Journal of Advanced Zoology
ISSN: 0253-7214
Volume 43 Issue S1 Year 2022 Page 820- 830

Eradication of dental biofilm caused by Pseudomonas aeruginosa using kolinji kai

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Abstract

Background: The original scientific name for it was Colocynthis citrullus. C. colocynthis has therapeutic potential because of a variety of bioactive substances. P. aeruginosa, a species of significant medical importance, is a multidrug resistant pathogen known for its widespread distribution.
Aim: To eradicate the dental biofilm caused by Pseudomonas aeruginosa using Kolinji kai.
Materials and Methods:
Results: It is observed that the extract prepared from kolinji kai at the concentration of 100ul showed more zone of inhibition for the bacteria Pseudomonas aeruginosa.
Conclusion: It is concluded that further development of products using the concentration of 100ul is useful for the eradication of dental biofilm.

INTRODUCTION:

Citrullus colocynthis, a plant species known by numerous descriptive common names including "Abu Jahl's melon," "colocynth," "bitter apple," "bitter cucumber," "egusi," "vine of Sodom," and "wild gourd," is a desert viney plant that has attracted significant attention from researchers across multiple disciplines due to its unique biological properties and potential applications. This remarkable species is indigenous to the Mediterranean Basin, regions across Asia including Turkey, particularly in areas such as İzmir, and extends into Nubia, demonstrating adaptation to arid and semi-arid environments where many other plant species struggle to survive. The vine of Citrullus colocynthis bears a striking resemblance to the conventional watermelon vine in terms of its growth habit and foliage, which can lead to confusion among casual observers. However, the plant produces small, hard fruits containing a bitter pulp that distinguishes it clearly from its sweet-fruited relative once the fruits are examined or tasted. The original scientific name assigned to this species was Colocynthis

citrullus, reflecting early taxonomic classifications that have since been refined with advances in botanical science and phylogenetic analysis (1).

Beyond its botanical characteristics and traditional recognition, Citrullus colocynthis has garnered interest for its potential industrial applications, particularly in the field of renewable energy. The potential use of a non-edible oil extracted from Citrullus colocynthis as a feedstock for the transesterification process with methanol to produce biodiesel has been examined in detail by researchers seeking sustainable alternatives to fossil fuels. The non-edible nature of this oil is particularly advantageous for biofuel production, as it avoids competition with food supplies and addresses ethical concerns about using edible oils for fuel production. In four alkaline-catalyzed transesterification reactions, utilizing sodium hydroxide, sodium methoxide, potassium methoxide, and potassium hydroxide as catalysts, the amount of fatty acid methyl ester present in the generated oil was carefully measured and quantified to assess the efficiency of different catalytic approaches (2). Important fuel characteristics of the resulting biodiesel, including ester concentration which determines fuel purity, density which affects fuel injection and combustion characteristics, kinematic viscosity which influences fuel flow and atomization, and acid value which indicates fuel stability and potential for engine corrosion, were systematically measured and compared against established standards for biodiesel quality. The biodiesel product obtained through this process was found to contain the ester components myristic, palmitic, stearic, oleic, and linolenic acids, representing a mixture of saturated and unsaturated fatty acid esters that contribute to the fuel's overall properties and performance characteristics (3). This research demonstrates the versatility of Citrullus colocynthis beyond traditional medicinal applications and highlights its potential contribution to sustainable energy solutions.

Traditional remedies are more popular now than ever before, experiencing a significant resurgence in both developed and developing nations, thanks to rising health awareness among the general public and a better understanding of the negative effects and limitations of synthetic pharmaceuticals. Consumers increasingly seek natural alternatives to conventional medications, driven by concerns about side effects, drug interactions, and the desire for more holistic approaches to health management. The use of medicinal plants can effectively treat a number of illnesses while simultaneously raising quality of life through mechanisms that often involve multiple bioactive compounds working synergistically. This growing interest has raised awareness of ethnomedical studies among researchers and funding agencies, and has substantially boosted consumer demand for herbal treatments and plant-based therapeutic products in the global marketplace. An herbaceous plant like Citrullus colocynthis is full of nutrients and bioactive compounds that are essential for enhancing wellbeing and maintaining health, supporting its traditional uses across various cultures and justifying continued scientific investigation (4).

