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PHYTOCHEMICAL EVALUATION OF ANTI-INFLAMMATORY AGENTS OF STARFRUIT LEAF EXTRACT (*Averrhoa bilimbi*) FROM URBAN AREA

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Abstract

Inflammation is the body's natural defense mechanism against infection or injury.This study aims to evaluate the phytochemical compounds contained as anti-inflammatory agents in starfruit leaves grown in urban areas. The methods used in the study include laboratory tests to obtain soil characteristics including physical and chemical properties, phytochemical screening tests and GC-MS to obtain accurate data related to the bioactive compounds contained in starfruit leaves. The results showed that starfruit leaves contain alkaloids, steroids, saponins, flavonoids, phenolics, and terpenoids, but no tannins were found. This may be due to the clay soil structure where the plant grows which affects the secondary metabolites produced. Furthermore, the GC-MS test obtained thirteen compounds which are anti-inflammatory agents including 2-(p-tolymethyl)-p-xylene; cis-tricyclo [7.5.0.0(2,8)]tetradeca-7,9-diene; (2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol; (2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol; Dimethyl (3S,4R,5S,6R)-3,6-dimethylcyclohexene-4,5-dicarboxylate; 9,12,15-Octadecatrienol; Palmitic acid; 2-amino-4,4,6,6-tetramethyl-4,6-dihydrothieno[2,3-c]furan-3-carbonitrile; 2(3H)-Phenanthrenone, 4,4a-dihydro-4a-methyl-; 4,8-Dimethyl-3,7-nonadien-2-ol; Benzofuran, 2,3-dihydro-; Acrylic acid; 4-Hydroxy-3-methyl-2H-pyran-2-one.

Keywords: anti-inflammatory agents, characteristics of soil, GC-MS, phytochemical compounds, starfruit leaves

INTRODUCTION

Inflammation is the body's natural defense mechanism against infection or injury. However, ongoing (chronic) inflammation can cause various serious health problems such as heart disease, type 2 diabetes mellitus, arthritis, autoimmune diseases, neurological disorders and cancer (Pahwa et al., 2025). Chronic inflammatory diseases are the most significant cause of death worldwide. The World Health Organization (WHO) places chronic diseases as the biggest threat to public health with prevalence continuing to increase over the next 30 years (Kompas.com, 2022). Acute to chronic inflammation is characterized by dilation of blood vessels (vasodilation), increased blood flow, capillary permeability and migration of neutrophils to infected tissue through capillary walls (diapedesis), and infiltration of primary inflammatory cells such as macrophages, lymphocytes and plasma cells at tissue sites, as well as producing inflammatory cytokines, growth factors, enzymes, causing widespread tissue damage and granuloma formation (Milenkovic et al., 2019; Yousuf et al., 2019). Current inflammatory treatments still use synthetic NSAIDs (Nonsteroidal Anti-Inflammatory Drugs) or NSAIDs (Nonsteroidal Anti-Inflammatory Drugs) (Candra et al., 2022; Santi, 2025). This class of drugs is useful in relieving pain, fever and inflammation by inhibiting enzymes that produce hormones that trigger inflammation (prostaglandins). The use of synthetic drugs can have negative effects on the body including impaired kidney function, bleeding in the gastrointestinal tract, edema, and hypertension with long-term use. This is what has encouraged the discovery of anti-inflammatory drugs that are safe, do not cause side effects, and are accessible to the public

(Candra et al., 2024; Santi et al., 2022). Treatment with herbal sources has become an option and is developing as a treatment trend and one of the herbal plants (Candra & Santi, 2017; Kamal et al., 2024; Santi, 2025) that is believed to have anti-inflammatory potential is starfruit (*Averrhoa bilimbi*) leaves (Sarker & Chowdhury, 2024). Medicinal plants have been traditionally used for traditional medicine to cure various diseases. However, the phytochemical compounds of plants grown in urban areas are not yet fully understood, thus the mechanism of action of these plants is also not yet clear (Santi et al., 2025). The aim of the research was to evaluate phytochemical compounds from starfruit leaves that live in urban areas.

