Research Article

Development of Repetitive Synergism in *Calitropis procera* Extract with Ampicillin for Combating Drug Resistance in Clinical Bacteria and Identification of its Bioactive Components using GS-MS Analysis.

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ABSTRACT

The pursuit of innovative approaches to develop lead compounds that exhibit selectivity, efficacy, and safety as potential candidates for clinical trials poses a significant scientific challenge. 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 consistent and repetitive 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 under heat and acidic conditions. The resultant sample exhibited pronounced 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.

Utilizing gas chromatography-mass spectrometry (GC-MS) analysis, the study identified 53 phytochemicals in the extract, with 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%) emerging as the top five most abundant phytochemicals. Notably, 18 out of the 53 phytochemicals were associated with known biological activities. Some underwent molecular transformations, generating new molecules or analogues of existing biologically active compounds under the reaction conditions applied.

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.

The study's findings underscore the potential of medicinal plant phytochemicals in synthetic combination reactions, either with themselves or other drugs/reagents, to yield a diverse array of compounds exhibiting significant pharmacological activities. 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.

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1. INTRODUCTION

The increasing global apprehension regarding the dwindling efficacy of antibiotics in the treatment of infections and diseases, largely attributed to bacterial resistance, underscores the imperative for the identification of novel approaches to develop lead compounds characterized by selectivity, efficacy, and safety for subsequent clinical trials [1]. As the pace of discovering new antibiotics falters, researchers confront the formidable challenge of devising innovative strategies to counteract drug resistance in bacteria. A promising avenue in this quest is the exploration of synergistic drug combinations, wherein two or more drugs collaborate to generate a more potent and targeted antimicrobial effect. This involves leveraging different mechanisms of action to concurrently target multiple pathways [2].

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

The process of drug design, discovery, and development is an intricate and demanding task, necessitating the employment of various synthetic reactions. These encompass 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 contributing unique insights into optimizing therapeutic outcomes.

Natural products emerge as crucial contributors to drug design and development, offering distinctive pharmacophores, chemotypes, and scaffolds that can be harnessed to formulate effective drugs against a spectrum of infections and diseases [8-10]. The rich reservoir of chemical diversity present in natural products serves as a valuable source for inspiration, providing a wide array 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 foundation for numerous pharmaceuticals. These compounds possess an intrinsic capacity to undergo diverse chemical reactions, giving rise to 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, leading to 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,

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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 been traditionally administered orally for the treatment of Malaria and intermittent fever, while decoctions have been employed 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 exhibited effectiveness against various bacteria, including *Staphylococcus aureus, Klebsiella pneumonia,* 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, Methyl9,12-hepatadecadienoate, Tetradecanoic acid, (2,3-Diphenylcyclopropyl) methylphenyl sulfoxide, hexadecanoic acid, methyl ester, n-hexadecanoic acid, dodecanoic acid, linolenic acid, ethyl ester, among others [15][16][17][18][19][20][21][22]. The detailed GC-MS analysis provides a 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.

In this study, our primary objective is to devise a method that establishes consistent and repetitive synergism between medicinal plant extracts and ineffective antibiotics, aiming to counteract drug resistance in clinical bacteria. We intend to leverage combinatorial chemistry techniques to create a mixture exhibiting enhanced antimicrobial properties. Additionally, we plan to conduct antimicrobial screening to assess the effectiveness of the developed combination and employ GC-MS analysis to gain insights into the transformations occurring within the mixture. 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, Hematology, and Microbiology diagnostic laboratory of Oxford Hospital Makera,

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Kakuri, Kaduna State, Nigeria.

2.2 Experimental

The methodology outlined by [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, filtered using Whatman 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, followed by centrifugation, and then preserved for further analysis.

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, proven 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. The 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 phytochemicals present in the prepared sample, an Agilent 19091S-433UI Gas Chromatography-Mass Spectrometer (GC-MS) machine was utilized. The specifications included HP-5ms Ultra Inert, temperature range 0 °C—325 °C (350 °C), dimensions 30 m x 250 µm x 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.

