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ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF JUICE AND ETHANOLIC EXTRACTS OF *Garcinia mangostana* L. LEAVES

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Abstract

G. mangostana is a plant that can be used as a traditional medicine to treat various infectious diseases for the treatment of diarrhea, skin infection and chronic wounds. The activity as antifungal and antibacterial of juice and ethanolic extract from *G. mangostana* leaves were investigated. Juice and ethanolic extract were concentrated by using a rotary evaporator to get concentrated extract with rendemen 2.571 and 5.647 % (w/w). Fluid and ethanolic extract dilution method were employed to evaluate the antifungal activity against *Saccharomyces cerevisiae*. Ethanolic extract dilution method was used to assess the antibacterial activity against *Bacillus subtilis* and *Escherichia coli*. Results showed that juice, ethanolic extract was effective against *Saccharomyces cerevisiae*, the minimum inhibitory concentration (MIC) was 1000, and 500mg/ml. Antibacterial activity of the *G. mangostana* leaves ethanolic extract showed that the action was potential with the inhibition zone in *Bacillus subtilis* and *Escherichia coli*. The conclusion of this study is that juice, and ethanolic extract *of G. mangostana* leaves have possible antifungal and antibacterial activity.

Keywords: *G. mangostana*, antifungal, antibacterial, juice, ethanolic extract

INTRODUCTION

Mangosteen (*G. mangostana*) is one of the primary commodities of Indonesian export, known as the Queen of Tropical Fruits. Even though the fruit has been exported, the availability of good quality fruit is still inadequate [1]. There were many reports of biological activity of *G. mangostana.* Several studies have shown that obtained xanthones from mangosteen have remarkable biological activities such as antioxidant, antitumor, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral activities[2], [3]. The twig extract of *G. mangostana* inn. Was the most useful sample against platelet aggregation caused by arachidonic acid [4] and several studies had been designed to examine the anticancer activities, hepatocellular carcinoma human leukemia of xanthones from mangosteen-fruit pericarp [5].

The concentration of secondary metabolites can differ between parts of the plant [6]. However, leaves are the most potent part of the plant to be used because they are not dependent on the season and interfere with the growth of the plant. Mangosteen leaves are proven to have high phenolic compounds and have the potential as antibacterial and antitumor activity [7]. Juice of mangosteen leaves was shown to have free radical scavenging activity against DPPH radicals with

IC_{50} 19 ppm [8]. The results of the previous research show the great potential as of *Garcinia mangostana*. Mainly leaves, as compared with fruit and bark, leaves are not explored to prove antibacterial and antifungal activity. The use of mangosteen leaves as traditional medicine is by making juice. The aims from this research to compare the action of the extracted juice and ethanolic extract of *G. mangostana* leaves as antifungal and antibacterial.

MATERIALS AND METHODS

Materials

Plant material. The leaf of *G. mangostana* (mangosteen) collection was carried out in Somagede Village, Somagede District, Banyumas Regency, Central Java, Indonesia and identified in the laboratory of Botany and genetic Faculty of FKIP, Universitas Muhammadiyah Purwokerto, Indonesia.

Antifungal and Antibacterial Assay. Fungal and bacterial strains used were *Saccharomyces cerevisiae* and *Bacillus subtilis* FNCC 0059 then *Escherichia coli* ATCC 35218. The organism was obtained from of Microbiology laboratory, Universitas Muhamamdiyah Purwokerto.

Methods

Preparation of juice. Leaves of *G. mangostana* amount three (3) kilograms were added water 3 liters at blander after bland was pressed with a flannel cloth and was concentrated using rotary evaporator and with water bath during five days with a temperature less than 40 ° C to get concentrated extract produces.

Preparation ethanolic extract. *G. mangostana* leaves were collected, dried and pulverized using a mechanical grinder. 500 grams of powder were extracted by maceration method with solvent water: ethanol 50% (1:5) during 24 hours and re-maceration with solvent water: ethanol 50% (1:4). After extract exhaustive extraction ethanolic extract was collected, then concentrated under reduced pressure at 40°C by using rotary evaporator.

