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Purslane as a Natural Antimicrobial: Investigating the Bioactivity of Its Aqueous Extract

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ABSTRACT

Plants are among the most ancient life forms on Earth and have evolved sophisticated chemical defense mechanisms to protect themselves from microbial pathogens. In this study, the antibacterial potential of *Portulaca oleracea* was investigated, with a focus on evaluating the bioactivity of its aqueous extract against a range of bacterial species, including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium*, *Enterococcus faecalis*, *Streptococcus mutans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The plant material underwent extraction using the hot percolation process with distilled water as the solvent. A preliminary phytochemical analysis was conducted, revealing the presence of various bioactive compounds such as carbohydrates, amino acids, proteins, cardiac glycosides, alkaloids, flavonoids, tannins, and phenolic compounds.

The antibacterial efficacy of the aqueous extract varied across the tested bacterial strains. It exhibited the highest activity against *E.coli*, with an inhibition zone of 16 ± 0.57 mm. Moderate antibacterial effects were observed against *B. subtilis* and *K. pneumoniae*, each with an inhibition zone of 13 ± 0.57 mm, while the least activity was recorded against *P. aeruginosa*, with a zone of inhibition measuring 11 ± 0.57 mm.

Furthermore, the Minimum Inhibitory Concentration (MIC) assay revealed that *S. aureus* and *E.coli* were the most susceptible bacterial strain to the aqueous extract of *P. oleracea* leaves, showing complete growth inhibition at a minimal concentration of 12.5 µg/ml. These findings suggest that *P. oleracea* possesses promising antibacterial properties, against both Gram positive and Gram negative bacteria, highlighting its potential for further exploration in the development of natural antimicrobial agents.

Keywords: *Portulaca oleracea*, phytoconstituents, antibacterial activity.

INTRODUCTION

The food sector is a dynamic and complex field that employs an extensive variety of additives, including but not limited to preservatives, food colorants, antioxidants, stabilizers, and flavorings. These additives are utilized primarily to enhance the overall shelf life, flavor profile, color, and nutritional quality of the finished food products that reach consumers' tables. Additionally, pesticides and pharmaceutical drugs are regularly added to crops, livestock, and fish in order to control infestations, pests, and diseases that can arise in agricultural fields. This method serves to significantly increase yield and improve outputs; however, a notable fraction of these chemicals can remain on or within the agricultural products post-harvest. As a result, the subsequent transference of these chemicals to consumers may occur, potentially posing various toxicological challenges and health risks. Plants, on the other hand, represent natural wellsprings of distinctive bioactive metabolites that comprise a miscellaneous array of categories such as phenolics, alkaloids, terpenoids, and flavonoids. These metabolites are essential for plants, enabling them to secure defense mechanisms against a multitude of biotic threats, including pests and pathogens. It has been reported that an impressive number of approximately 3,000 plant species are known to produce secondary metabolites that exhibit significant antimicrobial potentiality. Research indicates that these compounds broadly function by destructing the cytoplasmic membrane, leading to increased cytoplasmic leakage and spillage, diminishing the P^H levels within the microbial cytoplasm, obstructing the establishment of ATP, impeding RNA synthesis, disrupting RNA functions, and hindering essential transduction chains vital for microbial life. Over the last few years, there has been a noticeable and boosting interest in the preservation of foodstuff through the use of botanical antimicrobial formulations. Furthermore, traditional practices and herbal remedies have garnered extensive approval and acceptance all around the globe, primarily due to their meager incidence of undesirable secondary effects and their economical feasibility. One notable plant, Purslane, has been touted for its remarkable potential in soothing various ailments including stomach inflammation, piles, troublesome bowel conditions, and urinary fatty excretion, as well as issues such as diarrhea, urethral excrescence, pituitary swellings, blood emission through the nose, and inflamed knuckles. The use of Purslane is said to stimulate the spleen and offer relief down through the chest and the belly. When applied, it possesses the ability to draw out pus and heat. Drinking a concoction made from its seeds combined with old wine is reputedly effective against sharp bites. Additionally, applying it with jumbuck's oil can alleviate the effects of a snake bite. A syrup derived from its seeds is known to curb bloating, while a placebo made with raw honey has been noted to enhance fertility for a span of five days. Such traditional uses emphasize the multifaceted benefits and applications of this remarkable plant in tackling various health concerns[1-3].

