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Identifying the Active Chemical Ingredients in Extracts of Certain Plants with Medicinal Properties used for the Treatment of Intestinal Diseases

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ABSTRACT

Traditionally, in many places, including Saudi Arabia, the plants Salvia officinalis, Artemisia herba alba, and Teucrium polium have been used to treat different diseases, especially intestinal ones. All three plant-specific water extracts were analysed using FTIR and GC-MS. Several flavonoids, phytosterol and phenolic acids have been identified. In a time-dependent growth suppression experiment, Teucrium polium (O1), Salvia officinalis (O2), and Artemisia herba-alba (O3) were examined for antibacterial activity. The growth curves of Pseudomonas aeruginosa and Staphylococcus aureus at O1, O2, and O3 revealed that these extracts may suppress bacterial growth and reproduction. O1, O2, and O3 had concentration-dependent effects on test microorganisms, with O1 inhibiting P. aeruginosa at 87% and S. aureus at 74% at the minimum inhibitory dose. At 0.5xMIC O1, very equal inhibition was observed. At MIC, the O_2 extract reduces 88%, but at 0.5xMIC, it reduces 63% and 60%. O_3 showed a 10% and 22% difference against P. aeruginosa and S. aureus at MIC and 0.5xMIC, respectively. It has also extended the plant's therapeutic advantages, opening up new applications.

Key words: Antibacterial Activity, Diseases, HPLC, Time-Dependent Growth. Biofilm Assays.

INTRODUCTION

Teucrium polium L.Teucrium polium L. (O1), sometimes known as Germander, is a perennial member of the Lamiaceae family[1, 2]. This plant originates in the Mediterranean, Southwest Asia, Turkey, and North Africa[3,4] and is also used in traditional medicine due to its medicinal characteristics. Antibacterial, anticancer, antioxidant, and analgesic. Its low-glycaemic properties make it an ideal diabetes treatment. Flavonoids, terpenoids, and essential oils all contribute to the plant's medicinal benefits. Antioxidants and anti-inflammatory substances enhance the plant's therapeutic properties[5-8].

Salvia officinalis.Salvia officinalis(O_2), also known as Sage, has been used for decorative, medicinal, and culinary purposes. This Lamiaceae plant has received much research owing to its biological functions and complicated chemical composition. Salvia officinalis is a versatile medicinal plant. Because of its complex chemical structure, it possesses antibacterial, antioxidant, antidiabetic, anti-inflammatory, and neuroprotective activities. Even though the current study has supported Salvia officinalis' traditional use[9-13].

Artemisia herba-alba.Artemisia herba-alba(O_3), often known as desert or white wormwood, is a perennial that has silvery-grey leaves and a pleasant odour. The plant grows 20–40 cm, depending on the environment. Artemisia herba-alba grows in dry and semi-arid regions of southern Europe, the Middle East, and North Africa. Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Jordan, Syria, Iraq, Saudi Arabia, and Spain often have it. The plant grows on hot, well-drained soils with minimal rainfall. Artemisia herba-alba is used in traditional medicine in North Africa and the Middle East. It treats parasites, skin, respiratory, and digestive problems. Plant flavonoids, volatile oils, and other bioactive compounds might explain its medicinal properties. Artemisia herba-alba is used in traditional medicine to alleviate diarrhoea, bloating, and stomach pain. Anthelmintic: kills parasitic worms. The plant's antimicrobial properties may help to heal skin and wounds. It alleviates coughs and bronchitis. Artemisia herba-alba has antibacterial, anti-inflammatory, and antioxidant activities that support its traditional use[14-18].

MATERIALS AND METHODS.

Chemical analysis begins with the collection and preparation of plant samples. The Tabuk region is utilised to collect different components of Salvia officinalis, Artemisia herba alba, and Teucrium polium, such as leaves, fruits, seeds, and roots, for the research[19]. To increase extraction efficiency and improve preservation, plant material is frequently dried after collection to reduce moisture content[20]. Researchers employed the oven-drying approach to speed up the drying process by working at controlled temperatures (such as 40-60°C). To increase the surface area for solvent extraction, the dried plant material is ground into a fine powder. Each extract sample was prepared for examination by dissolving 80 milli grammes (80 mg) of dried or finely powdered plant material in 250 millilitres of water as a solvent. Until further study, the powdered material is stored in airtight containers that are protected from moisture and light.

HPLC and gas chromatography-mass spectrometry (GC-MS) have been employed to identify bioactive components[21]. The researchers employed the GC-MS [Shimadzu of the United States] Method 8260 to analyse volatile organic chemicals and the Method 8270 to investigate semi-solid organic compounds.

Bioactivity

Bacterial Strains Used

We use *P aeruginosa* and *S aureus* from laboratory stock *S. aureus* (ATCC 25923) as standard strains to use control for various experiments. We selected well-characterised, pre-identified strains, cefoxitin-resistant, ESBL positive *P. aeruginosa*, and methicillin-resistant S. aureus from the stock culture. The *P. aeruginosa* was reassessed as cefoxitin resistant and ESBL positive, while S. aureus was for methicillin resistance using CLSI guidelines. The standard strains used were *P. aeruginosa* ATCC 27853 and S aureus ATCC 25923.

Biofilm Formation in 96-well Microtiter Plates

Biofilm formation was examined quantitatively as described elsewhere[22] using the method in 96-well flat bottom plates. Biofilm assays were performed in triplicate, and the mean biofilm absorbance value was determined. Biofilm formed were classified as weak (OD590 0.1 to $\leq$0.400), moderate (OD590 > 0.400) and strong (OD590 > 0.800).

