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Invitro Antibacterial Activity and GC-MS Analysis of *Acalypha Fruticosa* Leaf Extract Against Some Human Pathogens

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Abstract
Medicinal plants are of special interest to biotechnology researchers because most of the drug industry uses them to make pharmaceutical compounds. Most herbal medications and their derivative products have crude plant extracts as their main constituent, which are a complex mixture of several phytochemical substances (plant secondary metabolites). The chemical properties of these constituents differ substantially among species. The findings showed that the plant extracts affected all of the bacteria that were utilised. In the current investigation, which extracts the leaves from *Acalypha fruticos*a using various solvents, ethyl acetate showed high antibacterial activity against both Gram-positive and Gram-negative bacterial strains, in contrast to other solvent extracts. An intriguing technique for determining the concentration of certain active ingredients in herbs used in cosmetics, medications, and pharmaceuticals is the GC-MS method, which is employed to analyse the extracted materials.

Keywords: Antimicrobial Activity, *Enterobacter aerogens*, *Acalypha fruticosa,* GC-MS analysis.

1. Introduction

In an effort to reduce the danger of infectious diseases brought on by bacteria, fungi, viruses, and parasites that are harmful to humans, one of the most active areas of research has been the hunt for compounds with strong antimicrobial action. Many medicinal substances, such as antibacterial medicines used to treat infectious disorders, are still primarily derived from plant extracts (Touati *et al*., 2018). Since ancient times, people have utilised natural remedies to improve their health, and contemporary medicine has relied heavily on medications made from natural resources. In the past, many antimicrobial compounds were found in both natural and synthetic goods to cure and control infectious agents (Shriram *et al*.,2018). But only a small number of these compounds were available to the market in the underdeveloped globe (Poulakou *et al*.,2018). The availability and cost of many commonly prescribed antibiotics around the world have been further jeopardised by the advent of multidrug-resistant bacteria (Falcone *et al*., 2018). As a result, it raises morbidity, mortality, and medical expenses while decreasing the efficacy of treatment plans (Fair *et al*., 2018).
Globally, antimicrobial resistance is becoming a bigger issue. Antimicrobial resistance-related diseases claimed the lives of an estimated 4.95 million individuals in 2019 (Laxminarayan *et al*., 2022), according to a recent study. Additionally, it is particularly upsetting to see the rapid global expansion of multi-resistant bacteria. To combat the negative effects of multidrug-resistant bacteria on society and health, modern medicine is ultimately focused on developing novel antibiotics derived from natural sources (Bakal *et al*., 2017). Natural goods are a vast source of biologically active ingredients in this regard (Lautié *et al*., 2020). The synthesis of primary and secondary metabolites by plant and mammalian cells, as well as microbes, has impacted the creation of successful therapies for a variety of illnesses and ailments, such as cancer, inflammatory processes, and infectious diseases (Dejani *et al*., 2021). The WHO has identified more than 20,000 medicinal plant species and listed them as potential sources of new pharmaceuticals. More than 100 countries have developed regulations relating to medicinal plants. Apart from the about 30,000 antimicrobial compounds that have been found in plants, there are roughly 1340 plants that have antibacterial properties. Furthermore, it has been estimated that 14–28% of higher plant species had therapeutic qualities, and 74% of the bioactive compounds derived from plants were discovered to have ethnomedical uses. From sea level to an elevation of 1400 meters, Acalypha fruticosa can be found in humid areas, coastal and deciduous bushland, riverine grassland, and woodland grassland. Other names for *Acalypha fruticosa* include Cinnaku, Perim-munja, Siru sinni, Kittik-kilanku, Cinni, Chinee mara, and others. It is referred to as Sotthaachedi, Seethaatthazhai, Aathaathazhai, and so on in Tamil. *Acalypha fruticosa* is a monoecious, aromatic shrub with many branches that grows up to 4 meters tall. Its stems are hairy and initially green before turning reddish-brown. Simple spirally arranged leaves with narrowly lanceolate stipules, 3–4 mm long, brown, petiole 0.5–4.5 cm long, blade broadly ovate to rhombic–ovate, base cuneate to rounded, apex acuminate, margins toothed, sparingly to evenly just hairy on both surfaces, sparingly to evenly yellowish gland-dotted beneath, membranous, veined at the base, and with four to five pairs of lateral veins. The axillary, single spike inflorescence is up to 5 cm long, with 1-4 female flowers interspersed throughout the lower portion and densely packed male flowers at the top, ending with flowers that lack petals, are sessile, and are unisexual.

