EVALUATION OF ETHANOLIC EXTRACT AND FRACTIONS OF RAMBUTAN LEAVES (*Nephelium lappaceum*) AGAINST *Shigella dysenteriae* AND *Bacillus cereus* AS ANTI DIARRHEA AGENT

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ABSTRACT

Aim: To determine the antibacterial activity of Rambutan leaves ethanol extract and fractions to inhibits *S. dysenteriae* and *B. cereus* growth *in vitro*.

Methods : The ethanol extract and fractions obtained from the Rambutan leaves were studied for antibacterial activity against *S. dysenteriae* and *B. cereus* using the agar diffusion method. The simplicia of Rambutan leaves was extracted using maceration method and to obtained the fractions using liquid-liquid extractions. The phytochemical screening was taken using Farnsworth methods. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC) test were performed by a macrodilution method and following by subculturing the overnight result on to the surface of agar media.

Results : Phytochemical screening of Rambutan leaves ethanol extract and fraction revealed the presence of flavonoids, polyphenols, tannins and saponins, and had antibacterial activity against *S. dysenteriae* and *B. cereus*, which the ethyl acetate fraction was the most active fraction. The value of MIC and MBC extract for *S. dysentriae* was in the range concentration 2.5-5.0% w/v, and for *B. cereus* was in the range concentration 0.08-0.16% w/v, while for the ethyl acetate fraction for *S. dysenteriae* was in the range concentration 1.25-2.50% w/v, while for *B. cereus* was in the range concentration 0.04-0.08% w/v.

Conclusions : The Rambutan leaves gave potent and direct antibacterial effect on *S. dysenteriae* and *B. cereus.*

Keywords: Diarrhea, *Nephelium lappaceum,* Antibacterial, *Shigella dysenteriae, Bacillus cereus*

INTRODUCTION

Diarrhea is one of the most contagious diseases with a high rate of morbidity, mortality and malnutrition in infants and children worldwide [1,2]. Diarrhea disease causes 17.5-21% (equivalent to 1.5 million deaths per year) of all deaths in children under 5 years old [2]. Diarrhea infections caused by toxin producing bacteria. Bacteria that can produce toxins that cause diarrhea infections are *Shigella* dysentriae and *Bacillus cereus* [3,4]. The most dominant cause of diarrhea, *Shigella dysenteriae* and *Bacillus cereus* are two bacterias that can cause diarrhea.

S. dysenteriae will produce exotoxin that can affect the digestive tract and central nervous system. Exotoxin is an antigenic protein that stimulates antitoxin production that can kill the patient [5]. *S. dysentriae* can produces a potent cytotoxin called Shiga toxin [3]. *Shigella* occurs in 50% of cases of dysentery or bloody diarrhea [6]. Clinical features of bloody diarrhea by invading and causing destruction of the colonic epithelium with or without fever [3,6], abdominal pain and tenesmus [6], bloody stools, and some acute gastrointestinal infections [7].

Bacillus cereus is a gram-positive aerobic bacterium capable of forming spores and causing gastroenteritis due to its ability to form enterotoxin complexes [6]. *B. cereus* produces two types of toxins that cause two types of diseases. Emetic syndrome (nausea) is caused by emetic toxins produced by bacteria during the growth phase in the diet. Whilst diarrhea syndrome is caused by diarrhea toxin produced during the growth of bacteria in the small intestine [8].

Rambutan is a plant that grows in the tropical countries. Traditionally, rambutan leaves are commonly used to darken the hair, antidote for diarrhea and to reduce fever [9]. There has been much research to determine the antibacterial activity of rambutan leaves as antibacterial agent. The ethanol extract 70% of rambutan leaves (*N. lappaceum* L.) has antibacterial activity against *Staphylococcus aureus* ATCC 25925 and *Escherichia coli* ATCC 25922 bacteria [10], *Pseudomonas aeruginosa* multiresistant [11], and Methicillin Resistant *Staphylococcus aureus* (MRSA) [12]. This research was aimed to investigate antibacterial activities of the ethanolic extract and its fractions of *N. lappaceum* leaves against *S. dysentriae*.

MATERIALS AND METHODS.