Minimum Inhibitory Concentration (MIC) Activity of Extracts

MIC of Teucrium polium (O1), Salvia officinalis (O2), and Artemisiaherba alba (O3) against drug-resistant biofilm-positive strains of *P. aeruginosa* and *S. aureus* was determined using the standard micro-broth dilution method of CLSI[23]. The extract having an initial concentration of 99000mg/L for O1, 167000mg/L for O2 and 33000mg/L for O3. To examine the bacterial growth curve and inhibition in microtiter plate, inoculations were given from fresh colonies on MHA plates into 10 ml of Luria Bertaini (LB) culture medium. Growth was allowed till the optical density reached 0.1 at 580 nm (OD of 0.1 corresponds to 108 CFU/ml of medium). Subsequently, 2x108 CFU/ml from above was added to 1.5 ml of liquid LB media supplemented with 0, 0.5x, 0.25x, 0.125x and 0.0625x of initial Concentrations of Teucrium polium (O1), Salvia officinalis (O2), and Artemisiaherba alba (O3) extracts. The bacterial growth was determined by measuring optical density after every 2 h. The lowest concentration at which there was no exponential phase after 18-22 h incubation at 37°C at 580nm was considered for antibiofilm experiment at this concentration (MIC) and 0.5xMIC with slight modification.

Effect of Extract on Biofilm Formation

In the inhibition tests, bacteria inoculated in microtiter plates were exposed to MIC and 0.5xMIC. The treated mixtures were incubated for 48 hours at 37 °C. To quantify biofilm inhibition, the methods described in the previous section were employed. Positive control wells contained no extracts, while negative control wells were filled only with Tryptic Soy Broth (TSB). After the incubation, the plates were rinsed gently with 1X phosphate-buffered saline (PBS, pH 7.4) and stained for 30 minutes at room temperature using 100 µl of 0.1% crystal violet solution (Sigma-Aldrich, St. Louis, MO). The crystal violet was then solubilized in 95% ethanol, and any excess dye was removed through washing. Lastly, the biofilm's optical density was measured at 590 nm (OD590 nm) of the supernatant[22-23]. All experiment were performed in triplicate.

RESULTS AND DISCUSSIONS

The FTIR Results and Discussions

The FTIR study of these three plants constantly reveals considerable functional groups that reflect their phytochemical richness. Common outcomes include:

1. Alkane (C-H) stretches at 2918-2851 cm ^{1} indicate the presence of saturated hydrocarbons and fatty acids.
2. Carbonyl (C=O) stretches at 1729 cm ^{1} represent esters, ketones, and conjugated carbonyls.
3. Amine and amide groups (N-H) exist with varied frequency depending on hydrogen bonding patterns.
4. Identify aromatic compounds by C=C stretching vibrations in the 1500-1600 cm ^{1} range.
5. Identify the phenolic and alcoholic (O-H) groups. Broad absorption bands at 3000-3500 cm ^{1}.[24]

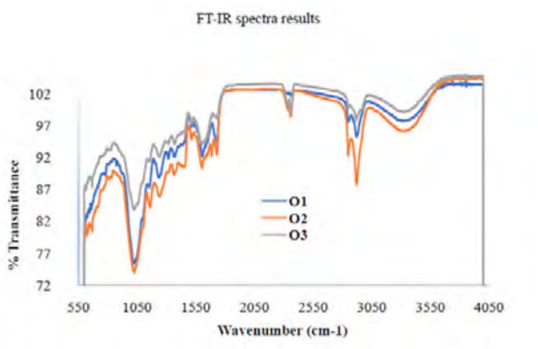


Fig. 1. FT-IR spectra of O1(Teucrium Polium Plant), O2 (Salvia_officinalis Plant), O3 (Artemisia_herba-alba plant)

FTIR spectroscopy detects functional groups, revealing the chemical profiles of Teucrium polium, Salvia officinalis, and Artemisia herba alba solutions. Organic compounds with unique resonance patterns show strong absorption bands in FTIR spectra. Plant extracts commonly have O-H stretching vibrations between 3700-3200 cm ^{1}, which indicate the presence of alcohols and phenols. C-H stretching vibrations in alkanes and aliphatic molecules range from 3000-2850 cm ^{1}. Carbonyl groups (C=O stretch) in compounds including aldehydes, ketones, carboxylic acids, and esters display strong absorption bands around 1750-1650 cm ^{1}, whereas C=C stretching vibrations emerge at 1680-1580cm ^{1} in alkenes and aromatic rings. Figure - 1 illustrates one of these examples.

Each plant has distinctive spectrum characteristics that reflect its chemical content. For example, Salvia officinalis extracts often exhibit peaks corresponding to carboxylic acids, alcohols, phenols, esters, ethers, and aldehydes, suggesting a diverse range of biomolecules implicated in the antioxidant capabilities. *Teucrium polium, Salvia officinalis,* and *Artemisia* herba alba's chemical profiles may be distinguished by the presence or absence of certain bands, as well as differences in their strength. These observed functional groupings correspond to recognised phytochemical classes. For example, O-H bonds are found in flavonoids and phenolic acids, C=O bonds are found in numerous terpenoids and flavonoids, and C-H bonds are required for alkaloids, terpenoids, and fatty acids, all of which are widely found in medicinal plants[25].

RESULTS AND DISCUSSIONS

GC-MS examination of *Teucrium polium, Salvia officinalis,* and Artemisia herba-alba plants revealed several chemical constituents. Terpenoids, lipid compounds, alcohol compounds, and ketones were among the volatile and non-volatile chemical substances discovered in this investigation at varying concentrations. One may be seen in Tables 1 to 6.

