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ISOLATION AND IDENTIFICATION OF SECONDARY METABOLITE COMPOUNDS OF LAOR WORM EXTRACT AS ANTIBACTERIAL

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Abstract

Laor worms (*Lysidice oela*) are worms that appear on the surface of the Maluku sea to reproduce in March or April. Laor worms are usually consumed by most people because they contain 13.92% protein, 81.51% water, 1.01% fat, and 2.41% ash and contain 9 types of essential amino acids. Secondary metabolite compounds (bromophenol) from marine worms are antimicrobial. This research aimed to determine the potential of secondary metabolites in inhibiting the growth of *E. coli* bacteria and to determine the secondary metabolites contained in laor worms. Laor worm is extracted maceration with ethanol, ethyl acetate and petroleum ether. The results of each extract were tested for antibacterial activity using the disc diffusion method with variations in concentrations of 25, 50, 75, 100, and 125 mg/mL against *E. coli* bacteria. The results of the extract which had the highest antibacterial activity were then tested for phytochemicals, then separated using TLC, and identified active compounds using UV-Vis spectrophotometers and their functional groups using FT-IR spectrophotometers. The results showed that laor worm extract could inhibit the growth of *E. coli* bacteria with an inhibition zone area of 13.4 mm for ethanol extract; 14.8 for ethyl acetate extract and 12.6 mm for petroleum ether extract. The phytochemical test showed that the ethyl acetate extract of la worms contained flavonoids, saponins, steroids, triterpenoids, and alkaloids. Identification using a UV-Vis spectrophotometer obtained λ_{max} was 203 nm which showed steroid compounds. The identification of active compounds using an FTIR spectrophotometer shows the functional groups O-H, C = O, C-C, C-OH primary, and C-H which are thought to be steroid compounds.

Keywords: Laor worms; Secondary Metabolite; Antibacterial; Escherichia coli

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INTRODUCTION

Infection is a type of disease caused by micro-organisms and affects most people in developing countries, including Indonesia (Ningsih & Zusfahair, 2016; Sari, 2012). Escherichia coli is usually a resident of the digestive tract. Several strains of E.coli are one of the causes of diarrhea in both children and adults, which can cause enterobacterial infections, for example diarrhea to meningitis. suffered by society.

To treat infectious diseases, antibiotics are the target. The irrational use of antibacterial has caused many pathogenic microbes to adapt to their environment and become resistant to these drugs (Candrasari et al., 2011).The problem of bacterial resistance to existing drugs has prompted the importance of extracting antibacterial compounds from

natural ingredients that are more patent, inexpensive, have fewer side effects and are continuously available in large quantities (Hartini, 2001).

Maluku is an area in Indonesia which consists of a very wide archipelago with seas that stretch around each island. This area has marine biodiversity and provides opportunities to utilize marine biota in the search for secondary metabolites of new bioactive compounds. Marine biota (marine organisms) are a very rich source of natural materials with unique biological activities (Handayani et al., 2012). Polychaeta are invertebrate animals that belong to the phylum Annelida. People in Indonesia know polychaeta by the name of sea worms, because most of their habitat is in the sea.

One of the worms in Worms Laor (Lysidice oela). This wormis one type that belongs to the family *Eunicidae*which is usually consumed by most people in Maluku (Pamungkas, 2009).These worms usually come to the surface of the water in March or April while moving his body or dancing. The spiral movement of the worms to the surface of the water allows these two worms of different sexes to meet, which eventually breaks off and sperm or egg cells come out. The meeting between the egg and sperm will become a zygote that will go to the seabed. It was at that time that the people caught the sea worms and until now it has become a tradition for the people of Maluku.

The abundant number of laor worms during the timbah laor activity is an indication that the laor worms have the ability to protect themselves from other creatures in the sea (Jekti et al., 2008). The ability to take care of itself is possible because the laor worm has active ingredients (natural products) that can kill or inhibit the growth of other living things. The ability of the Laor worm to inhibit the growth of benthos bacteria is related to the place where the Laor worm lives, namely in coral. The chemical components of sea worms encourage the development of isolating and identifying secondary metabolites found in sea worms. Secondary metabolites have the ability as bioactive compounds so they are very promising as lead compounds for materials that have pharmacological activity.

