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Research Article

Acid Induced Phytoconstituents of *Calotropis procera* Extract Combined with Ampicillin in Combating Clinical Resistant Isolates of *Staphylococcus aureus* and *Salmonella* spp.

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Abstract: Natural products, with their inherent diversity, offer unique pharmacophores, chemotypes, and scaffolds that can be harnessed to create effective drugs targeting various infections and diseases. This study introduces a straightforward, rudimentary, and environmentally sustainable method to enhance the antimicrobial activity of two less potent antimicrobial agents, fostering synergism against drug-resistant clinical bacteria. The aqueous extract of fresh leaves and flowers of *Calotropis procera* was subjected to a reaction with a 1 mg/mL ampicillin solution, resulting in synergy and heightened susceptibility against resistant strains of *Staphylococcus aureus* and *Salmonella* spp., augmenting their zones of inhibition from 0 mm to 16.8 mm and from 5.3 mm to 21.4 mm, respectively. Gas chromatography-mass spectrometry (GC-MS) analysis identified 53 phytochemicals in *C. procera* extract, revealing oleic acid (13.04%), 1,1,1,3,5,5,7,7,7-nonamethyl-3-(trimethylsiloxy) tetrasiloxane (9.50%), 9-heptadecanone (3.75%), cystamine (3.35%), and tetrahydro-4H-pyran-4-ol (3.15%) as the top five most abundant phytochemicals. Notably, 18 out of the 53 phytochemicals were associated with known reported biological activities. The analysis revealed the discovery of compounds such as farnesol, cystamine, cystine, metaraminol, dl-phenylephrine, and two distinct substituted amphetamine compounds. Three phytochemicals demonstrated anticancer properties, namely farnesol, 4-amino-1-pentanol, and an imidazole derivative resembling the drug Ribavir. These compounds may serve as valuable starting materials, intermediates, or derivatives in pharmaceutical production.

Keywords: *C. procera*, ampicillin, farnesol, cystamine, cystine, metaraminol, dl-phenylephrine, amphetamine

1. Introduction

The growing global concern regarding the diminishing effectiveness of antibiotics in treating infections, largely due to bacterial resistance, emphasizes the urgent need to identify novel approaches for developing lead compounds characterized by selectivity, efficacy, and safety for subsequent clinical trials.[1] With the pace of discovering new antibiotics slowing down, researchers face the daunting task of devising innovative strategies to combat drug resistance in bacteria. A promising avenue in this pursuit is exploring synergistic drug combinations, where two or more drugs collaborate to produce a more potent and targeted antimicrobial effect. This involves leveraging different mechanisms of action to simultaneously target multiple pathways.[2] Even minor alterations in the chemical structure of

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antimicrobial agents, such as changes in geometry, stereochemistry, or functional groups, can have profound impacts on their pharmacological activities.[3] These subtle changes play a crucial role in influencing the efficacy and specificity of antimicrobial agents, highlighting the intricate relationship between molecular structure and pharmacodynamics behaviour.

The process of drug design, discovery, and development is complex and demanding, requiring the use of various synthetic reactions. These include structural modifications derived from medicinal plants, semi-synthetic derivatives, prodrug synthesis, and approaches from combinatorial chemistry to enhance antimicrobial activities.[4-7] The utilization of diverse synthetic strategies underscores the multifaceted nature of drug development, with each approach providing unique insights into optimizing therapeutic outcomes.

Natural products emerge as important contributors to drug design and development, offering distinct pharmacophores, chemotypes, and scaffolds that can be utilized to formulate effective drugs against a range of infections and diseases.[8-10] The rich diversity of chemical compounds present in natural products serves as a valuable source of inspiration, providing a wide range of molecular frameworks that can be strategically modified to create novel pharmaceutical agents with enhanced efficacy and reduced side effects.

The chemical diversity and potential therapeutic properties inherent in natural products make them prolific sources of biologically active compounds, forming the basis for numerous pharmaceuticals. These compounds have an intrinsic ability to undergo diverse chemical reactions, leading to the generation of novel bioactive compounds.[11] The dynamic nature of natural products, coupled with their ability to undergo various chemical transformations, underscores their versatility as a valuable resource in the continuous pursuit of innovative drug development. Functional groups present in natural products often undergo chemical transformations such as oxidation, reduction, esterification, and alkylation, resulting in derivatives with altered biological activities.[12] These modifications, driven by the reactivity of specific functional moieties, contribute to the generation of analogues with improved pharmacological profiles, paving the way for the development of more potent and selective antimicrobial agents.

