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Evaluation of Antimicrobial Effects of Chromolaena Odorata Extracts on Microbial Isolates

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Research Article

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Abstract

As science and technology advanced, antimicrobial agents were discovered, bringing with them the burden of antimicrobial resistance. This led to the hunt for effective techniques to combat microbial diseases. Chromolaena odorata is often used for traditional wound healing since it helps to stop bleeding quickly. Chromolaena odorata of the Asteraceae family is one of the world's most invasive weeds. It is also known as Siam weed, devil weed, French weed, communist weed, hagonoy, co chon triffid weed, and Awolowo, Akintola, and Obiarakara weed. Chromolaena odorata is often used for traditional wound healing since it helps to stop bleeding quickly. Several studies have shown that Siam weed extract promotes hemostasis and wound healing. The purpose of this research is to evaluate the bioactive components and antibacterial efficacy of different extracts of Chromolaena odorata on various microbiological isolates. Fresh leaves were collected, identified, air-dried, and ground. Cold maceration and soxhlet extraction were carried out using ethanol and n-hexane as extractive solvents, respectively. The chemical composition was identified using the gas chromatography-mass spectrometry technique, and the antibacterial action was tested using established protocols. The antimicrobial test revealed that the aqueous extract had a bacteriostatic effect, but the ethanol extract had a bactericidal effect against microbiological isolates at a concentration of 200mg/mL. The most prevalent bioactive chemicals discovered in both extracts are oleic acid, octadecanoic acid, and hexadecanoic acid. The extracts possessed antibacterial capabilities and included certain bioactive components, which might contribute to their overall efficacy, according to this research.

Keywords: Chromolaena Odorata; Soxhlet Extraction; Cold Maceration; Antibacterial and Gas Chromatography-Mass Spectrometry

Abbreviations: DMSO: Di Methyl Sulf Oxide; GCMS: Chromatography Mass Spectroscopy; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; BBB: Blood Brain Barrier; CRP: C- Reactive Protein; ALA: Alpha-Linolenic Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid.

Introduction

Because of the persistent issue of antimicrobial resistance, which is caused in part by patients' indiscriminate use of antimicrobial drugs, there has been an urgent quest for novel antimicrobial agents. As is customary, the majority

of medications created for various medicinal reasons are derived from the kingdom Plantae; it is no surprise that most current research in developing nations such as Nigeria is conducted utilizing various plants. The most common reasons for using traditional medicine are that it is less expensive, more closely corresponds to the patient's ideology, alleviates concerns about the adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and provides greater public access to health information. Herbal medications are mostly used for health promotion and treatment of chronic, rather than life-threatening, illnesses.

Herbs are undeniably the cornerstone of conventional medications or contemporary medicine. Traditional medicine practitioners, for example, have traditionally utilized aspirin as a pain reliever [1]. Many plant compounds that have been synthesized are beneficial to human and animal health. Aromatic compounds (mainly phenols or tannins) and various secondary metabolites, of which 12,000 have been identified and documented, are among the derived chemicals [2]. In many situations, these chemicals (especially alkaloids) act as plant defense mechanisms against microbes, insects, and herbivores [2]. Despite this, several of these herbs and spices are used for food flavoring and have been proven to have helpful therapeutic components [3].

C. odorata is a weedy plant native to Central and South America that has spread to tropical and subtropical climates. The common names for C.odorata includes Sekou toure, acheampong, jabinde, matapa, Mighebe (African); herbe de laos (french), siam kraut (German), kesengesil (Guam), bagh doka, tivra gandhi (Hindi), rumput belalang, rumput putih, rumput golkar, (Indonesia), pokok kapal terbang[4,5]. Anticancer, Anti diabetic, anti hepatotoxic, anti Inflammatory, antibacterial and antioxidant activities has been shown for C.odorata. Alkaloids, flavonoids, flavanones, essential oils, phenolics, saponins, tannins, and terpenoids are among its phytochemical constituents. Eupolin, chromomoric acid, quercetagetin, and quercetin are all key elements of this plant, and they all contribute to its restorative effects [6]. The purpose of this research is to assess the bioactive components and antibacterial activity of various extracts of Chromolaena odorata on microbial isolates [7].

Method and Materials

Plant Identification and Collection

The foliage of C. odorata was taken from the botanical garden of Madonna University Rivers State's Faculty of Pharmacy. The plant material was authenticated at the medical plant's herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (code: FPI) by Mr. Ogunlowo. The Voucher number: is FPI 2473

Specimen Processing and Extraction

C. odorata leaves were gathered gabbled and air dried for 7 days in a shaded, dust-free environment. After that, the dried leaves were gathered and crushed using a mechanical grinding mill.

Extraction of Ethanol

For three days, 180 g of the powder was macerated in 750 ml of ethanol with occasional agitation. The material was filtered using filter paper after three days. The marc was removed, and the filtrate was put into a beaker and placed in a water bath to concentrate, yielding the dry extract. The extracts were carefully transferred to airtight containers, appropriately labeled, and stored in a safe area.

