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Phytochemical screening and determination of total phenolic content of *Dendophthoe pentandra* L. leaves ethanolic extract on mango host

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Abstract.
Context: Phytochemical screening and determination of total phenolic content of Loranthus leaves extract at different varieties of mango with a host *Manalagi*, *Arummanis*, and *Gadung*. **Aims**: To identify the highest total phenolic content of Loranthus leaves extract with different host. **Settings and Design**: *Dendophthoe pentandra* L. leaves extract were identified the phytochemical content and determined the total phenolic content. **Methods and Material**: Using Thin Layer Chromatographic (TLC) test and tube test to identify the chemical substance of *D. pentandra* leaves extract. Total phenolic contents were determined using spectrophotometry UV–vis method with the Folin–Ciocalteau reagent. **Statistical analysis used**: ANOVA. **Results**: TLC test and tube test showed that the extract of *D. pentandra* contained alkaloids, flavonoids, terpenoids, steroid, and polyphenols, but the tannin compound is found only in the ethanolic extract of Loranthus from *Gadung*. Total phenolic content in ethanolic extract of *D. pentandra* leaves with a host *Manalagi*, *Arummanis* and *Gadung* mangoes were respectively 358.03 mg GAE g^{-1} ± 4.445 mg GAE g^{-1}; 282.869 mg GAE g^{-1} ± 3.440 mg GAE g^{-1}; and 237.314 mg GAE g^{-1} ± 4,438 mg GAE g^{-1}. The statistic result of total phenolic content in the sample shows the value of $P < 0.05$ which means there is a significant difference between total phenolic content in the sample. **Conclusions**: The highest total phenolic content was *D. Pentandra* leaves extract from *Manalagi* mango host.

Keywords: Loranthus leaves, *Mangifera indica* L., the highest total phenolic

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Introduction

Loranthus plant has been used traditionally to cure various diseases. Loranthus plant (*Dendrophthoe pentandra* L.) is one of the plants that can be used as a source of natural antioxidants. Loranthus plants are known as semiparasitic plants with a variety of species that can grow in a variety of deciduous tree branches that are widespread in the world[1].

Loranthus plant part that is often used as a natural treatment by the community is the part of the parasite leaf[2]. The research of extract *D. pentandra* on different hosts has been known to have activity as an antioxidant, anticancer, immunomodulator, anti plasmodium, and antibacterial[3],[4]. Various activities of the parasite are influenced by secondary metabolites contained in the host, this is because the parasite obtains nutrients and compounds for defense from the parasite–host[5].

Several studies of Loranthus plants have been widely published, but research is still rarely done on the effect of differences in Loranthus plants varieties on total phenolic content and the content of secondary metabolite compounds produced. This study, total phenolic concentration and phytochemical screening of Loranthus from *Manalagi*, *Arummanis*, and *Gadung* mangoes host were determined. Mango (*Mangifera indica* L.) as a parasite–host was known contained vitamin C, vitamin E, carotenoids, and phenolics which can act as antioxidants and anticancer breast in rats[6].

Phytochemical screening is an initial selection stage to detect groups of chemical compounds found in plants. This method is one of several approaches that are commonly used to determine the components of plant chemical compounds that have biological activity. Detection of plant chemical compounds can be done by tube test with or without Thin Layer Chromatography (TLC) confirmation test [7].

Materials and Methods

Materials

The sample used was leaves of *D. pentandra* on *Arummanis*, *Manalagi* and *Gadung* mango hosts taken from Situbondo city, Indonesia. Ethanol 96 %, HCl 2N, Dragendroff reagent, Mayer reagent, acetic anhydride acid, concentrated sulfuric acid, chloroform, $FeCl_3$ 1 %, concentrated HCl, distilled water, standard gallic acid (Sigma–Aldrich), 1,1–diphenyl–2–picrylhydrazyl, Folin–Ciocalteu (Merck) reagent, Na_2CO_3, and methanol.

Extraction

Extraction of simplicia using maceration method by immersing 30 g of dried powder of Loranthus leaves on mango *Arummanis*, *Manalagi* and *Gadung* in solvents ethanol 96 % with a comparison simplicia and solvent 1 : 10. The solution is soaked for 24 h and shaken for some time, the solution is left to

stand at room temperature. The macerate is then filtered and the solvent is replaced twice. All macerates were collected and evaporated with a rotary evaporator at a temperature of 50 °C until a semi–thick extract was obtained. The half thick extract was dried in the oven so that thick extracts were obtained and then calculated percent and stored in the refrigerator for further testing.

