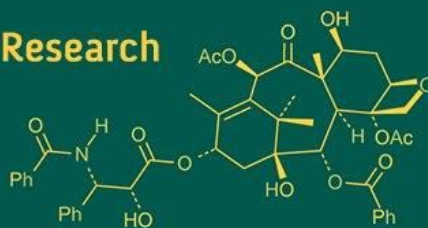
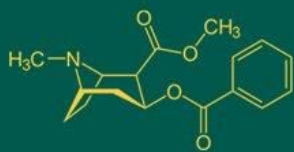


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Comparison between the effect of some plant extracts and ciprofloxacin and fluconazole against some bacterial and fungal species

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Abstract

Background: The growing incidence of antibiotic-resistant bacteria has prompted research into alternatives in natural medicine, as plants and their products are used to solve various health problems.

Objectives: The study compares the antimicrobial properties of plant extracts from *Allium sativum*, *Salvia officinalis*, and *Mentha piperita* with antibiotics Ciprofloxacin and Fluconazole against various bacterial and fungal species.

Materials & Methods: This study utilized three medicinal plants and five bacterial types from burn patients at Al-Fayhaa Hospital, notable resistant bacteria include *Escherichia Coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Results: Plants *A. sativum*, *S. officinalis*, and *M. piperita* showed antimicrobial activity against pathogens, while *C. tropicalis* showed significant antifungal effectiveness against *Candida* species, with *C. tropicalis* being more sensitive.

Conclusion: The study reveals that extracts from *A. sativum*, *S. officinalis*, and *M. piperita* effectively combat various pathogens, including bacteria and *Candida* species, using piperine, eucalyptol, and carvone.

Keywords: *Allium sativum*, *Salvia officinalis*, *Mentha piperita*, *Escherichia Coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*

Introduction

Each year hundreds of thousands of people die, due to a universal resistance for antibiotics when it comes to bacteria and new strains that no longer can be treated by standard medicine the consequences being recognized as serious global health risks. In 2014, the World Health Organisation identified increased mortality from communicable disease as a critical global public health issue [1]. Increasing glyconvenient access to potent antibiotics including colistin and carbapenem, used not only where necessary but throughout the agri-food system unchecked [2, 3] fuelled a huge rise in global antibiotic consumption from 2000-2015 (65%), helping create resistant bacterial strains that it is impossible or demanding (requiring doses of several other drugs together) to treat. In response to this increasing challenge, medicinal plants previously used for centuries in traditional medicine might be useful source of new antimicrobials [4, 5]. The bioactive compounds present in these plants could be potential sources of resistance-breaking pharmaceuticals. Despite the historical use of natural medicine for a long period, there has been little research, and their consumption is declining which highlights that caution should be applied in order to make awareness programs on herbal products with regard to plant active compounds taking into consideration.

Antibiotics have revolutionized medicine, particularly while treating bacterial illnesses such Amoxil, cephalosporin and ciprofloxacin. Nevertheless, when we see the reality of increasing antibiotic resistance it is one of a number of challenges that limit our ability to develop antibacterial drugs at the same time as these key bacterial pathogens become resistant [6,7]. This situation has also reawakened the attention to traditional medicine and herbal products, due to its historical role in human health [8].

Ciprofloxacin, a fluoroquinolone antibiotic, is FDA-approved for a number of bacterial illnesses, including as pneumonia, urinary tract infections, and several others [9]. Notably, *Neisseria gonorrhea* exhibits high resistance rates to ciprofloxacin, making it unsuitable as a first-line therapy for respiratory infections caused by *Streptococcus pneumoniae* that is responsive to penicillin. Nonetheless, patients with mixed infections or those at risk for Gram-negative infections should still take ciprofloxacin [10]. Ciprofloxacin is used off-label for Crohn's disease linked to perianal fistula and can be used to treat both acute and chronic bacterial prostatitis, according to the American Academy of Family Physicians guidelines [11].

