doi.org/10.36721/PJPS.2025.38.1.REG.219-232.1

The influence of molecular framework of phytochemicals from five Asteraceae species' extracts on their antibacterial and antioxidant activities

Abdelrahim Elbalola
Department of Biology, Faculty of Science, Tabuk University, Tabuk, KSA

Abstract: The structure of active compounds determines their functions. This study investigates the phytochemical composition of five plant species, tests their capacity to obstruct the growth of four pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*), and to scavenge free radicals, and inspects the relationship between the biological activities and the phytochemical structural frameworks. GC-MS analysis was employed for specifying phytochemicals. The plant methanolic and ethanolic extracts and the antibiotic (AMC30), were evaluated for antibacterial capability utilizing the disc diffusion method. For assessing antioxidant activity, the DPPH method was used. *Achillea fragrantissima* exhibited the highest phytochemical diversity. Gram-negative bacteria were less sensitive to plant extracts. *Pulicaria undulata* and *Pulicaria incisa* demonstrated greater efficacy in suppressing bacterial proliferation. *Artemisia judaica* and *P. undulata* extracts showed higher free radicle scavenging relative to other species. The antioxidant action, antibacterial properties against *B. subtilis*, *S. aureus* and *E. coli* exhibited a statistically significant correlation with aromatic monocyclic compounds. A significant negative correlation was also observed between aliphatic polycyclic compound contents and *E. coli* growth inhibition, indicating that this compound category may have an *E. coli* growth stimulating capacity. This study proposes research into phytochemical frameworks and their target-specific properties.

Keywords: Asteraceae, phytochemicals, antibacterial, antioxidant, molecular frameworks.

Submitted on 22-05-2024 – Revised on 09-08-2024 – Accepted on 12-08-2024

INTRODUCTION

Plants have a wide variety of compound classes with different molecular structures, including aromatic and aliphatic compounds, as well as monocyclic, polycyclic, and acyclic arrangements. The wide variety of phytochemicals available in nature makes them a valuable source of medicinal compounds for both humans and animals. These compounds are abundant and easily accessible. Throughout history and up to the present day, humanity has relied on phytochemicals for the prevention and treatment of numerous illnesses and health conditions. This is because many of these phytochemicals have extraordinary capabilities to fight against pathogenic bacteria, as well as other characteristics that support preventive and optimal health. The field of research investigating the relationship between chemical structure and biological activity is expanding. These studies provide valuable insights into the essential molecular components necessary for the development of potent and specific drugs. In addition, they can offer an understanding of the mechanism through which drugs produce their effects (Van Rossum 1966). Through comprehending the interplay between the structure and activity of compounds, it is possible to make targeted and precise alterations to achieve the desired function to the highest degree feasible (Lewandowski *et al.,* 2020). The Asteraceae family is renowned for its numerous traditional medicinal properties (Chithan *et al.,* 2012). The genus Artemisia consists of over 400 species, the majority of which are rich in powerful compounds. The genus Pulicaria plays a significant role in traditional herbal medicine, with numerous species like *P. undulata* and *P. incisa*. The intent of the present research is to examine potential correlations between the molecular and chemical compositions of active compounds found in five medicinal plants from Asteraceae family and the biological activities of phytochemicals derived from these plants. The species were chosen based on their importance as sources of herbal medicine for the local population of Tabuk area in KSA. The results of this investigation have the potential to offer valuable understanding regarding the possible therapeutic uses of these medicinal plants and contribute to the development of new natural products for the pharmaceutical and healthcare industries. Understanding the chemical composition and biological activities of these plants may also help in preserving traditional knowledge and promoting sustainable use of herbal resources in the region.

MATERIALS AND METHODS

Plant materials

The above-ground portions of five herbaceous species from the Asteraceae family, particularly *Pulicaria*

**Corresponding authors:* e-mails: aalbalolh@ut.edu.sa

undulata L., *Pulicaria incisa* Lam., *Artemisia herba-alba* Asso., *Artemisia judaica* L., and *Achillea fragrantissima* Forssk., were collected from different natural vegetation sites in the Tabuk region of Northwestern Saudi Arabia. This collection was assembled during the peak growth phase of these plant species. Aside from plants gathered for phytochemical analysis, voucher specimens of these samples (No. 9 - 13) were preserved in the departmental herbarium. Then, the samples were taken to the lab to be cleansed and dried in a sheltered location. The confirmation of species identities was carried out by referring to Chaudhary (1999) and the KEW database. Subsequently, the specimens were ground and stored for further analyses.

Extraction

The extraction process followed the conventional method. Each investigated plant material, weighing 20g, was mixed with 180 ml of distilled water. The mixtures were then heated at 90°C for 30 minutes and incubated overnight at 37°C and 150 rpm in a shaking incubator (Xu *et al*, 2008). In a similar manner, round bottom flasks were employed to mix 10g of the powdered plant materials being tested with a solution of ethanol and methanol in a ratio of 9:1. The mixtures were then incubated overnight at a temperature of 37°C and a speed of 150 revolutions per minute. After the liquid extracts were separated from the solid residue through filtration using a Whatman No. 1 filter, they were concentrated using a rotary evaporator.

Phytochemical analysis

Methanol extracts were analyzed using Thermo GC Trace Ultra version 5.0 gas chromatography and an MS DSQ II mass spectrometer for GC-MS. The investigation met requirements: Helium flowed at 1 ml/min through the 30 X 0.25mm X 0.25 m DB5-MS Capillary standard non-polar column. The oven was set at 70°C and increased by 6°C per minute to 260°C. Masses ranged from 50 to 650 m/z. It took 43 minutes to run. Classy Fire, an automated chemical categorization system, was used to classify all phytochemicals in plant extracts and determine their molecular structures and immediate ancestors (Djoumbou *et al.,* 2016). PubChem-NIH-National Library of Medicine provided the simplest synonyms for compounds (Kim *et al.,* 2023)

Antibacterial activity

The pathogenic bacterial strains selected for this study were *Bacillus subtilis* and *Staphylococcus aureus* (both Gram-positive) and *Escherichia coli* and *Pseudomonas aeruginosa* (both Gram-negative). These strains were obtained from the laboratory's stock culture and were grown on Mueller-Hinton broth agar at a temperature of 37°C for a duration of 24 hours. Prior to conducting any antimicrobial testing, the samples were subjected to sub-culturing following the incubation period. In order to create inoculums, bacteria were suspended in a sterile saline solution containing 0.85% NaCl. As stated in reference [20], the suspensions were kept at an optical density (OD) of 0.4 to 0.6 at a wavelength of 405 nm. This OD value corresponds to a cell density approximately equal to 0.5 McFarland, which is equivalent to an inoculum estimated to contain 106 to 108 colony forming units per mL (CFU/mL)

Disk diffusion was employed to evaluate antibiotic susceptibility (Zazharskyi *et al.,* 2019). The method involved applying the pre-prepared samples to Mueller-Hinton agar (MHA) plates with a sterile swab. After sterilization, 6 mm Whatman paper N5 discs were immersed in 5 L of the extracts.

The soaking solution was 10% dimethyl sulfoxide and 1% tween 80 in deionized water. Under the same conditions, the extract fractions were diluted with 5g/mL Augmentin AMC30 antibiotic and 10% v/v dimethyl sulfoxide and 1% v/v tween 80 in deionized water for control. Plates were kept at room temperature before being incubated at 37°C for 24 hours. Finally, the zones of inhibition and discs were measured in millimeters to determine antibacterial efficacy.

