Analgesic Activity of Thyme (*Thymus vulgaris* L.) Leaf Extract Using Chemical Stimulus Method in Male White Mice (Mus musculus)

Annik Megawati[1*], Yanulia Handayani[2], Dian Arsanti Palupi[3], Nazir Ahmad[4], Veny Ayu Andini[5]

[1-3,5]Department of Pharmacy, Institut Teknologi Kesehatan Cendekia Utama Kudus, Central Java, Indonesia

[4]Department of Pharmacy, Abasyn University Islamabad Campus - 44000, Pakistan

*Corresponding Author: annikmegawati33@gmail.com

Abstract. Pain is an uncomfortable feeling that can also be felt by every individual who has a subjective nature, which can affect the mind and can change life, pain or pain in a part of the body is a problematic pain in one part of the body, Pain can be overcome by the use of analgesic drugs, which can reduce or relieve pain without losing consciousness. Analgesic drugs have a working mechanism that is by affecting the threshold of awareness of the feeling of pain. Analgesic drug groups are divided into 2, namely opioid and non-opioid analgesics. The design of this study is *Post test Only Control group Design.* This study was conducted using 24 mice as test animals divided into 4 groups. K1 was given CMC-Na 0.5%, K2 Na-Diclofenac 13 mg, K3 thyme leaf extract dose 25 mg, K4 was given thyme leaf extract 50 mg, Mice were induced by 1% acetic acid intraperitoneally . Mice were treated orally, then the number of wriggles was counted every 5 minutes for 30 minutes and the % analgesic power was calculated. The data obtained were analyzed by One Way *Anova and Post Hoc* test . The number of wriggles was 151.67, the percentage of analgesic power was 25.28%, the results of statistical tests showed that each treatment group had an average number of mice wriggles within 1 hour that did not differ significantly (p = 0.654). From the results of the percentage of protection power of mice wriggling for 1 hour, it showed that a dose of 25 mg had a percentage analgesic power of 25.28%, which was the optimum dose that had the best analgesic ability. Thyme leaf extract *(Thymus vulgaris* L. *)* had analgesic activity in white male mice induced by 1% acetic acid. The optimum dose of 25 mg had the highest analgesic power of 25.28%.

Key words: 1% acetic acid, analgesic power, thyme leaves, number of writhing, mice *(Mus musculus)*

INTRODUCTION

Pain is an uncomfortable feeling that can also be felt by every individual who has a subjective nature, which can affect the mind and can change life, pain or pain in a part of the body is a problematic pain in one part of the body, pain stimulation can be caused by temperature stimulation and mechanisms or chemical stimulation that can cause damage to tissue and which can release substances commonly called mediators (Zulkifli & Octaviany, 2019).

According to *the International Association for the Study of Pain* (IASP), pain can be classified based on the region of the body involved (e.g., head, visceral), the duration of the pain (acute or chronic), or the dysfunction of the system that causes the pain (e.g., gastrointestinal, nervous). However, it is recommended that pain be classified based on only three characteristics: symptoms, mechanisms, and syndromes (Jamal *et al.*, 2022).

Pain can be managed with analgesic drugs, which can reduce or relieve pain without losing consciousness. Analgesic drugs work by affecting the threshold of awareness of pain. Analgesic drugs are divided into two groups: opioid analgesics, commonly known as narcotics, and parietal or non-opioid analgesics. Analgesics are a group of drugs that have properties similar to narcotic and non-narcotic analgesics (Mita & Husni, 2017).

