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Phytochemical Screening and Antimicrobial Activity Study of Portulaca oleracea Cultivated in Iraq

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ABSTRACT: An urgent reexamination of natural botanical reservoirs for new bioactive chemicals is required due to the growing worldwide challenge of antimicrobial resistance. The phytochemical profile and antibacterial effectiveness of *Portulaca oleracea* grown in *Iraq's* unique soil and climate conditions are thoroughly examined in this study. The plant's combined leaf and flower tissues were used to carefully create ethanolic extracts, which were then put through a rigorous analytical process. Qualitative phytochemical screening assays were used to identify the presence of main classes of secondary metabolites. Using the standardized agar well diffusion method, the extract's antimicrobial efficacy was thoroughly assessed against a panel of therapeutically important pathogenic pathogens, including both Gram-positive and Gram-negative bacterial strains and a fungal yeast. The abundance of important bioactive substances, such as flavonoids, alkaloids, saponins, tannins, and terpenoids, was definitively validated by the phytochemical screening. The extract showed strong antifungal activity against the opportunistic yeast pathogen *Candida albicans* and significant, concentration-dependent antibacterial activity against Gram-positive bacteria, particularly *Staphylococcus aureus* and *Staphylococcus epidermidis*, in antimicrobial assays. The combined results of this study firmly support *Portulaca oleracea* potential role as a promising, natural source of antimicrobial agents and offer strong scientific validation for the plant's traditional therapeutic uses, potentially opening up new approaches to the difficult problem of antibiotic resistance.

1. INTRODUCTION

Throughout history, medicinal plants have been an essential and fundamental source of bioactive compounds. They are the basis of many different cultures' ancient traditional therapeutic systems as well as vital lead compounds for contemporary pharmaceutical research and development. In order to bridge the gap between ethnobotanical wisdom and evidence-based pharmacology, the empirical information gathered over millennia about the therapeutic qualities of flora is increasingly being examined and validated by modern scientific approaches. The genus Portulaca has garnered substantial and increasing attention because of its proven durability, capacity to adapt to challenging conditions, and remarkable pharmacological flexibility. *Portulaca oleracea L.*, commonly known as purslane. This resilient succulent, belonging to the Portulacaceae family, has a long-standing history of use in traditional medicine systems across diverse cultures [1]. This family is comprised of succulent plants predominantly found in tropical and subtropical regions. These plants are well-adapted to arid environments due to their specialized water-storing tissues, which allow them to survive in conditions of low rainfall. Species within this family, including *Portulaca oleracea,* typically feature fleshy leaves and stems, a key adaptation for drought tolerance. The family holds significant importance in ethnobotany, with many of its members, such as Portulaca oleracea, being utilized in both traditional and modern medicine for their recognized therapeutic properties correlated with the biosynthesis of unique and potent secondary metabolites as adaptive survival strategies [2]. These very metabolites including alkaloids, flavonoids, terpenoids and essential oils

are frequently the source of significant pharmacological potential, offering antioxidant, anti-inflammatory, neuroprotective, and inherent antimicrobial properties. Recent and ongoing phytochemical investigations have begun to systematically document the intricate chemical richness of *Portulaca oleracea*, identifying a broad spectrum of critical secondary metabolites such as flavonoids (e.g., quercetin [3], kaempferol [4]), alkaloids, organic acids (e.g., malic acid, oxalic acid), and essential oils (e.g., geraniol, linalool, myrcene).[1] These compounds are known to act not in isolation, but rather in synergistic or additive manners, collectively contributing to the plant's significant and multifaceted biological efficacy, which may surpass the activity of individual purified compounds. In the urgent contemporary context, the global surge in antimicrobial resistance (AMR) represents one of the most pressing public health crises of the 21st century [5]. The relentless evolution of bacterial, fungal, and viral pathogens has rendered many conventional synthetic antibiotics increasingly ineffective, leading to treatment failures, higher healthcare costs, and increased mortality [6]. This alarming scenario has intensified and refocused the scientific exploration of plant-derived natural products as promising, sustainable, and often mechanistically novel alternatives or adjuncts to synthetic antimicrobial drugs. Plants like *Portulaca oleracea* with a history of ethno medicinal use for infections, are now viewed as valuable resources that may offer new chemical scaffolds capable of bypassing existing microbial resistance pathways, disrupting biofilms, or modulating host immune responses.