The chemical composition of Teucrium polium is mostly established by GC-MS analysis, which displays many terpenoids. Plant biology makes use of monoterpenes and sesquiterpenes. Teucrium polium's essential oils include -pinene, germacrene D, and -pinene. Compounds including caryophyllene, -cadinene, and 3-carene have been used to treat gastrointestinal disorders and inflammation.

The GC-MS analysis of Salvia officinalis (common sage) consistently reveals a diversified monoterpene and diterpene-dominated essential oil composition. Understanding its many traditional and therapeutic use requires a complicated chemical fingerprint. Salvia officinalis essential oils include -thujone, camphor, 1,8-cineole, and borneol, which have antibacterial, antioxidant, and memory-enhancing properties. This chemical richness is linked to the plant's culinary and medicinal functions.

The GC-MS analysis of Artemisia herba alba essential oil often yields oxygenated monoterpenes and sesquiterpenes. This comprises key compounds such cis-chrysanthenyl acetate, -thujone, camphor, chrysanthenone, 1,8-cineole, and trans-dihydroterpineol. Its antibacterial and antifungal characteristics make it ideal for therapeutic use[26].

The FTIR and GC-MS analysis reveal that these three plant species contain an extensive range of bioactive compounds with great therapeutic potential. Functional group identification via FTIR reveals the presence of phenolic compounds, flavonoids, and terpenoids, while GC-MS identifies specific chemicals quantitatively. These findings support the plants' historic use while also emphasising their potential for pharmacological and nutraceutical uses[27-28]

Bioactivity result and discussion

Antibacterial effect: The antibacterial efficacy of Teucrium polium (O1), Salvia officinalis (O2), and Artemisia herba-alba (O3) was assessed using a time-dependent growth inhibition experiment. The growth curves of Pseudomonas aeruginosa and Staphylococcus aureus subjected to O1, O2, and O3 demonstrated that these extracts possess the capability to limit bacterial growth and reproduction. Table - 7 and Figure-2 illustrates the proliferation of P. aeruginosa in LB broth inoculated with 10^8 CFU/mL, subjected to 0.5x, 0.25x, 0.125x, and 0.0625x concentrations of O1 (99,000 mg/L), O2 (167,000 mg/L), and O3 (33,000 mg/L). All concentrations exhibited a growth delay in the tested strains. The growth curves displayed three stages: lag, exponential, and stabilisation; however, decline phases were absent, as OD580 values included both viable and non-viable bacteria. In the absence of extract, P. aeruginosa swiftly entered the exponential growth phase; however, treatment with 0.5x O1, 0.125x O2, and 0.0625x O3 postponed growth for as long as 4 hours, demonstrating significant bacteriostatic action at lower doses and bactericidal effects at elevated concentrations. Likewise, S. aureus exhibited three phases, characterised by rapid exponential development in the control group. Treatments with 0.5x O1, 0.25x O2, and 0.25x O3 successfully suppressed growth for 3–6 hours,

Table 1: Volatile organic compounds (VOCS) analysis Sample code:Q1 Teucrium polum

S. No.	Compounds detected	Results &/l unit: mg
1	Benzene	0.5877
2	Ethane, 1,1-dichloro-	0.5797
3	p-Isopropylbenzene (o-Cymene)	0.5007
4	Ethylbenzene	0.403
5	Ethylene, 1,2-dichloro-, (Z)-	0.3915
6	o-Xylene	0.3742
7	Benzene, 1,2-dichloro-	0.0411
8	Propane, 1,2,3-trichloro-	0.0001
9	Methane, bromodichloro-	0.0001
10	Methane, dibromo-	0.0001
11	Methane, dibromochloro-	0.0001
12	Methylene chloride	0.0001
13	m-Xylene	0.0001
14	Naphthalene	0.0001
15	Propane, 1,2-dibromo-3-chloro-	0.0001
16	Isopropylbenzene	0.0001
17	Propane, 1,2-dichloro-	0.0001
18	Propane, 1,3-dichloro--	0.0001
19	Propene, 1,1-dichloro-	0.0001
20	p-Xylene	0.0001
21	Sec- butylbenzene	0.0001
22	Trichloroethylene	0.0001
23	Toluene	0.0001
24	trans-1,2-dichloro Ethene	0.0001
25	trans-1,3-Dichloropropene	0.0001
26	Methylene chloride	0.0001
27	Ethene, 1,1-dichloro-	0.0001
28	Hexachlorobutadiene	0.0001
29	Benzene, chloro-	0.0001
30	2-Chlorotoluene	0.0001
31	2-Pentanone	0.0001
32	Benzene, 1,2,3-trichloro-	0.0001
33	Benzene, 1,2,4-trichloro-	0.0001
34	Benzene, 1,2,4-trimethyl-	0.0001
35	Benzene, 1,2,5-trimethyl-	0.0001
36	Benzene, 1,3-dichloro-	0.0001
37	Benzene, bromo-	0.0001
38	Benzene, propyl-	0.0001
39	cis-1,3-Dichloro-Propene	0.0001
40	Benzene, propyl-	0.0001
41	Benzene, tert-butyl-	0.0001
42	Bromoform	0.0001
43	Chloroform	0.0001
44	Cyclopentane, methyl-	0.0001
45	Ethane, 1,1,1-trichloro-	0.0001
46	Ethane, 1,1,2-trichloro-	0.0001
47	Ethane, 1,2-dibromo-	0.0001
48	Tetrachloroethylene	0.0001

