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Antimicrobial activity of *Portulaca oleracea* leaves extracts

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Abstract
Throughout all medical traditions, folk medicine has utilized plants since ancient times. Oral hygiene folklore uses a variety of herbs and plant-derived antibacterial components. Portulaca oleracea extracts, both water and ethanolic, were evaluated for their antimicrobial activity against a range of microbial strains, including four bacterial species: *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Additionally, the extracts were tested against two fungal species, *Aspergillus niger* and *Fusarium exosporium*, as well as *Candida albicans*.

Key Words: Aspergillus niger, C. albicans, Escherichia coli, Fusarium exosporium, Listeria monocytogenes, Pseudomonas aeruginosa, Portulaca oleracea, Staphylococcus aureus

1.Introduction

There are 25–30 genera and 450–500 species in the Portulacaceae family, with Portulaca being the biggest genus.[1]. *Portulaca oleracea L.*(PO) is an annual grassy [2] plant with tiny black seeds, yellow or white flowers, succulent leaves, and a meaty stem. It can be found all across the world, but particularly in tropical and subtropical regions. Countries in the Mediterranean, central Europe, and Asia have long employed purslane as a medicinal herb. [4] and has been given the term Global Panacea [5].

One of the most beneficial medicinal herbs is purslane, which is categorized as a C4 plant. [6]. In many nations, it is utilized as a herbal remedy with pharmacological properties such as analgesic, antibacterial, skeletal muscle relaxant, wound-healing, and diuretic, febrifuge, vermifuge, antiseptic, and anti-spasmodic effects. [7], anti-inflammatory [8], radical scavenger [2] and anti-convulsant [9]. Additionally, it has been used to treat a variety of illnesses, such as headaches, fevers, sleeplessness, liver inflammation, kidney and bladder ulcers, respiratory conditions, and gastrointestinal disorders.

[4]. Moreover, PO is known to regulate the lipid and sugar metabolism in the body [6]. Similar in flavor to spinach, the stems and leaves of *Portulaca oleracea* (purslane) are edible. The aerial parts of this plant have medicinal uses, serving as an antiseptic and helping to reduce pain and swelling. In China, the dried plant is often boiled to make soup or tea. Studies have shown that the aqueous extract of *Portulaca oleracea* exhibits no cytotoxicity or genotoxicity, which further supports its use as a safe daily vegetable. Additionally, the methanolic extract of *Portulaca oleracea* has demonstrated antimicrobial activity, particularly against *Bacillus subtilis*, showcasing its potential as a natural antimicrobial agent [10]. Omega-3 fatty acids, which are usually found in fish oil and animal fats but not in most plants, are also present in *Portulaca oleracea* (purslane). These fatty acids are known for their significant role in boosting immune function, along with a range of other health benefits. The presence of omega-3s in purslane makes it a noteworthy plant-based source of these essential nutrients, enhancing its potential as a health-benefiting food [15] in the avoidance and management of cancer, heart disease, hypertension, and other autoimmune and inflammatory diseases [16]. *Portulaca oleracea* contains essential fatty acids, including linoleic acid and α-linolenic acid, which are crucial for promoting human health, supporting proper growth, and preventing disease. The polysaccharides found in *Portulaca oleracea* have the potential to serve as therapeutic agents for treating diabetes mellitus, as they can influence blood lipids, metabolism, and blood glucose levels. Additionally, the plant contains monoterpenes such as portulosides A and B, diterpenes like portulene, and β-amyrin-type triterpenoids, all of which contribute to its medicinal properties. [19, 17]; Additionally, vitamins have been extracted from this plant's leaves. Of all the green leafy vegetables, it has the highest concentration of vitamin A, a naturally occurring antioxidant that is crucial for healthy mucous membranes, eyesight, and the prevention of malignancies of the mouth and lungs. Ascorbic acid, α-tocopherol, and B-complex vitamins like riboflavin, pyridoxine, and niacin are also present in this plant.

