Pharmacology and Clinical Pharmacy Research **ISSN:2527-7322 | e-ISSN: 2614-0020**
Volume 6 No. 3 December 2021 **doi: 10.15416/pcpr.v4i3.31666**

Antibacterial Activities of Ethanol Extract of Jernang Resin (*Daemonorops draco* Blume)

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Abstract

Bacterial resistance to several antibiotics adds to the list of unresolved problems so that alternatives and renewal or development of natural medicines are needed to kill bacteria and prevent resistance, one of which is jernang resin. The resin compound is obtained from Jernang (*Daemonorops draco* blume), a plant that grows in the tropical rainforests of Indonesia. This study was undertaken to determine the antibacterial ability of ethanol extract of jernang resin against both *Salmonella typhi* and *Streptococcus mutans*. The crude extract was prepared by soxhlet extraction in ethanol, was then fractionated by vacuum liquid chromatography (VLC) with increasing solvent polarity starting from n-hexane (100%); n-hexane: ethyl acetate (80:20); ethyl acetate: methanol (40:60) up to methanol (100%). The antibacterial activity were evaluated using the disc diffusion method, these were compared with that of standards chloramphenicol as positive control and dimethyl sulfoxide (DMSO) as a negative control. Determination of the antibacterial activity were verified by counting the diameter of the zone of inhibition against *Salmonella typhi* at concentration 0.00mm/10ppm, 9.03mm/50ppm; 8.4mm/100ppm; and 11.73mm/500ppm. These were compared with that of standards chloramphenicol (21.03mm/300ppm) and DMSO (0.00mm/300ppm). On the other hands, the diameter of inhibition of growth zone against *Streptococcus mutans* at the variation of the extract concentration were 9.33mm/50ppm, 9.83mm/75ppm, 10.67mm/100ppm, 11.5mm/125ppm, and 13.33mm/150ppm. These were compared with that of standards chloramphenicol (25.7mm/300ppm) and DMSO (0.00mm/300ppm). The ethanol extract of jernang resin exhibited antibacterial effects on *Salmonella typhi* and *Streptococcus mutans*, these effects were less than chloramphenicol. The antibacterial effect increased with an increase in the concentration of the extract.

Keywords: Jernang Resin, Antibacterial, *Salmonella thypi, Streptococus mutans*

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Received: 12 January 2021. **Revised**: 13 April 2021 . **Published:** 20 November 2021

Introduction

Indonesia has abundant rattan resources. Out of 530 world types of rattan, 316 of them are found in Indonesia, originating from the genus of Calamus, Daemonorops, Ceratolobus, Korthalsia, Plectocomia, Plectocomiopsis, Cornera, and Miryalepis.[1,2] Rattan plants producing jernang of *Daemonorops draco* (*D. draco)* that are easily found or grown in Jambi, Sumatera Island. Previous studies reported that the clear resin of *D. draco* contains flavonoids, triterpenoids, and tannins. These secondary metabolites may potentially use for both antibacterial and antifungal.[3]

D. draco has been used as an antiseptic, stimulating blood circulation, antiviral, antitumor, wound medicine, and others.[4–6] The Anak Dalam tribe in Jambi was utilizing a clear resin as a drug for dysentery, wound medicine, toothache medicine, and childbirth medicine.[7] An infection in the mouth can occur due to the role of pathogenic bacteria that caused toothache. *Streptococcus mutants* (*S. mutans*) can cause dental caries, and the clear resin shows the presence of antibacterial activity against it.[8–10] Besides, the pathogenic bacteria such as *Salmonella typhi (S. typhi)* may infect the small intestine and cause typhoid fever.[11–13] Moreover, jernang resin is also useful for antifungals[14,15] where n-hexane extract shows high sensitivity against *Candida albicans*.[6]

Several studies have not explored antibacterial bioactive compounds from jernang resin extracts with different polarity levels, thus, it is necessary to carry out specific research for the class of bioactive compounds acting as antibacterials.

