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Phytochemical Profiling and Bioactivity Assessment of Xanthium orientale: Evidence from HPLC, GC /MS and Antibacterial activity

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ABSTRACT

Patients *Xanthium orientale* L. is an annual, medicinal plant that is an association of the Asteraceae family and has been extensively known to have been widely utilized in the traditional medicine in treating health conditions, especially inflammatory diseases, skin diseases, infections, and certain central nervous system disturbances. Plant phytochemicals Screenings and chromatography analysis reported presence of diverse secondary metabolites. The plant is native to the American continent but has spread widely across the Asian continent. In the present study, phytochemical screening of *Xanthium orientale*, Chromatographic (HPLC and GC/MS) identification and preliminary evaluation the antibacterial activity of against Escherichia coli and klebsiella were performed. The GC/MS finding revealed presence of fatty acids and major monoterpenes such as limonene, and thujene while, The Lactone sesquiterpene, xanthatine was detected by HPLC analysis. Other secondary metabolites screened through preliminary tests; flavonoids, steroids, glycosides, and phenolic compounds. The results show good zones of inhibition were observed around wells containing the extract depend on highest concentrations (75-100 mg/ml), However, Klebsiella pneumoniae and E. coli shows sensitive to the extract with high concentration. To the best of our knowledge, this work considers the first phytochemical profiling, chromatographic and evaluation of its antibacterial activity evidence for *Xanthium orientale* L. grown in Iraq. Over all, the findings of this work support the therapeutic application corresponding to previous studies. Nevertheless, further phytochemical and pharmacological assessment are required.

Keywords: Xanthium, phytochemical, Antibacterial, Chromatography, Inhibition zone.

I. INTRODUCTION

Xanthium orientale is an annual herbaceous plant belong to family (Asteraceae), Genus *Xanthium* has long been utilized in folk medicines belongs to it's the phytochemical composition [1]. The plant is native to north and South America, but widely distributed across Asia, especially in the temperate regions, even in the agricultural fields. *Xanthium orientale* is an annual herb that typically grows to a height of 1–1.5 m, with rough, ribbed, and branched stems. The leaves are triangular to ovate, coarse-textured, and may be lobed or toothed [2]. Herbal products derived from this plant are prone to adulteration and variability, highlighting the need for proper quality control and authentication through microscopical, physicochemical and phytochemical analyses [3]. It is also toxic to livestock, especially cattle, when consumed in large quantities due to the presence of toxic glycosides and sesquiterpene lactones [4]. Studies on phytochemicals have indicated that some species of the genus Xanthium carry an extensive group of secondary metabolites, which comprise sesquiterpenoids, phenylpropanoids, flavonoids, anthraquinones, naphthoquinones coumarins, steroids, lignans, and glycosides [5-7]. Among them, Xanthanides are a characteristic group of bicyclic sesquiterpene lactones and serve as chemotaxonomic markers of the genus Xanthium [8]. Structurally, they consist of a seven-membered carbocyclic ring fused to a five-membered γ-lactone ring, which often contains a α-methylene-γ-lactone moiety responsible for biological activity [9]. Structural diversity arises from variations in functional groups and stereochemistry, which strongly influence their pharmacological properties [10]. More than thirty xantholides have been identified in Xanthium species, including xanthin, which exhibit important biological activities such as cytotoxic, anti-inflammatory, antifungal, antiulcer, and insecticidal effects [11]. The aeries part of *X.oriantle* have pharmacologically effect includes: diuretic, anthelmintic, antifungal, dermatologic

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conditions, treat of rheumatism, antidiabetic, and anticancer effects [12, 13]. The Ethnobotanical Chinese Medicine (TCM), the plant is known as Cang ER Zi, which commonly used for sinusitis, nasal congestion, respiratory allergies, headache and fever related conditions [14]. It has also been used to treat vitiligo, fever, epilepsy, rheumatism, and dermatological disorders [15, 16]. In addition, Xanthium extracts exhibit antibacterial and antifungal activities, mainly due to terpenoids, phenolic compounds, and lactones that can disrupt microbial membranes and inhibit enzyme systems [17, 18].