Table 2: Semi Volatile Organic Compounds (SVOCs) Analysis Report Q1 - Teucrium polum

S. No.	Compounds	Concentration (mg/l)
1	Benzo[a]pyrene	0.0271
2	Benzo[k]fluoranthene	0.0135
3	Dimethyl phthalate	0.0102
4	Di-n-octyl phthalate	0.0099
5	Naphthalene, 2-methyl-	0.0093
6	Diethyl Phthalate	0.0083
7	Anthracene	0.0049
8	4-Nitroaniline	0.0044
9	Azobenzene	0.0012
10	2-Nitroaniline	0.0015
11	Carbazole	0.0013
12	Phenol, 2-nitro-	0.0014
13	Dibutyl phthalate	0.0010
14	2,4-Dinitrotoluene	0.0010
15	Isophorone	0.0008
16	Phenol, 4-methyl (p-Cresol)	0.0007
17	Phenanthrene	0.0007
18	Naphthalene	0.0006
19	Benzene, 1,2-dichloro-	0.0003
20	Benzene, 1,4-dichloro-	0.0003
21	Bis(2-ethylhexyl) phthalate	0.0003
	Undetectable Compounds (<0.0001 mg/l)	< 0.0001
22	1,1'-Biphenyl, 2-methyl-	< 0.0001
23	2,6-Dinitrotoluene	< 0.0001
24	3-Nitroaniline	< 0.0001
25	4-Bromophenyl ether	< 0.0001
26	4-Chloroaniline	< 0.0001
27	Acenaphthene	< 0.0001
28	Acenaphthylene	< 0.0001
29	Benz[a]anthracene	< 0.0001
30	Benzene, 1,3,4-trichloro-	< 0.0001
31	Benzene, 1,3-dichloro-	< 0.0001
32	Benzene, 1-chloro-3-phenoxy-	< 0.0001
33	Benzene, hexachloro-	< 0.0001
34	Benzene, nitro-	< 0.0001
35	Benzo[b]fluoranthene	< 0.0001

36	Benzo[ghi]perylene	< 0.0001
37	Benzyl butyl phthalate	< 0.0001
38	Bis(2-chloroethyl) ether	< 0.0001
39	Chrysene	< 0.0001
40	Dibenzofuran	< 0.0001
41	Ethane, hexachloro-	< 0.0001
42	Fluoranthene	< 0.0001
43	Fluorene	< 0.0001
44	Hexachlorobutadiene	< 0.0001
45	Hexachlorocyclopentadiene	< 0.0001
46	Indeno[1,2,3-cd]pyrene	< 0.0001
47	Methane, bis(2-chloroethoxy)-	< 0.0001
48	Naphthalene, 2-chloro-	< 0.0001
49	Pentacene	< 0.0001
50	Phenol	< 0.0001
51	Phenol, 2,4,5-trichloro-	< 0.0001
52	Phenol, 2,4,6-trichloro-	< 0.0001
53	Phenol, 2,4-dichloro-	< 0.0001
54	Phenol, 2,4-dimethyl-	< 0.0001
55	Phenol, 2,4-dinitro-	< 0.0001
56	Phenol, 2-chloro-	< 0.0001
57	Phenol, 2-methyl-	< 0.0001
58	Phenol, 2-methyl, 4,6-dinitro-	< 0.0001
59	Phenol, 4-chloro-3-methyl-	< 0.0001
60	Phenol, pentachloro-	< 0.0001
61	Pyrene	< 0.0001
62	Tetradecane	< 0.0001

Table : 3 Volatile Organic Compounds (VOCs) Analysis Report Q2 - *Salvia officinalis*

S. No.	Compounds detected	Result (mg/l)
1	Benzene	0.5867
2	Ethane, 1,1-dichloro-	0.5787
3	p-Isopropylbenzene (o-Cymene)	0.5015
4	Benzene, tert-butyl-	0.4739
5	Benzene, propyl-	0.4294
6	Ethylbenzene	0.4034
7	o-Xylene	0.3748
8	Propane, 1,2,3-trichloro-	0.2658
9	2-Chlorotoluene	0.2445
10	Benzene, 1,2,5-trimethyl-	0.1202
11	Benzene, 1,2-dichloro-	0.0403
12	Benzene, chloro-	0.0219
13	Propane, 1,2-dichloro-	<0.0001
14	Propane, 1,3-dichloro-	<0.0001
15	Propene, 1,1-dichloro-	<0.0001
16	p-Xylene	<0.0001
17	Sec- butylbenzene	<0.0001
18	Tetrachloroethylene	<0.0001
19	Toluene	<0.0001
20	trans-1,2-dichloro Ethene	<0.0001
21	trans-1,3-Dichloropropene	<0.0001
22	Trichloroethylene	<0.0001
23	Ethene, 1,1-dichloro-	<0.0001
24	cis-1,3-Dichloro-Propene	<0.0001
25	2-Pentanone	<0.0001
26	Benzene, 1,2,3-trichloro-	<0.0001
27	Benzene, 1,2,4-trichloro-	<0.0001
28	Benzene, 1,2,4-trimethyl-	<0.0001
29	Benzene, 1,3-dichloro-	<0.0001
30	Benzene, bromo-	<0.0001
31	Benzene, n-butyl-	<0.0001
32	Bromoform	<0.0001
33	Chloroform	<0.0001
34	Cyclopentane, methyl-	<0.0001
35	Ethane, 1,1,1-trichloro-	<0.0001
36	Ethane, 1,1,2-trichloro-	<0.0001
37	Ethane, 1,2-dibromo-	<0.0001
38	Ethylene, 1,2-dichloro-, (Z)-	<0.0001
39	Hexachlorobutadiene	<0.0001
40	Isopropylbenzene	<0.0001
41	Methane, bromochloro-	<0.0001
42	Methane, bromodichloro-	<0.0001
43	Methane, dibromo-	<0.0001
44	Methane, dibromochloro-	<0.0001
45	Methylene chloride	<0.0001
46	m-Xylene	<0.0001
47	Naphthalene	<0.0001
48	Propane, 1,2-dibromo-3-chloro-	<0.0001