Antifungal Assay

Identification of *Saccharomyces cerevisiae*. Identification of *S. cerevisiae*, conducted on PDA at room temperature for 48 hours to form colonies of soft cream-colored, having a smell like yeast. Youth culture will form seed tubes "germ tube" when placed in serum for 3 hours at temperature 37 ^{0} C.

Preparation of Medium

Potato Dextrose Agar (PDA). A total of 19.5 g of PDA (Potato Dextrose Agar) was weighed and 500 ml of distilled water heated on a hot plate, continually stirring until a homogeneous solution, distilled water is added to replace the volume lost due to heating precisely 500 mL. Further medium sterilized by autoclave at 121 ° C for 15 minutes (Aminiati, 2007).

Potato Dextrose Broth (PDB). 12 grams PDB (Potato Dextrose Broth) were weighed and 500 ml of distilled water heated on a hot plate, continually stirring until a homogeneous solution, distilled water is added to replace the volume lost due to heating precisely 500 mL. Further medium sterilized by autoclave at 121 ^{0} C for 15 minutes.

The culture of *Saccharomyces cerevisiae*. Cultures performed using methods that tilt, and all the tools that are used have been sterilized using an autoclave. The

yeast ose from 2 days streaking on PDA near a Bunsen flame, then closed with sterile cotton and incubated for 48 hours in an incubator with a temperature of 28 ^{0} C for later use in antifungal tests. All processes are carried out in the LAF (Laminar Air Flow), so to avoid contamination from the outside environment.

Calculation of Saccharomyces cerevisiae Yeast. One ose was of yeast *S.cerevisiae* 2 days old to be grown in a liquid medium of different PDB and then incubated for 48 hours at 28 ^{0} C. After 48 hours the number of colonies was calculated using the number of microbes indirectly, by using successive dilution of the concentration of 10^{-5} to 10^{-7} with distilled water. Then take one mL of the solution was added 15 mL PDA together and inserted into each petri dish and let it harden, and then incubated for 48 hours at 28 ^{0} C. The number of fungal colonies in a petri dish should meet the test of the 30-300 colonies (Lay, 1994).

Antifungal Activity Assay. The test is done using some fungal inoculums colony assay is 30-300 compliant. In a petri dish placed 7 paper disks that had been treated, as follows: one paper disk as negative control (10% DMSO), one paper disk with treatment (itraconazole), a paper disc with solvent control (distilled water) and four paper disks treatment with juice of mangosteen leaves each with concentration of 500 mg, 750mg, 1000mg, dan1250mg do replication 3 times. The medium used is made by taking as many suspension 1mL yeasts obtained dilution of test breeding, is poured into a petri dish. Then the media were thawed PDA was poured into a petri dish.

Furthermore, it was homogenized media culture with a shake form a figure 8. Hardening media was used as data to obtain the antifungal test by calculating the inhibitory zone. Paper disks placed on agar medium, then each poured by the positive control, negative control, distilled water control and each concentration using ten mL micropipette. Then incubated for 48 hours at 28 ° C. Inhibitory regions can be measured by looking at the diameter of the transparent area on each sample around the paper disk using calipers.

Antibacterial Assay

Preparation of Medium

Nutrient Agar (NA). 2.3 grams of NA, put it in Erlenmeyer and dissolved with 1000 mL of distilled water, then heated to evaporate completely. Furthermore, the NA solution which was still warm was poured into a test tube 20 mL 10 ml and 5 ml respectively, then sterilized in an autoclave at l2loC for 15 minutes.

Nutrient Borth (NB). 1.3 grams of NB, put it in Erlenmeyer and dissolved with 100 mL of distilled water, heated until it disappeared completely. Then the NB solution was poured into a test tube and sterilized in an autoclave at 12l^{o}C for 15 minutes.