Identification of secondary metabolite content is an important initial step in research to search for new bioactive compounds from natural ingredients that can be precursors for the synthesis of new drugs or prototypes of certain active drugs (Rasyidi, 2016). The group of secondary metabolites is very abundant and commonly found in organisms including alkaloids, flavonoids, phenols, steroids, and terpenoids (Marliana, 2007). This study aims to determine the potential of secondary metabolites from the Laor worm (Lysidice oela) in inhibiting the growth of Escherichia coli bacteria andTo find out the secondary metabolite compounds contained in Laor worms.

METHOD

Material

The materials used were Laor worm samples, 96% ethanol, 50% methanol, Mg metal, ethyl acetate, petroleum ether, 1 N HCl, concentrated HCl, 2% HCl, chloroform, DMSO, chloramphenicol, acetic acid anhydrous, concentrated H2SO4, Aquades, 1% FeCl3, Reagent solution (dragendorff, mayer), KBr, Medium NA, Medium NB, Aluminum foil, Escherichia coli, disc paper and cotton.

Method

The research method used is descriptive qualitative and quantitative research through two stages of experimental testing in the laboratory, the first stage aims to determine the effect of the type of solvent on the antibacterial activity of laor worm extract. Laor worms were extracted with a variety of solvents consisting of ethanol, ethyl acetate and petroleum ether. Extraction of active compounds was carried out using the maceration method, the extraction results

were tested for antibacterial using the agar diffusion method. The antibacterial test was carried out in duplicate and repeated 3 times. In the second stage, the best antibacterial activity of the extract will be tested for phytochemicals, then the active compounds will be separated using TLC. Identification of chemical compounds using a UV-Vis spectrometer while determining their functional groups using FTIR.

Work procedures

Sampling

Sampling of laor worms in Latuhalat Village, Ambon Island, Maluku Province.

Sample Preparation

Sampling results are included in the coolbox. Then soaked in cold water 14 ° C for 24 hours. Aerated at room temperature covered with aluminum foil. Next, the samples were ground using a blender. The milled products were dried in an oven at 50 °C for 12 hours and sieved (90 mesh) to obtain flour extract (Aninda, 2016).

Laor Worm Extraction

As much as 25 g of Laor worm powder was put into a 100 mL Erlenmeyer flask. Then each solvent was added, namely 96% ethanol, ethyl acetate and 50 mL petroleum ether. Shaking was carried out with a shaker for 3 hours at a speed of 120 rpm (rotation per minute) and macerated for 24 hours. Then filtered the maceration residue with a Buchner funnel and redissolved using the same solvent until it is clear. The filtrate obtained was combined in an extract storage container (Nurhayati & Purwaningsih, 2017). The ethanol, ethyl acetate and petroleum ether extracts obtained were concentrated using a rotary evaporator at a temperature of 30-40 ºC, and flowed with N2 gas until the solvent completely evaporated.

Antibacterial Activity Test

The NA (Nutrien Agar) media is heated until it melts, cooled to a temperature of 40°C. The NA solution was poured into sterile petri dishes, mixed with 0.1 mL of E. coli bacteria solution, then homogenized and allowed to solidify.Laor worm extract was prepared in various concentrations (25, 50, 75, 100 and 125 mg/mL). A total of 0.01 gram of Laor worm extract was dissolved in 10 mL of ethanol, ethyl acetate and petroleum ether as the main solution and then divided into several predetermined concentrations (Elayaraja et al., 2010). Paper discs with a diameter of 5 mm were soaked in the resulting laor worm extract and controls (positive controls were given chloramphenicol and negative were given DMSO solution). The disc paper is placed on the surface of the media using sterile tweezers and pressed slightly. Incubated at 37 °C for 24 hours until the inhibition area appears (Mulyadi et al., 2017). The antibacterial activity test for each solvent was carried out in duplicate and repeated 3 times. The inhibition zone was measured using a ruler to determine bacterial activity.

