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Research article

Assay for antimicrobial activity of mangosteen rind extracts

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Abstract

Mangosteen rind that just used to be discarded, in a fact, it possesses a benefit to be developed as a phytopharmacal product. It contains quite a lot of compounds with pharmacological activity, such as anti-inflammatory, antihistamine, antibacterial, antifungal even for therapy or treatment of heart disease and HIV. The purpose of this study was to determine antibacterial activity of mangosteen rind extract (MRE) on growth of *Escherichia coli*. Effectiveness of antibacterial effect of the MRE, a conical flask containing 250 ml of sterilized agar was added with 0.5% bacterial suspension and then poured onto a petri dish and paper discs that had been dipped prior into MRE solution at various concentrations were placed onto the media and then incubated at 37°C for 18-24h. Diameter of clear zones were performed around the paper discs were measured. The results showed that estimation amount of bacterial colonies of *E. coli* at dilution rate of 10^{-6} was $9.7x10^{-8}$ CFU/ ml. The results showed that the antibacterial activity of MRE at all levels of concentration effectively inhibited the growth of *E. Coli.* MRE showed more effectiveness and large areas of inhibitory against *E. coli* at a concentration of 10% which was equal to15 mm, while MIC at a concentration of 0.62% was equal to 5.75 mm. This suggests that increasing the concentration of MRE resulted in occurrence of the growth inhibition zones were greater. To learn more antibacterial activities of MRE, it must be tested against some gram positive bacterial strains and with different solvent extraction methods.

Key words: Antibacterial, Clear zone, *E. coli*, Mangosteen peel extract, Phytopharmaca.

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1. Introduction

As a tropical country in South East Asia, Malaysia and Indonesia have a potential natural resource that has not been much revealed. Tens of thousands species of plant that can be found in these two countries are potentially as medicinal and pharmaceuticals sources. However, utilization of these potential source has not yet optimal, even though utilization of some potential medicinal herbs may be

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important dealing with improving health and may generate income also towards local communities [11]. In additional, the utilization of herbal medicine is more economical and relatively have small side effects [12]. Today, utilization of extracts of plant as beverages made from spices and medicinal plants presented by-healthy drinks, herbs, soft drinks, juices and syrup has increased. Mangosteen rind is one of them. Mangosteen rind used as a healthy beverages that has been sold in drugs store or convenient store as instant drinks and beverage supplement [2]. Mangosteen (*Garcinia mangostana* Linn) is an exotic fruit and has attractive color with high nutrient content, consists of carbohydrates, protein, fat, calcium, phosphorus, iron, sodium, potassium, vitamin B1, vitamin C, and others. Moreover, in the thick skin is contained xanthone, resins, flavonoids and tannins. According to Ditjen POM and Paramawati [5] xanthone of mangosteen rind consist of mangostin, mangostenol, mangostin α, ß and γ mangostin, gartanin, mangostinon A, mangostinon B, garcinon, flavonoid, epicatechin, and some others. These active compounds that allegedly has some potential properties for health and beauty. The mangosteen rind that was used just thrown away, evidently it showed potential properties for development of medicinal herb, since it contains active compounds as pharmacological ingredients such as antibacterials [8,6]; antifungal [7], anti-inflammatory, antihistamine, as well as for therapeutic treatment of heart disease and HIV-AIDS [3,10]. The purpose of this study is to examine the effectiveness of antibacterial activity of mangosteen rind extract on bacterial growth of pathogenic microbial strain.

2. Materials and Methods

Simplisia of Mangosteen Rind: Plant material used was mangosteen rind obtained from market in Kranji, Bekasi, Indonesia, which had been identified at Herbarium Bogoriense, Research Center for Biology-LIPI, Bogor, Indonesia. Pulverizing simplicia was an initial process on production of mangosteen rind extract. Fresh mangosteen rinds were washed, drained and then weighed on wet weight based and then chopped or cut into pieces and dried into oven at 40 to 50°C and weighed on dry weight based. Moreover, crude fine powder of simplicia was prepared using a blender and weighed again before stored in plastic containers [4].

