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The Bioactive Compound of Cassava (*Manihot utillisima*) Leather as A Natural Antimicrobial By GC-MS

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Abstract. On the other hand, cassava leather contain antimicrobial compounds that can be used to reduce microbial contamination. This research aimed to determine active compound of Manalagi cassava leather by analysis using GC-MS. Therefore in this research, we aimed to study whether antimicrobial activity of extracts of cassava leather and leaves by GCMS. The result research showed that the most dominant compounds by analysis using GC-MS CP-3800 are Propane,1,1,3-trietoxy- (3,17%); 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2,97%); 2- furancarboxaldehyde, 5-(hydroxymethyl)- (21,29%); alpha. -D-Glucopyranoside, .alpha. -D-glucopyranosyl (56,61%); Propane,1,1,3-trietoxy- (3,17%) and Cholestan-22(26)-isoepoxy-3,16-dione (1,83%). alpha-D-glucopyranosyl; Propane,1,1,3-trietoxy-; and 2-furancarboxaldehyde, 5- (hydroxymethyl). alpha. -D-Glucopyranoside, .alpha. -D-glucopyranosyl and 2- furancarboxaldehyde, 5-(hydroxymethyl)- Compounds of cassava leather are secondary metabolites that play a role as an antimicrobial.

INTRODUCTION

One of the efforts to inhibit microbial damage and contamination is to store in a sterile place using freezing temperatures and the use of preservatives that are antimicrobial [1]. The current news about the misuse of hazardous chemicals as preservatives that are not suitable for their intended use, such as formaldehyde and borax, has made public uneasy . Formaline and borax are not allowed to be used as food preservatives, because they can cause acute food poisoning and the impact of accumulated chemicals that are carcinogens that can trigger cancer.

The results of a survey conducted by the Indonesian Food and Drug Administration in 2010 showed the use of formaldehyde in fish and marine products was in the top rank, namely, 66% of the total 786 samples. Given the danger that is caused by formalin, it is necessary to use materials preservatives naturally that is not harmful to health. Natural preservatives can be obtained from several plants that contain antimicrobial compounds by extracting these antimicrobial compounds. Cassava leaves are one of the ingredients that can be used as an antimicrobial.

Currently cassava leaves (*Manihot utillisima*) are only used for consumption and animal feed. Cassava leaves are known to contain active compounds which can be a natural antimicrobial that flavonoids, triterpenoids, tannins

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and saponins. Research result by Hartari [2] showed that the ethanol extract rubber cassava leaves (*Manihot glaziovii*) can reduce bacterial contamination *of Salmonella sp.* amounting to 6.4 x 10^5 cfu/g and *Escherichia coli* at 3.3 x 10^5 cfu/g. Information concerning the use of parts of the leaves of cassava (*Manihot utillisima*) as antimicrobial still very rarely found . By because it is , necessary research to determine the utilization of parts of the leaves of cassava potency as an antimicrobial by GC-MS test.

MATERIAL AND METHODES

Material

The main ingredients used in the research were cassava leaves and white parts of Manalagi cassava leather, which were obtained from Batanghari Nuban District, East Lampung Regency. While the auxiliary materials used are 70% alcohol, 96% ethanol, *Mac Conkey* Agar (MCA), aquadest, aluminum foil, cotton, filter paper, disc paper, and as well as other materials GC-MS analysis. The tools used in this research are scales, trays, ovens, *vacuum rotary evaporators*, drop pipettes, micrometers, Erlenmeyer, Beaker glass, measuring cups, tweezers, and other analytical tools.

Method

The study was conducted to find the active compound of cassava leather extract as an antimicrobial against. Each experiment used 5 replications. The data were descriptive analyzed. As supporting data, a quantitative test of the levels of active compounds was carried out. Identification of the chemical components of herbal extracts was carried out using *Gas Chromatography-Mass Spectrometry* (GC-MS). A total of 5 mL of extract was added to 5 mL of ether then shaken in a separating funnel for 5 minutes and the upper fraction (ether) was separated from the lower fraction. The upper fraction is then collected and into the lower fraction and 5 mL ether is added again, shaken again in a separating funnel as before. The upper fraction produced in this second extraction is mixed with the fraction on the first separation, then concentrated by blowing nitrogen gas until the remaining volume is about 1 mL. These results were then detected using GC-MS. GC-MS operating conditions at the time of testing were using the ionizer type EI (Electron Impact) 70 eV, injector temperature 290°C, detector temperature 280°C, column type Rtx-5MS (95% dimethyl polysiloxane; 5% diphenyl) with long column 30 meter, column temperature 70 ° C to 230 ° C with a temperature increase of 5°C per minute, helium carrier gas, and flow rate of 60 mL/minute at a pressure of 13.7 kPa.

