Antibacterial Activity Studies of Essential Oils from Red Sage (*Lantana Camara)*

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Research Article

Keywords: Essential oil, Lantana camara, Antibacterial activity

Posted Date: February 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1299145/v1

Abstract

Essential oils are important source of rich medicinal compounds. They are highly volatile, aromatic compounds from natural origins. The essential oils cause the hydrophobility of the bacteria cytoplasm, resulting to the cell wall degradation. The recent activity studies of *Lantana camara* have mapped out the need to investigate the antibacterial potency of its essential oil. Herein, the result and discussion of the characterization with their antibacterial activity of *Lantana camara* was reported; the Minimum inhibitory concentration (MIC) on the bacteria (Positive and Negative Gram) with Ciprofloxacin a known antibiotic as the positive control. The broth and agar diffusion method were used in estimating Zone of the inhibition. Oil show moderate antibacterial potency with respect to each of the bacterium used; the potency tends to increase considerably with increase in concentration (25% <50% < 80% < 100%). The zone of inhibition exhibited by the assay at 100% concentration of the essential oil on *E. coli* was the closest shown to that of the control (ciprofloxacin), also the MIC on *E. coli* at 100% concentration of the essential oil was greater than that of the control (ciprofloxacin). Consequently, essential oils from *lantana camara* becomes a wellspring of antibiotics for restraining bacteria action.

Introduction

Natural products are predominant for the design of novel drugs in the prevention and treatment of disease [1]. Over the years, the people have exploited their usages due to their availability, low cost, effectiveness, low toxicity [2]. Medicinal plants since ancient time have provided huge sources of medicine [3]; thereby hone the modern medicine with many plant-based therapeutic agents [4]. In India and Africa alone over 6000 plants are exploited for traditional medicine [5].

Essential oils are extracts from complex mixtures of diverse parts of certain plant and animal species [6]. They are highly concentrated volatile substances with peculiar and potent therapeutic activity [7]. Due to their low toxicity to mammals, we acknowledge for their pharmacological and therapeutic properties [8]. The chemicals constituent of essential oils are secondary metabolites; terpenes, terpenoids, phenylpropenes, and others based on chemical structure, which play a core role in their strong bactericidal activity [6,9]. They are fused into edible films as an active composition in food safety and packaging to enhance food quality and shelf live by inhibiting microbial growth [10].

The plant *Lantana camara* (Verbanaceae) is indigenous to subtropical and tropical regions and is generally known as red or wild sage [11]. It is an ever green intense smelling shrub with diverse flower colors and stout recurred prickles [12]. The therapeutic potential of *Lantana camara* comes as a result of its bioactive compounds which have been exploited for antibacterial, antipyretic, larvicidal, insecticidal, and antimicrobial purposes [13,14]. Essential oil extract of *Lantana camara* is used in conventional medication framework for the treatment of various diseases [15]. This include antiseptic for wounds [16], disorders [17], respiratory problems [8], antiseptic and antibiotic applications [18].

The antimicrobial activity of an essential oil involved disruption of bacterial structures by causing hydrophobicity between the cytoplasm and external envelope of the cell [19]. This increased permeability and degradation of the cell wall, essential oils is difficult to be removed from the bacterial cell membrane [20]. In addition, this resulted to the leakage of the cell contents and diminished the intracellular ATP pool [21].

Essential oil contains hydrophobic antimicrobial compounds; the Gram-negative bacteria have hydrophilic lipopolysaccharides (LPS) outer membranes which impede the action of the hydrophobic essential oil constituent making them less affected compare to the Gram-positive bacteria which show more affinity [22-24].

In view of this, this research study is mapped out to study the antibacterial activity of *lantana camara* essential oil through the determination of the chemical composition of essential oil, zone inhibition of the action of the bacteria and the characterization of the sample with Ciprofloxacin a known antibiotic as the positive control in order to obtain the antibacterial potential of the essential oil and its relation with the antibiotic.

Methodology

Sampling and collection of plants

The *Lantana camara* leaves were sourced locally within FUTA south-gate, around Abavicinity, Akure Nigeria, where it is serves as a weed and a plant of no interest. They were collected by carefully uprooting from the vegetation of different cluster of the weed around the bushes to ensure homogeneity of sample. The plant was authenticated at the Department of Crop, Soil, and Pest Management, The Federal University of Technology, Akure, Nigeria. Subsequently, the plant parts (leaves and part of the stem close to the leaves) were carefully separated and washed lightly to remove all forms of dirt.

