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Research Article

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Isolation and Elucidation Structure of Triterpenoids from *Hippobroma Longiflora* Leaf Extract and Tested of Antibacterial Activity

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ABSTRACT

Isolation and characterization of triterpenoid compounds from Hippobroma longiflora plants have been done. Hippobroma longiflora leaf powder was extracted by maceration method using hexane, ethyl acetate and methanol. Ethyl acetate fraction was columned by chromatography colomn using silica gel as a stationary phase and hexane, ethyl acetate, acetone and methanol as mobile phase. The isolation result is in the form of 22 mg white crystals and leave a single spot with some comparisons eluent on a thin layer chromatography. Isolated compound was characterized using UV spectroscopy, FTIR and NMR.

Keywords: *Hippobroma longiflora*; Antibacterials; Triterpenoids

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INTRODUCTION

Different types of plants contain several secondary metabolite compounds. Secondary metabolite compounds which contained in plants are bioactives substances related to chemical content in plants, so some plants can be used as a material of medicine. One of the thousands species of plants which attractive in terms of phytochemicals are family of *Campanulaceae.* According of Waliana, family of *Campanulacea* has 84 genuses, 2,000 species and has five subfamily [1]. This plant can be found at the tropics and sub-tropics [2]. Mabberley stated that the family of *Campanulacea*e had a chemical content of alkaloids, flavonoids, steroids, terpenoids, glycosides, tannins and saponins [3]. Literature study to one of the *Campanulacea* families, *Hippobroma longiflora*, reveal the secondary metabolites in these plants have biological activity as antioxidant, cytotoxic, anticancer, antimicrobial and anti-inflammatory [4], the treatment of neurological disorders such as Alzheimer's or Parkinson's disease [5]. Several types of isolated compounds from *Hipobroma longiflora* are anthocyanin [6,7], triterpenes palmitate [8] as well as the alkaloid named lobelin and lobetyolin [9]. Based on these considerations, then the isolation and elucidation structure of triterpenoids compounds from *Hippobroma longiflora* and antibacterial activity test because this plant is widely used as a drug eyes of ordinary people.

EXPERIMENTAL SECTION

Plant Material

Fresh leaf of *Hippobroma longiflora* samples taken from Andalas University environment at Limau Manis, subdistrict Pauh, Padang. Furthermore, the examination was done at Herbarium of Biology Department of Andalas University.

Chemical Material

The chemical used in the research were Acetone, methanol, hexane, ethyl acetate, dichloromethane, silica gel 60 (230-400 mesh) from Merck company. All chemicals used were in high grade.

Instruments

Rotary evaporator Heidolp WB, 2000, the general in organic laboratory glassware, melting point apparatus (John Fisher), oven, and vacuum desiccator. IR spectra were recorded on JASCOFT / IR-460. Plus spectrometer column chromatography (CC) was performed on silica gel 60 N, spherical, neutral, Kanto Chemical Co. INC and sephadex LH- 20LH-20 (GE Health care, Japan). UV spectra on a UV Spectrometer Secoman S 1000. Thin Layer Chromatography (TLC) was performed on silica gel 60 F254 for analytical chromatography (200 micrometer layer thickness (Merck) Preparative thin layer (PTLC) was performed on silica gel 60F254 (1 mm Thickness layer, merck) All the chemical used in this study were purchased from Merck.

