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Original Article

Comparative Phytochemical Study of Some Species of *Artemisia* in the Middle East: A Focus on Antimicrobial Activities and GC-MS Analysis in *A. absinthium* L. Jazan, KSA and *A. herba-alba* Asso Sinai, EGY

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ABSTRACT

This study deals with the potential antimicrobial activity of *Artemisia absinthium* L. (from Saudi Arabia) and *A. herba-alba* Asso. (from Egypt) extracts by using variety of solvents. The pathogenic microorganisms; *Candida albicans*, *Cronobacter sakazakii*, *Enterococcus faecalis* and *Salmonella enterica*, were manipulated. The MICs recorded different affinities for each solvent. The MBC and MFC were also determined. The chemical compositions of two plant species were determined by GC-MS analysis. A comparative study was conducted among *Artemisia* sp. belonging to the Middle East region. They are *A. absinthium*, *A. abyssinica*, *A. annua*, *A. herba-alba*, *A. judaica*, *A. monosperma*, *A. scoparia*, *A. sieberi* and *A. vulgaris*. The binary matrix included the chemical components of *Artemisia* sp. The phenogram and similarity matrix cleared the recent chemotaxonomic position of this genus in the Middle East. MIC values of both plant species were analyzed using the ANOVA test. Pearson correlation coefficients were calculated in the form of SLR curves. The two studied plant species were recommended as alternative natural antimicrobial inhibitor agents.

INTRODUCTION

The Middle East region is regarded as the most global biodiversity hotspot that is neglected and all theoretical and practical studies on it are scarce. The study of plant species in a wide-spread region summarizes human interference, climatic shifts, valuable plant species resources, geographical adaptation, and genetic changes on individual, species, population or community [1, 2].

Medicinal plants are the solutions to many problems facing the human race. The medicinal plants that belong to the Middle East region can treat carminatives, laxatives, anti-diarrhoeals, anthelmintics, swollen joints, muscle pain, burns, skin disorders, bruises, wounds, bites, stings, urinary disorders, Diuretics, Fertility, Coughs, Cold, Headaches, Fever etc [3].

Most potential secondary metabolites are derived as natural products from medicinal plants. They are classified into several groups according to their medical active materials. Biosynthetic phytochemicals can be tannins, alkaloids, volatile oils, steroids, fixed oils, phenols, glycosides, flavonoids and resins that have efficacy in healing many people from pathogenic diseases. They are situated in just definite parts in plants such as leaves, flowers, bark, seeds, fruits, and roots or the whole plant body [4, 5].

They are distinguished by their antibacterial and antifungal activities against many pathogenic and infectious microorganisms. Today, researchers utilize natural phytochemicals instead of synthetic additives because they avoid harmful side effects in coordination with the safety of the environments.

From this point on, many published articles focus on using plant extracts as natural antibiotics according to the recommendations from therapeutic physicians [6, 7].

Artemisia belongs to the Asteraceae family and seeks the most effective economic important value. It is considered antitumor, antispasmodic, and antirheumatic medicinal plant. Some species are allergenic but others are toxic [8, 9]. About 500 *Artemisia* species are distributed especially in the northern hemisphere of the earth in America, Europe, America, Asia and Middle East [10].

Artemisia plants have been exploited to contain several and variety of chemical components. The chemical composition varies considerably according to developmental stages and geographical locations [11]. They have been proven to be an alternative cure than synthetic ones [12].

The inquestrian taxonomy of this economically important genus is ongoing and ambiguous because of the complexity of plant structures. Hence, chemotaxonomy is useful for overcoming restricted related species and contributing classification on basis of modern tools [13, 14]. The present study focuses on two *Artemisia* sp.; *A. absinthium* L. (from Saudi Arabia) and *A. herba-alba* Asso. (from Egypt), which are representative to another *Artemisia* sp. in the Middle East. They are analyzed to demonstrate antimicrobial activities against some pathogenic fungi and bacteria strains in addition to describing the main chemical composition of their extracts. The other *Artemisia* sp., in addition to *A. absinthium* L. and *A. herba-alba* Asso. from different countries of the Middle East region, are also comparatively analyzed to determine the taxonomic position of the genus in this region.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The entire plant species were collected in July-September 2022. *A. absinthium* L. was found in the Jazan region, KSA at 17 °15 ′55.3″ attitude, 43 ° 06 ′ 47.1 ″ longitude, 383 m elevation while *A. herba-alba* Asso was in Sinai, Egypt at 28°50′15.3″ latitude, 33 ° 55 ′ 30.1 ″ longitude, 77 m elevation. Identification of two *Artemisia* sp. was identified by the herbarium of the Department of Biology, College of Science, Jazan University (JAZUH). The studied species were compared with the most common wild native *Artemisia* sp. distributed in the Middle East region; *A. abyssinica* Sch. Bip. (Yemen, Saudi Arabia), *A. annua* L. (Pakistan, Iran-Northern region, Egypt, Cyprus, Iraq, Libya, Morocco, Kazakhstan, Kirgizstan, Lebanon-Syria, Tunisia, Turkey) *A. judaica* L. (Algeria, Jordan, Egypt-Sinai Peninsula, Saudi Arabia, Libya, Israel-Negev) *A. monosperma* Delile (Saudi Arabia-Northern region, Egypt-Northern region, Kuwait, Lebanon-Syria, Libya, Oman, Palestine), *A. scoparia* Waldst. & Kit. (Pakistan, Iran-Northern region, Tajikistan, Turkey, Kazakhstan, Afghanistan, Egypt, Iraq, Kirgizstan), *A. sieberi* Besser (Iran, Saudi Arabia -North and Central regions, Turkey, Egypt, Afghanistan, Iraq, Pakistan, Lebanon-Syria, Libya, Oman, Palestine), *A. vulgaris* L. (Egypt, Pakistan-Northern region, Iran-Northern region, Afghanistan, Iraq, Kazakhstan, Kirgizstan, Tadzhikistan, Tunisia, Turkey, Uzbekistan) in addition to *A. absinthium* L. (Iran-Northern region, Pakistan-Northern region, Turkey, Algeria, Afghanistan, Iraq, Libya, Morocco, Lebanon-Syria, Uzbekistan) and *A. herba-alba* Asso (Morocco, Jordan-Southern region, Algeria, Pakistan, Israel-Negev, Libya, Tunisia) (Fig. 1).

Preparation of Plant Extracts for Antimicrobial Analysis

The entire plant materials were dried at 50^0 C for 24 hours and then powdered for solvent utilization; aqueous in addition to different organic solvents. Acetone, butanol, chloroform, diethyl ether, ethanol, and methanol were included in this investigation. Each plant species was extracted according to [15]. 20 g of the entire plant were placed in 250 ml of each solvent and then stirred in a water bath at 45^0 C for 10 hr. Filtration and evaporation of plant residue occurred under vacuum on a rotary evaporator at 40^0 C. The remaining residue was dissolved in standard negative solvent (DMSO). Replication was performed to obtain standard deviations for readings [16].

Microbial Samples

The antimicrobial potency of each plant extract was evaluated using three pathogenic bacterial strains in addition to one pathogenic fungal strain. They are isolated, identified, and international accredited by ATTC international accredited company. The American Type Culture Collection (ATCC) produces more than two thousands of microbial strains with cross-referencing of extensive complete information with trademarks. All summarized data from the studied microbial strains were obtained in

(Tables 1, 2). *C. albicans* (Robin) Berkhout (ATCC® 10231™) behaves like yeast; white. gray, opaque, and intestinal. It belongs to *Ascomycota*, *Saccharomycotina*, *Saccharomycetes*, *Saccharomycetes and Saccharomycetales*.

It has great diversity of forms; linear, chlamydospores, and sinusoidal. Pseudohyphae of *C. albicans* remain attached after cytokinesis and donate mycelia after several rounds of hyphal cell divisions. *C. albicans* can grow biofilms, single-cell cultures, and microcolonies. *C. albicans* asymptomatically colonizes the skin, oral mucosa, vagina, and gastrointestinal tract of healthy individuals. It invades normal tissues and organs in the event of a weakened immune system (Ignacio *et al*., 2022). *C. sakazakii* (Farmer *et al*.) Iversen *et al*. (ATCC®29544™) is a biofilm-forming, motile, rod-shaped, Gram-negative, facultative anaerobic foodborne bacillus of the family Enterobacteriaceae. It is the opportunistic pathogen associated with meningitis and other vigorous infections in immunosuppressed adults and children. These infections linked to epidemiologically fatal newborns and occur in all age groups (Jennifer *et al*., 2017; Hongxuan *et al*., 2023). *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC®29212™) is a lactic acid, Gram positive, nonspore-forming, facultatively anaerobic, fermentative, nosocomial bacterial coccus found in plant-associated environments, animal intestine, and a variety of food. It is occurred naturally in gastrointestinal tract, oral cavity, and vaginal vault. It can be potentially pathogenic to humans after exposure to severe conditions like stress, high-dose medicine, less efficient immunity, and other compatible diseases. Sequenced data from *C. albicans* contains 18S ribosomal RNA gene, internal transcribed spacer 1, internal transcribed spacer 2, 26S ribosomal RNA gene, 5.8S ribosomal RNA gene [18, 19]. *S. enterica* subsp. *arizonae* (Borman) Le Minor *et al*. [20] (ATCC®13314™) is a gram negative bacillus lactose fermented malonate with lactose bacillus utilized and a member of *Enterobacteriaceae*.

It can penetrate food chains through abiotic surfaces such as farms and contaminated food to reach human consumptions. The food that carries salmonellosis is food processing plants, broiler meat, eggs, etc. It causes gastroenteritis, severe dehydration, and cardiorespiratory diseases. The most common symptoms are developed shock, vomiting, abdominal pain, diarrhea and headache [21, 22].

Estimation of Antimicrobial Activity of *Artemisia* Extracts

The disc diffusion assay was performed to evaluate the antimicrobial activity of the extracts of each studied species. The media components for each microbe were demonstrated in (Table 3). Culture preparation was done using antiseptic handle procedure. 2 mg of plant extract was dripped and loaded onto sterile filter paper discs, placed on agar medium and then inoculated with referenced ATCC strains at 35°C±2°C for 24 h. The negative control paper discs were dimethyl sulfoxide (DMSO) on the other hand; the positive control discs were 0.01 mg of streptomycin. The diameter of the clear zone was measured in millimeter [7, 23].

