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# ANTIOXIDANT ACTIVITY OF TRADITIONAL BALINESE MEDICINE *LOLOH DON PIDUH* (*Centella asiatica (L.) Urban*) AND *LOLOH DON KAYU MANIS* (*Saoropus androgynus (L.) Merr*)

**Angelia Efraim Horas[1], I Gusti Made Gde Surya Chandra Trapika[2*)], Ida Ayu Alit Widhiartini[2], I Gusti Made Aman[2]**
[1]Bachelor of Medicine and Medical Profession, Faculty of Medicine, Udayana University
[2]Department of Pharmacology, Faculty of Medicine, Udayana University
[*)] Correspondence author: e-mail: surya_trapika@unud.ac.id

## ABSTRACT

*Loloh* is traditional Balinese medicine whose popularity has increased recently. Balinese people consume it as a fresh beverage to maintain their immune system. *Loloh Don Piduh* and *Loloh Don Kayu Manis* are two traditional formulas that are widely used today. Limited studies regarding their potential formulas activity in immune response and their antioxidant properties have not been reported. Several studies reported the antioxidant properties as a single herb but not in formula as a traditional *loloh*. Therefore, it is necessary to explore their antioxidant property. *Loloh* is formulated in the way of traditional process and lyophilized by freeze-drying to produce a stable dry extract. Gas chromatography-mass spectrum (GC-MS) was conducted to examine their chemical compounds. The antioxidant test was carried out using the DPPH method. Antioxidant test showed $IC_{50}$ values of 15.22 ppm and 33 ppm for *Loloh Don Kayu Manis* and *Loloh Don Piduh* respectively. There are 5 dominant compounds contained in *Loloh Don Piduh* namely *Hexadecanoic acid, ethyl ester, Neophytadiene, Caryophyllene oxide, (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene, Ethyl 9,12,15-octadecatrienoate*. While, *Octadecadienoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, alpha.-Copaene, Panaxynone, Neophytadiene* were found in *Loloh Don Kayu Manis.* Antioxidant test showed $IC_{50}$ values of 15.22 ppm and 33 ppm for *Loloh Don Kayu Manis* and *Loloh Don Piduh,* respectively. *Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid, ethyl ester, alpha copaene, neophytadiene* were reported to have antioxidant activity. This two loloh showed potencial antioxidant property and need further examination. In addition, they could be developed to enhance their activity emerges as promising traditional healthy food.

**Keywords :** loloh don piduh., loloh don kayu manis., antioxidant

## INTRODUCTION

*Loloh* is Balinese traditional medicine formulated from various parts of herbal medicine including leaves, roots, fruits, flowers and bark. This traditional beverage is consumed by the Balinese people to prevent disease, body fitness and beauty. Among the various kind of *loloh, Loloh Don Piduh* and *Loloh Don Kayu Manis* are two most popular one.[1] *Loloh Don Piduh* has the main raw material, namely gotu kola leaves. In making *Loloh Don Piduh*, gotu kola leaves are usually added with other ingredients such as lime.[2] Traditionally, gotu kola is used as a medicine for coughs, stomachaches, body aches, asthma, hemorrhoids, inflammation.[3] Gotu kola leaves as the main ingredient of *Loloh Don Piduh* contain metabolite compounds, namely saponins, steroids, and triterpenoids such as madecassoside, asiaticoside.[4,5]

*Loloh Don Kayu Manis* is made from katuk leaves as the main raw material and added with shallots. Katuk leaves are effective in healing wounds, curing urinary disorders, and relieving fever. The compounds contained in katuk leaves are alkaloids, triterpenoids, saponins, tannins, polyphenols, glycosides, and flavonoids.[6]

To our knowledge, a comparative study of antioxidant activity between *Loloh Don Piduh* and *Loloh Don Kayu Manis* has not been conducted. Therefore, it is necessary to explore and compare their antioxidant activity.

## MATERIALS AND METHODS

### Materials Collection

The ingredients for making *Loloh Don Piduh* and *Loloh Don Kayu Manis* were obtained from the Perkebunan Cijengkol Bukit Raya Atas, (Punclut) Ciumbuleuit, Cidadap District, Bandung, West Java in January. In this study, *loloh* will be prepared traditionally. Gotu kola leaves, katuk leaves, lime, and shallots were washed under running water. Forty grams of gotu kola leaves were mixed with 100 ml of water and 4 ml of lime using a blender. Forty grams of katuk leaves were mixed with 100 ml of water and 4 ml of shallots using a blender. Both Lolohs were filtered with *Whatman no.1* filter paper. Then to reduce the solvent from the solute in both loloh, a freeze dryer machine was used at Pusat Penelitian Nanosains dan Nano Teknologi Institut Teknologi Bandung. Both *lolohs* were reduced in solvent until a thick paste-like consistency was obtained. This research has received ethical feasibility with ethical feasibility number 226/Un14.2.2.VII.14/LT/2023.