Preparation for Mangosteen Rind Extract:

Simplisia powder of mangosteen rind was poured into Schott bottle and soaked with 1500 ml 70% ethanol and stirred well and then allowed for 18-24h at room temperature. Macerated simplisia were screened and separated from pulp through a filter paper. The screening process was repeated for several times or until the color of macerated rinds became yellowish resulted by the solvent. The whole macerated simplisia was then collected and evaporated using rotary vacuum evaporator at 40-50°C and 20-60 rpm, thus obtaining concentrated extract was then freeze dried at -42.20°C under 12.1pa thus to obtain the freeze dried extract [4].

Total Bacterial Colony Count:

The number of colonies were count using total plate count method by preparing a serial dilution of bacterial suspension. A respective tube for dilution containing 0.9 ml of 0.85% physiological NaCl. Bacterial

suspension that had been incubated for 24h was about 0.1 ml pipetted into respective tubes and then homogenized by vortexing and was continuously repeated to obtain serial dilutions of 10^{-3} to 10^{-8}. Approximately 0.1 ml bacterial suspension of 10^{-5} to 10^{-8} were then pipetted and inoculated onto media in a petri dish and then incubated at 37°C for 24h to observe the total plate count for the colonies.

Preparation for Stock Cultures:

Colonies of *E. coli* ATCC-25922 was inoculated into NA slant medium and then incubated at 37°C for 18-24h to be employed as stock culture. Colonies of the grown bacterial strain was then suspended into test tubes containing 5.0 ml NB medium, and then incubated at 37°C for 18-24h and its optical density was measured at 600 nm using Spectrophotometer to obtain OD of 0.5.

Antibacterial Activity Test:

Before the analysis, bacterial strains were cultured on nutrient agar (NA) pH 7.0, under aerobic conditions for 48 h, at 37 °C. This bacterial culture was used as inoculum in order to test the antibacterial activity of crude mangosteen rind extract. The antibacterial activity of the extract was estimated using the disk agar diffusion method, according to the Kirby-Bauer test [1]. Inocula of bacterial cells were suspended in sterile physiological saline solutions, in sterile tubes, and homogenized on Vortex, until the density of the test suspension was equivalent to a concentration of 1.5×10^{8}/mL. Petri dishes containing NA (pH 7.0) was inoculated with 0.1 mL of microbial suspension spread to ensure complete coverage. The plates were left for 5 min before excess fluid was removed using a sterile pipette. Then, the inoculated Petri dishes were dried at room temperature for a maximum of 20 min. Inoculation was carried out using a biological safety cabinet. Sterile paper discs impregnated with 20 µL crude mangosteen rind extracts were aseptically applied to the surface of each of the inoculated plates in a central position, using a sterile forceps, gently pressing to ensure even contact with the medium surface. Control discs impregnated with antibacterial substances and blank discs (impregnated with solvent) were also placed into the inoculated Petri dishes using a disc dispenser. Amoxicillin was used as reference antibacterial. The inoculated plates were incubated for 24 h, at 37 °C. The inhibition zones were expressed in mm, as the diameters of clear zones around the discs. The results of antimicrobial activity were expressed as the mean value of three independent analyses.

Preparation of Test Solution:

A total of 10g of mangosteen peel extract reconstituted with sterile akuadest of 100 ml, in order to obtain a concentration of 10% and diluted again in order to obtain extracts with concentrations 5%, 2.5%, 1.25% and 0.62%. For the positive control used amoxicillin 5mg, and a negative control akuadest used ster-ile. Making the concentration holding a 10% solution of 100 ml:

$$10\ \% = \frac{10}{100} \text{ x } 100 = 10$$

Dilution formula : V1 x N1 = V2 x N2

V1 = volume of tested extracts (ml)
N1 = initial extract concentration (%)
V2 = volume of prepared solution (ml)
N2 = concentration of tested extract (%)

Mangosteen Rind Extract Test:

Approximately 1.25 ml (0.5%) predetermined OD bacterial suspension was added into conical flask containing 250 ml sterilized liquid medium (45°C)

and then homogenized throughout media by shaking well. It was poured into sterilized petri dish and allowed to solidify. Some paper discs (∅9 mm) that had been soaked into respective test solution including positive and negative control solutions at various concentrations were then placed on the media that had been inoculated with bacterial suspension and then incubated at 37°C for 18-24 h. Furthermore, the diameter of the clear zone formed around the test solution was then observed and measured according to formula as follows;

