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Chemical Compound Profile of Infusion from *Crescentia cujete* Fruit Peel and Toxicity Test against *Artemia sallina* Leach

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Abstract

Cancer is one of the top causes of death around the world, thus we need to make safer and more selective anticancer drugs from natural sources. The objective of this work is to identify the chemical constituents and evaluate the toxicity of infusions derived from the fruit peel of *Crescentia cujete* L., a potential bioactive candidate. Samples were botanically identified, prepared as infusions using a water-heating method (1:10 w/v), then concentrated and analysed by phytochemical screening, LC–MS, and the Brine Shrimp Lethality Test (BSLT) on *Artemia salina* larvae at concentrations of 25–2000 ppm. Screening results showed the presence of alkaloids, flavonoids, saponins, and tannins The LC-MS analysis showed 105 compounds, and the main alkaloid was marmeline (m/z 351.1834). The BSLT test resulted in an LC_{50} of 187.15 ppm, indicating moderate toxicity and potential for bioactivity or cytotoxicity. The correlation between alkaloid dominance and the LC_{50} value suggests that the C. cujete fruit peel infusion has potential for further development as a source of natural-based anticancer compound candidates.

Keywords: Artemia salina; Brine Shrimp Lethality Test; Crescentia cujete L.; LC–MS; cytotoxic toxicity.

INTRODUCTION

One of the most common causes of death around the world is cancer, which is characterized by the uncontrolled growth of abnormal cells that can spread to other organs (Sung *et al*., 2021). The GLOBOCAN 2020 report states that cancer killed almost 10 million people over the world, which is a big health problem in many nations (Sung *et al*., 2021). The quest for new anticancer agents that are safer and more selective, especially those derived from natural sources, is driven by the considerable adverse effects commonly associated with conventional cancer treatments, such as chemotherapy and radiotherapy, which lack specificity for cancer cells (Newman & Cragg, 2020). More than 60% of today's cancer-fighting drugs come from or are based on natural sources. Plants are a big source of bioactive chemicals (Newman & Cragg, 2020). *Crescentia cujete* L. is a plant that has showed promise and has been used in traditional herbal therapy. Numerous research studies indicate that C. cujete possesses secondary metabolites such as flavonoids, phenols, tannins, saponins, and terpenoids, which demonstrate antioxidant and cytotoxic properties (Fatimah *et al*., 2022).

The Brine Shrimp Lethality Test (BSLT) using *Artemia salina* larvae is an efficient, rapid, and cost-effective preliminary screening technique for assessing the toxicity of an extract or chemical (Hamidi *et al*., 2014). The LC_{50} value derived from this method offers a preliminary assessment of bioactivity potential, with LC_{50} <1000 ppm typically classified as hazardous and possibly appropriate for future advancement as an anticancer candidate (Meyer *et al*., 1982; Mozhiarhasi *et al*., 2019).

According to previous research, *Crescentia cujete* extract exhibits antioxidant properties and cytotoxic potential against *Artemia salina*, indicating the presence of bioactive compounds (Mozhiarhasi *et al*., 2019). However, there is a lack of study that specifically examines the chemical components of *C. cujete* fruit peel infusion and evaluates its toxicity using the BSLT method. The goal of this study is to describe the chemical components of the *Crescentia cujete* fruit peel infusion and see how poisonous it is to *Artemia salina* larvae. This is the first time we are looking at its bioactive and possible anticancer effects.

MATERIALS AND METHODS

Sample Preparation

The fruit peel samples of *Crescentia cujete* L. were first identified at the UPT Materia Medica Batu, East Java, to ensure the authenticity of the botanical identity and guarantee the validity of the research, as species authentication is a crucial step in natural product studies (Abubakar & Haque, 2020). The fruit peel was selected in a fresh, green condition, subsequently subjected to wet sorting to remove dirt and superfluous materials, and rinsed with running water to eliminate pollutants while maintaining the bioactive chemical content (Peiris *et al*., 2023). The samples were then air-dried in a shaded area, away from direct sunlight, to keep the active compounds stable and prevent them from breaking down from heat and UV radiation. This is important because proper drying is necessary to keep the quality of the herbal material before further extraction and analysis (Cetinkaya *et al*., 2025).

Extraction

Infusions from the skin of the wajackson fruit (*Crescentia cujete* L.) were prepared using the infusion method, a water-based extraction technique suitable for polar compounds. A total of 100 grams of prepared fresh fruit skin was placed into a heat-resistant container, then 1000 mL of distilled water was added (ratio 1:10 w/v). To ensure the optimal diffusion of active compounds, the mixture was heated to approximately 90°C and agitated intermittently for 15 minutes. According to (Azwanida, 2015), the infusion method is effective in extracting water-soluble secondary metabolites, including flavonoids, tannins, and phenolic compounds, without causing excessive degradation at temperatures near the boiling point of water. After heating, the solution was promptly filtered while still hot to prevent the reprecipitation of dissolved compounds. Vacuum evaporation can reduce the thermal degradation of bioactive compounds, which is why the filtrate was concentrated using a rotary evaporator at a controlled temperature and reduced pressure to remove the water solvent and produce a thicker, more stable extract for further analysis (Abubakar & Haque, 2020).

