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Research Article

In Vitro Antibacterial Activity and Phytochemical Screening of Galoba (*Hornstedtia alliaceae*) Seeds Extract

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Abstract
Hornstedtia alliacea has been traditionally used by indigenous people for a variety of medicinal purposes. This study aimed to determine the antibacterial activity of ethanol extracts of Galoba seeds (*Hornstedtia alliacea*) against standard bacterial cultures of *Staphylococcus aureus* (ATCC-29213) and *Escherichia coli* (FNCC-6183) using Kirby-Bauer disk diffusion method. The phytochemical tests were carried out to determine the presence of active substances which are antibacterial in the results of the extraction. The color intensity or the precipitate formation was used as analytical response to these tests. The major phytochemical constituents identified in galoba seeds ethanol extracts were tannins, flavonoids, saponins, quinones, and steroids. The antibacterial assay showed that galoba seed extract inhibited the growth of *S. aureus* and *E. coli*. The diameter of the inhibition zone increased as the extract concentration increased. The largest inhibition zone for *S. aureus* was at a concentration of 100% with a diameter of 20.93 mm and the largest inhibition zone for *E. coli* was at a concentration of 100% with a diameter of 18.05 mm. The results of this study indicated that the plant contains some major bioactive compounds that inhibit the growth of microorganisms, thereby showing great potency as an effective source of drugs. The phytochemical analysis also reveals that the plant contains similar constituents useful for medicinal purposes.

Keywords: antibacterial, galoba, *Hornstedtia*, resistance

1. INTRODUCTION

Excessive, inappropriate and irregular use of antibiotics has resulted in the emergence of antimicrobial resistance, causing many currently available drugs to be ineffective [1]. This emerging trend is alarming and is considered by the WHO to be the most pressing problem facing medical science. Therefore, there is increasing development of new antimicrobial agents capable to reduce the use of antibiotics and develop resistance. This leads researchers to isolate and identify new plant bioactive chemicals to combat microbial resistance [2]-[5]. Considering that approximately 50% of current pharmaceuticals and nutraceuticals are natural products and their derivatives, medicinal plants produce an almost unlimited source of bioactive compounds, and their use as antimicrobial agents has been exploited in different ways [6].

Medicinal plants have been utilized for medicinal purposes since ancient times and are therefore a preferred source for a wide range of bioactive compounds [7]. These plants are extensively screened to determine their bioactivity and to isolate new bioactive compounds. The medicinal properties of plants are attributed to their phytochemical composition and toxicological profile [8]. As stated by Silva and Fernandes [9], the bioactive compounds produced during secondary vegetal metabolism are primarily responsible for the biological properties of plants.

Zingiberaceae fruits are found in 1,740 small islands, including five major islands, in the eastern part of Indonesia, specifically in the North Maluku and Maluku provinces [10]. There are at least four types of *Zingiberaceae* fruits from the North Maluku and Maluku provinces, including (1) Halmahera susu golobe (*Hornstedtia alliacea*), (2) Halmahera golobe or Ambon golobe (*Zingiberaceae alliacea*), (3) Halmahera rambutan golobe (*Amomum* sp.), and (4) Ambon halia golobe/ Halmahera kelereng golobe (*Etlinger alba* (Blume) A.D. Poulsen) [11]. These fruits typically grow in the lush forests in the North Maluku and Maluku provinces, with high moisture levels. However, it has become increasingly difficult to find these fruits near residential areas, as they are now mostly found growing wild in the forests.

