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Evaluation of Anti-cancer and Anti-inflammatory Properties of *Crescentia cujete* L. and *Citrullus vulgaris* Schrad. Methanol Seed Extracts Against MCF-7 Breast Cancer Cells and Molecular Docking

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Abstract

Breast cancer continues to be the leading form of cancer in women all over the world and one of the primary cancer killers of women. Crescentia cujete L. and Citrullus vulgaris Schrad. have longstanding uses in traditional medicine. This study was carried out to determine the anti-inflammatory and anti-cancer characteristics of the methanol extracts of seeds of C. cujete and C. vulgaris. The anti-cancer effects were tested on MCF-7 cells with MTT assay, Annexin V-FITC/PI staining, and cytokine quantification. The anti-inflammatory activity was evaluated using carrageenan and nitric-oxide induced rat model. Both extracts reduced cell viability in a dose dependent manner, showing an IC_{50} value of 24.5 ± 2.4 µg/mL for C. cujete and 12.6 ± 3.1 µg/mL for C. vulgaris. Flow cytometric analysis provided fluorescence images of MCF-7 cells containing both early and late apoptotic populations. The 200 µg/mL C. cujete treatment yielded an increase in MCF-7 cell apoptosis to 38.2% apoptosis compared to 42.16% for C. vulgaris and 14.24% for doxorubicin whereas the control was at 5.4%. ELISA quantification proved significant TNF-a diminishment along with the IL-1β, and IL-6 levels ($p < 0.05$). Carrageenan-induced paw edema and nitric oxide (NO) assays in Wistar rats were used to evaluate the in vivo anti-inflammatory activity. At 400 mg/kg, C. Cujete and C. vulgaris extracts, as opposed to 69.1% with Ibuprofen, decreased the volume of paw edema by 61.3% and 47.9%, respectively, at the fifth hour. Serum NO levels were significantly suppressed by 52.6% (C. cujete) and 41.8% (C. vulgaris). Strong binding affinities with important cancer-related targets, such as COX-2 (−9.1 kcal/mol), Bcl-2 (−8.4 kcal/mol), and EGFR (−7.6 kcal/mol), were demonstrated by molecular docking of identified phytochemicals (such as β-sitosterol and cucurbitacin B). The study showed that these plants' extracts possessed potential anti-cancer and anti-inflammation activities.

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Keywords: Anti-cancer, Anti-inflammatory, Crescentia cujete, Citrullus vulgaris, MCF-7 cells, Molecular docking.

INTRODUCTION

Breast cancer is a deeply impactful health issue, being the most frequently diagnosed cancer among women worldwide (Sung *et al.*, 2021). Each year, about 2.3 million women receive this daunting diagnosis, and tragically, more than 685,000 lose their lives to the disease, according to the World Health Organization (Sung *et al.*, 2021). It's important to recognize that breast cancer is not a single disease; it's highly heterogeneous, meaning it comes in various forms, with hormone receptor-positive tumors (like the MCF-7 subtype) being among the most common (Pillai *et al.*, 2022). While advancements in medical treatments for instance surgery, radiotherapy, cancer drugs as well as use of hormones have improved outcomes for numerous patients, challenges persist. Patients often experience therapeutic resistance, where their cancer becomes less responsive to treatment over time, as well as systemic side effects that can significantly affect quality of life. This is particularly concerning in low- and middle-income countries, where access to modern healthcare options may be limited. Alternative treatment approaches that are safe, economical, and effective are therefore desperately needed. Addressing this gap could make a critical difference in the lives of countless women battling breast cancer and their families.

The potential of medicinal plants to treat cancer has drawn more attention in recent years. This renewed focus highlights the treasure trove of bioactive compounds found in nature, which offer a variety of health benefits (Madan & Karole, 2022). These include not just anticancer properties, but also abilities to reduce inflammation, combat oxidative stress, and help regulate the immune system. Compounds like flavonoids, alkaloids, terpenoids, and phenolic acids have shown promise in fighting cancer through a multitude of ways (Madan & Karole, 2022). For example, they can halt the cell cycle, trigger programmed cell death, inhibit the formation of new blood vessels that tumors need to grow (a process called angiogenesis), and even prevent cancer cells from spreading to other parts of the body (Alfuraydi *et al*., 2024). A key aspect of these phytochemicals is their ability to address chronic inflammation, which is often found within tumors and can contribute to cancer progression (Lee *et al*., 2019). This inflammatory environment can enable cancer cells to grow and escape the body's immune defenses. Therefore, finding treatments that can both reduce inflammation and target cancer directly offers a hopeful path forward. Researchers and medical professionals can create novel, efficient cancer treatment methods by investigating the therapeutic potential of natural substances.. This approach not only broadens our treatment options but also taps into the wisdom of traditional medicine, potentially improving outcomes and quality of life for patients facing this challenging disease.

