



Antioxidant and Antifungal Activities of Temu mangga (*Curcuma mangga* Val.) Extract in Some Solvents

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Abstract

Temu mangga (*Curcuma mangga* Val.) has been widely used to overcome health problems in Indonesia, one of them used as the ingredient of Madurese herbal medicine. The active compounds contained in *C. mangga* extract have potential as a medicine to improve female fertility. This study aims to determine the content of phytochemicals, antifungal, and antioxidant activity of *C. mangga* rhizome extract in some solvents based on their polarity. *C. mangga* rhizome was extracted by a maceration method using ethanol (polar), chloroform (semi-polar), and n-hexane (non-polar) solvents. Standard phytochemical methods were used for preliminary phytochemical screening of the plant extracts. Antioxidant activity was tested using the DPPH method with extract concentrations of 25, 50, 100, 200, and 400 ppm. The antifungal activity test against *Candida albicans* used the Kirby Bauer method with a 100% concentration and microdilution method with percent concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39%. Phytochemical analysis of the ethanol and n-hexane extracts showed the presence of alkaloids and triterpenoids, while chloroform extracts revealed the presence of triterpenoid only. The antioxidant activity test of ethanol extract, chloroform, and n-hexane yielded IC₅₀ values of 99.33 ppm (active), 119.3 ppm (medium), and 192.1 ppm (medium) and the inhibitory zones of *C. albicans* were 5.17 ± 1.37 , 1.78 ± 1.09 , and 3.43 ± 1.40 mm, respectively. MIC values of all extracts studied were obtained at 0.78% concentration and MFC values at 1.56%. The rhizome of *C. mangga* extracts contain flavonoid, alkaloid, and triterpenoid that have antioxidant properties and antifungal activities against *C. albicans*. Ethanol is the most promising solvent for extracting the active compound of *C. mangga* rhizome as an antioxidant and antifungal.

Keywords: Antioxidant, Antifungal, *C. albicans*, *C. mangga*, Phytochemical.

1. Introduction

Temu mangga (*Curcuma mangga* Val.) is one of a medicinal herb that has been widely used to overcome health problems, especially in Indonesia. This plant was reported to have

some active compounds such as essential oils, alkaloids, flavonoids, tannins, terpenoid [1] and curcuminoid include curcumin, demethoxycurcumin and bisdemethoxycurcumin [2]. Phytochemical

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content, antioxidant and antimicrobial activity present in *C. mangga* extract have potential as a medicine to improve female fertility. The rhizome of *C. mangga* is often used in traditional medicine to treat vaginal discharge, diarrhea, acne remedies, and itching [3]. The content of compounds in *C. mangga* oil also reported has the highest and most broad-spectrum activity by inhibiting *C. albicans*, *C. neoformans*, *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Inhibition of pathogenic fungi is influenced by active compounds contained in rhizomes [4].

Infertility is a problem that often threatens the reproductive system in men and women, especially in Indonesia. Infertility is defined as the failure to achieve pregnancy after 12 months or more of sexual intercourse without contraception. The World Health Organization (WHO) states that the number of infertile couples is due to male abnormalities (36%) and female abnormalities (64%) [5].

Infertility can be corrected with synthetic drugs, alternative medicines, and physiotherapy, but this does not always result in pregnancy and live birth. Management of female infertility with Chinese herbal medicine can improve pregnancy rates two-

fold within a 3-6-month period with Western medical fertility drug therapy. Also, fertility indicators such as ovulation rates, cervical mucus scores, biphasic basal body temperatures, and appropriate thickness of the endometrial lining were positively influenced by Chinese herbal medicine therapy, indicating an ameliorating physiological effect via a viable pregnancy [6]. In developed countries that have widely used modern medicine, recently, there has been a tendency to use traditional medicines and medicines from plants [7]. WHO recommends the use of traditional medicines including herbs in public health maintenance, disease prevention, and treatment, especially for chronic degenerative diseases. WHO also promotes the safety, quality, and effectiveness of traditional medicines by developing national policies, regulatory frameworks, and strategic plans for traditional medicines products, practices, and practitioners [8].

As a developing country, Indonesia also has one of traditional medicine known as *jamu subur kandungan* to overcome the fertility problem in women. *C. mangga* rhizome extract is one of the basic ingredients of *jamu subur kandungan*. However, to date, the evaluation of *C. mangga* rhizome extract properties as the basic ingredient of *jamu subur kandungan* still needs further evaluation. Thus, this work aimed to probe the appropriate solvent that would promote antioxidant and antifungal activity of *C. mangga* rhizome extract. The results from this experiment could be used as guidance for

further standardization of Madurese herbs, particularly *jamu subur kandungan*.