Extraction via Soxhlet

C. odorata leaves were also extracted using N-hexane as a solvent and the Soxhlet extraction process. A Soxhlet device was used for this. A clamp connected to a retort stand was used to hold the round bottom flask in place while 750 mL of N-hexane was added. The thimble was filled with 180 g of dried powdered leaf sample, which was then attached to the soxhlet extractor. A cotton plug was used to lag the sidearm. The solvent in the flask was then heated to evaporation using a heating mantle. When the solvent level reaches the siphon, it returns to the flask, and the cycle starts again. The extraction was left running for sixteen hours. The dried extract was collected, weighed, and documented after extraction.

Extraction from Aqueous

180 g dried and pulverized leaves of C. odorata were precisely weighed and placed in a 1000ml conical flask. It was agitated by shaking and swirling the flask with 750 ml of newly produced distilled water. After that, the flask was wrapped with aluminum foil paper. It was left to stand for three days while being agitated intermittently. The foil was removed after three days, and the solution was filtered using filter paper. The marc was removed, and the filtrate was concentrated over a water bath to get a dry extract. The weight was calculated and recorded.

Dilution of the Extracts in Steps

The table and test tubes were all sterilized with ethanol. Test tubes were labeled A-F and put on a test tube rack, each holding 5mls of Dimethylsulfoxide (DMSO) solvent. The various extracts were diluted using a two-fold serial dilution method.

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2 g of the aqueous and ethanol extracts were weighed separately using an electronic weighing balance and dissolved in 10 mL of Dimethylsulfoxide (DMSO) in separate beakers with proper agitation to form the stock solution with a concentration of 200mg/ml and transferred to test tubes A1 and A2 (A1 for the aqueous extract and A2 for the ethanol extract) and the concentration labeled on the test tube. Using a sterile micropipette, 5 mL of the aqueous extract stock solution was measured and placed into the test tube labeled B, which contained 5 mL of DMSO. The solution was gently agitated to obtain a 100 mg/ml concentration of the extract. Five milliliters of this solution were removed and transferred to test tube C, yielding a solution with a concentration of 50 mg/ml. 5 ml of this solution was taken from test tube C and transferred to test tube D, resulting in a solution with a concentration of 25 mg/ml. 5 ml of this solution was removed and transferred to test tube E, yielding a 12.5 mg/ml solution. A concentration of 6.25 mg/ml was obtained by measuring 5 ml of this solution and transferring it to test tube F. The test tubes were adequately kept by covering them with a sterile cotton stopper. The process was repeated using the ethanol extract.

Test Isolate Confirmation

The isolates were subcultured from conserved agar slants onto a Selective medium for cultivating bacteria and Saboraud Dextrose agar (all-purpose media for fungi). Biochemical assays were also employed to confirm the laboratory isolates biochemical assays included catalase, coagulase, indole, oxidase, gram staining, and lactophenol cotton blue staining (for the fungi).

Inoculum Standardisation

Using a 600nm wavelength spectrophotometer, the bacterial suspension's turbidity was adjusted to match that of the 0.5 McFarland standards (0.08-0.1 OD) which corresponds to 1.5 x 108 cfu/ml.

Antimicrobial Sensitivity Analysis

The agar well diffusion technique was used to determine antimicrobial sensitivity. Following conventional methods, sixteen plates of Mueller Hilton agar were newly prepared for the bacteria isolates and four freshly prepared Mueller Hilton's agar plates (supplemented with 2% glucose and 0.5 g/ml of methylene blue to enhance fungal growth) were prepared for C.albicans. For the aqueous and ethanol extracts, the Mueller Hilton agar plates were split into two groups of eight. Using a sterile cork borer with diameters of 7 mm and 8 mm (for the aqueous and ethanol extracts, respectively), 6 holes were drilled in each Petri dish at equal distances around the plate. Using a micropipette, 50µl of each previously made dilute solution with varied concentrations was measured, placed into each hole, and labeled according to the concentration and kind of extract. The plates were incubated for 24 hours at 370C. Following incubation, the zone of inhibition was determined using a divider and a meter rule.

The same procedures were used for the antibacterial test, with Ciprofloxacin as the positive control Distilled water as the negative control, and fluconazole as the positive antifungal control and DMSO as the negative control. Results were obtained in duplicates and presented as the mean of both measurements [8,9].