Many people who use traditional medicine have a particular place in their hearts for *Crescentia cujete* L., also affectionately known as the calabash tree.This tropical plant is a testament to nature's bounty, offering remedies for a range of ailments like respiratory issues, infections, hypertension, and inflammation (Januário *et al*., 2022). The calabash tree is more than just a plant; it is a symbol of healing that has been passed down through generations. Its seeds, rich in polyphenols, flavonoids, and terpenoids, speak to its pharmacological properties, providing hope for those seeking natural alternatives to conventional medicines (Januário *et al*., 2022). On the other hand, *Citrullus vulgaris* Schrad., better known as watermelon, delights us not just with its refreshing sweetness but also as a treasure trove of health benefits (Januário *et al*., 2022). This beloved fruit brings joy on hot summer days, but its seeds often go unnoticed. Yet, these seeds are packed with valuable phytochemicals that have caught the attention of scientists (Ansori *et al.*, 2022). Their antimicrobial, anti-inflammatory, and antioxidant

qualities have been demonstrated by research, which makes them a topic of interest for those looking to fully utilize the plant (Ansori *et al*., 2022).

Despite the growing body of knowledge around these two plants, there remains a gap in research regarding their anticancer potential, particularly concerning hormone receptor-positive breast cancer. This is an area ripe for exploration, as understanding how these natural compounds could aid in cancer treatment might open new avenues for therapies. By delving deeper into the medicinal qualities of the calabash tree and watermelon seeds, we can honor their traditional uses while also paving the way for innovative approaches in modern medicine. It is this blending of age-old wisdom with contemporary science that can lead to impactful discoveries, ultimately improving health and well-being.

The anti-inflammatory and anti-cancer properties of methanol seed extracts from *C. vulgaris* and *C. cujete* were investigated in this work.The potential anti-cancer effects were examined using MCF-7 human breast cancer cells, with a focus on inhibiting cell viability, inducing apoptosis, and reducing the levels of pro-inflammatory cytokines. To provide further evidence for the anti-inflammatory effects, *in vivo* studies were evaluated using two established models: carrageenan-created paw inflammation, which helps assess acute inflammation, and nitric oxide (NO) production assays to evaluate the systemic inflammatory response. Additionally, docking of key molecules were conducted to identify main connections between key phytochemicals present in the extracts and various cancer-associated molecular targets, including cyclooxygenase-2 (COX-2), epidermal growth factor receptor (EGFR), and B-cell lymphoma 2 (Bcl-2).

This study took a holistic approach by combining laboratory experiments, animal studies, and computer modeling to explore the healing potential of these lesser-known plant seeds. By uncovering the mechanism of how these plants work, we not only validate the traditional uses of these plants in medicine but also pave the way for creating new plant-based treatments for breast cancer and inflammation. This study explored exciting advancements in natural therapies, making a meaningful impact on health and wellness.

MATERIALS AND METHODS

Collection and Extract Preparation of Plant Materials

During the lush fruiting season from July to September 2024, seeds of *C. cujete* and *C. vulgaris* were thoughtfully gathered from the local farmlands and home gardens in Fori, Maiduguri, Borno State, Nigeria. After harvest, these plant materials were transported to the Department of Pharmacognosy at the University of Maiduguri. Here, the expertise of plant taxonomist Dr. C.A. Ukwubile ensured that the seeds were carefully identified and authenticated. To preserve a lasting record, voucher specimen numbers (UMM/FPH/BIN/006 and UMM/FPH/CUB/011) were assigned and deposited in the departmental herbarium for future reference. Thereafter, skilled individuals meticulously separated the seeds from the fruits. They took care to wash them thoroughly with clean distilled water, ensuring any adhering debris was removed. To maintain the seeds' valuable phytochemical properties, they were air-dried in the shade at a comfortable ambient temperature of 28°C for three weeks. Once fully dried, the seeds underwent a transformation, being ground into a fine powder using an electronic blender (Model P500, China). The final step involved sifting the powdered seeds to achieve a uniform particle size, ready for use in further research and applications.

Subsequently, extraction was carried out using Soxhlet apparatus to ensure efficient recovery of both polar and moderately non-polar phytochemicals. Two hundred grams (200 g) of the powdered seeds from each plant were placed separately into cellulose thimbles and extracted

with 1.5 L of analytical-grade methanol for 8 hours at 65°C. The methanol extracts were filtered using filter paper (Whatman No. 1). To achieve complete dryness under reduced pressure, the samples were subjected to a rotary evaporator. This method effectively removed solvents from the extracts while minimizing heat exposure, which helped to preserve sensitive compounds. The samples were placed in the rotary evaporator's flask and rotated gently. As the flask was heated, the reduced pressure allowed the solvent to evaporate quickly and efficiently. This process continued until the extracts were concentrated to a dry residue, ready for further analysis. The use of the rotary evaporator ensures high-quality results while maintaining the integrity of the sampes. The final weights were noted, and the extracts were then kept in airtight glass containers at 4°C until further use for biological assays. The percentage yields were calculated using the formula (Luo *et al*., 2025):
Yield (%) = (Final weight of dried extract / Initial weight of powdered sample) × 100.
The percentage yields obtained were approximately 9.3% w/w for *C. cujete* and 7.8% w/w for *C. vulgaris*.

