



Phytochemical Screening of Ethanol Extract of Avocado Leaves (*Persea americana* Mill)

¹Rangga Sastra, ^{1*}Hardani, ¹En Purmafitriah, ¹Sri Idawati, ¹Adriyan Suhada,
²Sirajunnasih

¹Program Studi DIII Farmasi, Politeknik Medica Farma Husada Mataram (Indonesia)

²Program Studi DIV Teknologi Rekayasa Perangkat Lunak, Politeknik Medica Farma Husada
Mataram (Indonesia)

*Corresponding e-mail: (danylchild07@gmail.com)

ABSTRACT

Avocado is a popular fruit, popular in the community. Besides its delicious flesh, avocados are also beneficial in medicine. Almost all parts of this plant have medicinal properties. The part of the avocado that has many benefits is the leaves, which are used in herbal remedies. Boiling the leaves and drinking the resulting decoction is believed to significantly lower blood pressure in hypertension sufferers. This experimental laboratory study aimed to determine the active ingredients in ethanol extract of avocado leaves (*Persea americana* Mill). This study was conducted at the Biology Laboratory of the Politeknik Medica Farma Husada Mataram from May to July 2021. The population and sample in this study were avocado plants. The data in this study were primary data, namely direct observation of the active ingredients in avocado leaves (*Persea americana* Mill). Based on the results of phytochemical screening in this study, it can be seen that avocado leaves contain secondary metabolites, which are bioactive components in the form of alkaloids, flavonoids, tannins, and saponins.

KEYWORDS

Phytochemical Screening, Avocado Leaves, *Persea americana* Mill

INTRODUCTION

Indonesia is a country rich in biodiversity and is recognized as the second megabiodiversity country after Brazil (Kemendag, 2014). There are approximately 30,000 plant species in Indonesia, of which 7,200 have medicinal properties, and about 2,500 are classified as medicinal plants (Kemendag, 2014). The growing awareness of health quality and value has led people to increasingly prefer the use of traditional medicines derived from plants containing active compounds. The use of plants as traditional medicine is also expected to have economic value that could support the cultivation and processing of medicinal plants in the future.

In the last decade, there has been a significant increase in the use of traditional medicine as an alternative treatment across developing countries (Marbun & Restuati, 2015). In fact, traditional

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medicines are widely used in almost all countries worldwide. The utilization of traditional medicine has reached 60%–70% in Japan, 90% in China, 71% in Chile, 40% in Colombia, 49% in France, 70% in Canada, 40% in the United Kingdom, 42% in the United States, 80% in Africa, 76% in Singapore, and 86% in South Korea (WHO, 2013). One of the plants commonly used as traditional medicine is the avocado (*Persea americana* Mill).

Avocado is a fruit widely consumed by the public. In addition to its delicious pulp, the avocado also has medicinal benefits. Almost every part of this plant has pharmacological properties. The leaves, in particular, are highly beneficial and are commonly used in herbal remedies. Boiling the leaves and drinking the decoction are believed to significantly reduce blood pressure in patients with hypertension (Tamsuri, 2012).

According to a study by Owalabi et al. (2010), avocado leaves have antioxidant activity and can help prevent or slow down the progression of various oxidative stress-related diseases. Moreover, research by Rumiati (2012) suggests that avocado leaves also possess anticancer potential. These therapeutic effects are attributed to the presence of secondary metabolites—compounds that play an essential role in treatment. Secondary metabolites are biomolecules that function as lead compounds (molecules capable of producing biological effects such as therapeutic, toxic, or physiological regulatory effects) and are therefore widely used in the development of new drugs (Atun, 2014).

Research conducted by Kartika and Arsito (2017) reported that the ethanol fraction of avocado leaves exhibited antibacterial activity against *Shigella dysenteriae* at concentrations of 12.5%, 25%, and 50%. Similarly, Sarinastiti (2018) found that 96% ethanol extract of avocado leaves could inhibit *Staphylococcus aureus* and *Escherichia coli* at concentrations of 20%, 40%, 60%, and 80%. Therefore, phytochemical screening of avocado leaf ethanol extract (*Persea americana* Mill) is essential.

Phytochemical screening is a preliminary stage in phytochemical studies aimed at identifying the classes of compounds present in plants. This method involves testing chemical reactions, particularly color reactions using specific reagents. To date, no phytochemical screening has been reported on avocado leaf extract (*Persea americana* Mill) (Aloisius et al., 2020). Hence, it is important to conduct phytochemical screening of the ethanol extract of avocado leaves (*Persea americana* Mill) to identify the chemical constituents contained within. The purpose of this study is to determine which secondary metabolites are present in the ethanol extract of avocado leaves (*Persea americana* Mill).