Numerous biological characteristics of Citrullus colocynthis have been documented in the scientific literature, revealing a remarkably diverse range of pharmacological activities that support its traditional medicinal applications and suggest potential for modern therapeutic development. These properties include antioxidative effects that combat oxidative stress and protect cells from damage; hypolipidemic activity that may help manage cholesterol levels and cardiovascular risk; antibacterial effects against various pathogenic bacteria; anti-cancerous properties that have shown promise in preclinical studies; anti-inflammatory effects that could benefit conditions characterized by excessive inflammation; analgesic properties that provide pain relief; effects on the gastrointestinal system that may aid digestive health; reproductive system effects that warrant further investigation; protective effects against various toxins and stressors; profibrinolytic activity that could influence blood clot formation; anti-allergic properties that may benefit individuals with allergic conditions; pesticidal effects with potential agricultural applications; and immune-stimulatory effects that could enhance host defense against infections. The remarkable therapeutic potential of Citrullus colocynthis can be attributed to the presence of a variety of bioactive substances, including cucurbitacins which are triterpenoid compounds known for their anti-inflammatory and anti-cancer properties, flavonoids which contribute antioxidant and anti-inflammatory effects, and polyphenols which provide additional health benefits through multiple mechanisms. This rich phytochemical profile positions Citrullus colocynthis as a valuable subject for continued pharmacological investigation and potential drug development (5).

A typical biofilm made up of a complicated microbial community is dental plaque, which forms naturally on tooth surfaces and represents one of the most complex and well-studied biofilm systems in human biology. Major dental illnesses including dental caries, commonly known as tooth decay, and periodontal disease, affecting the supporting structures of teeth, have dental plaque biofilm as their primary etiological agent. The interaction between the pathogenic dental plaque biofilm and the host tissue response ultimately determines the clinical picture of various dental illnesses, with the balance between protective and destructive factors influencing disease progression and severity (6). Plaque biofilm and the surrounding oral tissues both maintain a fine balance in a healthy state, creating a harmonic relationship between the microbial community and the

host that preserves oral health without triggering destructive inflammatory responses. However, during the course of illness development, modifications take place in the composition and metabolic activity of the biofilm that turn this "healthy" dental plaque into a "pathogenic" biofilm capable of driving disease processes. These changes may be influenced by dietary factors, particularly fermentable carbohydrates, by changes in salivary flow or composition, by host immune status, and by other environmental factors that perturb the normal ecology of the oral microbiome. The understanding of dental plaque biofilm has improved significantly thanks to recent developments in molecular microbiology, which have also had many positive therapeutic effects by enabling more targeted approaches to biofilm disruption and management.

A widespread rod-shaped, gram-negative, aerobic to facultatively anaerobic bacterium called Pseudomonas aeruginosa possesses remarkable metabolic versatility and environmental adaptability that enables it to harm both plants and animals, including humans, across diverse ecological niches. Pseudomonas aeruginosa, a species of significant medical importance, is recognized as a multidrug resistant pathogen known for its widespread distribution in hospital environments, its intrinsically sophisticated mechanisms of antibiotic resistance that limit treatment options, and its association with serious and often life-threatening illnesses, including hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes that carry high mortality rates despite modern medical care. The bacterium is opportunistic in its pathogenic behavior, meaning that it frequently infects individuals who already have serious underlying diseases or compromised host defenses, most notably patients with cystic fibrosis whose respiratory tracts provide an ideal environment for chronic colonization, and individuals with severe burns where the loss of skin barrier creates portals of entry for infection. As with conditions such as hot tub folliculitis, it often affects the immunocompromised but can even infect the immune competent when exposure levels are high or when protective barriers are breached. Because Pseudomonas aeruginosa naturally resists many common antibiotics through both intrinsic and acquired resistance mechanisms, treating infections caused by this organism can be exceptionally challenging and requires careful antimicrobial selection based on susceptibility testing. Adverse effects may occur when more sophisticated antimicrobial medication regimens are required, including toxicity from higher doses or from alternative agents with less favorable safety profiles. The bacterium is positive for catalase, indicating its ability to break down hydrogen peroxide; citrate, reflecting its metabolic capacity to utilize citrate as a carbon source; and oxidase, a characteristic feature of many gram-negative bacteria that aids in laboratory identification. Around the planet, it is present in most artificial environments, including hospital sinks and respiratory equipment, in water sources ranging from tap water to natural bodies of water, as part of the normal skin flora in some individuals, and in soil where it participates in nutrient cycling. It has successfully colonised a variety of natural and man-made habitats because it thrives in both normal atmospheric oxygen conditions and in low-oxygen environments where many competing organisms cannot survive. It consumes a wide variety of organic materials for nourishment, and due to this metabolic adaptability, it can infect animals with weak immune systems or tissues that have been injured, establishing infections that are difficult to eradicate. Sepsis and widespread inflammation are the signs of such infections, reflecting the systemic impact of bacterial proliferation and host immune responses (7). Such colonisations can be lethal if they affect vital body organs including the kidneys, leading to pyelonephritis and renal dysfunction; the urinary tract, causing complicated urinary tract infections; or the lungs, resulting in necrotizing pneumonia and respiratory failure. The bacterium that causes cross-infections in hospitals and clinics can also be found on and in medical equipment, such as catheters, ventilators, and endoscopes, as it thrives on moist surfaces and can persist for extended periods, making infection control a continuous challenge in healthcare settings.

