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Antibacterial Activities of N-Hexan Ethyl Acetate Fraction and Water From Ethanol Extract of Yodium Leaves (Jatrophora Multifida L.) Against Pseudomonas Aeruginosa ATCC 27853

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ABSTRACT
Yodium leaf (J. multifida) is a plant of Euphorbiaceae. Yodium leaf chemical constituents are alkaloids, flavonoids, saponins and tannins that are antibacterial. In this study, yodium leaf explored its use as an antibacterial against P. aeruginosa ATCC 27853. The aim of the study was to find out the effectiveness of ethanol extract, n-hexana, ethyl acetate and water fraction of yodium leaf against P. aeruginosa ATCC 27853.
The extraction of yodium leaf by maceration method used ethanol 70% solvent, followed by fractionation with n-hexana, ethyl acetate and water solvents. The antibacterial activity test performed by the diffusion method and dilusion method. Diffution method by consentration ethanol extracts and fraction are used 50%, 25% and 12,5%. Dilution method by consentration ethyl acetate fraction are used 50%, 25%, 12,5%, 6,25%, 3,125%, 1,5625%, 0,78125%, dan 0,390625%. The chemical contens of the most active fraction were tested by TLC.
The results showed that the ethanol extract of yodium leaf (J. multifida), of n-hexana, ethyl acetate and water fraction had antibacterial activity against P. aeruginosa ATCC 27853. Ethyl acetate fraction was the most active fraction with an average of inhibition diameter at concentrations of 50% was 14,7 mm, concentration of 25% was 14,4 mm and concentration 12,5% was 11,7 mm. Minimum kill concentration ethyl acetate fraction against P. aeruginosa ATCC 27853 of 12,5%. The chemical contents of the most effective ethyl acetate fraction were tested by TLC. The result of identification showed that ethyl acetate fraction positively contained alkaloid, flavonoids, saponin and tannin.

Keywords: Yodium Leaf (J. multifida), P. Aeruginosa ATCC 27853, n-hexana, Ethyl Acetate and Water Fraction

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BACKGROUND

Indonesia is a mega-biodiversity country that has a wealth of natural plants in Indonesia, covering 30,000 plant species out of a total of 40,000 plant species in the world. Based on research results, of the many types of medicinal plants, only 20-22% are cultivated. Efforts to improve community health status are carried out by various development efforts in the health sector. However, there are many challenges and obstacles that must be faced, including the high number of infectious diseases in the community (Wahjono 2007).

This infectious disease can be caused by bacteria, viruses, parasites or fungi (Muhaimin 2003). P. aeruginosa bacteria are Gram-negative, mobile, aerobic bacteria, some of which produce pigments that are soluble in water and often enter the tissue that is affected by wounds or burns, causing pus (Jawetz 2005). To overcome this problem, the use of medicines from natural ingredients has been developed. One of them is the iodine plant (J. multifida).

The J. multifida plant is a medicinal plant originating from the Euphorbiaceae family. The chemical content of iodine plants is campesterol, alpha amirin, stigmaterol, 7 alpha diol, HCN and beta-sitosterol, the content in iodine stalks is alkaloids (which are called blood clots), flavonoids, saponins and tannins (Syarfati et al. 2011). In addition, this iodine plant also contains sulfur and iodine which act as an antiseptic agent and accelerate the healing of burns (Ilmi 2009)

In a study conducted by Aiyelaagbe et al. (2008) extracts and fractions showed potential antimicrobial activity of various organisms with minimum inhibitory concentrations ranging from 0.75-12.5 -1 for the fractions. Research on the antibacterial activity of iodine leaves that has been studied is the inhibition of Chinese jatropha (J. multifida) against S. aureus in vitro with a concentration value of 50% inhibition zone of 13.5 mm (Darmawi et al. 2013), while according to (Maryati) 2013) 100% diameter concentration in the extract was 7.67 mm.

The extraction method used is maceration using 70% ethanol as solvent. Meanwhile, in fractionation, n-hexane, ethyl acetate and water were used to separate the compounds based on their polarity (Susilowati 2010). The antibacterial activity in this study was carried out by diffusion and dilution methods. The most active fraction was tested for chemical content by TLC.

METHODS

Tools and Materials

Tool. Several kinds of tools used in this study include blenders, maceration bottles, Buchner funnels, ovens, moisture balances, analytical balances, volume pipettes, other glassware used such as Erlenmeyer flasks, measuring cups, stirring rods, test tubes. The antibacterial activity tools used were autoclave, incubator, sterile cotton swab, tweezers, oven, spirit burner, gauze, tripod, measuring pipette, petri dish, syringe, platinum loop, object glass.

