TLC SCREENING FOR ANTIOXIDANT ACTIVITY OF HENNA (*Lawsonia inermis* L.) LEAF EXTRACT

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Abstract

Background. *Antioxidants have an important role to health, for example is the implications of the oxidation reaction in the body that can lead to cardiovascular disease, cancer, and aging. Natural antioxidants in plants can come from one or more components such as in Henna leaf, its compound can inhibit the oxidation reaction.*

Objective. *The aims of this study is to determine the chemical compound groups in Henna leaf extract that have antioxidant activity.*

Methods. *Powder of Henna leaf were macerated and frequently shaken with various of solvent such as water, methanol and chloroform for an hour. The filtrate of each extract was made up to concentration 10% w/v. Five microliter of each extract were spotted on silica gel F254 plates and eluated with mixture of chloroform: methanol 17:3 v/v as mobile phase. Spots that appear on the chromatogram were identified for flavonoids, naphthoquinone, polyphenols and also were sprayed with DPPH reagent to determine the spot that active as antioxidants.*

Outcome measured. *The active antioxidant Henna leaf constituents were detected as yellowish white spots produced by bleaching of DPPH by resolved bands on the TLC plates. All spot that have active antioxidant were identified by Rf value, characteristics spot under UV254, UV365, and spot colour after sprayed with specific spray reagent.*

Result. *The results of chromatogram showed that water extract, methanol extract and chloroform extract have antioxidant activity and were identified as flavonoids, naphthoquinone and polyphenols group. Potential antioxidant from the lowest respectively were chloroform extracts, water extract and methanol extract. Methanol extract have 4 spots, water extract have 3 spots and chloroform extract only have 1 spot were detected have antioxidant activity.*

Conclusion. Methanolic extract of Henna leaf had the greatest antioxidant activity than water or chloroform extract. The active compounds as antioxidant were flavonoids, naphthoquinones and polyphenols group.

Keyword : *Lawsonia inermis L, antioxidant, DPPH, TLC.*

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INTRODUCTION

Antioxidants have an important role to health, for example the implications of the oxidation reaction in the body that can lead to cardiovascular disease, cancer, and aging (Nelson *et al.*, 2003). Some synthetic antioxidant such as BHA and BHT are carcinogent and potentially toxic. Previous studies indicated that a high intake of antioxidants was positively associated with the reduced risk of coronary heart diseases and ageing related diseases (Flora, 2007; Valko *et al*., 2007). Indonesia has a fairly extensive tropical forests and biodiversity, both flora and fauna. One of the plants with the potential to be developed as antioxidant is henna (*Lawsonia inermis* L.). Natural antioxidants, such as in henna leaves, can inhibit the oxidation reaction.

L. Inermis L. have many biological activities besides used as a dye. Water and methanol extracts of the henna leaves have a high potential as an antioxidant and can simultaneously inhibit oxidative cell toxicity of MDA-MB-435S and pBR322 DNA induced Cr (VI) (Guha *et al*, 2009). The antioxidant activity of the methanol extract is higher than the water extract. This compares with total phenolic compounds extracted with methanol greater than the water extract of 2.56 mg / g and 1.45 mg / g calculated as tannins with Folin-Ciocalteu method (Hosein & Zinab, 2007). Henna plant have broad antimicrobial activity including as antibacterial, antiviral, antimycotic and antiparasitic (Babu and Subhasree, 2009). Chloroform extract can inhibit the growth of Malassezia in concentrations of 3 and 4 (v / v%), methanol extract at 0.25 and 3 (v / v%), while the water extract at 0.25 and 0.5 (v / v%) (Berenji, *et.al.*, 2010). Based on the description above, so in this experiment were used various of solvent such as water, ethanol and chloroform for henna leaves powders extraction to produce extract that have antioxidant activity. So the results of this study can be used as a reference for the preparation of henna extracts with high antioxidant activity.

MATERIALS AND METHODS

1. General

Silica gel 60 F254 TLC plates (Merck, Germany) were used for TLC bioautography analysis. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH_) purchased from Sigma-Aldrich (Steinheim, Germany). All solvents used for chromatography were pro analytical grade and extraction solvent used pharmaceutical grade.

2. Plant Materials

Leaves of *L. inermis* L. was collected from Yogyakarta province, Indonesia, in April 2012, and authenticated in Biology Phaculty of Ahmad Dahlan University, Yogyakarta, Indonesia.

3. Preparation of Henna Leaves Powder

Fresh free disease henna leaves was collected from Celeban Baru, UmbulHarjo, Yogyakarta then washed with running water and drained. The leaves material was then dried in oven at temperature 50^{o}C for 2 day until completely dry or easily crushed by hands. Dried leaves were powder by mechanicaly.