Antioxidant activity

According to Liyana-Pathiranan *et al.,* (2005), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test assessed the plants' antioxidant activity. This experiment mixed 1mL of extract with 200–1000 g/mL of 0.135mM DPPH. While gently stirring, the mixture was kept at room temperature without light for 40 minutes. Experimental positive control was ascorbic acid. The formula for DPPH scavenging activity (%) is [(Absorbance control - Absorbance sample)/Absorbance control] * 100. "Abs control" means DPPH + methanol absorbance, while "Abs sample" means DPPH radical absorbance. The absorbance of samples and control solutions was measured at 517 nm. The extracts' DPPH scavenging percentage was calculated using the equation.

Calculate DPPH scavenging activity (%) using the formula: [(Abs control - Abs sample)/Abs control] × 100. “Abs control” measures DPPH + methanol absorbance, while “Abs sample” measures DPPH radical absorbance.

The EC50, the concentration between the maximum and minimum response, was calculated using dose response models. The calculator is the 2023 Quest Graph™ EC50 Calculator from AATBioquest, Inc. The most powerful compound has the lowest EC_{50} value. In natural products chemistry, an increase in potency should likely be accompanied by an increase in a parameter that directly indicates potency. Singh *et al.* (2020) defined “effective dilution volume (EDV50)” as the inverse of the EC50 (1/EC50).

STATISTICAL ANALYSIS

PAST statistical analysis program version 4.13 was used to create a stacked chart that displays the distribution of phytochemical structural frameworks among the plant extracts. The peak areas of compounds obtained from GC-MS analysis were utilized as the foundation for determining the percentage of compounds belonging to each structural framework. This is because the peak area accurately represents the quantity of the chemical present in the extract. A bar chart was generated to display the antioxidant activity of the plants being studied. A linear Pearson correlation was carried out and a matrix was generated using JASP software version 0.18.3.0 to examine the potential correlations between the percentages of different phytochemical frameworks, antibacterial activities (measured as inhibitory zones in millimeters), and antioxidant activities (measured as EDV50 values).

RESULTS

Phytochemical composition of plant extracts

Epianastrephin (-) was the most abundant of 48 phytochemicals in *A. fragrantissima* extract (table 2 and fig. 1). *A. herba-alba* (table 2 and fig. 2) and *A. judaica* (table 3 and fig. 3) extracts contained 17 phytochemicals each, with 3-Thujanone and Xanthoxylin being the most respectively abundant. *P. incisa* extract contained 25 phytochemicals (table 4 and fig. 4), with caryophyllene oxide being the most abundant. Spathulenol was the most abundant of 24 phytochemicals in *P. undulata* extract (table 5 and fig. 5). Fig. (6) showed plant extracts with varying amounts of molecular frameworks.

The two Pulicaria species' extracts were mostly aliphatic homo-monocyclic compounds. *A. judiaca* extract was mostly aromatic homo-monocyclic compounds. However, *A. herba-alba* extract was mostly aliphatic homo-polycyclic compounds. *A. fragrantissima* extract contained aliphatic and aromatic homo-monocyclic compounds. *P. incisa* and *A. herba–alba* extracts contained a few aromatic homo-monocyclic chemicals. Only the *A. judaica* extract contained aromatic heteromonocyclic compounds, while *P. undulata* contained aromatic homopolycyclic compounds. Some examples of compounds from different molecular frameworks identifies were shown in fig. 9.

Antibacterial activity

Table (6) showed that all plant extracts demonstrated higher effectiveness against gram-positive bacteria in comparison to gram-negative bacteria. The extract from *P. undulata* demonstrated the highest efficacy against *B. subtilis,* whereas the extract from *P. incisa* exhibited the greatest effectiveness against *S. aureus*. The *A. judaica* extract exhibited the highest efficacy against *E. coli*, while *A. herba-alba* extract was most effective against *P. aeruginosa*.

Antioxidant activity

EDV50 values showed that *P. undulata*, *A. judaica*, and *A. fragrantissima* extracts scavenged free radicals better than *P. incisa* and *A. herba-alba* (fig. 7).

Molecular framework/biological activity relationship

Fig. (8) demonstrated a notable positive correlation ($p< 0.05$) between aromatic monocyclic compounds and the inhibition ability against *E. coli*, *B. subtilis* and *S. aureus*, as well as the antioxidant activity of plant extracts. The association study uncovered a significant positive association ($p<0.05$) between aromatic heteromonocyclic compound content and *E. coli* bacteria inhibition. A significant negative correlation was also observed between aliphatic polycyclic compound contents and *E. coli* growth inhibition, indicating that this compound category may have an *E. coli* growth stimulating capacity.

DISCUSSION

According to previous studies, *A. fragrantissima*, among Asteraceae species, had the highest taxa_S, an alpha phytochemical diversity index that measures the number of identified compounds in the extract. Additionally, it exhibited the greatest Shannon phytochemical diversity, while *P. incisa* showed the lowest phytochemical diversity (Elbalola *et al.,* 2023). The most striking finding was that the positive control antibiotic AMC30 was inactive against *P. aeruginosa*. Gram-negative bacteria are less susceptible to antimicrobials than gram-positive bacteria because they have an external membrane.

Thus, they are expected to significantly increase global illness and death rates. Gram-negative bacteria are a major concern for the WHO (Breijyeh *et al.,* 2020). Multiple studies have found antibiotic resistance in *P. aeruginosa* (Lister *et al.,* 2009; Mulcahy *et al.,* 2008). Ruiz-Garbajosa *et al.,* (2017) attributed the phenomenon to the microorganism's remarkable ability to acquire resistance to common antibacterial medications. Mutations in chromosome genes or microbe-gained resistance can cause resistance. *P. undulata* and *P. incisa* have many aliphatic homo polycyclic compounds, which make them antibacterial. Spathulenol dominates *P. undulata*, while Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- dominates *P. incisa*. Myriactis nepalensis essential oil contained spathulenol, a potent gram-positive bacteria inhibitor.

Many authors reported that *P. undulata* phytochemical constituents showed better free radicle scavenging compared to the other species (Rav *et al.,* 2011; Hussein 2017; Mohammed 2021). Due to their high aromatic homo monocyclic chemical content, *P. undulata*, *A. judaica* and *A. fragrantissima* are better antioxidants than other species.

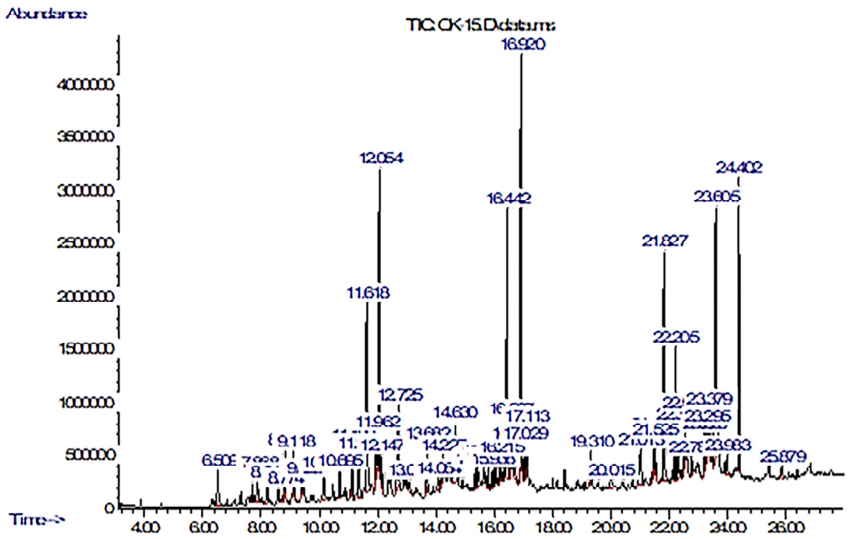


Fig. 1: GC-MS chromatogram of *A. fragrantissima* methanolic extract.