Unfortunately, there is lack of study about the antibacterial potential effect of jernang resin *Daemonorops*, thus, we conducted this study to determine of bioactive potential as antioxidants or antibacterial. In this study we analyzed the potential of *D. draco* jernang resin in ethanol extract against *S.typhi* (gram-negative bacteria) and *S.mutants* (gram-positive bacteria) as an antibacteria.

Methods

Sample Collection and Extraction

Jernang (*Daemonorops draco*) were obtained from Kabupaten Batanghari, Sarolangun, Merangin, Muara Tebo, Muara Bungo, dan Tanjung Jabung Barat, Jambi. Jernang resin was extracted from rattan fruit using ethanol. Clear rattan fruit was put into a brown bottle container for 3x24 hours with 10L of ethanol solvent, filtered until the solvent from maceration was clear, then tput in the evaporator until the thick ethanol is formed.

Thick ethanol extract was obtained. The resin was produced by the plant and collected. We ware adjusted up to 700 gram of Jernang Resin samples and macerated by ethanol 70%, then concentrated by rotary evaporator. Ethanol was separated to by evaporated until a concentrated ethanol extract was obtained. The separation (fractionation) was carried out by vacuum liquid column chromatography (VLC) using a stationary phase of silica gel with a sample: silica gel ratio (1: 4). The sample extract is impregnated using silica gel, then added to a column that contains a stationary phase. While the mobile phase used is in accordance with the best separation in Thin Layer Chromatography (TLC). The fractions obtained are collected in vials, eluquites are accommodated based on each band obtained and then evaporated. The resultant from column chromatography were performed TLC again. Eluates which have identical stain patterns are combined based on the Rf value on the chromatogram.[17]

Separation of Compounds

Separation of compounds was done by using VLC to simplify the compounds present in the total extract. The stationary phase used is a 1x10 cm silica plate and eluent variying polarity (mobile phase) in the form of n-hexane: ethyl acetate and ethyl acetate: methanol. The best effluent was obtained by n-hexane: ethyl acetate in a ratio of 7: 3. The comparison is a reference in the separation of compounds by using column chromatography.

A total of 1.4425 grams of Ethanol extract was impregnated using 20.8636 grams (1: 4) silica gel with a size of 230-400 mesh. VLC was carried out using a 5 cm diameter column with 65 grams of vacuum silica gel as a stationary phase and solvents with multi-level polarity starting from n-hexane 100%; n-hexane: ethyl acetate; ethyl acetate: methanol to 100% methanol as the mobile phase.

Antibacterial Activity Assays

All of media and chemical materials were purchased from Sigma-Aldrich and MERCK. We used ethanol as solution test and the concentrations were; 10; 50; 100; 500; 1000 µg / mL (ppm) as an antibacterial active fractions with a concentration of 50; 75; 100; 125; 150 µg / mL (ppm) in ethanol solvents. The positive control of antibacterial is chloramphenicol 0.3% w / v in ethanol solvent.

The bacterial rejuvenation was carried out using the aseptic and uniform scratch method. The disc paper was dipped in the test solution, the positive control and blank are then affixed to the agar media, then incubated for 24 hours. After incubating, the diameter of the inhibition zone was measured, ie the area that was not attacked by bacteria using a calipers. Moreover, we compared with positive control of chloramphenicol for antibacterial. The tests were conducted by three repetitions, and the best of antibacterial activity was indicated by the largest of diameter inhibition.[18] The criteria of antibacterial activity refers to previous studied who classified it into: low (<5 mm), moderate (5-10 mm), strong (10-19 mm), and very strong (>20mm).[19]

Results and Discussion

The jernang resin was extracted using a single extraction method using ethanol as a solvent. We adjusted 700 grams of Jernang resin samples (*D. draco*) added into 69.34 grams of ethanol extract and was obtain 9.91% of yield. The phytochemical screening results of Jernang resin ethanol extract (*D. draco*) is presented in Table 1.

Separation of compounds was done using VLC to simplify the compounds present in the total extract. The stationary phase used is a 1x10 cm silica plate and eluent variying polarity (mobile phase). The use of a solvent ratio is used to separate compounds based on their polarity properties. The separation process with VLC can be seen in Figure 1.