Purslane (*Portulaca oleracea*) is a herbaceous plant classified as an annual, reproducing via seeds. Purslane is consumed as a vegetable in Turkey[4]. Fresh Purslane has high amounts of minerals: potassium, calcium, magnesium, and phosphor, vitamins: ascorbic acid, nicotinamide, and folic acid, carotenoid derivatives, omega-3 fatty acids, glutathione, and phenolic compounds. Purslane has been used in a wide ethnic level as it is written in herb books in ancient medicine. It has been prescribed as a laxative agent, hemorrhoidal disorder treatment and removal of helminths in the gastrointestinal tract. Fresh Purslane has remarkable antioxidant activity, mostly due to the existence of ascorbic acid and glutathione. Knockdown Purslane extract has higher antioxidant activity than Fresh Purslane. Potent DPPH• scavenging and tyrosinase preparations have been attributed to the phenolic materials of extract-based prior articles. Additionally, Purslane has antibacterial activities. *E. coli* production in milk at 4°C for 72h was reduced by 2 logs by the extract. The treatment of cancer cells, named MCF-7, located from breast with Purslane extract triggered the Degradation of mitochondria and destructed the membrane of mitochondria[5-9].

The pharmaceutical industry has focused its attention on antioxidants due to their locative hurdle policy against the development of cancer and cardiovascular diseases. As the oldest human ailments, disease-causing microbes are prevented by support of useful microbes. In Asia, existing commensality among health promotion and disease prevention agglomeration, yogurt, was designed by L. delbrueckii subsp. bulgaricus and S. thermophilus. Since the neutraceutical industry has risen, the supported balance has turned into additional risk for health. Thus, due to the need to nourish novel hazardous forms, exploration has been catered to herbal commixture supplements. However, edible plants have not been studied in yogurt applications. *P. oleracea* is used folk remedy for various diseases like inflammation, fever, spasmodic pain, and microbial infections. Antioxidant activity of *P. oleracea* plant extract suggests its marketing potential as a health promoting food supplement. Purslane has been largely farmed because of its healing, antibacterial, antiviral, and anticancer activities for many years. Given its broad industrial potential, Purslane plant is a biologically valuable medicinal and aliments rich in mineral food. At present, there is no published study exploring the corollary of Purslane powder supplementation on yogurt fermentation and quality. Purslane-started punch yogurt may attract consumer interest, especially inhabitants, with its fruity aroma and antioxidant. Pomological interest in the cultivation and production of this noteworthy fruit with its wide industrial uses may be increased[9-11].

This study focuses on purslane (*Portulaca oleracea* L.), a succulent annual plant that comes from the family Portulacaceae. The plant grows naturally in Iran and has spread round the world. Purslane has been employed traditionally for numerous activities, e.g., antiepileptic, antioxidant, antiulcerogenic, wound healing, and for protection against somnolent heart failure[4]. Purslane is affluent in a wide range of bioactive elements that have antioxidant characteristics, which suggests halting or decelerating the oxidative process. Purslane can be consumed in salads, pickles, or various foods in both fresh and dried forms. Purslane seed contains oil that is considered to have health benefits, including omega-3, galacturonic acid, and some vitamins.

The goal of this study is to evaluate the bioactivity of *Portulaca oleracea* aqueous extract as antibacterial after identifying its bioactive components and antibacterial properties. This is important to explore the antibacterial propertiesso that this potential can be utilized for housing or other applications as antimicrobial material.

Purslane is a prostrate spreading annual weed which grows abundantly as a wild plant in open areas with a wide range of temperatures. It is considered as a medicinal plant in some parts of the world. Surprisingly, the plant may contain more of some beneficial substances than many household vegetables as well as some known medicinal plants. Scientific analysis of its chemical components has shown that this common weed has uncommon nutritional value, making it one of the potentially important foods for the future[12]. Purslane is already commercially fixed as an herbal plant and used in food products. One of the most widespread commercial uses of purslane is a source of oil, which is used as a smooth muscle relaxant, a cryopreservative, an edible oil, and a salve. Kalyn Oil is produced from the seeds and is used in a number of cosmetics. Recent studies have demonstrated that the Kalyn herb plant extract inhibits human neutrophil elastase, a protease that plays a critical role in the disease pathology of emphysema. The use of Purslane topical anti-inflammatory and mucoprotective extract composition is disclosed as a treatment for chronic obstructive lung disease and inflammatory diseases[13,14].