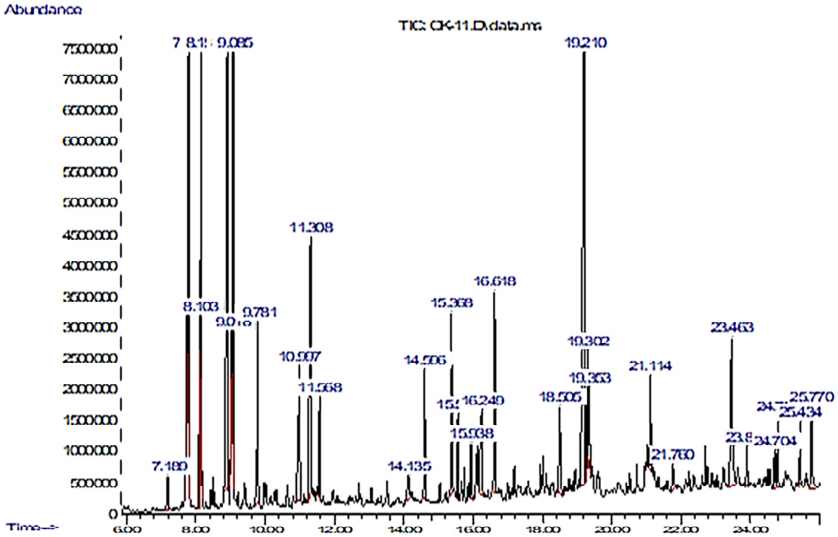


Fig. 2: GC-MS chromatogram of *A. herba-alba* methanolic extract.

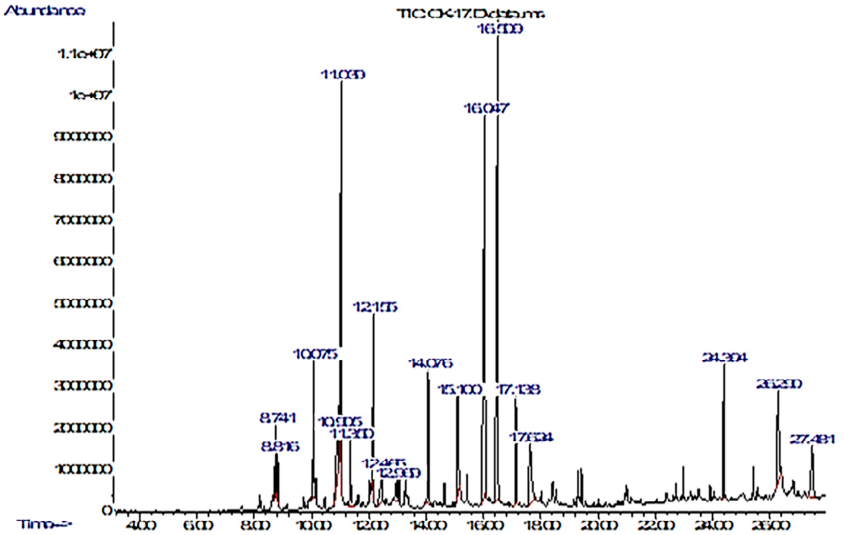


Fig. 1: GC-MS chromatogram of *A. judaica* methanolic extract.

Table 1: Phytochemical composition of *A. fragrantissima* methanolic extract, compound peak areas, direct parents and molecular frameworks.

No.	Compound identifier	Area	Direct parent	Molecular framework
1	cis-Carvyl acetate	739791	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
2	Verbenyl acetate (trans)	780536	bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
3	Thymol	4264559	Aromatic monoterpenoids,	Aromatic homomonocyclic compounds
4	(+)-trans-Isolimonene	570815	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
5	Myrcenylacetat	591868	Acyclic monoterpenoids	Aliphatic acyclic compounds
6	8-Chlortheobromin	641529	Monocyclic monoterpenoids	Aliphatic heteromonocyclic compounds
7	Patchoulane	1894513	Sesquiterpenoids	Aliphatic homopolycyclic compounds
8	trans-Shisool	707843	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
9	.alpha.-Farnesene	828854	Sesquiterpenoids	Aliphatic acyclic compounds
10	Cadabicilone	5098896	Eudesmanolides, secoeudesmanolides and derivatives	Aliphatic heteropolycyclic compounds
11	Longifolenaldehyde	1303878	Sesquiterpenoids	Aliphatic homopolycyclic compounds
12	p-Menth-1-en-9-ol	996266	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
13	Ambrosin	471385	Ambrosanolides and secoambrosanolides	Aliphatic heteropolycyclic compounds
14	IZQBEEISLWNFIO-KBPBESRZSA-N	845271	Monocyclic monoterpenoids	Aliphatic homomonocyclic compounds
15	alpha-Bulnesene	1116184	Sesquiterpenoids	Aliphatic homopolycyclic compounds
16	2(5H)-Furanone, 5,5-dimethyl-	1084960	Butenolides	Aliphatic heteromonocyclic compounds
17	Lavender lactone	761731	Gamma butyrolactones	Aliphatic heteromonocyclic compounds
18	1,4-Cyclohexadiene, 3,3,6,6-tetramethyl-	369466	Cyclic olefins	Aliphatic homomonocyclic compounds
19	2,4-Cyclopentadiene-1-ethanamine	393449	Monoalkylamines	Aliphatic homomonocyclic compounds
20	Bicyclo[2.2.2]octenone	657382	Cyclohexenones	Aliphatic homopolycyclic compounds
21	Isophorone	848661	Cyclohexenones	Aliphatic homomonocyclic compounds
22	Ketoisophorone	546061	Cyclohexenones	Aliphatic homomonocyclic compounds
23	1-Butanone, 1-cyclohexyl-	569920	Ketones	Aliphatic homomonocyclic compounds
24	2-Cyclohexen-1-one, 3,5-dimethyl-	1150833	Cyclohexenones	Aliphatic homomonocyclic compounds
25	3,3-Dimethyl-6-methylenecyclohexene	1319543	Branched unsaturated hydrocarbons	Aliphatic homomonocyclic compounds
26	Bmlyrjoyqdjmor-uhfffaoysa-n	6277059	Branched unsaturated hydrocarbons	Aliphatic homomonocyclic compounds

