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Development and characterization of polyherbal formulation from *Allium sativum, Cuminum cyminum, Cinnamomum verum, Elettaria cardamomum and Zingiber officinale* with therapeutic potential against sinusitis-associated pathogens

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Abstract: **Background**: One of the most common diseases in the world is sinusitis, which is an inflammation of the membranes lining the paranasal sinuses. The bacteria involved are highly resistant to antibiotics, prompting scientists to explore green bioactives as potential biofilm inhibitors. **Objectives**: The current study investigated the antioxidant potential, phytochemical screening, antibacterial and biofilm inhibition potential of polyherbal extracts prepared using distilled water, ethanol, hydroethanol and *n*-hexane solvents. However, no prior research has specifically addressed their use in treating sinusitis. **Methods**: Samples were prepared by maceration method followed by antioxidant and phytochemical analysis. Chemical fingerprinting was conducted using FTIR, GC-MS and LC-MS analysis. Antibacterial activity, Minimum inhibitory concentration (MIC) and biofilm inhibition was checked. **Results**: Among the extracts, polyherbal ethanol extract showed the highest antioxidant activity, followed by *n*-hexane, distilled water and hydroethanol. The distilled water extract showed the highest flavonoid and phenolic content, followed by hydroethanol. Different functional groups were identified in all extracts. Bioactive compounds such as quercitrin and kaempferol derivatives were identified. Antibacterial profile showed that hydroethanol extract was the most effective against sinusitis isolates (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus and Enterococcus faecalis*) with the lowest MIC against *Proteus mirabilis*. Biofilm inhibition assay revealed the highest inhibition by hydroethanol extract against *E. faecalis* followed by ethanol extract against *E. coli.* **Conclusion**: The findings suggest that polyherbal extracts, particularly in hydroethanol and ethanol, possess significant antioxidant, antibacterial and biofilm inhibiting properties and could serve as promising candidates for the effective management of sinusitis.

Keywords: Antioxidant; Antibacterial; Biofilm inhibition; Polyherbal extracts; Sinusitis isolates

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INTRODUCTION

Sinusitis is characterized by inflammation of the mucosal lining of paranasal sinuses. Since sinuses are part of the upper respiratory tract, it is closely linked to upper respiratory tract infections (Kaur and Kabra, 2023). Each year, approximately 2.7 million physician visits are recorded for chronic sinusitis (Prevention, 2022). Sinusitis involves inflammation of the air-filled sinuses around the nose, causing pain, congestion and infection. Sinusitis is classified based on physiological location, duration and allergic etiology. The most common causes of sinusitis are pollutants, viruses, bacteria and fungi. Chronic rhinosinusitis (CRS) is marked by prolonged inflammation of sinonasal mucosa. CRS persistence and pathogenicity are implicated with the bacterial ability to form biofilms (Drago *et al*., 2019). The predominant bacteria linked to biofilm formation in chronic rhinosinusitis include *Pseudomonas aeruginosa, Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus* (Fastenberg *et al*., 2016).

Biofilm refers to an interconnected network of microbes bound to living or non-living surfaces encased within a protective layer made of extracellular polymeric substances (EPS). Proteins, carbohydrates and organic compounds are the chief chemical constituents of EPS (Samrot *et al*., 2021). The extensive use of antibiotics increases resistance in biofilm-producing bacteria. Biofilm forming bacteria grow quickly and pass resistance genes to other bacteria making them hard to treat with existing antibiotics (Gebreyohannes *et al*., 2019). Other factors include limited supply of oxygen, reduced sensitivity of dormant bacteria within the biofilm's deeper layers with slow growth and metabolism (Tuli *et al*., 2024).

Antibiotics are one of the most commonly used medical treatments for CRS and biofilm inhibition. Antibiotic resistance, side effects and a lack of research supporting antibiotic efficacy have all contributed to the search for other treatment methods. However, overuse of antibiotics leads to resistance and surgery carries risks, particularly in children. Therefore, new treatment options are urgently needed (Poudineh *et al*., 2024). Many plants, like herbs and spices, produce small organic molecules that include flavonoids, tannins, phenolics, quinones and phenolic acids (Sun and Shahrajabian, 2023). These secondary metabolites exhibit antioxidant, antimicrobial, anticancer and anti-inflammatory properties (Shahid *et al*., 2021).

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The World Health Organization (WHO) indicated that over 80% of the global population utilizes various herbal remedies for disease treatment. A combination of two or more herbs is called a polyherbal formulation, which often has greater efficacy than a single herb due to synergistic effect. Combining herbal extracts augments therapeutic efficacy, amplifies pharmacological effects, facilitates expedited relief and diminishes adverse effects in comparison to conventional medications, with lower dose (Aladejana, 2023). The selection of *Allium sativum, Cuminum cyminum, Cinnamomum verum, Elettaria cardamomum and Zingiber officinale* was guided by their traditional use in managing respiratory and sinus infections and their reported antimicrobial and antibiofilm activities (Sagar *et al*., 2024; Bhatwalkar *et al*., 2024; Yassin *et al*., 2022; Osanlo *et al*., 2023; Jasim *et al*., 2025). However, a literature review revealed no study has been reported about the development of polyherbal products from *Allium sativum, Cuminum cyminum, Cinnamomum verum, Elettaria cardamomum and Zingiber officinale* for the treatment of sinusitis. The current study describes the antioxidant potential, phytochemical profile, biofilm inhibition and antibacterial efficacy of synthesized polyherbal products against sinusitis isolates.

MATERIALS AND METHODS

Extraction of phytochemicals

Collection, authentication and preparation of polyherbal extracts

Specimens of *Allium sativum, Cuminum cyminum, Cinnamomum verum, Elettaria cardamomum and Zingiber officinale* were procured from Imtiaz Mall, Faisalabad, Pakistan and authenticated by Dr. Farooq Ahmed (Associate Professor, Department of Botany, University of Agriculture, Faisalabad) in January 2024 and voucher numbers were issued: *Zingiber officinale* (234-4-24), *Allium sativum* (234-5-24), *Elettaria cardamomum* (234-2-24), *Cuminum cyminum* (234-1-24) and *Cinnamomum verum* (234-3-24). The Fresh samples of *Allium sativum* and *Zingiber officinale* were air dried in the shade and ground. The extraction procedure utilized distilled water, ethanol, hydroethanol and *n*-hexane as solvents. Briefly, a 1:1:1:1 ratio of each plant powder was mixed with 750 mL of 96% ethanol, hydroethanol, *n*-hexane and distilled water separately, placed on shaker for 3 days. Filtration was done with Whatman filter paper and the filtrates were concentrated on a rotary evaporator. Finally, the concentrates were weighed and stored at 4 ℃ (Wolde *et al*., 2018; Chen *et al*., 2016).

Phytochemical analysis

Determination of antioxidant potential of polyherbal extracts

A modified spectrophotometric method was used to measure the polyherbal extracts' total phenolic and flavonoid content (Fatima *et al*., 2022). The Folin-Ciocalteu method was used to measure the total phenolic content (TPC) of the extracts in mg GAE/mL, using gallic acid as a standard ($y=0.0032x+0.03461$ with $R^2=0.9497$). The Aluminum Chloride method was used to measure the total flavonoid content (TFC) in µg CE/mL, using catechin as a reference ($y=0.0038x+0.0285$ with $R^2=0.9835$). 99.9% methanol was used to make the 0.1 mM DPPH reagent solution. For the prepared extracts and DPPH solution, 1:9 ratios were employed. Using 190 µL of DPPH solution and 10 µL of each extract was incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm (Ihsanpuro *et al*., 2022).

Fourier transform infrared spectroscopy (FTIR)

A Bruker IFS 125HR spectrophotometer (Germany) was used for FTIR analysis in order to determine the functional groups present in the polyherbal extracts. Using a mortar and pestle, the finely ground extracts were first dried before being thoroughly combined with potassium bromide. A wavelength range of 4000 to 650 cm^{-1} was used to record the FTIR spectra. Then, using references, characteristic peaks corresponding to different functional groups were found (Chaudhary *et al*., 2019; Agulei *et al*., 2023).

