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Antibacterial effects of *Pheretima javanica* extract and bioactive chemical analysis using Gas Chromatography Mass Spectrum

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Antibacterial effects of *Pheretima javanica* extract and bioactive chemical analysis using Gas Chromatography Mass Spectrum

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Abstract. *Pheretima* sp is an earthworm from the Oligochaeta group found mostly in Java. The characteristics has segments reaching 95-150 segments. Clitellum is located in segment 14-16. The body fluids contain protein, amino acids and various enzymes. The purpose of this study was to determine the composition of bioactive compounds and evaluate antibacterial activity. The method used was maceration, antibacterial test against *Salmonella typhi* and GCMS analysis to identify bioactive compounds. Antibacterial test showed the inhibition zone diameter ranged from 15 to 20 mm. The identification of bioactive compounds is based on the percentage area, percentage peak height, retention time, molecular weight and pharmacological action. GC-MS analysis showed the presence of 50 peaks of compounds. Bioactive compounds which are antibacterial are 1) Nitrogen oxide (N2O) (CAS) Nitrous oxide with an area 2.03%, height 7.36%, retention time 1.361, molecular weight 44.013 g/mol; 2) Acetic acid (CAS) Ethylic acid with an area 17.02%, height 29.03%, retention time 1.789, and molecular weight 60.05 g/mol; 3) Butanoic acid, 3-methyl- (CAS) Isovaleric acid with an area of 3.27%, height 2.04%, 3.456, molecular weight 102.13 g/mol; 4) 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) with an area 0.95%, height 1.32%, retention time 36.306 and molecular weight 222.24 g/mol.

Keyword: antibacterial, GC-MS, *Pheretima sp*, *Salmonella typhi*, bioactive compound.

1. Introduction

Typhoid fever cases are still a public health problem with as many as 22 million cases per year in the world and causing 216,000–600,000 deaths[1]. In 2008, the number of typhoid fever sufferers in Indonesia was reported at 81.7 per 100,000 population, with the distribution according to the age group of 0.0 / 100,000 population (0–1 years), 148.7 / 100,000 population (2–4 years), 180.3 / 100,000 (5-15 years), and 51.2 / 100,000 (≥16 years). This data shows that most sufferers are in the 2-15 years age group. The results of case studies in major hospitals in Indonesia show a tendency to increase the number of typhoid cases from year to year with an average morbidity of 500 / 100,000 population and mortality estimated at around 0.6–5% [2]. Efforts to control transmission have been carried out by the

government with prevention and treatment. Prevention in the form of vaccination is less efficient and there are contradictions. Treatment with antibiotics still causes relapse and resistance [3].

Microbial resistance to drugs occurs due to genetic changes and is followed by a series of selection processes by antimicrobial drugs[4]. The mechanism of inhibiting pathogenic bacteria by producing cytotoxic and antibacterial compounds from extracellular product. The antibacterial compound will damage bacterial cell wall and causes bacteria dead [5]. So that the use of *Pheretima javanica* earthworm extract can be used as an alternative treatment in the prevention and treatment of *Salmonella typhi* infection.

Pheretima javanica is an earthworm from the Oligochaeta group that is commonly found in Java. The characteristics of the Pheretima javanica earthworm have a mouth on the anterior part of the first segment and anus on the posterior segment reaching 95-150 segments. The annular clit is located in segment 14-16[6]. Earthworms respire through their skin and their digestive system occurs throughout their body. Its transport system consists of coelomic fluid which moves in the coelom with a simple closed circulatory system[7]. Several studies have also proven the antibacterial power of the protein extract of the earthworm *Pheretima* sp.

The fluid from the coelom in earthworms has an antimicrobial activity. Coelom fluid in earthworms contains active compounds that have biological activity in the form of antibacterials. the contents of the coelom fluid in the form of enzymes and proteins. the liquid is able to inhibit the growth of several pathogenic bacteria. therefore earthworm extract can be used to kill certain pathogenic bacteria. Bioactive compounds are used to control bacterial growth in order to prevent the spread of disease and infection. Antibacterial protein mechanism by creating pores and inhibiting cell wall synthesis inhibits the integrity of bacterial cell wall permeability, inhibits enzyme action, and inhibits the synthesis of nucleic acids and proteins, so that the bacterial cytoplasm is exposed to the external environment and disrupts the activity inside bacterial cells and causes death [8].

Bioactive compounds are compounds that have various benefits for human life. This compound is found in both animal and plant bodies. Some of the benefits include being antibacterial, antioxidant, anti-inflammatory and anti-cancer. Antibacterial is a drug or chemical compound that is used to kill bacteria, especially bacteria that are harmful to humans or pathogens [9].