Furthermore, the semi-synthesis approach involves modifying a natural product through chemical reactions while retaining a portion of its original structure.[13] This strategic approach allows for the preservation of key pharmacophores while introducing targeted modifications, striking a balance between harnessing the inherent therapeutic potential of natural products and enhancing their pharmacological properties through chemical manipulation. The application of combinatorial chemistry techniques to natural product libraries enables the creation of diverse compound libraries with potential bioactivity.[14] By systematically exploring vast chemical space through combinatorial methods, researchers can efficiently identify novel compounds with desirable antimicrobial properties, accelerating the drug discovery process and expanding the arsenal of potential therapeutic agents. *Calotropis procera*, commonly known as milkweed, holds historical significance in traditional medicine. Its leaves and flowers have traditionally been administered orally for the treatment of malaria and intermittent fever, while decoctions have been used for treating gonorrhoea.[15] The historical usage of *Calotropis procera* highlights its traditional role in addressing infectious diseases, providing a cultural and historical context to its potential therapeutic applications.

Antibacterial screening of *Calotropis procera* leaf extracts has demonstrated inhibitory effects on *Escherichia coli* and *Staphylococcus aureus*.[16] Furthermore, the plant extract has shown effectiveness against various bacteria, including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, suggesting its potential as an antimicrobial agent.[17] These findings underscore the broad-spectrum antimicrobial activity of *Calotropis procera*, positioning it as a promising candidate for further exploration in the development of novel antimicrobial agents.

Gas chromatography-mass spectrometry (GC-MS) analysis of different extracts from *Calotropis procera*, such as methanol stem, aqueous stem, chloroform leaf, pet-ether acetone leaf, methanol leaf, and aqueous leaf, has identified a myriad of phytochemicals. These include Methyl palmitate, 9,12-Octadecadienoic acid (z,z)-methyl ester, 9-octadecadienoic acid, octadecadienoic acid, Methy-l9,12-hepatadecadienoate, Tetradecanoic acid, (2,3-Diphenylcyclopropyl) methylphenyl sulfoxide, hexadecanoic acid, methyl ester, n-hexadecanoic acid, dodecanoic acid, linolenic acid, ethyl ester, among others as depicted in Table 1.[15-22] The detailed GC-MS analysis provides comprehensive insight into the chemical composition of *Calotropis procera*, highlighting the diverse array of phytochemicals present in different plant extracts. These identified compounds serve as potential leads for further investigation, offering valuable information for understanding the plant's pharmacological properties and guiding future drug development efforts.

Table 1. Some phytochemicals present in *C. procera* leaf and latex extract by GC-MS analysis[15]

No.	Phytoconstituents	No.	Phytoconstituents
1	Tetratetracontane ($C_{44}H_{90}$)	25	1,3,5-Tri-isopropylbenzene ($C_{15}H_{24}$)
2	Ergost-5-en-3-ol ($C_{28}H_{48}O$)	26	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene ($C_{30}H_{50}$)
3	Farnesol isomer ($C_{15}H_{26}O$)	27	3,7,11-Trimethy l-2,6,10,12-pentadecatrien-1-ol ($C_{18}H_{30}O$)
4	(6Z,9Z)-Pentadecadien-1-ol ($C_{15}H_{28}O$)	28	*n*-Eicosane ($C_{20}H_{42}$)
5	6,10,14-Trimethyl-pentadecanone-2 ($C_{18}H_{36}O$)	29	2,6-Dimethyl tetra-1,5-decaene ($C_{16}H_{28}$)
6	9-Octadecenoic acid, (Z)-($C_{18}H_{34}O$)	30	*n*-Pentadecane ($C_{15}H_{32}$)
7	4-Hydroxy-4-methylpentan-2-one ($C_6H_{12}O_2$)	31	2,3,4-Trimethylhexane (C_9H_{20})
8	Decane ($C_{10}H_{22}$)	32	*n*-Pentadecane ($C_{15}H_{32}$)
9	Neophytadiene ($C_{20}H_{38}$)	33	Hexadecanoic Acid ($C_{17}H_{34}O_2$)
10	8-Heptadecyne, 1-Bromo-$C_{17}H_{31}Br$	34	7,9-Di-Tert-Butyl-1-oxaspiro(4,5) DeCa-6,9-Diene-2,8-Dione ($C_{17}H_{24}O_3$)
11	2-Pentadecanone ($C_{18}H_{36}O$)	35	Phytol ($C_{20}H_{40}O$)
12	Tert-Hexadecanethiol ($C_{16}H_{34}S$)	36	17-Octadecenoic Acid Methyl ester ($C_{19}H_{32}O_2$)
13	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester ($C_{19}H_{34}O_2$)	37	Methanesulfonic Acid ($C_{26}H_{43}DO_4S$)
14	α-Linolenic acid ($C_{19}H_{32}O_2$)	38	9-Octadecenoic Acid (Z)-Ethyl ester ($C_{20}H_{38}O_2$)
15	Linoleic Acid Ethyl Ester ($C_{20}H_{36}O_2$)	39	À-Tocospiro A ($C_{29}H_{50}O_4$)
16	À-D-Glucopyranoside, Methyl 2-(Acetylamino)-2-Deoxy-3-O-(TrimEthylsilyl)-, cyclic butylboronate ($C_{16}H_{32}BNO_6Si$)	40	Picrotin ($C_{15}H_{18}O_7$)
17	Promecarb ($C_{16}H_{16}N_2O_5$)	41	14-Hydroxy-14-Methyl-Hex Adec-15-Enoic Acid Methyl Ester ($C_{18}H_{34}O_3$)
18	Stigmasterol ($C_{29}H_{48}O$)	42	Boroxin ($C_{21}H_{12}B_3F_9O_3$)
19	Tetrakis (4-Methylphenyl) Thieno 3,2-BThiophene ($C_{34}H_{28}S_2$)	43	Astilbin ($C_{21}H_{22}O_{11}$)
20	Thieno 3,4-CPyridine, 1,3,4,7-Tetrephenyl ($C_{31}H_{21}NS$)	44	Nicotiflorin ($C_{27}H_{30}O_{15}$)
21	Momordicinin ($C_{30}H_{46}O_2$)	45	Gombasterol A ($C_{28}H_{48}O_7$)
22	25-Hydroxy-24-Epi-Brassinolide ($C_{28}H_{48}O_7$)	46	1,2-Dilinoleoyl-Sn-Glycero-3-Phosph Oethanolamine ($C_{41}H_{74}NO_8P$)
23	α-Amyrin ($C_{30}H_{50}O$)	47	Lupeol Acetate ($C_{32}H_{52}O_2$)
24	Methyl Commate D ($C_{31}H_{50}O_4$)	48	Among others

