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Evaluation of the Antibacterial Activity of *Chromolaena Odorata* in Wistar Rats and its Chemical Characterization

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Abstract

Chromolaena odorata is a plant that has been used traditionally in the treatment of diverse disease conditions. This study sort to evaluate the anti-*Salmonella typhi* potential of *Chromolaena odorata* compared to ciprofloxacin in Wistar rats and to identify the phytochemical constituents present in the plant that could be responsible for the antibacterial activity exhibited by the plant. The animals were divided into six groups: group 1 was normal control, group 2 was infected with *Salmonella typhi* without treatment, groups 3, 4 and 5 were *Salmonella typhi* infected and treated with 100mg/kg, 200mg/kg and 400mg/kg of the extract respectively and group 6 was infected and treated with 500mg/70kg of ciprofloxacin. The animals were inoculated with a single infective dose of *Salmonella typhi* bacteria and subsequently, treated with the graded doses of methanol leaf extract of *Chromolaena odorat*a and 500mg/70kg dose of ciprofloxacin for a period of fifteen days, when the animals were confirmed infected. Fecal matter was collected from each group on day 0 (when the animals were confirmed infected) and on every five days interval for the period of the study, for *Salmonella typhi* load assay. This was followed by phytochemical screening and GC-MS analysis of the plant extract. Treatment of infected animals with the extract and standard drug, resulted in a dose and time dependent eradication of the *Salmonella typhi* from the stool, while the phytochemical screening and the GC-MS analysis revealed the presence of phenolic, flavonoid, alkaloid, steroid and Geijerene, 3-methyl-3-vinyl- cyclopropene; 4, 6-Dimethyl-3-nitro-2(1H)-pyridinone, 2-Hexyn-1-ol, 1,2,3,4-Tetrahydro-2,3-dimethylquinoxaline, nonyl prop-1-en-2-yl ester Carbonic acid respectively. Thus the observed antibacterial activity could be attributed to the presence of these phytochemicals.

Keywords: Chromolaena odorata; Phytochemical Screening; GC-MS analysis; Salmonella typhi; Wistar Rats.

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1. Introduction

Salmonella enterica subsp. Enterica, is a ‘’rod-shaped, gram-negative, non-spore-forming, predominantly motile entero-bacteria with cell diameters between approximately 0.7 and 1.5 µm, and lengths of 2 to 5 µm, and peritrichous flagella’’ [1]. They are non-capsulated, non-sporulating, gram-negative bacilli, which have characteristic flagella, somatic, and outer coat antigens [2]. Salmonella primarily occurs in the gastrointestinal tracts of birds, insects, reptiles, and mammals, and environments polluted with human or animal excreta [3]. A huge majority of prescription drugs used in the world contains compounds that are directly or indirectly (via semi-synthesis) derived from plants [4] and plant extracts and plant-derived medicines have made immense contributions over the years to the overall health and well-being of human beings [5]. Additionally, 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during the period 1981–2002 were inspired by natural product research [6]. *Chromolaena odorata* leaf extract is said to possess great medicinal value: for instance, the leaf extracts are used to treat skin diseases and indigestion [7]. In northern Africa, aqueous extracts are used to cause clotting of blood, treat abdominal pains and to abort foetuses during the early stages of pregnancy [8]. *C. odorata* leaf extracts display antibacterial activities against *Pseudomonas aeruginosa* and *Streptococcus faecalis* [9] and Gram negative bacteria, *Escherichia coli* [10].

1.1 Methods

1.2 Collection of Samples

Leaves of *Chromolena odorata* were fetched from Omuoko-Aluu community and cleaned of soil and dust by washing. The leaves were identified at the Department of Plant Science and Biotechnology, University of Pot Harcourt

1.3 Plant Extraction

After collection, the leaves of the plant were shade-dried at room temperature (32-35^{o}C) to constant weight over a period of seven (7) days. The method employed a little modification of the cold maceration extraction method as described by [11]. 50g of powdered leaves of *Chromolaena odorata* was dissolved in 1000 ml of seventy percent methanol in a 2 litre conical flask and was intermittently shaken vigorously at 1 hour intervals for a period of 12 hours and left to stand over-night at room temperature for effective extraction. Afterwards, the extract was filtered using a 0.45 millipore filter paper. The clear solution obtained was then concentrated with a rotary evaporator at 40^{o}C and 200 rpm and subsequently, on a steam bath at 40^{o}C. The semi-solid extract obtained was stored in sterile pre-weighed screw capped bottles and labeled accordingly. The extracts were stored in desiccators at room temperature until needed.