Morphologically, *Portulaca oleracea* is a hardy, fast-growing annual succulent plant that typically grows to a height of 15–40 cm. It is characterized by its fleshy, succulent leaves that are obovate to spatulate in shape, and smooth, often reddish, prostrate to ascending stems. These water-storing features enable the plant to thrive in arid and hot environments with minimal water requirements. The plant produces small, vibrant flowers, predominantly yellow, which notably bloom in the morning and close by midday, leading to its common name "Eleven O'clock Flower."

Its adaptability makes it suitable for cultivation in drought-prone regions and a popular ornamental plant, while also contributing to its resilience for medicinal applications 7(Figure 1).

A B

Figure 1. *Portulaca oleracea* (A) Stem, (B) Succulent leaves and a developing flower bud.

Among the plants of notable ethnobotanical and pharmacological interest is Portulaca oleracea L., commonly known as purslane. This resilient succulent, belonging to the Portulacaceae family, has a long-standing history of use in traditional medicine systems across diverse cultures 1. It has been traditionally employed for a wide spectrum of conditions, including the treatment of skin infections, acceleration of wound healing, and alleviation of inflammatory conditions 7,8. Contemporary scientific research has increasingly validated these traditional applications, highlighting Portulaca oleracea L., as a promising source of bioactive compounds with significant potential for applications in the pharmaceutical and cosmetic industries 1,8,9. The plant is also traditionally used as a poultice or wash to reduce swelling, inflammation, and alleviate pain associated with arthritis and muscle inflammation by inhibiting key inflammatory mediators 10,11. Furthermore, it finds application in skin

disorders which explained vy plant's rich antioxidant compounds contribute to its efficacy in treating skin ailments like rashes, eczema, and psoriasis by reducing oxidative stress and promoting cellular regeneration 8, 10,11. The plant's historically recognized broad-spectrum antimicrobial and antifungal properties further solidify its utility in treating a spectrum of localized and systemic infections, particularly against dermatophytes ,this activity is attributed to compounds that disrupt fungal cell membranes, inhibiting their growth 1and historically the leaves and stems of the plant are often applied topically to cuts, wounds, and burns. This practice is supported by its antimicrobial and anti-inflammatory properties, which promote tissue repair and reduce the risk of infection 8, 12.

This vast and historically acknowledged pharmacological potential is directly attributable to its complex and diverse chemical profile, which includes alkaloids, organic acids (e.g., malic acid, oxalic acid), and essential oils (e.g., geraniol, linalool, myrcene).1. Moreover, the presence of potent flavonoids (e.g., quercetin and kaempferol) confers not only vivid pigmentation but also strong antioxidant, anti-inflammatory, and antibacterial properties. These phytochemicals are vital not only in direct antimicrobial action via membrane disruption or enzyme inhibition but also in combating oxidative stress a fundamental pathological process implicated in aging, chronic inflammation, neurodegenerative diseases, diabetes, and numerous degenerative disorders. The antioxidant activity neutralizes reactive oxygen species (ROS), thereby protecting cellular components from damage and modulating inflammatory cascades 13,14.