(t) (r-1) ≥ 20

(t) is number of treatment (= 7), (r) is number replication applied, and 20 is value factor for degree of freedom

3. Results and Discussion

Estimation of grown colony of *E. coli*

Figure 1 showed yield of mangosteen rind extract that was obtained from extracting 500g simplisia powder of mangosteen rind by application of maceration method using 70% ethanol and continued with evaporation of solvent using vacuum rotary evaporator and then freeze drying method was the extract of mangosteen rind that could be implemented as antibacterial active compounds against *E. coli* growth (Figure 2). However, prior to the antibacterial activity test, the total bacterial colony counts should have been determined at initial stage by application of standard plate counts (SPC). This method is to determine the sample of bacterial culture in petri dish that should be chosen for further test is based on petri dish containing 30-300 grown colonies. Some colonies may be grown and merged into one big colony as well as grown in line as a chain that may be seen as a thick line, where the number of grown colonies are in doubt, thus they may be considered as one large colony [13].

Formula for estimation number of colonies:

> Σ bacterial cell (CFU/ml) = $\bar{x}$. X
> Noted :
> $\bar{x}$ = Σ counted colonies
> X = dilution factor

Σ bacterial cells (CFU/ml) at dilution rate 10^{-4} = $\bar{x}$. X

$$1800 \times \frac{1}{10^{-4}} \times \frac{1}{10^{-1}}$$

$$\frac{1800}{10^{-5}} = 1800 \times 10^{5} = 1800 \times 10^{3} \times 10^{5} = 1800 \times 10^{8} \text{ CFU / ml}$$

Further estimation of Σ bacterial cells (CFU) at dilution rate of 10^{-5} to 10^{-7} as the same as above.



A



B

Figure 1: Extract of mangosteen rind before (*A*) and after concentrated (*B*).

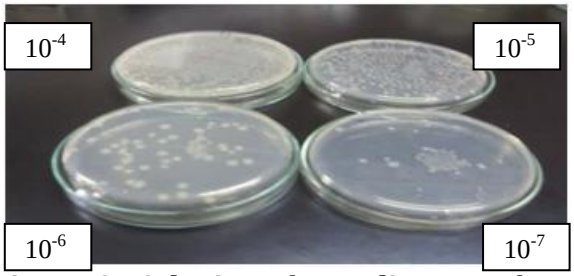


Figure 2: Colonies of *E. coli* grown from serial diluted culture suspension.

Table 1. Estimation of *E. coli* strain colony count

Dilution rate	Average ∑ Colony count	∑ Cell of CFU/ml	Description
10^{-4}	1800	1,800 x 10^{8}	> SPC
10^{-5}	490	4,90 x 108	> SPC
10^{-6}	97	9,7 x 10^{8}	= SPC
10^{-7}	29	2,9 x 10^{8}	< SPC

Based on the results obtained in bacterial count with cup count method in Table II, the result that the dilution 10^{-4}, 10^{-5} and 10^{-6}, the number of bacterial colonies growing on NA medium can be calculated, but the 10^{-4} dilution are the average number of colonies 1800 or 1,800 x 108 CFU/ml and the dilution 10^{-5} are the average number of colonies of 490 or 4.90 x 10^{8} CFU/ml, so it does not meet the standards of SPC, because the number of colonies greater than the standard SPC, whereas the number of colonies that dilution 10^{-7} too few grow on media that is 29 or 2.9 x 10^{9}, so it does not qualify SPC [12]. At 10^{-6} di-lution of the number of colonies SPC eligible bacterial colonies that contained 97 or 9.7 x 10^{8} CFU / ml.