Isolation of L. camara essential oils

The hydro-distillation method was engaged for the extraction process. A glass clevenger-type apparatus was connected to a heating mantle; this consists of 2000ml round bottom flask enclosing 2.70 kg of the raw material and 1.5 liters of water. The setup was for 4 hours at an optimum temperature 750^0C, which was repeated five consecutive times to have maximum yield. The resulting liquid (essential oil) was collected in a scaled, sterile amber glass vial [25]. The essential oil was kept in the refrigerator to avoid vaporization until used for bactericidal activities, UV analysis and GC-MS analysis.

The yield of the essential oil is estimated as;

Yield = wt. of oil x 100(g/g)/ Wt. Of *lantana camara* leaf collected

CHARACTERIZATION OF LANTANA CAMARA **ESSENTIAL OIL**

The oil extract from the *lantana camara* leaveswas analyzed and characterized by UV-visible spectroscopy and GC-MS. The whole reagent used for this analysis was of analytical grade and prepared according to the manufacturer's specification.

GC-MS Analysis

The essential oil was scanned over UV/Visible spectrophotometer within the range of 280nm to 900nm to measure the absorbance using UV-Vis 1800 series machine.

GC-MS Analysis

A report was adopted for the GC-MS analysis of L. camera oil [26]. The GCMS-QP2010 obtained from Japan was employed for the characterization. The initially oven temperature was set at 50°C, which was raised gradually to 250°C. The column was injected1µl of the L. camera oil extract and 99.99% pure helium carrier gas was passed through for 1.2 ml/min. The MS detector pickup the distinctive peak for indentified compounds in conformity with the retention time and was quantified my measuring the peak are. The data obtained from the peaks spectra were compared with data from the library (NIST14).

Method Of Antibacterial Assay

Sterilization of materials

Materials: Petri dishes, syringe, swab stick, sterile borer Autoclave, Test tubes, conical flask, cotton wool.
Media: Mueller-Hinton agar (MHA)

All materials and media used were sterilized in order to avoid contamination. The agar and broth media employed were also prepared as stated by the manufacturer. The media were boiled and sterilized by autoclaving at 121^0C for 15 minutes. Also, all the glass wares were washed with soap, rinsed and dried with sterilized towel.

Bacteria used

Four (4) bacteria obtained from the Department of Microbiology, FUTA were used in the antibacterial assay of the *lantana camara* essential oil; *Bacillus subtilis* and *Staphylococcus aureus* which are Gram positive and *Salmonella typhi and Escherichia coli II* (Gram negative bacteria).

Preparation of Mueller Hinton agar

Mueller-Hinton agar (MHA) engaged for the routine antimicrobial susceptibility testing. 3.8g of Mueller Hinton agar powder was dissolved with 100ml of de-ionized water according to manufacturer's specification. The medium was shaken well to dissolve the agar powder in the water and then sterilized in the autoclave at 121℃ for 15 minutes [25].

Antibacterial assay of oil extract

The potential antibacterial activity of plant oil extract was carried out by modification of Geetha et al.,[27] 25ml of Muller Hinton agar was placed on the sterile petri dishes to solidify. A 5mm diameter well was punch on the medium using a sterile borer followed with streaking the plates with bacterial culture. Upon solidification of the agar, sterile cotton swabs were used for inoculation of the bacterial cultures by spreading on the petri dish. Afterward, 0.5ml of varying concentration (20, 50, 80 and 100%) of the Oil extract was added to the well surface (Muller Hinton agar containing bacterial lawn). Antibiotic ciprofloxacin and wells containing distilled water was used as the positive and negative control respectively. To estimate the bacterial growth, the inoculated plates were incubated overnight at 37^{o}C and the diameter in mm was estimated as the zone of inhibition.

Minimum inhibitory concentration (MIC)

The tube dilution method for Mueller Hinton broth was used to estimate the Minimum inhibitory concentration. 0.5ml of standardized density of the test organisms was added to different concentrations (20-100%) of the plant oil extract in a series of Mueller-Hinton broth tubes for 24 hours incubation period. Hence, following visual inspection, the extract with the concentration resulting in no growth was read using a spectrophotometer (UV-Vis 1800 series machine) at 620nm as the MIC.

