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RESEARCH ARTICLE

Chemoprofiling of Active n-Hexane Fraction as Alpha-Glucosidase Inhibitors from Kanunang (*Cordia myxa* L.) Leaves from Enrekang South Sulawesi

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Abstract

Kanunang (Cordia myxa L.) is a native plant from tropical Asia. This study aims to determine the profile of chemical compounds contained in leaves of Kanunang (Cordia myxa) which can inhibit alpha-glucosidase enzyme. Extraction of Kanunang leaves was done by maceration method using ethanol 96% solvent for 3 days then filtered and thickened with rotary vacuum evaporator. Resulting extract with yield value of 5%. The extract was fractionated using conventional column method with n-hexane: ethyl acetate (10: 0) eluent. The n-hexane fraction was tested using the GC-MS instrument with the following condition; injector temperature 250°C, initial oven temperature 70°C, up to 325°C increasing 10°C/minute. The results obtained was 24 compounds which have alpha-glucosidase enzyme inhibitors activity with maximum abundance at RT 12:43, as 9,12,15-Octadecatrienoic Acid, methyl ester (C19H32O2) compound.

Keywords: *Cordia myxa L.; Chemical profile; GC-MS; Alpha-glucosidase.*

Introduction

Medicinal plants are a gift to humankind to achieve a healthy life, free from disease. One way that can be done to achieve these goals, among others, is by optimizing the use of medicinal plants that have been widely used and proven empirically to give treatment effect. *Cordia myxa* L. has several benefits in medicine.

In several studies *Cordia myxa* L. has been reported to have pharmacological effects such as anti-gastric ulcer, hepatoprotective, anti-inflammatory, anti-diabetic, degenerative, anti-microbial and has potential as an antioxidant [1].

Previous research by Syarif [2] showed that the leaves of Kanunang (*Cordia myxa* L.) plants has chemical content such as phenols, steroids, flavonoids, and saponins. This is also reassured by a review from Ali Esmail Al-Snafi [3] which states that leaves of several plants with genus Cordia, such as *C.martinicensis*, *C.myxa*, *C.serratifolia,* and *C.ulmifolia* contain four flavonoid glycosides, robinin, routine, hesperidin, one flavonoid aglycone, and two phenolic derivatives. Based on the description above, further research will be carried out regarding the identification of chemical profiles in the n-hexane fraction of Kanunang leaf (*Cordia myxa* L.) to determine the chemical components of alpha-glucosidase inhibiting compounds contained in the leaf, using GC-MS instruments.

Material and Method

Sample Processing

Sample of *Cordia myxa* L. leaf was taken in Kab. Enrekang. The leaves that have been taken then cleaned from the dirt that attached by using tap water and then dried using drying cabinet with temperature of ± 50^0 C. Then grinded, and ready to be extracted [2].

Sample Extraction and Fractionation

Cordia myxa L. leaf powder is inserted into the maceration container, then ethanol is added until the sample is submerged, left for 3 days in a closed jar and protected from direct sunlight while stirred periodically, after 3 x 24 hours filtering is done and the residue is macerated again with new solvent. The extract then evaporated using rotary vacuum evaporator, then thick extracts would be obtained [2].

Analysis Using GCMS (Gas Chromatography-Mass Spectrometry)

The analysis that used to determine the chemical profile in this study was a GCMS instrument (Gas Chromatography-Mass Spectrometry). The content of each compound in the sample has different retention time and peak area in the chromatogram according to the type of compound analyzed [4]. The condition of the GC-MS used is as follows: oven with a maximum temperature of 325°C, with an initial temperature of 70°C and raised gradually 10°C / minute to 325°C with the time taken is 40 minutes. Mobile phase is He (Helium), injection of 1 microliter using split injection temperature of 250°C, capillary column with a column length of 30 m, diameter 25 mm and particle size of 0.25 micro liters [4]. Detection of chemical content is based on the computer's mass spectrum evaluation from the sample through a ratio of peaks and retention time and matching, also by following the fragmentation characteristics of the pattern of mass spectra of certain classes of compounds [5].

