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Evaluating the Active Chemical Compounds in Extracts from Various Medicinal Herbs used for the Treatment of Skin Rashes

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ABSTRACT

Many countries use the herb to treat ailments. Antioxidants are beneficial for treating skin rashes. Calligonum comosum, Ziziphus spina-christi, and Ruta graveolens have historically treated skin rashes. Saudi Arabia and its neighbours utilise it extensively. The FTIR and GC-MS examined all three plant-specific water extracts. The three plants vary in phytosterol and phenolics. Numerous flavonoids and phenolic acids were identified. Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus spina-christi (N3) were tested for antibacterial activity utilising time-dependent growth inhibition. P. aeruginosa and Staphylococcus aureus growth curves treated with N1, N2, and N3 revealed bacterial growth and reproduction suppression. N1 and N3 inhibited test microorganisms' concentration-dependently, with N2 inhibiting S. aureus by 94% and P. aeruginosa by 83% at MIC. N2 showed minimal effect at 0.5xMIC. S. aureus and P. aeruginosa are decreased by 91% and 85% at MIC and 65% and 61% at 0.5xMIC by N2 extract. In contrast, N1's 2xMIC and MIC against P. aeruginosa and S. aureus dropped 91%, 87%, 72%, and 59%. This research may reveal Calligonum comosum, Ziziphus spina-christi, and Ruta graveolens' bioactive substances, molecular processes, and therapeutic impacts on human health. This discovery has increased plant bioactivity understanding and medical uses.

Key words: - Skin Rash, Identified, Quantified, Phytochemical, FTIR and GC-MS.

INTRODUCTION

Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) are three herbs that have been used throughout history for the treatment of skin rashes. It is also used in Saudi Arabia and the neighbouring countries.

Calligonum comosum (N2)

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The plant in question has been traditionally used medicinally. The polygonaceous perennial shrub, Calligonum comosum, grows in the sandy deserts of North Africa, Western Asia, and the Middle East. This plant, called "Arta" in certain places, has been used in folk medicine to cure numerous diseases in dry climates. C. comosum's antioxidant polyphenol concentration makes it a valuable medicinal plant in Saudi Arabia and other Middle Eastern nations. This Egyptian desert plant includes bioactive components that boost its medicinal properties[1-3].Medical professionals in Oman use C. comosum to treat inflammation, tooth pain, gum lesions, and ulcers. Rural Tunisians have used this plant to treat microbiological diseases. The physiological properties of this plant include antibacterial, antioxidant, anti-inflammatory, anti-gastric ulcer, and anti-hyperglycaemic effects. Additionally, it treats gastrointestinal issues and tooth discomfort[4-].In addition, it is utilised in traditional medicine to treat skin diseases[6].

Ruta graveolens (N1)

The plant known as rue, or Ruta graveolens L., has a long history of traditional usage and a wide range of pharmacological qualities. A member of the Rutaceae family, this plant is known for its strong bitter taste and unique smell. Numerous communities have utilised it for medicinal purposes to cure diseases ranging from gynaecological issues to pain relief. These effects are affected by the plant's phytochemical composition, which includes flavonoids, coumarins, alkaloids, and essential oils. Among these are the alkaloids gravacridondiol, rutacridone, and rutacridone epoxide. Coumarins consist of psoralen, methoxypsoralen, bergapten, isoimperatorin, isopimpinellin, and xanthotoxin. Quercetin and rutin are flavonoids. Essential oils: The main monoterpene components are 1,8-cineole, limonene, and αpinene. The major ingredients of volatile oils extracted from plant aerial sections are 2-ketones, namely undecanone-2, 2-nonanone, 2-acetoxy tetradecanone, and nonyl cyclopropane carboxylate[7-10].

Ziziphus spina-christi (N3)

Christian Thorn Jujube, also known as Ziziphus spina-christi (L.) Desf., is a tree native to the Middle East's tropics and subtropics. It is well-known for its delicious fruits, as well as its health advantages. The medicinal properties of this widely distributed plant make it an important subject for research in the Middle East, South Asia, and East Asia. The plant has been linked to diabetes, malaria, typhoid fever, liver problems, skin infections, urinary issues, obesity, diarrhoea, and fatigue. Traditional uses include treating asthma, fever, pain, wounds, ulcers, eye problems, and dandruff. The traditional usage of Z. spina-christi has prompted more investigation into its pharmacological and bioactive chemical characteristics. Z. spina-christi is one of 193 chemicals discovered in different sections of plants. It has an abundance of polyphenols and flavonoids. These chemicals are beneficial to plants, making them an ideal target for medication. A recent study confirms Z. spina-christi's antibacterial, antifungal, antioxidant, antihyperglycemic, and antinociceptive properties. The present scientific research examines the phytochemical constitution, and bioactivities of Z. spina-christi to see if they may be employed for medicinal reasons[11-14].

