



## Preliminary Analysis of Bioactive Compounds and Antibacterial Properties in Methanolic Extract of *Achillea fragrantissima*

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### ABSTRACT

*Achillea fragrantissima*, a desert plant traditionally used in Arabian medicine to treat various ailments, is recognized as a rich source of biologically active metabolites. In this study, a methanolic extract of *A. fragrantissima* leaves was analyzed using GC-MS & LC-MS to identify its bioactive compounds. The extract's effectiveness against microbes was tested using the agar well diffusion method. The results indicated the presence of several biologically active components, including fatty acids, flavonoids, and steroids. This research confirms the antibacterial potential of the methanolic extract from *A. fragrantissima* leaves and provides the first detailed phytochemical analysis. However, further studies are necessary to investigate the active components and their wider biological activities.

**Keywords:** *Achillea fragrantissima*, Antibacterial, GC-MS analysis, LC-MS, Bioactive compounds.

### INTRODUCTION

Medicinal plants remain a primary source of drugs in both modern and traditional medicine worldwide<sup>1,2</sup>. For centuries, plants have provided valuable natural products essential for maintaining the health of both animals and humans. Natural products derived from higher plants present a promising source of antimicrobial agents, potentially offering novel mechanisms for treating infectious diseases<sup>3,4</sup>. Medicinal plants are rich in active compounds that can provide effective herbal alternatives for treating common bacterial infections. These plants are a valuable reservoir of diverse drugs and bioactive substances. Therefore, they should be extensively

researched to better understand their properties, safety, and effectiveness. The genus *Achillea*, part of the Asteraceae family, is commonly found across various Middle Eastern countries. Several studies have demonstrated the diverse pharmacological effects of its hydro-distilled volatile oils, which are effective in treating various diseases, both when applied topically and taken orally<sup>5</sup>.

Researchers have explored the antibacterial properties of various plants against both *Gram-negative* and *Gram-positive* bacterial strains, but there are limited reports on their activity against drug-resistant bacteria. Our study was designed to assess, *in vitro*, the antibacterial properties of



the methanolic extract of *A. fragrantissima* against bacterial pathogens.

## MATERIALS AND METHODS

### Preparation of Plant Materials

Leaves of *A. fragrantissima* were harvested during the spring season Gathered in the Hail province of Saudi Arabia. Plant identification was carried out by Dr. Naila Alkafei from the University of Hafer Albatin, KSA. The collected leaves were left to dry naturally in a shaded area at room temperature for a period of two weeks. After drying, the plant material was finely ground into powder form for extraction purposes<sup>6</sup>.

### Extraction Procedure

Only the dried leaves were used for the extraction process. These were separately ground into a fine powder. A total of 30 g of this powder was mixed with 250 mL of methanol at room temperature. Extraction was enhanced using ultrasonic treatment, applied in four 20-min cycles spread over a 24-h period in a water bath. Following extraction, the mixture was filtered, and both the filtrate and residue were preserved for subsequent analyses.

### GC–MS Analysis

This method was employed to identify the major bioactive compounds in the methanolic leaf extract. The analysis was performed using a Gas Chromatography–Mass Spectrometry system equipped with AS 3000 autosampler and an Ion Quantification System detector. A non-polar fused silica capillary column was utilized, with helium gas as the mobile phase at a constant flow rate of 1.2 milliliters per minute. For the analysis, 2 milliliters of the methanol-diluted extract were injected to enable partial separation of its chemical constituents. Spectral data were collected via mass spectrometry and analyzed using Xcalibur software. The resulting mass spectra were compared with reference data from the NIST and MAINLIB libraries for compound identification<sup>7</sup>.

### Liquid Chromatography–Mass Spectrometry (LC–MS) Analysis

This method was employed to analyze and identify the bioactive constituents present in the methanolic extracts of the selected plant leaves.

Chromatographic separation was performed using a Shimadzu ExionLC system, with the mobile phase comprising 0.1% formic acid in water and acetonitrile. A Gas & Liquid column (100 × 2.1 mm, 3 μm particle size) was used for separation, with a flow rate maintained at 0.35 mL/min. The gradient elution profile was set as follows: 5% solvent B from 0 to 5 min, a linear increase from 5% to 95% B between 5 and 30 min, and a return to 5% B from 30 to 40 minutes. Mass spectral data were acquired using a SCIEX X500R QTOF system, using an electrospray ionization source, the analysis was carried out in both positive and negative ionization modes. The compounds were identified by cross-referencing the acquired mass spectra with entries in the NIST spectral library database.

