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Evaluation of antimicrobial effect of *Juniperus procera* and *Coleus forskohlii* extract

Mona S. Ashoor *and Amal A. Abdulbaqi

Biology Department, Darb University College, Jazan University, Saudi Arabia

Abstract

Because of the existence of different active components and their limited side effects, several plant extracts and phytoconstituents are known as an alternative to chemically synthesised chemicals used as anticancer and antimicrobials. The study's goal is to evaluate the antibacterial activity and zone of inhibition of extracts on some bacterial and fungal strains. The antibacterial efficacy of hydroalcohol extracts of *Juniperus excelsa* and *Coleus forskohlii* leaves was investigated in this study for possible antimicrobial activity against pathogenic bacterial and fungus strains. The extracts' antibacterial activity was tested using the agar disc diffusion method. *Juniperus excelsa* and *Coleus forskohlii* leaf extracts were evaluated against two Gram-positive strains—*Staphylococcus aureus* and *Streptococcus pyogenes*—as well as two fungal strains—*Aspergillus fumigates* and *Penicillium notatum.* These plant extracts were subjected to phytochemical analysis. The antibacterial and antifungal activity of extracts of *Juniperus excelsa* and *Coleus forskohlii* leaves (5, 25, 50, 100, and 250 g/ml) were demonstrated by a high inhibition zone against pathogenic microorganisms. The zones of inhibition of extracts were compared to those of other standards for antibacterial activity, such as ampicillin, ciprofloxacin, norfloxacin, and chloramphenicol, and nystatin and griseofulvin for antifungal activity. The results demonstrated significant reduction of bacterial and fungal growth. The presence of secondary metabolites in *Juniperus excelsa* and *Coleus forskohlii* is responsible for their antibacterial activity. As a result, these plants can be used to identify bioactive natural compounds that can aid in the creation of novel pharmaceutical research activities.

Keywords: *Juniperus excelsa, Coleus forskohlii* , Antibacterial activity, Antifungal activity, Secondary metabolites.

Email of corresponding author: - drmonasaad213@gmail.com

1. Introduction

According to the countries Health Organisation (WHO), traditional medicines derived from medicinal plants continue to help 80% of the poor countries. In compared to the 28,187 medicinal species used by humans, the total estimated number of plants is roughly 374,000. WHO has also documented the names of over 20,000 medical plant species and identified medicinal plants as a potential source of new medications. Medicinal plant rules have been developed in over 100 nations. Over 1340 plants have been identified as having antimicrobial action, and over 30,000 antimicrobial molecules have been extracted from plants. Furthermore, 14-28% of higher plant species are medicinal, and 74% of bioactive plant-derived chemicals were discovered based on ethnomedicinal applications [1]. Plant-derived natural products have been employed for their medicinal capabilities and as a key source of pharmaceuticals since early human civilization. Medicinal plants have antioxidant qualities that contribute in the creation of new drugs. The growing interest in plant secondary metabolites has revealed their important biological activities as well as the significance of their structural arrangements and variation in their medicinal qualities [16].Human health and disease treatment advancements are increasing on a daily basis. Synthetic materials are also hazardous to human health and the environment when used in industry, medicine, and agriculture. As a result, many new diseases are arising and becoming a worry. Changing synthetic materials that cause such disorders to natural herbal products has yet to be accomplished adequately to treat such illnesses. The potential for herbal medicinal benefits due to secondary metabolites is immense, with many such plants still needing to be more active [7].According to various literatures, *Juniperus* is related with about 65 species that are found all over the world. J. procera extracts were analysed to establish the presence of several components that may represent its pharmacological characteristics. J. *communis*, for example, is traditionally used to treat urinary infections, while J. *oxycedrus* is used to treat dermatological diseases. Plants that produce non-phenolic essential oils, such as several *Juniperus* species, are also employed as antiseptics in folk medicine [19].

The genus *Juniperus* (*Cupressaceae*) has around 60 species native to the northern hemisphere, especially North America, Europe, and Asia. *Juniperus excelsa*, a member of the Cupressaceae family and locally known as "boyluardç," is one of these species. Because of its usage in folk medicine

to treat abdominal spasms, asthma, diarrhoea, fever, headache, gonorrhoea, leucorrhoea, and is regarded effective as an antihypertensive, appetiser, diuretic, carminative, stimulant and anticonvulsant, J. *Excelsa* essential oils also has antibacterial, antimicrobial, antioxidant and antifungal properties. It grows in Turkey at altitudes ranging from 300 m at the lowest to 2300 m at the highest [21]. This *juniper* species which is resistant to harsh growth conditions, is known to have a variety of biochemical qualities in its numerous organs, including tannin, resin, and essential oil, phenolics and antioxidants. The high presence of -pinene, cedrol, and sabinene in *J. excelsa* essential oils determines their significant key components carene, -pinene, -cedrol, and -pinene. These essential oils have strong antimicrobial activity [2].*Coleus forskohlii* (Family: Lamiaceae) has a long history of use in many traditional herbal treatments, particularly in the healing arts of Ayurveda. It has antianaphylactic, antiobesity, amebic, gastroprotective, bronchodilating, antiaging, antioxidant, anti-inflammatory, and anti-cancer properties. It is only used for weight management and hypotension. [18].*Coleus forskohlii* is a Lamiaceae (previously Labiatae) family plant native to India. It is the most important Coleus species, known in Karnataka as Mainamool, Manganiberu, or Makandi Beru, and in Maharashtra as Garmar. It can be found up to 2500m above mean sea level in the subtropical Himalayas, from Gharwal to Nepal, including Pakistan and Sri Lanka. Ayurvedic medical institutions have historically used the roots for therapeutic purposes. In skin care, *Coleus* volatile oil contains anti-inflammatory, antiphlogistic, antiseptic, astringent, cicatrisant, cytophylactic, diuretic, and tonic properties. The fresh leaves have medicinal characteristics and are used in a decoction with other drugs to treat a variety of diseases [10].*Coleus forskohlii* (CF), also called simply as *Coleus*, is a medicinal herb with numerous ethnopharmacological applications. In Ayurvedic medicine, CF is used to treat a number of problems such as inflammatory diseases, hypertension, respiratory disorders, ageing, and weight management. Secondary metabolites such as terpenoids, flavonoids, and alkaloids are abundant in CF. Forskolin, a labdane diterpene with weight-loss properties, is the most important bioactive ingredient in *Coleus* root [3]. This plant has a high antioxidant activity value when compared to plants growing in other places, thus it can be employed in the biological, pharmaceutical, and food industries. Furthermore, phytochemicals (phenols, flavonoids, tannins, alkaloids, proteins, carbohydrates, steroids, saponins, and glycosides) have been identified in the leaves' ethanol extract [4] and [15].

