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Research Paper

Phytochemical Profile Analysis of Ethanol from Jamaican Cherry Leaf (*Muntingia calabura L.*) and Spanish Cherry Leaf (*Mimusops elengi L.*)

Fatmawati, Siswanto, Siti Aisiah, Sucipto

Aquaculture Study Program, Lambung Mangkurat University, Banjarmasin, Indonesia

Corresponding Author: Fatmawati

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ABSTRACT

Jamaican cherry and Spanish cherry plants are widely used as shade trees. These plants are commonly found on the grounds of Lambung Mangkurat University, but they have not yet been utilized as sources of antioxidants or for treating fish diseases. These plants produce phytochemical compounds in the form of secondary metabolites that can be used as antioxidants and to treat diseases in fish. This study aims to identify the phytochemical secondary metabolites present in Jamaican cherry and Spanish cherry leaves. The method employed was a qualitative analysis. Based on the test results of this study, Jamaican cherry leaf contain secondary metabolites in the form of alkaloids, flavonoids, tannins, and hydroquinones; saponins were not detected in Jamaican cherry leaf in this study, whereas Spanish leaf contain alkaloids, flavonoids, tannins, saponins, and hydroquinones.

Keywords: *Phytochemicals, jamaican cherry leaf, spanish cherry leaf, qualitative, ethanol.*

INTRODUCTION

Indonesia is a tropical country with extremely high biodiversity, including various plant species that have the potential to serve as sources of natural bioactive compounds. The use of plants as ingredients in traditional medicine and health supplements continues to grow in line with the public's increasing demand for natural products that are relatively safe and environmentally friendly. One important group of compounds extensively studied in plants is phytochemicals secondary metabolites that include alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. These compounds are known to possess various biological activities, such as antioxidant, antibacterial, anti-inflammatory, antifungal, and immunostimulatory properties, which hold potential for application in the fields of health, food, and aquaculture. (Panche et al., 2016).

Indonesia is a tropical region with a rich diversity of flora. Efforts to develop medicinal plants are ongoing. One of the challenges in developing medicinal plants is the limited availability of raw materials, as well as the fact that cultivation is not yet intensive. One plant with potential for development as a medicinal plant is the jamaican cherry plant (*Muntingia calabura* L.) (Nurholis & Saleh, 2019).

In addition to jamaican cherry leaf, spanish cherry leaf (*Mimusops elengi* L.) are also a plant with significant phytochemical potential, yet their use remains relatively limited. The spanish cherry plant is known as a shade tree and ornamental plant that grows widely in tropical regions. According to research Kadam et al. (2012), extracts from the leaves and other parts of the spanish cherry plant exhibit significant biological activity, including antioxidant, anti-inflammatory, and antibacterial properties.

Oxidative stress is a significant challenge in aquaculture, often triggered by environmental factors such as poor water quality, high stocking density, temperature fluctuations, and exposure to contaminants like ammonia and heavy metals. These conditions lead to the excessive production of harmful molecules known as reactive oxygen species (ROS), which can overwhelm the body's natural defenses and cause cellular damage. Natural antioxidants found in plants and other natural sources have the potential to reduce oxidative stress by neutralizing ROS and protecting aquatic species. These antioxidants not only enhance immune function and stress tolerance but also improve growth and product quality. These findings provide insights into how natural antioxidants can serve as an environmentally friendly alternative to synthetic additives, supporting sustainable aquaculture practices (Hu et al., 2025).

Geographical factors such as elevation, temperature, soil pH, and humidity are reported to influence the accumulation of secondary metabolites (Lingga et al., 2025). Phytochemical screening is a fundamental first step in the exploration of bioactive compounds in plants. This process aims to provide a qualitative overview of the classes of secondary metabolites present in plant crude extracts or extracts, such as alkaloids, flavonoids, tannins, terpenoids, and hydroquinones (Safitri et al., 2025).