Phytochemical Test of Active Compounds in Laor Worms

The extract which has the widest inhibition zone will be identified for its chemical content, including flavonoids, terpenoids, saponins, tannins, phenolics and alkaloids (Kristianti et al., 2008).

Classification of Compounds with Analytical TLC

Extracts that showed antibacterial activity were then tested chemically by Thin Layer Chromatography (TLC) which was carried out using silica gel GF254 plates as the stationary phase. Silica gel plates are made with a width of 1x10 cm2 at the top and bottom ends with a limit of 1 cm. 0.001 gram of the extract to be tested was dissolved in 2.5 mL of the solvent (4,000 ppm) used, then spotted 10 times ± 1 cm from the bottom of the plate. Developers (mobile phase) used for each compound were: steroids (n-hexane 1 : 1 ethyl acetate), alkaloids (chloroform 4 : 1 methanol), saponins (chloroform 13 : 7 methanol : 2 water), triterpenoids (n- butanol 4 : 1

Ammonia), flavonoids (ethyl acetate 4.5 : 0.5 methanol) and phenolics (Toluene 3 : 3 ethyl acetate : 0.2 formic acid). Then it was dried and eluted with eluent in a TLC flask which had been saturated and tightly closed. After the eluent reaches the top line, the plate is removed and dried (Jekti et al., 2008).The spots were observed visually with a UV lamp at a wavelength of 245 nm and 366 nm and using a universal spray reagent to reveal the spots with the Liebermann-Burchard reagent. Then the Rf value is calculated and the spot shape is observed in various eluents.

Identification of Compounds Using a UV-Vis Spectrophotometer

Laor worm extract which had been dissolved based on the solvent with a concentration of 100 ppm was then put into a cuvette and analyzed using a UV-Vis spectrophotometer at a wavelength range of 200-800 nm (Maharani et al., 2016).

Functional Group Identification Using FTIR

The characterization in this study used FTIR (Fourier Transform Infra Red) spectroscopy. The target compound in solid form was crushed with KBr salt (2:98) and then pelleted using a diameter of 7 mm. The pellet was then placed in the sample holder and its absorption was measured using an FTIR spectrometer in the area of 4000-400 cm-1. The sample to be analyzed must be ensured that it is dry and free from impurities around the container. The data from the analysis results were then processed using Ms. Excel 2010 (Sigee et al., 2002).

Data analysis

The data obtained from the first stage of the research were in the form of inhibition zones from the results of the antibacterial activity test of each variation of solvent, the best solvent was indicated by the widest diameter of the inhibition zone and the second stage of research was the identification of chemical compounds and functional groups from extracts that had the widest inhibition zones. Each data is presented in tabular form and interpreted according to the results obtained

RESULTS AND DISCUSSION

Sample preparation

Wet laor worm samples 256 g were washed thoroughly using running water. Then the samples were dried by means of laor worm samples placed on top of aluminum foil and dried in the laboratory for ± 2 weeks. After drying, a sample weight of 121.82 g was obtained which was then refined using a 90 mesh sieve. Smoothing is done so that the surface area of the sample is greater so that the sample contact with the solvent is maximized. In addition, the refining process also allows the cells to rupture, thus facilitating the uptake of active compounds by solvents. Laor worms have a moisture content of 47.58%. Water content analysis aims to determine the water content contained in laor worm biomass.

Extraction of Laor worms by maceration

Sample extraction in this study was carried out by maceration. Extraction was carried out using 3 solvents namely ethanol, ethyl acetate and petroleum ether. Laor worm powder was added to each solvent in the ratio (1 : 3) and macerated for 24 hours at room temperature. The filtrate obtained is then concentrated using a rotary evaporator and N2 gas, this aims to remove the solvent. The results of observations on the color of the filtrate, extract color, measurement of crude extract weight and extract yield extract yield can be seen in Table 1.