2. Materials and Methods

2.1. Sample Preparation

Simplicia of *C. mangga* was obtained from UPT Materia Medica Batu Indonesia, whereas *C. albicant* 41-SV isolate was purchased from the Microbiology Laboratory of Medical Faculty of Brawijaya University. Plant identification was confirmed according to the taxonomical book of Flora of Java [9]. Extract preparation method was referred to the research of Muchtaromah *et al.* [10] with variations of polarity solvent, ethanol (polar), chloroform (semi-polar), and n-hexane (nonpolar).

2.2. Moisture Content

A total of 5 g rhizome was dried in the oven (Model Memert) at 104 °C until reaching constant weight. The initial weight and final weight were taken; the weight difference for each sample represents its moisture content. The moisture content determination process was repeated three times. The percent of moisture content was reported in this study. The thermogravimetric method was used to determine the water content of the simplicia before the extraction process [11].

2.3. Extraction Using Maceration Method

The process of maceration was done by weighing the sample then immersing it in the solvent for 24 h. During the immersion process, samples were shaken several times using a shaker at 150 rpm for 9 h. The extracts

obtained were then filtered. Solvent evaporation was done using a rotary evaporator vacuum at 50 °C. The evaporation was stopped until the concentrated extract was obtained and then characterized by the cessation of the solvent flow in the container flask [10].

2.4. Phytochemical Screening Test

All tests of phytochemical screening (alkaloids, flavonoids, terpenoids, saponins, tannins, steroids) followed the procedure described by Tiwari *et al.* [12].

2.4.1. Alkaloid Test

Samples were inserted in the test tube, 0.5 ml of 2% HCl was added, and the solution was divided into two tubes. Three drops of Dragendorff reagent were added to the first tube and three drops of Meyer reagent were added to the second. If an orange precipitate formed in the first tube and a yellow or white precipitate formed in the second tube, this indicated the presence of alkaloids.

2.4.2. Flavonoid Test

Samples were inserted in a test tube then dissolved in 1-2 ml of 50% hot methanol. After that Mg metal and 0.5 mL concentrated HCl was added. If a red or orange solution is formed, this indicates the presence of flavonoids.

2.4.3. Triterpenoid and Steroid Test

The sample was put into a test tube, dissolved in 0.5 mL of chloroform, and then 0.5 mL of anhydrous acetic acid was added.

This mixture was further coupled with 1-2 mL of concentrated H₂SO₄ through the tube wall. If the results obtained are in the form of a brown or violet ring on the border of two solvents, this indicates the presence of triterpenoids, whereas if a bluish-green color appears, it indicates the presence of steroids.

2.4.4. Saponin Test

The samples were inserted into the test tube, and water was added (1:1) while shaking for 1 minute. Sample foam was added to 2 drops of HCl 1 N and left for 10 minutes. If the formed foam can remain stable, then the positive extract contains saponin.

2.4.5. Tanin Test

The sample was put into a test tube, and 2-3 drops of 1% FeCl₃ solution were added. If the solution produces a blackish-green color, that indicates the presence of the catechol tannin compound and the blackish-blue color indicates the presence of a tannin galate compound.

2.5. Antioxidant Activity Assay

The antioxidant activity of the extract was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as conducted by Sabahannur *et al.* with some modifications [13]. The DPPH solution was prepared in ethanol, chloroform, and n-hexane. Each extract was prepared in various concentrations (25, 50, 100, 200, and 400 ppm). The extract (2 mL) was added to 2 mL of DPPH solution (50 mg/mL) to initiate the reaction for obtaining a calibration curve. The absorbance

at 515 nm was measured after incubation for 30 min by using an ultraviolet UV-Vis spectrophotometer (Beckman Coulter DU 720). DPPH (50 mg/mL) was used as the control, ascorbic acid (Vit C) as the standard, and ethanol as the blank. The analysis was conducted in triplicate for the standard and each extract. The antioxidant activity was revealed as IC₅₀ of DPPH scavenging activity by observing the 50% inhibitory concentration for each extract using the calibration curve. According to Jun *et al.* [14] antioxidant activity was categorized as strong (IC₅₀ 0 < 50 ppm), active (IC₅₀ 50-100 ppm), moderate (IC₅₀ 101-250 ppm), weak (IC₅₀ 250-500 ppm), and not active (IC₅₀ > 500 ppm).

2.6. Antifungal Activity Assay

Antifungal activity of *C. mangga* extract included a diameter of inhibition zone, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC).

Determination of inhibition zone diameter uses a diffusion technique (Kirby Bauer method) with a concentration of 100% according to the CLSI procedure with some modifications [15]. A total of 0.1 g of *C. mangga* extract using several solvents (ethanol, chloroform, and n-hexane) was diluted to a total volume of 100 µl into the final concentration of 10⁶ ppm. Three sterile disc papers (6 mm) were inserted into a *C. mangga* extract solution and saturated for 30 minutes. The disc paper was then inserted into the SDA (Sabouraud Dextrose Agar) that already dispersed with *C. albicans*. It was then incubated at a temperature of 35 ± 2°C for 18-

24 hours. The classification of the inhibitory zone is classified as strong (> 6mm), moderate (3-6 mm), and weak (0-3 mm) [16].

