to evaporate without being heated so that the solvent can evaporate leaving the extract which was used for further analysis. The n-Hexane extract weighed 8.7 g giving a percentage yield of 4.35%.

Preparing the serial dilution

The ethanol extract was serially diluted using the two-fold dilution procedure. Two grams of the ethanol extracts were weighed into beakers and diluted in 10 mL of dimethylsulphoxide (DMSO) to make a stock solution with a concentration of 200 mg/mL. Dilute solutions were prepared from the stock, and six test tubes were labeled A-F as follows A = 100 mg/mL, B = 50 mg/mL, C = 25 mg/mL, D = 12.5 mg/mL, E = 6.25 mg/mL, and F = 200 mg/mL. Ciprofloxacin (5 μg) and fluconazole (10 μg) were used as positive controls for bacteria and fungi isolates, respectively. The negative controls used were DMSO.

Test isolate confirmation

The isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*) were subcultured from conserved agar slants onto selective media (media for bacteria) and Sabouraud Dextrose agar (all-purpose medium for fungus) for 24 h and 72 h, respectively, before use. Biochemical assays such as catalase test, coagulase test, oxidase test, indole test, lactophenol cotton blue staining, and Gram staining were also employed to confirm the laboratory isolates.[15] The duly identified pure cultures were standardized to an inoculum size of 1.5×10^8 CFU/mL which corresponds to the 0.5 McFarland

standards. This was accomplished by diluting the cells to 0.08–0.1 optical density at 600 nm using a spectrophotometer.

Antimicrobial studies

The sensitivities of bacterial isolates to the extracts were tested using the agar well diffusion method – the punch-hole agar diffusion technique. To avoid contamination, Mueller–Hinton agar plates were prepared, and the isolates were meticulously swabbed with their designated swab sticks on the surface of the sterile plates. A sterile cork borer with an 8 mm diameter was used to drill holes in the solidified agar plates, and 50 mL of the extract was put into the bored holes.

Mueller–Hinton supplementary agar was prepared using Mueller–Hinton agar and supplemented with 2% glucose and 0.5 g/mL methylene blue to stimulate fungal growth in order to measure *C. albicans* susceptibility. The isolate of fungus was swabbed over the plates. Wells were drilled at similar distances around the plates using a sterilized conventional cork borer of 8 mm. Each extract concentration was aseptically placed in each well of the agar plates in a 50 mL volume. The extracts were allowed to diffuse over the agar plates for 30 min. The inoculation plates were then turned over and left to incubate for 24 h. A well-calibrated metric rule was used to measure the inhibition zone diameter for each well.[16]

Minimum inhibitory concentration (MIC)

Using the agar dilution method, the lowest inhibitory concentration of the ethanol extract on the isolates was determined. Pour plate method was used to make (6) agar plates. Six bijou bottles of nutrient agar of 19 mL each were autoclaved for this process, after which the mixture was shaken slightly for proper mixing before being poured into the Petri dishes, 1 mL of each concentration of ethanol extract was added to the bijou bottles to make up for 20 mLs in volume. The Petri dishes were marked into four equal parts, with each quadrant swabbed with the matching bacterial organism. The agar used for MIC of the *C. albicans* was Mueller–Hinton supplemental agar. The plates were incubated for 24 h to check for microbial growth.[16]

Determination of minimum bactericidal and minimum fungicidal concentration (MBC and MFC)

The plates from MIC were further incubated for an additional 24 h making 48 h of incubation. The lowest concentration of the extract that showed no growth after 48 h was reported as the MBC or MFC of the extract.

Gas chromatography-mass spectrometry (GC-MS) analysis

Using a Shimadzu GC-MS-QP 2010 Plus system and a gas chromatograph interfaced with a mass spectrometer system, GC-MS analysis was carried out under the following conditions. Elite: 1 capillary column made of fused silica (30 m × 0.25 mm, 1 D × L, 100% dimethyl polysiloxane). An electron ionization device with an ionization energy of 70 eV was used. With an injection volume of 2 l and a flow rate of 1 mL/min, the carrier gas was 99.99% helium gas. The ion source and injection temperatures were both set to 280°C. The oven was preheated at 110°C. Data from the National Institute of Standards and Technology collection was compared to the relative percentage amount of each component.[14]

Data analysis

After two runs of the antimicrobial susceptibility test, the results were expressed as mean ± standard error of the mean. The data generated from the study was analyzed using GraphPad Prism version 5. The difference between the values was analyzed using analysis of variance (ANOVA). $P \leq 0.05$ is considered statistically significant.