Results

Essential Oil Yield

The variations in the quantity (yield) of the *Lantana camara* essential oil composition are due to variations in the genetic, climate and geographical location [28]. The *L. camara* extract obtained via hydro distillation gave a pale yellow essential oil with an optimum yield of 0.20 ± 0.04% (v/w). This extraction yield is higher than the yield of 0.19% obtained by Jawonsi and Adoga [29], 0.125% by Elansary *et al.*[30], in Egypt and 0.13% by Rabindra and Balendra [31] in India. This yield is less than that of 0.5% obtained by Adjou *et al.*[32], in Benin.

UV-Visible spectra of Lantana camara essential oil

UV-visible spectroscopy is a fast and cost-effective technique. It is used for qualitative and quantitative analysis of compounds with powerful chromophores [33]. Phenolic compounds are detected by the UV-Visible spectroscopy. They give a strong color by forming complexes with iron [34]. Figure 1 and the results presented in Table 1, show the spectra of *Lantana camara.* The important peaks at 280nm, 320nm and 360nm are synonymous with presence of Xanthones, flavonoids (including flavonones, flavones and catechins) and phenolic acids respectively [35].

Chemical composition of L. camara essential oil

A reported that the variation in chemical composition characteristics and yield of an essential oil are affected with the drying method been used [36]. Similarly, the chemical profile of essential oil is affected by the time of the plant sample collection [37]. Herein, from the GC-Ms analysis report of oil extract of *L.*

camara (figure 2) a total of 51 chemical components were reported (Table 2). However, oxygenated compounds (96.69%) were predominant of which about 34.23% are simple sesquiterpenes. The composition of the studied *Lanatana camara* species is similar to a study reported Sonibare and Effiong [38]. Although, the sesquiterpenes reported in this study is lower than 37.30% and 56% reported, Saikia and Sahoo [39] and Sousa *et al.*,[40] respectively. Generally the major composition of the *L. camara* essential oil in this study was found to be β-phellandrene about 9.28% which is similar to report from Iran by Zandi-Sohani *et al.* [41] However the species from North Brazil shows limonene, macrene, α-phellandrene, gercurcumene α-zingiberen and α-humulene has the major constituents [42]. Other major components were eucalyptol (6.68%), α-pinene (6.26%), 3-Carene (6.64%), camphor (3.24%), camphene (4.04%), endoborneol (5.04%), caryophyllene (4.95%) humulene (3.09%), bycyclogermacrene (4.11%) and nerolidol (2.33%). The results obtained were similar to the chemical composition of *L. camara* reported Marongiu *et al.* [43] and Khan *et al.* [44].

Zone Inhibition of Lantana camara **essential oil on selected bacteria**

The antimicrobial activity of *L. camara* oil extracts could also related to the various phytochemicals and peroxidases composition [45]. *L. camara* essential oil contains hydrophobic antimicrobial compounds [46]. Generally the Gram-positive bacteria examined have hydrophobic outer membrane which indicates strong affinity for the hydrophobic composition of the essential oil. In contrast, the outer membrane of the Gram-negative bacteria which is hydrophilic lipopolysaccharides (LPS) alters anyadaptive response to the hydrophobic essential oil [46,47].

The study shows that the essential oils of extract (Figure 3) have varying antibacterial potency against the bacteria (*Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli*) at different concentration. The control (ciprofloxacin) showed significantly high zone of inhibition (16mm for *Bacillus subtilus*, 18mm for staphylococcus and Salmonella typhi and 20mm for *E.coli*).

The antibacterial activity exhibited increases by increasing the concentration of the oil extract. 100% concentration of the essential oil showed the best antibacterial activity against all the bacteria, which is an agreement with an earlier study Sangeetha *et al.*[48] and Tiwari *et al.*[49] *Bacillus subtilis* shows zone of inhibition of 12mm, *Staphylococcus aureus*shows zone of inhibition of 16mm, which is identical to the zone of inhibition observed for *Salmonella typhi*, and *Escherichia coli* shows the highest amongst the bacteria (18mm) and closest zone of inhibition to the positive control (ciprofloxacin). At 25% concentration, the oil had it weakest antibacterial activity on the bacteria. The essential oil extract of *L. camara* antimicrobial activity is as a result of its constituent; Caryophyllene, caryophyllene oxide and eucalyptol have been reported to exhibit moderate to strong activities against a wide range of bacteria [50]. Furthermore, Zoubiri and Baaliouamer [50] reported that *β*-caryophyllene and caryophyllene oxide possessed antimicrobial activity and caryophyllene oxide had a high activity against Candida albicans. *E.coli* showed the highest zone of inhibition (19mm). With further studies *lantana camara* essential oil could be modified as a potential antibacterial for *E.coli*.

