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Penetration Test of Gel Ethanol Extract of *Cyperus Rotundus* L. Rhizomes

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ABSTRACT

In vitro penetration has been done on ethanol extract from tuber root of teki grass (Cyperus rotundus L.) with concentration 7% in gel. The penetration test was conducted by two membranes that are whatman paper No.1 with Spangler fluid modification and skin of mice. The test was observed every hour for 3 hours by using Franz diffusion cells. Extract compounds and compounds in penetration liquid were analyzed by GCMS and compared to profile of extract compound. Profile of compounds of both extract and penetration test are same and showed contain some volatile compounds such caryophyllene oxide, Sphatulenol, Alloaromadendrene, Iso-velerenal, 1-Limonene, Globulol, Beta-Guaiene, and Beta-Citronellol. Based on this assay can be concluded that ethanol extract of Cyperus rotundus in gel formula can be penetrated through two membranes that are Spangler and skin of mice.

***KEYWORDS**: Cyperus Rotundus L, Penetration Test, GC-MS.*

INTRODUCTION

Drug delivery system through the skin that known as Transdermal Drug Delivery System is currently very widespread research topic. Transdermal preparation is a preparation which provides an alternative route for delivering drugs through the skin and reach the blood circulation to avoid the possibility of first pass metabolic [1]. Skin as body cover provide widest surface for the absorption of the drug, but the skin is a barrier that can limit the penetration of various substances that penetrate the skin. Transdermal dosage form will affect the release and penetration of active ingredients through the skin to reach the systemic circulation and cause effects such as oral administration [2]. Transdermal dosage forms are generally in the form of creams, gels, patches and so forth.

The results of previous studies have been formulated transdermal gel dosage of the ethanol extract of *Cyperus rotundus* L. rhizome with a concentration of 3%, 5%, 7%, and tested it analgesic effect foot mice that had been induced with 1% AgNO3 solution. The resulting formula shows the analgesic effect, it is observed on the amount of pain reflex at various observation time after administration of nut-grass rhizome extract gel. Conclusions of previous research stated that the formula with a concentration of 7% is the best formula [3]. Results of other studies mention that the identification of the TLC show the results of isolation of essential oils of tubers positive puzzles containing sesquiterpenoids hydrocarbon group. Seskuiterpen class of compounds of the literature search turned out to have the ability of pharmacological effects, namely as analgesics [4].

Under these conditions, further research is taken to examine the penetration of ethanol rhizome extract gel sedges, a formula that is taken is 7% which is the best formulation that provides better analgesic effect than with the comparator. The test was done in-vitro using Franz diffusion cell apparatus. Using Whatman paper No. 1 with Spangler liquid modification and skin of mice as a membrane penetration. Analyzed using GC-MS (Gas Chromatography -Mass Spectrometry) to determine the components of the gel ethanol extract of *Cyperus rotundus* L. rhizome that penetrates through the membrane.

TOOLS AND MATERIALS

Materials

The materials used in this study is a rhizome sedges, ethanol 96%, HPMC, propilenglikol, nipagin, distilled water, powders Mg, HCl (concentrated), norit, H_2SO_4 (concentrated), H2SO4 2 N, acetic anhydride, chloroform, ammonia 0.05 N, reagents mayer, Whatman® No.1 paper, liquid Spangler modifications (15% oleic acid, stearic acid 5%, VCO 15%, 10% squalene, paraffin 10%, koleterol 5%, 15% white Vaseline, olive oil 25%), physiological saline (0.9%), skin of mice.

Tools

The tools used are glass bottles, funnels, filter paper, vacuum distillation, rotary evaporator, analytical balance, knives, measuring cups, measuring flask, glass beaker, test tubes, Erlenmeyer, cup vaporizer, watch glass, glass objects, glass size of 5 x 50 mm, spatula, ladle, parchment paper, pipette, plate drops, the reaction tube, rod stirrer, mortar and pestle, cloth napkins, pot of ointment, crucible incandescent, transparent plastic, magnetic stirrer, franz diffusion cell, GC-MS (Gas Chromatography-Mass Spectrometry).