Female flowers have three ovate-lanceolate blooms, whereas males have four lobed, tiny, thickly white, hairy calyxes and eight stamens. Fruit: 3-lobed capsule with yellow glands scattered throughout, densely short hairs that split into three cocci, each with two valves and one seed (Gupta *et al*., 2003). Seeds are ellipsoid, ovoid, smooth, brown, caruncle, and elliptical. There are also potential medical uses for perennial Acalypha species that bear both male and female flowers on the same inflorescence. Headaches are treated by rubbing a leaf extract on the head. The entire plant is mashed and used to treat scabies, and the root juice is administered to sores. When a cough gets really bad, ground leaves are inhaled. One uses the root infusion as a purgative. For toothaches, the stem and root are chewed. In Southern Africa, a root infusion is used to cure venereal ulcers, fever, and snakebite. An infusion of leaves is used to relieve body swellings and stomach issues. Eye drops made from a maceration of leaves are used to treat eye infections. Stems that have been crushed in water are administered to animal wounds (Farell, 2002). The present study examined the potential antibacterial activity of ethyl acetate, methanol, and petroleum ether extracts of *Acalypha fruticosa* families used by herbalists for primary care against a variety of multidrug-resistant uropathogenic bacteria that can cause catastrophic illnesses in hospitals and the general public.

2. MATERIALS AND METHODS

2.1. Sample Collection

In the Kanyakumari District of Tamil Nadu, India, in the Western Ghats, at Vanniyoor, *Acalypha fruticosa* leaves were gathered. The disease-free samples were taken in the winter season. Taxonomically, the gathered plant was recognised and verified.

2.2. Preparation of plant extracts:

The leaf samples were air-dried after being properly cleaned two or three times with running tap water and once with sterile water. Using a mortar and pestle, five grams of fresh leaves were weighed and ground with five millilitres of ethyl acetate, methanol, petroleum ether, and water solution, respectively. After 15 minutes of centrifugation at 5000 rpm, the solutions were collected, filtered through Whatman number 1 filter paper, and exposed to UV light for an hour. Extracts were diluted in 10% dimethyl sulphoxide (DMSO) (Merck (India) Ltd., Mumbai, India) to a final concentration of 50 mg/ml following full solvent evaporation. They were then kept for later use at 5°C in labelled, sterile screw-capped bottles.

2.3 Preparing and Maintaining the Test Organisms:

The study employed eight human pathogenic microbial strains: *Klebsiella pneumoniae* (MTCC 4030), *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 2990), *Bacillus megaterium* (MTCC 453), *Streptococcus pyogenes* (MTCC 1928), Proteus vulgaris (MTCC 744), *Lactococcus lactis* (MTCC 440), and *Enterobactor aerogens* (MTCC 111). On nutrient agar slants, the bacterial strains were kept alive while being grown in the nutrient broth. After being injected into a Nutrient Agar plate, the pure culture from the plate was subcultured for 24 hours at 37ºC. The fresh culture was aseptically added to a 2 ml sterile 0.145 mol/L saline tube, and the cell density was then adjusted to match the 0.5 McFarland turbidity that standardised inoculum used for antimicrobial test, yielding a bacterial suspension of 1.5×108 cfu/ml.