Table 4: Semi Volatile Organic Compounds (SVOCs) Analysis Report Q2 - Salvia officinalis

S. No.	Compounds	Concentration (mg/l)
1	Di-n-octyl phthalate	0.0338
2	Dimethyl phthalate	0.0046
3	4-Nitroaniline	0.0021
4	Carbazole	0.0018
5	Fluoranthene	0.0007
6	2-Nitroaniline	0.0006
7	Pyrene	0.0006
8	Naphthalene	0.0005
9	Bis(2-ethylhexyl) phthalate	0.0005
10	Diethyl Phthalate	0.0004
11	Phenol, 2-nitro-	0.0003

12	Dibutyl phthalate	0.0003
13	Benzene, 1,2-dichloro-	0.0002
14	Benzene, 1,4-dichloro-	0.0002
15	Isophorone	0.0002
16	Benzo[k]fluoranthene	<0.0001
17	Benzene, 1,3-dichloro-	<0.0001
18	Acenaphthylene	<0.0001
19	Anthracene	<0.0001
20	Azobenzene	<0.0001
21	Dibenzofuran	<0.0001
22	Benz[a]anthracene	<0.0001
23	Chrysene	<0.0001
24	Benzene, 1,3,4-trichloro-	<0.0001
25	Ethane, hexachloro-	<0.0001
26	Benzo[ghi]perylene	<0.0001
27	Bis(2-chloroethyl) ether	<0.0001
28	Benzene, 1-chloro-3-phenoxy-	<0.0001
29	Benzene, hexachloro-	<0.0001
30	Benzene, nitro-	<0.0001
31	Benzyl butyl phthalate	<0.0001
32	Benzo[a]pyrene	<0.0001
33	Acenaphtherne	<0.0001
34	Benzo[b]fluoranthene	<0.0001
35	Fluorene	<0.0001
36	4-Chloroaniline	<0.0001
37	Phenol, 2,4-dinitro-	<0.0001
38	Hexachlorocyclopentadiene	<0.0001
39	Indeno[1,2,3-cd]pyrene	<0.0001
40	Methane, bis(2-chloroethoxy)-	<0.0001
41	Naphthalene, 2-chloro-	<0.0001
42	Naphthalene, 2-methyl-	<0.0001
43	Pentacene	<0.0001
44	Phenanthrene	<0.0001
45	Phenol, 2,4,5-trichloro-	<0.0001
46	Phenol, 2,4,6-trichloro-	<0.0001
47	Phenol, 2,4-dichloro-	<0.0001
48	Phenol, 2,4-dimethyl-	<0.0001
49	Phenol, 2-chloro-	<0.0001
50	Hexachlorobutadiene	<0.0001
51	Phenol, 2-methyl-	<0.0001
52	Phenol, 2-methyl, 4,6-dinitro-	<0.0001
53	Phenol, 4-chloro-3-methyl-	<0.0001
54	Phenol, 4-methyl (p-Cresol)	<0.0001
55	Phenol, pentachloro-	<0.0001
56	Tetradecane	<0.0001
57	Phenol	<0.0001
58	1,1'-Biphenyl, 2-methyl-	<0.0001
59	2,4-Dinitrotoluene	<0.0001
60	2,6-Dinitrotoluene	<0.0001
61	3-Nitroaniline	<0.0001
62	4-Bromophenyl ether	<0.0001

Table 5: Volatile Organic Compounds (VOCs) AnalysisQ3 -Artemisiaherba alba

S. No.	Compounds detected	Result (mg/l)
1	Benzene	0.5841 mg/l
2	Isopropylbenzene	0.4666 mg/l
3	Ethylbenzene	0.4007 mg/l
4	o-Xylene	0.3726 mg/l
5	Benzene, 1,2,5-trimethyl-	0.121 mg/l
6	Benzene, 1,2-dichloro-	0.0367 mg/l
7	Ethene, 1,1-dichloro-	0.0001 mg/l
8	cis-1,3-Dichloro-Propene	0.0001 mg/l
9	2-Chlorotoluene	0.0001 mg/l
10	2-Pentanone	0.0001 mg/l
11	Benzene, 1,2,3-trichloro-	0.0001 mg/l
12	Benzene, 1,2,4-trichloro-	0.0001 mg/l
13	Benzene, 1,2,4-trimethyl-	0.0001 mg/l
14	Benzene, 1,3-dichloro-	0.0001 mg/l
15	Benzene, bromo-	0.0001 mg/l
16	Benzene, chloro-	0.0001 mg/l
17	Benzene, n-butyl-	0.0001 mg/l
18	Benzene, propyl-	0.0001 mg/l
19	Benzene, tert-butyl-	0.0001 mg/l
20	Bromoform	0.0001 mg/l
21	Chloroform	0.0001 mg/l
22	Cyclopentane, methyl-	0.0001 mg/l
23	Ethane, 1,1,1-trichloro-	0.0001 mg/l
24	Ethane, 1,1,2-trichloro-	0.0001 mg/l
25	Ethane, 1,1-dichloro-	0.0001 mg/l
26	Ethane, 1,2-dibromo-	0.0001 mg/l
27	Ethylene, 1,2-dichloro-, (Z)-	0.0001 mg/l
28	Hexachlorobutadiene	0.0001 mg/l
29	Methane, bromochloro-	0.0001 mg/l
30	Methane, bromodichloro-	0.0001 mg/l
31	Methane, dibromo-	0.0001 mg/l
32	Methane, dibromochloro-	0.0001 mg/l
33	Methylene chloride	0.0001 mg/l
34	m-Xylene	0.0001 mg/l
35	Naphthalene	0.0001 mg/l
36	p-Isopropylbenzene (o-Cymene)	0.0001 mg/l
37	Propane, 1,2,3-trichloro-	0.0001 mg/l
38	Propane, 1,2-dibromo-3-chloro-	0.0001 mg/l
39	Propane, 1,2-dichloro-	0.0001 mg/l
40	Propane, 1,3-dichloro-	0.0001 mg/l
41	Propene, 1,1-dichloro-	0.0001 mg/l
42	p-Xylene	0.0001 mg/l