Sample preparation

Sampling

The sample used is a nut-grass rhizome. Taken in the Air Tawar, Padang. Identification of the samples was done in the Herbarium of the Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA) Andalas University in Padang.

b. Sample extraction

Cleared and smalled Rhizome sedges and included in a dark bottle the sedges is macerated with 96% ethanol for 3x24 hours, with each maceration using 1 litre of ethanol 96%. Results of maceration is filtered and all the combined filtrate is then evaporated with a rotary evaporator to obtain a thick extract ethanol.

Evaluation of Ethanol Extract Samples

Test Phytochemicals

Sedges rhizome extract ethanol was added to the test tube, add 5 ml of distilled water and 5 ml of chloroform, shaken and allowed to form two layers of water and chloroform.

Test Flavonoids (Sianidin Test Method)

Taken layer of water 1-2 drops, drops dripped on the plate and then added Mg powder and HCl (p), the formation of the red colour indicates the flavonoids.

Test Saponins Harbone [5].

Taken layer of water, shaken vigorously in a test tube, foaming permanent (± 15 min) showed a saponins.

Test Terpenoids and Steroids (Method Simes)

Taken little chloroform layer was filtered with norit, then included in the drip plate allowed to dry, add 2 drops of H_2SO_4 (p), acetic acid anhydride is added, the formation of blue-purple colour indicates steroid, whereas when formed red colour indicates the presence of terpenoids.

Test Alkaloids (Method Culvenore - Fitzgerald)

Taken little chloroform layer was added 10 ml of chloroform 0.05 N ammonia, stirring slowly added a few drops of H_2SO_4 2N then shaken gently, allowed to separate. Taken layer of water included in the test tube (acid mantle) was added a few drops of reagent mayer, positive reaction alkaloids characterized by a white fog to the white blob.

Organoleptic Inspection

Done visually by observing the shape, colour, and odour.

Examination Solubility

Examination solubility by dissolving the viscous extract in water and 96% ethanol.

The Yield Determination Extract

The yield of the extract is calculated by comparing the weight of viscous extract obtained by the weight of dry rhizome.

% Yield = (weight of extract) / (initial sample weight) x 100%

Dust Content Examination (Ministry of Health, 2000)

Weighed 1 gram of viscous extract was added to thick porcelain crucible which has blazed and weighed. Then put in a furnace for 6 hours at a temperature of 600ºC. Then after 6 hours put in a desiccator and weighed.

Drying Shrinkage Inspection (Ministry of Health, 1995)

Weighed 1 gram of viscous extract inserted into porcelain crucible which had previously been heated at a temperature of 105ºC for 30 minutes and had tared. Then inserted into the oven at 105ºC for 2 hours, then cooled in a desiccator and weighed to obtain the weight remains.

Extract pH probe [6]

By using a pH meter. Tool previously calibrated by using a buffer solution of pH 4 and pH 7. The digits that appear on the device is on the pH value of the solution. Then, the electrode was washed with distilled water and dried with a tissue. Extract pH measurements and fractions condensed by diluting 1 gram of extract thick with up to 10 ml of distilled water in a suitable container. Electrode is dipped into the container and the numbers allowed to move up to a constant position.

Identification of Essential Oils [7]

The extract is diluted with hexane, diluted extract is left for 1x24 hours. Subsequently extract samples were analyzed by GC-MS.

Preparation of gel with *Cyperus rotundus* L. Rhizomes

Formula of gel ethanol extract *Cyperus rotundus* L. rhizomes consist of ethanol extract *Cyperus rotundus* L. rhizomes 7%, HPMC 5%, propilenglycol 10%, nipagin 0,1% and aquadest ad 100%.

Preparation: HPMC sowed at the remaining water then let stand for 15-30 minute, after swelling added a solution of nipagin, stirred, ethanol extract *Cyperus rotundus* L. rhizomes dispersed in propilenglycol, then stir until homogeneous.

Penetration Test

The test was done using a Franz diffusion cell. The membranes used were back skin of mice and Whatman® paper No. 1 with Spangler liquid modification. First performed on mice deslokasi neck until dead and then fur of mice on the back shaved. After that the skin of mice was slashed in the back with a thickness of 0.6 ± 0.1 mm and fats in the subcutaneous sticking carefully removed. Then the mouse skin is immersed in the medium to be used (physiological NaCl 0.9%) for 30 minutes. Whatman® paper No.1 soaked with liquid Spangler before use.

