The phytochemical potential of *Averrhoa bilimbi* – A review

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Abstract. *Averrhoa bilimbi* grows in tropical and subtropical countries and has the potential to be used as phytopharmaceuticals. *Averrhoa bilimbi* leaves and fruits contain phytochemicals, such as alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids. These compounds are mostly obtained by extraction and can be used for their pharmacological properties in the form of antibacterial, antiviral, total cholesterol level reducer, and antioxidant. Further research and development need to be carried out on the effectiveness and performance of *Averrhoa bilimbi*.

1. Introduction
Phytopharmaceuticals are herbal medicine that contains phytochemicals and has been proven their safety and efficacy. Phytopharmaceuticals raw materials consist of simplicial or galenic preparations that have met the applicable requirements and standards. One of the fruits be used as raw material for phytopharmaceuticals is *Averrhoa bilimbi*, or in Indonesia, it is called *Belimbing Wuluh* [1]. *Averrhoa bilimbi* is one of the traditional medicinal plants that can grow in tropical and subtropical countries [2]. In Indonesia, *Averrhoa bilimbi* can be found at the height of up to 500 meters above sea level (MASL) and can bear fruit throughout the year [3].

*Averrhoa bilimbi* is a member of the Oxalidaceae family, with 3-10 m in height and multiple branching stems. The leaves are compound (imparipinnate) that break into the ends of branches and are arranged alternately at 30-60 cm in length. The leaf’s shape is oblong strands, but taper ends, rounded leaf base, medium green on the top surface, pale green on the bottom, young leaves brown-green, 3-10 cm long, and 2-2.5 cm wide. The flowers appear on the stem near the soil’s surface to the upper branches, scented, with small flowers, 1-1.5 cm long, purple crowns attached to 5 petals. The fruit is cylindrical, wavy, smooth five corners, 4-10 cm long, and light green when it is not yet ripe. The flesh is crispy, but when it is cooked, the colour is increasingly yellowed and is soft, has a shiny rind, gel-like structure, and has a sour taste. It has 3-15 seeds, plate-shaped, flat, 0.5 cm wide, smooth, light brown [4].

The useful parts of the *Averrhoa bilimbi* plant are leaves, flowers, and fruits. The fruit is used for sweets, syrups, and paste for itching [5]. It is useful for treating thrush, colic, mumps, rheumatism, coughing, bleeding gums, painful cavities, healthy digestive function, fever relief, and inflammation, stop bleeding in the rectum, reduce haemorrhoids, and control obesity [11]. Other uses of *Averrhoa bilimbi* is for removing fabric stain, overcoming fishy odours, repairing shiny goods made of brass [1]. Despite the abundance of traditional usage of this fruit, the scientific information is scattered. Therefore,
the present review aimed to collect information on the types of phytochemicals contained in *Averrhoa bilimbi* and their respective functions.

2. **Phytochemicals in *Averrhoa bilimbi***

The *Averrhoa bilimbi*'s phytochemical compounds that have been identified are alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids [6]. Table 1 and Figure 1 show the phytochemicals extracted from its fruit and the leaf [12].

| Part of plant | IC₅₀ value µg.ml⁻¹ | Phytochemicals                                                   |
|---------------|---------------------|-----------------------------------------------------------------|
| Fruit         | 154.9               | Tannins, Phlobatannins, Saponins, Flavonoids, Terpenoids,       |
|                |                     | Cardiac glycosides, Anthocyanins                                |
| Leaf          | 668.3               | Flavonoid, Alkaloids, Phenols                                    |

*Source: Nair et al., 2016 [12]*

![Chemical structure of alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids.](image)

Figure 1. Chemical structure of alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids.

2.1. **Alkaloids**

Alkaloids are a group of natural chemical compounds that contain mostly basic nitrogen atoms with neutral and weakly acidic or amphoteric properties. In their molecular structure, alkaloids also have oxygen (O₂), sulphur (S), and other elements such as chlorine (Cl), bromine (Br), and phosphorus (P). Alkaloids containing oxygen compounds are typically crystals and colourless, while alkaloids with oxygen-free usually volatile, colourless, oily liquid in the form of yellow (berberine) or orange (sanguinarine). Alkaloids may readily dissolve in organic solvents, such as 1, 2-dichloroethane, chloroform, or diethyl ether, and poorly dissolve in water [13].

Phytochemical examination of alkaloid compounds of *Averrhoa bilimbi* leaves showed that it contains potassium-alkaloid and the complexes of potassium tetradomerkurate [7]. *Averrhoa bilimbi* leaf maceration using 70% ethanol showed that the extract contains alkaloids, forming white sediment in Meyer reagent and reddish-brown sediment in Dragendorf and Wagner reagents [8].