43	Sec-butylbenzene	0.0001 mg/l
44	Tetrachloroethylene	0.0001 mg/l
45	Toluene	0.0001 mg/l
46	trans-1,2-dichloro Ethene	0.0001 mg/l
47	trans-1,3-Dichloropropene	0.0001 mg/l
48	Trichloroethylene	0.0001 mg/l

Table 6: Semi volatile Organic Compounds (SVOCs) Analysis Report Sample Code: Q3 - Artemisia Erba alba

S. No.	Compounds detected	Result (mg/l)
1	Nitroaniline	0.0402
2	Naphthalene,2-methyl-	0.0400
3	Fluorene	0.0275
4	Di-n-octyl phthalate	0.0225
5	Diethyl Phthalate	0.0216
6	Isophorone	0.0111
7	Phenol, 2-nitro-	0.0108
8	Acenaphthylene	0.0088
9	Dibutyl phthalate	0.0050
10	Azobenzen	< 0.0044
11	Phenol, 4methyl (p-Cresol)	< 0.0027
12	Phenol	0.0022
13	chloroaniline	0.0022
14	Dimethyl phthalate	0.0017
15	Phenol, 2,4-dimethyle	0.0017
16	Benzene, nitro-	0.0013
17	Bis(2-ethylhexyl) phthalate	0.0013
18	Naphthalene, 2-chloro-	0.0013
19	2,4-Dinitrotoluene	0.0012
20	Anthracene	0.0004
21	Bis(2-chloroethyle) ether	0.0003
22	Naphthalene	0.0003
23	Benzyl butyl phthalate	0.0001
24	1,1'-Biphenyl, 2-methyl-	< 0.0001
25	2,6-Dinitrotoluene	< 0.0001
26	2-Nitroaniline	< 0.0001
27	3- Nitroaniline	< 0.0001
28	4-Bromophenyl ether	< 0.0001
29	Acenaphthene	< 0.0001
30	Acenaphthylene	< 0.0001
31	Benz[a]anthrance	< 0.0001
32	Benzene, 1,2-dichloro-	< 0.0001
33	Benzene, 1,3,4-trichloro-	< 0.0001
34	Benzene, 1,3-dichloro-	< 0.0001
35	Benzene, 1,4-dichloro-	< 0.0001
36	Benzene, 1-chloro-3-phenoxy	< 0.0001
37	Benzene, hexachloro-	< 0.0001
38	Benzo[a] pyrene	< 0.0001
39	Benzo[b] fluoranthene	< 0.0001
40	Benzo[ghi] perylene	< 0.0001
41	Benzo[k] fluoranthene	< 0.0001
42	Carbazole	< 0.0001
43	chrysene	< 0.0001
44	Dibenzofuran	< 0.0001
45	Ethane, hexachloro	< 0.0001
46	fluoranthene	< 0.0001
47	Hexachlorobutadiene	< 0.0001
48	Hexachlorocyclopentadiene	< 0.0001
49	Indeno[1m2,3-cd]pyrene	< 0.0001
50	Methane, bis(2-chloroethoxy)-	< 0.0001
51	Naphthalene,2-methyl-	< 0.0001
52	Pentacene	< 0.0001
53	phenanthrene	< 0.0001
54	Phenol, 2,4,5-trichloro-	< 0.0001
55	Phenol, 2,4,6-trichloro-	< 0.0001
56	Phenol, 2,4-dichloro-	< 0.0001
57	Phenol, 2,4-dinitro-	< 0.0001
58	Phenol, 2-chloro-	< 0.0001
59	Phenol, 2-methyl-	< 0.0001
60	Phenol, 2-methyl, 4,6-dinitro-	< 0.0001
61	Phenol, 4-chloro-3-methyl-	< 0.0001
62	Phenol, pentachloro	< 0.0001
63	Pyrene	< 0.0001
64	Tetradecane	< 0.0001

whereas lower concentrations initially delayed growth before transitioning to exponential growth, indicating substantial bacteriostatic activity at reduced doses and bactericidal activity at elevated doses [Table - 8] and (Figure -2).

Antibiofilm Effect

The potential of herbal extract of Teucrium polium (O1), Salvia officinalis (O2), and Artemisia herba-alba (O3) to inhibit the biofilm formation at MIC and 0.5xMIC from the antimicrobial growth curve. The results of the biofilm inhibition study are presented in Figure. 3. Concentration-dependent effect of O1, O2 and O3 was observed against test bacteria and maximum inhibition was 87% by O1 against P aeruginosa and 74% for S aureus at MIC. Whereas almost similar inhibition was recorded at 0.5xMIC of O1. O2 extract shows 88% for both at MIC and 63% and 60% reduction at 0.5xMIC. Whereas, there was a difference of 10% and 22% at MIC and 0.5xMIC of O3 against P aeruginosa and S aureus. Can be seen in the Table -9.