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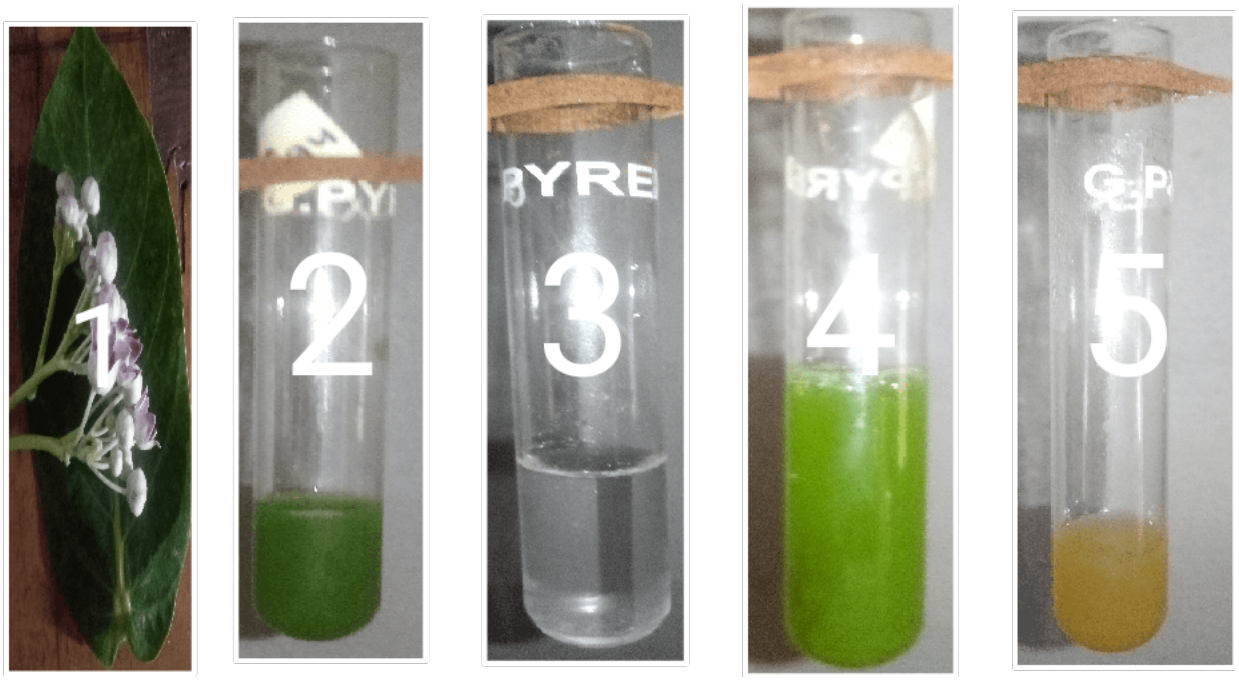

Figure 1. Sampling and stages in the preparation of the sample. 1: *C. procera* leaf and flowers, 2: *C. procera* extract, 3: Ampicillin solution, 4: Mixture before 5: Mixture after acid addition and heating.

3.0 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 labelled 5 in Figure 1. The chromatogram identified 53 phytochemicals as constituents (Table 1 and Figure 2).