Determination of the MIC of Plant Extracts

Minimum Inhibitory Concentration (MIC) evaluation is the lowest antimicrobial concentration that can inhibit microbial growth after 24 h, of incubation treatment. It was calculated for each microorganism tested by different serial micro-dilutions of plant extract in Dimethyl Sulphoxide (DMSO) solution in the range of 6.25 to 390.0 mg/L. according to the protocol described by [24]. The inhibition zones were determined and recorded for each of the concentrations of the plant species extracts. Streptomycin (10μg) was used as a positive control and DMSO was used as a negative control.

Evaluation of (MBC) and (MFC)

MBC (minimum bacterial concentration) and MFC (minimum fungal concentration) are the lowest concentration of plant extract that exhibited no microbial growth after the minimum inhibitory concentration approaches. At 37°C, the inoculated agar plates were then incubated for 24 h. Three separate biological replicates were performed without recognition of any colonies [25].

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

GC–MS analysis of the methanol extract of two whole studied plant materials was performed using TRACE GC Ultra capillary gas chromatograph interfaced to Flame Ionization Detector (FID) mass detector.

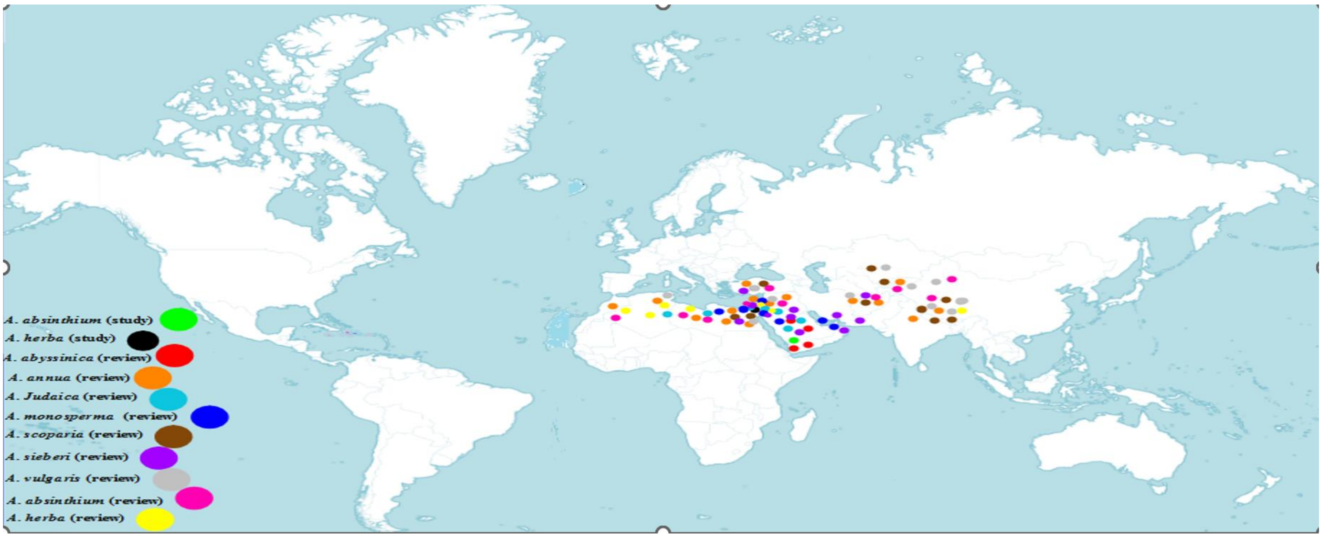

Fig. 1. All studied and reviewed *Artemisia* sp. in the Middle East region.

The gas chromatograph was equipped with an on-column injector (OCI) of 5% phenyl, 95% dimethyl-poly- siloxane (SE: 52, length: 15 m, ID: 0.25 mm, film thickness: 0.25 µm).

I The GC parameters were programmed as follows: (Oven Parameters): initial temperature; 40 °C, initial time; 2.00 min, final temperature; 310 °C, hold time; 2.00 min, rate; 15.0 °C/min. Carrier gas helium; 1.2 ml/min flow mode; constant pressure (250 kPa). njection parameters: volume; 100 µl, speed; 5 µl/s. Detector parameters: Det. Base temperature; 320 °C, Det. Gas; H_2 35 ml/min, Air 350 ml/min, M-up 30 mL/min. PTV parameters (programmable temperature vacuum): base temperature; 30 °C, splitless time; 1.00 min, solvent valve temperature; 120°C, inject time; 0.3 min, vent flow; 100 ml/min, transfer rate; 10 °C/sec, transfer temperature; 275 °C, transfer time; 15 min. The identification of constituents was determined by comparing their peak areas of mass spectra with the relative abundance of known compounds from NIST Mass Spectral Library [11]. All chemical information, classifications, physicochemical properties, and biological effects were revealed by the Canadian Institutes of Health Research, the Canada Foundation for Innovation, and by The Metabolomics Innovation center (TMIC).

Statistical Analysis

The Pearson's correlation coefficient for MICs of two extracts of studied plant species versus the bacterial and fungal strains was done according to [26, 27]. The determination of P values as significance tests based on degrees of freedom according to [28] approach was achieved. MICs were subjected to statistical analysis to carry out the ANOVA test and standard errors [29].

SPSS software (version 22) was utilized to conduct statistical analysis [14]. The representations of (SLR) represented the significant relationships between microbial strains versus *Artemisia* sp [30, 31]. They pave the way for comparative data among bacterial and fungal strains against one side and plant species from another side. They were explored using linear regression approaches to achieve the effect of a valuable parameter.

Scoring of Data and Evaluation of the Phenogram

The phytochemical traits of all *Artemisia* sp. Belonging to this investigation were scored to establish a phenetic analysis. Cluster analysis and similarity matrix were constructed by P class method [32]. Distances were calculated according to the Gower coefficient [33]. The Nei genetic similarity index (SI) was used to estimate the pairwise similarity between the operational taxonomic units (OUT) based on the equation SI = 2Nij / (Ni + Nj), where Ni and Nj are the total number of phytochemical characters for each species i and j, respectively, besides Nij is the number of common ones shared between them. The phenogram was performed based on a sequential agglomerative hierarchical nested clustering where series of successive mergers were used to aggregate *Artemisia* sp. with similar characters in a method called unweight pair group mathematical averages (UPGMA) [34].

Table 1 ATCC clinical database of studied microbial strains [17].

Property	*C. albicans* (ATCC® 10231™)	*C.sakazakii* (ATCC® 29544™)	*E. faecalis* (ATCC® 29212™)	*S. enterica* (ATCC® 13314™)
Specific applications	Assay of amphotericin B fungizone Assay of antimicrobial preservatives Assay of haloprogin Assay of nystatin fungicidin Media testing Membrane filter testing Preparatory test control Produces D-arabinolactone oxidase Produces DNA topoisomerase Food testing Pharmaceutical and Personal Care Produces aspartic proteinases aspartyl proteinases Produces estrogen-binding protein Produces lanosterol synthase 2,3-oxidosqualene lanosterol cyclase Produces phenethyl alcohol Produces polyamine oxidase Produces tryptophol Quality control strain Sterility testing Testing Testing fungicides Produces farnesoi	Food test	Food testing Control strain Evaluation of Mueller-Hinton agar Media testing Quality control Quality control strain Susceptibility disc testing Susceptibility testing Quality control strain for API, bioMerieux VITEK, IDS, Micro-Media, MicroScan, and Sensititre products, UTI assay development	Enteric Research Emerging infectious disease research
Temperature	24-26 °C	30 °C	37 °C	37 °C
Atmosphere	Aerobic	Aerobic	Aerobic	Aerobic
Isolation source	Man with bronchomycosis	Throat of a human child	Urine	Reproductive tracts of domestic animals
Applications	Agricultural research Antimicrobial resistance research Drug development Food testing Media testing Quality control Pharmaceutical testing	Food testing Quality control disease research	Bioinformatics Food testing Media testing Quality control Water testing Urinary tract infection research	Enteric disease research Infectious disease research Zoonotic disease research
Product format	Freeze & drying	Freeze & drying	Freeze & drying	Freeze & drying
Storage conditions	3 °C to 7 °C	3 °C to 7 °C	3 °C to 7 °C	3 °C to 7 °C
Susceptibility profile	-	-	-	-

Table 2 ATCC database genome of the bacterial strains [17].

Subunits of genome	*C. sakazakii*	*E. faecalis*	*S. enterica*
CDS No.	4423	2906	4195
Hypothetical Proteins No.	1374	1223	1048
tRNAs	84	60	84
5s rRNAs	8	4	8
16s rRNAs	7	4	7
23s rRNAs	7	4	7

Table 3 The media of ATCC accredited selective bacterial strains [23].

ATCC Medium	*C. albicans* (ATCC® 10231™)	*C. sakazakii* (ATCC® 29544™)	*E. faecalis* (ATCC® 29212™)	*S. enterica* (ATCC® 13314™)
Medium Name	Sabouraud Dextrose Agar/Broth, Emmons Modification	3 Nutrient Agar/Broth	44 Brain Heart Infusion Agar/Broth	3 Nutrient Agar/Broth
Agar Medium Composition	50 g Sabouraud Agar Modified (BD 274720) with1000 ml DI Water	23 g Nutrient Agar (BD 213000) with1000 ml DI Water	52 g Brain Heart Infusion Agar (BD 211065) with1000 ml DI Water	23 g Nutrient Agar (BD 213000), 1000 ml DI Water
Broth Medium Composition	30 g Sabouraud Broth (BD cat 238230) with 1000 ml DI Water	8 g Nutrient Broth (BD cat 234000) with 1000 ml DI Water	37 g Brain Heart Infusion Broth (BD 237500) with1000 ml DI Water	8 g Nutrient Broth (BD cat 234000) with 1000 ml DI Water
Sabouraud Dextrose Agar, Emmons Modification Composition	10 g Neopeptone, 20 g Dextrose, 20 g Agar, 1000 ml DI Water	Nil	Nil	Nil
Nutrient Agar Composition	Nil	3 g Beef Extract, 5 g Peptone and15 g Agar	Nil	3 g Beef Extract, 5 g Peptone, 15 g Agar
Brain Heart Infusion Composition	Nil	Nil	200 g Calf Brains infusion, 250 g Beef Hearts infusion, 10 g Proteose Peptone, 2 g Dextrose, 5 g NaCl, 2.5 g Na_2HPO_4, 1000 ml DI Water	Nil

RESULTS

Antimicrobial Analysis

Different organic solvent extracts in addition to aqueous one of the two *Artemisia* species exhibited different antimicrobial activities that were represented in terms of inhibition zones (IZ), (MICs), and (MBCs/MFCs) as shown in (Tables 4-9) (Fig. 1-6) labeled with alphabetical English letters written as abbreviations on microbial cultural plates.