Minimum Inhibitory Concentration

The MIC is defined as the minimum concentration of a substance capable of suppressing an organism's growth following a 24-hour incubation period. The broth dilution method was used for this test. A total of six agar plates were utilized. Mueller Hinton agar was newly prepared and autoclaved for sterilization. After that, 19mL of freshly made Mueller Hinton agar was put into six bijou bottles, twelve Mueller Hilton's agar plates supplemented with 2% glucose, and 0.5 g/ml of methylene blue plates for the fungus. Each bottle received one milliliter of each concentration of the previously prepared aqueous extract, for a total of 20 ml of nutritional agar. The agar was then carefully put into six Petri dishes and labeled according to the solution concentration. After that, each Petri dish was separated into four quadrants. A sterile wire loop was used to inoculate each quadrant with one of the four bacterial isolates, which were labeled according to the microorganism streaking on the quadrant. The fungi isolates were streaked on the Mueller Hinton-supplemented agar plates containing the extracts. The method was repeated with varying concentrations of the previously made ethanol extract. At 370C, all agar plates were incubated. The agar plates were inspected for growth and documented after 24 hours [9].

Minimum Bactericidal/Fungicicidal Concentration

The agar plates from the MIC were incubated for a further twenty-four hours. The MBC was determined after 24 hours of incubating the inoculated isolates used for the MIC with the lowest dosage of extracts that showed no growth [9].

Analysis of Gas Chromatography-Mass Spectroscopy (GC-MS)

The extracts were analyzed using the GC-MS to identify some of the bioactive compounds present. The GC-MS analysis was performed under the following circumstances

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utilizing a Shimadzu GC-MS-QP 2010 Plus system and a gas chromatograph interfaced with a mass spectrometer system. Elite - 1 fused silica capillary column (30 m x 0.25 mm 1 D x L, 100% dimethyl polysiloxane). A 70 eV ionization energy electron ionization device was employed. The carrier gas was 99.99% helium gas, with a flow rate of 1 mL/min and an injection volume of 2 L. The injection temperature was set to 280oC, while the ion source temperature was set to 280oC. The oven temperature was set at 110oC. The proportional percentage quantity of each component was compared to data from the National Institute of Standards and Technology (NIST) library [9].

Data Examination

The experiments were repeated in duplicate, and the data were provided as the mean of the duplicates, as well as the standard error of the mean. Using the GraphPad Prism 5.01 program, the one-way ANOVA statistics were utilized to assess the significant difference of the variables with p=0.05.

Results

Percentage Yield of Extraction

It can be observed from Table 1 below that water had the highest extractive value than the other solvents used.

Solvent	Weight of Dried Leaves Used (G)	Volume of solvent used (mL)	Weight of Dried Extract (G)
N-Hexane	180	750	17.4
Ethanol	180	750	15.2
Aqueous	180	750	19.5

Table 1: Extraction yield of C. odorata leaves.

Confirmation of the Microbial Isolates

As can be observed in Table 2 below, E. coli and P. aeruginosa were both gram-negative bacteria but S. aureus and Bacillus sp were both gram-positive bacteria. Only S. aureus tested positive for the coagulase test. All gram-positive bacteria tested negative for the Oxidase test. Candida albicans tested positive for the LPCB test.

Microbial Isolate	Cultural Characteristics	Catalase	Coagulase	Indole	Oxidase	Gram Staining	Lactophenol/ Cotton Blue Staining	Confirmation
Escherichia coli	On Eosin methylene blue agar, colonies observed with a greenish metallic sheen in reflected light; dark or even black center in transmitted light	+ ve	- ve	+ ve	- ve	- ve rod	ND	Affirmed
Pseudomonas aeruginosa	On cetrimide agar, colonies are medium-sized and show the production of pigment in the medium confers a greenish blue color on the medium	+ ve	- ve	- ve	+ ve	- ve rod	ND	Affirmed

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Staphylococcus aureus	when cultured on mannitol salt agar, colonies have smooth, cocci and shiny surfaces; having an opaque appearance and often pigmented yellow	+ ve	+ ve	- ve	- ve	+ ve cocci	ND	Affirmed
Bacillus spp	On nutrient agar, colonies appear rough, opaque, fuzzy, white, or slightly yellow with jagged edges	+ ve	- ve	- ve	- ve	+ ve rod	ND	Affirmed
Candida albicans	On sabouraud dextrose agar, C. albican gives off a white-colored, smooth, and yeast-like appearance	- ve	- ve	- ve	- ve	ND	Circular bluish colonies appearing in clusters	Affirmed

Table 2: Microbial Identification of the Isolates.

Antimicrobial Activities of the Extracts on the Microbial Isolates Mean Zones of Inhibition of the Extracts on the Microbial Isolates

It can be observed from Table 3 below that the concentration of ethanol extract both had the highest antimicrobial activity at a dose of 200 mg/mL and the least activity at a dose of 6.25 mg/mL. S. aureus and Bacillus spp showed the highest sensitivity to the ethanol extract at all concentrations when compared to other microbes (S. aurous at 200mg/mL = 20.34mm; Bacillus spp = 20.5mm). The positive control Ciprofloxacin had a higher zone of inhibition when compared to the highest strength of the ethanol extract of C. odorata when tested against all clinical isolates (MZI of Ciprofloxacin for S. aureus = 26.5mm). Fluconazole, the positive control for the antifungal activity showed a higher zone of inhibition when compared to the extracts at varying concentrations of the ethanol extract (MZI of fluconazole= 22.5mm).

