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by Nurbaiti Br. Singarimbun

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Phytochemicals of Extract N-Hexane Leaves Bangun-Bangun (*Plectranthus Amboinicus*) (Lour.) Spreng and Antibacterial Activity Causes Diarrhea

Nurbaiti Br. Singarimbun[1*], Defacto Firmawati Zega[1], Helen Anjelina Simanjuntak[2], Hermawan Purba[2], Kasta Gurning[2]

[1]Departemen of Midwifery, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Indonesia

[2]Departemen of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Indonesia

ABSTRACT

Objective: This research aims to determine the secondary metabolites contained in the n-hexane extract of *P. ambionicus* leaves and to determine the effective concentration as an antibacterial causing diarrhea.

Methods: The research method was carried out experimentally with the stages of making simplicia, maceration, extract making, phytochemical screening, and antibacterial activity testing.

Results: The results of phytochemical screening showed the presence of flavonoids, saponins, tannins, steroids/triterpenoids. The results of the antibacterial activity test showed that the n-hexane extract of *P.amboinicus* leaves at all concentrations (50%, 25%, 12.5%) had antibacterial activity in the strong category (10-20 mm).

Conclusion: The n-hexane extract of *P.amboinicus* leaves has the potential as an antibacterial with a strong category.

Keywords: Phytochemicals, Extract, *P.amboinicus*, Antibacterial, Diarrhea

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*Address for Correspondence:
Nurbaiti Br. Singarimbun, Departemen of Midwifery, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Indonesia

INTRODUCTION

Developing countries have faced childhood morbidity and mortality with diarrhea and other gastrointestinal problems[1]. In Indonesia, diarrheal disease is one of the public health problems because it is closely related to physical, cognitive development, susceptibility to disease[2] accompanied by high mortality, especially in Eastern Indonesia. Some causes of diarrhea are gastrointestinal tract infections caused by bacteria (*Vibrio cholera, Salmonella, Shigella* and *Eschericia coli*), viruses (rotavirus and enterovirus) and parasites (worms, protozoa and fungi)[3].

Escherichia coli is a gram-negative bacterium belonging to the family Enterobacteriaceae[4], including normal flora found in the digestive tract of humans and animals as well as symbionts in vitamin synthesis[5]. *Escherichia coli* pathogens can cause urinary tract infections, digestive tract infections (diarrhea), meningitis, sepsis and bacteremia[4]. *Salmonella typhi* is a gram-negative bacterium, does not ferment lactose and produces H_2S, a habitat in the human large intestine, transmitted by the oral-fecal route. Endotoxin-producing bacteria in cell walls that trigger fever, virulence occurs because of the capsule (Vi antigen). The genus Salmonella can cause enterocolitis, typhoid fever and diarrhea by infecting the digestive tract[6].

In general, the treatment of diarrheal disease used is antibiotics, but in its use it has an impact on the occurrence of antibiotic resistance, and there are side effects. To prevent this, it is necessary to use alternative natural ingredients as medicine[7]. In many countries, people still rely on herbal medicines to control diarrhea even though all antidiarrheal drugs are growing rapidly in the world[1]. Around 80% of the world's population still relies on natural medicines for health needs, because about 25% of medical medicines are plant-based and their derivatives[8]. Plants used are plants that are easy to find, such as daun bangun-bangun or *Plectranthus amboinicus*.

Bangun-bangun leaves or *Plectranthus amboinicus* (Lour) Spreng (Synonym *Plectranthus aromaticus* Roxb, *Coleus aromaticus* Benth and *Coleus amboinicus* Lour)[9] is an annual aromatic plant that is distribution in Afrika, Asia, Autralia, Amerika and Brazil[10]. This plant has been widely used in traditional medicine such as treating coughs, asthma, antispasmodics, abdominal pain, headaches, fever, epilepsy, and dyspepsia[11]. In India *P.amboinicus* leaves are used for skin ulcerations, scorpion bites, skin allergies, wounds, diarrhea, and are hepatoprotective. In Indonesia, *P. amboinicus* leaves are used to treat diarrhea and stimulate lactation after childbirth. In Cambodia the leaves of *P. amboinicus* are used to treat flu in children. In Brazil it is used to treat skin ulcerations[12]. In Uganda it is used to treat diarrhea, coughs, fungal infections of the skin and boost immunity[13]. In the Comoros Islands, a decoction of the leaves is used for abdominal pain, treatment of dysuria and laxative[14]. In Africa the leaves are used to treat headaches[15].

