ORIGINAL ARTICLE **Open Access**

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences (IJFAS)

PHYTOCHEMICAL CONSTITUENTS AND BIOACTIVE POTENTIAL OF *ACHILLEA LANULOSA* EXTRACT

Vinay Pratap[1,2#], Shivangi Singh[1#], Ashwini Kumar Nigam[2], Jalaj Kumar Gour[1]*

[1]Department of Biochemistry, Faculty of Science, University of Allahabad, Prayagraj, Uttar Pradesh - 211002, India

[2]Department of Zoology, Udai Pratap College (Autonomous), Varanasi, Uttar Pradesh - 221002, India

***Corresponding author email address**: jalaj19biochem@gmail.com

[#]These authors contributed equally to this work

Paper Received: 19.12.2025 | Revised: 14.01.2026 | Accepted: 13.03.2026

DOI: https://doi.org/10.59415/ijfas.326 | ARK: https://n2t.net/ark:/26340/IJFAS.v15i1.326

Abstract

Achillea is a genus known for its potent medicinal properties and, hence plants of this genus have been used traditionally for the treatment of fever, pain, wounds healing and inflammation in different parts of the world. Considering the use of this plant and its products at such large scale in treating different ailments, the scientific validation of such claims becomes very important. *A. lanulosa* is one of the least studied varieties from *Achillea* genus that might have great medicinal importance. Therefore, the current study has been undertaken to explore the phytochemical constituents, antioxidant and antibacterial properties of *A. lanulosa* extract prepared in 80% methanol. Phytochemical profiling of the extract by qualitative and quantitative assays has revealed the presence of phytoconstituents including phenolics and flavonoids. Gas chromatography and Mass spectrometry of the extract showed significant concentration of 20 compounds including polyhydroxy acids, flavonoids and tocopherols and different type of terpenes. The DPPH scavenging assay had shown the significant antioxidant potential of the extract with IC_{50} of 24.5 ± 3.56 µg/ml. Further, the well diffusion assay demonstrated a pronouncedantibacterial activity in the plant extract against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes,* and *Enterobacter sp.* The findings of this study highlight the therapeutic potential of *A. lanulosa*, which may be helpful and promising option for additional research to fight bacterial infections and enhance human health.

Keywords: *A.lanulosa*, Phytochemical analysis, Antibacterial activity, DPPH Assay, GCMS profiling

1. INTRODUCTION

Before the use of syntheticchemicals as therapeutics began, plants were the main or primary source of treatment of diseases and ailments. The chemical composition of medicinal plants is associated with their pharmacologicalproperties such as anti-inflammatory, antioxidative, antimicrobial, antispasmodic and antibacterial properties(Latif & Nawaz, 2025; Petrovska, 2012). The genus *Achillea* is one such plant of the Asteraceae family that is widely used by people and communities to treat ailments such as inflammation, pain, and wounds (Raudone et al., 2022a). Species of *Achillea,* like *A. millefolium* have been reported to have remarkable medicinal properties. A closely related plant of the same genus, *Achillea lanulosa*or native North American Yarrow is considered as a part of *Achillea millefolium* species complex (Ehrendorfer, 1973; Shah &Peethambaran, 2018). This proximity in taxonomical classification might also reflect in their pharmacological properties, making*A. lanulosa*a suitable choice for source of natural products that might help in health improvement (Farasati Far et al., 2023). *A. millefolium* from different parts of the world has been reported to have phytochemicals particularly phenolics, flavonoids, terpenoids, and other secondary metabolites that are responsible for itspharmacological properties including antioxidant and antimicrobial properties contributing to its health benefits. The type and relative proportion of secondary metabolites in plants are affected by change in climate, geography, soil, and natural stress. Among the important classes of secondary metabolites are terpenes/terpenoids, phenolics/phenols, alkaloids and flavonoids (Teoh, 2015; Wu et al., 2025).

Modern techniques such as GC-MS allow accurate identification of various phytoconstituents, enablingthe researchers to establish the correlation of biological effects with various phytoconstituents of the plants,a key aspect of study on plant extracts. (Konappa et al., 2020). Although importance of species *Achillea* as a pharmacologically important genus is evident from review, however studies on phytochemical composition and therapeutic properties of *A. lanulosa* specifically is sporadic and scanty(Farasati Far et al., 2023; Villalva et al., 2022). Therefore, this study has been intended to analyse the phytochemical constituents, antioxidants potential and antibacterial properties of methanolic extract of *A. lanulosa* collected from Bhowali region of Uttarakhand, India. The current study could provide a scientific validation for traditional medicinal applications of *A. lanulosa*.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Mueller–Hinton Agar (MHA), gentamicin and analytical-grade chemicals were used. Molisch's reagent, Fehling's solution, 10% NaOH, ferric chloride solution, Borntrager's reagent, Salkowski's reagent, frothing test solution, Shinoda test reagents, and Dragendorff's reagent were prepared in the laboratory using analytical-grade chemicals.

2.2. Plant Material and Extraction

The plant was collected and authenticated as *A. lanulosa* from ICAR, NBPGR Regional Station, Bhowali, Uttarakhand, India. The fresh aerial parts of the plant were obtained and were washed with water from tap to remove soil and debris followed by washing them two times with double distilled water (DW), then with the disinfectant 5% Tween-20 solution and finally twice with double distilled water (DDW) for further processing. The aerial parts were shade-dried for 15 days at ambient temperature (25–28°C), then cut into small pieces, ground to a granular fine particle using a mechanical grinder. Extraction was carried out using 80% methanol by maceration method(Abbas et al., 2021; Abubakar & Haque, 2020) for 48 hours with agitation (122 rpm). Briefly, 40 g of the milled plant material was macerated in 400 ml of 80% methanol. The mixture produced after maceration was filtered by using Whatman No. 1 filter paper. The filtrate was allowed for solvent evaporation at room temperature (37°C) to collect and store the concentrated extract for further analysis. The obtained extract was designated AL80. Percentage yield of the extract was determined using the formula mentioned formula (Abbas et al., 2021).

$$\boldsymbol{Yield}\ (\%)\ \boldsymbol{extract} = (\boldsymbol{Solvent\ free\ extract} \div \boldsymbol{Dried\ mass\ of\ the\ extract}) \times \mathbf{100}$$

