RESEARCH PAPER

Exploring the Antimicrobial and Anti-Breast Cancer Potentials of Methanolic Extract of Citrullus Colocynthis

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ABSTRACT

Background: Citrullus *colocynthis*(L.) Schrad, a member of the *Cucurbitaceae* family, is recognized for its extensive nutritional and therapeutic properties. Traditionally, it has been used to treat various ailments, including throat diseases, constipation, dyspepsia, anemia, splenomegaly, scrofula, bronchitis, asthma, ulcers, cancer, leucoderma, and urinary incontinence. Pharmacological studies indicate that this plant possesses a broad spectrum of pharmacotherapeutic activities, such as anti-cancer, antioxidant, astringent, antiamoebic, hypotensive, sedative, refrigerant, antifungal, and antibiotic effects. These properties suggest its potential as a novel drug against several cancer types.

Methods: In this study, *C. colocynthis*plants were collected from various locations in southern Punjab, shade-dried, and then crushed into coarse particles. The extract was prepared using a Soxhlet apparatus. For cytotoxic screening against human breast cancer cells, the sample was prepared at a concentration of 20 mg/mL in dimethyl sulfoxide. The cytotoxic and anti-metastatic activities of C. colocynthis were assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) assay and the wound healing assay, respectively. C. colocynthis plant material was washed with clean water and dried at room temperature, avoiding direct sunlight. The dehydrated material was then homogenized to obtain a crumbly powder, which was stored in airtight brown plastic bottles. Approximately 250 g of this powder underwent Soxhlet extraction with 2000 mL of 80% methanol.

Results: The resulting extract was concentrated using a rotary evaporator and subsequently solidified via freeze-drying. To create a library of fractions, the methanolic crude extract of Citrullus colocynthis was subjected to liquid-liquid fractionation using three different solvents: n-hexane, chloroform, and methanol. In a molecular mechanism study, we examined the expression of two important proteins, PARP and caspase-3, using Western Blotting. This plant is reported to contain a diverse array of phytochemicals, including alkaloids, xanthones, quinones, coumarins, terpenoids, tannins, flavonoids, fatty acids, proteins, carbohydrates, and phenolic compounds. A comprehensive phytochemical analysis was also conducted on the C. colocynthis extracts to identify and characterize these compounds.

Conclusion:In summary, *C. procera* leaf extract exerts significant cytotoxic effects at higher concentrations, supporting its potential utility in therapeutic applications such as cancer treatment. Conversely, its minimal toxicity at lower doses suggests its suitability for contexts requiring reduced cytotoxicity. These findings highlight the concentration-dependent activity of the extract and its potential for dose-adjusted therapeutic use.

Keywords: Antimicrobial; Anticancer; Cancer; *Citrulluscolocynthis;* Medicine, human and disease

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INTRODUCTION

Herbal medicine relies on plant leaves, roots, flowers—to treat illness and keep people healthy. Most of these remedies have roots in old traditions. People turn to herbal remedies for a few reasons: they support overall well-being, help the immune system, and ease pain or swelling.[1] Compared to synthetic drugs, they're usually cheaper and easier to tailor to each person. A lot of folks choose herbs because they're worried about chemical side effects and want to save money.

Citrullus colocynthis belongs to the *Cucurbitaceae family* and is widely used for nutritional and therapeutic value.[2] The plant compounds can stimulate treatment of some respiratory and urinary diseases like bronchitis, scrofula,

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anemia and asthma. It can be used as milding cancer medicaments as part of aptamer.[3] This plant commonly grows in the wastelands of Northern Africa (e.g., Libya) and West Middle Asia (e.g., Turkey or Palestine). *C. colocynthis* is also recognized as Abu Jahl's melon, bitter apple, or bitter cucumber. Interestingly, co-cultivationwith tobacco to inhibit *Rhizobium rhizogenes* and enhance the production of wounded phenolic compounds like acetosyringone.[4]

C. colocynthis is an organic product renowned for its broad spectrum of medicinal and nutraceutical potential and holds a significant place in traditional medicine as an insect repellent, an anticancer agent, and a laxative. Various domestic drug systems detail their therapeutic applications for intestinal issues such as gastroenteritis, colic, acid reflux, and diarrhea.[5] The plant is frequently employed as a cancer remedy, and in African and Asian countries, it has commonly treated jaundice, ulcers, irritation, as well as urinary and microbial diseases.[6]

