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Phytochemical Profile and Antioxidant Assay of Ethyl Acetateof *Lawsonia inermis* (Linn) Leaf Extract

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Abstract : Objectives.Previous study showed that tannin, flavonoid, saponin, antraquinone and glycoside were traced in ethyl acetate (EAE) of*Lawsonia inermis* Linn. leaf extract. EAE was also shown to have antihyperglycemic activity in streptozotocin-induced diabetic rats. Present study was conducted to identify chemical compounds in EAE by using spectrophotometry methods and evaluate its another bioactivity ie. as antioxidant.
Material and methods. EAE was obtained by serial extraction with *n*-hexane (HE) and ethyl acetate (EAE). EAE then analyzed using Ultraviolet-Visible Spectropscopy (UV-Vis), Fourier Transform Infra Red Spectropscopy (FTIR), and Gass-Chromatography Mass Spectrum (GCMS). Antioxidant assay was conducetd using DPPH method.
Results.The UV-Vis spectrophotometer illustrated that compounds in EAE had conjugated double bond. The FTIR analysis showed that EAE of *L. inermis* contained compounds with aliphatic, hydroxyl and carbonyl groups. The GC-MS analysis demonstrated that there were 4 chromatograms with molecular weight of (a)177, (b)222, (c)129 and (d) 95) with different fragmentation respectively. The antioxidant activity of EAE was strong (IC50=97.68 μg/ml, whereas vitamin C as standard was very strong (IC50=2.79 μg/ml).
Conclusions. EAE consists of compounds withconjugated double bond that have aliphatic, hydroxyl and carbonyl groups moieties. These compounds were suggested to contribute of EAE' antioxidant activity.
Keywords : phytochemical, antioxidant, *Lawsonia inermis* (Linn), leaf, ethyl acetate.

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Introduction

Medicinal plants are part of human society to combat diseases, one of which is *Lawsonia inermis* (Linn.) as antidiabetic (Chaudary, et al, 2010; Widyawati et al, 2015) . This plant is commonly known as Henna and much growing in tropical areas including Indonesia (Chaudary, et al, 2010; Widyawati et al, 2017).Free radicals, highly reactive,able to damage molecules, are known increasingly as an underlying mechanism of cells injury that affecting human health and diseasesuch as diabetes (Flora, 2007). Thus, antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Young and Woodside, 2001; Sies, 2015). Our previous study showed antidiabetic activity of ethyl acetate of *L.inermis* (Linn) (EAE) leaf extract on streptozotocin-induced diabetic rats. Phytocemical qualitative assay of EAE identified the present of tannin, flavonoid, saponin, antraquinone and glycoside. So far, there is no data available of antioxidant activity of EAE. Therefore, the present study was conducted to investigate EAE' phytocemical compounds using spectrophotometry methods and to evaluate its antioxidant activity.

Material and methods

Plant Material Collection and Preparation of Extracts

The leaves of *L. inermis* Linn. were collected from Titi Kuning, Medan, Indonesia (Coordinate: 3.526093, 98.684528). The plant was identified at "Herbarium Bogoriense", the Research Centre for Biology-Indonesian Institute of Science, Bogor, Indonesia and given a herbarium identification number - No.924/IPH.1.01/If.07/III/2017. The fresh leaves were dried under shade and ground into powder. About 1.5 kg of the powdered leaf was extracted serially by maceration in *n*-hexane and ethyl acetate (EAE). The freeze-dried extracts were kept in the freezer (-20°C) before used.

Phytochemical Identification using Spectrophotometry Methods

Ultraviolet-Visible Spectropscopy(UV-Vis)

A number of EAE was dissolved in ethyl acetate and was measured using UV-Vis (Shimadzu UV 1800) at a wavelength range of 200-800 nm and the obtaines spectrum was observed.

Fourier Transform Infra Red Spectroscopy (FTIR)

A number of EAE that had been water free was forged with KBr powder andwas measured using FTIR (Shimadzu FT-IR Prestige-21).

Gass-Chromatography Mass Spectrum (GCMS)

As much as 1 microliter of EAE was injeted at GCMS (Shimadzu QP 2010-Plus)using programmed column temperature starts from 100 °C with a temperature rise every 10 °C until a temperature of 300 ° C was obtained, injector- and detector-temperature were 320^{o}C, respectively.

Antioxidant Assay : DPPH Radical-Scavenging Assay

The DPPH radical-scavenging activities of EAE was determined according to Molyneux (Molyneux, 2014).