### Antioxidant Activity Test

Antioxidant activity test was conducted at Adventist University of Indonesia, Bandung, where this test is used to measure the ability of a chemical compound to fight free radicals using a spectrophotometer. Antioxidant activity in this study was carried out by the DPPH *(2-2,2-diphenyl-1-picrylhydrazyl)* method quantitatively. A total of 3 ml of 40 ppm DPPH was added to 1 ml of *loloh* solution dissolved in concentrations of 1,2,3,4 ppm and incubated for 30 minutes in a dark room. A total of 0.3 ml of 40 ppm DPPH was added to 0.1 ml of Loloh solution dissolved in concentrations of 0.1,0.2,0.3,0.4 ppm and incubated for 30 minutes in a dark room. Vitamin C used as positive control. The maximum wavelength of 517 nm was obtained in the 40 ppm DPPH solution. The wavelength of 517 nm will be used in the measurement of antioxidant absorbance. [7,8]

Then, $IC_{50}$ value were compared between *Loloh Don Piduh* and *Loloh Don Kayu Manis*. $IC_{50}$ intensity below 50 ppm is classified as very strong antioxidant, 50-100 ppm is classified as strong antioxidant, 100-150 ppm is classified as moderate antioxidant, above 200 ppm is classified as weak antioxidant.[8]

**Gas Chromatography-Mass Spectrometry (GC-MS)**

The GCMS test was conducted at Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada. It may analyze complex compounds sensitively. This analytical method combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. In the GC system, compounds will be separated according to their polarity in a capillary tube with silica as a stationary phase. The larger mass will move slower, and the time the compound passes through the capillary tube, the retention time will be varied. Retention time is a measuring parameter produced by the compound separation process. After the elution process through the GC system, the compounds will then pass into the MS system. They will be ionized and fragmented to be ion fragments, detected by the MS detector and computed to be a mass spectrum in which compounds will be identified based on their unique fragmentation of each compound. The difference in charge and mass of each compound will produce a different ratio of mass (m) to charge (z), so measurements in the MS system can be obtained and compounds can be identified. The retention time parameters of the GC system and the mass-to-charge ratio of the MS system for the compound will be matched with the National Institute of Standards and Technology Mass Spectral Database (NIST-MS) parameters.[9]

## RESULTS

**The Antioxidant Activity Test Results**

DPPH assay showed that *Loloh Don Piduh* and *Loloh Don Kayu Manis* have very strong antioxidant activity. Antioxidant activity test results can be seen in the Table 1 below.

**Table 1.** The DPPH test results of Loloh Don Piduh and Loloh Don Kayu Manis

| Loloh | $IC_{50}$ (ppm) | Category |
|---|---|---|
| Loloh Don Piduh | 33,007 | Very Strong |
| Loloh Kayu Manis | 15,222 | Very Strong |
| Vitamin C | 1,277 | Very Strong |

The $IC_{50}$ value of *Loloh Don Kayu Manis* is 2.1 times greater than *Loloh Don Piduh.*

**The GCMS Results of *Loloh Don Piduh***

Figure 1 shows the total ion chromatogram of the *Loloh Don Piduh*. A total of 13 abundance peaks were obtained from the chromatogram with different retention times. From the results of this measurement, show that the *Loloh Don Piduh* contain several compounds as listed in Table 2 below**.**