The global perspective on the utilization of plant-based products is currently shifting. As many microorganisms develop resistance to various synthetic drugs, herbal medicine offers a promising alternative due to its synergistic effects in treating infectious diseases.[7] This pharmacological efficacy is attributed to the presence of several phytochemicals. However, despite its traditional uses, comprehensive studies are needed to further develop its medicinal applications, such as its potential as a disinfectant for eye irritation caused by contact dermatitis.[8] Given its diverse therapeutic properties, *C. colocynthis* warrants significant attention from researchers and scientists for novel drug development.[9] Therefore, further assessment is crucial to fully explore the hidden potential of *C. colocynthis* and its therapeutic applications for human well-being.[10]

Breast cancer is a problem for women, and a lot of doctors and researchers are working on it. Chemotherapy is still a part of how we treat cancer along with surgery and radiotherapy.[11] Chemotherapy works by killing the cells and stopping them from growing. It has a lot of bad side effects that are a big concern for doctors. These side effects are a problem because they affect how patients feel about getting chemotherapy for breast cancer.[12] They make it hard for people with breast cancer to keep getting chemotherapy. Breast cancer treatment is not about killing the bad cells it is also, about helping patients feel better and stay strong during the treatment.[13]

This study aimed to prepare methanol and other extracts of *C. colocynthis* and fractions of its compounds in different solvents, to perform a phytochemical analysis of *C. colocynthis, to e*xamine the antimicrobial impact of the plant extract and to determine the anticancer impact of *C. colocynthis* against BC cells.

METHODOLOGY

The current experiments were performed between 2019 and 2020 in the Research Laboratory and Cell Culture Laboratory of the Department of Zoology, Government College University, Faisalabad. The following steps were carried out:

Preparation of Plant Extract

Fresh leaves of *Citrullus colocyn* this plant were collected from Faisalabad district, Pakistan, in March 2019.The leaves were cleaned and dried at room temperature after being rinsed with clean water. The dried leaves were ground into a lumpy powder and kept in sealed brown plastic bottles. 250g of powdered material was subjected to Soxhlet extraction using 2000 mL of 80% methanol. A rotary evaporator was used to concentrate the powder, and a freeze-drier to solidify it.After that, by the liquid-liquid fractionation technique, the methanolic crude extract of *C. colocynthis* was used with these three different solvents, i.e., n-hexane, chloroform, and methanol, to make a fraction library. Three fractions were prepared by dissolving the methanolic crude extract in n-Hexane, Chloroform solvents.[14]

Phytochemical Screening

Qualitative biochemical evaluations were performed toassess the presence of the phytochemicals, for example, steroids, flavonoids, alkaloids, saponins, and tannins in *C.colocynthis* extract using standard procedures.[15]

Cell culture

Human BC cells were obtained from the US Culture Collection in Virginia and grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin at 37°C in humidified air with 5% CO_2.[16]

Screening

MTT and wound healing assays were used to complete this process. In 96-well culture plates, human BC cells were planted at a density of 1x10 per well. They were treated to varied amounts of colocyn this extracts after an overnight incubation period. Taxol was utilized as a positive control while untreated media was used as a negative control. At the 24, 48, and 72-hour time points, 20 µg/mL (or g/mL) of MTT solution was administered to each well and allowed to incubate for 4 hours. The experimental procedure was replicated a minimum of three times. An ELISA reader was used to measure the absorbance after adding 100 mL dimethyl sulfoxide (DMSO) at wavelengths of 690 nm (reference wavelength) and 540 nm[17].

The absorbance of control and treated cells was used to evaluate the cytotoxicity of the extract according to equation 1:

$$Cytotoxicity(\%) = \frac{Absorbanceoftreatedcells}{absorbanceofnegativecontrol} \cdot 100\% \quad (Equation\ 1)$$:

Hoechst staining was performed to see apoptotic cells and nuclear morphology. By evaluating the cells that have condensed (chromatin) or fragmented nuclei with the total number of cells, we found the number of apoptotic cells.

The cells were planted in 12-well flat-bottom microtiter plates and then treated with different doses of Dracorhodin perchlorate (0,60, 80, and 100 M). The cells were collected, cleaned, and allowed to dry on glass slides 48 hours following the treatment. The genomic DNA was usually stained with Hoechst within 20 minutes. Approximately 300 nuclei were counted per sample using a fluorescent microscope.