Table 7: *Pseudomonas aeruginosa* growth time assay with MIC analysis

	0H	2H	4H	6H	8H	10H	12H	14H	16H	18H	20H	22H
O1	CONTROL	0.007	0.173	0.187	0.245	0.327	0.485	0.840	1.346	1.581	1.997	2.457
	0.5 CONC	1.200	1.400	1.440	1.100	1.120	1.140	1.000	1.000	1.000	1.000	0.940
	0.25 CONC	0.514	0.789	0.998	1.000	1.456	1.945	1.966	1.990	1.989	1.980	1.986
	0.125 CONC	0.138	0.212	0.114	0.588	0.800	0.960	1.336	1.732	1.897	1.815	1.958
	0.0625 CON	0.058	0.020	0.044	0.426	0.659	0.853	1.391	1.764	1.837	1.675	1.947
O2	CONTROL	1	0.173	0.187	0.245	0.327	0.485	0.84	1.346	1.581	1.997	2.457
	0.5 CONC	0.45	0.42	0.34	0.35	0.41	0.45	0.31	0.29	0.24	0.22	0.19
	0.25 CONC	0.594	0.721	0.679	1	1	0.95	0.84	0.74	0.45	0.34	0.2
	0.125 CONC	0.276	0.3	0.248	0.753	1.01	1.196	1.579	1.793	1.889	1.947	1.9772
	0.0625 CON	0.023	0.02	0.019	0.375	0.554	0.744	0.959	1.571	1.862	1.807	1.946
O3	CONTROL	0.007	0.173	0.187	0.245	0.327	0.485	0.84	1.346	1.581	1.997	2.457
	0.5 CONC	0.012	0.324	0.356	0.568	0.756	0.854	0.842	0.84	0.754	0.541	0.445
	0.25 CONC	0.265	0.379	0.485	1.138	1.34	1.59	1.96	1.933	1.965	1.994	1.956
	0.125 CONC	0.048	0.456	0.678	0.987	1.012	1.235	1.216	1.733	1.98	2.32	2.95
	0.0625 CON	0.079	0.108	0.745	1.123	1.134	1.321	1.354	1.84	2.01	3.01	3.42

Table 8: *S aureus* growth time assay with MIC analysis

		0H	2H	4H	6H	8H	10H	12H	14H	16H	18H	20H	22H
O1	CONTROL	0.770	0.187	0.157	0.196	0.285	0.410	0.418	0.808	1.271	1.527	1.828	1.913
	0.5 CONC	0.199	0.211	0.425	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.655	0.645
	0.25 CONC	0.193	0.849	0.664	0.679	0.679	0.679	0.679	0.679	0.679	1.675	1.650	1.639
	0.125 CONC	0.183	0.169	0.017	0.525	0.722	0.852	1.111	1.058	1.470	1.564	1.576	1.649
	0.0625 CONC	0.145	0.198	0.233	0.189	0.315	0.454	0.548	0.867	1.237	1.440	1.486	1.535
O2	CONTROL	0.17	0.187	0.157	0.196	0.285	0.41	0.418	0.808	1.271	1.527	1.828	1.913
	0.5 CONC	0.235	0.345	0.365	0.368	0.345	0.355	0.355	0.298	0.299	0.287	0.245	0.221
	0.25 CONC	0.273	0.154	0.168	0.198	0.321	0.398	0.41	0.456	0.51	0.623	0.655	0.67
	0.125 CONC	0.298	0.31	0.356	0.401	0.424	0.487	0.51	0.655	0.623	0.634	0.645	0.678
	0.0625 CONC	0.245	0.298	0.31	0.356	0.406	0.597	0.736	0.993	1.325	1.5	1.549	1.639
O3	CONTROL	0.629	0.187	0.157	0.196	0.285	0.410	0.418	0.808	1.271	1.527	1.828	1.913
	0.5 CONC	0.258	0.289	0.391	0.679	1.649	1.653	0.679	0.679	1.664	1.675	1.640	1.779
	0.25 CONC	0.055	0.012	0.137	0.689	0.759	0.991	1.287	1.452	1.574	1.628	1.622	1.919
	0.125 CONC	0.027	0.014	0.151	0.241	0.366	0.473	0.561	0.886	1.277	1.463	1.598	1.639
	0.0625 CONC	0.058	0.058	0.061	0.075	0.206	0.231	0.245	0.265	0.210	0.221	0.234	0.245

Table 9: *Pseudomonas aeruginosa*, and *S aureus* Biofilm Inhibition assay in microtiter plate

		NO GRWOTH BY MIC AT CONCENTRATION				
		PS	SA			
	C1	0.5x MIC	0.5x MIC			
	C2	0.125x MIC	0.25x MIC			
	C3	0.0625x MIC	0.25x MIC			
Percent (%) reduction of biofilm PRODUCTION						
	Mean CONTROL	Mean MIC	Mean 0.50 X MIC	SD CONTROL	SD MIC	SD 0.50 X MIC
SA-O1	91	74	54	3.21	2.95	2.9
SA-O2	95	88	63	5.21	3.45	2.56
SA-O3	91	69	42	3.14	4.58	2.99
PA-O1	93	87	55	4.12	3.54	3.01
PA-O2	94	88	60	5.01	2.98	1.98
PA-O3	96	79	64	3.54	4.32	3.74

