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Prospect Study of Anti-inflammatory Activity by Identification of *Muntingia calabura* Leaf Infusion

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Abstract

Many diseases occur due to inflammation that is not handled properly. One of the feature of inflammation is swelling or edema. Inflammation can be handled with traditional medicine, such as *Muntingia calabura L.* (*M. calabura L.*) Pharmacologically, this plant extract is reported to have antipyretic, analgesic, anti-inflammatory, antioxidant, and antibacterial. Metabolite compound contained in *M. calabura L.* has the potential as an anti-inflammatory agent. The objective of this study is to ascertain the secondary metabolites contained in *M. calabura L.* leaves infusion. This experimental research method includes the simplicia making and infusion of *M. calabura L.* leaves by cold infusion and phytochemical screening. This study also showed that the infusion of *M. calabura L.* leaves contained flavonoids, alkaloids, and triterpenoids. Overall, the findings indicate potential as an anti-inflammatory agent that requires further investigation, specifically in preclinical testing.

Keywords: Anti-inflammatory, infuse, *M. calabura L*, phytochemical screening

Introduction

Several diseases arise when inflammation symptoms are not promptly and accurately addressed. Osteoarthritis becomes a problem that arises due to inadequately managed inflammation. This condition is prevalent among racehorses and other equine athletes.

It occurs due to repetitive joint trauma, leading to cartilage damage and erosion of bone1. A study found that 33% of thoroughbred racehorses aged 2-3 years suffered from osteoarthritis and lesions in their articular cartilage.[2] Other diseases that can emerge from inflammation in horses is muscle and orthopedic pain, perioperative pain, corneal ulcers, uveitis, laminitis, and gastrointestinal pain (colic).[3] Efforts to prevent the worsening of trauma can be reduced using anti-inflammatory drugs. Indonesia has a wealth of natural medicines and traditional remedies that many people have been using for generations.[4]

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M. calabura L., also known as kersen, belongs to the family Muntingiaaceae and is widely found along the way. This plant typically grows in the wild. Nevertheless, due to its dense foliage, it is deliberately cultivated as a roadside shade tree and an air pollution absorber.[5] This plant is one of the potential candidates for further development as a traditional medicine for anti-inflammatory purposes. Some research journals state that flavonoids are the main constituents in kersen leaves. Flavonoids themselves are polyphenolic compounds with various activities, one of which is anti-inflammatory.[5]

One direct benefit of traditional medicine formulations for the community is the easy availability of these remedies. The demand for plant-based ingredients in traditional medicine is increasing because they are proven healthier and have fewer side effects than chemical-based alternatives. However, a challenge with traditional medicine is the limited knowledge and information about the different types of plants used as ingredients and how to use them effectively.[6]

Based on this information, the primary goal of this study is to identify the secondary metabolites present in the infusion of *M. calabura L.* leaf so that further tests can be carried out in vivo and in vitro as anti-alternative inflammatory drugs.

Methods

This study is a laboratory experiment to discover the secondary metabolites found in *M. calabura L.* leaf infusion (MCLI). This study was conducted at the Chemical Application Laboratory and Services, Universitas Padjadjaran. This research has received approval and authorization from the Research Ethics Committee of Universitas Padjadjaran (Document No. 1066/UN6.KEP/EC/2023).

Research Procedures

Collection and preparation of sample

Samples of *M. calabura L.* were collected from the Sukapura Village in Kiaracondong District, Bandung City, West Java Province. Before further investigation, the sample was recognize at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (Document No. No.212/LBM/IT/VIII/2023). The sample was cleaned of attached dirt, cut into small pieces, and dried in the dryer.

Preparation of M. calabura L. Infusion

The simplicia of *M. calabura L.* leaves (50 g) was extracted with 200mL distilled water (1:4). At room temperature (25-30°C), let it stays for 1 hour before being placed in the refrigerator for 19 hours. Next, the infusion substance was filtered and put into a falcon tube. Adaptive method for cold infusion was followed Farmakope Herbal Indonesia year 2017.