Ciprofloxacin is a bactericidal antibiotic in the fluoroquinolone class: Ciprofloxacin is a bactericidal antibiotic belonging to the fluoroquinolone class, primarily effective against gram-negative bacilli, such as Enterobacteriaceae (e.g., *E. coli*, *Salmonella spp.*, *Shigella spp.*, and *Neisseria*). It functions by blocking DNA-gyrase and bacterial DNA topoisomerase, crucial for DNA replication. Ciprofloxacin is particularly potent against *Pseudomonas aeruginosa*, although there are reports of decreasing susceptibility, especially in hospital settings [12]. It is commonly used for infections caused by *S. aureus*, but increasing resistance to fluoroquinolones has prompted efforts to enhance their efficacy or find alternatives [13]. The search for natural sources has led to the extraction of drugs from traditional herbs abundant in tropical regions [14].

Fluconazole exhibits fungistatic activity against various fungi, including *Candida* species and *Cryptococcus neoformans*. It interferes with cell wall synthesis and adhesion, treating fungal infections effectively [15]. The cytochrome P-450 enzyme 14-demethylase, which transforms lanosterol into ergosterol a crucial part of the fungal cell membrane is the target of fluconazole. Fluconazole enhances cellular permeability and interferes with fungal respiration and yeast development by preventing ergosterol synthesis. In fungi, the build-up of 14-methyl sterols corresponds with the loss of sterols, contributing to its fungistatic effects [16].

Materials and Methods

Sample collection

In this study, three medicinal plants were used: *A. sativum*, *S. officinalis*, and *M. piperita*. Five micro-organisms were isolated from burn patients who were admitted to Al-Fayhaa Hospital: also, two *Candida* species. Three medicinal plants were prepared by grinding garlic cloves, sage leaves, and mint leaves into fine powders, after which a Soxhlet

extractor was used in making the plant extracts.

Identification of bacterial & fungal species

The swabs from the patients and the healthy students were first cultured on MacConkey agar and blood agar media, after which an attempt at identification of five bacterial species was made using the automated VITEK2, followed by subculture on HiCrome *Candida* Differential Agar for rapid identification.

Evaluation of plant extracts' antibacterial efficacy

Disc diffusion method: The disc diffusion method was used to assess the antibacterial activity of studied plant extracts. Dissolved extracts were spread over Mueller Hinton agar plates, and discs impregnated with the extracts were placed on the media.

Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of plant extracts against gram negative, gram-positive bacteria, and *Candida* species was determined using the MHB broth micro-dilution method. Bacterial cell viability and MIC values were determined using turbidity tests. The MIC of the extracts was determined using a similar procedure, using bacteria that had been cultivated overnight suspended in sterile nutrient broth and incubated for two to four hours at 37 °C.

Results

The study assessed the antibacterial properties of the chosen herbs using seven pathogens. There were four species of bacterial pathogens were Gram negative (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*) and one was Gram positive (*S. aureus*), of which were identified by VITEK device. Also two *Candida* species have been used which identified by HiCrome *Candida* medium according to colonies color; *C. albicans* (green) and *C. tropicalis* (blue) (Figure 1). The study evaluated the antimicrobial effects of selected herbs against seven pathogens, various types of *Candida* and both Gram-positive and Gram-negative bacteria. The findings indicated that *A. sativum*, *S. officinalis*, and *M. piperita* shown antibacterial efficacy against every pathogen examined. Considerable antifungal efficacy was noted against *C. albicans* and *C. tropicalis*, with *C. tropicalis* being more sensitive. The highest activity was recorded against *C. tropicalis*, *C. albicans*, and *E. coli* (Table 1 & Figure 2).