The Taxonomic classification of plant reveled the plant under Xanthium genus and Asteraceae family as in Table 1

Table 1: Taxonomical classification of *Xanthium oriental*

kingdom	Plantae
Order	Asterales
phylum	Streptophyta
class	Equisetopsida
Subclass	Magnoliidae
Family	Asteraceae
Genus	Xanthium
Species	*Xanthium orientale*

Figure 1. *Xanthium oriental L.* grown in Baghdad- Al-Yusufiyah region.

II. METHODS AND MATERIAL

Collection and Authentication of *Xanthium orientale*:

Fresh leaves of *Xanthium orientale L.* were collected from the Al-Yusufiyah region, Baghdad, Iraq, during October 2025. The plant material was authenticated based on its morphological characteristics, and cleaned, washed to remove impurities materials. After cleaning, the collected leaves were placed in a shaded area at room temperature. When completely dried, they were ground into a coarse powder using a mechanical mill. The powdered sample was stored in a suitable container for further chemical analysis and investigation [19].

Preparation of plant extracts

Cold extraction:

About 100 g of coarse plant for 1000 ml was performed for maceration method, for each process was used 10 g of solid to (100 ml) of non-polar hexane for 48 hr., and this process repeated three times with fresh solvent to ensure extract complete non-polar compounds. The dried extract was yield about 3%, stored with optimum conditions for investigations. However, the marc was further extracted with hydro ethanolic extract 95% (10 g of the powdered plant sample mixed with 100 mL of solvent) for 72 hr. with intermittent shaking to enhance extraction efficiently. The filtrate was concentrated, dried and weighted about 10% about and transferred into suitable containers [20, 21].

Hot Extraction:

The crudes extract about 100 g /500 ml 95% hydroethanolic was subjected to reflux extraction at 60-70 °C for one hour to improve extraction yield about 13% of phytoconstituent [22].

Solvent -Extract Fractionation

In this method which is widely used for selective enrichment of bioactive compounds, the extract of hot method was performed Because of the resulting extract was viscous in cold method so, the hot solvent treatment method was used to ensure complete solubility. The aqueous solution of the alcoholic extract was suspended in distilled water and transferred to a separation funnel for liquid -liquid partitioning depend on solvent with different polarities. First the solution was extracted using chloroform three times (3 x100 ml) for HPLC analysis. This step was performed to remove nonpolar to semipolar components. The aqueous layer retained for further extraction ethyl acetate and N- butanol aqueous layer. All fractions stored at 4 °C in air tight containers [23].

Preliminary phytochemical screening

The wide range of detected compounds demonstrates the chemical nature of *Xanthium*, supporting its traditional and previous studies of medicinal use and represents its active secondary metabolites. The presence of multiple terpenoid and phenolic compounds suggests possible synergistic pharmacological effects. The ethanolic extract of *Xanthium orientale L.* leaves was performed using standard qualitative phytochemical procedures to identify the major classes of natural secondary metabolites [23, 24].

Wagner's method

A small quantity (2 ml) of reagent was added to the plant extract (5 ml). The appearance of a reddish-brown precipitate signified alkaloid.

Alkaline Reagent method
A yellow colorant appears after introduce of 3 ml of potassium hydroxide solution to the alcoholic extract 5 ml to examine the presence of flavonoids', the disappearing of color upon the addition of dilute acid signified a flavonoid.

Foam Test
The Assessment of saponins**,** The 4 ml of crude extract was agitated with distilled water about10 minutes. A steady foam lasting several minutes showed a good the presence of saponins.

Test for Carbohydrates (Fehling's Test)
An equal parts of Fehling's solution (A) and Fehling's solution (B), about 0.5 ml, assorted with filtrate. A brick-red precipitate will point to the reducing sugar.

Test for Tannins screening
The appearance of a blue or greenish-black color after addition of one ml of 1% solution were added to the three ml alcoholic extract, Indicates tannins and phenolic compounds.

Keller–Killiani Test
A Few milliliters of glacial acetic acid when added to filtrate, cooled and followed by drops of conc. sulfuric acid to result of brown reddish reaction color at interface referred to present of cardiac glycosides.

Salkowski Test
The formation of reddish-brown layer after reaction is important to indicate the terpenoids, Chloroform solution with filtrate is moderately to non-polar compounds present, and so the presence of concentrated sulfuric acid gives a positive result with terpenes.

