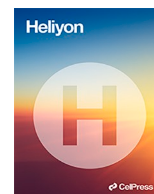
Heliyon 10 (2024) e23641

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Chemotaxonomy, antibacterial and antioxidant activities of selected aromatic plants from Tabuk region-KSA

Abdelrahim A. Elbalola *, Zahid Khorshid Abbas

Biology Department, Faculty of Science, Tabuk University, Tabuk, Saudi Arabia

A R T I C L E I N F O

Keywords:
Chemotaxonomy
Antibacterial
Antioxidant activity
Medicinal plants

A B S T R A C T

Chemotaxonomy is a valuable tool for obtaining taxonomic insights, which are most effectively employed in combination with other forms of data to establish a system of classification that closely reflects natural connections. The utilization of plant secondary metabolites possessing diverse therapeutic qualities signifies the growing exploitation of natural products in the medical discipline. The objectives of the current study encompassed the identification of phytochemicals in the extracts of nine species of medicinal plants, the examination of their chemotaxonomic properties, and the assessment of the antibacterial and antioxidant capabilities exhibited by the extracts. GC-MS technology was employed for the identification of phytochemical compounds. The study utilized ClassyFire, an automated chemical classification system that incorporates an extensive and computable classification, to categorize chemicals. The chemical classification of plants was examined by the application of principal component analysis (PCA) and cluster analysis (CA). The bactericidal properties of plants were assessed against four harmful bacterial species using the disc diffusion technique. The antioxidant properties of plant extracts were assessed employing the DPPH free radical scavenging methodology, and the half maximal effective concentration (EC50) was determined using dose response models. The calculator being referred to is the Quest Graph™ EC50 Calculator. In the plant extracts, the analysis disclosed the occurrence of 160 phytochemicals, classified into 36 phytochemical classes. The results of CA and PCA demonstrated the proximity and associations among Asteraceae species, while indicating the divergence of the two Lamiaceae species. *Achillea fragrantissima* and *Ducrosia flabellifolia* demonstrated the most diversity in phytochemical classes, while *Thymus vulgaris* displayed the highest level of dominance. *Pulicaria undulata* and *T. vulgaris* had the most notable antibacterial activity. *D. flabellifolia* and *P. incisa* demonstrated the highest levels of antioxidant activity. Ethanol exhibited superior antibacterial efficacy compared to other solvents. The remarkable biological activities exhibited by these plant extracts can be ascribed to the copious presence of certain chemicals, predominantly sesquiterpenoids, monoterpenoids, benzene and its derivatives, naphthalenes, fatty acyls, and phenols. The susceptibility of Gram-positive bacterial species to plant extracts was shown to be higher in comparison to Gram-negative bacterial species.

1. Introduction

Medicinal and aromatic plants from many regions of the world offer an abundant source of pharmacologically active chemicals [1].

* Corresponding author.
E-mail addresses: aalbalolh@ut.edu.sa (A.A. Elbalola), znourabbas@ut.edu.sa (Z.K. Abbas).

https://doi.org/10.1016/j.heliyon.2023.e23641
Received 1 June 2023; Received in revised form 23 November 2023; Accepted 8 December 2023
Available online 16 December 2023
2405-8440/© 2023 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

A diverse array of medicinal plants demonstrates remarkable antibacterial and antioxidant properties [2], which are contingent upon factors such as plant species, diversity, extraction and/or processing techniques, and their growing environment [3]. Antimicrobial agents have the ability to eliminate pathogenic bacteria, reduce their pathogenicity, and inhibit their metabolic activity [4]. Furthermore, these agents can also impact microbial respiration and hinder their reproductive potential. According to Ref. [5], an antioxidant is characterized as a chemical that possesses the ability to impede or postpone the process of oxidation in easily oxidizable materials, even when present in minimal quantities. Antioxidants prevent the process of oxidation in various substrates [6], including those containing free or non-free radicals, which can potentially induce damage to the plasma membrane, resulting in DNA mutations and lipid peroxidation, among other significant outcomes. In addition, phytochemicals are the focal point of chemotaxonomy, a field that is progressively being addressed as a pursuit to categorize plants by discernible distances and resemblances in their chemical makeup [7]. Chemotaxonomy is the study of how chemical compounds or groups of chemicals that are produced by living organisms are distributed among different plant species that are related or potentially related [8]. Chemotaxonomy possesses various benefits, such as directing natural product research towards specific plant groups [7], resolving existing taxonomical problems [9], serving as a complementary or essential tool for deciphering classification patterns and identifying potential synapomorphic chemical markers at different taxonomic levels [10], and facilitating evolutionary studies that examine phylogenetic and infra-taxonomic variations. The exclusive reliance on external morphological analysis is inadequate for a comprehensive assessment of a taxon's systematic classification and evolutionary relationships. Therefore, the significance of chemotaxonomy is increasing as it provides other avenues of investigation. Chemotaxonomy has made substantial taxonomic advances by providing support for the principles of categorization and phylogeny in an equitable manner. The advancement of highly advanced chemical analysis techniques capable of detecting even minuscule quantities of chemical substances has contributed a crucial role in the advancement of chemotaxonomy [11]. The Tabuk region, situated in Northwestern portion of Saudi Arabia, is renowned for its significant diversity of plant species. The geographical features of the region, such as wadies (valleys), mountains, sand dunes, and plateaus, provide conducive environments for the growth of plants. Moreover, these individuals provide backing for a diverse range of medicinal plant species that possess numerous traditional applications, which are deeply ingrained in the prevalent cultural heritage of the area [12]. Multiple investigations have been carried out on the utilization of medicinal plants in the Tabuk region for the treatment of various ailments [13–15]. The aforementioned research has not conducted quantitative analysis or identification of phytochemicals, explored their chemotaxonomy, or assessed their biological activities using established biological activity evaluation tests. This study aims to examine nine plant species that have been selected for investigation based on their perceived importance to the local community. These species are extensively exploited for their medicinal properties, as they are believed to possess the ability to treat a diverse array of ailments and health conditions. Furthermore, the chosen species exhibit a taxonomic mosaic derived from three distinct families and six genera, so offering a highly suitable topic for the investigation of chemotaxonomy. No previous research has been undertaken on the taxonomy of the region, specifically focusing on medicinal and aromatic plant species. This study aims to be the first of its type, pioneering inquiries in this highly diverse region.

2. Materials and methods

2.1. Plant materials

The aerial parts of nine plant species belonging to the Asteraceae, Apiaceae, and Lamiaceae families, namely *Pulicaria undulata* L., *Pulicaria incisa* Lam., *Artemisia herba-alba* Asso., *Artemisia monosperma* Delile, *Artemisia judaica* L., *Achillea fragrantissima* Forssk., *Ducrosia flabellifolia* Boiss., *Thymus vulgaris* L., and *Lavandula coronopifolia* Poir. The samples were gathered from several natural vegetation sites in the Tabuk region during the period of transitioning from Spring to Summer in the year 2022, when the majority of these plant species were at their stage of maximum growth. Subsequently, the specimens were individually packed within paper bags and subsequently transported to the laboratory for the purpose of undergoing a cleaning and drying process in a shaded environment. The verification of plant species identification was conducted by referencing authoritative sources such as the Flora of the Kingdom of Saudi Arabia [16] and Plants of the World Online, which is organized by the Royal Botanic Gardens, Kew. The plant samples were subsequently pulverized into fine powders and retained for subsequent testing.