From the results of VLC, 27 vials were obtained which were grouped into five fractions (Figure 2). The grouping of fractions is based on the similarity of stain patterns formed in the TLC test. Moreover, we carried out the phytochemical screening to verify the secondary metabolite compounds in each fraction and their activity. The total extract contains five secondary metabolites, alkaloids, flavonoids, steroids, and triterpenoids. These results are consistent with previous studies that positive jernang resin contains several bioactive compounds.[7,17]

The resins are components that can be obtained from jernang processing, which are traditionally used as a medicine. The results

(a) (b)

Figure 1. Jernang Resin.
(a) Jernang *Daemonorops draco* (b) Isolate of Jernang Resin

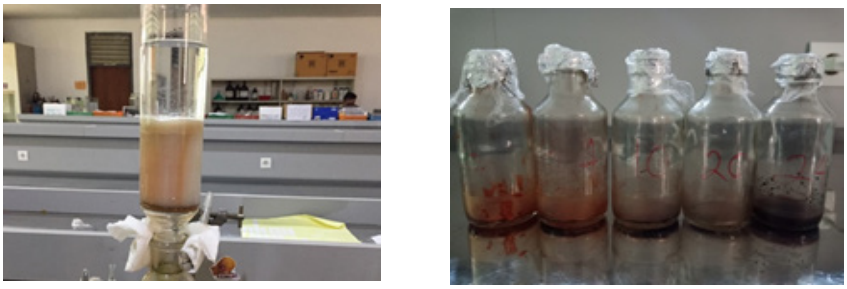

Figure 2. Vacuum Liquid Chromatography (VLC) Results
(a) VLC Apparatus (b) Five fractions after VLC

of phytochemical analysis (Table 1 and Table 2) of the total extracts and the fraction results contained metabolite compounds which were potential as antibacterial agents, namely alkaloids, flavonoids, and triterpenoids.

It is known that the results of phytochemical screening of jernang resin ethanol extract contain terpenoid as secondary metabolites which are marked by reddish color changes, flavonoids that are formed froth or orange and alkaloids dragendorff reagents are formed orange deposits. These results are in accordance with the study of Waluyo and Pasaribu (2013), also having the same secondary metabolite content, except for the positive dragendoff reagent alkaloids and tannins which were negative in this study.[6]

Phytochemical screening was carried out on fractions 1 to 5 to determine the distribution of secondary metabolites in each fraction Fractions 1 and 2 contained secondary metabolites of terpenoid, flavonoid,and alkaloids. In fraction 3, there were steroids, flavonoids and alkaloids, fraction 4 it contains steroid secondary metabolites and alkaloids, while fraction 5 it only contained secondary metabolites of dragendorff reagent alkaloids. This shows that each fraction contains different secondary metabolites. (Table 2)

The antibacterial activity were evaluated using the disc diffusion method by paper disc method. The inhibitory activity is indicated through the clear zone that appears around the test paper disc. The first screening was carried out to evaluate the antibacterial activity measured against the total ethanol extract of *D. draco* resin against *S. thypi* and *S. mutans* (Table 3 and Table 4).

In this study, few serial concentrations of extracts were carried out following several previous studies (1000 ppm, 500 ppm, 100 ppm, 50 ppm, and 10 ppm).[20–22] Based on

Table 1. Phytochemical result of Ethanol Extract of *Daemonorops draco* Resin

Secondary Metabolites	Result	Observation
Alkaloid		
- Dragendorf Reagent	+	Red till orange of Sediment
- Meyer Reagent	nd	Yellowish white of Sediment
Flavonoids	+	Orange Froth
Saponins	nd	Stable foam
Tanin	nd	Greenish black
Steroid	+	Blue or green color
Triterpenoid	+	Purple or orange

Table 2. The presence of Secondary Metabolite of VLC Fraction of *Daemonorops draco* resin

Secondary Metabolites	Results*				
	F1	F2	F3	F4	F5
Tannin/Phenolic	-	-	-	-	-
Terpenoids	+	+	-	-	-
Steroids	-	-	+	+	-
Flavonoids	+	+	+	-	-
Saponin	-	-	-	-	-
Alkaloids	+	+	+	+	+