Gas chromatography-mass spectroscopy (GC-MS) analysis

To determine the bioactive components of the polyherbal formulation, a QP 2010 Plus system (Shimadzu, Japan) was used to analyze the ethanolic extract using gas chromatography mass spectrometry (GC-MS). An HP-5 MS capillary column (30.0 m × 0.25 mm, 0.25 µm film thickness) with scan range (m/z 40-600) was used to separate the compounds. Using a micro-injector, a 0.5 µL aliquot of the extract was added to the system and the analysis was conducted between 0 and 37 minutes. The sample was ionized at 70 eV using the electron impact (EI) mode. The carrier gas, helium, flowed at a rate of 1.0 mL/min (Safdar *et al*., 2021).

Liquid chromatography-mass spectroscopy (LC/MS/MS) analysis

The analysis used a Shimadzu LCMS-8040 LC/MS/MS system with a Shimadzu Shim Pack FC-ODS column (2 mm x 150 mm, 3 µm) (Manuja *et al*., 2021). The column temperature was sustained at 35 °C and a sample injection volume of 1 µL was employed. Chromatographic separation was accomplished under isocratic conditions using a mobile phase of 90% methanol at a flow rate of 0.5 mL/min. The overall duration for the analysis was established at 80 minutes. Electrospray ionization (ESI) in positive ion mode performed mass spectrometric detection in positive ion mode, configuring the ion type to $[M]^+$. The capillary voltage was sustained at 3.0 kV, while the sampling cone voltage was established at 23.0 V. Gas flow rate of 60 mL/hr at a desolvation temperature of 350 °C enhanced desolvation, while the source temperature was maintained at 100°C. Low energy collision induced

dissociation (CID) was utilized with an impact energy of 5.0 V. Mass spectra were obtained across a mass to charge ratio (m/z) range of 10-1000, with a scan duration of 0.6 seconds for each scan.

Antibacterial assays

Antibacterial activity and minimum inhibitory concentration (MIC)

The microbial cultures of sinusitis isolates, including *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis and Escherichia coli* were collected and maintained by standardized method Zafar *et al*. (2022). The antibacterial activity of polyherbal extracts was evaluated by agar well diffusion method. Muller Hinton Agar (Oxoid, UK) was prepared under sterile conditions and bacterial strain was added. After adding a fresh culture of bacterial strain, media was poured into sterile petri plates, 6 mm wells were created using a borer and 100 µL of each extract was added to the respective labeled well. Ciprofloxacin served as a positive control and the zone of inhibition was measured in mm following 24 hours of incubation at 37 ℃. MIC value of polyherbal extracts was tested by the serial dilution method, as explained by Mathew *et al*. (2018). The first column of the microtiter plate was designated as the negative control, the second column as the positive control and the remaining columns for the samples (W, nH, E, & HE). All the wells of sterilized 96-well plates, except the first row, were filled with 50 µL of nutrient broth. In the first row, positive control (100 µL of ciprofloxacin), was introduced, while the negative control consisted of nutrient broth and the bacterial strain alone. 100 µL of each crude extract (10 mg/mL) was added to the respective labelled wells, and then 50 µL from each was shifted serially until the end. In the last well, 50 µL was discarded. 10 µL of bacterial strains were added to each plate, respectively, and incubated at 37 °C for 24 hours. Following incubation, 30 µL of resazurin solution was added to each well. Color change from blue to pink was recorded and pictures were taken. Then each row was labeled with the concerned concentration and MIC values were calculated.

Biofilm inhibition

Biofilm inhibition activity of polyherbal extracts was checked by following the method of Shahid *et al*. (2021). A microtiter plate assay was used for the analysis of bacterial biofilm inhibition. A microtiter plate having 96 wells with flat bottoms was used in this assay. Each well was filled with 100 µL nutrient broth, 100 µL of each extract and 20 µL of bacterial strain into respective wells. Positive control consisted of 100 µL nutrient broth, 100 µL of ciprofloxacin (antibiotic) and 20 µL of bacterial strain. The negative control consisted of nutrient broth and the bacterial strain. Plates were incubated at 37 °C for 24 hours. Following this period, plates were washed three times with 220 µL of sterile phosphate buffer (PBS, pH 7.2), after which residual bacteria were fixed by the addition of 220 µL of 99% methanol. Plates were dried after 15 minutes and stained by adding 220 µL of 0.1% crystal violet dye in each well. The biofilm was then re-solubilized by adding 220 µL of 33 % glacial acetic acid. Optical density was measured at 630 nm using a microplate reader (BioTek, USA).

Statistical analysis

All experiments were conducted in triplicate and the results are presented as mean values accompanied by their standard deviations (mean ± SD). Tukey's post hoc test was performed in Minitab 22.1.0 to determine statistically significant differences ($p < 0.05$) among group means.

RESULTS

Table 1 shows the percentage yield and antioxidant activities of polyherbal extracts. The distilled water extract had the highest yield, while *n*-hexane extract had the lowest. Various concentrations of the polyherbal extracts were employed to assess antioxidant activity using the DPPH radical scavenging assay and the IC_{50} value was calculated. The DPPH radical scavenging assay showed that the ethanol extract demonstrated the highest antioxidant activity. In terms of total flavonoid content (TFC), the distilled water extract exhibited the highest value, while total phenolic content (TPC) analysis revealed that the distilled water extract had the highest phenolic concentration. The $p<0.05$ indicated that all the results are statistically highly significant.

Characterization of the polyherbal extracts

Depending on the solvent polarity, the FTIR spectra of polyherbal extracts made with *n*-hexane (nH), hydroethanol (HE), water (W) and ethanol (E) showed different functional groups (Fig. 1). The polyherbal extracts of HE, W and E showed a broad absorption band at about 3269 cm^{-1}, suggesting the presence of O-H stretching vibrations that are characteristic of alcohols and phenolic compounds. The presence of aliphatic chains was indicated by peaks in all extracts that corresponded to C-H stretching and were located between 2920 and 2926 cm^{-1}. Since the solvent is non-polar when it comes to extracting lipophilic compounds, a sharp peak at 1741 cm^{-1} in the *n*-hexane extract verified the presence of carbonyl (C=O) functional groups, most likely from esters or ketones. Aromatic C=C stretching was evident around 1587-1607 cm^{-1} in polar solvent extracts, suggesting the presence of flavonoids and other aromatic phytochemicals. Furthermore, strong bands around 1026 cm^{-1} in HE, W and E indicated C-O stretching vibrations from glycosides or carbohydrates. These spectral differences highlight the role of solvent polarity in selectively extracting various bioactive compounds from polyherbal formulations, highlighting the diverse functional groups contributing to their biological activities.

Table 1. The percentage yield and antioxidant activities of prepared polyherbal extracts as evaluated by DPPH radical scavenging assay, TPC and TFC (Mean ±S.D)

Sample	Yield %	DPPH assay (IC_{50}) (µg/mL)	TFC (µg CE/mL)	TPC (µg GAE/mL)
*n*H	5.79±0.24^{c}	133.75 ± 0.85^{c}	88.12 ± 0.66^{d}	493.92 ± 0.47^{c}
W	17.95±0.96^{a}	146.59 ± 1.37^{b}	398.20 ± 0.55^{a}	718.30 ± 0.78^{a}
HE	10.93±0.54^{b}	216.94 ± 2.20^{a}	224.34 ± 0.52^{b}	702.26 ± 0.65^{b}
E	16.46±0.36^{a}	120.07 ± 1.15^{d}	97.15 ± 0.23^{c}	92.99 ± 0.78^{d}
p-value	<0.05	<0.05	<0.05	<0.05

**n*H= *n*-hexane extract, W= distilled water extract, HE= hydroethanol extract, and E= ethanol extract. Means that do not share a letter are significantly different.