The membranes placed between the donor compartment and the receptor compartment, samples have been weighed as much as 0.15 grams was applied to the membrane surface. Compartment receptors on Franz diffusion cell apparatus is filled with physiological NaCl 0.9% until full (119 ml) which maintained the temperature of about 37º C and stirred with a magnetic stirrer at a speed of 250 rpm. At a time of 1 hour, 2 hours, 3 hours, taken as many as 5

ml of the receptor compartment and is inserted into the test tube. Each 5-mL retrieval receptor compartment, was replaced with 5 ml of 0.9% NaCl physiological.

GC-MS analysis

Mix 5 mL of receiver fluid from penetration test results with measurable by 5 mL of hexane. Put it in a vial, shake a few times, and then leave it for a day and a night. Take 2 mL of fluid is injected into the gate injecting gas chromatography-mass spectrophotometer.

RESULTS AND DISCUSSION

In the examination of nut-grass rhizome extract phytochemicals which include tests that provide results ethanol extract of rhizome positive sedges contains flavonoids, alkaloids, terpenoids, steroids and saponins. Organoleptic inspection with ethanol thick rhizome extract sedges have a condensed form, dark brown colour and distinctive smell aromatic. The solubility results sedges rhizome extract ethanol practically insoluble in water and soluble in ethanol. The yield of 10.42% results meet the requirements of sedges rhizome yield of not less than 10.3%; ash content of 0.75% results meet the requirements of the ash content of nut-grass rhizome extract no more than 0.9% (MOH, 2008); drying shrinkage of 6.97% results meet the requirements of the drying shrinkage of nut-grass rhizome extract no more than 10%, and the measurement of pH with pH results of the ethanol extract of rhizomes sedges, namely 6.06 [7].

Data obtained from the identification of essential oils using GC-MS is used as a comparator for comparing the test data penetration, so it can be deduced whether the gel can be penetrated with a view of the same component among the components of the extract essential oil with the oil component Essential penetration test results. Data extracts can be seen in Table 1 and Figure 1.

Table-1: Data components of essential oils in the ethanol extract of the rhizome sedges analyzed by GCMS

No.	Retention Time (minute)	Peak (% area)	Base (m/z)	Compound Name
1.	2, 694	2 (0,02%)	68,05	1-Limonene
2.	4, 535	14 (0,33%)	57,10	Octane, 2,4,6-trimethyl
3.	9,113	48 (0,15 %)	108,15	Alloaromadendrene
4.	9,956	61 (0,30 %)	119,15	Isospathulenol
5.	10,056	62 (0,17 %)	119,15	Isospathulenol
6.	10,133	63 (0,12 %)	105,15	-beta-Guaiene
7.	10,219	64 (0,10 %)	69,10	Beta-Citronellol
8.	10,280	65 (0,08 %)	43,10	-(-)Caryophyllene oxide

9.	10,726	69 (1,48 %)	43,10	-(-)Caryophyllene oxide
10.	10,800	70 (0,71 %)	41,10	-(-)Caryophyllene oxide
11.	11,773	83 (0,18 %)	55,10	-(-)Caryophyllene oxide
12.	11,838	84 (0,27 %)	43,10	Globulol
13.	12,563	94 (0,39 %)	43,10	-(-)Caryophyllene oxide
14.	12,922	98 (1,69 %)	41,10	Isovelerenal
15.	13,597	104 (0,51%)	43,10	Dodecane, 2-methyl
16.	13,968	108 (1,65%)	57,10	Dodecane, 2,5-dimethyl
17.	14,658	116 (1,10 %)	43,10	Alloaromadendrene
18.	18,705	160 (0,18%)	57,10	Octadecane, 6-methyl
19.	18,869	162 (0,07%)	57,10	Eicosane
20.	19,390	170 (0,10%)	55,10	5-Eicosene
21.	20,110	176 (0,11%)	43,10	Eicosane, 2,4-dimethyl
22.	21,240	189 (0,13%)	57,10	Heptadecane 2,6,10,15-tetramethyl
23.	23,224	213 (0,33%)	57,10	Tetradecane, 1-bromo

Figure-1: Chromatogram Ethanol Extract of *Cyperus rotundus* L.

