ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Antioxidant and Antibacterial Activities of the Phenolic Compounds from the Fruit of *Toona Sureni*

M. Taufik Eka Prasada*
Dwimaryam Suciati, Polytechnic ATI Padang, West Sumatera, Indonesia
***Corresponding author: E-Mail: ekaprasada@yahoo.co.id**

ABSTRACT

Two compounds were isolated by silicagel in column chromatography with increasing percent ethyl acetate (EtOAc) in *n*-hexane. The structures were determined to be methyl 3, 4, 5-trihydroxybenzoate and 1, 2, 3-trihydroxybenzene, based on GCMS spectrum. They showed antioxidant activity in the *α,α*-diphenyl-*β*-picrylhydrazyl (DPPH) test, with IC_{50} value 33.33 ppm. Their antibacterial activity were evaluated by the disc diffusion agar method. Chloramphenicol was used as comparison. They were effective on the inactivation of two bacterial test (*Escherichia coli*, and *Staphylococcus aureus*). The minimal inhibition concentration (MIC) of the methyl 3, 4, 5-trihydroxybenzoate was 1.0 mg/mL against E. *coli and* 0.25 mg/mL against *Staphylococcus aureus*. MIC value of the 1,2,3-trihydroxybenzene was 0.125 mg/mL against *E. coli* and 0.25 mg/mL against *Staphylococcus aureus.*

KEY WORD: Antioxidant, Antibacterial, Phenolic, Toona sureni.

1. INTRODUCTION

Toona sureni is Meliaceae family. It also has common names e.i: suren (Indonesia), surian amba (Sumatera), surian wangi (Malaysia), danupra (Philippines), ye tama (Myanmar), surian (Thailand), surian, toon red, cedar, and limpaga (trade names). The distribution areas are found in India, Nepal, Buthan, Myanmar, Indo-China, China Southern, Thailand, Malaysia, and New Guinea. In Indonesia, the plant is found in Java, Sumatera and Sulawesi. The plant is found on riparian, hillsides and slopes, at 1,200-2,700 m with mean annual temperature around 22 °C, and requires fertile soil (Djama'an, 2002).

Some parts of the *Toona sureni* plant (Figure.1), specially the bark is often used for medicinal herbs for diarrhea, dysentery, fever, and kidney infection (Heyne, 1987). The bark contains of triterpenoid (Hudri, 2009) and flavonoids (Dartini, 2015). The leaves of the plant were reported to resist the insect and kill bedbug (*Cimex lectularius*) (Nurdin, 1978). The fruit of the plant contain phenolic compounds (Ekaprasada, 2015) and has insecticidal activities (Parvin, 2012). Another research of the plant revealed that leaves contain tetranortriterpenoid (Kraus, 1979, 1982), carotenoids (Nurdin, 2000, 2001; Ekaprasada, 2009) and methyl gallate (Ekaprasada, 2009). The fruits contain methyl gallate that can prevent damage the palm and coconut oil during storage (Ekaprasada, 2016).

Some literatures showed that the *Toona sureni* plant and the other species of the same genus from Meliaceae family also contains bioactive chemical compounds, but not harmful to health because this plant is also used for food flavoring. This fact showed the possibility of the compounds isolated from the plant can be utilized as natural antibacterial and antioxidant safely for human being. In this study, we report the isolation of phenolic compounds from the fruit exract of *T.sureni* and assay their activities as antioxidant and antibacterial.


Figure.1. The plant of *Toona sureni* (A) and the fruit of the plant (B)

2. MATERIALS AND METHODS

Materials: The fruit from the plant were collected in Padang, Indonesia. The plant was identified in the Herbarium of the Andalas University, Padang. Silica gel 60 (F254) was used as thin layer chromatography and Silica gel 60 (E7733) for column chromatography. DPPH (1,1-diphenyl-2-picrilhydrazyl) and gallic. All solvents were redistilled before used.

Microorganisms were purchased from Faculty of Pharmaceutical of STIFARM, Padang, Indonesia. A strain of gram-negative bacteria (*Escherichia coli*) and a strain of gram-positive bacteria (*Staphylococcus aureus*) were used. The cultures of test bacteria were saved at 4°C in the slant agars and used as stock cultures.