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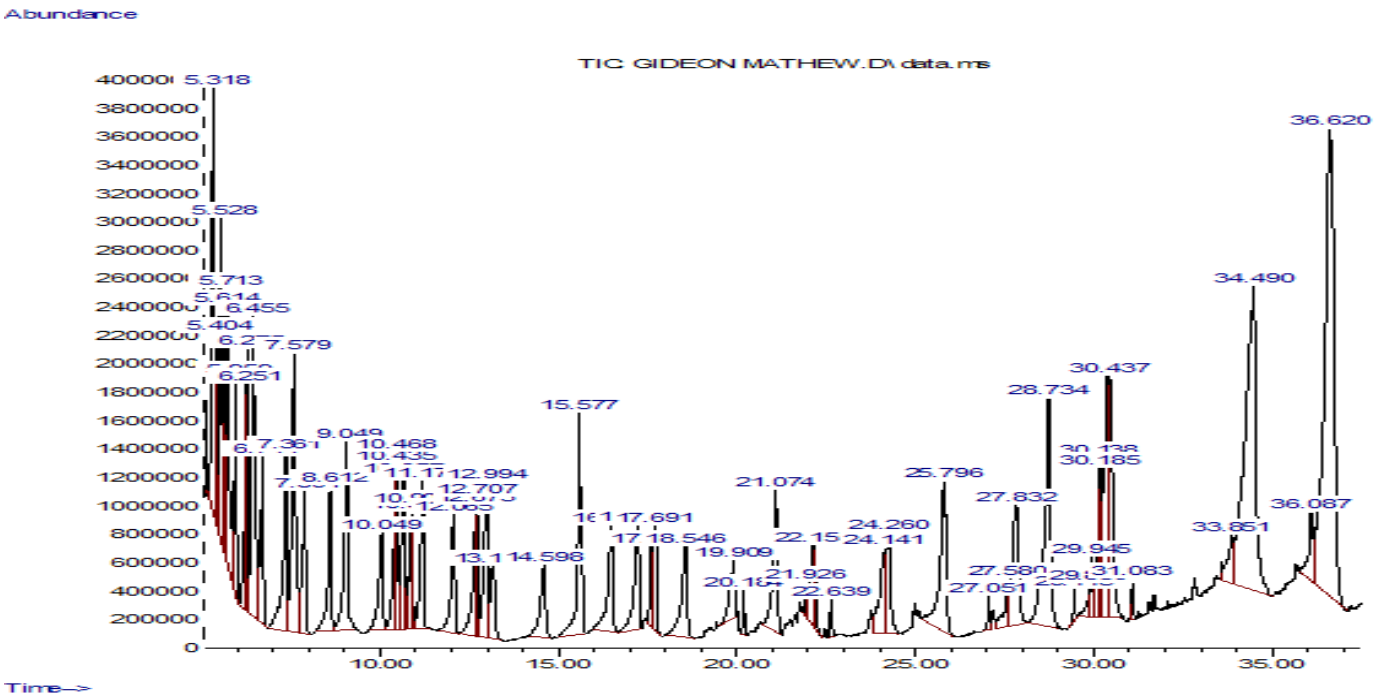

Figure 2. Chromatogram of the phytochemicals present in the prepared sample

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 closely is 1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane, contributing 9.50% and appearing at a retention time of 34.49 minutes, suggesting a significant presence during the analysis.

9-Heptadecanone emerges as another noteworthy component, comprising 3.72% and eluting at 28.73 minutes, while Cystamine is identified at 3.35%, with a corresponding retention time of 5.31 minutes. Tetrahydro-4H-pyran-4-ol follows closely, contributing 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. This detailed breakdown provides a comprehensive understanding of the diverse components present in the analyzed sample.

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 (TMS derivative), 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.

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Table 1 provides a comprehensive summary of the identified components, including their names, corresponding area percentages, retention times, and molecular weights. This tabulated information serves as a valuable reference for researchers and analysts seeking detailed insights into the composition of the sample. Additionally, Figure 2 visually depicts the relative abundances of these phytochemicals 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 1.0. 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

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28	17.691	0.98	N-(2-amin oethyl)- 1-hexadecanesulfonamide,	349.0
29	8.546	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

In Table 1, the comprehensive analysis of the C. procera extract using various solvents [15-19] reveals the presence of a diverse array of phytochemicals. Noteworthy constituents include Oleic Acid, 1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane, 9-Octadecenoic acid (Z)-methyl ester, 1-Docosene, Cycloeicosane, 1-Tridecene, 9-Eicosene, Pentadecane, (E)-, Dibutyl phthalate, Hexadecane, 13-Tetradecen-1-ol acetate, 10-Methyl-E-11-tridece-1-ol acetate, Z-8-Hexadecene, Alanyl-.beta.-alanine, TMS derivative, 9-Heptadecanone, Hexadecanoic acid, methyl ester, N,N-Dimethyl-dimethylphosphoric amide, dodecamethyl-Cyclohexasiloxane, and 2-methyl-Octadecane. Several compounds maintain their parent names while undergoing changes in 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 the antibiotic used [24]. Furthermore, the analysis unveils novel phytochemicals such as Cystamine, [S-(R*,R*)]-1,2,3,4-Butanetetrol, Cystine, dl-Phenylephrine, Metaraminol, N-methyl-1Benzeneethanamine, 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-

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methyl-Benzenemethanol, and 4-Cyclohexene-1,2-dicarboxylic acid, 4-chloro-, bis(trimethylsilyl) ester result from the reaction.

The remaining compounds undergo transformations in substituents and functional groups, posing challenges in determining their source, whether from the plant extract or antibiotic. Moving on to the antimicrobial screening (Table 2), two bacterial strains, *S. aureus* and *Salmonella spp,* exhibit 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 plant 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 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. (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.