Alkaloids have pharmacological activities, namely as anticancer and antiviral [6]. Alkaloids act as antibacterial by intercalating into cell walls and DNA [8]. Plant alkaloids can inhibit dihydrofolate reductase activity, an enzyme that is crucial for pyrimidine production, RNA and DNA biosynthesis, thereby inhibiting nucleic acid synthesis. Inhibition of nucleic acid synthesis causes undeveloped DNA cells of bacteria [14].

2.2. **Saponins**

Saponins are widely isolated from the medicinal plant, containing bioorganic molecules with considerable molecular weight [15]. This chemical compound includes 27 - 30 carbon atoms of a hydrophobic aglycone and 6-12 carbon atoms of one or two hydrophilic sugar moieties [16]. The
saponins’ chemical structure is triterpenoid (30 carbon atoms) and steroids (27 carbon atoms with a 6-ring spirostane or a 5-ring fuurostane skeleton). The presence of saponin depends on the tissue type, age, genetic background, physiology and environmental factors of the plant [17].

The saponin compounds contained in the Averrhoa bilimbi leaf is analysed using the Forth method. The results showed that the leaf extract formed a stable foam as high as 1 cm, confirming the saponin presence [7]. Other research confirmed saponins presence in Averrhoa bilimbi’s extract by creating foam 1-3 cm after the addition of hot water and HCl in the test.

Saponins have pharmacological activities as immunomodulators, anticarcinogens, anti-inflammatory, antiviral, antimicrobial, antiprotozoal, hypoglycemic, hypocholesterolemic, and antioxidant. There is a relationship between saponins and cholesterol metabolism because saponins can reduce cholesterol by binding to bile acids in the intestine. Saponins can inhibit the reabsorption of bile acids by intestinal cells which can be excreted together with faeces [3].

2.3. Tannins
Tannins are polymeric phenolics with high molecular weight from 500 up to 20,000 Daltons [18]. Tannins have a unique chemical structure, hydroxyls that can create a stable cross-linked bond association within different molecules, such as carbohydrates and proteins. This compound is divided into two main categories, which are hydrolysable and condensed ones (proanthocyanidins) [14].

The phytochemical testing of tannin compounds in the extract of Averrhoa bilimbi leaf was done by adding FeCl3 35% solution to the sample [7]. The sample contained polyphenols that will form complexes Fe³⁺ tannins/polyphenols with a coordination bond and colour changing. The Averrhoa bilimbi leaf extract sample appears to turn blackish-green, which shows that the tannin compound is condensed. The presence of tannin compounds is confirmed in Averrhoa bilimbi extract.

Tannin can bind to adhesin, inhibit bacterial enzymes, and be complex with metal ions to be used as an antibacterial [8]. The tannin antibacterial mechanism of Averrhoa bilimbi is carried out with the help of other compounds. After the bacterial wall undergoes lysis or rupture due to saponins and flavonoids, tannin compounds can quickly enter bacterial cells and target cell wall polypeptides to inhibit bacterial cell wall synthesis [25]. Tannins will inhibit bacterial growth by inactivating essential enzymes or genetic material and forming complexes with protein through hydrophobic interactions. This interaction will cause denaturation, and the bacterial cell will slowly die [26].

2.4. Flavonoids
Flavonoids are a group of natural substances with variable phenolic structures and have low-molecular-weight phenolic compounds. They are divided into several subgroups: chalcones, flavones, flavanols, and isoflavones [19]. Flavonoids are potent antioxidants as free radical scavengers, chelating metals, and inhibitors of fat oxidation. It is because flavonoids have a structure that consists of hydroxyl groups on the third carbon, have a double bond between the second and third carbon, have the carbonyl group in the fourth carbon position, and polyhydroxylated in the aromatic ring A and B [6].

The identification of flavonoid compounds in the extract of Averrhoa bilimbi leaf use magnesium (Mg) and concentrated hydrochloric acid (HCl) reagents [7]. The addition of Mg powder has the purpose of forming bonds with carbonyl groups in flavonoid compounds. The addition of HCl aims to create flavylum salt, characterised by changing the colour into orange-red. The results showed that yellow formed, which showed that the flavonoids were reduced by Mg metal and the formation of flavylum salts. The flavonoid test of Averrhoa bilimbi extract in addition to Mg and HCl indicates that there are red deposits, indicating the presence of flavonoid compounds in the sample [8].

