Readers Insight
Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X
Research Article | Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v7i3.3120

Vol. 7 No. 3, 2024: pp. 581-592
www.readersinsight.net/pjmls
Revised: September 11, 2024 **Accepted:** September 19, 2024
Submission: June 25, 2024 **Published Online:** September 30, 2024

PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ANALYSIS OF CITRULLUS COLOCYNTHIS

Mahganj Bakhshi[1], Saima Maher[1]*, Haneef Ur Rehman[2], Anum Abbas[5], Maria Essa[3], Shamim Khan[4], Shagufta Fahmid[1], Saleha Suleman Khan[1], Farah Mukhtar[1], Nusrat Nabi[1], Nadra Naheed[5]

[1]**Department of Chemistry,** Sardar Bahadur Khan Women University, Quetta, Pakistan
[2]**Department of Natural and Basic Science,** University of Turbat, Kech, Balochistan, Pakistan
[3]**Department of Environmental Chemistry**, Sardar Bahadur Khan Women University, Quetta, Pakistan
[4]**Department of Pharmacy,** Sardar Bahadur Khan Women University , Quetta, Pakistan
[5]**ICCBS,** University of Karachi, Karachi, 72500, Pakistan

***Corresponding Author:** Dr. Saima Maher. E. mail: saimamaher@yahoo.com

Abstract

Citrullus colocynthis, traditionally recognized for its medicinal properties, has been the subject of recent studies investigating its phytochemical analysis and antimicrobial activity. The study employed the Soxhlet extraction method with methanol, ethanol, and ether to extract compounds from different parts of the plant, including the stem, leaves, roots, and flowers. The phytochemical analysis revealed the presence of various compounds such as carbohydrates, glycosides, alkaloids, steroids, saponins, amino acids, phenols, and flavonoids in the methanolic, ethanolic, and chloroform extracts. The crude methanolic extract of Citrullus colocynthis showed strong DPPH radical scavenging activity, with a maximum of 96% at 0.8 mg/mL concentration, indicating significant antioxidant potential, the ethanolic extract of the flower demonstrated higher antibacterial activity compared to extracts from the leaves and stem. The methanol extract of Citrullus colocynthis flowers exhibited the highest against S. aureus (31 mm) and E. coli (19 mm), while showing moderate inhibition against P. aeruginosa (14 mm) and Bacillus subtilis (20 mm). In contrast, the stem extracts showed minimal or no inhibition across all organisms, including Klebsiella pneumoniae. The ethanolic extract exhibited significant effects, emphasizing the potential of C. colocynthis as a source of natural antimicrobial agents. This research was motivated by the growing need to find alternative antimicrobial agents due to increasing antibiotic resistance. The study aimed to address this gap by exploring Citrullus colocynthis for its potential as a source of natural antimicrobial compounds. However, detail research is required to purify and characterize its bioactive compounds. This will help better understand its therapeutic properties and uses. The outcomes of this research contribute to future study by prominence Citrullus colocynthis as a valuable source for determining natural antimicrobial agents and exploring bioactive compounds with medicinal properties.

Keywords: *Antimicrobial activity, Citrullus colocynthis, Medicinal plant, Phytochemicals*

INTRODUCTION

Medicinal chemistry is an interesting and rapidly evolving field that combines the principles of synthetic organic chemistry and pharmacology to facilitate the discovery and development of new therapeutic agents. This discipline focuses on the design, synthesis, and evaluation of bioactive molecules, primarily aimed at treating human and animal diseases. As one of the most stimulating branches of science, it attracts the interest of researchers who are passionate about improving healthcare outcomes through innovative drug development. At its core, medicinal chemistry involves understanding the intricate relationship between a compound's chemical structure and its biological activity (1). This knowledge is crucial for optimizing the efficacy and safety of new drugs. Medicinal chemists engage in various activities, including the synthesis of novel compounds, the isolation of active ingredients from natural sources, and the application of advanced techniques to enhance drug performance. The field also emphasizes the importance of structure-activity relationship (SAR) studies, which help researchers identify how modifications to a molecule can impact its therapeutic potential. By exploring these relationships, medicinal chemists can design more effective drugs with fewer side effects. In addition to traditional synthetic methods, medicinal chemistry increasingly incorporates biotechnological approaches, such as genetic engineering and synthetic biology. These innovations enable the sustainable production of bioactive compounds derived from natural sources, thereby enhancing the availability of potential therapeutic agents. Medicinal plants serve as a vital resource for addressing various health issues, including numerous medication-related challenges, ailments, and syndromes. Particularly prevalent in regions such as the Muslim Republic of Iran, China, India, and other Muslim countries in Asia, these plants have been utilized for centuries in traditional medicine (2, 3).

A key aspect of medicinal chemistry is the exploration of natural products, which have long been a vital resource in drug discovery due to their diverse and complex chemical structures. One such natural product is *Citrullus colocynthis*, commonly referred to as bitter apple or bitter cucumber, a member of the *Cucurbitaceae* family. This plant has been traditionally used in various regions, including the Mediterranean and Asia, for its medicinal properties. It is known for its bioactive components found in the seeds and fruits and characterized by its orange-colored fruit resembling that of an orange, typically found in arid regions of Mediterranean countries (4). *C. colocynthis*, locally known as Gunj or Kulkushta, is a xerophytic plant with significant medicinal properties (5). In India, indigenous communities have employed this plant as a natural treatment for bacterial infections, including tuberculosis and respiratory diseases. Pharmacological evaluations have confirmed its antimicrobial properties, making it effective against a range of pathogens (6). The medicinal applications of *C. colocynthis* are extensive. It is traditionally used to treat fever, abdominal disorders, intestinal parasites, and various forms of congestion (hepatic, visceral, and cerebral). Additionally, root extracts are utilized for conditions such as jaundice and urinary diseases. The fruit has been recognized for its potential as an anti-cancer agent and is also effective in alleviating joint pain. Studies indicate that both aqueous and methanol extracts of *C. colocynthis* exhibit significant antimicrobial activity against bacteria like *Escherichia coli* and *Staphylococcus aureus* (7, 8). Its significant pharmacological activities. Among its many uses, *C. colocynthis* is recognized for its antimalarial, antimicrobial, antioxidant, hepato-protective, anti-spermatogenic, and potential carcinogenic properties (9-11).