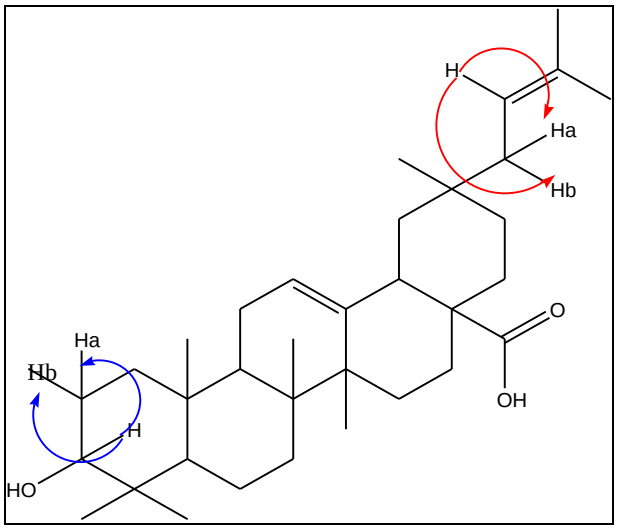
Extraction, Isolation and Purification

4000 gram *Hippobroma longiflora* leaf powder was extracted by maceration method using methanol, then done fractionation using hexane and ethyl acetate. Ethyl acetate fraction as much as 20 grams was chromatographed by 400 gram silica absorbent and eluted with step gradient polarity method using hexane 100% to 100% methanol. Each extract was monitored with TLC and the same stain pattern was combined so that 30 fractions were obtained. The I fraction was positively contained triterpen but is not pure yet as it has some simple stain patterns and continued purification by doing the recolom method to obtain a pure compound. Isolated compounds were obtained in the form of a white powder (22 mg) provides 1 stain on TLC with varying ratios of eluent. Furthermore, characterization was performed using UV, IR, NMR spectroscopy and calculated the melting point.

RESULTS AND DISCUSSION

Phytochemical studies on *Hippobroma longiflora* leaves showed that the leaves contain alkaloids, phenolics, flavonoids, triterpenoids, steroids, coumarins and saponins. The result of purifying by column chromatography obtained 22 mg of compound in the form of white powder and showed positive results of triterpenoids with the Liebermann Burchard reagent. Isolated compounds have a melting point of 214.4 - 215.9°C showed that the compound was purely because of the small temperature range of 2°C. UV spectra provide absorption at 307.40, 277.20 and 203.40 nm with wavelengths between 200-400 nm using methanol. UV spectrum of this compound indicates that the isolated compounds having conjugated double bonds. IR spectroscopy shows the important absorption at wave number 3395.77, 2931.33, 2631.61, 2327.61, 1692.12, 1453.83, 1375.82 and 1095.08 cm^{-1}. The presence of O and H atoms strain at the wave number 3395.77 cm^{-1} referring with the functional groups OH and supported by the vibrations of atoms C and O at 1095.08 cm^{-1}. Vibration of C and H atoms appear at 2931.61 cm^{-1} that refer to C-H primary and secondary bond aliphatic. The existence of geminal dimethyl groups as a hallmark of terpenoid compounds showed vibration between atoms C at 14537.83 and 1375.82 cm^{-1}. Spectroscopy ^{13}C NMR (125MHz, CD_3OD): 16:13, 16:48, 17.76, 19:57, 21.7, 24.20, 24.47, 25.4, 27.70, 28.04, 28.87, 29.3, 31.88, 33.6, 33.93, 34.4, 35.00, 38.20, 38.27, 38.4, 40.52, 40.64, 42.80, 42.98, 43.33, 47.35, 47.74, 54.45, 79.79, 123.74, 126.98, 139, 145.3, 181.79 ppm. The result of ^{13}C NMR measurements showed the chemical shifts with a signal that looks as much as 34 C atoms and spectrum DEPT ^{13}C NMR showed 10 C primary atoms, 10 C secondary atoms, 6 C tertiary atom and 8 C quarterner atoms. Spectrum ^{1}H NMR gave chemical shifts of 5:22, 3:13, 2:18, 1.97, 1.91, 1:51, 1:30, 1:21, 1:10, 0.94, 0.87, 0.84, 0.76 and 0.74 ppm indicating the number of protons attached to the carbon atom. Information that was obtained from spectral data ^{1}H NMR showed 49 protons derived from the integration of the spectrum. Meanwhile, ^{1}H - ^{1}H COSY (Correlated Spectroscopy) showed the correlation between H-3 to H-2, H-12 to H-13 and H-29 to H-34. The result of COSY spectrum and correlation with structure of compound could be seen on the following image (Scheme 1).