2.4. Antibacterial Assay of Plant Extracts:

The disc diffusion method was used to examine the antibacterial properties of *Acalypha fruticosa* leaf water, petroleum ether, methanol, and ethyl acetate extracts against various microorganisms. The medium used to cultivate these bacteria was nutrient agar (pH 7.4). After evenly filling the plates with agar medium to a depth of 5 mm, the medium was left to harden. Using a sterile L-rod, the microbial suspensions were applied to the media surface to guarantee the organisms' confluent growth. Whatman No. 1 filter sheets with a diameter of 5 mm were the disc that was utilized. At well-spaced intervals, 10µl aliquots of the leaf's ethyl acetate extract were aseptically applied to the agar's surface after being impregnated in filter paper discs. After 24 hours of incubation at 37°C, the plates were examined.

2.5. HPLC analysis of Plant Extracts:

The HPLC analysis of the high-antimicrobial-activity ethyl acetate extract of *Acalypha fruticosa* was performed. The purity of the crude sample was checked in RP-HPLC. Analysis was carried out in an RP-HPLC using a C_{18} column (250×4.6nm) equipped with a C_{18} guard column. The gel had an average pore size of 300 $A^{0,}$ and its particle size was 5µm. In order to prepare the column for the chromatographic run, the following procedure was carried out. The HPLC grade 65% of acetonitrile and 35% of distilled water with 0.1% of TFF was run for 8 minutes at 1ml/minute. The eluent was run until the column had been equilibrated, as indicated by the baseline stability. The crude sample was filtered through the 0.22mm syringe filter and loaded for each chromatographic run, and the column was run with isocratic elution. Fractions were eluted at the flow rate of 1ml/minute (Phyllis Brown *et al*., 1997).

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2.6. Methodology for Gas Chromatogram-Mass Spectrometry [GC-MS] Analysis

The second fraction of HPLC was analysed using Gas Chromatography-Mass Spectrometry [GC-MS] based on the HPLC results. The fraction was subjected to GC-MS analysis using a GC-MS Clarus 500 Perkin Elmer system, which included an AOC-20i autosampler and a gas chromatograph connected to a mass spectrometer [GC-MS] device under the following circumstances: Helium (99.999%) was used as the carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µl (split ratio of 10:1); injector temperature 2500C; column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 µMdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV. Initially set to 110°C (isothermal for 2 minutes), the oven temperature increased by 10°C/min to 200°C, then by 5°C/min to 280°C, culminating in a 9-minute isothermal at 280°C. At 70 eV, with a 0.5-second scan interval and fragments ranging from 40 to 550 Da, mass spectra were obtained. The National Institute of Standards and Technology (NIST) database was used to interpret the mass spectra of the GC-MS. The unknown component's mass spectrum was contrasted with the NIST library's collection of recognised components. The components of the test materials were identified by name, molecular weight, and structure (Subbarayan Gopalakrishnan et al., 2010).

3.RESULT

3.1 Antibacterial activity of leaf extracts of *Acalypha fruticosa*

The study employed eight human pathogenic microbial strains: *Klebsiella pneumoniae* (MTCC4030), *Salmonella typhi* (MTCC733), *Staphylococcus aureus* (MTCC 2990), *Bacillus megaterium* (MTCC 453), *Streptococcus pyogenes* (MTCC 1928), *Proteus vulgaris* (MTCC 744), *Lactococcus lactis* (MTCC 440), and *Enterobacter aerogens* (MTCC 111). In this regard, the potential of the presence of antibacterial activity of four crude extracts (*Acalypha fruticosa* leaf extracts: ethyl acetate, methanol, petroleum ether and water) was investigated. The extracts were subjected to antibacterial assays against eight different bacteria to determine and to compare the antibacterial activities between species. The antibacterial activity of plant extracts was detected by the indication of zone around the disc. The eight bacterial pathogens exposed to the plant extracts, *Enterobacter aerogens* showed higher inhibition, while the other bacterial strains showed lower inhibition. The results demonstrated differences in antibacterial activities as shown by the differences in the mean zone of inhibition. (Table 1 and Figure 1.) The results from this experiment were used to assess the antibacterial potential of each crude extract and to determine which extract would be used for further studies. The result shows high activity in ethyl acetate extract (3.6mm) against *Enterobacter aerogens,* followed by methanol extract (3mm) against the same bacterium. Ethyl acetate is the most effective of these four solvents.