In this study, our primary objective is to devise a method that establishes consistent synergism between *C. procera* plant extracts and ineffective antibiotic(ampicillin), aiming to counteract drug resistance in clinical bacteria. Leverage on combinatorial chemistry approach to create a mixture exhibiting enhanced antimicrobial properties. This comprehensive approach seeks to address the urgent need for innovative solutions in the battle against bacterial drug resistance, providing a potential avenue for the development of effective and sustainable antimicrobial agents.

2. Methods

2.1 *Sampling*

Ampicillin 500 mg in 10 capsules, manufactured by Sam-Ace Ltd. (Pharm. Man. Div.) at Plot 9/10 Block 7c Akoda Ind. Est. Osun State, Nigeria, was procured from pharmaceutical vendors in the Kaduna metropolis, Kaduna North, Kaduna, Nigeria.

Leaves and flowers of the *Calotropis procera* plant were collected in September 2023 at Sabon Tasha, Kaduna, and authenticated by a plant taxonomist at Ahmadu Bello University, Zaria, Kaduna State, with the voucher number V/N-ABU900086. Clinical isolates of *Salmonella* spp. (Stool) and *Staphylococcus aureus* (High Vaginal Swab) were collected at the Chemical Pathology, Haematology, and Microbiology diagnostic laboratory of Oxford Hospital Makera, Kakuri, Kaduna State, Nigeria.

2.2 *Experimental*

The methodology outlined by Gideon, et al.[1] was employed. Fresh leaves and flowers of *Calotropis procera* (10 g) were washed with distilled water, placed in a blending machine, and 100 mL of distilled water was added. The mixture was blended for homogeneity, and filtered using Whatmann No.1 filter paper to extract its juice. A 1 mg/mL Ampicillin solution was prepared. Subsequently, 2 mL of the Ampicillin solution was transferred into a test tube containing 2 mL of *C. procera* extract. To this, 0.2 mL of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added (as illustrated in Figure 1). The mixture was heated in a water bath at 110 °C for 10 minutes, it was centrifuged and the sediment/ precipitate was dried in an oven at 40 °C for 48 h, and then preserved for further analysis.

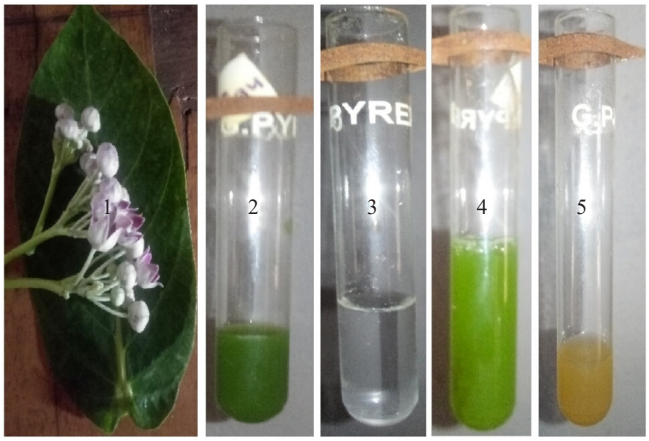

Figure 1. Sampling and stages in the preparation of the sample. 1: *C. procera* leaf and flowers, 2: *C. procera* extract, 3: Ampicillin solution, 4: Mixture before acid addition and heating, 5: Mixture after acid addition and heating