The fruit of *C. colocynthis* is particularly valued in traditional medicine. It is employed as an herbal remedy for conditions such as diabetes (13) and is believed to possess antitumor activity. Additionally, the fruit is rich in analgesic and anti-inflammatory compounds, making it effective for pain relief and inflammation management (14). The plant has also been noted for its antibacterial and anti-candida properties, which are beneficial for treating skin infections and gynecological issues caused by microbial infections. *C. colocynthis* exhibits remarkable antimicrobial activity against both Gram-negative and Gram-positive bacteria, demonstrating a broad spectrum of effectiveness against various pathogens. This includes its efficacy against *Mycobacterium tuberculosis*, the causative agent of tuberculosis, which is one of the major infectious diseases globally (World Health Organization). The seeds and fruits are particularly noted for their antimicrobial properties, further supporting their use in traditional medicine (15). In terms of environmental adaptability, *C. colocynthis* thrives in extreme xeric conditions, tolerating annual precipitation levels ranging from 250 to 1500 mm and temperatures between 14.8°C and 27.8°C (16). This resilience underscores its potential as a sustainable source of medicinal compounds in arid regions. The plant contains various chemical constituents, including glycosides, cucurbitacins (B, E, I, and E2 glycoside), alkaloids, resins, and gums. These components contribute to its effectiveness as an antimalarial, antimicrobial, antioxidant, hepato-protective, anti-spermatogenic, and even carcinogenic agent (17).

Additionally, *C. colocynthis* is traditionally used to treat poisonous bites from dogs and snakes and is employed in enemas. The seeds of *C. colocynthis* are particularly notable for their nutritional composition, containing approximately 25.2% oil, 28.4% protein (60% in defatted flour), 82% carbohydrates, 36% ash, and 2.7% fiber (18). They are rich sources of essential amino acids such as arginine, methionine, and tryptophan. The presence of various secondary metabolites including carbohydrates, saponins, tannins, essential oils, flavonoids, and triterpenoids underscores the plant's medicinal potential. Research has shown that several secondary metabolites from *C. colocynthis* exhibit significant biological activities. Flavonoids and cucurbitacins have been identified as key components contributing to the plant's medicinal properties (18). These chemical compounds are crucial for identifying beneficial agents that enhance the medicinal value of traditional plants (18). The bioactive constituents found in *C. colocynthis* include flavonoids, carbohydrates, tannins, alkaloids, triterpenoids, and steroids (19). Infusions made from the seeds and fruits of *C. colocynthis* are recommended for diabetic patients due to their effective antidiabetic properties. The plant's efficacy in managing diabetes is attributed to its alkaloids, polyphenols, polysaccharides, gums, and glycans (20). Overall, the diverse chemical composition of *Citrullus colocynthis* not only highlights its importance in traditional medicine but also emphasizes its potential for developing modern therapeutic agents against various health issues. This study aims to characterize the phytochemical analysis and anti-microbial properties of different extracts from *Citrullus colocynthis* to explore its potential as a natural antimicrobial agent and contribute to the ongoing search for substitutes to conventional antibiotics.

Table I. Nutritive value of *Citrullus colocynthis* (seeds)

Ingredients	Percentage/Amount	Description
Oil Content	25.2%	Rich in fatty acids, used in cooking and medicinal applications
Protein	28.4% (60% in defatted flour)	High protein content, essential for growth and repair
Carbohydrates	82%	Major energy source
Ash Content	36%	Represents the mineral content (calcium, magnesium, etc.)
Fiber	2.7%	Aids digestion and promotes gut health
Essential Amino Acids	Present	Includes arginine, methionine, and tryptophan
Calories	~550 kcal/100g (approximate)	High caloric value due to oil and carbohydrate content
Fatty Acids	Includes linoleic acid	Important for heart health
Minerals	Includes calcium, magnesium, phosphorus, potassium	Vital for bone health and metabolic functions

Table II. Highlights the important secondary metabolites of *C. colocynthis* and their medicinal significance

Secondary metabolites	Biological activities	Key components	Reported biological properties
Carbohydrates	Provide energy, support metabolic functions	-	Nutritional, energy source
Saponins	Antimicrobial, anti-inflammatory, antioxidant properties	-	Anticancer, immunomodulatory, cholesterol-lowering
Tannins	Astringent, antimicrobial, antioxidant properties	-	Antiviral, anti-diarrheal, hepatoprotective
Essential Oils	Antimicrobial, antifungal, anti-inflammatory activities	-	Antibacterial, insecticidal, analgesic
Flavonoids	Antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective activities	Identified as key contributors to medicinal properties	Cardioprotective, antidiabetic, neuroprotective
Triterpenoids	Anti-inflammatory, anticancer, antimicrobial activities	-	Antiviral, cytotoxic, antifungal
Cucurbitacins	Antitumor, anti-inflammatory, antioxidant effects	Identified as key contributors to medicinal properties	Anti-ulcer, anti-obesity, cytotoxic