Methods:

Extraction and Fractionation: Air-dried and powdered fruits (1500 g) were first macerated successively with 2 x 6 L of *n*-hexane for 2 days. After filtration, residue was macerated with 3 x 4 L acetone for 3 days and 2 x 4 L methanol (MeOH) for 5 days. Each fraction was concentrated using a rotary vacuum evaporator. Methanol extract (15 g) was put into column of silica gel and eluted by the increasing percent ethyl acetate to *n*-hexane (100: 0 (300

mL); 90:10 (300 mL); 80: 20 (300 mL); 70: 30 (300 mL); 60:40 (300 mL); 50:50 (300 mL); 40: 60 (300 mL); 30:70 (300 mL), 20:80 (300 mL), 10:90 (300 mL) and 0: 100 (200 mL) and ethyl acetate: methanol (90:10) (300 mL). Fractions that have same R_f in TLC were collected to be subjected on silica gel column and eluted by ethyl acetate - *n*-hexane and then recrystallized to yield white needle crystals.

Antioxidant Activity: The assay of antioxidant activity is performed by DPPH method, according to Samara (2014), with modifications. As the antioxidant standard was used gallic acid. DPPH method was based on using of the molecule 1,1-diphenyl-2-picrylhydrazyl, stable radicals, caused by the electron delocalization so that the molecules cannot form dimmer compounds like occurred in other radicals. The electron delocalization cause dark purple compound with absorption bands in ethanol solution at 520 nm. When the DPPH solution is mixed with a substance that can provide hydrogen atoms, the color turn from purple into yellow as the molar adsorptivity of DPPH that is reduced when the electron from DPPH become paired with a hydrogen from antioxidant to form DPPH-H compound.

DPPH and MeOH were used for free radical and blank respectively. The samples were dissolved by MeOH with a ratio of 1: 1 (w / v), and then made a series solution of 1000; 500; 250; 125; 62.50; 31.25; 15.63; 7.81; 3.91, and 1.95 mg/mL. 0.2 mL for each concentration was transferred to different vials. 3.8 mL DPPH (20 ppm) was added immediately into each vial. The absorbance of solutions were measured after 30 minutes at wavelength 517 nm. The IC_{50} value is number of antioxidant was used to decrease of 50% the initial concentration of DPPH. Total of Antioxidant Activity was the percent inhibition for DPPH and was calculated by using following equation.

$$\text{TAA (\%)} = \frac{(A_{\text{control}} - A_{\text{sampel}}) \times 100}{A_{\text{control}}}$$

Where, TAA = Total of antioxidant activity, A = Absorbance.

Antibacterial Activity: The assay of Antibacterial was measured by disc diffusion agar method. Paper discs contain a certain amount of antimicrobial substance was positioned on the solid agar medium previously inoculated test bacteria on its surface. After incubation, the inhibition zone around the disc used to measure the strength of a substance barrier against test organisms. This method is influenced by several factors, including antimicrobial substances and organisms (eg nature of the medium and the ability of diffusion, molecular size and stability of antimicrobial substances). Interpretation of the new diffusion test results are based on comparison to the dilution method. Some comparative data was used as the reference standard. Linear regression graph can show the relationship between log MIC on how large the dilution and diffusion diameter of inhibition on the disc. The single disc at any antibiotic with good standardization could show whether bacteria are sensitive or no by comparing with inhibition zone standards. To activate the bacterial strain, one loopfull of it was inoculated into 2.5 mL of Nutrient Broth in an incubator during 24 h, 37 °C. The inoculum of bacterial was diluted with the sterile saline solution (0.9 % NaCl) to obtain turbidity of a Mc.Farland No. 0.5 Standard (10^{6-8} CFU/mL). Nutrient Agar (NA) was sterilized and cooled at 40-50 °C and 15 mL NA was poured to petri dishes that have diameter 9 cm. NA was allowed to be harden at room temperature. Then, spread the homogenous distribution of 0,1 mL bacteria culture (10^{6-8} cfu/mL) onto medium in petri dishes. The steril filter paper disc (5 mm in diameter) was impregnated with 20 µL of the compound. The minimum inhibition concentration (MIC) was assigned for the lowest concentration that show by no visible growth culture after incubation at 37 °C in 48 h. Chloramphenicol; 3 mg/mL (antibiotic standard) was used to provide a control for the sensitivity test of organisms in the experiments.