2. Material and methods

2.1 Isolation and preparation of plant extracts

Fresh and healthy *Juniperus excelsa* and *Coleus forskohlii* leaves were obtained from the Jazan region. *Juniperus excelsa* and *Coleus forskohlii* leaves were shade-dried and ground in a mechanical grinder. The plant material powder (25.0 g) was first defatted with petroleum ether (60-80 °C), then with 900 ml of hydroalcohol in a Soxhlet extractor for 72 hours at a temperature not surpassing the boiling point of the solvent. The extracts were filtered while hot with Whatman filter paper (No. 1), concentrated in vacuum under reduced pressure with a rotary flask evaporator, and dried in a desiccator. The hydroalcoholic extract provides a 5.750 g dark greenish-solid residue (23.0% w/w). This extraction procedure produced greater extract yields. The extracts were then placed in sterile bottles until they were used. The total dry weight of the plant extracts was obtained using solvent evaporation, which was then used to compute the concentration in mg/mL. The extract was stored at a temperature of 2-4 °C. This crude hydroalcohol extract was used to conduct more investigation into its claimed antimicrobial qualities [5].

2. 2 Test Microorganisms and growth media

Staphylococcus aureus, Streptococcus pyogenes, and fungal strains (*Aspergillus fumigatus and Apergillus niger*) were studied for their clinical and pharmacological significance. The bacterial and fungal stock cultures were grown for 24 hours at 37°C on nutritional agar and potato dextrose agar (PDA) medium after being refrigerated at 4°C. The bacteria were grown on Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were cultured in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), while the moulds were grown in Sabouraud dextrose agar and PDA medium, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.3 Antimicrobial activity test

2.3.1 Determination of antimicrobial activity of the extracts

Hydroalcohol extracts were tested for antibacterial and antifungal activity in vitro. The agar disc diffusion method was used to test the antibacterial and antifungal properties of plant component extracts against two Gramme-positive pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes*) and two pathogenic fungi (*Aspergillus fumigatus* and *Apergillus niger*) [3]. The agar cup method was used to test antimicrobial activity. To determine the zone of inhibition, pure gram-positive and fungal strains were used as a standard antibiotic for comparison. Antibacterial and antifungal activity against *Aspergillus fumigatus* and *Apergillus niger* were assessed in all extracts. Using nutrient agar tubes, five dilutions (5, 25, 50, 100, and 250 g/ml) of *Juniperus excelsa*, *Coleus forskohlii*, and standard medicines were produced in double-distilled water. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and incubated for 3 hours at 37°C. Control experiments with common antibiotics ampicillin and chloramphenicol for antibacterial action and nystatin and griseofulvin for antifungal activity were undertaken under equivalent conditions. The zones of growth inhibition around the discs were measured after 18 to 24 hours of incubation at 37°C for bacteria and 24 to 48 hours at 28°C for fungus. The sensitivities of the microbe species to plant extracts were determined by measuring the widths of inhibitory zones on the agar surface around the discs, with values less than 8 mm deemed inactive against bacteria [6].

2.3.2 Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of *Juniperus excelsa* and *Coleus forskohlii* extracts were performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 C and then increased by 5°C /min to 230°C hold for 2 min. increased to the final

temperature 290°C by 30°C /min and hold for 2 min. The temperatures of the injector and MS transfer lines were fixed at 250 and 260°C, respectively; helium was utilised as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 minutes, and diluted samples of 1 l were automatically injected using an Autosampler AS1300 connected with a GC in split mode. In full scan mode, EI mass spectra were acquired at 70 eV ionisation voltages spanning the m/z 40-1000 range. The temperature of the ion source was fixed to 200 °C. The components were identified by comparing their retention durations and mass spectra to the mass spectral databases WILEY 09 and NIST 11 [18].

3. Results and Discussion

3.1 Antimicrobial activity test

The antimicrobial activity of the extracts of *Juniperus excelsa*, *Coleus forskohlii* were studied in different concentrations (5, 25, 50, 100, and 250 µg/ml) against two gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) pathogenic bacterial strains and two fungal strains (*Aspergillus fumigatus and Apergillus niger*). The antibacterial and antifungal properties of extracts was determined using the zone of inhibition of bacterial growth. The antibacterial and antifungal activity data are shown in Table (1) and Figure (1, 2). Tables (2 and 3) show the antibacterial and antifungal activity of chemical medicines. *Juniperus excelsa* extract outperformed *Coleus forskohlii* in antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*. Although *Juniperus excelsa* extract had excellent antifungal activity against *Aspergillus fumigatus*, *Coleus forskohlii* shown more antifungal activity against *Apergillus niger* than *Juniperus excelsa* extract. The extracts' antibacterial and antifungal capacities increased linearly with increasing extract concentration (g/ml). When compared to standard treatments, S. *aureus* was more responsive to extracts for bacterial activity than S. *pyogenes* employing *Coleus forskohlii* extract and *Juniperus excelsa*. *Aspergillus niger* surpasses *Aspergillus fumigatus* in terms of fungal activity. *Juniperus excelsa* extract's growth inhibition zone ranged from 3 to 15 mm for all sensitive bacteria and from 7 to 22 mm for fungal strains. All sensitive bacteria had growth inhibition zones ranging from 0 to 8 mm, while fungal strains had zones ranging from 9 to 23 mm. *Juniperus excelsa* extract is superior. The extracts from *Juniperus excelsa* and *Coleus forskohlii* demonstrated high action against gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and pathogenic fungus strains (*Aspergillus fumigatus* and *Apergillus niger*) in this study. *Juniperus excelsa* showed inhibition by 15mm, 12mm, 22mm and 16mm against *Staphylococcus aureus*, Streptococcus *pyogenes*, Aspergillus *fumigatus* and *Apergillus niger*, respectively. *Coleus forskohlii* showed inhibition by 8mm, 16mm and 23mm against *Streptococcus pyogenes*, *Aspergillus fumigatus* and *Apergillus niger*. Our findings are consistent with those of [7], who explained that the antimicrobial activity of methanolic and ethanolic extracts of *Juniperus sabina* that grows on the island of Cyprus has strong antimicrobial activity against two strains of gram-negative bacteria: *Escherichia coli* O157:H7 (932), *Salmonella typhimurium* (B-4420), gram-positive bacteria *Bacillus cereus* (ATCC 7064) and *Staphylococcus aureus* (6538 P) by inhibition 12mm, 17mm, 22mm and 15mm, respectively [14]. [17] Demonstrated that The methanolic extract of *Juniperus sabina* demonstrated antibacterial activity against numerous bacterial strains, including *E. coli* with an inhibition zone of 8.5 mm and S. *aureus* with an inhibition zone of 16.5 mm. Multiple-antibiotic-resistant *Staphylococci* and select strains of *Stenotrophomonas maltophilia* had the best results for reducing bacterial growth. Antibacterial activity of ethanolic and methanolic extracts against *E. coli* and S. *aureus* was discovered. *Juniperus excelsa* has been shown to have excellent antibacterial activity against *Staphylococcus aureus*, *Streptococus pyogenes*, *Candida albicans*, *Streptococus ppneumoniae*, *Mycobacterium smegnatis*, and *Candida crusei* by [17].Unlike [8], who reported no action against B. *subtilis*, *Candida albicans*, E. *coli*, P. *aeruginosa*, or S. *aureus*, the lack of activity might be due to essential oil decomposition during the antimicrobial assay, temperature, climate, and geographical location. Various doses of methanolic J. *procera* extract (30, 60, and 90 mg/ml) inhibited the growth of A. *flavus*, with inhibition rates of 50.86, 51.60, and 52.58%, respectively. *Juniperus* essential oils, particularly J. *communis* sp, J. *oxycedrus* ssp. *oxycedrus*, and J. *turbinata*, have been shown to have antifungal action against *Aspergillus* and dermatophytes [20]. *C. blumei* leaf extract shown antibacterial activity against both bacteria, *S. aureus* (14.56 mm) and S. mitis (13 mm), at a dose of 100 mg/ml, with a percentage of relative inhibition zone diameter (% RIZD) of 35% (*S. aureus*) and 65% (*S. mitis)*. With a 15.67 mm inhibitory zone and a% RIZD of 38% for S. *aureus* and a% RIZD of 75% for S. *mitis*, the extract was demonstrated to have similar effects as Oradex oral rinse (0.12% chlorhexidine). The MIC data demonstrate that the extract was most effective against S. *aureus*, with a MIC of 1.5625 mg/ml and MBCs of 100 mg, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml [24].

