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Isolation of β-sitosterol from n-Hexane Extract of *Picria fel-terrae* Lour. Leave and Study of Its Antidiabetic Effect in Alloxan Induced Diabetic Mice

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Abstract: *Picria fel-terrae* Lour., is an effective antidiabetic agent empirically. β-sitosterol is one of the steroid class of chemical constituents contained in the *n*-hexane extract of leave *Picria fel-terrae* Lour. The purpose of this study are to extract *Picria fel-terrae* Lour. leaves with *n*-hexane and study its antidiabetic effect and isolation of β-sitosterol from *n*-hexane extract. Structure elucidation of β-sitosterol was conducted by using spectroscopic methods (^{1}H and ^{13}C NMR, IR, and MS). *N*-hexane extract of *Picria fel-terrae* Lour. leaves shows good results as an antidiabetic agent, indicated by significant BGL depletion of alloxan induced diabetic mice for ten days, i.e. 44.47%, with metformin 50 mg/kg as a comparison.

Based on the results of the analysis, the isolate A_1 is steroid group, 24-ethyl-5α-cholest-5-en-3β-ol (β-sitosterol).

Keywords: *Picria fel–terrae* Lour., alloxan, column chromatography, preparative TLC, antidiabetic, spectrophotometer.

Introduction

Indonesia is a rich country of medicinal plants that have been used as traditional medicine[1,2,3,4]. In the present, the world of plants is still become one of the sources of drugs, used in herbs form, extracts and the isolation of chemical components which efficacious in the pure compounds form. Advances of science and modern technology was not ruled out the role of drugs derived from plants but rather as complementary. This is proved by the existence of many herbal medicine enthusiasts until now and the idea of “back to nature”. According to World Health Organization (WHO), plant was the best source for medicine and based on survey that has been

done, about 80% of the world population used traditional medicine[3,5]. There are needed scientific studies such as research in pharmacology, toxicology, identification and isolation of active chemical in plant, so the traditional medicine can be justified[6,7,8,9].

Diabetes mellitus (DM) is a heterogeneous syndrome that all of the symptoms are characterized by increased of blood sugar level that caused relative or absolute insulin deficiency. DM is divided by the need for insulin, i.e.: insulin-dependent diabetes mellitus (IDDM), called Type I, and non insulin-dependent diabetes mellitus (NIDDM), called Type II. DM is a dangerous degenerative disease, even considered as high risk disease because it can cause death[10].

Picria fel-terrae Lour., family Scrophulariaceae, is abundantly grows in Indonesia and traditionally has been used as stimulant, diuretic, malaria, and recently is used for the treatment of DM as an alternative medicine and empirically give satisfying results, but until now there was no scientific research which proves that *Picria fel-terrae* Lour. is effective as an antidiabetic agent and what chemical compounds contained in it.

Several researchers had studied the chemical compounds contained in *Picria fel - terrae* Lour. and its antioxidant effect[9,11,12], but isolation of its chemical compound and study of its antidiabetic effect has never been conducted.

In this study, preparation of simplicia, extraction of simplicia by using *n*-hexane, isolation of steroid compounds (β-sitosterol) from *n*-hexane extract, and test of antidiabetic effect were conducted and discussed.

Material and Methods

The materials used in this study were *Picria fel-terrae* Lour. leaves. The chemicals used unless otherwise stated are pro-analysis grade, i.e., α-naphthol, alloxan, ammonium hydroxide, acetic acid anhydride, concentrated acetic acid, concentrated hydrochloric acid, concentrated nitric acid, concentrated sulfuric acid, benzene, iron (III) chloride, bismuth (III) nitrate, CMC Na, chloroform, ethanol, ether, ethyl acetate, n-hexane, iodine, isopropanol, potassium iodide, methanol, sodium hydroxide, sodium sulfate anhydrous, petroleum ether, mercury (II) chloride, magnesium powder, powder zinkum, lead (II) acetate and toluene, technical ethanol and distilled water[1,2,7].

Sampling

Collection of samples was done purposively without comparing with the same plants from other regions. The samples used are *Picria fel-terrae* Lour leaves taken from Tiga Lingga Village, Dairi, North Sumatera.

Identification of Samples

Identification of *Picria fel-terrae* Lour. leaves was performed in Bogoriense Herbarium, LIPI, Jakarta, Indonesia**.**

Extraction and fraction isolation

Extraction was conducted with *n*-hexane by using maceration method and isolation of fraction was done with *n*-hexane:etylasetat by using column chromatography, obtained 85 fractions. Fractions with the same Rf (fractions 30 to 34) were combined, then further isolation was done by preparative TLC, the mobile phase was *n*-hexane-ethyl acetate (80:20) and the stationary phase was silica gel GF_{254}. The purity of isolate obtained was confirmed with two-way TLC[13,14].

Animal Preparation

The animals used in this study are male mice weighing 20-35 grams. Before the experiment, mice were maintained for 2 weeks in a good cage to match the environment, i.e., the reception of light, 12 hours dark and 12 hours light.

Preparation of Extract Suspension and Alloxan Solution

Suspension of *n*-hexane extract was prepared by using 2.0 % CMC-Na with certain concentration. Solution of alloxan was prepared by dissolving alloxan in cold NaCl 0.9% solution.

Preparation of Alloxan Induced Diabetic Mice

The mice were induced with alloxan solution 200 mg/kg intra-peritoneal (ip). The blood glucose level (BGL) of mice was measured on the third and the seventh day. On the seventh day, mice that have BGL higher than 200 mg/dl were separated and used as test animals. Animals with BGL lower than 200 mg/dl, were induced back with alloxan. If on the third day the BGL of the mice has been higher than 200 mg/dl, the animal is ready to be tested[15,16,17,18].