MIC was determined out using the microplate dilution method with Sabouraud Dextrose Broth (SDB) media, following the CLSI procedure [17]. Sterile microplates consisted of: well no.1 (line1-3/triplo) = microbial control (*C. albicans* suspension adjusted to McFarland standard), well no. 2 (line 1-3)= material control (*C. mangga* extract 100%, line 1: n-hexane extract, line 2: chloroform extract, line 3: ethanol extract), and well no. 3-10 = serial dilution of *C. mangga* extract (line 1-3 (triplo): n-hexane extract, line 4-6 (triplo): chloroform extract, line 7-10 (triplo): ethanol extract). Serial dilution was then performed to produce final concentrations (50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, and 0.39%). The final volume per well was 200 μ L. The microplate was incubated at 37 °C for 18-24 hours, then turbidity was observed and compared with control. No turbidity in the well indicated the MIC.

MFC determination was done by taking 10 μ L samples from each well then diluted to 10^6 and inoculated on solid SDA with a spread technique then incubated at 37 °C for 18-24 hours. The next day, the number of colonies grown on a plate was counted using a colony counter. MFC value was characterized by the absence of microbial growth in the SDA or the growth of the colony less than 0.1% of the original inoculum/OI colonies [17].

2.7. Data Analysis

The percentage of inhibition represented percent of antioxidant activity analyzed using a nonlinear regression test by SPSS 16.0. Data regarding inhibitory zone, MIC, and MFC were presented descriptively in the form of figures and tables.

3. Results and Discussion

3.1. Moisture Content and Extraction Result

The water content analysis aimed to determine the percentage of water in the sample. Based on the results of the measurement of the dry water content of the samples, the data obtained were 9.9%, 9.7%, and 9.6%, respectively, with an average of 9.73%. This indicated that the sample had good water content for the extraction process. The smaller moisture content of the dry matter leads to better extraction process. According to the National Agency of Drug and Food Control Republic of Indonesia, the standard water content for a dry matter is 10-12%, thus the extraction of the active compound by the solvent is not blocked by water and the process can run optimally.

Extraction is the process of attracting the active compound of a material by a suitable solvent and method so that the desired result can be perfectly extracted [18]. The extraction method used maceration with ethanol (polar) solvent, chloroform (semi-polar), and n-hexane (nonpolar). Each solvent will attract certain compounds based on the degree of polarity of the compound. Ethanol will attract polar and nonpolar compounds; chloroform will attract polar and nonpolar compounds,

while n-hexane will attract nonpolar compounds. The results of the maceration of dried powder of *C. mangga* were presented in Table 1.

The color difference between the extracts of the three solvents revealed that the types had different abilities to attract the bioactive compounds in a sample. Different solvents

Table 1. The yield of crude extract of *C. mangga* rhizome.

Solvents	Yield (%) (w/w)	Appearance
Ethanol	19.405	Dark brown, concentrated liquid
Chloroform	17.805	Dark brown, concentrated liquid
n-hexane	11.752	Brown, concentrated liquid

Table 1 showed that ethanol produced the highest yield of 19.405% with dark brown, concentrated liquid, followed by chloroform (17.805%), with dark brown concentrated liquid, and n-hexane (11.752%) with a concentrated thick liquid. The yield of the extract should depend on the polarity of the solvent used during preparation. Moreover, the solubility of the natural products and the choice of solvent could also determine the yield. A further test was qualitative phytochemical screening.

Biologically active compounds usually occur in low concentrations in plants. An extraction technique is able to obtain extracts with high yield with minimal changes to the functional properties of the extract required [19, 20]. Table 1 summarized that ethanol produced the highest yield of 19.40%, followed by chloroform of 17.80%, and n-hexane of 11.75%. This was because ethanol was a polar solvent and probably the *C. mangga* extract contained higher polar compounds than semi-polar and nonpolar compounds.

produced extracts with different weights, yields, textures, and colors [21]. The differences in the amount of this extract occurred because each solvent had different capabilities in attracting bioactive compounds in a sample. The texture of all the crude extracts of rhizome *C. mangga* was a concentrated liquid. This indicated that no more solvents were contained in the extract. The extract obtained is depend on several factors, namely the matrix properties of the sample, chemical properties of the analytes, matrix-analyte interaction, efficiency, and desired properties [22].