Table 1. Yield of Laor Worm Extract from various solvents

Solvent type	Filtrate Color	Extract Color	Crude Extract (g)	Yield (%)
Ethanol	Dark brown	Dark brown	6.9481	27,79
Ethyl acetate	Brown	Brown	2.8335	16.89
Petroleum ether	Brown	Brown	1.4081	5,63

The results in Table 1 show that the characteristics of the filtrate in ethanol are dark brown, while ethyl acetate and petroleum ether have the same color, namely brown. The type of solvent also affects the extraction results and yield. The highest extract value was ethanol solvent of 6.9481 g with a yield value of 27.79%. followed by 2.8335 g of ethyl acetate extract with a yield of 16.89% and petroleum ether extract with an extract value of 1.4081 g with a yield of 5.63%.

These results also indicated that the ethanol extract produced a higher yield when compared to the ethyl acetate and petroleum ether extracts. The high yield of extracts in polar solvents is because the active compounds are generally still in the form of glycoside bonds, namely compounds consisting of sugar compounds (glycones and primary metabolites) and non-sugar compounds (aglycones and secondary metabolites) (Utami et al., 2013).

Antibacterial Activity Test

The results of measuring the diameter of the inhibition zone showed that the Laor worm extract had activity in inhibiting Escherichia coli bacteria. The average inhibition zone resulting from the results of the antibacterial activity test can be seen in Table 2.

Table 2. The results of testing the antibacterial activity of laor worm extract against Escherichia coli bacteria

Concentration (mg/mL)	Average Inhibition Zone(mm)			Growth Response		
	Ethanol Extract	Ethyl Acetate Extract	Petroleum Ether Extract	Ethanol Extract	Ethyl Acetate Extract	Extract Petroleum Ether
25	6,6	7,1	5,6	Intermediate	Intermediate	Weak
50	9,2	8,8	9,2	Intermediate	Intermediate	Intermediate
75	10,7	12,1	10.5	Intermediate	Strong	Intermediate
100	12	13,8	11,8	Strong	Strong	Intermediate
125	13,4	14,8	12,6	Strong	Strong	Strong
Control (+)	34	34	35	Very strong	Very strong	Very strong
Control (-)		-		There isn't any	There isn't any	There isn't any

Based on Table 2 it can be seen that the ethyl acetate extract of the laor worm has a higher antibacterial activity when compared to the ethanol and petroleum ether extracts. This is indicated by the size of the inhibition zone at each concentration of each ethyl acetate extract. The higher extract concentration causes a higher inhibition effect so that the clear zone around the paper disk becomes wider.

Antibacterial activity in ethanol extract was identified at concentrations of 25 mg/mL (6.6 mm), 50 mg/mL (9.2 mm), 75 mg/mL (10.7 mm), 100 mg/mL (12 mm) and 125 mg/mL (13.4 mm). Ethyl acetate extract concentrations of 25 mg/mL (7.1 mm), 50 mg/mL (8.8 mm), 75 mg/mL (12.1 mm), 100 mg/mL (13.8 mm) and 125 mg /mL (14.8mm). Whereas in petroleum ether extract concentrations of 25 mg/mL (5.6 mm), 50 mg/mL (9.2 mm), 75 mg/mL (10.5 mm), 100 mg/mL (11.8 mm) and 125 mg/mL (12.6 mm). There was an increase in the diameter of the inhibition zone along with the increase in the concentration of the worm extract in all solvents.

The results of this study are different from the results of Nurhikmah's research (Nurhayati & Purwaningsih, 2017), where the ethanol and ethyl acetate extracts of the freeze-dried sea worm Siphonosoma australe-australe did not have antibacterial activity. While in research Elayaraja et al. (2010) ,Perinereis marine worm extract concentration*cultrifera* effective in inhibiting the growth of gram negative

bacteriaEscherichia coli bacterianamely the concentration of 25 mg/mL (7 mm) while the sea worm extract*Eunice siciliances*96% ethanol extract can inhibit the growth of bacteria such as E. coli bacteria (11 mm) at a concentration of 100 µg/mL (Jekti et al., 2008). The high value of the inhibition zone of the ethyl acetate extract in this study is thought to be due to the active compound that has the potential as an antibacterial from the Laor worm, which tends to be distributed in semipolar solvents. The results of observing the inhibition zones of Laor worm extract with various solvents against Escherichia coli bacteria can be seen in Figure 1.