Phytochemical Screening

Phenolic Test

Two grams of sample was mixed with 5 mL methanol. The solution was refined then 5% ferric chloride solution was added. When phenolic compounds are present, a positive outcome is indicated by the formation of a bluish-black color.[7]

Tannins Test

Roughly 2 grams of the plant extract were boiling with 5 mL of distilled water. After that, added 0.1% Ferric chloride to the mixture and watched for the appearance of a greenish-black color, which would suggest that tannins were present.[7]

Alkaloid Test (Wagner's Test)

Two grams of sample was mixed with 5 mL chloroform, then add 5 mL sulphuric acid. Next, put a few drops of Wagner's reagent

into the solution. If a reddish-brown or brown clumpy substance forms, it means the test is positive.[7]

Flavonoid Test

1. Shinoda test: combined 2 grams of the sample with 5 mL of methanol solution. Next, put in a tiny piece of magnesium chunk. Then, added 2 drops of concentrated HCl. When the color turned orange, it meant that there were flavonoids present.
2. Two grams of sample was mixed with 5 mL methanol solution; then plant solution was treated with 2-3 drops of 10% sodium hydroxide solution. The development of a strong yellow color suggests that flavonoids are present.
3. Mixed two grams of the sample with a 5 mL methanol solution and then reacted it with two drops of 2N hydrochloric acid. The sudden appearance of a acute yellow color shows that there are flavonoids present.[7]

Saponins (Foam Test)

Two grams of sample was mixed with 5 mL aquadest, filtered the solution, shaken vigorously for 2 minutes, then reacted with two drops hydrochloride acid 2N. The formation of a persistent froth for 10 minutes indicated the presence of saponins.[7]

Tripernoids and Steroids Tests

Mixed two grams of each sample with ethanol. 2 mL chloroform was extracted with 1 mL chloroform and water (1:1). Then added with 1 mL concentrated sulphuric acid. Reddish-brown coloration indicated the presence of tripernoids. Green, blue, and violet pigment indicated the presence of steroids.[7]

Results and Discussion

Extraction Yield

The cold infusion technique was employed in this research to extract compounds from the leaves of *M. calabura L.*, resulting in an extract yield of 0.1%. The cold infusion technique differs from the hot infusion technique. This method involves using refrigerator temperatures where the sample is left to steep for a minimum of 12-24 hours.[8] A non-thermal extraction method was selected to prevent any potential damage to heat-resistant active metabolits that may be present in the sample. Biological factors (plant part, plant species, location of growth, and harvesting time) and chemical factors (size, hardness, dryness of the material, levels and types of active compounds contained in plant material, type of solvent use, extraction methods) Can affect the quality of the extract.[9]

Table 1. Phytochemical Screening Results

No	Test	Testing Method	Result
1.	Phenolic	$FeCl_3$ reagent 5%	-
2.	Tannins	$FeCl_3$ reagent 1%	-
3.	Flavonoids	Concentrated HCL reagent + Mg	-
		Reagent H_2SO_4 2N	-
		NaOH reagent 10%	+
4.	Saponins	Reagent HCL 2N	-
5.	Triterpenoids		+
	Steroids	Concentrated H_2SO_4 reagent	-
6.	Alkaloids	Wagner's reagent	+

Note: (-) : Not detected | (+) : Detected

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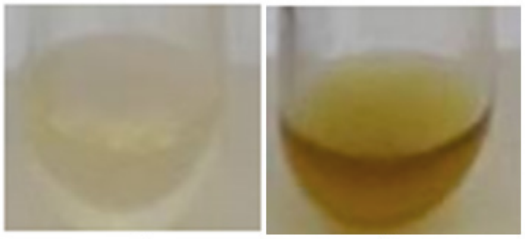

Figure 1. Phytochemical screening results of phenolics coumpounds with 5% ferric chloride solution: (a) before and (b) after adding the reagent.

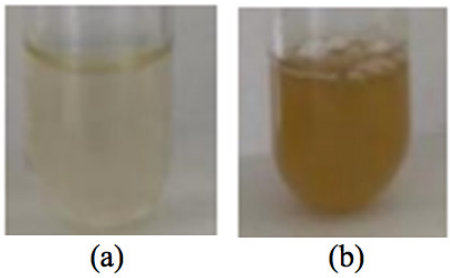

Figure 2. Phytochemical screening results of tannin coumpounds with 1% ferric chloride solution: (a) before and (b) after adding the reagent.

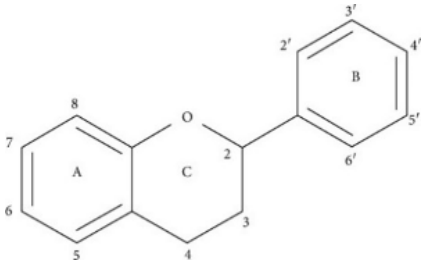

Figure 3. Basic Flavonoids Structure[14]

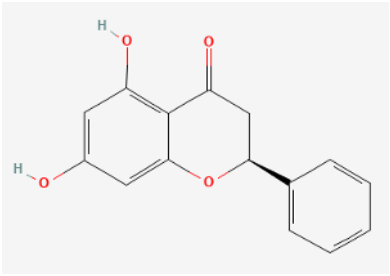

Figure 4. Pinocembrin Structure[21]