LITERATURE REVIEW

The World Health Organization (WHO) states that around 80% of people in Asia use traditional medicine for prevention, care and treatment (World Health Organization, 2023). This plant has been used as herbal medicine since the 14th century (ancient Egyptian times) in the form of decoction, powder, or cream and infusion. Current technological developments play a crucial role in scientifically uncovering the various bioactive compounds found in plants, including leaves, stems, roots, flowers, and other parts. These phytochemical compounds form the basis for selecting plants for specific ailments, as biologically active agents are responsible for the pharmacological activity of medicinal plants (Candra et al., 2025; Candra, Santi, et al., 2023; Santi, Candra, et al., 2023).

Starfruit is a plant from the Oxalidaceae family and is used as a traditional medicine by Indonesian people to treat coughs, colds, hypertension, diabetes, diarrhea, fever and inflammation. Starfruit is cultivated in tropical and subtropical countries for the purposes of treating diabetes mellitus, hypertension, and antimicrobials (Muhammad et al., 2016; Sá et al., 2019). Several previous studies have obtained information on bioactive compounds from starfruit. Phytochemical screening of starfruit leaf extract from Bangladesh includes flavonoids, alkaloids, triterpenes, phenols, tannins, saponins, glycosides. Other research states that starfruit leaves contain squalene, 3-(6,10,14-trimethylpentadecan-2-yl) furan-2 (5H)-one, phytol, 3,4-Dihydroxyhexanedioic acid, 2,3-bis (2,6,10-trimethylundeca-1,5,9-trienyl) oxirane, and 4,5-Dihydroxy-2-methylenehydroxybenzaldehyde, as well as malonic acid (Gunawan & A., 2013).

Starfruit is a woody plant with a tree height of up to 15 m with branches arranged not tightly. Wuluh starfruit leaves are compound, each stem is occupied by around twenty leaves with a length of 5–10 cm. The shape of starfruit leaves is pinnate with a hairy texture. (Alhassan & Ahmed, 2016). The starfruit plant has a stem that grows up to 10 m tall with few branches and odd-pinnate compound leaves. This plant is widely grown in home gardens as a local crop, and people use the fruit as a spice in traditional Acehnese cuisine and store it as asam sunti (Santi et al., 2022; Sarker & Chowdhury, 2024; Seebaluck-sandoram et al., 2019).

METHOD

The research is a qualitative study through phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS). Phytochemical screening is used to identify secondary metabolite compounds contained in starfruit leaves (Candra et al., 2024; Tahara et al., 2024).

Phytochemical screening test of ethanol extract of *Averrhoa bilimbi* leaves

The phytochemical test procedure is as follows (Santi, Siregar, et al., 2023; Santi, Zakaria, et al., 2023; Santi & Candra, 2023; Tahara et al., 2024):

a. Flavonoid Examination. 500 mg of starfruit leaf extract was placed in a test tube and mixed with 2 ml of ethanol. Homogenize by shaking, then heat and shake again, then filter. The addition of 0.2 g of Mg and 3 drops of concentrated HCl will produce red, green, and orange colors if the extract contains flavonoids (Huang et al., 2024; Nelly et al., 2024).
b. Alkaloid Examination. 10 g of starfruit leaf ethanol extract was taken and moistened with concentrated ammonia for 2 hours. The moistened sample was macerated with 5 mL of dichloromethane and shaken to accelerate the reaction process. The filtrate was filtered and concentrated to 1 mL. 5 mL of 5% hydrochloric acid was added, shaken vigorously, and then allowed to stand for a few moments until the

hydrochloric acid and dichloromethane solutions separated. The hydrochloric acid layer was collected and divided into three test tubes and tested with Mayer, Dragendorff, and Wagner reagents. A positive alkaloid test for Meyer's reagent is indicated by the formation of a white or yellow precipitate. The formation of a yellow, orange, or reddish-brown precipitate indicates the presence of alkaloids in Dragendorff's reagent, while for Wagner's reagent, a brown to black or yellow precipitate forms (Raal et al., 2020; Saidi et al., 2018; Santi, 2015).