Liebermann–Burchard Test
For detection of phytosterol**,** A Few milliliters of boiled extract filtrate with drops of glacial acetic acid, cooled and add the drops of conc. sulfuric acid was mixed to treated with extract. The changed color indicates phytosterols.

HPLC Identification
High-performance liquid chromatography analysis was carried out to detection of important constituents in the plant. The work was achieved depend on a UV–visible detector. Chloroform extract has different moderately to non-polar compounds can separate on C18 reversed-phase column (250 mm × 4.6 mm, 5 µm particle size). The solvent system (isocratic) consisted of acetonitrile (solvent A) and water (solvent B) with ratio (70:30, v/v). The flow rate was consisted at 1.0 mL/min, while the injection volume about 20 µL. the constituent's separation detected at a wavelength of 274 nm, which is suitable for the detection of some nonpolar compounds. The total run time was set to 15 minutes, the test was prepared by dissolving extract in 10 ml of HPLC-grade chloroform, filtered through very small size pores of membrane filter, and the filtrate was injected. [25-27].

GC–MS analysis
Gas chromatography–mass spectrometry investigation was done using chromatogram system. A carrier gas was used at a constant flow rate. The injection volume and injection temperature were set appropriately, and the was fixed. The oven temperature program was applied at 250 C to separate of volatile constituents. Electron ionization (EI) mode was used for Mass at 70 eV, with mass spectra recorded over ratio m/z range. Compound identification was achieved by comparing the obtained mass spectra of extract with those in the NIST mass spectral library. Relative percentages (high and area) of the detected compounds were calculated based on peak area [28-30].

Anti-Bacterial assay
Agar Well Diffusion is one of the interesting methods for investigate the inhibition effects of herbal medicines. Xanthium orientale is one of these plants using the hexane Extract to assess its effect. The materials were needed: hexane extract of *Xanthium orientale,* Mueller–Hinton Agar (MHA), Mueller–Hinton Broth, test bacterial strains, sterile swabs, cork borer (6–8 mm), micropipette, DMSO (solvent control), standard antibiotic (positive control).

Preparation of Extract Solutions
Agar media was prepared and boiled (38g / I litter D.W). The sterilization done through Autoclave.
Dried of hexane extract of Xanthium orientale was dissolved in sterile DMSO to prepare different concentrations.
Bacterial Inoculum
Test bacteria were cultured in Mueller–Hinton Broth at 37°C for 24 h and adjusted to 0.5 McFarland standard.
Plate Inoculation
MHA plates were uniformly swabbed with the bacterial suspension and allowed to dry for 5 min.
Well Formation
Wells (6–8 mm diameter) were pressed aseptically in the agar using a sterile cork borer
The sample prepared of Xanthium orientale (300 mg/mL) were made by dissolving 600 mg of dried hexane extract in 2 mL of 5% DMSO solution.
Loading Extract
Each well was occupied with 50–100 µL of the extract at different concentrations 100 mg/mL, 75 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL.
Solvent only (hexane) → negative control
Pre-diffusion
Plates were kept at 4°C for 30–60 min to allow extract flow.
Incubation

Plates were incubated at optimum temperature (37°C) for one day.

Measurement

The method repeated 3 times. Zones of inhibition were measured in millimeters. The inhibition zones according to concentration indicate the antibacterial activity. Data were calculated as mean ± standard deviation, p-values at a significance level ($p < 0.05$).[31,32]

III. RESULTS AND DISCUSSION

The preliminary screenings are performed for predicting the major classes of phytochemicals present in the plant extract. Table 2 revealed the presence of various phytochemical constituents, including flavonoids, terpenoids, steroids, tannins, saponins, and carbohydrates in the leaves of *Xanthium orientale L.* These secondary metabolites are known to contribute significantly to the pharmacological and biological activities of medicinal plants. The presence of flavonoids and phenolic compounds suggests potential antioxidant properties, while terpenoids and steroids are often associated with anti-inflammatory and antimicrobial activities. The findings are consistent with previously reported studies on plants belonging to the Xanthium genus, which confirmed the presence of similar classes of phytochemicals [33].