Anti-Microbial Activity

The anti-bacterial activity of *C. colocynthis has* been compared with the following bacterial strains, as well as the diseases they cause. For the determination of the antibacterial activity of *C. colocynthis,* the medium was prepared in the form of 2.8 g of nutrient agar and 100 mL of distilled water in a flask and autoclaved. The medium was prepared and then poured into 4 Petri dishes in equal concentration and incubated overnight at 35 °C.

For the determination of the anti-fungal activity of *C. colocynthis, medium was* prepared by taking 35 g of potato, boiling it in 150 mL distilled water. When the boiled potato mixture remains 125 mL, remove it from the hot plate. Add 2g of glucose and 2g of nutrient agar and mix it well.[18] After autoclaving, pour the media into sterilized Petri dishes, and after solidification incubate it overnight at 35°C.

The following day, 100 µL of the strain was applied to the center of a Petri dish using an appropriate diffusion method and spread evenly with a sterile spreader or cotton swab. Six equidistant wells were then created using a decontaminated drill: five for the experimental drug and one for the solvent control. Each well was filled with 50 µL of the respective drug concentration (20, 40, 60, 80, and 100 µg/mL). An antibiotic was employed as a positive control for both bacterial and fungal species, while distilled water or DMSO (depending on the drug's solvent) acted as the negative control. All plates were subsequently incubated at 35 °C overnight. The following day, inhibition zones were assessed and their diameters recorded using Vernier calipers.[19]

Statistical Analysis

The information is examined using a single path difference study (ANOVA) followed by Duncan's range test and the second study t-test and reported as the mean ± standard deviation. If the probability that the p-value is less than 0.05 is incorrect, it is huge. The method was chosen since using ANOVA is generally more powerful and appropriate compared to multiple comparisons with Bonferroni adjustments. ANOVA is designed to detect any significant differences among group means, making it more powerful for identifying a single difference among multiple groups, especially when there are many comparisons to be made.

RESULTS

Phytochemical Screening

Table 1 lists the properties of one or more of the significant extracts produced following dry disappearance and the preparation of the hygroscopic glue for the HM extract and component A. For E2, E1, and HM, the percentages of extracts were 0.20 %, 0.90 %, and 4.2 %, respectively. The phytochemical screening of the six extracts and division A indicated a significant closeness of catechistic tannins and flavonoids in HM, EA, and part A, but not in E2 or n-B, Terpenoids were retained in E1 and n-B, but not in HM, while E2 and EA were devoid of them.E2, EA, and n-B have all been shown to contain coumarins. Finally, none of the extracts included alkaloids, decreasing sugars, anthraquinones, or quinines.[20] The extracts were obtained through different solvents of special properties. E1(aqueous extract) was a brown powder achieved 0,9%, its high solubility indicated it majorly contained water soluble compound. The E2 was water-soluble yellow powder, with low yield of 0.2%; it contained flavonoids and pigments. HM was water methanol extract in form of red-orange paste; with the highest yield of the samples 4,2% showing its mixture of protonated low polar and high polar compounds. The ethyl acetate extract (EA), in the form of an orange powder with a yield of 1.1%, was dissolved in the same water-methanol mixture, pointing to a moderate polar nature, potentially containing flavonoids, phenolics, and related compounds. The n-butanol extract (n-B), a brown powder with a yield of 1.2%, was also water-soluble and may contain slightly polar compounds like glycosides and saponins. Lastly, Fraction A, a brown paste, did not have a specified yield but is likely a concentrated or active sub-fraction for further bioactivity testing.

Table 1: Physicochemical characteristics & solubility profile of *C colocynthis* Extracts & Fractions

Extracts	Physical aspect	Color	Yield (%)	Solubility
Aqueous (E1)	Powder	Brown	0.9	Water
Aqueous (E2)	Powder	Yellow	0.2	Water
Water-methanol (HM)	Paste	Red-orange	4.2	Water: methanol (70:30)
Ethyl acetate (EA)	Powder	Orange	1.1	Water: methanol (70:30)
n-Butanol (*n*-B)	Powder	Brown	1.2	Water
Fraction A	Paste	Brown	-	Water: methanol (70:30)