c. Tannin Test. 500 mg of starfruit leaf extract was placed in a test tube. 1 ml of distilled water was added, boiled for 15 minutes, and cooled. A drop of 1% ferric chloride was added, and a brownish-green color indicated the presence of tannins.
d. Saponin Test. 500 mg of starfruit leaf extract was added to 2 ml of 25% NaOH and boiled with 20 ml of distilled water in a water bath. The mixture was then shaken and allowed to stand for 15 minutes. A positive saponin test was indicated by the formation of a stable foam (Bhardwaj et al., 2019).
e. Steroid and Terpenoid Test. 500 mg of starfruit leaf extract was added to 3 drops of H2SO4 and 10 drops of anhydrous acetic acid. The formation of blue and green colors indicated the presence of steroids, while an orange or purple color indicated the presence of triterpenoids (Hou et al., 2020).
f. Phenolic Test. 1 mL of starfruit leaf extract was taken and added to 1 mL of 1% NaCl and 1 mL of 10% gelatin solution. A positive test result was determined by the appearance of a white precipitate (Sari et al., 2025).

Identification of active compounds of ethanol extract of *Averrhoa bilimbi* leaves through GC-MS

A total of 5 µL of ethanol extract of starfruit leaf was separately injected into the GC-MS using helium gas at a rate of 1.2 ml/min (ratio 8:1). The injector temperature was 2500C and the ion source temperature was 2300C, the oven temperature was from 800C to 2800C. The ionization energy was 70eV and the capillary column was 30x0.20 mm IDx0.11 m thick. When scanning, an interval of 2.42 seconds was used with a fragment weight of 40 to 650 Da. The fragmentation pattern followed the National Institute of Standards and Technology Mass Spectral (NIST-MS) (Candra et al., 2024; Labkesda, 2017).

Analysis of physical and chemical properties of urban soil

The methods used in this study were survey and laboratory analysis. Soil samples were collected in an urban area (Banda Aceh City) and then analyzed for physical properties in the Soil Analysis Laboratory. Evaluation of soil chemical characteristics included: soil pH determined by the H2O extraction method; organic carbon determined by the Walkley and Black method; total nitrogen determined by the Kjeldahl method; available phosphorus determined by the Bray I method; available potassium determined by the Bray I method; exchangeable base cations determined by the 1 N NH4Oac pH 7 extraction method; acid cations (Al and H) determined by the 1 N KCl extraction method; and base saturation (CEC) and base saturation are the sum of the free cations (S)/(CEC) x 100% (Candra, Fahrimal, et al., 2023; Isir et al., 2022; Santi & Candra, 2024).

RESULTS AND DISCUSSION

Identification, Taxonomy, and Plant Morphology

The results of the determination and identification of the plant indicate that the tested plant is indeed starfruit (*Averrhoa bilimbi*). Based on the identification of the herbarium samples, the plant taxonomic sequence is obtained, as presented in Table 1.

Table 1. Taxonomy of the starfruit plant

Taksonomi	Starfruit
Kingdom	Plantae
Sub Kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub Classis	Rosidae
Order	Geranniales
Family	Oxalidaceae
Genus	*Averrhoa* Adans
Species	*Averrhoa bilimbi* L

The characteristics of starfruit leaf samples obtained from urban areas are presented in Table 2. Macroscopic examination of starfruit leaves was carried out by observing the color, smell and taste which were then adjusted to the standards in the Indonesian Herbal Pharmacopoeia.

Table 2. Characteristics of starfruit leaf simplicial

Leaf	Length	Width	Form	Smell	Color	Flavor
Averrhoa bilimbi	8,5 cm	2,5 cm	Oval round, tapered tip	Typical	Green	Tasteless

Starfruit leaves are odd-pinnate compound leaves with 21-45 pairs of leaflets, with light brown tips. Starfruit leaves have short stalks and are ovate to oblong in shape, green on the upper surface and light green on the underside (Figure 1).