2. Method

2.1. Preparation and extraction

The research method begins with the selection of *Pheretima javanica* material by identifying it at the Biology Laboratory of the Faculty of Teacher Training and Education, University of Jember. Identification by looking at the characteristics of organs such as the number of segments, the location of the clitelium on the segment, body color, number and location of the seta, mouth shape and body shape. Identification using reference to Gates' identification book (1947).

Earthworm extracts are made by selecting healthy and mature earthworms *Pheretima javanica*. After cleaning with distilled water, it is weighed and then extracted with 70% ethanol as solvent. Before extraction, the earthworms are dried in the sun to dry. Extraction was carried out by means of worms in an oven until they reached constant dryness, a mixture of earthworms with 1: 3 solvent in a blender, then macerated by soaking in solvent for 24 hours in a shaker in a place that is protected by light. After that it is filtered and the filtration results are evaporated using a rotary evaporator to evaporate the remaining solvent, so that a thick extract is obtained[10].

After the extraction process, the next step is an antibacterial test to determine the activity of bioactive compounds that can inhibit the growth of *Salmonella typhi* bacteria. The steps of the bacterial activity test were carried out aseptically by providing three test tubes each containing 20 mL of liquid media. In each tube, 100 µl of *Salmonella typhi* was added then vortexed and then poured into a sterile petri dish and then allowed to solidify[11].

2.2. Antibacterial activity test

Antibacterial test on earthworm extracts was carried out after the agar medium solidified then three wells were made using a pipe molding. Then each well was filled with earthworm extract, positive

control solution with chloramphenicol and a standard solution of distilled water each of 1000 ppm. Petri dish is stored in an incubator for 24 hours at 37oC. Furthermore, observing and measuring the formed inhibition zone[12]. The earthworm extract was then analyzed for bioactive compounds that act as antibacterial by using Gas Chromatography Mass Spectrum.

2.3. Gas Chromatography Mass Spectrum (GCMS) Analysis

This study used GC-MS chromatography. Gas Chromathography-Mass Spectrometer is a combination of analytical methods between GC and MS to identify different compounds in sample analysis. There are two main blocks in the GCMS instrument, namely GC and MS. GC uses a capillary column which depends on the column dimensions (length, diameter, film thickness) as well as the nature of the phase. The different chemical properties between the different molecules in a solution can be separated by passing the sample along the column. The 70% ethanol earthworm extract was injected into the injector so that it turned into steam and scanning was carried out for 1 hour[13]. The gaseous sample is carried gas by the carrier gas with a constant flow rate towards the separation column. The sample components will separate as they pass through the column due to differences in the absorption of the stationary phase in the cell components[14]. When the instrument is running, the computer generates a graph of the signal called a chromatogram. Each peak in the chromatogram represents the signal generated when a compound is eluted from the gas chromatography column into the detector. Before analyzing the extracts using gas chromatography and mass spectroscopy, oven temperature, gas flow rate were used and the electron gun was programmed initially.

3. Result and Discussion

3.1 Extraction

Earthworm identification is done by observing the morphological characteristics of worms. 200 grams of earthworms after oven at 50^{o}C for 90 minutes obtained a dry weight of 35.841 grams. The dried earthworm was then crushed and obtained 34 grams of implisia. The decrease in weight of the earthworm powder produced can be caused by it being scattered and still stuck in the blender. The earthworm powder was then macerated with 70% ethanol (1: 3) solvent for 24 hours using a shaker with a speed of 100 rpm. The ethanol 70% solvent is a polar solvent used in this study because it has the ability to radiate with a wide polarity ranging from nonpolar to polar compounds. Extraction by maceration was chosen because it does not need heating so that the active compounds in the sample are not damaged. The result of maceration is filtered and the filtrate is concentrated using a rotary vacuum evaporator with a water bath temperature of 50^{o}C, a vacuum of 25 rpm and a speed of tube 3, until a thick extract is obtained. The results of the concentration in an oven at a temperature of 50^{o}C were obtained by extracting a weight of 0.536 grams.

3.2 Antibacterial activity test

Antibacterial activity test was carried out on *Salmonella typhi* bacteria, the results of the bacterial activity test showed an inhibition zone as shown in figure 1.