Fig 1: Colonies of *C. albicans* and *C. tropicalis* on HiCrome *Candida* agar

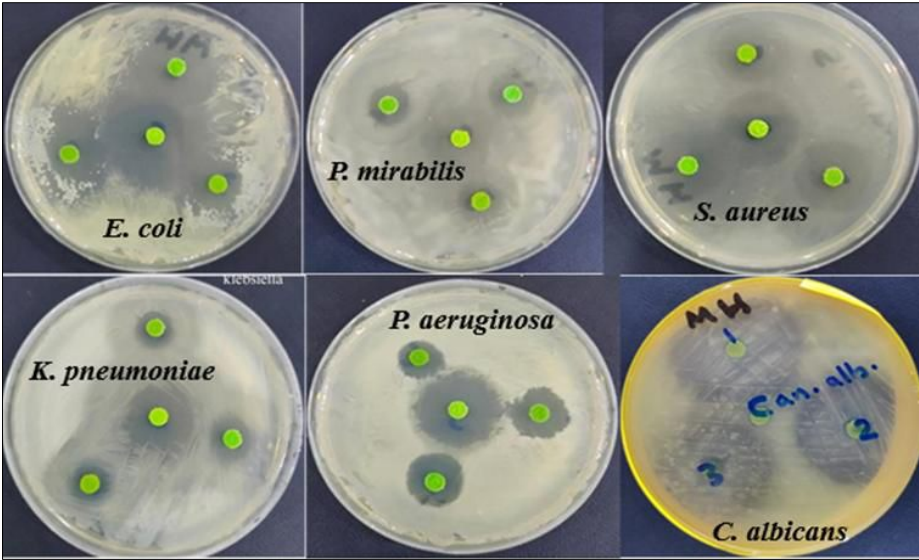


Fig 2: Growth inhibition of some microbial species caused by plant extracts

Table 1: Effects of antimicrobial agents against bacteria and fungi

Extract	Bacterial & Fungal Species						
	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
	Inhibition zone diameter in mm						
<i>A. sativum</i>	17	13	24	17	14	40	44
<i>S. officinalis</i>	13	10	22	18	13	35	40
<i>M. piperita</i>	9	13	15	14	11	30	40

The study found ciprofloxacin's effectiveness against bacterial species varied compared to plant extracts, while fluconazole demonstrated high efficacy against *C. tropicalis*, but *C. albicans* exhibited resistance (Table 2 & Figure 3).

Table 2: Antimicrobial agents' effects on bacteria and fungus

Antimicrobial Agent	Bacterial & fungal species						
	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
	Inhibition zone diameter in mm						
Ciprofloxacin	13	20	30	25	20		
Fluconazole						R	40

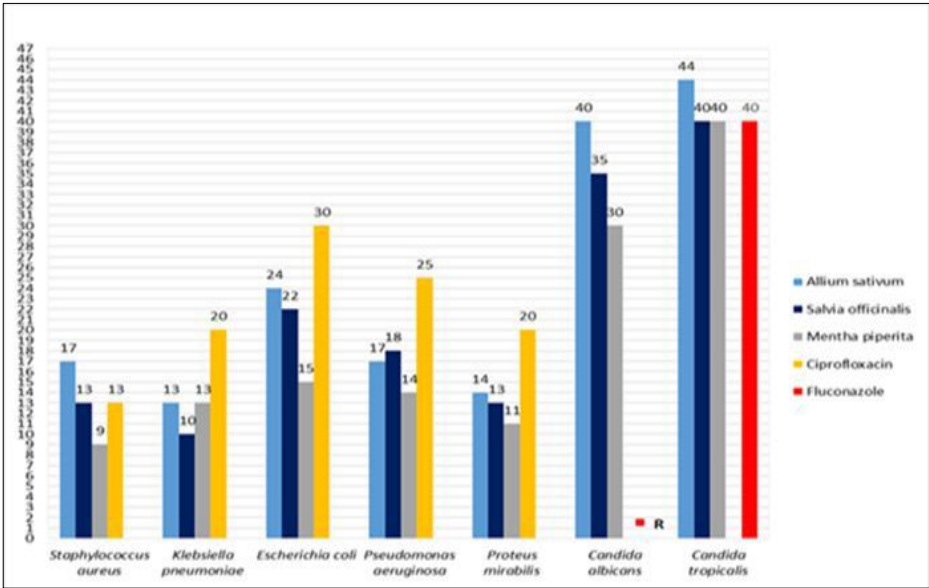


Fig 3: Effects of extracts and antimicrobial agents against bacteria and fungi

Determination MIC and *in vitro*

Table 3, displays the MIC of plant extracts, with *M. piperita* extract showing the lowest inhibitory concentration on *E. coli* and *S. officinalis* extract having the lowest inhibitory concentration against *C. tropicalis*.