Table 2: Phytochemical screening of Iraqi *xanthium oriental*

Test	Positive Indicator	Result
Wagner`s Test	Reddish brown ppt	-ve
Alkaline Reagent Test	Yellow color	+ve
Foam Test	Stable and persistent foam	+ve
Fehling`s Test	Brick-red ppt	+ve
Phenolic (Ferric Chloride Test)	Blue-black color	+ve
keller killliani Test	Blue-green ring	-ve
Salkowski Test	reddish-brown layer	+ve
Liebermann-Burchard reaction	Dark green layer	+ve

Chromatographic determination and identification

The HPLC chromatogram analysis of the chloroform extract monitored at 274 nm revealed multiple peaks represents different phytochemical constituents. The detection performed corresponding to of Xanthatin standard (retention time 7.2 min), as seen in figure 2 ,figure 3 and Table 3,the HPLC method showed acceptable peak resolution and reproducibility under the applied chromatographic conditions.

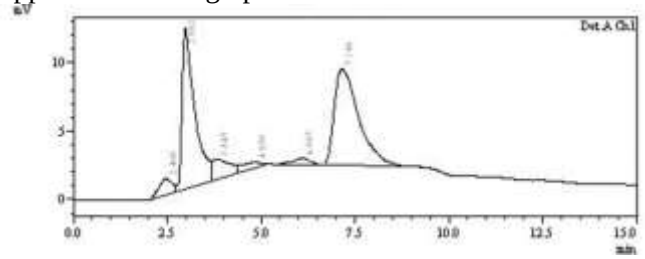

Figure 2: chromatogram analysis of chloroform extract showed the presence of Xanthatin.

Table 3: Separation of compounds in chloroform extracts with different retention time by HPLC.

Peak No.	Retention Time(min)	Area	Height	Area %	Height %
1	2.460	29668	1208	4.218	5.429
2	3.845	44416	1414	6.315	6.350
3	4.850	15412	358	2.191	1.609
4	6.085	16433	482	2.337	2.164
5	7.146	323164	7037	45.950	31.612

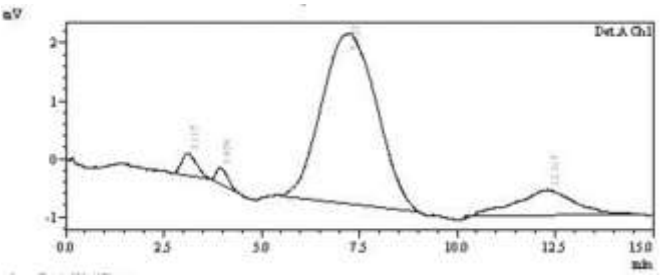

Figure 3: chromatogram analysis of Xanthatin standard.

Based on these findings the chromatogram exhibited distinct peaks, most prominent peaks at Rt 7.146 min corresponding to the peak around 7.1-7.2 min of Xanthatine sta The GC–MS profile findings of the *Xanthium* extract revealed a complex chromatographic profile with a large number of detected peaks, indicating the presence of diverse volatile and semi-volatile phytochemical constituents. A total of 56 compounds were detected across retention times ranging approximately from 4 to 35 minutes, accounting for 100% of the total chromatographic area. The major Identified phytochemicals by GC-MS were; Monoterpene hydrocarbons (limonene, thujene, phellandrene derivatives); monoterpenoids (verbenol, pinocarvone, fenchone), seen in Table 4 , sesquiterpenes Table 5, different fatty acids and non-polar compounds as in Table 6.the monoterpenes and fatty acids give a significant proportion of bioactive compounds in the hexane extract as seen in Figure 4. Several compounds showed relatively high peak areas, indicating their predominance in the extract. The most abundant constituents included long-chain oxygenated compounds and terpene-related

molecules, with individual compounds reaching relative abundances of more than 10% of total peak area as in Figure 5&6.

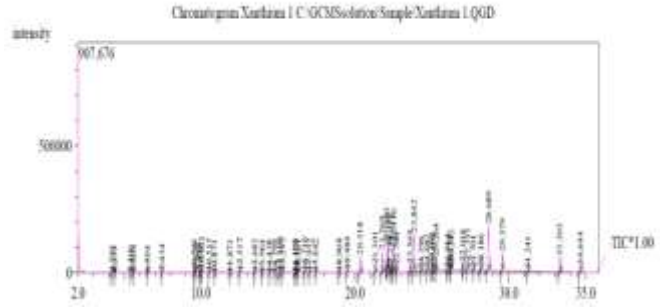

Figure 4: GC/MS chromatogram of hexane extract from *Xanthium orientale.*

Table 4: Monoterpenes in GCMS spectrum of hexane extract.