MATERIALS AND METHODS

IDENTIFICATION AND COLLECTION OF THE PLANT

The identification of *Citrullus colocynthis* was confirmed by a plant taxonomist at the Botany Department SBK, Quetta. Additionally, the herbarium specimen has been deposited in the Botany Department with the reference code CHEM/CC-108. The *Citrullus colocynthis* specimen was collected from the arid regions of Gwadar, Balochistan, Pakistan. The habitat is characterized by sandy and dry soil typical of desert environments during the months of July and August, ensuring that healthy and mature specimens were selected for analysis. (The maturity of *Citrullus colocynthis* was assessed by observing its flowering and fruiting stages. Specimens were considered mature if they exhibited fully developed flowers or fruits). Once the appropriate plant parts were collected, they underwent a thorough washing process with water. After washing the *Citrullus colocynthis* plant parts, they were carefully cut into smaller pieces measuring approximately 0.5–1.5 cm^3. This size was chosen to facilitate uniform drying and ensure efficient which ensures efficient moisture removal extraction of bioactive compounds analysis. These pieces were then placed on shade dry for a period of ten days. Shade drying was conducted under controlled environmental conditions with a temperature maintained at approximately 25–30°C and humidity levels around 40–60%. These conditions were chosen to enhance drying efficiency while preserving the stability of the bioactive compounds in the plant material. After initial drying period, the samples were further processed in a hot air oven set at 40 °C for one hour.

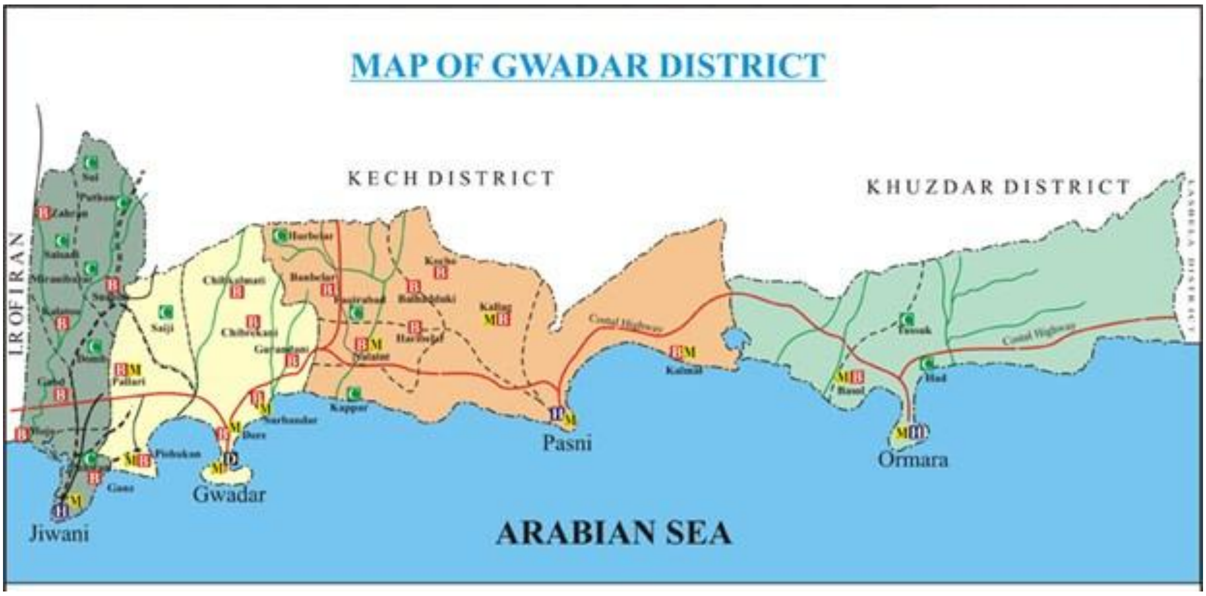

Fig. 1. Location of Gawader

Fig. 2. Experimental procedure

PREPARATION OF PLANT MATERIAL

The dried plant parts were converted into fine powder. This powder was then sieved through an 80-mesh size to ensure homogeneity in particle size, which is important for effective extraction. Approximately 40 grams of powdered plant material was used in the extraction process.

EXTRACTION OF *CITRULLUS COLOCYNTHIS*

The extraction of phytochemical compounds from *Citrullus colocynthis* was conducted using the Soxhlet extraction method, which is effective for obtaining concentrated methanolic crude extracts. 40 grams of powdered plant material from *Citrullus colocynthis* was subjected to extraction using methanol as the

solvent and extraction was performed using 200 ml of methanol as the solvent. At temperature of 45 °C, the resulting extract was collected. The concentrated extract was further dried in a vacuum oven at 45 °C to eliminate any remaining moisture (19). The yield of the extract was calculated using the formula:
% Yield = (Weight of Extract / Weight of Powdered Plant Material) × 100.

PHYTOCHEMICAL ANALYSIS

Phytochemical screening was performed to access the presence of major secondary metabolites alkaloids, flavonoids, terpenes, anthraquinone and phenol in methanolic extract. The secondary metabolites play a vital role to keep bioassay such as antibacterial, antifungal, antioxidant, analgesic and anticancer (18).

TEST FOR ALKALOIDS

The extracts of plant parts were mix distinctly in dilute HCL and then filter next that remains were individually subjected to Hager's, 1 mg/ml concentration of the plant extract and reagents, allowing reactions to develop for 10 minutes to check the presence of alkaloids.