Continue…

27	4-ethyl-3,4-dimethylcyclohexa-2,5-dien-1-one	395415	Cyclic ketones	Aliphatic homomonocyclic compounds
28	Lilac alcohol D	3145927	Tetrahydrofurans	Aliphatic heteromonocyclic compounds
29	2-pentynol	411645	Primary alcohols	Aliphatic acyclic compounds
30	3-Methyl-3-hexene	889484	Branched unsaturated hydrocarbons	Aliphatic acyclic compounds
31	1H-Indene, 1-ethylideneoctahydro-, trans-	441752	Branched unsaturated hydrocarbons	Aliphatic homopolycyclic compounds
32	Cyclohexanone, 2-(2-bromo-4,4-dichlorobutyl)-	520868	Cyclic ketones	Aliphatic homomonocyclic compounds
33	3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl-	2136381	Medium-chain aldehydes	Aliphatic acyclic compounds
34	Davanone B	395633	Tetrahydrofurans	Aliphatic heteromonocyclic compounds
35	Nuvzhuhadluejs-uhfffaoysa-n	433064	Branched unsaturated hydrocarbons	Aliphatic acyclic compounds
36	Ogkuljwtozzdjn-uhfffaoysa-n	402829	Primary alcohols	Aliphatic homomonocyclic compounds
37	1,5,9,13-Tetradecatetraene	454294	Alkatetraenes	Aliphatic acyclic compounds
38	Xanthoxylin	5703679	Alkyl-phenylketones	Aromatic homomonocyclic compounds
39	2,3,4-Trimethyl-hex-3-enal	722214	Medium-chain aldehydes	Aliphatic acyclic compounds
40	Lilac alcohol formate A	747131	Tetrahydrofurans	Aliphatic heteromonocyclic compounds
41	Bicyclo[6.1.0]non-1-ene	882736	Polycyclic hydrocarbons	Aliphatic homopolycyclic compounds
42	palmitic acid	522900	Long-chain fatty acids	Aliphatic acyclic compounds
43	Velleral	2351447	Organic oxides	Aliphatic homopolycyclic compounds
44	Podocarp-12-en-14-ol	834095	Hydrophenanthrenes	Aliphatic homopolycyclic compounds
45	Epianastrephin, (-)	8402652	Benzofurans	Aliphatic heteropolycyclic compounds
46	Diazoprogesterone	484466	Gluco/mineralocorticoids, progestogins and derivatives	Aliphatic homopolycyclic compounds
47	Mono(2-ethylhexyl) phthalate	4321176	Benzoic acid esters	Aromatic homomonocyclic compounds
48	Stigmasterol	434532	Stigmastanes and derivatives	Aliphatic homopolycyclic compounds

The strong positive association between antioxidant activity and the aromatic monocyclic content such as thymol, xanthoxylin, thymol acetate, alpha-Curcumene, p-Cymen-8-ol, Isoelemicin and other compounds revealed in this study supported this finding. All these phytochemicals are phenolics, which can act as antioxidants through various mechanisms (Valentão *et al.,* 2003; Valentão *et al.,* 2002a, 2002b, 2002c; Heim *et al.,* 2002; Payá *et al.,* 1992; Choi *et al.,* 2002; Parr *et al.,* 2002). Additionally, they can attach to metal ions that generate free radicals. Hydrophobic benzenoid rings and hydrogen bonding phenolic hydroxyl groups allow phenolics to interact with proteins. By inhibiting radical-producing enzymes like cytochromes P450, lipoxygenases, cyclooxygenase and xanthine oxidase, they act as antioxidants.

Heterocyclic compounds are important in medical chemistry and pharmacy (Łowicki *et al.,* 2022). Many biologically active chemicals, including natural and pharmaceutical drugs, have cyclic organic frameworks, especially heterocycles. These frameworks are crucial in organic and medicinal chemistry. Lu *et al.* (2005) and Kusumaningsih *et al.* (2017) found that heterocyclic compounds containing nitrogen, sulfur and oxygen are potent microbe-killers.

CONCLUSIONS

Aromatic monocyclic compounds are significantly effective against *E. coli*; aliphatic hetero and homo polycyclic compound structural frameworks are the cause of the antibacterial activities against gram-positive bacteria and the aromatic homo monocyclic compound contents are the potent antioxidant agents relative to other structures.

Table 2: Phytochemical composition of *A. herba-alba* methanolic extract, compound peak areas, direct parents and molecular frameworks

No.	Compound identifier	Area	Direct parent	Molecular framework
1	3-Thujanone	21740449	Bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
2	Thujone	17805330	Bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
3	cis-beta-Farnesene	3632623	Sesquiterpenoids	Aliphatic acyclic compounds
4	Sesquiphellandrene	5566314	Sesquiterpenoids	Aliphatic homomonocyclic compounds
5	(-)-Spathulenol	1705634	5,10-cycloaromadendrane sesquiterpenoids	Aliphatic homopolycyclic compounds
6	Nfzwwjwnbxicbw-uhfffaoysa-n	5988751	Eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids	Aliphatic homopolycyclic compounds
7	(4E)-2,7-Dimethyl-4,6-octadien-2-ol #	16584349	Tertiary alcohols	Aliphatic acyclic compounds
8	Artemisia ketone	6317033	Enones	Aliphatic acyclic compounds
9	palmitic acid	5511831	Long-chain fatty acids	Aliphatic acyclic compounds
10	(Z)-6-Dodecen-4-olide	7330029	Gamma butyrolactones	Aliphatic heteromonocyclic compounds
11	4-Methyl-1,3-pentadiene	13033701	Branched unsaturated hydrocarbons	Aliphatic acyclic compounds
12	Icvzmtigtxbihi-clfysbassa-n	2651220	Branched unsaturated hydrocarbons	Aliphatic acyclic compounds
13	Isobutylbenzene	1280395	Phenylpropanes	Aromatic homomonocyclic compounds
14	Sbtowvndplpksy-uhfffaoysa-n	5244980	Trialkylheterosilanes	Aliphatic heteromonocyclic compounds
15	Nigpdtpduaqpha-uhfffaoysa-n	2451344	Organobromides	Aliphatic homomonocyclic compounds
16	10-Methylanthracene-9-carboxaldehyde	9412869	Anthracenes	Aromatic homopolycyclic compounds
17	Spathulenol	3786275	5,10-cycloaromadendrane sesquiterpenoids	Aliphatic homopolycyclic compounds

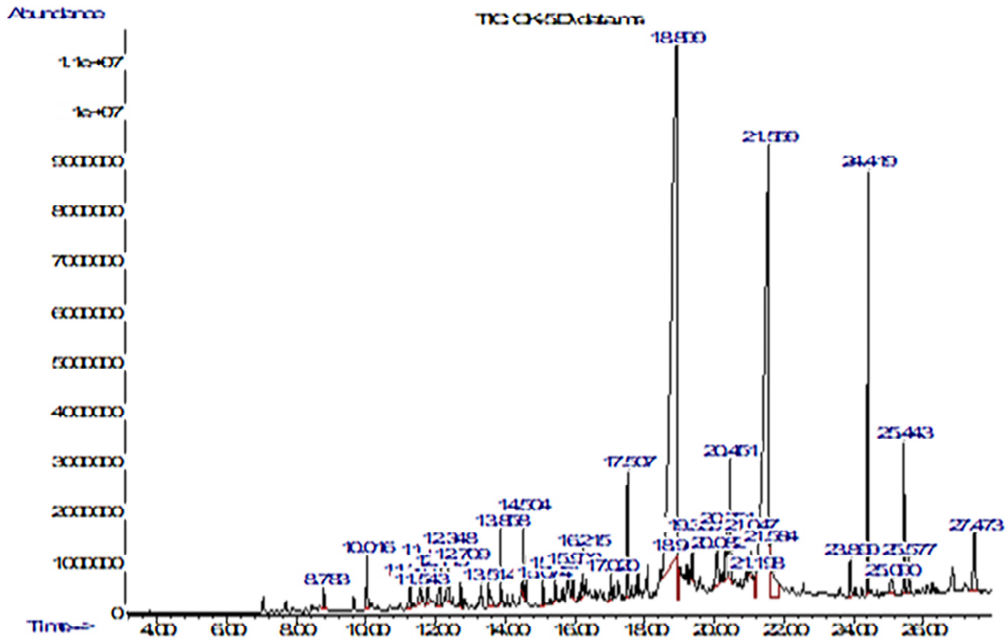


Fig. 2: GC-MS chromatogram of *P. incisa* methanolic extract.