Phytochemical Contents of Extracts
Phytochemical screening of the methanol seed extracts of *C. cujete* and *C. vulgaris* was conducted using standard qualitative/quantitative methods to detect the presence or absence of major bioactive constituents. The plant extracts were subjected to tests for alkaloids (using Mayer's and Wagner's reagents), flavonoids (with lead acetate and alkaline reagent tests), saponins (via frothing and emulsion formation methods), tannins (using ferric chloride), phenols (with Folin-Ciocalteu reagent), glycosides (using Keller-Killiani test), terpenoids (with Salkowski's test), and steroids (via Liebermann-Burchard reaction) (Consoli *et al*., 2023; Luo *et al*., 2025; Nakbi *et al*., 2020; Ukwubile *et al*., 2025).

Cell Culture for *in vitro* assays
The study utilized human breast cancer cells known as MCF-7, which are recognized as an estrogen receptor-positive (ER^+) adenocarcinoma cell line and were sourced from the American Type Culture Collection (ATCC) in Manassas, VA. To investigate their anti-cancer properties, these cells were cultured under optimal conditions. The culture medium used was Dulbecco's Modified Eagle's Medium (DMEM), enriched with a high concentration of glucose, sourced from Gibco, a part of Thermo Fisher Scientific. To further support cell growth and metabolic activity, the DMEM was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) from Sigma-Aldrich, along with 1% of a penicillin-streptomycin solution containing 100 U/mL penicillin and 100 µg/mL streptomycin. Additionally, 1% L-glutamine was included in the mix, providing essential nutrients for the cells to thrive in the laboratory setting (Wang *et al.,* 2019).

MTT cytotoxicity assay
The cytotoxic potentials of *C. cujete* and *C. vulgaris* methanol seed extracts against MCF-7 breast cancer cells were determined using the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, as described by Mosmann (1983), with slight modifications. In this experiment, MCF-7 breast cancer cells, which were in their logarithmic growth phase, were collected and placed into 96-well plates at a density of 10,000 cells per well in 100 µL of complete DMEM medium. The plates were then incubated at 37°C with 5% CO_2 for 24 hours to allow the cells to attach properly. After the 24-hour incubation, the old culture medium was removed, and each well was filled with 100 µL of freshly prepared DMEM containing varying concentrations (ranging from 1.56 to 200 µg/mL) of methanol seed extracts. These extracts had been prepared through a process of serial dilution in DMSO before being diluted into the culture medium, ensuring that the final concentration of DMSO remained at 0.5% or lower. For controls, cells treated with 0.5% DMSO were kept as the

negative control, while those treated with doxorubicin at 10 µg/mL served as the positive control. Each treatment was done in triplicate, and the entire experiment was repeated three times for accuracy. After 48 hours of treatment, MTT solution was added to each well. This solution (5 mg/mL in PBS) allows metabolically active cells to convert it into purple formazan crystals. The plates were returned to the incubator for an additional 4 hours to facilitate this reaction. Following incubation, the medium was carefully removed, and 100 µL of DMSO was added to dissolve the purple crystals. The plates were gently shaken for 10 minutes to ensure everything was fully minutes to ensure everything was fully dissolved. Finally, the absorbance at 570 nm was measured using a microplate reader (BioTek ELx800, USA), and the cell viability was calculated as a percentage of the untreated control group based on the measured absorbance values using the formula below:

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ control\ cells\ -\ Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells} x\ 100$$

Finally, dose-response curves to visualize how different concentrations of each extract affected cell viability was created. Using nonlinear regression analysis in MS Excel, the IC_{50} values for each extract was calculated. The IC_{50} represents the concentration at which 50% of the cells are inhibited, providing a key measurement of each extract's effectiveness against the cancer cells.

Apoptosis assay using Annexin V-FITC/PI staining technique

To evaluate whether the cytotoxic effects of *C. cujete* and *C. vulgaris* ssed methanol extracts on MCF-7 (breast cancer) cells were linked with apoptosis induction, dual staining methods using Annexin V and propidium iodide (PI) were employed. This fluorescence-based technique differentiates viable, early apoptotic, late apoptotic, and necrotic cells based on nuclear morphology and membrane integrity. Briefly, MCF-7 cells were seeded into 24-well plates at a cell density of 5×10^4 cells per well and allowed to adhere overnight in DMEM. After 24 hours, cells were treated with the IC_{50} concentrations of the respective methanol seed extracts (*C. cujete*: 42.7 µg/mL; *C. vulgaris*: 55.2 µg/mL), doxorubicin (10 µg/mL), and 0.5% DMSO (negative control). The treatment was continued for 24 hours at 37°C in a 5% CO_2 incubator. After the treatment, the culture medium was removed, and the cells were gently washed with a phosphate-buffered saline solution to prepare them for analysis. A staining solution was then prepared by mixing Annexin V and propidium iodide, both at a concentration of 100 µg/mL, and added 10 µL of this mix to each well. The cells were then incubated in the dark for 5 minutes at room temperature to allow the dyes to penetrate. Once stained, the cells were observed under a fluorescence microscope at 400× magnification. The cells were focused on randomly counting at least 200 cells from different fields to assess their viability and stage of cell death. Cells were classified the cells based on their appearance: viable cells had uniform green nuclei, early apoptotic cells showed bright green nuclei with condensed or fragmented chromatin, late apoptotic cells displayed orange to red nuclei with similar chromatin changes, and necrotic cells featured uniform red nuclei that were either intact or swollen.. The percentage of apoptotic cells (early + late apoptosis) was calculated as shown below (Wang *et al.*, 2019):