Table 3: MIC values of plant extracts on some bacterial and fungal species

Bacterial & Fungal Species	Plant Extract		
	<i>A. sativum</i>	<i>S. officinalis</i>	<i>M. piperita</i>
<i>S. aureus</i>	0.01	0.001	0.001
<i>E. coli</i>	0.1	0.01	0.00001
<i>K. pneumonia</i>	0.01	0.1	0.01
<i>P. aeruginosa</i>	0.1	-	-
<i>P. mirabilis</i>	0.01	0.01	0.1
<i>C. albicans</i>	0.1	0.001	0.1
<i>C. tropicalis</i>	0.001	0.00001	0.001

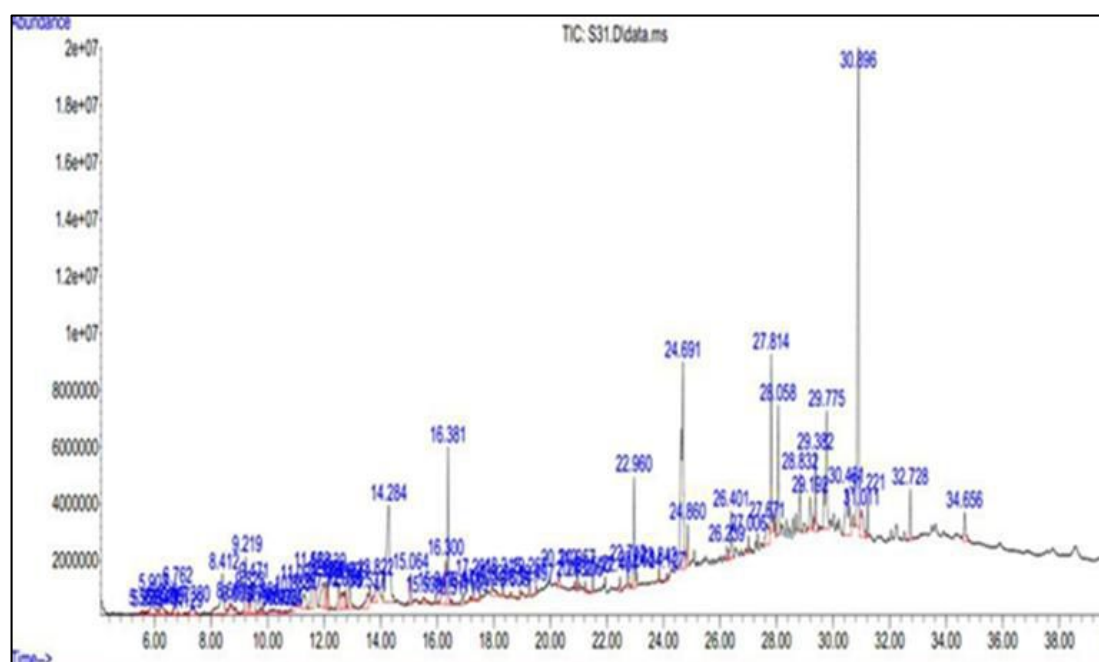
GC-MS Analysis

The analysis of *Allium sativum*, an ethanolic plant extract, was conducted using gas chromatography and spectrometry for precise compound detection. GC-MS for ethanolic

extract of *A. sativum* showed the presence of 45 peaks (Figure 4). The major peak belong to compound piperine that was found in considerable quantity (26.683%), (Table 4).