MATERIALS AND METHODS.

The first step of chemical analysis is to collect and prepare plant samples. The Tabuk area is utilised to collect different components of Ruta graveolens (N2), Calligonum comosum (N1), and Ziziphus spina-christi (N3), such as leaves, fruits, seeds, and roots, for the research [15]. To maximise extraction efficiency and improve preservation, plant material is usually dried after collection to reduce moisture content [16]. 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 milligrams (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[17]. 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

The antibacterial activity of Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) was determined using a time-dependent growth inhibition experiment.

Bacterial Strains Used: We *use P aeruginosa* and *S aureus* from laboratory stock. We selected well-characterized, 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 evaluated qualitatively using the method previously published[18] using the technique in 96-well flat-bottom plates. Biofilm experiments were done in triplicate, and the average biofilm absorbance value was calculated. Biofilm formation was categorized as weak (OD590 0.1-0.400), moderate (OD590 > 0.400), and strong (OD590 > 0.800).

Minimum Inhibitory Concentration (MIC) Activity of Extracts

Using the conventional CLSI micro-broth dilution method, the MIC of Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) against drug-resistant biofilm-positive strains of P. aeruginosa and S. aureus was ascertained[19]. The extract possesses initial concentrations of 66000mg/L for N1, 33000 mg/L for N2, and 33000 mg/L for N3. To investigate the bacterial growth curve and inhibition on a microtiter plate, inoculations were derived from fresh colonies on MHA plates into 10 ml of Luria Bertani (LB) culture media. Growth was permitted until the optical density attained 0.1 at 580 nm, which corresponds to 10^8 CFU/ml of media. Subsequently, $2x10^8$ CFU/ml from the aforementioned was added to 1.5 ml of liquid LB media supplemented with 0, 0.5x, 0.25x, 0.125x, and 0.0625x of the initial concentrations of Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) extracts. Bacterial growth was assessed by measuring optical density every 2 hours. The minimum concentration at which no exponential phase was observed after 18-22 hours of incubation at 37°C and 580nm was designated for the antibiofilm experiment, utilizing this concentration (MIC) and 0.5xMIC with minor modifications.

Effect of Extract on Biofilm Formation

In the inhibition assays, bacteria seeded on microtiter plates were subjected to MIC and 0.5xMIC. The treated mixes were incubated for 48 hours at 37 degrees Celsius. To measure biofilm inhibition, the procedures outlined in the previous section were used. While negative control wells were filled solely with Tryptic Soy Broth (TSB), positive control wells were devoid of extracts. Immediately following the incubation, the plates were carefully rinsed with 1X phosphate-buffered saline (PBS, pH 7.4) and stained for 30 minutes at room temperature with 100 µl of 0.1% crystal violet solution (Sigma-Aldrich, St. Louis, MO). The crystal violet was subsequently solubilized in 95% ethanol, and surplus dye was eliminated via washing. Finally, the optical density of the biofilm's supernatant was assessed at 590 nm (OD590 nm)[19]. Every experiment was carried out three times.

RESULTS AND DISCUSSIONS

The FTIR Results and Discussions

The FTIR analysis of these three plants consistently finds significant functional groups that represent their phytochemical diversity. Common results include:

1. Alkane (C-H) stretches often emerge at 2918-2851 cm^{-1}, suggesting the presence of saturated hydrocarbons and fatty acids.
2. Carbonyl (C=O) stretches at 1729 cm^{-1} indicate esters, ketones, and conjugated carbonyls.
3. Amine and amide groups (N-H) occur at varying frequency based on hydrogen bonding patterns.
4. Identify aromatic compounds using C=C stretching vibrations in the 1500-1600 cm^{-1} region. • Identify phenolic and alcoholic (O-H) groups. Broad absorption bands at 3000-3500 cm^{-1}. [20]