$$Apoptotic\ index\ (\%) = \frac{Number\ of\ apoptotic\ cells}{Total\ number\ of\ cells\ observed} x\ 100$$

***In Vivo* Anti-inflammatory Studies**

Experimental Animals and Grouping

Twent-five (25) healthy adult male Wistar rats was acquired, each weighing between 150 and 180 g, from the PJ Rat Farms Ltd in Jos. These little ones were settled into an environment that

mimics their natural habitat, with a 12-hour light and dark cycle, comfortable room temperature of around 22 ^{o}C, and humidity levels kept between 50 to 60%. They had full access to food and water, ensuring they were well-cared for. To help them adjust to their new surroundings and reduce any stress, the rats were allowed one week to acclimatize before beginning the experiment. After this period, the rats were randomly assigned to five different groups, each containing five rats as below, so the study could be conducted in a structured manner.

- Group I (normal control): received 0.5 mL normal saline.
- Group II (negative Control): received 0.5 mL of carrageenan or LPS (inflammatory inducer).
- Group III (standard Drug): received Ibuprofen (Alapharm., Nig., Ltd) at 10 mg/kg body weight.
- Group IV (treatment A): received *C. cujete* extract at 200 mg/kg.
- Group V (treatment B): received *C. vulgaris* extract at 200 mg/kg.

All treatments were administered orally once daily for five consecutive days before inflammation was induced. The anti-inflammatory activities of *C. cujete* and *C. vulgaris* methanol seed extracts were evaluated using two well-established models: carrageenan-induced paw edema and lipopolysaccharide (LPS)-induced nitric oxide (NO) production in Wistar rats.

Carrageenan-induced Paw Edema in rats model

In the carrageenan-induced paw edema model, acute inflammation was initiated in the rats, with the exception of the normal control group, by injecting 0.1 mL of a 1% carrageenan solution into the right hind paw on the sixth day. Measurements of paw volumes were taken immediately before the injection (0 hours) and then at 1, 2, 3, 4, and 5 hours afterward using a digital plethysmometer. The extent of inflammation was determined by calculating the changes in paw volume at each time interval, while the anti-inflammatory activity of the extracts was assessed by expressing the percentage inhibition of paw edema in comparison to the negative control group (Ukwubile *et al*., 2025).

Lipopolysaccharide (LPS)-induced Nitric Oxide (NO) Assay

To evaluate systemic inflammation and nitric oxide production, lipopolysaccharide (LPS) model was used. Briefly, on the sixth day, following the last dose of extract treatment, rats in Groups II to V were injected intraperitoneally with LPS at 1 mg/kg b.w. to induce an inflammatory response characterized by increased NO production. Six hours post-LPS administration, blood samples were collected via retro-orbital plexus under light anesthesia. The collected blood was allowed to clot, and serum was separated by centrifugation at 3,000 rpm for 10 minutes. The serum levels of nitric oxide were then quantified using the Griess reagent assay, which measures nitrite concentration, a stable end product of NO metabolism. The absorbance of the resulting purple-colored azo compound was read at 540 nm using a UV-vis spectrophotometer. The nitrite concentrations were calculated based on a sodium nitrite standard curve (Yemitan & Adeyemi, 2017).

Quantification of Inflammatory Cytokines

The evaluation of effects of *C. cujete* and *C. vulgaris* methanol seed extracts on inflammation was carried out by determining the levels of key pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) were determined in serum samples from treated rats using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience®, USA), following the manufacturer's instructions. Blood samples were collected at the end of the *in vivo* experiments (carrageenan and NO-induced models),

allowed to clot, and centrifuged at 3,000 rpm for 10 minutes to obtain serum. The cytokine concentrations were quantified by comparing absorbance values at 450 nm to a standard curve generated for each cytokine. Results were expressed in pg/mL (Ayertey *et al*., 2021).