Purslane is the sole vegetable source of alpha-linolenic acid, one of the nutrients used in the biosynthesis of omega-3 fatty acids. Besides its nutritional value, the plant has long been traditional in Asia as an herbal remedy for a wide variety of clinical conditions, particularly ways to prevent the formation of neuritic plaques, a nerve cell response that initiates the formation of Alzheimer's amyloid. High content of antioxidants (vitamins A and C, alpha-tocopherol, beta-carotene, and glutathione), omega-3 fatty acids, and its wound healing and antimicrobial effects as well as its traditional use in the topical treatment of inflammatory conditions suggest that purslane is a highly likely candidate as a useful cosmetic ingredient[15,16].

Portulaca oleracea has a very extensive geographical distribution. It flourishes in numerous biogeographical locations worldwide, which includes many countries throughout the Earth. Technology readily spreads natural flora to new locations. Since the Pleistocene period, P. oleracea has become widespread globally. Like a human commensal, incurably associated with human activity, it steadily and strategically disseminated itself throughout diverse biogeographical environments. It thrives in many regions of the world. For example, in Japan it is found in dry and disturbed sandy or gravel soils of riverbanks, vacant lots, stockyards, ports, gardens, and landscape areas throughout the country, except on cold northernmost isle, vast Mt. Aso caldera, and in seasonal snow areas. Purslane occupies the same habitat in many countries of the Earth. It survives in the dry sandy ruins of Antony in the South of France, yet also in the Giza desert sands of Egypt, both growing near ancient civilizations up to 5000 years old[12]. It is found abundantly in such geographically distant countries as the USA, France, South Africa, Australia, India, Iran, China, Indonesia, Syria, and Colombia[17]. Purslane is recognized worldwide for its claimed health benefits and has been used for different purposes such as food, healing, or an ornamental plant. The bioactive components in plants have a wide range of polarities; the major classes include phenolic compounds, fatty acids and lipids, nitrogen compounds, and terpenes, and all of these classes include a variety of chemicals. It is known that purslane contains different kinds and amounts of these chemicals in its composition. Purslane is an annual herbaceous plant species, generally known as a weed. This plant is culturally accepted as food in some countries due to the health benefits, as well as its antioxidant and antibacterial properties[18-20].

MATERIALS AND METHODS

Plant Material

The *P. oleracea* plants were collected from fields surrounding Guntur District, Andhra Pradesh, India. The plant specimen was authenticated by P. Satyanaraya Raju, Plant Taxonomy Consultant, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur. The plant specimen was identified as *P. oleracea*. The plant material was washed, dried in a shed and away from sunlight and water, then ground to a fine and uniform powder, and kept in a cool and dark place until use. The prepared powder was used for making the aqueous extract.

Extraction of phytoconstituents

The phytoconstituents present in the leaves of *P. oleracea* were extracted by hot percolation process. 100g powder of *P. oleracea* leaves was taken into conical flask. The phytoconstituents were extracted by adding 500ml of distilled water to the powder and boiled for 30 min. The extract was filtered through 5 layers of muslin cloth. The process was repeated twice. The collected extract was pooled and concentrated by evaporation. The extract was preserved in desiccator for further study.

Bacterial strains

In the study five Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Streptococcus mutans* and four Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris* were used.

Phytochemical screening

The phytochemical screening for crude extract of *Portulaca oleracea* was carried using standard methods[21,22].

Determination of Antibacterial activity

The antibacterial activity of the aqueous leaf extract of *Portulaca oleracea* was assessed using the agar well diffusion method. To evaluate bacterial susceptibility to the extract's compounds, an overnight culture grown in nutrient broth (Himedia, Mumbai, India) was used as the inoculum. Sterile Muller Hinton agar (Himedia, Mumbai, India) was melted and poured into sterile petri plates at 40–45°C, then allowed to solidify. The nutrient agar plates were inoculated with the overnight bacterial cultures. Wells of 6 mm diameter were evenly spaced on the inoculated plates. The extracts were dissolved in dimethyl sulfoxide (DMSO), and each well was loaded with 250 µg/mL of crude aqueous extract. Positive (Amoxicillin, 100 µg/mL) and negative (DMSO) controls were also included. After allowing the extract to diffuse into the medium, the plates were incubated upright at 37°C for 24 hours, and the zones of inhibition were measured[23,24].