2.3. Qualitative screening of Phytochemicals

Qualitative phytochemical analyses of 80% methanolic extract of *A. lanulosa*(AL80)were conducted using standard protocols (Akter et al., 2019; Dahanayake et al., 2019; Kancherla et al., 2019) to identify the major secondary metabolite classes in the crude plant extract. The presence of carbohydrates was supported by Molisch's and Fehling's tests. These tests indicated a violet ring and brick-red precipitate formation respectively, which confirmed the presence of carbohydrates. Coumarins were evidenced by yellow colouration with 10% NaOH. Phenolics and tannins produced dark blue to green colouration with ferric chloride. Glycoside's test was done by the Keller–Killiani test, where a brown ring at the interface in the confirmatory test. Borntrager's test for free anthraquinones yielded pink to violet colouration in the ammoniacal layer. Terpenoids were identified by reddish-brown colour at the interface using Salkowski's test. In the case of saponin, a stable froth formation confirmed its presence. Flavonoids showed orange to red colour when analysed by Shinoda's test, and alkaloids produced orange to reddish-brown precipitates with Dragendorff's reagent, confirming their presence.

2.4. Total phenolic content

Determination of total phenolic content in AL80 was done using colorimetric assay of Folin-Ciocalteu reagent (FCR) (Singleton & Rossi, 1965). To 0.5 ml of the plant extract (100µg/ml), an equal volume of DW and 0.4 ml of Na_2CO_3, further 0.75 ml of FCR in a 1:10 ratio was added. Finally, the whole volume was increased to 5.0 ml by diluting with distilled water. After incubation for 2 h, absorbance was recorded at 760 nm. To develop a standard curve, gallic acid was used at concentration ranging from 0-120 µg/ml. The phenolic content was quantified in µg gallic acid equivalents (GAE)/mg of *A. lanulosa* dried extract.

2.5. Total flavonoid content

The aluminum chloride colorimetric method was employed for the estimation of total flavonoid content (TFC)in the plant extract following standard protocol (Do et al., 2014) with slight adjustments. To 2 ml of 0.1 mg/ml plant

extract, 0.4 ml of 10 % $AlCl_3$ and 20 µl of 1M Potassium acetate were added. The final volume was brought up to 10 ml with DW. Quercetin at concentration of 0- 120 µg/ml was taken as the standard curve and the absorbance was recorded at 415 nm after 30-minute incubation period. Total flavonoid content was represented as µg quercetin equivalents (QE)/mg of *A. lanulosa* dried extract.

2.6 GC-MS Analysis

Gas Chromatography and Mass Spectrometry (GC-MS) analysis of the AL80 using a Shimadzu system was performed. The specific setup had a GC-2010 Plus gas chromatograph associated with a GCMS-QP2010 Ultra mass detector. The sample introduction was handled by an AOC-20i/s auto-sampler. The temperature of GC oven started at 60°C and was maintained for two minutes. After this the temperature was taken up to 300°C at a rate of 10°C per minute. The final temperature was held for 19 minutes. Helium was used as the mobile gas, set at a uniform linear velocity of 40.1 cm/s. The injector port temperature was fixed to 260°C for sample injection in split mode at a ratio of 10:1. Prior to GCMS analysis, the extract was derivatized by the analytical facility using a standard trimethylsilylation procedure to form TMS derivatives (Mami et al., 2016). The derivatization reagent and conditions were as per the facility's protocol. The temperature of the interface was set to 270°C at which the sample entry was taken and the ion source temperature was kept 220°C. This ensured smooth transition and efficient ionization in the mass spectrometer. The mass spectrometer was operated in electron impact ionization mode at 70eV, and data were measured in complete scan mode over a mass range of 40-600 m/z to enhance detection sensitivity. Before analysis, the extract was simply passed through a 0.22 µm membrane filter. Molecules were tentatively ascertained through comparing their mass spectra to source spectra in the National Institute of Standards and Technology (NIST) repository, with a similarity index (SI) greater than 90% considered a reliable match (Wakoli et al., 2024).

2.7. Assay of Antioxidant Potential

2.7.1. DPPH Assay

The free radical-quenching capacity of AL80 was measured employing DPPH assay following standard protocol (Kedare & Singh, 2011). DPPH is a purple coloured stable free radical, which change colour to yellow when treated with an antioxidant. The dried extract was dissolved in 80% methanol to prepare sample stock solutions. In the DPPH assay, 1 ml of each dilution from test samples (5–200 µg/ml) was blended with 1 ml of DPPH reagent solution (40 µg/mL) and 1 ml of DW to obtain a 3 ml final reaction volume. The reaction was permitted to proceed for 20 minutes briefly at room temperature. Throughout this incubation period, samples were kept in darkness. The absorbance was read at 517 nm with a UV-visible spectrophotometer. Ascorbic acid was employed as the standard. The control consisted of 1 ml DPPH working solution blended with 1 mL solvent (methanol) and 1 ml of DW. A blank (solvent without DPPH) was used to zero the spectrophotometer. The DPPH radical scavenging activity was determined using following equation:

$$\text{Scavenging (\%)} = \left[\frac{(A_0 - A_s)}{A_0}\right] \text{x } 100$$

where, A_0 is control solution absorbance (DPPH + Solvent), A_s is the sample reaction absorbance after subtracting the blank. Each experiment was done in triplicate. Results are represented as the Mean ± SD.

2.7.2. IC_{50} Calculation

Inhibitory concentration (IC_{50}) was determined by linear regression equation (y = mx +c) for each independent replicate and was reported as Mean ± SD.

2.7.3. Total antioxidant capacity

Phosphomolybdenum assay (Prieto et al., 1999) was employed to test total antioxidant capacity of the plant extract. 1 ml of plant extracts of 100 µg/ml concentration was added to 1 ml of reagent mixture containing 0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate in equal proportion. Further 1 ml DW was incorporated into the reaction mixture and allowed to react for 90 minutes in a water bath. Absorbance was taken at 695nm after cooling. Ascorbic acid (25-200 µg/ml) was utilized as standard positive control and results were represented in µg of ascorbic acid equivalent (AAE)/mg of *A. lanulosa* dried extract.

