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# Antioxidant Activity of Dominant Plants Species in *Obat Pahit* from Lingga Malay Ethnic in Riau Archipelago

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antioxidant; lingga malay; *obat pahit*

## Abstract

*Obat Pahit* is a potion that has been long commonly consumed by Lingga Malay society for generations as stamina keeper. The most dominant plants found in the packaging of the Obat Pahit were namely *Bauhunia semibifida, Cnestis palala* and Penawa Root (3 species). This research aimed to investigate and determine activity of antioxidant contents in *Obat Pahit* from five Traditional Medicine Practitioners (TMPs) in the district of Lingga. The tested samples were mashed then being soaked into 2 types of solvent: distilled water and methanol, containing HCl 1%. DPPH method was also used in this research. Quantitatively antioxidant activity test of *Obat Pahit* from the five TMPs by using methanol solvent had extremely highest activity compared to the distilled water solvent. The test, using TLC plate by spraying the extract from three dominant plants with 0.1 mM of DPPH solution, produced a pale-yellow spots at a wavelength of 366 nm. On the other hand, the test using HPLC at wavelengths of 230 nm and 280 nm showed the presence of two dominant secondary metabolites contents: flavonoid and phenolic. IC50 (ppm) of *Bauhinia semibifida* (6.6247), Penawa Root (5.0124) and *Cnestis palala* (5.9968) were much lower than IC50 of mangosteen's rind (41.7675), vitamin C (6.6612) and Stimuno drug (8.333). This antioxidant analysis has not been reported previously. This proof contributed greatly to uncovering potentially native natural resources as an indigenous Indonesian drug which is expected to decrease dependence on imported drugs especially imunomodulator, antihypertensive, antidiabet etc. This research would be beneficial and excellent manifestation for the development of natural antioxidant-based medicines from traditional knowledge of Indonesia's local ethnicities.

## How to Cite

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## INTRODUCTION

Antioxidants are compounds that can prevent from the oxidation of other compounds which occurs either in the body or interaction of other compounds which are easily oxidized. It also can block the oxidation of free radicals occurring inside our body cells by neutralizing or destroying (scavenging) them (Lautan, 1997). Free radicals are atoms or molecules that have one or more unpaired electrons. This causes them are chemically reactive, which can cause chemical changes and damage on various components of living cells. In the human body, free radicals are considered to play a role in the occurrence of some diseases, such as aging. Currently, exposured by them is quite widespread in society, ranging from pollution to unhealthy foods (Winarsi, 2007). Therefore, various studies to obtain a safe antioxidant from natural sources were mostly done. Alkaloids, Flavonoids, tannins, polyphenols, vitamin C, vitamin E, and carotenoids are classes of compounds group from natural materials that possess the potency as antioxidants (Miller, 1996; Prior, 2003; Pokorny et al. 2001; Teruna et al. 2007, Zamri et al. 2016)

One of potions that derived from natural ingredients is *Obat Pahit*, which is commonly consumed by Malay ethnicity in Lingga, Riau Archipelago. This potion is efficacious to maintain body's power and fitness. Each village in Lingga has a Traditional Medicine Practitioner (TMP), who still uses and mixes ingredients of *Obat Pahit* with different types of medicinal herbs based on the knowledge inherited by generations. Some TMPs who are famous in concocting of *Obat Pahit* are originally from Kalan Village, SP4 Village, Linau Village, Resun Village dan Musai Village. This knowledge will different from one village to another because village difference of residence, which is affected by customs and procedures and behavior of local society (Irawan *et al.* 2013). In packaging of the potion from TMPs, there are some dominant plants species mentioned Kangkang Valves Root "*Akar Kangkang Katup*" (*Bauhinia semibifida),* Seven Layers Root "*Akar Tujuh Lapis*" (*Cnestis palala*), and *Akar Penawa*, as well as other complementary plants.