2.3 *Screening for antibiotic resistance and antimicrobial test of prepared samples*

2.3.1 *Materials required*

Mueller-Hinton Agar (MHA), Antibiotic discs, Cotton swabs, Petri dishes, 0.5 McFarland Turbidity standard,

Inoculum, Forceps, Metric rule. *Salmonella* spp. (Stool) and *Staphylococcus aureus* (High Vaginal Swab) were isolated, characterized, and identified. The two bacterial isolates shown to be resistant to Amoxicillin, Septrin, Ampiclox, and Chloramphenicol in high-profile positive/negative 10-tipped multiple susceptibility antibiotic discs, were then cultured for the prepared sample antimicrobial test. Kirby-Bauer disk diffusion test, using Mueller-Hinton Agar (MHA), was employed.[1] After 24 hours of incubation, the Zone of Inhibition (ZOI) was measured in millimetres using a metric ruler, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[23]

2.4 *Gas chromatography-mass spectrometry (GC-MS) analysis of prepared sample*

To determine the phytoconstituents present in the prepared sample, an Agilent 19091S-433UI Gas chromatography-mass spectrometer (GC-MS) machine was used. The specifications included HP-5ms Ultra Inert, temperature range 0 °C-325 °C (350 °C), dimensions 30 m × 250 µm × 0.25 µm, pressure 7.3614 psi, flow rate 0.97414 mL/min, Helium as the carrier gas, injector temperature program set at 250 °C, column temperature at 500 °C, syringe size of 10 µL, and injection volume of 1 µL.