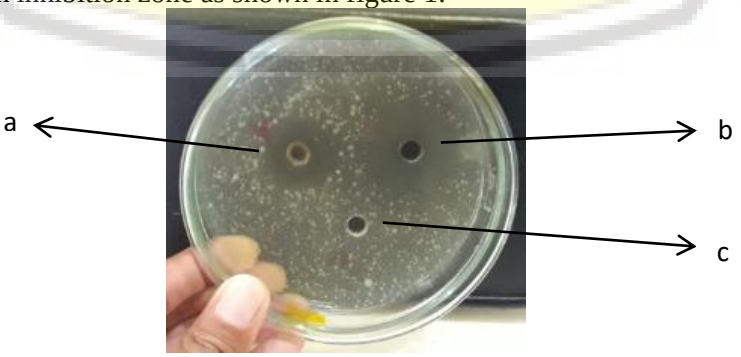


Figure 1. Inhibition zone results of *Salmonella typhi* antibacterial activity test, (a) Test solution: *Pheretima javanica* extract; (b) positive control: chloramphenicol; (c) negative control: distilled water

The bacterial activity test was carried out with three repetitions to obtain valid results. Activity test data can be seen in Table 1.

Table 1. The diameter of inhibition zone of *Pheretima javanica* extract against *Salmonella typhi*

Repetition	inhibition zone diameter (mm)		
	Pheretima javanica extract	Chloramphenicol	distilled water
1	15	35	-
2	20	25	-
3	20	25	-

Observation data from three repetitions of the antibacterial activity test against the growth of *Salmonella typhi* bacteria obtained an average inhibition zone for Pheretima javanica extract of 18,3 mm, chloramphenicol of 28,3 mm, while for distilled water there was no inhibition zone. The zone of inhibition in chloramphenicol is bigger because chloramphenicol is a positive control which is an antibiotic used in the treatment of infections caused by bacteria. So it can be concluded that *Pheretima javanica* extract has an inhibitory zone against the growth of *Salmonella typhoid* antibacterial which is the cause of typhoid fever. Previous research has been carried out by Waluyo regarding antibacterial activity and the resulting inhibition zone of *Pheretima javanica* against *Salmonella sp*. bacteria by using different solvents namely MOPS, Phosphate and NaCL with the inhibition zone in the solvent respectively 10 mm, 7 mm, and 8 mm [15]. Mathur et al also conducted a study on the antibacterial activity test using ethanol extract 95% *Eudrilus eugeniae* against *Streptococcus pyogens* with an inhibition zone of 19 mm [16].

3.3 Analysis of Bioactive Compounds using Gas Chromatography Mass Spectrum

The results of the GC-MS chromatogram consisting of 50 detected compound peaks are shown in Figure 2. The GC-MS chromatogram analysis of the *Pheretima javanica* extract showed that there were fifty main peaks and the components corresponding to the peaks were shown in Figure 3. Analysis of the compounds in the *Pheretima javanica* extract, shown in Table 2. The analysis used is the website pubchem.ncbi.nlm.nih.gov[17].

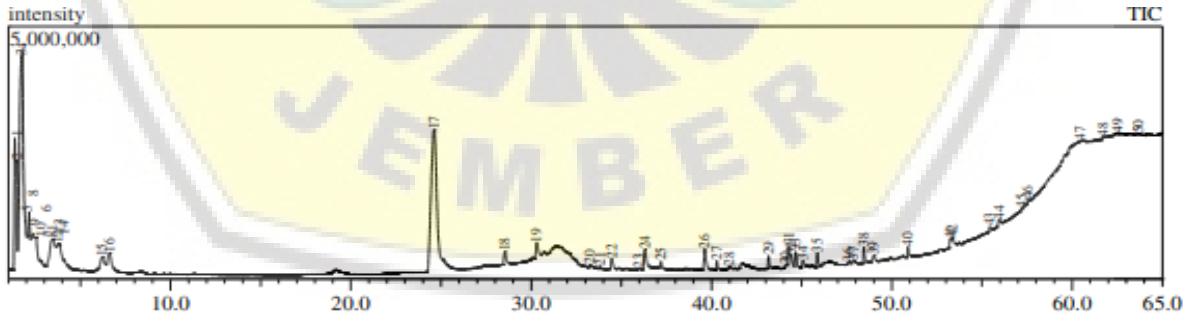
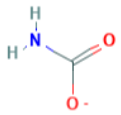
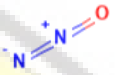
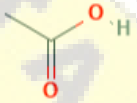
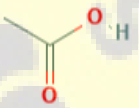
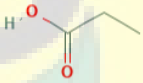
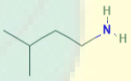
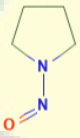
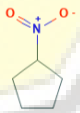
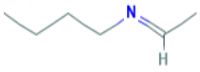
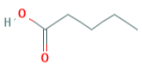
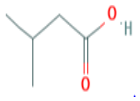
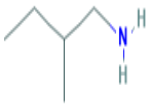
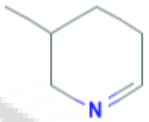
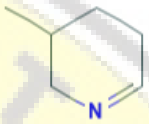
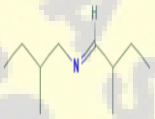
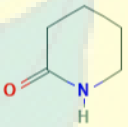


