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ANALGETIC POWER OF ETHANOL EXTRACT BROTOWALI (TINOSPORA CORDIFOLIA) IN SWISS MICE

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ABSTRACT
Brotowali (Tinospora cordifolia) plants are usually used for various kinds of treatment it is often used as a febrifuge, brotowali stems are proven to contain flavonoid compounds that have benefits in the treatment of various diseases, one of which is analgesic. This purpose is to determine the effectiveness of analgesics on male Swiss mice induced by acetic acid from the ethanol extract of brotowali stem (EETC) doses of 100 mg/Kg BW, 200 mg/Kg BW, and 400 mg/Kg BW. Brotowali stem extraction using maceration method with 70% ethanol solvent. EETC was tested by phytochemical screening and TLC to determine the content of active compounds. The test animals used Swiss male mice, the treatment group was divided into 5, negative control group, positive control, and the dose groups were 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. Testing is done by the writhing test method. Induction of pain stimuli using acetic acid, the number of mice writhing every 10 minutes for 1 hour, and the percentage of analgesic power. Research shows the yield of EETC is 16.35%. The percentage of analgetic power of dose I was 39%, dose II was 47% and dose III was 56% less than the percentage of analgesic power of methampyrone dose of 65 mg/KgBB which was 64%. Conclusion EETC doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW have analgesic activity equivalent to methampyrone at a dose of 65 mg/kg BW, with the largest percentage of analgesic protection at a dose of 400 mg/Kg BW of 56%.

Keywords: Brotowali; Analgesic; Acetic acid; Flavonoids

1. INTRODUCTION

Tinospora cordifolia is a member of the family Menispermaceae, also known as Giloe, Girchi (Hindi), and Amrta (Sanskrit). This plant can be found everywhere (Sumanlata et al., 2019). Tinospora cordifolia is widely used in the ayurvedic system and is known to treat various diseases including anti-spasmodic, anti-inflammatory, anti-rheumatic, hepatoprotective, anti-allergic, and anti-diabetic. Tinospora cordifolia is also used as an immune booster to increase the body's resistance to infection (Goel et al., 2014). Brotowali is also efficacious in the treatment of fractures (A. Sharma & Kaur, 2018).

The active compounds in this plant are diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds of essential oils, a mixture of fatty acids and polysaccharides (Khan et al., 2017). The results of phytochemical screening from several solvents were carried out on Tinspora cordifolia extracts including water and methanol extracts (alkaloids, saponins, phenols, flavonoids, carbohydrates, proteins, and steroids), chloroform extract, and petroleum ether (alkaloids, phenols, flavonoids, carbohydrates, proteins, steroids).), ethyl acetate extract (alkaloids, flavonoids, carbohydrates, proteins, steroids) (P. Sharma et al., 2019). In brotowali stems, there are also several compounds such as tinocordifomin, cordifolisade,

cordifolide, Aromaticamides-Trans-cinnamoyl-2-n-hexanyl-7-methoxynaphthyl amide, trans-cinnamoyl-2-n-pentanyl-6,7-dimethoxynaphthyl amide (methanol extract) (Singh et al., 2021).

In the past, it was very important to find medicinal plants and there was no synthesis of drugs, so only herbal medicines were used to treat all diseases. Biological studies are important to discover more medicinal properties of plants. Traditional plant-based medicines for primary health care in an area of approximately 80% of the world's population. Currently, much research on medicinal plants is being carried out, the main reason is that the synthetic drugs currently taken by humans have many side effects which often lead to serious complications. The development of traditional medicine is mostly done by a primary screening of compounds in plant extracts. Herbal medicine when compared to modern medicine saves a lot of lives. Among 400,000 plant species only 6% of plants are studied for their biological activity and only a few are studied phytochemically (Rukshana, M.S., Doss, A. and Kumari, 2017; Warsinah et al., 2020).

The degree of polarity in selecting the solvent used in the extraction process must be able to dissolve the compound being extracted, easily separated, and purified because it will affect the type and level of bioactive compounds and their activity (Firmansyah et al., 2019). This research is needed to determine its activity and pharmacological properties. Pharmacological activity tests for the analgesic effect of brotowali stems have been carried out (Prayitno & Ahung, 2020) from the water fraction, ether fraction, and ethanol fraction groups showing that the highest percentage of analgesic power was the water fraction with 61% compared to the positive control used, namely methampyrone with a percentage 91% analgesic power.

Based on this, research on brotowali stems (Tinospora cordifolia) was carried out by extraction using 70% ethanol solvent. This study aimed to investigate the analgesic activity of Tinospora cordifolia stems using male swiss albino mice as an experimental animal model. Variation of dose used 100 mg/Kg BW, 200 mg/Kg BW, 400 mg/Kg BW with acetic acid inducer as pain stimulant.