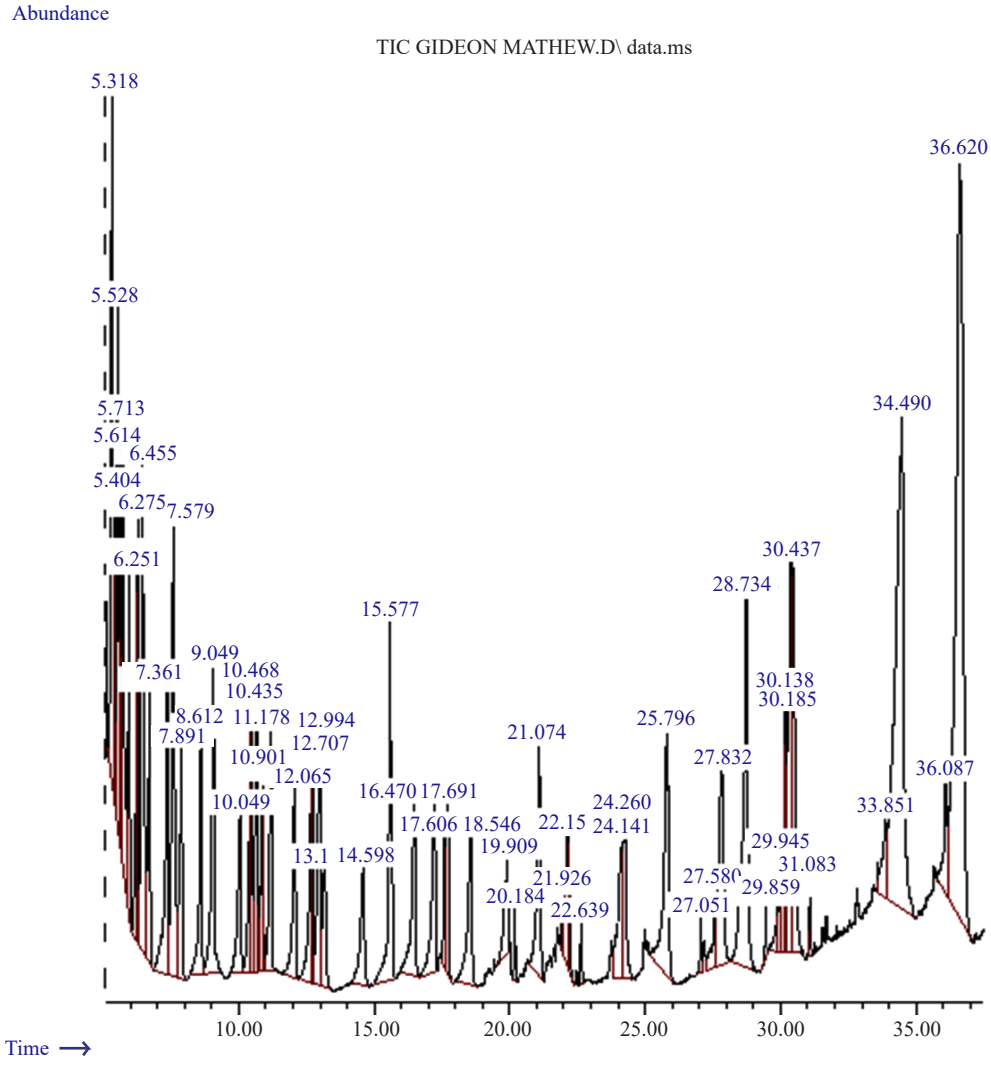

Figure 2. Chromatogram of the phytochemicals present in the prepared sample

Table 2. Phytochemicals obtained from the prepared sample

Pk#	RT	Area%	Compound name	Molecular weight
1	5.318	3.35	Cystamine	152.0
2	5.404	0.98	2-methyl-Piperazine	100.0
3	5.528	2.46	4-amino-1-Pentanol	103.0
4	5.614	1.20	2-Isopropoxyethylamine	104.0
5	5.959	1.42	N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide	155.0
6	6.275	0.73	1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide	284.0
7	6.455	3.15	Tetrahydro-4H-pyran-4-ol	102.0
8	6.711	1.34	8-[N-Aziridylethylamino]-2,6-dimethyloctene-2	224.0
9	7.361	1.94	3,3-dimethyl-4-(1-aminoethyl)-Azetidin-2-one	142.0
10	7.579	3.13	Acetic acid, [(aminocarbonyl)amino]	132.0
11	7.891	1.37	5-methyl-2-Heptanamine	128.0
12	8.612	1.42	2-chloro-Acetamide	93.0
13	9.049	1.95	N-methyl-1-Octadecanamine	283.0
14	10.049	1.18	hydroxy[(1-oxo-2-propenyl)amino]-Acetic acid	145.0
15	10.468	0.68	2-Formylhistamine	138
16	10.657	1.35	3,4-dibenzyloxy-2-fluoro-beta-hydroxy-N-methyl-Benzeneethanamine	381.0
17	10.874	0.80	[S-(R*,R*)]-1,2,3,4-Butanetetrol	122.0
18	10.901	0.42	Tetraacetyl-d-xylonic nitrile	343.0
19	11.178	1.40	N,N-Dimethyl-dimethylphosphoric amide	121.0
20	12.065	1.36	dl-Phenylephrine	167.0
21	12.673	1.13	2,5-difluoro-beta,3,4-trihydroxy-N-methyl-Benzeneethanamine	219.0
22	12.994	1.74	Ethyl oxamate	117.0
23	14.598	1.16	Cystine	240.0
24	15.577	2.46	dodecamethyl-Cyclohexasiloxane	429.0
25	16.470	1.62	Chlorodifluoroacetamide	129.0
26	17.241	1.53	3,4-dibenzyloxy-2-fluoro-beta-hydroxy-N-methyl-Benzeneethanamine	381.0
27	17.606	0.53	2-methyl-Octadecane	268.0
28	17.691	0.98	N-(2-amin oethyl)-1-hexadecanesulfonamide	349.0
29	18.546	1.56	N-methyl-1Benzeneethanamine	135.0
30	19.909	0.85	2-fluoro-2',4,5-trihydroxy-N-methyl-Benzenethanamine	201.0
31	20.184	0.23	Pentadecane	212.0
32	21.074	2.03	1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide	284.0
33	21.926	0.20	Hexadecane	226.0
34	22.127	0.73	Metaraminol	167.0
35	22.155	0.55	2-(2-aminopropoxy)-3-methyl-Benzenemethanol	195.0
36	24.141	1.30	13-Tetradecen-1-ol acetate	254.0

37	24.260	1.33	10-Methyl-E-11-tridece-1-ol acetate	254.0
38	25.796	2.83	3-Propoxyamphetamine	193.0
39	27.051	0.20	Z-8-Hexadecene	224.0
40	27.580	0.56	N-(3-aminopropy l)-1,4-Butanediamine	145.0
41	27.832	2.36	Alanyl-beta-alanine, TMS derivative	233.0
42	28.734	3.72	9-Heptadecanone	254.0
43	29.445	0.12	Hexadecanoic acid, methyl ester	270.0
44	29.859	0.27	4-Cyclohexene-1,2-dicarboxylic acid, 4-chloro-, bis(trimethylsilyl) ester	333.0
45	29.945	0.47	Dibutyl phthalate	278.0
46	30.138	0.99	9-Eicosene, (E)-	280.0
47	30.185	0.80	1-Tridecene	182.0
48	30.385	3.03	Cycloeicosane	280.0
49	30.437	2.68	1-Docosene	308
50	31.083	0.16	9-Octadecenoic acid (Z)-, methyl ester	296.0
51	34.490	9.50	1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane	369.0
52	36.087	0.78	3,7,11-trimethyl-2,6,10-Dodecatrien-1-ol	222.0
53	36.620	13.04	Oleic Acid	264.0

3. Results and discussion

3.1 *Gas chromatography-mass spectrometry (GC-MS)*

GC-MS analysis was carried out for the phytochemical study of the prepared sample labeled 5 in Figure 1. The chromatogram identified 53 phytoconstituents and different retention time (Table 2 and Figure 2).