Minimum inhibitory concentration (MIC)

The minimum concentration of antimicrobial agent that is required to completely microbial growth refers to as MIC [51,52]. From the result (Figure 4) the least concentration of the extract resulting in no growth following visual inspection after 24 hrs of incubation for Bacteria using a spectrophotometer at 620nm was recorded as the MIC. In comparing the MIC, ciprofloxacin (control used) showed the lowest MIC for all the bacteria except for E. coli at 100% concentration (0.015ug/ml). For all the bacteria MIC decreased progressively as the concentration increased. At 100%, *Salmonella typhi* had the highest MIC (0.026 ug/ml), followed by *B. subtilis* which had MIC value of 0.023 ug/ml, *E. coli* had MIC of 0.015 ug/ml then *Staph. aureus* (0.014 ug/ml). With respect to the control used, *E.coli* showed MIC value (0.015 ug/ml) less than that of ciprofloxacin (0.016 ug/ml), while MIC values for the rest of the bacteria were above that of the control.

Conclusion And Recommendation

The emergent of multi-drug resistant strains has launched the persistent examination of natural products for more potent new antibiotics. The activity of *lantana camara* essential oil extract on inhibiting bacteria growth was found to be concentration-dependent. The MIC bacteria assay, the essential oil extract showed effectiveness on the strains of bacteria in the following order *Staphylococcus aureus*>*Escherichia coli*> *Bacillus subtilis*>*Salmonella typhi*. Overall, the extract showed moderate growth inhibition action against bacteria when compared with standard antibiotic used Ciproflavin. The extract was found not to match the standard antibiotics already in use study, which show that there is potential for the extract, especially if the active compound is isolated, since they abound in the plant. This will be a novel way to explore indigenous plant biomass to their full potential and a sustainability way forward in unveiling new antibiotics residing in nature. However, extensive investigation of the unexplored bioactive composition of *Lantana camara* is needed to fully ascertain its antibacterial activity.

Declarations

Ethical Approval

The study do not involved experimentation on animals or man.

Consent to participate

N/A

Consent to Publish

All authors gave their approval for the manuscript publication.

Author's contribution

Omoyemi Oluwatosin Ajayi conceptualized the idea and was involved in performing the experiments, writing, reviewing and editing the original draft. Abdullahi Tunde Aborode; Opeyemi Isaac Subuloye; Abayomi Oyeyemi Ajagbe were involved in formal analysis and writing of the original draft

preparation**;** Mika Sillanpää; Iwuozor Kingsley Ogemdi; Babatunde Samuel Obadawo; Emmanuel Adebowale Fajemisin; Gaber El-Saber Batiha finally reviewed and edited the work. Emmanuel Adebowale Fajemisin made the submission with approval of all the authors.

Funding

No funding was received for this work.

Competing Interest

All authors have no conflict of interest to disclose.

Availability of data and materials

This data herein is original research work and has not been submitted for publication elsewhere.

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Tables

Table 1
UV Spectra Data for the Essential oil from *Lantana camara*

S/N	Wavelength (nm)	Absorbance (cm^{-1})
1	808.00	0.209
2	669.00	0.218
3	366.50	0.304
4	342.00	0.352
5	235.00	4.002
6	227.00	4.001
7	205.00	4.008
8	762.50	0.206
9	748.50	0.206
10	363.50	0.304
11	338.00	0.345
12	231.00	3.997
13	211.00	3.981

Table 2
Chemical composition of *Lantana camara* Essential oil

S/N	Retention time (min)	Phytochemical Constituent	Composition (%)
1	3.065	3-Hexen-1-ol	0.52
2	3.176	2-Hexen-1-ol	0.36
3	3.776	α-pinene	6.26
4	3.917	Camphene	4.04
5	4.361	β-phenandrene	9.28
6	4.435	β-mycrene	2.78
7	4.650	3-carene	6.46
8	4.902	2-butenylcyclopropane	3.72
9	5.087	Eucalyptol	6.61
10	5.206	β-ocimene	1.79
11	5.346	γ-terpinene	1.22
12	5.509	Terpinen-4-ol	2.22
13	5.746	2-carene	1.22
14	5.924	Linalool	2.31
15	6.065	Ipsdienol	0.09
16	6.213	Isopulegol	0.37
17	6.450	Camphor	3.24
18	6.731	Endoborneol	5.04
19	6.931	α-terpineol	2.56
20	7.043	Verbenone	0.51
21	7.257	Citral B/Neral	0.14
22	7.368	Piperitone	0.13
23	7.472	Citral A/Geranial	0.17
24	7.583	Selinene	0.06
25	7.650	Indole	0.07
26	7.717	Cumyl alcohol	0.11