RESULTS

Morphological and biochemical results of microbial isolates

From the results in Table 1, the biochemical tests on the organisms were used to confirm the presence of *C. albicans, P. aeruginosa, S. aureus, E. coli,* and *B. subtilis.*

Antimicrobial susceptibility testing of the ethanol extract

The antimicrobial susceptibility testing results of the extracts on the microbial isolates with respect to their mean zones of inhibition are shown in Table 2. The *E. coli* showed the greatest zone of inhibition at 200 mg/mL with a 9.5 mm diameter, followed by 100 mg/mL with a 7 mm diameter, and the lowest zone at 6.5 mg/mL with a 1.5 mm diameter. Regarding *Bacillus* sp., the largest zone of inhibition measured 200 mg/mL with a 6 mm diameter was followed by 100 mg/mL with a 5 mm diameter, and the lowest was 6.5 mg/mL with a 0.5 mm diameter. The zones of inhibition for *Pseudomonas* sp. were determined to be 200 mg/mL with a diameter of 6 mm, 25 mg/mL with a 5.5 mm diameter, and 6.5 mg/mL with a 1 mm diameter, which was the lowest. The maximum zone of inhibition for *S. aureus* was discovered to be 200 mg/mL with a 6 mm diameter followed by 100 mg/mL with a 5 mm diameter, and the lowest zone was discovered to be 6.5 mg/mL with a 1.5 mm diameter. The zones of inhibition for *Candida* sp. were discovered to be 200 mg/mL with a 17.5 mm diameter 100 mg/mL with a 16.5 mm diameter, and 6.5 mg/mL with an 11 mm diameter, which was the lowest. With $P = 0.9278$, there is no significant difference in the analysis conducted horizontally between the columns when comparing them using one-way ANOVA.

Table 1: Morphological and biochemical results of microbial test isolate.

Microbial isolate	Cultural characteristics	Gram staining	Catalase test	Coagulase test	Indole test	Oxidase test	Lactophenol cotton blue staining
Pseudomonas aeruginosa	Greenish blue size 2–4 mm in smooth surface in Cetrimide Agar	−(ve) rod	+(ve)	−(ve)	−(ve)	+(ve)	ND
Escherichia coli	Colonies on eosin methylene blue agar are 2–3 mm in diameter and have a metallic green sheen when reflected light is used.	− (ve) rod	+(ve)	−(ve)	+(ve)	−(ve)	ND
Bacillus subtilis	Whitish convex shape in Nutrient Agar	+(ve) rod	+(ve)	−(ve)	−(ve)	−(ve)	ND
Staphylococcus aureus	Golden yellow 2–3 mm smooth shiny surface in Mannitol Salt Agar	+(ve) cocci	+(ve)	+(ve)	−(ve)	−(ve)	ND
Candida albicans	White color on SDA with a smooth and yeast-like appearance	ND	ND	ND	ND	ND	Circular bluish colonies appearing in clusters

ND: Not determined, −ve: Negative, +ve: Positive, SDA: Saboraud dextrose agar

Table 2: Antimicrobial susceptibility testing of the ethanol extracts.

Concentration	Zone of Inhibition (mm) (X±SEM)				
	Escherichia coli	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida* sp.
200 mg/mL	9.5±1.5	6±0	6±3	6±1	17.5±0.5
100 mg/mL	7±1	5±0	4.5±3.5	5±0	16.5±0.5
50 mg/mL	5±1	3±1	4.5±2.5	3±1	15±0
25 mg/mL	3.5±0.5	2±1	5.5±0.5	3.5±0.5	14.5±0.5
12.5 mg/mL	2.5±0.5	1.5±0.5	3.5±2.5	2.5±1.5	12±1.0
6.25 mg/mL	1.5±0.5	0.5±0.5	1±1	1.5±0.5	11±0
Ciprofloxacin (5 µg)	22±2	18±4	15±1	26.5±0.5	0
Fluconazole (10 µg)	ND	ND	ND	ND	18±0
DMSO	0	0	0	0	0

The mean±SEM is used to represent the values. SEM: Standard error of the mean, DMSO: Dimethylsulfoxide, X: Mean, SEM: Standard error of mean.

MIC, MBC, and MFC of ethanol extracts on the isolates

The ethanol extract of *A. indica*'s MIC for *S. aureus, E. coli, C. albicans, and P. aeruginosa* was determined to be 12.5 mg/mL and 25 mg/mL, respectively, for *Bacillus* sp. as shown in Table 3.