TEST FOR CARBOHYDRATES

Plant extract 1 mg/ml concentration of the plant extract was mixed individually with 5 ml of purified water and then filtered. The filtrate was tested using Molish's reagent. The appearance of a violet color indicated the presence of carbohydrates.

TEST FOR SAPONINS

Plant extract was shaken thoroughly in a test tube with 5 mL of water for 5 minutes. The formation of two distinct layers indicated the presence of saponins.

TEST FOR GLYCOSIDES

Each plant extract was hydrolyzed with dilute hydrochloric acid (5 ml), and then treated with Brontrager's reagent. The appearance of a pink color indicated the presence of glycosides.

BRONTRAGER'S REAGENT TEST

Ferric chloride ($FeCl_3$) solution was mixed with the plant extract and transferred to boiling water for a few minutes. After cooling to room temperature, the solution was thoroughly mixed with an equal volume of benzene. The resulting solution was treated with ammonia, indicating the presence of glycosides if a pink color appeared.

GELATIN TEST

The extract was mixed with 5 ml of a 1% gelatin solution containing sodium chloride (NaCl). The formation of a precipitate indicated the presence of tannins.

TEST FOR FLAVONOIDS

Lead acetate test was used to detect flavonoids in the extract.

LEAD ACETATE TEST

Lead acetate solution was added to 5 ml of each extract. The appearance of a yellow precipitate indicated the presence of flavonoids.

TEST FOR AMINO ACIDS

The Ninhydrin test was employed for the detection of amino acids. Two drops of Ninhydrin solution were mixed with the extract. The appearance of a purple color indicated the presence of amino acids.

DPPH ASSAY

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is usually employed for evaluating the antioxidant activity, mostly plant extracts. The principle that antioxidants can scavenge free radicals, specifically the stable DPPH radical, which is considered by a deep violet color in solution result. A DPPH solution is prepared and mixed with different concentrations 0.2, 0.4, 0.6, and 0.8 µg/ml, of the antioxidant sample, and incubate. Not the absorbance and then decrease in absorbance, as (0.28, 0.033, 0.112, and 0.026) was used to assess the antioxidant activity. Each sample was incubated with the DPPH solution for 30 minutes at room temperature before the absorbance was measured at 517 nm using a spectrophotometer, reflects the reduction of DPPH radicals by the antioxidant compounds in the sample. The percentage of radical scavenging activity (% RSA) is calculated to quantify the antioxidant capacity, with higher values indicating greater efficacy in scavenging free radicals (13).

ANTI-BACTERIAL ASSAY

The antibacterial activity of *Citrullus colocynthis* crude extracts and their fractions was evaluated using the agar well diffusion method. The crude plant extracts were dissolved in dimethyl sulfoxide (DMSO) and filtered to remove impurities. For bacterial culture preparation, a nutrient broth medium was inoculated with target bacteria and incubated at 37°C for 24 hours. Mueller-Hinton agar plate the applying method of well diffusion. Well (6mm) were ready and 100µl of each extract injected in well. The plates were prepared for detection of anti-bacterial and incubated for 16-24h, after the day results of anti-bacterial was ready for measures the inhibition zones, mostly zones were measured by parameter in millimeter. For positive control, amoxicillin (Amoxil) discs were used, and the inhibitory zones of the plant extracts were compared against those of the antibiotic. The sterilization of bench and working surfaces was done to ensure a contamination-free environment. The crude methanolic extract of different parts of plant extracts were dissolved in DMSO at concentrations of 100 mg/ml, 50 mg/ml, and 25 mg/ml (% w/v) for the antibacterial assay. Using the well diffusion method for both assays, wells of 6 mm were made in Mueller-Hinton agar (for bacteria) into which 100 µL of each extract was injected. Amoxil discs were used as positive controls in both assays. The plates were incubated for 16-24 hours at 37°C, and the inhibition zones were measured in millimeters to evaluate the antibacterial efficacy of *Citrullus colocynthis* extracts against bacterial cultures.

ANTIFUNGAL ACTIVITY

The antifungal capability of *Citrullus colocynthis*, potato dextrose media was prepared by washing 200 g of potatoes thoroughly to remove any dirt. The peeled potatoes were then cut into small pieces and boiled in 300 ml of distilled water until they became soft (about 20-30 minutes). Then filtered through muslin cloth to obtain a clear potato extract. The volume of the filtrate was measured, and 20 g of dextrose and 15 g of agar were added to the extract. The mixture was then autoclaved at 121°C for 15 minutes to sterilize it, after which it was allowed to cool slightly before pouring into sterile Petri dishes. Plant extracts included respective methanol, ethanol, and ether. To inoculate the fungal strains, fresh colonies were obtained by sub culturing the fungi on PDA plates, which were incubated at 25-28°C for 2-3 days. After sufficient growth, a sterile inoculating loop was used to obtain a small amount of fungal mycelium, which was suspended in sterile saline or distilled water to create a homogeneous suspension (approximately 1 × 10^6 CFU/ml). The concentration of the fungal inoculum (1 × 10^6 CFU/ml) was standardized using a hemocytometer. Statistical analysis was performed using ANOVA in SPSS software, with a significance level set at $p < 0.05$. Positive controls included ketoconazole and fluconazole at 50 µg/ml, and the incubation duration was set for 48 hours for *Candida albicans* and 72 hours for *Aspergillus niger*. A sterile pipette was used to place 100 µl of the fungal suspension onto the surface of the prepared PDA plates, which was then spread evenly. Wells were created in the agar plates using a sterile cork-borer (about 6-8 mm in diameter), and each well was filled with 50 µl of the different plant extracts (methanol, ethanol, ether) as well as a control solvent (for example, methanol or ethanol alone) as a negative control. A positive control, such as an antifungal agent like ketoconazole or fluconazole, was included in separate wells for comparison. The plates were incubated at 25-28°C for 24-72 hours, depending on the growth rate of the fungus. After incubation, the plates were observed for zones of inhibition around the wells containing the plant extracts and controls. The diameter of the inhibition zones was measured using a ruler or caliper, and the results were recorded for further analysis. The average zone of inhibition for each extract was calculated and compared to control groups, with appropriate statistical analysis performed to determine the significance of the findings.