Based on the identification of research on herbal plants that can have benefits that are used as traditional plants in the field of medicine, and which are not widely known from the efficacy of this plant, one of them is the thyme leaf plant *(Thymus vulgaris* L. *)* is an aromatic herbal leaf plant that has a very wide source of benefits in the fields of health, herbal industry, cosmetics, and culinary. Thyme leaves are generally known to have benefits for relieving coughs and sore throats, as well as pain but can also be used to treat bronchitis, flu, asthma, and other upper respiratory tract infections. Thyme leaves can potentially be a medicine for diabetes and other related complications because they have

anti-hyperglycemic and antilipidemic effects, while in the food and cosmetics industry, thyme essential oil can be used as a preservative and antioxidant

The most important parts of thyme leaves that are used as raw materials for the pharmaceutical and cosmetic industries are the leaves and stems because these two parts are the ingredients for extracting thyme essential oil, the results of which will later be made into medicine in the form of oil that can be used as a pain reliever, in addition to its function as an analgesic, the essential oil content can also be used as an antioxidant, antibacterial, carminative, expectorant and antispasmodic (Safrina *et al.,* 2021).

METHODS

This research is an experimental study conducted in a laboratory to observe the analgesic effect of thyme leaves *(Thymus vulgaris* L. *)* on male white mice *(Mus Musculus).* The design in this study uses the *Randomized Control Group Posttest Only Design method.* Where there are 4 test groups in this study, namely the negative control group, the positive control group, treatment group 1 and treatment group 2, which were given the same treatment. This study only examined one independent variable, namely the variation in the dose of thyme leaf extract on the dependent variable, namely analgesics in test animals in the form of the number of writhing.

The materials used include: Male mice of the Mus Muscullus type (Sentat *et al.,* 2016), Sodium Diclofenac chemical, thyme leaves *(Thymus vulgaris* L*)* , pellet feed, 1% acetic acid, magnesium powder (Mg), 1 ml concentrated hydrochloric acid (HCl), and concentrated H_2SO_4 solvent, distilled water, CMC Na 0.5%. The tools that will be used include a drying cabinet, blender, mouse cage, mortar and stemper, glass beaker, Erlenmeyer, stirring rod, cup , *water bath* , dark glass jar, analytical balance, measuring cup, syringe, mouse probe, *hand scone* , dropper pipette, test tube, wooden test tube rack.

The preparation of thyme leaf extract is done by maceration using 70% ethanol. Prepare 609 grams of thyme leaf powder into the extractor container then add 70% ethanol solvent with a ratio of 1:10 (Ariawan *et al.*, 2022). Evaporator at 45°C. After that, pour the extract into a porcelain cup and evaporate the remaining 70% ethanol with a waterbatch water bath then weigh the obtained extract and put it into a glass container.

The phytochemical screening and analgesic activity test in this study included the flavonoid test. In the analgesic activity test, 24 mice were divided into 4 test groups: a negative control group, a positive control group, a 25 mg thyme leaf extract dose group, and a 50 mg thyme leaf extract dose group. The negative control group in this study used CMC-Na and the positive group used Na Diclofenac. Mice that were ready to be tested were given a 1% acetic acid preparation according to their respective groups intraperitoneally, then given the test preparation orally. Observe the mice's writhing at 5-minute intervals for 30 minutes. All data obtained were analyzed statistically using SPSS and calculated for analgesic power.

RESULTS AND DISCUSSION

The results of the water content test of the thyme leaf extract were 5.34%. These results indicate that the water content of the thyme leaf extract has met the existing requirements, which is less than 10%. Based on Voigt R.'s book, determining the content in a good extract should not be more than 10%. If the water content exceeds 10%, it can cause the growth of microbes and tends to be less stable and easily damaged (Voight, 1994).

The maceration method was used to extract thyme leaves in this study. This method was chosen because the procedure and equipment are simple and prevent damage to the compound components due to heating. Maceration optimizes the resulting compounds because the repeated addition of solvents allows for extraction of remaining compounds that may still be recoverable.

The resulting thick extract underwent qualitative chemical analysis to demonstrate that the thyme leaf extract contained flavonoid compounds, characterized by the formation of red, yellow, or orange colors. The flavonoid test results are presented in Figure 1.