Table 4: Components detected in fractions of *A. sativum* by GC-MS

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID
1	5.907	3498069	0.917	0.9165	Formamide, N-butyl-
2	6.762	3396256	0.89	0.8899	Furfural
3	7.38	2700032	0.707	0.7075	2-Furanmethanol
4	8.412	13040712	3.417	3.4169	Disulfide, methyl 2-propenyl
5	9.219	7555041	1.98	1.9795	3H-1,2-Dithiole
6	9.328	2435257	0.638	0.6381	2-Furancarboxaldehyde, 5-methyl-
7	9.471	5827376	1.527	1.5269	Dimethyl trisulfide
8	10.883	2185820	0.573	0.5727	Benzeneacetaldehyde
9	11.297	4143696	1.086	1.0857	2-Imidazolidinone, 1,3-dimethyl-
10	11.568	6558215	1.718	1.7184	Furaneol
11	11.969	7108549	1.863	1.8626	(Z)-1-Allyl-2-(prop-1-en-1-yl)disulfane
12	12.064	4516232	1.183	1.1833	3-Methyl-3H-1,2-dithiole
13	12.512	4750556	1.245	1.2447	Cyclopentasiloxane, decamethyl-
14	12.688	4191862	1.098	1.0983	Trisulfide, methyl 2-propenyl
15	13.822	3891291	1.02	1.0196	4H-1,2,3-Trithiine
16	14.284	30072174	7.879	7.8794	5-Hydroxymethylfurfural
17	15.064	3483693	0.913	0.9128	Cyclohexasiloxane, dodecamethyl-
18	16.3	10105535	2.648	2.6478	5-Methyl-1,2,3,4-tetrathiane
19	16.918	1866225	0.489	0.489	Caryophyllene
20	22.742	2299576	0.603	0.6025	2,4-Decadienamide, N-isobutyl-, (E,E)-
21	22.96	10801462	2.83	2.8302	n-Hexadecanoic acid
22	24.691	42763796	11.205	11.2048	Oleic Acid
23	24.86	3962741	1.038	1.0383	Octadecanoic acid
24	26.401	5154639	1.351	1.3506	Erucic acid
25	28.058	13072801	3.425	3.4253	Erucic acid
26	30.481	13946817	3.654	3.6543	1-Eicosene
27	30.896	60606381	15.88	15.8798	Piperine
28	31.011	6813317	1.785	1.7852	9-Octadecenoic acid, (E)-

**Fig 4:** GC-MS chromatogram peaks of *A. sativum*

Salvia officinalis

GC-MS for ethanolic extract of *S. officinalis* showed the presence of 59 peaks (Figure 5). The major peak belong to

compound eucalyptol that was found in considerable quantity (15.192%), (Table 5).

Table 5: Components detected in fractions of *Salvia officinalis* by GC-MS

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID
1	8.792	8240732	0.65	0.6499	alpha.-Pinene
2	9.26	13605453	1.073	1.0729	Camphene
3	10.047	11456406	0.903	0.9035	beta.-Myrcene
4	10.747	13814624	1.089	1.0894	p-Cymene
5	10.957	192644631	15.192	15.192	Eucalyptol
6	12.22	5905137	0.466	0.4657	Thujone
7	12.953	64297504	5.071	5.0705	(+)-2-Bornanone
8	13.34	18306651	1.444	1.4437	endo-Borneol
9	13.679	24072945	1.898	1.8984	alpha.-Terpineol
10	14.175	13382804	1.055	1.0554	5-Hydroxymethylfurfural
11	14.99	9052652	0.714	0.7139	Bornyl acetate
12	15.838	9253276	0.73	0.7297	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
13	15.947	24265965	1.914	1.9136	Eugenol
14	16.911	54782676	4.32	4.3202	Caryophyllene
15	17.216	33431005	2.636	2.6364	4-Vinylbenzene-1,2-diol
16	18.153	9359242	0.738	0.7381	cis-Calamenene
17	19.002	12067583	0.952	0.9517	Caryophyllene oxide
18	19.993	10868103	0.857	0.8571	14-Hydroxycaryophyllene
19	21.656	8872561	0.7	0.6997	Neophytadiene
20	22.987	28720177	2.265	2.2649	n-Hexadecanoic acid
21	23.38	6043989	0.477	0.4766	9-Hexadecenoic acid
22	24.27	8346775	0.658	0.6582	Labda-7,14-dien-13(R)-ol
23	24.697	60700193	4.787	4.7868	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
24	24.867	6820230	0.538	0.5378	Octadecanoic acid
25	25.994	7206941	0.568	0.5683	Kolavenol
26	26.395	7206396	0.568	0.5683	Ferruginol
27	26.748	11935283	0.941	0.9412	12-Methoxycarnosic acid
28	29.796	9597377	0.757	0.7569	5,7-Diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ol
29	30.617	6975448	0.55	0.5501	Nonacosane
30	30.895	20766064	1.638	1.6376	Piperine
31	32.206	8029830	0.633	0.6332	Hexadecane, 1-iodo-
32	32.769	6708028	0.529	0.529	delta.5-Androsten-3,17-dione
33	33.896	5810141	0.458	0.4582	Dotriacontane, 2-methyl-
34	34.358	14446956	1.139	1.1393	Triacontane
35	34.69	17973326	1.417	1.4174	gamma.-Sitosterol
36	35.417	23598550	1.861	1.861	Germanicol
37	35.756	6329853	0.499	0.4992	28-Norolean-17-en-3-ol
38	35.973	14179145	1.118	1.1182	Lupeol