Phytochemical screening

Identification of alkaloids

Each extract was taken 0.3 g each plus 5 mL 2N HCl, heated over a water bath for 2 min to 3 min while stirring. After cold, then 0.3 g of NaCl is added, stirred evenly then filtered. The filtrate is then added with 5 mL 2N HCl. After that was added NH_4OH 28 % until the solution became alkaline and then extracted with chloroform free of water, then filtered. The filtrate is evaporated to dryness then dissolved in methanol and ready for TLC examination[8].

Identification of flavonoids

Amounts 0.3 g of the extract was shaken with 3 mL of n–hexane. The residue is dissolved in ethanol and ready for TLC examination[8].

Saponin identification

Each extract was taken 0.1 g of extract added with 2 mL H_2O and heated for 5 min. The solution is cooled, then shaken until foam arises. Stable foam for 10 min shows the presence of saponins[8].

Identification of tannin

Amounts 0.3 g extract was added 10 mL of hot distilled water, plus a little gelatin solution and 5 mL of 10 % NaCl solution. If white deposits occur, there is tannin[8].

Identification of terpenoids and steroids

Amounts 0.3 g extract plus a few drops of ethanol, stirred until dissolved and bottled in the stationary phase for TLC examination[8].

Identification of polyphenols

Amounts 0.3 g extract plus 1 mL of hot aquadest, stirred and left to room temperature. Then add three to four drops of 10 % NaCl, stir and filter. Then it is bottled on the TLC plate[8].

Determination of total phenolic content

Determination of Phenol levels in total ethanol extract of *D. pentandra* leaves on *Arummanis* mango hosts, *Manalagi* and *Gadung* using Folin–Ciocalteu reagents. The test solution was taken 150 µL, then each 750 µL of Folin–Ciocalteu reagent was added which had been diluted with water (1 : 10 v/v) and left for 5 min. Then was added 600 µL 7.5 % Na_2CO_3. The mixture is shaken until homogeneous and incubated according to the optimized incubation time in a dark place. Uptake was measured using a visible spectrophotometer with a wavelength of 737 nm. Total phenol content expressed as milligram of gallic acid equivalent per gram of extract (mg GAE g^{-1}) was obtained from entering the absorbance value of each concentration of the test solution into the regression equation for the standard gallic acid solution.

Result

Extraction

In this study, ethanolic extract of Loranthus leaves on host *Manalagi* mango, *Arummanis* and *Gadung* using maceration method. The thick extract obtained is calculated as the yield weight. Results of the calculations yield of ethanol extract can be seen in Table 1.

Table 1. Results of the yield of ethanolic extract

Samples	Yield (%)
Loranthus of mango *Manalagi*	16.00
Loranthus of mango *Arummanis*	13.90
Loranthus of mango *Gadung*	11.03

Phytochemical screening

Based on the phytochemical screening test using TLC and tube inspection methods, the content of the ethanol extract of leaves *D. pentandra* mango on host *Manalagi*, *Arummanis* and *Gadung*, can be seen in Table 2.

The results screening of phytochemistry shows the compound alkaloids, flavonoids, terpenoids, steroids, and polyphenols, with color stains arising respectively, orange, yellow intensive, purple, and black. Saponin compounds were not found in the ethanolic extract of leaves *D. pentandra* in the host *Manalagi*, *Arummanis* and *Gadung*, this was indicated by the absence of a stable foam for 10 min during examination using a tube reaction. The phytochemical screening of tannin compounds not found in ethanolic extract leaves *D. pentandra* in the host *Manalagi* and *Arummanis*

mangoes, but were found in ethanolic extract of leaves *D. pentandra* in Gadung mango hosts that characterized by the appearance of white sediment deposits.

Table 2. Phytochemical compound of *D. pentandra*

Phytochemical compound	*Manalagi* mango Host	*Arummanis* mango host	*Gadung* mango Host
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	-	-	-
Terpenoid	+	+	+
Polyphenol	+	+	+
Tanin	-	-	+

Determination of total phenol content

Determination of total phenolic content using Folin–Ciocalteu reagent was carried out by measuring the absorbance of the standard solution of gallic acid with concentrations of 20 µg mL^{-1} to 100 µg mL^{-1} which were read at a wavelength of 737 nm.