Figure 1. Flavonoid Results

Description: results of flavonoid tests that have been tested

Table 1. Results of Compound Content Identification Thyme Leaf Extract

Compound Groups	Reagent	Interpretation	Results
Flavonoid *Wilstater* Test	Concentrated HCl + Mg powder	Orange	+

Information:
(+) : Indicates positive content of active substance compounds
(-) : Indicates negative for containing active substance compounds

After qualitative chemical analysis, analgesic activity was tested on four test groups that had been given induction and observed for writhing every 5 minutes for 30 minutes.

Table 2. Average Number of Mice Wriggling in Each Group For 30 Minutes

Treatment Group	Mean ± SD
Negative Control (-)	172.3 ±6.03
Positive Control (+)	135.83±6.38
Thyme Leaf Extract (P1)	151.67±4.77
Thyme Leaf Extract (P2)	147±5.00

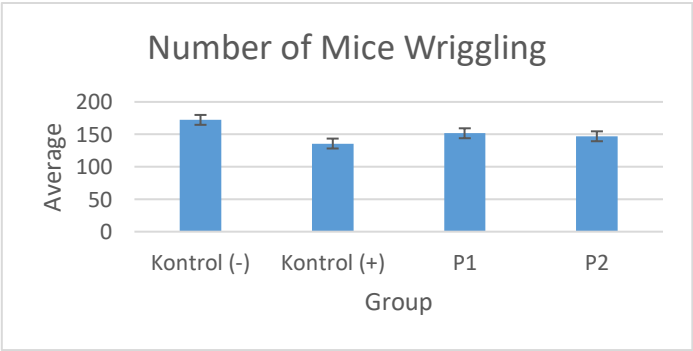

Figure 2. Average Number of Wriggles in Mice

Based on the data above, the highest total number of wriggles was observed within 5 to 30 minutes, and the average number of wriggles was calculated for the four groups. Group 1 had a higher number of wriggles than the other groups, with an average of 172.3. This was followed by group 3 with a total of 151.67 wriggles, using a dose of 25 mg. The highest wriggles were observed in group 1, where the pain was more dominant compared to the groups with doses of 25 mg and 50 mg.

Table 3. Results of Data Normality Test

Treatment group	*Shapiro Wilk*	Homogeneity	*One Way Annova Test*
K+	0.878		
k-	0, 463	0.276	0,000
P1	0.537		
P2	0.806		

In table 3 to determine whether the resulting data is normally distributed or not, the Shapiro-Wilk test was performed. The results obtained are normally distributed data with a significance value (p>0.005) and in the homogeneity test, (p>0.005). To determine the difference in the number of writhing between treatment groups, ANOVA statistical data analysis was performed with a 95% confidence level. The significance value obtained was p = 0.000 (p <0.05) which means Ho is rejected or the data on the number of writhing differs between each treatment group, namely the negative control group (CMC-Na), the positive control group (Na Diclofenac), the 25 mg dose thyme leaf extract group, and the 50 mg thyme leaf extract group. So it can be continued with *the Post Hoc test* LSD test which can be seen in table 4.

Table 4. LSD Test

Percentage of Analgesic Power	**Test Group**	**Signification**
Positive Control	Dose II	0.003
Dose I	Positive Control	0.003
	Dose II	0.654
LSD	Dose I	0.002
Dose II	Dose I	0.654
	Dose II	0.003

The results of the LSD test with a 95% confidence level in Table 4 show significant differences between the treatment groups, except for dose 1 with a dose of 25 mg and dose 2 with 50 mg. This is due to the number of writhing caused by each dose being different from the positive control group given Na Diclofenac. Therefore, it can be stated that the analgesic power of thyme leaf extract is equivalent to Na Diclofenac. Meanwhile, 50 mg thyme leaf extract has a smaller analgesic power compared to the positive control and 25 mg thyme leaf extract. From the group of thyme leaf extract with a dose of 25 mg in the LSD test above, there is a difference with the other groups. When compared with the positive group, there is a decrease in the number of writhing, as well as in the other groups. Therefore, it can be concluded that the analgesic power is better than the test group with a dose of thyme leaf extract with a dose of 50 mg.