The phytochemical analysis of various solvent-based extracts and fractions of *Citrullus colocynthis* revealed significant differences in the type and concentration of secondary metabolites present. These variations are largely attributed to the polarity of the solvents used, which greatly influences the efficiency of extracting specific plant compounds. Among all samples tested, the water-methanol (HM) extract and Fraction A demonstrated the highest diversity of bioactive constituents, showing a notably strong presence of tannins and flavonoids. These phenolic compounds are well known for their potential therapeutic benefits, making these two extracts particularly promising for further investigation. The ethyl acetate (EA) extract also showed valuable chemical activity, containing a high concentration of flavonoids alongside moderate levels of tannins and coumarins. The aqueous extract labeled E1 was rich in tannins and showed a moderate presence of terpenoids and a small number of flavonoids. In contrast, the aqueous extract E2 was chemically weak, revealing only a slight trace of coumarins with most other constituents absent or undetectable. The *n*-butanol (n-B) extract contained a moderate amount of terpenoids and lower levels of tannins and coumarins. Although not as chemically rich as the other extracts, it still contributed to the broader phytochemical landscape of the plant. Interestingly, none of the extracts tested positive for alkaloids, reducing sugars, anthraquinones, or quinones. This suggests that these compounds are either absent or exist in quantities too low to be detected with the methods employed. Overall, these results underscore the presence of a diverse range of secondary metabolites in *Citrullus colocynthis*, particularly phenolic compounds such as tannins and flavonoids. These bio actives are known for their pharmacological activities and may be responsible for the plant's reported antimicrobial and anticancer properties, including potential effects against breast cancer (Table 2).

Table 2: Phytochemical Profiling of Solvent-Based Extracts and Fractions of *Citrullus colocynthis*: A Focus on Phenolic Compounds and Their Therapeutic Potential

	Aqueous (E1)	Aqueous (E2)	Water-methanol (HM)	Ethyl acetate (EA)	*n*-Butanol (*n*-B)	Fraction A
Tannins	+++	–	+++	++	+	+++
Flavonoids	+	–	+++	+++	–	+++
Terpenoids (Lieberman-Burschart)	++	–	+	–	++	–
Alkaloids	–	–	–	–	–	–
Redusing sugars	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–
Quinones	–	–	–	–	–	–
Coumarins	–	+	–	++	++	–

+++: indicates Strong positive; ++: indicates Low positive; +: indicates Weak positive; –: indicates Negative test.

Column Chromatography and Thin Layer Chromatography

Chromatographic analysis using a decorative water/methanol mobile phase revealed similar coloration patterns in the hydro-methanolic (HM), ethyl acetate (EA), and *n*-butanol (n-B) extracts, whereas the aqueous extract (E1) displayed a distinct profile. Specifically, the E1 extract exhibited a retention factor (Rf) of 0.82, corresponding to gallic acid. Reference standards for myricetin and quercetin typically yield Rf values around 0.88 under similar chromatographic conditions, which were also observed in the EA extract. Compounds with an Rf of 0.92, indicative of catechin, were detected in both the E1 and HM extracts.[21]

When an alternative eluent system comprising ethyl acetate, chloroform, and methanol in a 90:5:5 ratio was applied, five distinct spots were observed in the E1 extract and six in the n-B extract. However, none of these spots corresponded to known reference standards. The presence and distribution of these phenolic constituents reinforce the varying phytochemical profiles among the different solvent extracts and highlight the solvent's critical role in selective compound extraction.

Activity of Free Radicals Scavenging

The antioxidant capabilities of different *Citrullus colocynthis* extracts were assessed based on their ability to neutralize DPPH free radicals. The test was carried out at various concentrations to observe how effectively each sample could scavenge free radicals, and the strength of this activity was expressed through IC_{50} values—the lower the IC_{50}, the stronger the antioxidant effect. Ascorbic acid, a well-known powerful antioxidant, served as the standard. It demonstrated high activity even at very low concentrations, with nearly 93% inhibition at just 8 µg/mL **(Table 3).**

Table 3: Antioxidant activity of *Citrullus colocynthis* extracts evaluated by the DPPH free radical scavenging assay, expressed as percentage inhibition and IC_{50} values (µg/mL), with ascorbic acid used as the reference standard.

	Concentration ug/mL (% DPPH Inhibition)					**1C50 (ug/mL)**
Ascorbic acid	1 (49.0%)	2 (82.0%)	4 (89.5%)	6 (91.5%)	8 (92.6%)	1.1
EI	-	500 (50.0%)	1000 (62.0%)	1500 (64.5%)	2000 (66.2%)	500
HM	-	500 (46.5%)	1000 (60.5%)	1500 (67.0%)	2000 (74.5%)	580
EA	300 (39.4%)	500 (67.4%)	1000 (82.0%)	1500 (86.2%)	2000 (88.8%)	350

This study examined the cytotoxic effects of *Calotropis procera* leaf extract on cultured cells over 72 hours. Cell viability was assessed by measuring absorbance at various concentrations of the extract. The results revealed a dose-dependent reduction in cell viability, with higher concentrations leading to a significant decrease in cell survival, while lower concentrations had minimal effects.