Materials

The materials used in this research are Rambutan leaves (*Nephelium lappaceum*), test bacteria *Shigella dysenteriae* ATCC 13313 and *Bacillus cereus* ATCC 13461. Dimethyl sulfoxide, ammonia, acetone, chloroform, HCl 2 N, 70% Ethanol, Mayer, Dragendorff, Mg powder, amyl alcohol, $FeCl_3$, ether, 10% vanillin in concentrated sulfuric acid, 1% gelatin, Lieberman, Burchard, KOH 5% , Methanol, Aquadest, SS (*Shigella-Salmonella*) Agar medium, MHA (Mueller-Hinton Agar) medium and MHB (Mueller-Hinton Broth) medium.

Extraction and Fractionation

Extraction was done by immersing 500 g of rambutan leaves simplicia in 70% ethanol for 3 x 24 hours with solvent substitution each day. Then, the liquid extract of the maceration was concentrated by using a rotatory evaporator and concentrated above the water bath. The result

of extracts obtained is then examined extract parameter, such as crude extract, organoleptic, weight and moisture content. The extract was then fractionated by using liquid-liquid extraction method. Twenty grams of viscous extract dissolved in 100 mL water, then added with 1:1 *n*-hexane solvent was shaken in separating funnel. The mixture was kept until it had separated and the n-hexane phase is accommodated, this process is carried out for 3 times and then continued by adding ethyl acetate solvent with the same volume ratio and process of *n*-hexane.

Phytochemical Screening

Phytochemical screening is performed to examine the compounds contained in the extract. The examinations include of alkaloid compounds, polyphenolates, tannins, flavonoids, monoterpenes and sesquiterpenes, steroids and triterpenoids, quinones, and saponins [13].

Thin Layer Chromatography (TLC) Profile

TLC was performed using Silica gel GF 254. Buthanol: Ethyl Acetate (3: 7) phase was then seen on UV lamps 254 and 366 and sprayed with $AlCl_3$

Test for Bacterial Confirmation

Bacterial confirmation tests included morphological observations of bacterial colonies in the media; Gram staining; and biochemical tests.

Antibacterial Activity Test of Extracts and Fractions

Activity test was performed by using agar diffusion method, by making a hole in the agar medium that has been mixed with bacteria using perforator. The extract and the tested fractions were made with varied concentrations.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of MIC and MBC were performed using microdilution method using microtiter plates. The concentration of extract and fraction used is the smallest concentration that still actively give activity to both of bacteria.

RESULTS AND DISCUSSION

From the research, it obtained ethanol extract of rambutan leaves with remaining extract equal to 23.39%, it was dark brown in color, with a distinctive odor and thick-hard shape. It had a moisture value of 4.95% (w/v) and had a weight of 1.05 g/mL. Fractionation results that can be seen in Table 1.

Table 1: Fractionation results of Rambutan leaves extract

Mass Extract (g)	Fraction	Mass Fraction (g)	Yield (%)
20	*n*-hexane	0.18	0.90
	Ethyl acetate	5.53	27.65
	water	8.98	44.90

In phytochemical screening tests it is known that rambutan leaves contains polyphenols, tannins, flavonoids, monoterpenes and sesquiterpenes, saponins. Based on screening results, secondary metabolites that are thought to be potentially antibacterial are polyphenols, flavonoids, and saponins.

The TLC profile resulted with varied Rf observed at visible light, UV λ254 & λ366 nm, and $AlCl_3$ spray reagent. The results showed that on rambutan leaves extract had 3 spots with one yellow spot and two red spots that could be seen when the plate is seen under UV λ366 nm, while in UV λ254 nm all spots of the extract did not show any color. Almost the similar with the extract, ethyl acetate fraction showed 3 spots with one yellow spot and two red color spots seen under UV λ366 and not visible color under UV λ254. It was also showed that the ethanol extract and ethyl acetate fraction of rambutan leaves that might be have contained flavonoids compound spotted in yellow colored spot that react with $AlCl_3$ reagent. Since the application of the spotting of $AlCl_3$ is a spotting agent for flavonoids and will usually give a distinctive color for flavonoid compounds in the TLC plate was yellow [14].

The results of the bacterial confirmation test showed that *S. dysenteriae* is grown in *Shigella-Salmonella* (SS) medium and was yellowish colored. While *B. cereus* is grown in MHA medium with murky white colored. The Gram staining results indicated *S. dysenteriae* is a Gram-negative bacteria with pink colored bacil form due to its inability to maintain a dye replaced by a counter dye. While *B. cereus* is a Gram-positive bacteria with purple bacil colored form because of its ability to maintain primary color and was not replaced by secondary dye when being washed.