Table 3: Phytochemical composition of *A. judaica* methanolic extract, compound peak areas, direct parents and molecular frameworks.

No.	Compound identifier	Area	Direct parent	Molecular framework
1	(+)-Linalool	3164432	Acyclic monoterpenoids	Aliphatic acyclic compounds
2	Thujyl alcohol	6370414	Bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
3	Cyclohexanone, 2-methylene-5-(1-methylethyl)-	27539848	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
4	Uuxrfmarqfdghe-yrnvussqsa-n	6211984	Acyclic monoterpenoids	Aliphatic acyclic compounds
5	Thymol acetate	6246479	Aromatic monoterpenoids	Aromatic homomonocyclic compounds
6	1,3-Cyclopentadiene, 1,2,5,5-tetramethyl-	1936166	Branched unsaturated hydrocarbons	Aliphatic homomonocyclic compounds
7	DKLRQEIICLZTQW-UHFFFAOYSA-N	3833852	Pyrazolines	Aliphatic heteromonocyclic compounds
8	Pyridine, 4-methyl-, 1-oxide	3894296	Methylpyridines	Aromatic heteromonocyclic compounds
9	Safranal	1919330	Organic oxides	Aliphatic homomonocyclic compounds
10	Ethanone, 1-(2-hydroxy-4-methoxyphenyl)-	7888694	Alkyl-phenylketones	Aromatic homomonocyclic compounds
11	Phenol, 4-methoxy-2,3,6-trimethyl-	35811264	Methoxyphenols	Aromatic homomonocyclic compounds
12	Xanthoxylin	44718187	Alkyl-phenylketones	Aromatic homomonocyclic compounds
13	Bicyclo[6.1.0]non-1-ene	4810218	Polycyclic hydrocarbons	Aliphatic homopolycyclic compounds
14	5-t-Butylpyrogallol	8509713	Pyrogallols and derivatives	Aromatic homomonocyclic compounds
15	Mono(2-ethylhexyl) phthalate	5127844	Benzoic acid esters	Aromatic homomonocyclic compounds
16	4',5-Dihydroxy-7-methoxyflavanone	9018832	7-O-methylated flavonoids	Aromatic heteropolycyclic compounds
17	3-Epimoretenol	6132472	Hopanoids	Aliphatic homopolycyclic compounds

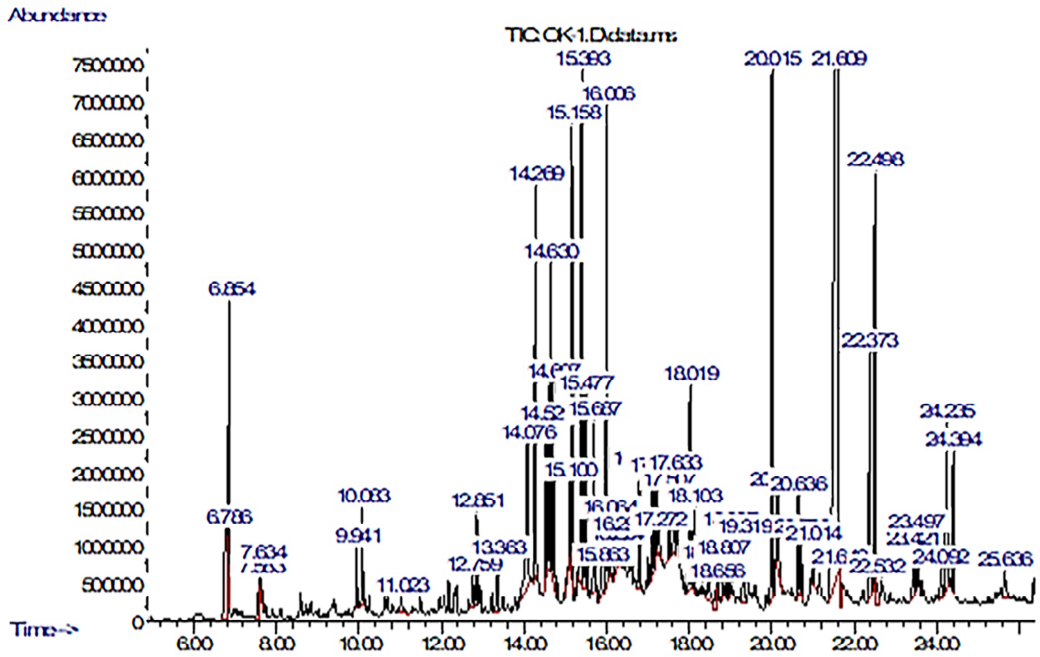


Fig. 3: GC-MS Chromatogram of *P. undulata* methanolic extract.

Table 4: Phytochemical composition of P. incisa methanolic extract, compound peak areas, direct parents and molecular frameworks.

No	Compound identifier	Area	Direct parent	Molecular framework
1	1-Methyltricyclo[2.2.1.0(2,6)]heptane	2258742	Monoterpenoids	Aliphatic homopolycyclic compounds
2	Geranylgeraniol	1426451	Acyclic diterpenoids	Aliphatic acyclic compounds
3	Bopimtnsywyzoc-uhfffaoysa-n	1920018	Eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids	Aliphatic homopolycyclic compounds
4	Caryophyllene oxide	6554418	Sesquiterpenoids	Aliphatic heteropolycyclic compounds
5	Farnesol (E), methyl ether	1204342	Sesquiterpenoids	Aliphatic acyclic compounds
6	alpha-Selinene	1689374	Eudesmane, isoeudesmane or cycloeudesmane sesquiterpenoids	Aliphatic homopolycyclic compounds
7	6-Methyl-3,5-heptadiene-2-one	1216804	Enones	Aliphatic acyclic compounds
8	1-ethyl-5,5-dimethylcyclopenta-1,3-diene	1825261	Branched unsaturated hydrocarbons	Aliphatic homomonocyclic compounds
9	2-Pentene, 2-methyl-5-nitro-	2412741	C-nitro compounds	Aliphatic acyclic compounds
10	6-Methyl-3-cyclohexen-1-carboxaldehyde	2801079	Organic oxides	Aliphatic homomonocyclic compounds
11	Methyl 4-methyl-3-cyclohexene-1-carboxylate	1506007	Methyl esters	Aliphatic homomonocyclic compounds
12	1,6-Octadiene, 2,5-dimethyl-, (E)-	2217752	Branched unsaturated hydrocarbons	Aliphatic acyclic compounds
13	2-Acetyl-5,6,7,8-tetrahydroquinoxaline	1280309	Aryl alkyl ketones	Aromatic heteropolycyclic compounds
14	1,3,6-Heptatriene, 5-methyl-	1146808	Alkatrienes	Aliphatic acyclic compounds
15	Cyclooctene, 3-(1-methylethenyl)-	1508600	Branched unsaturated hydrocarbons	Aliphatic homomonocyclic compounds
16	(6E,11E)-6,11-Tridecadienyl acetate #	1303888	Fatty alcohol esters	Aliphatic acyclic compounds
17	2,8,8-Trimethyl-4-methylene-2-vinylbicyclo[5.2.0]nonane #	107635687	Branched unsaturated hydrocarbons	Aliphatic homopolycyclic compounds
18	Palmitic Acid	1811981	Long-chain fatty acids	Aliphatic acyclic compounds
19	cis-Z-.alpha.-Bisabolene epoxide	3525528	Epoxides	Aliphatic heteromonocyclic compounds
20	2,5-Dimethoxybenzoic acid	3218024	O-methoxybenzoic acids and derivatives	Aromatic homomonocyclic compounds
21	Ulyktwolvhgahq-uhfffaoysa-n	5180442	Pyrroloazepines	Aromatic heteropolycyclic compounds
22	Methyl 8,11,14-heptadecatrienoate	1902493	Fatty acid methyl esters	Aliphatic acyclic compounds
23	Benzo[g]phthalazine-1,4(2H,3H)-dione, 4a,5,6,7,8,9,10,10a-octahydro-9,9-dimethyl-	57943630	Diazinanes	Aliphatic heteropolycyclic compounds
24	NIEPGDUXTWPJLS-RMKNXTFCSA-N	7441308	Medium-chain aldehydes	Aliphatic acyclic compounds
25	3-Eicosene, (E)-	1719328	Unsaturated aliphatic hydrocarbons	Aliphatic acyclic compounds