Ethanol, being organic and nontoxic, might have the highest frequency of use for extraction purposes. This solvent has good polarity and hence is used favorably to extract polar compounds such as phenolic compounds and flavonoids, which are believed to be effective antioxidants. Phytochemical processing of raw plant materials is essentially required to optimize the concentration of known constituents and also to maintain their activities [23]. Dhanani reported that extract

yield of *Withania somnifera* with ethanol (3.17%, 20 min) was about 3.74 times lower than the maximum extract yield (11.85%) obtained with water at 15 min. Possibly, this could be due to the higher polarity of water and also at elevated extraction temperature as in the case of ultrasound-assisted solvent extraction (UASE) and microwave-assisted solvent extraction (MASE), water has a similar dielectric constant as organic solvents such as methanol and acetonitrile [24].

In the case of MASE, the extract yield increased with the time of exposure to microwave radiation and water (13.02%, 20 min), and water-ethanol (13.75%, 20 min) was found to be comparable. The maximum yield of UASE and MASE was higher than the maximum extract yield using refluxing. The probable reasons for the highest yield in MASE may be due to direct heat generation within the volume with a significant impact on heating kinetics and also on the pressure effects on the cell wall membrane structure resulting in higher and faster diffusion or

partition rate of the solute from the solid matrix into solvent [25,26]. Besides, improved extract yield in the case of UASE may be explained in terms of the cavitation effects caused by high-intensity ultrasound [27, 28].

Hayouni *et al.* reported that water and organic solvents (acetone, chloroform, acetone/water/acetic acid [90/9.5/0.5] and ethyl acetate/methanol/water [60/30/10]) of *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts significantly affect the total of polyphenol content [22]. The results showed that solvents with different polarities had significant effects on antioxidant and antibacterial activities.

3.2. Qualitative Phytochemical Screening

Phytochemical testing of the extract is the first step that gives an overview of secondary metabolite compounds contained in each extract. The results of phytochemical screening were presented in Table 2.

This study revealed that the ethanol extract contains flavonoids and triterpenoids while the

Table 2. Phytochemical screening of *C mangga* rhizome extract.

Phytochemical	Reagen test	Extracts		
		Ethanol	Chloroform	n-hexane
Alkaloid	Dragendorff	-	-	+
	Mayer	-	-	-
Flavonoid	Wilstater	+	-	-
Triterpenoid	Lieberman-Burchard	+	+	+++
Steroid	Lieberman-Burchard	-	-	-
Saponin	Forth	-	-	-
Tannin	FeCl ₃	-	-	-

Note: (+++) very highly present, (++) highly present, (+) present, (-) not present

n-hexane extract contains alkaloids and triterpenoids. The chloroform extract contains triterpenoids only. The flavonoid compound was polar, dissolving in a polar solvent. The polarity of the compound was due to the flavonoids being polyhydroxy (having more than one hydroxyl group). Alkaloids were soluble in polar and nonpolar solvents. Triterpenoid/steroid compounds were soluble compounds in nonpolar solvents. This report was a bit different than that of Komala who reported that *C. mangga* was extracted by Soxhlet extraction using 96% ethanolic, which produced bioactive compounds consisting of flavonoids, saponins, quinone, and steroids. This difference might be due to the extraction method used. Thus, extraction was an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials. The selection of a suitable extraction technique was also important for the standardization of herbal products because they are used to dissolve the desired bioactive compounds [29].

3.3. Antioxidant Activity of *C. mangga* Extract

The antioxidant activity was determined using the DPPH assay. DPPH is a stable free radical compound and has an absorbance in its oxidized form, around 515-520 nm [30]. The DPPH assay is a relatively rapid and efficient method to evaluate free radical scavenging activity. DPPH is able to accept an electron or hydrogen radical to form a stable diamagnetic molecule. Changes in color, from purple to yellow, indicate a decrease in the absorbance of the DPPH radical. This demonstrates that the antioxidant found in a mixture solution interacts with the free radicals. In the present study, the percentage of inhibition (Table 3) was measured to determine the antioxidant activity of the extracts which were able to inhibit free radicals.

Based on the data in Table 3, the higher of solvent concentration leads to the higher percentage of inhibition. Five varying concentrations (25, 50, 100, 200, 400 ppm) of a different solvent extract of *C. mangga* demonstrated the different percentages of inhibition. Interestingly, the scavenging

Table 3. Data % antioxidant activity of *C. mangga* extract in some solvents compared to ascorbic acid (control).

(x) Concentration (ppm)	(y) Antioxidant Activity (% inhibition)			
	Ethanol extract	Chloroform extract	<i>n</i> -hexane extract	Ascorbic acid (Control)
25	26.022	13.404	8.644	39.853
50	34.578	25.550	17.162	93.025
100	47.033	36.138	28.632	92.909
200	69.737	69.883	50.061	92.181
400	73.315	89.091	73.427	91.203

activity of each extract was increased in a concentration-dependent manner. The 400 ppm of extracts showed the best antioxidant activity. The best antioxidant activity of the ethanol extract was 73.31, while in the chloroform extract, it was 89.09, and in n-hexane extract was 73.42. Ascorbic acid had the best antioxidant activity at 50 ppm of 93.03.