The formula is used to calculate the percentage inhibition of growth in a test sample compared to a control sample (Rusman, 2006).

% inhibition = 100 - (Linear growth in test / Linear growth in control) x 100

The experiment was carried out at Department of Livestock Management, Faculty of Animal Husbandry and Veterinary Sciences Sindh Agriculture University, Tandojam, located at 25° 25' 37.85" N latitude and 68° 32' 10.28" E longitude, in a semi-arid area, and 29 meters above sea level. The average annual ambient temperature ranges from 9.4 to 42.9 ºC. The study was conducted from April to June 2023.

RESULTS AND DISCUSSION

Phytochemical analysis of *Citrullus colocynthis* plant using different standard methods revealed the following parameters with variable ranges of values (Table III).

Table III. Phyochemical Screening of *Citrullus colocynthis* methanol extract

S. No	Tests	Stem	Leaves	Root	Fruit	Constituent
1. Carbohydrates						Presence of carbohydrate
1	Molish's test	+	-	+	+	
2. Flavonoid						Presence of flavonoid
1	Lead acetates	-	+/-	+	+	
3. Alkaloids						Presence of alkaloids
1	Hager's test	+/-	+/-	+/-	+	
4. Proteins and Amino Acid						Presence of proteins and amino acid in stem and leave
1	Ninhydrin test	+	+	-	-	
5. Phytosterols and Steroid						Presence of phytosterols and steroid
1	Salkwoski test	+	+	+	+	
6. Tannin and Phenolic compound						Presence of tannin and phenolic compound in stem leave and fruit
1	Ferric chloride test	+	+/-	-	+/-	
7. Fixed oil and Fats						Presence of fixed oil and fats only in root
1	Spot test	-	-	+	-	
8. Saponins by Foam Test						presence of saponins only in fruits
1	Foam test	-	-	-	+	
9. Glycosids Test						presence of glycoside only in fruit
1	Borntrager`s test	-	-	-	+	

The occurrence of carbohydrates was confirmed in the stem, root, and fruit through Molish's test, while the leaves showed a negative result. Flavonoids were detected in the leaves, root, and fruit using lead acetate, with the stem exhibiting a negative result. Alkaloids were slightly present in the stem, leaves, and root as indicated by Hager's test, whereas the fruit showed a positive response. Proteins and amino acids were identified in the stem and leaves through the Ninhydrin test, and absent in the root and fruit. Phytosterols and steroids were found in all parts of the plant, including the stem, leaves, root, and fruit, as determined by the Salkowski test. Tannins and phenolic compounds were present in the stem and fruit, with slight positivity in the leaves, but it was notpresent in the root. The presence of oils and fats was observed only in the root while the stem, leaves, and fruit were negative. Saponins were recognized completely in the fruit using the foam test, while no presence was detected in the stem, leaves, or root. Finally, glycosides were confirmed exclusively in the fruit by Borntrager's test, with the other parts showing negative results. These findings highlight the different phytochemical constituents present in different parts of the plant, signifying potential therapeutic applications. The DPPH activity of the crude methanolic extract (CME) of *Citrullus colocynthis* was evaluated at various concentrations (Table IV). At a concentration of 0.2 µg/ml, the net absorbance was 0.28, resulting in a radical scavenging activity (% RSA) of 58%. At 0.4 µg/ml, the absorbance significantly decreased to 0.033, corresponding to a 95% RSA. The 0.6 µg/ml concentration yielded an absorbance of 0.112, with an RSA of 83%. Finally, at 0.8 µg/ml, the absorbance was 0.026, demonstrating a remarkable 96% RSA. This data indicates a strong antioxidant potential of the CME at higher concentrations.

Table IV. DPPH assay of crude Methanolic extract of *C. colocynthis*

Crude Methanolic Extract (CME)	DPPH activity		
	Conc. of test solutions (CME)	Net Absorbance	% RSA
Citrullus colocynthis	0.2	0.28	58%
	0.4	0.033	95%
	0.6	0.112	83%
	0.8	0.026	96%