FTIR examination of Ruta graveolens

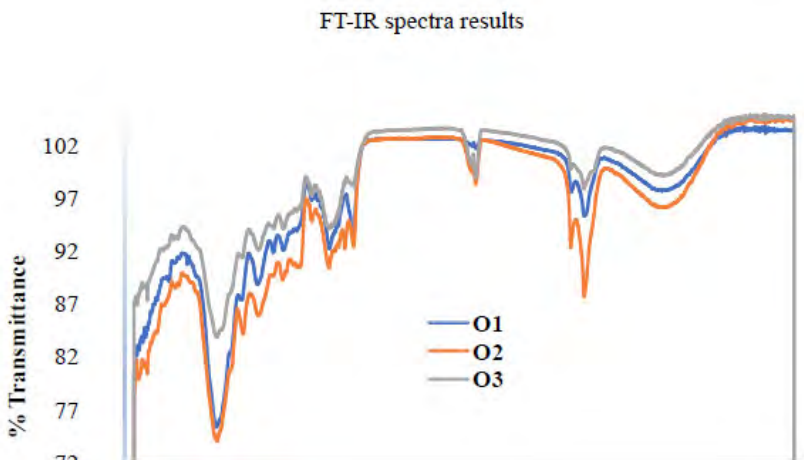


Fig. 1: FT-IR spectra of N2(Calligonum comosum plant), N1 (Ruta graveolens plant), and N3 (Ziziphus spina-christi plant)

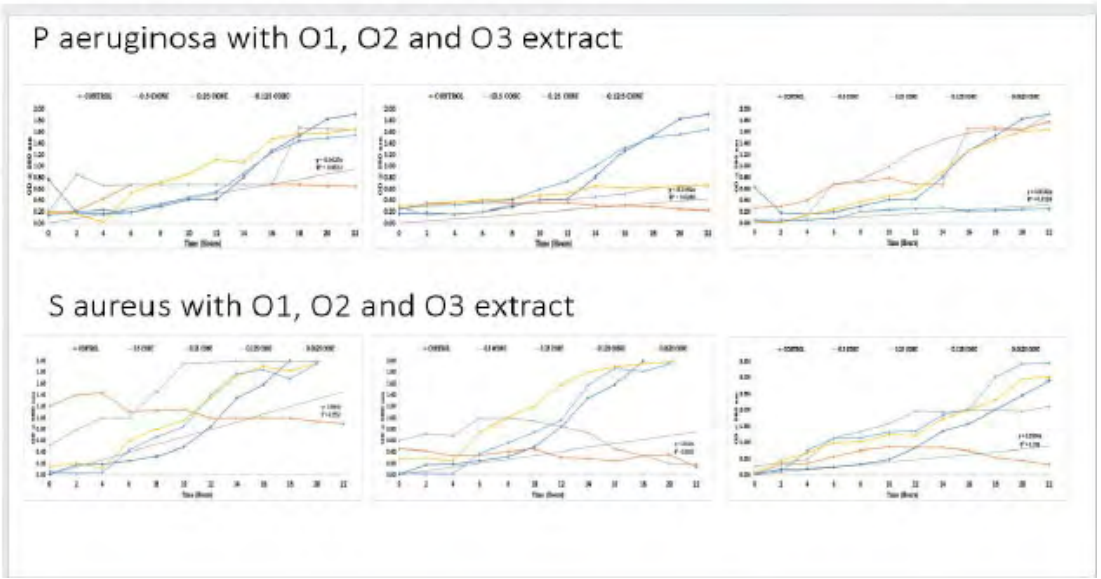


Fig. 2: *Pseudomonas aeruginosa* and *S aureus* growth curve over time when exposed to various concentrations with MIC

(N1) plant extracts shows functional groups of secondary metabolites such hydroxyl (-OH), carbonyl (C=O), aliphatic nitriles (CN), and amides, which contribute to the plant's therapeutic properties. For distinct functional groups, the FTIR spectrum shows separate absorption peaks at particular wavenumbers. Ruta graveolens, or rue, is a phenolic-rich plant having therapeutic benefits. Plants contain furanocoumarins and flavonoids.