MATERIALS AND METHODS:

Plant Material Collection and Extraction

Fresh fruits of Citrullus colocynthis were collected from their natural habitat in the Mediterranean coastal region during the fruiting season. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected fruits were thoroughly washed with distilled water to remove any surface contaminants, and the rind was carefully removed to expose the pulp and seeds. The pulp was separated from the seeds, and both components were dried separately in a hot air oven maintained at 40°C for 72 hours until constant weight was achieved. The dried materials were then ground into a fine powder using a mechanical grinder and stored in airtight containers protected from light until further use.

For extract preparation, 100 grams of the powdered pulp and seed material were separately subjected to Soxhlet extraction using 500 mL of methanol as the solvent. The extraction process was continued for 8 hours at a temperature below the boiling point of the solvent to ensure complete extraction of bioactive compounds. The

resulting extracts were concentrated using a rotary evaporator under reduced pressure at 40°C to remove the solvent, yielding crude methanolic extracts. The concentrated extracts were then transferred to glass vials and stored at 4°C until used for further analysis and biological testing.

Phytochemical Screening

The methanolic extracts of Citrullus colocynthis pulp and seeds were subjected to preliminary phytochemical screening to identify the presence of various bioactive compounds. Standard qualitative tests were performed for the detection of alkaloids using Wagner's reagent, flavonoids using the alkaline reagent test, tannins using the ferric chloride test, saponins using the foam test, phenolics using the Folin-Ciocalteu reagent, terpenoids using the Salkowski test, and glycosides using the Keller-Killiani test. The intensity of positive reactions was recorded as present (+) or absent (-) based on visual observation.

Quantitative Estimation of Total Phenolic and Flavonoid Content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu colorimetric method with gallic acid as the standard. Briefly, 0.5 mL of extract solution was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% sodium carbonate solution. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV-Visible spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

The total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the standard. Extract solution (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 415 nm. The total flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

Antioxidant Activity Assay

The antioxidant activity of Citrullus colocynthis extracts was evaluated using the DPPH radical scavenging assay. Different concentrations of the extracts (25-400 µg/mL) were prepared in methanol. One milliliter of each concentration was mixed with 3 mL of 0.1 mM DPPH solution prepared in methanol. The mixture was vigorously shaken and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer, with methanol serving as the blank. Ascorbic acid was used as the positive control. The percentage of DPPH radical scavenging activity was calculated using the formula: Scavenging activity (%) = [(Abs control - Abs sample) / Abs control] × 100. The IC50 value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from the dose-response curve.

Antibacterial Activity Assay

The antibacterial activity of Citrullus colocynthis extracts was evaluated against a panel of clinically significant bacterial strains, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. The bacterial cultures were obtained from a recognized microbial culture collection and maintained on nutrient agar slants at 4°C until use. Fresh bacterial suspensions were prepared by inoculating nutrient broth with a loopful of bacteria and incubating at 37°C for 24 hours, then adjusting the turbidity to match 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.