According to *A. absinthium*, *C. albicans* was regarded as the first highest sensitive level. It was inhibited with all different organic solvents to different degrees. The organic solvent with the most inhibitor was butanol (IZ 5.25±0.50 mm) while the less one was acetone (IZ 1.75±0.96 mm). The second highest sensitive level was *S. enterica*, which was inhibited, as well as *C. albicans* except for acetone extract that was resistant to it. The most inhibitory organic solvents and the least inhibitory were butanol (IZ 2.50±0.05 mm) and ethanol (IZ 0.5±0.01 mm) respectively. The rest of the studied micro-strains had less sensitive levels; *E. faecalis had* only two inhibitory responses (butanol and ethanol) and only one (butanol) for *C. sakazakii*.

On the other hand, *A. herba alba* expressed gradually inhibition unlike previous plant species. It was inhibited with five, three, two, and one organic solvents against *C. albicans***,** *C. sakazakii, S. enterica*, and *E. faecalis,* respectively. Butanol solvent was the superior inhibitor that appeared in all studied strains, while ethanol and methanol were the inferior ones that appeared in only one strain; *C. albicans*. However, the acetone solvent was regarded as the null response.

Although the aqueous solvent had no effect on the bacterial growth by using extracts of *A. herba alba,* it promoted the growth of *A. absinthium* extract against all selective bacteria except *Listeria monocytogenes*. The negative control; DMSO also had negative effect on all pathogenic bacterial strains. The positive control; Streptomycin exhibited predominantly antibacterial and antifungal activities on all microorganism tested in the same manner.

Taking into account the MIC values of *A. absinthium*, *C. albicans* showed the highest value (196.88±3.66 mg/ml) while *E. faecalis* and *S. enterica* showed the same lowest value (37.5 mg/ml). In contrast, *S. enterica* showed the highest value (300.0±2.03 mg/ml) while *C. albicans showed* the lowest value (75.0±1.01 mg/ml) in treatments with *A. herba alba* treatments. MFCs/MBCs ranged from 75.0±3.68 to 431.26±4.02 mg/ml in *A. absinthium* extract treatments of *A. absinthium* However, they ranged from 9.31±2.68 to 180.29±2.30 mg/ml of *A. herba alba*.

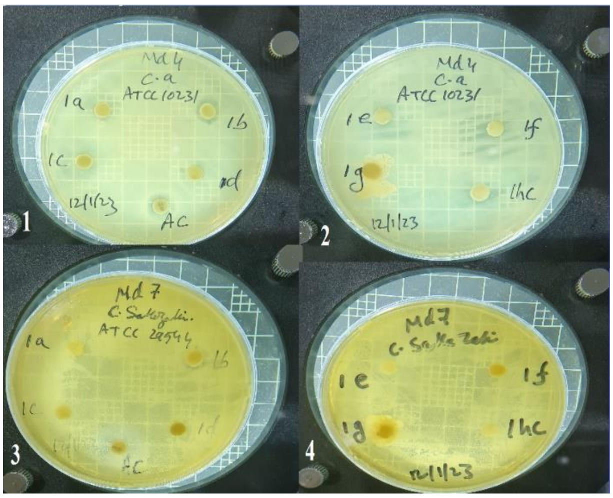

Fig. 1 Inhibition zone (IZ) diameter of *A. absinthium* against; 1-2. *C. albicans* (C a) and 3-4. *C. sakazakii* (C. *sakazakii*) was cultivated using different extract solvents.

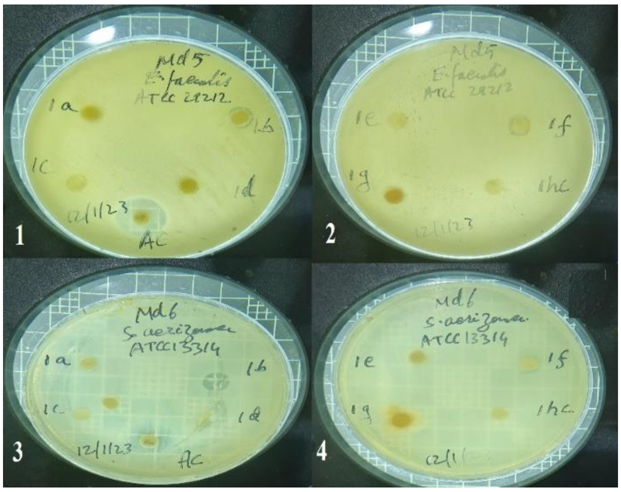

Fig. 2 Inhibition zone (IZ) diameter of *A. absinthium* against; 1-2. *E. faecalis* (E. *faecalis*) and 3-4. *S. enterica* (S. *arizonae*) was cultivated by using *different* extract solvents.

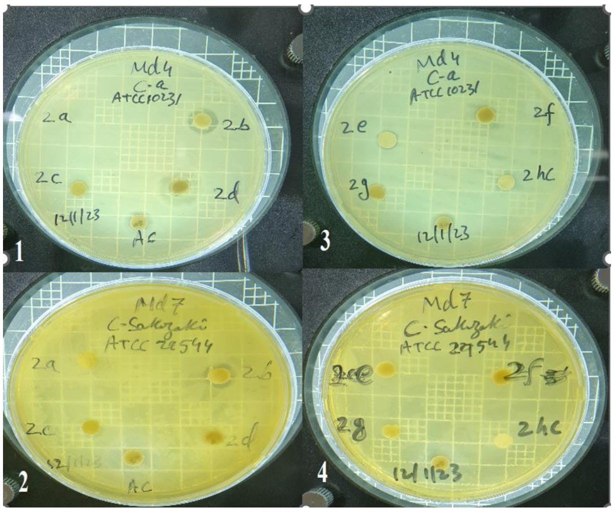

Fig. 3 Inhibition zone (IZ) diameter of *A. herba alba* against; 1-2. *C. albicans* (C a) and 3-4. *C. sakazakii* (C. *sakazakii*) was cultivated using different extract solvents.

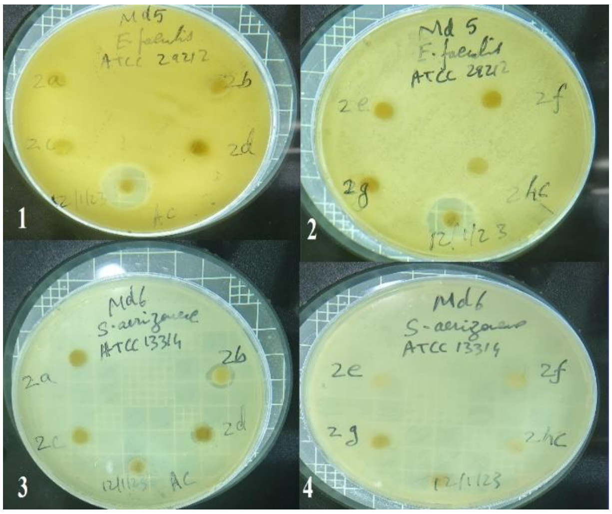

Fig. 4 Inhibition zone (ZI) diameter of *A. herba alba* against; 1-2. *E. faecalis* (E. *faecalis*) and 3-4. *S. enterica* (S. *arizonae*) by using *different* extract solvents.

Phytochemical Component Screening

During retention time (RT) 26.56 min, GC-MS analysis of *A. absinthium* extract resulted in the identification of 8 predominate main components where (-) caryophyllene oxide was considered as the main component (42.96%), however, (+) *β*-costol was the lowest (0.66%). 2,6,10-trimethyl-cis-7,10-oxido-dodeca-3E,11-dien-2-ol-5-one and (+) (1S,2S,5R)-4-isopropenyl-7-methyl-1-oxaspiro [2,5] octane was reported as the main component (15.78%) and (28.06%) respectively (Fig. 7-8, Table 10). On the other hand, GC-MS analysis of *A. herba-alba* extract within IR 27 min identified 15 phytocompounds that comprise 93.28% of total components where (-)-norephedrine and (-) caryophyllene oxide were the highest major ones (24.33%) and (19.13%) respectly, while others ranged from 1.72 to 9.00%. 4-hydroxy-cyclohexanone and isobutylethene were regarded the lowest (Fig. 9-10, Table 11).

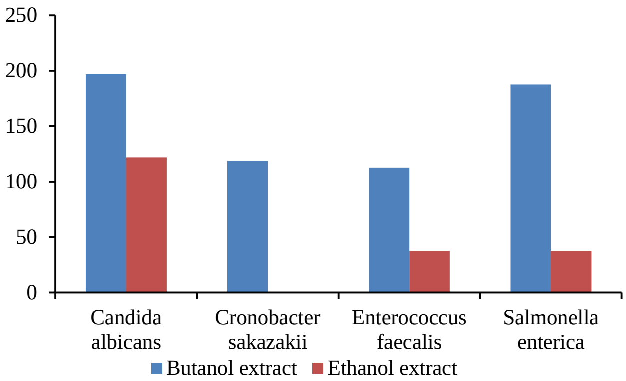

Fig. 5 MICs for *A. absinthium* extracts against selective microbial strains.