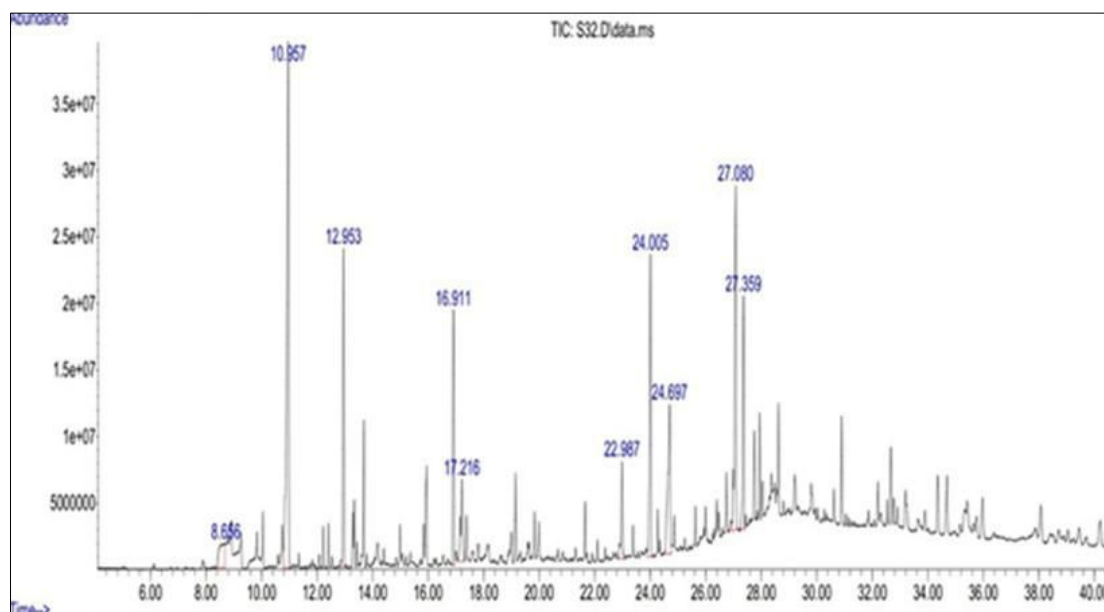


Fig 5: GC-MS chromatogram peaks of *S. officinalis*

Mentha piperita: GC-MS for ethanolic extract of *M. piperita* showed the presence of 54 peaks (Figure 6). The

major peak belong to compound D-carvone that was found in considerable quantity (12.852%), (Table 6).