S/N	Retention time (min)	Phytochemical Constituent	Composition (%)
27	7.931	Isoterpinolene	0.72
28	8.050	Eugenol	0.37
29	8.272	β-elemene	3.26
30	8.524	Caryophyllene	4.95
31	8.679	Humulene	3.09
32	8.783	β-copaene	2.17
33	8.887	Bicyclogermacrene	4.11
34	9.131	Nerolidol/Peruviol	3.92
36	9.376	Cyclopentanol 1-(1-methylene-2-propenyl)	2.34
37	9.472	2-octyne	3.04
38	9.561	1,3-Bis-(2-cyclopropyl-2-methylcyclopropyl)-but-2-en-1-one	4.18
39	9.635	2-butenylcyclopropane	2.46
40	9.842	Bisabolene epoxide	0.40
41	9.953	Dichlorovinyltrimethylsilane	0.83
42	10.146	Carophyllene oxide	0.23
43	10.316	Linolenic acid	0.27
44	10.524	Palmitic acid	0.13
45	10.768	Nerolidol	0.22
46	10.916	Hortrienol	0.26
47	11.020	Phytol	0.54
49	11.427	1-methyl bicycle (3,2,1) octane	0.09
50	11.590	Oleamide	0.05
51	11.990	9-octadenamide	0.06