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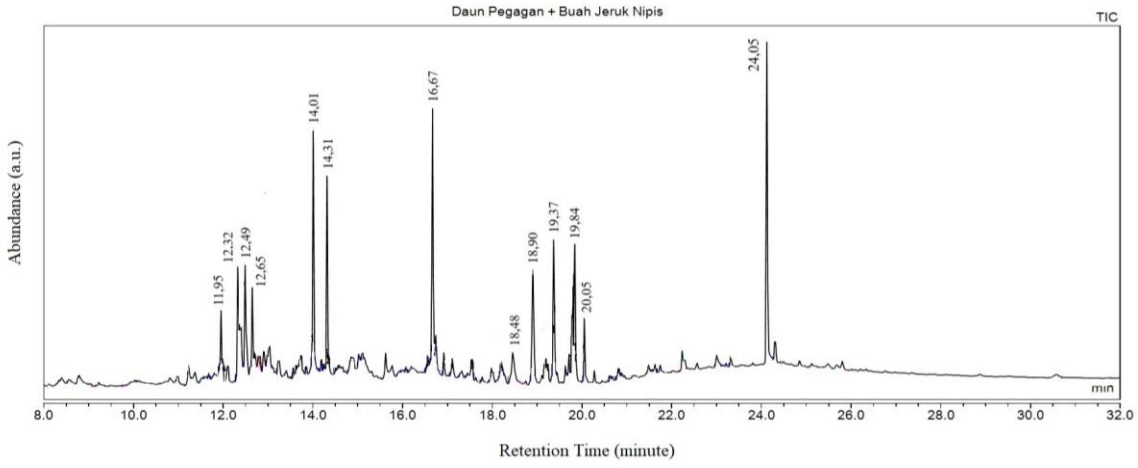


**Figure 1.** The Chromatogram of Loloh Don Piduh

**Table 2.** The GCMS Results of Loloh Don Piduh

| Peak # | Retention Time (Minute) | Peak Area | % Peak Area | Chemical Formula | Compound Name |
|---|---|---|---|---|---|
| 1 | 24,05053 | 38271275,65 | 18,26417611 | C18H36O2 | Hexadecanoic acid, ethyl ester |
| 2 | 16,66641 | 33974584,23 | 16,21366885 | C20H38 | Neophytadiene |
| 3 | 14,00677 | 26520817,73 | 12,65651268 | C15H24O | Caryophyllene oxide |
| 4 | 14,31286 | 23507351,73 | 11,21839826 | C15H24O | (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene |
| 5 | 19,83962 | 21269322,8 | 10,15034516 | C20H34O2 | Ethyl 9,12,15-octadecatrienoate |
| 6 | 18,90093 | 19619995,2 | 9,363237613 | C17H22O | Panaxynone |
| 7 | 19,36687 | 18642946,83 | 8,896961453 | C20H40O | Phytol |
| 8 | 12,48648 | 16997572,31 | 8,111740435 | C15H24 | (1R,9R, E)-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-4-ene |
| 9 | 12,32323 | 16807520,92 | 8,021042331 | C15H24 | cis-ß-Farnesene |
| 10 | 18,48000 | 10932426,202 | 5,217274682 | C17H24O2 | Panaxydol |
| 11 | 12,64633 | 8298567,293 | 3,960319899 | C15H24 | (1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl) spiro [4.5] dec-7-ene |
| 12 | 20,05049 | 7575184,227 | 3,615100267 | C20H40O2 | Heptadecanoic acid, 15-methyl-, ethyl ester |
| 13 | 11,95251 | 5396563,238 | 2,575398382 | C15H24 | Caryophyllene |
| | Total | 209542852,7 | 100 | | |

**The GCMS Results of *Loloh Don Kayu Manis***

Figure 2 shows the total ion chromatogram of the *Loloh Don Kayu Manis*. A total of 17 abundance peaks were obtained from the chromatogram with different retention times. From the results of this measurement, show that the *Loloh Don Kayu Manis* contain several compounds as listed in Table 3 below.