1,1 diphenyl-2-picrylhydrazyl (DPPH) preparation : 20 mg of DPPH was dissolved in methanol until 100 ml to obtain 200 mcg/ml of DPPH solution.

Determination of maximum wavelength : 5 ml of DPPH solution was put in a 25 ml of mixed flask and the volume was adjusted with methanol to the mark line, to obtain a solution with a concentration of 40 mcg/ml. The absorbance was measured at 400-800 nm wavelength. The spectrum obtained then was observed.

Measurement of DPPH absorbance after addition of EAE : 25 mg of EAE was dissolved in 25 ml of methanol to obatin1000 µg/ml (LIB). Different sample volumes of LIB (1, 1.5, 2, 2.5, 3, 3.5)were put in mixed flask (25 ml) + 5 ml DPPH (200 µg/ml) to obtain different extract concentration, ie 40, 60, 80, 100, 120, 140

and DPPH 40 mcg/ml. After 10-30 minutes, the absorbance was measured at maximum wavelenght 516 nm.

Measurement of DPPH absorbance after addition of vitamin C : 20 mg of vitamin C crystal was dissolved in methanol until 100 ml, to obtain 200 µg/ml (LIB). Different colume of LIB (0.5, 1, 1.5, 2, 2.5 ml) were put 100 ml mixed flask of each. 5 ml DPPH (200 µg/ml) was added to obatin different vitamin C consentrations, ie 1, 2, 3, 4, 5 and DPPH 40 µg/ml). The absorbance was measured at wavelength 516 nm after 10-30 minutes.

The assay was performed in triplicate. The percentage of DPPH radical scavenging activity was calculated as follows

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{ADPPH} - \text{A sample}}{\text{A DPPH}} \times 100$$

A DPPH = Absorbance of DPPH without sample
A sample = Absorbance of DPPH with sample
$IC_{50}$was determined using regression linear equation.

Result and Discussion

Ultraviolet-Visible Spectropscopy(UV-VIS) Analysis

Uv/Vis indicates the presence of compounds having a conjugated double bond which can absorb ultraviolet wavelengths and visible. As shown at Fig.1 and Tab.1, there are several peaks that were depicted at wavelength ranging 200-800 nm. Based on the spectrum, EAE contained 7 compounds. Each compound was able to absorb the radiation at ultraviolet area as well as visible. This shows that EAE contains several chemical compounds which have a chromophore group. Compound no. 2 located at 666 nm wavelength has the greatest absorbance that dominates other compounds which are also present in EAE.

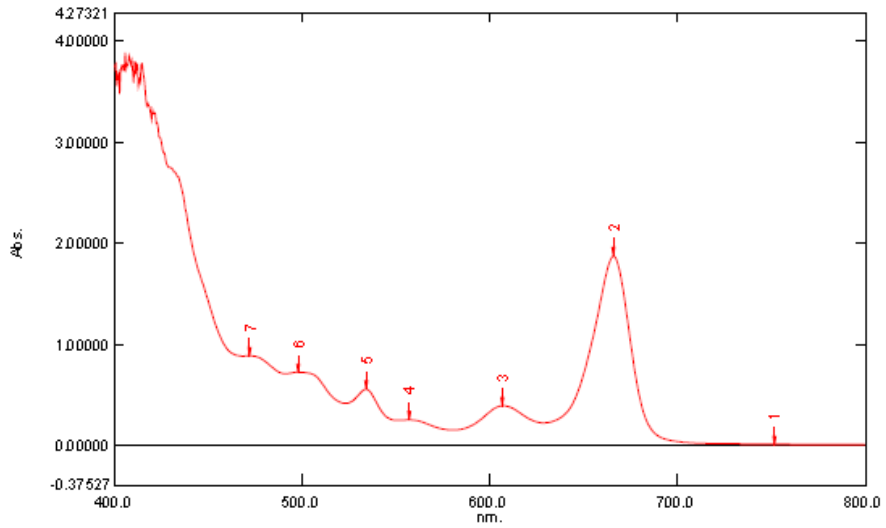

Fig.1. Spectrophotometry of ultraviolet-visible of ethyl acetate *L.inermis* Linn leaf extract

Tab.1. Absorption of ethyl acetate *L.inermis* Linn leaf extract

No	P/V	Wavelength	Absorbance
1	↑	752.0	0.01610
2	↑	666.0	1.87347
3	↑	607.0	0.39473
4	↑	557.0	0.25587
5	↑	534.0	0.55814
6	↑	497.5	0.72646
7	↑	472.0	0.88808