3.2 Gas chromatography-Mass spectrometery (GC-MS)

3.2.1 GC-MS analysis of Juniperus excelsa leaf extract.

GC-MS analysis of *Juniperus excelsa* leaf extract at figure (3) and table (4) show that the major compounds in *Juniperus excelsa* leaf extract are Epicubebol and Germacrene D-4-ol with molecular weight 222, Calamenen and Cadina-1,3,5-triene with molecular weight 202, and 5-cyclodecen-1-ol, 4,10 bis (methylene)-7-1(methylethyl) and Isospathulenol with molecular weight 220. The hydroalcohol extracts of *Juniperus excelsa* have significant antibacterial and antifungal action, according to the findings.

Table 1: Antibacterial activities of hydroalcoholic extracts of *Juniperus excelsa* and *Coleus forskohlii* leaves against pathogenic bacteria and fungi.

plant extracts / Microorganisms	*Juniperus excelsa* Hydroalcohol extract (ug/ml)					*Coleus forskohlii* Hydroalcohol extract (ug/ml)				
	5	25	50	100	250	5	25	50	100	250
Staphylococcus aureus	10	11	11	13	15	2	5	5	6	0
Streptococcus pyogenes	0	3	9	10	12	0	0	3	5	8
Aspergillus fumigatus	11	13	15	18	22	9	12	13	15	16
Apergillus niger	7	7	9	13	16	11	13	14	17	23

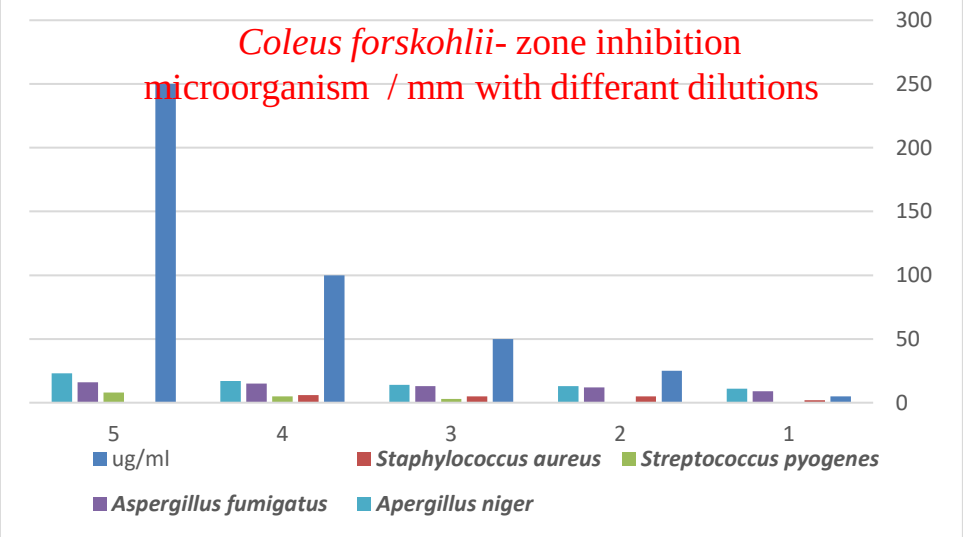


Figure 1: Antimicrobial activity of *Coleus forskohlii* against pathogenic microorganisms.

Table 2: Antifungal activity for chemical drugs (zone of inhibation).

Drugs	**Concentration (ug/ml)**	***Aspergillus fumigatus***	***Aspergillus niger***
Nystatin	**5**	**9**	**18**
	25	**11**	**19**
	50	**14**	**24**
	100	**20**	**28**
	250	**21**	**29**
Griseofulvin	**5**	**19**	**19**
	25	**23**	**24**
	50	**24**	**25**
	100	**25**	**26**
	250	**29**	**28**