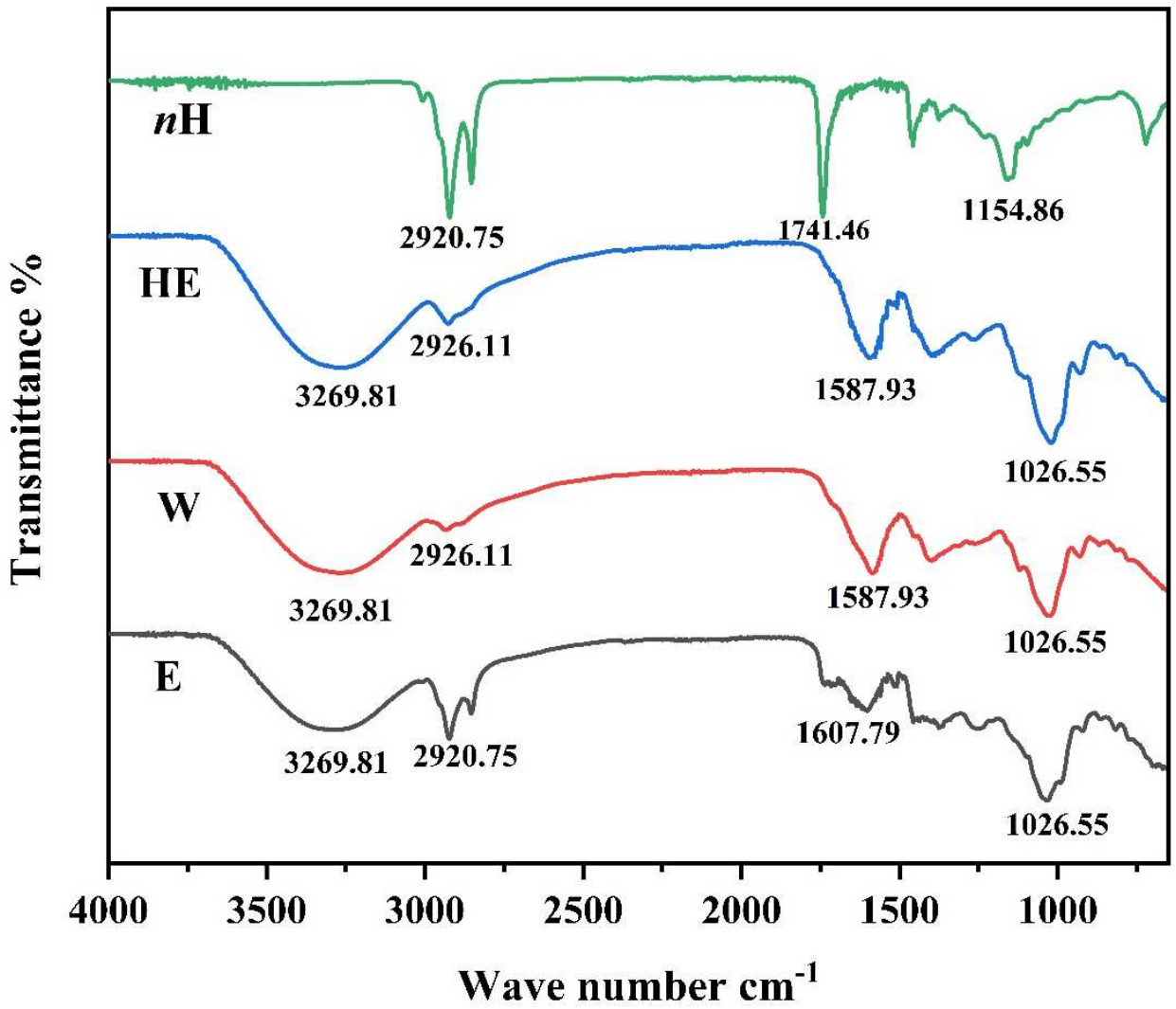

Fig. 1: FTIR spectra of polyherbal product extracts in *n*-hexane, hydroethanol, distilled water and ethanol solvents highlighting functional groups contributing to antioxidant activities.

Fig. 2 shows the chromatogram of GC-MS analysis of the polyherbal methanolic extract and relative content of each compound is shown in table 2. The total scan time for the sample was 0-40 minutes. The total number of peaks recorded includes 144 out of which only 26 peaks have compounds with 99% quality. Compounds have been listed in the table based on their peak number, retention time (minutes), % area, compound name, quality percentage and molecular formula. 9,12-octadecadienoic acid (Z, Z)-compound has the greatest area of 17.27 % out of these 26 compounds. Other major compounds with the highest percentage area include *n*-hexadecanoic acid (9.22%), (5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one (6.68%), cis-vaccenic acid (6.67%), (E)-1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one (4.28%) and octadecanoic acid (1.20%).

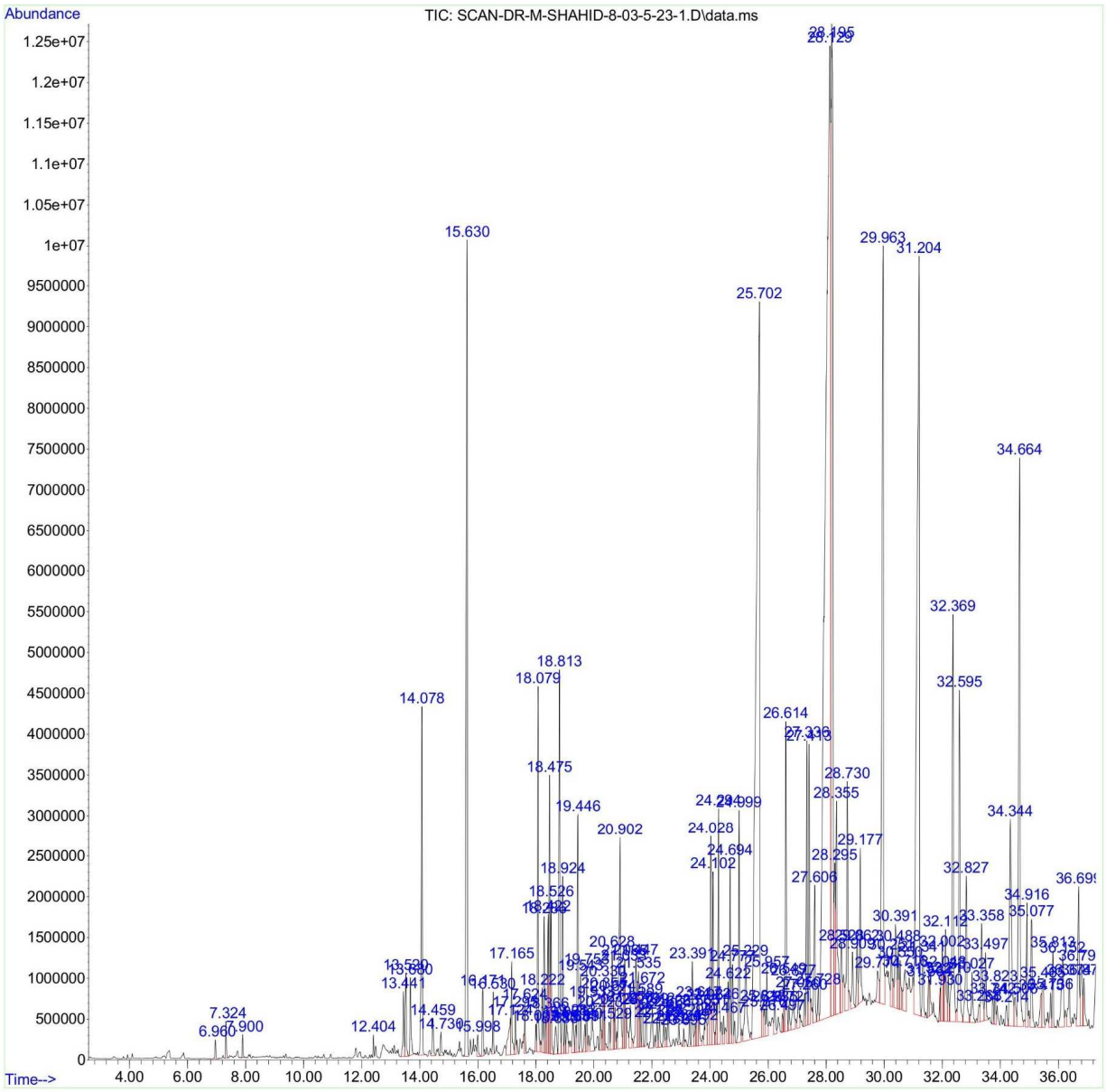

Fig. 2: GC-MS chromatogram of polyherbal methanolic extract for the analysis of volatile and semi-volatile compounds