[18]. is rich in essential amino acids such as isoleucine, proline, leucine, lysine, phenylalanine, methionine, cystine, valine, threonine, and tyrosine[20]. It also contains important minerals, including phosphorus, manganese, iron, calcium, and selenium [21]. In addition to these nutrients, the plant has been found to contain a variety of other bioactive compounds, such as beta-carotene, glutathione, melatonin, portulacerebroside A, catechol, and bergapten.

2.Materials and Methods

Plant material :-

The fresh leaves of *Portulaca oleracea* were collected from Qaliubia in 2023. Upon arrival at the laboratory, the samples were promptly cleaned by removing soil debris and unhealthy portions. The leaves were then air-dried under shaded conditions at room temperature until they reached a constant weight. After drying, the leaves were ground into a fine powder and stored for further analysis.

Selective Extraction Technique :-

The air plant dried powder was extracted completely once using Soxhlet apparatus with the following extraction :- Water and 70٪ ethanol.

To evaluate the antimicrobial activity of water and ethanolic extracts of *Portulaca oleracea*, the following test organisms were used: four bacterial strains—*Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*—as well as two fungal species, *Aspergillus niger* and *Fusarium exosporium*, and *Candida albicans* [3]. The antimicrobial assay was carried out in 96-well flat polystyrene plates. To each well, 10 µl of the test extracts (with a final concentration of 250 µg/ml) was added to 80 µl of lysogeny broth (LB broth), followed by the addition of 10 µl of microbial culture suspension in its logarithmic growth phase. The plates were incubated overnight at 37°C, allowing the extracts to interact with the microbes. After incubation, a positive antimicrobial response was evidenced by the formation of clear zones in the wells, indicating inhibition of microbial growth. In contrast, wells where no antimicrobial effect was observed showed opaque media due to continued bacterial growth. The control consisted of the pathogens without any treatment for comparison. Absorbance readings were taken around 20 hours later at OD600 using a Spectrostar Nano Microplate Reader (BMG LABTECH GmbH, Allmendgrun, Germany), providing an accurate measure of microbial activity.

Characterization of bimetallic ZnO-CuO NPs

The absorbance and spectral properties of the synthesized bimetallic ZnO-CuO nanoparticles (NPs) were analyzed using a UV-Vis spectrophotometer (JASCO V-560). A separate experiment, devoid of metal ions, was conducted for auto-zero calibration purposes. Initially, the optical characteristics of each sample were examined to determine the appropriate wavelengths necessary for accurate absorbance measurement.

The particle size distribution of the prepared bimetallic ZnO-CuO NPs was determined using dynamic light scattering (DLS) at the St. Barbara facility in California, USA, with the DLS-PSS-NICOMP 380-ZLS particle size system. A 100 µL sample of the nanoparticles was placed in a small cuvette, and after allowing it to equilibrate for two minutes at 25.0 ± 2°C, five measurements were conducted.

To further investigate the morphology, size, and overall structure of the bimetallic ZnO-CuO NPs, high-resolution transmission electron microscopy (HR-TEM, JEM2100, Jeol, Japan) was used. This technique enabled the analysis of the crystallization, crystallite size, and overall morphology of the nanoparticles. Additionally, X-ray diffraction (XRD) analysis was performed using a Shimadzu XRD-6000 system and SSI, Japan, with the 2θ diffraction angle providing information on the X-ray diffraction pattern.

The surface structure and arrangement of the bimetallic ZnO-CuO NPs were further evaluated by scanning electron microscopy (SEM, ZEISS EVO-MA10, Germany), particularly to study their interaction with the surrounding water extract of *Portulaca oleracea* leaves. Finally, the surface charges of the nanoparticles were measured using a Zeta potential analyzer from Malvern Instruments, UK, at the pH of preparation [24].