RESULTS

The results research showed that the ethanol extract of cassava leather had secondary metabolite compounds which functioned as antimicrobials. This is evidenced by quantitative tests on tannins, saponins, and HCN compounds. The levels of the active compound of the cassava leather ethanol extract can be seen in Fig. 1 and Table 1.

The results of the analysis using the GC-MS CP-3800 variant found 24 active compounds contained in the extract of cassava leather. Where the results show that in the ethanol extract of cassava leathers, the most dominant compounds are Propane,1,1,3-trietoxy- (3,17%); 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2,97%); 2-furancarboxaldehyde, 5-(hydroxymethyl)- (21,29%); alpha. -D-Glucopyranoside, .alpha. -D-glucopyranosyl (56,61%); Propane,1,1,3-trietoxy- (3,17%) and Cholestan-22(26)-isoepoxy-3,16-dione (1,83%). alpha-D-glucopyranosyl; Propane,1,1,3-trietoxy-; and 2-furancarboxaldehyde, 5- (hydroxymethyl) compounds are secondary metabolites that play a role as an antimicrobial [4,5,6]. Kusuma [7] stated that these compounds are present in the methanol extract of Jatropha, this water extract from Jatropha shows antimicrobial activity against gram-positive and gram-negative bacteria. The active compound was detected at 56.61% and 22%, while other compounds such as Propane, 1,1,3-trietoxy-, Cholestan-22 (26) -isoepoxy-3,16-dione, and Hexadecanoic acid are secondary metabolites that play a role as an antioxidant. The material content of cassava leather showed in Table 1.

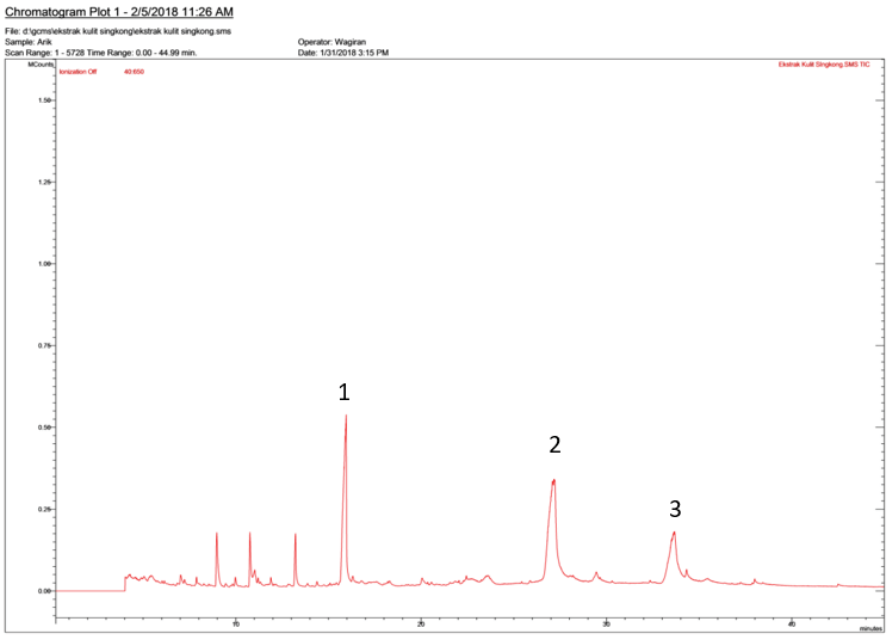

FIGURE 1. The GC-MS test results for the active compound content in the cassava leather: alpha. -D-Glucopyranoside, .alpha. -D-glucopyranosyl (1); 2- furancarboxaldehyde, 5-(hydroxymethyl)- (2); and Propane,1,1,3-trietoxy- (3).

TABLE 1. The GC-MS test results for the active compound content in the cassava leather

No	Rt	m/z	MW	Compound	R.Match	% Prob	Area	% Area
1.	5.443	42	90	N-((15)N-Nitro)-Dimethylamine	804	72.98	239.505	0,89
2.	6.154	98	169	2-Piperdinomethyl-Tetrahydrofuran	850	21.93	26.394	0,10
		98	98	2-Cyclopenten-1-One,2-Hydroxy-	888	14.60		
3.	7.234	110	110	2-Furancarboxcaldehyde, 5-Methyil-(Cas)	858	83.23	94.111	0,35
				5 Methyl Furfural	834	72,52		
4.	7.880	43	114	2,4-Dihydroxy-2,5-Dimethyl-3(2h)-Furan-3-One	790	55.10	107.082	0,40
		43		4h-Pyran-4-One,2,3-Dyhydro-3,5-Dihydroxy-6-Methyl-(Cas)	782	30.07		
		43		2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	749	10.07		
5.	9.456	68	136	Di-Limonene	899	37.34	61.438	0,23
		68		I-Limonene	884	27.11		
6.	9.730	43	114	1,3-Dioxol-2-One,4,5-Dimethyl-	732	20.83	22.449	0,08
		43	228	Ethyl2-(2-Acetoxy-3-Methylbuty)-2-Proponeate	862	19.22		
7.	9.838	43	114	4-Heptanone(Cas)	827	35.88	36.950	0,14
8.	10.767	59	176	Propane,1,1,3-Trietoxy-	888	95.73	855.138	3,17
9.	11.020	126	414	Cholestan-22(26)-Isoepoxy-3,16-Dione	722	14.89	494.581	1,83
		126	126	Maltol	717	25,36		
10.	11.203	95	126	Furyl Hydroxymethyl Ketone	920	55.04	120.773	0,45