With varying TMPs and composition of *Obat Pahit*'s ingredients, it was necessary to examine the activity of antioxidant contents in *Obat Pahit* obtained from five TMPs, who are originally from Lingga, Riau Archipelago, as well as the investigation of antioxidant contents over the most three dominant plants used in the packaging of *Obat Pahit*. Overall, it was expected that this research would be beneficial and excellent manifestation for the development of natural antioxidant-based medicines from traditional knowledge of Indonesia's local ethnicities.

## METHODS

Materials used in this study were *Obat Pahit* potion from the five TMPs, plants sample of *Akar Kangkang Katup* (*Bauhinia semibifida*), *Akar Tujuh Lapis* (*Cnestis palala*), *Penawar* root, mangosteen's rinds (*Garcinia mangostana*), vitamin C, Drug Stimuno (adjusted for human dose and converted according to mouse dose by serving in infusa form), distilled water, methanol, silica gel GF254, ethyl-acetate, and DPPH powder (1,1-Diphenyl-2-picrylhydrazyl).

### Sample Extraction

*Obat Pahit potion with aquades solvent*

Each samples of *Obat Pahit* ingredients, at weight of 250 g, was crushed to powder using a grinder. This powder was then used for the extraction of secondary metabolite constituents by adding 1 liter of distilled water into a jar containing 250 g of *Obat Pahit* powder until the powder was submerged thoroughly. After that, it was then soaked for 1 hour. The extraction was filtered with filter paper. The filtrate was then evaporated to form a solid-liquid extract which will be used for the future test of antioxidant activity.

*Obat Pahit potion with methanol solvent*

Potions of *Obat Pahit* potion from five TMPs was grinded by using a blender. Then, it was obtained a powder which was used for extraction. A 100 g of Simplicia powder was macerated with methanol until it was submerged, and being soaked for a day. All extract was collected and evaporated with a rotary vacuum evaporator at 50 ° C to obtain a solid-liquid extract. The resulted extract was then measured.

*The dominant plants of Obat Pahit (Akar Penawar, Akar Tujuh Lapis, Akar Kangkang Katup/Sebaju)*

Potions of three dominant plants from the packaging of *Obat Pahit* potion was then crushed to powder using a blender. A total of 5 grams of powder samples were soaked in methanol for 24 hours and then ultrasonized for 30 minutes, followed by the filtration to obtain a liquid extract. The liquid extract was then evaporated by rotary evaporator till getting a thick layer.

**Antioxidant Activity in Quantitatve and Qualitative Obat Pahit potion**

*Quantitatively antioxidant activity test of Obat Pahit ingredients from five TMPs and examination of three dominant plants with DPPH Method*

The test of antioxidant activity used a two-fold-delution microplate reader with DPPH method (1,1- diphenyl-2-picryl hydrazyl) (Zhang et al., 2006; Wahdaningsih et al. 2013) at a wavelength of 520 nm. A sample of 2 mg was dissolved in 2 mL of MeOH until the concentration of the sample became 1000 mg / mL. Line A was inserted a sample of 100 mL (plate consisting of rows A-H, respectively amounted to 12 wells). A total of 50 mL MeOH was inserted into each well on line B-F. 50 mL from row A was then put into row B, 50 mL from row B was subsequently inserted into the line C, the same preparation was continually done to the line F. However, 50 mL from line F was discarded in order to obtain a concentration of 1000, 500, 250, 125, 62.5, and 31.25 g / mL. On the other hand, line G to H was filled with only 50 mL of MeOH, specifically only wells on the line 1-6 of row H filled. Line A-G for DPPH method was added by 80 mL of MeOH with a concentration of 80 mg / mL, and then incubated for 30 minutes. The activity of radical bounding was measured as a decrease of DPPH absorbance by the presence of microplate reader and data processing. Positive control was used as a comparison for ascorbic acid gradient at a concentration of 50 ug / mL. The percentage of inhibition value was calculated by the following formula (Andayani et al. 2008):