2.8. Antibacterial Activity

The agar well diffusion method(Gonelimali et al., 2018) was employed for determination of antibacterial potential. Bacterial cultures (*P. aeruginosa, Klebsiella pneumoniae, Enterobacter sp., S. pyogenes*) were grown

in Mueller-Hinton broth an overnight. Cultures were normalized to 0.5 McFarland units of turbidity, containing about 1×10^8CFU/ml, and plated using a L-shaped glass spreader to evenly distribute a 100µL bacterial suspension over 90mm Mueller Hinton agar plates to achieve a confluent lawn. Later, 8 mm wells were created on the confluent lawn using a cork borer. The wells were loaded with 50 µL of reconstituted extract solution containing 40% extract concentrations for determination of antibacterial potential. After 24-hour incubation of culture plates at 37°C, the zone of inhibition (ZOI) was observed and documented in mm around each well to determine antibacterial activity. All tests were done in triplicate. For controls, gentamicin was utilized as a positive and the extraction solvent employed as a negative control.

2.9. Statistical Analysis

Complete experimental findings were presentedas mean ± SD from three independentreplicates ($n = 3$). For the analysis of antibacterial activity, zone of inhibition obtained for each bacterial strain were compared for statistical differences employing one way analysis of variance (One way-ANOVA) followed by S-N-K Post hoc test. Statistical analyseswere carried out using SPSS version 27 software. The threshold for statistical significance was agreed at the level $p<0.05$.

3. RESULTS

3.1. Phytochemical Constituents

The presence of a variety of natural compounds was confirmed by qualitative analyses of the plant extract (AL80) (**Table 1**). This diversity of compounds adds to the presence of a complex mixture in the extract. This suggests the possibility that the extracts may have multiple biological effects.

Table 1: Qualitative phytochemical screening of the AL80. (+++) strong positive reaction; (++) moderate; (+) weak**.**

S. No.	Phytochemical Reaction	Class of Compound Detected	Observation
1	Molisch's	Carbohydrates	++
2	Fehling's	Reducing sugars	+++
3	Coumarin	Coumarins	+++
4	Ferric chloride	Phenolic compounds and Tannins	+++
5	Glycoside	Glycosides	+++
6	Borntrager's	Anthraquinones	+
7	Salkowski	Terpenoids	+
8	Froth	Saponins	+++
9	Shinoda	Flavonoids	++
10	Dragendorff's	Alkaloids	++

3.2. Total phenolic content (TPC) and Total flavonoid content (TFC)

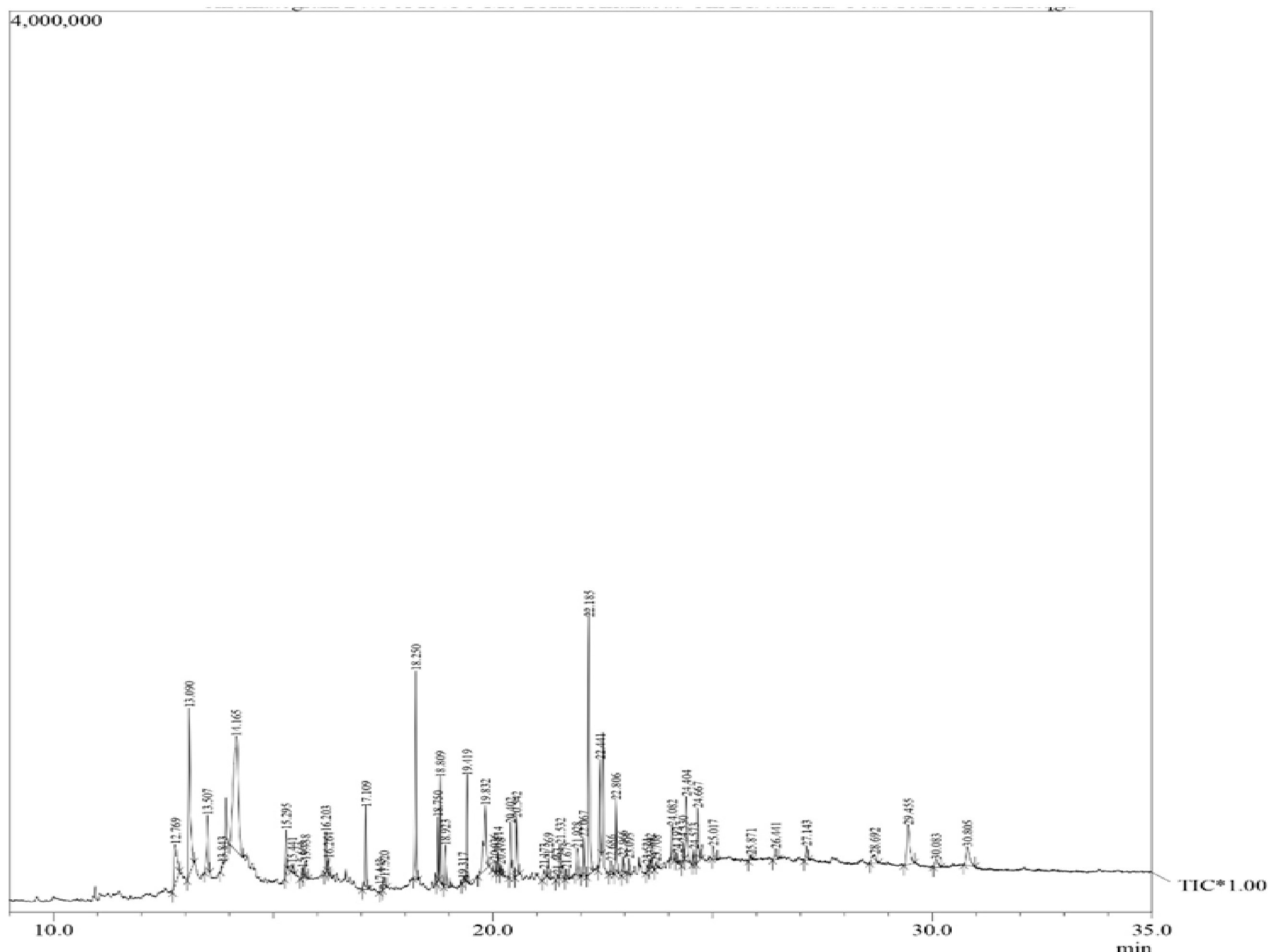
The AL80 produced an extraction yield of 9.09%. Despite having a moderate percentage yield, the extract has been found to have a high content of total phenolic compounds (418 ± 27.77 µg GAE/mg of dried plant extract) and total flavonoid compounds (400 ± 27.03 µg QE/mg of dried plant extract) (**Table 2**).