Despite this promising ethnobotanical background and growing scientific interest, there remains a distinct insufficiency of focused, region-specific research examining the precise chemical profile and consequent bioactivity of Portulaca oleracea cultivated under the unique environmental conditions of Iraq. Abiotic factors such as soil composition, pH, micronutrient availability, climatic stressors (high temperature, intense solar radiation, low humidity), and water availability can profoundly influence the biosynthesis, accumulation, and relative proportions of secondary metabolites in plants a phenomenon known as chemotypic variation. Therefore, a plant studied in one geographical region may possess a quantitatively or qualitatively different phytochemical profile when grown in another, leading to potentially different therapeutic potencies. This gap underscores the necessity for localized phytochemical screening to accurately assess the medicinal potential of flora within specific ecosystems15. Consequently, this study was explicitly designed to address this critical research gap. It aimed to perform a comprehensive qualitative phytochemical screening of the ethanolic extract of Iraqi-cultivated Portulaca oleracea to establish its unique chemical fingerprint. Concurrently, it sought to rigorously evaluate the extract's in vitro antibacterial and antifungal efficacy against a panel of pathogenic microorganisms highly relevant to community and healthcare-associated infections. These interconnected objectives were strategically formulated to achieve two primary goals: first, to provide scientific validation for its traditional ethnomedicinal uses within the regional context; and second, to critically assess its potential as a source of novel phytotherapeutic agents or lead compounds that could contribute to the arsenal of natural products in the ongoing global fight against resilient and multidrug-resistant microbial infections, offering potential solutions rooted in local biodiversity16 .

2. MATERIALS AND METHODS

2.1 Study Material

Fresh *Portulaca oleracea* specimens from several nurseries in *Baghdad, Iraq*, served as the plant material used in this investigation.

2.2 Sample Collection

The fresh plant material was meticulously handled after collection.
After being physically separated from the stems, the leaves and blooms were thoroughly cleaned under running tap water to r emove any remaining dust, soil particles, and epiphytic microbes.
The cleaned plant pieces were then thinly spread out on sterile trays and left to air dry at room temperature in a well-ventilated, shady environment. In order to preserve the original phytochemical integrity of the plant material and avoid the thermal degradation of heatlabile (thermolabile) bioactive chemicals, such as certain flavonoids and volatile terpenoids, this gentle drying process was purposefully chosen over oven-drying.

2.3 Extraction Procedure

The extraction protocol was based on the conventional soxhlet extraction technique, favoured for its simplicity and effectiveness in extracting a broad range of secondary metabolites. Approximately 50 grams of the dried, coarsely powdered plant material (comprising a 1:1 ratio of leaves and flowers) was accurately weighed and first extracted with 500 ml hexane

followed by extraction with 500 ml of 70% aqueous ethanol (v/v) was added as the extraction solvent. The choice of 70% ethanol represents a balanced solvent system, as it is sufficiently polar to extract glycosides and polar flavonoids while also being able to dissolve moderately non-polar compounds like terpenoids and alkaloids. After the extraction the mixture was filtered sequentially through several layers of muslin cloth followed by Whatman filter paper (No. 1) to obtain a clear, particle-free filtrate. The marc (the residual plant material) was pressed gently to recover any retained solvent. The combined filtrate was then concentrated using a rotary evaporator (Heidolph, Germany) operating under reduced pressure at a controlled temperature of 40°C. This process carefully removed the ethanol solvent, yielding a dense, semi-solid, dark greenish crude extract. The crude extract was further dried in a desiccator over anhydrous calcium chloride to remove any trace moisture, resulting in a final dry extract. The percentage yield of the extract was calculated based on the initial dry weight of the plant powder. The obtained dry extract was stored in a sterile, tightly sealed vial at 4°C in a refrigerator to inhibit any microbial growth or chemical degradation until it was required for subsequent phytochemical and biological analyses.[17] By mixing 100 mg of dried extract with 1 ml of ethanol solvent, a 100 ml/mg stock solution was gained. To perform antimicrobial testing, serial dilutions were produced at 50, 25, and 12.5 mg/ml concentrations.[18]

2.4 Analytical Methods

Ethanolic extract phytochemical screening: Qualitative phytochemical analyses of the 70% ethanolic extract was performed using a standard method to find out various secondary metabolites:

Test for saponins (Foam Test)

We put the fresh plant in a beaker and let it boil for five minutes, as showen in figure (2.2). Then, we put 1 ml of it in a test tube, added 5 ml of distilled water, and shaken it well until persistent foam appear. (19)

Test for terpenoids (Salkowski Test)

Ethanolic extract of the leaves (5 ml) was mixed with chloroform (2 ml) and concentrated sulphuric acid (3 ml) was carefully added. A reddish-brown colour of Interface will form to indicate the presence of terpenoids. (19)

Test for flavonoid

To 2 ml of ethanol extract, add 3 drops of sodium hydroxide. The color of extract becomes yellow in the tube (yellow colour indicates a positive result). (19)

Test for Alkaloid

1- Mayer test: A few drops of Mayer reagent were added to the ethanolic extract of the plant (2 ml) (Positive result is green precipitate).