H. alliacea (in Sumatra and Borneo-Pining

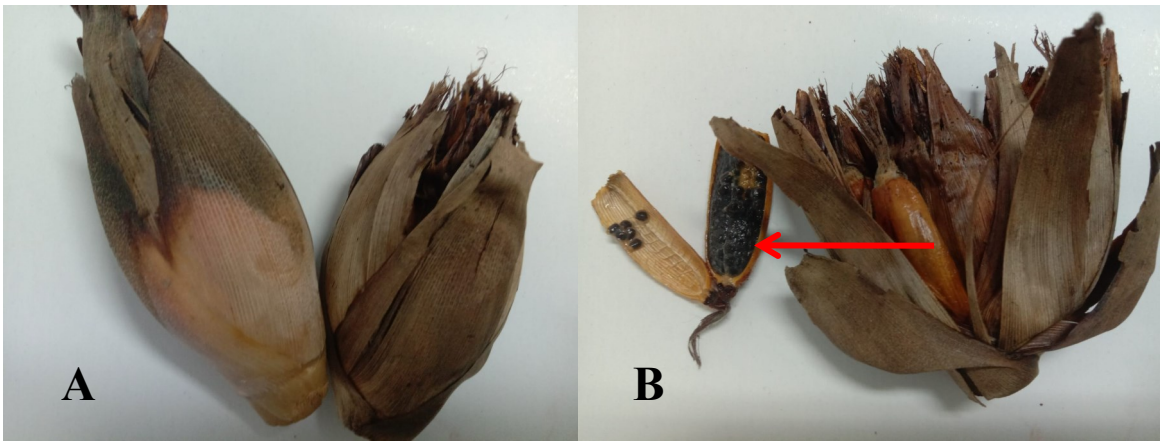

Figure 1. (A) Galoba (*Hornstedtia alliacea*); (B) Galoba seeds.

Bawang, in Maluku-Galoba, in North of Maluku-Goloba) has been traditionally used by indigenous people for a variety of medicinal purposes. Recent studies have shown that Pining bawang (*H. alliacea*) is a traditional medicinal plant that is used to treat burns. Active ingredients from the flavonoid group, including bioflavonoids, isoflavones, flavonols, and dihydrochalcones, show antioxidant and anti-inflammatory properties for treating skin burns [12]. Another study found that leaf extract of *H. alliacea* contains secondary metabolite compounds such as alkaloids, flavonoids, tannins, saponins, steroids, and phenolics, and has very strong antioxidant activity with an IC_{50} value of 3.87 µg/mL [13]. Currently, a lot of research has been carried out on *H. alliacea* as an antioxidant therapy. Still, this plant has not been widely used as a therapy for infectious diseases caused by bacteria.

Medicinal plant antimicrobial activity is a new hope to combat the dangerous threats posed by increasing evidence of antimicrobial resistance. Therefore, there is an urgent need to isolate and identify new bioactive compounds from medicinal plants, *H. alliacea*, which have yet to be adequately explored. The present study was aimed to determine the antibacterial activity of ethanol extracts of seeds of Galoba (*H. alliacea*), extracted by maceration methods, against standard bacterial cultures of *Staphylococcus aureus* (ATCC-29213) and *Escherichia coli* (FNCC-6183).

2. MATERIALS AND METHODS

2.1. Materials

Galoba seeds (*H. alliacea*, Figure 1), ethanol, Mueller Hinton agar (MHA) (oxoid), blank disc paper (oxoid), erythromycin (E) 15 µg discs (oxoid), aquadest, *S. aureus* (ATCC-29213), and *E. coli* (FNCC-6183) were used in this work.

2.2. Methods

2.2.1. Collection of Plant Materials

Galoba (*H. alliacea*) collected from Seith Village, Lehitu, Central Maluku, Indonesia. The Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, taxonomically identified and authenticated the plant materials. The galoba seeds were wet-sorted to separate the pulp that was still attached to the seeds and dried in an oven at 30–45 °C. The dried seeds were then sorted to separate the seeds that were not suitable for use and mashed using a blender (Figure 2).

2.2.2. Preparation of Ethanol Extract

Extraction of galoba seeds was carried out by weighing a sample of 300 g, putting it in a glass jar, and adding 3000 mL of 70% ethanol. The liquid was stored for 3 × 24 h with occasional stirring daily. The maceration results were then filtered using a filter paper, and then the macerate was evaporated using a rotary evaporator to obtain a thick extract. The concentrated extract was diluted with sterile distilled water to 20%, 40%, 60%, 80%, and 100%.