The use of *P. amboinicus* leaves as medicinal ingredients is related to the content of secondary metabolites such as flavonoids, terpenoids, alkaloids, glycosides, saponins, steroids/triterpenoids and tannins found in plants so that they can provide pharmacological effects such as antibacterial, antifungal, antitumor, antiepileptic, larvicidal, healing. wounds[16], antihyperlipidemic, antihyperglycemic, antilithiotic, immunostimulator, diuretic, anticancer, nephroprotective, antioxidant, and analgesic activity[17].

Previous research explained that the ethanolic extract of *Plectranthus amboinicus* leaves had antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia* coli at a concentration of 100 mg/ml[18]. Hot water extract of *P. amboinicus* leaves can inhibit the growth of *Escherichia coli* and *Salmonella typhimurium*[15]. Based on the literature above, the researchers were interested in examining the phytochemicals of the n-hexane extract of the leaves of Bangun-bangun (*Plectranthus amboinicus*) (Lour) Spreng and the antibacterial activity that causes diarrhea using *Eschericia coli* and *Salmonella typhi* bacteria.

METHODS AND MATERIALS

Preparation of Plant Samples

Fresh currant leaves *P. amboinicus* were collected from Simalingkar Village, Medan, North Sumatra. The sample was identified at the Herbarium Medanense Department of Biology, University of North Sumatra.

Extract Preparation

A total of 600 grams of P. amboinicus leaf simplicia was extracted by maceration method using n-hexane as solvent. Maceration was done by soaking the simplicia leaves of *P. amboinicus* for 3 days with occasional stirring. The procedure is repeated until the maserate is colorless (clear). The results of the maceration (maserat) were thickened using a rotary evaporator to obtain a pure extract.

Phytochemical Screening

Phytochemical screening was used to determine secondary metabolites contained in *P. amboinicus* leaf extract such as flavonoids, alkaloids, tannins, steroids/triterpenoids and saponins.

Antibacterial Metode Kirby Bauer Test

Determination of antibacterial activity was carried out using the scratch plate method. A total of 20 mL of sterilized MHA media was put into a sterile petri dish, then allowed to solidify. Take 1 ose suspension of bacteria (*Escherichia coli, Salmonella typhi*), then scratched on the media evenly. Then put the concentration of disc paper soaking (50%, 25%, 12.5%, chloramphenicol, and DMSO) on solid MHA media. Incubate the culture at 37°C for 1 x 24 hours, and 1 x 48 hours. The antibacterial activity test was carried out with three repetitions. Observations were made by measuring the diameter of the inhibition zone formed around the paper disc using a caliper. The results of the clear zone measurements were analyzed descriptively based on the Davis and Stout criteria.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening showed the presence of secondary metabolites contained in the leaf extract of *P. amboinicus* which can be seen in table 1.

Table 1: Results of Phytochemical Screening of Extract leaves Bangun-bangun (*P. amboinicus*)

No.	Screening	Reagent	Extract n-hexan leaves bangun-bangun
1	Flavonoids	Mg + HCl + AmilAlkohol	Positive
2	Alkaloids	Mayer	Negative
		Bouchardat	Negative
		Dragendorff	Negative
3	Saponins	Foam Test	Positive
4	Tannins	$FeCl_3$ 1%	Positive
5	Steroids/Triterpenoids	Lieberman-Buchard	Positive

Based on table 1, it can be seen that the secondary metabolites contained in P. amboinicus leaves consist of flavonoids, saponins, tannins and steroids/triterpenoids. The presence of secondary metabolites in plants is related to pharmacological properties[19].

Based on the results of previous studies, it was explained that there were chemical compounds from *P.amboinicus* leaves, namely essential oils (carvacrol, thymol,

caryophyllene, chlorogenic acid, rosmarinic acid, eugenol)12, terpenes (monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids)20, flavonoids (quercetin, epigenin, salvigenin, genkwanin and luteolin)[21]. The chemical composition of the aqueous extract of *P. amboinicus* leaves is reported to contain tannins, flavonoids, saponins, polyuronides and steroid glycosides that play a role in biological activities such as antibacterial[15]. The leaves contain carbohydrates, alkaloids, amino acids, flavonoids, tannins, quinones, glycosides, proteins, terpenoids and phenolic compounds[22] such as flavonoids, isoprenoids such as phytosterols and the abundance of volatile mono and sesquiterpenoids in essential oils, the presence of flavone glycosides and ancient diterpenoids such as royleanone. Phytochemical compounds from the class of diterpenes, phenolic acids, flavonoids, fatty acids, and docosapentaonic acid[23].