No	Rt	m/z	MW	Compound	R.Match	% Prob	Area	% Area
11.	13.215	43	144	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	728	38.12	802.194	2,97
		43		4h-Pyran-4-One,-Dihydro-3,5-Dihydroxy-6-Methyl-	721	29.90		
12.	14.384	142	142	4h-Pyran-4-One,3,5-Dihydroxy-2-Methyl-(Cas)	739	63.58	75.723	0,28
13.	14.997	57	144	Propanoic Acid,Pentyl Ester(CAS)	780	32.79	10.961	0,04
		57		1-Butanol,3-Methyl-,Propanoate(Cas)	765	21.84		
		57		Amylpropionate	740	17.60		
14.	15.085	41	156	Decanal(CAS)	852	30.79	27.993	0,10
15.	15.939	97	126	2- Furancarboxaldehyde,5-(Hydroxymethyl)-	744	63.63	5.743.000	21,29
16.	16.001	97	126	2-Furancarboxaldehyde,5-(Hydroxymethyl)-	848	91.51	237.353	0,88
17.	17.992	126	126	4h-Pyran-4-One,3-Hydroxy-2-Methyl-(CAS)	894	36,6	17.699	0,07
18.	18.198	150	150	Phenol,4-Ethenyl-2-Methoxy-	886	39.08		0,30
19.	19.315	73	370	Cyclopentasiloxane,decamethyl-(CAS)	893	16.61	8.707	0,03
		73	428	3,4-Dihydroxymandelic acid,ethyl ester,tri-TMS	842	13.05		
		73	370	benzoic acid,2,5-bis(trimethylsiloxy)-,trimethyilsilyl ester	839	10.26		
20.	20.053	84	143	Conydhrin	915	34.40	224.392	0,83
21.	21.851	73	518	Cycloheptasiloxane,tetradecamethyl-	880	98.06	57.291	0,21
22.	22.040	73	518	Cycloheptasiloxane,tetradecamethyl-	884	93.68	33.749	0,13
23.	23.258	191	206	Phenol,2,4-bis(1,1dimethylethyl-(CAS)	848	55.90	8.644	0,03
24.	23.536	60	180	D-allose	832	37.38	111.665	0,41
		60	162	1,6-anhydro-.beta.-d-	830	34.48		
		60	162	3,4-altrosan	805	10.51		
25.	23.630	60	162	1,6-anhydro-.beta.-d-glucopyranose(levoglucosan)	852	41.76	204.510	0,76
		60	180	D-allose	845	31.98		
26.	25.885	355	458	Silane,[[4-[1,2-bis[(trimethylsilyl)oxy]-1,2-phenylene]bis(oxy)]bis[trimethyl-(CAS)	964	21.29	93.415	0,35
		355	370	Benzoic acid,2,4-bis[(trimethylsilyl)oxy]-,trimethylsilyl ester	925	18.81		
27.	27.092	73	342	alpha.-D-Glucopyranoside,.alpha.-D-glucopyranosyl	891	29.00	5.710.000	21,17

		73	342	Lactose	868	12.18		
		73	342	D-Glucose,4-O-.beta.-D-galactopyrasnosyl-	868	12.18		
28.	27.212	73	342	.alpha.-D-Glucopyranoside,.alpha.-D-glucopyranosyl	889	36.74	5.725.000	21,22
		73	342	Lactose	865	12.31		
		73	342	D-Glucose,4-O-.beta.-D-galactopyrasnosyl-	865	12.31		
29.	28.189	43	284	Octadeconic acid(CAS)	840	12.40	42.278	0,16
30.	29.453	73	342	.alpha.-D-Glucopyranoside,.alpha.-D-glucopyranosyl	884	26.25	599.552	2,22
31.	30.303	178	178	Phenanthrene	920	32.27	63.637	0,24
		178	178	Anthracene	899	13.37		
32.	30.592	178	290	9,10-Ethanoanthracene,9,10-dihydro-11,12-diacetyl-	728	32.59	34.576	0,13
33.	32.041	73	150	1,2,3,4,5-cyclopentanol(CAS)	784	25.15	15.194	0,06
34.	32.215	73	180	Allo Insitols	783	21.11	30.309	0,11
		73	180	Neo Insitols	775	16.17		
35.	32.351	73	188	2,2'-Trimethylenebis-1,3-dioxolane	896	17.70	95.238	0,35
		73	130	1,3-Dioxolane,2-butyl-(CAS)	891	13.56		
36.	33.672	73	342	.alpha.-D-Glucopyranoside,.alpha.-D-glucopyranosyl	702	11.03		12,00