### *In vitro* Antibacterial Activity

The plant extracts were tested for their antibacterial potential against a panel of bacterial strains, including *S. aureus* ATCC 512477 (B-1), *S. epidermidis* ATCC 12228 (B-2), *E. faecalis* ATCC 29212 (B-3), *E. coli* ATCC 25922 (B-4), *K. pneumoniae* ATCC 700603 (B-5), *S. choleraesuis* ATCC 10708 (B-6), *P. aeruginosa* ATCC 27853 (B-7), and *P. mirabilis* ATCC 299 (B-8). Prior to testing, 24-h cultures were prepared from the respective stock strains. Antibacterial susceptibility testing was conducted following the standardized method described in<sup>8</sup>. Mueller-Hinton agar plates were prepared for the assays. Plant extracts were assessed for anti-bacterial properties via the agar well diffusion technique, whereas the disc diffusion approach was utilized for the reference antibiotic, ciprofloxacin (5 μg/disc). To ensure uniform distribution of the bacterial inoculum, sterile cotton swabs were dipped into standardized bacterial suspensions (based on CFU/mL) and uniformly distributed over the surface of Mueller-Hinton agar by gently rotating the Petri dishes. After allowing the plates to dry for around 10 min, wells were made in the agar using a sterile stainless-steel borer. Following sample application, the plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the diameters of the inhibition zones formed around the wells. The size of each zone indicated the effectiveness of the extract, with larger zones representing stronger antibacterial effects. The results are summarized in Table 3.

## RESULTS AND DISCUSSION

According to the results obtained from the fractionation process, a number of bioactive compounds were successfully isolated using both the GC-MS and LC-MS. These separated compounds were identified as the key contributors to the antibacterial activity observed in this plant. Their presence points to the plant's promise as a source of natural antimicrobial agents, supporting its traditional use and encouraging further pharmacological investigation. GC-MS analysis was done on a methanolic extract of *A. fragrantissima* leaves to find out what its main components and come up with an answer for the antibacterial activity that was seen in vitro. Table 1 & Fig. 1 shows the major chemical compositions of *A. fragrantissima* leaves methanolic extract via GC-MS. Thujone<sup>9</sup>,  $\alpha$ -Resorcylic acid<sup>10</sup>,

(+)- $\alpha$ -Funebrene<sup>11</sup>, Dihydroxanthin<sup>12</sup>, and Strophanthidin<sup>13</sup> were among the bioactive compounds identified in the extracts through GC-MS analysis and are believed to contribute to the observed antibacterial activity of the plant's extract in methanol.

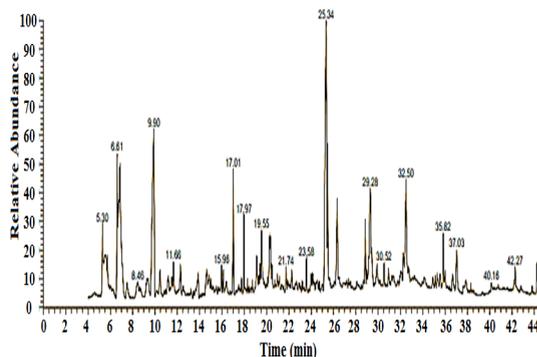


Fig. 1. GC-MS chromatogram of *A. fragrantissima* leaves extract in methanol

Table 1: Chemical compositions of *A. fragrantissima* leaves methanolic extract via GC-MS

Compound	Rt(min)*	Area (%)
T-2,7-Dimethyl-4,6-octadien-2-ol	5.29	1.55
4-Cyclohepten-1-amine	9.91	11.16
Thujone	6.61	4.44
cis-1,2-Cyclododecanediol	9.29	1.49
Myrtenyl acetate	11.18	0.77
$\alpha$ -Resorcylic acid	11.66	0.88
$\alpha$ -Copaene	15.96	0.72
17-Octadecynoic acid	12.28	0.97
(+)- $\alpha$ -Funebrene	17.01	3.24
(-)-Spathulenol	17.97	2.05
2-Naphthalenemethanol	19.55	1.62
Ledene oxide-(II)	20.28	1.60
Strophanthidin	20.35	1.12
Isoaromadendrene epoxide	22.25	0.61
Isoaromadendrene epoxide	23.58	0.91
n-Hexadecanoic acid	26.34	2.92
6,9-Octadecadienoic Acid, Methyl Ester	29.28	3.17
9,10-Secocholesta-5,7,10(19)-triene3,25,26-triol, (3 $\alpha$ ,5Z,7E)	30.52	0.73
Oxiraneoctanoic acid, 3-octyl-, cis-	32.25	0.82
Oleic Acid	35.30	0.56
Dihydroxanthin	35.54	0.53
Tris(2,6-dimethylphenyl)borane	37.03	1.43
Butanoic acid, ester	40.15	0.34
Sesamin	42.27	0.68
Stigmasterol	44.23	0.89
$\zeta$ -Sitosterol	45.07	1.80

\*Retention time.

The methanolic extracts of physiologically active *A. fragrantissima* leaves were analyzed using LC-MS to discover any chemical components that may be present. Many chemicals were discovered

utilizing both positive and negative ionization techniques (Table 2). Some of these chemicals may have been responsible for the observed antibacterial activity. 2-Oxovaleric acid<sup>14</sup>, chorismic

acid<sup>15</sup>, quercetagenin<sup>16</sup>, calcipotriol<sup>17</sup>, niacinamide<sup>18</sup>, and dihydromyricetin<sup>21</sup> were discovered to have butylated hydroxyanisole<sup>19</sup>, xanthurenic acid<sup>20</sup>, significant antibacterial action.