2.2.3. Phytochemical Screening

Galoba seeds extracts were subjected to phytochemical analysis by standard method [14]-[16]. Phytochemical tests were carried out to determine the presence of active substances which

are antibacterial agents in the results of the extraction. The color intensity or the precipitate formation was used as analytical response to these tests.

2.2.3.1. Test for Flavonoids

The Shinoda test was carried out to determine the presence of flavonoid compounds. A small amount of magnesium powder and a few drops of concentrated hydrochloric acid were added to 3 mL of the extracts. The compound belongs to the flavonoids if it results in a yellow, orange, red, or blue colour.

2.2.3.2. Test for Tannins (Ferric Chloride Test)

A few millilitres of the extract are mixed with a few millilitres of water and heated in a water bath. The mixture is filtered, and iron(III) chloride is added to the filtrate. The dark green colour formed indicates the presence of tannins.

2.2.3.3. Test for Saponins

The saponin test was carried out by the Forth method by adding 2 mL of sample into a test tube, then adding 10 mL of distilled water, shaking for 30 seconds, and observing the changes. If a steady foam is formed (does not disappear for 30 seconds), then the identification shows the presence of saponins.

2.2.3.4. Test for Quinone

Each 1 mL of extract was added with 1 mL of sodium hydroxide. The formation of blue, green, or red colours shows the presence of quinones.

2.2.3.5. Test for Steroids

A few drops of acetic anhydride were added to the extract, and the formation of purple to blue to green colour in the extract indicated the presence of steroids.

2.2.4. Antibacterial Activity

The antibacterial activity of the extracts was determined using the Kirby-Bauer disc paper diffusion method [17]. One loop of test bacteria was inoculated onto the surface of the MHA media. Sterile paper discs soaked in various concentrations of the extracts were placed on the inoculated agar surface with sterile forceps. Erythromycin was used as a positive control, and distilled water was used as a negative control. Incubation was performed for 24 h to observe the zone of inhibition. The ability of the extract to inhibit the growth of test bacteria is observed from the presence of a zone of inhibition formed around the disc paper.

2.2.5. Data Analysis

Processing and data analysis descriptively with the observation of diameter clear zone. Data is expressed as mean plus or minus standard deviation (mean ± SD).

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Screening

Standard preliminary phytochemical qualitative analyses of extracts were performed on various plant constituents and screened for the presence of biologically active compounds or secondary metabolites using standard methods. Phytochemicals can be divided into major classes depending on the chemical structures, botanical

Figure 2. Sample preparation (A) The drying stage of galoba seeds ; (B) Galoba seeds powder.

Table 1. Result of the phytocemical screening of ethanolic extract of galoba seeds (*Hornstedtia alliacea*).

Phytochemical	Occurrence
Tannins	+
Flavonoids	+
Saponins	+
Quinones	+
Steroids	+

origins, biosynthesis pathways or biological properties. Most phytochemical classification scheme is based on chemical structures such as phenolics, alkaloids, saponins, terpenoids, limonoids, polyacetylenes, and secoiridoids [18]. Numerous recent studies have been conducted *in vitro* and *in vivo* on the efficacy of plant phytochemicals as antimicrobial agents.

The major phytochemical constituents identified in the ethanolic extract of galoba seeds were tannins, flavonoids, saponins, quinones, and steroids (Table 1). Various chemical components in plants with antimicrobial effects include saponin, flavonoids, thiosulfate, glucosinolates, phenolics, and organic acids. However, the main components in plants with antimicrobial activity are phenolic compounds such as terpenes, aliphatic alcohols, aldehydes, ketones, acids, and isoflavonoids [19].

In most cases, bioactive plant extracts contain a complex mixture of ingredients that can lead to enhanced effects [20][21]. Microbial cells can be affected in different ways by these compounds. In general, the primary targets for bioactive compounds are cytoplasmic membranes, affecting their structure and integrity, permeability or functionality in various ways.