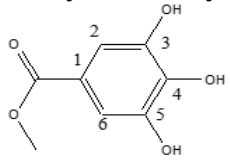
3. RESULTS AND DISCUSSION

Identification of the compounds: Isolation of the compounds from methanol fraction were obtained compound 1 (289.4 mg) and compound 2 (30 mg). Identification of the compounds by using GC-MS spectrum (Table.1**)**.

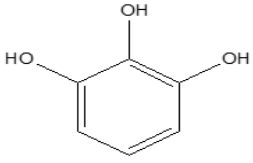
Table.1. Fragmentation pattern of GC-MS spectroscopy of compounds 1 and 2

No	Samples	Pattern of the fragmentations
1	Compound 1	m/z 184 $[M]^+$ (100), 153 (68), 125 (30), 107 (8), 79 (12), 51 (9)
2	Compound 2	m/z 126 $[M]^+$ (100), 108(29), 97(10), 80(38), 63(9), 52(30), 36 (8)

The fragmentation pattern of mass spectroscopy of the compound 1 significantly revealed of fragment ions $[C_6H_2(OH)_3CO]^+$ (m/ z153) reveal the loss of -OCH_3 form the molecular ions $[C_6H_2(OH)_3COOCH_3]^+$ (m/z 184) and fragment ions $[C_6H_2(OH)_3]^+$ (m/z 125) reveal the release CO from fragment ions (m/z 153) and reveal of ($COOCH_3$). The fragments were showed at *m/z* 107, 79 and 51 reveal the loss of H_2O and –CO from benzene ring. They reveal of presence of three hydroxyls from ring of benzene. Based on the fragmentations of GC-MS, it can be determined that compound 1 is methyl 3,4,5-trihydroxybenzoate.



Compound 1



Compound 2

The fragmentation pattern of the mass spectroscopy of the compound 2 showed a fragment ion (m/z 108) which caused the loss of H_2O molecule from the molecular ion $[C_6H_2(OH)_3]^+$ (m/z 126). Fragment ion (m/z 80) reveal CO from fragment ion (m/z 108), and the fragment (m/z 52) reveal the releasing of -CO from fragment ions (m z 80). Besides that, it also happen ion fragments of other fragments (m/z 97) showed loss of -COH from the molecular ion $[C_6H_2(OH)_3]^+$ (m/z 126), and the fragment (m/z 63) reveal the loss of -OH and fragment ions (m/z 80). They show three hydroxyl groups on benzene ring. Based on fragmentation of GC-MS, it can be assigned that compound 2 is 1,2,3- trihydroxybenzene.

Antioxidant activity: Figure 3 revea the concentration-response curve DPPH radical activity from compound 1, and compound 2, compared with gallic acid. The compounds of fruit the plant and gallic acid (standard) showed high antioxidant activity at 62.5 to 1000 ppm. At 62.5 ppm, the antioxidant activitiy from compound 1 was 93.45% and compound 2 was 94.14% while gallic acid was 93.45%.

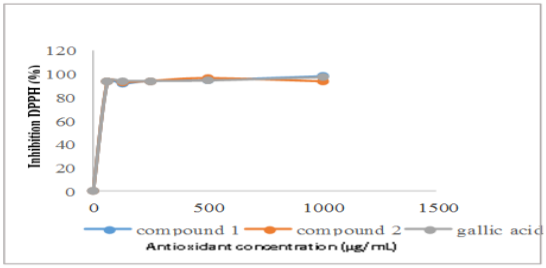


Figure.3. The curve of antioxidant activity of compound 1, compound 2 and gallic acid with using DPPH assay

Furthermore, at concentration of 125 to 1000 ppm, the activities of both compounds and standard remained high but did not increase significantly. IC_{50} value of compound 1, compound 2 and standard in this experiment are 33.33 ppm.