The analysis revealed that oleic acid constitutes the highest proportion, making up 13.04% of the overall composition. It is notably present at a retention time of 36.62 minutes, indicating a distinctive peak in the chromatogram. Following this, 1,1,1,3,5,5,7,7,7-nonamethyl-3-(trimethylsiloxy)tetrasiloxane contributes 9.50% and appears at a retention time of 34.49 minutes. 9-Heptadecanone emerges as another noteworthy component, comprising 3.72% and eluting at 28.73 minutes, while cystamine is identified at 3.35%, at a retention time of 5.31 minutes. Tetrahydro-4H-pyran-4-ol follows closely with 3.15% and eluting at 6.45 minutes, and acetic acid, [(aminocarbonyl)amino], accounts for 3.13% at a retention time of 7.57 minutes. The analysis further uncovers the presence of various compounds, each with distinct proportions and retention times. Notable examples include cycloeicosane at 3.03% and a retention time of 30.38 minutes, 3-propoxyamphetamine at 2.83% with a retention time of 25.79 minutes, and 1-docosene at 2.68%, eluting at 30.43 minutes. The presence of these compounds, along with others like 4-amino-1-pentanol, dodecamethyl-cyclohexasiloxane, alanyl-.beta.-alanine (Trimethylsilyl (TMS) derivatives), and 1,2,5-oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide, adds complexity to the chemical profile, enriching the understanding of the sample's composition.

Table 2 provides a comprehensive summary of the identified components, including their names, corresponding area percentages, retention times, and molecular weights. Figure 2 visually depicts the relative abundances of these phytoconstituents in the chromatogram, correlating each compound with its retention time. This graphical representation enhances the interpretability of the data, facilitating a quick and intuitive grasp of the overall chemical landscape.

Table 1 presents a comprehensive analysis of the *C. procera* extract utilizing various solvents and chromatography techniques,[15-19] revealing the presence of a diverse array of phytochemicals. Notably, in Table 2 which presents the phytochemicals obtained from the prepared sample, several compounds retain their parent names, while there have been alterations in their substituents and/or functional groups. Additionally, compounds such as 2-methyl-piperazine,

N-methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide, 1,2,5-oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide, 8-[N-aziridylethylamino]-2,6-dimethyloctene-2, and 3,3-dimethyl-4-(1-aminoethyl)-azetidin-2-one are likely derivatives originating from ampicillin.[24] Furthermore, the analysis unveils novel phytochemicals such as cystamine, [S-(R,R*)]-1,2,3,4-butanetetrol, cystine, dl-phenylephrine, metaraminol, N-methyl-1-benzeneethanamine, and 3-proproxyamphetamine. These compounds arise from synthetic reactions initiated by heat and concentrated sulfuric acid.[25] Additionally, complex organic compounds like 2,5-difluoro-beta,3,4-trihydroxy-N-methyl-benzeneethanamine, benzeneethanamine, 3,4-dibenzyloxy-2-fluoro-beta-hydroxy-N-methyl-benzeneethanamine, 2-fluoro-2',4,5-trihydroxy-N-methyl-benzenethanamine, 2-(2-aminopropoxy)-3-methyl-benzenemethanol, and 4-cyclohexene-1,2-dicarboxylic acid, 4-chloro-, bis(trimethylsilyl) ester possibly result from the reaction, while the remaining compounds undergo transformations in their substituents and/or functional groups, posing a significant challenge in determining their source either from the plant extract or ampicillin.

Table 3. Mean zone of inhibition, ZOI (mm) of resistant bacterial strains in response to prepared samples

Bacteria	Samples		
	C. Procera extract 5 mg/mL	Ampicillin 1 mg/mL	Prepared sample 100 µg/mL
Salmonella spp.	0 mm	5.30 mm	21.40 mm
Staphylococcus aureus	0 mm	0 mm	16.80 mm