The LC/MS/MS analysis of polyherbal hydroethanol extract identified a total of 156 peaks (Fig. 3 and Table 3). The major constituents include quercitrin (2.08%), quercetin-3-O-rhamnoside (1.89%), isorhamnetin-3-O-glucoside (1.72%), kaempferol 3-arabinopyranoside (1.71%), kaempferol-3-O-D-glucoside (1.65%), kaempferol (1.51%), kaempferol-3-O-rhamnoside (1.51%) and isoquercetin (1.51%). In addition to these major compounds, the sample contains many minor compounds, further illustrating the sample's rich and diverse phytochemical profile.

Antibacterial and biofilm inhibition potential of the polyherbal extracts

The antibacterial potential of polyherbal extracts was tested on both Gram-negative and Gram-positive bacterial strains by agar well diffusion method. A similar concentration (10 mg/mL) of these extracts was used on sinusitis isolates comprising three Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*) and two Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*). The antibacterial findings (Fig. 4) demonstrated that the polyherbal hydroethanolic extract has strong antibacterial efficacy against all bacterial strains, followed by distilled water, *n*-hexane while ciprofloxacin served as a positive control. These results indicated that hydroethanol, extract has a broad spectrum against both Gram-positive and Gram-negative bacteria.

The lowest inhibitory concentration of each extract was determined utilizing the resazurin assay.

Table 2: Secondary metabolites analyzed in polyherbal product by GC-MS analysis

Sr. no.	Peak number	Retention time (minutes)	Area %	Compound name	Quality	Molecular formula
1.	96	28.129	17.27	9,12-Octadecadienoic acid (Z,Z)-	99	$C_{18}H_{32}O_2$
2.	80	25.702	9.22	n-Hexadecanoic acid	99	$C_{16}H_{32}O_2$
3.	106	29.963	4.28	(E)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one	99	$C_{17}H_{24}O_3$
4.	122	32.595	1.36	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate	99	$C_{21}H_{32}O_6$
5.	20	18.079	1.21	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	99	$C_{15}H_{25}$
6.	99	28.355	1.20	Octadecanoic acid	99	$C_{18}H_{36}O_2$
7.	140	36.152	0.53	Stigmasterol	99	$C_{29}H_{48}O$
8.	126	33.358	0.52	Cannabinol	99	$C_{21}H_{26}O_2$
9.	26	18.526	0.48	beta-Bisabolene	99	$C_{15}H_{24}$
10.	127	33.497	0.28	5-Hydroxy-1-(4-hydroxy-3-methoxy	99	$C_{17}H_{26}O_4$
11.	124	33.027	0.26	Villosin	99	$C_{20}H_{28}O_2$
12.	21	18.222	0.20	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	99	$C_{15}H_{24}$
13.	14	16.530	0.19	Cinnamaldehyde dimethyl acetal	99	$C_{11}H_{14}O_2$
14.	52	21.589	0.17	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	99	$C_{15}H_{24}O$
15.	60	22.566	0.17	Tetradecanoic acid	99	$C_{14}H_{28}O_2$
16.	138	35.736	0.15	Squalene	99	$C_{30}H_{50}$
17.	10	14.730	0.08	2-Propen-1-ol, 3-phenyl-, (E)-	99	$C_9H_{10}O$
18.	19	18.002	0.07	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	99	$C_{15}H_{24}$
19.	121	32.369	1.87	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	99	$C_{19}H_{28}O_3$
20.	112	31.204	6.68	(5S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one ([6]-Gingerol))	99	$C_{17}H_{26}O_4$
21.	97	28.195	6.67	Cis-Vaccenic acid	99	$C_{18}H_{34}O_2$
22.	73	24.294	0.90	2,10-Bisaboladiene-1,4-diol	99	$C_{15}H_{26}O_2$
23.	78	24.999	0.80	Hexadecanoic acid, methyl ester	99	$C_{17}H_{34}O_2$
24.	79	25.229	0.64	6-Pentadecenoic acid, 13-methyl	99	$C_{16}H_{30}O_2$
25.	92	27.336	0.96	9,12-Octadecadienoic acid, methyl ester	99	$C_{19}H_{34}O_2$
26.	93	27.413	0.96	9-Octadecenoic acid (Z)-, methyl ester	99	$C_{19}H_{36}O_2$

The outcomes of antibacterial activity and minimal inhibitory concentration are correlated. Extracts having higher antibacterial potential have shown the lowest MIC values (Table 4). The lowest MIC value was depicted by ciprofloxacin (0.0195 mg/mL) followed by hydroethanol extract (0.625 mg/mL) against *Proteus mirabilis.* Ethanol extract has shown the lowest value (1.25 mg/mL) against *Escherichia coli* and *Staphylococcus aureus* while *n*-hexane extract has shown the greatest MIC value among all the extracts.

Biofilm inhibition potential of all extracts, along with ciprofloxacin as a positive control, was evaluated against sinusitis isolates at 10 mg/mL concentration, as shown in Fig. 5. Overall maximum biofilm inhibition activity of 73.65 ± 0.39 % was given by hydroethanol extract against *E. faecalis*. Ethanol and *n*-hexane extracts had also shown comparable biofilm inhibition activity, followed by distilled water extract, while ciprofloxacin was used as a positive control. All extracts exhibit biofilm inhibition capacity against all evaluated Gram-positive and Gram-negative bacterial strains, indicating that this polyherbal formulation had considerable biofilm inhibition potential against microbes.