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of *Portulaca oleracea* leaf aqueous extract was determined using a microtitre plate assay. A 100 µL aliquot of the test material (10% w/v in DMSO) was added to the first row of a 96-well microtitre plate, while the remaining wells were filled with 50 µL of sterile Muller Hinton broth (Himedia, Mumbai, India). A 50 µL portion of the test material was then transferred sequentially to the next well to achieve serial dilutions, ensuring each well contained 50 µL. Subsequently, 10 µL of the respective bacterial suspension, adjusted to MacFarland's standard (1×10^8 CFU/mL), and 30 µL of resazurin (0.02% w/v) were added. The plates were covered and incubated at 37°C for 24 hours. A color change from purple to pink indicated bacterial growth. The MIC was determined as the lowest concentration at which no color change was observed. Results were compared with a positive control (containing all solutions except the test compound) and a negative control (containing all solutions except the test compound and bacterial suspension)[25].

RESULTS

The potential antimicrobial bioactivity in the aqueous extract of *Portulaca oleracea* was evaluated, and tested against the Gram-positive bacteria; *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Enterococcus faecalis, Streptococcus mutans* and the Gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*.

Table-1: Preliminary phytochemical screening of leaves of *Portulaca oleracea*

Name of the Test	*P. oleracea* Aqueous Extract
Carbohydrates	+
Proteins	+
Amino acids	+
Steroids	+
Cardiac glycosides	+
Flavonoids	+
Alkaloids	+
Tannins & phenolic compounds	+

Table-2: Antibacterial activity of aqueous leaf extract of *Portulaca oleracea*

Name of the organism	Diameter of zone of inhibition in mm	
	Aqueous Extract 250µg/ml	Amoxicillin 100µg/ml
S.aureus	15±1.15	23±0.57
B.subtilis	13±0.57	17±0.57
E.faecalis	14±0.57	19±0.57
B. megaterium	12±0.57	16±1.15
S.mutans	12±0.57	17±0.57
E.coli	16±0.57	24±0.57
P. vulgaris	15±1.15	21±0.57
P.aeruginosa	11±0.57	17±1.15
K.pneumoniae	13±1.15	20±0.57

Table-3: Minimum inhibitory concentration of aqueous leaf extract of *Portulaca oleracea*

Name of the Organism	Minimum Inhibitory Concentration (MIC) (µg/ml)
S.aureus	12.5
B.subtilis	25
E.faecalis	50
B. megaterium	25
S.mutans	25
E.coli	12.5
P. vulgaris	25
P.aeruginosa	25
K.pneumoniae	50

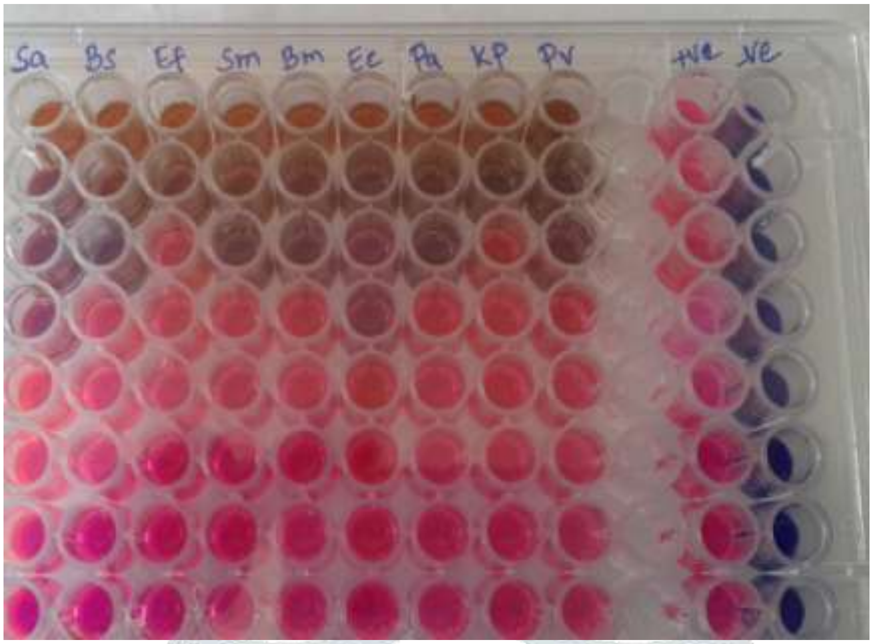

Figure-1: Minimum inhibitory concentration of aqueous leaf extract of *Portulaca oleracea*