LITERATURE REVIEW

Avocado leaves (*Persea americana* Mill.) have historically been used as an herbal medicine, particularly as a diuretic, for breaking down urinary tract stones, and as a remedy for mouth ulcers. The part most frequently used in traditional preparations is the leaf, as it contains gul, d-parsit, quercetin flavonoids, and sterol compounds. Avocado is a fruit widely consumed by society; aside from its delicious pulp, it also provides medicinal benefits. Almost every part of the



plant has therapeutic properties. The leaves, in particular, are believed to significantly reduce blood pressure in patients with hypertension when boiled and consumed as a decoction (Tamsuri, 2012).

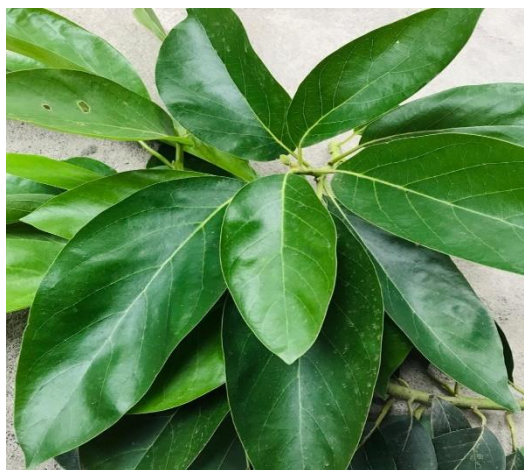


Figure 1. Avocado Leaves (*Persea americana* Mill.)

a. Classification of Avocado Plant

According to Martina (2015), the avocado plant is classified as follows:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Divisi	: Magnoliophyta
Kelas	: Magnoliopsida
Subkelas	: Magnoliidae
Ordo	: Laurales
Famili	: <i>Lauraceae</i>
Genus	: <i>Persea</i>
Species	: <i>Persea americana</i> Mill

b. Morphology of Avocado Plant

The avocado plant is classified as a small tree with a height of 3 to 10 m, with a taproot, woody stem, round, dirty brown in color, many branches, and twigs with fine hairs. The leaves are simple, ranging from oblong to oval-elongated, with a length of 10–20 cm, width of 3–10 cm, base and tip tapering, smooth edges, sometimes slightly rolled upward, green to brownish or purplish brown, pinnate venation, leaf stalk length 1.5–5 cm, odorless, bitter and astringent taste (Herbal Pharmacopoeia, 2017).

Extraction is a process of filtering active substances from medicinal plant parts, useful for extracting chemical components contained in those parts. Extraction is a process of separating substances from their mixture using a specific solvent (Marjoni, 2016).



The extraction process is basically a process of separating solid-phase components of simplicia into the organic solvent used. Extraction can be done with various methods and techniques depending on the purpose of the extraction itself. The sample to be extracted can be in the form of fresh or dried samples. Fresh samples are commonly used because solvent penetration occurs faster. The use of dried samples also has the advantage of reducing water content in the sample, thus preventing the possibility of compound damage due to microbial activity (Marjoni, 2016). Extract is a preparation of plant or animal material obtained by releasing active substances from each material, using a suitable solvent that is then evaporated, almost all or entirely, and the residue or powder is standardized. The form of the resulting extract can be thick or dry depending on the amount of solvent evaporated (Marjoni, 2016).

Plant chemistry or phytochemistry is a branch of organic chemistry located between natural product chemistry and plant biochemistry, and is closely related to both. The focus of phytochemistry is the diversity of organic compounds formed and stored by plants, including their chemical structure, biosynthesis, transformation, and metabolism, scientific distribution, and biological function. Phytochemical testing is one of the important steps in uncovering the potential of medicinal plant resources (Astuti et al., 2013).

Phytochemical analysis is one way to determine the content of secondary metabolites in a plant. Secondary metabolite compounds in plants include alkaloids, flavonoids, steroids and triterpenoids, tannins, and others. Basically, these secondary metabolites are toxic to plants and animals. In some plants, secondary metabolites are produced to defend themselves from enemies, but in certain doses, they can be used as medicine.

Phytochemical analysis or phytochemical testing is a preliminary test to determine the presence of specific chemical compounds such as alkaloids, phenolic compounds (including flavonoids), steroids, saponins, and terpenoids without producing biological screening. This test is very useful to provide information on the types of chemical compounds contained in plants. These compounds are secondary metabolites that may be used as medicinal ingredients. This analysis is the initial stage in isolating natural compounds so that it becomes a guide together with testing the biological activity of these compounds. One purpose of classifying these active compounds is to understand biosynthetic relationships and plant families. This information is very useful for organic synthesis chemists to predict or modify substituents of active compounds to increase their efficacy. Plants tested for phytochemistry can be in the form of fresh plants, dried slices, powders, extracts, or preparations.