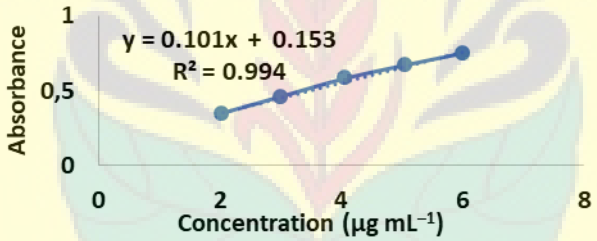

Figure 1. Standard curve of gallic acid

Based on the standard gallic acid calibration curve, the regression Equation(1):

$$y = 0.101x + 0.153 \quad (1)$$

with the r value was 0.997, as seen in Figure 1. Determination of the total phenolic content of the three ethanolic extracts was done by measuring the absorbance of each extract test solution which has been reacted before. The absorbance value obtained is entered into the gallic acid calibration curve. Total phenol content was interpreted in milligrams of equivalent gallic acid per gram of extract (mg GAE g^{-1}). The results of the determination of total phenolic content can be seen in Figure 2.

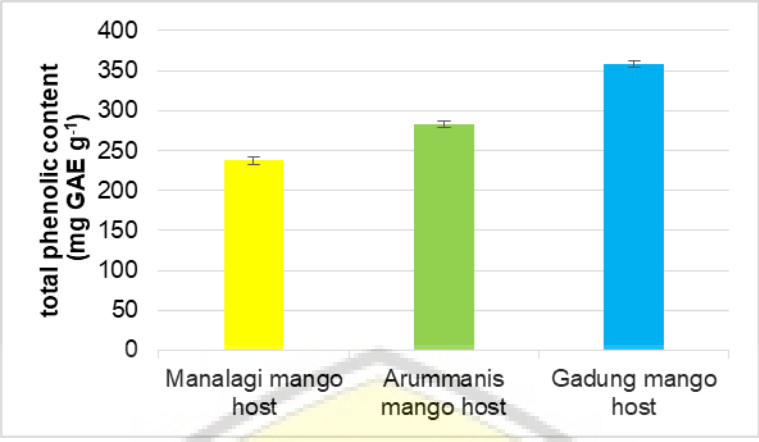

Figure 2. The result of total phenolic content of *D. pentandra* leaves extract

The highest total phenolic content was found in the ethanolic extract of Loranthus on *Gadung* mango host 358.203 mg GAE g^{-1} ± 4.445 mg GAE g^{-1}, followed by mango *Arummanis* host 282.869 mg GAE g^{-1} ± 3.440 mg GAE g^{-1}, and the lastis ethanolic extract of Loranthus on *Manalagi* mango host 237.314 mg GAE g^{-1} ± 4.438 mg GAE g^{-1}. The test results of each sample, have a significant difference one to the other as indicated by the *p value* < 0.05 in *one way ANOVA* and LSD tests.

Discussion

The results of ethanolic extract of Loranthus on mango *Manalagi* showed the highest yield, followed by Loranthus of mango *Arummanis* and *Gadung*. Based on chemotaxonomy, plant varieties in one species have the same chemical activity qualitatively and will differ quantitatively. Based on the results of the yield obtained, it shows that the yield of each extract is different. This difference can be caused by the amount of content of components of compounds that can dissolve in solvents with certain polarity[13].

The result of phytochemical screening showed that ethanolic extract of Loranthus on *Manalagi, Arummanis*, and *Gadung* mangoes host contain alkaloid, flavonoids, terpenoids or steroids, and polyphenols. Groups of alkaloid compounds, especially indoles, have the ability to stop the reaction chains of free radical efficiently[14]. Flavonoids are phenol group compounds which are commonly found in vascular plants, including Loranthus of mango[15]. The antioxidant properties of flavonoids come from the ability to transfer an electron to free radical compounds. The terpenoid compounds that have antioxidant activity are unsaturated terpenoids, have conjugated double bonds, so they can donate hydrogen atoms[16]. The activity of reducing radicals free of polyphenol compounds is believed to be influenced

by the number and position of hydrogen phenol in the molecule. Higher antioxidant activity will be produced in phenolic compounds which have more hydroxyl groups in the nucleus of their flavonoids[17]. Only extract of the Loranthus on *Gadung* mango host that contained of tannin compound. Tannin is a compound that has an –OH group which is bound to an aromatic carbon ring, which has antioxidant activity.

In the parasitic phytochemical screening study on mango hosts secondary metabolite compounds detected were polyphenols, tannins, flavonoids, steroids, triterpenoids, monoterpenoids and sesquiterpenoids, and quinones, while alkaloid and saponin compounds were not detected[19]. The difference in the results of this study is thought to be due to differences in the conditions of the growing environment which can cause differences in the types and quantities of secondary metabolites contained in plants[18]. In addition, things that cause differences in the content of secondary metabolites, namely genetics, and the time of sample collection[19].

