Determination of free radical scavenging activity from aqueous extract of *Curcuma mangga* by DPPH method

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**Abstract.** *Curcuma mangga* (mango ginger) belongs to the family of Zingiberaceae. The rhizome of *C. mangga* are morphologically similar to ginger (*Zingiber officinale*) with a little mango flavour. *C. mangga* can grow in tropical areas and easy found in Indonesia. The rhizomes of *C. mangga* were washed and cut into the small piece, then drying at room temperature for 6 days, and then grinded until get the powder of *C. mangga*. The powder of *C. mangga* was extracted with demineralized water by maceration for 6 hours. *C. mangga* extract was analysed with FTIR spectrophotometer to determine its functional groups. *C. mangga* extract was diluted at various of concentration (5, 10, 25, 50, 100, 250, 500 mg/L) using demineralized water. *C. mangga* extracts were tested the antioxidant activity using 0.002% DPPH at 517nm with UV-Vis spectrophotometer, and the IC₅₀ value of *C. mangga* extract is 212.70 mg/L.

1. Introduction

*C. Mangga* is the one kind of herbal medicinal plants. In long time ago, *C. mangga* was used as a traditional herbal medicine. The *C. mangga* extract was very useful for health, as antimicrobial, antidiarrheal, anti-inflammatory, anticancer, analgesic, and antioxidants [1-5]. That is because the *C. mangga* extract contain some active compounds such as curcuminoid and phenolic. The curcuminoid compounds are curcumin, bisdimethoxycurcumin, bismethoxycurcumin, and phenolic compounds are ferulic acid, p-coumaric acid, cinnamic acid, cafeic acid. In this research is focused into antioxidant activity of *C. mangga* extract. The general solvent for antioxidant extraction is using the organic solvents such as methanol, ethanol, and n-hexane, but in this research we use demineralized water, because the *C. mangga* extract will be applied for herbal medicine. The solvent must be kept safe and halal for consumption. In the previous research, Policegoudra (2007) reported about antioxidant activity from *C. mangga* extract [6]. The *C. mangga* rhizomes were washed, sliced and dried in a hot air oven at 50°C for 72 hours and powdered to 60 meshes using an apex grinder. About 100 g of dry *C. mangga* powder was extracted with chloroform at room temperature and at atmospheric pressure, for 48 hours by shaking at 100 rpm/min speed. The *C. mangga* extract was filtered and concentrated using rotary evaporator. The concentrated extract of *C. mangga* was freeze-dried and stored in refrigerator. The *C. mangga* extracts (10–100 µL) were mixed with 0.8 mL of Tris–HCl buffer (pH 7.4) to which 1 mL of DPPH (0.05 mM) in ethanol was added. The mixture was shaken vigorously and left to stand for 30 min to complete the reaction. And then absorbance of the resulting solution was measured at 517 nm using UV–vis spectrophotometer. The antioxidant activity of *C. mangga* extract was measured...
as a decrease in the absorbance of DPPH. Lower absorbance of the mixture, indicated the higher antioxidant activity. The antioxidant activity was expressed as IC\textsubscript{50} value, which showed as concentration of sample at which 50% of the DPPH radicals scavenged. The IC\textsubscript{50} value from the previous research is 178 mg/L [6]. The aim of this research is to study the free radical scavenging activity from aqueous extract from \textit{C. mangga} by DPPH method.

2. Experimental

2.1. Materials and chemicals
Fresh and healthy mango ginger (\textit{C. mangga}) rhizomes were purchased from the local market, Pucang, Surabaya, Indonesia, during July 2015. The rhizomes were washed, sliced and dried in a room temperature for 6 days and grinded until get the powder. The chemicals are demineralized water, ethanol absolute (Merck, Germany), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (Merck, Germany).

2.2. Extraction of \textit{C. mangga} with demineralized water
The rhizomes of \textit{C. mangga} were washed and cut into the small piece, then drying at room temperature for 6 days, and then grinded using mixer grinder (Philips, Indonesia) until getting the powder. 100 g of \textit{C. mangga} powder was extracted with 1000 mL demineralized water by maceration for 6 hours. The \textit{C. mangga} extract was filtered and concentrated using hot plate at temperature 50\textdegreeC for 12 hours. The concentrated extract were drying by vacuum freeze dryer (FD-1A-50 Series) and stored in desiccator. The extraction process of \textit{C. mangga} according to the method was described earlier [7].

2.3. Characterization of \textit{C. mangga} extract
The concentrate of \textit{C. mangga} extract was characterized by FTIR (Shimadzu-FTIR-8400S) using the KBr-disk technique. The little part of \textit{C. mangga} extract was mix with KBr powder until homogenous. The mixture obtained, was flattened and placed onto the sample holder for analysis.