Molecular Docking Study

Molecular docking studies were carried out to evaluate the potential interactions between phytoconstituents from *C. cujete* and *C. vulgaris* methanol seed extracts and selected protein targets implicated in inflammation and cancer. AutoDock Vina software was used to perform the docking simulations. The 3D structures of bioactive compounds previously identified or reported in the literature were retrieved from the PubChem database in SDF format and converted to PDBQT format using Open Babel. The crystal structures of the target proteins Bcl-2 (PDB ID: 1G5M), COX-2 (PDB ID: 5F1A), and EGFR (PDB ID: 1M17) were downloaded from the protein data bank and prepared by removing water molecules and adding polar hydrogens using AutoDock tools. Grid box parameters were set to cover the active sites of each protein, and Lamarckian Genetic Algorithm was applied for docking. Binding affinities (expressed in kcal/mol) and interaction patterns such as hydrogen bonds, hydrophobic interactions, and π-π stacking were analyzed to predict the strength and nature of the ligand-protein interactions. The docked complexes were visualized using PyMOL and Discovery Studio Visualizer to interpret the binding conformations. Compounds showing strong binding affinity and favorable interactions with Bcl-2, COX-2, and EGFR were regarded as promising candidates that caused the observed anti-cancer and anti-inflammatory activities of the plant extracts (Gonzales *et al*., 2023).

RESULTS

Phytochemical Screening

Phytochemical analysis of the methanol seed extracts revealed the presence of various bioactive compounds in both *C. cujete* and *C. vulgaris*. Flavonoids and phenols were particularly abundant, especially in *Crescentia cujete*, which may contribute to the observed anti-inflammatory and anticancer effects. Other constituents such as alkaloids, saponins, and terpenoids were also detected, supporting the pharmacological potential of these seeds in traditional medicine (Table 1).

Table 1: Phytochemical Contents of *C. cujete* and *C. vulgaris* Methanol Seed Extracts

Phytochemical	*Crescentia cujete*	*Citrullus vulgaris*
Alkaloids	+	+
Flavonoids	+++	++
Saponins	++	+
Tannins	+	+
Phenols	++	++
Terpenoids	+	++
Steroids	+	+
Glycosides	++	+

Legend: (+) = Present in low amount, (++) = Present in moderate amount and (+++) = Present in high amount.

Anti-cancer Activity of Extracts

Both *C. cujete* and *C. vulgaris* methanol seed extracts demonstrated potent cytotoxic effects against MCF-7 breast cancer cells in a dose-dependent manner. The IC_{50} value for *C. cujete* extract was 24.5± 2.4 µg/mL, while that of *C. vulgaris* extract was 12.6 ± 3.1 µg/mL, indicating stronger activity by the extracts (Table 2). The MTT assay confirmed a significant reduction in cell viability at increasing concentrations of both extracts, with the highest inhibition observed at 100 µg/mL (Figure 1). Apoptosis induction was further validated using Annexin V-FITC/Propidium Iodide (PI) dual staining and observed under fluorescence microscopy.

Treated MCF-7 cells exhibited classic signs of apoptosis, including chromatin condensation and membrane blebbing. Cells stained green (Annexin V-positive) and orange-red (Annexin V and PI double-positive) indicated early and late apoptotic populations, respectively, confirming the pro-apoptotic effect of both extracts (Figure 2). Fluorescence images revealed a higher proportion of apoptotic cells in *C. cujete*-treated samples compared to *C. vulgaris*, consistent with their IC_{50} values.

Table 2: Cytotoxicity of *C. cujete*, *C. vulgaris*, and Doxorubicin on MCF-7 Cells

Concentration (µg/mL)	*C. cujete* (% Viability)	*C. vulgaris* (% Viability)	Doxorubicin (% Viability)
6.25	85.2 ± 2.4	88.1 ± 2.7	70.5 ± 2.2
12.5	72.6 ± 2.1	75.4 ± 2.3*	52.3 ± 2.1
25.0	56.3 ± 1.9*	61.5 ± 2.0*	34.8 ± 1.8*
50.0	39.8 ± 1.7*	45.2 ± 1.6*	20.1 ± 1.3*
100.0	22.4 ± 1.5*	28.9 ± 1.4*	8.7 ± 1.0*

*P < 0.05 (one-way ANOVA followed by Turky's post hoc test). Estimated IC_{50} values: *C. cujete*: 24.5 µg/mL, *C. vulgaris*: 12.6 µg/mL, and Doxorubicin: 10.1 µg/mL.