Table 2: Extract yield percentage, total phenolic content and total flavonoid content of AL80 extract (Mean ± SD, n = 3). The extraction yield is expressed as percentage (%), defined as: grams of dried extract obtained total grams of dried plant material (w/w).

AL80	% Yield extract	TPC µg GAE/mg of dry extract	TFC µg QE/mg of dry extract
	9.09%	418±27.77	400±27.03

3.3. GC-MS Analysis

GC-MS analysis of AL80nidentified 56 phytoconstituents. Out of which 20 compounds (that had peak area



percentage higher than 1%) were selected. (**Table 3, Figure 1**). Compounds obtained from GC-MS (NIST library) were then confirmed based on the literature survey of the plant's phytochemistry. These components belong to diverse chemical classes such as polyhydroxy acids, dicarboxylic acids, fatty acids and esters, sesquiterpenes, diterpenes, triterpenes, flavonoids and tocopherols.

Figure 1: Chromatogram obtained afterGas Chromatography (GC) analysis of *A. lanulosa* extract prepared in 80% methanol

Table 3: Total detected compounds identified by GC-MS in the AL80 extract.
(MF, Molecular formula;MW, Molecular weight; RT, Retention time)

Peak	Compound Name	MF	MW (gram/mol)	RT (Min)	Peak Area%
1	Adipic Acid	$C_6H_{10}O_4$	146	13.09	8.01
2	5-Ethyl-1,3-Dioxane-5-Methanol	$C_7H_{14}O_3$	146	13.507	2.28
3	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$	192	14.165	15.26

4	Chamazulene	C14H16	184	15.295	1.31
5	Neophytadiene	C20H38	278.5	16.203	1.12
6	Methyl Palmitate	C17H34O2	270	18.25	6.03
7	Methyl Linolenate	C19H32O2	292	18.809	2.79
8	Phytol	C20H40O	296	19.419	2.71
9	A-Linolenic Acid,	C18H30O2	278	19.832	4.29
10	3-Decen-2-one, 10-phenyl-, (e)-	C16H22O	230	20.402	2.06
11	Matricarin	C17H20O5	304	21.532	1.63
12	2-(Dimethylamino)Ethyl 1-Adamantanecarb	C15H25NO2	251	21.928	1.07
13	2-Oxovaleric Acid	C5H8O3	116	22.067	1.12
14	3.Alpha.,4.Beta.-Dihydroxy-1,5,7.Alpha.(H),6.Beta.(H)-Guai-10(15),11(13)-Dien-6,12-Olide	C15H20O4	264	22.185	9.94
15	Ethanone, 1-(1,2,3,4,7,7a-Hexahydro-1,4,4,5-Tetramethyl-1,	C17H26O	246	22.441	3.42
16	2-Butenoic Acid	C4H6O2	346	22.806	2.22
17	Ionyl Angelate	C19H30O2	290	24.404	2.6
18	IonylTiglate	C19H30O2	290	24.667	2.19
19	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5-hydroxy-3,6,7-trimethoxy-	C20H20O8	388	29.455	3.22
20	A-Amyrin	C30H50O	426	30.805	1.76

3.4. DPPH scavenging assay

Both the AL80 as well as ascorbic acid demonstrated a tendency for increasing DPPH radical scavenging ability with increase inconcentration (**Table 4, Figure 2**). While the AL80 extract depicted a gradually increasing ability in lower concentrations, reaching about 76% at200 µg/ml concentration, ascorbic acid demonstrated nearly complete scavenging (about 90%) in the same concentration. Statistical analysis revealed a significant difference in the average scavenging ability between Ascorbic acid and the AL80 ($p<0.05$).

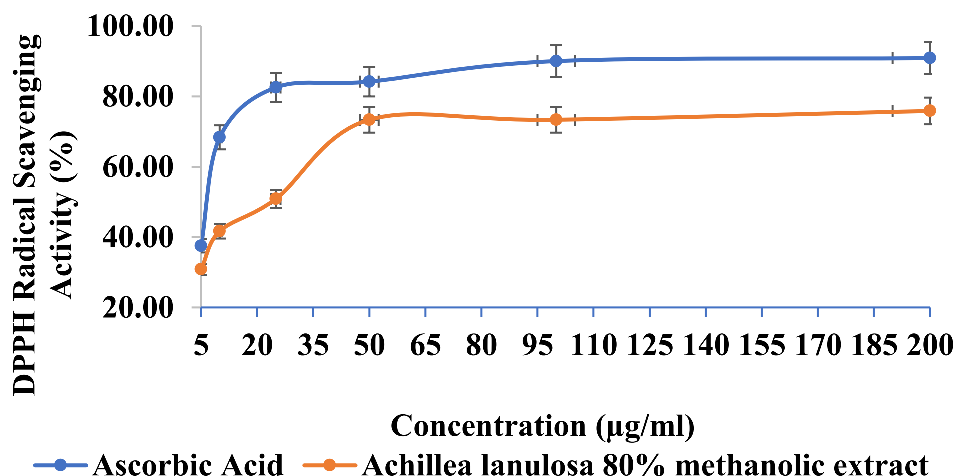


Figure 2: Dose dependent DPPH radical scavenging activity of AL80 extractand standard ascorbic acid

Table 4. DPPH radical scavenging activity (% inhibition, Mean ± SD, n = 3) and IC_{50} (µg/ml) of AL80 and standard Ascorbic acid

Concentration (µg/ml)	Ascorbic Acid (% scavenging ± SD)	AL80 (% scavenging ± SD)
5.0	37.5 ± 2.5	30.83 ± 1.44
10.0	68.33 ± 1.44	41.67 ± 3.82
25.0	82.5 ± 2.5	50.83 ± 3.82
50.0	84.17 ± 1.44	73.33 ± 2.89
100.0	90 ± 2.5	73.33 ± 2.89
200.0	90.83 ± 1.44	75.83 ± 1.44
IC_{50}	**7.02 ± 0.26 µg/ml**	**24.5 ± 3.56 µg/ml**

The IC_{50} values showed that ascorbic acid required the low concentration to achieve 50% DPPH scavenging (7.02 µg/ml), whereas the AL80 exhibited comparatively high IC_{50} values (24.50 µg/ml)

3.5. Total antioxidant capacity

AL80 exhibited a TAC value of 47.24 ± 4.93 µg AAE/mg of dried extract.