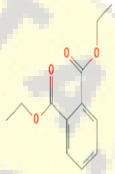
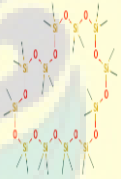
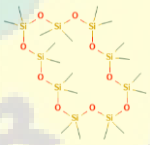
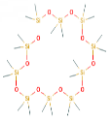
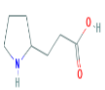
Figure 2. GC-MS Chromatogram of *Pheretima javanica* Earthworm Extract

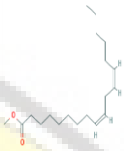
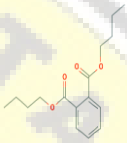
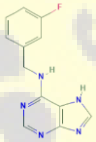
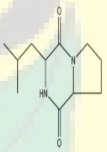
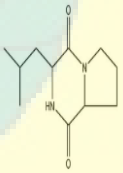
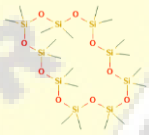
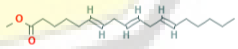
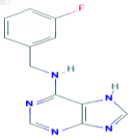
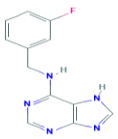
The electron flow causes the sample to split into fragments. The obtained fragments are actually charged with ions of a certain mass. The M / Z (mass / charge) ratio obtained is calibrated from the obtained graph, which is called a Mass spectrum graph which is the fingerprint of a molecule. Research on the analysis of bioactive compounds using GCMS has been carried out on the ethanol extract of *Zingiber officinale* to produce forty-eight bioactive phytochemical compounds. Identification of phytochemical compounds is based on peak area, molecular time, retention time, molecular weight, MS fragment-ion and pharmacological action[18].

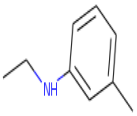
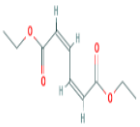
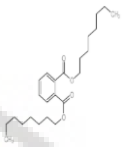
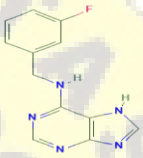
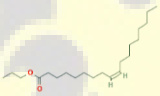
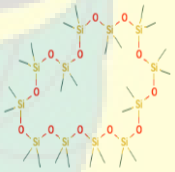
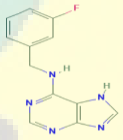
Table 2. Analysis of the compounds in the *Pheretima javanica* extract

No	Bioactive compound	Chemical formula	molecular weight	Structure	Function
1	Carbamic acid, monoammonium salt (CAS) A	$NH_4CO_2NH_2$ or $CH_6N_2O_2$	78.071 g/mol		soil fertilizer
2	Nitrogen oxide (N2O) (CAS) Nitrous oxide	N_2O	44.013 g/mol		Therapeutic antibacteria
3	Acetic acid (CAS) Ethylic acid	$C_2H_4O_2$ or CH_3COOH	60.05 g/mol		Antifungal Antibacteria
4	Acetic acid (CAS) Ethylic acid	$C_2H_4O_2$ or CH_3COOH	60.05 g/mol		Antifungal Antibacteria
5	Propanoic acid (CAS) Propionic acid	$C_3H_6O_2$ or CH_3CH_2COOH	74.08 g/mol		Anti cancer
6	1-Butanamine, 3-methyl- (CAS) Isoamylamino	$C_5H_{13}N$	87.16		Flavoring agents
7	Pyrrolidine, 1-nitroso- (CAS) N-nitrosopyrrol	$C_4H_8N_2O$	100.12		to induce tumors
8	Cyclopentane, nitro- (CAS) Nitrocyclopentane	$C_5H_9NO_2$	115.13		
9	1-Butanamine, N-ethylidene- (CAS) N-Ethyli	$C_6H_{13}N$	99.17		Flavoring agents
10	Pentanoic acid (CAS) Valeric acid	$C_5H_{10}O_2$ atau $CH_3(CH_2)_3COOH$	102.13		Food additives : Flavoring agents