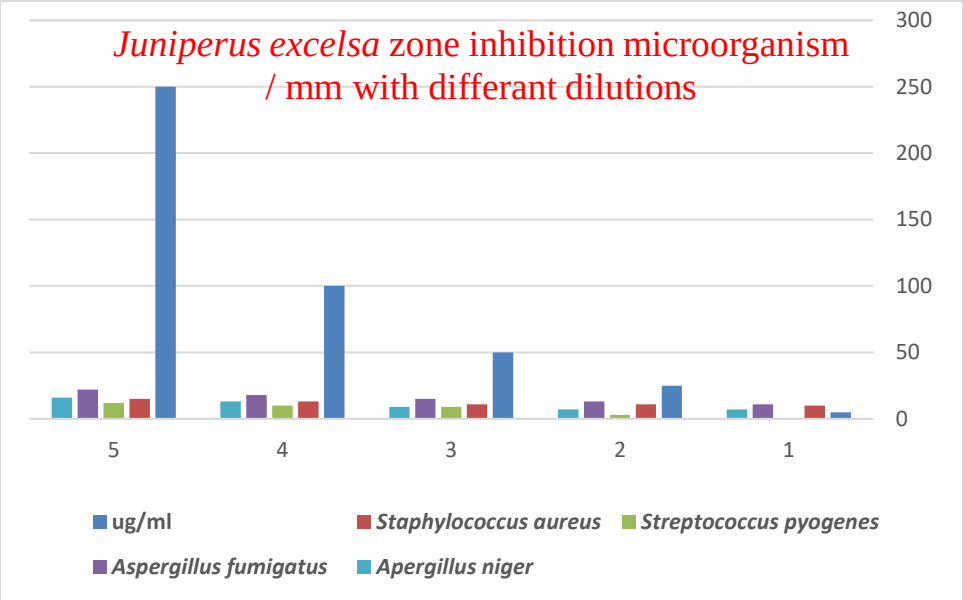


Figure 2: Antimicrobial activity of *Juniperus excelsa* against pathogenic microorganisms.

Table 3: Antibacterial activity for chemical drugs (zone of inhibation).

Drug	Concentration (ug/ml)	*Staphylococcus aureus*	*Streptococcus pyogenes*
Ciprafloxacin	5	17	16
	25	19	19
	50	21	20
	100	22	22
	250	22	23
Chloramphenicol	5	11	10
	25	11	13
	50	13	19
	100	19	20
	250	20	20
Ampicillin	5	9	10
	25	11	13
	50	13	15
	100	16	17
	250	18	19

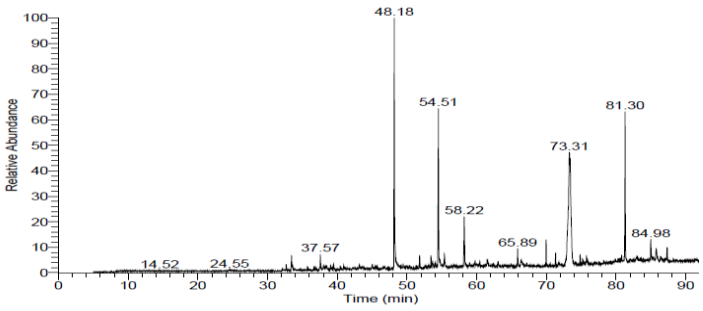


Figure 3: GC-MS analysis of *Juniperus excelsa* leaf extract.

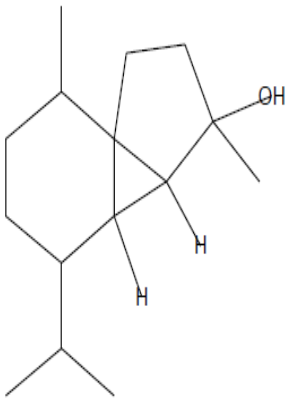
Table 4: GC-MS analysis of *Juniperus excelsa* leaf extract with its retention time.

33.59	8408.000000	24.12	4-ISOPROPYL-1,6-DIMETHYL-1,2,3,4-TETRAHYDRONAPHTHALENE	681	866	483-77-2	178275 59.65	0.57	WileyRegistry8e	
33.59	8408.000000	24.12	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	681	865	483-77-2	178275 59.65	0.57	mainlib	
33.59	8408.000000	20.37	trans-Calamenene	677	826	73209-42-4	178275 59.65	0.57	replib	
33.59	8408.000000	24.12	NAPHTHALENE, 1,2,3,4-TETRAHYDRO-1,6-DIMETHYL-4-(1-METHYLETHYL)-, (1S-CIS)-	676	859	483-77-2	178275 59.65	0.57	WileyRegistry8e	
33.59	8408.000000	19.58	cis-Calamenene	676	853	72937-55-4	178275 59.65	0.57	mainlib	
33.44	8363.000000	41.56	1,3-BENZENEDICARBOXYLIC ACID, 2-(METHOXYMETHYL)-, DIMETHYL ESTER	744	834	131980-49-9	441247 74.86	1.41	WileyRegistry8e	
33.44	8363.000000	12.45	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	718	890	23445-02-5	441247 74.86	1.41	mainlib	
33.44	8363.000000	9.53	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	711	891	38230-60-3	441247 74.86	1.41	mainlib	
33.44	8363.000000	6.54	1-(2,4-DIMETHYLPHENYL)-2-PHENYLETHNE	700	767	78594-13-5	441247 74.86	1.41	WileyRegistry8e	
33.44	8363.000000	12.45	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	698	841	23445-02-5	441247 74.86	1.41	replib	

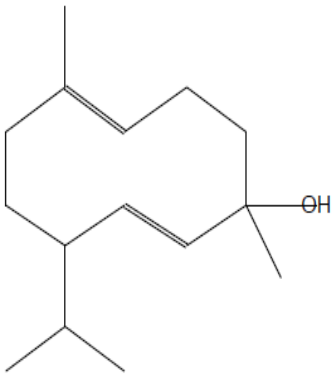
36.66	9309.000000	14.26	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	679	866	19888-34-7	8775462.93	0.28	replib
36.66	9309.000000	14.26	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	670	793	19888-34-7	8775462.93	0.28	mainlib
36.66	9309.000000	6.49	2,5,9-TRIMETHYL-4,8-CYCLOUNDECADIEN-1-ONE	659	735	NA	8775462.93	0.28	WileyRegistry8e
36.66	9309.000000	6.24	2,5,9-Trimethylcycloundeca-4,8-dienone	658	766	NA	8775462.93	0.28	mainlib
36.66	9309.000000	6.24	2,5,9-TRIMETHYL-4,8-CYCLOUNDECADIEN-1-ONE	658	766	NA	8775462.93	0.28	WileyRegistry8e
38.02	9709.000000	6.13	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	686	812	23445-02-5	112748 42.39	0.36	mainlib
38.02	9709.000000	6.13	1,1,4,7-TETRAMETHYLDECAHYDRO-1H-CYCLOPROPA[E]AZULEN-4-OL #	686	772	552-02-3	112748 42.39	0.36	WileyRegistry8e
38.02	9709.000000	5.89	1-NAPHTHALENOL, 1,2,3,4,4A,7,8,8A-OCTAHYDRO-1,6-DIMETHYL-4-(1-METHYLETHYL)-, [1R-(1à,4á,4Aá,8Aá)]-	685	768	19435-97-3	112748 42.39	0.36	WileyRegistry8e
38.02	9709.000000	5.66	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol	684	772	88728-58-9	112748 42.39	0.36	replib
38.02	9709.000000	5.66	1,1,4,7-TETRAMETHYLDECAHYDRO-1H-CYCLOPROPA[E]AZULEN-4-OL	684	772	NA	112748 42.39	0.36	WileyRegistry8e