Material. The sample used in this study was iodine (J. multifida) leaves. Taken randomly in fresh condition from young iodine plants (Hasibuan 2016). Obtained from Jatibarang Village, Brebes Central Java in January 2017. The test bacteria used in this study was P. aeruginosa obtained from the Microbiology Laboratory of Setia Budi University, Surakarta. The chemicals used in this study were 70% ethanol, n-hexane, ethyl acetate, sterile distilled water, concentrated sulfuric acid, acetic acid, and ciprofloxacin. The medium used was Brain Heart Infusion (BHI), Mueller Hinton Agar (MHA), Sulfide Indol Motility (SIM), Kligler Iron Agar (KIA), Lysine Iron Agar (LIA), Citrat, Pseudomonas Selective Agar (PSA)

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The research path

Plant determination

Determination was carried out at the Laboratory of Plant Morphology and Systematics of Setia Budi University, the result of the determination was that the plants used in this study were iodine leaves, Jatropha multifida L. species, Euphorbiaceae tribe (Backer 1965).

Material pick-up

The sample used in this study was iodine (J. multifida) leaves. Taken randomly in fresh condition from young iodine plants. (Hasibuan 2016). Iodine leaves were obtained from Jatibarang Village, Brebes, Central Java in January 2017.

Iodine leaf drying

Iodine leaves (J. multifida) were dried in an oven at 50^0C. Powdered dry leaves using a blender. Then sieved through a 40 mesh sieve.

Determination of moisture content of iodine leaf powder

Determination of moisture content of leaf powder iodine (J. multifida) was calculated using a moisture balance tool. The purpose of determining the moisture content of the powder is to determine the maximum limit of the amount of water content in the material (MOH 2000). Moisture determination should not be more than 10%. Moisture content of less than 10% can prevent mold growth and enzyme activity so that the material is more durable and the active substance content is not reduced (Katno et al. 2008).

Preparation of iodine leaf ethanol extract

The extraction method used is maceration using 70% ethanol. Iodine leaves are soaked in a brown bottle, 70% ethanol which is put into a brown bottle mixed with iodine leaf powder must be closed immediately so that the solvent does not evaporate, then let stand for 5 days with occasional shaking.

Iodine leaf extract fractionation

The fractionation process was carried out referring to the Windarwati method (2011), namely the partition process using an ethanol-water ratio (1:5) then dissolved with 10 ml of ethanol and 50 ml of aquadest in a glass beaker and then separated in a separating funnel by adding 60 ml of fractionated n-hexane until two layers are formed, so that the n-hexane fraction is obtained. The n-hexane fraction is at the top and the water is at the bottom because the density of the water is higher. The residue obtained was further extracted with ethyl acetate solvent.

Identification of the chemical content of powders, extracts and leaf fractions of iodine

Alkaloids. Identification of alkaloids was carried out by means of powder, extract and leaf fraction of iodine added 5 drops of HCl 2 N heated then added a few drops of Mayer's reagent positive reaction when a white / yellow clotted precipitate is formed. The addition of Dragendrof reagent forms red to orange deposits indicating the presence of alkaloids (Alamsyah et al. 2014).

Flavonoids. Identification of flavonoids was carried out by means of powder, extract and leaf fraction of iodine plus Mg powder then added with 5 ml of concentrated HCl with a positive reaction resulting in a yellow color indicating the presence of flavonoids (Puspasari et al. 2014).

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Saponins. Identification of saponins was carried out by means of powder, extract and leaf fraction of iodine added with hot water and then shaken vigorously or using a vortex for 10 seconds. A positive reaction when the addition of 2 N 2 drops of HCl forms a stable foam, it contains saponins (Puspasari et al. 2014).
Tannins. Identification of tannins was carried out by means of powder, extract and leaf fraction of iodine added with hot water and then filtered, the filtrate added with a few drops of 1% FeCl3 was homogenized until it experienced a change in color. The positive reaction is the emergence of blackish brown / blue-black color (Alamsyah et al. 2014).

Preparation of test bacteria suspension
Pure cultures of P. aeruginosa ATCC 27853 were taken with a sterile ossicle needle. Then it was put aseptically into a sterile test tube containing liquid BHI (Brain Heart Infusion) media whose turbidity was adjusted to the modified Mc Farland turbidity of 0.5, then incubated at 37 oC for 18-24 hours.

