Isolation of Antioxidant Compounds from *Mangiferaindica* L. Leaves

Fitria[1], Sri Fatmawati[1], Taslim Ersam[1]

Abstract – The free radical scavenging activity of Mangiferaindica L. Leaves had been performed. The methanol extract showed the highest 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity among other extracts. Bioassay guided fractionation was performed and yielded three isolated compounds. Their structures were identified as mangiferin (1), friedelin (2) and lupeol (3). Mangiferin exhibited free radical scavenging activity, with an IC_{50} value of 12.12 µg/mL in vitro.

Index Terms – Free radical scavenging, Mangifera indica L. Leaves., ABTS.

INTRODUCTION

Mangiferaindica L., known as mango, is the fruit tree belonging to the Anacardiaceae family. Mango is widely distributed in many tropical and sub-tropical regions of the world, including the countries of Indonesia, India, Thailand and China [1]. The previous investigation of ***M. indica*L.** Chemical composition had been reported the presence of triterpenoids [2], phenolic compounds [3], and diarylheptanoid compounds [4]. The crude extract from the seed kernels of ***M. indica***L showed antibacterialactivity [5] and antioxidant activity [6] while that of the peels displayed anti-inflammatory activity [7].

The aim of present study were to isolate and identify antioxidant compounds from methanol extract of *M. mangifera*L leaves. The compounds were identified by NMR spectroscopy. The antioxidant activity from four different extracts of ***M. indica*** **L.** and isolated compounds had also been studied. To the best of our knowledge, this is the first report on ABTS free radical scavenging activity of extracts and isolated compound of *M. mangifera*L leaves.

EXPERIMENTAL

A. Extraction and Isolation

The leaves of *M. indica* L. (650 g) were extracted with methanol (10 L) for 3 days at room temperature. The extracts were concentrated by vacuum rotary evaporator which resultedthe dark brown crude extract. The crude extract was suspended in 50 mL of 50% methanol then partitioned with 100 mL dichloromethane for 4 times.

The aqueous methanolic fraction was hydrolysed by reflux with 2 N sulphuric acid at pH 3 for an hour with continuous stirring. After cooled at room temperature, it was partitioned with 100 mL ethyl acetate for 3 times. Subsequently, the combined ethyl acetate layer was dried using a vacuum rotary evaporator. The dried ethyl acetate fraction was dissolved in methanol and left in a refrigerator (4-8°C) over night, then the filtration was done to produce compound **1**.

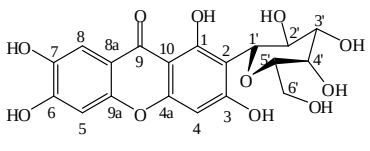
The dichloromethane phase was evaporated by rotary evaporator vacuum. The dichloromethane fraction residue was subjected to column chromatography to produce compound **2**(*n*-hexane-dichloromethane from 100:0 to3:7 as elute). Fraction D was subjected to column chromatography to give compound **3** (*n*-hexane- dichloromethane from 8:2 to 1:9 as elute).

B. 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity

Radical scavenging activity was validated by the UV absorbing method. Various concentration of sample were prepared by dissolving sample (10 mg) in DMSO (1 mL). The sample solution (10 µL) were added ABTS solution (1 mL). After 4-min incubation at 30°C, the absorbance of the resulting solution was measured at 734 nm with spectrophotometer.

RESULT AND DISCUSSION

The four different extracts of M.indica L. leaves and three isolated compounds were subjected to examination for potential free radical scavenging on ABTS. The results were summarized in Fig 1. Methanol extract showedthe highest antioxidant activity than that of other extracts, with IC50 value of 3,18 µg/mL.The abundant bioactive compounds plays an important role in antioxidant activity. Methanol extract includes many secondary metabolic compounds as flavonoid, saponin, tannin, steroid [8]. Based on the results, the methanol extract was further fractionated and yielded three compounds.



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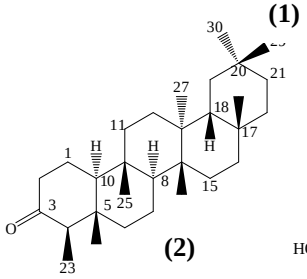

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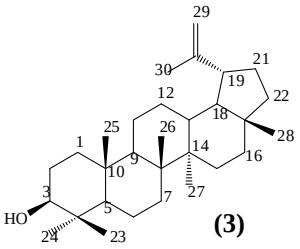

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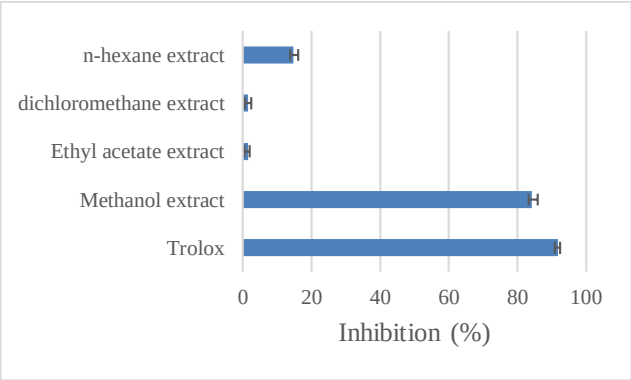

Figure 1. ABTS free radical scavenging activities of four *Mangiferaindica* L. Leaves extracts. All results presented as means ± standard deviations of three replicated determinations. Trolox as was a reference antioxidant.

The chemical structure elucidation of isolated compounds was then performed by using NMR data. The isolated compounds are identified asMangiferin (**1**), friedelin (**2**) and lupeol (**3**). The NMR data were accorded with the literature value of mangiferin [4], friedelin [2] and lupeol [2].

Mangiferin had higher ABTS free radical scavenging than that of compound 2 and 3. Mangiferin showed antioxidant activity with IC_{50} value of 12.12 µg/mL.Mangiferin is the xanthone derivative that has ortho-protected catechol. These result suggested that presence of the ortho-dihydroxy groups at C-6 and C-7 on ring B of xanthones might be involved in the enhanced antioxidant activity. The xanthones with catechol groups can be donating hydrogen radical and give a higher stability to their radical form [9]. The compound 2 and 3 showed low activity, with IC_{50}> 49.50 µg/mL.

CONCLUSION

In the present study, the antioxidant compounds were isolated from methanol extract of*M. indica*L.leaves. Mangiferin showed the highest ABTS free radical scavenging activity which can be developed as antioxidant source.

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