

## Cream Formulation of *Cyperus rotundus* L Rhizome Extract for Joint Pain Treatment

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### ABSTRACT

The research about formulation ethanol extract of *Cyperus rotundus* L. rhizomes in the dosage form of cream and preparation have been done to test the activity of joint pain. In this research uses three formulas (F1, F2, and F3) with concentration 3%, 5% and 7% of ethanol extract of *Cyperus rotundus* L rhizomes. The formulas were evaluated for their organoleptic, homogeneity, type of cream, pH, washability, stability of temperature, particle size distribution, skin irritation,. The test of activity of treatment to the joint pain has done to the male albino rats which induced by AgNO<sub>3</sub> 1% as the pain inductor via intraarticular. Parameters observation of pain reflex is amount of experimental animals given squeaks flexion movement 10 times for 1 min were observed in time to 30 minutes; 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, and 14 hours. Formula ethanol extract of *Cyperus rotundus* L rhizomes in cream dosage form physically stable at all concentrations. Based on the results of statistical calculations, faster loss of reflexes is given by a group of formula 3 in the 4th hour of observation compared with other groups. Furthermore, the penetration test formula 3 *in vitro* using Franz diffusion cells. This study uses two membrane penetration, which Spangler membranes and skin of mice were observed for 3 hours with an interval of 1 hour. The test was done to see penetration component of essential oil compounds ethanol extract of *Cyperus rotundus* L rhizomes in the cream. Essential oil of extract of *Cyperus rotundus* L rhizomes was analyzed by GC-MS (Gas Chromatography Mass Spectroscopy) is used as a comparison to the results of penetration tests. On the results of penetration tests detected some constituents of the essential oil in the fluid to extract the same recipient, meaning extract of *Cyperus rotundus* L rhizomes in cream can penetrate through the membrane.

**KEY WORDS:** Cream, *Cyperus rotundus* L. rhizomes, ethanol extract, joint pain, difusi Franz.

### 1. INTRODUCTION

The number of people with arthritis or other chronic joint disorders in the United States continues to increase. According to the Arthritis Foundation in 2006, in 1990 there were 38 million people and in 1998 nearly 43 million, or 1 in 6 people in America suffer from joint disorders and in 2005 the number of people with arthritis has reached 66 million, or 1 in 3 people suffer from joint disorders. A total of 42.7 million of which has been diagnosed as arthritis and the remaining 23.2 million are patients with chronic joint pain. While in Indonesia, according Nainggolan 2009 national prevalence of arthritis was 32.2%.

Arthritis or rheumatism is commonly referred to as one of the causes of joint pain, especially the small joints in the wrist and fingers. Arthritis is caused by damage to the body's autoimmune system produces substances that cause inflammation, especially in the joints. Various ways that can be done to get a pain reliever for example Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and other analgesic drugs. Analgesics or pain relievers are substances that reduce or block the pain without losing consciousness. But these drugs have side effects such as stomach ulcers, gastrointestinal bleeding, can also damage the kidneys, liver and others (Katzung, 1995).

Along with the growing awareness of the side effects of chemical products, then grow the awareness of the importance of natural medicine as a natural product that is considered safer, cheaper and a little side effects. One of the plant known as pain medication is *Cyperus rotundus* L. rhizomes. It's included in the family Cyperaceae, where part of the plant that is often used is the rhizome (Moh, 1980). Ethanol extract of *Cyperus rotundus* L. rhizomes contains chemical components include phenolic compounds, flavonoids, glycosides, saponins, alkaloids, and tannins (Sivalan, 2012). Meanwhile, according to Rahim, 2014 based on the results of GC-MS analysis of the preparation of ethanol extracts of *Cyperus rotundus* L. Rhizomes mask peel off ethanol extract contain compounds of  $\alpha$ -gurjunene, spatulenol, caryophyllene oxide and aristolenepoxide. It's found a derivatives compound of sesquiterpene in the result of 70% ethanol extract of *Cyperus rotundus* L. Rhizomes isolation. The sesquiterpenes proved to have analgesic effects (Jin, 2011).

