Research Article

A brief review on antibacterial activity of *Citrullus colocynthis*

Akash Sahar[1,2], Muhammad Khalid Afzal[2,3]

[1] Department of Zoology, Cholistan University of Veterinary & Animal Sciences Bahawalpur-63100, Pakistan
[2] College of Forestry, Beijing Forestry University, Tsinghua East Road No. 35, Haidian District Beijing-100083, P.R China
[3] Institute of Forest Sciences, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur- 63100, Pakistan

* Correspondence: mkhalidafzal007@gmail.com, mkhalidafzal@bjfu.edu.cn (+92-3442255880)

Abstract

The fruit, seeds, and leaves of *Citrullus colocynthis* were subjected to extraction using various solvents, including ethanol, methanol, and aqueous solutions. The resulting extracts were evaluated for antibacterial activity against a range of Gram-positive and Gram-negative bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, through disk diffusion assays and minimum inhibitory concentration (MIC) determination. *Citrullus colocynthis*, commonly known as bitter apple or colocynth, is a perennial herbaceous plant with a long history of use in traditional medicine. This study investigates the antibacterial properties of *Citrullus colocynthis*, focusing on its potential as a natural antimicrobial agent. The findings demonstrate significant antibacterial activity of the plant extracts, particularly against Gram-positive bacteria. Methanolic extracts exhibited the highest efficacy, suggesting that the antibacterial activity is primarily attributed to bioactive compounds such as phenolic acids, flavonoids, and cucurbitacins. These results highlight the potential of *Citrullus colocynthis* as a promising source of natural antibacterial agents, which could be valuable in the development of alternative treatments for bacterial infections, particularly given the increasing issue of antibiotic resistance.

Keywords: Antibacterial activity, bitter apple, *Citrullus colocynthis*, *E.coli*, Flavonoids, Phenolic acids

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1. Introduction

Citrullus colocynthis is commonly called a bitter apple or colocynth, a perennial herbaceous plant native to the Cucurbitaceae family. Native to arid and semi-arid areas, this plant has been grown in its natural environment in North Africa, the Middle East, India, and Mediterranean countries [1]. Traditionally, *Citrullus colocynthis* is utilized in folk medicine as a purgative, anti-inflammatory agent, and anti-diabetic agent. Over the past decade, it has increasingly been in focus regarding its potential as an antimicrobial agent-particularly in light of the increased antibiotic resistance and a general need for natural alternatives to synthetic antimicrobials [2].

The medicinal attributes of *Citrullus colocynthis* are greatly attributed to the rich phytochemical profile. Phytochemical studies have so far revealed several bioactive principles from the plant, which include cucurbitacins, alkaloids, flavonoids, saponins, tannins, and phenolic acids. Amongst the bioactive components of the plant, the cucurbitacins are highly oxygenated tetracyclic triterpenoids that represent the most active principles of the plant with potential antibacterial and anti-inflammatory and anticancer activities. These compounds have been reported to inhibit bacterial growth by disrupting cell membranes and interfering with important bacterial functions [3].

Phenolic compounds and flavonoids present in the plant also make significant contributions to its antibacterial activity. These compounds are known to chelate metal ions, scavenge free radicals, and disrupt bacterial cell walls, which gives a compound effectiveness against a wide range of pathogenic microorganisms. Additionally, the occurrence of alkaloids and saponins increases the plant's antimicrobial effects owing to the fact that such compounds may inhibit bacterial protein synthesis and membrane integrity.

Antimicrobial activities of *Citrullus colocynthis* were conducted against Gram-positive and Gram-negative bacterial strains. The methanolic extract of the fruit and seeds exhibited a strong bacteriostatic activity against *Escherichia coli* and *Staphylococcus aureus* that are common causes of human infections [4]. Methanol as an extracting solvent is effective in extracting polar compounds like phenolics and flavonoids, which are responsible for the plant's potent antibacterial activities.

In this regard, a study undertaken by [3] illustrated that fruit extracts from *Citrullus colocynthis* have shown very encouraging results for the presence of antibacterial activity against the bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* using disk diffusion assays. For both bacteria, the Minimum Inhibitory Concentration values were fairly low to reflect the good efficacy of the extracts. This therefore points to the prospect of bioactive compounds present in the plant for their possible development as natural anti-bacterial compounds.