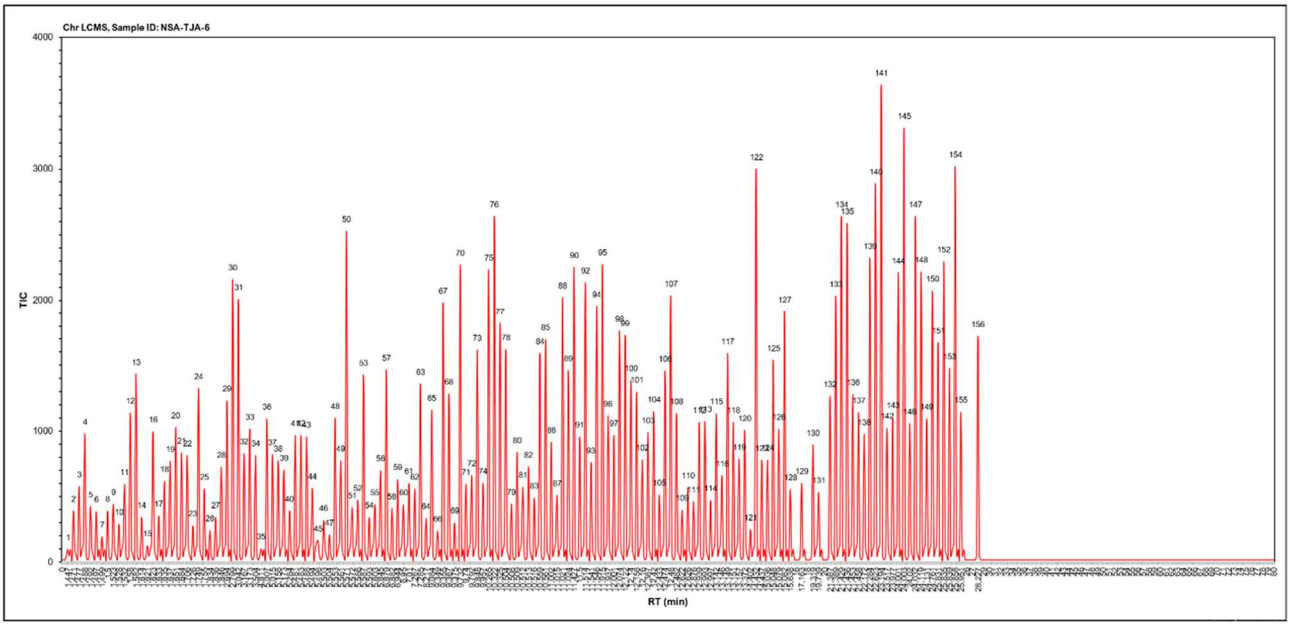

Fig. 3: LC/MS/MS chromatogram of polyherbal hydroethanol extract for the analysis of non-volatile phytochemicals.

Table 3: Secondary metabolites analyzed in polyherbal product by LC/MS/MS analysis

Serial #	Peak #	RT (min)	Curve area	Composition (%)	Compound name	Chemical formula
1.	141	23.194	3638.18965	2.08180	Quercitrin	$C_{21}H_{20}O_{11}$
2.	145	24.003	3310.13860	1.89409	Quercetin-3-O-rhamnoside	$C_{21}H_{20}O_{11}$
3.	154	25.858	3016.36590	1.72599	Isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$
4.	122	14.432	2999.71811	1.71646	Kaempferol 3-arabinopyranoside	$C_{20}H_{18}O_{10}$
5.	140	22.623	2889.42757	1.65335	Kaempferol-3-O-D-glucoside	$C_{21}H_{20}O_{11}$
6.	76	10.322	2639.94040	1.51060	Kaempferol	$C_{15}H_{10}O_6$
7.	134	21.429	2639.22690	1.51019	Kaempferol-3-O-rhamnoside	$C_{21}H_{20}O_{10}$
8.	147	24.032	2639.02312	1.51007	Isoquercetin	$C_{21}H_{20}O_{12}$
9.	135	21.436	2588.19053	1.48098	Quercetin-3-arabinoside	$C_{20}H_{18}O_{11}$
10.	50	5.571	2526.17021	1.44549	Eugenin	$C_{15}H_{10}O_4$
11.	139	22.284	2324.44890	1.33007	Botulin	$C_{30}H_{50}O_2$
12.	152	25.835	2296.84493	1.31427	Quercituron	$C_{21}H_{18}O_{13}$
13.	95	11.915	2274.42033	1.30144	Syzygiol	$C_{18}H_{18}O_5$
14.	70	9.732	2272.96980	1.30061	Naringenin	$C_{15}H_{12}O_5$
15.	90	11.427	2253.26420	1.28934	Quercetin	$C_{15}H_{10}O_7$
16.	75	10.265	2235.74430	1.27931	Luteolin	$C_{15}H_{10}O_6$
17.	148	24.119	2217.75525	1.26902	Myricitrin	$C_{21}H_{20}O_{12}$
18.	144	24.001	2213.30130	1.26647	Quercetin 3-glucoside	$C_{21}H_{19}O_{12}$
19.	30	2.799	2159.20834	1.23552	Vanillic acid	$C_8H_8O_4$
20.	92	11.514	2137.39069	1.22303	Myricetin	$C_{15}H_{10}O_8$
21.	150	24.761	2072.88003	1.18612	Maslinic acid	$C_{30}H_{48}O_4$
22.	107	12.48	2038.24963	1.16630	Zambesiacolactone A	$C_{20}H_{30}O_5$
23.	133	21.389	2034.38641	1.16409	Apigetrin	$C_{21}H_{20}O_{10}$
24.	88	11.021	2022.36258	1.15721	Kaempferide	$C_{16}H_{12}O_6$
25.	31	3.042	2010.13641	1.15022	Gallic acid	$C_7H_6O_5$
26.	67	9.355	1982.76117	1.13455	3,5,7-trihydroxyflavone	$C_{15}H_{10}O_5$
27.	94	11.546	1956.52159	1.11954	3,3',5,7-tetrahydroxy-4'-Methoxyflavone	$C_{16}H_{12}O_7$
28.	127	15.082	1913.63822	1.09500	3,7,4'-tri-O-methylkaempferol	$C_{18}H_{16}O_6$

Table. 3 is continue…….

Serial #	Peak #	RT (min)	Curve area	Composition (%)	Compound name	Chemical formula
29.	77	10.325	1822.22348	1.04269	Fisetin	$C_{15}H_{10}O_6$
30.	98	12.074	1763.32985	1.00899	Ayanin	$C_{18}H_{16}O_7$
31.	99	12.122	1729.37569	0.98956	Bergenin	$C_{14}H_{16}O_9$
32.	156	28.227	1721.86352	0.98526	Methyl 2-[(1R,2R,4S,7R,8S,11R,12R,13R, 16R)-7-[(2R)-2-hydroxy-5-oxo2H-furan-4-yl]-1,8,12,15,15-pentamethyl-5,18-dioxo3,6,14-Trioxapentacyclo [9.7.0.02, 4.02,8.012,16]octadecan-13-yl]acetate	$C_{27}H_{34}O_{10}$
33.	85	10.561	1699.36305	0.97239	[6]-gingerol	$C_{17}H_{26}O_4$
34.	151	25.831	1674.55544	0.95819	Tamarixetin-7-glucoside	$C_{22}H_{22}O_{12}$
35.	78	10.504	1620.26093	0.92713	[6]-gingerdione	$C_{17}H_{24}O_4$
36.	73	9.848	1617.13083	0.92534	[6]-shogaol	$C_{17}H_{24}O_3$
37.	84	10.559	1591.86498	0.91088	Galanganol B	$C_{18}H_{20}O_4$
38.	117	13.149	1591.76598	0.91082	Zambesiacolactone B	$C_{20}H_{30}O_6$
39.	125	15.048	1540.58227	0.88153	Campesterol	$C_{28}H_{48}O$
40.	153	25.839	1477.03393	0.84517	Mearnsitrin	$C_{22}H_{22}O_{12}$
41.	57	6.879	1464.31339	0.83789	Eugenetin	$C_{12}H_{12}O_4$
42.	89	11.024	1460.37906	0.83564	Rhamnocitrin	$C_{16}H_{12}O_6$
43.	106	12.474	1459.13562	0.83493	3,7-dihydroxy-5,3',4'-Trimethoxyflavone	$C_{18}H_{16}O_7$
44.	13	1.582	1433.36770	0.82018	Cinnamic acid	$C_9H_8O_2$
45.	53	5.587	1426.70799	0.81637	Eugenol acetate	$C_{12}H_{14}O_3$
46.	100	12.14	1385.60202	0.79285	Ombuin	$C_{17}H_{14}O_7$
47.	63	7.285	1359.06751	0.77767	Muricarpone B	$C_{19}H_{22}O_5$
48.	24	1.749	1324.84579	0.75809	3'-hydroxycinnamic acid	$C_9H_8O_3$
49.	101	12.158	1296.81194	0.74205	(2R,3R)-2-(1,3-benzodioxol-5-yl)-3,4-dihydro-5,7-dimethoxy2H-1-benzopyran-3-ol	$C_{18}H_{18}O_6$
50.	68	9.367	1283.22690	0.73427	Alpinetin	$C_{16}H_{14}O_4$
51.	136	21.453	1278.40592	0.73151	Dihydrokaempferol-3-O-α-Lrhamnopyranoside	$C_{21}H_{22}O_{10}$
52.	132	21.387	1265.87550	0.72434	Vitexin	$C_{21}H_{20}O_{10}$
53.	29	2.694	1231.15257	0.70448	Eugenol	$C_{10}H_{12}O_2$
54.	65	9.084	1159.69381	0.66359	Cardmomim	$C_{16}H_{14}O_4$
55.	104	12.42	1147.57349	0.65665	Isobiflorin	$C_{16}H_{18}O_9$
56.	155	25.951	1144.18555	0.65471	Ombuin-3-glucoside	$C_{23}H_{24}O_{12}$
57.	137	21.458	1142.80274	0.65392	Isoengeletin	$C_{21}H_{22}O_{10}$
58.	12	1.58	1138.27228	0.65133	Anethole	$C_{10}H_{12}O$
59.	108	12.482	1135.95865	0.65000	5-hydroxy-3,7,3',4'-Tetramethoxyflavone	$C_{19}H_{18}O_7$
60.	115	13.142	1132.84350	0.64822	Muricarpone A	$C_{20}H_{24}O_6$
61.	96	11.937	1117.05558	0.63919	(E,Z,Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-5,12,15-triene	$C_{23}H_{38}O_4$
62.	143	23.977	1103.12296	0.63122	Hirsutrin	$C_{22}H_{22}O_{11}$
63.	48	5.553	1098.64963	0.62866	α-ylangene	$C_{15}H_{24}$
64.	36	5.007	1094.44904	0.62625	Mycaminose	$C_8H_{17}NO_4$
65.	149	24.131	1093.72028	0.62584	β-amyrin acetate	$C_{32}H_{52}O_2$
66.	113	12.993	1075.76745	0.61556	Gingerenone B	$C_{22}H_{26}O_6$
67.	118	13.151	1067.58614	0.61088	3,5,7,3',4'-pentamethoxyflavone	$C_{20}H_{20}O_7$
68.	112	12.939	1067.32996	0.61073	3,5,7,4'-tetramethoxyflavone	$C_{19}H_{18}O_6$
69.	146	24.02	1056.54618	0.60456	Hyperoside	$C_{21}H_{20}O_{12}$
70.	20	1.651	1027.57465	0.58799	Citronellal	$C_{10}H_{18}O$
71.	142	23.517	1021.44258	0.58448	Betulinic acid	$C_{30}H_{48}O_3$
72.	33	3.173	1015.35918	0.58100	7-methoxycoumarin	$C_{10}H_8O_3$