Table 6: Components detected in fractions of *M. piperita* by GC-MS

Peak	R.T.	Area	Area Pct	Library/ID
1	9.844	3663590	0.44	beta.-Pinene
2	10.828	35608203	4.2763	D-Limonene
3	10.91	8326553	1	Eucalyptol
4	14.189	9586257	1.1512	5-Hydroxymethylfurfural
5	14.467	1.07E+08	12.852	D-Carvone
6	15.051	10121286	1.2155	Cyclohexasiloxane, dodecamethyl-
7	16.402	4283372	0.5144	(-)-.beta.-Bourbonene
8	16.897	20614090	2.4756	Caryophyllene
9	17.97	4600047	0.5524	Thymoquinone
10	18.439	4537704	0.5449	4,4'-Bipyridine
11	21.67	50424607	6.0556	Neophytadiene
12	21.914	7925072	0.9517	9-Octadecen-1-ol, (Z)-
13	22.104	16830741	2.0212	Neophytadiene
14	22.973	22217551	2.6682	n-Hexadecanoic acid
15	24.365	20736504	2.4903	Phytol
16	24.854	5745126	0.6899	Octadecanoic acid
17	26.239	3483311	0.4183	Oxazepam, 2TMS derivative
18	26.334	9746821	1.1705	(+)-Dihydrocarveol, trimethylacetate
19	26.707	14266186	1.7133	3-Carene
20	29.674	9519219	1.1432	Neophytadiene
21	29.776	6055552	0.7272	.beta.-Bisabolenol
22	30.027	12110878	1.4544	Squalene
23	30.366	3590303	0.4312	Octacosane, 2-methyl-
24	30.624	5789253	0.6952	1-Hexacosene
25	30.862	19271100	2.3143	Piperine
26	31.866	3833493	0.4604	2-Methyltriacontane
27	32.199	3515232	0.4222	Eicosane
28	32.538	32242705	3.8721	Vitamin E
29	33.631	5331647	0.6403	Campesterol
30	33.917	6801328	0.8168	Stigmasterol
31	34.738	81780577	9.8212	.gamma.-Sitosterol

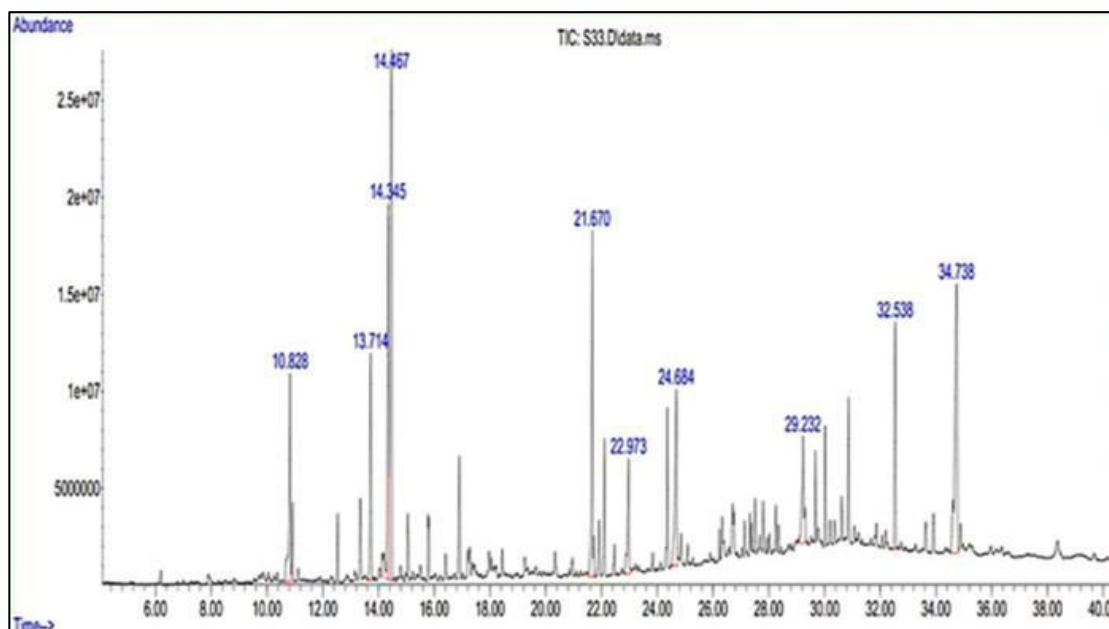


Fig 6: GC-MS chromatogram peaks of *M. piperita*

Discussion

The present investigation examined the antibacterial properties of extracts from *Allium sativum*, *Salvia officinalis*, and *Mentha piperita* against *Candida* species (*C. albicans* and *C. tropicalis*) and harmful bacteria (*Escherichia Coli*, *Klebsiella pneumoniae*, *Proteus*

mirabilis, *Pseudomonas aeruginosa*). As shown in (Table 1) and MIC values in (Table 3), all plant extracts inhibited both Gram-positive and Gram-negative bacteria, with significant action against the *Candida* species.