In addition, methanolic extracts of *Citrullus colocynthis* tested by [5] showed strong activity against Gram-positive *Staphylococcus aureus*, a well-known bacterium responsible for nosocomial infections that possesses acquired resistance to antibiotics. The activities of the plant against bacteria were mainly attributed to the high content of cucurbitacins and flavonoids which disrupt bacterial cell walls and inhibit bacterial enzyme activity.

The anti-*Citrullus colocynthis* bacterium is thought to exert several mechanisms of action. The different secondary metabolites of cucurbitacins and phenolic acids disrupt the bacterial cell wall and membrane, which subsequently promotes cell lysis and death. In contrast, flavonoids inhibit bacterial enzymes and proteins essential for microbial growth and replication via inhibiting action [6]. Besides, alkaloids in the plant interfere with the process of replication of DNA in bacteria; thus, the bacteria cannot multiply.

These also exhibit high antioxidant activity, which contributes indirectly to its action against microbial infection by reducing the oxidative stress in the infected tissue. Therefore, *Citrullus colocynthis* is a very good agent for developing new pharmacological remedies against bacterial infections because of rising cases of antibiotic resistance. In *Citrullus colocynthis*, dual antibacterial and antioxidant action can be achieved, and this plant is a promising candidate for developing new treatments against bacterial infections in view of the rising antibiotic resistance.

With increased antibiotic resistance worldwide, the need for the development of other forms of treatments, especially those from natural sources, is of high essence. *Citrullus colocynthis* hence presents a potential solution in that its bioactive phytochemicals have been reported in several literatures to exhibit strong activities against different bacteria resistant to several antibiotics. Its extracts could therefore be developed into topical formulations for wound infections or oral treatments for gastrointestinal infections caused by these resistant bacteria.

Besides the antibacterial action, *Citrullus colocynthis* exhibits antifungal, antiviral, and anti-inflammatory activity, providing wide scope to its application areas for therapeutic use. Due to the ability of acting against both types of pathogens, *Citrullus colocynthis* can be advanced as one of the most versatile plant-based remedies in fighting infectious diseases.

2. Botanical Properties

Citrullus colocynthis, a member of the Cucurbitaceae family, demonstrates considerable genetic diversity and is highly adapted to arid and semi-arid environments. Known for its strong resilience to both salinity and water stress, it thrives particularly well in alkaline soils. *C. colocynthis* is a perennial plant with morphological characteristics resembling that of a watermelon, featuring stiff, hairy green stalks. Its leaves are arranged alternately on long petioles, triangular in shape, and covered in fine hairs. These leaves exhibit varying degrees of indentation and possess a dark green upper surface and a paler, uneven underside. The plant produces solitary yellow flowers, which bloom from the axils of its leaves. The fruit is apple-sized, smooth, and extremely bitter, with a yellow, dry appearance. Upon ripening, the fruit develops a tough, coriaceous outer peel, encasing a spongy, white flesh filled with numerous ovate seeds. The seeds are shiny, oily, and compressed, measuring approximately 0.5 cm in width and 0.75 cm in length [7].

2.1. Botanical Classification

C. colocynthis belongs to the class *Magnoliopsida* under the kingdom *Plantae*, sub-kingdom *Tracheobionta*. It is part of the genus *Citrullus* within the Cucurbitaceae family. The plant's scientific name is *Citrullus colocynthis* (L.) Schrad., classified under the division *Magnoliophyta* and the order *Cucurbitales* [8].

2.2. Vernacular Names

C. colocynthis is known by various names across regions: in Kannada as *Hamekkae* and *Haramekkikayi*, in Malayalam as *Kattuvellari*, in Marathi as *Kaduindravani*, and in Sanskrit as *Brihadvani*, *Brihatphala*, and *Atmaraksha*. In Tamil, it is called *Kumatti* or *Peykomatti*, in Telugu as *Chittipapara*, and in Urdu as *Hanzal*, *Indyaran*, and *Shahmehinzal.* (Bnyan *et al.*, 2013).

2.3. Common Names

Common English names for *C. colocynthis* include "Vine of Sodom," "Colocynth," "Bitter Apple," and "Bitter Cucumber".

Fig.1. *Citrullus colocynthis*

2.4. Geographical Distribution

C. colocynthis is widely distributed across various regions, including the Islamic Republic of Iran, Pakistan, Afghanistan, India, Egypt, Kuwait, Saudi Arabia, Turkey, North Africa, and the Sahel region. The plant is also found in Cyprus, Lebanon, Jordan, and the Mediterranean region, as well as the Syrian Arab Republic [9]. Its wide adaptability to harsh environmental conditions contributes to its expansive geographical presence.