Based on the results of the above description, the researchers tried to formulate the ethanol extract of *Cyperus rotundus* L. rhizomes into a transdermal preparation in the form of a cream, so it can be easily applied to the treatment of pain. Transdermal is one way of giving a drug that is used on the skin surface, which is capable of delivering drugs into the body through the skin to obtain a systemic effect. The cream preparations is choosen because it has the advantage such as have attractive shape, simple in its construction, easy to use, giving a sense of cold on the skin (Moh, 1995). Methods of testing activity for the treatment of joint pain is using analgesics screening method for joint pain. In testing, the male rats is used as experimental animals. The best formula conducted a *in vitro* penetration test using Franz diffusion cells, with paper Whatman No 1 and the mouse skin as a membrane penetration and then analyzed for volatile oils using GC-MS instrument

**2. MATERIALS AND METHODS**

**Materials :** *Cyperus rotundus* L. rhizomes, 95% ethanol, aquadest, AgNO<sub>3</sub>, comparative preparation, stearic acid, glycerin, sodium tetraborat, triethanolamine, nipagin, nipasol and reagen of indentification for phytochemical test, Spangler liquid (oleat acid 15 %, stearic acid 5 %, VCO 15 %, squalen 5 %, parafin 10 %, cholesterol 5%, vaselinum album 15 %, olive oil 25%), whatman No 1, skin of mice.

**Test Animal:** Tes animal used were male albino rats, weight 200-300 g. They were acclimatized for at least one week.

**Preparation of ethanol extract of *Cyperus rotundus* L. Rhizomes:** *Cyperus rotundus* L. rhizomes was taken in the district of West Air Tawar, Padang. Samples are cleaned, ground, and weighed as much as 3.3 kg and then macerated with ethanol 95% for 3x24 hours. Results maceration is filtered and evaporated with a rotary evaporator to get a crude extract in thick mass.

**Preparation of cream base and cream with ethanol extract of *Cyperus rotundus* L Rhizomes:** The composition of the cream base and the cream with ethanol extract of *Cyperus rotundus* L Rhizomes is shown in Table 1 and Tabel 2.

**Preparation of Cream Base:** In this study, the cream base was prepared by the addition of aqueous phase to the oily phase with additional agitation. To prepare the base, an oily phase that consisted of VCO, stearic acid, and nipasol was heated up to 70°C. At the same time, aqueous phase consisting of aquadest, glycerin, sodium tetraborate, triethanolamine and nipagin was heated to the same temperature. Then, the prosedure was continued exactly as described above for preparation of the base.

**Preparation of Cream with *Cyperus rotundus* L. Rhizomes:** The ethanol extract of *Cyperus rotundus* L. Rhizomes put in a mortar, add the cream base for each formula bit by bit and then crushed until homogeneous. Then each formula is stored in a container of cream.

**Characterization of the cream base and cream with ethanol extract of *Cyperus rotundus* L. Rhizomes:** Characterization of the cream base and cream with ethanol extract of *Cyperus rotundus* L. rhizomes include ; organoleptic, homogeneity, type of cream, washability, stability of temperature, particle size distribution and skin irritation.

**Activity Test to Treatment of Joint Pain In Male Albino Rats:** Test animals that used were male albino rats were induced by injecting a solution of AgNO<sub>3</sub> 1% into the joint tibio tersienne. After 18 hours of inducing, do flexion in the swollen joint. Animals that squeak when performed flexion movement of the joints to swell as much as 10 times in 1 minute is an animal that can be used in this study. Animals that have been selected will be grouped into 6 groups: group I was just given a positive control inducers solution of AgNO<sub>3</sub> 1%, group II (base cream/F0), Group III (F1), group IV (F2), group V (F3 ) and group VI (comparative).