37.57	9578.000000	34.29	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4S,4aS,8aR)-	859	914	73365-7-2	404171 40.49	1.29	mainlib
37.57	9578.000000	24.21	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S,4R,4aS,8aR)-	849	906	19912-6-7-5	404171 40.49	1.29	mainlib
37.57	9578.000000	11.75	Cubenol	830	900	21284-2-2-0	404171 40.49	1.29	mainlib
37.57	9578.000000	5.00	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	809	849	23445-0-2-5	404171 40.49	1.29	replib
37.57	9578.000000	3.43	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	798	835	38230-6-0-3	404171 40.49	1.29	replib
36.87	9371.000000	26.84	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	627	773	81968-6-2-9	9199517 .27	0.29	mainlib
36.87	9371.000000	7.31	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan-4-ol	596	778	124753-7-6-0	9199517 .27	0.29	mainlib
36.87	9371.000000	6.46	Isospathulenol	593	704	88395-4-6-4	9199517 .27	0.29	mainlib
36.87	9371.000000	6.21	1-NAPHTHALENOL, 1,2,4A,5,6,7,8,8A-OCTAHYDRO-3-METHYL-8-METHYLENE-5-(1-METHYLETHYL)-	592	747	24268-3-4-6	9199517 .27	0.29	WileyRegistry8e

Formula C15H26O, MW 222, CAS# 38230-60-3, Entry# 166102
Epicubebol



(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol
Formula C15H26O, MW 222, CAS# 198991-79-6, Entry# 13159
Germacrene D-4-ol



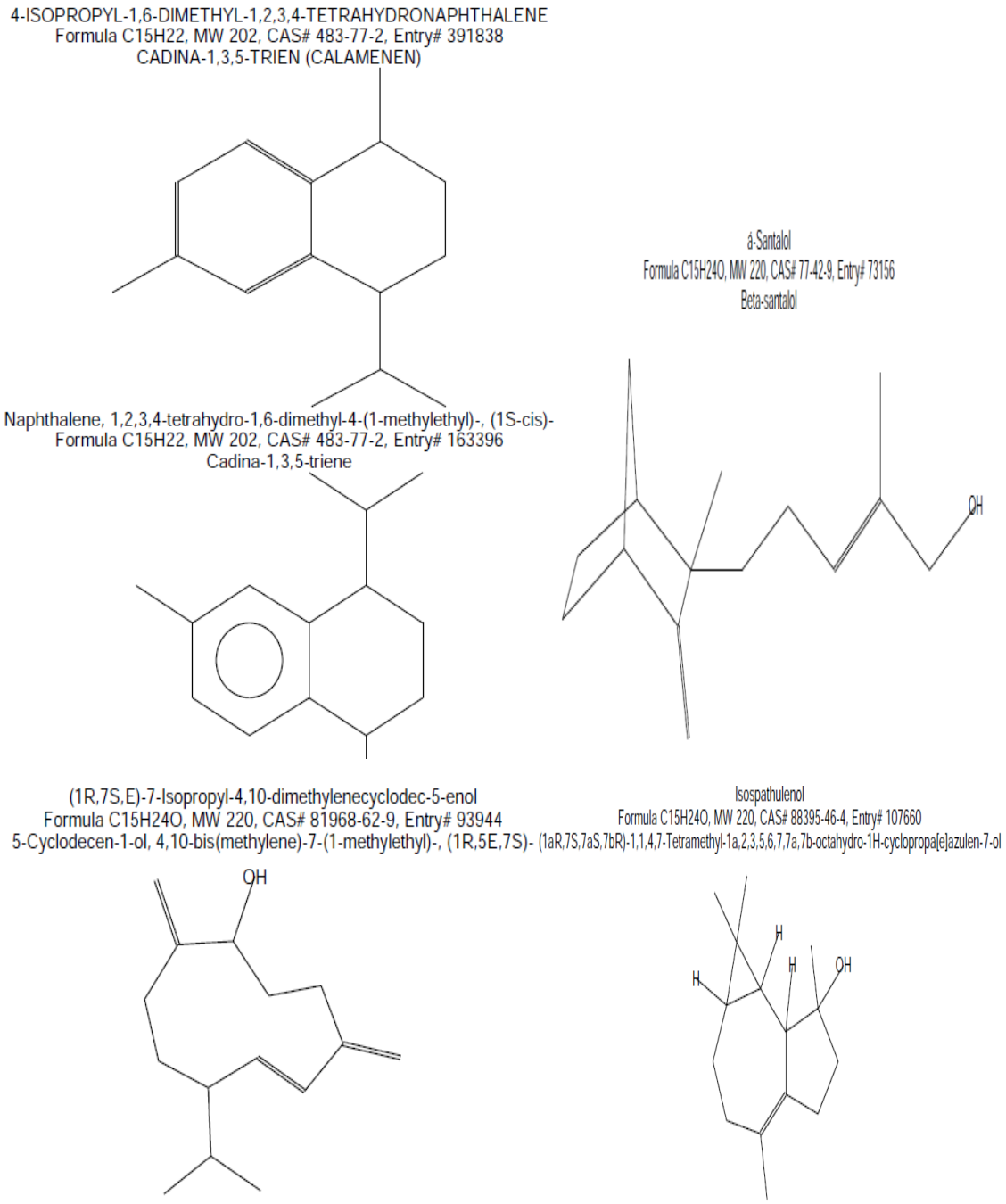


Figure 4: The major compounds of . *Juniperus excelsa* leaf extract with their formulas and molecular weights.

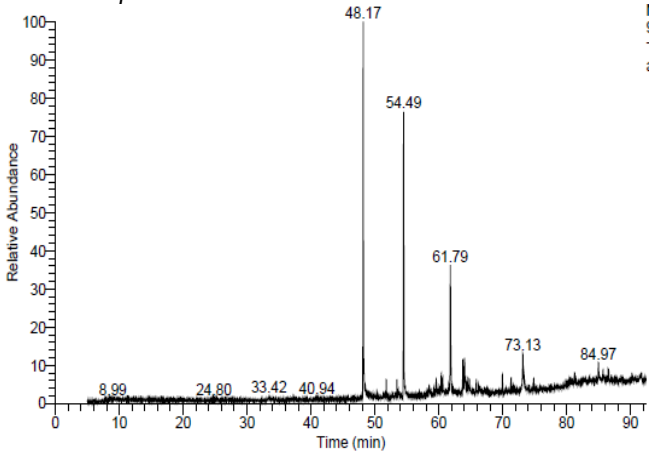


Figure 5: GC-MS analysis of *Coleus forskohlii* leaf extract.