Results

Kanunang with its Latin *name Cordia myxa* L. has chemical content of phenols, steroids, flavonoids, and saponins. This plant has pharmacological effects such as anti-gastric ulcer, hepatoprotective activity, anti-inflammatory, antidiabetic, degenerative diseases, anti-microbial, and potentially as an antioxidant [1]. The purpose of this study was to determine the chemical profile contained in the n-hexane fraction of Kanunang (*Cordia myxa* L.) leaves which can be used as an alpha-glucosidase enzyme inhibitor [7]. The results obtained from the extraction of Kanunang (*Cordia myxa* L.) leaves can be seen in the Table 1.

Table 1: Extraction Result from Kanunang (*Cordia myxa* L.) Leaf

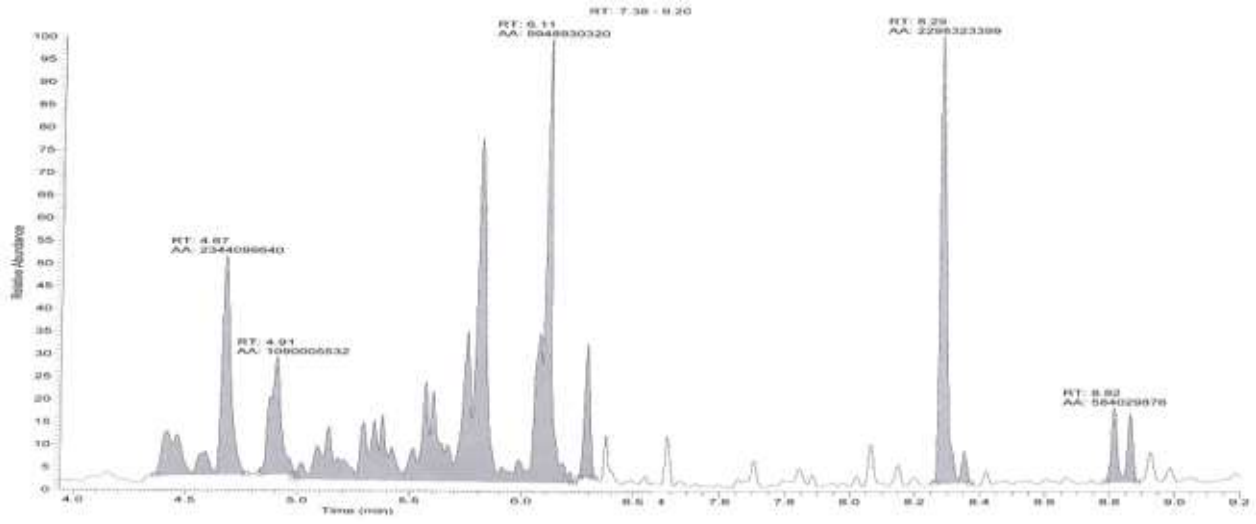
Sample	Weight (kg)	Ethanol Solvent (mL)	Extract (g)	Yield value
Kanunang (*Cordia myxa* L.) Leaf	2	2100	100	5%

The extraction results from kanunang (*Cordia myxa* L.) leave then fractionated using the Column Chromatography method, resulting in 1 active fraction of n-hexane: Ethyl acetate (10: 0) eluent, which was then tested on GC-MS as seen on Table 2.