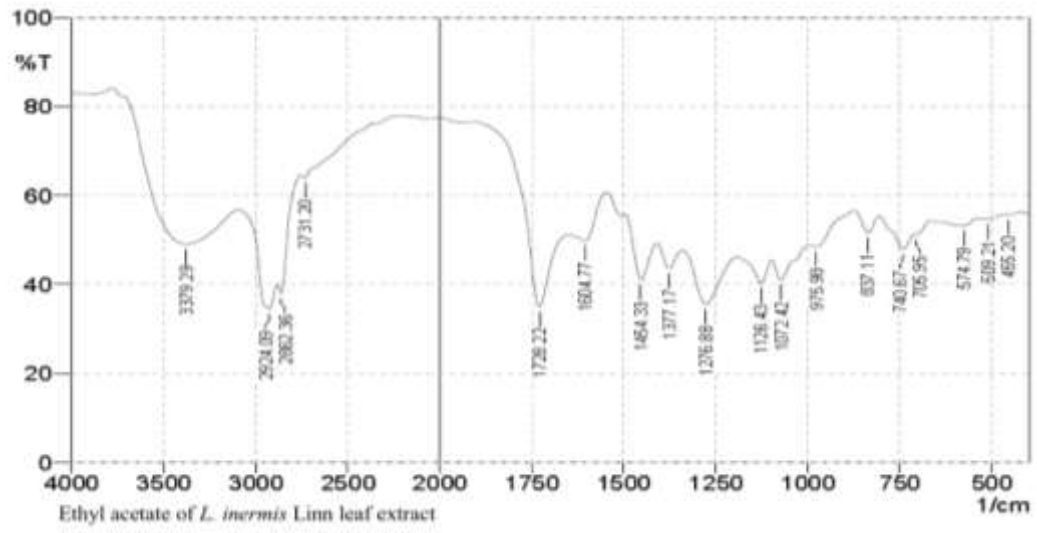

Fig.2. Spectrophotometry of infra red of ethyl acetate *L.inermis* Linn leaf extract

Fourier Transform Infra Red Spectroscopy (FTIR) Analysis

An assay using Infrared Spectroscopy was also performed to analyze functional groups of the chemical compounds that present in EAE. As shown at Fig. 2., EAE consists of several functional groups, namely: hydroxyl group (-OH) indicated by the presence of broad spectrum peak at wave number 3379.29 cm^{-1}, CH alifatis which was indicated by the presence of peak at wave number 2900-2700 cm^{-1} and was confirmed with peaks 1454.33 cm^{-1} and 1377.17 cm^{-1}. In addition, there was also C = O group which was indicated by the peak at wave number 1728.22 cm^{-1}.

Gass-Chromatography Mass Spectrum (GCMS) Analysis

GCMS analysis gives a result of a chromatogram profile and a molecular weight with a fragmentation pattern of a chromatogram of each compound contained in EAE. The chromatogram extract and GCMS analysis of EAE can be seen at Fig.3. and Tab.2.