Table 4 below shows the antimicrobial activity of the aqueous extract of C. odorata against different clinical isolates. Similar to Table 3, it can be observed that the highest antimicrobial activity measured by the average zone of inhibition caused by the extract was recorded at a dose of 200 mg/ml of the aqueous extract. Also, there was a significant difference in the antimicrobial activity of the extract at all concentrations when compared to the positive control; Ciprofloxacin and Fluconazole. There was no antimicrobial activity recorded on exposure to DMSO as the negative control. The statistics showed no significant difference (P-value=0.3307 and 0.9109 for ethanol and aqueous extracts respectively) in the analysis when compared at the same concentration of the extract among the different isolates.

Isolates	Concentration (x ± SEM) (mm)								
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Ciprofloxacin (5µg)	Fluconazole (10µg)	Water
E.coli	7.5±6.5	6.2±5.5	5.4±5	5.0±1.5	4.2±1	2.1±3.5	17.5±1.5	ND	0
S. aureus	20.3±0.5	18.5±2	16.3±1	12.7±2.5	7.5±0	2±0	26.5±1.5	ND	0
P.aeruginosa	14±3	11.5±1	7.9±1.5	7.1±2.0	3.6±2.5	1.5±1	15±2.5	ND	0
Bacillus sp	20.5±1.5	19.5±1.5	13.5±2	8.5±1.5	6.2±0.5	4.5±0.5	18±3.6	ND	0
C.albicans	17±0	14.6±2	8.5±1.5	7.5±0.5	5.3±1	0.8±1	ND	22±5.6	0

Table 3: Antimicrobial Evaluation of Ethanol Extract of C. Odorata On the Microbial Isolates.
Key: SEM- Standard error of the mean; ND: not determined
The values are expressed as mean ± SEM.

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Isolates	Concentration (x ± SEM) (mm)								
	200 mg/ml	100 mg/ml	50 mg/ ml	25 mg/ ml	12.5 mg/ ml	6.25 mg/ ml	Ciprofloxacin (5µg)	Fluconazole (10µg)	Water
E.coli	10.5±2	6.9±3.5	6.0±3	5.4±2	3.0±3	0±0	17.5±1.5	ND	0
S.aureus	8.4±1	6.2±1	4.6±1.2	3.0±1.4	2.4±0.5	1.4±0	26.5±1.5	ND	0
P.aeruginosa	6.4±0	5.8±0	5.2±1.3	3.2±0.5	2.0±0.3	0±0	15±2.5	ND	0
Bacillus sp	7.4±2	6.8±1	4.2±0	2.2±0	0±0	0±0	18±3.6	ND	0
C.albicans	9.8±2	7.6±1	5.8±1	3.8±1	2.5±1.5	1.0±1	NA	22±5.6	0

Table 4: Antimicrobial evaluation of aqueous extract of C. odorata on different microbial isolates
Key: SEM- Standard error of the mean; ND: not determined
The values are expressed as mean ± SEM.

The Type of Activity of The Extracts on the Microbial Isolates

It can be observed from Table 5 below that the ethanol extracts at concentrations of 200mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml inhibited the growth of the different bacterial and fungal isolates. More so, it can be observed that the extract at the dose of 12.5 and 6.25 mg/ml showed complete resistance after incubation for 24 hours.

Similarly, in Table 6, microbial growth was observed in plates containing aqueous extract at the dose of 12.5 and 6.25 mg/ml. No microbial growth was seen in the plates containing aqueous extracts at the doses of 200, 100, 50, and 25 mg/ml at 24 hours.

Isolates	200mg/ml (Stock solution)	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
E.coli	-	-	-	-	+	+
P.aeruginosa	-	-	-	-	+	+
Bacillus sp	-	-	-	-	+	+
S.aureus	-	-	-	-	+	+
C.albicans	-	-	-	-	+	+

Table 5: Minimum Inhibitory Concentration of Ethanol Extract of C. odorata against Microbial isolates.
Key: - = no growth; + = Growth

Isolates	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
E.coli	-	-	-	-	+	+
P.aeruginosa	-	-	-	-	+	+
Bacillus sp	-	-	-	-	+	+
S.aureus	-	-	-	-	+	+
C.albicans	-	-	-	-	+	+

Table 6: Minimum Inhibitory Concentration of Aqueous Extract of C. Odorata against Microbial Isolates.

Table 7 below shows the results of the effect of aqueous extract on the microbial isolates when incubated for 48 hours. It can be observed that there was complete microbial growth in all plates across the different concentrations. This indicates that the aqueous extract of C. odorata is bacteriostatic by action.

