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Phytochemical, Antimicrobial and Total Phenol Test of Coral Plants "Betadin" Leaf Methanol Extract (*Jatropha muitifida linn*)

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ABSTRACT

Coral Plants leaves were extracted with methanol and carried out by maceration then evaporated with a rotary evaporator. This extract contained several phytochemical compound such as alkaloid, flavonoid, tannin, phenol and terpenoid. Therefore, Coral Plants leaves has an antimicrobial function. Phenol compound has the ability to form complex compound with proteins through hydrogen bonds, which can damage bacterial cell membrane. Total phenol content was quite high in the leaf of 165.20 ± 33 mg GAE/mL. Antibacterial activity test results showed that the methanol extract of Coral Plants leaves can inhibit *E. coli*, *Gram-negative* bacteria with strong inhibition (10-20 mm) and *Gram-positive* bacteria (*Bacillus* sp) by 12.3 mm. Coral Plant leaves can inhibit the growth of penicillium and Tricodherma fungi, but cannot inhibit the growth of *Aspergillus niger* fungi. The inhibition against Penicillium was higher (7 mm) compared to Tricodherma of 1.8-4.5 mm.

Keywords: Coral Plants, Leaf, Total phenol, Antimicrobial, Phytochemical.

INTRODUCTION

The principle "back to nature" is increasingly popular in this modern era, with the advancement of science many people turn to traditional medicine. One of the plants that has benefit as an alternative treatment for disease is "betadin", *Jatropha multifida* L. derived from the family Euphorbiaceae which is widely grown in the continent of Africa and Asia. "Betadin" plant (*Jatropha multifida* L.) is a medicinal plant which is rarely used by people, especially in Indonesia. The benefit of this plant is not widely known by the public, especially in Indonesia. This plant can respond to climatic condition, are able to adapt to various agro-ecological and have accumula ted variations over the years.

This plant has many benefits for life that can combat microbial infections and leishmaniasis. In the future, it has a strong laxative activity. The gum of this plant can provide an inhibiting effect on the flow of blood in the wound incision. The stem extract can be used as a plant-based pesticide controlling pest Plutella xylostella in mustard plants (*Brassica juncea* L.)[1]

"Betadin" plants are known contained alkaloid and flavonoid. Flavonoid is secondary

metabolite in plants and is polar compound, and therefore soluble in alcohol[2]. This secondary metabolite can be used as an antimicrobial, infectious drug in wounds, antifungal, antibacterial, hypo-allergenic, cytotoxic, and anti-hypertensive. *Jatropha multifida* L. is widely used by people as a wound medicine, this is what causes this plant to be known as betadin plant[5]. In addition, flavonoid can be used as antioxidant, which are compound that protect cells against the effect of damage by reactive oxygen[3]. Flavonoid can also affect the increase in platelet counts and have anti-cancer, anti-virus, anti-bacterial, anti-inflammatory and anti-allergic bioactivity[5].

Fig. 1. Coral Plants (*Jatropha muitifida* Linn.)

Liana's research in 2016 also showed the antibacterial activity of betadin leaf active fraction against *Staphylococcus aureus*[3]. This can be caused by the presence of active ingredient flavonoid and phenol. This result was supported by a study of Nagaharika, *et al.,* in 2013 which showed betadin leaves can be used as anti-inflammatory[4]. Phenol compound has the ability to form complex compound with protein through hydrogen bond, which can damage bacterial cell membranes.

From the result of the previous study[3], it was known that the benefit of betadine leaf as a wound drug were related to the antibacterial activity against *Staphylococcus aureus* and had not been studied much in other types of bacteria or in types of fungi. Chemical compounds, especially phenol compounds, are active antimicrobial substances, so they need to know their levels and are expected to produce different antibacterial and antifungal activities. Therefore, researchers want to explore further about betadin plants whether they have the antibacterial activity of *E.coli* and *Bacillus subtilis* and types of fungi, such as *Aspergilus flavus* or *Aspergilus niger*. In this research, methanol solvent was used to extract betadin leaves, which then determined the total amount of phenol and antibacterial and antifungal activity test through growth inhibitory test.

The aim of this experiment was to obtain information on chemical content, phenol content and antimicrobial activity as an antibacterial and antifungal in extract methanol of betadin leaf (*Jatropha muitifida* Linn.). The result of this study are expected demonstrate the effectiveness of betadin leaf methanol extract in inhibiting microbial growth.