Figure 1. Starfruit leaves

Phytochemical Screening Test of ethanol extract of *Averrhoa bilimbi* leaves

The results of the phytochemical test of the ethanol extract of starfruit leaves are presented in Table 3. Phytochemical screening aims to test the content of active compounds, namely to identify flavonoids, alkaloids, tannins, saponins, phenolics, steroids, and terpenoids in starfruit leaves.

Table 3. Phytochemical Screening Test Results for Leaf Samples

Phytochemical compounds	Ethanol extract of *Averrhoa bilimbi* leaves
Flavonoid	+
Alkaloid	+
Tanin	-
Saponin	+
Fenolik	+
Steroid	+
Terpenoid	+

Flavonoids are the most abundant group of polyphenols found in plants and have pharmacological effects. The flavonoid test showed a positive result, indicating that starfruit leaves contain flavonoid compounds. A positive result was also obtained for tests on alkaloids, saponins, phenolics, steroids, and terpenoids. A negative result was only obtained for the tannin test, indicating that starfruit leaves do not contain tannins. Saponins are natural glycosides found in plants. Saponin testing results show a positive result, indicated by the formation of foam and an emulsion, indicating that starfruit leaves contain saponins. Starfruit leaves contain bioactive compounds, including alkaloids, flavonoids, and steroids, which act as anti-inflammatories. Flavonoids play a role in inhibiting COX, lipoxygenase, prostaglandins, and thromboxanes, thus inhibiting muscle damage (Candra & Santi, 2017; Santi, 2019).

Texture of urban soil

Soil texture is important to understand because it determines the physical, chemical, and biological properties of the soil. Laboratory analysis results consistently show that soil contains particles of varying sizes (Candra, Fahrimal, et al., 2023; Neina, 2019). The ratio of these particles varies from one soil to another. Data from the analysis of the physical properties of soil in urban areas are presented in Table 4.

Table 4. Soil texture analysis

No	Soil Particles	Urban Area (Banda Aceh)
1	Dust (%)	38
2	Clay (%)	10
3	Sand (%)	52
Texture Class		Loam

Laboratory analysis results indicate that soil in urban areas is predominantly sand, making it unable to retain water and lacking plastic and adhesive properties. Sand consists of individual grains, has a small surface area, and large pores, allowing for smooth aeration and acting as a soil framework (Bünemann et al., 2018). Table 4 shows that the soil texture in urban areas is clay. This soil is not good for plants because the soil composition is mostly clay which causes water to not flow easily so that the soil hardens easily in dry conditions.

Soil chemical characteristics from urban areas

The results of the analysis of soil chemical properties in urban areas are presented in Table 5.

Table 5. Results of soil chemical properties analysis

No	Characteristics	Urban Area
1	pH H_2O	7.1
2	C-organic (%)	0.79 %
3	N total (%)	0.11%
4	P available	27.65 mg kg^{-1}
5	Base cations can be exchanged	
	Ca^{++}	11.57 cmol kg^{-1}
	Mg^{++}	0.54 cmol kg^{-1}
	K^{+}	0.26 cmol kg^{-1}
	Na^{+}	0.24 cmol kg^{-1}
6	Cation exchange capacity	18.4 cmol kg^{-1}
7	Base saturation	68.53 %

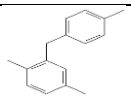
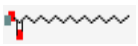
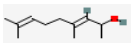
Soil analysis results in urban areas indicate a neutral pH of 7.1, H_2O. Hydrogen ions (H+) in the soil indicate the acidity and alkalinity of the soil. If the H+ and OH- concentrations are equal, the soil reacts neutrally, with a pH of 7. High H+ ions indicate acidity, while alkaline soils contain more OH- than H+. Neutral and alkaline pHs allow nutrients to be easily absorbed by plant roots because they dissolve in water. Acidic soils, on the other hand, allow the presence of toxic elements and affect the growth of microorganisms (Singh, 2014). Laboratory analysis results show that the organic carbon content of the soil in urban areas is very low, at 0.79%. Efforts to achieve optimal levels include the annual addition of organic matter. This will prevent soil compaction, which would reduce fertility (Nwachokor et al., 2009). Laboratory analysis results indicate that the total nitrogen content of the soil in urban areas is relatively low, at 0.11%.