These secondary metabolites are not essential for primary plant growth; rather, they function as defense mechanisms against biotic and abiotic stresses, such as pathogen attacks, ultraviolet radiation, and climate change (Safitri et al., 2025). The dynamics of the biosynthesis of these compounds are strongly influenced by internal (genetic) and external (environmental, geographic location, and seasonal) factors, so research conducted in different regions is likely to yield different chemical profiles (Lingga et al., 2025). Based on the above, tests were conducted to determine the phytochemical content for potential use in aquaculture for fish health management.

MATERIALS & METHODS

Phytochemical testing was conducted qualitatively in October 2025 at the Basic Laboratory of the Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarbaru.

1. Equipment: The equipment used in this study included a test tube rack, a blender, a knife, a Bunsen burner, test tubes, a measuring flask, an Erlenmeyer flask, a stirrer, sample bottles, a dropper, a strainer, and Whatman filter paper.
2. Materials: The materials used in this study were crude extracts of jamaican cherry leaves and spanish cherry leaves, Dragendroff, Meyer, and Wagner reagents, HCl, lead acetate, magnesium powder, $FeCl_3$, distilled water, ethanol, and magnesium powder.
3. Sample Preparation: The samples to be tested are jamaican cherry leaf and spanish cherry leaf obtained from the ULM Banjarbaru campus. The collected leaves were sorted to select fresh, mature bay leaves that were free of defects and disease. They were then washed under running water to remove any dirt. The jamaican cherry and spanish cherry leaves were then dried with paper towels. Once dry, they were chopped and left to dry at room temperature. Afterward, the leaves were blended into a powder and sifted to obtain a fine flour. Both types of crude extracts were macerated with 96% ethanol, then filtered using Whatman paper and heated at 50°C to produce a paste, which was then placed in sample bottles, ready for phytochemical testing. Phytochemical testing for alkaloids, flavonoids, tannins, saponins, and hydroquinones was conducted using the

testing guidelines established at the Basic Laboratory of the Faculty of Fisheries and Marine Sciences (FPIK) at ULM in 2025.

4. Phytochemical screening: The alkaloid test is performed by preparing a sample extract in the form of a paste diluted with 1% KOH as needed in three test tubes; each is then treated with a drop of Dragendorff's reagent. A positive result is indicated by a color change to brick red or brownish; Mayer's reagent turns white or yellow; and Wagner's reagent turns reddish or brick red.
5. Flavonoid Test: Prepare the sample extract in the form of a paste diluted with 1% KOH as needed in two test tubes; add Mg powder and HCl to one test tube. Boil water in a beaker, let it cool until warm, then immerse the test tubes in the warm water. To the other test tube, add a solution of lead acetate. If the result is positive, a color change will occur to yellow, orange-yellow, or brownish-red; a positive sample contains flavonoids.
6. Tannin Test: Place the sample extract into a test tube. Add a 1% iron (III) chloride solution. The formation of a dark blue or blackish-green color indicates the presence of tannin compounds.
7. Saponin Test: Prepare the required amount of sample extract in a test tube, add distilled water, shake vigorously for 10 minutes, then place the test tube in a shaker and shake again. Allow it to stand for 10 minutes. The formation of stable foam in the test tube indicates the presence of saponins. Add 1 drop of 1% HCl; if the foam remains stable and does not disappear, then the sample is positive for saponins.
8. Hydroquinone Test: Place the sample extract into a test tube. Add 1–2 drops of 10% KOH; if the color turns yellow or brownish-yellow, this indicates the presence of hydroquinone.

RESULT AND DISCUSSION

Studies on the phytochemical profiles of plants form the fundamental basis for the development of natural-product-based therapeutic agents. Two species with a long ethnobotanical history in traditional medicine in tropical regions are jamaican cherry (*Muntingia calabura* L.) and spanish cherry (*Mimusops elengi* L.). Jamaican cherry, a member of the Muntingiaceae family, and spanish cherry, from the Sapotaceae family, each possess a unique wealth of secondary metabolites. The qualitative analysis presented in Table 1 provides an initial overview of the differences and similarities in the phytochemical content of the leaves of these two species, which includes alkaloids, flavonoids, tannins, saponins, and hydroquinones. These differences in profiles not only reflect genetic variation and distinct biosynthetic pathways but also imply the pharmacological activities that each extract may exhibit.