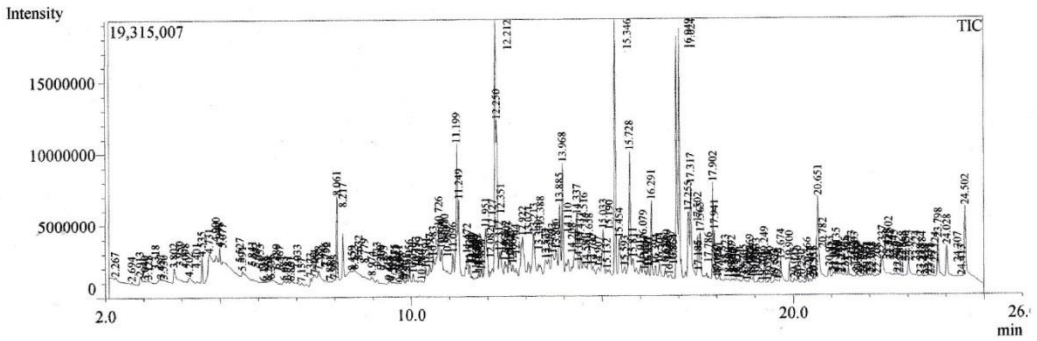


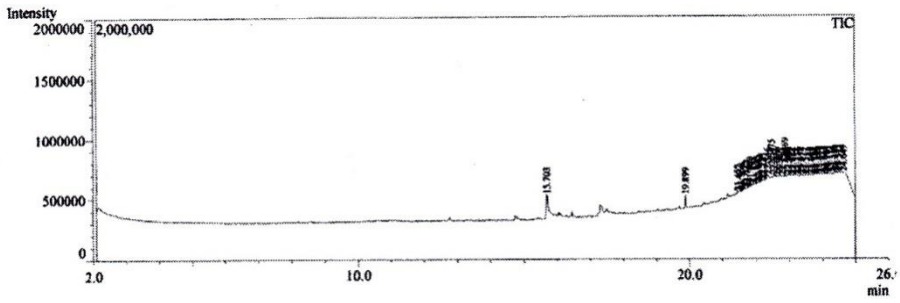
Table-2: Data M/Z of Ethanol Extract of *Cyperus rotundus* L.

No	Retention Time (Minute)	Peak (%area)	MW	Base m/z	Compound Name
1 a	9,956	61 (0,31 %)	220	41*,43,69,79,91,105,119,131,147,159,177,205, 220	Spathulenol
b	10,056	62 (0,17 %)			
2 a	10,280	65 (0,08 %)	220	39,41*,69, 79,93,109, 121,135,149,177	-(-) Caryophyllene oxide
b	10,726	69 (1,48 %)			
c	10,800	70 (0,17 %)			
d	11,773	83 (0,18 %)			
e	12,563	94 (0,39 %)			
f	15,891	127 (0,43 %)			
3 a	9,113	48 (0,15 %)	204	41,55,67,79,91*,105,119,133,147,161,175,189,204	Alloromadendrene
b	14,658	116 (1,10 %)			
4	12,922	98 (1,69 %)	232	41*,55,65,77,91,105,119,133,147,161,175,204,232	Iso-velerenal
5	2,694	2 (0,02 %)	136	38,39,53,68*,79,93,107,121,136	1-Limonene
6	11,838	84 (0,27 %)	222	27,41,43*,69,81,95,109,122,135, 161,189,204	Globulol
7	10,133	63 (0,12 %)	204	27,41,43,67,81,91,105,119,133,147,161*,175,189,204	Beta-Guaiene
8	10,219	64 (0,10 %)	156	39,41,55,69*,82,95,109,123,138, 158	Beta-Citronellol