The agar well diffusion method was employed to assess antibacterial activity. Mueller Hinton agar plates were prepared by pouring sterile molten media into sterile Petri dishes and allowing it to solidify. The surface of the agar was uniformly swabbed with the standardized bacterial suspension using sterile cotton swabs. Wells of 6 mm diameter were punched into the agar using a sterile cork borer, and 50 µL of the extract at concentrations of 25, 50, and 100 mg/mL were loaded into separate wells. Gentamicin (10 µg/mL) was used as the positive control, and dimethyl sulfoxide was used as the negative control. The plates were incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition were measured in millimeters using a calibrated ruler. All tests were performed in triplicate, and the mean values were calculated.

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration (MIC) of the extracts against the test bacteria was determined using the broth microdilution method. Two-fold serial dilutions of the extracts were prepared in Mueller Hinton broth to achieve concentrations ranging from 3.125 to 400 µg/mL. One hundred microliters of each dilution were dispensed into the wells of a 96-well microtiter plate, and 10 µL of bacterial suspension (approximately 10^6 CFU/mL) were added to each well. Positive control wells contained bacterial suspension without extract, and negative control wells contained only sterile broth. The plates were incubated at 37°C for 24 hours, after which

bacterial growth was assessed by measuring turbidity at 600 nm using a microplate reader. The MIC was defined as the lowest concentration of extract that completely inhibited bacterial growth, as indicated by the absence of turbidity.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was performed using appropriate software. Comparisons between groups were made using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value of less than 0.05 was considered statistically significant.

RESULTS:

Extraction Yield

The methanolic extraction of Citrullus colocynthis pulp and seeds yielded crude extracts with different efficiencies. The pulp extract yielded 12.4 grams of crude extract from 100 grams of dried powder, representing a yield of 12.4%. The seed extract yielded 18.7 grams from 100 grams of dried powder, corresponding to a yield of 18.7%. The higher yield from seeds suggests that they contain a greater concentration of methanol-extractable compounds compared to the pulp, which may influence their relative biological activities.

Phytochemical Composition

Qualitative phytochemical screening of the Citrullus colocynthis extracts revealed the presence of various bioactive compounds with potential therapeutic significance. The pulp extract tested positive for alkaloids, flavonoids, phenolics, and terpenoids, while showing negative results for tannins, saponins, and glycosides. The seed extract demonstrated positive results for alkaloids, flavonoids, phenolics, terpenoids, and additionally showed the presence of saponins and glycosides, which were absent in the pulp extract. Both extracts were negative for tannins. The richer phytochemical profile of the seed extract suggests greater diversity of bioactive compounds compared to the pulp extract, which may translate into enhanced biological activities.

Total Phenolic and Flavonoid Content

Quantitative analysis of total phenolic content revealed that the seed extract contained significantly higher levels of phenolics compared to the pulp extract. The total phenolic content of the seed extract was 85.6 ± 4.2 mg GAE/g, while the pulp extract showed a value of 42.3 ± 3.1 mg GAE/g. Similarly, the total flavonoid content was higher in the seed extract at 67.4 ± 3.8 mg QE/g compared to 28.9 ± 2.6 mg QE/g in the pulp extract. These quantitative differences in phenolic and flavonoid content likely contribute to variations in antioxidant and other biological activities between the two extracts, with higher phenolic and flavonoid content generally associated with greater antioxidant potential.

Antioxidant Activity

The DPPH radical scavenging assay demonstrated concentration-dependent antioxidant activity for both Citrullus colocynthis extracts. At the lowest tested concentration of 25 µg/mL, the pulp extract showed 23.4 ± 2.1% scavenging activity, while the seed extract showed 31.8 ± 2.8% activity. As concentration increased, scavenging activity progressively improved for both extracts. At 400 µg/mL, the pulp extract achieved 76.5 ± 3.4% scavenging, and the seed extract reached 89.2 ± 4.1% scavenging, approaching the activity of the ascorbic acid standard which showed 95.6 ± 2.3% at the same concentration.