Antioxidant activity can be expressed as a percent of inhibition of DPPH, but may also be expressed as a concentration causing 50% inhibition of DPPH activity (IC_{50}). The value of IC_{50} is considered a measure of the efficiency of antioxidants in pure compounds or extracts. The value of IC_{50} can be defined as the amount of concentration that can inhibit 50% of DPPH free radical activity. The smaller the value of IC_{50} in the test sample, the greater the antioxidant activity is produced.

IC_{50} values were obtained from the percentage of antioxidant activity. IC_{50} and R^2 values of each extract and control were presented in Table 4 and Figure 1. Table 4 revealed that the lowest IC_{50} value was for ethanol (99.33 mg/L), followed by chloroform (119.3 mg/L), and n-hexane (192.1 mg/L). The ethanol extract was included in the active category whereas chloroform and n-hexane

extracts were categorized as moderate. However, the antioxidant activity of ethanol extract, chloroform, and n-hexane of *C. mangga* was lower than that of vitamin C, which had an IC_{50} value of 27.59 mg/L, classified as a strong category. The results showed that the highest coefficient of determination was obtained by n-hexane extract (0.9951). This means that the concentration of extract (x) influenced the percent of antioxidant activity (y) of 0.9951. If other x variables influenced besides variables known in this study, they only gave a maximum contribution as 0.0049.

The results of the qualitative analysis of samples having antioxidant activity noted the decreased color intensity of DPPH. The extracts from the various concentrations that were incubated changed color from purple to yellow. The higher concentrations of antioxidant compounds in the sample meant more compounds would donate electrons or hydrogen atoms to DPPH free radicals, which also caused color changes in DPPH [31]. Yang *et al.* reported that IC_{50} extract ethanol of *C. mangga* (99.33 ppm) is higher than ethanol extract *C. xanthorrhiza* (*temulawak*), and *C. domestica* (turmeric) (58.45 ppm and 29.64 ppm), but lower than *C. pandurata* extract

Table 4. Results of the coefficient of determination (R^2) and IC_{50} of *C. mangga* extracts compared to control.

No.	Sample	R^2 value	IC_{50} (mg/L)	Catagorized[9]
1.	Ethanol extract	0.9689	99.33	Active
2.	Chloroform extract	0.9742	119.3	Moderate
3.	n-hexane extract	0.9951	192.1	Moderate
4.	Vitamin C (C+)	0.9172	27.71	Strong

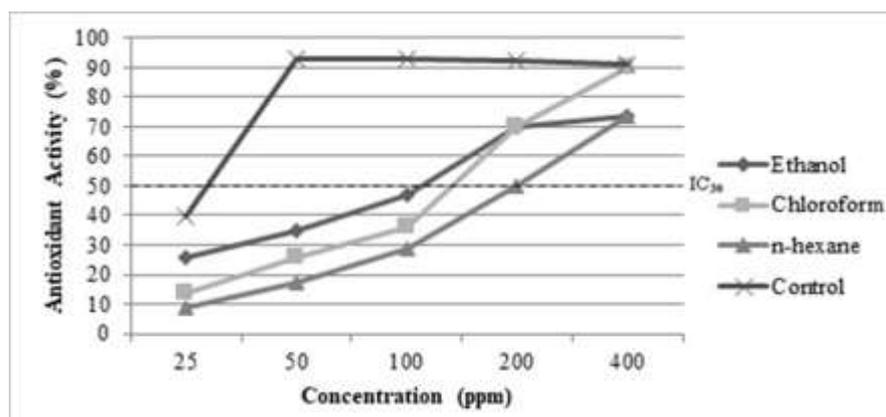


Figure 1. Percentage of antioxidant activity of *C. mangga* extract with various solvents compared to control (ascorbic acid).

(*temu kunci*) (140.21 ppm) [32].

Tables 3 and 4 summarized that ethanol extract of *C. mangga* had the highest antioxidant activity compared to chloroform and n-hexane extracts because of the active compound content of several classes of antioxidants, such as flavonoids and triterpenoids. Differences in antioxidant activity in each extract were thought to be related to the type of antioxidants contained therein. Yang *et al.* [32] reported that the type of antioxidant based on its solubility consisted of lipophilic antioxidants (soluble in nonpolar) and hydrophilic antioxidants (soluble in polar). According to Setzer *et al.* [33] triterpenoids or steroids are active compounds belonging to lipophilic antioxidants. Flavonoids are active compounds that are included in the type of antioxidant intermediates that act as hydrophilic and lipophilic antioxidants.