11	Butanoic acid, 3-methyl- (CAS) Isovaleric acid	$C_5H_{10}O_2$	102.13 g/mol		Antibacteria
12	2-butyl-(2-methylbutylidene)-amine	$C_5H_{13}N$	87.16 g/mol		Flavouring Agents
13	1-Butanol, 2-ethyl- (CAS) 2-Ethyl-1-butanol	$C_6H_{11}N$	97.16 g/mol		Commercial Activity Status
14	Pyridine, 2,3,4,5-tetrahydro- (CAS) Tetrahydro	$C_6H_{11}N$	97.16 g/mol		Commercial Activity Status
15	1-Butanamine, 2-methyl-N-(2-methylbutylide	$C_{10}H_{21}N$	155.28 g/mol		Flavoring agents
16	3-methylbutyl-(3-methylbutylidene)amine	$C_{10}H_{21}N$	155.28 g/mol		Flavoring Agents
17	2-Piperidinone (CAS) 2-Piperidone	C_5H_9NO	99.13 g/mol		Anti cancer Anti inflammation
18	Dodecanoic acid, methyl ester (CAS) Methyl l	$C_{13}H_{26}O_2$	214.34 g/mol		Flavoring Agents
19	Tridecanoic acid, methyl ester (CAS) Methyl tr	$C_{15}H_{30}O_2$	242.4 g/mol		flavouring agent and a fragrance
20	Hexadecanoic acid, 15-methyl-, methyl ester (CAS)	$C_{17}H_{34}O_2$	270.5 g/mol		source of calories, lowers cholesterol

21	Eicosamethylcyclodecasiloxan	$C_{20}H_{60}O_{10}Si_{10}$	741.5 g/mol		Prevent degenerative diseases
22	Tetradecanoic acid, methyl ester (CAS) Methyl	$C_{15}H_{30}O_2$	242.4 g/mol		Flavoring Agents
23	Hexadecanoic acid, methyl ester (CAS) Methyl	$C_{17}H_{34}O_2$	270.5 g/mol		Flavoring Agents Therapeutic nerve development
24	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	$C_6H_4(COOC_2H_5)_2$ or $C_{12}H_{14}O_4$	222.24 g/mol		antibacterial
25	Tetracosamethylcyclododecasil	$C_{24}H_{72}O_{12}Si_{12}$	889.8 g/mol		Antifungal
26	Hexadecanoic acid, methyl ester (CAS) Methyl	$C_{17}H_{34}O_2$	270.5 g/mol		Therapeutic nerve protection
27	Octadecamethylcyclononasilox	$C_{18}H_{54}O_9Si_9$	667.4 g/mol		Anti cancer
28	Cyclopentanetridecanoic acid, methyl ester (CAS)	$C_{19}H_{36}O_2$	296.5 g/mol		Prevent infertility in men
29	Eicosamethylcyclodecasiloxan	$C_{20}H_{60}O_{10}Si_{10}$	741.5 g/mol		Prevent degenerative diseases
30	3-PYRROLIDIN-2-YL-PROPIONIC ACID	$C_7H_{13}NO_2$	143.18 g/mol		Antrasiklin antimicroba

31	Cyclopentanetridecanoic acid, methyl ester (CAS)	C19H36O2	296.5 g/mol		Prevent infertility in men
32	OCTADEC-9-ENOIC ACID	$C_{18}H_{34}O_2$	282.5 g/mol		Anti Hama
33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.5 g/mol		Food additives
34	1,2-Benzenedicarboxylic acid, dibutyl ester (CAS)	$C_{16}H_{22}O_4$ or $C_6H_4(COOC_4H_9)_2$	278.34 g/mol		Indirect Additives
35	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	$C_{12}H_{10}FN_5$	243.24 g/mol		Anti oxidant
36	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]none	C11H18N2 O2	210		Protein pengawet makanan
37	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]none	C11H18N2 O2	210		Protein, food preservative
38	Octadecamethylcyclononasilox	$C_{18}H_{54}O_9Si_9$	667.4 g/mol		Anti cancer
39	6,9,12-Octadecatrienoic acid, methyl ester (CAS	$C_{19}H_{32}O_2$	292.5 g/mol		
40	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	$C_{12}H_{10}FN_5$	243.24 g/mol		Anti oxidant
41	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	$C_{12}H_{10}FN_5$	243.24 g/mol		Anti oxidant