Antibacterial Activity Test
The methods used are diffusion and dilution methods. The diffusion method was used to determine the diameter of the zone of inhibition against the test bacteria. Diffusion method by immersing a sterile cotton swab in the bacterial suspension that has been made and pressed at the end of the tube, then inoculated into the MHA medium by the spread plate method and the medium is allowed to stand for 10 minutes at room temperature so that the culture suspension diffuses into the media. The media is filled with 6 mm disc paper using tweezers at the same distance. Each disc paper that has been given an antimicrobial agent according to the concentration. Which contains ethanol extract, n-hexane fraction, ethyl acetate and water. Ciprofloxacin as a positive control and 5% DMSO solvent as a negative control. The extract and fraction concentrations were made 50%, 25% and 12.5%, respectively. The media containing disc paper was inserted into the incubator and incubated for 18-24 hours at 37 ° C and the results were observed, after which the diameter of the inhibition zone around the disc paper was measured which was expressed in units of mm. The dilution method uses 1 row of test tubes from 10 sterile tubes. Preparation of stock solutions of n-hexane, ethyl acetate and water fractions using DMSO 5% solvent, each tube has several dilution concentrations, namely 50%; 25%; 12.5%; 6.25%; 3.125%; 1.5625%; 0.78125%; 0.390625%. 0.5 ml of BHI medium was put into each test tube aseptically, 0.5 ml of the fraction was added to the first tube and then shaken then 0.5 ml was taken from the first tube and 0.5 ml was taken from the second tube. inserted into the third tube and so on until the tenth tube. The bacterial suspension in BHI medium was put into each test tube as much as 0.5 ml. The most active extract / fraction as a negative control, P. aeruginosa suspension as a positive control and ciprofloxacin as a comparative control. All tubes were incubated at room temperature for 18-24 hours, then observed the turbidity then determined MIC and KBM.

Thin layer chromatography
Thin layer chromatography was performed to test the active fraction in the ethanol extract of iodine leaves. The active extract or fraction is dissolved in a suitable solvent, then spotted on the chromatographic plate, after the spots are dry, the TLC plate is put in a vessel that has been saturated by the appropriate mobile phase. The development is carried out to a certain distance, then the detection is carried out under UV 254 nm and UV 366 nm rays and

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certain reagents. The detected spots are determined by the Rf value and the appearance of the color.

RESULTS AND DISCUSSION

Table 1. Percentage of dry weight to wet weight of iodine leaves.

Wet weight (gram)	Dry weight (gram)	Rendemen (% b/b)
5000	1700	34 %

The percentage of dry weight to wet weight of iodine leaves (J. multifida was carried out as much as 5000 grams of wet weight then dried and obtained a dry weight of 1700 grams, the yield of dry weight to wet weight was 34% (w/w).

Table 2. The weight percentage of iodine leaf macerated extract

Powder (gram)	Thick extract (gram)	Rendemen (%)
1500	700	46,67

The yield of the extract obtained was 46.67%, meaning that the higher the yield value produced, the more macerated extract value was produced. This is possible during the filtering process using a Buchner funnel, the powder is still being filtered

Table 3. The yield of n-hexane fraction of iodine leaves

Wet weight (gram)	Dry weight (gram)	Rendemen (% b/b)
120	1,85	1,54

Calculation of the percentage yield of the n-hexane fraction of iodine leaves obtained a percentage of 1.54%. The results of the n-hexane fraction obtained are small because at the time of fractionation there are 3 layers, namely the water phase, the n-hexane phase and the phase that has not been separated. The phase that has not been separated is accommodated in a separate container so that it is most likely that the active substance contained in the n-hexane fraction is lost.

Table 4. The yield of iodine leaf ethyl acetate fraction

Ethanol extract weight (gram)	Fraction weight (gram)	Rendemen %
120	6,32	5,26

Calculation of the percentage yield of ethyl acetate fraction of iodine leaves obtained a percentage of 5.26%.