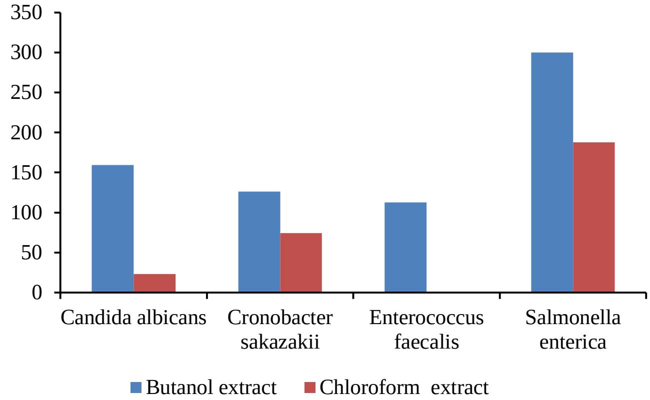

Fig. 6 MICs for extracts of *A. herba alba* against selective microbial strains.

Statistical Analysis

Analysis of variance for MICs performed by ANOVA tests showed significant differences for all selective microbial strains. In *A. absinthium* extracts, *C. albicans* showed the highest *F* test values with low significance versus *C. sakazakii* and *E. faecalis;* in contast, *C. sakazakii* showed *the* lowest value with high significance *vs. E. faecalis*. On the other hand, *C. sakazakii* and *E. faecalis E. faecalis* expressed the highest *F* test values in this study after treatment with *A. herba alba* extract *vs. S. enterica* with moderate significant. Moreover, *C. sakazakii vs. E. faecalis* showed the same statistical investigation as *A. absinthium* extract treatments except for moderate significance, pearson's correlation coefficients for MIC values of both plant species against selective microbial strains stated that *C. sakazakii* had a highly positive correlation, *C. albicans* and *E. faecalis* had moderate correlations and *S. enterica* had low correlation. In addition, regression was able to describe the co-variation among MIC variables. The SLR curves indicate the significant relationships among them. There was such an extremely high regression in *C. albicans* and *S. enterica*, while a high regressed in *C. sakazakii* and *E. faecalis* (Fig. 11, Table 12).

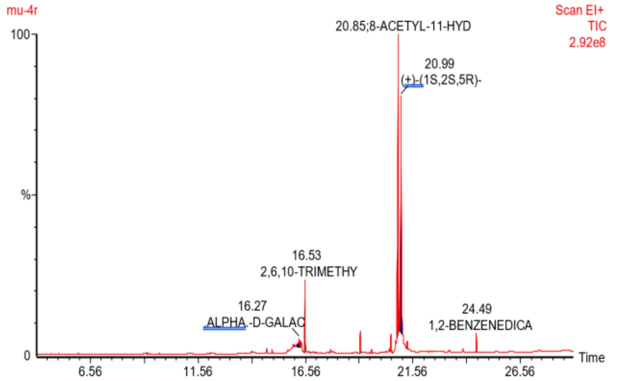

Fig. 7 Chromatogram analysis of *A. absinthium* extract.

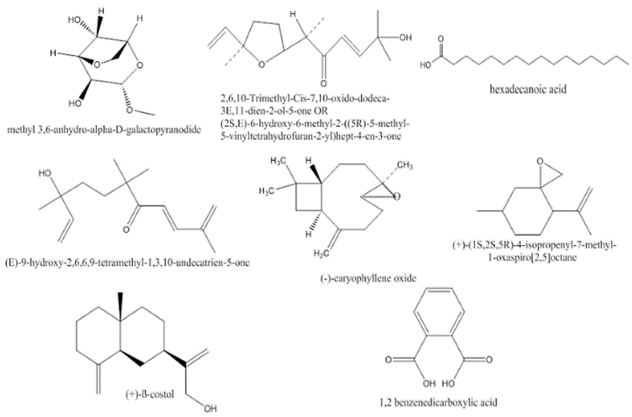

Fig. 8 Chemical structures of *A. absinthium* extract identified by GC-MS designed by ChemDraw

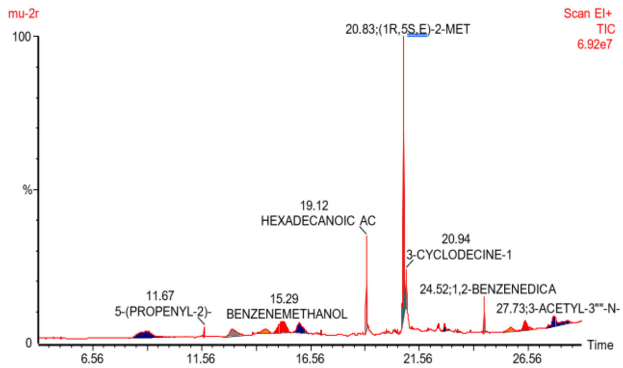

Fig. 9 Chromatogram analysis of *A. herba alba* extract.

Table 4 Antimicrobial activity of *A. absinthium* extracts against selective strains

Microorganism strain	Diameter of Inhibition Zone (IZ) in mm								
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)	Streptomycin (Positive control) (AC)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)			
C. albicans	1.75±0.96	5.25±0.50	4.75±0.50	2.38±2.06	3.25±1.50	5.75±0.96	-10.25±4.92	1.25±1.71	7.0±0.01
C. sakazakii	-	4.0±0.01	-	-	-	-	-9.50±1.73	-	9.0±0.01
E. faecalis	-	3.0±0.01	-	-	1.0±1.16	-	-6.75±1.50	-	10.0±0.01
S. enterica	-	2.50±0.05	1.0±0.01	1.0±0.15	0.5±0.01	2.0±0.03	-	-	12.0±0.01

Table 5 Antimicrobial activity of extracts of *A. herba alba* against selective strains

Microorganism strain	Diameter of Inhibition Zone (IZ) in mm								
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)	Streptomycin (Positive control) (AC)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)			
C. albicans	-	4.25±0.96	0.62±0.48	1.50±0.58	2.0±0.01	2.25±0.96	1.25±0.29	0.50±0.25	5.50±0.01
C. sakazakii	-	4.25±0.50	2.50±0.58	1.0±0.01	-	-	-	-	2.0±0.01
E. faecalis	-	3.0±0.01	-	-	-	-	-	-	12.0±0.01
S. enterica	-	4.0±0.01	2.5±0.01	-	-	-	-	-	10.0±0.05

Table 6 MICs for *A. absinthium* extracts against selective microbial strains.

Microorganism strain	MIC (mg/ml)							
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)		
C. albicans	65.62±2.32	196.88±3.66	178.12±4.02	89.25±2.36	121.88±1.02	215.63±2.99	-	196.88±3.05
C. sakazakii	-	118.67±2.45	-	-	-	-	-	-
E. faecalis	-	112.5±0.99	-	-	37.5±2.78	-	-	-
S. enterica	-	187.5±0.01	75.0±2.66	75.0±0.05	37.5±0.25	150.0±1.66	-	-
F test	Ca vs Cs 3.35	Ca vs Ef 3.67	Ca vs Se 1.15	Cs vs Ef 1.10		Cs vs Se 2.91		Ef vs Se 3.18
P<0.05 *, *P*<0.01**, *P*<0.001***	*	*	*	***		***		*

Table 7 MICs for *A. herba alba* extracts against selective microbial strains.

Microorganism strain	MIC (mg/ml)							
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)		
C. albicans	-	159.38±2.05	23.25±0.05	56.25±5.01	75.0±1.01	84.38±2.66	-	195.23±1.65
C, sakazakii	-	126.09±2.00	74.17±4.01	29.67±0.24	-	-	-	-
E, faecalis	-	112.5±5.02	-	-	-	-	-	-
S, enterica	-	300±2.03	187.5±5.36	-	-	-	-	-
F test	Ca vs Cs 2.29	Ca vs Ef 3.24	Ca vs Se 2.66	Cs vs Ef 1.42		Cs vs Se 6.09		Ef vs Se 8.62
P<0.05 *, *P*<0.01**, *P*<0.001***	*	*	*	**		**		**

Table 8 MBCs for selective microbial strains against *A. absinthium* extracts.

Microorganism strain	MFC/MBC (mg/ml)							
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)		
C. albicans	131.24±2.03	393.76±2.56	356.24±5.01	178.5±0.98	243.76±2.08	431.26±4.02	-	393.76±2.03
C. sakazakii	-	196.20±2.05	-	-	-	-	-	-
E. faecalis	-	225.0±2.36	-	-	75.0±3.68	-	-	-
S. enterica	-	375.0±5.02	150.0±2.69	150.0±4.36	75.0±8.01	300.0±1.09	-	-

Table 9 MBCs for selective microbial strains against *A. herba alba* extracts.

Microorganism strain	MFC/MBC (mg/ml)							
	Organic solvents						Aqueous solvent (g)	DMSO (Negative control) (h)
	Acetone (a)	Butanol (b)	Chloroform (c)	Diethyl ether (d)	Ethanol (e)	Methanol (f)		
C. albicans	-	63.85±2.59	9.31±2.68	22.54±5.06	30.05±2.01	33.81±5.36	-	18.78±2.02
C. sakazakii	-	120.78±5.78	71.04±3.01	28.42±4.98	-	-	-	-
E. faecalis	-	180.29±2.30	-	-	-	-	-	-
S. enterica	-	120.19±8.65	75.12±0.89	-	-	-	-	-

Table 10 Chemical composition of *A. absinthium* extract identified by GC-MS.

Peak	Compound name	RT	Area %	Area	Similarity MS	kovats index	Molecular Formula	Molecular Weight g/mol.
1	methyl 3,6-anhydro-alpha-D-galactopyranoside	16.28	1.720	38313	92	786	$C_7H_{12}O_5$	176.17
2	2,6,10-trimethyl-cis-7,10-oxido-dodeca-3E,11-dien-2-ol-5-one	16.53	15.780	350713	88	711	$C_{15}H_{23}O_3$	251.3423
3	hexadecanoic acid	19.10	5.220	115941	95	1240	$C_{16}H_{32}O_2$	256.4241
4	(E)-9-hydroxy-2,6,6,9-tetramethyl-1,3,10-undecatrien-5-one	20.53	1.590	35249	90	1348	$C_{15}H_{24}O_2$	236.35000
5	(-)-caryophyllene oxide	20.85	42.960	954633	93	1358	$C_{15}H_{24}O$	220.35
6	(+)-(1S,2S,5R)-4-isopropenyl-7-methyl-1-oxaspiro[2,5]octane	20.99	28.060	623405	95	1145	$C_{11}H_{18}O$	166.135765
7	(+)-β-costol	21.09	0.660	14766	98	1498	$C_{15}H_{24}O$	220.3505
8	1,2-benzenedicarboxylic acid	24.49	4.010	89027	95	1516	$C_8H_6O_4$	166.1308

Table 11 Chemical composition of *A. herba alba* extract identified by GC-MS.