The MBC for *Pseudomonas* sp. is 12.5 mg/mL. For *E. Coli,* the MBC is 100 mg/mL. For *Bacillus* sp., there is no bactericidal activity as there was growth at all concentrations. For *S. aureus,* concentrations 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL showed the absence of *S. aureus* but concentration 6.25 mg/mL showed the presence of *S. aureus;* hence, the MBC is 12.5 mg/mL. For *C. albicans*, the MBC is 12.5 mg/mL.

Table 3: Result for MIC and MBCor MFC of the ethanol extract.

Isolates	MIC (mg/mL)	MBC or MFC (mg/mL)
Pseudomonas aeruginosa	12.5	12.5
Escherichia coli	12.5	100
Bacillus subtilis	25	0
Staphylococcus aureus	12.5	12.5
Candida sp.	12.5	12.5

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration, MBC: Minimum bactericidal concentration

GC-MS

In this work, the GC-MS analysis was carried out on the ethanol and n-hexane extract of the dried bark of *A. indica* commonly known as the Neem plant to analytically identify, quantify, and characterize the bioactive constituents of this extract.

The GC-MS analysis was carried out and the analytical results of N-Hexane and ethanol extract are shown in Tables 4 and 5, respectively. In the N-Hexane GC-MS result, it was observed that some constituents were more prominent in quantity than other constituents which made 6H-Purin-6-one,2-(dimethylamino)-1,7-dihydro the highest in terms of

quantity based on the area percentage that it possesses which is 29.30% and the lowest quantity is the gamma-sitosterol occupying 1.25% in area, while from the ethanol extract, the result shows that constituent with the highest quantity occupied based on area percentage is linoelaidic acid occupying 20.45% and the lowest quantity occupied in area percentage is the 2H-1,4rbenzoxazin-3 (4H)-one with 0.19%.

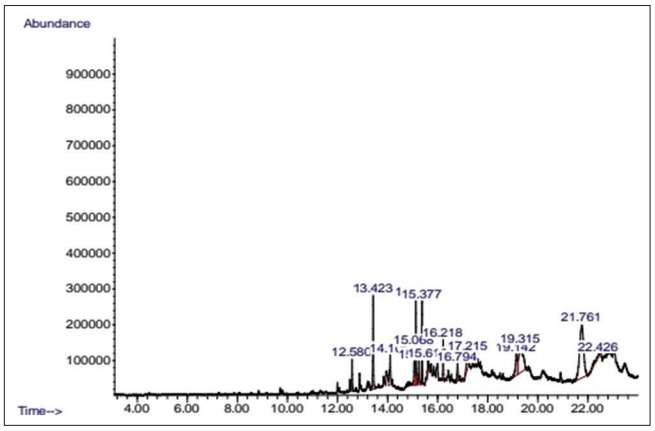

Figure 1: Chromatogram of n-hexane extract of neem bark.

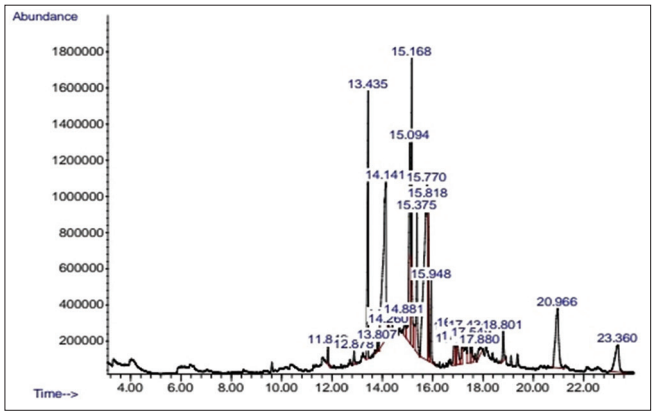

Figure 2: Chromatogram of ethanol extract of neem bark.