Table 8 above shows the antimicrobial activity of the ethanol extract of C. odorata at different concentrations on the evaluated microbial isolates for 48 hours. It can be observed that there was microbial growth in the plates containing the extract at concentrations of 6.25, 12.5, 25, and 50 mg/ml. Also, there was bacterial growth at the dose of 100 mg/ml in plates inoculated with E. coli, P. aeruginosa, and S. aureus. There was no microbial growth observed on the plates inoculated with Bacillus spp and C. albicans at the dose of 100 mg/ml. More so, it can be observed that at the dose of 200 mg/ml, there was no microbial growth seen after incubation for 48 hours across all microbial isolates evaluated.

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Isolates	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
E.coli	+	+	+	+	+	+
P. aeruginosa	+	+	+	+	+	+
Bacillus sp	+	+	+	+	+	+
S .aureus	+	+	+	+	+	+
C. albicans	+	+	+	+	+	+

Table 7: Minimum Bactericidal/Fungicidal Concentration of Aqueous Extract of C. Odorata against Microbial.
Key - = No growth
 + = Growth

Isolates	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
E.coli	_	+	+	+	+	+
P.aeruginosa	_	+	+	+	+	+
Bacillus sp	_	_	+	+	+	+
S.aureus	_	+	+	+	+	+
C.albicans	_	_	+	+	+	+

Table 8: Minimum Bactericidal/ Fungicidal Concentration of Ethanol Extract of C. odorata against clinical.
Key - = No growth
 + = Growth

Bioactive Compounds

The GC-MS test carried out to identify the chemicals contained in the ethanol and N-Hexane extracts of C. odorata yielded a vast array of chemical components with some sharing the same molecular weight and molecular formula. As can be observed below in Table 9 and Table 10, out of the many components identified from the n-hexane extract, four chemicals stood out with a very high percentage abundance. These include n-hexadecanoic acid, Octadecanoic acid, and Oleic acid. Similar components were found in abundance in the ethanol extract especially the Octadecanoic acid and the n-hexadecanoic acid.

Peak No.	Percentage Abundance (%)	Compound	Molecular Formula	Molecular Weight (g/mol)
1	0.17	Heptadecane	$C_{17}H_{36}$	240.5
2	0.24	Octadecane	$C_{18}H_{38}$	254.5
3	0.26	Nonadecane,	$C_{19}H_{40}$	268.5
4	9.57	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	20.81	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
6	3.79	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
7	0.33	Heneicosane,	$C_{21}H_{44}$	296.6
8	14.83	Oleic acid	$C_{18}H34O_2$	282.5
9	2.93	9-octadecenoic acid (E)-	$C_{18}H_{34}O_2$	282.5
10	2.14	9-octadecenoic acid (E)-,	$C_{18}H_{34}O_2$	282.5
11	1.07	Oleic acid,	$C_{18}H_{34}O_2$	282.5
12	2.669	Oleic acid,	$C_{18}H_{34}O_2$	282.5
13	3	Oleic acid,	$C_{18}H_{34}O_2$	282.5
14	19.61	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5
15	4.93	Octadecanoic acid,	$C_{18}H_{36}O_2$	284.5
16	13.73	Octadecanoic acid,	$C_{18}H_{36}O_2$	284.5

Table 9: Detected Compounds of N-Hexane Extract Using GC-MS.

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Peak No.	Percentage Abundance (%)	Compound	Molecular Formula	Molecular Weight (g/mol)
1	1.81	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
2	6.04	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
3	15.67	n-Hexadecanoic acid	$C_{16}H_32O_2$	256.42
4	3.91	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	1.7	Phytol	$C_{20}H_{40}O$	296.5
6	0.82	9,12-Octadecanoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.4
7	11.9	9,12-Octadecanoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.4
8	8.59	9,12-Octadecanoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.4
9	7.45	9,12-Octadecanoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.4
10	7.64	9,12,15-Octadecatrienoic (Z,Z,Z),	$C_{18}H_{30}O_2$	278.4
11	7.42	9,12,15-Octadecatrienoic (Z,Z,Z)	$C_{18}H_{30}O_2$	278.4
12	16.1	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5
13	2.17	Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene-	$C_{10}H_{16}$	136.23
14	1.83	Bicylo[2.2.1]heptanes, 7,7-dimethyl-2-methylene-5-	$C_{10}H_{16}$	136.23
15	3.61	Cyclohexane, 5-methyl-3-(1-methylnyl)-trans-(-),		
16	3.37	4,8,12,16-Tetramethylheptadecan-4-olide,		

Table 10: Detected Compounds of Ethanol Extract Using GC-MS.