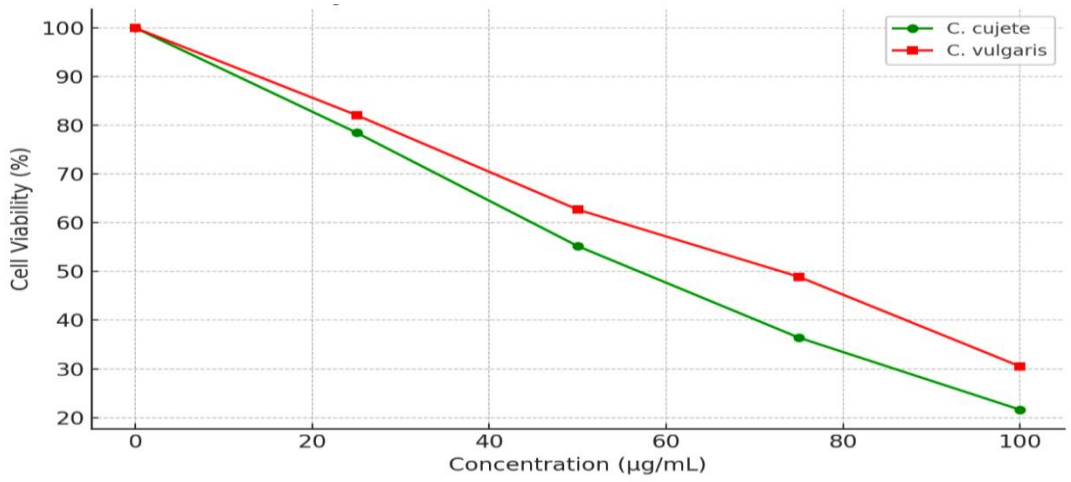

Figure 1: Cytotoxicity of MCF-7 cells exposed to *C. cujete* and *C. vulgaris* at 100 µg/mL concentration after 48 hours.

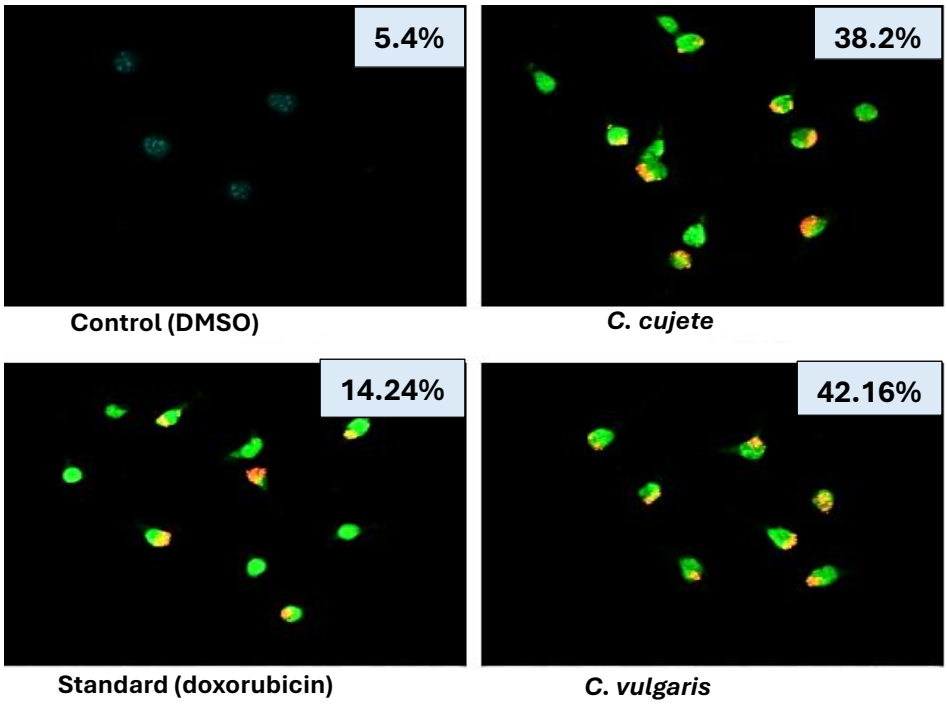

Figure 2: Fluorescence images of MCF-7 cancer cells exposed to extracts from *C. cujete* and *C. vulgaris* at 200 µg/mL concentration after 48 hours.

In Vivo Anti-inflammatory Effect of Extracts

Carrageenan-induced Paw Edema in Rats

The paw volume of rats in the carrageenan-induced group (Group II) significantly increased over 5 hours, indicating acute inflammation. Pre-treatment with ibuprofen (Group III), *C. cujete* extract (Group IV), and *C. vulgaris* extract (Group V) markedly reduced paw edema compared to the negative control. Notably, the *C. cujete* extract showed slightly better anti-inflammatory activity than *C. vulgaris*, particularly from the third hour onward (Table 3).

Table 3: Carrageenan-induced Paw Edema (Paw Volume in mL, Mean ± SD)

Time (h)	Group I (Normal)	Group II (Carrageenan)	Group III (Ibuprofen 10 mg/kg)	Group IV (*C. cujete* 200 mg/kg)	Group V (*C. vulgaris* 200 mg/kg)
0	0.25 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
1	0.26 ± 0.02	0.45 ± 0.04	0.34 ± 0.03	0.36 ± 0.03*	0.38 ± 0.04
2	0.26 ± 0.01	0.61 ± 0.05	0.41 ± 0.02*	0.44 ± 0.03	0.47 ± 0.04*
3	0.25 ± 0.01	0.73 ± 0.06*	0.46 ± 0.04	0.51 ± 0.03*	0.56 ± 0.04
4	0.25 ± 0.01	0.80 ± 0.07*	0.49 ± 0.03*	0.53 ± 0.03*	0.58 ± 0.05*
5	0.25 ± 0.01	0.83 ± 0.06*	0.50 ± 0.02	0.54 ± 0.03*	0.59 ± 0.04*

*P < 0.05 (one-way ANOVA followed by Turky's post hoc test).