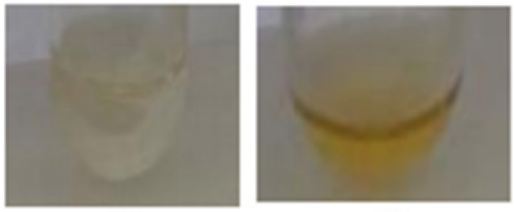

Figure 5. Phytochemical screening results of flavonoids compounds found in 10% sodium hydroxide solution reagent: (a) before and (b) after adding the reagent.

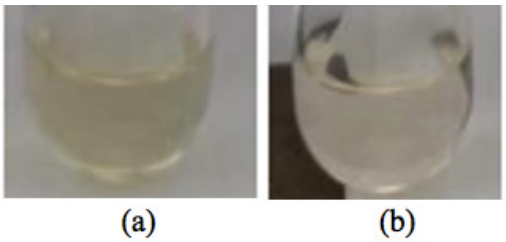

Figure 6. Phytochemical screening results of saponins compounds with hydrochloride acid 2N: (a) and (b) after adding the reagent.

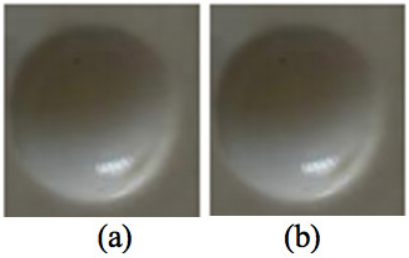

Figure 7. Phytochemical screening results of triterpenoids and steroids compounds with concentrated sulphuric reagent: (a) before and (b) after adding the reagent.

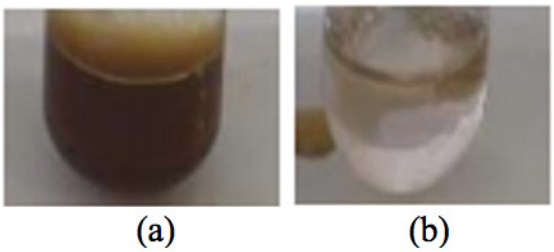

Figure 8. Phytochemical screening results of alkaloids compounds using Wagner's reagent: (a) before and (b) after adding the reagent

Meanwhile, the extraction process employs the infusion method due to its relatively shorter and faster preparation time and the easy availability of materials and tools.[10] Water is a commonly used solvent for extracting active constituents soluble in water from plant materials, despite the drawback of the infusion being susceptible to mold growth.

Phytochemical Screening

The content of secondary metabolite compounds in the leaves through phytochemical screening for the test was phenolics, tannins, flavonoids, saponin, triterpenoids, steroids, and alkaloids showed the following results as in Table 1.

Phenolics

Test for phenolic compounds obtained negative results (Fig. 1). The level of phenolics in plant sources rests on factors such as maturation processes, cultivation techniques, cultivars, growing conditions, and processing and storage conditions, among others.[11]

Phenolic compounds of MCLI that were not detected may have been influenced by extraction method factors. According to a study, Many traditional methods used to extract phenolic compounds from plants, like soxhlet extraction, percolation, and maceration, have their own problems. These include getting only small amounts of the compounds, using a lot of extraction solvents, taking a long time, and creating a lot of waste.

Because of these issues, new methods like Microwave Assisted Extraction. (MAE), (Ulrasonic Assisted Extracytion) Ulrasonic Assisted Extracytion (UAE) , Supercritical Carbon Dioxide (SC-CO2), Enzyme Assisted Extraction (EAE), and Pressure Liquid Extraction (PLE) have come up. These techniques are unconventional but help fix the problems of the methods before12. Therefore, further testing is necessary to identify the phenolic compounds in *M. calabura L.* leaf.

Tannins

Based on the results of a phytochemical test of tannin compounds, MCLI showed negative results. The results showed an acute yellow solution was formed after adding 1% ferric chloride solution, while positive results were obtained if a greenish-black coloration solution was formed. The results of the study can be viewed in Figure 2.