1.4 Isolation and culturing of the Organisms

All media used in this study were prepared following the manufacturers’ instructions. *Salmonella typhi* was isolated from typhoid patients in University of Port Harcourt Teaching Hospital (UPTH). Bile salt broth (broth culture)[12] and streptokinase broth (clot culture) [13] were used for enrichment. The enriched samples showing

visible turbidity were streaked on Mac-Conkey agar media. The isolates producing characteristic colonies were identified by conventional biochemical tests.

1.5 Screening for the antibacterial potential of the plant

The agar well diffusion procedure used in this experiment was similar to that used by [14] and [15]. A single colony of the organisms was picked from the culture and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 37^0C. 5 ml of plain Mueller Hinton broth was incubated alongside the organisms to ensure that the medium was not contaminated. The spectrophotometer was set to 625 nm wavelength and each of the microbial cultures was pipetted into cuvettes to measure the absorbance. A cuvette of plain Mueller Hinton broth was used as a blank at 0.000 absorbance. The absorbance of the microorganisms was measured. The bacterial organisms exceeding 0.1 absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08-0.10, matching the McFarland Standard. All the organisms, therefore, reached a cell density of 1.5×10^8 cfu/ml [16]. 100µl of each of the organisms were then inoculated onto agar plates for the bioassay [17].

1.6 Preliminary Anti-Microbial Sensitivity Test

The agar well diffusion technique was used in this study to determine the antibacterial activity of the plant extracts as described by [18]. The Nutrient agar (Diffco) used was prepared according to the manufacturer's specification and the extracts were tested against *Salmonella typhi*. Nutrient agar was inoculated with the study microorganisms by spreading it on the media. Three wells of diameter 6mm were bored in the agar using sterile cork borer and filled with 0.5ml of the diluted plant extracts (methanol, ethyl acetate and n-hexane). Control wells containing neat solvents (vehicle control) were also run in parallel with the positive controls using ciprofloxacin as standard drug, in the same plate. One thousand microliters of the standard drug, Ciprofloxacin was placed in one well, the extract in another well and dimethyl sulfoxide (DMSO) was placed in the third well on each plate. The experiment was run in triplicate for each extract tested. All plates were incubated at 37^oC for 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a meter rule. The diameter of the zones of inhibition in the triplicate plates was measured by calculating the difference between the cork borer (6 mm) and the diameters of inhibition as described by [19].The activity indices were calculated as the division of zone of inhibition of the extract by that of the standard drugs following the method of [20]. The results are presented as the mean values of the triplicate plates.

1.7 Isolation, identification and enumeration of the microbial flora in the GIT of apparently healthy rats

Feces of animals from the various groups were septically taken and 1 g each was introduced into separate McCartney bottles and serially diluted. One milliliter of each suspension was plated separately using pour plate technique on nutrient agar for bacterial growth and the plates were incubated at 37°C for 24 h and at room temperature (30 ± 2°C), for 72 hours respectively, after which the resulting number of colonies were counted.

1.8 Experimental design

Eighty five (85) animals were divided into 6 groups numbered 1-6. Group 1 (normal) had five (5) animals; Group 2 (negative control) had twenty (20) animals, while groups 3-6 had fifteen (15) animals each. Group 1 animals were not treated throughout the experimental period but were given free access to normal animal feed and water, *ad labitum*. Group 2 (negative control) contained *Salmonella typhi*-infected rats not treated after disease induction. Groups 3, 4 and 5 consisted of *Salmonella typhi*-infected rats treated with100mg/kg (low dose), 200mg/kg (medium dose) and 400mg/kg (high dose) of methanol extract of *Chromolena odorata* leaves respectively. Group 6 contained *Salmonella typhi*-infected rats treated with 500mg/70kg of a standard antibiotic drug (Ciprofloxacin). On day 0, (when the animals were confirmed infected), on day 6 and at 5 days intervals and at the end of the fifteen days of treatment, fecal matter was collected from each group and were subjected to *Salmonella typhi* load assay

1.9 Challenging apparently healthy animals with Salmonella typhi

Eighty (80) animals (groups 2-6) were orogastrically challenged with the infective dose (1.0 - 2.0 x 10^8cfu/ml) of *Salmonella typhi* and fecal matter were assayed to determine when infection had set in, thereafter, the animals were treated with the three different doses of the methanol leaf extract of *Chromolena odorata and the standard antibiotic (ciprofloxacin)* as described earlier, once daily, for fifteen days.

1.10 Preparation of the Extract Concentrations and Antibiotic

Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500mg of Ciprofloxacin in sterile distilled water. DMSO was also used as vehicle control in the study.

1.11 Phytochemical Screening

The extracts were analyzed by following the procedures of [21] to test for the presence of saponins, terpenoids, alkaloids, glycosides, tannins, flavonoids, reducing sugars and volatile oils.