3.Results

Bioassay against bacteria and fungi

Antibacterial activity

The presented data outlines the antimicrobial efficacy of different compounds water extract of *Portulaca oleracea* (A1) and 70 ٪ethanolic extract of *Portulaca oleracea* (A2) against four bacterial strains: Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, and Staphylococcus aureus. The antibacterial activity was quantified in terms of percentage inhibition, where higher values indicate greater efficacy.

For water extract of *Portulaca oleracea* (A1) Escherichia coli exhibited a moderate inhibition of 30.30% In the case of Listeria monocytogenes, water extract of *Portulaca oleracea* (A1) demonstrated substantial inhibition, Against Pseudomonas aeruginosa, water extract of *Portulaca oleracea* (A1) displayed the highest activity among all compounds, with an inhibition of In the case of Staphylococcus aureus, water extract of *Portulaca oleracea* (A1) exhibited moderate inhibition (23.00%)60.44% Moving on to70 ٪ethanolic extract of *Portulaca oleracea(* A2) displayed improved antibacterial activity compared to their A1 counterparts across all tested strains

Table(1) :Antibacterial activity of water and ethanolic extracts of *Portulaca oleracea* :-

Sample	Antibacterial activity (%)			
	Staphylococcus aureus	*Pseudomonas auruginose*	*Listeria monocytogenes*	*Escherichia coli*
(water extract of *Portulaca oleracea*) A1	23.00000	60.44000	23.63320	30.30250
(70 ٪ethanolic extract of *Portulaca oleracea*) A2	53.10000	55.24608	50.21000	60.50230
Ciprofloxacin	98.00223	99.23651	97.93214	98.36987

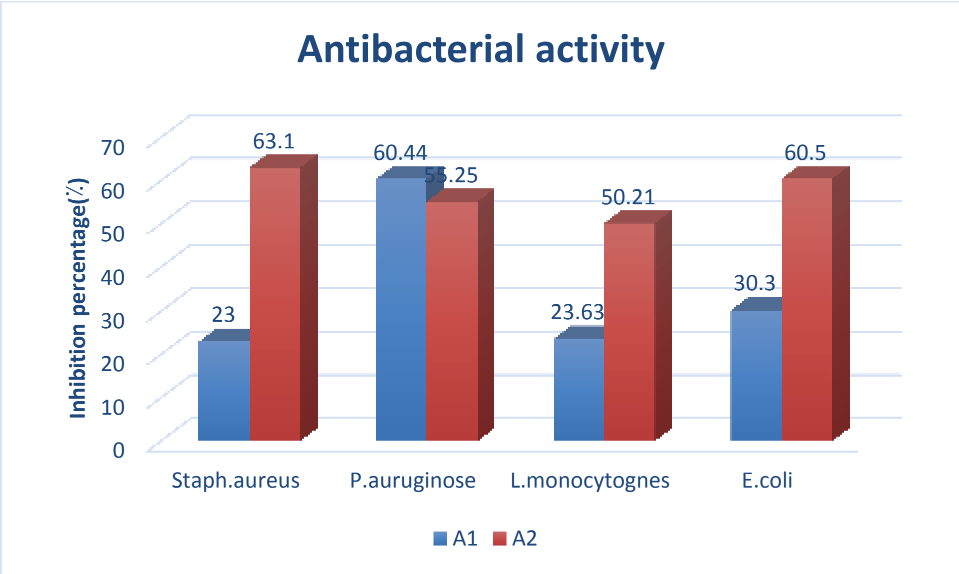

Fig.(1) Antibacterial activity of water and ethanolic extracts of *Portulaca oleracea* :-

ANTIFUNGAL activity:-

The provided data illustrates the antimicrobial activity of different compounds water extract of *Portulaca oleracea* (A1),and 70 ٪ethanolic extract of *Portulaca oleracea(* A2), against two fungal strains: *Aspergillus niger* and *Fusarium exosporium*

The values in the table represent the percentage of inhibition or activity against each fungal strain. water extract of *Portulaca oleracea* (A1) shows no activity against *Aspergillus niger* but exhibits significant inhibition (84.48%) against *Fusarium exosporium*. 70 ٪ethanolic extract of *Portulaca oleracea* (A2) has no activity against *Aspergillus niger* and shows moderate inhibition (84.14%) against *Fusarium exosporium*.