The antibacterial activity test results showed that the inhibitory zone for each sample can be seen in Table 2.

Table 2: Test Results of Extract and Fraction Activity

Sample	Concentration (%w/v)	*S. dysenteriae* Diameter Zone of Inhibition (mm)	*B. cereus* Diameter Zone of Inhibition (mm)
ethanolic extract	40	13.8 ± 0.02	11.4 ± 0.02
	20	10.9 ± 0.02	9.8 ± 0.02
	10	9.4 ± 0.02	6.7 ± 0.02
	5	6.7 ± 0.02	4.2 ± 0.02
ethyl acetate fraction	40	13.4 ± 0.02	13.5 ± 0.02
	20	12.1 ± 0.02	11.5 ± 0.02
	10	10.7 ± 0.02	8.5 ± 0.02
	5	7.7 ± 0.02	7.5 ± 0.02
n-hexane fraction	40	-	-
	20	-	-
	10	-	-
	5	-	-
Water fraction	40	5.6 ± 0.02	11.9 ± 0.02
	20	2.3 ± 0.02	9.5 ± 0.02
	10	1.5 ± 0.02	6.7 ± 0.02
	5	1.4 ± 0.02	4.3 ± 0.02
Dimethyl sulfoxide (DMSO)	1% v/v	-	-

Tabel 2 showed that the ethanol extract of rambutan leaves has an antibacterial activity at concentrations of 5% (w/v) for both bacterias, which is the smallest concentration tested that still contained activity. While the fractions that were tested, ethyl acetate and water fraction has an antibacterial activity at concentration of 5% (w/v) for both bacterias with the greatest activity was shown by the fraction of ethyl acetate. Whereas for n-hexane fraction showed that has not antibacterial activity for *S. dysenteriae* and *B. cereus* bacteria. From every sample that provide antibacterial activity, it can be concluded that as concentration tested was increasing, the greater the zone of inhibition being given.

Based on the test results of the activity of extracts and fractions of rambutan leaves, ethanol extract of rambutan leaves and ethyl acetate fraction as a fraction of the most active to do the determination of MIC and MBC from ethanol extract of rambutan leaves against *S. dysenteriae* and *B. cereus*. MIC and MBC testing was performed by using microdilution method with varying concentrations starting from a concentration of 5%(w/v), which is then diluted to a concentration of 0.04%(w/v). The results of determination of MIC and MBC from ethanol extract and ethyl acetate fraction of rambutan leaves against both bacteria test can be seen in Table 3.

Table 3: Results Determination of MIC and MBC

Sample	Concentration (%w/v)	Bacterial Growth of *S. dysenteriae**	Bacterial Growth of *B. cereus**
Ethanolic Extract	5	-	-
	2.5	+	-
	1.25	+	-
	0.62	+	-
	0.31	+	-
	0.16	+	-
	0.08	+	-
	0.04	+	+
Ethyl acetate Fraction	5	-	-
	2.5	-	-
	1.25	+	-
	0.62	+	-
	0.31	+	-
	0.16	+	-
	0.08	+	-
	0.04	+	+

* (-): absence; (+): presence

The results (Table 3) showed that *Shigella dysenteriae* bacteria can growth at a concentration of 1.25%(w/v) which makes this concentration as the value of MIC of the ethyl acetate fraction and at concentration 2.5%(w/v) was set as MBC value of ethyl fraction because has no bacterial growth at this concentration. While in *Bacillus cereus* bacteria, MIC value was at concentration of 0.04%(w/v) because at this concentration the growth of *Bacillus cereus* could be seen, the MBC value was seen at concentration of 0.078%(w/v) where *Bacillus cereus* has not growth at this concentration. The antibacterial activity indicated from the secondary metabolites compounds that contained in the extract or fraction can inhibit *S. dysentriae* and *B. cereus* such as flavonoid, polyphenols, tannins, saponins, monoterpens,

sesquiterpens. Flavonoid and polyphenols are natural compounds that contained in the extract and fractions which are phenolic compound [15]. Monoterpene or sesquiterpene as essential oils that contained in plant that have antibacterial activity [16].

CONCLUSIONS

From the research it can be concluded that the ethanolic extract and ethyl acetate fraction of the *Nephelium lappaceum* leaves are active for antibacterial activity and gave the potent and direct antibacterial effect on *Shigella dysenteriae* and *Bacillus cereus* that causing diarrhea infection.

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