2.2. Extraction

The extraction process adhered to the conventional protocol, wherein 180 ml of distilled water was mixed with 20 g of each plant material under investigation. Subsequently, the solutions were subjected to heating at 90 °C for a duration of 30 min, followed by overnight incubation at 37 °C and 150 rpm in a shaking incubator [17]. In a similar manner, the utilization of round bottom flasks facilitated the amalgamation of 10 g of powdered plant materials under examination with a solution consisting of ethanol and methanol in a ratio of 9:1. Following that, the mixtures were subjected to an overnight incubation period at a temperature of 37 °C and a rotational speed of 150 revolutions per minute. The liquid extracts obtained were subsequently concentrated using a rotary evaporator following their separation from the solid residue through filtration utilizing a Whatman No. 1 filter paper.

2.3. Phytochemical analysis and taxonomy

The GC-MS analysis of ethanol extracts of the test plant species was conducted using a Thermo GC Trace Ultra version 5.0 gas chromatography system in combination with a Thermo MS DSQ II mass spectrometer. The analysis was carried out under the specified circumstances. The DB5-MS Capillary standard non-polar column (30×0.25 mm $\times$ 0.25 m) used helium as the carrier gas, flowing at a

constant rate of 1 ml/min. The oven's temperature was consistently held at 70 °C and programmed to rise by 6 °C each minute until reaching 260 °C. The mass range was from 50 to 650 *m/z*. The whole duration of the running time was 43 min. The study utilized ClassyFire, an automated chemical classification system, to classify all phytochemicals found in the plant extracts at the class level [18]. The shortest synonyms of specific compounds were obtained from the PubChem-NIH-National Library of Medicine [19].

2.4. 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 stock culture and were cultured on Mueller-Hinton broth agar at a temperature of 37 °C for a duration of 24 h. Prior to conducting any antimicrobial tests, the samples were subjected to sub-culturing following the incubation period. In order to establish inoculums, bacteria were suspended in a sterile saline solution containing 0.85% NaCl. As reported by Ref. [20], the suspensions were kept at an optical density (OD) ranging from 0.4 to 0.6 at a wavelength of 405 nm. This corresponds to a cell density about equal to 0.5 McFarland and corresponds to an inoculum estimated to be between 106 and 108 colony forming units per mL (CFU/mL). The antibiotic susceptibility test was conducted using the conventional disk diffusion technique as described by Ref. [20]. Using a sterile swab, previously produced inoculums were streaked onto Mueller-Hinton agar (MHA) plates using this approach. Subsequently, 6 mm Whatman paper N5 discs underwent sterilization and were saturated with 5 L of the ethanol, methanol, and aqueous fractions. The solvent used for impregnation consisted of 10% v/v dimethyl sulfoxide and 1% v/v tween 80 in deionized water. The Augmentin AMC30 antibiotic (5 g/mL) and solvent (10% v/v dimethyl sulfoxide and 1% v/v tween 80 in deionized water) were used to dilute the extract fractions, which served as the control, under identical circumstances. The plates were stored at ambient temperature prior to being placed in an incubator set at 37 °C for a duration of 24 h. Ultimately, the closest outside boundary of the inhibitory zones, which includes the diameter of the discs, was measured in millimeters to evaluate the antibacterial effectiveness.

2.5. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was followed to evaluate the antioxidant activity of the plants under investigation [21]. In this experiment, different doses of 0.135 mM DPPH (200–1000 g/mL) were mixed with 1 mL of the extract. The mixture was held at room temperature in the dark for 40 min while being gently stirred. A positive control was ascorbic acid. DPPH scavenging activity (%) = [(Abs control Abs sample)/Abs control] 100, where Abs control is the absorbance of DPPH + methanol and Abs sample is the absorbance of DPPH radical. The absorbance of the samples and the control solutions were measured at 517 nm and % of DPPH scavenging activities of the extracts were calculated using the following equation:

DPPH scavenging activity (%) = [(Abs control − Abs sample)/Abs control] × 100, where Abs control is the absorbance of DPPH + methanol and Abs sample is the absorbance of DPPH radical.

2.6. Statistical analysis

2.6.1. Chemotaxonomy

A matrix of phytochemical class distribution expressed as a percentages in plant extracts was created after categorizing all phytochemicals found in each plant extract. Since abundance (acquired from peak areas of the GC-MS analysis) depicts the amount of a compound in the extract, it was used as the basis for computing percentages of compound classes. The matrix was then used to create a network plot, Euclidean cluster analysis dendrogram, and a scatter plot (biplot) of principal component analysis, all of which classify and group plant species and highlight similarities, linkages, and differences between them based on their contents of phytochemical classes. Additionally, a phytochemical class diversity profile plot was created.

2.6.2. Antibacterial analysis

A connecting line graph was produced after conducting a two-way repeated measure ANOVA test to examine the significance of differences ($p < 0.05$) between plant species in terms of their capacity to inhibit bacterial growth, as well as between the ethanolic, methanolic, and aqueous extracts and the antibiotic in terms of their effects on bacterial growth. Graphical representation of the sensitivity of bacterial species to plant extracts was also obtained.

2.6.3. Antioxidant activity

The EC50 value, which represents the concentration needed to achieve a 50% antioxidant effect, was used to quantify the antioxidant capacity and to compare the efficacy of various plant extracts. They were studied using the dose response models in the [22]. Statistical analyses were carried out using PAST (Paleontological Statistics) 4.12.

3. Results

3.1. Phytochemical constituents and chemotaxonomy

The examined plant extracts contained 160 phytochemicals in total, distributed among 36 different phytochemical classes (Fig. 1); chromatograms were given in figures (a) through (i). The main classes in the plant extracts were monoterpenoids, sesquiterpenoids,

fatty acyls, organooxygen compounds, benzene and its substituted derivatives, steroids, and unsaturated hydrocarbons. Some phytochemical classes were abundant and characteristic to certain plant extracts (Appendix Table 1) including benzene and its derivatives in *D. flabellifolia* extract (41.53%), monoterpenoids in *T. vulgaris* extract (84.29%), fatty acyls and steroids in *L. coronopifolia* (52.19 and 42.5% respectively), sesquiterpenoids and coumarins in *P. incisa* (45.21 and 38.17% respectively), and organooxygen compounds in *A. monosperma* (40.11%). The most abundant compounds in plant extracts were shown in Table 1. *A. fragrantissima* had the highest richness (number of classes = 17), in addition to 8 exclusive classes not detected in other plant extracts. Carboxylic acids & derivatives and flavonoids were confined to *A. judaica* extract; thiophenes, quinone & hydroquinone lipids, coumarins and hopanoids to *A. monosperma* extract; benzenoids, sesquiterpene lactones and pyridines & derivatives to *D. flabellifolia* extract; glycerolipids in *L. coronopifolia* extract; sulfonyls, naphthalenes, linear-1-3, diarylpropanoids in *P. incisa*; phenyl ethers, thiochromines and quinolines & derivatives to *P. undulata* extract. Paired group (UPGMA) classical clustering dendrogram (Fig. 2) showed that the Asteraceae species *A. herba-alba* and *A. fragrantissima* were the closest to each other's in their content of phytochemical classes, followed by the two Pulicaria species (*P. undulata* and *P. incisa*). *A. judaica* and *D. flabellifolia* were linked and close to the former two pairs, *T. vulgaris* and *L. coronopifolia* (Lamiaceae) with *A. monosperma* were far from other species. This taxonomic grouping was aided by the principal component analysis (PCA) scattered plot (biplot), which showed the same pattern of positioning the taxa relative to each other's (Fig. 3). It was also very useful in locating and distributing phytochemical classes between plant extracts, showing that sesquiterpenoids and benzene & derivatives were directed towards *P. incisa, P. undulata* and *D. flabellifolia*; Naphthopyrans and thiochromones were located beside *A. fragrantissima*; unsaturated hydrocarbons towards *A. herba-alba*; monoterpenoids were positioned close to *T. vulgaris*; steroids and fatty acyls towards *L. coronopifolia* and coumarins towards *A. monosperma*. The Network diagram (Fig. 4), which showed the interconnections between the set of species based on chemical composition, where each species was represented by a node and connections between species represented through links. The nodes (species) were scaled by phytochemical class richness, and links were scaled by number of common classes. It affirmed closeness of Asteraceae species *P. undulata, P. incisa, A. fragrantissima, A. judaica* and *A. herba-alba*, and the Apiaceae species *D. flabellifolia*. Lamiaceae species *T. vulgaris* and *L. coronopifolia* and *A. monosperma* (Asteraceae) were disconnected and exhibited relative uniqueness in their chemical composition. Fig. (5) showed the phytochemical diversity profile of the plant species, *A. fragrantissima* followed by *D. flabellifolia* were the most diverse in phytochemical classes, while *T. vulgaris* and *L. coronopifolia* (Lamiaceae) had the lowest phytochemical richness.