Analyzed with Penetration Test GC-MS

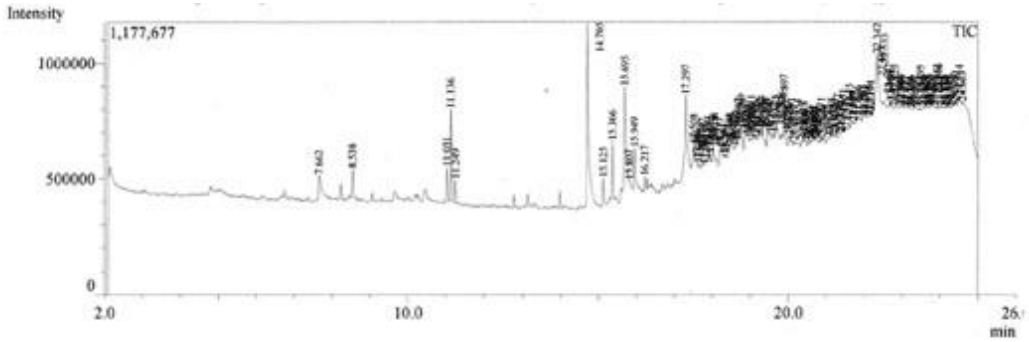
From the analysis of the penetration of the gel in each liquid receiver S1 results showed no detectable compound that is equal to the extract, can be seen in Figure 2 and Table 3.

Figure-2: Chromatogram S1 (Whatman No 1)



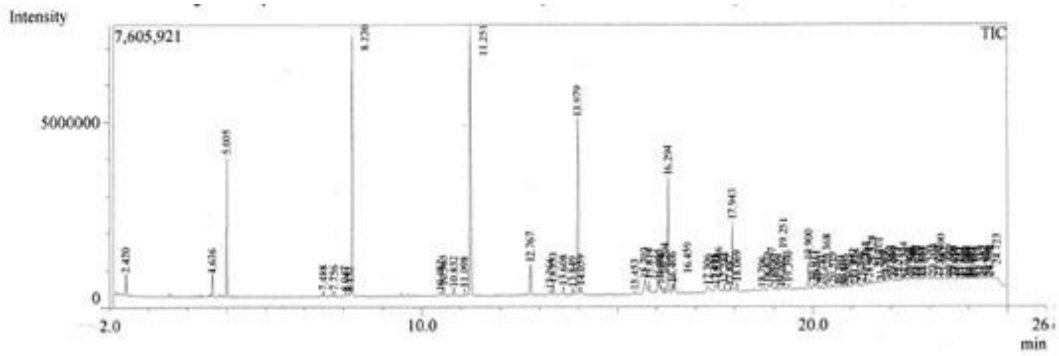
In S2 detected 1 the same compound with an extract that is Heptadecane 2,6,10,15-tetramethyl retention time 18.017 minutes with the percent area of 0.62% and a retention time of 18.867 minutes with an area of 1.04% percent, can be seen in table Figure 3 and Table 3.

Figure-3: Chromatogram S2 (Whatman No.1)



S3 detected on 4 the same compound with an extract that is Eicosane at a retention time of 10.565 minutes with the percent area of 0.36%; Dodecane, 2,5-dimethyl at a retention time of 11.251 minutes with the percent area of 9.92%; 5-Eicosane row at a retention time of 13.849 minutes with 0.32% peak area, retention time 16.204 minutes with the percent area of 0.25%, the retention time of 17.882 minutes with the percent area of 0.58%, the retention time of 19.390 with pesen area 0, 45%, retention time of 20.519 minutes with an area of 0.58% percent, can be seen in figure 4 and table 3.

Figure-4: Chromatogram S3 (Whatman No.1)



In S4 no detectable same compound with the extract. At S5 no detectable same compound with the extract. In S6 detected five compounds are 1-Limonene at a retention time of 2.071 minutes with a peak area of 0.24%; Octane,

2,4,6-trimethyl at a retention time of 5.003 minutes with 0.11% peak area and retention time of 8.218 minutes with a peak area of 1.83%; Eicosane at a retention time of 10.566 minutes with 0.23% peak area and retention time of 24.568 minutes with a peak area of 2.10%; 5-Eicosene at a retention time of 13.851 minutes with the percent area of 0.14%, the retention time of 16.207 minutes with the percent area of 0.40%, the retention time of 17.882 minutes with the percent area of 0.37%, the retention time of 18.070 minutes with an area of 0.80 percent %, retention time 19.391 minutes with the percent area of 0.53%, the retention time of 50.521 minutes with the percent area of 0.54%. The result can be seen in Table 3 and Figure 5,6 and 7.