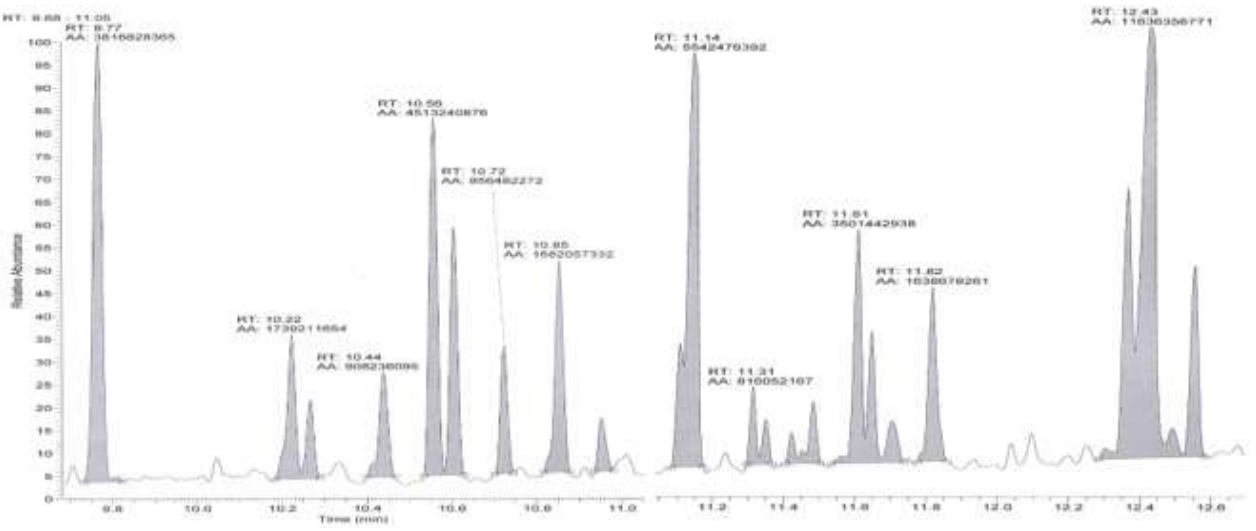
Table 2: The results of identification of alpha-glucosidase enzyme inhibitors from the Kanunang (*Cordia myxa* L.) leaf fraction using GCMS instrument

No	RT (Retention Time)	% Area	Suspected Compound
1	4,67	17,3 %	Benzene, 1-etil-3-metil
2	4,91	20,8 %	Benzene,1-2-3-trimetil
3	6,11	3,65 %	7-Tetradecyne
4	8,29	68,4 %	Fenol, 2,4-bis (1,1-dimetiletil)
5	8,82	7,79%	Cetene
6	9,77	13,7%	Dekana 5,6 bis (2,2 dimetilpropilida)
7	10,22	5,72%	1-Nonadekana
8	10,44	54,9%	Isopropil myristate
9	10,56	52,5%	3,7,11,15-Tetrametil-2-Heksadekana-1-ol
10	10,85	42,6%	3,7,11,15-Tetrametil-2-Heksadekana-1-ol
11	10,72	50,6%	3,7,11,15-Tetrametil-2-Heksadekana-1-ol
12	11,14	82%	As. Heksadekanoid, metil ester
13	11,31	68,3%	Isophytol
14	11,61	22,1%	As.Oktadekanoid, etil ester
15	11,82	71,4%	Isopropil Palmitat
16	12,43	54,8%	9,12,15 As.Oktadekatrianoid, metil ester
17	13,03	7,35%	1-Docosene
18	17,78	8,92%	Heptacosane
19	14,05	75,5%	1-As.Propenoid, 3-(4-metoksifenil)-2-etilheksil ester 1-Heksadekanal 2 Metil
20	14,47	5,02%	Bis (2-etilheksil) ftalat
21	15,67	42,4%	1-Heksadekanal 2 Metil
22	15,90	9,78%	1,3 As.Benzenedikarboksilat, bis 2-etil heksil ester
23	17,04	35,4%	2,2,4-Trimetil-3-(3,8,2,16-tetrametilheptadeka, 3,7,11,15, tetraenil-sikloheksanol)
24	17,60	15,7%	