Figures

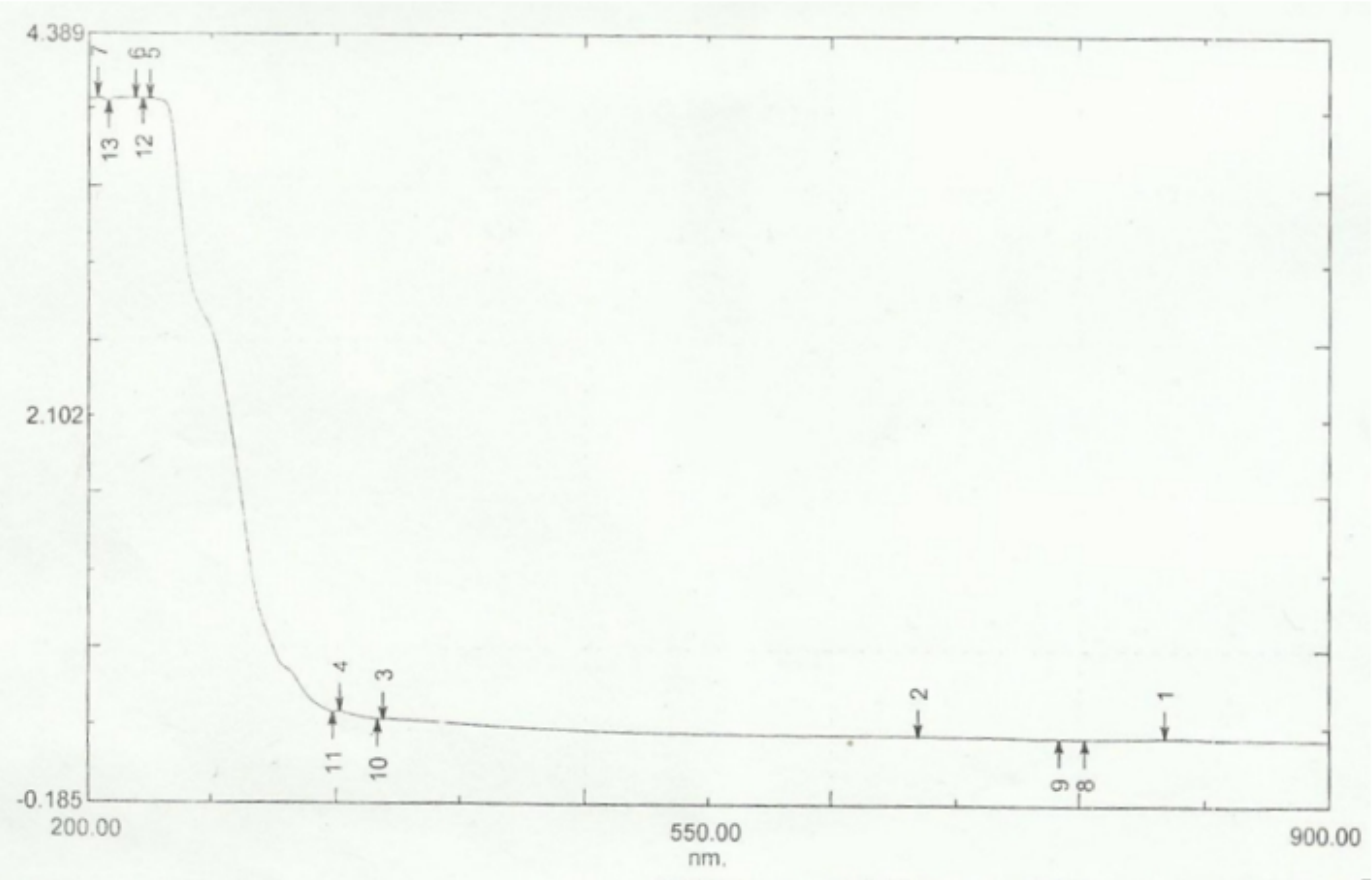

Figure 1

UV-Visible spectra of *Lantana camara* essential oil

UV-Visible spectrophotometer Result and Analysis

UV-Visible spectra analysis

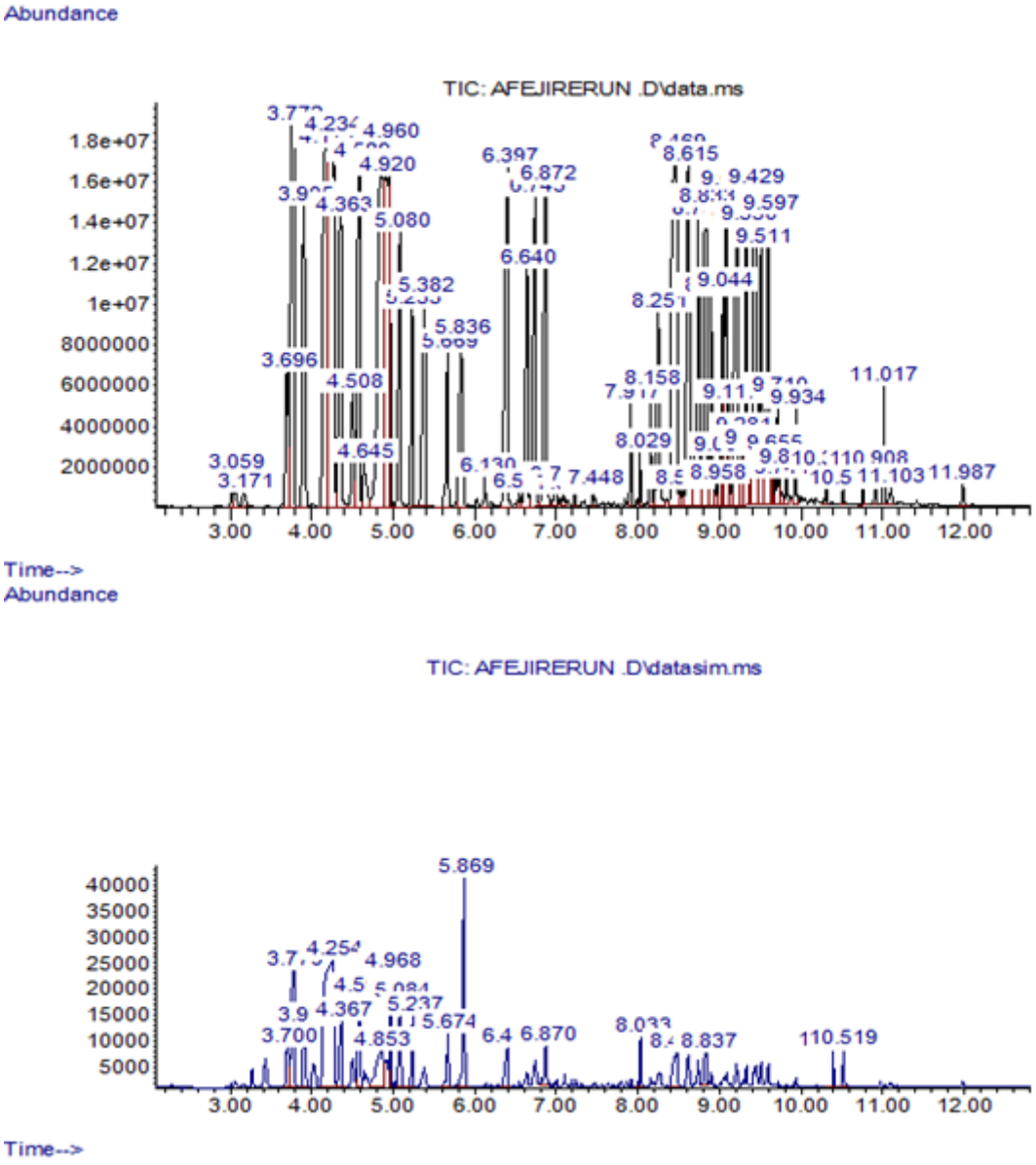

Figure 2

GC-MS graph of *lantana camara* essential oil