Peak	Compound name	RT	Area %	Area	Similarity MS	kovats index	Molecular Formula	Molecular Weight g/mol.
1	5-(propenyl-2)-1,3,7-nonatriene	11.67	1.890	29457	92	1254	$C_{12}H_{19}$	163.28
2	N,N-dimethyl-hydroxylamine	12.98	9.000	140050	95	650	C_2H_7NO	61.08
3	N,N-dimethyl-10-undecen-1-amine	14.53	8.850	137709	88	1354	$C_{13}H_{27}N$	197.36
4	(-)-norephedrine	15.28	24.330	378622	98	1090	$C_9H_{13}NO$	151.21
5	2-(aminooxy)-propanoic acid	16.06	2.310	36027	95	781	$C_3H_7NO_3$	105.09
6	4-hydroxy-cyclohexanone	17.03	0.780	12212	85	799	$C_6H_{10}O_2$	114.14
7	hexadecanoic acid	19.12	8.620	134190	93	1754	$C_{16}H_{32}O_2$	256.4241
8	(-)-caryophyllene oxide	20.83	19.130	297724	98	1652	$C_{15}H_{24}O$	220.35
9	3-cyclodecine-1-ol	20.94	3.670	57082	87	1127	$C_{10}H_{16}O$	152.233
10	undecanal	22.44	4.600	71526	92	1175	$C_{11}H_{22}O$	170.29
11	3,8-nonadien-2-one	22.72	1.720	26800	95	1066	$C_9H_{14}O$	138.21
12	isobutylethene	23.30	0.580	9009	87	847	C_6H_{12}	84.16
13	1,2-benzenedicarboxylic acid	24.52	3.730	58123	90	967	$C_8H_6O_4$	166.1308
14	*cis*-sabinene hydrate	25.70	4.070	63383	91	1144	$C_{10}H_{18}O$	154.25
15	4-(5’,5’-dimethyl-2’-methylidene-3’,8’-dioxabicyclo[5.1.0]oct-4-ylidene)-2-butanone	26.40	3.580	55762	91	1429	$C_{13}H_{18}O_3$	222.28

Table 12 Pearson’s correlation coefficients integrated in the MICs for each studied plant species against selective microbial strains.

	C. albicans	*C. sakazakii*	*E. faecalis*	*S. enterica*
C. albicans	0.710	-	-	-
C. sakazakii	-	0.831	-	-
E. faecalis	-	-	0.744	-
S. enterica	-	-	-	0.655

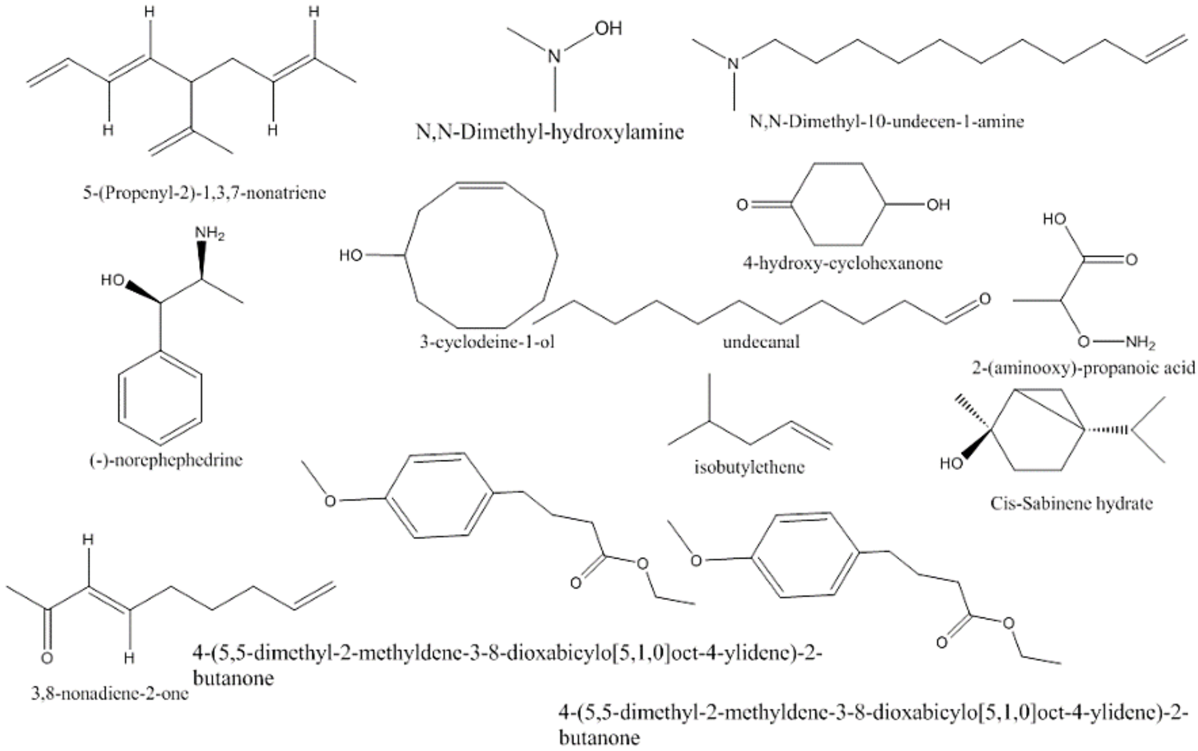

Fig. 10 Chemical structures of *A. absinthium* extract identified by GC-MS designed by ChemDraw

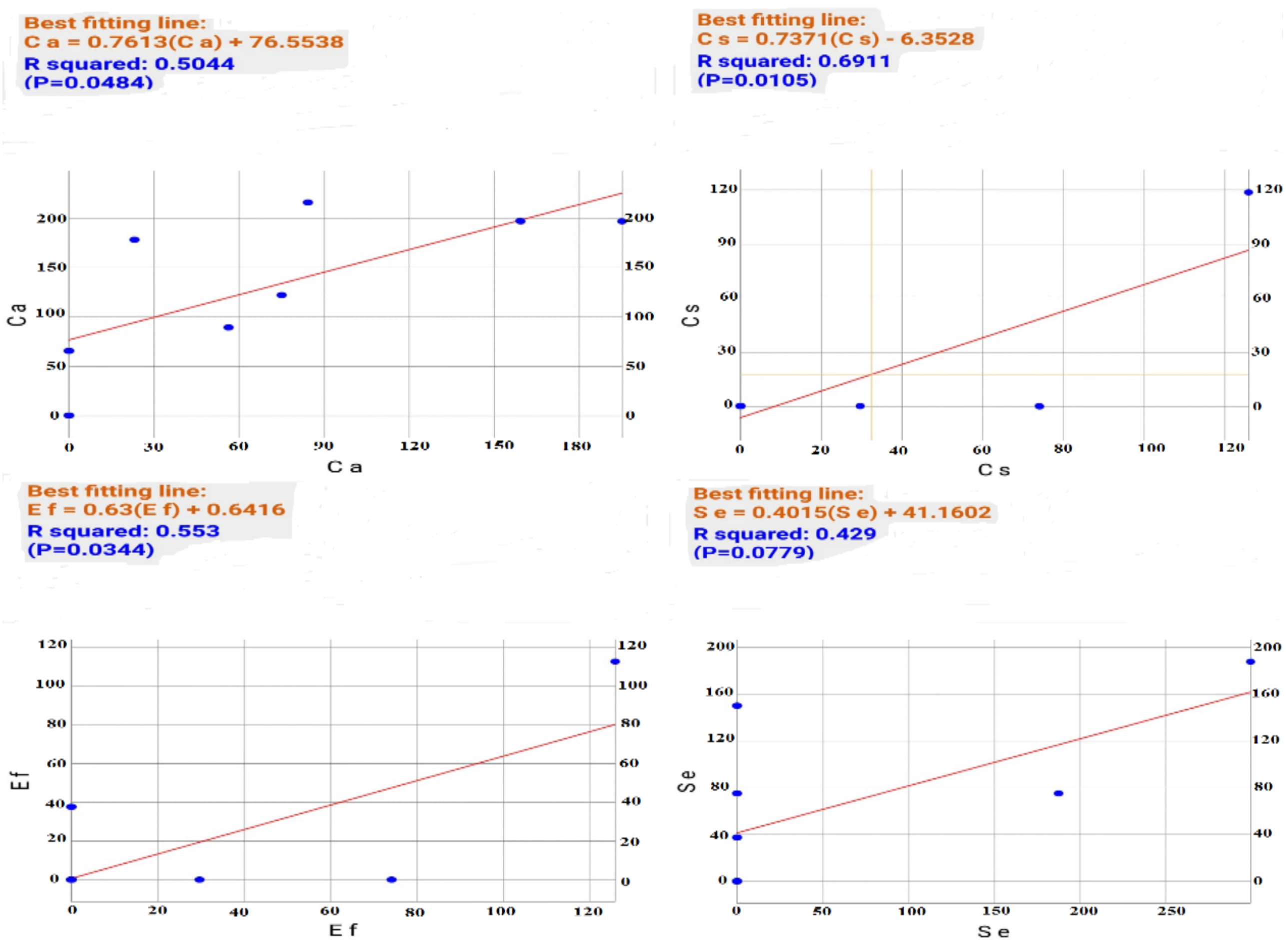

Fig. 11 SLR curves for MICs of each studied plant species extract against the selective microbial strains

Cluster Analysis

223 phytochemical traits of all reviewed *Artemisia* sp. were scored as binary matrix parameter (-) and (+). Unique parameters can be defined as the parameters that specifically identify a specific species from the other species by their presence or absence among them. Parameters that are present in a specific species but not found in the others are called positive unique parameters (PUP). They are 85 distributed within 7 species where *A. absinthium* scores highest percentage (40%) while *A. sieberi* scores the lowest one (1.18%). On the other hand, the negative unique parameters (NUP) are another term that is absent in a specific species but present in others. Only 6 and 3 parameters are registered as NUP and common parameter, respectively. These parameters could be used for species differentiation (Fig.12-14, Table 13). The resulting phenogram revealed that the studied species had an average taxonomic distance of 1.06. At this level, *A. absinthium* is separated as a delimited species. At 1.095, *A. scoparia* was split off as a sub cluster while the remaining species were differentiated at 1.123 into two clusters. The first cluster included five species where *A. herba-alba* presented as a single clad at 1.162 and other species were grouped into a sub cluster which differentiated into two clades; one included *A. monosperma* at 1.266 and another included *A. abyssinica* as a single subclad at 1.344 in addition to *A. judaica* and *A. sieberi* as another subclad. The second cluster included two species; *A. annua* and *A. vulgaris* at 1.202. (Fig. 15). As shown in Table 14, the estimated similarity matrix consisted of 36 numbers in 5 categories; ranged from (0.46 to 0.47), (0.50 to 0.57), (0.60 to 0.69), (0.70 to 0.79) and (0.80 to 0.81).