3.2. Antibacterial activity

Differences between the studied plant species and that for interactions between different plant species and the type of extract used in bacterial growth inhibition ability were not statistically significant at $p < 0.05$, while the extract types (ethanolic, methanolic and

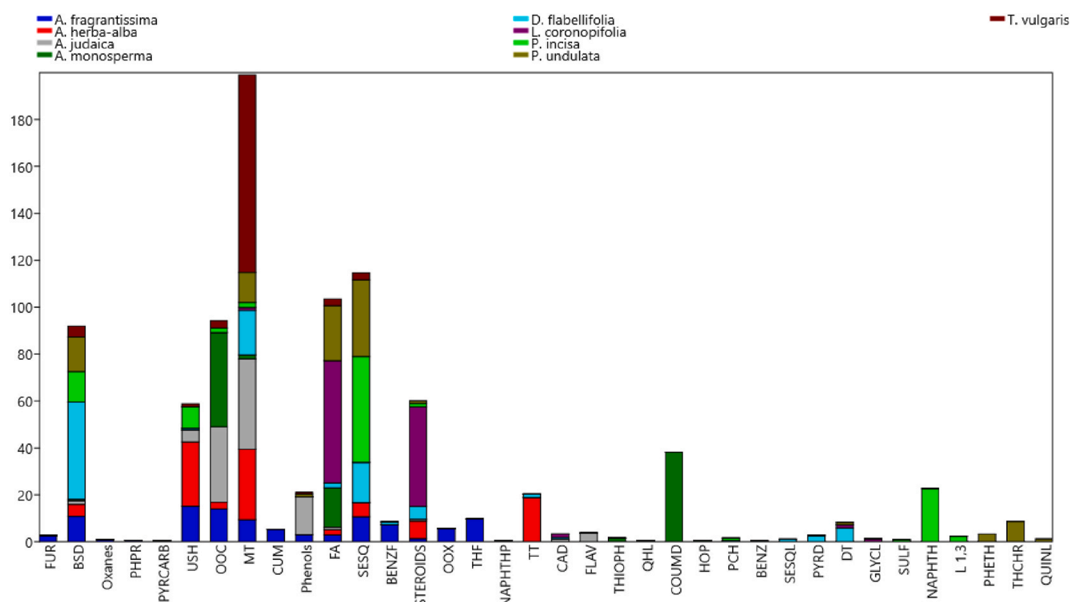


Fig. 1. Stacked chart showing distribution of different phytochemical classes between plant extracts. FUR = furanones; BSD = benzene and substituted derivatives; PHPR = phenyl propanes; PYRCARB = pyridinecarboxamides; USH = unsaturated hydrocarbons; OOC = organooxygen compounds; MT = monoterpenoids; CUM = cumenes; FA = fatty acyls; SESQ = sesquiterpenoids; BENZF = benzofurans; OOx = organic oxides; THF = tetrahydrofurans; NAPHTHP = naphthopyrans; TT = triterpenoids; CAD = carboxylic acids and derivatives; FLAV = flavonoids; THIOPH = thiophenes; QHL = quinone and hydroquinone lipids; COUMD = coumarins and derivatives; HOP = hopanoids; PCH = polycyclic hydrocarbons; BENZ = benzenoids; SESQL = sesquiterpene lactones; PYRD = pyridines and derivatives; DT = diterpenoids; GLYCL = glycerolipids; SULF = sulfonyls; NAPHTH = naphthalenes; L 1,3 = Linear 1,3-diarylpropanoids; PHETH = phenol ethers; THCHR = thiochromenes; QUINL = quinolides and derivatives.

Table 1
The most abundant compounds in plant extracts and their classification.

Plant species	Compound	Compound class
A. fragrantissima	Mono(2-ethylhexyl) phthalate	Benzene and substituted derivatives
	Lilac alcohol A	Tetrahydrofurans
A. herba-alba	Thujone	Monoterpenoids
	2,4-Hexadiene, 3-methyl-	Unsaturated hydrocarbons
	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	Triterpenoids
A. judaica	Xanthoxylin	Organooxygen compounds
	p-Menth-1(7)-en-2-one	Monoterpenoids
	Phenol, 4-methoxy-2,3,6-trimethyl-	Phenols
A. monosperma	Pinacol	Organooxygen compounds
	Bergaptol	Coumarins and derivatives
D. flabellifolia	2,2',4-Trihydroxybenzophenone	Benzene and substituted derivatives
	(Z,Z)-.alpha.-Farnesene	Sesquiterpenoids
	Longifolene-(V4)	Monoterpenoids
L. coronopifolia	Palmitic acid	Fatty Acyls
	25,26-Dihydroelasterol	Steroids and steroid derivatives
	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	Steroids and steroid derivatives
P. incisa	Patchoulane	Sesquiterpenoids
	1,4-Naphthoquinone, 6-acetyl-2,5,7-trihydroxy-	Naphthalenes
	2-(((2-Ethylhexyl)oxy)carbonyl)benzoic acid	Benzene and substituted derivatives
P. undulata	Falcarinol	Fatty Acyls
	alpha-Curcumene	Sesquiterpenoids
	Spathulenol	Sesquiterpenoids
T. vulgaris	Thymol	Monoterpenoids
	Phenol, 2-methyl-5-(1-methylethyl)-	Monoterpenoids
	p-CYMENE	Monoterpenoids