% Inhibition = (A Control-A Sample)/(A Control) x 100 %

Note: A control = Absorbansi uncontained sample

A sample = Absorbansi sample

*Qualitatively antioxidant activity test of three dominant plants with Thin Layer Chromatography Methods (TLC)*

0.5 ml of liquid extract (from point A) was then inserted into the vial. 5 samples were prepared for a TLC plate with a length of 5 cm elution. The respective samples were applied at the start layer in the elution from the chamber to the finish lines. The pattern of spots was appeared on the lamp of Uv ray at separation λ = 254 nm and 336 nm.

*Qualitatively antioxidant activity test of three dominant plants with High Performance Liquid Chromatography (HPLC)*

HPLC analysis was performed using elution method. Samples were dissolved in methanol (HPLC grade) (Susanti et al., 2017) (1 mg in 1mL methanol), then filtered with a 0.45 µ of PTFE 13mm. Filtrat as much as 20 mL were injected into the column, and then the samples were analyzed for 25 minutes using water:asettonitril (HPLC grade at 1 mg in 1mL methanol). ODS column was detailed with a length and a diameter of 150 x 4.6 mm.

## RESULTS AND DISCUSSION

**Quantitatively antioxidant activity test of Obat Pahit from five TMPs with two types of solvent: Distilled water and methanol**

Based on the results of quantitatively antioxidant activity test with the solvent of distilled water, only *Obat Pahit* from TMP's Linau village had the lowest value of IC50 compared to the IC50 value of *Obat Pahit* from TMP's other villages, at 73.6347. This indicated that the antioxidant constituents of *Obat Pahit* potion from Linau village were belonged to the category of powerful antioxidants. On the other hand, by using methanol solvent on quantitatively antioxidant activity, *Obat Pahit* potion from all TMPs had a very low value of IC50 as following: TMP from Kalan (4.9285), TMP from SP4 (13.1546), TMP from Linau (11.1490), TMP from Resun (27.9204) and TMP from Musai (9.2948); therefore this *Obat Pahit* potions, in other words, were classified as a very powerful antioxidant. IC50 values of each *Obat Pahit* potions from the five TMPs on the result test of quantitatively antioxidant activity by using DPPH method stated in Table 1.

**Antioxidant Activity Test of Three Dominant Medicinal Plants in the packaging of Obat Pahit potion**

*Qualitatively Antioxidant Activity test of three dominant medicinal plants using TLC Method*

The qualitatively antioxidants activity experiment was done by using TLC method. The extraction of *Obat Pahit* was spotted on TLC plate and then eluted by using eluen n-Heksan : ethyl acetate (5:5), eluen ethyl acetate : methanol (8:2), eluen ethyl acetate (100%), eluen ethyl acetate : methanol (6:4). The reason of using these eluen n-Heksan : ethyl acetate (5:5) was in order to be able to eluting by the clear appearence of colours and spot distance when spotted on TLC plate. This was according to its polarity, eluen n-Heksan : ethyl acetate n-Heksan : ethyl acetate (5:5), eluen ethyl acetate : methanol (8:2), eluen ethyl acetate (100%), eluen ethyl acetate : methanol (6:4). The reason of using eluen n-Heksan : et-

hyl acatete (5:5) was because of their attributes which are more non-polar so that compounds which were separated were also non-polar, this association based on the TLC principle, "like dissolve like". After the TLC plate eluted, it was then sprayed by DPPH and next observed under UV light with different wave lengths. The spot appearing with yellow colour showed the occurence of free anti-radicals activity. Antioxidant compounds will react to DPPH radicals through the mechanism of hydrogen atoms donation and also cause the occurence of decaying colour from purple to yellow (Molyneux, 2004).