Compound name	Chemical class	Molecular formula	Molecular weight (g/mol)	SI
α-Phellandrene	Monoterpene hydrocarbon	$C_{10}H_{16}$	136	85
α-Thujene	Monoterpene hydrocarbon	$C_{10}H_{16}$	136	94
β-Thujene	Monoterpene hydrocarbon	$C_{10}H_{16}$	136	89
Sabinene	Monoterpene hydrocarbon	$C_{10}H_{16}$	136	92
Limonene	Monoterpene hydrocarbon	$C_{10}H_{16}$	136	67
Fenchone	Oxygenated monoterpene	$C_{10}H_{16}O$	152	64
Pinocarvone	Oxygenated monoterpene	$C_{10}H_{14}O$	150	89
Verbenol	Oxygenated monoterpene	$C_{10}H_{16}O$	152	80
Borneol (endo-)	Oxygenated monoterpene	$C_{10}H_{18}O$	154	85

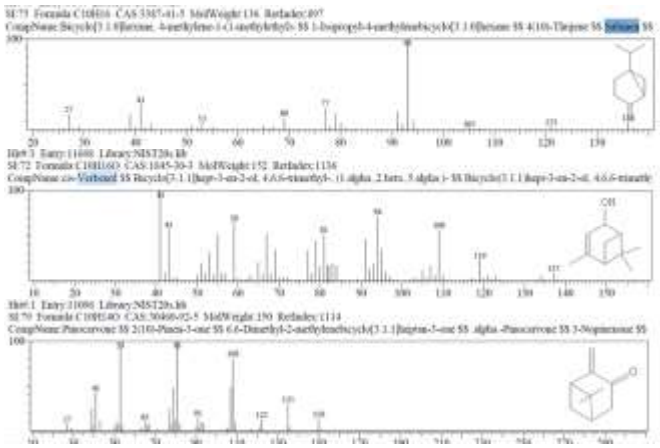

Figure 5: GC/MS Spectrum of Monoterpenes in hexane extract.

Table 5: Sesquiterpenes (C_{15} skeleton) in GCMS spectrum of hexane extract.

Compound name	Chemical class	Molecular formula	Molecular weight (g/mol)	SI
cis-Muurola-4(15),5-diene	Sesquiterpene hydrocarbon	$C_{15}H_{24}$	204	65
Long-chain terpenoid derivatives (unresolved isomers)	Sesquiterpene-related	$C_{15}H_{24}$ – $C_{15}H_{26}$	~204–206	60–70

Table 6: Fatty Acids and Fatty Acid Derivatives in GCMS spectrum of hexane extract.

Compound name	Chemical class	Molecular formula	Molecular weight (g/mol)	SI
Isovaleric acid (3-Methylbutanoic acid)	Fatty acid	$C_5H_{10}O_2$	102	79
Propanoic acid	Short-chain fatty acid	$C_3H_6O_2$	74	99
4-Chlorobutanoic acid	Fatty acid derivative	$C_4H_7ClO_2$	122	74
Long-chain alkyl phenols (anacardol-type)	Fatty acid–related phenolics	$C_{21}H_{36}O$	304	65

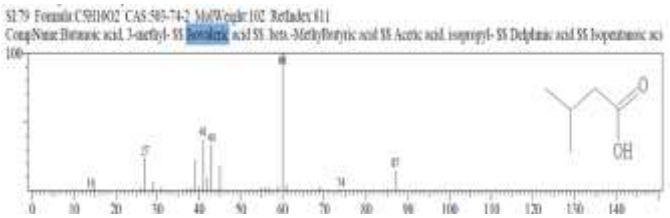

Figure 6: GCMS spectrum of fatty acid in hexane extract.

Antibacterial activity

The antibacterial activity of the *xanthium oriental* extract was evaluated using the agar well diffusion method against Escherichia coli and klebsiella pneumonia. The results show good zones of inhibition were observed around wells containing the extract depend on highest concentrations (75-100 mg/ml), as seen in Figure 7and Table 7. However, Klebsiella pneumoniae and E. coli shows sensitive to the extract with high concentration. However, higher susceptibility was observed in Klebsiella pneumoniae compared with Escherichia coli. However, the hexane extract exhibited that E. coli has highest inhibition zones was about (17 mm), while the lowest

effect observed (12 mm). In contrast, the bacteria strains of Klebsiella pneumoniae reveals inhibition of 19 mm with concentration at 100 mg/ml.