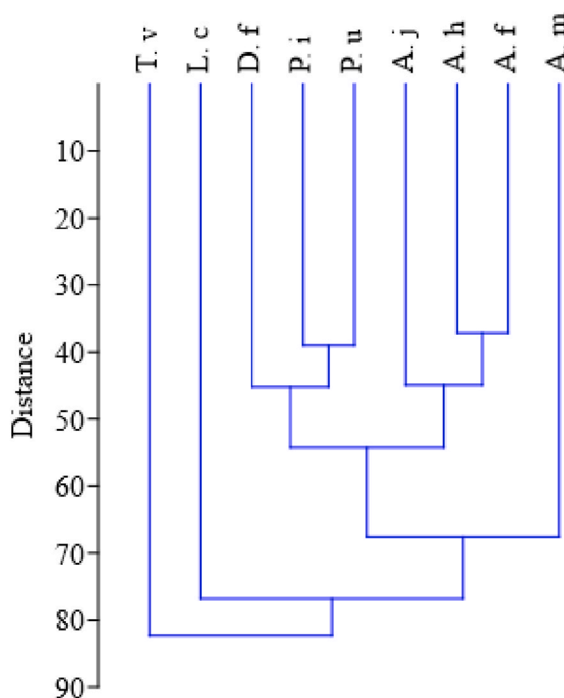


Fig. 2. Euclidian Paired group (UPGMA) classical clustering dendrogram of plant species based on their contents of phytochemical classes. T. v = *Thymus vulgaris*; L. c = *Lavandula coronopifolia*; D. f = *Ducrosia flabellifolia*; P. i = *Pulicaria incisa*; P. u = *Pulicaria undulata*; A. j = *Artemisia judaica*; A. h = *Artemisia herba-alba*; A. f = *Achillea fragrantissima*; A. m = *Artemisia monosperma*.

aqueous) and the antibiotic tested against the four bacterial species differed significantly (Table 2). Graph depicting the average size of inhibition zones caused by plant extracts and antibiotic on four different bacterial species (Fig. 8) showed that the ethanolic extract, in average, was more efficient in antibacterial activity for four plant species (*P. undulata, T. vulgaris, D. flabellifolia* and *P. incisa*. For *A. monosperma and A. herba-alba*, ethanolic and methanolic extracts exhibited similar average antibacterial activities, while for *L. coronopifolia* and *A. judaica*, ethanolic extracts and the antibiotic also showed similar activity while the antibiotic was better than

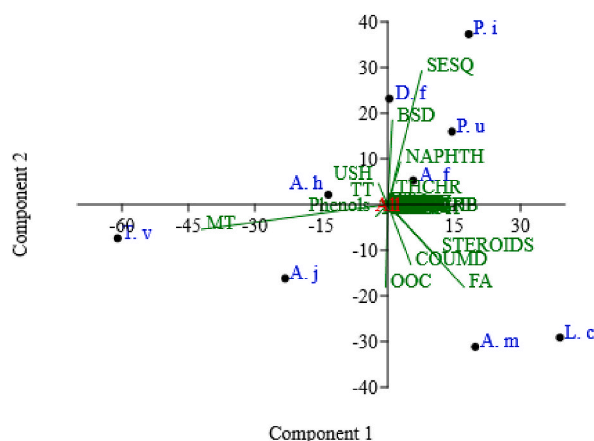


Fig. 3. Principal component analysis (PCA) Scattered plot (biplot) of plant species and phytochemical classes. T. v = *Thymus vulgaris*; L. c = *Lavandula coronopifolia*; D. f = *Ducrosia flabellifolia*; P. i = *Pulicaria incisa*; P. u = *Pulicaria undulata*; A. j = *Artemisia judaica*; A. h = *Artemisia herba-alba*; A. f = *Achillea fragrantissima*; A. m = *Artemisia monosperma*. FUR = furanones; BSD = benzene and substituted derivatives; PHPR = phenyl propanes; PYRCARB = pyridinecarboxamides; USH = unsaturated hydrocarbons; OOC = organooxygen compounds; MT = monoterpenoids; CUM = cumenes; FA = fatty acyls; SESQ = sesquiterpenoids; BENZF = benzofurans; OOx = organic oxides; THF = tetrahydrofurans; NAPHTHP = naphthopyrans; TT = triterpenoids; CAD = carboxylic acids and derivatives; FLAV = flavonoids; THIOPH = thiophenes; QHL = quinone and hydroquinone lipids; COUMD = coumarins and derivatives; HOP = hopanoids; PCH = polycyclic hydrocarbons; BENZ = benzenoids; SESQL = sesquiterpene lactones; PYRD = pyridines and derivatives; DT = diterpenoids; GLYCL = glycerolipids; SULF = sulfonyls; NAPHTH = naphthalenes; L 1,3 = Linear 1,3-diarylpropanoids; PHETH = phenol ethers; THCHR = thiochromenes; QUINL = quinolides and derivatives.

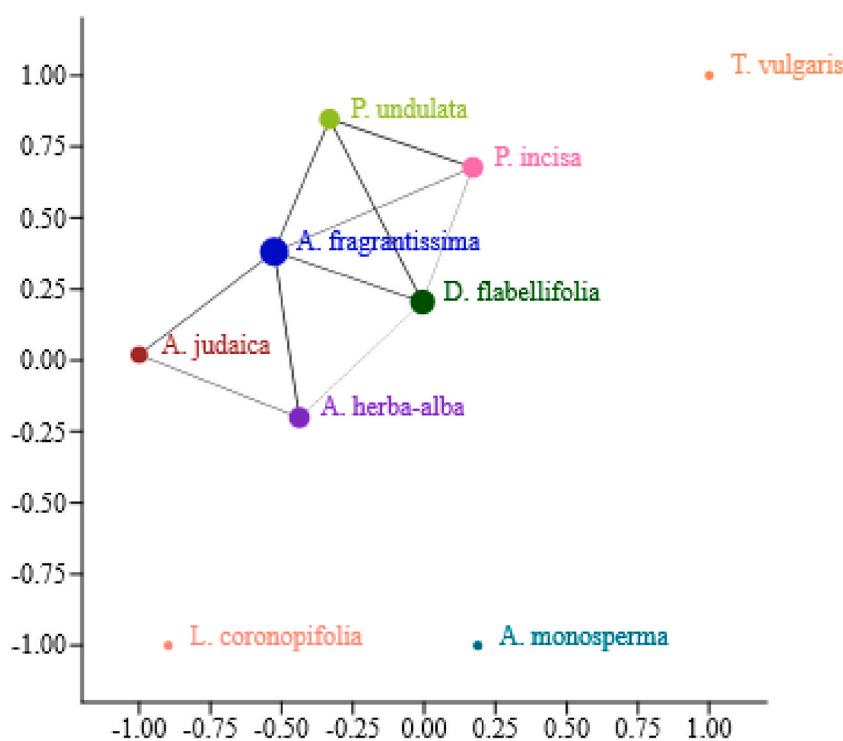


Fig. 4. Network diagram of plant species based on their contents of phytochemical classes.

plant extracts for *A. monosperma*. Aqueous plant extracts were the least efficient and exhibited activity only for *A. monosperma* and *P. undulata*. Antibacterial activities of plant ethanolic extracts were plotted in Fig. 6. *P. undulata* and *T. vulgaris* were the most efficient, while D. flabellifolia was the least efficient one. The plant ethanolic extracts displayed greater effect on Gram positive bacterial species (*S. aureus* and *B. subtilis*) in comparison to Gram negative species (*P. aeruginosa* and *E. coli*) (Fig. 7). Notably, *P. aeruginosa* demonstrated the highest level of resistance, whereas *S. aureus* displayed the highest level of sensitivity.