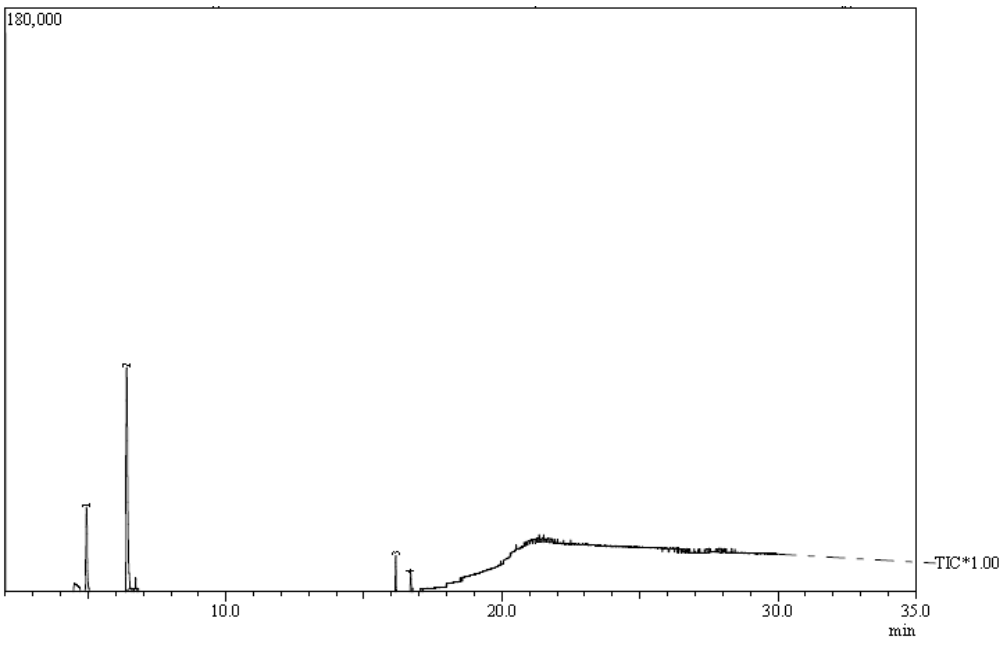

Fig.3. Chromatogram of ethyl acetate *L.inermis* Linn leaf extract

Tab.2. GCMS analysis of ethyl acetate *L.inermis* Linn leaf extract

Peak	R.Time	Area	Area %	Height
1	4.948	80243	22.26	26153
2	6.404	252673	70.08	68811
3	16.149	15815	4.39	11160
4	16.695	11797	3.27	5624
		360528	100.00	111748

The results showed that EAE consists of 4 chromatograms, indicating there were several chemical compounds contained in EAE. The chromatogram showed incomplete separation of the compound that shoed as an asymmetrical chromatogram.

It is known that the retention time of each compound detected in EAE were (1) 4.948, (2) 6.404, (3) 16.149, and (4) 16.695 minutes with different percentage of area. Chromatogram no 2 showed a high ercentage of area at 70.08%.In addition, GCMS assay showed the molecular weight of each peak that can be seen at Fig.4. The result shows that each peak has different molecular weight, ie. (1) 177, (2) 222, (3) 129, (4) 95 with different model of fragmentation. It means characteristic of a chemical compound.