Antibacterial activity

The results of the antibacterial activity test that causes diarrhea can be seen by the diameter of the inhibition zone of the n-hexane extract of *P.amboinicus* leaf extract against the growth of *Eschercihia coli* and *Salmonella typhi* in Table 2 and Figure 1.

Table 2: Antibacterial Activity Test Results Cause Diarrhea

Bacteria	Inhibition Zone Diameter (mm)				
	Sample Concentration				
	12.5%	25%	50%	Chloramphenicol 3%	DMSO 1%
Escherichia coli	10.2	11.3	12.53	31.25	0
Salmonella typhi	11.16	11.68	13.67	25.15	0

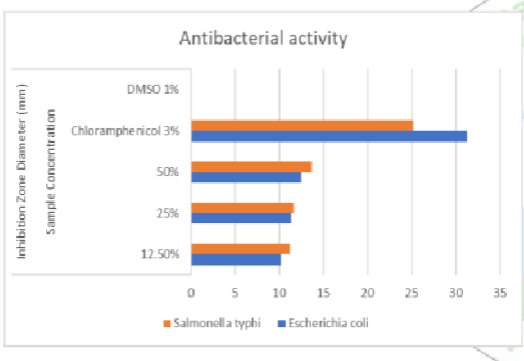

Figure 1: Antibacterial activity that causes diarrhea

Based on table 2 and figure 1, it can be seen that the n-hexane extract of *P. amboinicus* leaves has antibacterial activity against *Escherichia coli* and *Salmonella typhi* that cause diarrhea. The higher the concentration of the extract, the higher the diameter of the inhibition zone formed. Based on the diameter of the inhibition zone compared to the David and Stout categories, it was concluded that the *P. amboinicus* leaf extract at all concentrations was in the strong category (10-20 mm). This is related to the secondary metabolites found in *P. amboinicus* leaves which have pharmacological effects such as antibacterial. This is supported by the phytochemical results of *P. amboinicus* leaves having flavonoids, saponins, tannins, steroids/triterpenoids that have potential as antibacterial.

Flavonoids isolated from *P.amboinicus* have various biological activities, especially antioxidant, diuretic, anti-inflammatory, cytotoxic and antimicrobial activities[9], antimutagenic, hypocholestemic, and antiplatelet agglomeration properties[11]. Flavonoid compounds can act as antibacterial because they are very effective in inhibiting bacterial growth by penetrating the peptidoglycan layer which is more polar than the non-polar lipid layer, causing cell wall disruption and will result in cell lysis, besides that flavonoids can inhibit cell membrane function by disrupting cell function. cell membrane permeability and inhibit the binding of enzymes such as ATPase and phospholipase, inhibit cell membrane function by forming complex compounds with extracellular proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds[24].

Saponin compounds have antibacterial properties by reducing the surface tension of bacterial cell walls and disrupting membrane permeability. Saponins that are absorbed on the cell surface will cause damage by increasing cell membrane leakage, so that essential ingredients for microbial life will be lost and cause cell death[26].

Tannin compounds are a complex class of polyphenolic biomolecules produced in various types of plants that are often used as pesticides[26]. Tannins are polyphenol compounds that have a high molecular weight and can bind to proteins. Tannins can affect the permeability of the cytoplasmic membrane[27].

Triterpenoid compounds are reported to have antibacterial and antifungal activity[28]. Triterpenoid compounds have an antibacterial mechanism by damaging the bacterial cell membrane. Damage to bacterial cell membranes can occur when antibacterial active compounds react with the active site of the membrane or by dissolving lipid constituents and increasing their permeability. The bacterial cell membrane is composed of phospholipids and protein molecules. There is an increase in the permeability of the aktibacterial compounds can enter the cell and can lyse the cell membrane or coagulate the cytoplasm of the bacterial cell. In addition, the presence of terpene groups such as thymol and carvacrol in *P. amboinicus* has antibacterial activity against *S. mutans*. Terpenes such as carvacrol, thymol and germacren D are capable of changing cell walls or disrupting cell membranes facilitating drug

absorption[29]. The essential oil from the leaves of *P. ambionicus* showed activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*[30].

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that the n-hexane extract of the leaves of Bangun-bangun *P.amboinicus* has potential as an antibacterial causing diarrhea with a strong category (10-20 mm).

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