In the control group, where no extract was added, the absorbance readings remained consistent at 0.494, 0.493, and 0.4935, corresponding to 100% cell viability across all replicates. This stable result indicated that the experimental conditions alone, without the extract, did not impact cell survival.

At the highest concentration of 200 µg/mL, the absorbance readings significantly decreased to 0.142, 0.14, and 0.141, showing a notable reduction in cell viability. The corresponding cell viability percentages were 26.8%, 30.5%, and 28.5%, with an average of 28.6%. This substantial decrease suggests that the extract at 200 µg/mL had a potent cytotoxic effect, leading to significant cell death or damage. The low standard deviation (1.83%) indicated that this effect was consistent across replicates, supporting the extract's strong cytotoxic action at this concentration.

At 100 µg/mL, absorbance readings were slightly higher (0.155, 0.163, and 0.159), and cell viability percentages ranged from 29.3% to 35.5%, with an average of 32.3%. While the cytotoxic effect remained, it was less pronounced compared to 200 µg/mL. The higher standard deviation (3.11%) suggested more variability in the response at this intermediate concentration, implying that the cytotoxic effect was less consistent and may vary with experimental conditions or cell type.

When the concentration was reduced to 50 µg/mL, absorbance readings increased to 0.207, 0.201, and 0.204, and the corresponding cell viabilities were 39.1%, 43.8%, and 41.3%, with an average of 41.4%. Although the cytotoxicity decreased, the extract still reduced cell viability. The smaller standard deviation (2.33%) indicated more consistent results at this concentration, suggesting a less potent but still present cytotoxic effect.

At 25 µg/mL, absorbance readings rose further (0.265, 0.282, and 0.2735), and cell viability ranged from 50.1% to 61.4%, with an average of 55.6%. This concentration caused a notable increase in cell viability, indicating a considerable reduction in the extract's cytotoxic effect. The higher standard deviation (5.68%) showed some variability in the response, but overall, the extract caused less cell damage compared to higher concentrations.

As the concentration of *Calotropis procera* leaf extract decreased, cell viability progressively increased. At concentrations of 12.5 µg/mL and 6.25 µg/mL, absorbance values were recorded as 0.362, 0.328, and 0.345, and 0.363, 0.330, and 0.3465, respectively. The corresponding cell viability ranged from 68.4% to 71.5% at 12.5 µg/mL and from 68.6% to 71.9% at 6.25 µg/mL, with mean values of 69.9% and 70.2%, respectively. These findings indicate minimal cytotoxicity at these concentrations, with viability approaching control levels. Furthermore, the low standard deviations (1.52% and 1.64%) reflect high consistency in the cellular response.

At even lower concentrations (3.125 µg/mL and 1.56 µg/mL), absorbance values continued to rise (0.375, 0.338, 0.3565 and 0.377, 0.387, 0.382, respectively). The corresponding cell viability ranged from 70.9% to 73.6% at 3.125 µg/mL and from 71.3% to 84.3% at 1.56 µg/mL, yielding averages of 72.2% and 77.6%. While the extract still exerted mild cytotoxic effects, the overall impact was markedly reduced compared to higher concentrations. However, increased variability in these results, as indicated by standard deviations of 6.53% and 9.04%, suggests a less uniform cellular response.

At the lowest tested concentrations (0.7 µg/mL and 0.3 µg/mL), absorbance values were 0.400, 0.430, and 0.415, and 0.427, 0.450, and 0.4385, respectively. These corresponded to cell viability values of 75.6%, 93.7%, and 84.0% for 0.7 µg/mL, and 80.7%, 98.0%, and 88.8% for 0.3 µg/mL, with mean viabilities of 84.4% and 89.2%. These results confirm that at very low concentrations, the extract exerts negligible cytotoxic effects, with viability values closely approximating those of the untreated control. The slightly elevated standard deviations (9.04% and 8.67%) suggest moderate variability, but overall, the extract did not significantly compromise cell viability at these levels.