Table(2) :Antifungal activity of water and ethanolic extracts of *Portulaca oleracea* :-

Sample	**Antifungal activity (%)**		
	F. exosporium	*A .niger*	*C. albicans*
(water extract of *Portulaca oleracea*) A1	84.47775629	0	25.3666511
(70 ٪ethanolic extract of *Portulaca oleracea*) A2	84.13926499	0	0
Nystatin (10ug/ml)	98.12531255	99.33662534	98.42241130

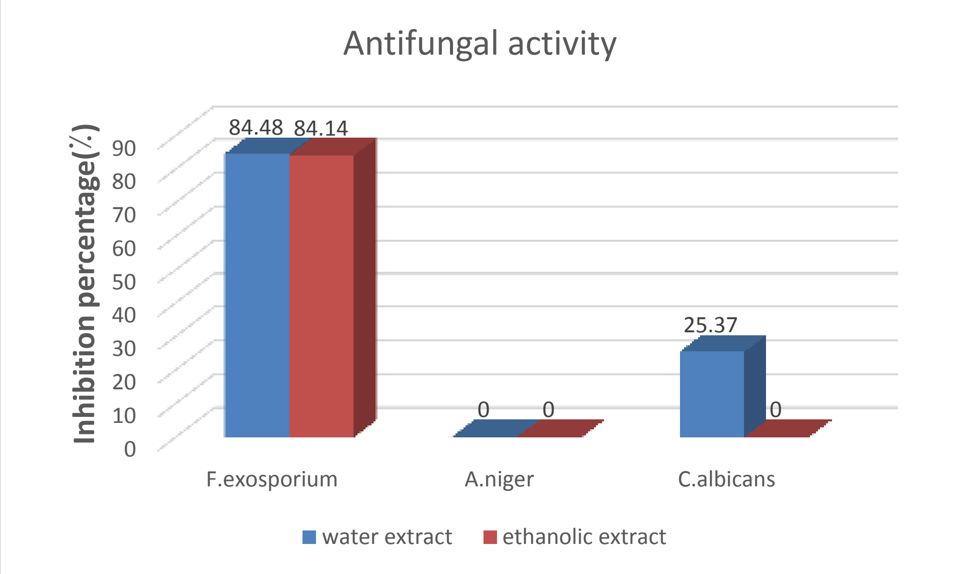

Fig.(2) Antifungal activity of water and ethanolic extracts of *Portulaca oleracea* :-

Synthesis and characterization of bimetallic ZnO-CuO NPs

The UV-Vis spectra revealed an optical density (OD) peak at 2.23, indicating that the synthesized bimetallic ZnO-CuO NPs were small, with absorbance visible at 420 nm. The spectrum exhibited a broad range from 270 to 500 nm, signifying the formation of poly-dispersed nanoparticles. This broad peak is attributed to factors such as the particle size distribution, polydispersity index (PDI) (as observed from dynamic light scattering [DLS] measurements), surface defects (seen in scanning electron microscopy [SEM] images), particle size variation (evident in high-resolution transmission electron microscopy [HR-TEM] results), and the nature of the bimetallic interaction between Zn and Cu.

HR-TEM analysis indicated that the bimetallic ZnO-CuO NPs were stabilized by the water extract of *Portulaca oleracea* leaves and exhibited a semi-spherical shape. The sizes of the nanoparticles varied from 4.9 nm to 18.4 nm, with a median diameter of 12.8 ± 1.5 nm. The size reduction is likely due to the radical-multi-position mechanism introduced by the *Portulaca oleracea* leaf extract, as discussed and compared in previous studies. The nanoparticles were found to be poly-dispersed, with a significant portion of them exhibiting spherical shapes. The asymmetry of some nanoparticles could be attributed to the influence of the leaf extract, which might contribute to various morphologies during the synthesis process.