Flavonoids can bind to bacterial extracellular proteins, inactivate proteins to inhibit bacterial cell metabolism, and involved with bacterial cell walls [8]. The separation of flavonoids can be done by utilising the difference in polarity between the lipid constituents of bacterial cells with alcohol groups in flavonoid compounds [25]. Both will form complexes with bacterial lipids through hydrogen bonds. This situation will cause an unstable structure of the cell wall and cytoplasmic membrane so that the bacterial cell loses biological activity. Furthermore, bacterial cell permeability will be disrupted, and
bacterial cells will undergo lysis or rupture, resulting in bacterial cell death [26]. Flavonoids can also be one of the compounds that can reduce total cholesterol levels and prevent hypercholesterolemia. Hypercholesterolemia is a condition in which the concentration of total cholesterol in the blood increases above average and is at risk of heart and blood vessel disease [3].

2.5. Phenols
Phenolic compounds constitute a group of secondary metabolites that have low toxicity. Phenolic compounds contain a variety of molecules with one phenol ring such as phenolic acids and phenolic alcohols and polyphenol structure such as hydroxyl groups on aromatic rings. The phenolic compound, usually formed by at least one phenyl ring, can also be replaced by a more active residue such as methyl, hydroxyl, or acetyl [20].

The study of phenolic content in the extract of *Averrhoa bilimbi* leaf was carried out research [6] by the Follin Ciocalteu method. The principle of the Follin Ciocalteu method is the formation of blue complexes, which can be measured at a wavelength of 765 nm using a spectrophotometer. The results showed that in ethanol, the extract of *Averrhoa bilimbi* leaf had 39.03±0.25 µg GAE / mg total phenolic with gallic acid derivatives.

Phenolic compounds have pharmacological activities such as antibacterial, anti-inflammatory, antithrombotic, antiviral, hepatoprotective, anticaner, antiallergic, antioxidant properties. When the pathogen cell cytoplasmic membrane damage, H + ions from phenol compounds and flavonoids will attack the phosphate group so that the phospholipid molecules will break down into glycerol, carboxylic acids, and phosphoric acids. This results in a damaged cytoplasmic pathogen cell membrane. The cytoplasm damage will prevent the entry of food ingredients or nutrients needed for pathogen cell metabolism [26]. In plants, phenolic compounds can provide specific colours, tastes, and aromas on certain plant parts [6].

2.6. Triterpenoids
Terpenoids are hydrocarbons obtained from oxygenated, hydrogenated, and dehydrogenated derivatives. Triterpenes are a part of terpenoid composed of hydrocarbons and possess no heteroatoms. Triterpene that is functionalised can be called triterpenoids. These chemical compounds have not to steam volatile properties [21].

The extract of *Averrhoa bilimbi* has triterpenoid content, evidenced by the formation of red colour in the solution after the addition of CHCl₃, anhydrous acetic acid, and H₂SO₄ in the triterpenoid test [8]. Triterpenoids are antibacterial compounds that can inhibit bacterial cell division by inhibiting DNA synthesis and macromolecular synthesis.

Triterpenoid compounds from Ligustrum could inhibit the activity of the cyclooxygenase enzyme in converting arachidonic acid into prostaglandins as an inflammatory mediator [6]. Inhibition of macromolecular synthesis in bacteria can also destroy the bacterial cell membrane [8]. The antimicrobial activity mechanism of triterpenoids can be done by damaging the cytoplasmic membrane lipid fraction. This will cause the membrane or cell wall to be lysis, not formed, or formed imperfectly. The mechanism of tannins, flavonoids, phenols, and triterpenoids can damage the cytoplasmic membrane with different mechanisms [26].

3. Utilisations of *Averrhoa bilimbi*

3.1. Antibacterial
Based on the phytochemical compounds found in *Averrhoa bilimbi*, studies prove that *Averrhoa bilimbi* can be used as antibacterial [11]. The extract from *Averrhoa bilimbi* is used for recurrent aphthous stomatitis [1]. In short, the preparation method [1] is as follows: The *Averrhoa bilimbi* was extracted by maceration using 70% ethanol and evaporated. The *Averrhoa bilimbi* extract gel was tested on *Staphylococcus aureus*, a bacterium that attacks the oral mucosa in a state of necrosis, inflammation, and abscess. The antibacterial activity of *Averrhoa bilimbi* extract gel was measured using the amount
of inhibition zone produced. The results showed that the average diameter of the inhibition zone of bacterial growth increase with the increasing concentration of Averrhoa bilimbi extracts used. This is caused by the presence of flavonoid, tannin, and saponin, which has disinfectant properties [1].