In the antimicrobial screening (Table 3), two bacterial strains, *S. aureus* and *Salmonella* spp., display resistance to both *C. procera* extract at 5 mg/mL and Ampicillin at 1 mg/mL. This resistance is attributed to the inherent resistance of the bacterial isolates and the low concentration of the *C. procera* extract used.[2] Conversely, the prepared sample at 100 µg/mL successfully inhibits the growth of *S. aureus* and *Salmonella* spp., with inhibition zones (ZOI) of 16.8 mm and 21.4 mm, respectively. This observed increase in ZOI may be attributed to the synergistic effects of the new, transformed, and/or existing phytoconstituents present in *C. procera* and ampicillin. These compounds are known and reported to possess antimicrobial, antifungal, anti-inflammatory, antiviral, and anticancer activities, such as 4-Amino-1-pentanol, 2-(3-Propoxyphenyl)ethanamine, 1-Docosene, 3,3-dimethyl-4-(1-aminoethyl)-Azetidin-2-one, Cystine, 3,7,11-trimethyl-2,6,10-Dodecatrien-1-ol, Hexadecenoic acid, octadecanoic acid, methyl ester, as well as other compounds like N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide, 1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide, and 1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide (Table 1). These compounds potentially inhibit bacterial growth by targeting multiple pathways, enhancing uptake or accumulation, inhibiting resistance mechanisms, pharmacokinetic interactions, and suppressing biofilm formation. The mechanisms include targeting the bacterial cell wall, disrupting cell lysis, affecting the bacterial cell membrane, preventing bacterial replication and growth by inhibiting nucleic acid synthesis, and inducing oxidative stress through the generation of reactive oxygen species (ROS) or interference with antioxidant systems.[1-3,13,17-18] This outcome aligns with previous studies where the addition of sulfuric acid to a mixture of plant extract and antibiotic increased the zone of inhibition against resistant clinical isolates of *Streptococcus* spp. (High Vaginal Swab (HVS)), *Salmonella typhi* (stool), *E. coli* (urine), *Shigella* spp. (stool), and *S. aureus* (HVS).[1] Similarly, another study[2] reported increased inhibition zones for resistant *Salmonella* spp. following the addition of sodium hydroxide and sulfuric acid to the mixture of plant extract and aspirin. This is consistent with,[3] where Guava Aspirin Guava (GAG), at 0.1 mg/mL, inhibited the growth of *E. coli* and *Streptococcus* spp., with a zone of inhibition of 5.0 mm. Guava Guava (GG) extract reacting with concentrated sulfuric acid at 0.1 mg/mL inhibited the growth of *E. coli*, *S. aureus*, *Salmonella* spp., and *Streptococcus* spp. with inhibition zones of 12.0 mm, 7.0 mm, 9.0 mm, and 10.0 mm, respectively. These findings emphasize the potential synergistic antimicrobial effects achieved through strategic combinations of plant extracts and chemical additives.

3.2 *Pharmacological activities of some of the identified phytoconstituents in Table 2*

This phenomenon emphasizes the intricate interplay between transformed bioactive compounds and natural phytochemicals, resulting in an enhanced inhibitory effect on bacterial growth. One such compound, Cystamine (functionally related to cysteamine), serves as an inhibitor for EC 2.3.2.13 (protein-glutamine gamma-glutamyltransferase) and may also provide protection against liver damage.[26] This dual functionality of Cystamine underscores its potential therapeutic significance, not only as an enzyme inhibitor but also as a protective agent against specific organ damage, such as liver injury. 4-Amino-1-pentanol is reported to have anticancer activity and antiherpetic properties. It has demonstrated efficacy in inhibiting viral replication and preventing Herpes Simplex Virus (HSV) from entering cells.[27] Meanwhile, 2-(3-Propoxyphenyl) ethanamine, also known as 3-Propoxyamphetamine, recognized as a psychoactive drug. The diverse activities of these compounds, ranging from anticancer and antiviral properties to psychoactive effects, underscore their potential versatility in therapeutic applications. Nevertheless, they have been the subject of medical research due to their potential therapeutic effects.[28]