Figure-5: Chromatogram S4 (skin of mice)

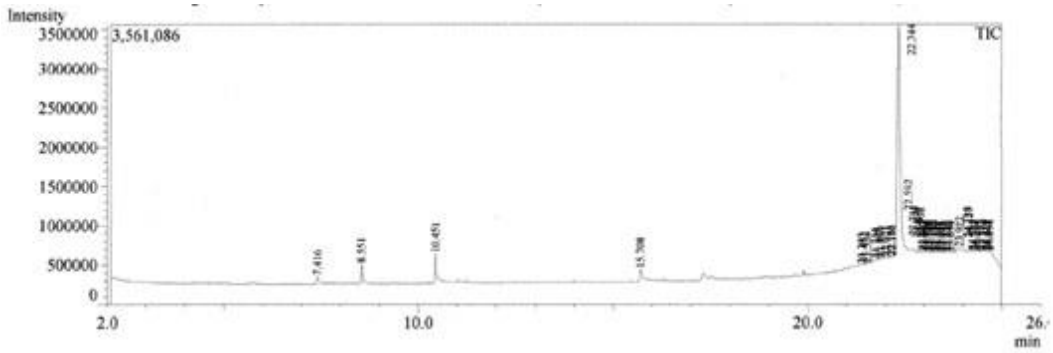


Figure-6: Chromatogram S5 (skin of mice)

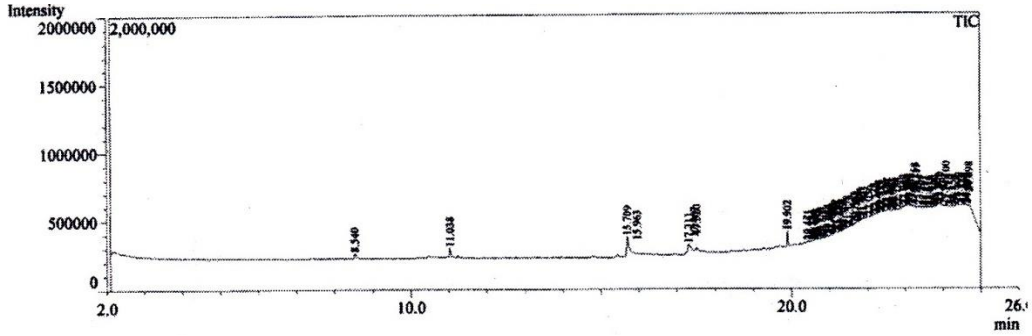
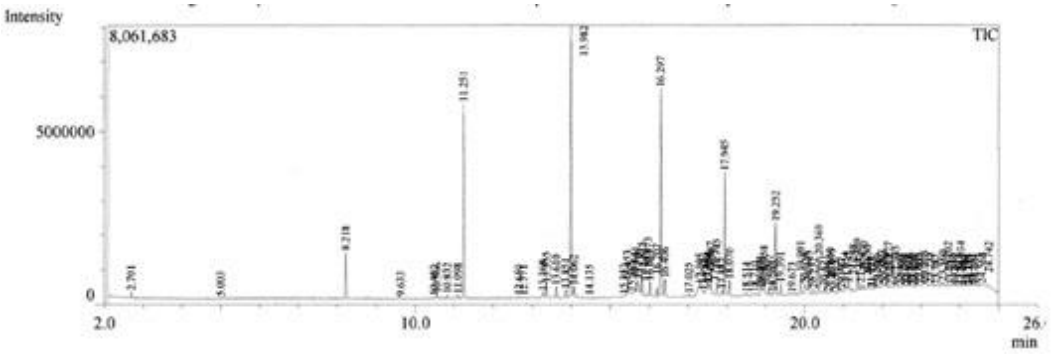


Figure-7: Chromatogram S6 (skin of mice)



The intensity of the compound detected is different in each sample being analyzed, it is probably due to the penetration of different on each sample, the evaporation of essential oils for sample storage for too long, the levels of the compounds that are too small so it is not readable by the device when analyzed, compound name data on the device is not complete so there are some compounds that are detected but not known the name of the compound. Data on the S2, S3 and S6 shows that ethanol rhizome extract gel can penetrate sedges penetrate the membrane. Factors affecting the penetration of the compound into the skin, the physical and chemical properties of drugs such as molecular weight, solubility, partition coefficient and dissociation constants; nature of the carrier material and the skin condition, but that with increasing concentrations of the drug, the amount of drug absorbed higher, broad basting, drugs that are non-polar will be faster penetrated caused by skin that has a fat content which is nonpolar, and also the application of the drug is also will affect penetration where basting on the skin which has a thin layer of horn will enhance penetration [8-10]. Data penetration gel grass rhizomes extract ethanol puzzle analyzed by GCMS seen in table 3.