The assay showed that both of the compounds have a strong ability as an antioxidant and can work as a barrier or seek (Scavenge) free radicals as primary antioxidants. Similarities activity both of the compounds and standard were caused by the phenyl ring that has three hydroxyl groups, like as methyl 3,4-dihydroxybenzoic, ethyl 3,4-dihydroxybenzoic and butyl or acid 3,4-dihydroxy-trans-sinnamic, ethyl 3,4-dihydroxy-trans-sinnamic, and pentil 3,4-dihydroxy-trans-sinnamic show their similarities as an antioxidant activity (Mokbel, 2005).

They showed no difference with gallic acid (IC_{50} 33.33 ppm) which was known as an antioxidant standard as control (Table 2).

Table.2. Antioxidant activity of compound 1, compound 2 and gallic acid by using DPPH assay*

Sample	concentration (ppm)						IC_{50}** (ppm)
	1000	500	250	125	62.25	0	
compound 1	98.13	94.82	94.14	91.72	93.45	0	33.33
compound 2	93.80	96.55	94.14	93.10	94.14	0	33.33
gallic acid	97.24	94.48	94.14	94.14	93.45	0	33.33

* The values represent percent inhibition of the DPPH radical.

** Inhibition activity was revealed as 50% inhibition concentration. It was determined by interpolation of concentration-inhibition curves

Antibacterial Activity: Antibacterial activity from *E. coli*, and *S. aureus* are showed in Table 3 and Table 4. The assay showed antibacterial activity both of the compound against all bacterial strains that was used in this study. The growth of *E. coli* was inhibited by compound 1 start from 1 mg/mL concentration with 6 mm in diameter. It mean that MIC of the compound against *E. coli* was 1 mg / mL. The growth *S. Aureus* was inhibited by compound 1 start from 0.25 mg/mL concentration with a resistance diameter of 6 mm. The minimal inhibition concentration value of the compound to *S. aureus* was 0.25 mg/mL. The growth of *E. coli* was inbibited by compound 2 at 0.125 mg/mL concentration with 6 mm in diameter. It means the value of MIC the compound 2 against *E. coli* is 0.125 mg/mL. The growth *S. Aureus* was inhibited by compound 2 began at 0.25 mg/mL concentration with diameter 6 mm. The the minimal inhibition concentration value of the compound to *S. aureus* was 0.25 mg/mL.

Table.3. Antibacterial activity of compound 1 and chloramphenicol against *E.coli* and *S.aureus*[x]

Bacterial species	Concentration of compound 1 (mg/mL)								Chloramphenicol	
	4	2	1	0.5	0.25	0.125	0.062	MIC	3 (mg/mL)	MIC[z]
E. coli	8.2	6.0	6.0	Y	Y	Y	Y	1	22	5.5
S. aureus	8.0	8.0	6.5	6.5	6.0	Y	Y	0.25	22	5.5

[x] the values represent inhibition diameter (mm), [y] Not active (the inhibition diameter is lower or equal to 5 mm),

[z] MIC of chloramphenicol by literature (Tung, 2009)

Table.4. Antibacterial activity of compound 2 and chloramphenicol against *E.coli* and *S.aureus*[x]

Bacterial species	Concentration of compound 2 (mg/mL)								Chloramphenicol	
	4	2	1	0.5	0.25	0.125	0.062	MIC	3 (mg/ mL)	MIC[z]
E. coli	9.0	7.0	6.2	6.0	0.6	6.0	y	0.125	22	5.5
S. aureus	8.9	7.9	7.8	6.2	6.0	y	y	0.250	22	5.5

[x] the values represent inhibition diameter (mm), [y] Not active (the inhibition diameter is lower or equal to 5 mm), [z] MIC of chloramphenicol by literature (Tung, 2009)

4. CONCLUSION

Two compounds isolated from *Toona sureni* showed antioxidant and antibacterial activity and support using of the part of this plant for medicinal purposes.