DISCUSSION

The *A. indica* plant is a natural medication store. Antibacterial activity was reported in all concentrations of *A. indica* bark extract, with antibacterial activity against *S. aureus* seen at higher doses of >6.12 mg/mL. At all doses except 6.12 mg/mL, the bark of *A. indica* demonstrated a zone of clearance against *P. aeruginosa*. Higher doses produced a zone of inhibition against *S. aureus*. The method by which bacteria typically withstand the action of antimicrobial drugs is unknown and controversial. The findings of this study support Okemo *et al.'s* claim that *A. indica* components might be utilized safely as chemotherapeutic agents at predefined quantities.[17] The antibacterial action of *A. indica* extracts utilized in this investigation is demonstrated in Tables 2 and 3 used in the interpretation of the findings. In all instances, ethanol extracts were the most effective against *S. aureus*. The bactericidal activity of all extracts used in this study increased with increasing extract concentration, suggesting that on plates containing extract with a low dilution factor, the inhibitory zone was bigger. This was also noted by Esimone *et al*., who opined that varying concentrations of neem plant extract are inhibitory to bacterial growth.[18] This is in line with the investigation's results, which showed that the diameter of inhibitory zones increased in proportion to an increase in extract concentration.[19] *S. aureus* and *P. aeruginosa* were unaffected by low concentrations of neem

Table 4: Compounds identified from the n-hexane extract.

Peak	Retention time (s)	Area (%)	Compound ID	Molecular weight (g/mol)	Molecular formula
1	12.582	3.04	2-undecone, 6,10-dimethyl	198.34	$C_{13}H_{26}O$
2	13.425	9.38	Pentadecanoic acid, 14-methyl-methyl ester	270.4507	$C_{17}H_{34}O$
3	14.104	4.49	Hexadecanoic acid, ethyl ester	284.4772	$C_{18}H_{36}O_2$
4	15.068	3.77	9,12-octadecadienoic acid, methyl ester	294.4721	$C_{19}H_{34}O$
5	15.130	8.52	9-octadecanoic acid (z) methyl ester	294.4879	$C_{19}H_{36}O$
6	15.254	2.64	Phytol	294.5310	$C_{20}H_{40}O$
7	15.377	7.43	Methyl stearate	298.50	$C_{19}H_{38}O_2$
8	15.620	1.87	9,17-octadecadienal (z)	264.4461	$C_{18}H_{32}O$
9	16.215	3.33	3,7,11,15-tetramethyl-2-hexadecon-1-ol	294.5319	$C_{20}H_{40}O$
10	16.792	1.97	1,1-biphenyl, 2,2',4,4'-tetrachloro	291.9880	$C_{12}H_6Cl_4$
11	17.215	3.11	4,5,6,7-tetrafluorobenzimidazol (1,2–9) pyrazolo (3,2-C) quinazoline-3-carboxylcacid, ethylester	240.2800	$C_{10}H_{12}N_2O_3S$
12	19.144	4.82	Bis (2-ethylhexyl) phthalate	390.5561	$C_{24}H_{38}O_4$
13	19.316	15.05	Stigmasterol	412.69	$C_{29}H_{48}O$
14	21.763	29.30	6H-Purin-6-one, 2-(dimethylamino)-1,7-dihydro	400.0661	$C_{10}H_{12}N_{10}O_6$
15	22.425	1.25	Gamma-sitosferol	414.7067	$C_{29}H_{50}O$

Table 5: Compounds identified from the ethanol extract.