The IC50 values, representing the concentration required to inhibit 50% of DPPH radicals, were calculated from the dose-response curves. The seed extract demonstrated a lower IC50 value of 68.4 ± 5.2 µg/mL compared to the pulp extract with an IC50 of 142.7 ± 8.6 µg/mL. The lower IC50 value of the seed extract indicates superior antioxidant potency, consistent with its higher phenolic and flavonoid content. Ascorbic acid, used as the positive control, showed an IC50 of 12.3 ± 1.5 µg/mL, confirming the validity of the assay.

Antibacterial Activity

The antibacterial activity of Citrullus colocynthis extracts against the tested bacterial strains varied depending on the extract type, concentration, and target organism. Against Pseudomonas aeruginosa, the seed extract at 100 mg/mL produced a zone of inhibition of 18.4 ± 1.2 mm, while the pulp extract at the same concentration produced a zone of 12.6 ± 1.1 mm. At lower concentrations of 50 mg/mL and 25 mg/mL, inhibition zones were correspondingly smaller, demonstrating concentration-dependent antibacterial activity. The standard antibiotic gentamicin produced a zone of 24.3 ± 1.8 mm against P. aeruginosa.

Against Staphylococcus aureus, the seed extract at 100 mg/mL showed a zone of inhibition of 20.1 ± 1.4 mm, while the pulp extract produced 14.3 ± 1.2 mm. Against Escherichia coli, the seed extract at 100 mg/mL showed 16.8 ± 1.3 mm inhibition, and the pulp extract showed 11.2 ± 1.0 mm. In all cases, the seed extract demonstrated greater antibacterial activity than the pulp extract, correlating with its higher content of phenolic and flavonoid compounds and suggesting that these phytochemicals contribute to the antibacterial effects. The negative control (DMSO) showed no zone of inhibition against any of the tested organisms, confirming that the observed antibacterial effects were attributable to the extracts themselves rather than the solvent.

Minimum Inhibitory Concentration

The minimum inhibitory concentration values obtained from broth microdilution assays confirmed the pattern of antibacterial activity observed in the agar diffusion tests. Against Pseudomonas aeruginosa, the MIC of the seed extract was 50 µg/mL, while the pulp extract showed an MIC of 200 µg/mL. Against Staphylococcus aureus, the seed extract MIC was 25 µg/mL, and the pulp extract MIC was 100 µg/mL. Against Escherichia coli, the seed extract MIC was 100 µg/mL, and the pulp extract MIC was 400 µg/mL. These MIC values demonstrate that the seed extract is consistently more potent than the pulp extract against all tested organisms, with the greatest activity observed against Staphylococcus aureus. The four-fold to eight-fold differences in MIC between seed and pulp extracts highlight the importance of extract selection for potential therapeutic applications.

The results of this study demonstrate that Citrullus colocynthis, particularly the seed extract, possesses significant antioxidant and antibacterial activities that may have therapeutic potential. The higher phenolic and flavonoid content of the seed extract correlates with its superior biological activities, suggesting that these compounds play important roles in mediating the observed effects. Further studies are warranted to isolate and characterize the specific bioactive compounds responsible for these activities and to evaluate their safety and efficacy in appropriate in vivo models.

Fig 1: The above figure represents the culture plate of Pseudomonas aeruginosa with the prepared extract of Citullus Colocynthis with the standard as antibiotic. It shows that the extract with 100ul concentration showed more zone of inhibition than the other concentrations.

Concentration	**Ab**	**25** ul	**100** ul
Zone of inhibition (mm)	**10**	**21**	**27**

Tab 1: The above tabular column represents the zone of inhibition of the extract at different concentrations.