Then each group was given a treatment that is applied to the preparation on the swollen joints. Parameters measured were the number of rats squeaks by flexion 10 times in 1 minute which made the hour-30 minute, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 14 hours after administration preparations. Test preparation is stated to be analgesics for joint pain when the animals do not squeak in pain by flexion movements performed.

**Penetration Testing: a.** Penetration Test creamy of extract *Cyperus rotundus* L rhizomes. Diffusion tool used is the Franz diffusion cell. The receptor compartment filled with a solution of NaCl 0.9% until full. Enter a stirrer, weigh as much as 150 mg dosage then applied to each membrane and placed on a Franz diffusion cell apparatus. After that, cover with a glass lid equipped with a clamp. Franz diffusion cell is placed in a glass vessel equipped with a thermometer. The glass vessel placed on a magnetic stirrer. Through a plastic hose stream of water from a thermostat with circulation at  $37 \pm 0.5$  °C in to glass vessel. Magnetic stirrer is turned on and set a scale rotation of 100 rpm, and then the samples were taken on the hour to 1, 2, and 3 by 5 mL of the receptor compartment using a measuring pipette, each sampling immediately added a number of fluid equal with the same volume. Then the sample is introduced into a 5 mL volumetric flask.

**b. GC-MS analysis:** Test Ethanol Extracts Essential Oils *Cyperus rotundus* L rhizomes. 1 gram of ethanol extract of *Cyperus rotundus* L rhizomes mixed with 5 mL of hexane. Enter into the vial, shake a few times, and then let up overnight. Take 2 mL of fluid is injected into the gate injecting Gas Chromatography-Mass Spectrophotometer.

Penetration Test Results Ethanol Extract Cream *Cyperus rotundus* L rhizomes Mix 5 mL of receiver fluid penetration test results with measurable by 5 mL of hexane. Put it in a vial, shake a few times, and then let up overnight. Take 2 mL of fluid is injected into the gate injecting Gas Chromatography-Mass Spectrophotometer.

**GC-MS instrument conditions as follows:**

Type column	:	RTU5MS
Column length	:	30meters
Diameter column	:	0:25millimeters

Column temperature	:	60-300°C
Injector temperature	:	300°C
Detector temperature	:	300°C
The speed of temperature rise	:	10°C/min
Carrier gas	:	He 123.3 Kpa

### 3. RESULTS

From the extraction process 3.3 kg of *Cyperus rotundus* L. rhizomes thick extract obtained as much as 353.8 g with yield 10.72%. The yield obtained accordance with requirements Herbal Pharmacopoeia Indonesia in 2008, not less than 10.3%. Identification of the ethanol extract of the *Cyperus rotundus* L. rhizomes consecutive observations obtained as follows: the form of the thick extract, brown, specific smell and taste somewhat bitter. Ash content obtained in identification was 0.85%, the loss drying is 8.26% and the results of pH measurement ethanol extract of *Cyperus rotundus* L. rhizomes is 5.43. Test results of chemical constituents of ethanol extract of *Cyperus rotundus* L. rhizomes positively to flavonoids, saponins, terpenoids and alkaloids, in accordance with the results shown by Sivalan 2012.

At this formula is added virgin coconut oil (VCO) as penetration enhancers. VCO contains 92% saturated fatty acids comprising 48-53% lauric acid (C12), 1.5-2.5% oleic acid and other fatty acids such as 8% caprylic acid (C: 8) and 7% capric acid (C: 10) (Enig, 2007). According Santoyo and Pygartua, 2000, oleic acid and lauric acid can increase the percutaneous absorption of piroxicam *in vitro*. Allegedly VCO can increase the penetration through increased hydration of the skin or through the help of fatty acids short chain easily across the skin membrane (Lucida, 2008).

