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Research Article

# ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF JUICE AND ETHANOLIC EXTRACTS OF *GARCINIA MANGOSTANA* L. LEAVES

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**ABSTRACT**

**Objective:** *Garcinia 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.

**Methods:** Juice and ethanolic extract were concentrated using a rotary evaporator to get concentrated extract with rendemen 2.571 and 5.647% (w/w). Juice 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:** The results of this research showed that juice and ethanolic extract were effective against *S. cerevisiae*, and the minimum inhibitory concentration was 1000, and 500 mg/mL. Antibacterial activity of the *G. mangostana* leaves ethanolic extract showed that the action was potential with the inhibition zone in *B. subtilis* and *E. coli.*

**Conclusions:** The conclusion of this study is that juice and ethanolic extract of *G. mangostana* leaves have possible antifungal and antibacterial activity.

**Keywords:** *Garcinia mangostana*, Antifungal, Antibacterial, Juice, Ethanolic extract.

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## INTRODUCTION

Mangosteen (*Garcinia 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 *G. mangostana* have remarkable biological activities such as antioxidant, antitumor, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral activities [2,3]. The twig extract of *G. mangostana* 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. The leaves of *G. mangostana* are proven to have high phenolic compounds and have the potential as antibacterial and antitumor activity [7]. Juice of leaves of *G. mangostana* was shown to have free radical scavenging activity against α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals with $IC_{50}$ 19 ppm [8]. The results of the previous research show the great potential as of *G. mangostana*. Mainly leaves, as compared with fruit and bark, leaves are not explored to prove antibacterial and antifungal activity. The use of leaves of *G. mangostana* as traditional medicine is by making juice. The aims from this research are to compare the action of the extracted juice and ethanolic extract of leaves of *G. mangostana* 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 Muhammadiyah Purwokerto.

### Methods

#### *Preparation of juice*

Fresh leaves of *G. mangostana* amount 3 kg were added water 3 l at blander after bland was pressed with a flannel cloth and was concentrated using rotary evaporator and with water bath during 5 days with a temperature <40°C to get concentrated extract produces.

*Preparation ethanolic extract*

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

**Antifungal assay**

*Identification of S. cerevisiae*

Identification of *S. cerevisiae* conducted on potato dextrose agar (PDA) at room temperature for 48 h 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 h at temperature 37°C.

**Preparation of medium**

*PDA*

A total of 19.5 g of PDA were weighed and 500 mL of distilled water heated on a hot plate, continually stirring until a homogeneous solution, and 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 min (Aminiati, 2007).

*Potato dextrose broth (PDB)*

12 g PDB were weighed and 500 mL of distilled water heated on a hot plate, continually stirring until a homogeneous solution, and 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 min.

*The culture of S. 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 h in an incubator with a temperature of 28°C for later use in antifungal tests. All processes are carried out in the laminar air flow, so to avoid contamination from the outside environment.

*Calculation of S. 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 h at 28°C. After 48 h, the number of colonies was calculated using the number of microbes indirectly, using successive dilution of the concentration of $10^{-5}$–$10^{-7}$ with distilled water. Then, take 1 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 h at 28°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 inoculum 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% dimethyl sulfoxide [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, 750 mg, 1000 mg, and 1250 mg do replication 3 times. The medium used is made by taking as many suspension 1 mL yeasts obtained dilution of test breeding and is poured into a petri dish. Then, the media were thawed and PDA was poured into a petri dish. Furthermore, it was homogenized media culture with a shake form. 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 10 mL micropipette. Then, incubated for 48 h 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 g 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 121°C for 15 min.

Nutrient broth (NB)

1.3 g 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 121°C for 15 min.

*The culture of B. subtilis and E. coli*

5 mL NA medium that is still liquid and put in a test tube and tilted let it solidify. *B. subtilis* and *E. 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 h. Growing isolates was carried out by isolating *B. subtilis*, and *E. 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 h.

*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 1 µg/µl. Then, in each petri dish which contained 20 mL NA media and a suspension of *B. subtilis* and *E. 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 h. 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 and also to determine the antibacterial activities of ethanolic extract of *G. mangostana.* Extract the juice of leaves of *G. mangostana* we get by way of blending fresh leaves of *G. mangostana* 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

**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% |

*G. mangostana: Garcinia mangostana*

for 24 h with a comparison between simplicia. Extracts derived either from the juice or extract were 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.

The calculation of the number of colony *S. cerevisiae* using the total plate count in Table 2 showed that fungal cultures are a qualified suspension in dilution $10^{-6}$. The results of the test antifungal activity

**Table 2: Calculation of the number of colonies *S. cerevisiae***

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

*S. cerevisiae: Saccharomyces cerevisiae*

**Table 3: Antifungal activity from juice and ethanolic extract**

| Concentration (mg/ml) | Zone of inhibition (cm) | | | Average±SD |
|---|---|---|---|---|
| | I | II | III | |
| 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 |

SD: Standard deviation

**Table 4: Antibacterial activity ethanolic extract against *B. subtilis***

| Concentration (mg/ml) | Zone of inhibition (cm) | | | Average±SD |
|---|---|---|---|---|
| | I | II | III | |
| 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 |

SD: Standard deviation, *B. subtilis: Bacillus subtilis*

**Table 5: Antibacterial activity ethanolic extract against *E. coli***

| Concentration (mg/ml) | Zone of inhibition (cm) | | | Average±SD |
|---|---|---|---|---|
| | I | II | III | |
| 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 |

SD: Standard deviation, *E. coli: Escherichia coli*

juice and ethanol extracts in Table 3 showed that ethanolic extract had the greater inhibitory power of the *G. mangostana* leaves with minimum inhibition concentration (MIC) 500 and 1000 mg/mL.

Tables 4 and 5 show that in the ethanolic extract of mangosteen leaves had antibacterial activity against the *B. subtilis* as Gram-positive bacteria and *E. coli* bacteria as Gram-negative bacteria in each extract concentration and positive control of Streptomycin 1 µg/µl gave the zone of inhibition. The DMSO solvent control as suspending agent of extract and 70% ethanol solvent control 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 aeruginosa, and Staphylococcus aureus*.

*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 contains α-mangostin compounds which are proven to have potential antifungal and antibacterial activity [11]. Xanthone is the major compound of *G. mangostana* showed high-antifungal activity (against *Candida albicans* and *Aspergillus niger*) and antibacterial activity (against *B. subtilis*, *E. coli, S. aureus*, and *P. 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 are formed. Changing the permeability of the cytoplasmic membrane which will cause inhibit growth or death 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 *B. subtilis* is bactericidal while *E. coli* is bacteriostatic. This difference is likely to occur due to differences in cell wall composition in *B. subtilis* as Gram-positive bacteria and *E. coli* as Gram-negative bacteria. Single-layered Gram positive bacterial cell wall with 1-4% lipid content. Three-layered cell wall Gram negative bacteria consisting of lipoproteins. Outer membrane contain phospholipids and lipopolysaccharides, with lipid content in cell walls ranging from 11 to 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 *S. cerevisiae*. Antibacterial activity from the ethanolic extract of *G. mangostana* leaves was potential with the inhibition zone in *B. subtilis* and *E. coli*. Needs further confirmation of chemical compounds that are containing in juice of mangosteen leaves.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declared that they had no conflicts of interest with respect to their authorship or the publication of this article.

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