Table 5: Phytochemical composition of P. undulata methanolic extract, compound peak areas, direct parents and molecular frameworks.

No.	Compound identifier	Area	Direct parent	Molecular framework
1	beta-Pinene	6294811	Bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
2	D-Limonene	957595	Menthane monoterpenoids	Aliphatic homomonocyclic compounds
3	2-Isopropenyl-5-methylhex-4-enal	1401405	Acyclic monoterpenoids	Aliphatic acyclic compounds
4	Myrcene	813107	Acyclic monoterpenoids	Aliphatic acyclic compounds
5	Cedrene	884215	Cedrane and isocedrane sesquiterpenoids	Aliphatic homopolycyclic compounds
6	Hooyrfuyendqnp-uhfffaoysa-n	2468082	Monoterpenoids	Aliphatic homopolycyclic compounds
7	Caryophyllene	1701118	Sesquiterpenoids	Aliphatic homopolycyclic compounds
8	alpha-Curcumene	6391414	Sesquiterpenoids, Toluene - Benzenoid	Aromatic homomonocyclic compounds
9	(+)-Calarene	3645452	Aristolane sesquiterpenoids	Aliphatic homopolycyclic compounds
10	Cadina-1(10),4-diene	7680498	Sesquiterpenoids	Aliphatic homopolycyclic compounds
11	Upwudjzkosqirq-uhfffaoysa-n	4763547	Bicyclic monoterpenoids	Aliphatic homopolycyclic compounds
12	2,6-Octadiene, 2,6-dimethyl-	11633014	Acyclic monoterpenoids	Aliphatic acyclic compounds
13	Globulol	852290	5,10-cycloaromadendrane sesquiterpenoids	Aliphatic homopolycyclic compounds
14	Dehydroxy-isocalamendiol	12731716	Sesquiterpenoids	Aliphatic homopolycyclic compounds
15	Phytol	2673801	Acyclic diterpenoids	Aliphatic acyclic compounds
16	Isopropyl p-tolyl sulfide	1531479	Aryl thioethers, Thiophenol ether	Aromatic homomonocyclic compounds
17	p-Cymen-8-ol	2477886	Phenylpropanes	Aromatic homomonocyclic compounds
18	Methylisoeugenol	9078334	Dimethoxybenzenes, Phenol ether	Aromatic homomonocyclic compounds
19	Spathulenol	13280356	Aromadendrane sesquiterpenoids	Aliphatic homopolycyclic compounds
20	Isoelemicin	1679786	Anisoles, Phenol ethers	Aromatic homomonocyclic compounds
21	Nntinehydbksdv-uhfffaoysa-n	3299054	Anisoles, Phenol ethers	Aromatic homomonocyclic compounds
22	Palmitic Acid	2211513	Long-chain fatty acids	Aliphatic acyclic compounds
23	linolenic acid	907453	Lineolic acids and derivatives	Aliphatic acyclic compounds
24	Estriol	1399013	Estrogens and derivatives	Aromatic homopolycyclic compounds

Table 6: The disc diffusion assays of the plant methanolic extracts, water and the antibiotic against the test bacteria.

Sample	Treatment	Zone of inhibition (mm) mean ± SE			
		P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*
P. undulata	Methanolic extract	8.5±0.173	12±1.154	25±0.577	10±0.155
	Water	0.00	18.50±0.088	14±0.152	0.00
	Antibiotic (AMC30)	0.00	27±0.115	20±0.1527	8±0.1154
P. incisa	Methanolic extract	9±0.1527	19.5±0.252	19.5±0.265	10±0.200
	Water	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	30±0.057	20±0.251	7±0.305
A. herba-alba	Methanolic extract	12±0.00	14±0.100	12.5±0.152	10.5±0.057
	Water	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	31.5±0.251	13.5±0.00	8±0.176
A. fragrantissima	Methanolic extract	10±0.057	14.5±0.202	14±0.115	10.5±0.176
	Water	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	30±0.057	17±0.306	7±0.173
A. judaica	Methanolic extract	11±0.00	14±0.850	12±057	12±0.00
	Water	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	28±0.360	17±0.115	8.5±0.057