*(+) : Detected; ND: not detected

Table 3 and 4, concentrations of 1000 ppm and 500 ppm possessed strong effect in inhibiting growth of *S. thypi*, whereas, 5000 ppm and 100 ppm possessed strong effect against *S. mutans*. Concentrations of 100 ppm and 50 ppm were classified as moderate effect, whereas concentrations 10 ppm did not show any antibacterial activity. These results confirmed that the ethanol extract of *D. draco* resin has the potential to be used as an antibacterial agent. The extract response to *S. mutants* was stronger than *S. typhi*, because the outer membrane of gram-negative bacteria is the main reason for resistance to a wide range of antibiotics including beta-lactams, quinolones, colistins, and other antibiotics.[23]

Ethanol fraction which has the strongest antibacterial activity was fraction 5. In fraction 5, the antibacterial test was carried out again using concentrations of 50, 75, 100, 125 and 150 ppm. This was done to determine at concentrations above or below 100 ppm that the fraction was active antibacterial.

Antibacterial Activity of Fraction 5

Fractionation aimed to obtain a single active compound then investigated its activity, whether the total activity of the extract had the same activity as the fraction. Testing for antibacterial activity in addition to ethanol extract was also tested for fraction against *S. typhi* and *S. mutans*, the results was obtained present in Table 5 and Table 6

Table 3. The Diameter of Inhibition of Growth Zone Ethanol Extract against *Salmonella thypi*

Ethanol Extract (ppm)	The diameter of the zone of inhibition (mm)			Average (mm) ± SD	Response
	1	2	3		
1000	13.5	11.4	13	12.63 ± 1.09	***
500	12	9.2	14	11.73 ± 2.41	***
100	10	8.2	7	8.4 ± 1.50	**
50	9.1	8	10	9.03 ± 1.00	**
10	0	0	0	0 ± 0	ND
C+	16.1	23	24	21.03 ± 4.30	****
C-	0	0	0	0 ± 0	ND

Table 4. Antibacterial activity of Ethanol extract against *Streptococcus mutans*

Ethanol Extract (ppm)	The diameter of the zone of inhibition (mm)			Average (mm) ± SD	Response
	1	2	3		
1000	16.7	14.65	16	15.78 ± 1.04	***
500	15.3	13.7	15	14.66 ± 0.85	***
100	11	10.8	11	10.93 ± 0.11	***
50	9	9.3	9.5	9.26 ± 0.11	**
10	0	0	0	0 ± 0	ND
C+	26.1	27	24	25.70 ± 1.53	****
C-	0	0	0	0 ± 0	ND

* Inhibition ability level; C+ Positive control; C- Negative control
ND: not detected

respectively. Based on Table 6, concentration of 150 ppm, 125 ppm, and 100 ppm had a strong antibacterial activity, while other concentrations had moderate effects. This showed that all concentrations have antibacterial activity.

In ethanol fraction, fraction 5 had the strongest antibacterial activity. Fraction 1 and fraction 2 had relatively moderate antibacterial activity. Whereas, fraction 3 and fraction 4 did not show any antibacterial activity (not shown). Its seems that the fraction does not contain enough bioactive compounds in inhibiting bacterial growth.[19] Furthermore, in this study, we focus on exploring the antibacterial activity.

Table 5 and 6 showed that antibacterial testing returned to fraction 5 due to the fraction had greater antibacterial strength or inhibition compared to other fractions. At concentrations of 150, 125 and 100 ppm the antibacterial inhibition was classified as strong, while at concentrations 75 and 50 included in the medium category.[24] This

Table 5. Antibacterial activity of Ethanol extract against *Salmonella thypii*

Fraction 5	The diameter of the zone of inhibition (mm)			Average (mm) ± SD	Response
	1	2	3		
150 ppm	15	12	13	13.33 ± 1.53	***
125 ppm	12	11	11.5	11.5 ± 0.5	***
100 ppm	11	10.5	10.5	10.67 ± 0.28	***
75 ppm	10	10	9.5	9.83 ± 0.28	**
50 ppm	9.5	9.5	9	9.33 ± 0.28	**
C+	16.1	23	24	21.03 ± 4.30	****
C-	0	0	0	0 ± 0	ND