Table 7: zone of inhibitions (mm) of hexane extract of *xanthium orientale* against Two different bacterial strains.

Bacterial Strains	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Escherichia coli	12.0±0.5	14.0±0.6	15.0±0.5	17.0±0.4
Klebsiella Pneumoniae	15.0±0.4	16.0±0.5	18.0±0.5	19.0±0.6

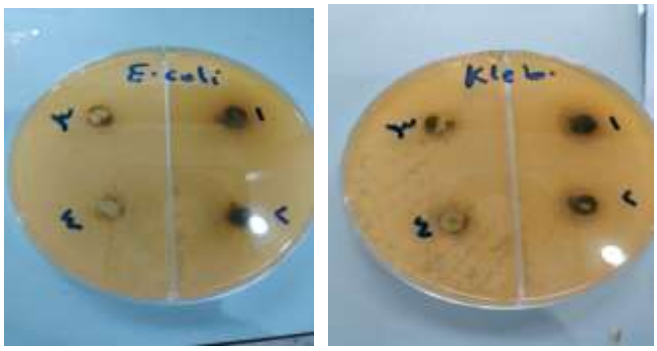

Figure 7: Agar well diffusion method for screening the antibacterial activity of hexane extract Iraqi *Xanthium oriental le*aves on the Agar Mueller- Hinton.

Based on presented findings, the HPLC results revealed Xanthatine is a major compound in this plant represents the sesquiterpene lactone, which has different pharmacological activity with significant antimicrobial and cytotoxic potential. [34]. Moreover, GC-MS analysis exhibited the presence of monoterpenes in *xanthium oriental as* the dominant chemical class, followed by sesquiterpene hydrocarbons and fatty acid derivatives. Oxygenated monoterpenes such as borneol, verbenol, and fenchone were also detected. Overall considered the prominent chemicals reported in the genus xanthium [35]. These dominant compounds are known to contribute to antimicrobial, antioxidant, and anti-inflammatory activities reported for *Xanthium* species in previous studies [34,35]. Furthermore, the obtained antibacterial activity results against the investigated bacterial strains (K. Pneumoniae and E. coli) showed dose -dependent sensitivity, using disc diffusion assay. The observed evidence may affect the bacterial metabolic activity due to presence of bioactive phytochemicals. Moreover, Klebsiella pneumoniae exhibited greater sensitivity than Escherichia coli, which may be related to the ability of extract to alter the integrity of membrane, disrupting bacterial cell, and altering the structural components. The secondary metabolites found in plants, such as terpenes and phenolic compounds, have been reported have different effects on mechanisms of bacterial action through multiple mechanisms. Consequently, interference of cell wall synthesis, membrane damage, disruption of nucleic acid synthesis, and affect the permeability cause to variations in outer membrane and then cell death [36]. For this reason, the knowledge of plant antibacterial action is important in different applications of infectious treatment and resistance. In summary, the study indicates that the plant extract possesses a significant antibacterial activity. Therefore, further studies are needed to improve its effectiveness through combination with other medicinal plants have potent activity.

V. CONCLUSION

The presented study demonstrated that Iraqi *Xanthium orientale L.* leaves contain a diverse class of secondary metabolites, including flavonoids, terpenoids, steroids, tannins, saponins, and carbohydrates. Chromatographic analysis using HPLC, and GC/MS confirmed the several non-polar compounds, particularly monoterpenes, fatty acid derivatives, sesquiterpenes and lactone xanthatin. The detected concentration -dependent antibacterial effect of hexane extract as against Escherichia coli and Klebsiella pneumoniae may be associated with synergistic effect of these constituents. The hexane extract exhibited concentration-dependent antibacterial activity against, with greater susceptibility observed in Klebsiella pneumoniae. The detected antibacterial effect may be associated with the synergistic action of the identified phytochemical constituents, particularly terpenoids and phenolic compounds. According to the available literature, this study represents the first phytochemical investigations for this species in Iraq. Nevertheless, further phytochemical analysis, isolations and pharmacological assessment are required.

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