Evaluation of cream base and cream with ethanol extract of *Cyperus rotundus* L. rhizomes 3%, 5% and 7%, which includes the organoleptic, homogeneity, type of cream, cream pH, washability, stability of temperature, particle size distribution and the irritation test. The observation of the evaluation showed that the formula is physically stable during storage of six weeks and no formulas that result in irritation. Recapitulation of the results of the evaluation of base cream and cream with extract ethanol of *Cyperus rotundus* L. rhizomes can be seen in Table 3.

The pH human skin typically range from 4.5 to 6.5 (Osol, 1975). The result of pH measurement of cream base above normal skin pH is 7.52. While cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained pH values in the range of normal skin pH. Irritation test results on volunteers showed none of the cream with ethanol extract of *Cyperus rotundus* L. rhizomes that cause irritation to the skin of volunteers.

Test on the stability of the cream at cold temperatures (4-8°C) and at room temperature is applied on cream base formula and cream of extract ethanol of *Cyperus rotundus* L. rhizomes. According to Lachman, 1994 emulsion systems at high temperatures can lead to an increase in kinetic energy of the droplets of the dispersed phase so as to facilitate merger and an increase in the size of the diameter of the globule. While the cold temperature solubility and emulsifying the oil phase in the water phase will be reduced so that the effectiveness of the emulsifier to coat globules to be reduced. Test of the stability of the base cream and cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained results of the test of all formulas is stable at room temperature and cold temperatures.

From test of the both type of the cream, then obtained cream with the type of o/w. This test used methylene blue which have water soluble properties. The result shows the uneven spread of methylene blue after dropped on a layer of cream on the glass objects. Test washability using distilled water and the result show that greater the concentration of ethanol extract of *Cyperus rotundus* L. rhizomes, the more amount of distilled water needed.

Measurement of particle size distribution is done by using a tool microscope equipped with a microscope eyepiece. Particle measurement cream of ethanol extract of *Cyperus rotundus* L. rhizomes obtained tends to have the distribution is not symmetrical but this result still qualify stable particle size is 1-50 µm (Lachman, 1994).

The formula of cream cream of ethanol extract *Cyperus rotundus* L. rhizome was tested on the activity of joint pain treatment by using the screening method analgesics for joint pain. Parameters measured were amount of male albino rats squeak by flexion in the joints that have been induced by 1% AgNO<sub>3</sub>. AgNO<sub>3</sub> is a heavy metal that can precipitate proteins. These deposits will cause pain in the joints, AgNO<sub>3</sub> can also decompose into NO<sub>2</sub> which is a free radical. The free radicals which, if it formed would trigger an inflammatory response in the joints. Results of calculation of the number of rats squeaks after administration cream of ethanol extract of *Cyperus rotundus* L. rhizomes can be seen in Figure 1.

Observations average number of the most rapid disappearance of pain reflex occurs in the formula F3 with a concentration of 10% during the 4<sup>th</sup> hour, so it can be concluded that the rat had recovered from joint pain. Compared to the control (+) and F0 were given a cream base alone during the 14th hour observation still provide pain reflexes with the average amount squeaks respectively 4 and 2.33. While the formula F2 (5%) healing joint pain occur at the 6th hour and F1 (3%) recovery time to 10 hours. Rats that were given a comparative, pain reflex was lost at the 8th hour.

Based on the results of one-way ANOVA statistical analysis significant difference from cream of ethanol extract of *Cyperus rotundus* L. rhizomes with elevated levels of the extract given by ( $p < 0.05$ ). Results of calculation followed by Duncan test where in the 30th minute and 1<sup>st</sup> hour from F2 and F3 significantly different from the control, comparative, F0 and F1. At the 1st hour F1 and comparative significantly different from the control and F0. In observation hours 2nd and 4th hour, significantly different from the F3 F2, F1, F0, comparative and control. F2 is also significantly different from F1, F0, comparison and control. While F1 and the comparative was not significantly different, F0 and controls did not differ significantly. At the 6th hour F3 and F2 did not differ significantly, F0 and controls did not differ significantly, while F1 and comparative significantly different. Observations on the hour to-8 F3, F2 and the comparative were not significantly different, whereas F1 significantly different from F0 and control, F0 significantly different from controls. Observations average number of the most rapid disappearance of pain reflex occurs in the formula F3 with a concentration of 7% during the 4th hour, so it can be concluded that the rats had recovered from joint pain. Compared to the control (+) and F0 were given a cream base alone during the 14-hour observation still provide pain reflexes with the average amount of squeaks respectively 4 and 2.33. While the formula F2 (5%) healing joint pain occur at the 6th hour and F1 (3%) recovery time to 10 hours. Rats that were given a dosage comparative, pain reflex was lost at the 8<sup>th</sup> hour.