Table 1. Results of Phytochemical Analysis of Jamaican cherry Leaf and Spanish cherry Leaf Extracts

Extract ingredients	Alkaloids			Flavonoids		Tannins	Saponins	Hydroquinones
	D	M	W	Mg+Hcl	Lead acetate	$FeCl_3$	Aquades+HCl	KOH 10%
Jamaican cherry Leaf	-	+	-	+	+	+	-	+
Spanish cherry Leaf	-	+	+	+	+	+	+	+

Ket: + = exist; - not exist; D=Dragendorff; M=Mayer's; W=Wagner

Result of Phytochemical Screening

Alkaloid

The results of the Shapiro-Wilk Table 1 shows that jamaican cherry leaf yield a positive result only in the Mayer test (+), while negative results were found in the Dragendorff test (-) and the Wagner test (-). In contrast, spanish cherry leaf yield positive results in the Mayer test (+) and the Wagner test (+), but negative in the Dragendorff test (-). This difference indicates variations in the concentration or type of alkaloids contained in the two species. This suggests that the success of the Mayer reagent in detecting alkaloids indicated by the formation of a white or yellowish precipitate in both plant samples (jamaican cherry and spanish cherry) indicates that both plants are positive for alkaloid compounds.

The success of Mayer's reagent in detecting alkaloids in both plants indicates that both jamaican cherry and spanish cherry contain basic nitrogen compounds. However, the failure of Wagner's reagent to detect alkaloids in jamaican cherry leaf (result: negative) provides important insight into the sensitivity of the reagent or the type of alkaloids present.

Alkaloids at very low concentrations may not be sufficient to form a visible precipitate with Wagner's reagent but are still detected by Mayer's reagent due to differences in the stability constants of the complexes formed (Kaushik, 2026). A study by notes that the alkaloids in *Mimusops elengi* L. may be more stable when forming complexes with various iodide reagents, which explains why the extract yielded positive results in two of the three alkaloid tests conducted (Purnima et al., 2010). On the other hand, several studies on *Muntingia calabura* L. have often shown the absence of alkaloids in certain fractions, such as in methanol extracts, indicating that the presence of alkaloids in jamaican cherry is greatly influenced by environmental factors or extraction methods (Pungot et al., 2020).

Flavonoid

Flavonoids are one of the most abundant groups of secondary metabolites in cherry and tamarind leaf, as indicated by positive results for both test materials using the Mg+HCl reagent (Rezeki et al., 2023). The similarity in the positive results for both leaves confirms that polyphenols are the primary constituents supporting the traditional use of both plants as herbal medicines. Because, according to (Zuraida et al., 2017) Flavonoids are secondary metabolites that belong to the polyphenol group. The presence of flavonoids in cherries has been documented by several researchers who have identified specific compounds such as flavones, flavanols, and aurones (Pungot et al., 2020).

In the leaf of the spanish cherry plant, flavonoids such as quercetin, myricitrin, and kaempferol play a key role in its pharmacological activity (Gami et al., 2012). Kaempferol and quercetin in spanish cherry have been linked to anti-inflammatory activity and potential as anticancer agents due to their ability to inhibit tumor cell proliferation by inducing apoptosis (Dixit, 2026).

Tannin

The data in Table 1 show that both jamaican cherry and spanish cherry contain significant amounts of tannins. In jamaican cherry, tannins work synergistically with flavonoids to produce an antimicrobial effect (Krishnaveni & Dhanalakshmi, 2014). Tannins are able to bind to proteins on the cell walls of bacteria, causing protein coagulation and ultimately the death of the microbial cells (Sowjanya et al., 2023). This supports the use of cherry leaf for treating open wounds or skin infections.

In the spanish cherry plant, tannins are an important component of the bark and leaves that provide an astringent effect; this property is used in traditional dentistry to tighten loose gums and stop gum bleeding (Bhavikatti et al., 2021) Research by (Zahid et al., 2016), confirms that the high levels of tannins and phenols in the bark directly contribute to free radical scavenging activity comparable to that of commercial antioxidants.

