Antibacterial Compounds Characterization in Chloroform Extract Leaves of Tahiayam Plant (*Lantana Camara* Liin.)

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Abstract. The research has been conducted to determine the compound has antibacterial activity in chloroform extract of leaves of *Lantana camara* Linn.. Using the screening, isolation by column chromatography fractionation method and the structure was determined by IR and NMR. The results showed that the chloroform extract has bacterial activity and isolated two compounds where are 22-β-dimethylakriloiloxy-3-oxoolean-12-ene-28-oic and pektolinarigenine flavonoid compounds. Both have the activity against E. coli and S. aureus.

Keywords: antibacterial, *L. camara*. Linn., pektolinarigenin, Lantaden B

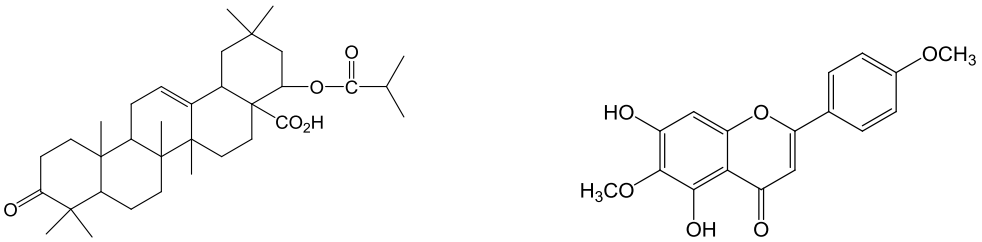

Fig 1. [1] 22-β-dimethylakriloiloxy-3-oxoolean-12-ene-28-oic/lantadene B

and [2] pektolinarigenine

INTRODUCTION

A lot of plants species in Indonesia be able to synthesize various of chemical compounds that have an interesting bioactivity but have not studied in intensively. Natural products was proven in scientifically have pharmacological effects of various plants. Millions of organic compounds of natural products that was isolated from plants have commercialized as medicines and various extracts concoction of plants and have been believed as a medicine. The plant has the prospect to be used in the medical field.

Health problems such as drug resistance in certain diseases, the search for new medicines, food problems, and energy sources alternative are as a challenge as well as opportunities for researchers of natural products. The high of evolution of the plant will be able to produce of new compounds in vary (Achmad SA, 2006). Therefore, it is need efforts in continuously to answer these challenges, through research, search and discovery of new chemical compounds in Indonesia plants.

L. camara Linn. is a wild plant that resistant to climate change (Figure 2) and is wide known in many areas in South Sulawesi with name “tahiayam” and is one of the medicinal plants in Indonesia. This plant, in Indonesia generally known as tembelekang. This plant can be used as an object of research as a secondary metabolites resources that potential as an antibacterial drug for traditionally prevention in particular one of the tropical diseases, namely wound infections.

Fig. 2. Lantana Camara Linn.

This potential is seen by ethnobotany approach that based on the knowledge and practices of traditional communities in utilizing plants for the treatment of certain diseases. In the community, especially in South Sulawesi, this plant is used as an anti wounds and is believed to cure various types of wounds on the skin very quickly. The second potential of this plant species is easy to grow, can be easily found in all area, especially in South Sulawesi, a wild plant, growing very rapidly, and able to cultivate.

This plant is the most potential as an anti-bacterial. This potential is seen from the use of traditional medicinal plants as beneficial that can improve the public health, especially diseases caused by bacteria. Search of bioactive compounds in plants and use as phitofarmaca to control the certain diseases are considered more advantageous because of the absence of the negative impacts of the application. Essential oils from the leaves of L. camara Linn is used as anti-bacterial that large enough to growth of bakleri S. pygenes, (Tedjo Narko, 1996). The potential as a medicine according to surveys ethnomedikal, L. camara Linn. is able to develop as medicine plants (Kalita, S., et al., 2012). Methanol extract of the leaves of L. camara Linn. have IC50 <20 µg / mL so that it can be said, have a relatively high toxicity potential as antitumor (R. Bulan, 2003).