MATERIAL AND METHODS

Materials and Chemicals

Coral Plants "Betadin" (*Jatropha mulitifida* Linn) leaves, methanol (pa), concentrated Hydrochloric Acid (HCl), ammonia, chloroform, Dragendorf reagent, Mayer reagent, Mg powder, Amyl alcohol, Diethyl ether, Libermen reagent, $FeCl_3$, Agar Nutrient media, Potatos Dextros Agar media, Folin-Ciocalteu reagent, anhydrous Na_2CO_3, test suspension (bacteria and fungi), penicillin and ketomidazole standards. The tools used were: UV-Vis spectrophotometer, rotary evaporators, ceramic containers, petri dishes, magnetic stirers, vortices, paper discs, centrifuges, incubators, erlenmeyer, goblets, vial bottles, test tubes, ose, bunsen, tweezers, and other glassware.

Coral Plants Leaf Extraction

Coral Plants and stalk leaves were separated and washed, then drained and dried in direct sunlight for 4 hours. A total of 50 g of simplicia Coral Plant leaf was extracted with a solvent ratio of 1:10 (w/v). The solvent used was methanol. Extraction was carried out as much as 2x24 h by maceration. The maceration result was filtered, then evaporated with a rotary evaporator at a temperature of 50°C. Furthermore, the calculation of the yield content of the extract obtained.

Extract yield rate (%) = (Extract weight/ Simplification weight) x 100%

Phytochemical Test of Coral Plant Leaf Extract

a. **Alkaloid Identification**

A total of 100 mg of Coral Plant leaf methanol extract, added 5 mL of 10% HCl and diluted ammonia to pH 8, then extracted with 20 mL chloroform, then the extract was evaporated. The extract was dissolved with 2 mL HCl 2%

and divided into 3 tubes. The first tube was used as a comparison, the second tube was added by Mayer reagent and the third tube was added by Dragendorff reagent. If there is a white precipitate with a Mayer reagent, an orange-red precipitate with a Dragendorf reagent in the sample[7].

b. **Flavonoid Identification**
As much as 100 mg of methanol extract Coral Plant leaves were dissolved in 100 mL of hot water, then boiled for 5 min then filtered. As much as 5 mL of filtrate was added 0.1 mg of Mg powder, 1 mL of concentrated HCL and 1 mL of amyl alcohol and then shaken vigorously. The presence of flavonoids was shown by the formation of red, yellow or orange in the amyl alcohol layer[7].

c. **Triterpenoid and Steroid Identification**
A total of 100 mg Coral Plants leaves extract was added with 25 mL of diethyl ether and then shaken. The diethyl ether layer was separated and 2-3 Lieberman-Burchard reagent were added. Triterpenoids were present when blue solutions were formed and steroid were present when green solutions are formed[7]

d. **Saponin Identification**
A total of 100 mg Coral Plants leaves extract was added with 10 mL of hot distilled water, cooled, and shaken vigorously for 10 minutes. Saponins were present when a solid foam was formed and on the addition of 1 drop of HCl 2 N (the foam remains stable)[7]

e. **Tanin Identification**
A total of 100 mg Coral Plant leaf extract was extracted using 1 mL ethanol and 1 mL aquadest. The filtrate obtained was then added a few drops of $FeCl_3$ 1%. The presence of tannin compounds was indicated by the formation of green, blue or purple[7]

Antimicrobial Activity Test

Petri dish containing Nutrient agar media was added 200 µL suspension of one of the test bacteria (*E.coli*, and *B.cereus*) and then spread using a string. To test the antifungal media used was a PDA (Potatos dextrose agar) with *A.plavus*, and *A. niger* test fungi next, sterile tracing paper (6 mm diameter discs) was prepared to be placed on the surface so that the petri dish was prepared. The discs were previously soaked in the test solution for 30 min in a sterile petri dish. Then the incubation process was carried out at 37°C for 24 hours. Observations were made by measuring the zone of resistance formed around paper discs in the form of clear areas using a ruler then recorded. This applied also to standard using antibiotics p.a.

Inhibition Zone = Diameter (clear area + discs) - Diameter of disc paper

Analysis of Total Phenols (Folin Method)

Each sample was piped as much as 2 mL, then macerated with ethanol as much as 3 mL, and shaken. The extract was dropped on a drip plate and added with 5% $FeCl_3$ solution of 3-5 drops. A positive test for the presence of phenolic compounds was shown if the color changes to green, blue, purple or black. Gallic acid stock solution with a concentration of 100 ppm (mg/L), which can be made by dissolving 0.01 g gallic acid in a 100 mL volumetric flask and adding distilled water to the mark. Then made a series of standard solutions with a concentration of 0; 2; 4; 8 ppm. Stock of 100 ppm gallic acid stock as much as 0; 0.2; 0.4; 0.8 mL each was added with 0.8 mL folin reagent, placed in a 10 mL volumetric flask next 5% Na_2CO_3 was added to the boundary mark, so as to produce a standard solution with a concentration of 0; 2; 4; 8 ppm. Each solution was allowed to stand for 60 min, and its absorption was measured at the maximum wavelength. By channeling absorbance to concentration, a calibration curve can be obtained with the regression equation $y = bx + a$.