Table 5. Percentage of Analgesic Power

Test Group	**Treatment**	**Percentage of Analgesic Power**
II	Positive control (+) Na-Diclofenac	22.64%
III	Thyme Leaf Extract (P1)	25.28%
IV	Thyme Leaf Extract (P2)	24.50%

From the data in Table 5 above, it can be seen that the thyme leaf extract administered was able to suppress pain stimuli in group 3 at a dose of 25 mg. The analgesic power of thyme leaf extract was proven to reduce the number of writhing in two dose variations that have been tested. The content of secondary metabolites contained in thyme leaf extract, such as flavonoids, can affect the number of writhing in test animals. Flavonoids have the potential as analgesics by inhibiting the enzyme cyclooxygenase 1, which plays a role in prostaglandin biosynthesis as an indicator of pain formation, thereby inhibiting COX 1, which can cause inhibition of pain (Safrina *et al.*, 2021).

There are several differences between this study and previous studies, namely environmental conditions, the room temperature used at the start of the test, differences in dosage administration, pharmacokinetic phases that can vary in each test animal, and the ability of the active compounds in thyme leaves. The reason why these varying doses are used is as a benchmark for the ability of thyme

leaf extract when administered to the test animals themselves. If a test material provides a dose-effect relationship, meaning that the higher the dose given, the greater the effect received. From the average number of writhing in this study, namely at a dose of 25 mg thyme leaf extract was able to provide an analgesic effect on test animals.

CONCLUSIONS

Thyme leaf extract *(Thymus vulgaris* L. *)* has been shown to contain flavonoid compounds, which have analgesic activity. Thyme leaf extract *(Thymus vulgaris* L. *)* has been shown to have analgesic activity in mice using a chemical stimulation method. A 25 mg dose of thyme leaf extract *(Thymus vulgaris* L. *)* is the optimal dose as an analgesic inhibitor.

REFERENCES

Ariawan, M. W., Indrayati, A., & Supriyadi, S. (2022). Aktivitas Iron Chelator Ekstrak Etanol Dan Fraksi Daun Putri Malu (Mimosa pudica L) Terhadap Pasien Thalasemia Menggunakan Metode Fic (Ferrous Ion Chelating). Jurnal Fitofarmaka Indonesia, 9(2).

Jamal, F., Andika, T. D., dan Adhiany, E. (2022). Penilaian dan Modalitas Tatalaksana Nyeri. Ked. N. Med, 5(3), 66–73.

Mita, R. S., dan Husni, P. (2017). Pemberian Pemahaman Mengenai Penggunaan Obat Analgesik Secara Rasional Pada Masyarakat Di Arjasari Kabupaten Bandung. (skripsi). Aplikasi Ipteks Untuk Masyarakat, 6(3): 193–194.

Ramadhan, M. F. (2023). Uji Aktivitas Antibakteri Minyak Atsiri Herba Timi (Thymus vulgaris) Terhadap Bakteri Patogen Penyebab Jerawat. (skripsi).

Safrina, D., Herera, B. P., dan Kusumadewi, N. R. R. (2021). Pengaruh lama penyimpanan simplisia Thymus vulgaris L). terhadap rendemen minyak atsiri dan kadar sari. Jurnal Penelitian Agronomi, 5(1): 88–93.

Sentat, T., Pangestu, S., dan Samarinda, A. F. (2016). Uji efek analgesik ekstrak. (skripsi).

Voight,R. (1994). Buku Teknologi Sediaan Farmasi (Edisi V). UGM-Press

Zulkifli, dan Octaviany, E. E. (2019). Uji efek analgetik ekstrak akar binasa (Plumbago indica L.) asal Kabupaten Sidenreng Rappang terhadap mencit dengan metode Writhing Reflex Test. Jurnal Herbal Indonesia, 1(1): 43–49.