Low nitrogen in the soil is caused by nitrogen utilization by plants, even though plants shed leaves and twigs, turning them into organic matter that is decomposed by microorganisms in the nitrogen cycle and then reused by plants. Total nitrogen (N) functions in protein formation, and optimal levels can be maintained through fertilization (White & Brown, 2010). Laboratory analysis results show that the soil phosphorus content in urban areas is 27.65 (high). Phosphorus is essential for plants; a deficiency will inhibit cell division and stunt plant growth (Zhang et al., 2014). Analysis of the exchangeable base cations Ca++, Mg++, K+, and Na+ in urban areas, respectively, categorized as moderate, low, normal, and low. Calcium functions in root and seed formation, strengthens stems, and aids pollination. Magnesium plays a role in chlorophyll formation. Sodium plays a role in plant growth in dry areas, and potassium plays a crucial role in neutralizing negative electrical charges (Jakovljevic et al., 2003).

Identification of active compounds in strfruit leaves using GC-MS

The phytochemical content of starfruit leaves obtained from GC-MS analysis consists of thirteen compounds which are presented in Table 6.

Table 6. Bioactive compounds of ethanol extract of *Averrhoa bilimbi* leaves

No	Bioactive compounds	Molecular Formula	Structure	%	Biological activities
1	2-(p-tolymethyl)-p-xylene	$C_{16}H_{18}$		58	Anti-inflammatory, antinociceptive, antioxidant
2	cis-tricyclo [7.5.0.0(2,8)]tetradeca-7,9-diene	$C_{14}H_20$		16.50	Anti-inflammatory, antioxidant
3	(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol	$C_20H_{34}O$		10.31	Anti-inflammatory, antiviral
4	(2E,7R,11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$		9.36	Anti-inflammatory, anti-cancer, anti-inflammatory
5	Dimethyl (3S,4R,5S,6R)-3,6-dimethylcyclohexene-4,5-dicarboxylate	$C_{12}H_{18}O_4$		8.34	Anti-cancer, anti-inflammatory
6	9,12,15-Octadecatrienol	$C_{18}H_{32}O$		6.96	Anti-inflammatory, antioxidant, antibacterial, antifungal, pesticide, antiviral
7	Palmitic acid	$C_{16}H_{32}O_2$		6.83	Anti-inflammatory, analgesic, antipyretic, and antimicrobial
8	2-amino-4,4,6,6-tetramethyl-4,6-dihydrothieno[2,3-c]furan-3-carbonitrile	$C_{11}H_{14}N_2OS$		5.40	Antioxidant, prevents cellular damage
9	2(3H)-Phenanthrenone, 4,4a-dihydro-4a-methyl-	$C_{15}H_{14}O$		5.35	Antioxidant, anti-inflammatory, anticancer, immunomodulatory effects
10	4,8-Dimethyl-3,7-nonadien-2-ol	$C_{11}H_{20}O$		3.73	Antipsoriatic, anti-inflammatory, antibacterial
11	Benzofuran, 2,3-dihydro-	C_8H_8O		3.52	Analgesic, anti-inflammatory, antibacterial, antimicrobial, antifungal, antihyperglycemic
12	Acrylic acid	$C_3H_4O_2$		3.46	Antibacterial, anti-inflammatory, anti-diabetic, antioxidant
13	4-Hydroxy-3-methyl-2H-pyran-2-one	$C_6H_6O_3$		2.12	Anticancer, antioxidant, anti-inflammatory, analgesic

Structure of compound from (National Library of Medicine, 2025)

Examination of starfruit leaves using GC-MS identified that the phytochemical content obtained was thirteen compounds which have antibacterial, antioxidant, anti-inflammatory, anticancer, analgesic, antibacterial and antimicrobial activities.

CONCLUSION

Starfruit leaves contain thirteen bioactive compounds that are influenced by the chemical and physical characteristics of urban soil. The compounds found in starfruit leaves have diverse pharmacological effects, particularly anti-inflammatory and antioxidant properties.

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