Study of Antidiabetic Effect

Study of the antidiabetic effect of *n*- hexane extract of *Picria fel-terrae* Lour. leaves was conducted by using alloxan induced diabetic mice by single dose of *n*-hexane extract. Mice were divided into 3 groups and each group consisting of 6 mice, they were:

Group I : Diabetes mice were given suspension of 2 % CMC, dose 1 % of body weight (BW)

Group II : Diabetic mice were given suspension of Metformin® with dose 50 mg/kg BW

Group III : Diabetic mice were given suspension of *n*-hexane ectract of *Picria fel-terrae* Lour. Leaves with dose 200 mg/kg BW.

Suspension of test material (*n*-hexane extract) was administered for 5 consecutive days orally and the BGL of mice were measured on the third, fifth, seventh, and tenth days after administration of the test material[10,15,16,17].

Data analysis

All of the data were analyzed statistically by analysis of variance (ANOVA) method, using SPSS (Statistical Product and Service Solutions) 17.0 software.

Results and Discussion

Isolation and characterization of β – sitosterol.

The purity of β-sitosterol obtained was analyzed by using TLC. The chromatogram gave positive results with Libermann-Burrchad reagent. Rf 0.54 indicated steroid group and confirmed with two-way TLC (mobile phase I: *n*-hexane:ethyl acetate 80:20 and mobile phase II: Toluene:ethyl acetate 90:10). Finally, elucidation of the structure of β-sitosterol with the data of UV spectrum (ethanol) at λ-max 268.5 nm; infra red (KBr) with wave number 3433.29; 2939.52; 2345.44; 1635.64; 1450.47; 1373.32, and 1118.71 cm^{-1}. Carbon NMR spectrum (75 MHz, $CDCl_3$) of pure isolates indicated by the chemical shift data A_1 isolate ^{13}C - NMR spectrum showed the presence of hydroxyl group δC = 71.8172 ppm (C3) and two group ena δC 121.7172 (C6) and δC 140 , 7564 (C5). ^1H-NMR spectrum (300 MHz, $CDCl_3$) showed a multiplet δH 3.5261 to 3.6854 ppm of the H-3 and the characteristics of the C-3 atom; singlet peak at δH1, 0524 ppm and δH 0.6514 ppm; doublet at δH 0.9523 ppm and 0.9306 ppm. Mass spectra of m/z 414 $C_{29}H_{50}O$ showed fragmentation patterns of 396; 273; 255; 273; 231; 213[19,20,21].

Antidiabetic test

Antidiabetic effect is determined with depletion of blood glucose level (BGL) in the alloxan induce diabetic mice. The results showed that the BGL depletion of the mice for ten days was 44.47 %. This effect is predicted due to the group of steroid compounds on *n*-hexane extract of *Picria fel-terrae* Lour. leaves. Fasting BGL of the mice before and after induced by alloxan was shown in Table 1.

Table 1. Fasting BGL before and after alloxan induction

Test Group	Fasting BGL before alloxan induction (mg/dl)	Fasting BGL after alloxan induction (mg/dl)
2% CMC Sodium	81,67±1,02	413,17±6,14
Metformin®, 50 mg/kg BW	82,67±1,74	491,17±3,81
n-heksana extract 200 mg/kg BW	82,67±1,80	540±16,79

The BGL of the mice after treatment was shown in Table 2.

Table 2. The BGL of the mice after treatment

Test Group	BGL after treatment (mg/dL)			
	Day 3	Day 5	Day 7	Day10
2% CMC Sodium	403±6,68	403,1±2,75	395±3,13	394,1±3,15
Metformin®, 50 mg/kg BW	447,17± 6,92	378,33± 7,72	226,83± 15,66	135± 11,35
n-hexane extract, 200 mg/kg BW	513± 15,71	428,67± 13,79	276,33± 14,50	167± 4,76

The effect of treatment to BGL of alloxan induced diabetic mice was shown in Figure 1.

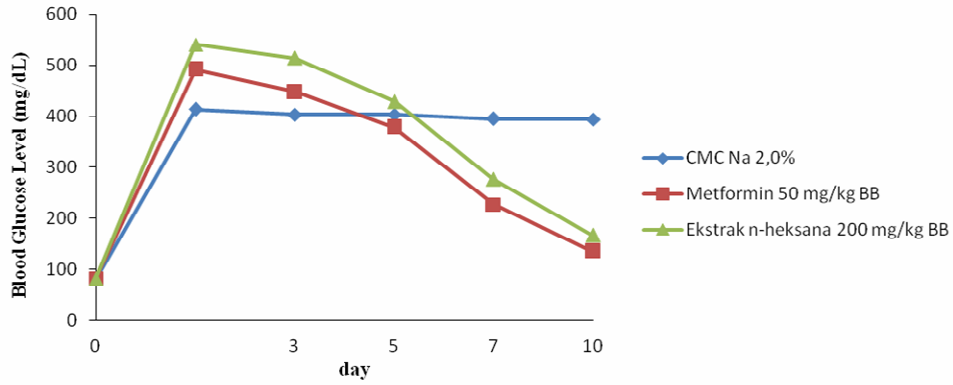

Figure 1. Effect of various treatment to the blood glucose level in alloxan induced diabetic mice

Conclusion

From the analysis of the data above it is concluded that the *n*-hexane extract of *Picria fel-terrae* Lour. Leaves is effective as an antidiabetic agent. It has the ability to reduce BGL, and the reduction for 10 was 44.47%. The chemical compounds isolated from *n*-hexane of *Picria fel-terrae* Lour. leaves is a steroid compound, 24-ethyl-5α-cholest-5-en-3β-ol (β-sitosterol).

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