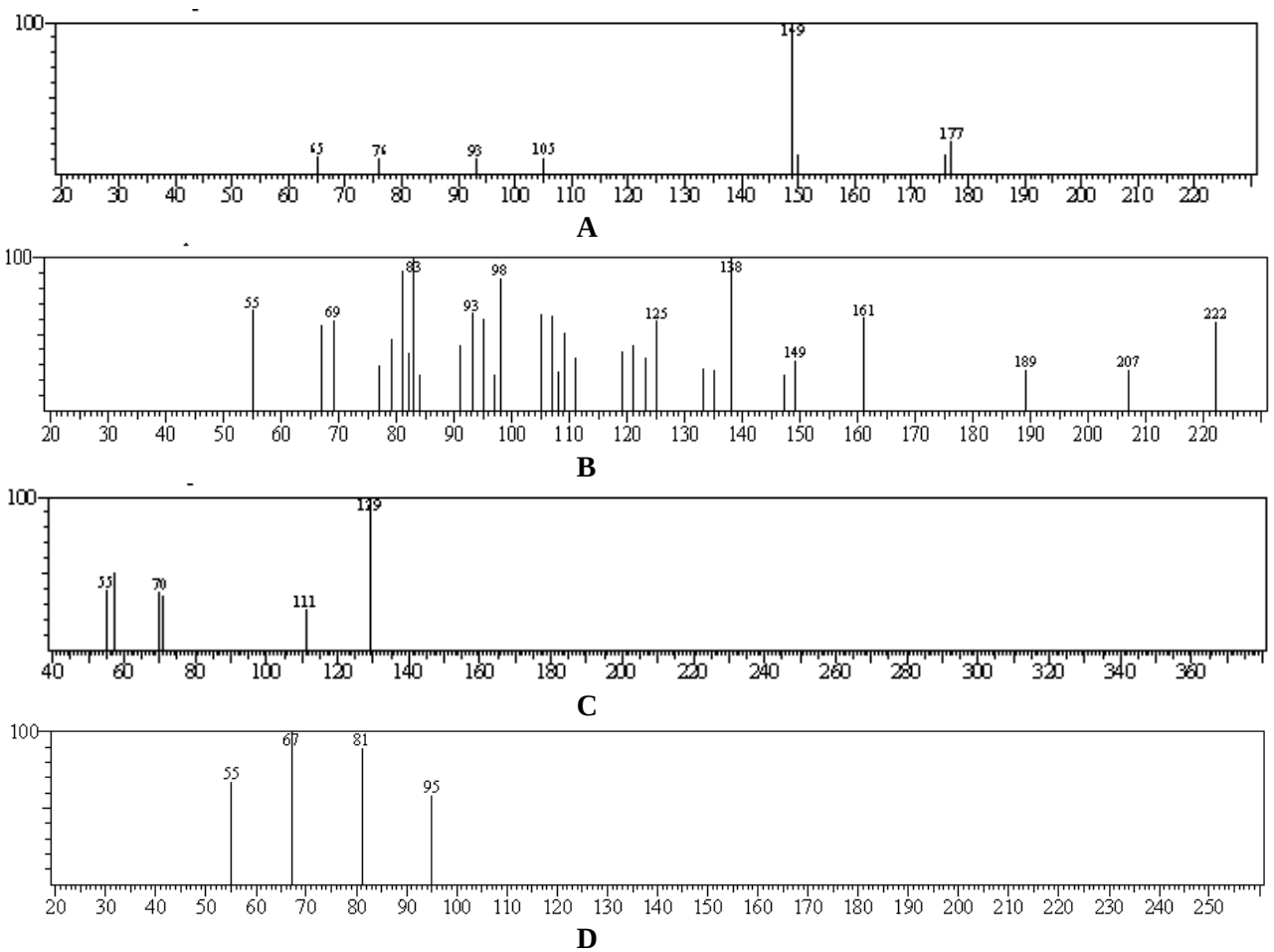

Fig.4. Pattern of fragmentation and molecular weight of each peak.

Spectrophotometric analysis results above suggested that there are several compounds contained in EAE that have a chromophore group. This notions that EAE may have antioxidant properties. A double bond increases antioxidant activity by affording a more stable flavonoid radical through conjugation and electron delocalization (Heim, et al, 2002).Conjugated double bonds in the molecule imparts pro-oxidant properties, making it very susceptible to attack by the addition of peroxyl radical (Burton, 1989; Wu, 1999). Multiple hydroxyl groups confer upon the molecule substantial antioxidant (Heim, et al, 2002). Thus, the present study continued with antioxidant activity test using DPPH radical-scavenging method. This assay was used to

evaluate the strength of antioxidant properties of EAE compare to vitamin C.Vitamin C, is a water-soluble free radical scavenger that can change to the ascorbate radical by donating an electron to the lipid radical in order to terminate the lipid peroxidation chain reaction (Nimse and Pal, 2015).The roles of many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to the protective effects (Rice-Evans, 1997)The more polar ones (ethyl acetate and n-butanol) are those that generally have higher antioxidant activity (Mensor, et al, 2001).

Tab.3. DPPH radical scavenging activity of ethyl acetate *L.inermis* Linn (EAE) leaf extract

EAE (µg/ml)	Absorbance	DPPH radical scavenging activity (%) (x± SD)
0 (blank)	0,850	0± 0.
40	0,775	8.82± 0.002
60	0,611	28.12 ± 0.
80	0,498	41.41± 0.0006
100	0,341	59.88± 0.0006
120	0,275	67.64± 0.0006
140	0,271	68.11± 0.001

Tab.4. DPPH radical scavenging activity of vitamin C

Vitamin C (µg/ml)	Absorbance	DPPH radical scavenging activity (%) (x± SD)
0	0.850	0± 0.0006
1	0.745	12.35± 0.0006
2	0.550	35.29± 0.001
3	0.395	53.52.± 0.001
4	0.226	73.41± 0.
5	0.071	91.64± 0.0006

Tab.4. IC50 of ethyl acetate *L.inermis* Linn. (EAE) leaf extract and vitamin C on DPPH free radical

Sample	IC_{50} (mcg/ml)
EAE	97,68
Vitamin C	2,79

Tab.3 and 4 showed the activity of EAE and vitamin C as radical scavengers. The higher of EAE lowered the absorbance which means the scavenging activity of EAE increased following the higher concentration of EAE. The IC50 values showed the inhibition concentration of DPPH at 50%. The results showed that IC50 of EAE was 97.68 µg/ml, whereas vitamin C as standard was at 2.79 µg/ml. A compound claimed to have antioxidant activity when the IC50 as the following categories: <50 µg/ml (very strong), 50-100 µg/ml (strong), 101-150 µg/ml (moderate), and 151- 200 µg/ ml (weak). Therefore, the present study concluded that EAE has strong antioxidant propertieswhile vitamin C showed a very strong antioxidant activity. Finally, the results show that the relative abilities of EAE to scavenge the radical are afffected by the presence of functional groups with increasing polarities, such as carbonyl and hydroxyl groups, as well as by the number of conjugated double bonds.

Conclusion

EAE consists of compounds withconjugated double bond that have aliphatic, hydroxyl and carbonyl groups moieties. These compounds were suggested to contribute to the strength of EAE' antioxidant activity**.**

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