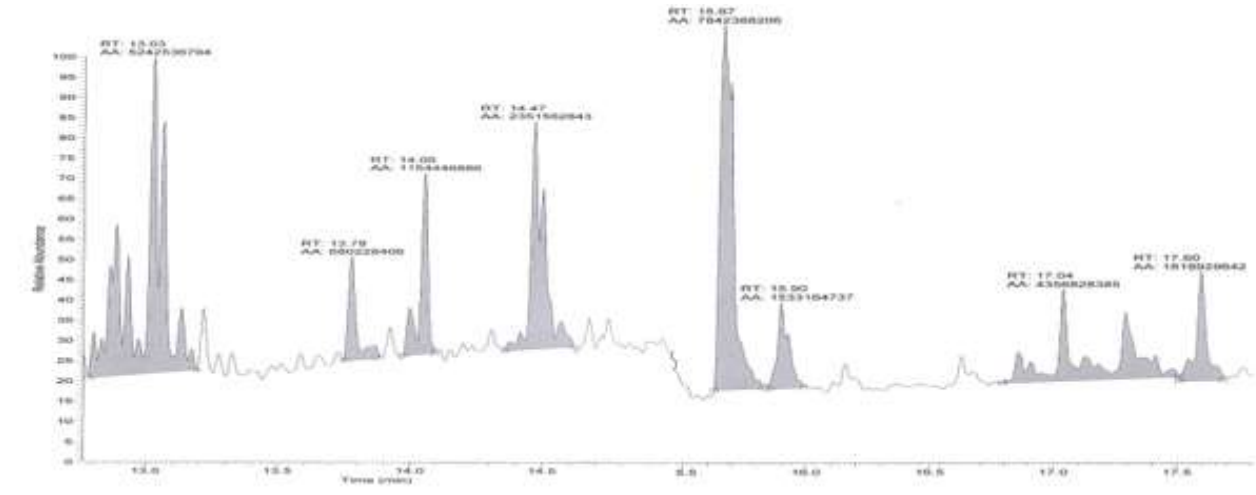
Then the results of identification of alpha-glucosidase enzyme inhibitors can be seen more specifically based on GCMS instrument data in the Figure 1 (a-c):



(a)



(b)

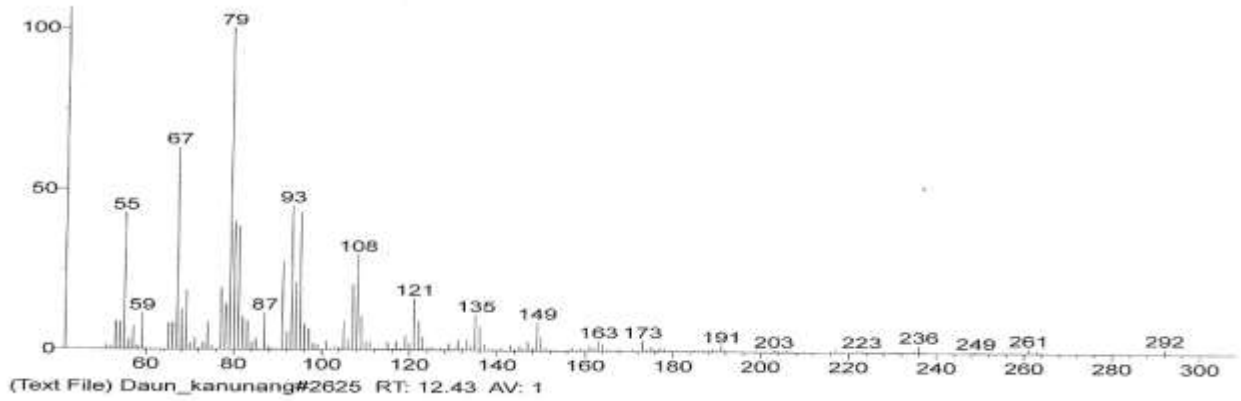


(c)

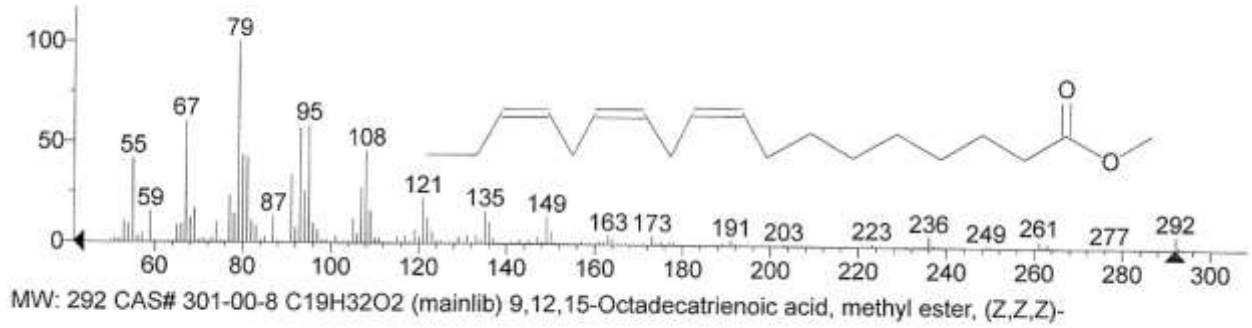
Figure 1: The chemical profile measured by GCMS in the sample of Kanunang leaf fraction with Retention Time (RT) as follows: (a) 4.67 – 8.82, (b) 9.77-12.43, and (c) 13.03-17.60