The highest total phenolic content was found in the ethanolic extract of Loranthus on *Gadung* mango host according to the phytochemical test results which show that Loranthus on *Gadung* mango host has more types of phytochemical components. Phenolic compounds have activities as antioxidants, antibacterial, anticancer, and various other activities. The difference in total phenolic content of Loranthus is influenced by the host, because the parasite obtains nutrients and compounds for defense from the parasite host[5].

Conclusion

The highest total phenolic content was *D. Pentandra* leaves extract from *Manalagi* mango host.

References

[1] Mathiasen RL, Nickrent DL, Shaw DC, Watson DM. Mistlethoe pathology, systematics, ecology and management. The American Phytopathological Society. 2008;92(7):988–1006. https://apsjournals.apsnet.org/doi/10.1094/PDIS-92-7-0988

[2] Anita A, Khomariah S, Yanti AH. Aktivitas antibakteri ekstrak daun benalu jambu air (*Dendropthoe petandra* (L.) Miq) terhadap pertumbuhan *Salmonella thyphi* [Antibacterial activity of water guava (*Dendropthoe petandra* (L.) Miq) leaf extract on the growth of *Salmonella thyphi*]. Jurnal Protobiont. 2014;3(2):268–72.[in Bahasa Indonesia]. http://jurnal.untan.ac.id/index.php/jprb/article/view/6834

[3] Bulan R, Aliyah F. Uji aktivitas antioksidan dari flavonoid total daun benalu (*Dendrophthoe pentandra* (L.) Miq) dari pohon glodokan

(*Polyalthia longifolia*) [Antioxidant activity test of total flavonoids of benalu leaves (*Dendrophthoe Pentandra* (L.) Miq) from glodokan (*Polyalthia longifolia*) Trees]. Seminar Nasional Kimia dan Pendidikan Kimia. 2016;30–1 [in Bahasa Indonesia]. http://digilib.unimed.ac.id/23786/

[4] Nur J, Dwyana Z, Abdullah A. Bioaktivitas getah pelepah pisang ambon *Musa paradisiaca* var. sapientum terhadap pertumbuhan bakteri *Staphylococcus aureus*, *Pseudomonas aeroginosa* dan *Escherichia coli* [The bioactivity of ambon banana sap from (*Musa paradisiaca* var. sapientum L.) on the bacterial mutation of *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Escherichia coli*]. Fakultas Biologi, Universitas Hasanuddin. 2013. [in Bahasa Indonesia] https://pdfs.semanticscholar.org/b2a2/be840777ed23a172ea8ba49fbf2434e265e9.pdf

[5] Sembiring HB, Lenny S, Marpaung L. Aktivitas antioksi dan senyawa flavonoida dari daun benalu kakao (*Dendrophthoe pentandra* (L.) Miq.) [Antioxidant activity and flavonoidal compounds from cocoa parasite leaves (*Dendrophthoe pentandra* (L.) Miq.)]. Chimica et Natura Acta. 2016;4:117–122. [in Bahasa Indonesia] http://jurnal.unpad.ac.id/jcena/article/view/10920

[6] García–Solís P, Yahia, EM, Aceves C. Study of the effect of Ataulfo mango (*Mangifera indica* L.) intake on mammary carcinogenesis and antioxidant capacity in plasma of N–methyl–N–nitrosourea (MNU) treated rats. J Food Chemistry. 2008;111(2):309–315. https://www.researchgate.net/publication/222677503_Study_of_the_effect_of_'Ataulfo'_mango_Mangifera_indica_L_intake_on_mammary_carcinogenesis_and_antioxidant_capacity_in_plasma_of_N-methyl-N-nitrosourea_MNU-treated_rats

[7] Gritter RJ, Bobbit JM, Schwarting AE. Introduction to chromatography. second edition. California: International Business Machines Inc. Traslate : Panduwinata K, Niksolihin S. 1991. Pengantar kromatografi [Introduction to Chromatography]. Bandung: ITB.1968;266. [in Bahasa Indonesia]. http://library.um.ac.id/free-contents/index.php/buku/detail/pengantar-kromatografi-roy-j-gritter-james-m-bobbitt-arthur-e-schwarting-penerjemah-kosasih-padmawinata-penyunting-sofia-niksolihin-11850.html