The methanolic extract from *Citrullus colocynthis* was investigated for antibacterial activity. The results show in table demonstrates the antibacterial potential of essential oil of *Citrullus Colocynthis* using both disk-diffusion and micro dilution methods. Present investigation reveals the proof of antibacterial activity was evaluated through four different bacterial strains (*E. coli, P. aeruginosa, S. aureus*). The extracts of the plants showed low antibacterial activity against *E. coli, P. aeruginosa, S. aureus* Standard antibiotics as positive control (cifrofloxicin) restricted the growth of microorganism to a significant level than the subject essential oil content. Likewise, DMSO used as a negative control does not exhibited any inhibition as well. The antifungal activity of extracts of *Citrullus colocynthis* was carried out using the standard protocol of Baur et al., (1966) and Mahesh & Satish (2008) method with slight modifications. The selection of this d*isc* diffusion method was used. Plenty of fungal strains can be examined through this and the results obtained might contribute in the search of antifungal metabolites for the eradication of human and plant pathogens. In this study four different fungal strains (*Aspergillusflavus, Fusarium* sp., *Acromonium* sp., *Aspergillus niger.*) are selected to study the subject extracts for its possible potential against fungus caused pathogens. The antimicrobial activities of methanol, ethanol and ether of extracts of *Citrullus colocynthis* plant parts including leave, stem and flower were investigated using disc diffusion method and agar well diffusion method against selected pathogens such as *Escherichia coli, Pseudomonas aeruginosa* and *S. aureus* (Table V). All aforementioned extracts were used and showed varied degree of antimicrobial activity against the pathogens. The present preliminary screening for antimicrobial activity showed that the methanolic extract of leaves of *Citrullus colocynthis* produced maximum inhibitory zone (14 mm) against *P. aeruginosa,* as compared to others (Fig. 3). While the ether extracts of *Citrullus colocynthis* leaves indicated maximum inhibitory zone (14 mm) against *E. coli*. The ethanolic extract of *Citrullus colocynthis* leaves showed least antimicrobial activity. The methanolic extracts of flower of *C. colocynthis* showed higher antimicrobial zone activity of 31mm against *S. aureus*, 19 mm zone of inhibition activity against *E. col* and 14 mm against *P. aeruginosa.* The ethanolic extract of flower of *C. colocynthis* only showed antimicrobial activity by producing about 28 mm zone of inhibition against *S. aureus* and no inhibition was observed against *E. coli* and *P. aeruginosa* (Fig. 4 a, b, c). The extract of flower from ether showed no antimicrobial activity. The ethanolic and ether extracts of stem of *Citrullus colocynthis* plant showed 8mm of zone of inhibition against *S. aureus* and 14mm zone of inhibition against *E. coli*, respectively. The antimicrobial assay by agar-well diffusion

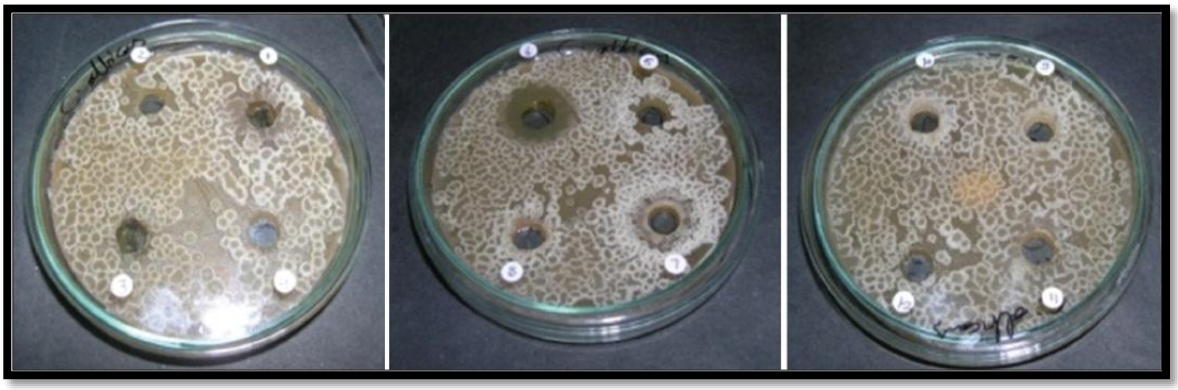

Fig 3. Antibacterial activity against *E. coli, S. aureus* and *P. aeruginosa*

method revealed that ethanolic extracts of *C. colocynthis* flower exhibited broad spectrum activity against tested isolates as compared to other parts of plant.

This study highlights that *Citrullus colocynthis* may contain novel compounds with significant antimicrobial activity, offering promising potential for the development of new therapies against antibiotic-resistant bacteria. This discovery is crucial given the increasing prevalence of antibiotic resistance and emphasizes the need for alternative treatments. The findings contribute to pharmacognosy and encourage further exploration in natural product drug development.