Figure 1: Antibacterial activity of various solvent extracts

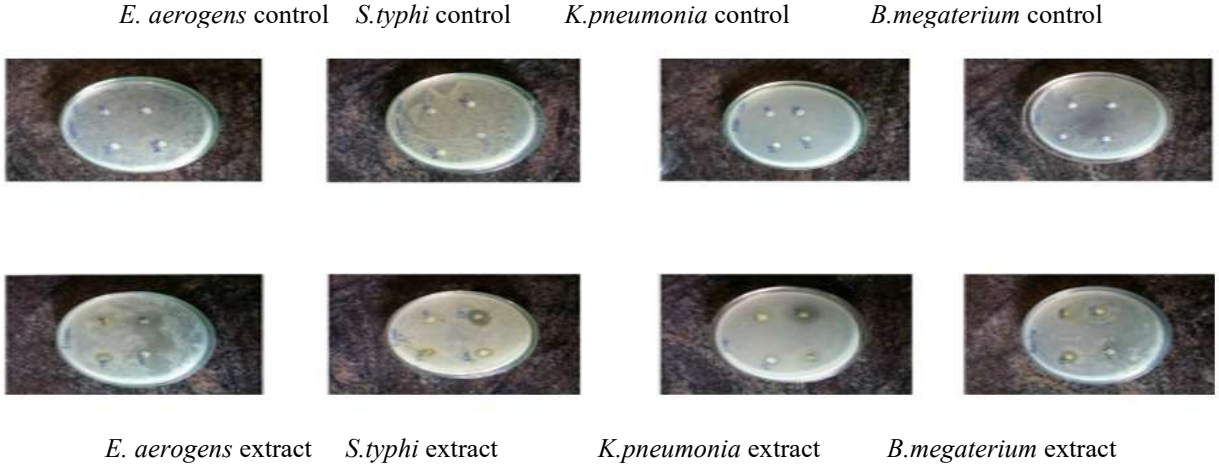

Table- 1: Antibacterial activity of various solvent extracts of *Acalypha fruticosa*

Microorganisms	**Zone of inhibition in 10µl Sample(mm)**			
	Ethyl Acetate	**Methanol**	**Petroleum Ether**	**Water**
Enterobacter aerogens	**3.6**	**3.0**	**1.2**	**0.5**
Salmonella typhi	**2.2**	**1.2**	**1.2**	**0.6**

Klebsiella pneumonia	**1.0**	**0.5**	**1.3**	**0.6**
Bacillus megaterium	**1.8**	**0.6**	**1.6**	**0.9**
Streptococcus pyrogens	**2.3**	**0.5**	**0.5**	**0.5**
Proteus vulgaris	**3.2**	**0.5**	**2.3**	**0.7**
Staphylococcus aureus	**1.8**	**0.6**	**0.6**	**0.5**
Lactobacillus lactis	**2.0**	**3.0**	**0.5**	**0.5**