2.4. DPPH free radical scavenging activity of \textit{C. mangga} extracts
1,1-Diphenyl-2-picryl-hydrazyl radical scavenging activity was determined according to the method described earlier [7]. The concentrate of \textit{C. mangga} extract (100 mg) was mixed with 100 mL of demineralized water (1000 mg/L of stock solution). From the stock solution, diluted to various concentration (5, 10, 25, 50, 100, 250, 500 mg/L). 0.002% DPPH (w/w) made from 2 mg DPPH was mixed with 126.75 mL ethanol absolute (\(\rho = 0.789\)g/mL). 2 mL of \textit{C. mangga} extracts (5–500 mg/L) were mixed with 2 mL of 0.002% DPPH. The mixture was shaken and left to stand for 10 min to complete the reaction. Absorbance of the resulting solution was measured at 517 nm using UV–Vis spectrophotometer (Genesys 10S UV-Vis). The radical scavenging activity of \textit{C. mangga} extract was measured as a decrease in the absorbance of 0.002% DPPH. Lower absorbance of the mixture, indicated the higher of radical scavenging activity. The radical scavenging activity was expressed as IC\textsubscript{50} value, which represents the sample concentration at 50% of the DPPH radicals scavenged.

3. Result and Discussion

3.1. Characterization of \textit{C. mangga} extract
The functional groups of \textit{C. mangga} extract was characterization using FTIR spectrophotometer. The spectr FTIR of \textit{C. mangga} extract was shown at \textbf{Figure 1}. It shows N-H stretching (amine group) at 3712 cm\(^{-1}\), O-H stretching and C-O stretching (alcohol group) at 3493 cm\(^{-1}\) and 1210 cm\(^{-1}\), C-H sp\(^{3}\) stretching (alkyl group) at 2947 cm\(^{-1}\), C=O stretching (carbonyl group) at in 1702 cm\(^{-1}\), and C=C stretching (aromatic group) at 1597 cm\(^{-1}\). FTIR spectrum of \textit{C. mangga} extract from the previous research was shown at \textbf{Figure 2}. It shows O-H stretching and C-O stretching (alcohol group) at 3403cm\(^{-1}\) and 1202 cm\(^{-1}\), C-H sp\(^{3}\) stretching and C-H sp\(^{3}\) bending (alkyl group) at 2912 cm\(^{-1}\) and 2996
cm$^{-1}$, C=O stretching (carbonyl group) at 1657 cm$^{-1}$. There are 2 differences on FTIR spectra at Figure 1 and Figure 2. Figure 2 does not show N-H and C=C functional groups, because the extraction method between the previous research and in this work is different.

![FTIR spectra of C. mangga extract](image1)

**Fig. 1.** FTIR spectra of *C. mangga* extract

![FTIR spectra of C. mangga extracts from the previous research](image2)

**Fig. 2.** FTIR spectra of *C. mangga* extracts from the previous research [6]

### 3.2. DPPH free radical scavenging activity of *C. mangga* extracts

The maximum wavelength of 0.002% DPPH measured using UV-Vis spectrophotometer. The result is shown at **Figure 3**. It shows that the maximum wavelength of 0.002% DPPH is 517 nm. The maximum wavelength of 0.004% and 0.1 mM DPPH from the previous research are 517 nm [8,9]. The
result is DPPH solutions at different concentrations have the same wavelength. The results of free radical scavenging activity from *C. mangga* extracts (5–500 mg/L) using 0.002% DPPH is shown at Figure 4. It shows the IC$_{50}$ value of *C. mangga* extract is 212.70 mg/L, with the linear regression equation is $y = 0.0919x + 30.453$ with linear correlation of 0.9906.

![Fig. 3. UV-Vis spectra of 0.002% DPPH solution](image)

Smaller IC$_{50}$ value indicates higher free radical scavenging activity [10]. The result obtained in this research provide information about the IC$_{50}$ value from the aqueous extract of *C. mangga*, it can be used as a standard to determine the dose of traditional herbal medicine from *C. mangga* extract.

![Fig. 4. 0.002% DPPH free radical scavenging activity of *C. mangga* extracts](image)
4. Conclusion
The aqueous extracts of C. mangga have a good free radical scavenging activity with the IC\textsubscript{50} value is 212.70 mg/L, the linear regression equation is y = 0.0919\textit{x} + 30.453 with linear correlation of 0.9906. This result can be used as a standard to determine the dose of traditional herbal medicine from C. mangga extract

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