The DLS analysis showed that the average particle size of the synthesized ZnO-CuO NPs was 49.67 nm. According to the International Standards Organization (ISO), nanoparticles are considered monodisperse when the PDI value is below 0.05, and the DLS results indicated that the synthesized particles were not monodisperse. Furthermore, it was observed that HR-TEM imaging resulted in smaller particle size estimates compared to the average and dominant sizes found in DLS measurements.

To evaluate the surface morphology and properties of the synthesized nanoparticles, scanning electron microscopy (SEM) was utilized. The surfaces of the ZnO-CuO NPs appeared smooth and clear in combination with the water extract of *Portulaca oleracea* leaves. SEM images also revealed bright spherical particles, demonstrating that the leaf extract effectively partitioned the NPs into their spheroidal components. In contrast to earlier studies, the bimetallic ZnO-CuO NPs synthesized in this work exhibited uniform distribution, controlled sizes, and well-defined spherical shapes.

X-ray diffraction (XRD) analysis of the ZnO-CuO NPs demonstrated both amorphous and crystalline phases within the final nanocomposite. To distinguish the specific properties of the synthesized nanoparticles, the XRD data for individual ZnO and CuO NPs were analyzed. The XRD results for the bimetallic ZnO-CuO NPs revealed prominent diffraction peaks associated with ZnO, indicating the presence of crystallization in the nanoparticles. The XRD data also confirmed that the ZnO-CuO NPs exhibited a face-centered cubic (fcc) crystal structure

Disscussion:-

Ercisli and colleagues [11] demonstrated that the methanol extract of *P. oleracea* exhibits antimicrobial properties against *Pseudomonas syringae* pv. *tomato*, *Bacillus subtilis*, *Vibrio cholerae*, and *Yersinia pseudotuberculosis*.

Similarly, Cho and co-authors [12] found that both water and ethanol extracts of this plant possess antimicrobial activity against *Helicobacter pylori*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus mutans*.

Elkhayat and collaborators [13] reported that the methanol extract of *P. oleracea* also demonstrates antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *S. aureus*, *B. subtilis*, *Streptococcus faecalis*, and the fungus *Candida albicans*.

These findings collectively indicate that *P. oleracea* has significant antimicrobial effects against various pathogens responsible for human infections.

Because medicinal plants typically have less side effects, are inexpensive, and are more effective against a wide range of antibiotic-resistant bacteria, scientists and the pharmaceutical industry have thought they are a promising option. In many regions of the world, therapeutic plant extracts are used for their antiviral, antifungal, and antibacterial qualities.

[14]. The antifungal action of Portulaca oleracea against dermatophytes of the genera indicates that it has antibacterial, antifungal, and antiviral properties. Trichophyton [22]. Because it inhibits virus penetration rather than virus adsorption, a pectic polysaccharide that was extracted from this plant's aerial portion exhibits antiherpes properties against simplex virus type 2. [23]. The 70% methanol extract of *Portulaca oleracea* demonstrated notable antimicrobial activity, effectively inhibiting *Candida albicans* with a 12 mm inhibition zone. It also exhibited strong antibacterial properties against Gram-positive bacteria, producing inhibition zones of 13 mm, 14 mm, and 15 mm against *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus faecalis*, respectively. Additionally, the extract showed impressive antibacterial effects against Gram-negative bacteria, with inhibition zones of 14 mm, 15 mm, and 15 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae*, respectively. [19]

Synthesis and characterization of bimetallic ZnO-CuO NPs

The UV-Vis spectra of the precursor compounds, zinc acetate and copper acetate, displayed distinct absorption peaks, with zinc acetate showing a peak at 240 nm [25] and copper acetate at 210 nm [26].