1.12 GC-MS Data analysis

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanol extract of *C.odorata* was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of length 30m, diameter and film thickness of 0.25mm and 0.25μm respectively. The oven temperature of the column was programmed between 50°C to 300°C for every 2°C per minute. The ionization of the components of the sample was accomplished in an electron impact mode (EI, 70 eV). The injector temperature was fixed to 240°C, while that of the detector was fixed to 200°C. The carrier gas was Helium of 99.9995% purity, and was fixed at a flow rate of 1.5 ml per minute. The scanning of the mass ranging from 40-1000 m/z was done at a rate of 3.0 scans/s. The process was initiated by the injection of 1.0 μl of the methanol extract of *C. odorata* with a Hamilton syringe in to the GC-MS manually for total ion chromatographic analysis in split injection technique. The total GC-MS activity time for the analysis was 35min and the relative percentage of the extract constituents was expressed as percentage with peak area normalization.

1.13 Identification of Components

The identification of the bioactive compounds in the methanol leaf extracts of *Chromolaenaodorata* was executed using Mass Spectroscopy based on the comparison of their retention indices and mass spectra fragmentation patterns and matching the identified components with those from various sources including: those stored on the computer library, published literatures, Wiley Registry of Mass Spectral Data, National Institute of Standards Technology (NIST08s), Fatty Acid Methyl Esters Library version 1.0 (FAME library) and New York (Wiley 8).

1.14 Statistical analysis

The results are presented as Mean ± Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) and post test using LSD multiple comparison test [22].

2. Results

2.1 Effect of methanol leaf extract of Chromolaena odorata on Salmonella typhi load (x10^{5}cfu) in Salmonella typhi infected Wistar Rats

The result of the effect of methanol leaf extract of *Chromolaena odorata* on *Salmonella typhi* load in animals exposed to a single infective dose of *Salmonella typhi* is summarized in table 1. It was observed that exposure of animals to the bacterial inoculums caused a significant increase ($p<0.05$) in the *Salmonella typhi* load on day 0 ranging from 0.00±0.00 x10^{5}cfu - 25.67±2.40 x10^{5}cfu. Following administration of the extract however, the *Salmonella typhi* load decreased in a dose dependent manner from 25.67±2.40 x10^{5}cfu on day 0 to 0.025±0.002 x10^{5}cfu on day 16, for the low dose and from 25.67±2.40 x10^{5}cfu on day 0 to 00.00±0.00 x10^{5}cfu on day 16 and from 25.67±2.40 x10^{5}cfu on day 0 to 00.00±0.00 x10^{5}cfu on day 16 for both the medium and high doses respectively. Ciprofloxacin caused a decrease from 25.67±2.40 x10^{5}cfu on day 0 to 0.06±0.005 x10^{5}cfu on day 16.We observed a significant difference in the salmonella type load between the negative control and normal rats, and between the negative control and the treatment groups on days 6, 11 and 16.

2.2 Minimum Inhibitory Concentration (MIC) of Chromolaena odorata in Salmonella typhi bacteria

The standard drug had the lowest minimum inhibitory concentration followed by the methanol and n-hexane fractions of the extract, while ethyl acetate had the highest value of the minimum inhibitory concentrations (Table 2).

2.3 Zone of Inhibition of Chromolaena odorata in Salmonella typhi bacteria after 24 hours of culture

Table 3 presents the result of the diameter of the zone of inhibition, The ethyl acetate fraction at all concentrations produced the largest zones of inhibition followed by the methanol fraction, while the n-hexane fraction produced the lowest value of the zone of inhibition. Both the different fractions of the extract and the standard drug all showed a dose dependent effect on the diameter of the zone of inhibition.

2.4 Phytochemical analysis of Chromolaena odorata

The result of the phytochemical analysis revealed the presence of alkaloids, tannins, saponin, carbohydrate, flavonoids, anthraquinones, cardiac glycosides, phlobatanin and phenol (Table 4).

2.5 Percentage and Qualitative yield of Chromolaena odorata

The percentage and qualitative yield of the various constituents of C*hromolaena odorata are as outlined in Table 5*. Flavonoids were the most abundant followed by alkaloids. Anthraquinones were the least abundant in the methanol extract of the plant.

2.6 Gas Chromatography and Mass Spectrometer (GC-MS) analysis

Gas Chromatography and Mass Spectrometer (GC-MS) analysis of the plant extract, revealed the presence of the compounds listed below in figures 1and 2.