2. METHODS

2.1. Extraction brotowali stem (Tinospora cordifolia)

Brotowali stem Simplicia (Tinospora cordifolia) was obtained from Bugis village, Indramayu Regency, West Java, Indonesia, and the plants were determined at the Mathematics and Natural Sciences Laboratory Unit, Islamic Institute of Sheikh Nurjati Cirebon, West Java, Indonesia. 400 grams of Simplicia was extracted by maceration using 70% ethanol as a solvent, which was obtained and then concentrated using a vacuum rotary evaporator (IKA®), and evaporation of the solvent using a water bath to obtain a viscous extract (EETC) of 65.42 grams with yield 16.35% w/w.

2.2. Phytochemical Screening

In this study, EETC was subjected to phytochemical screening tests, including tests for alkaloids, glycosides, saponins, phenols, tannins, diterpenes, and flavonoids (Pawar et al., 2014).

In alkaloid detection, the extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (potassium mercury iodide), Wagner's reagent (iodine in potassium iodide), and Dragendroff's reagent (potassium bismuth iodide solution).

Saponin detection was carried out by two tests, namely the foam test: the extract was added to 20 mL of distilled water and shaken for 15 minutes. The presence of a 1 cm layer of foam indicates the presence of saponins, and the foam test: 0.5 grams of the extract is added to 2 mL of water and shaken. If the resulting foam persists for 10 minutes, it indicates the presence of saponins.

Phenol detection was carried out by ferric chloride test, the extract was added with 3-4 drops of iron (III) chloride. The presence of a bluish-black color indicates the presence of phenol.

Tannin detection was carried out by the gelatin test, the extract was added with 1% gelatin solution containing sodium chloride. The formation of a white precipitate indicates the presence of tannins.

Flavonoid detection was carried out by two tests, namely the alkaline reagent test: the extract was added with a few drops of sodium hydroxide solution. The formation of a deep yellow color which becomes colorless with the addition of dilute acid indicates the presence of flavonoids, and the lead acetate test: the extract is added with a few drops of lead acetate solution. The formation of yellow color indicates the presence of flavonoids.

2.3. Thin Layers Chromatography

Identification of flavonoids as active compounds that have analgesic activity was carried out using Thin Layer Chromatography (TLC) with silica gel GF_{254} stationary phase and mobile phase using a solution of n-hexane: ethyl acetate: acetic acid ratio of 5:4:1. The results of the TLC plates before and after sprayed with $FeCl_3$ (Warsinah et al., 2020).

2.4. Preparation of Animal Test

The animal's tested in this study were Swiss strain male white mice obtained from the Pharmacology Laboratory of Muhammadiyah School of Pharmacy Cirebon. The test animals were acclimatized in the study room for ± 1 week in cages at room temperature and underwent a 12-hour day-night cycle. Animals are given enough food and drink and have received ethical approval to use test animals with document No. 074/ec.02/kepk-bth/VII/2022 from the Health Research Ethics Committee of Bakti Tunas Husada University, Tawang District, Tasikmalaya Regency, West Java, Indonesia

2.5. Analgesics Activity with Abdominal Writhing Test

Animals tested were grouped into 5 groups, each group consisting of 5 mice. Group I positive control (methampyrone dose 65 mg/Kg BW), group II negative control (Na CMC 0.5%), group III dose 100 mg/Kg BW (EETC100), group IV dose 200 mg/Kg BW (EETC200), and Group V dose of 400 mg/Kg BW(EETC400). Each treatment group was given orally, group I (methampyrone dose 65 mg/Kg BW), group II (Na CMC 0.5%), group III (EETC 100 mg/Kg BW), group IV (EETC 200 mg/Kg BW), and Group V (EETC 400 mg/Kg BW). After 5 minutes of administration, each mouse was induced intraperitoneally with 4% acetic acid. Testing the analgesic activity causing contractions in the abdominal wall, this method is known as the "stretching method test" or the Writhing test. The goal is that the stomach touches the floor of the space it occupies (Adedapo et al., 2014). Evaluation by observing the amount of stretching that occurs every 10 minutes for 60 minutes and calculating the percentage of analgesic power for each group with the Eq 1:

$$\% \text{ Analgesic power} = 100\% - (p/k \times 100\%) \quad (1)$$

P = Cumulative number of mice in the treatment group; K = cumulative number of mice in the negative control group (Hijazi et al., 2017; Kiromah et al., 2021; Syamsul et al., 2016).