Calligonum comosum (N2) FTIR indicates alkanes, aromatics, aliphatic amines, and carboxylic acids. FTIR shows that C. comosum's ethyl acetate fraction includes several phytochemical profiles and bioactive classes. The spectral data showed C-I stretch, C=O stretch, C-H stretch, and C-N bond peaks, indicating plant chemical structures.

FTIR analysis of Ziziphus spina-christi (N3) revealed functional groups, suggesting bioactive chemicals. Spectroscopic analysis identified hydroxyl groups, carbonyl functionalities, and aromatic

Table 1: Volatile organic compounds (VOCS) analysis Sample code: Q1 TeucriumpolumBio Activity

S. No.	Compounds detected	Results &unit: mg /l
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 – Teucriumpolum

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	1,1αBiphenyl, 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	1,1αBiphenyl, 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) Analysis Q3 – Artemisiaherbaalba

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

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	1,1αBiphenyl, 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

compounds connected to the plant's antioxidant and anti-inflammatory properties. Figure -1 illustrates one for each herb.

The fingerprint region (600-1500 cm^{-1}) of FTIR spectra helps distinguish these three substances. Identification and quality control are achievable since each plant has unique absorption patterns that reflect its phytochemical composition. The region also provides information on C-O-C, C=O, and C-N stretching vibrations, which highlight secondary metabolite structure.

FTIR shows hydroxyl and carbonyl functionalities, which indicate antibacterial, antioxidant, and anti-inflammatory action. The spectroscopic characterisation of all three species supports their traditional medicinal uses and indicates the presence of molecules responsible for their pharmacological properties [20].

GC- MS Results and Discussions

Different chemical compounds were found in GC-MS analysis of Calligonum comosum (N2), Ruta graveolens (N1), and Ziziphus spina-christi (N3)

Terpenoids, lipid molecules, alcohol compounds, and ketones were found in this investigation at various quantities. One may be seen in Tables 1 to 6.

The GC-MS investigation of Calligonum comosum (N2) yielded a large number of volatile and non-volatile chemical compounds with different concentrations. The main components are viridiflorol, palmitic acid, and 9-octadecenoic acid. The principal ingredients of the essential oil were found to be sesquiterpenes, monoterpenes, and aliphatic hydrocarbons. Additionally, the plant's protective volatile oil includes a significant amount of benzaldehyde derivative. The GC-MS investigation of Ziziphus spina-christi stem bark extracts revealed a large number of volatile and

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

Condition	0H	2H	4H	6H	8H	10H	12H	14H	16H	18H	20H	22H
CONTROL	0.038	0.187	0.157	0.196	0.236	0.41	0.418	0.838	1.271	1.527	1.832	1.913
0.5 CONC	0.278	0.278	0.278	0.278	0.191	0.336	0.529	0.842	1.259	1.445	1.604	1.889
0.25 CONC	0.187	0.187	0.187	0.187	0.145	0.306	0.453	0.822	1.249	1.447	1.598	1.879
0.125 CONC	0.155	0.1847	0.196	0.234	0.258	0.268	0.333	0.717	1.158	1.333	1.556	2.029
0.0625 CONC	0.125	0.135	0.146	0.165	0.198	0.211	0.232	0.275	0.301	0.333	0.334	0.31
CONTROL	0.01	0.187	0.157	0.196	0.284	0.41	0.418	0.838	1.271	1.527	1.832	1.913
0.5 CONC	1.579	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679
0.25 CONC	1.48	1.339	1.179	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.677	1.879
0.125 CONC	0.574	0.509	0.551	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.663	2.039
0.0625 CONC	0.029	0.147	0.214	0.559	0.759	0.863	1.044	1.162	1.44	1.553	1.624	2.529
Condition	0H	2H	4H	6H	8H	10H	12H	14H	16H	18H	20H	22H
CONTROL	0.75	0.187	0.157	0.196	0.268	0.41	0.418	0.838	1.271	1.527	1.832	1.913
0.5 CONC	0.35	0.328	0.46	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.67	1.779
0.25 CONC	0.012	0.32	0.36	0.568	0.756	0.654	0.842	0.84	0.754	0.51	0.445	0.51
0.125 CONC	-0.311	-0.298	-0.279	0.133	0.294	0.44	0.536	0.85	1.22	1.42	1.583	2.659