In one way or another, medicinal shrubs are plants that give people medicine to keep them healthy and prevent disease. According to Jayaweera (1981), there are further uses in ritual healing, body care, and nutrition. According to Despande R. Ret al. (2011), plants have chemicals that participate in metabolic processes and help combat bacterial infections. Since they can accomplish their intended function without the negative side effects that are frequently connected to synthetic antimicrobials, plant-based antimicrobial molecules offer immense therapeutic promise. This study showed that the leaf extract of *Acalypha fruticosa* has antibacterial properties. Except for ethyl Acetate, all other solvent extracts showed a lower inhibition zone diameter against all bacterial strains. *Acalypha fruticosa* exhibits strong antibacterial activity against Enterobacter aerogens in ethyl acetate extract, followed by methanol extract against the same bacterium. Thambi Raj et al. (2011) reported that three alcoholic extracts of the stem portion of *Acalypha fruticosa* exhibited strong antibacterial activity against the organisms under test. Additionally, using the agar diffusion method, Mothana et al. (2010) reported that the methanolic extracts of *Acalypha fruticosa* demonstrated antimicrobial activity against three Gram-positive bacteria, two Gram-negative bacteria, one yeast species, and three multiresistant *Staphylococcus* strains, with inhibition zones greater than 14 mm. *Acalypha fruticosa* was discovered to have a higher potential as an antibacterial agent against a variety of Gram-negative bacteria, including *Klebsiella pneumoniae* and *Salmonella typhi*. *Acalypha fruticosa's* methanolic extract can be used as an antibacterial agent to treat infectious diseases brought on by microorganisms (Duraiarasan et al., 2011). Additionally, an investigation into the antibacterial properties of various Indian medicinal plants revealed that *A. fruticosa* was among the most effective plants tested (Duraipandiyan et al., 2006). Additionally, Shubashini et al. (2010) demonstrated that *Acalypha fruticosa* has the strongest antibacterial activity against *Streptococci sp*., *Escherichia coli,* and *Proteus sp*.

3.2. High-Performance Liquid Chromatography (HPLC) of *Acalypha fruticosa*

The crude extract of *Acalypha fruticosa* in ethyl acetate solvent was tested to purify the compounds. From the crude extract, seven fractions were obtained. The second fraction showed the highest peak, and the retention time is 2.917 minutes. The results were shown in Table 2 and Figure 2. The crude ethyl acetate extract of *Acalypha fruticosa* was subjected to HPLC analysis. The purity of the extract was analysed. Seven fractions were obtained, and the second fraction showed the highest peak. Monajjemi et al. (2012) reported that four Iranian medicinal plants showed highly different phenolic content based on HPLC analysis