4. Preparation of water, methanol, and chloroform extract from henna leaves.

Five hundred milligrams of henna leaf powder was macerated using 5 ml of a suitable solvent while shaken for 1 h. The filtrate obtained by filtration using filter paper then volume was made to 5.0 ml by adding appropriate solvent.

5. Preparation of standard solution

Standard solution was made 0.1% w/v concentration in methanol.

6. Thin Layer Chromatography of Bioautographic Antioxidant Assays

A set of four plates of silica gel 60 F254 were used one plate for detection of bioautographic antioxidant assay and three another plates for detection of flavonoids,

polyphenols and naphthoquinones groups in each extract. Five µl 20% w/v of water, MeOH, and chloroform extract, were applied on TLC plate 1 and also 5.0 µl of naphthoquinone (lawsone) standard solution. On TLC plate 2 and 3 was used 5.0 µl of quercetine as standard solution. On TLC plate 4 was used lawsone and quercetine as standard solution. The plates were then developed with chloroform-MeOH (17:3, v/v) (Zainab, 2012). The dried TLC plates 1-4 were inspected under UV light (254 nm and 366 nm). TLC plate 1 was visualization with 5% KOH methanolic for naphthoquinone identification. TLC plate 2 was visualization with 1% $AlCl_3$ for flavonoids identification. TLC plate 3 was visualization with 1% $FeCl_3$ for polyphenol identification. TLC plate 4 was sprayed with 0.05% DPPH solution in methanol and allowed to stand for 30 minutes at room temperature. Spots which active compound as an antioxidant will be detected as a yellowish white spot with a purple background. All spots that have antioxidant activity were recorded the Rf values, characteristic of the spots under UV and given a score. Quercetin and lawsone were used as a positive control, and blank TLC plate was taken as negative control (Kannan *et al,* 2010).

RESULTS AND DISCUSSION

1. Determination plant

Determination was performed at Faculty of Biology, University of Ahmad Dahlan, Yogyakarta. The Results of determination is: 1b - 2b - 3b - 4b - 12b - 13b - 14b - 17b - 18b - 19b - 20b - 21b - 22b - 23b - 30b - 31a - 32a - 33a - 34a - 35b - 37b - 38b - 39b - 41b - 42b - 44b - 45b - 46a - 50a - 51b - 53b - 54b - 56a - 57b - 58b - 59b - 72B - 73b - 74a - 75b - 76a - 77a - 78b - 103A - 104b - 106b - 107b - 186b - 287b - 288b - 289a - 290b - 291a - 292b - 293a - Lythraceae - 1a - 2a - Lawsonia inermis L. (Backer, 1965).

Based on the result of determination, it can be seen that the plant will be determined and used in this study is correct species of *Lawsonia inermis* L.

2. Preparation of henna leaves powder

The Henna leaf was taken on April 2012 in Celeban Baru, Umbulharjo, Yogyakarta. The main material is taken from a certain place designed to avoid variations in the chemical constituents of plants. If the plants are taken from different places, then the influence of climatic and environmental conditions can lead to variations of active compounds in plants.

The dried leaves was powdered with blender and then sieved with sieve flour to get uniform powder size. The powder size was influence the effectivity of solvent contact with the powder, more small powder more effective so the active substance more extracted to the solvent. From 316.0 g of henna fresh leaves obtained 98.6 g of dried henna leaves, so the obtained yield was 31.20%. Dried henna leaf powder then was measured by graphimetric method and obtained 3.45% value of lost on drying.

3. Phytochemical Screening with Thin Layer Chromatography

3.1. The results of TLC screening for naphthoquinone identification

Identification of naphthoquinone groups compounds in water, methanol and chloroform extract was done by TLC with silica gel F254 as stationary phase and mixture of chloroform: methanol (17:3) v/v as mobile phase. The results of TLC test can be seen in Table I. All the spots of naphthoquinone showed quenching in UV-254 nm. After sprayed with 10% methanolic KOH reagent, naphthoquinone showed red fluorescence in UV-366 nm and red to red-brown in visible light (Wagner and Bladt, 1996).

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Table I. Result of thin layer chromatography of naphthoquinone identification

No	Rf value			Detector			Naphtho-quinone
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + KOH	
1	-	0,05	-	Q	orange	red orange	+
2	0,09	0,09	-	Q	orange	red orange	+
3	-	0,14	-	Q	yellow	yellow	-
4	0,19	0,19	0,19	Q	orange	red orange	+
5	0,23	0,23	-	Q	blue	yellow	-
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	red orange	+
8	-	0,47	0,47	Q	blue	yellow	-
9	-	-	0,66	Q	blue	yellow	-
Rf standard of lawsone : 0,19				Q	orange	red orange	+

Note:
Q = Quenching

The chromatogram results of naphthoquinone identification showed that water, methanol and chloroform extract contained of naphthoquinone groups. The water extract showed 2 spots at Rf 0.09 and 0.19, methanol extract showed 4 spots at Rf 0.05, 0.09; 0.19, 0.39 and chloroform extracts showed 2 spots at Rf 0.19; 0.39 which is detected as naphthoquinone groups.