The culture of *Bacillus subtilis* and *Escherichia coli*. Five mL NA medium that is still liquid and put in a test tube and tilted let it solidify. *Bacillus subtilis* and *Escherichia coli* derived from stock were taken with a sterile ose needle and put into a test tube containing aseptically solid NA and incubated at 37 ° C for 24 hours. Growing isolates was carried out by isolating *Bacillus subtilis*, and *Escherichia coli* derived from stock taken with a sterile ose needle then suspended in a test tube containing NB aseptically, then incubated at 37 ° C for 24 hours.

Antibacterial Activity Assay. The test is done using some bacteria inoculums colony assay is 30-300 compliant. 12 paper disc with a diameter of 2 mm prepares for six petri dish. In 12 disc paper dripped each concentration of juice and ethanolic extract of mangosteen leaves with a solvent with 10% DMSO, positive control was trickled with streptomycin 1µg/µl. Then in each petri dish which contained 20 ml NA media and a suspension of *Bacillus subtilis* and *Escherichia coli* as much as 1 ml placed sequentially six paper discs which had been penetrated by different concentrations of ethanolic extract and juice of mangosteen leaves. There also put paper discs for negative control, positive control, and solvent control. Then placed in an incubator at 37 ° C for 24 hours. The diameter of the inhibitory zone is observed.

RESULTS AND DISCUSSION

This study was designed to assess the antifungal activities from juice and ethanolic extract, also to determine the antibacterial activities of ethanolic extract of *G. mangostana.* Extract the juice of mangosteen leaves we get by way of blending fresh mangosteen leaves after pressed; the filtrate was concentrated used rotary evaporator to get the concentrated extract. The ethanolic extract obtained from fresh which dried in the sun covered with black cloth, then was bland with a blender to minimize the surface area so that the contact surface of the particles with ethanol as solvent bigger bulbs and extraction more optimal. The method to extraction was used is maceration, by soaking the powder in the liquid botanicals solvent, is done stirring and re-maceration to improve the effectiveness of the extraction, macerated for 24 hours with a comparison between simplicia. Extracts derived either from the juice and extract was evaporated with a rotary evaporator and over a water bath until thick consistency. Fading of the extract was done to eliminate solvent solution so as not to affect the antifungal, antibacterial activities assay.

Leaves from *G. mangostana* was bland and then concentrated and got thick extract 77.14 g and ethanolic extract obtains 116,94 g with rendemen 2.571 and 5,647 % (w/w) shown in Table 1. The results of an organoleptic extract of juice and the ethanolic extract are scent typical, bitter taste, and the color is brown.

Table 1. Rendement juice and extract *G. mangostana* leaves

	Juice		**Ethanolic extract**		
Fresh Leaves (g)	Thick extract (g)	Rendement (% w/w)	Simplisia (g)	thick extract (g)	Rendement (% w/w)
3000	77.14	2.571	500 (from 2 kg leaves)	116,94 g	5,647 %

The calculation of the number of colony *Saccharomyces cerevisiae* using the Total Plate Count (TPC) in Table 2 showed fungal cultures are a qualified suspension in dilution 10^{-6}. The results of the test antifungal activity juice and ethanol extracts in Table 3 shows that ethanolic extract had the greater inhibitory power of the *G. mangostana* leaves with MIC 500, and 1000 mg/ml.

Table 2. Calculation of the number of colonies *Saccharomyces cerevisiae*

Dilutions	The number of colonies in a petri dish	
	1	2
10^{-5}	310	325
10^{-6}	110	90
10^{-7}	10	22

Table 3. Antifungal Activity from Juice and Ethanolic Extract

Concentration (mg/ml)	Zone of Inhibition (cm)			
	I	II	III	Average ± SD
Juice				
500	1.855	1.635	1.745	1.745±0.110
750	2.210	1.935	1.895	2.013±0.171
1000	2.145	2.095	2.080	2.107±0.034
1250	2.145	2.310	2.025	2.160±0.143
Positive Control	1.370	1.025	1.790	1.395±0.383
Ethanolic	**Extract**			
500	-	-	-	-
750	-	-	-	-
1000	0.970	0.955	1.10	1.008±0.079
1250	1.00	1.11	1.115	1.075±0.065
Positive Control	1.025	1.52	1.09	1.212±0.269