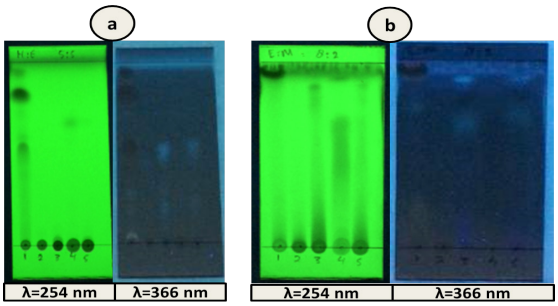



**Figure 1**. KLT Profile of plant was sprayed DPPH with Eluen N-Heksana : Etil asetat = 5:5(a) and Eluen Etil asetat : Methanol = 8:2(b)

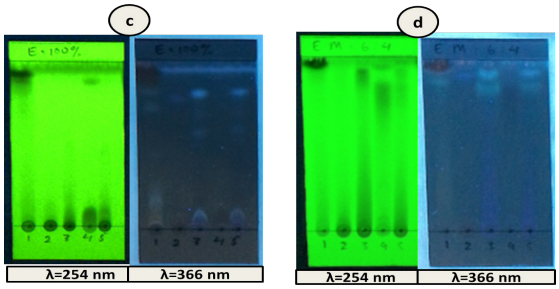
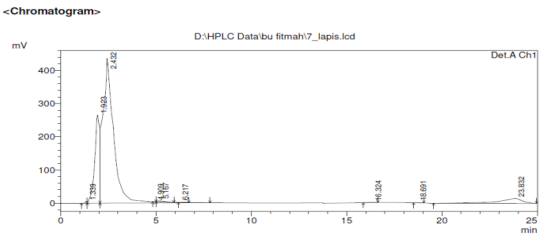



**Figure 2**. KLT Profile of plant was sprayed DPPH with Eluen Etil asetat = 100 % (c) dan Eluen Etil asetat : Metanol = 6:4 (d)
Note:1 = Mangosteen peel; 4 = *Penawar* Roots; 2 = *Cnestis palala;* 5 = Mixture; 3 = *Bauhinia semibifida*

Based on the TLC results, it can be compared between patches or spots from the five plant extracts with two different wavelengths, namely λ = 254 nm and λ = 366 nm. On the samples, more than one dots indicated that the samples had an organic compound or antioxidant compound. At a wavelength λ = 254 nm, antioxidant compounds such as terpenoids and flavonoids were not visible. However, at the wavelength λ = 366 nm, they are visible. One of them that can be seen from the TLC plate by using eluen ethylacetate:methanol (6:4) at a wavelength λ = 366 nm was more vivid than other eluens.

*Qualitatively Antioxidant Activity test of three dominant medicinal plants using HLPC Method*

Based on the HPLC assay with wavelengths of 230 nm and 280 nm, it was obtained some compounds which were dominant on the three plant samples and mixture sample. Wavelengths of 230 nm and 280 nm are the wavelengths to identify flavonoids and phenolic contents. It was also proved by the test of three dominant plants on antioxidant activity that showing a high antioxidant activity, because of phenolic and flavonoids contents are categorized as antioxidants (Figure 3; Figure 4; Figure 5; Figure 6).

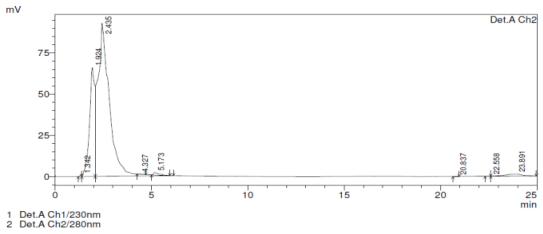



(a)

**Table 1**. Results of Quantitatively Antioxidant Activity Test of *Obat Pahit* potion from each TMPs with Distilled Water and Methanol Solvent