Antibacterial test and search of active compounds of L. camara Linn. based inspection and test phytochemical antibacterial activity to obtain the extract has potential as an anti-bacterial (Pian Sopyan Nurochman 1996). Chloroform extract of the leaves of L. camara is dominant compared to other tissue extracts in obstruction of bacterial growth of S. aureus and E. coli as gram-positive and gram-negative with area of inhibition zone of 17.2 mm and 31.7 mm respectively. Formulations in the form of a cream crude extract of chloroform and in vitro test on rabbit back skin showing the ability to heal and prevent wound infection (Muharram, et al., 2010). The studies revealed through the literature provide evidence of it. Furthermore, it has provided contribution evidence bioactive chemical compounds that are metabolized by these plants.

At this time, the plants of L. camara Linn. is interesting to investigated the potential use of this plant in particular for potential cure and prevention of skin wound infections in related to utilization in Sulawesi society as a cure skin wounds and prevent a strong infection. Our preliminary results of secondary metabolites we found triterpenoids and flavonoids that have potent anti bacteria in the plant leaf chloroform extract of L. camara Linn.

MATERIALS AND METHODS

Tools and materials

Elektrotermal stuart®SMP11, FTIR shimadzu® prestige-21. Agilent 500 MHz NMR with DD2 console system, 500 MHz (^{1}H) and 125 MHz (^{13}C) for characterization. Laminar Air Flow, culture freezer, freezer medium, analytical balance, incubator, autoclave, oven, hot plate, Erlenmeyer flask, beakers, measuring cups, calipers, rod, wire loop, tweezers, cup petridish, test tubes, racks, and spoit To test the antibacterial. Rotary evaporator, chromatography column, ovens, UV lamp 254-365 nm, and analytical balance for isolation and fractionation.

Extraction and fractionation

Extraction was done by maceration of the plant leaf sample of L. camara Linn. that was obtained from Sunggumanai, District Parangloe, Gowa, South Sulawesi Indonesia. Fractionation by vacuum and flash column chromatography techniques.

Characterization and Anti Bacterial test

Characterization using Elektrotermal stuart®SMP11, FTIR shimadzu® prestige-21. Agilent 500 MHz NMR with DD2 console system, 500 MHz (1H) and 125 MHz (13C). Anti-bacterial test using agar diffusion method and inhibitory metabolites based Iguchi 1982 procedures.

RESULT AND DISCUSSION

Extraction and Fractionation

4.7 kg of finely powdered leaves of L. camara Linn. was macerated with methanol and evaporated by the rotary evaporator (at 40 °C) and we obtain 493 mL of methanol extract thick solid green. Then the extract was partitioned with chloroform 1: 1, volume 100 mL. Chloroform extract (9.00 grams) was fractionated by vacuum liquid chromatography column with silica gel GF254 7730 as the stationary phase and as mobile phase is some kind of solvent of n-hexane, ethyl acetate, and chloroform with a certain ratio with a gradient properties. The combined fractions of F and G was further fractionated by flash column chromatography with silikagel 7734 G 60 as stationary phase and eluent n-hexane are combined ethyl acetate, chloroform and acetone as the mobile phase and was obtained two isolates. The first isolate was obtained from fraction F and we call as the compound **3** (12.2 mg) and the second isolate was obtained from fraction G as compound **4** (11.8 mg).

Characterization of compounds

Compound **3**; a solid white powder with a melting point of 236-238 ° C. IR spectra (KBr): υ 3442.94 and 3317.56 cm^{-1} wide peak and moderate intensity were identified as OH stretching vibration. υ 2926.01 and 2858.51 cm^{-1} with a strong intensity identified as stretching vibration of C-H in -CH2 -CH3 and is supported by stretching vibration at 3000-2700 cm^{-1}, identified as methyl and methylene aliphatic , the absorption peak of υ 1695.43 cm^{-1} is the stretching vibration of the carbonyl group C = O ester (lit. 1820-1600 cm-1) and are supported by a C-O absorption at υ 1230.58 cm^{-1} region of moderate intensity, whereas in υ 1737.86 cm^{-1} is the stretching vibration of C = O ketones. Absorption at υ 1004.91 cm^{-1} with a weak intensity as bending group = C-H and moderate intensity at υ 1647.21 cm^{-1} is the bending vibration of C = C non-conjugation. The IR data show the presence of functional groups on which the isolates as in Table 1.