The intensity of the resulting brown color was closely linked to the successful biosynthesis of the bimetallic ZnO-CuO nanoparticles, serving as a visual cue for their formation [27-28]. Surface Plasmon resonance (SPR), a critical phenomenon governing the optical characteristics of nanoparticles, is shaped by multiple factors, including the absorption intensity, particle size, morphology, structural configuration, and dielectric properties of the nanoparticles [29, 30].

The synthesis of bimetallic ZnO-CuO nanoparticles (NPs) was characterized by examining their hydrodynamic diameter, particle size distribution, and polydispersity index (PDI) through density-functional theory (DLS) analysis. By comparing the DLS data with high-resolution transmission electron microscopy (HR-TEM) findings, we determined the average particle size of the produced nanoparticles [31].

The water extract from *Portulaca oleracea* leaves, used in the biosynthesis, was rich in active functional groups. These groups served as capping agents that stabilized the nanoparticles and reduced the concentration of the functional groups, contributing to the creation of polydispersed NPs [32].

HR-TEM analysis revealed that the produced nanoparticles exhibited uniform line spacing, indicating a consistent grading system. Copper was evenly distributed throughout the zinc matrix, leading to the formation of a unique alloy [33].

The polydispersed nature of the nanoparticles signifies their long-term stability, as the water extract of *Portulaca oleracea* leaves acted as the most effective reducing and capping agent in our synthesis approach [34]. This approach aligns with studies by Mohsin and colleagues [39] who synthesized bimetallic silver and gold core-shell nanoparticles by manipulating the pH and temperature conditions during the citrate reduction process. The synthesis process is highly sensitive to pH and temperature, as these factors influence the morphological shape and particle size, with the desired specifications indicating that the particles should be spheroidal and maintain a size range of 50 to 65 nm. Additionally, it is important to note that the 2θ values correspond to the water extract of *Portulaca oleracea* leaves within the 2θ range of 5° to 21° [11].

PDI results showed that particles with a variable distribution were likely to be generated, as PDI values above 0.7 indicate a broad distribution [35]. . In this study, the PDI of the biosynthesized ZnO-CuO NPs was found to be 0.91, which is considered within the acceptable range for polymorphic nanoparticle formation [36]. As noted in previous research [37], the large size of the nanocomposite can be attributed to its internal hydrodynamic radius and the surrounding water layers. Figure 3b presents the Zeta potential analysis of the ZnO-CuO NPs at a pH of 7.1 during preparation. The results indicated that the Zeta potential of the nanoparticles remained negative, likely due to the negative charge of the water extract of *Portulaca oleracea* leaves, with a Zeta potential of -4.5 mV at a neutral pH of 7.8. the large size of the nanocomposite can be attributed to its internal hydrodynamic radius and the surrounding water layers. Figure 3b presents the Zeta potential analysis of the ZnO-CuO NPs at a pH of 7.1 during preparation. The results indicated that the Zeta potential of the nanoparticles remained negative, likely due to the negative charge of the water extract of *Portulaca oleracea* leaves, with a Zeta potential of -4.5 mV at a neutral pH of 7.8 [42].

Lastly, the crystallite size of the synthesized bimetallic ZnO-CuO NPs was calculated using the Williamson-Hall (W H) equation [43, 44], which yielded a crystallite size of 8.23 nm, consistent with the expected properties of the nanoparticles.

5.Conclusion

This study investigates the antimicrobial properties of water and ethanolic extracts of *Portulaca oleracea*. The testing of microbial activity revealed that extracts of *Portulaca oleracea* are rich in essential compounds and phytochemicals, offering a wide range of therapeutic potential. These extracts demonstrate promise as viable alternatives to traditional antibiotics for treating bacterial and fungal infections, highlighting their significance in alternative medicine.

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