Table 5: Total antioxidant capacity (TAC) AL80. Values are expressed as Mean ± SD of three independent replicates (n = 3)

Plant species	TAC (Mean ± SD (µg AAE/mg of dry extract)
A. lanulosa	47.24 ± 4.93

3.6. Antibacterial Activity

Agar well diffusion method demonstrated a significantly ($p < 0.05$) varied extent of antibacterial activity in methanolic extract of *A. lanulosa* against all the bacterial strain tested as evidenced from clear zone of inhibition around sample wells (**Table 6**).

Table 6: Antimicrobial efficacy of AL80 and gentamicin, against four pathogenic bacteria, using agar well diffusion

technique (Mean ± SD, n = 3). The zone of inhibition (ZOI) is presented in mm.

Bacterial strains	ZOI for Gentamicin Mean ± SD (mm)	ZOI for AL80 Mean ± SD (mm)
P. aeruginosa	30.00 ± 2.0[a]	28.00 ± 2.65[a, b]
K. pneumoniae	32 ± 1.70[a]	16.17 ± 0.76[c]
Enterobacter sp.	31.66 ± 0.58[a]	19.83 ± 1.04[d]
S. pyogenes	33.33 ± 1.53[a]	13.17 ± 0.76[e]
Values are expressed as mean ± SD (n = 3). for gentamycin and AL80, ZOI values having different superscript letters indicate statistically significant differences among groups according to one-way ANOVA followed by SNK post-hoc test at $p < 0.05$.		

The activity of gentamicin and the extract was found to be different against all the four bacteria. The antibacterial activity showed by AL80 was highest (28.00 ± 2.65 mm ZOI) against *P. aeruginosa* strain which is comparable to reference drug gentamicin (30.00 ± 2 mm ZOI). However, the antibacterial activity of the AL80 extract was shown to be highest against *P. aeruginosa* (28.00 ± 2.65 mm ZOI) followed by *Enterobacter sp.* (19.83 ± 1.04 mm ZOI), *K. pneumoniae* (16.17 ± 0.76 mm ZOI), and *S. pyogenes* (13.17 ± 0.76 mm ZOI). Nevertheless, the extent of antibacterial activity of plant extract was significant ($p < 0.05$) lower than that of gentamycin **(Table 6)**.

4. DISCUSSION

It is well known that plant products are rich sources of natural antioxidants, antimicrobial agents along with wide ranges of medicinal properties against different health related problems(Kumar et al., 2021).Qualitative analysis of the AL80 has shown the presence of a variety of secondary metabolites. The secondary metabolites like phenolics, coumarins, glycosides, tannins, and saponins are in high concentration, and the alkaloids, flavonoids and occur in moderate concentrations (Table 1). The major portion of phenolics and flavonoids in *A. lanulosa* is supported by related species such as *A. millefolium* or *Achillea santolina*, which have exerted strong antioxidant and anti-inflammatory activity in previous studies (Radušienė et al., 2023). Phenolic and flavonoid components have shown greater effects in eliminating free radicals(Sonboli et al., 2010), decreasing microbe infection and reducing inflammation (Al-Khayri et al., 2022; Ecevit et al., 2022), while tannins aregood against microbes, tissue damage, and wounds (Buzzini et al., 2008; Orlowski et al., 2018). Coumarins and saponins are considered to be associated with anticoagulant action, expectorant benefits, and lipid-lowering effects which support their considerable presence (Flores-Morales et al., 2023; Olas et al., 2020; Tejada et al., 2017; Timilsena et al., 2023; Xiao et al., 2025)[e,]. While the number of glycosides and alkaloids were moderate. Nevertheless, they exhibit cardioprotective, analgesic, and cytoprotective action similar to those of diverse *Achillea* species (Heinrich et al., 2021; Khan et al., 2020; Ponce et al., 2025; Sasikumar et al., 2021).

The AL80 extract showed extraction yield of 9.09 %, but it contained high total phenolic content (TPC: 418 ± 27.77 µg GAE/mg of plant extract) and total flavonoid content (TFC: 400 ± 27.03 µg QE/mg of dry extract). Although the yield was below 20%, the extract was rich in useful phenolics and flavonoids. This depicts that 80% methanol concentrates antioxidant-related secondary metabolites rather than increasing bulk yield. It was observed that there was a close correlation between total phenolic content and total flavonoid content, indicating that flavonoids are major contributors towards the phenolic content of AL80 extract (Table 2).

The phytochemicals that were found to be present in AL80 with analysis of GC-MS showed diversity (Table 3, Figure1). The entire profile with identifiable mass spectral matches was reported. The major component ‘‘1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid (Quinic acid)’’ at 15.26%, a polyhydroxy acid is recognized for its antioxidant effects, anti-proliferative and anti-inflammatory activities(Benali et al., 2024; Iqra et al., 2025). The adipic acid and palmitic acid further support bioactivity associated with lipid metabolism, anticancer and antimicrobial properties(Benali et al., 2024; Wang et al., 2023). As results informs, the powerful antioxidant activity of AL80 may be credited to combined effects of phenolics and flavonoids metabolites. The credit of the antioxidant potential of these components could be confirmed by the previous studies on various *Achillea* species (Saeidnia et al., 2011). In summary, AL80 gives information about a complex and significantly relevant phytochemical profile that support its medicinal properties (Raudone et al., 2022).