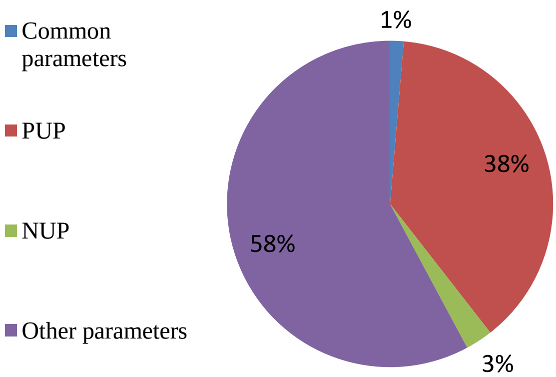

Fig. 12 Binary matrix parameters of reviewed *Artemisia* sp. in the Middle East region.

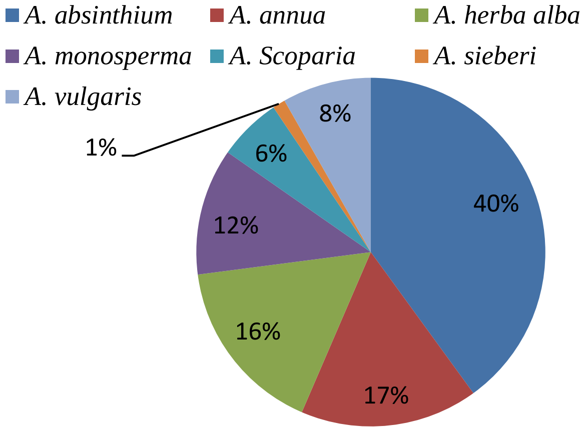

Fig. 13 PUP parameters of reviewed *Artemisia* sp. in the Middle East region.

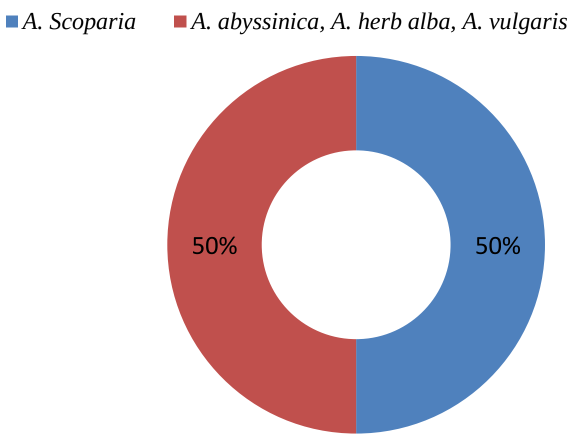

Fig. 14 NUP parameters of reviewed *Artemisia* sp. in the Middle East region.

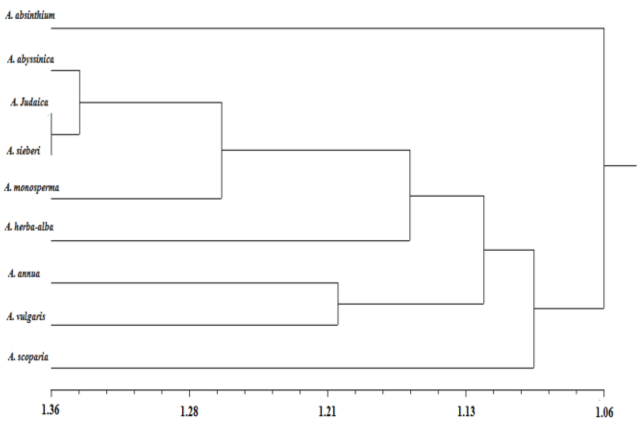

Fig. 15 Phenogram of all reviewed *Artemisia* sp.

The highest similarity value was recorded between *A. abyssinica* and *A. judaica*. On the other hand, the lowest similarity value was recorded between *A. abyssinica* and *A. monosperma*.

Table 13 Binary matrix of reviewed *Artemisia* sp. in the Middle East region.

	A.absinthium	*A. abyssinica*	*A.annua*	*A. herba-alba*	*A.Judaica*	*A.monosperma*	*A.scoparia*	*A.sieberi*	*A.vulgaris*	Ref.
Absinthin	+	-	-	-	-	-	-	-	-	35
Acenaphthene	-	-	-	-	+	-	+	+	-	36, 37, 38
2-Acetoxyundecane	-	-	-	-	-	-	+	-	-	39
β-Acoradiene	-	-	+	+	-	-	-	-	-	40, 41
alkhanin	-	-	-	+	-	-	-	-	-	42
Allo-ocimene	+	-	-	-	-	-	-	-	-	43
Arabsin	+	-	-	-	-	-	-	-	-	35
Apigenin	+	-	-	-	-	-	+	-	-	44. 45, 46
Artanoic acid	-	-	-	-	-	-	-	-	+	47
Artemetin	+	-	+	-	-	-	-	-	+	35, 42
Artemisia triene	+	-	+	-	-	-	-	-	-	40, 48
artemisyl acetate	-	+	+	-	+	-	-	-	+	49. 50, 37
Artemisia ketone	+	-	+	-	+	-	-	-	+	51, 40, 52
Artemisinin	+	-	+	+	-	+	+	-	+	53, 54, 55, 56
Artemitin	+	-	+	-	-	-	-	-	+	57, 40
Artemolin Isoabsinthin	+	-	+	-	-	-	-	-	+	35
Artenolide	+	-	+	-	-	-	-	-	+	58
Artemisitene	+	-	+	+	-	+	-	-	+	55, 59, 60
Artemisinic acid	+	-	+	+	-	-	-	-	+	61, 56
α-bisabolol	+	-	-	-	-	-	-	-	-	35
α-Bisabolol oxide	-	-	-	+	-	-	-	-	-	62
Benzyl isovalerate	-	-	+	-	-	-	-	-	-	40
α-bergamotene	-	-	-	+	-	-	-	-	-	62
Bergamotol	-	-	-	+	-	-	-	-	-	62
Z-Bisabolol	-	-	-	+	-	-	-	-	-	41
bornan-2-one	+	-	+	+	+	+	-	-	+	63, 64, 42, 12
Borneol	+	+	+	+	+	+	-	+	+	65, 66, 67, 43, 12, 40
Bornyl acetate	+	+	+	+	+	+	-	+	+	39, 42, 40, 12, 66
α-bulnesene	-	-	-	+	-	-	-	-	-	62
Butanoic acid	+	+	-	-	+	+	-	-	-	36, 66, 68, 37
Cadinene	+	-	+	-	-	+	-	-	-	12, 69, 40
α-Cadinol	+	-	+	-	+	+	+	-	-	43, 12, 59, 70
caffeic acid	+	-	-	-	-	-	+	-	-	71, 68, 16
Calamenene	-	-	+	-	-	-	-	-	-	40
Calarene	-	-	+	-	-	-	-	-	-	40
Campesterol	+	-	-	-	-	-	-	-	-	57
camphene	+	+	+	+	+	+	-	+	-	72, 12, 66, 64, 40
Camphor	+	+	+	+	+	+	+	+	+	73, 74, 66, 43, 40, 12
Carvone	-	-	+	-	+	+	-	+	+	51, 12, 64, 43, 40, 75
Caryophyllene	+	-	+	-	+	+	+	+	+	65, 76, 12, 59
α-Caryophyllene oxide	+	-	+	-	+	+	+	+	+	43, 59
Chamazulene	+	+	-	-	+	-	-	+	-	77, 66, 64
α-Chamigrene	-	-	-	+	-	-	-	-	-	41
δ-3-Carene	+	-	+	+	+	-	-	-	-	78, 52, 79, 80
Carotol	-	-	+	-	-	-	-	-	-	40
Casticin	+	-	-	-	-	-	-	-	-	46
Catechin	+	-	+	-	-	-	-	-	-	16
α-Cedrene	-	-	+	-	-	-	-	-	-	40
chlorogenic acid	+	-	-	-	-	-	-	-	-	44, 46
Chrysanthenyl acetate	+	-	+	+	+	-	-	-	-	10, 62, 59, 52
Chrysanthenyl propionate	-	-	+	+	-	-	-	-	-	81, 48
Chrysoeriol	+	-	-	-	-	-	-	-	-	82