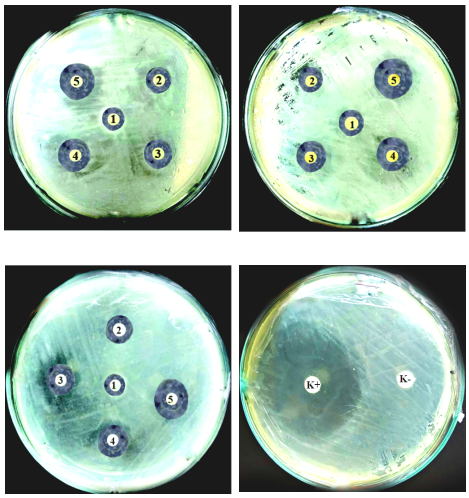

Figure 1. Zone of Inhibition of Laor worm extract from various solvents against Escherichia coli bacteria. (A) Ethanol, (B) Ethyl acetate, (C) Petroleum ether and (D) Control. (1) 25 mg/mL, (2) 50 mg/mL, (3) 75 mg/mL, (4)100 mg/mL, (5)125 mg/mL, (K+) positive control and (K-) negative control

The positive control used in this study ischloramphenicol. The use of chloramphenicol is due to:Chloramphenicol is a broad spectrum antibiotic (Nuria, 2016). Chloramphenicol is bacteriostatic or inhibits bacterial growth (Cahyono & Indrayudha, 2013). The test results show that the average inhibition zone formed onchloramphenicol was larger when compared to the zone of inhibition in ethanol, ethyl acetate and petroleum ether extracts. The large inhibition zone in the positive control was due to the fact that the active ingredient in chloramphenicol was pure, while the ethanol, ethyl acetate and petroleum ether extracts were not pure because they were mixed with other compounds so that the inhibition of bacteria was not effective.

The negative control used was DMSO. According to Pratiwi, Dimethyl Sulfoxide (DMSO) is a solvent that can dissolve both polar and nonpolar compounds that dissolve in organic solvents and water. The absence of an inhibition zone in the negative control in this study proved that the inhibition zone formed was not influenced by the type of solvent but due to the activity of the active compounds present in the laor worm extract as antibacterial.

Identification of Laor Worm Secondary Metabolite Compounds

The results of the antibacterial activity test showed that the ethyl acetate extract of the laor worm had the highest antibacterial activity, so the ethyl acetate extract was then identified as a secondary metabolite. The results of the secondary metabolic compounds test for the ethyl acetate extract of the Laor worm qualitatively can be seen in Table 3.

Table 3. Test results for the content of secondary metabolites of ethyl acetate extract of Laor worms

No.	Compound Class	Formed color/precipitate	Results
1	Flavonoids	Yellow	+
2	Steroids	Bluish green	++
3	Triterpenoids	Browning Ring	++
4	Saponins	Foam turbid	++
5	tannins	Yellow	-
6	Phenol	Dark brown	+
7	Alkaloids		
	a. Mayer	Yellowish white precipitate	+
	b. Dragendroff	Orange precipitate	+

Note: (+) There is a group of active compounds and (-) There is no group of active compounds.

Based on Table 3, it can be seen that the ethyl acetate extract of the laor worm contains flavonoids, steroids, triterpenoids, saponins, phenols and alkaloids. The secondary metabolites found in this study were not much different from those found by Erviani and Arif (2017) where the results of the phytochemical screening of sea worm extract Perinereis aibuhitensis also contain alkaloids, flavonoids, saponins, triterpenoids/steroids and tannins. In addition, the Siphonosoma austral sea worm contains saponins, flavonoids, steroids and alkaloids (Aninda, 2016). The results of the phytochemical screening of the sea worm extract of Eunice siciliensis contain alkaloids, flavonoids, saponins, triterpenoids/steroids and tannins (Erviani et al., 2019).