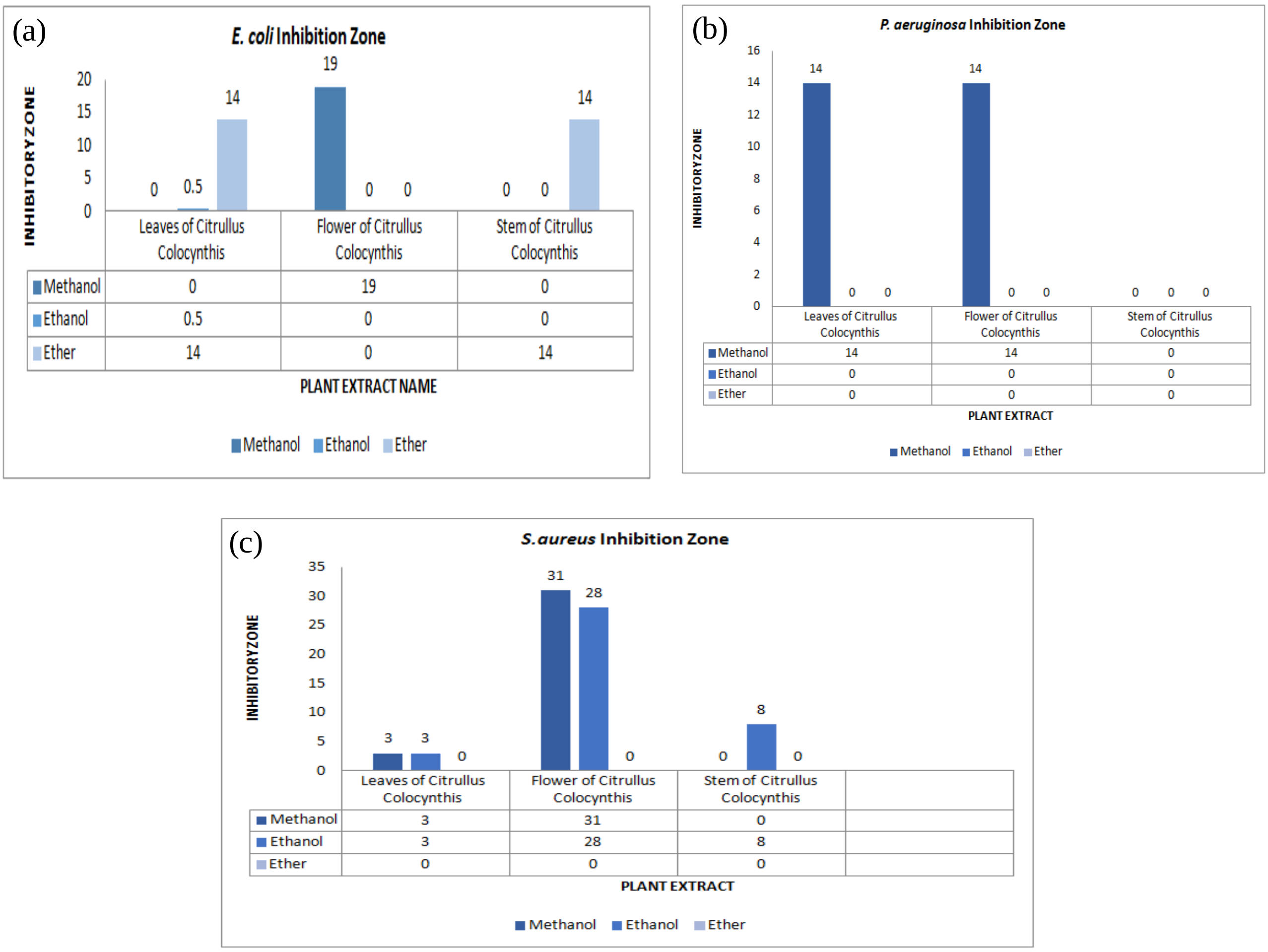

Fig. 4 (a). The zone of inhibition caused by extracts from different parts of *Citrullus colocynthis* against *E. coli*, **(b).** the zone of inhibition caused by extracts from different parts of *Citrullus colocynthis* against *P. aeruginosa*, **(c).** the zone of inhibition caused by extracts from different parts of *Citrullus colocynthis* against *S. aureus*

Table V. Antimicrobial activities of methanolic, ethanolic and ether extracts of leaves, flower and stem of *Citrullus Colocynthis* plants

S. No	Plant Extract Name	Solvent	Organism	Inhibitory zone-(mm) Control zone
01	**Leaves of *Citrullus Colocynthis***	Methanol	*E. coli*	0
			P. aeruginosa	14
			S. aureus	3
		Ethanol	*E. coli*	0.5
			P. aeruginosa	0
			S. aureus	3
		Ether	*E. coli*	14
			P. aeruginosa	0
			S. aureus	0
02	**Flower of *Citrullus***	Methanol	*E.coli*	19.0
			P. aeruginosa	14.0
			S. aureus	31
		Ethanol	*E.coli*	0

	Colocynthis		*P. aeruginosa*	0
			S. aureus	28
		Ether	*E. coli*	0
			P. aeruginosa	0
			S. aureus	0
03	**Stem of *Citrullus Colocynthis***	Methanol	*E. coli*	0
			P. aeruginosa	0
			S. aureus	0
		Ethanol	*E. coli*	0
			P. aeruginosa	0
			S. aureus	8
		Ether	*E. coli*	14
			P. aeruginosa	0
			S. aureus	0

CONCLUSION

The current research was carried to explore the chemical constituents of *Citrullus colocynthis* including carbohydrate, glycosides, alkaloids, steroid, saponin, amino acids, phenols and flavonoids. The presence of said phytochemicals was investigated in different parts of *C. colocynthis* by Soxhlet method using methanol, ethanol and ether as extraction solvent in the stem, leave, root and flower of indigenous plant of *C. colocynthis***.** The methanolic, ethanolic and chloroform extracts showed the presence of various phytochemical compounds. Furthermore, the antimicrobial activities of these extracts were determined against various pathogens. The ethanolic extracts of flower of *C. colocynthis* showed higher antibacterial activity as compared to leaves and stem. The DPPH assay was determined to crude methanolic extract of *C. colocynthis* the experiment was performed with different concentration the DPPH assay showed significance antioxidant activity in high concentration the plant have a good potential of antioxidant properties Further researches are needed to carry out on the purification of phytochemicals of said medicinal plant to obtain purified chemicals which may will enhance their antimicrobial activities. The phytochemical analysis and antimicrobial studies of *C. colocynthis* showed that this plant is a potential source of natural compounds with various medicinal characteristics. Further research for the purification and characterization of its phytochemicals will provide a good source of natural medicine for curing of various diseases in effective way.