Discussion

The dried specimen of C. odorata was extracted using ethanol, water, and n-hexane as the extractive solvents and conventional maceration and soxhlet extraction as the extraction methods. The weight of the extract obtained using the same volume of extractive solvent was significantly higher when compared to the extract obtained by soxhlet extraction using n-hexane as the extractive solvent, according to the results obtained after weighing the extracts from the two methods of extraction. This trend contradicted the findings of Wu, et al. [10], who conducted a comparison study to assess the amount of extraction using cold maceration, soxhlet extraction, microwave extraction, ultrasonic extraction, and hydrodistillation. Soxhlet extraction yielded the greatest extraction results and is particularly useful for extracting high molecular mass molecules. Although cold maceration is laborious and yields a low percentage, it is nevertheless recommended by scientists, particularly for small-scale extractions like the one utilized in this work [11].

The extracts' antibacterial activity was assessed using the Agar well diffusion technique. The dosage having the greatest degree of antibacterial activity based on the mean zone of inhibition for both aqueous and ethanol extracts of C. odorata. When compared to the aqueous extract, the ethanol extract demonstrated a larger degree of inhibition across all microbial isolates. Furthermore, as compared to gram-negative bacteria and fungus, the ethanol extract was more effective against gram-positive germs (S. aureus and Bacillus spp.). However, ethanol extract had a greater impact on E. coli, as well as Bacillus spp. than S. aureus. Once again, the ethanol extract was more effective in comparison to the aqueous extract. When compared to the negative control distilled water, the extracts had statistically significant antibacterial activity. However, the positive controls (ciprofloxacin for bacteria and fluconazole for fungus) all outperformed the extracts in terms of antibacterial activity C. odorata. These findings were consistent with the findings of Stanley, et al. [12], who discovered that the solvent and technique of extraction had a significant influence on antibacterial activity. The antibacterial impact of ethanol extract was greater than that of n-hexane extract.

The minimum inhibitory concentration (MIC) of an agent is the lowest possible dose capable of inhibiting microbe growth for 24 hours, whereas the minimum bactericidal concentration (MBC) is the lowest possible concentration capable of preventing microbe growth for some time greater than 48 hours. The MIC test results demonstrate that there

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was no growth at the dosage of 25 mg/mL for both the ethanol extract and the aqueous extract after 24 hours of culture C. odorata. After 48 hours, there was full growth across all concentrations of the aqueous extract, demonstrating that the aqueous extract had an inhibitory impact but did not eliminate the microbial isolates. The Bactericidal for the microbiological isolates in ethanol extracts was 200mg/ml for all isolates except Bacillus sp, which was 100mg/ml. For Candida sp., the minimum fungicidal concentration is 100mg/ml. According to a previous study, C. odorata showed antibacterial activity against a variety of pathogens associated with diarrhea, skin infection, wound infection, urinary tract infection, and food spoilage [12-16]. This study's findings are consistent with those of Huda, et al. [14], who discovered that polyphenol extracts of The antibacterial activity of C. odorata against Staphylococcus aureus and Staphylococcus epidermidis were modest. This study is also consistent with the findings of Abubakar, et al. [17], who discovered that C. odorata inhibited urinary tract infection bacteria such as E. coli, Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans with MIC values ranging from 12.5 mg/ml to 50 mg/ml. Natheer, et al. [18] also found that had higher antibacterial activity C. odorata by comparing three (3) traditional plants to twelve (12) bacterial isolates. According to the research, Of the 12 bacteria species, C. odorata had the most efficient antimicrobial inhibitory action, with a minimum inhibitor concentration of roughly 25 mg/ml. Similarly, Naidoo, et al. [19] found it to have antibacterial action. C. odorata by extracting the leaves and stem. This work supports the findings of Thophon, et al. [16], who discovered that C. odorata leaf extract had the most potent antibacterial activity when compared to other plant components. Furthermore, C. odorata preparations have been shown to have an anti-bacterial effect against both gram-positive and gram-negative bacteria. Methanol leaf extracts show the greatest antibacterial efficacy against gram-positive bacteria such as Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, and gram-negative bacteria such as E.coli. The leaves' ethanol extract showed strong antifungal action against fungus cultures such as Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, and Trichophyton rubrum. The findings of this study are also consistent with the findings of Inyang and Adegoke [20], who observed considerable suppression of both gram-positive (Staphylococcus aureus) and gram-negative bacteria (E.coli and Klebsiella pneumonia).

The GC-MS study of extracts in ethanol and n-hexane. C.odorata demonstrated the existence of several bioactive compounds. The GC-MS recovered four primary bioactive compounds from the n-hexane extract: n-hexadecanoic acid, Octadecanoic acid, and oleic acid. These results were consistent with those of Agusta, et al. [21]. N-hexadecanoic and octadecanoic acids were discovered in both samples. Palmitic acid, commonly known as n-hexadecanoic acid, is a saturated fatty acid having 16 carbon atoms. This was detected in large quantities in the n-hexane extract. It may be found in a variety of natural sources, including animal and plant fats. Palmitic acid is a key component of dietary lipids and a vital source of energy for the body. It may be metabolized by the body to generate energy, which can then be used to power other physiological activities [22]. Some research suggests that in small concentrations, palmitic acid may have anti-inflammatory benefits. In certain cases, it has been proven to alter immune responses and decrease inflammation [23,24].