Antibacterial Activity Test

The results on antibacterial activity test of the mangosteen rind extracts against the growth of *E. coli* strain was shown in Table 2. The result indicated that negative control sample that was only using distilled water definitely not to result any inhibition zone against the growing of *E. coli* strain, thus that the growth of this bacterial strain evenly reached around the paper disc. This was indicated that the bacteria could grow well in the absence of any antibacterial compound to inhibit its growth. While on the sample with paper disc containing Amoxicillin as a positive control, it apparently could play a role effectively as a broad-spectrum antibacterial compound, thus it could provide effective inhibition of 20.75mm against the growth of *E. coli* and it could apparently cause a damage of bacterial cell membrane. The result showed that the antibacterial activity of mangosteen rinds extracts at all concentration were able to inhibit the growth of *E. coli* strain with a considerable large inhibition spectrum (15 mm) and the most effective was demonstrated at a concentration of 10%. Inhibition zone is considered to be effective and convincing when it shows the diameter of clear zone between 14 to16 mm. The minimum inhibitory concentration (MIC) of the mangosteen rind extract against the *E. coli* strain at concentration of 0.62% was 5.75 mm (Figure 3). Figure 4 showed that the higher concentration of mangosteen rind extract was used, the greater the diameter of inhibition zone resulted from. This was caused by high content of chemical compounds in the extract of mangosteen rind, including flavonoids, tannins and xanton that may act as effective antibacterial, thus it may play a role to inhibit or prevent the bacterial cells from growing.

Table 2. Antibacterial activity test of mangosteen rind extract against *E. coli* strain.

Replication	Diameter of Inhibition Zone (mm)*						
	C (+)	C (-)	Concentration Mangosteen Rind Extract (%)				
			0.6	1.25	2.5	5	10
I	21	-	6	7	10	14	14
II	20	-	9	10	11	13	14
III	20	-	3	9	11	14	16
IV	22	-	5	9	11	11	16
Average	20.8	-	5.8	8.8	10.8	13	15

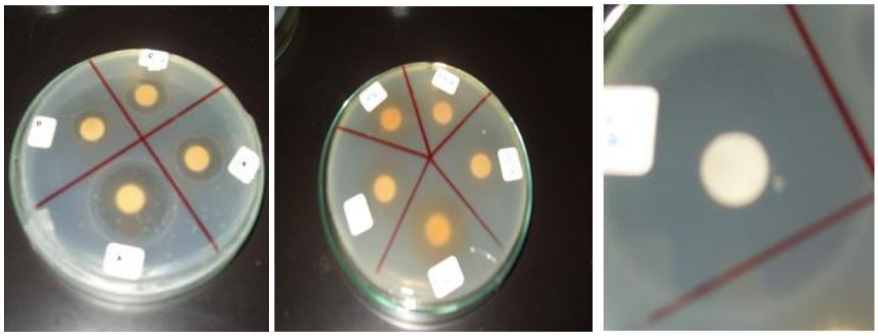


Figure 3: Inhibitory zone of antibacterial activity against *E. coli* strain.

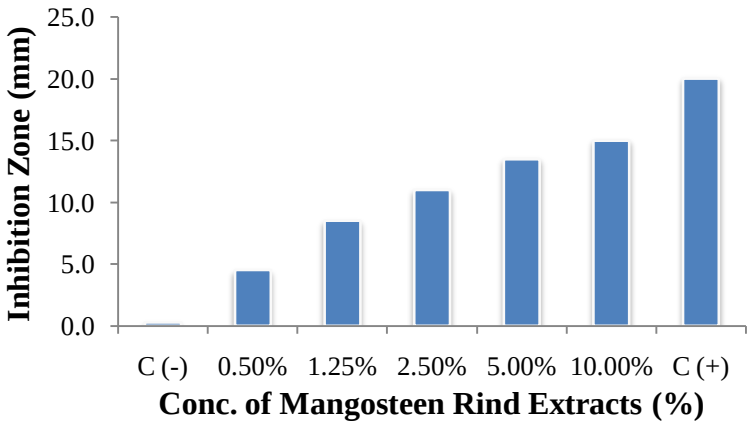


Figure 4: Effect of mangosteen peel extract against the growth of *E. coli*.

Conclusion

From the research that has been done, it can be concluded that the antibacterial activity test of mango-steen peel extract is able to inhibit the growth of the bacterium *Escherichia coli*. Crude mangosteen rind extract showed large inhibition zone and is effective against *E. coli* at a concentration of 10%, ie by 15 mm with the MIC of 0.62% capable of forming inhibitory zone of 5.75 mm. Zone of inhibition on the growth of the bacteria E. coli more in line with increasing concentrations mangosteen peel extract were tested.

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