Phytochemical Screening

Phytochemical screening is an approach to find groups of secondary metabolites in extracts. This is accomplished by creating precipitates or color shifts using specific reagents. This method is often the first step in figuring out what natural items are because it can tell you what compounds are in them (Peiris *et al*., 2023). To check for alkaloids, you first use chloroform and ammonia to get the material out. After that, concentrated sulfuric acid and the reagents Mayer's and Dragendorff's are added. A positive result is indicated by the formation of a white precipitate (Mayer) or a reddish-yellow precipitate (Dragendorff). The process of identifying flavonoids involves the addition of ethanol and $FeCl_3$ solution, which results in the production of distinctive color variations such as green, blue, purple, or black when the test is positive. The saponin test is conducted by creating a stable froth in an acidic environment, while the tannins are identified by adding $FeCl_2$ or $FeCl_3$, which results in a dark green to bluish-black color. These reactions, which create changes that can be seen, are based on how some functional groups in secondary metabolites can react with particular chemical reagents (Bandiola & Alday, 2019).

Compound Analysis

The chemical compound profile analysis of *Crescentia cujete* fruit peel extract was carried out using Liquid Chromatography Mass Spectrometry (LC–MS) technique. Before analysis, 2 mg of the extract was diluted in pro-analysis methanol to a final volume of 10 mL, resulting in a 100 ppm concentration. The solution was filtered over a 0.45 µm cellulose acetate membrane and degassed to remove any particles or air bubbles that could damage the chromatographic system (Zhang *et al*., 2020). The chemicals were separated using a UPLC system connected to a Quadrupole Time-of-Flight (QTOF) mass spectrometer equipped with Electrospray Ionization (ESI) in positive mode. This approach is sensitive and accurate for detecting secondary metabolites in complex mixtures (El-Aneed *et al*., 2019). The UPLC C18 column (Shim-Pack FC-ODS, 2 mm × 150 mm, 3 µm) was used to combine methanol, acetonitrile, and water in a 40:15:45 v/v/v ratio. The flow rate was 0.5 mL/min for 9 minutes. The mass spectrometer was calibrated for a range of 100 to 5000 m/z and a capillary temperature of 350°C. This produced high-resolution mass spectra, which could be used to identify compounds based on their molecular weight and fragmentation patterns.

Toxicity Effect Testing on *Artemia sallina* Leach

The extract's toxicity was tested using the Brine Shrimp Lethality Test (BSLT) method on newly hatched Artemia salina larvae. To assess acute toxic effects, 10 larvae were exposed to each treatment concentration for 24 hours. The extract was tested at several graded concentrations, including 2000, 1000, 500, 250, 100, 50, and 25 ppm, to evaluate the dose-response relationship. After a 24-hour incubation period, living and dead larvae were counted to determine the mortality percentage, which was calculated by dividing the number of dead larvae by the initial number of larvae and multiplying by 100% (Rajabi *et al*., 2015).

Testing was carried out in triplicate to increase data reliability, with potassium dichromate as the positive control and seawater as the negative control. The LC_{50} (Lethal Concentration 50%) value was determined by probit analysis to estimate the concentration of the extract required to cause 50% larval mortality. The extract is categorized as having toxic activity if its LC_{50} value is <1000 ppm, indicating potential bioactivity, including possible cytotoxicity (Hamidi *et al*., 2014). Toxicity is defined as very toxic (<30 ppm), severely toxic (30-100 ppm), moderately toxic (100-250 ppm), weakly toxic (250-1000 ppm), or non-toxic (>1000 ppm) (Rajabi *et al*., 2015). The BSLT approach is commonly employed as an initial screening for bioactive chemicals since it is simple, quick, inexpensive, and highly correlated with more advanced cellular cytotoxicity tests (Hamidi *et al*., 2014).

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening results indicated that the extract of *majapahit* fruit peel (*Crescentia cujete*) contains several important groups of secondary metabolites, namely alkaloids, flavonoids, saponins, and tannins, as evidenced by the formation of precipitates or specific color changes after the addition of certain reagents.

Table 1. Phytochemical Screening Results.

Compound	Reagent	Result
Alkaloid	$CHCl_3$ + H_2SO_4 + Mayer + Dragendrorf	+
Flavonoid	C_2H_5OH + $FeCl_3$	+
Saponin	HCl pekat	+
Tannin	$FeCl_2$	+

Compound Analysis

LC–MS analysis of the infusion of Crescentia cujete L. fruit peel identified a total of 105 compounds, reflecting the complexity of the secondary metabolite composition in the water-based extract. The chemical with the highest concentration was marmeline ($C_{22}H_{25}NO_3$; MW 351.4460; m/z 351.1834 [100%]), which eluted after 12.414 minutes. In reversed-phase chromatography, retention duration is affected by interactions between the chemical and the non-polar stationary phase, with more polar compounds eluting faster than less polar ones (El-Aneed *et al*., 2019; Zhang *et al*., 2020).