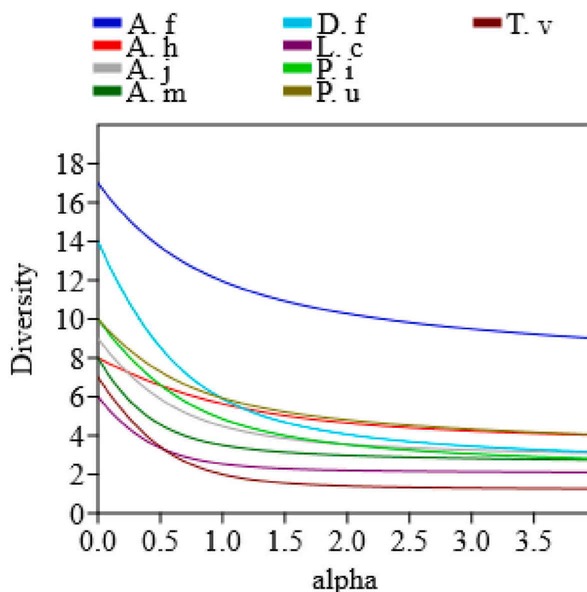


Fig. 5. Phytochemical class diversity profile of plant species. T. v = *Thymus vulgaris*; L. c = *Lavandula coronopifolia*; D. f = *Ducrosia flabellifolia*; P. i = *Pulicaria incisa*; P. u = *Pulicaria undulata*; A. j = *Artemisia judaica*; A. h = *Artemisia herba-alba*; A. f = *Achillea fragrantissima*; A. m = *Artemisia monosperma*.
a GC-MS Chromatogram of *A. fragrantissima* ethanolic extract.
b GC-MS Chromatogram of *A. herba-alba* ethanolic extract.
c GC-MS Chromatogram of *A. judaica* ethanolic extract.
d GC-MS Chromatogram of *A. monosperma* ethanolic extract.
e GC-MS Chromatogram of *D. flabellifolia* ethanolic extract.
f GC-MS Chromatogram of *L. coronopifolia* ethanolic extract.
g GC-MS Chromatogram of *P. incisa* ethanolic extract.
h GC-MS Chromatogram of *P. undulata* ethanolic extract.
i GC-MS Chromatogram of *T. vulgaris* ethanolic extract.

Table 2
Two-way repeated measure ANOVA table testing significance of difference between plants, between extracts and antibiotic and the extract/plant species interaction on bacterial growth.

Source of variation	Sum of squares	*df*	Mean square	F	*p (same)*
Factor A: Plants	292.156	8	36.5195	2.067	0.08084 NS
Factor B: Extracts &Antibiotic	3738.02	3	1246.01	5.292	0.02235*
A x B: interaction	246.885	24	10.2869	1.575	0.07238 NS
A x subj:	423.983	24	17.6659		
B x subj:	2118.91	9	235.434		
A x B x subj:	470.309	72	6.53207		
Total:	9516.44	143			

NS = non-significant difference, * = significant difference ($p < 0.05$).

3.3. Antioxidant activity

Fig. 9 highlighted the variation between the investigated plant extracts in their free radical scavenging efficiencies based on the EC_{50} values. *D. flabellifolia* extract followed by *P. incisa* and *A. monosperma* exhibited the highest values, while *T. vulgaris* was the least efficient one. It also showed that the pairs of plant species *A. judaica/L. coronopifolia* and *A. fragrantissima/A. herba-alba* were close to each other's in antioxidant activity.

4. Discussion

Because monoterpenoids represent the most extensive category of secondary metabolites [23], and have a significant role on chemotaxonomy, they predominated and were distributed widely in the examined plant extracts. Monoterpenoids have been proposed as chemotaxonomic markers for the Lamiaceae family at the family, genus, and species levels [24], therefore, *T. vulgaris* (Lamiaceae) outperformed other species. Sesquiterpenoids were the co-dominant phytochemical class especially in Asteraceae species (mainly

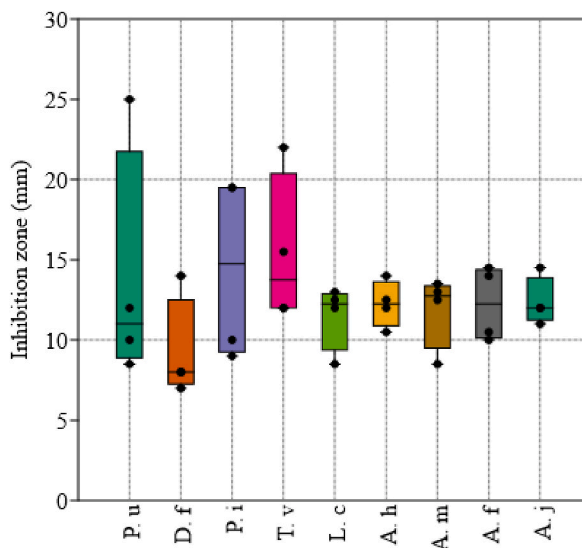


Fig. 6. Bar chart showing efficiencies of plant species in bacterial growth inhibition. A.f = *Achillea fragrantissima*; A.h = *Artemisia herba-alba*; A.j = *Artemisia judaica*; A.m = *Artemisia monosperma*; D.f = *Ducrosia flabellifolia*; L.c = *Lavandula coronopifolia*.

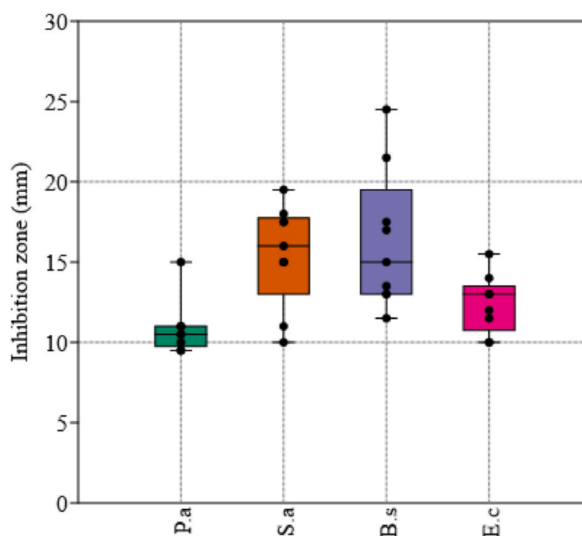


Fig. 7. Sensitivity of bacterial species to plant extracts and the antibiotic. P. a = *Pseudomonas aeruginosa*; S. a = *Staphylococcus aureus*; B. s = *Bacillus subtilis*; E. c = *Escherichia coli*.

P. incisa and *P. undulata*), for that [25], considered sesquiterpenoids as taxonomic markers of Asteraceae family. Fatty acyls showed a strong occurrence in the plant extracts with *L. coronopifolia* having remarkable amounts of fatty acyls along with steroids. Fatty acyls were also suggested previously by Refs. [26,27] to have a potential chemotaxonomic value. Coumarins represented by six compounds detected in this analysis were confined to *A. monosperma*, and this makes them a distinctive feature of this species. The phytochemical variety of the plant species under investigation indicates the presence of a well-developed and stable plant biosynthetic system that promotes health and stability and complex biosynthetic pathways of *A. fragrantissima* and *D. flabellifolia* [28] relative to other plants, that guarantees effective utilization of the distinct plant components by different customers. Furthermore, it suggests that these species exhibit greater resilience against invasive species and allelopathy. They are also capable of withstanding herbivory, pests, and pathogenicity due to their high phytochemical diversity. This diversity plays a crucial role in enhancing the likelihood of producing a small number of biologically active compounds, which can be utilized to bolster plant defense when ecological conditions require it [29]. Ref. [30] stated that plants with a high absolute diversity of secondary metabolites have a greater probability of producing one or more active compounds at any time than plants with a low diversity. In contrast, the high dominance of *T. vulgaris* indicated that few compounds, particularly monoterpenoids, dominated its extract completely. Cluster analysis and network diagram provided evidence that classifying the studied taxa on phytochemical composition basis was in line with their known taxonomic positions within the plant