5. ACKNOWLEDGMENT

We would like to express our deep thanks to the Centre of Industry Training and Education of Republic of Indonesia and Director of Polytechnic ATI Padang who have provided financial support and facilties in this research.

REFERENCES

Dartini, Nurdin H, Afrizal, Ekaprasada M.T and Suciati D, Isolation and characterization Flavonoids from The Bark of *Toona Sureni* (Blume) Merr, Journal of Chemical and Pharmaceutical Research, 8 (6), 2016, 156-159.

Djama'an D.F, *Toona sureni* (Blume) Merr, Informasi Singkat Benih, Indonesia Forest Seed Project (IFSP), Balai Penelitian Dan Pengem-bangan Teknologi Pembenihan, 24, 2002, 1-2.

Ekaprasada M.T, Isolation of methyl gallate from the fruit of *Toona sureni* and its utilization in preventing damage the vegetables oil during storage, Der Pharma Chemica, 8 (12), 2016, 7-11.

Ekaprasada M.T, Nurdin H, Ibrahim S, Dachriyanus β, ε- Carotene 3, 3'-diol, a carotenoid from *Toona sureni*, The *Bulletin of* The Indonesian Society of Natural Products Chemistry, 9 (1-2), 2009, 21-25.

Ekaprasada M.T, Nurdin H, Ibrahim S, Dachriyanus, Antioxidant activity of methyl gallate isolated from the leaves of Toona sureni, International Journal on Advanced Science Engineering Information Technology, 5 (4), 2015, 280-282.

Ekaprasada M.T, Suciati D and Dartini, Utilization of the phenolic compounds from the fruit of *Toona Sureni* (blume) Merr as an antioxidant in coconut oil, Journal of Chemical and Pharmaceutical Research, 7 (12), 2015, 886-890.

Heyne K, Tumbuhan Berguna Indonesia (*De nuttige planten van Nederlandsch Indie*), Jilid II, Yayasan Sarana Wana Jaya, Jakarta, 1987, 1112-1114.

Hudri S, Harneti D and Supratman U, Toxic triterpenoid from the bark os suren (*Toona sureni*), Proceeding of The International Seminar on Chemistry, Jatinangor, 2008, 599-600.

Kraus W, Kypke K, Bokel M, Grimminger W, Sawitzki G, Schwinger G, Surenolactone, a novel tetranortriterpenoid A/B-dilactone from *Toona sureni* (Blume) Merrill (Meliaceae). Liebigs Annalen,1982, 87-88.

Kraus W, Kypke K, Surenone and surenin, two novel tetranortriterpenoids from *Toona sureni* (blume) Merrill, Tetrahedron Letters, 20 (29), 1979, 2715-2716.

Mokbel M.S and Hashinaga F, Antibacterial and Antioxidant Activities of Banana (*Musa*, AAA cv. Cavendish) Fruits Peel, American J. of Biochemistry and Biotechnology, 1 (3), 2005, 126-132.

Nurdin H, Ibrahim S, Darma A, Rajagukguk S, Muchtar M.H, Sugiyanto, Proceeding International Symposium on the Role of Chemistry in Industry and Environment, Andalas University, Padang, 2000,133-137.

Nurdin H, Muchtar M.H, Sugiyanto Dewi V, International Seminar on Tropical Rainforest Plants and Their Utilization for Development, Andalas University, Padang, 2001, 1-12.

Nurdin H, Presented on Seminar and Workshop on Essential Oils, Essential Oil in West Sumatera, Bangkok, 1978.

Parvin S, Zeng, X.N, Islam M.T, Bioactivity of Indonesian mahogany, Toona sureni (Blume) (Meliaceae), against the red flour beetle, Tribolium castaneum (Coleoptera, Tenebrionidae), Revista Brasileira de Entomologia, 56 (3), 2012, 354–358.

Samara, A Evaluation of Antioxidant Activity, Total Flavonoids, Tannins, and Phenolic Compounds in *Psychotria* Leaves Extracts, Antioxidant, 3, 2014, 745-757.

Tung Y.T, Wu J.H, Huang C.Y, Kuo Y.H, Chang S.T, Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark, Bioresource Technology, 100, 2009, 509–514.