Furthermore, to support the spectroscopic analysis of the isolated compounds were compared with the results of the NMR spectrum of the comparative compound. The comparison data can be seen in Table 1.

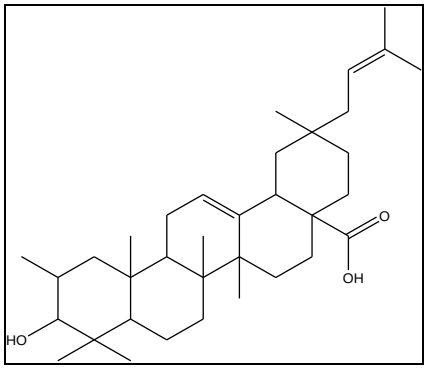


Scheme 1: COSY spectrum and correlation

Table 1: ^{13}C NMR chemical shifts of isolated compounds and prosapogenin compounds

No. C	δC of isolated compounds	Carbon type	δC prosapogenin
1	38.2	CH2	38.6
2	27.7	CH2	26.5
3	79.79	CH	88.9
4	38.27	Cq	38.4
5	54.45	CH	55.8
6	19.57	CH2	18.4
7	33.93	CH2	33.4
8	40.64	Cq	39.8
9	42.8	CH	47
10	43.33	Cq	36.9
11	24.47	CH2	23.7
12	123.74	CH	122.3
13	145.3	Cq	145
14	42.98	Cq	42
15	38.4	CH2	36.1
16	29.3	CH2	74.7
17	47.74	Cq	48.8
18	40.52	CH	41.3
19	47.35	CH2	47.2
20	28.04	Cq	31
21	35	CH2	36.1
22	31.88	CH2	32.8
23	28.87	CH3	28.1
24	16.48	CH3	16.9
25	16.13	CH3	15.5
26	17.76	CH3	17.4
27	33.6	CH3	27.2
28	181.79	CH3	179.9
29	33.6	CH3	33.3
30	24.2	CH3	24.6
31	126.98	CH	
32	139	Cq	
33	25.4	CH3	
34	21.7	CH3	
GlcA1			107.1
2			75.5
3			78.1
4			73.4
5			77.6
6			172.8

Based on UV spectrochopy analysis, IR, and NMR and comparison with NMR spectrum results from known compounds, it can be concluded that the isolated compound which obtained is a triterpenoid compound with the following structure (Scheme 2).



Scheme 2: Triterpenoid

CONCLUSION

Based on data analysis of UV, FTIR and NMR, the isolated compound from ethyl acetate fraction of Hippobroma longiflora leaf extract is triterpenoid

ACKNOWLEDGEMENTS

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REFERENCES

[1] I Waliana, Secondary Metabolites of One Indonesian Medicinal Plants: Hippobroma longiflora, G. Don (Campanulaceae), ITB, **2010**.
[2] EHA Rahman, ARA Monem. Cholinesterase Inhibiting Activity and A New Piperidine Alkaloid from Lobelia laxiflora L. Roots *(Campanulaceae)*, Cairo, **2014**.
[3] DJ Mabberley; CM Pannell; AM Sing. *Flowering Plants*. **1995**, 12(1), 1-407.
[4] S Philipov; R Istatkova; N Ivanovska; P Denkova; K Tosheva; H Navas; J Villegas. *Naturforsch*. **1998**, 53c, 311-317.
[5] PA Newhouse; A Potter; M Kelton; J Corwin. *Biol Psychiatr*. **2001**, 49, 268-278.
[6] T Kondo; J Yamashiki; K Kawahori; T Goto. *Tetrahedron Lett.* **1989**, 30, 6055-6058.
[7] N Saito; K Toki; H Kuwano; H Moriyama; A Shigihara; T Honda. *Phytochem*. **1995**, 39, 423-426.
[8] A Subarna; Y Oshima; Sidik; YJ Ohizumi. *Phar Sci*. **1992**, 81, 620-621.
[9] JR Kesting; IL Tolderlund; AF Pedersen; M Witt; JW Jaroszewski; D Staerk. *J Nat Prod*. **2009**, 72, 312-315.