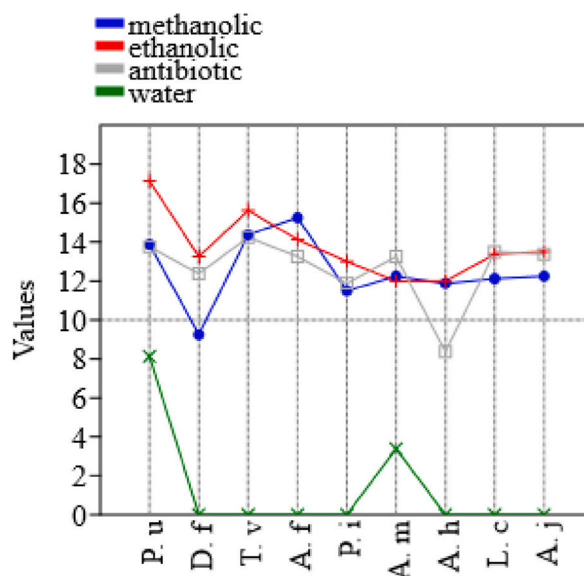


Fig. 8. Graph of means (inhibition zones) resulted from application of ethanolic, methanolic and aqueous extracts and the antibiotic on the test bacterial species.

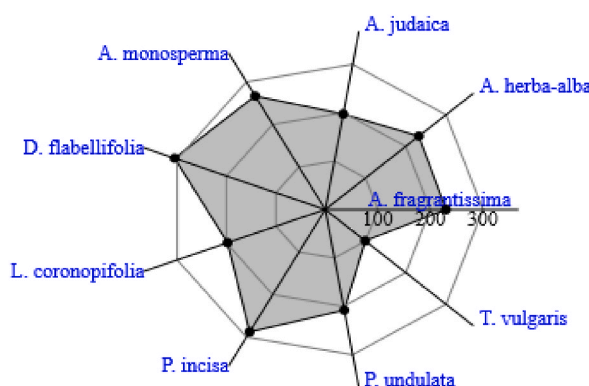


Fig. 9. Radar chart showing antioxidant activity of plant extracts based on EC_{50} values.

kingdom, except for *A. monosperma* which was disconnected and far from the rest of Asteraceae species. Principal component analysis (PCA) affirmed the taxonomic grouping and dimensionality of taxa based on their chemical composition and was further useful in outlining the distribution of phytochemical classes between different plants. Significant difference between ethanolic, methanolic and aqueous extracts and the antibiotic in antibacterial efficiencies was due to differences between extraction solvents in their abilities to pull active compounds from plant material, which, in turn, is determined by many factors including affinity between solvent and constituents, contact time and many others. Stronger antibacterial activities of *P. undulata* and *T. vulgaris* and stronger antioxidant activities of *D. flabellifolia* and *P. incisa* relative to other plants is mostly probably due to high contents of certain compounds that belong to sesquiterpenoids, fatty acyls, monoterpenoids, benzene and substituted derivatives, unsaturated hydrocarbons and organooxygen compounds in their extracts. Fatty acyls exhibit remarkable antibiotic action [31], with Falcarinol being the predominant compound in the *P. undulata* extract. Falcarinol had antibacterial activity against drug-resistant strains of Gram-positive bacteria, specifically *S. aureus*, and shown antimycobacterial properties [32]. The sesquiterpenoid α-Curcumene, which was present in high abundance in the *P. undulata* extract, was obtained from *Senicio selloi* and *Curcuma* sp. It exhibited antimicrobial action against clinical strains of *E. coli*, *S. aureus, P. aeruginosa*, and *Salmonella enteria* [33,34]. Furthermore, *P. undulata* was shown to have a significant abundance of Spathulenol, which is a sesquiterpenoid. Previously, *Myriactis nepalensis* essential oil was discovered to possess potent antibacterial properties against Gram positive bacteria [35]. Thymol and p-cymene, which are the predominant components found in *T. vulgaris* extract, were identified by Refs. [36,37] in Thyme and *Zataria multiflora* essential oils. These oils have the ability to inhibit the growth of *E. coli* and *S. aureus* due to their antibacterial properties. [38–40], and [41] showed the outstanding antioxidant properties of 2,2',4-Trihydroxybenzophenone, (Z,Z)-.alpha.-Farnesene, Patchoulane, and 2-(((2-Ethylhexyl)oxy)carbonyl) benzoic acid, which were found in high quantities in *D. flabellifolia* and *P. incisa*. Gram-negative bacteria exhibit greater resistance compared to

Gram-positive bacteria because to their unique structure. Consequently, they are anticipated to contribute significantly to global morbidity and death. As a result, the World Health Organization has prioritized addressing this issue [42]. The phenomenon of antibacterial resistance in *P. aeruginosa* has been documented in numerous investigations [43,44]. The exceptional ability of [45] to develop resistance to routinely used antimicrobials is mostly due to the selection of mutations in chromosomal genes or the acquisition of resistant determinants by horizontal transfer. The ethanolic extract of *D. flabellifolia* shown superior antipseudomonal action in comparison to other plants (see Appendix Table 2). Augmentin was used as the reference medication to assess the antibacterial effectiveness against four different bacterial strains, including *Pseudomonas aeruginosa*. While Augmentin may not be highly effective against *P. aeruginosa*, its use as a standard treatment in the study did not prevent the feasibility of conducting a comparative analysis. The selection was made based on the primary goal of the study, which was to evaluate the comparative effectiveness of the medicinal plant species being investigated. The phytochemical profile of *D. flabellifolia* established in this work is anticipated to be the focus of future investigations aimed at identifying the specific chemicals responsible for its antipseudomonal properties and understanding their mode of action. Chemotaxonomy is best understood by considering the variations in phytochemical composition of a plant species across different ecological zones, as well as its response to factors like elevation, climate, and geophysical conditions. To effectively study chemotaxonomy, it is important to examine ecotypes and other related aspects of plant taxonomy.

5. Conclusions

The investigation of chemotaxonomy in plant species has proven to be essential in elucidating significant associations and disparities among them, mostly based on their phytochemical makeup. Furthermore, it proved to be advantageous in the identification of certain plant species by designating certain phytochemical classes as chemotaxonomic markers. The chemotaxonomy of the plants aligned with their established taxonomic classification within the plant kingdom. Specifically, the two Pulicaria species exhibited the highest degree of similarity to one another, while the Artemisia species also displayed a close proximity. In contrast, the two Lamiaceae species were distinct and exhibited no phytochemical relationship to the other species. The species under investigation exhibited a significant degree of diversity in terms of their phytochemical contents. This finding suggests the presence of well-developed and robust plant biosynthesis mechanisms, as well as intricate biosynthetic pathways. The plants that were examined in the study demonstrated diverse levels of antibacterial and antioxidant capabilities, and these activities were shown to be correlated with the concentrations of specific phytochemical groups. This study proposes the introduction of a novel concept termed "eco-chemotype" or "eco-chemo species" to delineate a separate population or geographic variant within a species based on phytochemical characteristics.