Table 1: Effect of methanol leaf extract of *Chromolaena odorata* on*Salmonella typhi* load (x10^5cfu) in *Salmonella typhi infected* Wistar Rats

Group	Day 0	Day 6	Day 12	Day 18
Control	0.00±00	0.00±00	0.00±00	0.00±00
Neg. Control	25.67±2.40**	25.00±1.15**	24.00±1.73//	5.80±2.11**
Low Dose	*25.67±2.40	*6.57±1.39***	*0.57±0.04***	*0.025±0.002***
Medium Dose	*25.67±2.40	*4.67±0.95***	*0.83±0.06***	*0.00±00***
High Dose	*25.67±2.40	*2.63±0.85***	*0.24±0.02***	*0.00±00***
Ciprofloxacin	*25.67±2.40	*6.83±0.58***	*0.91±0.02***	*0.06±0.005***

* = Significant difference between normal and test groups

** = Significant difference between normal and negative control

*** = Significant difference between and negative control and test groups

- Normal (Animals not exposed to any form of treatment but were fed *ad libitum*)
- Negative Control (Animals inoculated with *Salmonella typhi* without treatment)
- Low Dose (100mg/kg of extract)
- Medium Dose (200mg/kg of extract)
- High Dose (400mg/kg of extract)
- Ciprofloxacin (500mg/70kg)

Table 2: Minimum Inhibitory Concentration (MIC) of *Chromolaena odorata* in *Salmonella typhi* bacteria

Extract	Concentration
Methanol	0.78±0.02 mg/ml
Ethyl Acetate	3.12±0.15 mg/ml
n-hexane	1.25±0.09 mg/ml
Ciprofloxacin	0.25±0.11 mg/l

Table 3: Zone of Inhibition of Chromolaena odorata in Salmonella typhi bacteria after 24 hours of culture

Concentration	Extract zone of inhibition (mm)			Ciprofloxacin (mm)
	Methanol	Ethyl Acetate	n-hexane	
50mg/ml	2.8±0.06	7.1±0.24	1.4±0.34	30±0.15 for (0. 125µg/ml)
100mg/ml	4.5±0.18	15.0±1.04	2.1±0.23	35±0.10 for (0.25µg/ml)
150mg/ml	6.8±0.28	18.3±1.23	3.2±0.12	
200mg/ml	7.9±0.26	21.1±1.45	4.4±0.21	
250mg/ml	11.7±0.69	28.1±1.56	6.7±0.24	

