Open Access Indonesian Journal of Medical Reviews

Journal Homepage: https://hmpublisher.com/index.php/OAIJMR

Antioxidant Activity of *Leucaena leucocephala Lmk.de Wit.* N-Hexane Fraction

Ricka Islamiyati[1*], Yanulia Handayani[1], Kadar Ismah[1], Gendis Purno Yudanti[1], Heni Setyoningsih[1], Sri Fitrianingsih[1]

[1]Bachelor of Pharmacy Study Program, ITEKES Cendekia Utama Kudus, Kudus, Indonesia

ARTICLE INFO

Keywords:
Antioxidant
DPPH
IC_{50}
Leucaena leucocephala
N-hexane fraction

***Corresponding author:**
Ricka Islamiyati

E-mail address:
rickaislamiyati494@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/oaijmr.v3i1.286

A B S T R A C T

Antioxidants are chemical compounds that can neutralize free radical agents. These compounds work by donating electrons to achieve a stable form, thereby inhibiting the oxidative mechanisms that cause degenerative disease. Lamtoro seeds contain many active compounds that can be used as a source of natural antioxidants and antibacterials. This study aimed to determine the antioxidant effect of the n-hexane fraction of lamtoro seeds (*Leucaena leucocephala Lmk. de Wit.*). This study is an in vitro experimental study. The object of study was the n-hexane fraction of lamtoro seeds, where antioxidant activity was examined using the DPPH method. Analysis was performed using a UV-Vis spectrophotometer. The n-hexane fraction contained a total flavonoid content of 9.224% and had antioxidant activity with an IC_{50} value of 67.94 ppm. In conclusion, the n-hexane fraction of lamtoro seeds (*Leucaena leucocephala Lmk.de Wit.)* has strong antioxidant activity.

1. Introduction

The role of free radicals damaging cells and tissues occupies the most important position in the body's metabolism. Free radical reactions occur due to oxidation reactions of stable compounds to become unstable and also reactive compounds. These reactive species can react with other compounds in the body and cause tissue damage which will lead to diseases such as cancer, Alzheimer's disease, heart reperfusion, and disorders. Free radicals such as peroxides, hydroperoxides, or peroxyl lipids can oxidize nucleic acids, proteins, lipids, DNA and can initiate degenerative disease. Physical and chemical factors such as heavy metals, heating, radiation, dyes, and preservatives play an important role in the excessive occurrence of oxidation reactions.[1-5]

Antioxidants are chemical compounds that can neutralize free radical agents. These compounds work by donating electrons to achieve a stable form, thereby inhibiting the oxidative mechanisms that cause degenerative disease. Antioxidant compounds can be natural ingredients and synthetic compounds. Synthetic antioxidants have several side effects and are carcinogenic agents. Therefore, many studies have developed antioxidant compounds from natural ingredients. Most of the antioxidant compounds obtained from these plants such as vitamin C, vitamin E, carotenoids, and phenolic acids. Various groups of compounds with broad physical and chemical

properties were isolated, such as gallic acid having strong antioxidant activity.[6-8]

Indonesia is a country with abundant biological wealth. Indonesia is a country with the second largest natural wealth in the world after Brazil. Of course, the potential of this biological wealth is great potential to be developed into a modality of antioxidants new natural to improve health status. lamtoro seeds (*Leucaena leucocephala Lmk.de Wit.*) are one of the most common plants found in Indonesia. However, the utilization of lamtoro seeds as vegetables is still very low. Lamtoro seeds contain many active compounds that can be used as a source of natural antioxidants and antibacterials. One of the compounds contained in lamtoro seeds viz flavonoid, besides that lamtoro seeds, contain various active compounds, namely alkaloids, saponins, mimosine, and soil.[9-12] This study aimed to determine the antioxidant effect of the n-hexane fraction of lamtoro seeds (*Leucaena leucocephala Lmk.de Wit.*).

2. Methods

Materials

Lamtoro seeds (*Leucaena leucocephala Lmk.de Wit.*) used in this study were obtained from Bogoharjo, Kaliori, Rembang Regency, Central Java, Indonesia. Solvents (methanol, ethanol, ethyl acetate, dichloromethane, dimethylsulfoxide, and n-hexane) are of the analytical class. DPPH (2,2-diphenyl-2-picrylhydrazyl; Sigma-Aldrich, Steinheim, Germany), 1 M sodium acetate, 10% aluminum chloride, concentrated hydrochloric acid, Mayer's reagent, Wagner and Dragendorff, aquades, quercetin, $FeCl_3$, HCl 2N, concentrated sulfuric acid, NaOH.