Phytochemical testing is carried out based on reactions that produce color or precipitate. Over the years, simple color tests and drop reactions have been developed to indicate the presence of certain compounds or groups, as they have proven to be characteristic and sensitive. Phytochemical testing is still often used in compound characterization because it is simple and does not require complex equipment, although sometimes it cannot provide satisfactory results. Triterpenoid compounds have cyclic alcohol structures that make them tend to be semi-polar. The tannin group is a phenolic compound that tends to dissolve in water and polar solvents, saponins



are triterpenoid glycosides with polar properties due to their glycosidic bonds. Flavonoids have sugar groups that make them polar. Ethanol solvent has a polarity index of 5.2, so in extraction it can increase the permeability of simplicia cell walls, making the extraction process more efficient in attracting polar to semi-polar components (Sangi et al., 2013).

Some phytochemicals such as alkaloids, flavonoids, glycosides, glycolipids, polysaccharides, peptidoglycans, carbohydrates, amino acids, and saponins obtained from plant sources have shown hypoglycemic activity. In addition, there are still various phytochemicals as active substances that are widely used in the treatment of diseases.

a) Alkaloid

Alkaloids are a group of organic compounds most widely found in nature. Almost all alkaloids come from plants and are widely distributed among various types of plants. All alkaloids contain at least one nitrogen atom, usually basic, which is part of a heterocyclic ring (Dewi et al., 2013). Almost all naturally occurring alkaloids have specific biological activity, some are very toxic but others are very useful in medicine. For example, quinine, morphine, and strychnine are well-known alkaloids with significant physiological and psychological effects. Alkaloids can be found in various parts of plants such as seeds, leaves, twigs, and bark. Naturally, alkaloids are amines that have biological effects.

Alkaloids are basic compounds depending on the electron pairs on nitrogen. If the functional groups adjacent to nitrogen release electrons, the electron availability on nitrogen increases and the compound becomes more basic. If the adjacent functional groups attract electrons, the availability of electron pairs decreases, and the effect of the alkaloid may be neutral or even slightly acidic. The basicity of alkaloids makes them very susceptible to decomposition, especially by heat and light in the presence of oxygen. This reaction often produces N-oxides. Decomposition of alkaloids during or after isolation can cause problems if stored for a long time. Salt formation with organic or inorganic compounds often prevents decomposition (Dewi et al., 2013).

b) Flavonoid

Flavonoids (bioflavonoids) from the Latin word *flavus* meaning yellow, are secondary plant metabolites. Flavonoids were once called Vitamin P (possibly due to their effects on capillary permeability) from the mid-1930s to the early 1950s, but the term has since fallen out of use. According to IUPAC nomenclature, they can be classified into:

- 1) Flavonoids or bioflavonoids.
- 2) Isoflavonoids, derived from the 3-phenylchromen-4-one structure.
- 3) Neoflavonoids, derived from the 4-phenylcoumarin structure.

c) Tanin

Tannins are phenolic plant compounds with an astringent taste. Chemically, tannins are divided into two groups: condensed tannins or catechin tannins, and hydrolyzable tannins. Condensed tannins are found in ferns, gymnosperms, and angiosperms, especially woody plants. Hydrolyzable tannins are limited to dicotyledonous plants. Tannins are polymeric glycoside



derivatives found in various plants, especially dicots. The monomers of tannins are digallic acid and D-glucose. Tannin extracts consist of complex mixtures of polyphenolic compounds, usually combined with low carbohydrates. Because of their phenolic groups, tannins can condense with formaldehyde. Condensed tannins are highly reactive to formaldehyde and can form condensation products (Putri et al., 2015).

d) Saponin

Saponins are glycosides of steroids, steroid alkaloids, or steroids with a nitrogen function or triterpenoids found in plants. Charantin, a steroidal saponin isolated from *Momordica charantia*, has been reported to exhibit insulin-like activity by increasing insulin release and slowing gluconeogenesis. β -sitosterol, a steroid found in *Azadirachta indica*, andrographolide, a diterpenoid lactone isolated from *Andrographis paniculata*, and gymnemic acid saponins isolated from *Gymnema sylvestre* can potentially induce hypoglycemic activity in animals.