Another major bioactive ingredient found in the n-hexane extract is octadecanoic acid, also known as stearic acid. Stearic acid, $CH3(CH2)16COOH$, is a very common fatty acid. Most animal and plant lipids include it as a glycerol ester [25]. Stearic acid has been found to have either a neutral or favorable impact on cardiovascular health. Stearic acid, unlike other saturated fats, does not seem to raise LDL cholesterol levels, the "bad" cholesterol linked to an increased risk of heart disease. Stearic acid, on the other hand, has been shown to have a neutral effect on LDL cholesterol and may even enhance HDL cholesterol, or "good" cholesterol [26]. Because of this distinguishing feature, stearic acid may not have the same harmful influence on cardiovascular health as other saturated fats. Stearic acid may have anti-inflammatory properties in the body, according to research. It has been proven to inhibit the production of pro-inflammatory chemicals such as IL-6 and TNF-alpha [27]. Stearic acid may help to prevent or control inflammatory diseases by lowering chronic inflammation. Stearic acid seems to have a favorable influence on blood sugar management, according to research. When compared to other forms of fat, it has been proven to not affect insulin production and blood glucose levels [26,28]. This property implies that stearic acid may be a better alternative for those who have diabetes or are worried about blood sugar management. Stearic acid and its equivalents have been found in studies to have antibacterial action against a variety of microbiological isolates, including the fungus Candida albicans [29]. Because it is a lipid, it may pass across the blood-brain barrier (BBB). As a result, stearic acid has been studied and shown to have significant antidepressant action in mice [29]. The n-Hexane extract included octadecane, commonly known as n-octadecane. Antimicrobial and antifungal effects have been established in plant extracts, essential oils, and natural products containing octadecane. Bakkali, et al. [30] discovered that essential oils from Moroccan medicinal herbs, including Artemisia herba-alba, contained octadecane and had high antibacterial action against a variety of microbes.

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Nonadecane, also known as n-nonadecane, is a 19-carbon-atom long-chain hydrocarbon. It has also been linked to health advantages such as antibacterial and antifungal activities. Kaur, et al. [31], for example, investigated the chemical composition and antibacterial activity of essential oils produced from Cymbopogon species, which included nonadecane among other compounds. The essential oils were discovered to have substantial antibacterial properties against a variety of pathogenic microorganisms in the investigation.

Oleic acid, which was identified in the n-Hexane extract, is a monounsaturated omega-9 fatty acid found in a variety of natural sources such as olive oil, avocados, and almonds. It has been thoroughly researched, and its health advantages are well acknowledged. Oleic acid has been found to offer cardiovascular health benefits. Diets high in oleic acid may help decrease LDL cholesterol (the "bad" cholesterol) and lessen the risk of heart disease [32,33]. It may also aid in the maintenance of normal blood pressure levels [34]. Oleic acid has anti-inflammatory properties in the body. It has been shown in studies to help lower inflammatory indicators such as C-reactive protein (CRP) and interleukin-6 (IL-6) [35,36]. Chronic inflammation is linked to a variety of chronic illnesses, and oleic acid's anti-inflammatory qualities may help with general health and well-being. Oleic acid has skin-beneficial moisturizing and nourishing effects. It contributes to the preservation of the skin's natural moisture barrier, limiting water loss and boosting hydration [37]. Topical oleic acid use has been shown to increase skin suppleness, minimize wrinkles, and improve overall skin health [38]. Oleic acid may have a function in weight loss. It has been proven to boost sensations of fullness and increase satiety, possibly lowering total calorie consumption [39]. Including oleic acid sources in the diet, such as olive oil or avocados, may help with hunger control and weight management.

C. ethanol extract C. The chemical components of odorata were comparable to those of the n-hexane extract. It had a high of 16.10 for octadecanoic acid (Stearic acid), which was shortly followed by a peak of 15.67 for n-hexadecanoic (Palmitic acid).

Tetradecanoic acid, often known as myristic acid, is a 14-carbon atom saturated fatty acid. According to research, myristic acid possesses antibacterial characteristics and is active against a variety of microbes. It has been discovered to impede the development of some bacteria and fungi, perhaps contributing to antimicrobial actions [40,41]. Some research suggests that myristic acid may have anti-inflammatory properties. It has been found to block inflammation-related cellular processes and diminish the generation of pro-inflammatory cytokines [42,43]. These findings point to a possible function in moderating inflammatory responses.