Toxicity Analysis

The Brine Shrimp Lethality Test (BSLT) found that the infusion extract of the peel of the Crescentia cujete fruit was the most lethal at 2000 ppm. The LC_{50} value of 187.15 ppm from linear regression analysis puts it in the moderate toxicity range and shows that it has a lot of bioactivity potential. In BSLT testing, extracts with LC_{50} values below 1000 ppm are often classified as physiologically active and may be considered for development as cytotoxic drug candidates (Rajabi *et al*., 2015). It is thought that this toxic activity is linked to the presence of secondary metabolites found through phytochemical screening, such as alkaloids, flavonoids, saponins, and tannins. This is further supported by LC–MS analysis results that show that marmeline, an alkaloid, is the compound that makes up the most of the extract.

Table 2. Results of Toxicity Analysis.

Extract	Concentration (ppm)	LC50 (ppm)	Toxicity Category
Infusion of Crescentia cujete L fruit peel	25-2000	187.15	Moderate

Discussion

Phytochemical screening shows that *Crescentia cujete* fruit peel extract contains alkaloids, flavonoids, saponins, and tannins. Identification used specific reagents: white precipitate (Mayer) and orange-red (Dragendorff) for alkaloids, a reddish color with $FeCl_3$ for flavonoids, stable foam for saponins, and bluish-black with $FeCl_2$ for tannins. This qualitative screening effectively detects plant secondary metabolites during initial exploration (Harborne, 1998; Tiwari *et al*., 2011).

The presence of these secondary metabolites suggests that they may be biologically active. Alkaloids have been shown to have a variety of pharmacological properties, including cytotoxic and anticancer effects via interactions with DNA and cellular structural proteins (Rani *et al*., 2022). Flavonoids are potent antioxidants that trap free radicals and reduce cancer cell proliferation (Panche *et al*., 2016). Saponins have been shown to have lethal effect by increasing cell membrane permeability and inducing apoptosis (Man *et al*., 2010), whereas tannins, as polyphenol chemicals, can bind to proteins and enzymes, altering the biological functioning of target species.

The LC-MS analysis of the infusion of *C. cujete* fruit peel showed 105 compounds, revealing the complex chemical composition of the aqueous extract. The molecule with the highest intensity was marmeline ($C_{22}H_{25}NO_3$; m/z 351.1834 [100%]) with a retention time of 12.414. In reverse-phase chromatography, the analyte's polarity and hydrophobic interactions with the nonpolar stationary phase influence retention time, with more polar compounds eluting faster than their less polar counterparts (El-Aneed *et al*., 2019; Zhang *et al*., 2020).

The dominance of marmeline, an alkaloid, indicates that the main component of the extract is a bioactive compound. LC–MS-based studies show that peaks with the highest intensity or area percentage often correlate with the greatest contribution to the extract's biological activity, including antioxidant and cytotoxic activities (Mondal *et al*., 2019). Therefore, the presence of marmeline, the dominant component, is suspected to play an important role in the observed toxic activity. Alkaloids decrease cell growth by influencing microtubule dynamics. These chemicals can bind to tubulin and block microtubule polymerization, interrupting spindle formation during mitosis, arresting the cell cycle at the metaphase stage, and inducing death (Rani *et al*., 2022).

The Brine Shrimp Lethality Test (BSLT) found that Artemia salina larvae were moderately dangerous, with an LC_{50} of 187.15 ppm. The BSLT method is a rapid, easy, and cheap way to see if natural product extracts are hazardous to cells (Meyer *et al*., 1982). Extracts with LC_{50} values under 1000 ppm are often thought to be physiologically active and may be tested as possible cancer treatments (Rajabi *et al*., 2015).

The LC_{50} value of 187.15 ppm indicates that the *C. cujete* fruit peel infusion has significant bioactivity. This toxic activity is suspected to be closely related to the alkaloid content, which is the dominant component, supported by the presence of flavonoids, saponins, and tannins. Alkaloids can operate as stomach poisons in the context of BSLT, affecting the larval digestive system and sensory receptors, preventing food absorption and resulting in death (Meyer *et al*., 1982). The phytochemical screening results, high marmeline concentration in LC-MS analysis, and modest LC_{50} value suggest that the alkaloid component is responsible for the material's toxic properties.

CONCLUSIONS

Based on the research results, it can be concluded that the extract of *Crescentia cujete* fruit peel contains primary and secondary metabolites, including alkaloids, flavonoids, saponins, and tannins, which have the potential to exhibit biological activity. The LC-MS investigation identified 105 compounds, with marmeline being the most abundant alkaloid, showing that it plays a significant role in the extract's bioactivity. The Brine Shrimp Lethality Test (BSLT) on Artemia salina larvae yielded an LC_{50} of 187.15 ppm, demonstrating high toxicity and cytotoxic potential. The correlation between the phytochemical screening results, the dominance of marmeline in the LC–MS analysis, and the obtained LC_{50} value demonstrates consistency, suggesting that alkaloids are likely the main contributors to the observed toxic effects. As a result, the fruit peel infusion of C. cujete has the potential to be further developed as a source of natural product-based anticancer compounds.

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