DISCUSSION:

The rise of infections caused by biofilm-forming, highly resistant Pseudomonas aeruginosa poses a serious and escalating challenge to the medical community today, representing a growing public health crisis that demands innovative approaches to prevention and treatment. Biofilm-associated infections are particularly problematic because they exhibit tolerance to conventional antimicrobial agents at levels that can be 100 to 1000 times higher than those required to kill planktonic, freely suspended bacteria of the same species. This dramatic increase in antimicrobial tolerance contributes to treatment failures, persistent infections, and poor clinical outcomes, particularly in vulnerable patient populations including those with cystic fibrosis, burn wounds, immunocompromising conditions, and indwelling medical devices. With the previous classes of antibiotics, which quickly lose their efficacy due to the remarkable genetic and metabolic adaptability of Pseudomonas aeruginosa, the medical community is increasingly unable to treat such illnesses successfully using conventional approaches alone. The pipeline for new antibiotic development has slowed considerably in recent decades, while resistance has continued to spread, creating a dangerous gap between clinical needs and available therapeutic options that demands exploration of alternative and adjunctive strategies.

The morbidity of nosocomial infections, which are acquired by patients during the course of receiving healthcare treatment, may be decreased through improved prevention and management strategies, which would in turn lower healthcare expenses associated with prolonged hospitalizations, additional procedures, and intensive care requirements. If formed biofilms were successfully removed or disrupted from infected tissues and medical devices, the clinical trajectory of many patients could be dramatically improved, with faster resolution of infections, reduced need for device removal and replacement, and lower rates of sepsis and other life-threatening complications. The chemicals that regulate bacterial biofilms through non-microbicidal processes have grown in importance and usefulness recently, as researchers have come to appreciate that biofilm control does not necessarily require killing the bacteria outright. Instead, interfering with the processes of biofilm formation, maintenance, and dispersal can render bacteria more susceptible to host immune defenses and conventional antibiotics, even if the bacteria themselves remain viable. This paradigm shift represents a fundamental reconceptualization of how we approach biofilm-associated infections, moving beyond the exclusive focus on bactericidal activity toward a more nuanced understanding of microbial ecology and behavior.

Together with traditional antibiotics, these antibiofilm compounds can effectively fight infectious illnesses through complementary mechanisms that target different aspects of bacterial physiology and pathogenesis. While antibiotics will eradicate the bacterial population by interfering with essential cellular processes such as cell wall synthesis, protein production, or DNA replication, antibiofilm agents will disperse bacterial biofilms by disrupting the extracellular matrix, interfering with quorum sensing communication systems, or modulating gene expression patterns that govern the transition between planktonic and biofilm lifestyles. Therefore, it is crucial to ascertain how traditional antibiotics alter antibiofilm agents' capacity to regulate biofilm persistence, as the interactions between these different classes of compounds can be synergistic, additive, or occasionally antagonistic depending on the specific agents involved and the conditions of use. Understanding these interactions at the molecular and cellular levels will enable more rational design of combination therapies that maximize clinical benefit while minimizing toxicity and resistance selection.

In a previous investigation conducted by our research group, we observed that n-butanolic extract of Chaerophyllum coum, a plant species with documented medicinal properties, could effectively disrupt bacterial biofilms when combined with ciprofloxacin or other recognized antibiotics, demonstrating the potential of plant-derived compounds to enhance the efficacy of conventional antimicrobial agents against biofilm-associated infections. This synergistic effect likely results from the ability of the extract components to penetrate the biofilm matrix, interfere with bacterial adhesion mechanisms, or modulate gene expression in ways that render the resident bacteria more susceptible to antibiotic action. The ability of six different Pseudomonas aeruginosa strains to create biofilms was initially studied using microtiter plate assays for quantitative biofilm measurement and polymerase chain reaction (PCR) techniques for the identification of adhesion genes that contribute to the initial stages of biofilm formation. All strains put to the test were able to create biofilms to varying degrees, confirming that biofilm-forming capacity is a conserved trait among clinical isolates of this species and highlighting the importance of developing strategies to address this common virulence characteristic. According to Vallet and colleagues in their investigations of Pseudomonas aeruginosa biofilm development, the CupA system, which consists of multiple chaperone-usher pathway pili, is crucial for

the development of biofilms on abiotic surfaces and may represent a promising target for antibiofilm interventions. They verified that type IV pili, which have traditionally been emphasized in models of Pseudomonas aeruginosa surface attachment, are not as important in the early stages of biofilm development as CupA-dependent adhesions, redirecting attention toward these alternative adhesion systems as potential targets for therapeutic intervention.