Antioxidants can protect the body's cells from damage caused by free radicals. Free radicals can damage cells and body tissues. The body has natural antioxidants, but not in large quantities, so it requires antioxidants from external sources. Antioxidants from external sources can be derived from various natural ingredients native to Indonesia that possess antioxidant potential, one of which comes from jamaican cherry leaf (*Muntingia calabura* L.) and salam leaf (*Syzygium polyanthum*). (Nurfitri et al., 2023).

Saponin

The difference in the profiles of cherry leaf and spanish cherry leaf in this test lies in their saponin content. Table 1 shows that spanish cherry leaf contain saponins (+), whereas no saponins were detected in cherry leaf (-); this is consistent with the results of the study (Nurfitri et al., 2023). The positive results for Spanish cherry leaf are fully consistent with the botanical characteristics of the Sapotaceae family. Plants in this family are widely known as a rich source of triterpenoid saponins (Gami et al., 2012).

Saponins in spanish cherry, like glycosides, have been shown to possess significant antinociceptive activity, which explains why spanish cherry extract is highly effective in relieving pain (Sani et al., 2012). Saponins work by interacting with the lipids in cell membranes; in medical treatment, this can increase membrane permeability to facilitate the absorption of other drugs or directly inhibit the growth of fungi and bacteria (Bhavikatti et al., 2021).

Research findings (Cheong et al., 2022) contrary to the findings of this study, namely the detection of saponins in cherry leaf extract. The discrepancy in the results of this study may be attributed to several technical and biological factors, such as the extraction method and solvent; since saponins are polar compounds, they are more efficiently extracted using solvents such as water or methanol (Gami et al., 2012; Sowjanya et al 2023). If the tests in Table 1 are performed on extracts using less polar solvents or through extreme drying procedures, the saponin content may fall below the detection limit (Gilbis & Yasmin, 2022).

Another factor contributing to the absence of saponins is seasonal and geographical variation, which causes the content of secondary metabolites to fluctuate significantly depending on leaf age, harvest time, and environmental stress conditions (Pungot et al., 2020). Cherry trees growing in highly alkaline soil or exposed to high levels of pollution may shift their biosynthetic pathways from saponins to flavonoids as a mechanism of oxidative protection (Gilbis & Yasmin, 2022) or due to the sensitivity of the foam test, which requires a critical concentration to form stable micelles. If the saponin concentration in the cherry sample is very low, the foam formed may dissipate in less than a minute, resulting in a negative result according to standard operating procedures (Pratiwi & Dewi, 2022).

Hydroquinone

The positive hydroquinone test results for both extracts using 10% KOH indicate the presence of simple phenolic compounds. The presence of natural hydroquinone in jamaican cherry and spanish cherry is most likely due to (Silalahi, 2025) in relation to their role as precursors in the biosynthesis of more complex polyphenols or as defense agents against herbivores. Quantitative analysis of jamaican cherry revealed relatively low levels of hydroquinone (approximately 0.244 mg/L), which theoretically remain within safe limits for mild topical applications, but still require caution in pharmaceutical formulations (Silalahi, 2025). Hydroquinone or the arbutin precursor is found in various food and medicinal plants, such as vegetables, fruits, coffee, tea, and pear leaf (Panda & Reynders, 2025; Ma et al., 2019; Ribeiro et al., 2025). Some plant extracts rich in phenolic compounds (including hydroquinone and its derivatives) exhibit activity against pathogenic bacteria, although this activity is not always specific to fish bacteria (Panda & Reynders, 2025;

Ma et al., 2019; Tadese et al., 2021; Ribeiro et al., 2025).

The Relationship Between the Potential of Screened Compounds and Fish Health

Results of phytochemical analysis using qualitative methods in this study. Cherry leaves were found to contain alkaloids, flavonoids, tannins, and hydroquinones, with no saponins detected, while Spanish cherry leaf contained alkaloids, flavonoids, tannins, saponins, and hydroquinones. Several studies have shown that phytochemical compounds are important bioactive compounds with antimicrobial and antioxidant properties and as feed additives, such as (Sathiyamoorthi et al., 2023) found that the flavonoids and tannins in spanish cherry leaf exhibit strong inhibitory activity against Vibrio by disrupting the cell-to-cell communication system (*quorum sensing*) that regulates the expression of hemolysin.