Thin Layer Chromatography Profile

The ethyl acetate extract obtained was then analyzed by Thin Layer Chromatography (TLC). Tests were carried out with a combination of ethyl acetate: methanol, n-hexane: ethyl acetate, n-butanol: NH4OH, the TLC test can be seen in Table 4 and Figure 2

Table 4. Color and Rf value of loar worm ethyl acetate extract elution results

Compound Class	Mobile Phase	Stain	Rf value	Stain Color	Compound Suspect
Alkaloids	Chloro: meta (4 : 1)	1	0.91	Orange	Alkaloids
Saponins	Chloro : meta : water (13 : 7 : 2)	2	0.80 0.83	Dark Purple Red	Saponins Triterpenoids
Triterpenoids	n-Butanol : NH4OH (4 : 1)	4	0.20 0.37 0.50 0.89	Greenish blue Greenish blue. Greenish blue Violet	Flavonoids Flavonoids Flavonoids Triterpenoids
Steroids	n-Hexane : EA (1 : 1)	6	0.39 0.58 0.64 0.7 0.83 0.93	Blue Red Red Red bluish green Red	Flavonoids Triterpenoids Triterpenoids Steroids Triterpenoids Triterpenoids
Flavonoids	EA : Methanol (4.5 : 0.5)	2	0.83 0.95	Dark purple Red	Saponins Triterpenoids
Phenol	Toluene : EA : Formic acid (3:3:0,2)	4	0.52 0.57 0.90 0.96	Red Red Dark blue Dark purple	Triterpenoids Triterpenoids Flavonoids Saponins

Notes : EA (Ethyl Acetate), Meta (Methanol) and Chloro (Chloroform).

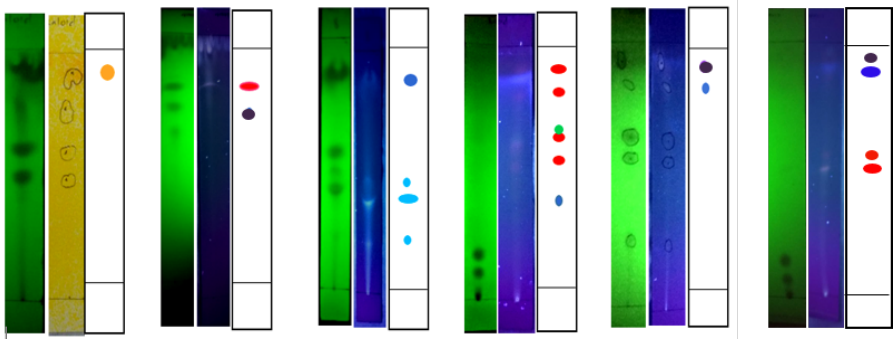

Figure 2. TLC profile of Laor worm ethyl acetate extract

Based on the results of the TLC in Table 4, it was shown that the ethyl acetate extract of the laor worm contained flavonoids, steroids, triterpenoids, saponins and alkaloids. The TLC test with ethyl acetate: methanol (4.5 : 0.5) eluent produced two spot stains at a wavelength of 366 nm, namely blue and purple with an Rf value of 0.83; 0.95, blue color indicates flavonoids and purple indicates saponins. In the n-hexane eluent: ethyl acetate (1:1) six spots were obtained at a wavelength of 366 nm with an Rf value of 0.39; 0.58; 0.71; 0.64; 0.67; 0.83 and 0.93. It is suspected that at Rf 0.67 it is a steroid compound due to the presence of a bluish-green stain after being sprayed with the Liberman-Buchard reagent, the blue color indicates flavoid compounds with Rf 0.39 while the red color indicates triterpenoid compounds with Rf 0.58; 0.64; 0.83 and 0.93.

In the n-butanol eluent: NH4OH (4:1) at a wavelength of 366 nm produced four spots with an Rf value of 0.20; 0.37; 0.50 and 0.89 with blue-green color indicates flavonoid compounds respectively and light purple is a triterpenoid. In testing with the mobile phase of chloroform: methanol: water (13:7:2) it produced two dark purple spots which indicated saponin compounds with an Rf value of 0.80 and the red color indicated triterpenoids with Rf 0.83 at a wavelength of 366 nm.