Flavonoids are compounds that act as antioxidants. The antioxidant mechanism of flavonoids is the direct capture of ROS, preventing ROS regeneration and indirectly can increase the antioxidant activity of cellular

antioxidant enzymes. Flavonoids are the most effective compounds as the scavenger of reactive species, for example, super dioxide, peroxy radicals, and peroxy nitrite, by transferring H^+ atoms. Flavonoids prevent the formation of ROS by inhibiting the action of xanthine oxidase enzymes and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and chelating metals (Fe^{2+} and Cu^{2+}), thus preventing the redox reactions that can produce free radicals [34].

Atmani *et al.* stated that flavonoids are antioxidants that play a role in protecting lipophilic antioxidants to strengthen cellular antioxidants [35]. Some studies have also shown that flavonoid antioxidant activity is closely related to the prevention of several diseases, such as cardiovascular disease, cancer or tumors, and liver disease [34]. Triterpenoids or steroids are compounds that play an antioxidant role. The antioxidant mechanism of triterpenoids is achieved by capturing or scavenging reactive species, for example, superoxide, and metal chelating (Fe^{2+} and Cu^{2+}). Triterpenoid compounds from

Annurca apple have activity as antioxidants and may inhibit lipid peroxidation. The biological activity of triterpenoids/steroids as an addition to antioxidants, serves as a hepatoprotection and analgesic, antitumor, antiproliferative, and provides immunomodulatory effects [35, 37].

Simplicia powders of *C. zedoaria*, *C. mangga*, and *C. xanthorrhiza* rhizome contain flavonoids, saponins, terpenoids, and essential oils. Pujimulyani reported that processed *C. mangga* is capable of inhibiting oxidation because it contains curcuminoid. The level of curcumin in ethanol extract of *C. mangga* is 0.19%. Curcuminoid is a group of phenolic compounds that have antioxidant and anti-inflammatory properties [38]. Melannisa reported that phenolic compounds that have been shown to have radical scavenging activity from rhizome of *C. domestica*, *C. xanthorrhiza*, *C. zedoaria*, *Boesenbergia pandurata* are curcuminoid compounds (*curcumin*, *demetoxicurcumin* and *bisdemetoxicurcumin*), xanthorizol, and panduratin A. The secondary metabolite compounds present in the extract of a plant are classified as secondary antioxidants [37]. Some flavonoids have inhibitory activity against organisms, and flavonoids can inhibit *Fusarium oxysporum* that causes pain to the plant [39, 40]. Rafael states, in general, the mechanism of action of secondary antioxidants is achieved by the cutting off of the chain oxidation reaction of free radicals or by capturing free radicals such that they will not react with cellular components [41]. The curcumin compound has been known to have

antioxidant activity and acts as a radical scavenger. Also, curcumin acts as a catalyst for the formation of hydroxyl radicals. This ability makes curcumin able to act as a radical scavenger to the reactive intermediate compound from carcinogen, thereby reducing the incidence of cancer. Curcumin is a potent antioxidant and an antidote to oxygen and nitrogen radicals from biological processes occurring in the body. Curcumin is also potent as a lipid peroxidase inhibitor induced by various cellular or foreign agents. This property may have an important role in the mechanism of action of curcumin as an antioxidant, antibacterial, anti-inflammatory, antitumor, and other pharmacological activities [42].

Chloroform and n-hexane extracts also have antioxidant activity even in moderate categories. The difference in activity between extracts is probably due to differences in the type and amount of active compounds contained in the extract so that its antioxidant activity in capturing DPPH free radicals is also different. Antioxidant activity is not only played by a class of polar compounds but also can be played by a class of nonpolar compounds, including the class of nonpolar flavonoids, alkaloids, and triterpenoids. Flavonoid glycosides in the form of nonpolar aglycons have higher antioxidant activity when compared with polar glycons [43].

Rita reports that phytochemical screening of n-hexane extract of *C. mangga* produces alkaloids, flavonoids, saponins, and triterpenoids [43]. The alkaloid and triterpenoid compounds have more OH (polar)

groups than CH (nonpolar). This OH group has a role in donating its hydrogen atom so that the DPPH radical becomes stable and the antioxidant compound serves as a radical. Alkaloid compounds, flavonoids, and triterpenoids in ethanol extract of *temu mangga* are thought to be able for increasing super oxide dismutase (endogen antioxidant) secretion in cells to counteract free radicals by activating Nrf2 (Nuclear Factor Erythroid 2). Nrf2 plays an important role in normal activity and induces genes coding for some antioxidants [44]. Cells that experience oxidative stress will trigger Nrf2 to move to the nucleus and bind to ARE (Antioxidant Response Elements). ARE is a promoter of genes producing endogenous antioxidant enzymes. Binding between Nrf2 and ARE will enable transcription and translation for protein synthesis of endogenous antioxidant enzymes such as SOD to fight oxidative stress induced by free radicals [45].