42	BENZENAMINE, N-METHYL-N-OCTYL-	C9H13N	135.21 g/mol		Flavoring agent
43	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarbonic acid, diethyl est	$C_{10}H_{14}O_4$	198.22 g/mol		
44	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate	C24H38O4	390.55 g/mol		
45	Pentadecanoic acid, 14-bromo- (CAS)	$C_{15}H_{29}BrO_2$	321.29 g/mol		
46	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	$C_{12}H_{10}FN_5$	243.24 g/mol		Antioxidant
47	OLEIC ACID, PROPYL ESTER	$C_{21}H_{40}O_2$	324.5 g/mol		Indirect Additives
48	Tetracosamethylcyclododecasil	$C_{24}H_{72}O_{12}Si_{12}$	889.8 g/mol		anti fungal
49	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	$C_{12}H_{10}FN_5$	243.24 g/mol		antioxidant
50	1'H-Androst-2-eno[3,2-b]indol-17-one, 1'-(phenylmethyl)-, (5.alpha.)- (CAS) 17-OXO	$C_{19}H_{30}O_2$	290.4 g/mol		therapeutic

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	1.361	17371818	4.30	2616727	8.85	Carbamic acid, monoammonium salt (CAS) A
2	1.459	8188347	2.03	2177607	7.36	Nitrogen oxide (N2O) (CAS) Nitrous oxide
3	1.725	34718272	8.59	4246243	14.36	Acetic acid (CAS) Ethylic acid
4	1.789	34060086	8.43	4338787	14.67	Acetic acid (CAS) Ethylic acid
5	2.067	2316335	0.57	668504	2.26	Propanoic acid (CAS) Propionic acid
6	2.158	7993913	1.98	1152435	3.90	1-Butanamine, 3-methyl- (CAS) Isoamylamin
7	2.292	2856048	0.71	660278	2.23	Pyrrolidine, 1-nitroso- (CAS) N-nitrosopyrroli
8	2.367	5393588	1.34	729555	2.47	Cyclopentane, nitro- (CAS) Nitrocyclopentane
9	2.486	7950580	1.97	641626	2.17	1-Butanamine, N-ethylidene- (CAS) N-Ethylic
10	2.800	1890971	0.47	169171	0.57	Pentanoic acid (CAS) Valeric acid
11	3.456	13219445	3.27	604628	2.04	Butanoic acid, 3-methyl- (CAS) Isovaleric aci
12	3.736	5697726	1.41	520338	1.76	2-butyl-(2-methylbutylidene)-amine
13	3.867	4315952	1.07	493681	1.67	1-Butanol, 2-ethyl- (CAS) 2-Ethyl-1-butanol
14	4.092	2807705	0.70	187631	0.63	Pyridine, 2,3,4,5-tetrahydro- (CAS) Tetrahydr
15	6.212	3314372	0.82	241192	0.82	1-Butanamine, 2-methyl-N-(2-methylbutylider
16	6.605	5789906	1.43	348908	1.18	3-methylbutyl-(3-methylbutylidene)amine
17	24.602	63848672	15.81	2792008	9.44	2-Piperidinone (CAS) 2-Piperidone
18	28.540	2123231	0.53	256413	0.87	Dodecanoic acid, methyl ester (CAS) Methyl l
19	30.310	2565665	0.64	301166	1.02	Tridecanoic acid, methyl ester (CAS) Methyl t
20	33.247	594330	0.15	62585	0.21	Hexadecanoic acid, 15-methyl-, methyl ester (
21	33.808	290745	0.07	67269	0.23	EICOSAMETHYLCYCLODECASILOXANI
22	34.468	1700539	0.42	221015	0.75	Tetradecanoic acid, methyl ester (CAS) Methy
23	35.961	270167	0.07	40626	0.14	Hexadecanoic acid, methyl ester (CAS) Methy
24	36.306	3833976	0.95	391690	1.32	1,2-Benzenedicarboxylic acid, diethyl ester (C
25	37.195	906664	0.22	139478	0.47	TETRACOSAMETHYLCYCLODODECASI
26	39.629	3421110	0.85	423527	1.43	Hexadecanoic acid, methyl ester (CAS) Methy
27	40.295	778535	0.19	177704	0.60	OCTADECAMETHYLCYCLONONASILO
28	40.983	387642	0.10	48968	0.17	Cyclopentanetridecanoic acid, methyl ester (C.
29	43.165	1259247	0.31	257118	0.87	EICOSAMETHYLCYCLODECASILOXANI
30	44.117	444428	0.11	61737	0.21	3-PYRROLIDIN-2-YL-PROPIONIC ACID
31	44.283	3272706	0.81	443587	1.50	Cyclopentanetridecanoic acid, methyl ester (C.
32	44.408	1526891	0.38	245543	0.83	OCTADEC-9-ENOIC ACID
33	44.666	2244418	0.56	314881	1.06	9,12-Octadecadienoic acid (Z,Z)-, methyl este
34	45.073	1000133	0.25	133369	0.45	1,2-Benzenedicarboxylic acid, dibutyl ester (C
35	45.874	1315494	0.33	285365	0.97	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
36	47.589	446922	0.11	52859	0.18	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]n
37	47.861	684912	0.17	72008	0.24	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]n
38	48.450	1663824	0.41	326669	1.10	OCTADECAMETHYLCYCLONONASILO
39	48.990	1124277	0.28	127479	0.43	6,9,12-Octadecatrienoic acid, methyl ester (C/
40	50.894	945750	0.23	197714	0.67	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
41	53.217	863008	0.21	154796	0.52	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
42	53.341	1551230	0.38	170053	0.58	BENZENAMINE, N-METHYL-N-OCTYL-
43	55.433	392113	0.10	87924	0.30	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarb
44	55.943	761302	0.19	104385	0.35	1,2-Benzenedicarboxylic acid, dioctyl ester (C
45	57.200	298701	0.07	43259	0.15	Pentadecanoic acid, 14-bromo- (CAS)
46	57.533	1918388	0.47	142865	0.48	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
47	60.467	97556562	24.15	661450	2.24	OLEIC ACID, PROPYL ESTER
48	61.717	18890440	4.68	480220	1.62	TETRACOSAMETHYLCYCLODODECASI
49	62.507	24193217	5.99	369128	1.25	1H-Purin-6-amine, [(2-fluorophenyl)methyl]-
50	63.661	2989019	0.74	116181	0.39	1'H-Androst-2-eno[3,2-b]indol-17-one, 1'-(phe
		403949322	100.00	29568350	100.00	