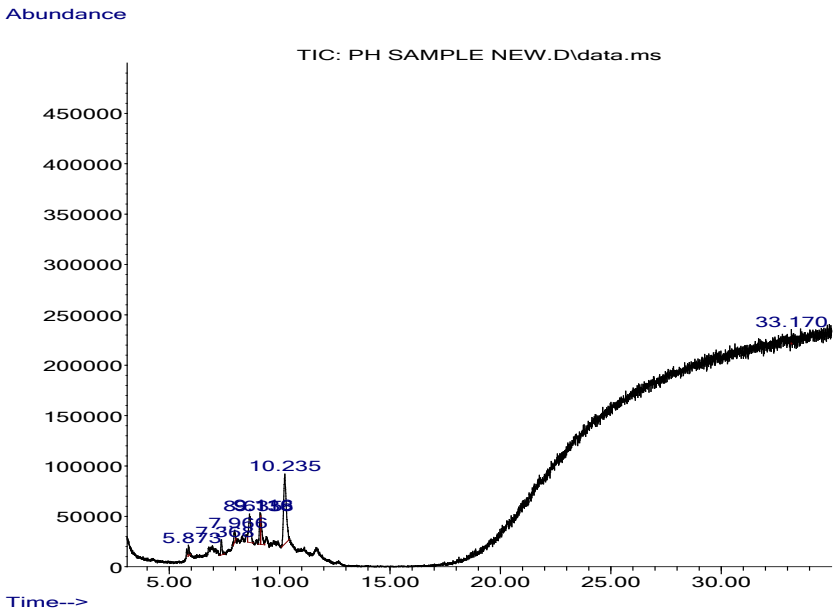


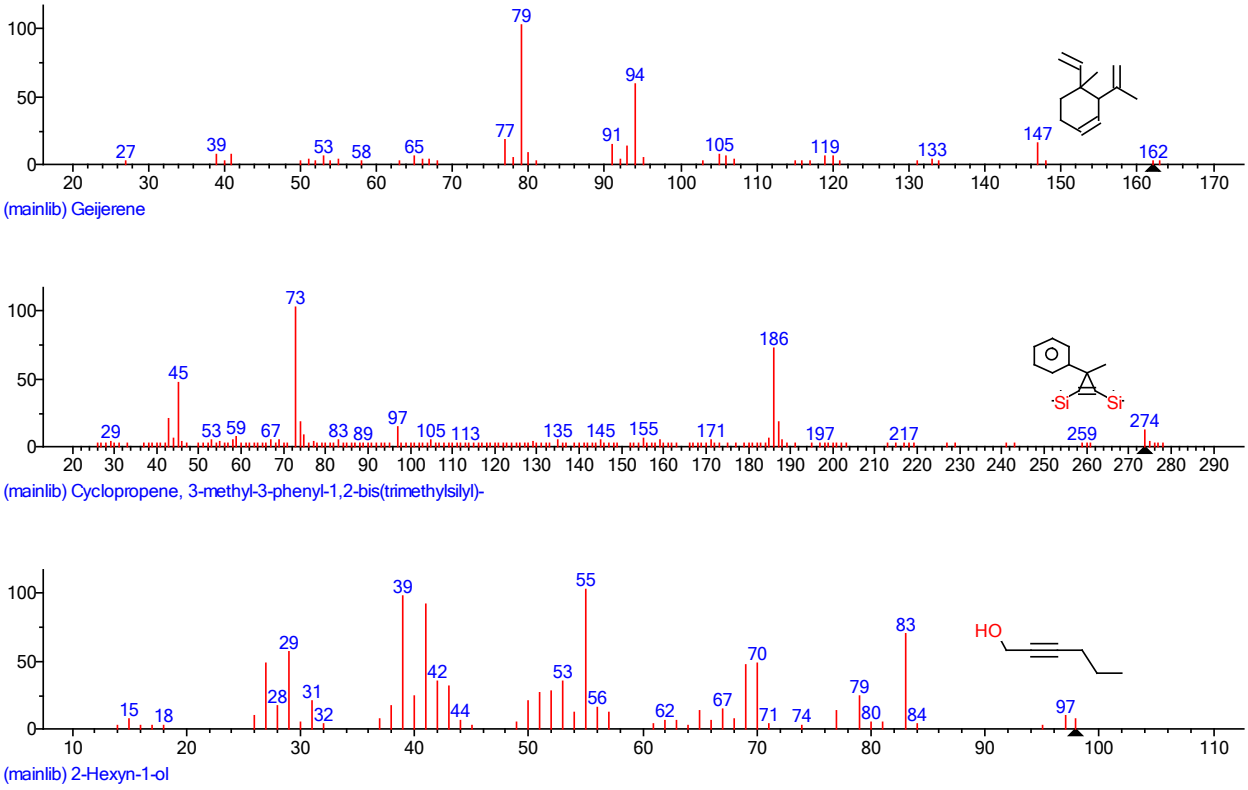
Figure 1: GC-MS original tracings and graphs Library Search Report

Table 4: Phytochemical result on methanol extract of *Chromolena odorata*

S/No	Constituent	Test	Result
1.	Alkaloid	Meyer's Test	+ve
		Drangendorf Test	+ve
		Hager's Test	+ve
2.	Tannins	Bromine water Test	+ve
		Ferric Chloride Test	+ve
3.	Saponin	Frothing Test	+ve
		Emulsion Test	+ve
4.	Carbohydrates	Molisch Test	+ve
		Fehling Test for sugar reduction	+ve
5.	Flavonoids	Selivanoff Test	+ve
		Shinoda Test	+ve
6.	Anthraquinones	Free Anthraquinones	-ve
		Combined Anthraquinones	-ve
7.	Cardiac Glycosides	Keller Killiani	+ve
		Salwooki	+ve
		Legal	+ve
		Liebermann	+ve
8.	Phlobataanin	Hydrochloric Acid Test	+ve
		Sodium Bicarbonate Test	+ve
9.	Phenoli	Phenolic compound Test	+ve

Table 5: Percentage and Qualitative yield of *Chromolena odoarata*

S/No	Constituent	Result	Percentage yield (%)
1.	Alkaloid	++	17.91
2.	Tannins	++	11.04
3.	Saponin	++	8.84
4.	Carbohydrates	++	15.64
5.	Flavonoids	++	18.79
6.	Anthraquinones	--	0.007
7.	Cardiac Glycosides	++	8.55
8.	Phlobataanin	++	6.14
9.	Phenoli	++	13.08
Total			100.00