Figure-2: HPLC Analysis of *Acalypha fruticosa* in Ethyl Acetate Extract

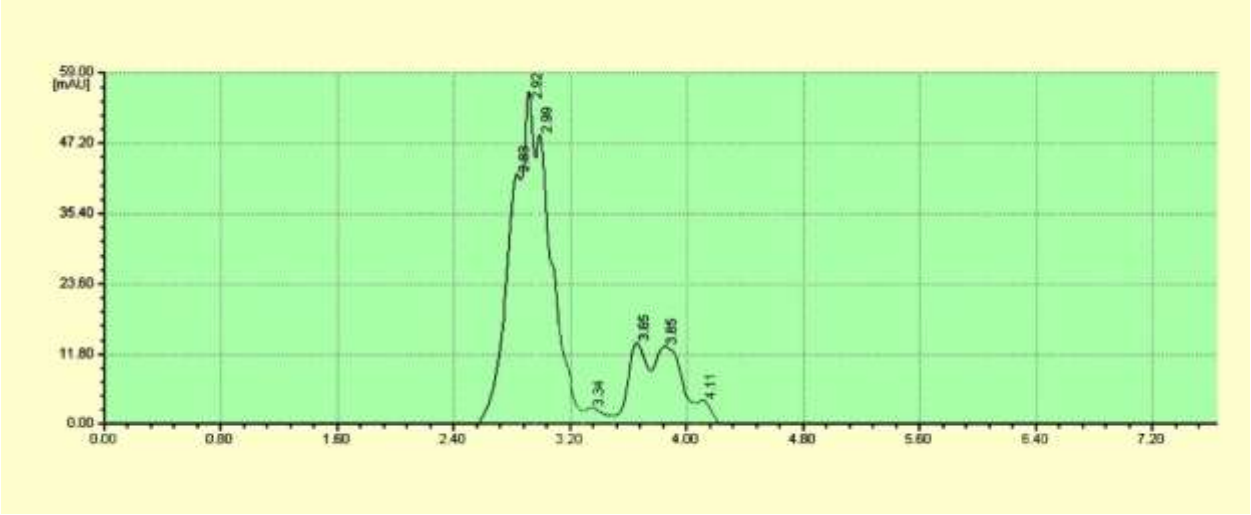

Table-2: HPLC Analysis of *Acalypha fruticosa* in Ethyl Acetate Extract fractions values

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Parameter		Result	Group Result	Sample Inf	Conc	Half width (S)	Res	Theo Plate	Tail Fact
ID	Name	RT (min)	Height	Area					
6	4	2.833	4391	35759.5	24.1955	8.14	0.00	2412.48	0.55
2		2.917	5772	25869.8	17.5039	4.48	0.47	8440.42	0.92
3		2.992	5045	43299.6	29.2972	8.58	0.41	2421.62	5.00
4		3.342	456	4406.3	2.9814	9.66	1.35	2383.55	2.00
5		3.653	1529	14714.1	9.9558	9.62	1.14	2872.51	0.81
6		3.845	1462	19396.0	13.1236	13.27	0.59	1674.17	1.68
7		4.112	551	4349.0	2.9426	7.89	0.89	5408.63	4.08
		xU1/4E:	i/E=19206	i/E=147794.4	i/E=100.0000				

3.3. GC-MS ANALYSIS

GC-MS analysis indicated that the second fraction of high-performance chromatography contained about 20 peaks obtained from *Acalypha fruticosa* leaves. Twenty compounds were found in the ethyl acetate leaf extract of *Acalypha fruticosa*, according to the GC-MS analysis. The compounds have been displayed (Table 3 and Figure 3) along with their Retention Time (RT) and Peak Area (%). The chromatogram's % Peak Area was used to identify the main phytochemicals. The major constituents were identified as 3, 3-Difluropentane, N-Methoxy-n-methylacetamide, Timonacic, Ethyl 3-ethyl pentanoate, 1,2-Ethanediol, monoacetate, 2-pentanol, Oxime, methoxy-phenyl, 1, 2, 4-Trithiolane, 3, 5-bis (1-methylethyl), Clovane, Myristoleic acid methyl ester, Pent-4-enal oxime, Ethanone, 1-cyclopropyl, oxime, 2-aminoadamantane - 2 - thioamide, 2 – Methoxy – 4 - methylbenzaldehyde, Dictyocertin-A and 1-Dodecanol. Minor constituents were identified as 3-Chlorooctylacetate, Malonamic acid, Acetic acid, ethyl ester, Thiambutosine and 1-Allylazetidine. The results were shown in Table 3 and Figure 3. As shown in Figure 3 and Table 3, the GC-MS spectra verified the existence of many components with varying retention periods. To determine the nature and structure of the molecules, the mass spectrometer examines the chemicals that elute at various times. The formation of peaks at various m/z ratios results from the big compound breaking up into smaller compounds. These mass spectra serve as the compound's fingerprint, which the data library can identify. The current study aids in the formulation and structural prediction of twenty biomolecules. Additional research may result in the isolation of bioactive compounds, and subsequent medication development will benefit from the structural clarification of these compounds as well as the screening of their pharmacological activity. Alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are examples of secondary metabolites of plants that act as a defence mechanism against predictions made by numerous insects, microbes, and other herbivores. Alkaloids had significant biological impacts on both human and animal organisms at very low dosages. Along with neuropharmacological, antibacterial, antifungal, and other actions, they displayed anti-inflammatory, anticancer, analgesic, local anaesthetic, and pain-relieving qualities.