2- 2- Wagner test: A few drops of Wagner reagent were added to the ethanolic extract of the plant (2 ml) (The positive result is brown reddish precipitate). (20)

2.5 Statistical Analysis

Microorganisms and culture conditions: Antimicrobial evaluation was undergone at the College of Science, Al-Mustansiriyah University.

Experimental creatures included:

•Gram-positive bacteria: *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus* species

•Gram-negative bacteria: *Escherichia coli, Klebsiella* species

•Fungal strain: *Candida albicans*

Assay of antibacterial and antifungal activities: For the measurement of the efficacy against microbes, the agar well diffusion was utilized. Standardized microbiological suspensions were utilized to seed Mueller-Hinton Agar plates (for bacteria) and Sabouraud Dextrose Agar plates (for fungus), at 1.5 × 10^8 CFU/mL concentration (0.5 McFarland standard). Agar was punctured with wells of 6 mm diameter. After that, 100 µL of the ethanolic extract with doses of 100, 50, 25 and 12.5mg/mL

was introduced. The negative control was methanol. At 37°C, the plates were maintained for full day to evaluate the antibacterial activity. The inhibition zones were determined in millimetres (Fig. 2). [19]

3. RESULTS AND DISCUSSION

3.1 Preliminary Analysis

Initial screening for phytochemical properties: Alkaloids, terpenoids, Tannins and flavonoids found in *Portulaca oleracea* are shown in Table (1):

Table 1. Qualitative profile of phytochemicals in *Portulaca oleracea*

Tests	Alkaloid	Flavonoid	Terpenoids	saponins
Result	+	+	+	-

3.2 Biological Activity

Antimicrobial and antifungal activities of the ethanolic extract: The aqueous ethanol (70%) extract showed concentration-dependent antimicrobial activity against the tested microorganisms Table (2):

Table 2. Inhibition zones (mm) of ethanolic extract of leaf and flower parts of *Portulaca oleracea* cultivated in *Iraq* against tested microorganisms (Agar well diffusion method).

Bacterial species	Concentrations of aqueous ethanol 70% extract of leaf and flower parts of *Portulaca oleracea* cultivated in *Iraq* (mg/ml) /inhibition zone in millimeters by agar well diffusion method			
	S1	S2	S3	S4
Staphylococcus aureus	38	14	12	0
Staphylococcus epidermidis	18	12	0	0
Escherichia coli	0	0	0	0
Candida albicans	36	14	12	0

The qualitative phytochemical screening of the aqueous ethanolic 70% extract of *Portulaca oleracea* indicated the presence of alkaloids, flavonoids, and terpenoids/steroids. Saponins were found to be absent based on the negative froth test. The positive results for alkaloids, flavonoids, and terpenoids/steroids are consistent with the extensive literature on *Portulaca oleracea*, which documents it as a rich source of these bioactive compounds.[21] Alkaloids such as oleraceins, various flavonoids like quercetin and kaempferol, and diverse terpenoids contribute significantly to the plant's medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory effects.[21,22,23]The methods used for these tests are standard qualitative procedures for detecting these phytochemical classes. The negative result for saponins in this particular analysis is noteworthy. While many studies report the presence of saponins in *Portulaca oleracea* [20] their detectability can be influenced by various factors including the specific plant chemo type, environmental conditions during growth, and the extraction and testing procedures. It is possible that the concentration of saponins in the prepared 70% ethanolic extract was below the detection limit of the froth test, or that this particular sample had a naturally low saponins content. The absence of significant froth formation is a clear indicator for this test.