Instrumentation

Digital scales (Ohaus®), UV-Vis spectrophotometer (Biobase®), cuvette, water bath, bunsen, tripod, blender (Getra®), scissors, aluminum foil, test tubes (Herma®), thermometer, beaker (Herma®), measuring glass (Herma®), Erlenmeyer tube (Herma®), glass bottle, glass funnel (Herma®), oven (Mammert®), separatory funnel (Herma®), dropper pipette, micropipette, stir bar, volumetric flask (Herma®), evaporating cups, spatulas, horn spoons, wooden tongs, and filter cloths.

Preparation of lamtoro seed fraction

The dried simplicia was then macerated with a ratio of 1: 3. The maceration was carried out for 1 x 24 hours every 6 hours, stirring and repeated 2 times. The results of maceration are then collected together and concentrated water bath at a temperature of 40°C. As much as 24 grams of ethanol extract from lamtoro seeds was fractionated using n-hexane, ethanol, and water as solvents. Fractionation was carried out by dissolving the viscous extract in a mixture of ethanol and water. The solution was put into a separatory funnel and added n-hexane the amount of n-hexane was proportional to the amount of ethanol-water which was added to the ethanol extract in a ratio of 1: 1. The n-hexane fraction formed was separated, and the ethanol-water fraction was shaken again until the n-hexane fraction was clear. The n-hexane fraction obtained was concentrated using a water bath at 40°C.

Determination of water level

Determination of water content is carried out using the gravimetric method by heating using an oven with a temperature of 105°C for 3 hours.

Phytochemical screening

Flavonoid test: 10% NaOH reagent as much as 20 mg of sample added with 2-5 drops of 10% NaOH reagent. The reagent is positive if there is an orange or orange color change. Reactor Wilstater: as much as 20 mg of sample added a little Mg metal powder and 2-5 drops of concentrated HCl. A yellow color change indicates the presence of flavonoids. Reactor Bate Smite-Metcalfe: as much as 20 mg of sample added with 2-5 drops of concentrated HCl, then heated using a water bath for 15 minutes. The positive reaction showed a red color change.

Saponin test: 20 mg sample is weighed into a test tube added with aquades heat and 2-5 drops of concentrated HCl, then shaken. The presence of

saponins was indicated by the appearance of foam for 15 minutes. Phenol test: sample weighed 20 mg added 2-5 drops of $FeCl_3$ solution. The formation of bluish-green and black color identified the presence of phenol.

Alkaloid test: sample weighing as much as 20 mg was added with 1.5 mL of chloroform, 2-5 drops ammonia, then added 2-5 drops of sulfuric acid until 2 layers were formed, the acid layer was reacted with Mayer's reagent, Dragendorff, Wagner. Orange color change in Mayer's reagent, green precipitate Dragendorff, red in Wagner.

Determination of total flavonoid levels

As much as 25 mg of the extract was then dissolved with pro-analytical ethanol up to the mark of 25 mL. Pipette 0.5 mL of this solution and then add 0.1 mL of $AlCl_3$ solution 10% and 0.1 mL of 1 M sodium acetate added with pro-analytical ethanol to the mark of the 10 mL volumetric flask. The mixture was shaken until homogeneous and left for 8 minutes. Then the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 444.0 nm repititions three times up to the level of flavonoids obtained as equivalent quercetin.

Antioxidant activity testing with the DPPH method

10 mg of DPPH powder was weighed, put into a 50 mL volumetric flask, then added ethanol p.a up to the mark to obtain a solution with a concentration of 0.5 mM. Take 1.0 mL of 0.5 mM DPPH solution, put it in a volumetric flask added with pro-analytical ethanol up to 10 mL mark, shake until homogeneous, and the absorbance is measured at a wavelength of 400-800 nm.

To determine the operating time, 0.1 mL of lamtoro seed n-hexane fraction solution with 0.4 mL DPPH was put in a 10 mL volumetric flask. Pro-analytic ethanol was added to the mark. Shake until homogeneous, then observe the absorbance with a maximum wavelength of 515.0 nm using a spectrophotometer UV-Vis every 1-minute time interval.