In addition to exogenous antioxidant

zinc (Zn), and manganese (Mn) so that if the metal content is available in sufficient quantities, it will trigger the maximum SOD work. The active side of SOD in the form of Cu-Zn minerals is important in the free radical catalyst process. *C. mangga* extract can be used as a source of natural antioxidants. A lot of synthetic antioxidants have been found, although cheap and able to be produced in large quantities, this material can cause unwanted side effects, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene that can damage the liver [45].

3.4. Antifungal activity of *C. mangga* extract

Table 5 revealed that the highest values for the inhibition were for ethanol extract (5.17 ± 1.37), followed by n-hexane (3.43 ± 1.40), and chloroform (1.78 ± 1.09). Nystatin, as is a standard antifungal agent, produced a higher inhibition zone than *C. mangga* extracts. The next step was a well plate diffusion test. This method was used because in preliminary tests

Table 5. Inhibition zone of *C. mangga* extract with various solvents and control.

No.	Sample	Inhibition zone (mm) ±SD	Categorized[16]
1	Ethanol extract	5.17 ± 1.37	Moderate
2	Chloroform extract	1.78 ± 1.09	Weak
3	n-hexane extract	3.43 ± 1.40	Moderate
4	Nystatin (C+)	18.43 ± 0.46	Strong

compounds, the content of ions in exogenous antioxidants is thought to increase SOD production. This is because SOD is a metalloenzyme that has an active side derived from metal elements, namely copper (Cu),

using the tubular dilution method, the turbidity of the extracts could not be observed because all the tubes were cloudy when compared with C+ (*C. albican* concentration 100%) (figure2).

Table 6. Total colony account of *C. albicans*, MIC and MFC value of *C. mangga* extracts with various solvents.

Sample test	Total Colony Account (CFU/mL)		
	Ethanol extract	Chloroform extract	n-hexane extract
C+	123 x 10 ⁹	123 x 10 ⁹	123 x 10 ⁹
0.39 %	105 x 10 ⁹	78 x 10 ⁹	92 x 10 ⁹
0.78 %	36 x 10 ⁶ (MIC)	65 x 10 ⁶ (MIC)	66 x 10 ⁶ (MIC)
1.56 %	0 (MFC)	0 (MFC)	0 (MFC)
3.13 %	0	0	0
6.25 %	0	0	0
12.50 %	0	0	0
25.00 %	0	0	0
50.00 %	0	0	0
C-	0	0	0

Based on the microplate dilution plate assay after incubation, it was found that the turbidity of *C. albicans* could only be observed directly at well 10 and well 1 (Figure 2). Therefore, all samples were grown on solid media with a spread plate method.

The result of the spread plate technique was presented in Table 6. The MIC values of each extract were found at 0.78% concentration. This concentration was the smallest concentration that could still inhibit microbial growth, characterized by the ability of *C. albicans* to still grow after spread plating and calculated by using a colony counter. The best extract to kill *C. albicans* was ethanol extract (3.6 x 10⁷), followed by chloroform extract (6.5 x 10⁷), and n-hexane extract (6.5 x 10⁷). The determination of MFC from *C. gallate* extracts had a requirement of a total colony count of $\leq 0.1\%$ of original inoculum (OI) which is $\leq 1.23 \times 10^8$ CFU/ml. The MFC value

of *C. mangga* extracts was obtained at a concentration of 1.56%.

The size of the inhibition zone of *C. mangga* extract is due to the active compound content which acts as an antifungal, the compounds that play a role in inhibiting the growth of *C. albicans*. The solvent difference is one of the factors that can influence the extraction result [4]. In this research, ethanol extract of *C. mangga* was found to contain flavonoid and triterpenoid compounds, while chloroform extract contains triterpenoids, and n-hexane extracts contain alkaloids and triterpenoids. Each compound probably has antimicrobial activity with different mechanisms. Borman *et al.* revealed *C. mangga* proved to have antifungal activity against *C. albicans*, *C. kruseii*, and *C. parapsilopsis* species [46]. Ariviani *et al.* [47] found that *C. mangga* extract has several secondary metabolite compounds that have