IR spectra in area υ 2926.01 and 2858.51 cm^{-1} with a strong intensity identified as stretching vibration of C-H as -CH2 -CH3 and is supported by stretching vibration at 3000-2700 cm^{-}1, as methyl and methylene aliphatic. The existence of methyl and methylene reinforced by the bending vibration on the area υ 1463.97 and 1362.96 cm-1 which is the characteristic absorption band for bending vibrations geminal dimethyl group -CH(CH3)2 from triterpenoids (Cresswell et al., 1982) , The analysis followed by Liberman-Burchard reagent test was supported the identification of compounds **3** as triterpenoid with the change in color to red-purple in this test.

Table 1. IR spectrum of compound 3

(ν, cm^{-1})	Peaks	Functional group	Intensity
3442,94;3317,56	Shape	−OH	Moderate
2926,01	Widen	−CH pada $-CH_3$	Strong
2858,51	Sharp	−CH pada $-CH_2$	Moderate
1737,86;1695,43	Sharp	C=O	Strong
1647,21	Sharp	C=C	Moderate
1463,97;1362,96	Sharp	−CH pada $-CH_3$ dan $-CH_2$	Moderate
1230,58	Sharp	C−O ester	Moderate
1072,42	Weak	$-CH_2-$	weak

Source: Nurrahmania, 2014.

Tabel 2. ^{13}C-NMR spectra data of compounds 3

No. peak	δ (ppm)	No. peak	δ (ppm)
1	15,1	19	38,8
2	16,8	20	39,2
3	19,6	21	39,3
4	20,2	22	42,1

5	21,5	23	46,0
6	23,6	24	46,9
7	24,1	25	47,5
8	25,8	26	50,6
9	26,3	27	55,4
10	26,5	28	75,1
11	27,4	29	116,0
12	27,7	30	122,5
13	30,1	31	143,1
14	32,3	32	157,3
15	33,8	33	165,3
16	34,2	34	176,3
17	36,8	35	217,6
18	37,8	$CDCl_3$	77,0

^{13}C-NMR spectra of the compound **3** show in Table 2 that indicate the presence of 35 carbon absorption peak. This data give information about compound **3** which is composed of 35 carbon as the main characteristic for triterpenoid. In Table 2, the three chemical shifts at low field (downfield), indicate the presence of C = O ketones in δC 217.6 ppm, the signal for the C = O carboxylate group on δC 176.3 ppm, and the signal at 165.3 ppm is δC chemical shift for the group C = O ester this result suggests that the compound **3** is triterpenoid with three carbonyl groups, that are ketones, esters and acids carbonyl.

From the literature study, we found that there was the similarity of ^{1}H-NMR and ^{13}C-NMR compound **3** with a compound lantaden B, such as H multiplet at δ 2.3417 to 2.6039 ppm for proton methylene (-CH2-) in the atom C-2 , the compound **3** in δ 2.353 to 2.591 ppm; δH duplet peak at 3.0072 to 3.0488 ppm proton metin (-CH-) in atom C-18, the compound **3** peak duplet at δ 3.044 to 3.065 ppm. From the ^{1}H-NMR and ^{13}C-NMR compound **3** showed similarities with lantaden B.