Saponins are amphiphilic compounds with hydrophilic and hydrophobic groups. When shaken with water, the hydrophilic groups bind to water while the hydrophobic groups interact with air, resulting in foam. In micelle structures, the polar groups face outward while the non-polar groups face inward, forming bubbles. However, in this analysis, the sample did not contain saponins because it lacked the ability to form foam (Simaremare et al., 2014).

e) Triterpenoid/ steroid

Triterpenoids are compounds whose carbon skeletons are derived from six isoprene units and biosynthetically from the acyclic C₃₀ hydrocarbon squalene. These compounds have relatively complex cyclic structures, most often in the form of alcohols, aldehydes, or carboxylic acids. Triterpenoids are colorless, crystalline compounds, often with high melting points and optical activity. They are divided into four groups: true triterpenes, steroids, saponins, and cardiac glycosides. The most commonly used qualitative test is the Liebermann–Burchard reaction (anhydrous acetic acid–concentrated H₂SO₄). Most triterpenes and steroids produce a blue-green coloration (Tarziah, 2013).

METHOD

The design of this research is Laboratory Experimental, which is a test conducted in the laboratory. The purpose of this study is to determine the active compounds of the ethanol extract of avocado leaves (*Persea americana* Mill).

The population is the entire object of the study, by determining the population researchers can process the data (Sugiyono, 2016). The population in this study is avocado plants. The sample is a part of the population taken in a certain, clear, and complete way that can be considered to represent the population (Sugiyono, 2016). The sample to be used in this study is avocado leaves. The data in this study are primary data, namely data from direct observation of the active compounds of avocado leaves (*Persea americana* Mill). Data analysis in this study was obtained from the results of screening made in the form of tables and graphs, then the results were described.

RESULT AND DISCUSSION

RESEARCH RESULTS

Avocado leaves (*Persea americana* Mill.) used in this study were extracted using 96% ethanol, then evaporated to obtain a thick extract. The thick extract of avocado leaves (*Persea americana* Mill.) was then subjected to qualitative phytochemical tests to identify the chemical compounds contained in the avocado leaf extract (*Persea americana* Mill.). The phytochemical tests carried out included tests for flavonoids, alkaloids, saponins, and tannins. The results of the phytochemical tests of the ethanol extract of avocado leaves (*Persea americana* Mill.) can be seen in the table.

Table 1. Results of phytochemical test of ethanol extract of avocado leaves (*Persea americana* Mill.)

No	Test	Reagent	Result	
			(+)	(-)
1	Alkaloid	Sampel + dragendorff	✓	
2	Flavonoid	Sample + concentrated HCl + 3–4 pieces of Mg metal	✓	
3	Tannin	Sampel + FeCl ₃	✓	
4	Saponin	Sample + 10 ml distilled water + 10 ml alcohol	✓	
5	Triterpenoid/steroid	Sample + acetic acid + sulfuric acid		✓

Based on the results of phytochemical tests of the ethanol extract of avocado leaves (*Persea americana* Mill.) in the table, it is seen that avocado leaves contain chemical compounds in the form of alkaloids, flavonoids, tannins, and saponins. The presence of these chemical compounds is identified by the color changes that occur after the addition of reagents.



Figure 2. Phytochemical test results of alkaloid compounds using Dragendorff reagent

The results of the phytochemical test of alkaloid compounds by reacting ethanol extract of avocado leaves (*Persea americana* Mill.) with Dragendorff reagent showed a color change in the precipitate to orange-red.



Figure 3. Phytochemical test results of flavonoid compounds using concentrated HCl and magnesium

The results of the phytochemical test of flavonoid compounds by reacting ethanol extract of avocado leaves (*Persea americana* Mill.) with concentrated HCl and 3–4 pieces of Mg metal showed a color change in the precipitate to red or orange.



Figure 4. Phytochemical test results of tannin compounds using FeCl_3

The results of the phytochemical test of tannin compounds by reacting ethanol extract of avocado leaves (*Persea americana* Mill.) with FeCl_3 showed a green or bluish-black color change.



Figure 5. Phytochemical test results of saponin compounds using 10 ml distilled water and 10 ml alcohol



The results of the phytochemical test of saponin compounds by reacting ethanol extract of avocado leaves (*Persea americana* Mill.) with 10 ml distilled water and adding 10 ml alcohol showed foam with a height of 3.5 cm that remained stable for 10 minutes.



Figure 6. Phytochemical test results of triterpenoid/steroid compounds using acetic acid and sulfuric acid

The results of the phytochemical test of triterpenoid/steroid compounds by reacting ethanol extract of avocado leaves (*Persea americana* Mill.) with acetic acid and sulfuric acid showed no red-orange or purple color indicating the presence of triterpenoids, nor was there a blue color indicating the presence of steroids.