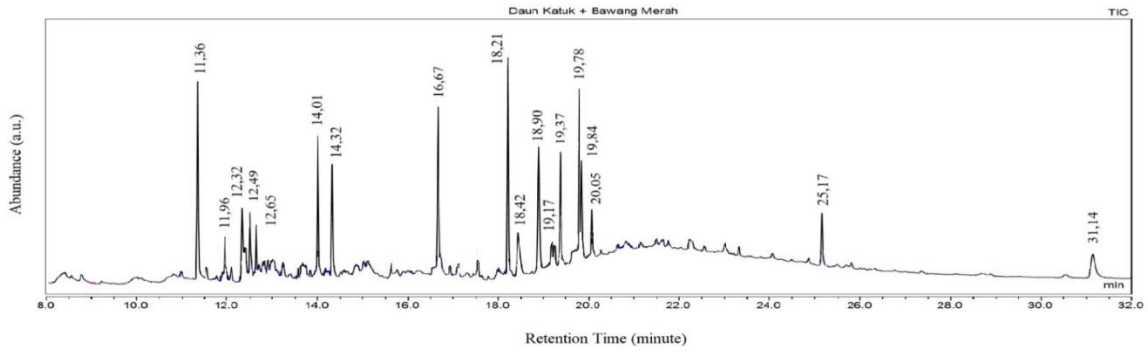


**Figure 2.** The Chromatogram of Loloh Don Kayu Manis

**Table 3.** The GCMS Results of Loloh Kayu Manis

| Peak # | Retention Time (Minute) | Peak Area | % Peak Area | Chemical Formula | Compound Name |
|---|---|---|---|---|---|
| 1 | 19,78 | 43265442,149 | 11,251896 | C20H36O2 | 9,12-Octadecadienoic acid, ethyl ester |
| 2 | 18,21 | 40592512,489 | 10,556756 | C18H36O2 | Hexadecanoic acid, ethyl ester |
| 3 | 11,36 | 40166318,841 | 10,445917 | C15H24 | alfa-Copaene |
| 4 | 18,90 | 32200218,070 | 8,3742006 | C17H22O | Panaxynone |
| 5 | 16,67 | 30679588,155 | 7,9787356 | C20H38 | Neophytadiene |
| 6 | 14,01 | 26494972,589 | 6,8904569 | C15H24O | Caryophyllene oxide |
| 7 | 19,84 | 25606882,038 | 6,6594942 | C20H34O2 | Ethyl 9,12,15-octadecatrienoate |
| 8 | 14,32 | 23293295,911 | 6,0578078 | C15H24O | (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene |
| 9 | 19,37 | 21554421,254 | 5,6055846 | C20H40O | Phytol |
| 10 | 12,32 | 19305401,157 | 5,0206896 | C15H24 | cis-ß-Farnesene |
| 11 | 31,14 | 18776135,603 | 4,8830454 | C29H48O | Stigmasterol |
| 12 | 12,49 | 17041133,351 | 4,4318293 | C15H24 | (1R,9R, E)-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-4-ene |
| 13 | 25,17 | 11891048,336 | 3,0924643 | C30H50 | Squalene |
| 14 | 20,05 | 10178927,000 | 2,6471987 | C20H40O2 | Octadecanoic acid, ethyl ester |
| 15 | 19,17 | 10031585,254 | 2,6088801 | C19H34O2 | 9,12-Octadecadienoic acid, methyl ester, (E, E)- |
| 16 | 12,65 | 7938969,564 | 2,0646607 | C15H24 | R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl) spiro [4.5] dec-7-ene |
| 17 | 11,96 | 5500067,561 | 1,4303838 | C15H24 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- |
| Total | | 384516919,325 | 100 | | |

## DISCUSSION

### Antioxidant Activity of *Loloh Don Piduh* and *Loloh Don Kayu Manis*

Antioxidant activity test using DPPH (*α-diphenyl-β-picrylhydrazyl*) is based on donating electrons from antioxidants to neutralize DPPH radicals. The reaction is seen from the color change of DPPH from purple to yellowish at a wavelength of 517 nm and the color changed is an indicator of antioxidant activity. Antioxidant activity by DPPH is indicated by the $IC_{50}$ value. The $IC_{50}$ value describes the amount of antioxidant concentration that can reduce 50% of DPPH concentration. DPPH is a simple test to measure antioxidant activity using a spectrophotometer.[10,11]

Previous study on gotu kola ethanolic extract showed that an $IC_{50}$ value of 191.19 ppm which is classified as a weak antioxidant. When compared to *Loloh Don Piduh* which is a combination of gotu kola leaves and lime which has an $IC_{50}$ of 33.00 ppm, it can be concluded that the addition of lime has a major influence on increasing the value of its antioxidant activity.[12]

The katuk leaf extract showed an $IC_{50}$ value of 50.62 ppm which is classified as a strong antioxidant.[13] combination of katuk leaves and shallots made into *Loloh Don Kayu Manis* has a greater antioxidant value of 15.22 ppm. This is because shallots have antioxidant activity that will increase the $IC_{50}$ value of katuk leaves when combined.

The antioxidant activity of Vitamin C at concentrations of 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm is 25.8 times greater than the IC50 result of *Loloh Don Piduh* and 11.9 times greater when compared to the $IC_{50}$ result of *Loloh Don Kayu Manis*. This is because vitamin C is a pure compound that is the result of isolation while *loloh* extract is still a mixture of compounds that have various properties. In an effort to produce the same antioxidant activity as Vitamin C, it is necessary to increase the amount of concentration in both *loloh*.