The aqueous ethanolic 70% extract of *Portulaca oleracea* demonstrated notable antimicrobial activity against the majority of the tested microorganisms, with the inhibitory effect generally appearing to be concentration-dependent. Activity against Gram-Positive Bacteria: The extract exhibited strong activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, which progressively decreased with subsequent dilutions. This potent activity against pathogenic *Staphylococci* is significant and aligns with findings in the literature that attribute such effects to the presence of flavonoids and alkaloids in *Portulaca oleracea* [20,23], which were detected in our phytochemical screening Table (1).

Activity against Gram-Negative Bacteria: no discernible activity was observed against *Escherichia coli* with any of the tested concentrations. The outer membrane of Gram-negative bacteria like *Escherichia coli* often presents a significant barrier to antimicrobial agents. [21] While some studies report activity of *Portulaca oleracea* against *Escherichia coli*, [21] the lack of activity in this instance could be due to strain specificity, insufficient concentration of active components effective against this particular barrier, or the specific phytochemical profile of the extract used.

Activity against Fungi: The extract displayed strong antifungal activity against *Candida albicans*, with large inhibition zones observed across tested concentrations. This is a particularly important finding given the prevalence of candidiasis. Although saponins (often associated with antifungal activity [24] were not detected in our phytochemical analysis, other compounds present in *Portulaca oleracea*, such as certain flavonoids and terpenoids (both detected in our extract), are also known to possess antifungal properties [21,24], and could be responsible for this significant inhibition.

Correlation with Phytochemical Profile and Literature: The observed broad-spectrum antimicrobial activity (excluding *Escherichia coli*) is well supported by the phytochemical profile indicating the presence of alkaloids, flavonoids, and terpenoids. These classes of compounds are widely reported to exert antimicrobial effects through various mechanisms, including disruption of cell membranes, inhibition of essential enzymes, or interference with nucleic acid synthesis. [21,25] The concentration-dependent nature of the activity is typical for antimicrobial agents.

The phytochemical richness of *Portulaca oleracea* is well-documented in scientific literature, with each class of compounds contributing distinct therapeutic properties. antibacterial activity. Specific research highlights the plant's antibacterial properties against key pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa.* [26,27] Beyond antimicrobial activity, the high concentration of flavonoids and phenolic compounds in *Portulaca oleracea* establishes it as an effective antioxidant agent. These compounds scavenge free radicals and chelate pro-oxidant metal ions, thereby preventing oxidative stress, which is a key contributor to chronic illnesses such as cardiovascular diseases, diabetes, and cancer. Furthermore, extracts of *Portulaca oleracea* have shown compelling anti-inflammatory properties in experimental models, specifically through the inhibition of pro-inflammatory cytokines like TNF-α and IL-6. This specific activity has prompted researchers to investigate its potential utility in the management of autoimmune and chronic inflammatory disorders.

3.4 Discussion

The results of this comprehensive study into *Portulaca oleracea* cultivated in *Iraq* highlight the plants potential as a source of novel bioactive compounds and provide significant insights into the phytochemical basis of its traditional therapeutic uses. the combination of standardized antimicrobial and qualitative phytochemical screening offer a solid, multifaceted validation of the plant's pharmacological potential while also highlighting significant issues for more research. [28] The verified presence of terpenoids, saponins, tannins, and alkaloids is consistent with the recorded phytochemistry of the Po rtulacaceae family and directly connects to the claimed traditional uses of the plant. [16] Terpenoids, a vast and structurally diverse class, are often implicated in antimicrobial and anti-inflammatory activities through mechanisms such as membrane disruption and inhibition of pro-inflammatory mediators like prostaglandins and leukotrienes. It is also noteworthy that both Mayer's and Wagner's tests for alkaloids yielded very favorable results. Alkaloids, such as morphine, quinine, and vincristine, are among the most therapeutically significant groups of plant metabolites. Given that many alkaloids disrupt nucleic acid synthesis, cell division, or neurotransmitter systems, their significant concentration in *Portulaca oleracea* indicates a high potential for neuroactive, analgesic, antibacterial, and anticancer activities. Since the extraction solvent (70% ethanol) or the particular test used may not have been ideal for their isolation or detection, the lack of detected glycosides in this particular extract does not completely rule out their presence; further research using different solvents or enzymatic hydrolysis may shed light on this point. These phytoconstituents are evolutionary adaptations for plant defense against herbivores and pathogens, however they inadvertently provide a wide range of medical benefits to humans, including antioxidant, anti-inflammatory, antibacterial, and