Determination of total phenolic compound

Determination of the total content of these phenolic compounds was carried out based on the Folin-Ciocalteu method. Coral Plants leaf extract was dissolved with water then filtered using filter paper, then the filtrate was pipetted 1.0 mL in a 10 mL volumetric flask and added with 0.8 mL folin reagent. After that the mixture was shaken. Next 5% Na_2CO_3 was added to the mark, so the total volume of the solution becomes 10 mL. The solution was allowed to stand for 60 min, and its absorption was measured at a maximum wavelength of 750 nm. Measurement were repeated 3 times. The concentration of the compound was determined by converting the absorbance of the sample to the calibration curve and calculated as total phenol content in units of percent (%).

RESULTS AND DISCUSSION

Phytochemical properties

Coral Plants leaves were thought to have a high alkaloid compound and a little bit flavonoid and phenol. Phytochemical screening test result showed that the methanol extract from the leaves of Coral Plants (*Jatropha mulitifida* Linn) contained several active compounds, namely alkaloid, flavonoid, tannin, total phenols and terpenoids. The leaves had the function as an anti microbial. Flavonoid was anti-inflammatory so they can reduce inflammation and help reduce pain, if bleeding or swelling occurs in the wound. Phenol had the ability to form complex compounds with proteins through hydrogen bonds, which can damage bacterial cell membranes[6].

Total Phenol

Total phenol was set on the leaves to determine level of phenol. Phenol level can show the nature of active substances as antioxidant and antimicrobial. total phenol was quite high found in the leaves average of 165,20 ± 33 mg GAE/mL. This showed that Coral Plants leaves have the potential to reduce free radical (antioxidant) and inhibit microbial growth. Polyphenol derivatives were thought to be antioxidant compound that can stabilize free radical by completing the lack of electrons possessed by free radical, and inhibit the chain reaction of free radical formation. Polyphenol were component which responsible for antioxidant activity in fruits and vegetable[8]. Besides, total phenol can act as an antimirobial compound. Phenol compounds had the ability to form complex compound with protein through hydrogen bond, which can damage bacterial cell membrane[9].

Table 1: Total Phenol of Coral Plants leaves

No	Total Phenol (mg GAE/mL)*
1	164, 16 ± 32
2	165, 34 ± 21
3	166,11 ± 46
Average	165,20 ± 33

Antimicrobial Activity of Coral Plants leaves Methanol Extract

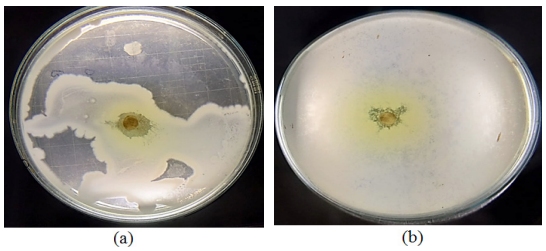
To determine the antimicrobial activity of methanol extract on the Coral Plants leaf, an antimicrobial test (antibacterial and antifungal test) was conducted using the disc method. Antibacterial activity test was carried out on 2 types of bacteria (*E.coli* and *Basillus* sp) which can be seen in Table 2.

Table 2: Inhibitory Potency (Anti-bacterial) of Coral Plants leaves

Sample	Inhibitory Potency (mm)*	
	E. Coli	*Bacillus* sp
Leaf	11,6	12,3
Standard Amoxcillin	9	9

*} Average of 3 replication

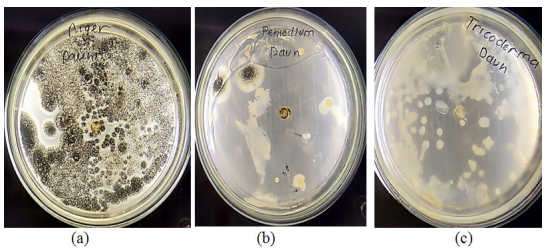
Table 2 showed the methanol extract of Coral Plants leaf can inhibit *E.coli Gram-negative* bacteria with strong inhibition (10-20 mm) exceeding Amoxicilin standard, while gram positive bacteria (*Bacillus* sp) can only be inhibited by Coral Plants leaves methanol extract by 12.3 mm. According to Davis Stout in Rita, the antimicrobial category is weak (<5mm), moderate (5-10mm), strong (10-20mm) and very strong (> 20mm). Coral Plants leaf is the most active part in inhibiting the growth of *Gram-positive* and negative bacteria. This property has been utilized by the community to utilize Coral Plants leaves as a wound medicine.