Table. 3 is continue……

Serial #	Peak #	RT (min)	Curve area	Composition (%)	Compound name	Chemical formula
73.	126	15.079	1015.36355	0.58100	Tsaokoarylone	$C_{20}H_{20}O_4$
74.	120	13.372	1006.17991	0.57574	(2R,3R)-2,3-bis[(3,4-dimethoxyphenyl) methyl]-1,4-butanediol	$C_{22}H_{30}O_6$
75.	16	1.623	996.76423	0.57036	Vanillin	$C_8H_8O_3$
76.	103	12.387	990.54112	0.56680	Methyl 2-(1-hydroxy-2-oxopropyl) hexadec-2-enoate	$C_{20}H_{36}O_4$
77.	4	1.488	978.61754	0.55997	Limonene	$C_{10}H_{16}$
78.	138	22.172	973.63567	0.55712	Orientin	$C_{21}H_{20}O_{11}$
79.	41	5.481	966.59827	0.55310	α-cubebene	$C_{15}H_{24}$
80.	97	12.001	966.61321	0.55310	Rhamnetin	$C_{16}H_{12}O_7$
81.	42	5.483	966.59562	0.55309	Germacrene B	$C_{15}H_{24}$
82.	43	5.488	954.61373	0.54624	α-copaene	$C_{15}H_{24}$
83.	91	11.5	954.43830	0.54614	Capsaicin	$C_{18}H_{27}NO_3$
84.	86	11.002	914.37323	0.52321	[6]-gingerdiol	$C_{17}H_{28}O_4$
85.	130	19.319	894.62412	0.51191	β-amyrin	$C_{30}H_{50}O$
86.	80	10.509	838.24996	0.47965	Galanganol A	$C_{18}H_{20}O_4$
87.	21	1.684	833.77698	0.47709	Umbelliferone	$C_9H_6O_3$
88.	32	3.167	828.79559	0.47424	Cinnamyl acetate	$C_{11}H_{12}O_2$
89.	37	5.015	822.78316	0.47080	Scopoletin	$C_{10}H_8O_4$
90.	34	3.504	815.42777	0.46659	Methyleugenol	$C_{11}H_{14}O_3$
91.	22	1.706	813.47057	0.46547	Geranial	$C_{10}H_{16}O$
92.	119	13.157	786.66563	0.45014	Aulacocarpin B	$C_{21}H_{32}O_6$
93.	102	12.16	781.38438	0.44711	Trans-(-)-2-(1,3-benzodioxol-5-yl)-3,4-dihydro-5,7-dimethoxy2H-1-benzopyran-3-ol	$C_{18}H_{18}O_6$
94.	124	15.036	779.82631	0.44622	Aframodial	$C_{20}H_{30}O_3$
95.	123	14.437	779.54558	0.44606	Citrusoside A	$C_{21}H_{36}O_7$
96.	49	5.561	774.44222	0.44314	α-gurjunene	$C_{15}H_{24}$
97.	38	5.158	774.02582	0.44290	Geranyl acetate	$C_{12}H_{20}O_2$
98.	19	1.637	771.90498	0.44169	Linalool	$C_{10}H_{18}O$
99.	93	11.541	760.57966	0.43521	Gingerenone C	$C_{20}H_{22}O_4$
100.	82	10.513	727.12125	0.41606	Naringenin 7,4'-dimethyl ether	$C_{17}H_{16}O_5$
101.	28	1.846	725.20034	0.41497	Tsaokoin	$C_{10}H_{14}O_2$
102.	39	5.173	703.86978	0.40276	Linalyl acetate	$C_{12}H_{20}O_2$
103.	56	5.845	697.96237	0.39938	Jasmonic acid	$C_{12}H_{18}O_3$
104.	72	9.792	664.21222	0.38007	[6]-dehydroshogaol	$C_{17}H_{22}O_3$
105.	116	13.146	659.86156	0.37758	Aulacocarpin A	$C_{21}H_{32}O_5$
106.	59	6.944	631.58428	0.36140	Apiole	$C_{12}H_{14}O_4$
107.	18	1.635	616.38442	0.35270	Borneol	$C_{10}H_{18}O$
108.	129	17.163	604.38306	0.34583	β-sitosterol	$C_{29}H_{50}O$
109.	61	7.01	602.03984	0.34449	Viridiflorol	$C_{15}H_{26}O$
110.	74	9.957	601.88694	0.34440	[6]-paradol	$C_{17}H_{26}O_3$
111.	11	1.532	594.97962	0.34045	α-phellandrene	$C_{10}H_{16}$
112.	71	9.74	594.04454	0.33992	Uvangoletin	$C_{16}H_{16}O_4$
113.	3	1.477	578.42603	0.33098	α-pinene	$C_{10}H_{16}$
114.	81	10.512	572.16259	0.32740	Auraptene	$C_{19}H_{22}O_3$
115.	110	12.626	569.19013	0.32570	Gingerenone A	$C_{21}H_{24}O_5$
116.	44	5.494	563.82316	0.32262	β-caryophyllene	$C_{15}H_{24}$
117.	25	1.755	559.86434	0.32036	Isosafrole	$C_{10}H_{10}O_2$
118.	62	7.267	559.20378	0.31998	Xanthyletin	$C_{14}H_{12}O_3$
119.	128	15.638	555.30070	0.31775	Stigmasterol	$C_{29}H_{48}O$
120.	131	19.739	532.63809	0.30478	Friedelinol	$C_{30}H_{52}O$
121.	105	12.433	513.84788	0.29403	Sesamin	$C_{20}H_{18}O_6$