2.5. Cultivation

C. colocynthis can be propagated both vegetatively and generatively. In the wild, it is a perennial herb that flourishes during the Indian summer, from January to October. The plant thrives best in sandy soils and is often found in arid desert environments. Due to

its high tolerance to drought and salinity, *C. colocynthis* has been successfully cultivated in regions characterized by extreme climates.

3. Morphological Description

C. colocynthis is a perennial herbaceous vine with flocculent, delicate shoots, and obliquely branching stems. Its leaves are acutely lobed with sterile segments. The leaves are arranged alternately along lengthy petioles and are typically divided into three to seven lobes, each measuring approximately five to ten centimeters in length. The flowers are small, yellow, and unisexual, blooming at the leaf axils. The plant produces large, globoid fruits, which are green with white variegations, measuring about 5–7.5 cm in diameter [10]

The seeds are oblong, compact, and compressed, approximately 6 mm in length, located on the parietal placentation within the fruit. The root system is large and robust, enabling the plant to anchor itself in sandy soils, while the vine-like stems spread across the ground and climb over adjacent vegetation with the help of axillary tendrils [2].

4. Chemical Components

The chemical composition of *C. colocynthis* is crucial in determining its pharmacological and toxicological effects. The plant contains a range of bioactive compounds, most notably cucurbitacins, which are oxygenated tetracyclic triterpenes responsible for its bitter taste and significant medicinal properties. Additionally, the plant contains glycosides that can be hydrolyzed by enzymes into cucurbitacin E, J, and L, among others. These compounds have been shown to possess potent anti-inflammatory and anti-cancer properties [11].

Beyond cucurbitacins, the plant also contains alkaloids, flavonoids, phenolic acids, and tocopherols. Flavonoids and phenolic acids are known for their antioxidant and antimicrobial effects, contributing to the plant's traditional uses in treating infections. Furthermore, quercetin has been isolated from *C. colocynthis*, which is widely recognized for its anti-inflammatory and antioxidant properties [3].

Cucurbitacins are primarily tetracyclic triterpenoids derived from the cucurbitane molecule. They are highly oxygenated compounds that exhibit a distinct bitter taste. Cucurbitacins have been extensively studied for their pharmacological effects, including their anti-inflammatory, hepatoprotective, and anticancer activities**.** These compounds are abundant within the Cucurbitaceae family, and in *C. colocynthis*, they are responsible for the plant's strong bioactivity. Cucurbitacin E, J, and L, in particular, are the most prevalent in *C. colocynthis* and have demonstrated significant antibacterial and anticancer potential [10]

5. Antimicrobial Activity of *Citrullus colocynthis*

Citrullus colocynthis, which is commonly referred to as bitter apple or colocynth, is one plant that has been under much consideration in the recent years on account of its effective antimicrobial activities. Though traditionally used in various forms and manners for a variety of illnesses, modern research proves that this plant, being a natural agent, has effective antimicrobial action against a broad spectrum of pathogens such as bacteria, fungi, and viruses.

5.1. Antibacterial Activity

The antibacterial activity of *C. colocynthis* has been documented by several studies against Gram-positive and Gram-negative bacteria. Methanolic extracts of the plant, especially those from the fruits and seeds, have been widely reported in various studies to exhibit strong inhibition activities against various pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. MICs in these studies further demonstrate *that C. colocynthis* extracts can be highly effective at very low concentrations, which makes it a promising candidate for developing natural antibacterial agents.

[7] conducted an experiment where methanolic extracts from *C. colocynthis* demonstrated high antibacterial action against *Staphylococcus aureus* and *Escherichia coli*, which falls in the range between 0.5 to 2 mg/mL. Those modes of antibacterial activities are believed to be largely due to cucurbitacins and phenolic compounds, since these are known to disrupt bacterial cell walls and inhibit enzymatic activity that is crucial for survival in bacteria.

In this regard, [5] presented related research in which the methanolic extracts of *C. colocynthis* were found to display potent antibacterial activity against *Pseudomonas aeruginosa* and *Salmonella typhi*-two well-known Gram-negative pathogens known to cause a wide range of infections. These extracts were especially active in the inhibition of bacterial growth, further establishing this plant as a potential source of natural antibacterial compounds. Similarly [12, 13] has also found the antimicrobial potential in several medicinal plants extract.