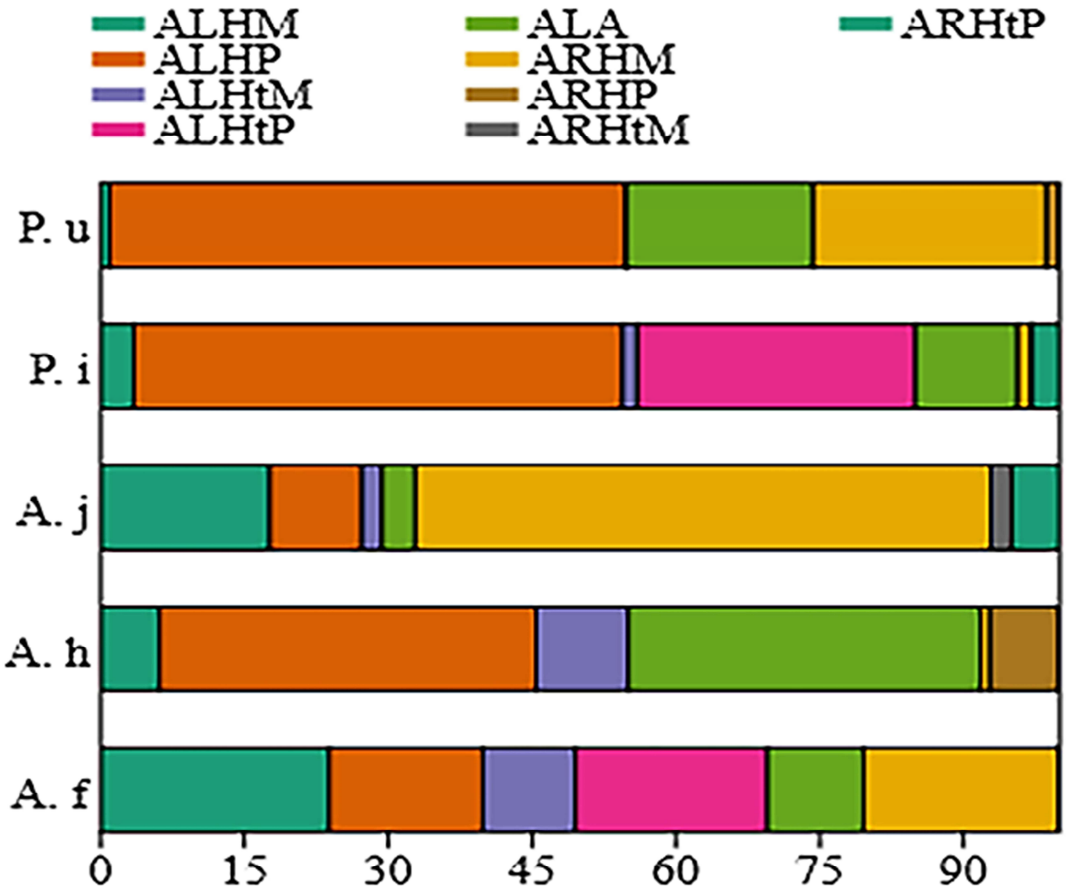


Fig. 4: Stacked chart (color scheme) showing the distribution of phytochemical frameworks between plant extracts. P.u = *P. undulata*; P.i = *P. incisa*; A. j = *A. judaica*; A. h = *A. herba-alba*; A .f = *A. fragrantissima*; ALHM = aliphatic homo monocyclic; ALHP = aliphatic homo polycyclic; ALHtM = aliphatic hetero monocyclic; ALHtP = aliphatic hetero polycyclic; ALA = aliphatic acyclic; ARHM = aromatic homo monocyclic; ARHP = aromatic homo polycyclic; ARHtM = aromatic hetero monocyclic; ARHtP = aromatic hetero polycyclic.

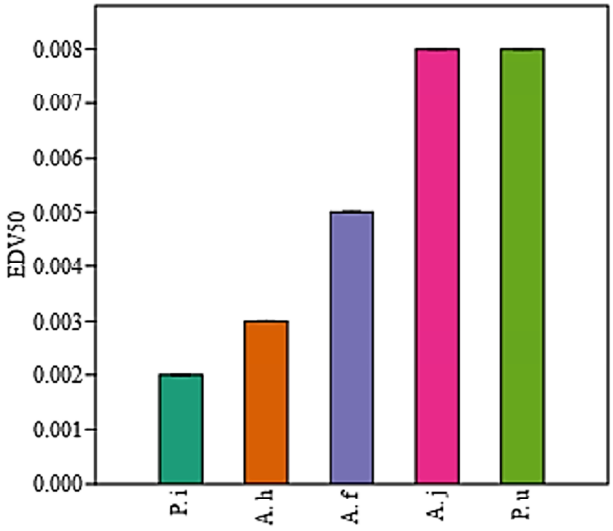
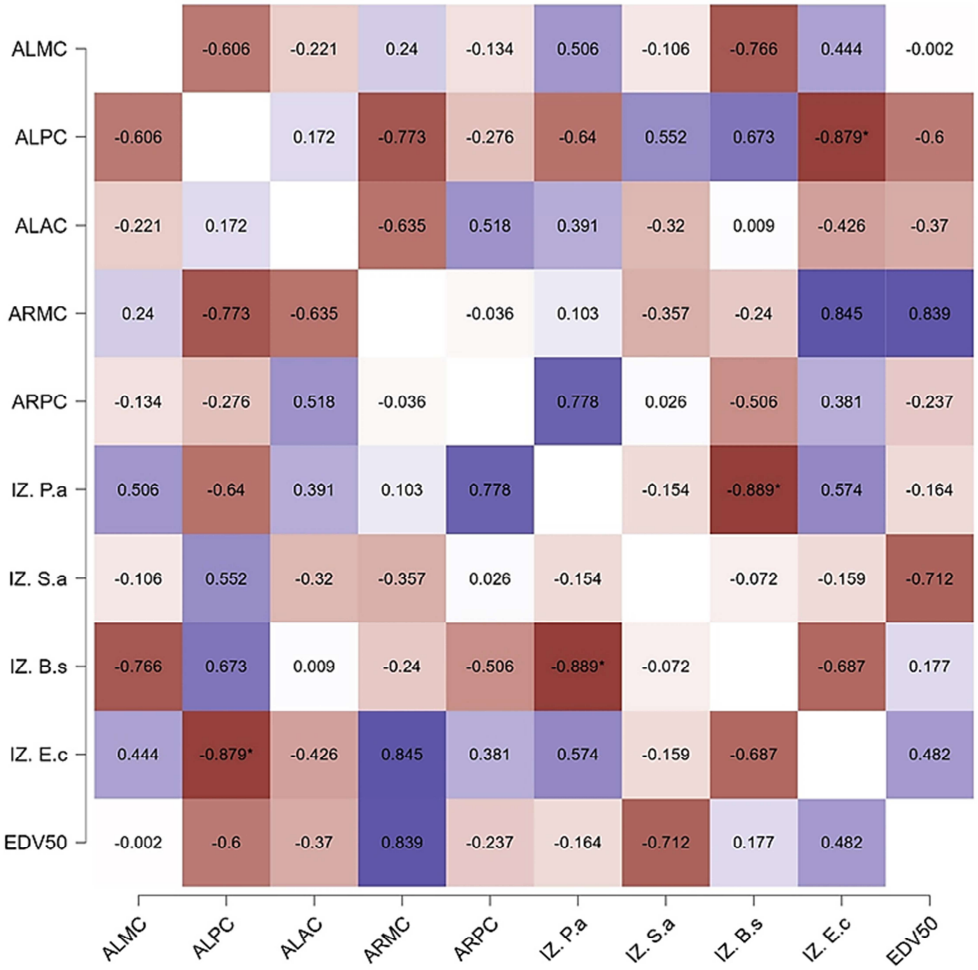


Fig. 7: Bar chart showing the antioxidant activities (free radical scavenging ability) of plant extracts based on EDV50 values. P.u = *P. undulata*; P.i = *P. incisa*; A. j = *A. judaica*; A. h = *A. herba-alba*; A.f = *A. fragrantissima*.



ALMC = aliphatic monocyclic, ALPC = aliphatic polycyclic, ALAC = aliphatic acyclic, ARMC = aromatic monocyclic, ARPC = aromatic polycyclic. IZ.P.a = inhibition zone for *P. aeruginosa*, IZ. S.a = inhibition zone for S. aureus, IZ.B.s = inhibition zone for *B. subtilis*, IZ.E.c = inhibition zone for *E. coli.*

Fig. 8: r Pearson's correlation matrix plot (heatmap) showing the degrees and natures of association (from blue – positive, to red – negative) between concentrations of different molecular frameworks and antibacterial/ antioxidant activities of plant extracts.

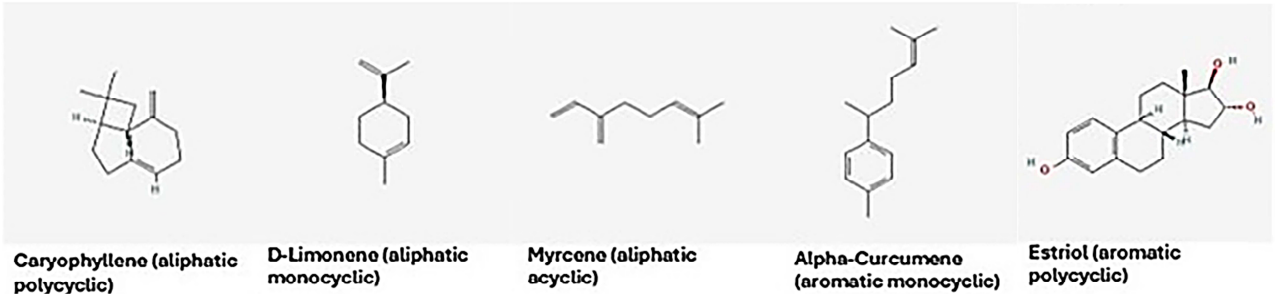


Fig. 9: examples of compounds of different molecular frameworks identified in the plant extracts.