Table 5. Iodine leaf water fraction yield

Ethanol extract weight (gram)	Fraction weight (gram)	Rendemen %
120	23,36	19,46

Calculation of the percentage yield of the water fraction of iodine leaves obtained a percentage of 19.46 %. The results of the water fraction obtained were more than the other fractions because maybe most of the compounds in iodine leaves were polar. The yield of each solvent is different because the ability of each solvent to extract compounds contained in the ethanol extract of iodine leaves is different. The yield results obtained were far from expected, namely 100% or close to 100%

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Table 6. Inhibitory diameter on leaf iodine antibacterial test against P. aeruginosa ATCC 27853 by diffusion

Sample	Concentration	Inhibition Diameter (mm)			
		Replication			
		1	2	3	Mean
n-heksana	50%	10,7	10,3	10,3	10,4 ± 0,23
Ethyl Acetate	50%	16,0	13,6	14,3	14,7 ± 1,23
Water	50%	12,7	14,0	12,7	13,1 ± 0,75
Extract	50%	11,5	12,0	12,0	11,8 ± 0,28
n-heksana	25%	9,3	9,7	9,7	9,5 ± 0,23
etil asetat	25%	14,0	15,3	14,0	14,4 ± 0,75
Water	25%	12,6	13,0	12,6	12,7 ± 0,23
Extract	25%	11,6	12,0	11,6	11,7 ± 0,23
n-heksana	12,5%	8,0	8,7	8,0	8,2 ± 0,40
etil asetat	12,5%	12,0	12,0	11,3	11,7 ± 0,40
Water	12,5%	8,7	9,0	8,7	8,8 ± 0,17
Extract	12,5%	9,0	9,0	8,7	8,9 ± 0,17
Control (+) Siprofloksasin		27,0	27,0	28,0	27,3 ± 0,57
Control (-) DMSO 5%		0	0	0	0

Table 7. Results of the antibacterial activity of the most active fraction of iodine leaf extract against P. aeruginosa ATCC 27853

No	Concentration (%)	Ethyl acetate fraction		
		I	II	III
1	Control (-)	-	-	-
2	50	-	-	-
3	25	-	-	-
4	12,5	-	-	-
5	6,25	+	+	+
6	3,125	+	+	+
7	1,5625	+	+	+
8	0,78125	+	+	+
9	0,390625	+	+	+
10	Control (+)	+	+	+

Information:
(-) : No bacterial growth
(+) : There is bacterial growth
Control (-) : Ethyl acetate fraction
Control (+) : Bacterial suspension

Table 8. Results of identification of alkaloids by TLC

Rf	The resulting color is formed		
	UV 254 nm	UV 366 nm	Dragendorph spray reagent
0,51 cm	Dark brown	Green	Orange to dark red

The identification results of alkaloid compounds using silica gel stationary phase and mobile phase toluene: ethyl acetate: diethylamine (7:2:1). The results of TLC are aerated and observations on UV light 254 nm are blackish brown, while on UV light 366 nm are green.

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The addition of a spray reagent using Dragendorph produces an orange to dark red color. Based on this test, it shows that it contains alkaloids

Table 9. Results of flavonoid identification by TLC

Compound	Rf	The resulting color is formed		
		UV 254 nm	UV 366 nm	Sitroborat spray reagent
Quersetin	0,98 cm	Muffle	Blue	Yellow
Sample	0,98 cm	Muffle	Blue	Yellow

The results of identification of flavonoids using silica gel stationary phase and mobile phase n-butanol: acetic acid: water (4: 5: 1). The selection of the BAA mobile phase because it is able to provide the best separation, it can be seen from its composition, namely the eluent is very polar so that it can separate flavonoid compounds which are also polar.

Table 10. Saponin identification results by TLC

Rf	The resulting color is formed		
	UV 254 nm	UV 366 nm	Anisaldehid spray reagent
0,93 cm	Yellow	Green	Purple and under regular light spots are blue

The results of identification of saponin compounds using silica gel stationary phase and mobile phase chloroform: methanol: water (6: 3: 1). The results of observations on UV light 254 nm are yellow, while those on UV light 366 nm are green. Addition of spray reagent using anisaldehyde with purple results and under ordinary light blue spots.

Table 11. Results of identification of tannins by TLC

Rf	The resulting color is formed		
	UV 254 nm	UV 366 nm	$FeCl_3$ spray reagent
0,48 cm	Dark	Blue black	Yellow

CONCLUSION

It can be concluded that: First, the ethanolic extract, n-hexane, ethyl acetate and water fractions from leaf iodine (J. multifida) have antibacterial activity against P. aeruginosa ATCC 27853.

Second, the ethyl acetate fraction was the most active fraction in inhibiting P. aeruginosa ATCC 27853 which showed an average diameter of inhibition at 50% concentration of 14.7 mm, 25% concentration of 14.4 mm and at 12.5% concentration of 11 , 7 mm.

Third, the ethyl acetate fraction has a Minimum Kill Concentration (KBM), which is at a concentration of 12.5%..