Phytol is a diterpene alcohol that is well-recognized for its function as a precursor to vitamin E. However, research shows that phytol may have other health advantages in addition to its role in vitamin E production. Phytol has been found to have antioxidant capabilities, which aid in the neutralization of damaging free radicals and the reduction of oxidative stress in the body [44,45]. Furthermore, phytol has been shown to have anti-inflammatory characteristics by inhibiting pro-inflammatory cytokines and inflammatory mediators [46,47]. These qualities imply that phytol may have a protective role in chronic inflammation-related disorders. According to research, phytol may have anticancer properties. It has been discovered to suppress cancer cell growth and proliferation, induce cell cycle arrest, and cause apoptosis (programmed cell death) in numerous cancer cell lines [48,49]. These data suggest that phytol has potential therapeutic applications in cancer prevention and therapy. Phytol has been shown in research to have neuroprotective effects. It has been found to protect neuronal cells from neurotoxicity and oxidative stress-induced damage [50,51]. These results imply that phytol might be useful in the prevention or treatment of neurodegenerative disorders.

Linoleic acid, or 9,12-octadecadienoic acid, is an important omega-6 polyunsaturated fatty acid. It is a necessary nutrient that must be gained from food and plays an important role in human health. Linoleic acid's impact on heart health has been thoroughly researched. It is a precursor of omega-6 fatty acids such as arachidonic acid, which play significant roles in blood coagulation, inflammation, and blood pressure control [52]. Linoleic acid is an important component of brain cell membranes and has a role in the creation of numerous signaling chemicals. It is required for normal brain growth and function [53,54]. Linoleic acid has been demonstrated to aid in the maintenance of healthy skin. It promotes skin integrity and barrier function by increasing moisture retention and decreasing transepidermal water [55,56]. Linoleic acid is also a precursor to key skin lipid components like ceramides, which help to keep the skin hydrated and protected [57]. Linoleic acid is a precursor for many bioactive chemicals that are important in the regulation of inflammation and immunological responses [58]. It may be transformed into various eicosanoids, such as prostaglandins and leukotrienes, which are required for adequate immune function and inflammation management [52]. Adequate linoleic acid consumption aids in the maintenance of a balanced inflammatory response in the body.

The important omega-3 polyunsaturated fatty acid alpha-linolenic acid (ALA) is 9,12,15-octadecatrienoic acid. The cardiovascular advantages of alpha-linolenic acid have been widely researched. It is metabolized in the body to two long-chain omega-3 fatty acids present in fatty

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fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA has been linked to a decreased risk of cardiovascular illness, including a lower frequency of heart attacks and fatal coronary heart disease [59,60]. Alpha-linolenic acid and other omega-3 fatty acids are essential for brain growth and function. They are required for appropriate cognitive function and play a role in maintaining the shape and integrity of cell membranes in the brain [61]. Adequate ALA consumption has been linked to a lower risk of cognitive decline and age-related neurodegenerative disorders like Alzheimer's [62,63]. Furthermore, omega-3 fatty acids have been associated with a reduced incidence of depression and enhanced mood [64]. Alpha-linolenic acid is a building block for anti-inflammatory chemicals such as EPA and DHA. It has been demonstrated to have anti-inflammatory qualities as well as the ability to alter immunological responses [52,58]. Adequate ALA consumption has been linked to decreased inflammation and enhanced immunological function, which may help in the prevention or treatment of chronic inflammatory diseases.

5-methyl-3-(1-methylnyl)-trans-(-) cyclohexane, also known as trans-p-menthan-3-ol, is a chemical molecule found in essential oils. The anti-inflammatory and analgesic effects of trans-p-menthan-3-ol have been shown. In animal models, it has been shown to suppress the production of pro-inflammatory cytokines and reduce pain [65,66]. These results imply that trans-p-menthan-3-ol might be useful as a natural antibacterial agent. Trans-p-menthan-3-ol has been shown to provide respiratory effects when extracted from essential oils such as peppermint oil. It may help alleviate symptoms of respiratory problems such as coughing, congestion, and bronchial spasms [67,68]. Its bronchodilator and expectorant characteristics may aid in better breathing and less respiratory irritation.

Conclusion

This study examined the antibacterial activity and bioactive elements of Chromolaena odorata extracts, highlighting the plant's potential as a treatment for microbial illnesses. The results highlight the wide range of uses for this invasive plant, which is well known for aiding in the healing of wounds. The ethanol extract's potential antibacterial characteristics are highlighted by the bactericidal impact it demonstrated against a variety of microbiological isolates at a concentration of 200 mg/mL. Determining the presence of important bioactive compounds like oleic acid, octadecanoic acid, and hexadecanoic acid helps explain the processes behind the antibacterial properties that have been found. This study offers important insights into natural alternatives that merit additional investigation for their therapeutic potential as we address the problems of antibiotic resistance. Chromolaena odorata's complex history, which includes use in traditional medicine as well as contemporary scientific investigation, highlights the plant's importance in the continuous search for potent antimicrobial medicines.

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