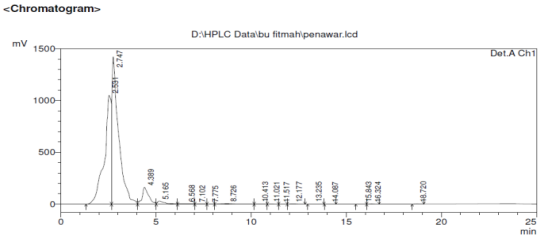
| TMP | IC50 Value (ppm) Distilled Water | Antioxidant Activity Criteria* | IC50 Value ppm) Methanol | Antioxidant Activity Criteria* |
|---|---|---|---|---|
| Kalan | 150.2199 | Moderate | 4.9285 | Very Strong |
| SP4 | 122.3022 | Moderate | 13.1546 | Very Strong |
| Linau | 73.6347 | Strong | 11.1490 | Very Strong |
| Resun | 202.9095 | Very Weak | 27.9204 | Very Strong |
| Musai | 161.1920 | Weak | 9.2948 | Very Strong |

*Antioxidant activity criteria base on (Zuhra, 2008)

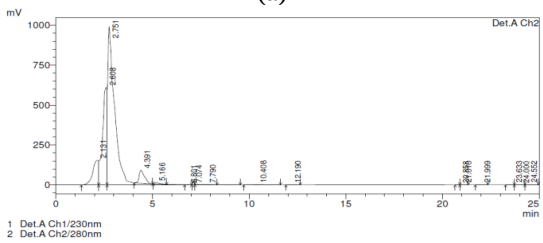



(b)

**Figure 3**. Result of HPLC from *Akar Tujuh Lapis* with Wavelength Detector (A) 230 nm and (B) 280 nm

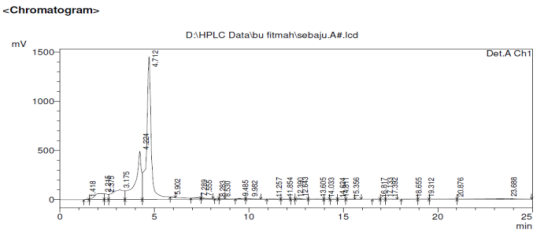



(a)

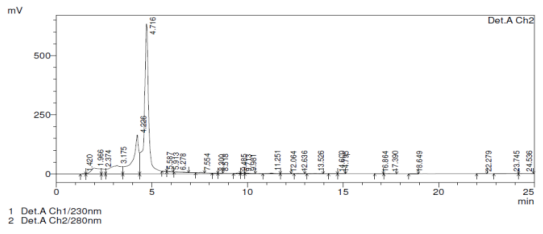



(b)

**Figure 4.** Result of HPLC from *Akar Penawar* with Wavelength Detector (A) 230 nm and (B) 280 nm

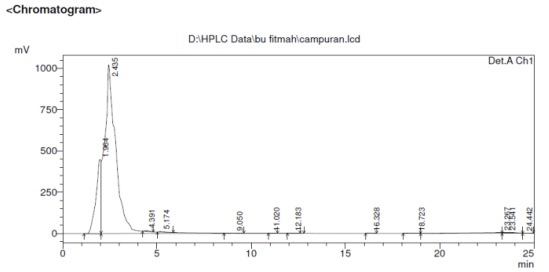



(a)

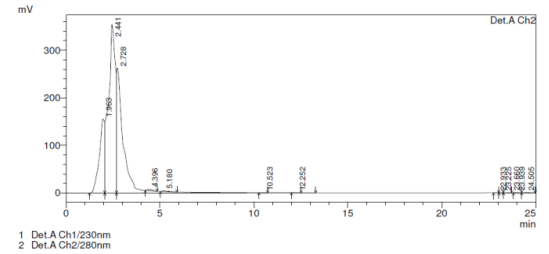



(b)

**Figure 5**. Result of HPLC from *Akar Kangkang Katup* with Wavelength Detector (A) 230 nm and (B) 280 nm




(a)




(b)