Essential oil of ethanol extract of *Cyperus rotundus* L rhizomes analyzed using GC-MS that is used as a comparison to the results of penetration tests, at the extract was detected 16 compounds which 8 kinds of essential oils and 8 types of constituents of the essential oil. Essential oils that are detected are Spathulenol, - (-) caryophyllene oxide, Alloaromadendrene, Iso-veleranal, 1-Limonene, Globulol, Beta-guainene, and beta-citronellol, where spathulenol, - (-) caryophyllene oxide, alloaromadendrene, iso-veleranal, globulol, and beta-guine is a compound essential oils class sesquiterpen while beta-citronellol and 1-limonene is an essential oil group monoterpenes, which is an essential oil group hydrocarbon sesquiterpene that has efficacy as an analgesic that is Spathulenol, - (-) caryophyllene oxide (Rahim, 2014).

Spathulenol detected by two peaks, the first peak at a retention time of 9.956(0.30%) and a second peak at a retention time of 10.056 (0.17%) with a molecular weight of 220, caryophyllene detected with 6 peak is at a retention time of 10.280 (0.08%); The second peak with a retention time of 10.726 (1.48%); The third peak with a retention time of 10.800 (0.71%); The fourth peak retention time of 11.773 (0.18%); fifth with 12.563 (0.39%); and the sixth peak with a retention time of 15.893 (0.43%) with a molecular weight of 220. Alloaromadendrene 2 peak is detected at a retention time 9.113(0.15%) and 14.658 (1.10%) with a molecular weight of 204, hereinafter that isoveleral the first peak is at a retention time of 12.922(1.69%) and a molecular weight of 232. the 1-limonene 1 peak with the retention time 2,694 (0.02%) with a molecular weight of 136. Globulol had a retention time of 11.838 (0.27%) and a molecular weight of 222. beta-guine had a retention time of 10.133 (0.12%) and a molecular weight of 204, while the beta-citronellol with a retention time of 10.219 (0.1%) and a molecular weight of 156, can see in table 4-5, Figure 2-9.

Furthermore, fluids of the recipient of the membrane Whatman No. 1 and the skin of mice was analyzed using GCMS, so that detected some constituents of the essential oil as detected on extract of *Cyperus rotundus* L rhizomes, on the chromatogram S1 detected five types of constituents of the essential oil that is equal to the extract that is, Decane, Dodecane, Heptadecane, octadecane, Eicosane, In the chromatogram S2 detected one type of constituents of the essential oil extract that is the same with Tridecane, this may occur because of agitation. S3 on the chromatogram detected five types of constituents of the essential oil that is equal to the extract that is, Hexadecane, Eicosane, Decane, Dodecane, and Tridecane. This indicates that the ethanol extract of *Cyperus rotundus* L rhizomes in the cream can pass through the membrane Whatman no 1, as indicated by the constituents of essential oil compounds were detected in the fluid penetration time receiver with 1, 2, and 3 hours.

In the chromatogram S1 ' no essential oil component is detected, the chromatogram S2' detected six types of constituents of the essential oil extract that is similar to Decane, Eicosane, octadecane, Heptadecane, Dodecane, and Tridecane. In the chromatogram S3 ' detected two types of constituents of the essential oil extract that is similar to Decane and Dodecane. This indicates that the ethanol extract in creams can penetrate through the membrane of mouse skin, which penetrates the cream preparation begins at 2 and 3 hours.