Funding

We would like to express our deepest gratitude to the deanship of scientific research in the university of Tabuk, we are extremely grateful to them our completion of this research project would not have been possible without their support and nurturing.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

CRediT authorship contribution statement

Abdelrahim A. Elbalola: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Zahid Khorshid Abbas:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix Tables

Appendix Table 1
Percentage distribution of phytochemical classes between plant extracts

No	Phytochemical classes	% in Plant extracts

(*continued on next page*)

Appendix Table 1 (*continued*)

No	Phytochemical classes	% in Plant extracts								
		A. f.	A. h-a	A. j.	A. m.	D. f.	L. c.	P. i.	P. u.	T. v.
		A. f.	A. h-a	A. j.	A. m.	D. f.	L. c.	P. i.	P. u.	T. v.
1	Dihydrofurans	2.54	0	0	0	0	0	0	0	0
2	Benzene & substituted derivatives	10.84	5.02	1.61	0.54	41.53	0	13.02	14.66	4.73
3	Oxanes	0.98	0	0	0	0	0	0	0	0
4	Phenylpropanes	0.6	0	0	0	0	0	0	0	0
5	Pyridinecarboxamides	0.49	0	0	0	0	0	0	0	0
6	Unsaturated hydrocarbons	15.09	27.52	4.99	0	0.69	0	9.3	0	1.21
7	Organooxygen compounds	13.89	2.92	32.2	40.11	0	0	1.99		3.13
8	Monoterpenoids	9.31	30.16	38.44	1.71	19.02	1.21	2.15	12.8	84.29
9	Lactones	5.26	0	0	0	0	0	0	0	0
10	Phenols	2.99	0	16.13	0	0	0	0	1.26	0.69
11	Fatty Acyls	2.9	2.19	1.05	16.8	2.07	52.19	0	23.4	2.93
12	Sesquiterpenoids	10.67	5.98	0	0	17.12	0	45.21	32.62	3.01
13	Benzofurans	7.27	0	0	0	1.21	0	0	0	0
14	Steroids and steroid derivatives	1.32	7.36	0.8	0	5.55	42.51	1.41	1.1	0
15	Organic oxides	5.67	0	0	0	0	0	0	0	0
16	Tetrahydrofurans	9.69	0	0	0	0	0	0	0	0
17	Naphthopyrans	0.49	0	0	0	0	0	0	0	0
18	Triterpenoids	0	18.84	0	0	1.47	0	0	0	0
19	Carboxylic acids and derivatives	0	0	1.05	0	0.73	1.49	0	0	0
20	Flavonoids	0	0	3.74	0	0	0	0	0	0
21	Thiophenes	0	0	0	1.61	0	0	0	0	0
22	Quinone and hydroquinone lipids	0	0	0	0.48	0	0	0	0	0
23	Coumarins and derivatives	0	0	0	38.17	0	0	0	0	0
24	Hopanoids	0	0	0	0.58	0	0	0	0	0
25	Polycyclic hydrocarbons	0	0	0	0	0.52	0	1.07	0	0
26	Benzenoids	0	0	0	0	0.48	0	0	0	0
27	Sesquiterpene lactones	0	0	0	0	1.14	0	0	0	0
28	Pyridines and derivatives	0	0	0	0	2.6	0	0	0	0
29	Diterpenoids	0	0	0	0	5.87	1.31	0	1.06	0
30	Glycerolipids	0	0	0	0	0	1.29	0	0	0
31	Sulfonyls	0	0	0	0	0	0	0.91	0	0
32	Naphthalenes	0	0	0	0	0	0	22.64	0	0
33	Linear 1,3-diarylpropanoids	0	0	0	0	0	0	2.31	0	0
34	Phenol ethers	0	0	0	0	0	0	0	3.25	0
35	Thiochromenes	0	0	0	0	0	0	0	8.61	0
36	Quinolines and derivatives	0	0	0	0	0	0	0	1.23	0

Appendix Table 2
Inhibition zone diameters resulted from the disc diffusion assays for plant extracts and antibiotic against the test bacterial species.

Plant species	Treatment	Inhibition zone (mm)			
		P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*
P. undulata	Methanolic extract	8.5	12.00	25.00	10.00
	Ethanolic extract	10.50	18.00	24.50	15.50
	Aquatic extract	0.00	18.50	14.00	0.00
	Antibiotic (AMC30)	0.00	27.00	20.00	8.00
D. flabellifolia	Methanolic extract	8.00	7.00	14.00	8.00
	Ethanolic extract	15.00	10.00	15.00	13.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	24.50	16.00	9.00
P. incisa	Methanolic extract	9.00	19.50	19.50	10.00
	Ethanolic extract	9.50	19.50	21.50	12.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	30.00	20.00	7.00
T. vulgaris	Methanolic extract	12.00	22.00	15.50	12.00
	Ethanolic extract	10.50	17.50	17.00	11.50
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	32.00	12.00	9.00
L. coronopifolia	Methanolic extract	8.50	12.00	13.00	12.50
	Ethanolic extract	9.50	15.00	17.50	10.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	27.00	13.00	7.50
A. herba-alba	Methanolic extract	12.00	14.00	12.50	10.50
	Ethanolic extract	11.00	11.00	13.00	13.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	31.50	13.50	8.00

(*continued on next page*)

Appendix Table 2 (*continued*)

Plant species	Treatment	Inhibition zone (mm)			
		P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*
A. monosperma	Methanolic extract	8.50	13.00	12.50	13.50
	Ethanolic extract	10.00	15.00	13.00	10.00
	Aquatic extract	0.00	13.50	0.00	0.00
	Antibiotic (AMC30)	0.00	22.00	11.50	0.00
A. fragrantissima	Methanolic extract	10.00	14.50	14.00	10.50
	Ethanolic extract	11.00	16.00	13.50	13.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	30.00	17.00	7.00
A. judaica	Methanolic extract	11.00	14.50	12.00	12.00
	Ethanolic extract	11.00	17.50	11.50	14.00
	Aquatic extract	0.00	0.00	0.00	0.00
	Antibiotic (AMC30)	0.00	28.00	17.00	8.50

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23641.