**Table 2: LC-MS examination of the methanolic extract of *A. fragrantissima* leaves**

Compound	Rt(min)*	Ionization mode	Calculated mass	Experimental mass
2-Oxovaleric acid (NIST)	2.20	Negative	116.0000	114.993 [M-H]-
Chorismic acid (NIST)	2.38	Negative	207.9867	206.9802 [M-H]-
DL-Ornithine (NIST)	2.51	Negative	113.9965	112.990 [M-H]-
Dulcitol (NIST)	2.51	Negative	181.9863	180.9806 [M-H]-
Quercetagenin (NIST)	2.63	Negative	317.74080	316.7340 [M-H]-
Dihydromyricetin (NIST)	2.63	Negative	319.7379	318.7313 [M-H]-
D-Arabinonic acid (NIST)	2.93	Negative	166.0533	165.0475 [M-H]-
Threonic acid (NIST)	3.16	Negative	136.04164	135.0353 [M-H]-
2-Amino-3-methoxybenzoic acid (NIST)	3.52	Negative	167.03056	166.0238[M-H]-
Lactitol (NIST)	10.70	Negative	344.0881	343.0804 [M-H]-
3-Hydroxybenzyl alcohol (NIST)	11.93	Negative	124.0558	123.0491 [M-H]-
2',4'-Dihydroxydihydrochalcone (NIST)	2.51	Positive	241.9175	242.9242 [M+H]+
Calcipotriol (NIST)	2.87	Positive	394.9814	356.0184 [M+K]+
D-Ornithine (NIST)	3.22	Positive	115.0621	116.0630[M+H]+
6-Azathymine (NIST)	3.56	Positive	127.0628	128.0696 [M+H]+
Cyclobutylamine (NIST)	4.36	Positive	71.0730	72.0798 [M+K]+
p-Hydroxy-o-toluidine (NIST)	5.95	Positive	123.0315	124.0383 [M+H]+
Niacinamide (NIST)	6.89	Positive	122.0472	123.0539 [M+H]+
Oleic Acid( NIST)	11.74	Positive	281.4650	282.4670 [M+H]+
Butylated hydroxyanisole (NIST)	11.40	Positive	180.0895	181.0963 [M+H]+
Xanthurenic acid (NIST)	11.59	Positive	206.0443	205.0375 [M+H]+
5-Methyluridine (NIST)	16.58	Positive	259.0963	258.0895 [M+K]+
5,6-Dehydroarachidonic acid (NIST)	19.57	Positive	302.2254	335.2584 [M+CH3OH+H]+

\*Retention time.

As presented in Table 3, the methanolic extract of *A. fragrantissima* leaves exhibited the highest antibacterial activity against *S. aureus*,

*E. coli*, *P. mirabilis*, and *S. choleraesuis*. However, its efficacy was significantly lower compared to the standard antibiotic ciprofloxacin.

**Table 3: Results of *In vitro* antibacterial activity**

	<i>Staphylococcus aureus</i> ATCC 512477	<i>Staphylococcus epidermidis</i> ATCC12228	<i>Escherichia coli</i> ATCC25922	<i>Proteus mirabilis</i> ATCC 299	<i>Salmonella choleraesuis</i> ATCC 10708	<i>Klebsiella faecalis</i> ATCC700603	<i>Enterococcus faecalis</i> ATCC 29212	<i>Pseudomonas aeruginosa</i> ATCC 27853
	18	10	16	17	14	11	7	9
	15	11	18	16	13	12	8	12
	18	10	19	18	14	9	12	14
Ciprofloxacin (5 mg/Disc)	33.66±1.24	33.3±1.24	35.66±0.9	23.66±1.24	33.3±1.24	26±1.4	25.33±0.4	33.3±0.8
Std	0.577350269	0.577350269	1.527525232	1	0.577350269	1.527525232	2.64575131	2.516611478

\*Each value is the mean of 6 batches with standard deviation. All the values are compared to the standard ciprofloxacin disc by performing Tukey Kramer test (post hoc). All the test values are significantly lesser than the standard ciprofloxacin disc at p<0.05. Std: Standard deviation

## CONCLUSION

The methanolic extract of *A. fragrantissima* leaves grown in Hail, KSA has a range of components that exhibit antibacterial properties against *S. aureus*, *E. coli*, *P. mirabilis*, and *Salmonella choleraesuis*. Our findings, together with earlier research, confirm the

plant's antibacterial qualities in the face of rising antibiotic resistance. It can serve as an antibacterial complement for the creation of novel medicinal medicines. Further research is needed to confirm the plant's potential as an antibacterial agent in topical or therapeutic applications, as well as to assess the effects of its active components *In vivo*.

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**Conflicts of Interest**

There are no conflicts of interest declared by the authors.

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