Nitric oxide (NO) and Serum Cytokine Levels in Rats

The results showed that rats treated with LPS alone (Group II) showed significantly elevated levels of NO, TNF-α, IL-6, and IL-1β, indicating systemic inflammation. Treatment with *C. cujete* and *C. vulgaris* extracts significantly ($p<0.05$) reduced these inflammatory markers, with *C. cujete* demonstrating a slightly superior anti-inflammatory effect, closely comparable to the standard drug (Table 4).

Table 4: Nitric Oxide (NO) and Cytokine Levels in Serum (Mean ± SD, n = 5)

Groups	NO (µM)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
Group I (normal)	12.5 ± 1.3	18.6 ± 2.1	22.4 ± 2.8	15.2 ± 1.7
Group II (LPS)	39.8 ± 3.6	76.5 ± 4.7	83.2 ± 5.3	69.4 ± 4.9
Group III (Ibuprofen)	18.7 ± 1.5*	28.2 ± 2.6*	30.7 ± 2.4*	24.6 ± 2.0*
Group IV (*C. cujete*)	21.3 ± 2.2*	33.1 ± 2.8*	37.5 ± 2.9*	29.3 ± 2.1
Group V (*C. vulgaris*)	23.5 ± 2.4*	35.4 ± 3.0	40.8 ± 3.1*	31.7 ± 2.5*

*P < 0.05 (one-way ANOVA followed by Turky's post hoc test).

Molecular Docking Studies

Figure 3 below shows the molecular docking interactions between bioactive compounds from *C. cujete* and *C. vulgaris* extracts and the active sites of some key cancer and inflammatory-related proteins (Bcl-2, COX-2, and EGFR). The docked complexes show stable hydrogen bonding and hydrophobic interactions, indicating strong binding affinities. These interactions suggest that the phytochemicals may inhibit cancer progression by modulating apoptotic (Bcl-2), inflammatory (COX-2), and growth signaling (EGFR) pathways, supporting the experimental findings of the extracts' anti-cancer and anti-inflammatory activities.

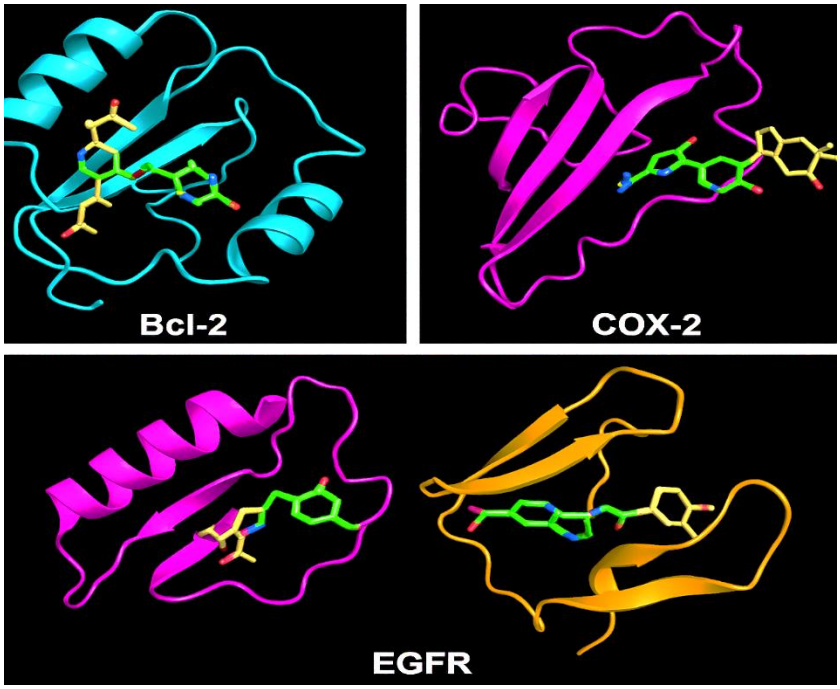

Figure 3: Molecular docking interactions of key phytochemicals from *C. cujete* and *C. vulgaris* with cancer and inflammatory-related proteins (Bcl-2, COX-2, and EGFR).

DISCUSSION

Cancer and inflammation are intricately linked biological processes, with inflammation often acting as a precursor or promoter of tumorigenesis (Famurewa *et al*., 2024). In particular, chronic inflammation contributes to cancer progression by fostering an environment rich in cytokines, growth factors, and reactive species that can promote DNA damage and cellular proliferation (Kandilarov *et al*., 2023). Given the limitations and side effects associated with conventional chemotherapeutics, there has been increasing interest in exploring natural plant-based agents with dual anti-inflammatory and anticancer properties (Nelson *et al*., 2020). In this study, we investigated the methanol seed extracts of *Crescentia cujete* and *Citrullus vulgaris*, traditionally used in ethnomedicine, for their phytochemical content, anticancer activity against MCF-7 breast cancer cells, anti-inflammatory potential, and molecular interactions with cancer-related targets.