Sa - *S.aureus*, Bs - *B.subtilis*, Ef - *E.faecalis*, Sm - *S.mutans*, Bm- *B.megaterium*, Ec - *E.coli*, Pa - *P.aeruginosa*, Kp - *K.pneumoniae*, Pv - *P.vulgaris*

DISCUSSION

Purslane is an easily cultivated plant found globally and has been used for centuries both as a food source and in folk medicine. This plant exhibits potential antimicrobial properties. This work investigates the bioactivity of aqueous Purslane extract on selected Gram-positive and Gram-negative bacteria. The Disc Diffusion Method is applied with the Purslane extract. The results show that the investigated concentration of Purslane extract successfully inhibits the growth of all the tested bacteria. Purslane extract exhibits strong antibacterial activity. This study demonstrates the use of Purslane as an antimicrobial on various bacteria.

The phytochemical screening of *Portulaca oleracea* aqueous extract revealed the presence of several bioactive compounds, including carbohydrates, proteins, amino acids, steroids, cardiac glycosides, flavonoids, alkaloids, tannins, and phenolic compounds (Table: 1). These compounds are known to possess various pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and cardioprotective effects.

The presence of carbohydrates, proteins, and amino acids suggests that the extract contains essential nutrients that may contribute to its biological activity. Proteins and amino acids can play roles in cellular metabolism and may contribute to the overall bioavailability of the extract's active components.

The detection of steroids and cardiac glycosides is particularly significant, as these compounds are known for their cardioprotective and anti-inflammatory properties. Cardiac glycosides, in particular, have been widely studied for their role in heart disease treatment by influencing sodium-potassium pump activity, which regulates cardiac contractions.

The identification of flavonoids, alkaloids, tannins, and phenolic compounds is of great interest due to their well-documented bioactivities. Flavonoids are potent antioxidants that help scavenge free radicals, reducing oxidative stress and inflammation. They also possess antimicrobial properties, which could explain the antibacterial effects observed in previous studies of *P. oleracea* extracts. Alkaloids are known for their diverse pharmacological activities, including antimicrobial, analgesic, and anticancer effects. Tannins and phenolic compounds contribute to astringency and are known to exhibit antimicrobial and antioxidant properties, which may enhance the extract's medicinal potential.

The presence of these phytochemicals suggests that the aqueous extract of *P. oleracea* may have therapeutic applications in treating infections, cardiovascular disorders, and oxidative stress-related conditions. Future studies should focus on isolating and characterizing the active compounds, determining their mechanisms of action, and evaluating their efficacy in preclinical and clinical settings.

The antibacterial activity of the aqueous extract (250 µg/ml) was compared with amoxicillin (100 µg/ml) against nine bacterial species, including both Gram-positive and Gram-negative bacteria. The results indicate that the aqueous extract exhibited antimicrobial properties, although its inhibitory effect was consistently lower than that of amoxicillin.

Among the tested bacteria, E. coli showed the highest sensitivity to both the aqueous extract (16 ± 0.57 mm) and amoxicillin (24 ± 0.57 mm). Similarly, S. aureus and P. vulgaris displayed notable susceptibility to the aqueous extract, with inhibition zones of 15 ± 1.15 mm each. This suggests that the extract contains bioactive compounds capable of inhibiting bacterial growth, particularly in these species.

Gram-positive bacteria such as *S. aureus* (15 ± 1.15 mm), *B. subtilis* (13 ± 0.57 mm), *E. faecalis* (14 ± 0.57 mm), and *S. mutans* (12 ± 0.57 mm) exhibited moderate sensitivity to the extract. However, their inhibition zones were still smaller than those caused by amoxicillin, indicating the latter's superior antibacterial efficacy.

Among Gram-negative bacteria, *P. aeruginosa* exhibited the least sensitivity to both the aqueous extract (11 ± 0.57 mm) and amoxicillin (17 ± 1.15 mm). This aligns with the known resistance of *P. aeruginosa* to many antibiotics. *K. pneumoniae* also showed moderate inhibition (13 ± 1.15 mm for the extract and 20 ± 0.57 mm for amoxicillin), indicating partial effectiveness of the extract against this pathogen.