Meanwhile, using a phenolic eluent using toluene as mobile phase: ethyl acetate: formic acid (3:3:0,2) produces 4 stain spots, namely red (triterpenoid), red (triterpenoid), dark blue (flavonoid) and purple (saponin).) with Rf values of 0.51 each; 0.57; 0.90 and 0.96 at a wavelength of 366 nm. In the eluent chloroform: methanol (4:1) produced a spot of orange stain which is an alkaloid compound with an Rf value of 0.91 at a wavelength of 366 nm after being sprayed with Dragendroff's reagent.

The TLC profile obtained from Laor worms identified a flavonoid compound from Curcuma aeruginosa Roxb with an Rf of 0.8 which was close to that of the study, namely 0.9(Nugrahaningtyas et al., 2005). The saponin compound from Smilax rotundifolia leaf wrap has an Rf value of 0.79 which is close to the TLC profile Rf value of 0.8(Firawati & PRATAMA, 2018). In alkaloid compounds, an Rf value of 0.91 was obtained which was in accordance with Rahmawati's research (2015) who carried out the separation of alkaloid compounds from Alstonia scholaris L. with an Rf value of 0.9. Identification of steroid compounds from Eucheuma spinosum obtained an Rf value of 0.694 exactly with the above observation, namely 0.7 (Laili, 2016).

Analysis of Compounds with a UV-Vis Spectrophotometer

UV-Vis spectrophotometer is an analysis based on measuring the absorption of a solution through which monochromatic radiation passes. In a UV-Vis spectrophotometer the interaction between electromagnetic radiation and molecules causes electronic transitions. The spectrum can be seen in Figure 3.

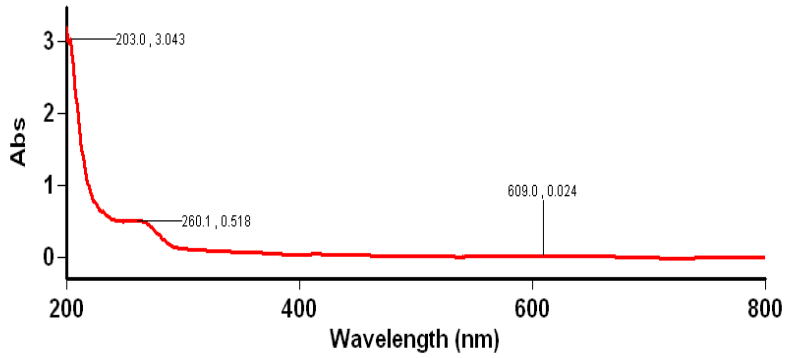

Figure 3.UV-Vis results of Laor worm ethyl acetate extract

Table 5. The results of qualitative analysis of the ethyl acetate extract of Laor worms

Wavelength (nm)	absorbance
609.0	0.024
260,1	0.518
203.0	3,043

Based on the results of UV-Vis spectrum measurements, there is a maximum absorption at a wavelength of 203 nm. This absorption peak is a typical absorption for steroid compounds indicating the presence of π π* electronic transitions caused by unconjugated double bonds (C=C) in the isolated compound. The resulting spectrum pattern is similar to Baderos's research(2017)who identified steroid compounds from the sponge Xestospongia sp de L. and the red algae Eucheuma cottonii which produced a UV-Vis spectrum at a wavelength of 203 nm indicating the presence of steroid compounds that have hydroxyl groups. At a wavelength of 260 nm. According to Maulidiyah(2011)absorption at a wavelength of 260 nm comes from the C=O chromophore group withtype of transition n π*. Meanwhile, at a wavelength of 609 nm, it shows flavonoid compounds that are in accordance with Suarsa's research (2011) who identified flavonoid compounds from Musa paradiasiaca L. Cv kapok and Musa paradiasiaca L. Cv Susu. The transition at a wavelength of 609 nm shows a bathochromic shift, namely a shift in absorption towards longer wavelengths, due to substituents or the influence of solventsSastrohamidjojo (1996).