From the results of penetration testing ethanol extract of *Cyperus rotundus* L rhizomes in cream that has been analyzed using gas chromatography mass spectrometry is apparently a difference where the membrane that uses Whatman No. 1 results fast penetration better than using mouse skin, wherein the membrane Whatman No. 1 shows the essential oil component is detected on the receiving fluid the first hour, the second and third hours. While in the skin membrane of mice in the first hour, the compound components essential oil is not detected, but in the second hour and three components of essential oils has been shown, this occurs because one of the factors that affect the penetration that the thickness of the skin, where the membrane mouse skin thicker and also its structure is more complex than Whatman no. 1, so that the active substance or a longer time to penetrate the skin of mice.

Compounds detected from the entire receiver fluid that has been analyzed using Gas Chromatography Mass Spectrometer only constituent of essential oil compounds, because the essential oil is not a pure compound but rather a mixture of organic compounds which usually consists of several components that the majority come from terpenoids.

**Table.1. Composition of Cream Base**

Compound	Formulation (Weight %)
VCO	10
Stearic acid	14,136
Glycerin	9,995
Sodium tertraborate	0,248
Triethanolamine	0,995
Nipagin	0,1
Nipasol	0,05
Aquadest ad	100

**Table.2. Composition of Cream with Extract Ethanol of *Cyperus rotundus* L Rhizomes**

Compound	Formulation			
	F0	F1	F2	F3
Ethanol Extract of <i>Cyperus rotundus</i> L. rhizomes	-	3%	5%	7%
Base ad	100%	100%	100%	100%

**Table.3. Recapitulation of the evaluation**

No	Evaluasi	Pengamatan			
		F0	F1	F2	F3
1.	Organoleptic				
	-Form	SS	SS	SS	SS
	-Smell	NO	S	S	S
	-Color	W	B	B	B
2.	Homogeneity	H	H	H	H
3.	pH	7,52	5,51	5,48	5,49
5.	Irritation Test	-	-	-	-
6.	Stability :				
	-Room temperature	St	St	St	St
	-temperature 0 - 4°C	St	St	St	St
7.	Type of cream	o/m	o/m	o/m	o/m
8.	Particle size distribution	20.643µm	19,346µm	19,673µm	21,299µm
9.	Washability	12 ml	16 ml	18 ml	22 ml

**Information:** SS: Semisolid, NO: No smile, B: Brown, W: White, S: Spesific, - : No irritation, H: Homogeneous, St: Stable.

**Table.4. Component of essential oil of ethanol extract of *Cyperus rotundus* L rhizomes analysed using GC-MS**

No.	Retention time (minute)	Peak (% area)	Base (m/z)	Component
1.	2.694	2(0.02%)	68.05	1-Limonene
2.	4,998	20(0.71 %)	57.10	Decane
3.	8.061	45(1.11 %)	57.10	Tridecane
4.	8.217	46(0.88 %)	57.10	Tridecane
5.	9.113	48(0.15 %)	108.15	Alloaromadendrene
6.	9.956	61(0.30 %)	119.15	Spathulenol
7.	10.056	62(0.17 %)	119.15	Spathulenol
8.	10.133	63(0.12 %)	105.15	-beta-Guaiene
9.	10.219	64(0.10 %)	69.10	Beta-Citronellol
10.	10.280	65(0.08 %)	43.10	-(-)Caryophyllene oxide
11.	10.726	69(1.48 %)	43.10	-(-)Caryophyllene oxide
12.	10.800	70(0.71 %)	41.10	-(-)Caryophyllene oxide