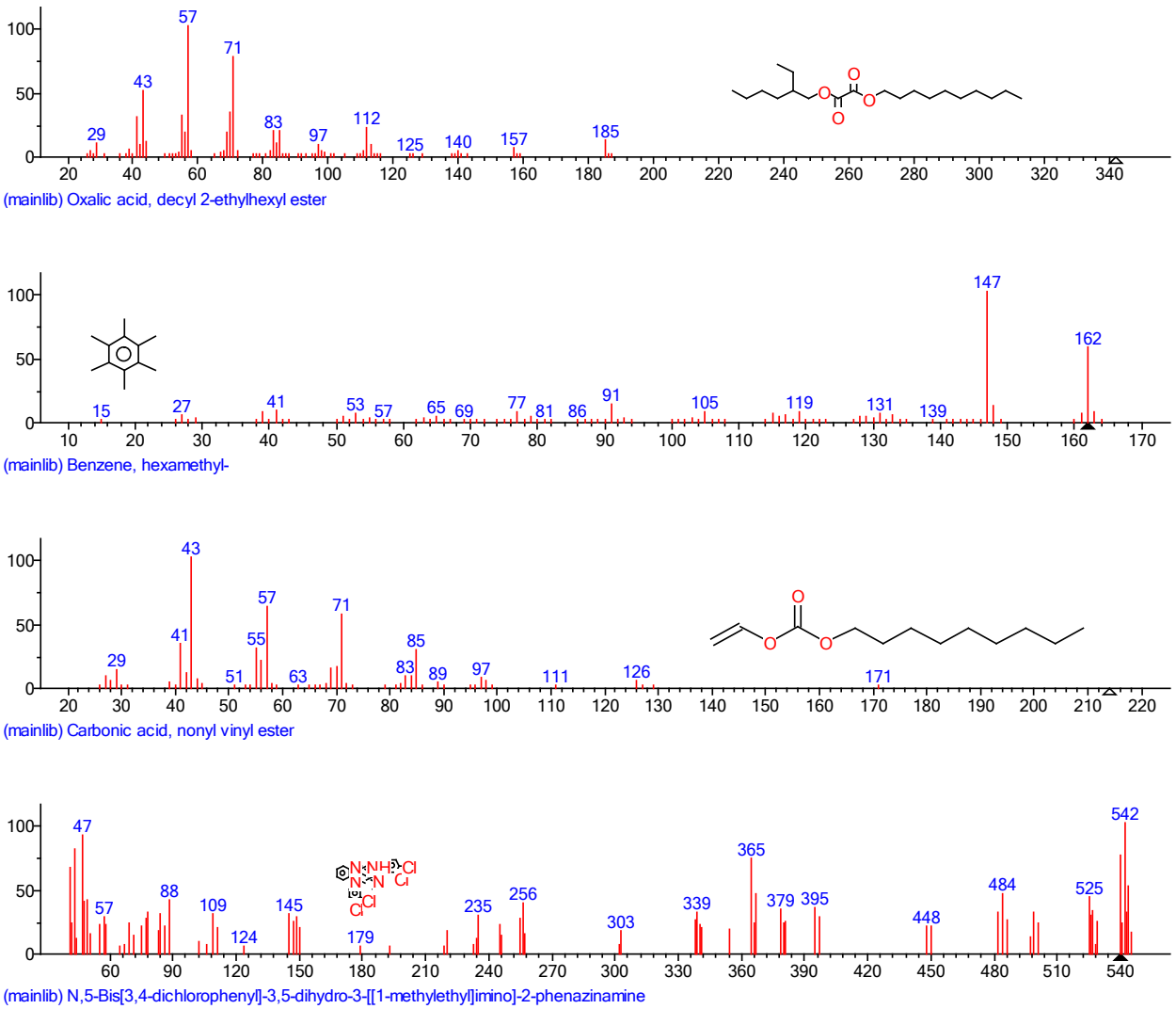
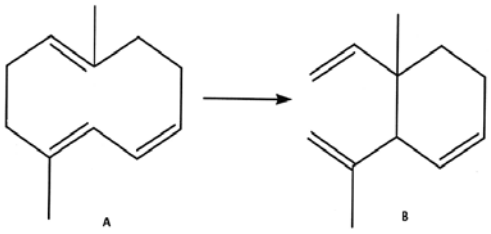
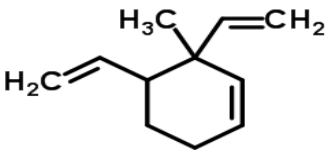


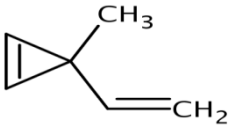
Figure 2: Structures of Chemicals Identified in Chromolaena odorata using GCMS