Table 3. Penetration Gel Grass Rhizome Extract Ethanol Puzzle Analyzed by GC-MS

No	Sample	Retention Time (minute)	Peak (% area)	Base (m/z)	Compound Name
1.	S1	19, 899	2 (0,64%)	149,10	1,2-Benzenedicarboxyclic acid
2.	S2	18,017	23 (0,62%)	57,10	Heptadecane 2,6,10,15-tetramethyl
		18,867	35 (1,04%)	57,10	Heptadecane 2,6,10,15-tetramethyl
3.	S3	10,565	10 (0,36%)	57,10	Eicosane
		11,251	13 (9,92%)	57,10	Dodecane, 2,5-dimethyl
		13,849	18 (0,32%)	57,10	5-Eicosane
		16,204	26 (0,25%)	57,10	5-Eicosane
		17,882	35 (0,32%)	55,10	5-Eicosane
		18,069	37 (0,58%)	55,10	5-Eicosane
		19,390	44 (0,45%)	55,10	5-Eicosane
		20,519	50 (0,58%)	55,10	5-Eicosane
		21.135	55 (0,61%)	57,10	Eicosane, 2,4-dimethyl
		22,094	64 (0,89%)	57,10	Eicosane, 2,4-dimethyl

4.	S4	23,922	33 (8,02%)	57,10	Dodecane, 2,6,10-trimethyl-Farnesane
5.	S5	8,540	1 (0,40%)	121,10	Benzoic Acid
		17,311	5 (1,27%)	55,10	9-Octadecanoic acid
		19,902	8 (0,57%)	149,10	1,2-Benzenedicarboxyclic acid
6.	S6	2,701	1 (0,24%)	68,05	1-Limonene
		5,003	2 (0,11%)	57,10	Octane, 2,4,6-trimethyl
		8,218	3 (1,83%)	57,10	Octane, 2,4,6-trimethyl
		10,566	6 (0,23%)	57,10	Eicosane
		11,251	9 (8,31%)	57,10	Dodecane, 2,5-dimethyl
		13,851	15 (0,41%)	55,10	5-Eicosane
		16,207	28 (0,40%)	57,10	5-Eicosane
		17,882	39 (0,37%)	55,10	5-Eicosane
		18,070	41 (0,80%)	55,10	5-Eicosane
		19,391	50 (0,53%)	55,10	5-Eicosane
		50,521	57 (0,54%)	55,10	5-Eicosane
		24,568	100 (2,10%)	57,10	Eicosane

Description:

S1 = Penetration Testing on artificial membranes Whatman first hour.

S2 = Penetration Testing on artificial membranes Whatman second clock.

S3 = Penetration Testing on artificial membranes Whatman third hour

S4 = Penetration Testing in mouse skin first hour

S5 = Penetration Testing on a second clock mouse skin

S6 = Penetration Testing in mouse skin third hour

CONCLUSION

From the research that has been done can be concluded that the ethanol extract gel of nut-grass rhizome can penetrate the membrane, seen from the data component equal penetration test results with extracts. In the extracts,

various components of the essential oil were detected among which caryophyllene Oxide, Sphatulenol, Alloaromadendrene, Iso - velerenal, 1 - Limonene, Globulol, Beta - Guaiene, and beta - citronellol and other constituents of the essential oil. On the results of penetration tests detected some constituents of the essential oil that is equal to the extract. That is, the essential oils of rhizome sedges in the preparation of the gel can penetrate through the membrane, namely, membranes Whatman No. 1 and the skin of mice.

SUGGESTION

Advised on further research to test the penetration power rhizome extract gel sedges by the addition of penetration enhancers and analyzed quantitatively.

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