13.	11.773	83(0.18 %)	55.10	-(-)Caryophyllene oxide
14.	11.838	84(0.27 %)	43.10	GLOBULOL
15.	12.563	94(0.39 %)	43.10	-(-)Caryophyllene oxide
16.	12.925	98(1.69 %)	41.10	Iso-Velerenal
17.	13.597	108(0.51 %)	43.10	Dodecane
18.	13.968	111(1.27 %)	43.10	Dodecane
19.	14.658	116(1.10 %)	41.10	Alloaromadendrene
20.	15.811	130(0.47 %)	57.10	Octadecane
21.	15.891	127(0.43 %)	149.20	-(-)Caryophyllene oxide
22.	16.754	141(0.11 %)	57.10	Hexadecane
23.	18.705	163(0.10 %)	57.10	Octadecane
24.	18.869	166(0.07 %)	57.10	Eicosane
25.	19.089	170(0.09 %)	57.10	Hexadecane
26.	20.110	180(0.11 %)	43.10	Eicosane
27.	20.230	181(0.07 %)	57.10	Hexadecane
28.	21.240	193(0.13 %)	57.10	Heptadecane
29.	21.523	197(0.21 %)	55.10	Decane
30.	24.307	226(0.26 %)	57.10	Oxirane

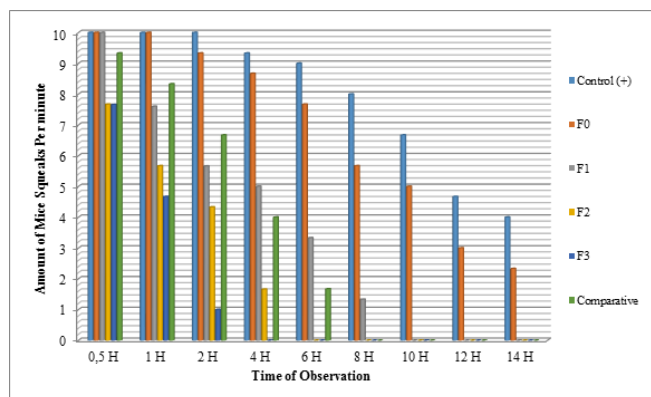
Table.5.M/Z data of essential oil in ethanol extract *Cyperus rotundus* L rhizomes detected GC-MS

No	Retention time (Minute)	Peak (%area)	BM	Base m/z	Component
1a	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 %)			
3a	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*,82,95,109,123,138,156	Beta-Citronellol

Table.6. Composer components recapitulation essential oil of penetration fluid detected with GC-MS

Component	S1	S2	S3	S1'	S2'	S3'
Decane	+	-	+	-	+	+
Dodecane	+	-	+	-	+	+
Tridecane	-	+	+	-	+	-
Heptadecane	+	-	-	-	+	-
Hexadecane	-	-	+	-	-	-
Eicosane	+	-	+	-	+	-
Oxirane	-	-	-	-	-	-
Octadecane	+	-	-	-	+	-

**Information:** S1 = Membranes Whatman at first clock, S2 = Membranes Whatman at the second clock, S3 = Membranes Whatman at the third clock, S1 ' = Membrane skin of mice at first clock, S2 ' = Membrane skin of mice at the second clock, S3 ' = Membrane skin of mice at the third clock, + = Detected, - = Not Detected



**Figure.1. The Result of the Test of Activity for Treatment of Joint Pain In Male Albino Rats**

#### 4. CONCLUSION

Based on research that has been done, it can be concluded that the ethanol extract of *Cyperus rotundus* L. rhizome can be formulated in cream dosage forms physically stable at all concentration. In the reflex decrease joint pain in F3 group with 7% extract concentration can treat joint pain better than other groups F0, F1 (3%), F2 (5%) and comparison. In the penetration testing the components of the extract in cream can penetrate *in vitro* using Franz diffusion cells with paper Whatman no 1 and the mouse skin as a membrane penetration is shown in the recipient liquid chromatogram. Of the two membranes used the ethanol extract of the rhizome in cream faster penetrated the membrane Whatman no 1 than mouse skin membran.

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