(a) (b)

Fig. 2. Inhibition Potency of Coral Plants Leaf Methanol Extract against (a) *Bacillus* sp and (b) *E. coli*

Antifungal activity test was carried out on 3 types of fungi namely *Aspergillus* flavus, *Tricodherma* and *Aspergillus* niger by measuring the amount of inhibitory potency referring to the amount of clear zone formed around the disk. The result of antifungal test on the methanol extract of Coral Plants leaf can be seen in Table 3.



(a) (b) (c)

Fig. 3. Inhibition Potency of Coral Plants Leaf Methanol Extract against (a) *Aspergillus* niger, (b) Penicillium and (c) *Tricodherma* sp

Table 3: Inhibitory Potency (Antifungal) of Coral Plants Leaf

Sample	Inhibitory Potency *)		
	Penicillium	*A. niger*	*Tricoderma*
Leaf	7	--	4,5
Standard Ketokonazole	13	18	13,5

*} Average of 3 replication

Coral Plant leaves can inhibit the growth of Penicillium and Tricodherma fungi, but cannot inhibit the growth of *Aspergillus* niger fungi. Inhibition of fungal growth is in the medium category (5-10 mm) and lower than inhibition zone in the ketoconazole standard. Leaves can be used as treatment materials to heal wounds and as anti-inflammatory[4] because they contain flavonoid which are able to reduce inflammation and help reduce pain, if bleeding or swelling occurs in the wound. In addition, the presence of phenol compound has the ability to form complex compound with protein through hydrogen bonds, so that these compounds can synergize in healing wounds. Antimicrobial activity on Betadin leaf had been investigated by Liana and Putinah in 2016, which showed limited antibacterial activity from active fraction of Betadin leaf against *Staphylococcus aureus*.

CONCLUSION

Total phenol content was quite high in the leaf average of 165,20 ± 33 mg GAE/mL. This showed that Coral Plant leaves had the potential to reduce free radicals (antioxidants) and inhibit microbial growth. Antibacterial activity test result showed that the methanol extract of Coral Plants leaves can inhibit *E.coli Gram-negative* bacteria with strong inhibition (10-20 mm) and *Gram-positive* bacteria (*Bacillus* sp) by 12.3 mm. Based on the results of antifungal activity test, it was known that Coral Plants leaves can inhibit the growth of *Penicillium* and *Tricodherma* fungi, but cannot inhibit the growth of *Aspergillus niger fungi*. The inhibition against *Penicillium* was higher (7 mm) compared to *Tricodherma* of 1.8 - 4.5 mm.

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Conflict of interest

The authors have no conflicts of interest related to this article.

REFERENCES

1. Abdurrahmat, A. S.; Gorontalo State University *ENTOPI Journal*, **2014**, *9*(1),721-840.
2. Apriyanti, M.;Yogyakarta: New Library Pres, **2010**, *2.*
3. Liana, Y. and Putinah; *Bina Husada Journal of Health.*, **2016**, *11*, 479-485
4. Nagaharika, Y.; Kalyani, V.; Rasheed S.; and Karthikeyan R.; *Journal of Acute Disease.*, **2013**, *2*(2),156–158.
5. Sudaryono, A.; *Media Medika Indonesia Journal.*, **2011**, *45*(2).
6. Iwan, J. and Nur, A.; *Bandung Medical Magazine.*, **2010**, *42*(2), 77.
7. Coolborn, A.F. and Bolatito, B.; *Journal of Natural Products.*, **2010**, *3*, 27-34.
8. Junior, M.R.M.; Leite A.V.; and Dragano N.R.V., *The Open Chemical Engineering Journal.*, **2010**, *4*, 51-60.
9. Balachandar, R.; Saran, P.L.; Ashok K.K., Ragavi A., and Gurumoorthy, P.; *Journal of Chemical and Pharmaceutical Sciences.*, **2014**, *1*(2), 100–3.
10. Rita, W. S.; *Journal of Chemistry.*, **2010**, *4*, 20-26.