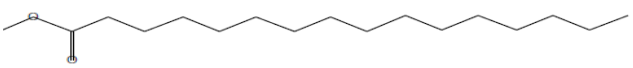
Table 5: GC-MS analysis of Coleus *forskohlii* leaf extract with its retention time.

RT	Scan #	Probability	Compound Name	SI	RSI	Cas #	Area	Area %	Library
48.17	12693.000000	74.66	Hexadecanoic acid, methyl ester	929	930	112-39-0	360065618.36	26.53	mainlib
48.17	12693.000000	74.66	HEXADECANOIC ACID, METHYL ESTER	928	949	112-39-0	360065618.36	26.53	WileyRegistry8e
48.17	12693.000000	74.66	HEXADECANOIC ACID, METHYL ESTER	924	927	112-39-0	360065618.36	26.53	WileyRegistry8e
48.17	12693.000000	74.66	Hexadecanoic acid, methyl ester	921	925	112-39-0	360065618.36	26.53	replib
48.17	12693.000000	74.66	HEXADECANOIC ACID, METHYL ESTER	916	924	112-39-0	360065618.36	26.53	WileyRegistry8e
50.36	13338.000000	14.94	OCTADECANOIC ACID, ETHYL ESTER	598	653	111-61-5	4893683.59	0.36	WileyRegistry8e
50.36	13338.000000	8.15	HEXADECANOIC ACID, ETHYL ESTER	581	700	628-97-7	4893683.59	0.36	WileyRegistry8e
50.36	13338.000000	8.15	HEXADECANOIC ACID, ETHYL ESTER	580	700	628-97-7	4893683.59	0.36	WileyRegistry8e
50.36	13338.000000	8.15	Hexadecanoic acid, ethyl ester	575	706	628-97-7	4893683.59	0.36	mainlib
50.36	13338.000000	6.24	Ethyl 14-methyl-hexadecanoate	574	711	NA	4893683.59	0.36	mainlib
51.34	13624.000000	39.22	Hexadecanoic acid, 15-methyl-, methyl ester	683	750	6929-04-0	5565384.57	0.41	mainlib
51.34	13624.000000	39.22	HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER	683	750	6929-04-0	5565384.57	0.41	WileyRegistry8e
51.34	13624.000000	30.83	HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER	677	734	2490-49-5	5565384.57	0.41	WileyRegistry8e
51.34	13624.000000	30.83	Hexadecanoic acid, 14-methyl-, methyl ester	676	734	2490-49-5	5565384.57	0.41	mainlib
51.34	13624.000000	9.40	HEPTADECANOIC ACID, METHYL ESTER	652	752	1731-92-6	5565384.57	0.41	WileyRegistry8e

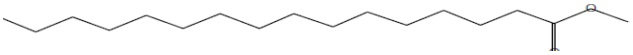
51.79	13757.000000	34.25	1-Naphthalenepropanol, à-ethenyldecahydro-à,5,5,8a-tetramethyl-2-methylene-, [1S-[1à(R*),4aá,8aà]]-	761	858	1438-62-6	19034797.98	1.40	replib
51.79	13757.000000	34.25	1-NAPHTHALENEPROPANOL, à-ETHENYLDECAHYDRO-à,5,5,8A-TETRAMETHYL-2-METHYLENE-, [1S-[1à(R*),4Aá,8Aà]]-	743	834	1438-62-6	19034797.98	1.40	WileyRegistry8e
51.79	13757.000000	17.66	1-Naphthalenepropanol, à-ethenyldecahydro-à,5,5,8a-tetramethyl-2-methylene-, [1S-[1à(S*),4aá,8aà]]-	743	806	596-85-0	19034797.98	1.40	mainlib
51.79	13757.000000	34.25	1-Naphthalenepropanol, à-ethenyldecahydro-à,5,5,8a-tetramethyl-2-methylene-, [1S-[1à(R*),4aá,8aà]]-	742	833	1438-62-6	19034797.98	1.40	mainlib
51.79	13757.000000	17.66	1-NAPHTHALENEPROPANOL, à-ETHENYLDECAHYDRO-à,5,5,8A-TETRAMETHYL-2-METHYLENE-, [1S-[1à(S*),4Aá,8Aà]]-	742	804	596-85-0	19034797.98	1.40	WileyRegistry8e
53.25	14186.000000	20.70	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	697	774	56051-53-7	4182651.44	0.31	mainlib
53.25	14186.000000	20.70	CYCLOPROPANEBUTANOIC ACID, 2-[[2-[[2-[(2-PENTYLCYCLOPROPYL)METHYL]CYCLOPROPYL]METHYL]CYCLOPROPYL]METHYL]-, METHYL ESTER	697	774	56051-53-7	4182651.44	0.31	WileyRegistry8e
53.25	14186.000000	6.93	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	673	748	18465-99-1	4182651.44	0.31	mainlib
53.25	14186.000000	6.93	9,12,15-OCTADECATRIENOIC ACID, 2,3-DIHYDROXYPROPYL ESTER, (Z,Z,Z)-	672	747	18465-99-1	4182651.44	0.31	WileyRegistry8e
53.25	14186.000000	4.48	7,10,13-Eicosatrienoic acid, methyl ester	660	696	30223-51-9	4182651.44	0.31	replib
53.44	14244.000000	4.98	9-Octadecenoic acid (Z)-, methyl ester	797	834	112-62-9	16240618.43	1.20	mainlib
53.44	14244.000000	4.98	9-Octadecenoic acid, methyl ester, (E)-	797	829	1937-62-8	16240618.43	1.20	mainlib
53.44	14244.000000	4.98	9-Octadecenoic acid, methyl ester, (E)-	796	835	1937-62-8	16240618.43	1.20	replib
53.44	14244.000000	4.79	10-Octadecenoic acid, methyl ester	796	822	13481-95-3	16240618.43	1.20	mainlib
53.44	14244.000000	4.79	10-OCTADECENOIC ACID, METHYL ESTER	796	822	13481-95-3	16240618.43	1.20	WileyRegistry8e