Peak	Retention time (s)	Area (%)	Compound ID	Molecular weight (g/mol)	Molecular formula
1	11.844	0.58	Tetradecanoic acid	228.3709	$C_{14}H_{28}O_2$
2	12.877	0.50	Maltose	342.2965	$C_{12}H_{22}O_{11}$
3	13.435	8.08	Pentadecanoicacid, 14-methyl-methyl ester	270.4507	$C_{17}H_{34}O_2$
4	13.806	0.26	Dibutyl phthalate	278.3435	$C_{16}H_{22}O_4$
5	14.139	16.95	N-hexadecanoic acid	256.4241	$C_{16}H_{32}O_2$
6	14.220	0.43	2-acetyl-3-nitrobenzoic acid	209.1560	$C_9H_7NO_5$
7	14.258	0.19	2h-1,4rbenzoxazin-3 (4h)-on	149.15	$C_8H_7NO_2$
8	14.882	0.90	1-deoxy-d-altriol	166.17	$C_6H_{14}NO_5$
9	15.096	9.41	9,12,-octadecadienoicacid, methyl ester	299.4721	$C_{19}H_{34}O$
10	15.168	9.78	7- octadecadienoicacid, methyl ester	296.4879	$C_{19}H_{36}O_2$
11	15.377	4.91	Methyl stearate	298.50	$C_{19}H_{38}O_2$
12	15.768	20.45	Linoelaidic acid	280.4455	$C_{18}H_{32}O_2$
13	15.820	4.98	Cis-vaccenic acid	282.5	$C_{18}H_{34}O_2$
14	15.949	2.59	Octadecanoic acid	284.4772	$C_{18}H_{36}O_2$
15	16.849	1.43	Ethanone, 1-(6-hydroxy-2-(-1-methylethenyl)-5-benzofuranyl	216.2756	$C_{14}H_{16}O_2$
16	16.920	0.66	5-(1,1-ethylenedioxyethyl)-1,3 diazaazulene	302.4	$C_{21}H_{18}O_2$
17	14.982	1.85	5-(1,1-ethylenedioxyethyl) 1,3-diazaazulene	302.4	$C_{21}H_{18}O_2$
18	17.154	0.71	Methyl-18-methylnonadecanoate	326.5570	$C_{21}H_{42}O_2$
19	17.435	2.21	3-phenylthio-2h-chromen-2-one	238.24	$C_{15}H_{10}O_3$
20	17.549	0.97	Eicosanoic acid	312.5304	$C_{20}H_{40}O_2$
21	17.882	0.78	8h-imidazo (4,5-)-2,1,3,-benzothiadiazole-7-methyl	190.22	$C_8H_6N_4S$
22	18.801	1.16	Glycerol-1-palmitate	330.5026	$C_{19}H_{38}O$
23	20.968	6.50	9-octadecenoic acid (z)-2-hydroxy-1-(hydroxymethyl) ethyl ester	354.5240	$C_{21}H_{40}O_4$
24	23.358	3.72	Vitamin E	430.7041	$C_{29}H_{50}O_2$

extract; however, when the dilution level dropped, the effect of the extract concentration increased. These studies' findings are in line with those of Alorzohairy, who stated that traditional Indian medicine has utilized various neem tree parts, such as the bark (*A. indica*), for their purported bioactive, therapeutic, and preventive qualities.[20] The results of Wylie and Merrell's study, which found that a variety of *A. indica* components, including seeds, barks, and leaves produce extracts with moderate to strong antimicrobial activity against a range of pathogens, including *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *Salmonella*. Typhi, *Shigella boydii*, *B. subtilis*, *Candida tropicalis, K. pneumoniae, and Streptococcus agalactiae.*[21] The reports of this work are also in line with the work of Singaravelu *et al.* which reported the antimicrobial effect of *A. indica* bark extracts on *P. aeruginosa* and *Pseudomonas mirabilis.*[22] The results of this work are also consistent with the work of other authors, who reported the antimicrobial effect of *A. indica* extracts on *S. aureus, P. aeruginosa,* and *E. coli.*[23]

Some compounds identified in the *A. indica* have been previously reported to have useful characteristics. Compounds, like Linoelaidic acid found in the ethanol extract, have been previously reported to possess antibacterial effect on gram-positive isolates as can be seen in the work of Dilika *et al.*[24] Dewi *et al.*[25] reported antimicrobial effects on *S. aureus and B. subtilis*. Desbois and Smith[26] reported antibacterial activity in pathogenic bacteria. Stigmasterol molecules from the n-hexane extract have been reported to have antimicrobial effect as can be seen in the report of Yusuf *et al.* on *S. aureus*, *E. coli*, and *C. albicans*.[27] Yohanna *et al.*[28] reported the antimicrobial effect of stigmastrol on bacteria isolates. Hexadecanoic acid, also known as palmitic acid, was present in both extracts. It has also been previously reported to possess an antibacterial effect.[29,30]

CONCLUSION

This research found that an ethanol extract of *A. indica* bark has antimicrobial properties against *P. aeruginosa*, *S. aureus*, *E. coli*, *C. albicans*, and *B. subtilis.* The extracts contained some bioactive compounds. Some of the compounds identified including Linoleic acid, Stigmasterol, and Hexadecanoic acid have previously been identified as antimicrobial compounds. The other compounds can potentially contribute to the antimicrobial effect of the extracts. More research is needed to determine the individual compounds that actually have antimicrobial properties.

Authors' contributions

Oluchi Judith Osuala: Conceptualization, Literature collection and curation, writing–original draft.

Samuel Igwe: Literature collection and curation. Chinyere Constance Ezemba: Literature collection and curation. Chukwuma Chukwuemeka Chukwuma: Literature collection and curation. Angus Nnamdi Oli: Literature collection and curation, writing–original draft, review and editing.

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Ethical approval

The Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

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None.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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