[8] Departemen Kesehatan Republik Indonesia. Materia medika Indonesia jilid v [Materia medika Indonesia volume v]. Jakarta: Direktorat Pengawasan Obat dan Makanan.1989:116. [in Bahasa Indonesia]. https://www.academia.edu/37429450/MATERIA_MEDIKA_INDONESIA

[9] Badan Pengawasan Obat dan Makanan. Standardisasi ekstrak tumbuhan obat Indonesia, salah satu tahapan penting dalam pengembangan obat asli Indonesia [Standardization of Indonesian medicinal plant extracts, one of the important stages in the development of native Indonesian medicines]. Info POM. 2005;6(4):1–12. [in Bahasa Indonesia]. https://perpustakaan.pom.go.id/index.php

[10] Fu L, Xu BT, Xu XR, Gan RY, Zhang Y, Xia EQ, et al. Antioxidant capacities and total phenolic contents of 62 fruits. J Food Chemistry, 2011;129(2):345–50. https://www.sciencedirect.com/science/article/pii/S0308814611006455

[11] Yu LL. Wheat antioxidants. New Jersey: John Wiley & Sons,Inc. 2008. https://books.google.co.id/books?id=VN3a5YsM31IC&pg=PR4&dq=%5B11%5D%09Yu,+L..+Wheat+Antioxidants.+New+Jersey:+John+Wiley+%26+Sons,Inc.2008&hl=id&sa=X&ved=0ahUKEwjLq86arqflAhWRaCsKHb2pAkAQ6AEIKTAA#v=onepage&q=%5B11%5D%09Yu%2C%20L..%20Wheat%20Antioxidants.%20New%20Jersey%3A%20John%20Wiley%20%26%20Sons%2CInc.2008&f=false

[12] Blainski A, Lopes GC, de Mello JCP. Application and analysis of the Folin–Ciocalteu for the determination of the total phenolic content from *Limonium brasiliense* (L.). J Molecules. 2013;18:6852–65. https://www.mdpi.com/1420-3049/18/6/6852

[13] Dai J, Mumper RJ. Plat phenolic: extraction, analysis and their antioxidant anticancer properties. J Molecules. 2010;15(1):7313–52. https://www.mdpi.com/1420-3049/15/10/7313

[14] Yuhernita, Juniarti. Analisis senyawa metabolit sekunder dari ekstrak metanol daun Surian yang berpotensi sebagai antioksidan [Analysis of secondary metabolite compounds from Surian leaf methanol extract that has potential as an antioxidant]. Makara J of Sceince. 2011;15(1). [in Bahasa Indonesia]. http://www.ijil.ui.ac.id/index.php/science/article/viewArticle/877

[15] Artanti N, Ma'arifa Y, Hanafi M. Isolation and identification of active antioxidant compound from star fruit (*Averrhoa carambola* L.) Mistletoe (*Dendrophthoe petandra* (L.) Miq.) ethanolextract. J of Applied Sciences. 2006;6(8):1659–63. https://scialert.net/fulltextmobile/?doi=jas.2006.1659.1663

[16] Capelli B, Cysewski G. Natural astaxanthin: kingdom of carotenoid. Cyanotech Corporation. 2007;78:7. http://www.purehealingfoods.com/docs/NaturalAstaxanthin_King_of_the_Carotenoids.pdf

[17] Es–Safi NE, Ghidouche S, Ducrot PH. Flavonoids: hemisynthesis,reactivity, characterization and free radical scavenging activity. J Molecule. 2007;12(9):2228–58.https://www.mdpi.com/1420-3049/12/9/2228

[18] Dewi IDADY, Astuti KW, Warditiani NK. Identifikasi kandungan kimia ekstrak kulit buah manggis (*Garcinia mangostana* L.) [identification of the chemical content of mangosteen rind extract (*Garcinia mangostana* L.)]. J Farmasi Udayana 2008;2(4):13–18. [in Bahasa Indonesia]. https://ojs.unud.ac.id/index.php/jfu/article/view/8404

[19] Nurfaat LD, Wiwiek I. Uji toksisitas akut ekstrak etanol benalu mangga (*Dendrophthoe petandra* L.) terhadap mencit Swiss Webster [acute toxicity test of mango parasite (*Dendrophthoe petandra* L.) ethanol extract on Swiss Webster mice]. Indonesian J of Pharmaceutical Science and Technology. 2016;3(2):53–65. [in Bahasa Indonesia]. http://jurnal.unpad.ac.id/ijpst/article/view/7941