REFERENCES

Aiyelaagbe O.O, Oguntuase B.J, Arimah B.D and Adeniyi B.A 2008. *The antimicrobial activity of jatropha multifida extract and chromatographic fractions against sexually transmitted infections*. *J. Med,Sci.,* 8(2):143-147.

Alamsyah, Heru Kurniawan *et al.* 2014. *Aktivitas Antibakteri Ekstrak Rumput Laut* Sargassum cinereum (J.G.Agardh) *dari Perairan Pulau Panjang Jepara* terhadap Bakteri *Escherichia coli* dan *Staphylococcus epidermidis*, Semarang: Universitas Diponegoro. Vol 3. No 2.

Backer C.A. & Brink R.C.B. (1965); *Flora of Java* (Spermatophytes only). N.V.P. Noordhoff-Groningen-The Netherlands.

DOI: 10.30994/sjik.v10i1.552
ISSN: 2252-3847 (print); 2614-350X (online) Vol.10 No.1 May 2021 Page. 1242-1249

Darmawi. *et al.*2013. Daya Hambat Getah Jarak cina (*Jatropha multifida* L.) terhadap Staphylococcus aureus Secara In Vitro. *Jurnal Medika Veterinaria*: Vol. 7 No. 2.

[Departemen Kesehatan RI]. 2000. *Parameter Standar Umum Ekstrak Tumbuhan Obat.* Jakarta: Direktorat Jenderal Pengawasan Obat dan Makanan.

Ilmi, A. N. 2009. *Tanaman Yodium Jatropha multifida Sebagai Bahan Fortifikasi Bath (Abstrak)*. Karya Tulis Ilmiah Strata Satu. Fakultas Sains dan Teknologi Universitas Airlangga. Surabaya.

Jawetz, E., Melnick., J.L., E.A., 2005. *Medical Microbiology,* 23 Th Ed. Elferia Nr, Penerjemah: Jakarta, hlm 170, 229

Hasibuan, S.A. 2016. Pebandingan Daya Hambat Ekstrak Daun Jarak Pagar (*Jatropha curcas Linn*) Terhadap Pertumbuhan Bakteri *Staphylococcus aureus* Dan Escherichia coli Secara In Vitro.[Skripsi]. Lampung: Fakultas Kedokteran,Universitas Lampung.

Maryati, C. 2013. Uji Aktivitas Antibakteri Ekstrak Jarak Tintir (*Jatropha multifida* L.) Terhadap Pertumbuhan *Staphylococcus aureus* Secara InVitro.

Muhaimin, *et al*. 2003. Optimasi Proses Over produksi, Pemurnian dan Karakterisasi Protein Mga Sebagai Molekul Target Untuk Pencegahan Infeksi Oleh Streptococcus Pyogenes. *Jurnal Matematika dan Sains.* Vol. 8,No 3. Hal. 117-123.

Puspasari R.K.FM, *et al*. 2014. *Studi Aktivitas Antibakteri dari Ekstrak Daun Sukun (Artocarpus Altilis) Terhadap pertumbuhan Bakteri Pseudomonas aeruginosa.* Jurnal: Sains dan Teknologi Kimia, Jilid 5 No. 2.

Sari FP, Shofi MS 2011. *Ekstraksi Zat Aktif Antimikroba Dari Tanaman Yodium (Jatropha multifida Linn) Sebagai Bahan Baku Alternatif Antibiotik Alami*. Laporan Penelitian. Fakultas Kimia dan Teknik Universitas Diponegoro.

Syarfati, Eriani, K, *et al*. 2011. *The Potensial of Jarak Cina (Jatropha multifida L.) Secretion in Healing New-wounded Mice.* Jurusan Biologi. FMIPA Universitas Syiah Kuala Darussalam - Banda Aceh. *Jurnal Natural.Vol. 11, No. 1,* 2011. Diakses pada 23 September 2013.

Susilowati N. 2010. Aktivitas antioksidan fraksi fraksi ekstrak metanolik daun seligi (*Phyllanthus buxifolius.* Muell, arg) terhadap radikal bebas DPPH [skripsi]. Surakarta : Fakultas Farmasi Universitas Setia Budi.

Wahjono, H. 2007. *Peran Mikrobiologi Klinik Pada Penanganan Penyakit Infeksi*. Semarang: Badan Penerbit Universitas Diponegoro.

Windarwati S.2011. *Pemanfaatan Fraksi Aktif Ekstrak Tanaman Jarak Pagar (Jatropha curcas Linn) Sebagai Zat Antimikroba dan Antioksidan Dalam Sediaan*