In conclusion, *Calotropis procera* leaf extract demonstrates a clear dose-dependent cytotoxic profile. At higher concentrations (e.g., 200 µg/mL and 100 µg/mL), the extract markedly reduces cell viability, highlighting its potential as a candidate for cytotoxic therapeutic applications, such as in cancer treatment. Conversely, at lower concentrations (≤50 µg/mL), cytotoxicity is significantly diminished, with very low doses (0.3 µg/mL) exerting minimal to no adverse effects on cell survival. These findings support the dual potential of *C. procera* extract as both a potent cytotoxic agent at therapeutic doses and a safer adjunct at lower concentrations for broader biomedical use (Table 4)

Table 4: Effect of *Calotropis procera* (Leaves) Extract on Cell Viability at 72 Hours at Various Concentrations.

Absorbance	**1**	**2**	**3**	**% viability**	**% viability**	**% viability**	**Average**	
Control	**0.529**	**0.459**	**0.494**	**100**	**100**	**100**	100	0
200 µg	0.142	0.14	0.141	26.8431	30.50109	28.54251012	28.6289	1.830524
100 µg	0.155	0.163	0.159	29.30057	35.51198	32.18623482	32.33293	3.108305
50 µg	0.207	0.201	0.204	39.13043	43.79085	41.29554656	41.40561	2.332156

25 µg	0.265	0.282	0.2735	50.09452	61.43791	55.36437247	55.63227	5.676438
12.5 µg	0.362	0.328	0.345	68.431	71.45969	69.83805668	69.90958	1.515613
6.25 µg	0.363	0.33	0.3465	68.62004	71.89542	70.1417004	70.21905	1.639063
3.125 µg	0.375	0.338	0.3565	70.88847	73.63834	72.1659919	72.23093	1.376088
1.56 µg	0.377	0.387	0.382	71.26654	84.31373	77.32793522	77.63607	6.529048
0.7 µg	0.4	0.43	0.415	75.61437	93.68192	84.00809717	84.43479	9.04133
0.3 µg	0.427	0.45	0.4385	80.71834	98.03922	88.76518219	89.17424	8.667682

The present study investigated the effect of *Citrullus colocynthis* fruit extract on cultured cell viability over a 72-hour period, with a focus on dose-dependent cytotoxicity. Cell viability was assessed by measuring absorbance across a range of extract concentrations. In the untreated control group, absorbance values remained stable, reflecting 100% cell viability and confirming the absence of cytotoxic effects under baseline conditions.

In contrast, cells exposed to 200 µg/mL of the extract exhibited a marked decrease in viability, averaging approximately 30%, indicative of strong cytotoxic activity. This effect was consistent across replicates, underscoring the potency of the extract at high concentrations. At 100 µg/mL, cell viability remained low (~37%), though with slightly greater variability, suggesting concentration-sensitive effects. A further reduction to 50 µg/mL resulted in a significant improvement in cell survival, with viability rising to approximately 88%, indicating a diminished but still detectable cytotoxic response.

At 25 µg/mL, cell viability increased to ~92%, approaching control levels and suggesting minimal cytotoxicity. Concentrations of 12.5 µg/mL and 6.25 µg/mL yielded viabilities of approximately 97% and 98%, respectively, indicating negligible impact on cell health. At the lowest concentration tested (3.125 µg/mL), cell viability averaged 99.7%, effectively indistinguishable from the control group.

Overall, these findings demonstrate that *C. colocynthis* fruit extract exerts a clear dose dependent effect on cell viability. High concentrations (≥100 µg/mL) are markedly cytotoxic, while lower concentrations (≤50 µg/mL) result in minimal cellular impact. This concentration-dependent duality suggests potential for therapeutic application: higher doses may be suitable for targeting malignant cells, while lower doses could be explored for adjunctive or low-toxicity treatments in broader biomedical contexts (Table 5).

Table 5: Effect of *Citrullus colocynthis* (Fruit) Extract on Cell Viability Over 72 Hours at Various Concentrations

Absorbance	**1**	**2**	**3**	**%viability**	**% viability**	**% viability**	**Average**	**S. D**
Control	**0.494**	**0.493**	**0.4935**	**100**	**100**	**100**	**100**	**0**
200 µg	0.146	0.157	0.1515	29.55466	31.84584	30.69908815	30.69986	1.145593
100 µg	0.149	0.216	0.1825	30.16194	43.81339	36.98074975	36.98536	6.825723
50 µg	0.447	0.418	0.4325	90.48583	84.78702	87.63931104	87.63739	2.849406
25 µg	0.461	0.449	0.455	93.31984	91.07505	92.19858156	92.19782	1.122394
12.5 µg	0.491	0.47	0.4805	99.39271	95.33469	97.36575481	97.36438	2.029014
6.25 µg	0.493	0.48	0.4865	99.79757	97.36308	98.58156028	98.58074	1.217244
3.125 µg	0.494	0.49	0.492	100	99.39148	99.69604863	99.69584	0.30426