GC-MS results and Chemical Composition of Essential oil

GC-MS graph

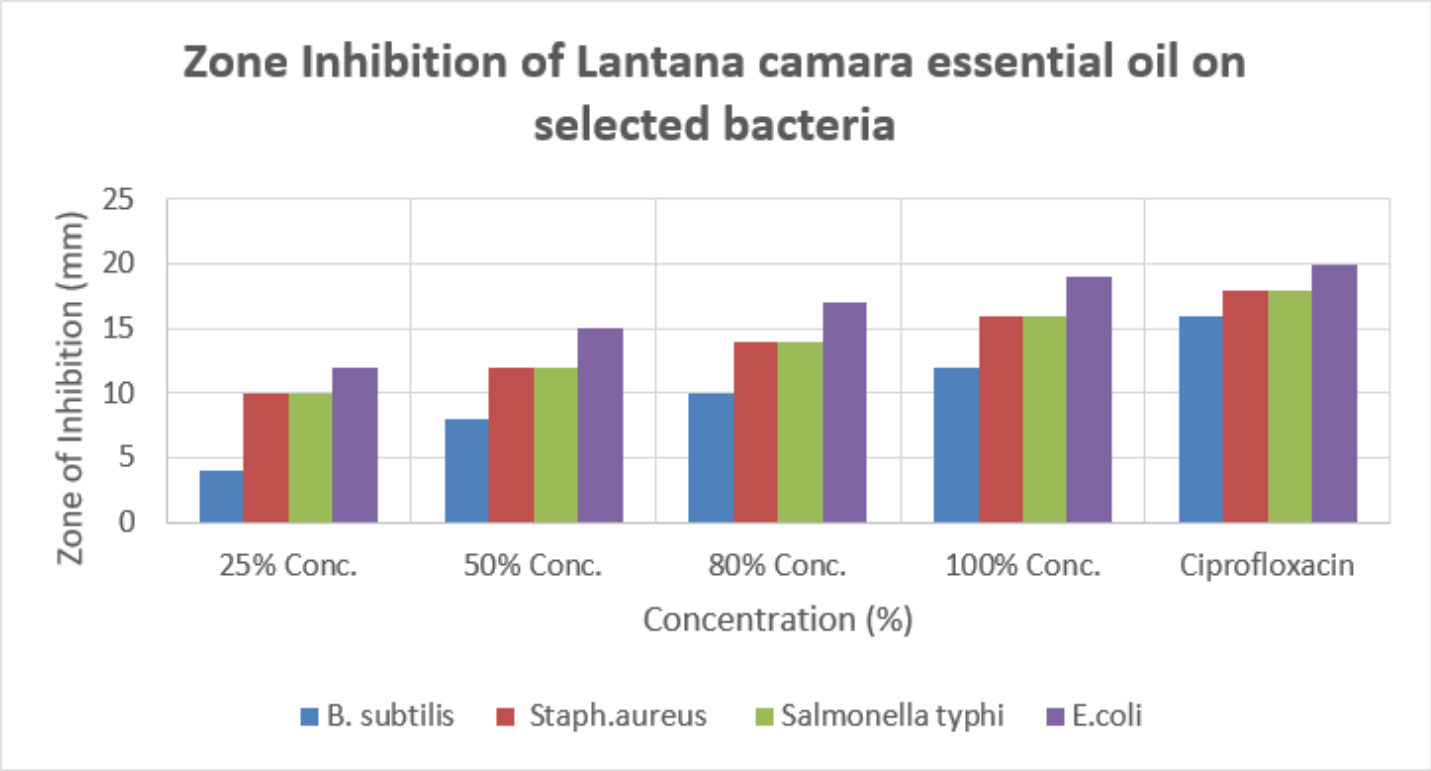

Figure 3

Zone Inhibition of Lantana camara essential oil of selected bacteria

Zone of inhibition exhibited by Oil extracts

The potency of antibiotics to inhibit bacterial growth is quantifying by agar diffusion assay.[52]