The tannin content in *M. calabura L.* leaves might be low in amounts detectable through cold infusion methods. The analytical methods employed should be highly sensitive to detect tannins at low concentrations. Several advanced technologies, such as microwaves and ultrasonication, have demonstrated the potential to extract tannins efficiently. Additionally, controlling factors like temperature, solid-to-solvent ratio, particle size, source of the material, and extraction time contribute to obtaining higher-quality tannins.[13]

Flavonoid

Previous study reported that *M. calabura L.* leaves extract rich in flavonoid active metabolits, which show a potential anti-inflammatory activity. Flavonoids direct to inhibiting the biosynthesis of prostaglandins (PGE) and lipooxygenase (LOX), which are enzymes involved in the inflammatory process.

Flavonoid compounds are categorized into various subgroups, such as flavones, flavanones, flavans, and biflavans.[15] One of the flavanone compounds found in kersen leaves is pinocembrin.[16] The study states that pinocembrin possesses anti-inflammatory activity and has been demonstrated in various

disease models.[17] Pinocembrin works by decrease of c-Jun N-terminal Kinase levels, p38/MAPK, and NF-kB, thereby inhibiting cytokines products.

In a rheumatoid arthritis rat model, pinocembrin decrease joint erosion and the percentage of inflammatory cells.[18] In the ulcerative colitis animal study, administering high doses of pinocembrin demonstrated therapeutic and anti-inflammatory effects, likely due to inhibiting the NF-kB pathway.[19] Additionally, pinocembrin has shown promising results in respiratory allergic inflammation by reducing Th2-type cytokines (IL-4, IL-5, and IL-13) in bronchoalveolar lavage fluid from sensitized rats through the inhibition of NF-kB activation blocking).[20]

Based on the phytochemical screening test results, positive results were found in a 10% sodium hydroxide solution reagent characterized by acute yellow formation. In the other reagent (Table 1.), negative results were obtained because there was no formation of acute yellow color in the sample. The results of the flavanoid compound test using a 10% sodium hydroxide reagent be seen in Figure 5.

Saponin

Saponins are potent surface-active substances that lead to the formation of foam upon being heated in water. The saponins compounds were not detected in MCLI. The saponins test results can be seen in Figure 6.

These results might occur due to insufficient extraction time and temperature. According to a study, higher temperatures and longer extraction durations increase saponin levels.[22] Additionally, active saponin compounds are more effectively generated when extracted using methanol as a solvent. This results because methanol's universal nature allows it to capture saponins with polar and non-polar groups.[23]

Triterpenoids and steroids

The test results of triterpenoid compounds in *M. calabura L.* leaves using the 1 mL concentrated sulphuric reagent showed that the leaves contained triterpenoids but not steroid compounds in MCLI.

In a study, it is mentioned that the compound group belongs to the triterpenoid class treatment effects on inflammation complex range, which persists for an onset and duration treatment to treated of chronic diseases, including periodontitis, cerebral edema, sepsis, liver injury, gastric ulcer, acute lung injury, allergic reaction.[24] Another group (Escin) showed a significant reduction in inflammation induced in mice in vivo. Moreover, its effects better compared to the positive treatment by NSAID. The Escin mechanism is linked to the glucocorticoid receptor (GR). Through GR activation, escin inhibits the activation of NF-κB, subsequently reducing the release of inflammatory cytokines (TNF-α and IL-1β) along with nitric oxide (NO).

Alkaloids

Based on phytochemical screening results, the MCLI tested showed the presence of alkaloid compounds. This was seen from the formation of brown sediment at the bottom of the test tube from the MCLI. The alkaloid compound test resulting from using Wagner's reagents can be seen in Figure 7.

Several studies have indicated that alkaloids can inhibit various pro-inflammatory factors expression, such as histamine, lipid mediators, cytokines, and enzymes involved in the inflammatory response.[25] Some alkaloids direct mechanism to treated dermatomyositis rheumatoid arthritis, ankylosing spondylitis, myasthenia gravis, ankylosing spondylitis, systemic lupus erythematosus, Behcet's

disease, and other rheumatic immune diseases.[26]

Conclusion

The investigation of this study showed that local *M. calabura L.* contains flavonoids, triterpenoids, and alkaloids and each has the potential to developing for alternative inflammation traetment by natural products.

Prospect Future Studies

The study revealed that the anti-inflammatory potential of the studied medicinal plants. The cold maceration extraction method can be use to obtain more secondary metabolites without damaging heat-sensitive compounds. *Muntingia calabura L.* leaves extract contains a wide range of non-polar, semi-polar, and polar secondary metabolite chemicals. Using the fractional method to separate some of these compounds based on their polarity is better. As anti-inflammatory drugs, further tests can be carried out in vivo and in vitro. By purifying and separating the beneficial components from these plant extracts, we could gain a better understanding of how they function and discover potential ingredients for developing new drugs.

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Conflict of Interest

None declared.

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