Saponins are well-established candidates for use as feed supplements because they have been shown to increase the permeability of small intestinal mucosal cells, thereby enhancing the absorption of nutrients, particularly macromolecules. In addition, their detergent-like activity improves carbohydrate digestibility by reducing viscosity (Acosta et al., 2019). In this study, the absence of saponins in cherry leaf makes them a very safe ingredient for use in high doses via the soaking method (dipping/bath) (Azzaria et al., 2025). This allows for the treatment of external wounds in fish fry and larvae, which are typically highly sensitive to chemical irritants (Azzaria et al., 2025).

Although saponins in spanish cherry leaf are potentially toxic to gills, they can be put to good use when applied as a feed additive. At controlled low doses (less than 300 mg/kg of feed), saponins can increase intestinal permeability, aiding the absorption of macronutrients and other antibacterial compounds into the bloodstream (Acosta et al., 2019). A vaccine adjuvant designed to enhance the specific antibody response when used in combination with inactivated bacterial antigens (Wang et al., 2016). Ammonia control: for example, saponins from the Yucca schidigera herb can inhibit the urease enzyme, helping to reduce ammonia levels in aquaculture ponds (Biovet, 2023).

Therefore, the strategy for applying spanish cherry leaf should focus more on enteral administration (feed) to avoid direct contact with the gills, while jamaican cherry leaf are more versatile and can be used for both soaking and oral administration (Acosta et al., 2019). The main advantages of using these natural ingredients are their high biodegradability and the low risk of residue accumulation in fish tissue. Cherry and spanish cherry extracts not only kill pathogenic bacteria directly but also inhibit biofilm formation, which is a key strategy bacteria use to survive attacks by the immune system and antibiotics (Ma et al., 2019).

In brackish-water and marine fish, Vibrio species such as *V. alginolyticus* and *V. parahaemolyticus* are major threats that cause septicemia. The flavonoids and tannins in spanish cherry leaf exhibit strong inhibitory activity against Vibrio by disrupting the cell-to-cell communication system (*quorum sensing*) that regulates the expression of hemolysin toxins (Sathiyamoorthi et al., 2023). The synergy between the alkaloids and hydroquinone in these two leaves also helps disrupt existing bacterial biofilms, making the bacteria more susceptible to attack by the fish’s immune cells (Sathiyamoorthi et al., 2023).

Different results are often found in specimens extracted using different solvent concentrations or originating from other geographic regions (Surjowardojo et al., 2014) For example, research in Indonesia shows that cherry leaf extract prepared with 70% ethanol contains saponins, whereas in the 96% ethanol extract, no saponins were detected (Heradewi & Azzahra, 2025). This can be explained by the principle that “like dissolves like”; saponins are polar compounds that are more easily extracted by solvents with a higher water content. The use of 96% ethanol, which is semi-polar, may not be potent enough to extract all saponin fractions from the plant cell matrix compared to 70% ethanol, which is more polar (Heradewi & Azzahra, 2025).

CONCLUSION

The results of phytochemical tests on cherry and spanish cherry leaf revealed both differences and similarities in the content of secondary metabolites. The success of the Mayer reagent in detecting alkaloids in both plants indicates that both jamaican cherry and spanish cherry contain basic nitrogen compounds. However, the failure of the Wagner reagent to detect alkaloids in jamaican cherry leaf (negative result) provides an important indication regarding the sensitivity or type of alkaloids present, whereas the Wagner reagent tested positive on spanish cherry leaf (positive result). Flavonoids are one of the most abundant groups of secondary metabolites in jamaican cherry and spanish cherry leaf, as indicated by positive results in both test materials using the Mg+HCl reagent. The similarity of positive results in both leaf confirms that the polyphenol group is the primary constituent supporting the traditional use of these two plants as herbal medicines.

Declaration by Authors

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