The antimicrobial effects of *A. sativum* are attributed to high concentrations of piperine (26.683%), a major alkaloid

known for its bioavailability-enhancing, antioxidant, and antimicrobial properties [17]. The extract showed maximum efficacy against *C. tropicalis*, *C. albicans*, and *E. coli*, with inhibitory zones measuring 44 mm, 40 mm, and 24 mm, respectively, consistent with findings by Aldaly [18]. The sensitivity of *E. coli* to piperine may be due to its highly permeable membrane, which allows piperine to alter cell wall permeability [19]. Additionally, piperine inhibits lipid accumulation in yeast, reducing cell growth [20], and inhibits DNA and protein synthesis [21]. Additionally, it inhibits efflux pumps, contributing to bacterial resistance to antibiotics [22].

The antimicrobial activities of *S. officinalis* are linked to high concentrations of eucalyptol (15.192%), a bicyclic terpenoid present in various essential oils [23]. Eucalyptol's effectiveness against both Gram-negative and Gram-positive bacteria, such as *S. aureus* and Gram-negative strains including *E. coli* and *P. aeruginosa*, is mainly due to its ability to permeabilise cell membranes [24, 25]. Given the increasing problem of antimicrobial resistance and biofilm formation among bacteria, natural compounds like eucalyptol are gaining attention for their potential to address these challenges [26]. Eucalyptol displays antibacterial properties against Gram-positive and Gram-negative pathogenic strains, including *Proteus mirabilis*, because of its capacity to cause cell membrane permeabilisation [27]. Its hydrophobic nature has been found to effectively combat *Klebsiella pneumoniae* isolates, demonstrating antimicrobial properties against bacteria with outer lipopolysaccharide membranes [28]. Carvone, a naturally occurring compound, has shown promise as an antibacterial and antifungal agent [29, 30]. It exhibits antibacterial activity against various bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) [29], by increasing cell permeability and disrupting cell membrane integrity [29, 30]. Carvone also demonstrates an antibiofilm effect against *S. aureus* [30]. Its antifungal properties have been studied against *Candida* species, mycotoxigenic fungi such as *Fusarium*, *Aspergillus*, and *Penicillium*, and dermatophytes such as *Microsporum*, *Trichophyton*, and *Epidermophyton* [31]. Like *Trichophyton*, *Epidermophyton*, and *Microsporum* [31].

While some studies have shown carvone's effectiveness against certain bacterial strains [29, 30], others have found no activity against *Staphylococcus* strains [32]. Previous research from Ethiopia focused on antibacterial activity but excluded antifungal activity and methanol in the extraction process [33-35]. However, studies from Ghana and Pakistan have reported that methanol extracts exhibited strong antimicrobial activity [33, 36].

Carvone and other natural antibacterial substances have a great deal of potential for treating infections brought on by drug-resistant bacteria [34]. Natural antimicrobial compounds offer the Global public health is seriously threatened by the rise of multidrug-resistant bacteria such as *S. aureus*, *E. coli*, and *P. aeruginosa* [34]. Advantage of lower resistance development compared to traditional antibiotics [23]. This raises the question of whether natural compounds could serve as viable alternatives to conventional drugs and whether they will prove effective [23].

Conclusions

According to the study, extracts of *A. sativum*, *S. officinalis*, and *M. piperita* inhibit a variety of infections, such as

bacteria and species of *Candida*, through piperine, eucalyptol, and carvone, respectively.

Recommendations

Further studies on plant extracts are needed for isolation, purification, and identification of bioactive compounds for antibiotic and antifungal activities. Further studies have to be conducted against other pathogens and also *in vivo* to confirm their effectiveness.

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