5.2. Antifungal Activity

Besides its antibacterial activities, antifungal activity has also been established for *C. colocynthis*. That is very important, considering the increase in fungal infections and the inefficiency of currently available drugs that act against fungi. This extract has also proven effective against certain pathogenic fungi, which include *Candida albicans*, *Aspergillus niger, and Trichophyton mentagrophytes*, as reported by [14].

A study conducted by [15] demonstrated that the ethanolic extracts from *C. colocynthis* exhibited efficiency in the suppression of *Candida albicans*, a widely opportunistic agent involved in infections among immunocompromised patients. In this regard, antifungal

action is attributed to phenolic acids and flavonoids that interfere with cell membrane integrity in fungal pathogens.

5.3. Antiviral Activity

Compared to antibacterial and antifungal activities, fewer studies have actually considered the antiviral potentials of *C. colocynthis*. Preliminary studies show that colocynthis may equally possess some antiviral properties. In the context of the present global search for new agents with antiviral activity from natural sources, particularly against new emerging viral infections, this is very important. Flavonoids and cucurbitacins are constituents in *C. colocynthis* recognized for their antiviral activity and have been reported to inhibit the replication of viruses in vitro [2]. However, more in-depth studies are necessary for the revelation of the complete anti-viral potential of *C. colocynthis.*

6. Mechanism of Antimicrobial Action

The very diverse array of bioactive compounds in *C. colocynthis* is considered responsible for its antimicrobial activities. Cucurbitacins are the most studied constituent of this plant and represent highly oxygenated tetracyclic triterpenoids with significant antimicrobial action. Such types of compounds are known to disrupt microbial cell membranes, interfere with protein synthesis, and inhibit essential enzymes that microorganisms require for their growth.

It also includes phenolic acids and flavonoids-antioxidants and free radical scavengers that contribute to the antimicrobial potential of this plant. These compounds can exert their action by cell membrane damage or interference with cellular metabolic pathways of the pathogens. Alkaloids and essential oils present within *C. colocynthis* also possess antimicrobial activity, possibly through interactions with bacterial DNA and proteins, thus further hindering microbial replication and survival.

7. Potential Applications in Medicine

The antimicrobial potential of *C. colocynthis*, considered in relation to the increasing problem of antibiotic resistance, is immense. Topical formulations for treating skin infections or wound healing, or oral medications for gastrointestinal infections, could be developed using the plant extracts. More importantly, its antifungal and antiviral spectrum is broadened and can be utilized against infections when conventional antimicrobial treatments have failed. *C. colocynthis*, therefore, merits an alternative or adjuvant therapy to existing antimicrobial agents to reduce further the spread of multidrug-resistant pathogens. Future studies are needed in the isolation and characterization of the active compounds of the plant for the development of standardized and effective antimicrobial preparations from *C. colocynthis.*

8. Conclusion

Citrullus colocynthis (L.), also known as bitter apple and colocynth, among other common names, is a plant with enormous perspectives for its antimicrobial action against a wide

range of pathogens such as Gram-positive and Gram-negative bacteria. Its remarkable antibacterial and antifungal, probably antiviral activities, can be attributed to its rich phytochemical profile underlined by high concentrations of cucurbitacins, phenolic acids, and flavonoids. Several studies reported that extracts taken from different parts of *C. Colocynths*, especially those prepared with methanol, showed remarkable inhibitory activity against a wide spectrum of pathogens that included the following: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans.*

In view of the growing menace posed by antibiotic-resistant bacteria worldwide, *C. colocynthis* provides a potentially good alternative or supplement to conventional antimicrobial therapies. Such dual action-antioxidant and antimicrobial-considerably enforces its chance of being developed into effective remedies for infections caused by drug-resistant pathogens. Besides, the general activities of the plant against a wide variety of pathogens offer great versatility for this natural antimicrobial agent.

The isolation and characterization of its active principles, followed by the formulation of standardized products for clinical use, would *need further studies. With such a wide pharmacological spectrum, C. colocynthis* could provide new sources of natural antimicrobial compounds, offering an alternative to global strategies against infectious diseases resulting from increased antimicrobial resistance.

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Akash Sahar used his personal finances to complete this project.

Conflict of interest

Both authors do not have any conflict of interest.

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