3.2. The result of TLC screening for flavonoids identification

Identification of flavonoids groups compounds in water, methanol and chloroform extract was done by TLC with silica gel F254 as stationary phase and mixture of chloroform: methanol (17:3) v/v as mobile phase. The results of TLC test can be seen in Table II. All the spots of flavonoids on the chromatogram shows

Table II. Result of thin layer chromatography of flavonoids identification

No	Rf value			Detector			Flavonoids
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + $AlCl_3$	
1	-	0,05	-	Q	orange	-	-
2	0,09	0,09	-	Q	orange	-	-
3	-	0,14	-	Q	yellow	yellow	+
4	0,19	0,19	0,19	Q	orange	-	-
5	0,23	0,23	-	Q	blue	yellow	+
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	-	-
8	-	0,47	0,47	Q	blue	yellow	+
9	-	-	0,66	Q	blue	yellow	+
Rf Quercetin Standard: 0,25				Q	yellow	yellow	+

Note:
Q : Quenching

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Table III. Result of thin layer chromatography of polyphenol identification

No	Rf value			Detector			Polyphenol
	Water Extract	MeOH Extract	$CHCl_3$ Extract	UV_{254}	UV_{366}	Vis + $AlCl_3$	
1	-	0,05	-	Q	orange	grey	+
2	0,09	0,09	-	Q	orange	grey	+
3	-	0,14	-	Q	yellow	grey	+
4	0,19	0,19	0,19	Q	orange	grey	+
5	0,23	0,23	-	Q	blue	grey	+
6	-	0,26	-	Q	-	-	-
7	-	0,39	0,39	Q	orange	grey	+
8	-	0,47	0,47	Q	blue	green	+
9	-	-	0,66	Q	blue	green	+
Rf standar Quercetin : 0,25				Q	yellow	grey	+

Note
Q : Quenching

queching under UV-254 nm light. Depending on the type of flavonoids structure, flavonoid spots will show yellow, green-yellow, green, blue, dark violet, orange or red fluorescence under UV-366 nm and turn yellow after sprayed with citroborat or $AlCl_3$ reagent in visible light (Wagner and Bladt, 1996; Markham, 1988).

The results of chromatogram showed that water, methanol and chloroform extract contained of flavonoid groups. Water extract had one spot Rf 0.23, methanol extract had three spots at Rf 0.14; 0.23; 0.47 and chloroform extracts had two spots at Rf 0.47; 0.66 were detected as flavonoids groups.

3.3. The result of TLC screening for polyphenol identification

Identification of class of polyphenolic compounds content in water extract, methanol and chloroform was done by TLC with silica gel F254 stationary phase and mobile phase mixture of chloroform: methanol (17:3) v / v. TLC test results can be seen in Table III.

All the spots on the chromatogram shows the outage polyphenols under UV-254 nm. Depending on the type of structure of polyphenols, spots of polyphenols can fluorescence or not under UV-366 nm and blue-black or green-black after being sprayed with $FeCl_3$ reagent under visible light (Wagner and Bladt, 1996; Markham, 1988).

The results of polyphenolic identification on water, methanol and chloroform extract are shown in Table III. All of extract contained polyphenols group. The water extract had three spots Rf 0.09; 0.19 and 0.23 that detected as polyphenol. Methanolic extracts had seven spots that detected as polyphenols at Rf 0.05, 0.09; 0.14; 0.19, 0.23; 0.39 and 0.47. Chloroform extracts had four spots were detected as polyphenols at Rf 0.19, 0.39; 0.47 and 0.66.

3.4. The Results of Bioautografi Antioxidant Henna Leaves

Thin-layer chromatography bioautography antioxidant was used to rapidly detect chemical components in the extract that have antioxidant activity. This method components that have antioxidant activity with the ability to capture free radicals DPPH marked as yellowish white spots with purple background (Gu, *et al.*, 2009). Capture reactions of free radicals by antioxidant compounds can be seen in Figure 1. Results of thin-layer chromatography detection

of antioxidant compounds can be seen in Table IV.