References

[1] M.P. Junqueira-Gonçalves, L. Yáñez, C. Morales, M. Navarro, R.A. Contreras, G.E. Zúñiga, Molecules 20 (4) (2015) 5698–5713.
[2] S. Razack, K.H. Kumar, I. Nallamuthu, M. Naika, F. Khanum. Antiox 4 (2015) 185–203.
[3] Z. Ayoub, M. Archana, K. Siddhartha, L. Mishra, J. Bot. Soc. 48 (2017).
[4] M. Pirmoradian, T. Hooshmand, Remineralization and antibacterial capabilities of resin-based dental nanocomposites, in: M. Abdullah, M. Asiri, Inamuddin, Mohammad Ali (Eds.), In Woodhead Publishing Series in Biomaterials, Applications of Nanocomposite Materials in Dentistry, Woodhead Publishing, 2019, pp. 237–269.
[5] I. Bhatt, S. Rawat, R. Rawal, Antioxidants in Medicinal Plants, 2013, https://doi.org/10.1007/978-3-642-29974-2_13.
[6] B. Halliwell, How to characterize a biological antioxidant, Free Radic. Res. Commun. 9 (1990) 1–32.
[7] Da-Cheng Hao, X.J. Gu, Pei-Gen Xiao, Medicinal Plants: Chemistry, Biology and Omics, 2015, pp. 1–681.
[8] V.V. Bhargava, S.C. Patel, K.S. Desai, Int. J. Herbal Med. 1 (2013) 14–21.
[9] Ram Singh, J. Med Plants Stud. 4 (2016) 90–93.
[10] P. Cárdenas, T. Pérez, N. Boury-Esnault, Chapter two - sponge systematics facing new challenges, in: Mikel A. Becerro, Maria J. Uriz (Eds.), Manuel Maldonado, Xavier Turon, Advances in Marine Biology vol. 61, Academic Press, 2012, pp. 79–209.
[11] A. N. Kalia. Textbook of Industrial Pharmacognosy, sixth ed., Navneet Publication, Mumbai, 201,131-162..
[12] A.A. Elbalola, G.A. Al-Atawi, Pakistan J. Bot. 54 (6) (2022) 2263–2271.
[13] N. Al-Harbi, J. Med. Plants Res. 11 (2017) 549–555.
[14] A.H. Rajasab, Lamber Academic Publishing, Germany, 2011.
[15] A. Fakhry, M. Migahid, Int. J. Current Microb. App. Sci. 5 (2016) 258–270, https://doi.org/10.20546/ijcmas.2016.506.029.
[16] S.A. Chaudhary, National Herbarium, National Agriculture and Water Research, Riyadh, KSA, 1999 vols. 1–3.
[17] G.H. Xu, J.C. Chen, D.H. Liu, Y.H. Zhang, P. Jiang, X.Q. Ye, J. Food Sci. 73 (2008) C11–C18.
[18] Y. Djoumbou Feunang, R. Eisner, C. Knox ClassyFire, Automated chemical classification with a comprehensive, computable taxonomy, J Chem. Inform. 8 (2016) 61, https://doi.org/10.1186/s13321-016-0174-y.
[19] S. Kim, J. Chen, T. Cheng, PubChem update, Nucleic Acids Res. 51 (D1) (2023) D1373–D1380, https://doi.org/10.1093/nar/gkac956.
[20] V.V. Zazharskyi, P.O. Davydenko, M. Kulishenko, I.V. Borovik, V.V. Brygadyrenko, Biosyst. Divers. 27 (2) (2019) 163–169, https://doi.org/10.15421/011922.
[21] C.M. Liyana-Pathiranan, F. Shahidi, J. Agric. Food Chem. 53 (2005) 2433–2440.
[22] Quest Graph™ EC50 Calculator, AAT Bioquest, Inc., 30 May 2023. https://www.aatbio.com/tools/ec50-calculator.
[23] M. Zielińska-Błajet, J. Feder-Kubis, Int. J. Mol. Sci. 21 (19) (2020) 7078, https://doi.org/10.3390/ijms21197078. PMID: 32992914; PMCID: PMC7582973.
[24] Ferdinand Bohlmann, Christa Zdero, Dieter Bergert, Suwita Albert, Mahanta Pradip, C. Jeffrey, Claudio Frezza, Alessandro Venditti, Mauro Serafini, Armandodoriano Bianco, Phytochemistry, Chemotaxonomy, Ethnopharmacology, and Nutraceutics of Lamiaceae, 2019, https://doi.org/10.1016/B978-0-444-64185-4.00004-6.
[25] F. Bohlman , J. Jakupovic, C. Zdero, R. M. King.. Phytochemistry, 18, 625-630..
[26] L. Velasco, F.D. Goffman, H.C. Becker Hc, 1999. J. American Oil Chemists Soc. 76 (1979) 25–30.
[27] S. Mongrand, A. Badoc, P. Brigitte, L. Chantal, C. Marie, B. Jean-Jacques, Phytochemistry (Elsevier) 66 (2005) 549–559.
[28] W.C. Wetzel, S.R. Whitehead, Ecol. Lett. 23 (2020) 16–32, https://doi.org/10.1111/ele.13422.
[29] M.R. Berenbaum, A.R. Zangerl, Phytochemical diversity, in: J.T. Romeo, J.A. Saunders, P. Barbosa (Eds.), Phytochemical Diversity and Redundancy in Ecological Interactions. Recent Advances in Phytochemistry, vol. 30, Springer, Boston, MA, 1996, https://doi.org/10.1007/978-1-4899-1754-6_1.
[30] C.G. Jones, R.D. Firn, Phil. Trans. Roy. Soc. 333 (1991) 273–280.
[31] C. Grellet-Bournonville, M. Filippone, P. Di Peto, T. María, A. Couto, A. Marchese, J. Ricci, B. Welin, A. Castagnaro, Sci. Rep. 10 (2020) 8196, https://doi.org/10.1038/s41598-020-65125-7.
[32] P. Lars Christensen, Chapter 20 - Bioactivity of Polyacetylenes in Food Plants, in: Ronald Ross Watson, Victor R. Preedy (Eds.), Bioactive Foods in Promoting Health, Academic Press, 2010, pp. 285–306.
[33] G. Silva, P. Pozzatti, F. Rigatti, R. Hörner, S.H. Alves, C. Mallmann, B. Heinzmann, J. Microbiol. Biotechnol. Food Sci. 4 (2015) 434–436, https://doi.org/10.15414/jmbfs.2015.4.5.434-436.
[34] J.J. Albaqami, H. Hamdi, A. Narayanankutty, N.U. Visakh, A. Sasidharan, A.M. Kuttithodi, A.C. Famurewa, B. Pathrose. Antibiotics 11 (2022) 1547, https://doi.org/10.3390/antibiotics11111547.
[35] J. Fu, Y. Gao, X. Xing, 27, Molecules 20 (14) (2022) 4631, https://doi.org/10.3390/molecules27144631. PMID: 35889501; PMCID: PMC9324352.
[36] D. Santurio, F. Kunz de Jesus, R. Zanette, K. Schlemmer, A. Fraton, L. Fries, Acta Sci. Vet. 42 (2014).
[37] S. Pourhosseini, H. Ahadi, A. Aliahmadi, M. Mirjalili, J. Oil-Bearing pl 23 (2020) 779–787, https://doi.org/10.1080/0972060X.2020.1824688.

[38] A.C. Doriguetto, F.T. Martins, J. Ellena, R. Salloum, M.H. dos Santos, M.E.C. Moreira, Biodiversity 4 (2007) 488–499, https://doi.org/10.1002/cbdv.200790041.
[39] P. Lai, H. Rao, Y. Gao, Record Nat. Prod. 12 (2018) 290–294.
[40] Z.A. Wani, A. Farooq, S. Sarwar, V.S. Negi, A.A. Shah, B. Singh, S. Siddiqui, S. Pant, H. Alghamdi, M. Mustafa, Biol. 11 (2022) 1415, https://doi.org/10.3390/biology11101415.
[41] N. Denga, S.T. Vuyisile, S.G. Sechene, Biochem. Res. Intl. 9 (2022), https://doi.org/10.1155/2022/5365024.
[42] Z. Breijyeh, B. Jubeh, R. Karaman, 16, Molecules 25 (6) (2020) 1340, https://doi.org/10.3390/molecules25061340. PMID: 32187986; PMCID: PMC7144564.
[43] P.D. Lister, D.J. Wolter, N.D. Hanson, Clin. Microbiol. Rev. 4 (2009) 582–610, https://doi.org/10.1128/CMR.00040-09. PMID: 19822890; PMCID: PMC2772362.
[44] H. Mulcahy, L. Charron-Mazenod, S. Lewenza, PLoS Pathog. 4 (2008), e1000213.
[45] P. Ruiz-Garbajosa, R. Cantón, Rev. Española Quimioter. 1 (2017) 8–12. PMID: 28882007.