3.2. Antibacterial Activity

The test results showed that galoba seed extract inhibited the growth of *S. aureus* (Figure 3) and *E. coli* (Figure 4). It can be observed by forming an inhibition zone around the disc paper. The inhibition zone measurements (Table 2) show that galoba seed ethanol extract can inhibit the growth of *S. aureus* and *E. coli* at all concentrations. The ability of galoba seed ethanol extract to inhibit the growth of test bacteria is due to the content of phytochemical compounds (Table 1). Phenolic compounds, including flavones, flavanols, flavonoids, quinones, and tannins, are the diverse groups of bioactive secondary metabolites found in medicinal plants, which are widely used against pathogenic bacteria [22]-[24]. However, their activity could be more robust and specific [25]. Chemically, saponins are a group of high molecular -weight glycosides in which saccharide chain units (1–8 residues) are linked to a triterpene (triterpene saponins) or steroidal (steroid saponins) aglycone moiety, i.e. sapogenin. Many plant extracts containing saponins from various plants and purified saponins show antimicrobial activities at different concentrations [26]. However, the types of saponins exhibit different spectra of antimicrobial effects. Some saponins, in general, exhibit stronger antimicrobial activity against Gram-positive bacteria than against Gram-negative bacteria [18].

Medicinal plant extracts have been reported to exhibit various biological properties, including antibacterial, anti-inflammatory, and antioxidant activities [27]. Antimicrobial compounds from medicinal plants inhibit the growth of bacteria, fungi, viruses, and protozoa through mechanisms different from those of currently available antimicrobials and have important therapeutic value in the treatment of resistant strains [28]. Furthermore, the effectiveness of medicinal plant extracts in inhibiting bacterial growth is also associated with the synergistic effects between the active ingredients of the extracts and synergism can

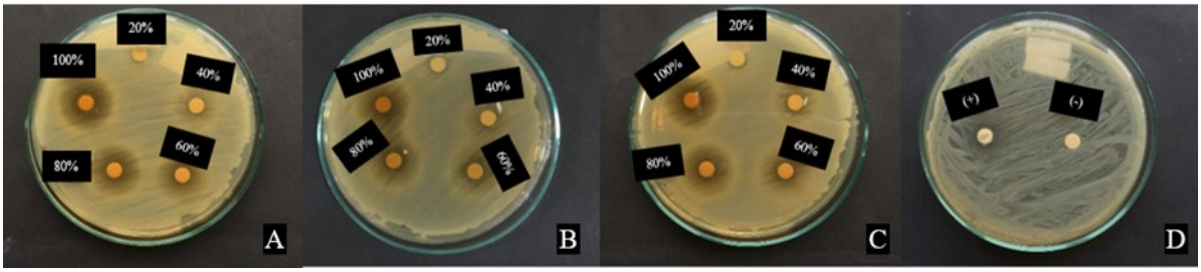

Figure 3. Antibacterial assay of galoba seeds ethanol extract against *S. aureus* for (A) repetition 1; (B) repetition 2; (C) repetition 3; and (D) control.

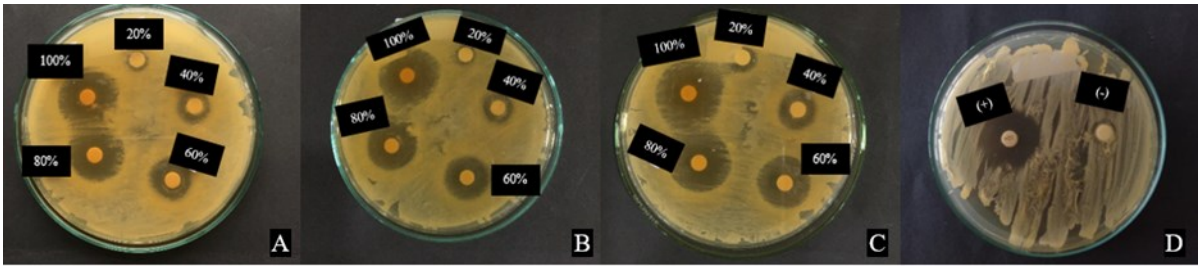

Figure 4. Antibacterial assay of galoba seeds ethanol extract against *E. coli* for (A) repetition 1; (B) repetition 2; (C) repetition 3; and (D) control.

be attributed to a variety of actions, i.e., emergence of multi-target mechanisms, presence of compounds capable of inhibiting bacterial resistance mechanisms, pharmacokinetic or physicochemical effects leading to improved bioavailability, solubility and absorption, among side effects and decreased toxicity [29].