Cinnamic acid	+	-	-	+	+	-	+	+	-	64, 82, 37, 41
Z-Ethyl cinnamate	+	-	-	-	-	-	-	+	-	39
1,8-cineole**	+	+	+	+	-	-	+	+	+	51. 83. 84, 85
Citronellyl acetate	-	-	+	-	+	+	-	-	-	12, 40, 86
α-Copaene	+	-	+	-	-	-	-	-	+	11, 86, 80
p-coumaric	+	-	+	-	-	-	-	+	+	44, 47, 16, 87
β-Cubebene	+	-	+	-	-	-	-	-	-	40, 48, 80
Cubenol	+	-	+	-	-	-	-	-	-	40, 48, 80
Cumin aldehyde	-	-	+	-	-	-	+	+	-	40, 48, 88
β-curcumin	+	-	-	-	-	+	-	-	-	41, 12
2-cyclohexen-1-one	+	-	-	-	+	-	-	+	-	37, 38, 80
p-cymene	+	+	+	-	+	+	+	+	-	89, 12, 66, 40, 52, 75
davanone	+	+	-	-	+	+	+	+	+	49, 72, 12, 38, 90
2-Decanone	-	-	+	-	-	-	+	-	-	39, 40
1,2-dehydro acenaphthylene	-	-	-	-	+	-	+	+	-	43
Dehydrosabinaketone	-	-	-	+	-	-	-	-	-	40
Dicaffeoylquinic acid isomer	+	-	-	-	-	-	+	-	-	46
22-dien-3b-ol	+	-	+	-	-	-	-	-	-	58, 59
Diepoxyhexadecane butyl ester	-	-	-	+	-	-	-	-	-	62
Dihydroartemisinic acid	-	-	-	+	-	+	-	-	-	61, 55
Dihydroartemisinic alcohol	-	-	-	-	-	+	-	-	-	61
Dihydroartemisinic aldehyde	-	-	-	-	-	+	-	-	-	61
Dihydroartemisinin epimer	-	-	-	-	-	+	-	-	-	91
dihydrochamazulene isomer	+	-	-	-	-	-	-	-	-	92
Dihydroxybenzoic acid hexoside	+	-	-	-	-	-	-	-	-	46
m-Diisopropylbenzene	-	-	+	-	-	-	-	-	-	40
6,7 dimethoxycoumarin	+	-	+	-	-	-	+	-	+	82
1,3-Dimethyladamantane	-	-	+	-	-	-	-	-	-	40
6-Dodecanone	-	-	-	-	-	-	+	-	-	39
γ-Elemene	+	-	+	-	-	+	+	-	+	12, 40, 12, 93, 80
Elixene	-	-	-	+	-	-	-	-	-	81
Epicatechin	+	-	+	-	-	-	-	-	-	16
Trans-epoxyocimene	+	-	-	-	-	-	-	-	-	22
Ergosterol	+	-	-	-	-	-	-	-	-	57
Esculin	+	-	-	-	-	-	-	-	-	46
24X-ethylcholesta-7	+	-	-	-	-	-	-	-	-	58
ethyl cinnamate	+	-	-	+	+	-	+	+	-	94
Ethyl hydrocinnamate	+	-	-	+	+	-	+	+	-	39
Ethyl isovalerate	+	-	-	-	-	+	-	+	-	67, 39
Ethyl linoleate	-	-	+	+	-	-	-	-	+	62
Ethyl 2-methylbutyrate	+	-	-	-	-	-	+	+	-	39
α-Eudesmol	-	-	+	-	+	+	-	-	-	12, 67, 40, 95

β-Eudesmol	-	-	+	-	+	+	-	-	-	12, 67, 40, 95
Eugenol	-	-	+	-	-	-	+	-	-	43, 40
eupatilin	-	-	-	-	-	-	-	-	+	47
Eupatorin	+	-	-	-	-	-	-	-	-	46
Farnesene epoxide	-	-	+	+	-	+	+	-	-	62, 85, 12, 96
α & *β* –fellandrene	+	-	+	-	-	+	-	-	-	97, 12, 85
Fenchol	-	-	-	+	-	+	-	-	-	62, 12
Ferulic Acid	+	-	+	-	-	-	-	-	-	16
Feruloylquinic acid	+	-	-	-	-	-	-	-	-	46
Gallic Acid	-	-	+	-	-	-	-	-	-	16
Germacrene D	+	-	+	+	+	+	+	+	+	92, 76, 12, 85, 37, 38, 90, 99, 40
geranyl acetate	+	-	-	-	+	+	-	+	-	100. 12. 64
geranyl bromide	+	-	-	-	+	+	-	+	-	49, 12, 64
guaiazulene	+	-	-	-	-	-	-	-	-	69
β-Guaiene	-	-	+	-	-	-	-	-	-	40
Guaiol	-	-	+	-	-	-	-	-	-	40
β –gurjunene	+	-	+	-	-	+	-	-	-	65, 67, 40
hanphillin	+	+	-	+	-	-	-	-	-	42, 66, 80
Cis-3-Hexenyl valerate	-	-	+	-	+	+	-	-	+	40, 12, 52, 93
Hotrienol	-	-	+	-	-	-	-	+	-	40, 75
5-Humulene	-	-	+	-	-	+	+	+	+	12, 43, 38, 59
7,4'-hydroxyflavone	+	-	-	-	-	-	-	-	-	57
p-hydroxyphenylacetic	+	-	-	-	-	-	-	-	-	44
5-hydroxy-3,3',4',6,7-pentamethoxyflavone	+	-	-	-	-	-	-	-	-	57
4-hydroxyphenyl acetate	-	-	-	-	-	-	-	-	+	47
isoaromadendrene	-	-	+	-	+	+	-	-	-	67, 64
Isoaromadendrene epoxide	-	-	+	-	+	+	-	-	-	40
Isorhamnetin 3-O	+	-	-	-	-	-	-	-	-	46
iso-valerate	+	-	-	-	-	+	-	+	-	67, 39
Kaempferide	+	-	-	-	-	-	+	-	-	46
Kaempferol	+	-	-	-	-	-	+	-	-	101, 120
Khusimone	-	-	-	+	-	-	-	-	-	41
Limonen-10-yl acetate	+	+	-	+	-	+	-	+	-	39
Limonene	-	+	-	+	-	+	+	+	-	102, 76, 66, 41, 88
Linalool	+	+	+	+	+	+	+	+	+	35. 62, 12, 64, 40
α-longipinene	-	-	+	+	-	-	-	-	-	62
Longiverbenone	-	-	+	+	+	-	-	-	-	40, 70
Luteolin	+	-	+	-	-	-	-	-	+	44, 47, 16
β-maaliene	-	-	-	-	-	+	-	-	-	102
Matricin	+	-	-	-	-	-	-	-	-	35
cis-*p*-Menth-2-en-1-ol	+	+	+	-	-	-	-	+	-	39, 59, 66
trans-*p*-Menth-2-en-1-ol	+	+	+	-	-	-	-	+	-	39, 59, 66
p-Mentha-1,3,8-triene	-	-	+	-	-	+	-	-	-	12, 59
Mesitylene	-	-	-	-	-	+	-	-	-	12

Methyl cinnamate	+	-	-	-	-	-	+	+	-	43
methyl hinokiate	+	-	-	-	-	-	-	-	-	65
cis-Methyl isoeugenol	-	-	+	-	-	+	+	-	-	12
Methyl jasmonate	+	-	+	+	+	-	-	+	-	39, 99, 40, 70
3-methyl-,1.2-15, trans-(Z)-α bisabolene epoxide	-	-	-	+	-	-	-	-	-	62
Miscellaneous	+	-	-	-	-	-	+	+	-	39
Monoterpenes	+	-	-	-	-	-	+	+	-	51, 39
γ-muurolene	-	-	+	+	+	+	-	-	+	83, 12, 40, 70, 93
Myrcene	+	+	+	-	+	+	+	+	+	35, 89, 12, 64, 125, 60, 75
Myricetin	+	-	-	+	-	-	-	-	-	44, 41
Myrtanol acetate	+	-	+	+	-	-	-	+	+	39, 40, 93, 79
naphthalene	-	-	-	-	-	+	-	-	-	67
neoclovenoxidalcohol	-	-	-	-	-	+	-	-	-	67
Nerolidol-epoxyacetate	-	+	+	-	+	+	-	-	-	12, 66, 64
Neryl butyrate	+	-	-	-	-	-	-	-	-	51
Neryl 2-methylbutanoate	+	-	-	-	-	-	-	-	-	51
Neryl 3-methylbutanoate	+	-	-	-	-	-	-	-	-	51
Nerolidol	-	+	+	+	+	+	-	-	-	40, 41
Nonanal	-	-	+	-	-	-	+	-	-	39, 40
2-Nonanol	-	-	+	-	-	-	+	-	-	39, 103
2-Nonanol acetate	-	-	+	-	-	-	+	-	-	39
2-Nonanone	-	-	+	-	-	-	+	+	-	39
Nonyl acetate	-	-	+	-	-	-	+	-	-	39
cis-β-ocimene	-	-	-	+	-	+	+	-	-	76, 12, 79
2-Octanol acetate	-	-	-	-	-	-	+	-	+	39, 60
2-Octanone	-	-	-	-	-	-	+	-	+	39, 60
Oxygenated monoterpenes	+	-	+	+	+	-	+	+	-	39, 41, 59, 70
Oxygenated sesquiterpenes	+	-	+	+	+	-	+	+	-	39, 41, 59, 70, 96
Parishin B	+	-	-	-	-	-	+	-	-	58, 96
Parishin C	+	-	-	-	-	-	-	-	-	58
α-pinene	+	+	+	-	+	+	+	+	-	43, 40, 95, 66, 75
β-pinene	-	+	+	+	+	+	-	+	-	102. 104. 92, 105, 40, 52, 75
α- phellandrene	+	-	-	+	+	+	-	+	-	92, 12, 41, 52, 75
Pinocarveol	-	-	+	+	-	-	+	-	+	43, 99, 40
Piperitone	-	-	-	+	+	+	+	-	+	62, 12, 86, 96
polyacetylene glucosides	-	-	-	-	-	-	+	-	-	82
Propyl 2-methylbutyrate	+	-	-	-	-	-	-	+	-	39
protocatechuic acid	-	-	-	-	-	-	-	-	+	47
Pseudolimonen	+	-	-	-	-	-	-	-	-	97
4-Pyridone glucoside	-	-	-	-	-	-	+	-	-	82
Quercetin	+	-	-	-	-	-	+	-	-	44, 82
Rosmarinic acid	+	-	-	-	-	-	-	-	-	46
Rutin	+	-	+	-	-	-	+	-	-	44, 16