Table. 3 is continue……

Serial #	Peak #	RT (min)	Curve area	Composition (%)	Compound name	Chemical formula
122.	87	11.015	510.90129	0.29234	Aurentiacin	$C_{18}H_{18}O_4$
123.	83	10.516	491.86118	0.28145	Naringenin 5-methyl ether	$C_{16}H_{14}O_5$
124.	52	5.586	474.57183	0.27155	Isoeugenitol	$C_{11}H_{10}O_4$
125.	114	12.997	471.66782	0.26989	Isogingerenone B	$C_{22}H_{26}O_6$
126.	111	12.876	462.62234	0.26472	Isosinensetin	$C_{20}H_{20}O_7$
127.	9	1.523	443.81719	0.25396	γ-terpinene	$C_{10}H_{16}$
128.	79	10.508	443.81354	0.25395	Phytol	$C_{20}H_{40}O$
129.	55	5.603	439.97298	0.25176	α-cedrene	$C_{15}H_{24}$
130.	60	6.95	438.65634	0.25100	Nerolidol	$C_{15}H_{26}O$
131.	5	1.492	426.57794	0.24409	Terpinolene	$C_{10}H_{16}$
132.	51	5.572	416.25782	0.23819	5,7-dimethoxycoumarin	$C_{11}H_{10}O_4$
133.	58	6.882	409.85122	0.23452	Isoeugenitin	$C_{12}H_{12}O_4$
134.	109	12.622	397.21776	0.22729	[1,7-bis(4-hydroxy-3-methoxyphenyl)hepten-3-one]	$C_{21}H_{24}O_5$
135.	2	1.471	391.99350	0.22430	p-cymene	$C_{10}H_{14}$
136.	40	5.284	391.93457	0.22427	Calamenene	$C_{15}H_{22}$
137.	8	1.5	391.81506	0.22420	Myrcene	$C_{10}H_{16}$
138.	6	1.497	386.53985	0.22118	Camphene	$C_{10}H_{16}$
139.	17	1.633	353.73782	0.20241	Geraniol	$C_{10}H_{18}O$
140.	27	1.836	343.93590	0.19680	Tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)furan	$C_{10}H_{18}O$
141.	14	1.614	343.73536	0.19669	Citral	$C_{10}H_{16}O$
142.	54	5.593	342.73590	0.19612	α-trans-bergamotene	$C_{15}H_{24}$
143.	64	8.217	334.88496	0.19162	Pinocembrin	$C_{15}H_{12}O_4$
144.	46	5.503	319.57819	0.18287	β-bisabolene	$C_{15}H_{24}$
145.	69	9.375	301.63830	0.17260	8-methylpinocembrin	$C_{16}H_{14}O_4$
146.	10	1.528	289.67839	0.16576	Azulene	$C_{10}H_8$
147.	23	1.726	278.88023	0.15958	Teresantanane	$C_{10}H_{16}$
148.	121	14.402	250.65478	0.14343	Stellasterol	$C_{28}H_{46}O$
149.	26	1.834	241.76622	0.13834	p-menth-3-en-1-ol	$C_{10}H_{18}O$
150.	66	9.349	238.40032	0.13641	(−)-strobopinin	$C_{16}H_{14}O_4$
151.	47	5.504	210.61994	0.12052	δ-elemene	$C_{15}H_{24}$
152.	7	1.499	195.85825	0.11207	α-thujene	$C_{10}H_{16}$
153.	45	5.498	166.34782	0.09519	β-farnesene	$C_{15}H_{24}$
154.	15	1.621	126.97358	0.07266	Nerol	$C_{10}H_{18}O$
155.	35	4.811	103.98182	0.05950	Psoralen	$C_{11}H_6O_3$
156.	1	1.447	99.46195	0.05691	6-methyl-5-hepten-2-one	$C_8H_{14}O$

Table 4: Minimum inhibitory concentration of distilled water, ethanol, hydroethanol and *n*-hexane extracts of polyherbal product against sinusitis isolates using resazurin assay.

Bacterial strains	MIC of different extracts µg/mL				
	W	E	HE	*n*H	Ciprofloxacin
Escherichia coli	2.50	1.25	1.25	10.0	0.039
Staphylococcus aureus	1.25	1.25	1.25	2.50	0.039
Klebsiella pneumoniae	2.50	2.50	2.50	10.0	0.078
Proteus mirabilis	10.0	2.50	0.625	2.50	0.0195
Enterococcus faecalis	5	2.5	1.25	10.0	0.312

*W= Distilled water, E= Ethanol extract, HE= Hydroethanol extract, and *n*H= *n*-hexane extract.

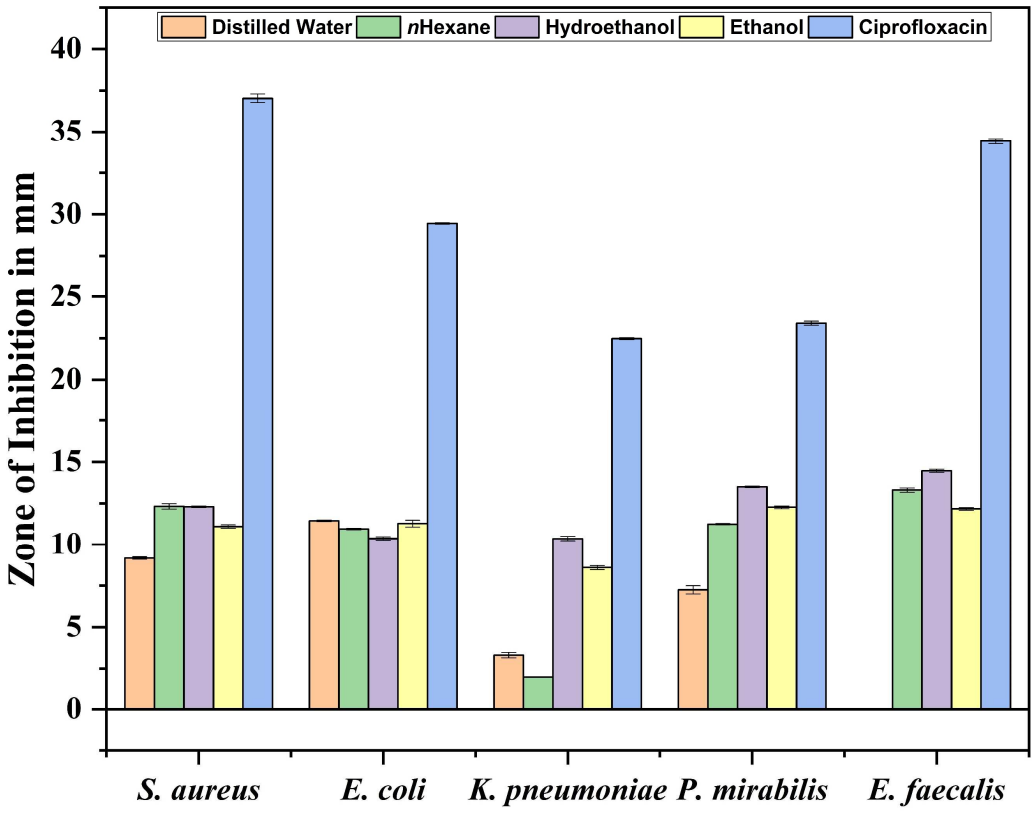

Fig. 4: Antibacterial potential of polyherbal product against sinusitis isolates (Mean ±S.D).