Table 2. Mean zone of inhibition, ZOI (mm) of resistant bacterial strains in response to prepared samples

	Samples		
Bacteria	***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

The observed inhibition of growth in resistant bacteria can be attributed to the presence of newly transformed bioactive compounds within the mixture. These compounds synergistically interact with some of the phytochemicals initially present in the *C. procera* extract, enhancing the overall antimicrobial activities of the mixture. This phenomenon highlights the intricate interplay between the transformed bioactive compounds and the natural phytochemicals, resulting in a heightened inhibitory effect on bacterial growth.

One such compound, Cystamine (functionally related to cysteamine) as illustrated in Figure 3, serves as an inhibitor for EC 2.3.2.13 (protein-glutamine gamma-glutamyltransferase) and may additionally 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.

Figure 4 represents 4-Amino-1-pentanol, known for its reported anticancer activity and antiherpetic properties. It has demonstrated efficacy in inhibiting viral replication and preventing HSV from entering cells [27]. Meanwhile, Figure 5

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showcases 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, it has been the subject of medical research due to its potential therapeutic effects [28]. Another compound, 1-Docosene, recognized for its antibacterial, antifungal, and anti-inflammatory activities [29]. This compound's multifaceted nature, encompassing antibacterial, antifungal, and anti-inflammatory properties, positions it as a promising candidate for further exploration in the realm of medicinal research and drug development.

Oleic acid, represented in Figure 6 and identified as the most abundant phytoconstituent in the sample, 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 (Figure 7) 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, 4,4'-azobis-, 2,2'-dioxide, possess antioxidant, anti-inflammatory, and antimicrobial activities [33]. The latter group, being imidazole derivatives, has been reported to exhibit antibacterial, anticancer, antitubercular, 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), depicted in Figure 8, finds its indication in the prevention and treatment of acute hypotensive states resulting from spinal anesthesia. It is also used as 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, shown in Figure 9, 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.

Figure 10 illustrates 3,7,11-trimethyl- 2,6,10-Dodecatrien-1-ol, commonly known as Farnesol. This compound has been

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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.

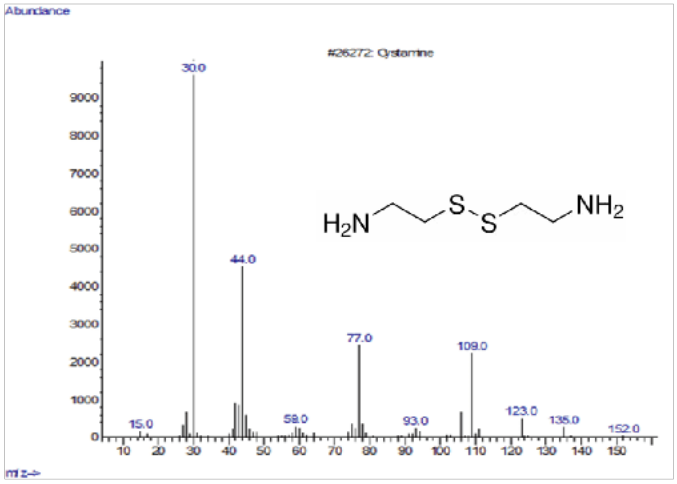

Figure 3. Spectrum and structure of Cystamine.

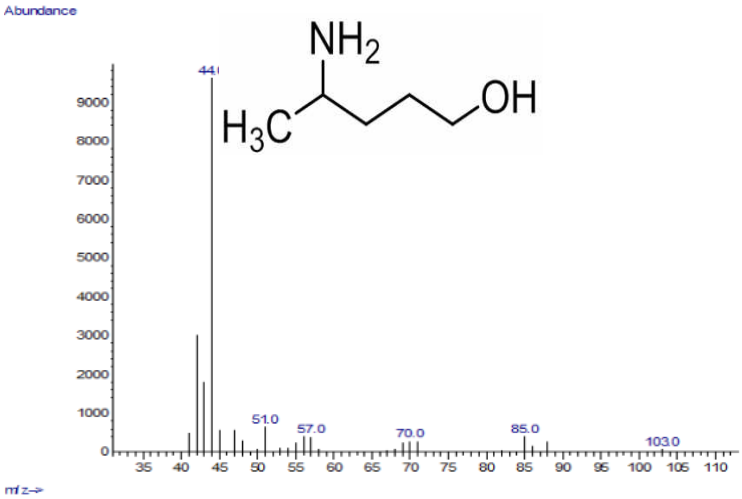

Figure 4. Spectrum and structure of 4-amino-1-pentanol.