Authors Contribution:

MGB & ME collected the plant samples, prepared extracts and HR, concept and directed the work. SK, performed the antibacterial and cell culture experiments. SF performed Anti-fungal experiments. NM, performed the chemical analysis and anti-oxidant activity. SM, designed and directed the study. FK, analyzed the data and wrote the manuscript. SSK, Critical review the manuscript. The All authors read, revised and permitted the final manuscript

Acknowledgement:

Author would like to extend to Special thanks to Dr.Tahira Mengal, Associate professor of Botany Department at Sardar Bahadur Khan Womens University, Quetta Balochistan, Pakistan, for her invaluable assistance in the identification of plant material.

Conflict of Interest:

Authors have no conflict of interest.

References:

1. Fadzen CM, Wolfe JM, Zhou W, Cho CF, Von Spreckelsen N, Hutchinson KT, Lee YC, Chiocca EA, Lawler SE, Yilmaz OH, Lippard SJ. A platinum (IV) prodrug—perfluoroaryl macrocyclic peptide conjugate enhances platinum uptake in the brain. Journal of medicinal chemistry. 2020;63(13):6741-7.

2. Ekor M. The growing popularity of herbal medicines: A global perspective. Journal of Herbal Medicine. 2014;4(2):1-5.
3. Khattak SG, Gilani SN, Ikram M. Antipyretic studies on some indigenous Pakistani medicinal plants. Journal of Ethnopharmacology. 1985;14(1):45-51.
4. Sebbagh N, Cruciani-Guglielmacci C, Ouali F, Berthault MF, Rouch C, Sari DC, Magnan C. Comparative effects of Citrullus colocynthis, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. Diabetes & Metabolism. 2009;35(3):178-84.
5. Akobundu ENT, Cherry JP, Simmons JG. Chemical, functional, and nutritional properties of egusi Colocynthis citrullus L. seed protein products. Journal of Food Science. 1982;47(3):829-35.
6. Cincinnati O. Citrullus Colocynthis. The Western Druggist, Chicago, USA. 1989.
7. Salama HM. Alkaloids and flavonoids from the air-dried aerial parts of Citrullus colocynthis. Journal of Medicinal Plants Research. 2012;6(38):5150-5.
8. Idan SA, Al-marzoqi AH, Hameed IH. Spectral analysis and anti-bacterial activity of methanolic fruit extract of Citrullus colocynthis using gas chromatography-mass spectrometry. African Journal of Biotechnology. 2015;14(46):3131-8.
9. Marzouk B, Marzouk Z, Décor R, Edziri H, Haloui E, Fenina N, Aouni M. Antibacterial and anticandidal screening of Tunisian Citrullus colocynthis Schrad. from Medenine. Journal of Ethnopharmacology. 2009;125(2):344-9.
10. Uma C, Sekar KG. Phytochemical analysis of a folklore medicinal plant Citrullus colocynthis L. (bitter apple). Journal of Pharmacognosy and Phytochemistry. 2014;2(6).
11. Marzouk B, Marzouk Z, Décor R, Mhadhebi L, Fenina N, Aouni M. Antibacterial and antifungal activities of several populations of Tunisian Citrullus colocynthis Schrad. immature fruits and seeds. Journal of Medical Mycology. 2010;20(3):179-84.
12. Mehar S, Anam I, Naheed N, Naheed S, Khan SS, Ullah N, Iqbal S, Khan N. GC-MS analysis of antibacterial compounds from floral part of methanolic extract of Rosa damascena. Pure and Applied Biology. 2019;8(1):479-88.
13. Mehar S, Zamina B, Riaz M, Riaz S, Khalid N, Imran M, Fahmid S, Ishtiaq H, Parveen S. Green synthesis of Withania coagulans extract-mediated zinc oxide nanoparticles as photocatalysts and biological agents. ACS Omega. 2023;8:46715-27.
14. Shawkey AM, Rabeh MA, Abdellatif AO. Biofunctional molecules from Citrullus colocynthis: An HPLC/MS analysis in correlation to antimicrobial and anticancer activities. Advances in Life Science and Technology. 2014;17:51-61.
15. Fallah N, Ullah N, Khan MA, Asif AH, Khan T, Ahmad W. Citrullus colocynthis failed to combat against renal derangements, in spite of its strong antioxidant properties. Acta Poloniae Pharmaceutica. 2013;70(3):533-8.
16. Rajamanickam E, Gurudeeban S, Ramanathan T, Satyavani K. Anti-inflammatory activity of Citrullus colocynthis. International Journal of Current Research. 2010.
17. Reddy VP, Pallavi K, Ravi Kumar Y, Gopinath C, Srinivasa Rao A. Anti-ulcer activity of Citrullus colocynthis fruit in male Wistar rats using a pylorus ligation model. International Journal of Pharmacy and Pharmaceutical Sciences. 2012.
18. Salama HM, Talole B, Salve P, Waje M. Phytochemical screening and determination of total phenolic content of Citrullus colocynthis Linn. International Journal of Pharmaceutical and Phytopharmacological Research. 2017;3(1):44-5.
19. Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from Citrullus colocynthis on human breast cancer cells. Biochemical Pharmacology. 2007;73(1):56-67.
20. Uma C, Sekar KG. Phytochemical analysis of a folklore medicinal plant Citrullus colocynthis L. (bitter apple). Journal of Pharmacognosy and Phytochemistry. 2014;2(6).
21. Zaini NAM, Anwar F, Hamid AA, Saari N. Kundur [Benincasa hispida (Thunb.) Cogn.]: A potential source for valuable nutrients and functional foods. Food Research International. 2011;44(7):2368-76.
22. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. Journal of Ethnopharmacology. 1997;58:45-54.