Another compound, 1-Docosene, known for its antibacterial, antifungal, and anti-inflammatory activities,[29] positions it as a promising candidate for further exploration in medicinal research and drug development. Oleic acid is identified as the most abundant phytoconstituent in the sample, and serves multiple roles. It acts as an EC 3.1.1.1 (carboxylesterase) inhibitor, an *Escherichia coli* metabolite, a plant metabolite, a Daphnia galeata metabolite, an antioxidant, and a mouse metabolite.[30-31] Additionally, dl-Phenylephrine is commonly employed for the temporary relief of symptoms such as stuffy nose, sinus congestion, and ear discomfort associated with flu, common cold, allergies, or respiratory illnesses like sinusitis and bronchitis.[32] The diverse functions of oleic acid, from inhibiting enzymes to its varied roles across different organisms, and the common application of dl-Phenylephrine in relieving respiratory symptoms, highlight the broad impact of these compounds in both biological and therapeutic contexts. Hexadecenoic acid, octadecanoic acid, methyl ester, as well as other compounds, such as N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide, 1,2,5-Oxadiazol-3-carboxamide, 4,4'-azobis-, 2,2'-dioxide, and 1,2,5-Oxadiazol-3-carboxamide, possess antioxidant, anti-inflammatory, and antimicrobial activities.[33] The latter group, being imidazole derivatives, has been reported to exhibit antibacterial, anticancer, anti-tubercular, antifungal, analgesic, and anti-HIV activities.[34] The collective antioxidant, anti-inflammatory, and antimicrobial properties of these compounds, especially the imidazole derivatives, highlight their potential as therapeutic agents with a broad spectrum of applications. Aramine (metaraminol), finds its indication in the prevention and treatment of acute hypotensive states resulting from spinal anesthesia. It is also used as an adjunctive treatment for hypotension caused by hemorrhage, reactions to medications, surgical complications, and shock associated with brain damage due to trauma or tumor.[35-36] The diverse applications of Aramine in treating various hypotensive conditions emphasize its significance as a versatile pharmaceutical agent with potential benefits in a range of medical scenarios. Another compound, 3,3-dimethyl-4-(1-aminoethyl)-Azetidin-2-one, has exhibited notable antimicrobial activity.[37] This finding underscores the potential of the mentioned compound as an effective antimicrobial agent, suggesting its exploration in further research for developing novel therapeutic interventions against microbial infections.

Cystine plays a crucial role in proper vitamin B6 utilization. It is beneficial in burns and wound healing, breaks down mucus deposits in conditions like bronchitis and cystic fibrosis, aids in insulin supply to the pancreas, and increases glutathione levels in the liver, lungs, kidneys, and bone marrow.[38] The multifaceted roles of cystine in various physiological processes, from wound healing to mucus breakdown and insulin regulation, highlight its importance in maintaining overall health and well-being.

Methamphetamine, chemically similar to amphetamine and represented here, is utilized in the treatment of attention-deficit hyperactivity disorder (ADHD) and narcolepsy, a sleep disorder.[39] The recognition of methamphetamine as a therapeutic agent for ADHD and narcolepsy underscores its pharmacological significance in addressing neurological disorders, emphasizing its role in improving cognitive function and managing sleep-related conditions. 3,7,11-trimethyl-2,6,10-Dodecatrien-1-ol, commonly known as Farnesol has been reported to exhibit anti-cancer and anti-inflammatory effects while also providing relief in conditions such as allergic asthma, gliosis, and edema.[40] The diverse therapeutic effects of Farnesol, ranging from anticancer and anti-inflammatory properties to its potential in alleviating conditions like allergic asthma and edema, highlight its versatility and potential in various medical applications.

4. Conclusion

This study presents a straightforward, fundamental, and environmentally friendly approach to enhancing the antimicrobial efficacy of two less potent agents through synergism, thereby inhibiting the growth of resistant clinical bacterial isolates. The prepared sample demonstrated increased susceptibility to resistant *S. aureus*, resulting in an expansion of the Zone of Inhibition (ZOI) from 0 mm to 1 mm. Additionally, the ZOI of resistant *Salmonella* spp. increased significantly from 5.1 mm to 21.4 mm. Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of 53 phytochemicals, with the five most abundant being oleic acid (13.04%), 1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane (9.50%), 9-Heptadecanone (3.75%), Cystamine (3.35%), and Tetrahydro-4H-pyran-4-ol (3.15%). Among these, 18 phytochemicals were identified with known biological activities. Some underwent molecular transformations, generating new molecules or analogues of existing biologically active compounds under the reaction conditions used. Three of the identified phytochemicals were reported to possess anticancer properties, namely Farnesol, 4-amino-1-pentanol, and an imidazole derivative resembling the drug Ribavir. The findings of this study underscore the potential of medicinal plant phytochemicals in synthetic combination reactions with themselves, other drugs, or reagents, yielding a diverse range of compounds with robust pharmacological activities. These compounds may serve as valuable starting materials, intermediates, or derivatives in pharmaceutical production processes.

The comprehensive phytochemical analysis of *C. procera* extract reveals a diverse range of compounds, some of which are novel and others derived from the antibiotic used in the extraction process. The antimicrobial screening results highlight the efficacy of the prepared sample against resistant bacterial strains, underscoring the potential of plant extract-antibiotic combinations as antimicrobial agents. Further research is warranted to elucidate the mechanisms behind these synergistic effects and to explore the therapeutic applications of the identified phytochemicals.

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Legal ethics

The study obtained ethical approval from the Ministry of Health in Kaduna State, Nigeria. Adherence to ethical guidelines was strictly observed, starting from the sampling of clinical isolates to the antimicrobial testing.

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Conflict of interest

The authors declare that they have no competing financial interests.

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