Figure 3. GC-MS Chromatogram analysis of *Pheretima javanica* extract

The results of GCMS analysis observations on 70% ethanol extract of *Pheretima javanica* detected 50 bioactive compound peaks which were shown in the chromatogram. The mechanism of GCMS is that the sample is injected into the injector so that it turns into steam or gas. The gaseous sample will be carried by the carrier gas to the separation column. The sample components that pass through the column will be separated because there are differences in the absorption power of the mobile phase of the sample components. Then the sample component will come out of the column along with the mobile phase and the concentration will be measured by the detector that produces the signal and sent to the recorder which produces the curves in the chromatogram. Analysis of the quality of the separation results measured based on the retention time.

In accordance with the research objectives to test the anti-bacterial activity, the earthworm extract has the potential to be anti-bacterial which is indicated by the presence of an inhibition zone against the growth of *Salmonella typhi* bacteria as shown in Figure 1.The results of GCMS analysis of the 70% ethanol extract of *Pheretima javanica* in table 2, there are bioactive compounds. as an anti-

bacterial, namely 1) Nitrogen oxide (N2O) (CAS) Nitrous oxide with an area of 2.03, height 7.36%, retention time 1.361, molecular weight 44.013 g / mol; 2) Acetic acid (CAS) Ethylic acid with an area of 17.02%, a height of 29.03%, a retention time of 1.789, and a molecular weight of 60.05 g / mol; 3) Butanoic acid, 3-methyl- (CAS) Isovaleric acid with an area of 3.27%, height 2.04%, 3.456, molecular weight 102.13 g / mol; 4) 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) with an area of 0.95%, a height of 1.32%, a retention time of 36.306 and a molecular weight of 222.24 g / mol.

Bioactive compounds Nitrogen oxide (N2O) (CAS) Nitrous oxide) and Acetic acid (CAS) Ethylic acid contained in *Pheretima javanica* extract acts as antibacterial. The content of Nitrogen oxide (N2O) (CAS) Nitrous oxide) and Acetic acid (CAS) Ethylic acid was also found in symbiont bacteria found in molluscs in previous studies[19]. The bioactive compounds Butanoic acid, 3-methyl- (CAS) Isovaleric acid and 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) have potential as antibacterial properties which were also found in bacterial isolates of gastropda symbionts in previous studies[20]. its pharmacological activity is clear and is an attractive candidate for a new drug especially in the field of antibacterial medicine[21].

4. Conclusion

Pheretima javanica extract has antibacterial activity against *Salmonella typhi* with an inhibition zone diameter ranging from 15 to 20 mm. GC-MS analysis showed the presence of 50 peaks of the compound contained. Bioactive compounds which are antibacterial are 1) Nitrogen oxide (N2O) (CAS) Nitrous oxide with an area 2.03%, height 7.36%, retention time 1.361, molecular weight 44.013 g/mol; 2) Acetic acid (CAS) Ethylic acid with an area 17.02%, height 29.03%, retention time 1.789, and molecular weight 60.05 g/mol; 3) Butanoic acid, 3-methyl- (CAS) Isovaleric acid with an area of 3.27%, height 2.04%, 3.456, molecular weight 102.13 g/mol; 4) 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) with an area 0.95%, height 1.32%, retention time 36.306 and molecular weight 222.24 g/mol. *Pheretima javanica* extract has the potential to be used as a natural remedy to cure typhoid fever. required a process to purify the active compound and make it a drug.