Discussion

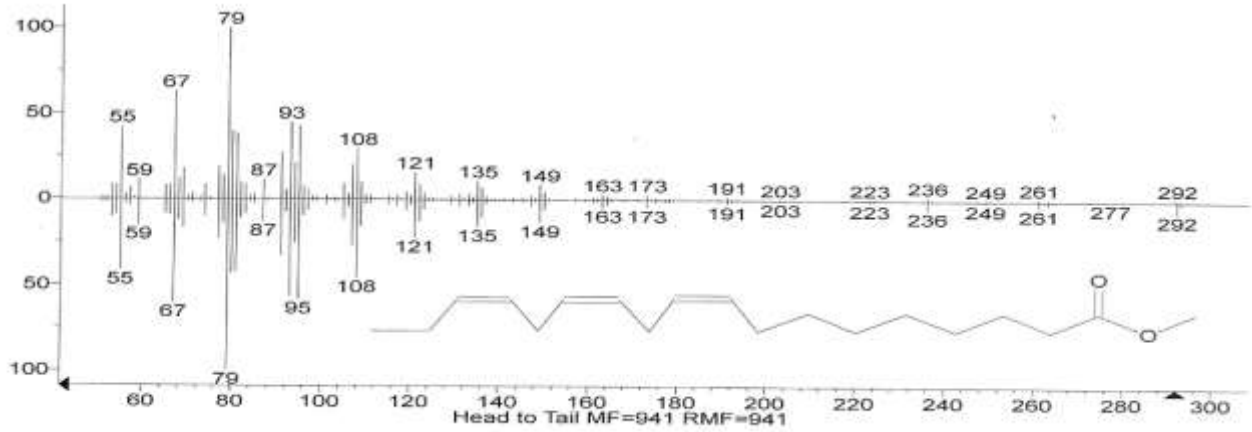
Based on the data, it was shown that the compounds appeared in measurements using GCMS are compounds that can inhibit alpha-glucosidase enzymes as anti-diabetes. From several compounds that appear, one of the compounds has a maximum abundance Figure 2a-c.



(a)



(b)



(c)

Figure 2: Fragmentation of alpha-glucosidase enzyme inhibitors with an area of maximum abundance (a), comparative standard fragmentation (b), and the results of comparison of alpha-glucosidase enzyme inhibitors with maximum abundance and comparative standard (c)

Diabetes mellitus is a hyperglycemia disease which is marked by the absolute absence of insulin or a decrease in relative cell insensitivity to insulin [8]. Diabetes arises because the body is unable to control the level of sugar (glucose) in the blood. Diabetics fail to produce adequate amounts of insulin. Insulin produced by the pancreatic gland works to control blood sugar levels. As a result, there will be excess sugar in the body [9]. Alpha-glucosidase inhibitors are one of the antidiabetic agents that work by inhibiting the action of alpha-glucosidase enzymes. Reducing carbohydrate absorption from food by the intestine is a therapeutic approach for postprandial hyperglycemia. Complex polysaccharides will be further hydrolyzed into glucose by alpha-glucosidase enzymes before entering the blood circulation through absorption of the epithelium. Synthetic amylase and alpha-glucosidase inhibitors such as acarbose have been widely used to treat patients with diabetes type II but this drug is also reported to cause various side effects [10]. The results of the analysis after analyzed with GCMS is in the form of

24 peaks, where the peak with the largest peak area was seen at Retention Time (RT) 12.43, which was seen as 9,12,15 Octadecatrienoic Acid methyl ester compound.

Conclusıon

Based on the research that has been done, it can be concluded that there are 24 chemical compounds that can work as alpha-glucosidase enzyme inhibitors found in the Kanunang (*Cordia myxa* L.) Leaf fraction, where the compound with the widest peak area is seen in RT 12.43, which is 9, 12, 15 Octadecatrienoic Acid methyl ester compound.

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