The DPPH assay is largely accepted in vitro method to examine the free radical scavenging capacity of components derived from the plants(Blois, 1958; Brand-Williams et al., 1995; Kumar et al., 2021). In this study, we observed

that as the concentration of ascorbic acid and AL80 increased, their antioxidant activity also increased. The findings, together with the significantly lower IC_{50} value of Ascorbic acid compared with the extract, indicate that ascorbic acid has greater antioxidant potency. The antioxidant activity of both the extract and antioxidant went up to 50µg/ml. After this, a plateau phase started. This showed the plateau phase in the extract and the ascorbic acid. The IC_{50} of ascorbic acid was 7.02 µg/ml showing more potency than the extract (IC_{50} = 24.50µg/ml). Additionally, the IC_{50} value still categorizes the extract that it can act as potent antioxidant according to the criteria set since the extracts having IC_{50} < 50 µg/ml fall into the potent category. These extracts exhibit high effectiveness in DPPH-scavenging activity when considering natural materials currently in use in the pharmaceutical aspects of pharmacognosy. It is strong evidence for the ability of the extract to make free radicals inactive and inhibit oxidative stress, even if the values surpass those of the ascorbic acid standard (Fidrianny et al., 2016). The general implications of the results obtained are that there are antioxidant compounds in the extract, though not to the same extent as ascorbic acid. Further study is needed to obtain the knowledge of constituents in the extract that cause this.

The TAC of AL80 was 47.24 ± 4.93 µg AAE per mg of dried extract, revealing its moderate antioxidant effect. Indeed, polar antioxidant compounds such as phenolic acids and flavonoids, which highly contribute to the TAC, are effectively extracted by methanol–water mixtures. The standard deviation assures acceptable experimental consistency. Such results suggest that *A. lanulosa* contains bioactive compounds capable of donating electrons or hydrogen, supporting its potential as a natural antioxidant, as well as the need for further detailed studies. AL80 extract has a rich composition of phenolic compounds, flavonoids, tannins, and terpenoids, as shown by qualitative analysis of the extract suggests. The existence of diverse phytochemicals, as screened in related species such as *A. millefolium* and *A. santolina*, may contribute to the antioxidant property of *A. lanulosa* (Candan et al., 2003; Motavalizadehkakhky et al., 2013). The antioxidant activity of AL80 is likely attributable to the availability of phenolic compounds, flavonoids, tannins and terpenoids, which were detected qualitatively in the extract. Similar classes of compounds have been reported in other *Achillea* species where they contribute to antioxidant effects (Dabbaghi et al., 2025).

The antibacterial activity of the AL80 was found to be strain-dependent (Table 6). With extract showing its highest antibacterial activity against *P. aeruginosa*, producing the largest inhibition zone (28 ± 2.65 mm), followed by *Enterobacter* (19.83 ± 1.04 mm), *K. pneumoniae* (16.17 ± 0.76 mm) while against *S. pyogenes* AL80 had least efficacy with ZOI of 13.17 ± 0.76 mm**.** This strain-specific zone inhibition may reflect differences in cell envelope structure or target accessibility, particularly in *Pseudomonas*. However, the present study did not investigate the underlying mechanism. The remaining organisms were more susceptible to gentamicin. As compared to the AL80 extract *P. aeruginosa* (30.00 ± 2 mm), *K. pneumoniae* (32 ± 1.7 mm), *Enterobacter sp.* (31.66 ± 0.58 mm), and *S. pyogenes* (33.33 ± 1.53 mm), producing inhibition zones, confirming the broader and more potent antibacterial spectrum of the gentamicin under the present assay conditions. These outcomes indicate that the AL80 shows promising selective antibacterial activity against *P. aeruginosa*.

5. CONCLUSION

This study has shown that the AL80 exhibited significantantioxidant and antibacterial activities. Phytochemical profiling analysis revealed the diverse chemicals in *A. lanulosa*, which may contribute to the observed bioactivities. The *in vitro* assays conducted provided preliminary evidence supporting its medicinal potential. Further studies such as *in vivo* experiments, isolation and characterization of the bioactive compounds responsible for the therapeutic activity, are necessary to confirm the therapeutic properties of *A. lanulosa.*

6. ACKNOWLEDGEMENTS

We are thankful to the Department of Biochemistry, University of Allahabad, Prayagraj-211002 for providing necessary facilities and infrastructure to conduct the experimental work. The financial support from UGC, New Delhi to Vinay Pratap and Shivangi Singh is kindly acknowledged. Authors are also thankful to Dr. Krishna Madhav Rai, Scientist, Fruit Science, ICAR-NBPGR Regional Station Bhowali– 263132, Uttarakhand, India for providing and authenticationof plant sampleto carry out this research work. Authors are very much thankful to Professor Shanthy Sundaram, Centre of Biotechnology, University of Allahabad, Prayagraj for providing bacterial strains used in this study. The Advanced Instrumentation Research Facility (AIRF) of Jawaharlal Nehru University, New Delhi, is greatly acknowledged for providing GC-MS facility.

7. STATEMENTS AND DECLARATIONS

AI Statement: NIL

Ethical Standards: All the ethical Research standards were followed while writing this Research Article.

Conflict of Interest: The authors state that they do not have any conflict of interest.

Human or animal involvement in the article: Nil

Data Availability: NA

8. REFERENCES

Abbas, A., Naqvi, S. A. R., Rasool, M. H., Noureen, A., Mubarik, M. S., & Tareen, R. B. (2021). Phytochemical Analysis, Antioxidant and Antimicrobial Screening of *Seriphidium Oliverianum* Plant Extracts. *Dose-Response*, *19*(1), 15593258211004739. https://doi.org/10.1177/15593258211004739

Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy and Bioallied Sciences*, *12*(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19

Akter, S., Begum, T., Begum, R., Tonny, T., Yasmin, M., Shifa, S., Afroze, F., & Farabi, F. (2019). PHYTOCHEMICAL ANALYSIS AND INVESTIGATION OF ANTI-INFLAMMATORY AND ANTI-ULCER ACTIVITY OF TERMINALIA BELLIRICA LEAVES EXTRACT. *International Journal of Pharmacognosy*, *6*, 54–65. https://doi.org/10.13040/IJPSR.0975-8232.IJP.6(2).54-65

Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules*, *27*(9). https://doi.org/10.3390/molecules27092901

Benali, T., Bakrim, S., Ghchime, R., Benkhaira, N., El Omari, N., Balahbib, A., Taha, D., Zengin, G., Hasan, M. M., Bibi, S., &Bouyahya, A. (2024). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews*, *40*(4), 3408–3437. https://doi.org/10.1080/02648725.2022.2122303

Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, *181*(4617), 1199–1200. https://doi.org/10.1038/1811199a0

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/https://doi.org/10.1016/S0023-6438(95)80008-5

Buzzini, P., Arapitsas, P., Goretti, M., Branda, E., Turchetti, B., Pinelli, P., Ieri, F., & Romani, A. (2008).

Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Reviews in Medicinal Chemistry*, *8*(12), 1179–1187. https://doi.org/10.2174/138955708786140990

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., & Akpulat, H. A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. Millefolium Afan. (Asteraceae). *Journal of Ethnopharmacology*, *87*(2–3), 215–220. https://doi.org/10.1016/s0378-8741(03)00149-1

Dabbaghi, M. M., Fadaei, M. S., Goldoozian, M., Fadaei, M. R., Baradaran Rahimi, V., & Askari, V. R. (2025). Promising impacts of *Achillea* spp., beyond A medicinal plant, against toxins, toxicities, and injuries: *In vivo* and *in vitro* mechanisms. *Biochemistry and Biophysics Reports*, *42*, 102023. https://doi.org/10.1016/j.bbrep.2025.102023

Dahanayake, J. M., Perera, P. K., Galappatty, P., Melshandi Perera, H. D. S., &Arawwawala, L. D. A. M. (2019). Comparative Phytochemical Analysis and Antioxidant Activities of Tamalakyadi Decoction with Its Modified Dosage Forms. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 1–9. https://doi.org/10.1155/2019/6037137

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophilaaromatica. *Journal of Food and Drug Analysis*, *22*(3), 296–302. https://doi.org/10.1016/j.jfda.2013.11.001

Ecevit, K., Barros, A. A., Silva, J. M., & Reis, R. L. (2022). Preventing Microbial Infections with Natural Phenolic Compounds. *Future Pharmacology*, *2*(4), 460–498. https://doi.org/10.3390/futurepharmacol2040030

Ehrendorfer, F. (1973). New chromosome numbers and remarks on theAchillea millefolium polyploid complex in North America. *ÖsterreichischeBotanischeZeitschrift*, *122*(3), 133–143. https://doi.org/10.1007/BF01624798

Farasati Far, B., Behzad, G., & Khalili, H. (2023a). Achillea millefolium: Mechanism of action, pharmacokinetic, clinical drug-drug interactions and tolerability. *Heliyon*, *9*(12), e22841. https://doi.org/10.1016/j.heliyon.2023.e22841

Farasati Far, B., Behzad, G., & Khalili, H. (2023b). Achillea millefolium: Mechanism of action, pharmacokinetic, clinical drug-drug interactions and tolerability. *Heliyon*, *9*(12), e22841. https://doi.org/10.1016/j.heliyon.2023.e22841

Fidrianny, I., Kusmardiyani, S., & Novita, G. (2016). ANTIOXIDANT ACTIVITIES FROM VARIOUS EXTRACTS OF DIFFERENT PARTS OF KELAKAI (STENOCHLAENA PALUSTRIS) GROWN IN CENTRAL KALIMANTAN - INDONESIA. *Asian Journal of Pharmaceutical and Clinical Research*, 215. https://doi.org/10.22159/ajpcr.2016.v9s2.13630

Flores-Morales, V., Villasana-Ruíz, A. P., Garza-Veloz, I., González-Delgado, S., & Martinez-Fierro, M. L. (2023). Therapeutic Effects of Coumarins with Different Substitution Patterns. *Molecules*, *28*(5), 2413. https://doi.org/10.3390/molecules28052413

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., &Hatab, S. R. (2018). Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Frontiers in Microbiology*, *9*, 1639. https://doi.org/10.3389/fmicb.2018.01639

Heinrich, M., Mah, J., &Amirkia, V. (2021). Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity—An Update and Forward Look. *Molecules*, *26*(7). https://doi.org/10.3390/molecules26071836

Iqra, Sharif, A., Akhtar, B., Shao, C., Wang, S., & Younas, A. (2025). Quinic acid alleviates inflammatory responses and oxidative stress in Freund's complete adjuvant-induced arthritic rat model and associated risk factors of atherosclerosis. *Inflammopharmacology*, *33*(11), 6669–6690. https://doi.org/10.1007/s10787-025-01930-8

Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). *Maedica - A Journal of Clinical Medicine*, *14*(4). https://doi.org/10.26574/maedica.2019.14.4.350

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Khan, H., Pervaiz, A., Intagliata, S., Das, N., Nagulapalli Venkata, K. C., Atanasov, A. G., Najda, A., Nabavi, S. M., Wang, D., Pittalà, V., &Bishayee, A. (2020). The analgesic potential of glycosides derived from medicinal plants. *Daru: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, *28*(1), 387–401. https://doi.org/10.1007/s40199-019-00319-7

Konappa, N., Udayashankar, A. C., Krishnamurthy, S., Pradeep, C. K., Chowdappa, S., &Jogaiah, S. (2020). GC–MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. *Scientific Reports*, *10*(1), 16438. https://doi.org/10.1038/s41598-020-73442-0

Kumar, M., Pratap, V., Singh, M. K., Nigam, A. K., Kumar, P., & Gour, J. K. (2021). Evaluation of Antioxidant Potential of Hedychium spicatum Rhizome Extracts from Bhowali Region, Uttarakhand, India. *SSR Institute of International Journal of Life Sciences*, *7*(6), 2901–2907. https://doi.org/10.21276/SSR-IIJLS.2021.7.6.1

Latif, R., & Nawaz, T. (2025). Medicinal plants and human health: A comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochemistry Reviews*. https://doi.org/10.1007/s11101-025-10194-7

Mami, O., Takanobu, J., Sato, M., yamaki, S., Sawada, Y., & Yokota Hirai, masami. (2016). *Application for Plant Metabolome Analysis Using the GC/MS/MS Smart Metabolites Database*.