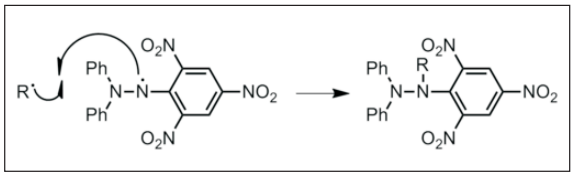

Figure 1. DPPH free radical capture reaction by an antioxidant compounds
(Gu, *et al.*, 2009)

Table IV showed that the water, methanol and chloroform extract contained an active component as an antioxidant. In the water extracts was detected 3 spots that active as an antioxidant. There were 2 spots that identified as naphthoquinone with Rf 0.09 and 0.19 and 1 spots detected as flavonoids with Rf 0.23. In the methanol extracts was detected 4 spots that active antioxidant. There were 2 spots with Rf 0.05, 0.09 detected as naphthoquinone and 2 spots were detected as flavonoids with Rf 0.19 and 0.23. In the chloroform extract was detected only 1 spot that has antioxidant activity at Rf 0.19 and was detected as naphthoquinone. Compared among of the three extracts the components of methanol extract have greates of antioxidant activity with the highest intensity of the yellow color. Results of other studies also showed that the water extract and methanol extract of henna leaves has great potential as an antioxidant and can simultaneously inhibit oxidative cell toxicity of MDA-MB-435S and pBR322 DNA induced Cr (VI). This compares with total phenolic compounds extracted with methanol greater than the water extract of 2.56 mg / g and 1.45 mg / g of tannins equivalen with Folin-Ciocalteu method (Guha *et al,* 2009; Hosein & Zinab, 2007). So that the methanol extract was more potent as an antioxidant than the water and chloroform extract.

CONCLUSION

In general water, methanol and chloroform extract of henna leaves had antioxidant activity and were identified as flavonoids, naphthoquinone and polyphenols groups. The potency of antioxidant from the lowest respectively were chloroform extracts, water extract and methanol extract. Methanol extract have 4 spots, water extract have 3 spots and chloroform extract only have 1 spot were detected have antioxidant activity. Our finding suggested that methanolic extract of henna

Table IV. Result of thin layer chromatography of antioxidant identification

No	Rf value			Vis + DPPH
	Water Extract	MeOH Extract	$CHCl_3$ Extract	
1	-	0,05	-	yellow
2	0,09	0,09	-	yellow
3	-	0,14	-	-
4	0,19	0,19	0,19	yellow
5	0,23	0,23	-	yellow
6	-	0,26	-	-
7	-	0,39	0,39	-
8	-	0,47	0,47	-
9	-	-	0,66	-
Spots active antioxidant	3 spots	4 spots	1 spot	
Score	+++	++++	+	

leaves more suitable to used as further research for antioxidant activity.

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REFERENCES

Babu, D.P. And Subhasree, R.S., 2009, Antimicrobial Activities of Lawsonia inermis - A Review, *Acad. J. Plant Sci.* 2 (4): 231-232.

Backer, C.A., Van Den Brink Jr., R.C.B., 1965, Flora of Java, vol. 1, 251, 256, Published under The Auspices of The Rijksherbarium, Leyden.

Berenji, F., Rakhshandeh, H., Ebrahimipour, H., 2010, *In vitro* study of the effects of henna extracts (*Lawsonia inermis*) on *Malassezia* species, *Jundishapur Journal of Microbiology*, 3(3): 125-128 http://jjm.ajums.ac.ir.

Gu, L., Wu, T. And Wang, Z., 2009, TLC bioautography guided isolation of antioxidants from fruit of Perilla frustescens var acuta, Food. Sci. Tech, 42:131-136.

Guha, G., Rajkumar, V., and Mathew L., 2009, Antioxidant Activity of *Lawsonia inermis* Extract Inhibits Cromium (VI)-Induced Cellular and DNA Toxicity, *eCAM*, http://creativecommons.org/licenses/by-nc/2.5

Hosein, H.K.M., and Zinab, D., 2007, Phenolic Compounds and AntiOxidant Activity of Henna Leaves Extracts (*Lawsonia inermis* L.), *World Journal of Dairy & Food Sciences* 2(1): 38-41, IDOSI Publications.

Kannan, R.R.R., Arumugam, R., Meenakshi, S., and Anantharaman, P., 2010, Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South., *International Journal of ChemTech Research CODEN(USA)*: IJCRGG ISSN : 0974-4290 Vol.2, No.3, pp 1526-1530, www.sphinxsai.com.

Nelson, J.L., P.S. Bernstein, M.C. Schmidt, M.S. Von Tress, dan E.W. Askew. 2003. Dietary modification and moderate antioxidant supplementation defferently affect serum carotenoids, antioxidants level and marker of oxidative stress in older humans. J. Nutr. 133: 3117-3123.

Valco M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M., Telser J., 2007, Review Free radicals and antioxidants in normal physiological functions and human disease, *The International Journal of Biochemistry & Cell Biology* vol. 39 p 44–84, www.sciencedirect.com.

Zainab, 2012, *Pengaruh konsentrasi etanol sebagai pelarut pengekstraksi terhadap kadar naftokinon dalam ekstrak daun Pacar Kuku*, research report of Research and Development Departement of Ahmad Dahlan University.