DISCUSSION

Avocado plants belong to the Lauraceae family and grow widely in the highlands of Indonesia. Avocado fruit is rich in protein, fat, and vitamins. Avocados are widely consumed fresh, processed into various food products, and used in cosmetics. Other parts of the avocado plant that can be utilized are the leaves, which are used as traditional medicine (Duarte et al., 2016).

Based on the phytochemical screening results in this study, as shown in Table 1, avocado leaves contain secondary metabolite compounds, which are bioactive components in the form of alkaloids, flavonoids, tannins, and saponins. This finding is consistent with the study by Arukwe et al. (2012), which reported that avocado leaves contain flavonoids, phenols, saponins, tannins, and alkaloids.

The phytochemical screening results (Table 1) showed that the ethanol extract of avocado leaves (*Persea americana* Mill.) was positive for flavonoids, as indicated by a color change to reddish-black after the extract was added with concentrated HCl. The reddish-black color in the flavonoid test is due to the formation of flavilium salts (Purba, Michael, 2007). Flavonoids are the most abundant compounds in avocado leaves (Arukwe et al., 2012). Flavonoid compounds in avocado leaves have strong electron-donating ability, can react with free radicals to form more stable compounds, terminate radical chain reactions, and act as chemopreventive agents (Asolu et al., 2010).

Flavonoids are compounds in plants that have been proven to inhibit the proliferation of several cancer cells, with low toxicity or even non-toxic to normal cells. The anticancer mechanisms of flavonoids include carcinogen activation, cell proliferation inhibition, cell cycle arrest, induction of apoptosis and cell differentiation, inhibition of angiogenesis, and antioxidant activity (Ren et



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al., 2003). Flavonoids are a group of phenolic compounds most abundant in nature, responsible for red, purple, and blue pigments, as well as yellow coloration in plants.

In addition to flavonoids, the ethanol extract of avocado leaves (*Persea americana* Mill.) was also positive for alkaloids, as indicated by the formation of a yellowish-white precipitate after the extract was added with Mayer's reagent. It is presumed that nitrogen in alkaloids reacted with K⁺ ions from potassium tetraiodomercurate (II) to form an insoluble potassium-alkaloid complex. Alkaloid compounds also play a role in inhibiting cancer cell growth, such as vincristine and vinblastine found in periwinkle leaves, which act as antimitotic agents by binding tubulin dimers, interfering with microtubule formation during metaphase, thereby inhibiting mitosis and cell proliferation (Ewesuedo and Ratain, 2003; Dewi and Saraswati, 2009).

The phytochemical screening results also indicated that the ethanol extract of avocado leaves (*Persea americana* Mill.) contained tannins, as shown by a green color change when 1% FeCl₃ solution was added. The FeCl₃ solution is widely used to identify phenolic compounds, including tannins (Robinson, 1995). Tannins are widely distributed compounds in plants and serve as protection against predators (pesticidal effect) and growth regulators. They have therapeutic uses as antiseptics for tissue injuries such as burns, by precipitating proteins (Nadjeeb, 2009).

Furthermore, the phytochemical screening showed that the ethanol extract of avocado leaves (*Persea americana* Mill.) contained saponins, as indicated by foam formation lasting about 10 minutes after adding distilled water. Saponins are amphipathic glycosides that form micelles when shaken with water, with polar groups facing outward and non-polar groups inward, resulting in foam formation (Robinson, 1995). Saponins are known to inhibit increased blood glucose levels by reducing glucose absorption in the small intestine and delaying gastric emptying (Bruneton, 1999; Matsuda et al., 1999; Mahendra and Fauzi, 2005).

The final phytochemical screening test of the ethanol extract of avocado leaves (*Persea americana* Mill.) with acetic acid and sulfuric acid showed no color change, indicating the absence of triterpenoid/steroid compounds. This aligns with Harborne (1987), who stated that triterpenoid/steroid compounds can be tested using the Liebermann–Burchard method, which produces red-orange or purple coloration for triterpenoids and blue coloration for steroids.

The results of phytochemical analysis can provide clues about the presence of chemical components (compounds) such as steroids/terpenoids, alkaloids, phenolics, flavonoids, saponins, and tannins in plants.

CONCLUSION

Based on the research that has been conducted, it can be concluded that the secondary metabolite compounds contained in the ethanol extract of avocado leaves (*Persea americana* Mill.) are Alkaloids, Flavonoids, Tannins, and Saponins.



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