Table 6. Antibacterial activity of Ethanol extract against *Streptococcus mutans*

Fraction 5	The diameter of the zone of inhibition (mm)			Average (mm) ± SD	Response
	1	2	3		
150 ppm	15	10	14	13.0 ± 8.38	***
125 ppm	13	10	11	11.33 ± 1.53	***
100 ppm	12	9.5	10.5	10.67 ± 1.26	***
75 ppm	11	9	10	10 ± 1	**
50 ppm	10.5	8.5	9	9.33 ± 1.04	**
C+	16.1	23	24	21.03 ± 4.30	****
C-	0	0	0	0 ± 0	ND

* Inhibition ability level; C+ Positive control; C- Negative control
ND: not detected

showed that all concentrations of fraction 5 which contained single alkaloids compound that confirmed has potent as antibacterial agent. The mechanism of inhibition of bacterial growth by alkaloids can be in the form of damage to cell walls by inhibiting their formation or changing them after they are formed, changes in permeability of cytoplasmic membranes causing food release from the cell, changes in protein and nucleic acid molecules, inhibition of enzyme work, and inhibition of synthesis nucleic acids and proteins. The presence of alkaloid and flavonoid compounds in the ethanol extract of *D. draco* resin shows an antibacterial activity. Flavonoids work on bacteria by damaging the cytoplasmic membrane.[24,25] Bacterial cytoplasmic membrane functions to regulate the entry of food ingredients or nutrients, if the cytoplasmic membrane is damaged then important metabolites in bacteria will come out and food ingredients to produce energy can't enter so there is an inability of bacterial cells to grow.

Moreover, the mechanism of action of alkaloids as antibacterial is by interfering with the components of peptidoglycan

(the polysaccharide consisting of N-acetyl glycosamine acid and N-acetylmuramate on bacterial cells, so that the cell wall layer is not formed intact and causes cell death. Other mechanisms of alkaloid antibacterial namely alkaloid component are known inhibits bacterial cell topoisomerase enzymes.[26,27]
Alkaloid have inspired the development of several antibacterial drugs, present as scaffold substuctures. Antibacterial mechanism of action (MOA) has been investigated for alkaloids in the indolizidine, isoquinoline, quinolone, agelasine, and polyamine classes. In the indolizidine class, it has been proposed that the alkaloids pergularinine and tylophorinidine act by inhibiting nucleic acid synthesis, as they inhibit the enzyme dihydrofolate reductase in cell-free assays. Alkaloids have great potential in pharmaceuticals such as as feeding deterrents, allelochemicals, autoinducers, and siderophores. In plants, the inhibitory effects of alkaloids on glycosidase and trehalose metabolism deters herbivores and the ability to quench singlet oxygen confers protection against this toxic photosynthetic byproduct.

Alkaloids also act as phytoanticipins and phytoalexins, protecting plants against infection. In the animal kingdom, rove beetles release the surface-active alkaloid stenusine to 'skim' across water away from danger, poison dart frogs secrete batrachotoxin as a defense against snake predation, and arctiid moths use pyrrolizidine alkaloids as a courtship pheromone. In sponges, alkaloids deter feeding and protect against infection, while in scleractinia they act as allelochemicals.[26,28]

Conclusion

Our findings, the separation of the active compound from the ethanol extract of Jernang resin (*D. draco*) through VLC, there are 5 combined fractions. The class of compounds found in resin ethanol of *D. draco* extract are flavonoids, triterpenoids, and alkaloids while remains alkaloids in Fraction V. In ethanol extract of *D. draco*'s resin showed the presence of antibacterial activity against *S. thypi* and *S. mutans* which were classified as strong both in extracts and fractions V.

Acknowledgement

None

Funding

None

Conflict of Interest

None declared

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