Table 3. Comparison of the 1H-NMR of compound 3 with Lantaden B (Suthar., 2014)

No.	Compound 3 in $CDCl_3$	Lantaden B in $CDCl_3$	
	δ (ppm)	δ (ppm)	H Atom
1.	5,584	5,5577	H at C-32
2.	5,399 – 5,413	5,3785	H at C-12
3.	5,055 – 5,067	5,0404	H at C-22
4.	3,044 – 3,065	3,0072 – 3,0488	H at C-18
5.	2,353 – 2,591	2,3417 – 2,6039	H at C-2
6.	1,182	1,1754	H at C-30
7.	1,092	1,0906	H at C-29
8.	1,067	1,0656	H at C-24
9.	1,051	1,0486	H at C-23
10.	1,021	1,0027	H at C-27
11.	0,892	0,8845	H at C-26
12.	0,863	0,8388	H at C-25

Table 4. Comparison of the ^{13}C-NMR of compound 3 with Lantaden B (Suthar., 2014)

Peak	δC (ppm) compound 3	δC (ppm) Lantaden B	C	Peak	δC (ppm) compound 3	δC (ppm) Lantaden B	C
1	15,1	15,16	C-25	19	38,8	38,54	C-18
2	16,8	16,85	C-24	20	39,2	39,17	C-1
3	19,6	19,52	C-26	21	39,3	39,24	C-8
4	20,2	20,25	C-34	22	42,1	42,05	C-14
5	21,5	21,50	C-6	23	46,0	45,97	C-19
6	23,6	23,56	C-11	24	46,9	46,87	C-4
7	24,1	24,13	C-16	25	47,5	47,45	C-9
8	25,8	25,77	C-30	26	50,6	50,57	C-17
9	26,3	26,28	C-27	27	55,4	55,30	C-5
10	26,5	26,44	C-23	28	75,1	75,20	C-22
11	27,4	27,46	C-35	29	116,0	115,96	C-32
12	27,7	27,59	C-15	30	122,5	122,37	C-12
13	30,1	30,07	C-20	31	143,1	143,09	C-13
14	32,3	32,26	C-7	32	157,3	157,16	C-33
15	33,8	33,75	C-29	33	165,3	165,33	C-31
16	34,2	34,16	C-2	34	176,3	178,84	C-28
17	36,8	36,77	C-10	35	217,6	217,77	C-3
18	37,8	37,63	C-21				

More details can be seen in Table 3 and Table 4. Based on the characterization of test reagents, test melting point, IR and NMR spectroscopy, the compound **3** chloroform extract that obtained from the leaves of plants Lantana camara is lantaden compound B (Figure 1) as triterpenoid. This compound had previously been isolated from the same plant by (Sousa & Jose in 2012 and Husain, H., et al., 2011)

Compound **4**; is obtained as needle-shaped crystals yellowish white with a melting point of 212-213 ^{0}C, these compounds fluoresce under UV254 nm, with cerium sulfate (CeSO4) reagent shows stain first and then light blue, light brown and then faded. The result of identification with the FeCl3 reagent show that compound is flavonoid with the change in color of solution becomes yellowish red. IR spectra (KBr): υ 3300.20cm wide band of medium intensity as a free OH group, and 2978.09, 2949.16, and 2926.01 cm 1 respectively -CH stretching vibration of the -CH3 and -CH2.

IR spectrum of the compound **4** shows the methyl and methylene aliphatic reinforced bending vibration at 1645.90 and 1381.03 cm 1; at 1737.86, and 1708.93 cm 1 as stretching vibration (C = O), enhanced uptake of C-O at 1230.58 cm-1 and a medium intensity as the C-O bending phenol. Sharp absorption band of moderate intensity at 1649.14 cm-1 stretching vibration of C = C aromatic. Sharp absorption band at 1138.00 cm-1 as functional groups C-O, and 1072.42 cm-1 as bending aryl ether. IR spectral data of compounds (4) indicate the presence of aromatic C and some other functional group as in Table 5.