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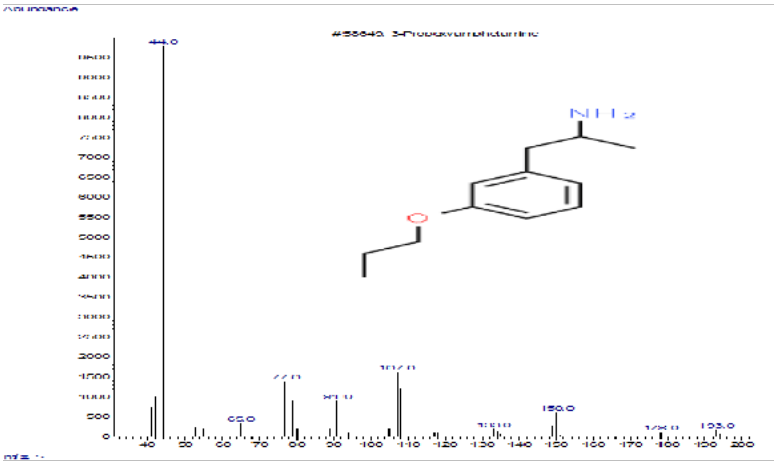

Figure 5. Spectrum and structure of 3-Propoxyamphetamine.

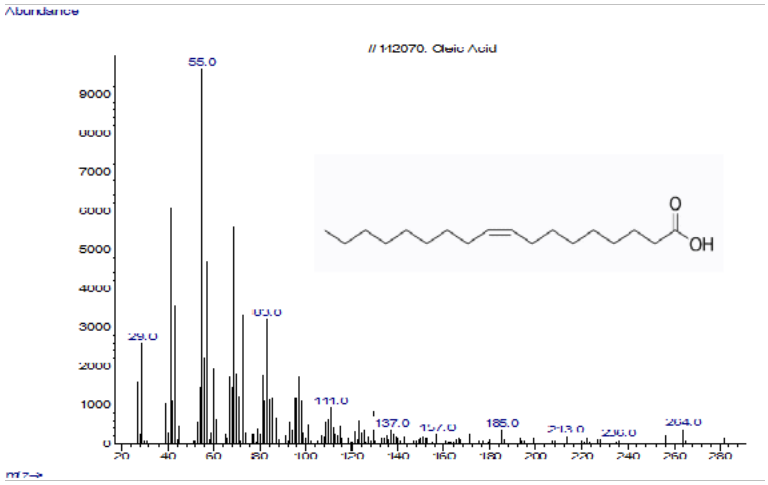

Figure 6. Spectrum and structure of Oleic acid.

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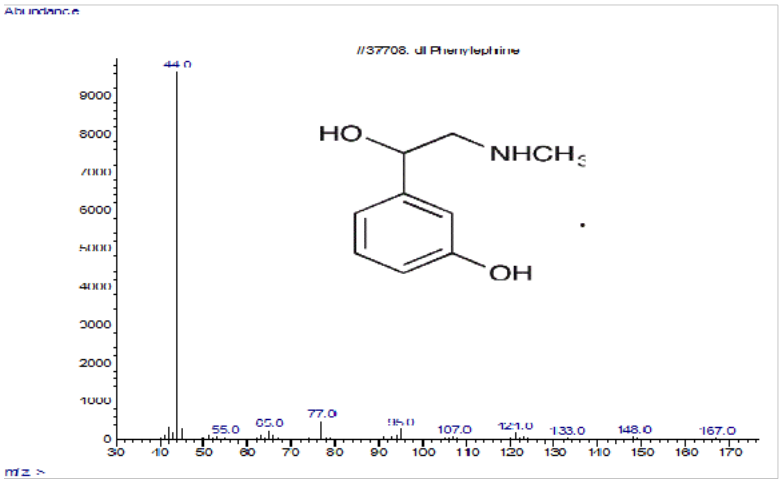

Figure 7. Spectrum and structure of dl-phenylephrine.