*Loloh Don Piduh* and *Loloh Don Kayu Manis* were shown to have very strong antioxidant activity, this strengthens the Balinese habit of drinking *loloh* as a health drink to increase endurance, especially for *Loloh Don Piduh*.[14] Meanwhile, *Loloh Don Kayu Manis* is often used for pain relief, fever reduction, cough medicine, dizziness, and fever.[15]

### Gas Chromatography-Mass Spectrometry

GCMS results show that there are 13 compounds contained in *Loloh Don Piduh* extract which is a combination of gotu kola leaves and lime, where there are 5 dominant compounds namely *Hexadecanoic acid, ethyl ester, Neophytadiene, Caryophyllene oxide, (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene, Ethyl 9,12,15-octadecatrienoate*. These GCMS results strengthen the research where in the study it was stated that gotu kola leaf extract contains *Hexadecanoic acid, ethyl ester,*

*Neophytadiene* compounds.[16] GCMS results of piduh leaves show that piduh leaves contain monoterpenoids (C10) and sesquiterpene (C15) including *caryophyllene, gemarcrene-D, alpha humulene, menthone, caryopyllene oxide*.[17] The study also states that the largest content in piduh leaves is pentacyclic triterpenoids (C30) (saponins, madekasosida, asiaticosida) or commonly called "*centelloids*". The results of research state that lime extract contains compounds of *d-limonene*, *Caryophyllene oxide, cis-ß-Farnesene, Caryophyllene.*[18] The difference in GCMS test results is caused by several reasons. Based on studies that have been conducted, it shows that differences in the place of growth of piduh leaves can affect the secondary metabolite compounds contained.[17]

*Loloh Don Kayu Manis* extract contains 17 compounds, of which there are 5 dominant compounds namely *Octadecadienoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, alpha.-Copaene, Panaxynone, Neophytadiene*. The results of this GCMS test strengthen the research where katuk leaves contain compounds of *Hexadecanoic acid, ethyl ester, Octadecadienoic acid, ethyl ester, Neophytadiene, Squalene, Phytol*.[19] The results of the GCMS test of shallot extract strengthen the results of the gcms test in this study, where one of the dominant compounds contained in shallots *is 9,12-Octadecadienoic acid, methyl ester, (E,E)-*.[20]

*Hexadecanoic acid, ethyl ester* has antioxidant activity.[21] 9,12-*Octadecadienoic acid, ethyl ester* is a chemical compound known as ethyl linoleate. Studies have shown that ethyl linoleate can inhibit inflammation.[21] Based on research, *neophytadiene* is a compound that has antioxidant activity, as well as protective against the heart because lipopolysaccharides cause acute inflammation and can injure the heart.[22]

## CONCLUSIONS AND SUGGESTIONS

*Loloh* used by the Balinese people to prevent disease, body fitness and beauty. One of the *Loloh* consumed by the community is *Loloh Don Piduh*. *Loloh Don Piduh* has the main raw material, namely gotu kola leaves. In making *Loloh Don Piduh*, gotu kola leaves are usually added with other ingredients such as lime. Gotu kola leaves as the main ingredient of *Loloh Don Piduh* contain metabolite compounds, namely saponins, triterpenoids, flavonoids, alkaloids, and tannins.

Besides *Loloh Don Piduh, Loloh Don Kayu Manis* is often consumed by the Balinese people. *Loloh Don Kayu Manis* is made from katuk leaves as the main raw material and added with shallots. The compounds contained in katuk leaves are alkaloids, triterpenoids, saponins, tannins, polyphenols, glycosides, and flavonoids.

Among the two *lolohs*, it was found that *Loloh Don Kayu Manis* had higher antioxidant activity compared to *Loloh Don Piduh*. *Loloh Don Piduh* has an $IC_{50}$ value of 33.00 ppm while *Loloh Don Kayu Manis* has an $IC_{50}$ value of 15.22 ppm. *Loloh Don Piduh* contains *Hexadecanoic acid, ethyl ester* and *Neophytadiene* compounds that have been studied to have antioxidant activity. While *Loloh Don Kayu Manis* contains *Octadecadienoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, alpha.-Copaene, and Neophytadiene* which also have antioxidant activity.

In this study the preparation was carried out traditionally taken from fresh ingredients and then directly pulverized using a blender. In future studies it is hoped that the material will be dried first, blended and soaked in water as its processing is commonly done scientifically.

This study has some shortcomings where the sample collection did not come from Bali so there is a possibility of differences in the quality of ingredients in *loloh* commonly made in Bali. There is a possibility of reducing antioxidant activity and metabolite compounds due to traditional processing. The GCMS test carried out was a combined test of two ingredients in each *loloh* so that it could not be specifically known which compounds were obtained from which ingredients.

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