Reference

[1] WHO, 2011. Guidelines for the Management of Typhoid Fever. Permanent Secretary Ministry of Health and Child Welfare. Pp 10
[2] Purba I E, Wandra T, Nugrahini N, Nawawi S and Kandun N 2016 Typhoid fever control program in Indonesia: challenges and opportunities *Media Litbangkes* **26** 99-108
[3] KEPMENKES, 2006. Guidelines for Typoid Fever Control Jakarta.Depkes Pp 3
[4] Levinson W E, Chin-Hong P, Joyce E, Nussbaum J and Schwartz B 2018 *Review of Medical Microbiology and Immunology, Fifteenth Edition* (San Francisco: McGraw Hill Professional) p 103
[5] Gily P, Gulo Y, Lailani D, Soraya A, Wardhani F M and Nasution S W 2020 Analyze effectiveness extract of worm *Lumbricus rubellus* and *Pheretima* based on bacteria *Salmonella typhi* and *Staphylococcus Int. J. Sci. Eng. Sci.* **4** 1-5
[6] Gates, G E 1940 One Some Earthworms From The Buintenzorg Museum. Burma,Judson College. 5. Pp 413-4.
[7] Edwards CA 2004 *Earthworm Ecology Second Edition* (Boca Raton: CRC Press)
[8] Tutar U and Karaman İ 2017 Investigation of antibacterial properties of mucus and coelomic fluid obtained from E*isenia Fetida Cumhuriyet Sci. J.* **38** 427-434
[9] Guaadaoui A, Benaicha S, Elmajdoub N, Bellaoui M and Hamal A 2014 What is a bioactive compound? Acombined definition for a preliminary consensus *Int. J. Food. Sci. Nutr.* **3** 174-9
[10] Chatterjee S, Sugilal G and Prabhu S V 2019 Heat transfer in a partially filled rotary evaporator *Int. J. Threm Sci*. **142** 407-21
[11] Dharmawati I G A A, Mahadewa T G B and Widyadharma I P E 2019 Antibacterial activity of *Lumbricus rubellus* earthworm.extract against *Porphyromonas gingivalis* as the bacterial cause periodontitis *Maced. J. Med. Sci.* **7** 1032-6

[12] Fu Y T, Chen K Y, Chen Y S and Yao C H 2014 Earthworm (*Pheretima aspergillum*) extract stimulates osteoblast activity and inhibits osteoclast differentiation *BMC Complement. Altern. Med*. **14** 440
[13] Hübschmann H J 2015 *Handbook of GC-MS: Fundamentals and Applications* (India: Wiley & Sons) Pp 4-7.
[14] Ferrer I and Thurman E M 2013 *Handbook Advanced techniques in gas chromatography-mass spectrometry (GC-MS-MS and GC-TOF-MS) for environmental chemistry* (Colorado: Elsevier) p 57
[15] Waluyo J 2004 Purification and Characterization of Antibacterial Proteins from earthworms. Program Pasca Sarjana. Universitas Airlangga. Surabaya. Pp 56
[16] Mathur A, Verma S K, Bhat R, Singh S K, Prakash A, Prasad G B K S and Dua V K 2010 Antimicrobial activity of earthworm extracts *J. Chem. Pharm. Res*. **2** 364-70
[17] Pubchem.ncbi.nlm.nih.gov/compound
[18] Shareef H K, Haidar J M, Hussein H M and Hameed I H 2016 Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum *Orient. J. Chem*. **32** 817-37
[19] Pringgenies D 2010 Characteristics of the mollusc symbiont bacteria bioactive compound by GC-MS Jurnal Ilmu dan Teknologi Kelautan Tropis **2** 34-40
[20] Hasanah N F, Pringgenies D and Wulandari S R 2012 Characterization of the secondary metabolites of the Gastropod Conus miles symbiont bacteria using the GC-MS method as antibacterial MDR (Multi Drug Resistant) *J. Mar. Res.* **1** 197-202
[21] Nyinoh I W, Atu B O and Oluma H O A 2018 The use of medical plants as alternatives for typhoid fever and bacterial gastroenteritis therapy in Abwa-Mbagen, Nigeria *Eur. J. Med. Plants*. **24** 1–12