cytotoxic actions. This chemical arsenal offers a credible mechanistic foundation for the plant's historical application in addressing infections, inflammation, and dermatological conditions. [29] The combined presence of these compounds, even in minor quantities, within the ethanolic extract of Iraqi *Portulaca oleracea* reinforces its status as a chemically diverse repository and indicates that the overall biological effect is likely due to synergistic or additive interactions among various active principles, a typical and beneficial characteristic of whole plant extracts. The outcomes of the antibacterial evaluation employing the agar well diffusion technique offer persuasive, practical proof endorsing the plant's ethno medicinal applicability. The extract demonstrated significant in vitro antimicrobial activity against the Gram-positive Bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*, and the fungi *Candida albicans*. The activity was generally concentration-dependent. No activity was observed against *Escherichia coli.* These findings are consistent with the ethnopharmacological uses of *Portulaca oleracea* and are supported by its rich phytochemical composition. The identified compounds likely contribute synergistically to the observed antimicrobial efficacy. The study underscores the potential of *Portulaca oleracea* cultivated in the region as a source of natural antimicrobial agents.

4. CONCLUSION

This study was undertaken to investigate the phytochemical composition and evaluate the in vitro antimicrobial activity of an aqueous ethanolic 70% extract of *Portulaca oleracea* cultivated in the region. The research successfully achieved its objectives, leading to the following key conclusions: 1. Phytochemical Profile: The qualitative phytochemical screening of the aqueous ethanolic 70% extract of *Portulaca oleracea* confirmed the presence of several important classes of bioactive secondary metabolites. These include alkaloids, flavonoids, and terpenoids/steroids. Under the conditions of this study, saponins were not detected. This phytochemical profile is largely consistent with existing literature, underscoring the plant's richness in compounds known for their therapeutic potential activity including antimicrobial Activity as the aqueous ethanolic 70% extract of *Portulaca oleracea* demonstrated significant and broad-spectrum antimicrobial activity against the tested microorganisms. The extract exhibited strong inhibitory effects against the Gram positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* and potent antifungal activity was demonstrated against *Candida albicans*. The antimicrobial effects were generally concentration dependent, with higher concentrations of the extract (at 100 mg/mL) showing greater zones of inhibition. However, under the experimental conditions and at the concentrations tested, the extract did not show discernible activity against *Escherichia coli*. The demonstrated antimicrobial activities, particularly against common pathogenic bacteria and fungi, provide scientific support for some of the traditional medicinal uses of *Portulaca oleracea* in treating various infections. The presence of key phytochemical groups known for their antimicrobial properties (alkaloids, flavonoids, terpenoids) further substantiates these findings. The results highlight *Portulaca oleracea* as a promising natural source for the development of new antimicrobial agents. Its efficacy against a range of microbes, including those of clinical relevance, suggests its potential utility in phyto medicine or as a source for isolating lead antimicrobial compounds. In summary, this research contributes to the scientific understanding of the phytochemical constituents and antimicrobial properties of *Portulaca oleracea*, reinforcing its value as a medicinal plant and suggesting its potential for further pharmacological exploration.

AUTHOR CONTRIBUTIONS

ZAINEB AZIZ ALI AL-ABBASI: Conceptualization, Investigation, Methodology, Laboratory Analysis.

Omar Waleed Abduljaleel Albasri: Data Curation, Formal Analysis.

Ruaa Aziz Jassim: : Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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