Preparation of an antioxidant test solution of the n-hexane fraction of lamtoro seed solutions with concentrations of 40 ppm, 60 ppm, 80 ppm, 100 ppm, and 120 ppm, then from each concentration, 0.1 mL was taken, and 0.4 mL of 0.5 mM DPPH solution was added. Put in a 10 mL volumetric flask, added with pro-analytical ethanol up to the mark. Then the wavelength absorption was measured using a UV-Vis spectrophotometer with a wavelength of 515.0 nm. Repetition was performed 3 times after that, the percent attenuation was calculated and included in the linear regression equation, and the lC_{50} value was obtained.

$$\% \text{ inhibition} = \frac{\text{Absorbance control} - \text{Absorbance of the test substance}}{\text{Absorbance control}} \; x\, 100\ \%$$

3. Results and Discussion

Results of drying simplicia lamtoro seeds *Leucaena leucocephala Lmk.de Wit* can be seen in Table 1.

Table 1. Results of drying shrinkage calculations.

Information	Wet sample (grams)	Dry simplicia (grams)	Dry powder (grams)	Weight loss (%)
Weight	3000	925	475	69,16
Color	Green	Brown	Light brown	-

The results of determining the moisture content of lamtoro seeds *Leucaena leucocephala Lmk.de Wit* can be seen in Table 2.

Table 2. Results of the water content test.

Simplicia	Simplicia powder (gram)	Empty cup (grams)	Initial sample (grams)	Final sample (grams)	Water content (%)	Level ± SD (%)
Lamtoro seeds	1,000	60,488	61,488	61,418	7	5,4 ± 2,48
	1,000	58,738	59,738	59,670	6,8	
	1,000	59,927	59,901	58,927	2,6	

The results of making the n-hexane fraction of the ethanol extract of lamtoro seeds *Leucaena leucocepala Lmk.de Wit* can be seen in Table 3.

Table 3. The yield of the N-Hexane fraction of lamtoro seeds.

Information	Condensed extract (grams)	N-Hexane condensed fraction (grams)	Yield (%)
Weight	24	2,1	8,75
Color	Dark brown	Brown	

The results of the determination of the moisture content of lamtoro seeds *Leuchaena leucocephala Lmk.de Wit* can be seen in Table 4.

Table 4. Identification results of the N-Hexane fraction of lamtoro seeds.

Compound	Information	Results
Flavonoid: NaOH 10% reagent	Orange	+
Wilstatter reagent	Yellow	+
Bate Smite Metcalfe	Red	+
Saponin test	There is foam	+
Phenolic test	Blackish-green	+
Alkaloid: Mayer's reagent	There is no green precipitate	-
Dragendroff's reagent	There is a green precipitate	-
Wagner's reagent	Red	-

Results of the determination of the total flavonoid content of lamtoro seeds *Leucaena leucocephala Lmk.de Wit* can be seen in Table 5.

Table 5. Results of determination of total flavonoid content of the N-Hexane fraction.

Concentration	Absorbance	Equivalent levels (ppm)	Total flavonoid levels	Level ± SD (%)
1000 ppm	0,304	92,528	9,252	9,224 ± 0,052
	0,305	92,808	9,280	
	0,300	91,404	9,140	

The results of reducing DPPH by extracting the n-hexane fraction of lamtoro seeds *Leucaena leucocephala Lmk.de Wit* can be seen in Table 6.

Table 6. Results of reducing DPPH by the n-hexane fraction from lamtoro seeds.

Levels	**Average attenuation ± SD (%)**		
40 ppm	35,88 ± 0,2		
60 ppm	46,73 ± 0,57	IC_{50}: 67,94ppm	Absorbance control: 0.992
80 ppm	55,94 ± 0,32		
100 ppm	66,76 ± 0,152		
120 ppm	73,28 ± 0,1		

Research results show that lamtoro seeds contain flavonoid compounds characterized by a change in orange color, using 10% NaOH reagent, Wilstater, Bate Smite-Metcalfe. Flavonoid compounds are compounds that are easily dissolved or extracted in ethanol solvents that are polar because of the presence of hydroxyl groups so that a hydrogen bond can form. In addition to flavonoids, lamtoro seeds also contain saponins and phenolic compounds.[13-16]

In the analysis of determining the potential antioxidant activity of a sample using the method measurement to (%) scavenging of free radicals, which is then used to calculate the lC_{50} value. IC_{50} is the number indicating the concentration of the extract, which is capable of inhibiting 50% oxidation. The IC_{50} value is inversely related to antioxidant activity. The higher the IC_{50} value, the smaller the antioxidant activity, and vice versa, the smaller the IC_{50} value, the higher the antioxidant activity.[17,18]

The results showed that the extract of the n-hexane fraction of lamtoro seeds had strong antioxidant activity at lC_{50} values of 67.94 ppm. Meanwhile, according to previous research, lamtoro seeds have very strong antioxidant activity, namely with an lC_{50} value of 8.726 ppm. The smaller the total flavonoid value, the greater the lC_{50} value, which means that the lower the total flavonoid value, the lower the antioxidant activity.[19,20]

4. **Conclusion**

The n-hexane fraction of lamtoro seeds (*Leucaena leucocephala Lmk.de Wit.*) has strong antioxidant activity.