**Figure 6**. Result of HPLC from Mixture of *Akar Kangkang Katup*, *Akar Tujuh Lapis* dan *Akar Penawa* with Wavelength Detector (A) 230 nm and (B) 280 nm

*Quantitatively Antioxidant Activity test of three dominant medicinal plants using DPPH Method*

Quantitatively antioxidant activity test was also conducted on the single-most dominant herbs. This was carried out to observe the antioxidants activity on the herbal plants within *Obat Pahit* potion stated in Table 2 below:

**Table 2**. Results of Quantitatively Antioxidant Activity Test of Three Dominant Medicinal Plants used in *Obat Pahit* potion Packaging with Methanol Solvent

| Samples' name | IC50 Value (ppm) | Antioxidant Activity Criteria* |
|---|---|---|
| Bauhinia semibifida | 6.6247 | Very strong |
| *Penawar* root | 5.0124 | Very strong |
| Cnestis palala | 5.9968 | Very strong |
| Mixture | 29.6644 | Very strong |
| Garcinia mangostana | 41.7675 | Very strong |
| Asam Askorbat | 6.6612 | Very strong |
| (*Phyllanthus niruri*) | 8.333 | Very strong |

*Antioxidant activity criteria base on (Zuhra, 2008)

Positive controls used in this study were vitamin C (Ascorbic Acid), Mangosteen's rinds and immunomodulatory drugs (Stimuno). Vitamin C is an antioxidant that is soluble in water. The use

of positive control of this test is to find out how strong antioxidant potential exist in three types of methanol extract obtained from the plants, when compared to vitamin C. When the IC50 value of the samples is equal or close to the IC50 value of the positive control, it can be considered that the sample could potentially become as a powerful alternative antioxidant.

Based on the results of this test, the IC50 values of Penawar Roots, Seven-Layer Roots and Kangkang-Valve Root are 5.0124; 5.9968; 6.6247 (ppm), respectively. The three plants can be considered to have a very strong antioxidant activity, because it has a lower IC50 values than positive controls, i.e mangosteen peel (41.7675 ppm), IC50 in pure ascorbic acid (6.6612) and IC50 Stimuno (8.333 ppm). This indicates that the Penawar Roots, Seven-Layer Roots and Kangkang-Valve Root have a great potential as an antioxidant.

Since substantial evidences indicate that numerous human disorders and diseases present the involvement of oxidative stress (Teixeira et al*,* 2015). The attention of scientists has turned on the prospection and evaluation of antioxidant agents for the prevention and treatment of several diseases such as diabetes, atherosclerosis, aging, immune suppression and neurodegeneration (Saeed et al, 2012). The results from this study demonstrate high potential of these plant species also as sources of natural antioxidants. Natural antioxidants derived from plants, either in the form of raw extracts or their chemical constituents, are accepted to be very effective preventing the deleterious processes caused by oxidative stress (Cruz et all*,* 2014). This antioxidant analysis was proof contributed greatly to uncovering potentially native natural resources as an indigenous Indonesian drug which is expected to decrease dependence on imported drugs especially imunomodulator, antihypertensive, antidiabet etc.

## CONCLUSION

The antioxidant activity of *Obat Pahit* potion from the five TMPs using methanol was significantly very strong compared to the solvent of distilled water. The qualitatively antioxidants test of *Bauhinia semibifida*, *Cnestis palala* and Penawar Roots by TLC and HPLC methods indicated the presence of flavonoid and terpenoids contents. Qualitatively antioxidant activity of IC50 values obtained from Penawar Roots, *Cnestis palala* and *Bauhinia semibifida*, respectively 5.0124 mg / mL; 5.9968 mg / mL: 6.6247 mg / mL, is much lower than the IC50 value of ascorbic acid (6.6612 mg / mL) and Stimuno (8.3333), even 4 times the mangosteen peel extract (41.7675 g / mL). Future studies are expected to examine the antioxidant contents of the active compounds of *Obat Pahit* potion and the three dominant plants in packaging.

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