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

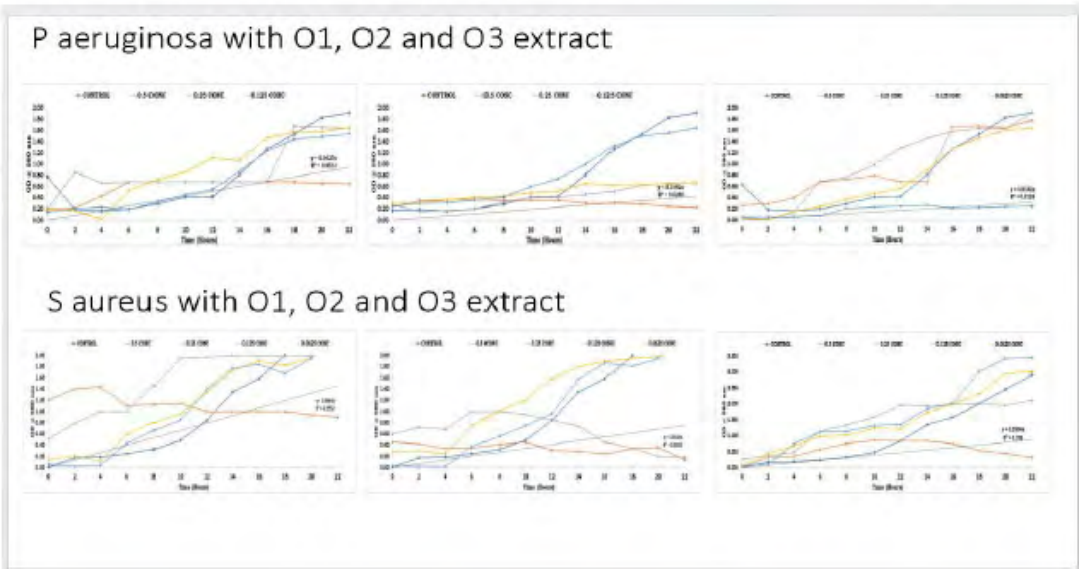
Sample	Type	0H	2H	4H	6H	8H	10H	12H	14H	16H	18H	20H	22H
N1	CONTROL	0.038	0.187	0.157	0.196	0.285	0.41	0.418	0.808	1.271	1.527	1.828	1.913
N1	0.5 CONC	0.278	0.278	0.278	0.278	0.191	0.335	0.529	0.842	1.269	1.445	1.604	1.889
N1	0.25 CONC	0.187	0.187	0.187	0.187	0.145	0.303	0.453	0.822	1.249	1.447	1.598	1.879
N1	0.125 CONC	0.155	0.1847	0.198	0.234	0.255	0.268	0.33	0.717	1.158	1.383	1.586	2.029
N1	0.0625 CONC	0.125	0.135	0.145	0.165	0.198	0.211	0.232	0.275	0.321	0.333	0.334	0.31
N2	CONTROL	0.01	0.187	0.157	0.196	0.284	0.41	0.418	0.808	1.271	1.527	1.828	1.913
N2	0.5 CONC	1.579	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679	0.679
N2	0.25 CONC	1.449	1.339	1.179	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.677	1.879
N2	0.125 CONC	0.574	0.509	0.561	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.663	2.039
N2	0.0625 CONC	0.029	0.147	0.214	0.569	0.759	0.863	1.044	1.162	1.44	1.558	1.624	2.529
N3	CONTROL	0.756	0.187	0.157	0.196	0.285	0.41	0.418	0.808	1.271	1.527	1.828	1.913
N3	0.5 CONC	0.325	0.328	0.496	0.679	0.679	0.679	0.679	0.679	0.679	0.679	1.67	1.779
N3	0.25 CONC	0.012	0.324	0.356	0.568	0.756	0.854	0.842	0.84	0.754	0.541	0.445	0.51
N3	0.125 CONC	-0.311	-0.293	-0.277	0.133	0.294	0.44	0.536	0.825	1.222	1.423	1.588	2.659
N3	0.0625 CONC	-0.226	-0.262	-0.259	0.018	0.142	0.273	0.324	0.666	1.092	1.333	1.554	2.679

non-volatile chemical compounds with different concentrations, including of alkaloids, tannins, flavonoids, glycosides, phenols, and terpenoids, and phytol. Gallic acid, ellagic acid, quercetin, phenol 2,5-bis(1,1-dimethylethyl), and decane, 2-methyl were found. The major components of essential oils were found to be trans-caryophyllene, alpha-pinene, and beta-caryophyllene. These three plant species contain a variety of bioactive compounds with great therapeutic potential, according to FTIR and GC-MS examinations. Functional group identification via FTIR detects phenolic compounds, flavonoids, and terpenoids, whereas GC-MS quantifies substances. These findings support the plants' historic use and its pharmacological and nutraceutical potential [21-22].