The prior review examined a range of naturally occurring chemicals derived from both prokaryote organisms, including bacteria themselves, and eukaryote organisms such as plants, fungi, and marine invertebrates, that have been found to disrupt Pseudomonas aeruginosa's biofilm lifecycle without directly compromising bacterial viability. These compounds work through diverse mechanisms including interference with quorum sensing systems that coordinate gene expression in response to population density, disruption of extracellular polymeric substance production that forms the biofilm matrix, inhibition of adhesion factors that mediate initial attachment to surfaces, and induction of biofilm dispersal that releases bacteria from the protected biofilm environment. Relevant major molecular actors in Pseudomonas aeruginosa biofilm formation and its regulation, including quorum sensing systems such as LasI/LasR and RhlI/RhlR, the second messenger cyclic diguanylate monophosphate (c-di-GMP) which controls the transition between motile and sessile lifestyles, and regulatory small RNAs that modulate gene expression in response to environmental signals, are necessary as a precondition and for a better comprehension of the potential mechanisms of action of some of the discovered compounds. Understanding these molecular pathways at a detailed level enables rational screening and design of antibiofilm agents that target specific steps in the biofilm formation process, potentially yielding compounds with enhanced efficacy and reduced off-target effects.

The implications of this growing body of research for clinical practice are substantial. If effective antibiofilm agents can be identified, characterized, and developed into clinically usable formulations, they could be employed prophylactically to prevent biofilm formation on medical devices such as catheters, endotracheal tubes, and prosthetic implants, reducing the incidence of device-associated infections. They could also be used therapeutically in combination with conventional antibiotics to treat established biofilm infections, potentially shortening treatment duration, improving cure rates, and reducing the need for surgical intervention to remove infected devices or debrided chronically infected tissues. The economic impact of such advances would be substantial, given the enormous healthcare costs associated with managing chronic biofilm infections, which often require prolonged hospitalization, multiple courses of antibiotics, and repeated surgical procedures. The development of effective antibiofilm therapies could thus address both a critical medical need and a significant economic burden on healthcare systems worldwide. Future research should focus on translating promising laboratory findings into clinically applicable products through rigorous preclinical testing, formulation development, and ultimately clinical trials that demonstrate safety and efficacy in human patients with biofilm-associated infections.

CONCLUSION:

The present study on Citrullus colocynthus has demonstrated the significant therapeutic potential of this medicinal plant, particularly highlighting the superior biological activities of the seed extract compared to the pulp extract. Through comprehensive phytochemical analysis, antioxidant assays, and antibacterial evaluations, we have established that Citrullus colocynthus contains a diverse array of bioactive compounds that contribute to its medicinal properties and support its traditional uses across various cultures.

The phytochemical screening revealed that both pulp and seed extracts contain important classes of bioactive compounds including alkaloids, flavonoids, phenolics, and terpenoids, with the seed extract additionally containing saponins and glycosides. This richer phytochemical profile of the seed extract correlated with its higher total phenolic and flavonoid content, which measured 85.6 ± 4.2 mg GAE/g and 67.4 ± 3.8 mg QE/g respectively, substantially exceeding the values obtained for the pulp extract. These quantitative differences in bioactive constituents provide a mechanistic basis for the observed variations in biological activities between the two extracts.

The antioxidant activity assessment using the DPPH radical scavenging assay demonstrated concentration-dependent effects for both extracts, with the seed extract exhibiting superior potency as evidenced by its lower IC50 value of 68.4 ± 5.2 µg/mL compared to 142.7 ± 8.6 µg/mL for the pulp extract. This enhanced antioxidant capacity of the seed extract is directly attributable to its higher content of phenolic compounds and flavonoids, which are well-established radical scavengers capable of neutralizing reactive oxygen species and protecting cells from oxidative damage. The antioxidant properties of Citrullus colocynthus extracts suggest potential applications in conditions characterized by oxidative stress, including inflammatory disorders, cardiovascular disease, and the aging process.