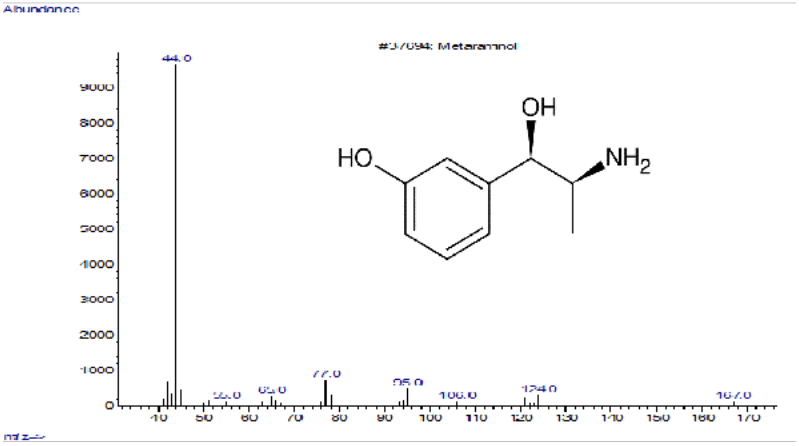

Figure 8. Spectrum and structure of Metaraminol.

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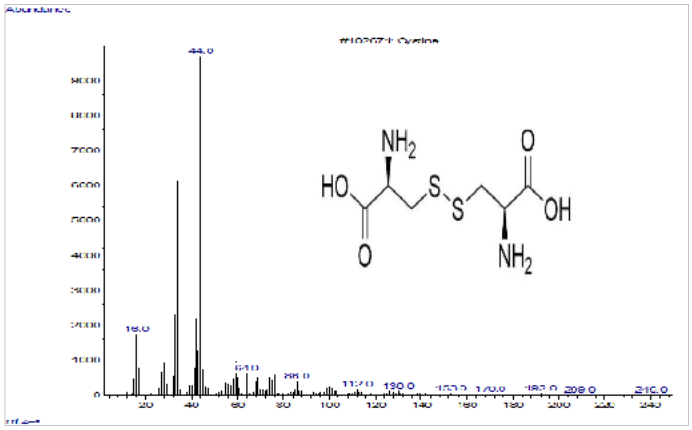

Figure 9. Spectrum and structure of Cystine.

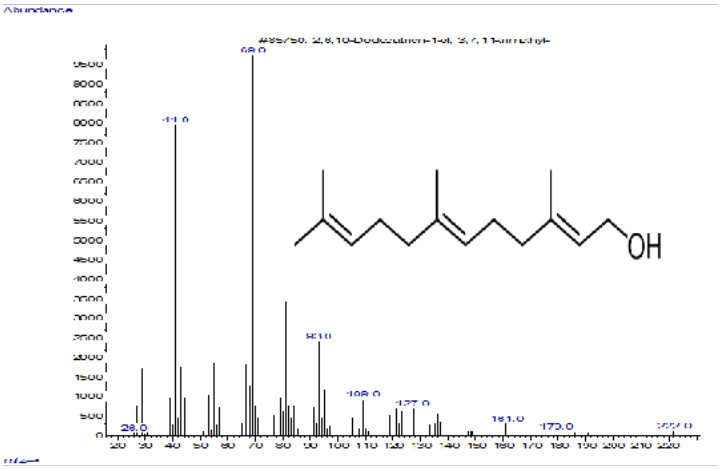

Figure 10. Spectrum and structure of Farnesol.

CONCLUSION

This study introduces a straightforward, fundamental, and environmentally friendly approach to enhance the antimicrobial efficacy of two less potent agents through synergism, thereby inhibiting the growth of resistant clinical bacterial isolates. The prepared sample exhibited heightened susceptibility to resistant *S. aureus*, expanding 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 unveiled 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-

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(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 explore the therapeutic applications of the identified phytochemicals.

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Conflict of Interests:

The authors declare that they have no competing financial interests.

Author Contributions:

Gideon Mathew contributed to the research design, development, and experimentation.

Zakari Ladan served as the major supervisor, while Yahaya Yakubu acted as the minor supervisor.

Ethics Approval and Consent to Participate:

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.

Consent for Publication:

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Not applicable.

Availability of Data:

Data availability is not applicable.

Further Disclosure:

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