Phytochemical analyses revealed the presence of diverse secondary metabolites including flavonoids, phenolics, alkaloids, saponins, tannins, and terpenoids in both plant extracts. These classes of compounds are widely recognized for their therapeutic properties, particularly in cancer prevention and inflammatory modulation (Adenowo *et al*., 2024). Notably, the abundance of flavonoids and phenolic compounds, known for their strong antioxidant and apoptotic-inducing properties, may underlie the biological activities observed in this study. Alkaloids and saponins, which were also detected, have been implicated in membrane disruption and immune regulation, supporting their role in both cytotoxic and anti-inflammatory pathways (Bencheikh *et al*., 2023).

The MTT assay demonstrated that both *C. cujete* and *C. vulgaris* methanol seed extracts exerted dose-dependent cytotoxic effects on MCF-7 breast cancer cells. The IC_{50} values were significantly lower than that of untreated controls and showed statistical differences when compared to doxorubicin, the standard control drug, confirming the extracts' cytotoxic potency. These findings are consistent with earlier reports that highlighted the cytotoxicity of

C. cujete leaf extracts on human leukemia cells (Januário *et al*., 2022), and the antiproliferative effects of *C. vulgaris* seed extracts on colorectal carcinoma cells (Ansori *et al*., 2022). Apoptosis assays using Annexin V and propidium iodide staining revealed characteristic morphological features of apoptosis, including nuclear fragmentation and membrane blebbing. This confirms that the reduction in cell viability was not due to nonspecific cytotoxicity, but rather a programmed cell death mechanism. The observed apoptotic activity can be linked to the presence of flavonoids and phenolics, which have been shown to activate intrinsic apoptotic pathways through modulation of mitochondrial membrane potential and caspase activation (Alfuraydi *et al*., 2024).

To better understand the anti-inflammatory potential of the extracts, we conducted a series of evaluations using both live animal tests (in vivo) and laboratory experiments (in vitro). In the carrageenan-induced paw edema model, both extracts produced significant inhibition of edema formation over time, indicating a suppression of acute inflammation. This model is a classic tool for evaluating anti-inflammatory agents, where inhibition of prostaglandin synthesis, histamine release, and leukocyte migration are key mechanisms (Ayertey *et al*., 2021). In the in vitro nitric oxide (NO) assay, both extracts demonstrated concentration-dependent inhibition of NO production. Since excessive NO production is associated with inflammation and cancer progression via angiogenesis and immune suppression (Ayertey *et al*., 2021), its reduction by the plant extracts further supports their anti-inflammatory and anticancer capabilities.

Biochemical assays further revealed that treatment with both *C. cujete* and *C. vulgaris* significantly ($p<0.05$) reduced serum levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while upregulating anti-inflammatory IL-10. This modulation of the cytokine profile aligns with existing literature on natural compounds that regulate immune responses and suppress inflammatory signaling pathways such as NF-κB and MAPK (Zhao *et al*., 2021). The anti-inflammatory activity observed complements the cytotoxic and pro-apoptotic effects, highlighting the therapeutic potential of the extracts in inflammation-driven cancers.

Molecular docking analyses provided mechanistic insights into the bioactivity of the key phytochemicals identified in both extracts. Selected compounds exhibited strong binding affinities for cancer-related protein targets including B-cell lymphoma 2 (Bcl-2), cyclooxygenase-2 (COX-2), and epidermal growth factor receptor (EGFR). Bcl-2, an anti-apoptotic protein overexpressed in various cancers, was effectively bound by phytochemicals from both extracts. This suggests a potential for these compounds to induce apoptosis via the mitochondrial pathway by antagonizing Bcl-2 activity (Saeed *et al*., 2022). COX-2, a key mediator in prostaglandin synthesis and inflammation, was also targeted. Inhibition of COX-2 supports the anti-inflammatory findings from the paw edema and cytokine studies and is consistent with previous research showing that COX-2 inhibitors can suppress tumor growth and metastasis (Alam *et al*., 2020). EGFR, frequently overexpressed in breast cancer, regulates cell proliferation and survival. Strong binding interactions between EGFR and the phytochemicals suggest that the extracts may also exert anti-proliferative effects through inhibition of EGFR signaling, a validated therapeutic target in oncology (Azar *et al*., 2021). These docking results not only validate the biological findings but also propose potential molecular targets for the observed bioactivities, paving the way for further mechanistic and structural biology investigations.

CONCLUSION AND RECOMMENDATIONS

The findings of this study demonstrate that methanol seed extracts of *C. cujete* and *C. vulgaris* possess rich phytochemical compositions, and could exhibit significant anticancer and anti-inflammatory properties. Their effects are mediated through apoptosis induction, suppression of pro-inflammatory cytokines, inhibition of nitric oxide, and potential molecular interactions with critical cancer-related targets. These results affirm the traditional uses of these plants and provide scientific justification for their continued exploration as sources of novel bioactive agents. Further studies, including compound isolation, mechanistic studies, and clinical evaluations, are warranted to fully harness their treatment capabilities.

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