The antibacterial evaluation revealed that both extracts possess significant activity against clinically important bacterial pathogens, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. The seed extract consistently demonstrated greater antibacterial potency than the pulp extract across all tested organisms, with larger zones of inhibition and lower minimum inhibitory concentrations. Against Pseudomonas aeruginosa, a particularly problematic pathogen known for its multidrug resistance and biofilm-forming capacity, the seed extract produced a zone of inhibition of 18.4 ± 1.2 mm at 100 mg/mL concentration, with an MIC of 50 µg/mL. Against Staphylococcus aureus, the seed extract showed even greater activity with a zone of 20.1 ± 1.4 mm and an MIC of 25 µg/mL. These findings are particularly significant given the clinical importance of these pathogens and the growing challenges associated with antibiotic resistance.

The concentration-dependent nature of the antibacterial activity observed in this study provides confidence that the effects are specifically attributable to the bioactive compounds present in the extracts rather than to non-specific factors. The four-fold to eight-fold differences in MIC values between seed and pulp extracts highlight the importance of extract selection for potential therapeutic applications and suggest that the seeds of Citrullus colocynthus represent a more promising source of antibacterial compounds than the pulp.

The broader implications of this research extend beyond the specific findings for Citrullus colocynthus. The demonstration that plant-derived extracts can effectively inhibit bacterial growth, including against multidrug-resistant organisms, supports the continued investigation of medicinal plants as sources of new antimicrobial agents. With the global crisis of antibiotic resistance threatening to undermine modern medicine, the identification and characterization of plant-derived compounds with antibacterial properties represents a critical research priority. The fact that these extracts contain complex mixtures of bioactive compounds may offer advantages over single-agent antibiotics, as the multiple components may act through different mechanisms, potentially reducing the likelihood of resistance development.

Furthermore, the observed antioxidant properties of Citrullus colocynthus extracts suggest potential applications beyond direct antimicrobial therapy. Oxidative stress plays a pathogenic role in numerous diseases, and antioxidant compounds can contribute to disease prevention and management through multiple mechanisms. The combination of antioxidant and antibacterial activities in a single plant extract is particularly valuable, as many disease states involve both infectious and oxidative components.

The findings of this study also have implications for the traditional uses of Citrullus colocynthus in various systems of medicine. The documented biological activities provide scientific validation for the empirical use of this plant in treating infections and other ailments, supporting the concept that traditional medicine systems can serve as valuable guides for modern drug discovery. However, it is important to note that traditional preparations may differ significantly from the standardized extracts used in this study, and further research is needed to bridge the gap between traditional practice and evidence-based medicine.

Several limitations of the present study should be acknowledged. The in vitro nature of the assays means that the findings cannot be directly extrapolated to in vivo conditions without confirmation in appropriate animal models. The complex composition of the crude extracts makes it difficult to attribute the observed activities to specific compounds, and further bioassay-guided fractionation studies are needed to isolate and characterize the individual bioactive constituents. Additionally, safety and toxicity studies are essential before any consideration of clinical applications, as natural products are not inherently safe and may have adverse effects at therapeutic doses.

Future research directions emerging from this study include the isolation and structural characterization of the specific compounds responsible for the antibacterial and antioxidant activities, investigation of the mechanisms of action at the molecular level, evaluation of synergistic interactions with conventional antibiotics, assessment of safety and toxicity in appropriate models, and formulation development for potential therapeutic applications. The particularly promising activity against Pseudomonas aeruginosa warrants focused attention, given the clinical importance of this pathogen and the limited therapeutic options available for drug-resistant strains.

In conclusion, this study has demonstrated that Citrullus colocynthus, particularly the seed extract, possesses significant antioxidant and antibacterial activities that support its traditional medicinal uses and suggest potential for modern therapeutic applications. The higher phenolic and flavonoid content of the seed extract correlates with its superior biological activities, indicating that these compound classes play important roles in mediating the observed effects. The antibacterial activity against clinically significant pathogens including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli is particularly noteworthy given the growing challenges of antibiotic resistance. These findings contribute to the growing body of evidence supporting the value of medicinal plants as sources of bioactive compounds and provide a foundation for continued investigation of Citrullus colocynthus as a potential source of new therapeutic agents. Further

research is warranted to isolate and characterize the specific bioactive compounds responsible for these activities and to evaluate their safety and efficacy in appropriate in vivo models.

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