53.66	14308.000000	4.70	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	660	791	112-62-9	113974 12.05	0.84	WileyRegistry8e
53.66	14308.000000	3.51	13-Octadecenoic acid, methyl ester	652	826	56554-47-3	113974 12.05	0.84	replib
53.66	14308.000000	3.51	ELAIDINSAEUREMETHYLESTER	652	778	NA	113974 12.05	0.84	WileyRegistry8e
53.66	14308.000000	4.70	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	651	784	112-62-9	113974 12.05	0.84	WileyRegistry8e
53.66	14308.000000	3.10	11-Octadecenoic acid, methyl ester	649	745	52380-33-3	113974 12.05	0.84	replib
54.49	14553.000000	71.47	Methyl stearate	929	948	112-61-8	281432 476.96	20.74	replib
54.49	14553.000000	71.47	OCTADECANOIC ACID, METHYL ESTER	924	943	112-61-8	281432 476.96	20.74	WileyRegistry8e
54.49	14553.000000	71.47	Methyl stearate	918	963	112-61-8	281432 476.96	20.74	replib
54.49	14553.000000	71.47	Methyl stearate	910	924	112-61-8	281432 476.96	20.74	replib
54.49	14553.000000	71.47	Methyl stearate	909	911	112-61-8	281432 476.96	20.74	mainlib
58.51	15734.000000	54.18	N,N'-DICYANO-3-METHYLBENZO[B]NAPHTHO[2,3-E][1,4]OXATHIIN-6,11-QUINONEDIIMINE	831	909	NA	166555 61.97	1.23	WileyRegistry8e
58.51	15734.000000	18.15	1-(2',5'-DIMETHOXYPHENYL)-4,4,8,9-TETRAMETHYLPERHYDRONAPHTHALENE	807	855	NA	166555 61.97	1.23	WileyRegistry8e
58.51	15734.000000	16.74	N,N'-DICYANO-2-METHYLBENZO[B]NAPHTHO[2,3-E][1,4]OXATHIIN-6,11-QUINONEDIIMINE	805	882	NA	166555 61.97	1.23	WileyRegistry8e
58.51	15734.000000	3.69	2,2'(1H,1'H)-SPIROBI-S-INDACENE, ETHANONE DERIV.	762	832	115171-70-5	166555 61.97	1.23	WileyRegistry8e
58.51	15734.000000	1.46	NAPHTHO[2,3-C]FURAN-1,3-DIONE, 6,7-BIS(TRIMETHYLSILYL)-	740	785	80964-24-5	166555 61.97	1.23	WileyRegistry8e
59.21	15938.000000	58.00	9H-14B,8A,12-ETHANYLYLIDENE-6,8-METHANO-8AH-1,3-DIOXOLO[1,8A]NAPHTH[2,3-B]AZOCINE, ACONITAN-6-OL DERIV.	718	934	509-28-4	103408 08.18	0.76	WileyRegistry8e
59.21	15938.000000	3.57	CHOLESTAN-6-ONE, 3,5-DIHYDROXY-, (3á,5à)-	605	821	13027-33-3	103408 08.18	0.76	WileyRegistry8e
59.21	15938.000000	2.52	Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-hexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester	595	608	NA	103408 08.18	0.76	mainlib
59.21	15938.000000	2.52	17-(ACETYLOXY)-3-(HYDROXYIMINO)-4,4-DIMETHYLANDROSTAN-19-YL ACETATE	595	608	NA	103408 08.18	0.76	WileyRegistry8e
59.21	15938.000000	2.32	STIGMAST-5-EN-3-OL, (3á,24S)-	593	640	83-47-6	103408 08.18	0.76	WileyRegistry8e

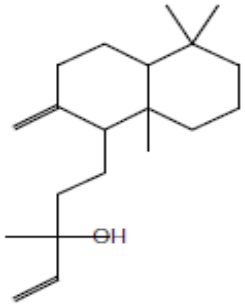
Hexadecanoic acid, methyl ester
Formula C17H34O2, MW 270, CAS# 112-39-0, Entry# 48957
Palmitic acid, methyl ester



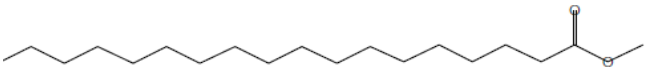
HEXADECANOIC ACID, METHYL ESTER
Formula C17H34O2, MW 270, CAS# 112-39-0, Entry# 161287
METHYL HEXADECANOATE



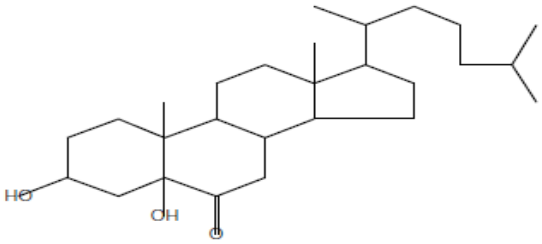
Formula C20H34O, MW 290, CAS# 1438-62-6, Entry# 25336
Labda-8(20),14-dien-13-ol, (13S)-



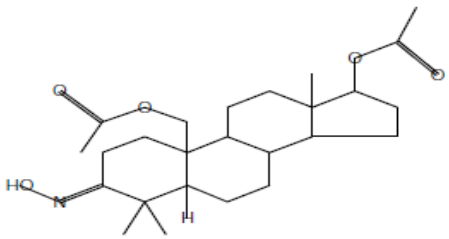
Methyl stearate
Formula C19H38O2, MW 298, CAS# 112-61-8, Entry# 12080
Octadecanoic acid, methyl ester



CHOLESTAN-6-ONE, 3,5-DIHYDROXY-, (3a,5a)-
Formula C27H46O3, MW 418, CAS# 13027-33-3, Entry# 264027
3,5-DIHYDROXYCHOLESTAN-6-ONE #



Formula C25H39NO5, MW 433, CAS# NA, Entry# 10264
17-(Acetyloxy)-3-(hydroxyimino)-4,4-dimethylandrostan-19-yl acetate #



Formula C25H42O2, MW 374, CAS# 56051-53-7, Entry# 48820

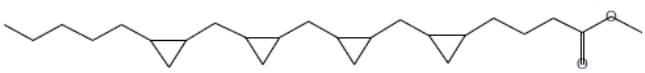


Figure 6: The major compounds of. *Coleus forskohlii* leaf extract with their formulas and molecular weights.