Secondary metabolite compounds such as dimethyl amine, have same characteristic with amonia is able to damage microbial cells by preventing permeability of the cytoplasmic membrane causing leakage of intracellular materials, then denaturing protein proteins and activating enzymes. Glucopyranosyl compound (Table 1) has an anti imflammatory characteristic. In mechanism, antimicrobial compounds can also break cross-links of peptidoglycan by breaking through the cell walls, causing leakage of cell nutrients. It due to damage to hydrophobic bonds. These process will inhibit activity and biosynthesis of specific enzymes that was required in the process of cell metabolism microbe [8].

DISCUSSION

Ehsan et al. [9] in their research found the presence of gallic acid and pyrogallol compounds (phenolic group), routine and myrcetin (flavonoid group), and daidzein (isoflavonoid group) from the results of HPLC analysis of the methanol extract of jatropha. Other metabolites detected by GC-MS are 2-(hydroxymethyl) 2nitro-1,3-propanediol, β-sitosterol, 2 furancarboxaldehyde 5-(hydroxymethy) and acetic acid in methanol extract.

As for the water extract, 2-furancarboxaldehyde, 5 (hydroxymethy), acetic acid and furfural (2-furancarboxaldehyde) were found. The methanol extract and water extract of Jatropha showed antimicrobial activity against gram-positive and gram-negative bacteria. In addition, research conducted also shows that the methanol fraction of Jatropha leaf extract contains compounds in the form of 2,3-dihydro-3,5 -dihydroxy-6-methyl 4-Phyran-4-one which is a product of the Maillard reaction [10] which is responsible for the antioxidant activity of this fraction. In addition, there are also n-hexadecanoid acid compounds and 2 furancarboxaldehyde, 5-hydroxymethyl which are also responsible for antioxidant activity.

In the ethanol extract of cassava leathers, the most dominant compounds are alpha-D-Glucopyranoside, alpha-D-glucopyranosyl and 2-furancarboxaldehyde, 5- (hydroxymethyl) -, both compounds are secondary

metabolites that act as antimicrobials. Ehsan et al. [9] stated that these compounds are present in the methanol extract of Jatropha, this water extract from Jatropha shows antimicrobial activity against gram-positive and gram-negative bacteria.

The ethanol extract of cassava leathers has an HCN level of 10.60 mg / L. The results of Atman's [6] study showed that Manalagi cassava had HCN levels of 19.5 ppm. According to Lenny,[8] cyanide acid (HCN) is toxic so that cyanide enters the cell structure of *Staphylococcus aureus* and poisons it so that it interferes with metabolic processes in c ells and even kills cells. Levels of HCN (cyanide acid) in cassava leathers range <50-250 ppm. The safe limit for HCN levels for consumption according to the FAO is <50 ppm. Based on the results of the study, the HCN level of Manalagi cassava leather was 10.60 ppm, which means it is safe for consumption . The previous study showed that cassava leather (*Manihot utillisima*), have a anti microbial characteristic [11]. The cassava leather have a secondary metabolite compounds such as tannins, saponins and HCN are able to damage microbial cells by preventing permeability of the cytoplasmic membrane causing leakage of intracellular materials, then denaturing protein proteins and activating enzymes [11]. The ethanol extract of cassava leather has antimicrobial activity against *Salmonella sp.* and *Eschericia coli* [11].

CONCLUSION

The most dominant compounds by analysis using GC-MS CP-3800 are Propane,1,1,3-trietoxy- (3,17%); 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2,97%); 2- furancarboxaldehyde, 5-(hydroxymethyl)- (21,29%); alpha. -D-Glucopyranoside, .alpha. -D-glucopyranosyl (56,61%); Propane,1,1,3-trietoxy- (3,17%) and Cholestan-22(26)-isoepoxy-3,16-dione (1,83%). alpha-D-glucopyranosyl; Propane,1,1,3-trietoxy-; and 2-furancarboxaldehyde, 5- (hydroxymethyl). The most dominant compounds of cassava leather are secondary metabolites that play a role as an antimicrobial.

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