Bio Activity

MIC by Growth curve time assay

The antibacterial activity of Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) was tested utilizing a time-dependent growth inhibition assay. The growth



SA: ***S aureus***

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

curves of P aeruginosa and Staphylococcus aureus treated with N1, N2, and N3 showed that these extracts have the capacity to inhibit bacterial growth and reproduction. Table - 7 and Figure-2 shows the growth of P. aeruginosa in LB broth inoculated with 10^8 CFU/mL and exposed to 0.5x, 0.25x, 0.125x, and 0.0625x concentrations of N1 (66000 mg/L), N2 (33000 mg/L), and N3 (33,000 mg/L). There was a delay in exponential and growth at all concentrations: lag, exponential, and stabilization; however, decline phases were absent, as OD580 values included both viable and non-viable bacteria. In the absence of extract, P. aeruginosa rapidly entered the exponential growth phase with control; however, treatment with 0.0625x of N1, 0.5x of N2 and 0.25x N3 delayed growth for up to 4-5 hours, demonstrating significant bacteriostatic action at lower doses and bactericidal effects at higher concentrations. Similarly for S. aureus, treatments with 0.125x N2 and 0.5x N3 show decline and stagnation of growth, respectively. While N1 shows variable results without any clear sign of inhibition or stagnant phase, which indicates no effect at these concentrations [Table - 8] and (Figure -2). Doubling the concentration of N1 at 2x MIC shows a decline in the exponential phase, suggesting higher concentrations.

Biofilm Inhibition assay

Herbal extracts of Ruta graveolens (N1), Calligonum comosum (N2), and Ziziphus apina-christi (N3) was suppress biofilm formation at MIC and 0.5xMIC levels, according to antimicrobial growth curves. Figure 3 & 4 illustrates the outcomes of the biofilm inhibition investigation. N1, N2, and N3 had concentration-dependent effects on test microorganisms, with N2 inhibiting S. aureus by 94% and P. aeruginosa by 83% at MIC. In contrast, there were not much difference at 0.5xMIC for N2. The N2 extract exhibits a reduction of 91% & 85% at the MIC and 65% and 61% at the 0.5xMIC for S aureus and P seruginosa respectively. In contrast, the 2xMIC and MIC of N1 against P. aeruginosa and S aureus exhibited reductions of 91% & 87% at the 2xMIC and 72% and 59% at the MIC, respectively. Can be seen in the Table -9.

CONCLUSIONS

FTIR and GC-MS studies reveal that Ziziphus spina-christi, Calligonum comosum, and Ruta graveolens contain bioactive compounds that may treat skin rashes. GC-MS quantifies compounds, whereas FTIR functional group identification identifies phenolic molecules, flavonoids, and terpenoids. P. aeruginosa proliferated rapidly in the absence of extract, while 0.0625x N1, 0.5x N2, and 0.25x N3 suppressed growth for 4-5 hours, indicating bacteriostatic and bactericidal effects at lower doses and higher concentrations. S. aureus growth plateaus around 0.125x N2 and 0.5x N3. Despite mixed effects, no inhibition, or stationary phase, N1 has no effect at these concentrations. The result of a biofilm inhibition experiment. S. aureus was suppressed by 94% and P. aeruginosa by 83% at the concentration-dependent MIC of N2. N2 changed very little at 0.5xMIC. The N2 extract lowers S aureus and P seruginosa by 91%, 85%, 65%, and 61%, respectively, at MIC and 0.5xMIC. However, N1's 2xMIC and MIC against P. aeruginosa and S. aureus decreased by 91%, 87%, and 72%, 59%, respectively. This study may illuminate Calligonum comosum, Ziziphus spina-christi, and Ruta graveolens' bioactive compounds, molecular mechanisms, and therapeutic effects on human health, and clarified the plant's bioactivities and expanded medicinal applications.

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