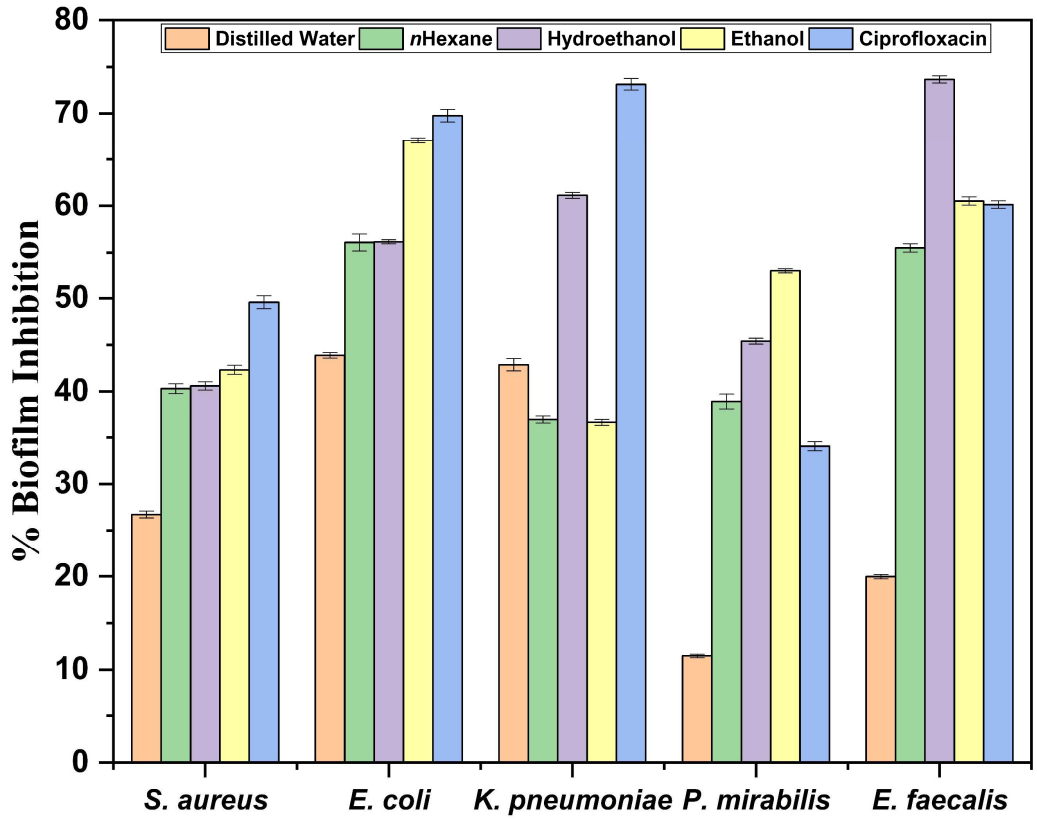

Fig. 5: Biofilm inhibition potential of polyherbal product against sinusitis isolates (Mean ±S.D).

DISCUSSION

This study is the first to develop a polyherbal formulation specifically targeting sinusitis isolates. The efficiency of phytochemical and antioxidant extraction depends on several factors, including extraction time, temperature, solvent characteristics and the particle size of plant material (Monteiro *et al*., 2020). Among these, solvent selection plays a critical role, as secondary metabolites vary in polarity and require solvents that match their chemical nature for effective extraction (Nawaz *et al*., 2020). To ensure the possible recovery of compounds, solvents from non-polar (*n*-hexane) to highly polar (distilled water) were used. Solvent polarity has a marked effect on both extraction yield and the antioxidant potential of phenolic compounds. As shown in table 1, the type of solvent significantly influenced extract yield, with the highest yield obtained using distilled water, a highly polar solvent (Abdelbaky, 2021).

The excessive production of free radicals is widely recognized as a key factor in the development and progression of many chronic diseases. Numerous studies have shown that natural products such as fruits, vegetables, edible flowers, cereal grains, medicinal plants and herbal infusions possess significant antioxidant activities (Mao *et al*., 2019). Plants are particularly valuable sources due to their strong antioxidant capabilities (Mohammed *et al*., 2024). Additionally, phenolic aldehydes are commonly associated with protection against oxidative stress (Hilal *et al*., 2024). Different varieties of *Allium sativum* which have significant DPPH radical scavenging activity besides the comparable flavonoid content. A study reported that ethanolic extract of *Zingiber officinale* has higher antioxidant activity than ethyl acetate and water extracts (Shahid *et al*., 2021; Yousfi *et al*., 2021). 6-Gingerol analyzed by GC-MS analysis is recognized for its several biological functions including antioxidant, anticancer and anti-inflammatory effects (Wang *et al*., 2014). Combining plant extracts with antibiotics and metal oxide nanoparticles enhances antibacterial and biofilm inhibition activity (Bataineh *et al*., 2024).

Various mechanisms have been proposed to explain the antibacterial activity of plant extracts and their bioactive compounds. One such mechanism involves the disruption of bacterial cell membranes, which impairs the bacteria's ability to form biofilms by reducing surface adhesion (Mao *et al*., 2019). Methanolic and water extracts of various *Allium sativum* types are known to have potent broad-spectrum antibacterial effects against similar bacterial strains, similar to this study (Shahid *et al*., 2021). Ethyl acetate and ethanolic extracts of *Zingiber officinale* have shown strong antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli* whereas the water extract exhibited the weakest activity (Yousfi *et al*., 2021). The ethanolic extract of *Elettaria cardamomum* possesses antimicrobial activity against *S. aureus, E. coli, P. gingivalis, P. intermedia, F. nucleatum and A. actinomycetemcomitans* (Souissi *et al*., 2020). The *n*-hexane extract and zinc oxide nanoparticles derived from *Cinnamomum verum* demonstrated antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Ansari *et al*., 2020). Cumin extract exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas species* (Balaram *et al*., 2021).

A study reported MIC values for *Zingiber officinale* extracts, which ranged from 0.62 to 1.25 mg/mL against *Staphylococcus aureus* and *E. coli* (Yousfi *et al*., 2021). Ethanolic extract from different varieties of *Allium sativum* has been reported to have remarkable biofilm inhibition activity against sinusitis isolates compared to its water extract (Shahid *et al*., 2021). Quercetin inhibits biofilm formation in bacteria by reducing viable cells, total protein and biofilm associated genes (*icaA* and *icaD*). It also inhibits the expression of *sigB, sarA* and *agrA* genes, which are related to virulence and quorum sensing. This disruption impairs bacterial communication and biofilm formation (Elsayed *et al*., 2024). Kaempferol and its derivatives (analyzed in the polyherbal product by LC/MS/MS analysis) also inhibit the biofilm formation by hindering microbial adhesion to the surfaces and interacting with the polar head groups of cell membranes, causing disruption and leakage of cell (Rajeswari *et al*., 2024). This study is particularly significant as it tackles the growing concern of antibiotic resistance in bacteria responsible for sinusitis by investigating polyherbal extracts as potential alternative therapies. It highlights the inherent antioxidant, antibacterial and biofilm inhibiting properties of the polyherbal formulation prepared using four different solvents. Additional studies, including cytotoxicity and *in-vivo* efficacy, are required before any translational applications can be considered.

CONCLUSION

This study demonstrates the development of a novel polyherbal formulation exhibiting significant antioxidant, antibacterial and antibiofilm activities against sinusitis associated isolates. The formulation contains diverse bioactive constituents, including phenolics, flavonoids and volatile compounds, as confirmed by FTIR, GC-MS and LC-MS/MS analysis. These results highlight the potential of this polyherbal product as a promising natural therapeutic approach for managing sinusitis. Future work will focus on pharmacological validation, formulation optimization and clinical evaluation to translate these findings into practical therapeutic applications.

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Authors' contributions

The conceptualization of the current study was created by Muhammad Tjammal Rehman and Muhammad Shahid, while the formal analysis was conducted by Muhammad Tjammal Rehman. Resources for the research were provided by Muhammad Shahid. The original draft of the manuscript was written by Muhammad Tjammal Rehman, while the intensive review and editing process was undertaken by Muhammad Shahid, Fatma Hussain and Bushra Akhtar. Additionally, the data and figures were visualized by Muhammad Tjammal Rehman.

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Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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