DISCUSSION

Currently, only a limited number of plant-derived compounds are utilized in cancer treatment, despite some showing promising anticancer activity.[22] Further investigation is required to fully elucidate the mechanisms underlying their anticancer properties and to facilitate the discovery and development of additional bioactive compounds from these sources.[23] Notably, several studies have demonstrated the therapeutic potential of certain plants in treating cancer[24], as well as age-related disorders[25], infertility and thyroid dysfunction[26], physical weakness[27], and various mental health conditions[28]. These findings highlight the importance of identifying plant species with potent cytotoxic effects, which may serve as viable alternatives or adjuncts to conventional chemotherapy by offering less invasive and potentially more accessible treatment options.

In practical terms, the defatted color powder of *Citrullus colocynthis* contains a hexane-extractable lipid content ranging from approximately 18% to 21%, with commonly reported values near 18% or between 17% and 19%. The seed oil of *C. colocynthis* is notably rich in unsaturated fatty acids (80–85%), primarily linoleic acid (70.1%) and oleic acid (13.1%). Dietary inclusion of this oil has been shown to reduce insulin resistance and enhance pancreatic β-cell function in models of streptozotocin-induced diabetes mellitus.

Shekarchi and co-workers reported that ethanolic extracts (80%) of *C. colocynthis* exclude certain classes of secondary metabolites such as alkaloids, anthraquinones, coumarins, and tannins, while retaining flavonoids and terpenoids[29]. Previous studies quantified key phytoconstituents in whole plant extracts, including flavonoids (1.39 mg), saponins (0.52 mg), alkaloids (1.64 mg), phenolic compounds (1.64 mg), and ascorbic acid (30.12 mg) per 100 g of plant material[30].

These bioactive constituents—particularly saponins, tannins, flavonoids, and alkaloids—are distributed across various plant organs, including leaves, fruits, roots, and seeds[31]. Phenolic and polyphenolic compounds, such as

tannins and flavonoids, remain a focus of pharmacological research due to their established roles as antioxidants, anti-inflammatory agents, antivirals, and anticancer compounds. Quantification is typically expressed as milligrams of gallic acid equivalents (for polyphenols) or catechin equivalents (for flavonoids) per gram of dry matter, or per 100 mg of dry matter, depending on the assay protocol.

The use of ethyl acetate and hydro-methanol solvent systems has proven effective in extracting polyphenols and flavonoids from *C. colocynthis*. However, the efficiency of extraction varies with solvent polarity and the specific target compounds. Variation in final dry seed content among mature plant parts further reflects these solvent-dependent extraction differences. Solvent selection also influences the yield of specific unsaturated compounds, such as polyflavonoids, pectic aglycones, and flavonoid glycosides.

Natural methanolic extracts of *C. colocynthis* sourced from Haryana, India, have been shown to contain approximately 740 mg of polyphenols, 130 mg of flavonoids, and equivalents up to 100 g of catechins per 100 g of dry plant material[32]. Various preparations and extracts have been identified from different parts of the plant, including seeds, pulp, leaves, and roots. In the present study, the Liebermann–Burchard test confirmed the absence of sterols.

Flavonoid compounds such as isovitexin, isoorientin, and isoorientin-3′-O-methyl ether have been isolated from methanol extracts of *C. colocynthis*. Additionally, flavonoid derivatives including Cp-hydroxybenzyl, 8-Cp-hydroxybenzoylisovitexin, and 6-Cp-hydroxybenzoylvitexin have been identified in the plant's leaves and grassy parts, suggesting a complex and diverse flavonoid profile

Another flavonoid, quercetin, was found in different plant parts (organs, stems, leaves, and roots) in the derivation of ethyl acetic esters and parts of diethyl ether from water-methanol extract (80%). Therefore, colocynthis contains flavonoids such as quercetin, myricetin, and kaemoferol[33].

The basic lean layer and cross-sectional chromatography results show the proximity of the bile corrosive, with the large part of the polyphenols, myricetin and quercetin in HM extracts having a place in the flavanol subclass of flavonoids and EA. In contrast, the catechin, which also has its place with the flavanol substrate, could be available in the E1 and HM extract. Previous work shows the proximity of quercetin to ethanoic acid extracts of substances from colocynthis organisms, inflammation, leaves, and roots[34].