This research also shows the presence of numerous phytochemicals with biological activity, which may have a therapeutic index. Epicubebol and Germacrene D-4-ol, Calamenen and Cadina-1, 3,5-triene and 5-cyclodecen-1-ol, 4,10 bis(methylene)-7-1(methylethyl), and Isospathulenol were discovered in the phytochemical investigation of *Juniperus excelsa* extract. These findings differed from those of [2], who discovered that the principal chemical components of J. *excelsa* were -terpinene, limonene, fenchene, and camphene (6%), -3-carene, 4-terpineol, germacrene B, myrcene, -pinene, -pinene, and abietatriene. Although the essential oil of J. *excelsa* was tested for antibacterial activity against one Gram-positive and two Gram-negative foodborne pathogenic bacteria, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, there was no action present. [7] found according to Gas chromatography/mass spectrometry (GC-MS) examination, the most frequent components in the Juniperus sp ethanolic extract were inositol, (-)-germacrene D, (+)-totarol, and cedrol, whereas in the methanolic extract were inositol, (+)-totarol, and alpha-terpinyl acetate. Both methanolic and ethanolic extracts contained substantial levels of sesquiterpenes. The most abundant components in the methanolic extract were 1, 3, 4, 5-tetrahydroxycyclohexanecarboxylic acid, octadecanoic acid, and cytidine. The majority of these copmpounds are the same as those found in our Juniperus excelsa investigation. The existence of 46 elements related to distinct secondary metabolites was shown by GC/MS analysis of J. *procera* extract. The obtained results demonstrated the existence of monoterpenes known as thymol in J. *procera* extract with RC 3.13%. Thymol exhibited the greatest antibacterial and antifungal efficacy against plant pathogenic fungi as well as grey moulds in horticultural products caused by *Botrytis cinerea*. Trans-Caryophyllene, -Caryophyllene, and Epoxy caryophyllene were found in J. *procera* stems with RC values of 0.475, 1.387, and 5.962%, respectively. [22] Discovered caryophyllene in J. *excels*. Caryophyllene was found in aerial sections of J. virginiana and J. *communis* using GC/MS, particularly in the branches. Numerous investigations have shown that caryophyllene and caryophyllene oxide have antibacterial and antifungal properties. The GC/MS results revealed the presence of pinene derivatives such as -Pinene, -Pinene, and -Terpinene in J. procera extract. -pinene was discovered to be a component of the active components of J. *communis* and to have fungistatic properties. B-Elemene, γ-Elemene, Camphene, β-Pinene and α-cubebene were identified with highest concentration compared with other components, these components with caryophyllene and pinene displayed great potential of antifungal activity as a mycelial growth inhibitor against the tested phytopathogenic fungi such as *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium solani*, *Phytophthora capsici* and *Colletotricum capsici* [20].

3.2.2. GC-MS analysis of Coleus forskohlii leaf extract

GC-MS analysis of *Coleus forskohlii* leaf extract at figure (5) and table (5) showed the major compounds in this extract were Hexadecanoic acid methylester at molecular weight 270, Labdana-8 (20), 1,4- dien-13 ol at molecular weight 290, Methyl stearate at molecular weight 298, Cholestan -6-one,3,5- dihydroxy at molecular weight 418 and 17-(acetyloxy)-3 (hydroxyimino) -4,4- dimethylandrostan-19- yl- acetate at molecular weight 433, this showed at figure (6). The researchers discovered that hydroalcohol extracts of *Coleus forskohlii* showed significant antibacterial and antifungal action. This study also indicates the presence of numerous phytochemicals with biological activity (flavonoids, alkaloids, glycosides, steroids, phenols, terpenoids, saponins, tannins, cardiac glycosides, and carboxyclic) that may have a useful therapeutic index. These findings were consistent with those of [24].*C. forskohlii* phytochemical research revealed the presence of terpenoids, flavonoids, tannins, reducing sugars, and alkaloids. Flavonoids are polyphenolic chemicals that are renowned for their health-promoting qualities such as antioxidant, antiallergic, anti-inflammatory, antibacterial, and anticancer activities [18].*Coleus forskohlii* has antimicrobial activity against *Bacillus subtilis, Pseudomonas fluorescence, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus*, and *Streptococcus pneumoniae*, according to [16].The major bioactive element of C. *forskohlii*, according to [11] is a labdane diterpenoid, which is used to treat heart disease, high blood pressure, and respiratory ailments. Antibacterial activity of C. *forskohlii* extract against gram-positive and gram-negative bacteria such as S. *aureus*, *Streptococcus mutans*, *Salmonella typhi*, and *Escherichia coli* has been reported. All constituents were found in aqueous and methanol extracts, whereas proteins, carbohydrates, and cardiac glycosides were found in chloroform extract and cardiac glycosides were found in hexane extracts, indicating that water and methanol were able to extract the majority of the phytochemicals found in the shoot [21].

The potential of flavonoid-based antimicrobial drugs to disrupt enzymatic action in cell division, platelet aggregation, immunological responses, and complex building in the bacterial cell wall, as well as extracellular and soluble proteins, is suggested to underpin their antimicrobial activity. Plants employ flavonoids to defend themselves against microbial infections. Terpenoids are active due to their capacity to disrupt membranes, whereas tannins work by interfering with protein synthesis by adhering to proline-rich areas. Similary, C. *forskohlii* preparations also tested positive for phenolic components. The phenolic compounds are aromatic secondary metabolites that contribute colour and smell and have been related to health benefits such as a lower risk of heart disease and stroke. Plant phenolic compounds are responsible for the majority of antioxidant action. Alkaloids have been demonstrated to have analgesic, antispasmodic, antibacterial, antimalarial, and analgesic effects [18].*Coleus aromaticus* alcoholic extract contains streroids, triterpenoids, alkaloids, phenolic compounds, tannins, amino acids, phyosterol, and reducing sugars that have antibacterial action against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Escherichia coli*, according to [23].According to [24], *Coleus blumei* alcoholic extract contains phenolics, flavonoids, and a novel compound of diasteremeric diterpenes that has activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans*.

4. Conclusion

The hydroalcoholic extracts of *Juniperus excelsa* and *Coleus forskohlii* leaves had antibacterial and antifungal activity against (*Staphylococcus aureus, Streptococcus pyogenes, Aspergillus fumigatus* and *Apergillus niger*), anticancer effects and phytochemical examination revealed the presence of terpenoids, flavonoids, tannins, reducing agents, phenols and alkaloids. Flavonoids are polyphenolic chemicals that are renowned for their health-promoting qualities such as antioxidant, antiallergic, anti-inflammatory, antibacterial, and anticancer activities.

Declaration

Normative Consent

From March 202 2to December 2023.

Competing interest

Authors do not have any conflict of interest to declare.

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Authors' contributions

The authors have equally contributed to writing, designing, compiling and final editing of the manuscript

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