Figure 3: GC-MS Chromatogram for *Acalypha fruticosa*

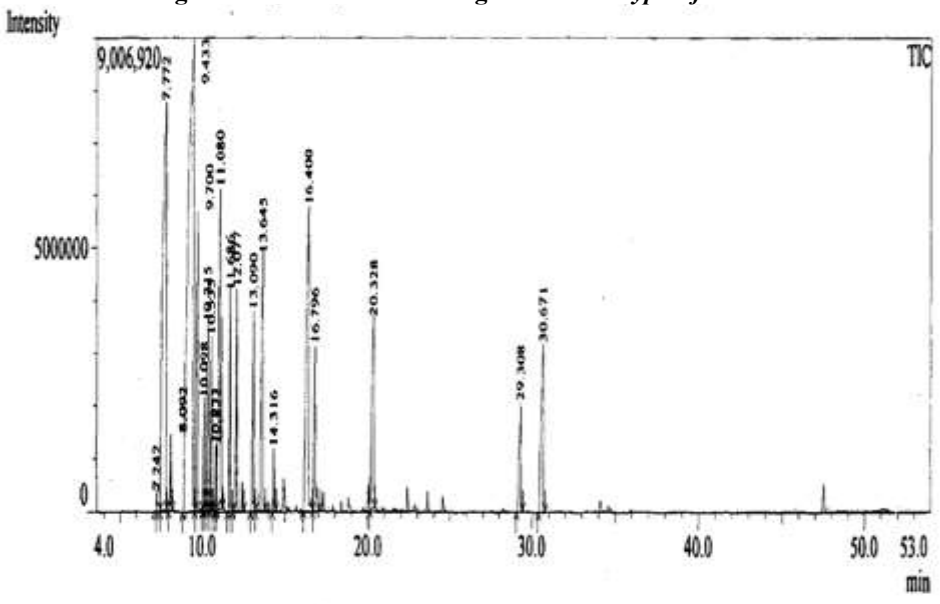

Table-3: GC-MS Chromatogram for *Acalypha fruticosa*

Sl.No	Compound	Percentage(%)	RT
1	3,3-Difluropentane,N-Methoxy-n-methylacetamide	13.93	16.40
2	3-Chlorooctylacetate	14.06	1.24
3	Timonacic	3.92	13.64
4	Ethyl 3-ethyl pentanoate	22.69	16.79
5	Malonamic acid	4.80	7.77
6	1,2-Ethanediol,monoacetate	3.15	14.31
7	Acetic acid,ethyl ester	3.54	8.09
8	2-pentanol	3.32	29.30
9	Thiambutosine	6.86	9.43
10	Oxime,methoxy-phenyl	9.55	12.07
11	1,2,4-Trithiolane,3,5-bis(1-methylethyl)	43.73	13.09
12	Clovane	4.74	20.20.2
13	Myristoleic acid methyl ester	4.35	10.09
14	Pent-4-enal oxime	7.40	10.33
15	Ethanone, 1-cyclopropyl,oxime	3.29	11.68
16	2-aminoadamantane-2-thioamide	10.50	10.17
17	2-Methoxy-4-methylbenzaldehyde	6.00	30.67
18	Dictyocertin-A	8.30	10.74
19	1-Allylazetidine	7.19	9.70
20	1-Dodecanol	9.83	19.02

4. CONCLUSION

This study showed that the *Acalypha fruticosa* leaf extract inhibited the growth of various tested species of Gram-positive and Gram-negative bacteria. Generally, I can conclude that *Acalypha fruticosa* leaves, ethyl acetate crude extract, have antibacterial activity. The *Acalypha fruticosa* leaves have an effective source for making drugs against lower-respiratory infections, pneumonia, urinary tract infections, infections of the skin and underlying tissues, prolonged fever, vomiting, diarrhoea, muscle pain, and diseases caused by pathogenic microorganisms, especially the ethyl acetate showed high activity against *Enterobacter aerogens* bacterial strain.

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