Sabinene	+	+	+	+	+	+	+	+	-	51. 62, 12,64, 59, 88
(E)-sabinyl acetate	+	-	-	+	-	-	-	-	+	51. 62, 60
Trans-sabinyl acetate	+	-	-	-	-	-	-	-	+	51
Trans-Salvene	-	-	-	-	-	-	-	-	+	60
α-santonin	-	-	+	+	+	-	-	-	-	42, 52, 103
Santolinatriene	-	-	+	+	+	-	-	-	-	40, 52, 48
Sativene	-	-	+	-	+	-	-	-	-	40, 70
Saturated fatty acids	+	-	+	+	-	+	+	+	-	39, 40, 106
Scoparone	-	-	+	-	-	-	-	-	-	40
scopoletin	-	-	+	-	-	-	-	-	-	56
β-Selinene	+	-	+	-	-	-	-	+	-	40, 108, 48
Sesquiterpene hydrocarbons	+	-	-	+	-	-	-	+	+	39, 41, 86
Shyobunone	-	-	-	-	-	+	-	-	-	102, 12
Sinapic Acid	-	-	+	-	-	-	-	-	-	16
spathulenol	+	-	+	-	+	+	+	+	+	35, 76, 12, 103, 11
Spinacetin	+	-	-	-	-	-	-	-	-	44
Stigmasterol	+	-	-	-	-	-	-	-	-	57
β- Sitosterol	+	-	-	-	-	-	-	-	-	57
Syringic acid hexoside	+	-	-	-	-	-	-	-	-	46
Terpinen-4-ol	+	+	+	+	+	+	+	+	+	39, 42, 12, 59, 52, 93, 66
α-Terpineol	+	+	+	+	+	+	-	+	+	39, 105, 40, 52, 86, 66
Terpinolene	+	+	+	+	+	+	-	+	-	39, 12, 59, 52, 66
α-Thujenal	-	-	+	+	-	+	-	-	-	40, 12, 79
Thujopsene	-	-	+	-	-	-	-	-	+	40, 93
Thymol	+	-	+	-	-	+	+	-	-	40, 12, 80, 96
Tricyclene	-	-	+	-	-	-	-	-	-	40
tetramethoxy	+	-	-	-	-	-	-	-	-	57
Thymol	-	-	+	-	-	+	-	-	-	12, 103
α-thujone	+	-	+	+	-	+	-	+	-	73, 51. 62. 98, 90
β-thujone	+	-	+	+	-	+	+	+	+	107, 51. 62. 98,48, 86
α-Thujplicin	-	-	-	+	-	-	-	-	-	41
Umbellulone	-	-	-	+	-	-	-	-	-	41
2-Undecanone	-	-	-	-	-	-	+	-	-	39
Vanillic acid	+	-	+	-	-	-	-	-	+	44, 47, 16
Trans-verbenol	+	-	+	+	-	-	-	+	+	51, 62, 40, 86, 75
β-Vinyl naphthalene	-	-	-	-	-	+	-	-	-	12
viridiflorol	-	-	-	-	-	+	-	-	-	67
vulgarin	-	-	-	-	-	-	-	-	+	47
Widdrene	-	-	-	+	-	+	-	-	-	62, 67
α-Ylangene	-	-	-	-	-	-	-	-	+	93
Yogomi alcohol	-	+	+	-	-	-	-	-	-	49. 50, 48
Zingiberene	-	-	-	-	-	-	-	+	-	75

Table 14 Similarity matrix of reviewed *Artemisia* sp. in the Middle East region.

	A. absinthium	*A. abyssinica*	*A. annua*	*A. herba-alba*	*A. Judaica*	*A. monosperma*	*A. scoparia*	*A. sieberi*	*A. vulgaris*
A. absinthium	1.00	-	-	-	-	-	-	-	-
A. abyssinica	0.51	1.00	-	-	-	-	-	-	-
A. annua	0.47	0.57	1.00	-	-	-	-	-	-
A. herba-alba	0.45	0.72	0.55	1.00	-	-	-	-	-
A. Judaica	0.51	0.81	0.63	0.70	1.00	-	-	-	-
A. monosperma	0.46	0.74	0.60	0.64	0.76	1.00	-	-	-
A. scoparia	0.50	0.70	0.54	0.60	0.68	0.63	1.00	-	-
A. sieberi	0.61	0.80	0.56	0.68	0.79	0.69	0.74	1.00	
A. vulgaris	0.52	0.74	0.61	0.65	0.68	0.65	0.66	0.69	1.00

DISCUSSION

The present study gives an obvious picture on the status of the most common *Artemisia* sp. distributed in the Middle East region. This region shows more or less the same climatic zone, topographic areas, human interference, and biodiversity. It is one of the few regions which has stationary climatic predictions, precipitations and temperature [109]. The study of evolutionary trends, origin of ancestors and reasons of bio-adaptation should be done on a large scale to obtain the perfect image on the real life and to improve the reality for the better conditions. The main goal of using wild plant species in the bio-experiments is to evaluate the medicinal properties which can solve our present or future problems. The choice of such a solvent is regarded as the best way to extract any bio-resource. Furthermore, the use of several numbers of different solvents in plant extract is the best guide for this novel analysis. The butanol solvent was the most suitable extract in the present study due to the moderate polarity index (4) with low solubility in water (0.43%) compared to other organic solvents. Chloroform ranked the second best effective organic solvent due to its lower viscosity (0.57 cP). The third convenient extract was ethanol. It had the highest solubility in water (100%) [110].

For all MICs values, *C. albicans* was the most susceptible microorganism because it possessed different cell wall composition than other bacterial strains. However, *S. enterica* was the most susceptible bacteria after treatment with *A. absinthium* than *A. herba alba*. It reflects the different active ingredients presented in *A. absinthium* in addition to the importance of using different organic solvents. The gradual susceptibility for other bacterial strains reported the idea of a natural combination of different plant resources for better results.

For MFC/MBC values, *C. albicans was* needed as a general high inoculum dose to propagate; moreover, *S. enterica* was needed when it was extracted with butanol and methanol; however, *E. faecalis* and *S. enterica* needed the lowest dose using ethanol that can denote to be more virulent than before previous in treatments with *A. absinthium.*

GC-MS analysis enhances the proper manner of discovering the active ingredients that promote or inhibit this biological case. In this case, (-)-caryophyllene oxide was the principal component of *A. absinthium* as a whole plant extract with highest percentage; otherwise, (-)-norephedrine was the same in *A. herba alba as* the preceding besides (-)-caryophyllene oxide had the highest percentage as well as confirmed that the idea of a natural combination may produce different results than using them separately.

The *P*-values reported that *Artemisia* sp. was more effective in this investigation. They are encouraged to be used as natural alternatives to many pathogenic microbial infections due to their important natural contents. Furthermore, Pearson's correlation coefficients emphasized that both *Artemisia* sp. could be used as the best inhibitory agent against *C. sakazakii* Moreover, (SLR) equations indicated the data analysis in the form of exponential curves showing that *Artemisia* sp. were more compatible with each other to one extent and between microbial strains to another extent.

Cluster analysis comprises the total visions about the experimental studies and confirms the interrelationships among studied species to evaluate the taxonomical position and assess the species role. It relies on some parameters such as PUP and NUP to crystallize the importance of the species. PUP gives priority to *A. absinthium* as the most common plant species distributed in the Middle East, on the contrary, *A. sieberi* with low PUP values neither distributed in North Africa nor in the Southern Arabian Peninsula. On the other hand, NUP gives a unique and odd place for *A. scoparia* to be a transition species among *Artemisia* sp.

The binary matrix revealed various classes of chemical compounds; sesquiterpene, polycyclic aromatic hydrocarbon, wax monoesters, terpenoids, bioflavonoid, plant polyphenol, oxanes, sesquiterpene alcohol, phenolic compound, aromatic oil, ketones, unsaturated bicyclic monoterpenes, flavonoid glycosides, sesquiterpene lactones, etc. Most of the extracted compounds have medicinal values like Chrysanthone, α-pinene. Therefore, it stated that the medicinal and vital role of *Artemisia* in biological approaches should be taken into consideration all over the world [111]. The variability of the chemical composition of the same plant distributed in different geographical locations was due to several factors such as chemotypes and geographical origin of plants besides other abiotic climatic factors [112]. Recent medicinal discoveries illustrated that the molecular coupling of some compounds of *Artemisia* sp. presented in the Middle East region had different affinities with the receptor binding domain (RBD) of the encoded SARS-CoV-2 protein S encoded that would open new perspectives in dealing with COVID variants; alpha, beta, gamma delta such as Chrysanthenyl acetate and Chrysanthenyl propionate [79, 113, 114].

The output phenogram illustrated the taxonomic positions of the reviewed species studied. *A. abyssinica*, *A. judaica*, and *A. sieberi* were presented in a group. Similarly, *A. annua* and *A. vulgaris* were in another. The two groups were intersected with two species; *A. monosperma* and *A. herba-alba*. *A. scoparia* was regarded above as a transition species between all other Artemisia sp. and *A. absinthum*, which was far from them because it was the most cosmopolitan and sustainable in different adapted habitats. This study concentrated on two specific species; *A. absinthium* and *A. herba-alba*.

Despite phenogram analysis, the similarity matrix recorded that *A. abyssinica* and *A. monosperma* were closely related to each other while *A. absinthium* and *A. monosperma* were distantly related species.

Referring to this investigation, in addition all aforementioned studies, we can confirm that modern therapeutic protocols should be applied in dealing with infectious pathogenic diseases. It was obvious to use *Artemisia* extract as a natural alternative example instead of other synthetic pharmaceutical supplies.

CONCLUSIONS

1-For a new pure and safe life, natural alternatives must be taken into account.

2- *Artemisia* sp. possesses the best effective antimicrobial activities

3- Butanol solvent records the most favorable plant extract.

4. Different geographical areas influence on the active chemical composition of any wild plant.

5. *Artemisia* sp. includes active ingredients to solve our problems in healing new discovered diseases.

Data Availability

All data sets were documented, used, and analyzed in the present study. They participated in this manuscript.

Conflicts of Interest

The authors confirmed that the following interest relationships and financial personality were potential competing interests without conflicts.

Authors' Contributions

Abdullah Mashraqi, Mohamed A. Al Abboud, Khatib Sayeed Ismail, Yosra Modafer, Mukul Sharma, and A. El-Shabasy contributed in this work equally.

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