Motavalizadehkakhky, A., Ebrahimi, Z., Emamiyan, R., Mohamadian, A., & Abedi, F. (2013). Chemical Compositions of Essential Oils of Different Parts and Extract of Achillea santolina L. from Iran. *Asian Journal of Chemistry*, *25*(11), 6372–6376. https://doi.org/10.14233/ajchem.2013.14647

Olas, B., Urbańska, K., &Bryś, M. (2020). Saponins as Modulators of the Blood Coagulation System and Perspectives Regarding Their Use in the Prevention of Venous Thromboembolic Incidents. *Molecules*, *25*(21). https://doi.org/10.3390/molecules25215171

Orlowski, P., Zmigrodzka, M., Tomaszewska, E., Ranoszek-Soliwoda, K., Czupryn, M., Antos-Bielska, M., Szemraj, J., Celichowski, G., Grobelny, J., &Krzyzowska, M. (2018). Tannic acid-modified silver nanoparticles for wound healing: The importance of size. *International Journal of Nanomedicine*, *13*, 991–1007. https://doi.org/10.2147/IJN.S154797

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, *6*(11), 1–5. https://doi.org/10.4103/0973-7847.95849

Ponce, A., Flores-Maldonado, C., & Contreras, R. G. (2025). Cardiac Glycosides: From Natural Defense Molecules to Emerging Therapeutic Agents. *Biomolecules*, *15*(6), 885. https://doi.org/10.3390/biom15060885

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, *269*(2), 337–341. https://doi.org/10.1006/abio.1999.4019

Radušienė, J., Karpavičienė, B., Raudone, L., Vilkickyte, G., Çırak, C., Seyis, F., Yayla, F., Marksa, M., Rimkienė, L., &Ivanauskas, L. (2023). Trends in Phenolic Profiles of Achillea millefolium from Different Geographical Gradients. *Plants*, *12*(4), 746. https://doi.org/10.3390/plants12040746

Raudone, L., Radušiene, J., Seyis, F., Yayla, F., Vilkickyte, G., Marksa, M., Ivanauskas, L., &Cırak, C. (2022a). Distribution of Phenolic Compounds and Antioxidant Activity in Plant Parts and Populations of Seven Underutilized Wild Achillea Species. *Plants*, *11*(3), 447. https://doi.org/10.3390/plants11030447

Saeidnia, S., Gohari, A., Mokhber-Dezfuli, N., & Kiuchi, F. (2011). A review on phytochemistry and medicinal properties of the genus Achillea. *Daru : Journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, *19*(3), 173–186.

Sasikumar, R., Jyoti Das, A., & Chandra Deka, S. (2021). In vitro cytoprotective activity of cyanidin 3-glucoside extracts from Haematocarpusvalidus pomace on streptozotocin induced oxidative damage in pancreatic β-cells. *Saudi Journal of Biological Sciences*, *28*(9), 5338–5348. https://doi.org/10.1016/j.sjbs.2021.05.065

Shah, R., &Peethambaran, B. (2018). *Chapter 19—Anti-inflammatory and Anti-microbial Properties of Achillea millefolium in Acne Treatment* (S. Chatterjee, W. Jungraithmayr, & D. B. T.-I. and I. in H. and D. Bagchi, Eds.; pp. 241–248). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-805417-8.00019-6

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Sonboli, A., Mojarrad, M., Nejad Ebrahimi, S., & Enayat, S. (2010). Free Radical Scavenging Activity and Total Phenolic Content of Methanolic Extracts from Male Inflorescence of Salix aegyptiaca Grown in Iran. *Iranian Journal of Pharmaceutical Research: IJPR*, *9*(3), 293–296.

Tejada, S., Martorell, M., Capo, X., Tur, J. A., Pons, A., & Sureda, A. (2017). Coumarin and Derivates as Lipid Lowering Agents. *Current Topics in Medicinal Chemistry*, *17*(4), 391–398. https://doi.org/10.2174/1568026616666160824102322

Teoh, E. S. (2015). Secondary Metabolites of Plants. *Medicinal Orchids of Asia*, 59–73. https://doi.org/10.1007/978-3-319-24274-3_5

Timilsena, Y. P., Phosanam, A., & Stockmann, R. (2023). Perspectives on Saponins: Food Functionality and Applications. *International Journal of Molecular Sciences*, *24*(17). https://doi.org/10.3390/ijms241713538

Villalva, M., Silvan, J. M., Alarcón-Cavero, T., Villanueva-Bermejo, D., Jaime, L., Santoyo, S., & Martinez-Rodriguez, A. J. (2022). Antioxidant, Anti-Inflammatory, and Antibacterial Properties of an Achillea millefolium L. Extract and Its Fractions Obtained by Supercritical Anti-Solvent Fractionation against Helicobacter pylori. *Antioxidants*, *11*(10), 1849. https://doi.org/10.3390/antiox11101849

Wakoli, J., Anjum, A., Sajed, T., Oler, E., Wang, F., Gautam, V., LeVatte, M., & Wishart, D. S. (2024). GCMS-ID: A webserver for identifying compounds from gas chromatography mass spectrometry experiments. *Nucleic Acids Research*, *52*(W1), W381–W389. https://doi.org/10.1093/nar/gkae425

Wang, X., Zhang, C., & Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. *Frontiers in Oncology*, *13*. https://doi.org/10.3389/fonc.2023.1224125

Wu, W., Wu, H., Liang, R., Huang, S., Meng, L., Zhang, M., Xie, F., & Zhu, H. (2025). Light regulates the synthesis and accumulation of plant secondary metabolites. *Frontiers in Plant Science*, *16*, 1644472. https://doi.org/10.3389/fpls.2025.1644472

Xiao, M.-Y., Li, S., Pei, W.-J., Gu, Y.-L., & Piao, X.-L. (2025). Natural Saponins on Cholesterol-Related Diseases: Treatment and Mechanism. *Phytotherapy Research: PTR*, *39*(3), 1292–1318. https://doi.org/10.1002/ptr.8432