This study suggests these phytochemical frameworks and their target-specific activities be subject to further investigation to better understand their action and probable applications in pharmaceutical and medical fields. Also, studying how combining different structural frameworks can work together might help create new compounds that are better at killing bacteria and preserving cells from harm.

REFERENCES

Bianco A, Venditti A, Foddai S, Toniolo C and Nicoletti M (2014). A new problem. Contamination of botanicals by phthalates. Rapid detection tests. *Nat. Prod. Res.,* **28**(2): 134-137.

Breijyeh Z, Jubeh B and Karaman R (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules,* **25**(6):1340.

Chaudhary SA (1999). Flora of the Kingdom of Saudi Arabia. National Herbarium, National Agriculture and Water Research, Riyadh, KSA, pp.273-412.

Chithan J, Sampath KK, Shailasree S and Prakash HS (2012). Antioxidant, antibacterial and NDA protecting activity of selected medicinally important Asteraceae plants. *Int. J. Pharm. & Pharm. Sci*. **4**(2): pp. 257-261

Choi HR, Choi JS, Han YN, Bae SJ and Chung HY (2002). Peroxynitrite scavenging activity of herb extracts. *Phytother. Res.,* **16**: 364-367.

Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel BV, Pieters L, Vlietinck AJ and Berghe DV (1988). Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.,* **6**: 171-76.

Djoumbou YF, Eisner R and Knox C (2016). Classy Fire: Automated chemical classification with a comprehensive, computable taxonomy. *J. Chem. Inform.,* **8**: 61.

Elbalola AA and Al-Atawi GA (2022). Diversity and distribution of soil seed banks and plant litter in AL Wadi Alakhdar (the green valley) of Tabuk region – Saudi Arabia. *Pak. J. Bot.,* **54**(6): 2263-2271.

Heim KE, Tagliaferro AR and Bobilya DJ (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutrit. Biochem.,* **13**: 572-584.

Hussein SR, Marzouk MM, Soltan MM, Ahmed EK, Said MM and Hamed AR (2017). Phenolic constituents of *Pulicaria undulate* (L.) CA Mey. sub sp. *undulata* (Asteraceae): Antioxidant protective effects and chemosystematic significances. *J. Food Drug Anal.,* **25**: 333-339.

Kim S, Chen J and Cheng T (2023). PubChem update. *Nucleic Acids Res.,* **51**(D1): D1373-D1380.

Kusumaningsih T, Firdaus M and Suparjo H (2017). Preparation and antibacterial activity of inclusion complex sulfonatocalix[4]arene-xanthone against *Escherichia coli*. Presented at the IOP Conference Series: *Mat. Sci. Engin.,* **172**(1): 012059.

Lewandowski W, Lewandowska H, Golonko A, Świderski G, Świsłocka R and Kalinowska M (2020). Correlations between molecular structure and biological activity in "logical series" of dietary chromone derivatives. *PLoS One,* **15**(8): e0229477.

Lister PD, Wolter DJ and Hanson ND (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin. Microbiol. Rev.,* **22**(4): 582-610.

Liyana-Pathiranan CM and Shahidi F (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L) as affected by gastric pH conditions. *J. Agric Food Chem.,* **53**: 2433-2440.

Łowicki D and Przybyls PT (2022). Construction of biological relevant aliphatic 5-membered N-heterocycles. *Eur. J. Med. Chem.,* **235**: 114303.

Lu SM and Alper H (2005). Intramolecular carbonylation reactions with recyclable palladium-complexed dendrimers on silica: synthesis of oxygen, nitrogen, or sulfur-containing medium ring fused heterocycles. *J. American Chem. Soc.,* **127**(42): 14776-14784.

Mohammed HA, Al-Omar MS, Khan RA, Mohammed SA, Qureshi KA, Abbas MM, Al Rugaie O, Abd-Elmoniem E, Ahmad AM and Kandil YI (2021). Chemical profile, antioxidant, antimicrobial and anticancer activities of the water-ethanol extract of *pulicaria undulata* growing in the oasis of central Saudi Arabian desert. *Plants,* **10**: 1811.

Mulcahy H, Charron-Mazenod L and Lewenza S (2008). Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *PLoS Pathog.,* **4**: e1000213.

Parr AJ and Bolwell JP (2002). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.,* **80**: 985-1012.

Payá M, Halliwell B and Hoult JRS (1992). Interactions of a series of coumarins with reactive oxygen species. Scavenging of super oxide, hypochlorous acid and hydroxyl radicals. *Biochem. Pharmacol.,* **44**: 205-214.

Rav M, Valizadeh J, Noroozifar M and Khorasani-Motlagh M (2011). Screening of chemical composition of essential oil, mineral elements, and antioxidant activity in *Pulicaria undulata* (L.) CA Mey from Iran. *J. Med. Plants Res.,* **5**: 2035-2040.

Ruiz-Garbajosa P and Cantón R (2017). Epidemiology of antibiotic resistance in *Pseudomonas aeruginosa.* Implications for empiric and definitive therapy. *Rev. Esp. Quimioter.,* **1**: 8-12.

Singh A, Raju R, Mrad, M, Reddell P and Muench G (2020). The reciprocal EC_{50} value as a convenient measure of the potency of a compound in bioactivity-guided purification of natural products. *Fitoterapia.,* P. 143.

Thiemann T (2021). Isolation of Phthalates and Terephthalates from Plant Material Natural Products or Contaminants? *Open Chem J.,* **8**: pp.1-36.

Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM and Bastos ML (2003). Hydroxyl radical and hypochlorous acid scavenging activity of small centaury (*Centaurium erythraea*) infusion: A comparative study with green tea (*Camellia sinensis*). *Phytomed.,* **10**: 517-522.

Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM and Bastos ML (2002a). Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. *J. Agric. Food Chem.,* **50**: 4989-4993.

Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM and Bastos ML (2002b). Antioxidant activity of *Hypericum androsaemum* infusion: Scavenging activity against super oxide radical, hydroxyl radical and hypochlorous acid. *Biol. Pharm. Bull.,* **25**: 1320-1323.

Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM and Bastos ML (2002c). Studies on the antioxidant activity of *Lippia citriodora* infusion: scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. *Biol. Pharm. Bull.,* **25**: 1324-1327.

Van Rossum JM (1966). Limitations of molecular pharmacology. Some implications of the basic assumptions underlying calculations on drug-receptor interactions and the significance of biological drug parameters. *Adv. Drug. Res.,* **3**: 189-234.

Xu GH, Chen JC, Liu DH, Zhang YH, Jiang P and Ye XQ (2008). Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. *J. Food Sci.,* **73**: C11-C18.

Zazharskyi VV, Davydenko PO, Kulishenko OM, Borovik IV and Brygadyrenko VV (2019). Antimicrobial activity of 50 plant extracts. *Biosyst. Divers.,* **27**: 163-169.