Results of Identification of Compounds by FTIR Spectroscopy

Ethyl acetate marine worm extract containing alkaloids, flavonoids, saponins, steroids and triterpenoids in KLTA was then followed by identification with FTIR instruments. The results of the FTIR spectrum are shown in Figure 4.

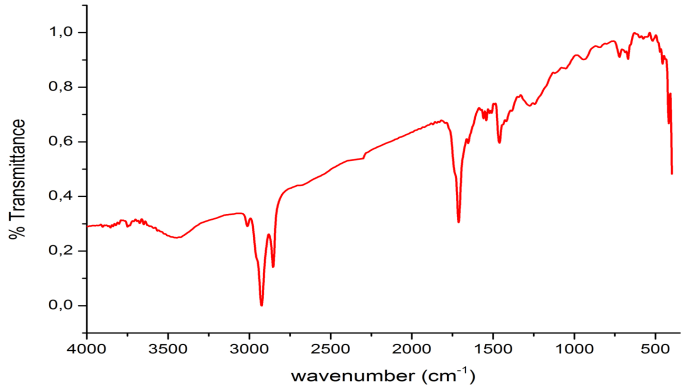

Figure 4. FTIR results of Laor worm ethyl acetate extract

Figure 6 shows the FTIR spectrum in the form of transmittance data from wave numbers 4000–400 cm-1. A relatively high transmittance value indicates that the signal from the sample is mostly transmitted, while a relatively low transmittance value indicates that the signal from the sample is mostly absorbed. The results of the spectrum functional group analysis can be seen in Table 5.

Table 5. The functional group of the FTIR spectrum of ethyl acetate extract of sea worms

No	Wavenumber (cm-1)	Range (cm-1)	Intensity	vibration
1	3445,377	4000-3200	Intermediate	-OH Stretch
2	3019,660	3100-3000	Intermediate	CH Aromatic Stretch
3	2924,766	3000-2800	Strong	-CH3 Stretch ash
4	2853,846	3000-2800	Strong	-CH2- Stretch sym
5	1711,306	1870-1550	Strong	C=O Stretch
6	1638.0	1680-1600	Intermediate	C=C Stretch
7	1541,025	1600-1450	Weak	C=C Stretch
8	1460,495	1467-1420	Intermediate	-CH2- bend (scissoring)
9	1273,214	1275-1225	Intermediate	CO Stretch
10	1243,214	1275-1225	Intermediate	CO Stretch
11	1048.0	1085-1030	Intermediate	Primary alcohol CO
12	942,773	995-675	Intermediate	CH Stretch

The results of the IR characterization of the crude extract of the Laor worm showed that there were specific absorptions for several functional groups, including in the wave number region of 3445.377 cm-1 indicating a weak absorption as vibrational alcohol (OH) which often appeared very weak and gave rise to a band and supported by the presence of primary alcohol (CO) Stretch vibrations at wave number 1048 cm-1. In addition, there is absorption at wave number 3019.66 cm-1 which indicates an aromatic Stretch CH vibration which is supported by the appearance of absorption at wave number 942.773 cm-1 with moderate intensity. The presence of aliphatic CH groups at wave numbers 2924.766 cm-1 and 2853.846 cm-1 indicates the asymmetric Stretch vibration of CH3 (methyl) and the Stretch symmetry vibration of CH2 (methylene).

CONCLUSION

Based on the IR spectroscopy data, it can be seen that the compounds contained in the crude ethyl acetate extract of Laor worms are thought to be steroid compounds. The FTIR spectra in this study are similar to Dewi's research (2008) who identified steroids in percolation extraction of sand sea cucumber (Holuthuria scabra) where at a wavelength of 3400.97 cm-1 1 showed absorption as vibrational alcohol (OH) which was supported by the presence of stretching vibrations of secondary alcohol (CO) at wave number 1097.03 cm -1. Then at wave numbers 1637.45 cm-1 and 1711.06 cm-1 there are C=C and C=O carbonyl functional groups.

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