5. References

1. Annu AA, Ahmed S. Green synthesis of metal, metal oxide nanoparticles, and their various applications. In: Martínez L, Kharissova O, Kharisov B, editors. Handbook of Ecomaterials. Springer; Cham, Switzerland: 2018.
2. Zhu L, Zeng W. Room-temperature gas sensing of ZnO-based gas sensor: A review. Sens. Actuators A Phys. 2017; 267: 242–61.
3. Jin H, Zhao X, Wu Z, Cao C, Guo L. Supercritical water synthesis of nano-particle catalyst on TiO_2 and its application in supercritical water gasification of biomass. J Exp Nanosci. 2017; 12: 72–82.
4. Das B, Moumita S, Ghosh S, Khan MI, Indira D, Jayabalan R, et al. Biosynthesis of magnesium oxide (MgO) nanoflakes by using leaf extract of *Bauhinia purpurea* and evaluation of its antibacterial property against *Staphylococcus aureus*. Mater Sci Eng C. 2018; 91: 436–44.
5. Amrulloh H, Fatiqin A. Synthesis of MgO nanoparticles using moringa oleifera extract and antibacterial activity test against *Escherichia coli* and *Staphylococcus aureus*. 1st ed. Pustaka Learning Center; Malang, Indonesia. 2020; 145.
6. Leung YH, Ng AMC, Xu X, Shen Z, Gethings LA, Wong MT, et al. Mechanisms of antibacterial activity of MgO: Non-ros mediated toxicity of MgO nanoparticles towards *Escherichia coli*. Small. 2014; 10: 1171–83.
7. Shekhar S, Liu Y, Wang S, Zhang H, Fang X, Zhang J, et al. Novel mechanistic insights and potential therapeutic impact of TRPC6 in neurovascular coupling and ischemic stroke. Int J Mol Sci. 2021; 22: 2074.
8. Chen K, Lu P, Beeraka NM, Sukocheva OA, Madhunapantula SV, Liu J, et al. Seminars in cancer biology. Academic Press; Cambridge,

MA, USA: 2020. Mitochondrial mutations and mitoepigenetics: Focus on regulation of oxidative stress-induced responses in breast cancers.

9. Patil AB, Bhanage BM. Novel and green approach for the nanocrystalline magnesium oxide synthesis and its catalytic performance in Claisen-Schmidt condensation. Catal Commun. 2013; 36: 79–83.
10. Pilarska AA, Klapiszewski Ł, Jesionowski T. Recent development in the synthesis, modification and application of $Mg(OH)_2$ and MgO: A review. Powder Technol. 2017; 319: 373–407.
11. Park J-W, Lee W-K, Lee C-H. Preparation of nano-magnesium oxide from seawater bittern using decarboxylation/precipitation method. Mol Cryst Liq Cryst. 2016; 636: 142–8.
12. Amrulloh H, Simanjuntak W, Situmeang RTM, Sagala SL, Bramawanto R, Fatiqin A, et al. Preparation of nano-magnesium oxide from Indonesia local seawater bittern using the electrochemical method. Inorg Nano-Met Chem. 2020; 50: 693–8.
13. Gudikandula K, Maringanti SC. Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. J Exp Nanosci. 2016; 11: 714–21.
14. Moorthy SK, Ashok CH, Rao KV, Viswanathan C. Synthesis and characterization of MgO nanoparticles by neem leaves through green method. Mater Today Proc. 2015; 2: 4360–8.
15. Manjula R, Thenmozhi M, Thilagavathi S, Srinivasan R, Kathirvel A. Green synthesis and characterization of manganese oxide nanoparticles from *Gardenia resinifera* leaves. Mater Today Proc. 2019; 26: 3559–63.
16. Halliwell B. Free radicals and antioxidants–Quo vadis? Trends Pharmacol Sci. 2011; 32: 125–30.
17. Ye ZW, Zhang J, Townsend DM, Tew KD. Oxidative stress, redox regulation and diseases of cellular differentiation. Biochim Biophys Acta. 2015; 1850: 1607–21.
18. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C. Roberfroid M, et al. Functional food science and defence against reactive oxygen species. Br J Nutr. 1998; 80: S77–S112.
19. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39: 44–84.
20. Speakman JR, Selman C. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. BioEssays. 2011; 33: 255–9.