Bioactive compounds (phytochemicals) derived from plants are mostly of therapeutic value secondary metabolites for medical purposes. Secondary metabolites are the result of secondary metabolism in plants and can occur as intermediates or end-products [30]. They have a wide spectrum of antibacterial activity, depending on their structure, number and position of substituents, presence of glycosidic bonds, alkylation of OH groups, and topography and climate of their country of origin. Indeed, variations in the quality and quantity of bioactive secondary metabolites alter their antimicrobial activity against different microbial strains [14][22][23].

The results of the inhibition zone measurements (Table 2) show that galoba seeds ethanol extract can inhibit the growth of *S. aureus* and *E. coli* at all concentrations. Galoba seeds ethanol extract can inhibit *S. aureus* at the smallest concentration of 20% with an average inhibition zone diameter of 1.80 mm with a low category and at the largest concentration of 100% with an average inhibition zone diameter of 20.93 mm. In *E. coli*, galoba seeds ethanol extract with the smallest concentration of 20% has an average inhibition zone diameter of 1.00 mm (low) and at the largest concentration, 100%, the average inhibition zone diameter is 18.05

Table 2. Antibacterial activity of galoba seeds ethanol extract against *S. Aureus* and *E. coli*.

Pathogen	Galoba seeds ethanol extract		
	Concentration	Inhibition zone (mm)	Inhibition zone category
S. aureus	20%	3.27±2.24	Weak
	40%	5.35±1.52	Moderate
	60%	9.65±2.03	Moderate
	80%	15.35±1.83	Strong
	100%	17.70±1.62	Strong
	C+	18.60	Strong
	C-	0	No inhibition zone
E. coli	20%	0.69±0.27	Weak
	40%	9.05±1.66	Moderate
	60%	13.48±1.63	Strong
	80%	15.00±1.24	Strong
	100%	16.77±1.11	Strong
	C+	2.60	Weak
	C-	0	No inhibition zone

Note: C+ and C- represent positive control and negative control, respectively

mm with a very strong category.

The present study demonstrated a greater inhibitory effect of galoba seeds on *E. coli* than *S. aureus*. Structural differences in the bacterial strain may also play a role in bacterial susceptibility to galoba seed constituents. However, the exact mechanisms of action of these antimicrobial products have not been well documented. Gram-positive reactions to plant extracts are generally much stronger than Gram-negative reactions [31] [32]. This is mainly because of the presence of lipopolysaccharide cell walls, which prevent the diffusion of hydrophobic compounds in Gram-negative bacteria [33]. Hashim *et al*. [34] found that the antimicrobial activities of the rhizome and flower oils of *H. havilandii* showed varying degrees of antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis* and *S. aureus*), Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) and yeasts (*Candida albicans* and *C. glabrata*). Both oils showed moderate activity against *S. aureus*, but weak activity against *B. subtilis*. Research with different *Hornstedtia* species showed the essential oils from leaves, rhizomes and whole plant of *H. bella* displayed the strongest inhibition effects against *S. aureus*, MRSA, *S. epidermidis*, *C. tropicalis* and *C. Parapsilosis* [35].

4. CONCLUSIONS

The results of this study indicated that Galoba seeds (*Hornstedtia alliacea*) contain some major bioactive compounds that inhibit *Staphylococcus aureus* and *Escherichia coli*, thereby showing great potency as an effective source of drugs. The phytochemical analysis also reveals that the plant contains tannins, flavonoids, saponins, quinones, and steroids, similar constituents useful for medicinal purposes. Further studies are needed to isolate, characterize and elucidate the structure of the bioactive compounds for industrial drug formulation.

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Conflicts of Interest

The authors declare no conflict of interest.

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