Result

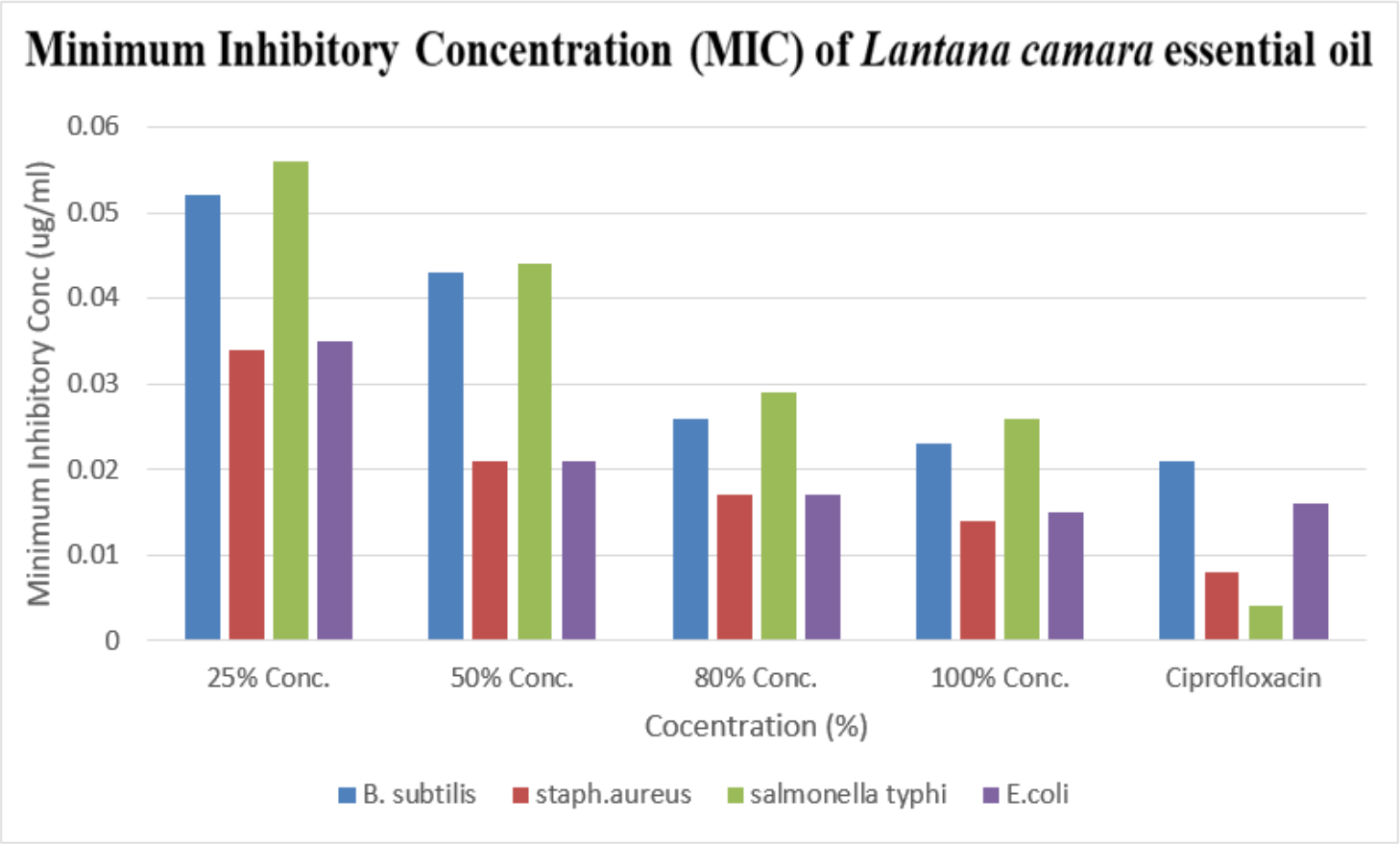

Figure 4

Minimum Inhibitory Concentration of Lantana *camara* essential oil