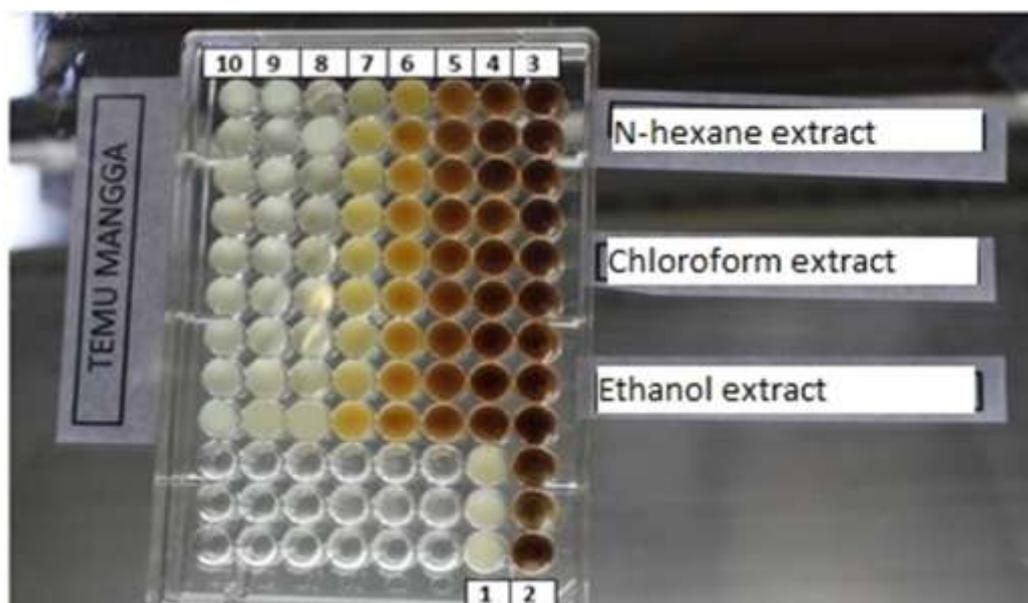


Figure 2. The turbidity level of *C. mangga* extracts with various solvents using the microdilution liquid method. well 1 (triplo): C+/C. albicans solution adjusted to Mc Farland standard, well 2: C-/extract 100% (Line (L) from up L1: n-hexane, L2: chloroform, L3: ethanol), well 3-10: extract stratified dilution 50%, 25%, 12.5%, 6.25% , 3.13%, 1.56%, 0.78%, 0.39% (L1-3 (triplo): n-hexane, L4-6: chloroform, L7-9: ethanol).

proved to have antifungal activity. They are flavonoids, alkaloids, triterpenoids, curcumin, and essential oils. The main target of antifungal content in *C. mangga* is the cell wall. The cells of *C. albicans* cells are composed of polysaccharides (mannan, glucan, chitin), proteins, and lipids with subcutaneous cell membranes containing sterols [48]. Denaturation of *C. albicans* cell wall proteins will lead to the fragility of the fungus cell walls that can be easily penetrated by fungistatic substances [36].

Curcumin is a group of phenolic compounds. Phenol compounds cause coagulation or protein clumping. The clumped protein is denatured, and in such a condition,

the protein does not work anymore. Also, reduction in proteinase secretion and alteration of membrane-associated properties of ATPase activity are other possible critical factors for the antifungal activity of curcumin [49]. Pandey and Kumar stated that the cytoplasmic membrane is composed primarily of proteins and fats, and the membrane is susceptible to phenol. Phenols can reduce surface tension and in high concentrations, cause total cytoplasmic membrane damage and precipitate proteins. In low concentrations, phenol can damage the cytoplasmic membrane leading to leaking important metabolites and inactivating some microbial enzyme systems [36].

Flavonoids are compounds that have pharmacological effects as antifungals. Flavonoids can form complexes with proteins and damage cell membranes by denaturing the membrane protein bonds so that cell membranes undergo lysis. Nonpolar triterpenoid compounds will more easily penetrate the fungi cell wall because many are composed of lipids [19]. Ahmad states that triterpenoid is a terpenoid group compound, which also has the potential as an antifungal [50]. Terpenoid compounds dissolve in fat, which can penetrate the fungi cell membrane then affect the permeability and cause disruption to the structure and function of cell membranes.

Intracellular acidification through inhibition of H⁺ extraction was identified as a possible mechanism for cell death of *Candida* species [51]. A medicinal substance is categorized as antimicrobial if it has functioned as fungistatic and fungicidal. Fungistatic is the ability of a drug to inhibit the growth of bacteria at certain levels, while fungicidal is the ability of drugs to kill bacteria at certain levels. According to Borman et al., the fungistatic activity can be increased to a fungicide if the antifungal level is increased beyond the MIC value. MFC observations of *C. albicans* showed that extracts with 0.156% content were bactericidal (microbial killing) because microbial growth was not visible, as was extract at higher levels (3.13%) [46].

4. Conclusion

The rhizome of *C. mangga* extracts contains flavonoids, alkaloids, and

triterpenoids that have antioxidant properties and antifungal activities against *C. albicans*. Ethanol is the most promising solvent for extracting the active compound of *C. mangga* rhizome, which functions as an antioxidant and antifungal proven to produce the highest antioxidant and antifungal activity when compared to chloroform and n-hexane.

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