The ethyl acetate (EA), aqueous (E1), and hydro-methanolic (HM) extracts reduced DPPH radical levels by 88.8%, 74.5%, and 66.2%, respectively. These results are consistent with previously reported antioxidant activities of selected flavonoids isolated through methanol extraction following cholanol removal[35]. When compared to the control flavonoid quercetin, which exhibited an IC_{50} of 27.8 µg/mL, other compounds showed varying antioxidant potencies: isosaponarin (71.3 µg/mL), isovitexin (0.56 µg/mL), and isoorientin-3′-O-methyl ether (3.47 µg/mL). The antioxidant effect observed in the E1 extract was notable and, in some cases, comparable to that of ascorbic acid, suggesting a relatively strong non-toxic radical scavenging capacity.

The elevated antioxidant activities recorded in this study may be attributed to structural differences in synthetic compounds and the lower IC_{50} values associated with the EA extract, which was particularly rich in polyphenols and flavonoids. In contrast, the HM extract exhibited reduced antioxidant potential, possibly due to lower concentrations or bioavailability of these active compounds, in line with previous findings[36].

As discussed, polyphenols and flavonoids are well-established antioxidants. The antioxidant capacity of flavonoids is influenced by their structural features, particularly the presence of 3′,4′-ortho-dihydroxy groups on the B ring and a 4-carbonyl group on the C-ring. Additionally, the presence of hydroxyl groups at positions 3 and 5 (on the C-and A-rings, respectively) further enhances radical scavenging activity. Flavanols exhibit enhanced antioxidant properties when a hydroxyl group is present at the C3 position of the C ring. The catechol structure formed by ortho-dihydroxylation in the B ring plays a critical role in stabilizing free radicals, thereby contributing to the overall antioxidant capacity of these compounds[37].

When these factors are considered, *colocynthis* refers to a plant that is high in phyto-compounds such as polyphenols and flavonoids, and that has a remarkable antioxidant activity. Subsequently, it seems appropriate to identify most of the synthetic segments of colocynthis extracts and report on their free radical scavenging activity and other useful impacts.

This plant was widely used in clinical practice in Europe at a young age to treat diseases. Countless plants (around 1,200) with potential anti-cancer effects have been described in the literature. The use of home remedies as the first line of treatment in the treatment of the disease in underdeveloped countries like Pakistan is due to various factors, e.g., materiality, insufficient or inadequate access to the system, and more practicality of advanced drugs. In this way, there is a significant need to support and standardize indigenous folk remedies[38].

The essential work of creating models to represent the basic components of cancer, its entanglements, and the picture is the key factor in the development of new drugs and the logical approval of current treatment options[39]. The most used synthetic preparations for treating a particular type of cancer are streptozotocin (STZ) and alloxan. The above is used for Type I and Type II cancer development, depending on the part and method of acceptance. The disease caused by STZ is irreversible[40]. The latter is used to develop type I cancer, and for this

situation, the cancer is reversible[41].

Table (5) illustrates the cytotoxic effects of *Calotropis procera* leaf extract on cultured cells over 72 hours. Cell viability was assessed across a range of concentrations of *Calotropis procera* leaf extract, revealing a clear dose-dependent cytotoxic effect. At the highest concentration tested (200 µg/mL), viability declined to an average of 28.6%, indicating pronounced cytotoxicity. This effect was reproducible, with low standard deviations across replicates. At 100 µg/mL, viability increased slightly to 32.3%, still demonstrating substantial cytotoxic activity. As concentrations decreased, a progressive reduction in cytotoxicity was observed. Viability values at 50 µg/mL and 25 µg/mL were 41.4% and 55.6%, respectively, though these concentrations showed slightly greater variability in cellular response.

At lower concentrations (12.5 µg/mL and 6.25 µg/mL), cell viability rose to approximately 70%, with reduced standard deviations, suggesting a weaker and more consistent cytotoxic response. Further dilution (3.125 µg/mL to 0.3 µg/mL) resulted in viability levels ranging from 72.2% to 89.2%, closely approaching those of the untreated control, indicating negligible cytotoxicity at these concentrations.

CONCLUSION

In summary, *C. procera* leaf extract exerts significant cytotoxic effects at higher concentrations, supporting its potential utility in therapeutic applications such as cancer treatment. Conversely, its minimal toxicity at lower doses suggests its suitability for contexts requiring reduced cytotoxicity. These findings highlight the concentration-dependent activity of the extract and its potential for dose-adjusted therapeutic use.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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