The variation in antibacterial activity suggests that the aqueous extract contains active compounds that may be effective against both Gram-positive and Gram-negative bacteria, but with lower potency compared to amoxicillin. The differences in inhibition zones among bacterial species could be attributed to variations in their cell wall composition, permeability, and intrinsic resistance mechanisms.

The Minimum Inhibitory Concentration (MIC) values indicate the lowest concentration of the aqueous extract required to inhibit the visible growth of each bacterial species. The results show variability in bacterial susceptibility, with MIC values ranging from 12.5 µg/ml to 50 µg/ml.
Among the tested organisms, *S. aureus* and *E. coli* exhibited the highest sensitivity to the extract, with the lowest MIC value of 12.5 µg/ml. This suggests that the extract contains potent bioactive compounds that can effectively inhibit these bacteria at relatively low concentrations.

Moderate susceptibility was observed in *B. subtilis*, *B. megaterium*, *S. mutans*, *P. vulgaris*, and *P. aeruginosa*, each requiring 25 µg/ml of the extract for inhibition. These findings indicate a moderate antimicrobial effect, suggesting that the extract might interfere with bacterial metabolism or cell wall integrity, but at a slightly higher concentration than required for *S. aureus* and *E. coli*.

The least susceptible organisms were *E. faecalis* and *K. pneumoniae*, both of which had MIC values of 50 µg/ml. This suggests that these species have a higher resistance to the extract, possibly due to structural differences in their cell walls or intrinsic resistance mechanisms. *K. pneumoniae*, a known multidrug-resistant pathogen, may have efflux pumps or enzymatic defenses that reduce the extract's effectiveness. Similarly, *E. faecalis* is known for its natural resistance to many antimicrobials. The results were depicted in table-3 and figure-1.

The variation in MIC values highlights differences in bacterial cell wall composition and defense mechanisms. Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*) generally have an outer membrane that acts as a barrier to antimicrobial agents, whereas Gram-positive bacteria (*S. aureus*, *B. subtilis*, *E. faecalis*, *B. megaterium*, *S. mutans*) lack this outer membrane but may have other resistance factors. The relatively low MIC values for *S. aureus* and *E. coli* suggest that the extract might target essential cellular processes in these bacteria effectively.

CONCLUSION

The aqueous extract demonstrated antibacterial activity against all tested bacterial species, with inhibition zones ranging from 11 ± 0.57 mm (*P. aeruginosa*) to 16 ± 0.57 mm (*E. coli*). However, its effectiveness was consistently lower than that of amoxicillin, which produced larger inhibition zones across all bacteria.

These findings suggest that the extract possesses bioactive compounds with antimicrobial potential, making it a candidate for further investigation. Future studies should focus on identifying the active components of the extract, optimizing its concentration, and evaluating its mode of action. Additionally, synergistic effects with standard antibiotics could be explored to enhance its efficacy against resistant bacterial strains.

Since ancient times, herbs and other natural products have been used to treat infections. Currently, a wide range of global ethnopharmacologically used plants have been screened for their antimicrobial activities. Many medicinal plants have potential antimicrobial effects that are expressed by a bioactive compound. They are recognized as a co-therapy where their assistance is greatly needed in overcoming the increased bacterial resistance.

One such herb widely used in traditional medicine, purslane, has been described to have antimicrobial and antioxidant properties stemming from its bioactive compounds. In the current research, it was aimed to investigate the bioactivity of its aqueous extract. It was found that the aqueous extract of purslane increased growth curves and biofilm production in bacteria tested and decreased cell viability.

Rapidly spreading infectious diseases, especially in urban communities with high population density, are a serious public health problem. Therefore, there are many studies to prevent the proliferation of microorganisms that cause infectious diseases. Increasing the bioactivity of edible products in the environment after the consumption of these products prevents the replication of microorganisms in the salivary environment. Purslane is one of the most commonly consumed herbs in the Mediterranean region. The inhalation of fresh purslane or its dry product will prevent the replication of microorganism in the oral environment after consumption and reduce the occurrence of infectious diseases that may threaten human health. In this context, the effect of the aqueous extract of the purslane brought by different research's on the salivary microorganisms that most frequently because infectious disease was investigated.

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