PA: Pseudomonas aeruginosa. SA: S aureus

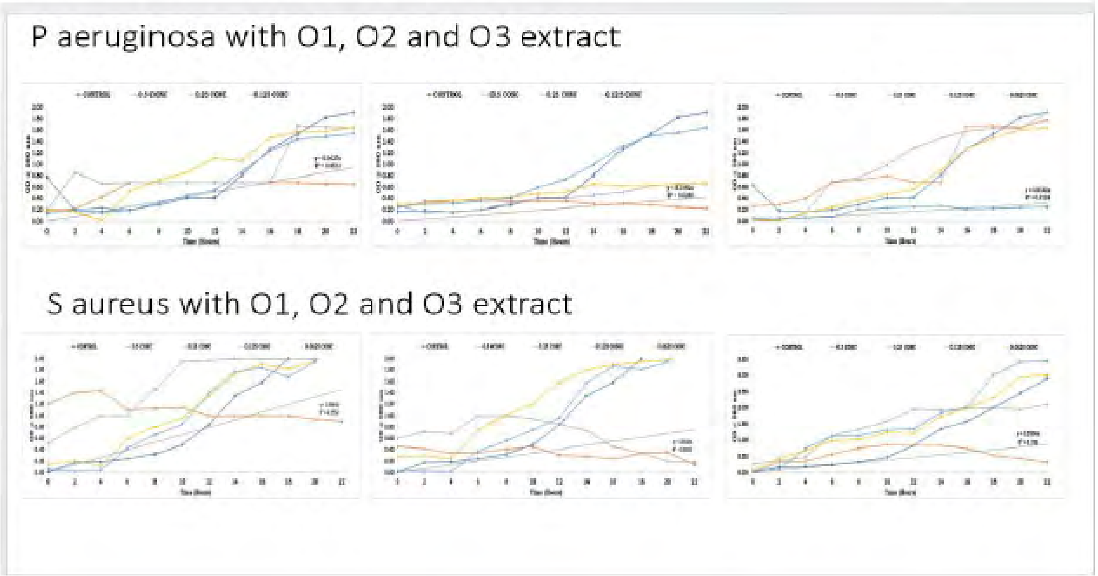
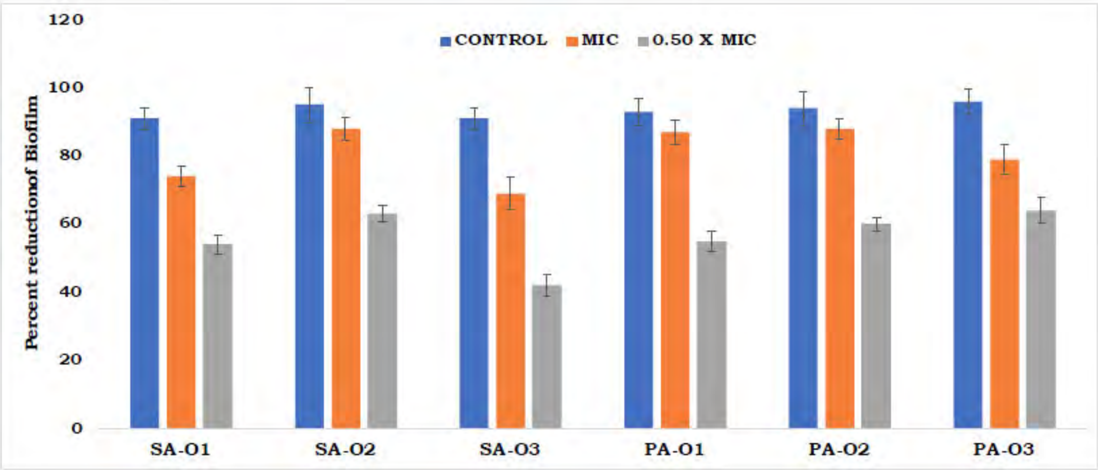


Fig. 2. *Pseudomonas aeruginosa* and *S aureus* growth curve over time when exposed to various concentrations with MIC



CONCLUSIONS

The FTIR and GC-MS analyses show that Salvia officinalis, Artemisia herba alba, and Teucrium polium contain a wide variety of bioactive chemicals with high medicinal potential. Functional group identification by FTIR indicates the existence of phenolic compounds, flavonoids, and terpenoids, while GC-MS identifies individual chemicals quantitatively.

P. aeruginosa immediately underwent exponential growth without extract, whereas 0.5x O1, 0.125x O2, and 0.0625x O3 reduced growth for 4 hours, exhibiting bacteriostatic and bactericidal effects at lower dosages and greater concentrations. S. aureus has three phases, including rapid exponential growth in controls. 0.5x O1, 0.25x O2, and 0.25x O3 inhibited growth for 3–6 hours, although lower doses delayed exponential growth, demonstrating bacteriostatic and bactericidal effects. The concentration-dependent biofilm inhibition investigation indicated that O1 inhibited P. aeruginosa 87% and S. aureus 74% at the MIC. Similar suppression was seen at 0.5x O1 MIC. O2 extract reduces by 88% at MIC, 63% at 0.5xMIC, and 60% at The MIC and 0.5xMIC of O3 varied by 10% and 22% against *P. aeruginosa* and *S. aureus*, respectively.

This study reveals the bioactive compounds, molecular processes, and health benefits of Salvia officinalis, Artemisia herba alba, and Teucrium polium. The finding has broadened the understanding of the plant's bioactivities and medicinal applications, especially for the treatment of intestinal diseases.

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