Table 4 and 5 showed that in the ethanolic extract of mangosteen leaves had antibacterial activity against the *Bacillus subtilis* as gram-positive bacteria and *Escherichia coli* bacteria as gram-negative bacteria in each extract concentration and positive control of Streptomycin 1 µg/µl gave the zone of inhibition. While the 10% DMSO solvent control as extract solvent and 70% ethanol control as a Simplicia solvent did not have antibacterial activity because it could not produce clear around the disc paper. Geetha et al. [7] reported that antibacterial activity from aqueous and ethanolic extract of *G. mangostana* fruit rinds was potential with the inhibition zone in *E.coli, Shigella dysenteriae, Vibrio cholerae, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aerugenosa, Staphylococcus aureus*.

Table 4. Antibacterial Activity Ethanolic Extract against *Bacillus subtilis*

Concentration (mg/ml)	Zone of Inhibition (cm)			
	I	II	III	Average ± SD
250	11.15	11.27	12.40	11.607±0.690
500	16.30	16.25	15.00	15.850±0.737
750	19.20	19.10	19.25	19.183±0.076
Positive Control	23.35	24.50	24.45	24.100±0.650

Table 5. Antibacterial Activity Ethanolic Extract against *Escherichia coli*

Concentration (mg/ml)	Zone of Inhibition (cm)			
	I	II	III	Average ± SD
250	12.05	11.05	11.30	11.467±0.520
500	17.05	18.07	16.10	17.073±0.985
750	20.15	21.00	20.50	20.550±0.427
Positive Control	23.35	12.40	18.00	17.917±5.475

G. mangostana leaves contain polyphenolic major role in the prevention of various diseases [8]. Juice of *G. mangostana* contains polyphenolic compound such as flavonoid and tannin [9]. *G. mangostana* leaves have more potential activity than peel. Bark. And essential oils for against DPPH as radical [10]. *G. mangostana* also contain α-mangostin compounds which are proven to have potential antifungal and antibacterial activity [11]. Xanthon is the major compound of *G. mangostana* showed high-antifungal activity (against *Candida albicans* and *Aspergillus niger*) and antibacterial activity (against *Bacillus subtilis*. *Escherichia coli*. *Staphylococcus aureus*. *Pseudomonas aeruginosa*) [12].

Juice and ethanolic extract of *G. mangostana* leaves have the effect of inhibiting the growth of fungi cause food spoilage as *S. cerevisiae* FNCC 3012. Mechanisms of antimicrobial compounds in inhibiting the growth of mold in some way damage the structure of the cell wall by inhibiting the formation or cause lysis of the cell wall is formed. Changing the permeability of the cytoplasmic membrane which will cause poor growth or death of cells. Denaturation of proteins. As well as inhibiting the enzyme in resulting in disruption of cell metabolism or cell death.

Antibacterial activity of ethanolic extract *G. mangostana* on *Bacillus subtilis* is bactericidal while *Escherichia coli* are bacteriostatic. This difference is likely to occur due to differences in cell wall composition in *Bacillus subtilis* as gram-positive bacteria and *Escherichia coli* as gram-negative bacteria. Single-layered gram-positive bacterial cell wall with 1-4% lipid content. Moderate to

three-layered cell wall gram-negative bacteria consisting of lipoproteins. Outer membrane phospholipids and lipopolysaccharides. and lipid content in cell walls ranging from 11-22%. The outer phospholipid membrane causes antibacterial chemical components that are difficult to penetrate the cell wall of gram-negative bacteria.

CONCLUSIONS

Antifungal activities of juice and ethanolic extract of *G. mangostana* leaves were 1000 and 500 mg/ml in MIC against *Saccharomyces cerevisiae*. Antibacterial activity from the ethanolic extract of *G. mangostana* leaves was potential with the inhibition zone in *Bacillus subtilis* and *Escherichia coli*. Needs further confirmation of compounds that is containing in juice and ethanolic extract.

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