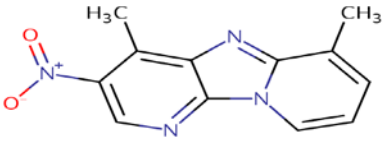
Geijerene



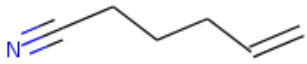
3, 4-diethenyl-3-methyl- Cyclohexene



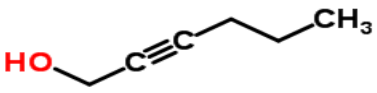
3-methyl-3-vinyl- Cyclopropene



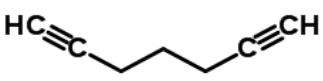
4, 6-Dimethyl-3-nitro-2(1H)-pyridinone



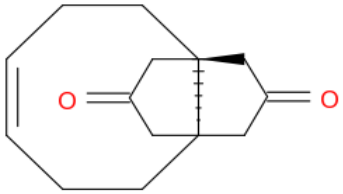
5-Cyano-1-pentene



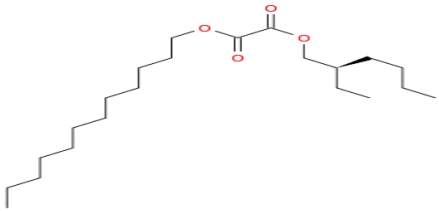
2-Hexyn-1-ol



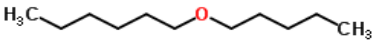
1, 6-Heptadiyne



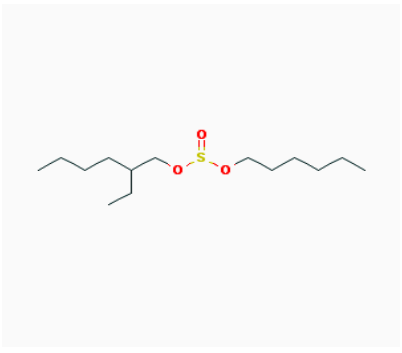
13-dioxo- Tricyclo [6.3.3.0] tetradec-4-ene



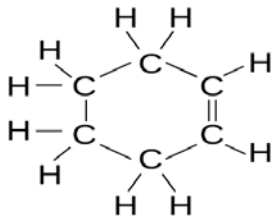
Oxalic acid, decyl 2-ethylhexyl ester



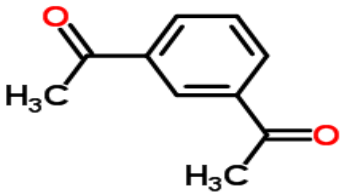
Hexyl-pentyl ether



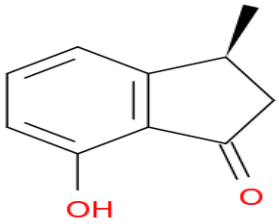
2-ethylhexyl hepta-decyl ester Sulfurous acid



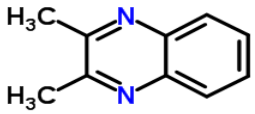
hexamethyl- Benzene



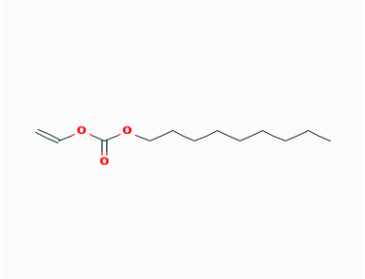
1,1'-(1,4-phenylene)bis- Ethanone



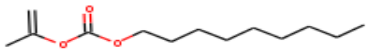
2, 3-dihydro-7-hydroxy-3-methyl-1H-Inden-1-one



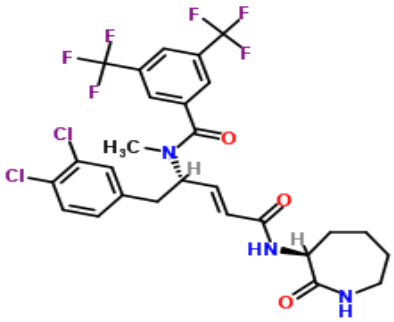
1,2,3,4-Tetrahydro-2,3-dimethylquinoxaline



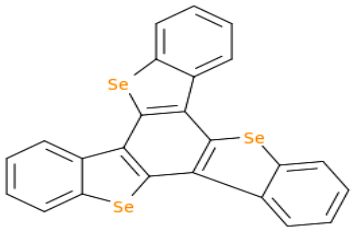
nonyl vinyl ester Tridecane Carbonic acid



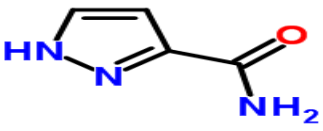
nonyl prop-1-en-2-yl ester Carbonic acid



N,5-Bis[3,4-dichlorophenyl]-3,5-dihydro-3-[1-methylethyl]imino]-2-p henazinamine



Tris (benzo[b] selenopheno)[2,3:2',':2'',3'']benzene



1H-pyrazole-3-carboxamide

Figure 3

3. Discussion

Administration of the methanol leaf extract of *Chromolaena odorata* to animals infected with *Salmonella typhi* caused a decrease in the levels of *Salmonella typhi* load, this could be a bactericidal effect as was evidenced in the in vitro study, showing growth inhibitory properties in the result of the minimum inhibitory concentration,