Averrhoa bilimbi, as an antibacterial is also beneficial for animal growth. Averrhoa bilimbi fruit juice is used to inhibit Aeromonas salmonicida Smithia bacteria’s growth in Sangkuriang catfish [9]. The experiment [9], in short, was started by injecting Aeromonas hydrophila into the Sangkuriang catfish to get the same sick fish. The parameters observed were wound diameter, percentage of fish healed, blood count (calculation of total leukocytes and total erythrocytes), survival and growth in absolute length, and absolute weight growth. The results showed that Sangkuriang catfish that only rely on their immunity require a longer recovery time than that are soaked in Averrhoa bilimbi fruit solution. The highest percentage of recovered Sangkuriang catfish was found in treatment with a 0.2% concentration of Averrhoa bilimbi solution. The number of red blood cells (erythrocytes) of Sangkuriang catfish that were previously reduced began to increase after being soaked in Averrhoa bilimbi solution and inversely proportional to the number of white blood cells (leukocytes). Averrhoa bilimbi fruit that has been studied contains vitamin C, and flavonoids can increase the immune system, play a role in the formation of collagen, immune system enhancer, and antibacterial [9].

3.2. Agriculture
Averrhoa bilimbi is used as a biopesticide for vegetable species to control armyworm (Spodoptera litura) [10]. This pesticide acts as a single active compound which functions as a repellent, antifertility (barren), poison, and various other forms. The parameters of Spodoptera litura observed in the study were mortality (LC50), antifeedant (appetite), and development (instar 3 to pupae). The results showed that after 24 hours, sample treatment with Averrhoa bilimbi leaf extract concentration of 50-70% had the lowest mortality value of S. litura larvae and showed the death of S. litura larvae in samples with extract concentrations of 80-90%. Death of the larvae is indicated by changes in the body that are blackening, lengthwise, and contracting, which was caused by the chemical content of Averrhoa bilimbi extract. The phenol, saponin, and alkaloid are toxic compounds for larvae. This compound can irritate to the digestion and obstruction of electron transport in the mitochondria so that the larval cells cannot activate and eventually die. It can kill larvae through the leaves that are eaten or seep into the body wall (integument), skin, and insect body gaps [10].

3.3. Antidiabetic
The antidiabetic activity of Averrhoa bilimbi leaf extracts was studied on diabetogenic substances-induced mice. In short, the study used alloxan monohydrates, which can cause mice to experience diabetes mellitus. This substance was dissolved in 0.9% sodium chloride and then injected 250 mg/kg bb in mice intraperitoneally in a single dose. The results showed that Averrhoa bilimbi leaf extract using an ethanol solvent at a dose of 125 mg/kg could reduce blood glucose levels to normal levels. This is caused by the presence of flavonoid compounds in extracts that can lower blood glucose levels in experimental animals by inhibiting the absorption of glucose in the intestine and stimulating insulin secretion by pancreatic beta cells through the regulation of calcium metabolism. Flavonoids also have antioxidant properties which can prevent free radicals from alloxan monohydrate [23].

3.4. Liver recovery
Averrhoa bilimbi with various types of polyphenols has outstanding potential to be developed as one of the raw materials for liver healing. For example, Averrhoa bilimbi was used in an experiment to cure liver damage in mice [24]. Most liver damage is caused by acetaminophen (paracetamol), environmental toxins, poor drug habits, alcohol, and other conditions [22]. Paracetamol, which is an antipyretic agent, produces excessive liver necrosis in humans so that the liver can produce enzymes and eventually induce hepatotoxicity. The experimental results show that Averrhoa bilimbi has a hepatoprotective activity, which can be seen from the structure of the liver tissue, glomeruli, and myocardial tissue, improving and appearing to be expected [22].
4. Future/Potential Research

*Averrhoa bilimbi* has the potential to be used as an antibacterial, pest control in agriculture, antidiabetic, and liver medicine. However, further research and development need to be carried out on its effectiveness and performance. Research on creating the medicine product necessary to find the correct dose is limited.

5. Conclusions

*Averrhoa bilimbi* is a source of phytopharmaceuticals production. Phytochemicals contained in *Averrhoa bilimbi* include alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids. This plant’s pharmacological is used as an antibacterial to reduce the activity of *Staphylococcus aureus*, which infected human mouth, and *Aeromonas hydrophila*, which infected Sangkuriang catfish. In agriculture, the *Averrhoa bilimbi* leaf extract can be used as a biopesticide to control pest attacks and plant disease in the form of *Spodoptera litura* larvae. The antidiabetic activity of *Averrhoa bilimbi* leaf extracts has been proven to reduce blood glucose levels to normal levels. This plant also has a hepatoprotective activity to cure liver failure.

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