Table 5. IR spectra of compound 4

(cm^{-1})		peak	Fungsional groups
Compund 4	**literature**		
3300,20	~3300	Shape	O-H
2926,01; 2866,22	2960 - 2850$^{*)}$	Sharp	C-H at CH_3, and CH_2
1708,93; 1695,43	1715 - 1680$^{*)}$	Sharp	C=O ketone
1649,14	1680 - 1620$^{*)}$	Sharp	C=C aromatic
1465,90; 1433,11	1470 - 1430$^{*)}$	Sharp	C-H at CH_3 and CH_2
1230,58	~1230$^{**)}$	Sharp	C-O phenol
1138,00	1300 - 1000$^{*)}$	Sharp	C-O
1072,42	1020 - 1075$^{*)}$	Sharp	C-O aril eter
821,68; 725,23	690 - 840$^{*)}$	Sharp	CH aromatic

Sumber : $^{*)}$ Williams & Fleming, 1973

1H-NMR spectra of the compound **4** as in Table 6 compare with the literature shows similarities with pektolinarigenin compound. From data of the melting point of the compound **4** is 212-2130 C and the melting point of 210-211oC pektolinarigenin (Juang et al, 2005). From the characterization, the compound **4** was concluded as pektolinarigenin compounds (Figure 1) a flavonoid derivatives.

Table 6. ^{1}H-NMR specta compound 4 and Pektolinarigenin (Juang *et al*, 2005)

No	Compound 4 δH, (ppm)	Pektolinarigenin, δH, (ppm)	Multiplisitas (J)	H position
1	13,086	13,08	1H, singlet	H C- 5
2	7,851& 7,833	7,77	2H, duplet	H C-2'& C-6'
3	7,029& 7,012	6,94	2H, duplet	H C-3'& C-5'
4	6,591	6,48	IH, singlet	H C-8
5	6,577	6,47	1H, singlet	H C-3
6	4,042	3,86	3H, singlet	H C-6
7	3,894	3,81	3H, singlet	H C-4'

Antibacterial test

Antibacterial activity test results demonstrate the potential of these compounds as anti-bacterial, especially in bacteria S. aureus and E. coli. The test results in Table 7.

Table 7. Antibacterialal activity of compound 3 and 4 for *S.aureus* dan *E.coli*

No.	Test	Inhibition zone (mm)	
		S.aureus	*E. coli*
1	Kloramfenikol (+ controle)	14,75	24,60
2	Kloroform (- controle)	0,00	0,00
3	Compound 3	8,97	10,60
4	Compound 4	9,20	9,40

Previous phytochemical test results showed that the extract kloroforom L. camara Linn. is able to inhibit the growth of bacteria with broad zones of inhibition were in strong category (Muharram, et al., 2010). It is understood that an antibacterial baha can inhibit the growth of bacteria in several ways, namely by destroying the cell wall, changing the permeability of cells, changing the protein molecules and nucleic acids, inhibits the action of enzymes, and inhibit the synthesis of nucleic acids and proteins and conversely, if there is a foreign organic materials can also be decrease the effectiveness of antibacterial chemicals to inactivate the way these materials or protect the microorganisms from antimicrobial substances (Pelczar, MJ, 1988).

Two compounds found in the chloroform extract also has potential as an anti-bacterial, indicating that the chloroform extract of leaves of L. camara Linn. contains antibacterial compounds visible for several potential functional groups that can serve as an anti-bacterial, namely; 22-β-dimetilakriloiloksi-3-oksooleana-12-ene-28-oat / lantaden B and pektolinarigenin.

CONCLUSION

In studies of *L. camara* Linn. has discovered two pure isolates from the chloroform extracts that are bioactive against *S. Aureus* and *E. coli*. That compounds is; 22-β-dimetilakriloiloksi-3-oksooleana-12-ene-28-oat or lantaden B and pektolinarigenin a flavone derivative compounds. The test against *S. aureus* and *E. coli* shows inhibition zone respectively 8.97 mm and 10.60 mm and compound pektolinarigenin respectively 9.20 mm and 9.40 mm.

ACKNOWLEDGEMENTS

To the LP2M DIKTI Fund Research Grant Stranas, fiscal year 2015 and Head of the Laboratory of Chemical and Biology FMIPA UNM who have facilitated this research.

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