which was as low as 0.78±0.02 mg/ml. This is inevitably due to the presence of the various phytochemicals including phenolics, flavonoid, alkaloid and steroid [23]. Antibacterial activity of *chromolaena odorata* has been reported against *Vibrio harveyi* [24], *Bacillus subtilis, Staphilococcus aureus* and *Salmonella typhimurium* [25]. Our results also corroborate the report of [26], who observed that the methanolic extracts of *C. odorata* showed positive inhibition against *Bacillus subtilis, Klebsiella Pneumoniae, Staphylococcus aureus,* which are associated with superficial infections, as well as those of [27]. The mechanisms of action of *Chromolaena odorata* could be revealed by the examination of the different mechanisms of action by the various constituents. [28,29] reported that phenolic compounds disturbs the function of cytoplasm membrane. This report was corroborated in studies by [24], who revealed that at low concentration, phenolic compound damages cytoplasm membrane causing the leakage of important metabolite thereby inactivating bacterial enzymatic system, and at high concentration, the compound damages cytoplasm cell membrane and as well precipitate the cell protein. They added that phenolic compound interact with the component of bacterial cell wall which causes higher permeability of the bacterial cell [24]. Also, [30] added that some flavonoids such as robinetin, myricetin and epigallocatechin, interfere with the intercalation bond or hydrogen bond at the nucleate acid assembly preventing the activity of DNA and RNA synthetases. Further studies also implicated the steroid compound to also play a role in damaging plasma membrane leading to leakage of cytoplasm constituent and cell death [31]. The anti-bacterial activity of *Chromolaena odorata* may be contributed by the presence of Geijerene which was one of the main compounds found in *Chromolaena odorata*. This is because Geijerene, one of the main constituent of oil extract of the plant "*Pimpinella affinis"* possess antibacterial and antifungal properties against *Vibrio Parahaemoliticus, B. Subtilis, E. Coli, L. Monocytogenes, and St. Aureus* [32]. In another study by [33], it was also reported that major components of *Pimpinella affinis* oil consisting of geijerene (17.68%), limonene (12.86%), Pregeijerene (9.92%), germacrene D (8.54%) and trans- β-cimene (4.94%) showed pronounced antimicrobial activity. Thus, the antibacterial activity observed in our study from *Chromolaena odorata* leaf extract may have been contributed by the presence of this geijerene. The compound 3-methyl-3-vinyl-cyclopropene, may have also contributed to the anti-*salmonella typhi* properties observed with *Chromolaena odorata* leaf extract in our study, because, a close relative, of this compound, 3-methyl-2-buten-1-yl cyclopropane–carboxylate, found in a similar plant oil extract of the same family "*Anthemis nobilis L.* (Asteraceae)" with 29.3% fraction yield was shown to exhibit antibacterial properties against *E. coli, S. aureus, P. Aeruginosa, Salmonella enteric, Klebsiella pneumonia* and *Shighuelle sonnei* [34]. Also, the compound 4, 6-Dimethyl-3-nitro-2(1H)-pyridinone may also contributed to the antibacterial effect of *C. odorata* because it contains both imidazole and benzene rings in its structure which are reported to show prominent antibacterial activity [35,36].

Similarly, 2-Hexyn-1-ol may also contribute to the antibacterial activity of *C. odorata* because, 4.9% yield of 3-Hexyn-1-ol fraction in the plant *Anthemis mixta L.* (Asteraceae) oil extract in a GC-MS analysis, exhibited significant antibacterial potentials, against *E. coli, S. aureus, P. Aeruginosa, Salmonella enteric, Klebsiella pneumonia* and *Shighuelle sonnei* [34].

Other components, that may have contributed to the anti-bacterial activity of *C. odorata* include 1,2,3,4-Tetrahydro-2,3-dimethylquinoxaline and nonyl prop-1-en-2-yl ester Carbonic acid because of the presence of quinoxaline [37,38] and Carbonic acid [39] respectively.

4. Conclusion

The bactericidal activity of *Chromolaena odorata* is due to the presence of some phytochemicals such as phenolics, flavonoid, alkaloid and steroid which damages bacterial cell membrane, increase membrane permeability, cause leakage of cytoplasm constituents and subsequent cell death. The various compounds found in these phytochemicals with these properties include; Geijerene, 3-methyl-3-vinyl- cyclopropene, 4, 6-Dimethyl-3-nitro-2(1H)-pyridinone, 2-Hexyn-1-ol, 1,2,3,4-Tetrahydro-2,3-dimethylquinoxaline and nonyl prop-1-en-2-yl ester Carbonic acid.

5. Constraints/Limitation

This study was limited to anti-bacterial assay of the extract of *Chromolaena odorata* on *Salmonella typhi* in Wistar rats, determination of the phytochemical constituents as well as Chemical characterization of the extract using Gas Chromatography and Mass Spectrometer (GCMS).

6. Recommendations

Further studies in this area should include

1. Isolation of the major chemical constituents from the extract.

2. Initial In vitro bioassay of the major chemical constituents of the extracts against *Salmonella typhi*.

3. From the outcome of the result of the In vitro bioassay, a further In vivo bioassay should be carried out against *Salmonella typhi* in Wistar rats using the constituents with observed anti-bacterial properties.

4. Further studies to develop the Chemical constituents with anti-bacterial properties into drugs should be initiated and be continued through the process to have alternative sources of drugs for the treatment of *Salmonella typhi* in endemic areas.

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