2.6. Statistical Analysis

The observations were analyzed using one-way ANOVA with the LSD post hoc test wherever needed and p-value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening and Thin Layer Chromatography

The results of the phytochemical screening of the EETC can be seen in **Table 1**. The results showed the presence of alkaloids, flavonoids, tannins, and saponins.

Table 1. Phytochemical Screening EETC

No.	Tests	Results
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+

The TLC results are depicted in **Table 2**. The Rf value at the EETC spot obtained was almost in agreement with the Rf value of the quercetin standard. The presence of other spots in EETC is due to the presence of other chemical compounds in EETC. The use of quercetin as a comparison is because quercetin is a flavonoid compound that is commonly found in plants that have very strong antioxidant activity (Firmansyah et al., 2019).

Table 2. TLC Result of EETC

Sample	R_f
Quercetine	Spot = 0,925
EETC	Spot 1 = 0,1 Spot 2 = 0,825

3.2. Analgesic activity with acetic acid-induced writhing response

This study examined the analgesic effect of EETC on Swiss male mice. Provision of a pain stimulant, namely acetic acid 4%, because it provides the best pain stimulus from variations in concentration, tested 1% (Hijazi et al., 2017; Kiromah et al., 2021); 0.5% (Syamsul et al., 2016); 0.7% (Jo et al., 2021); and 5% (Winarti & Wantiyah, 2011). Intraperitoneal injection because absorption occurs quickly and constantly, and the effects can last a long time (Mandal et al., 2020).

Analgesic activity testing with the acetic acid-induced writhing test in experimental animals is suitable for detecting central and peripheral analgesia. Acetic acid causes the sensation of pain due to the release of certain endogenous substances such as serotonin, histamine, bradykinin, and prostaglandins (Hussain et al., 2015). Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators such as PGE2 and PGF2alfa. Stomach constriction resulting from acetic acid administration is associated with the sensitization of nociceptive receptors to prostaglandins. The isolate is active as an analgesic, namely the flavonoids contained in the extract provide an analgesic effect by inhibiting the synthesis or action of prostaglandins (Hossain et al., 2009).

Table 3. The Average Number of Mice Writhing for 1 Hour

Animal tests	Number of Mice Writhing for 1 Hour				
	Negative control	Positive control	EETC100	EETC200	EETC400
1	109	27	37	45	33
2	129	20	50	30	41
3	102	94	67	47	45
4	114	35	103	90	55
5	91	15	73	76	67
Average	109	38,2	66*	57,6*	48,2*
SD	14,124	32,089	25,080	24,603	13,161

*p>0,05: there is no significant difference to the positive control

The results showed (**Table 3**) that the negative control group with the highest average number of writhing, 109 ± 14.124 to be precise, came from the average number of mice that stretched for one hour. This happens because the negative control group does not contain active ingredients in its administration. The amount of wriggling of the mice in the positive control group had an average writhing of 38.2 ± 32.089, while the first dose of 100 mg/Kg BW was 66 ± 25.080; dose II 200 mg/Kg BW 57.6 ± 24.603; and dose III 400 mg/Kg BW 48.2 ± 13.161.

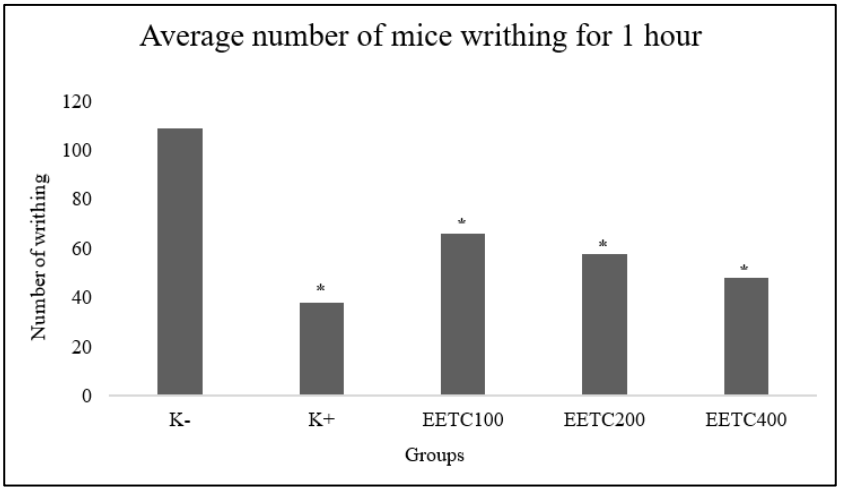

Figure 1. Diagram of The Average Number of Mice Stretching For 1 Hour. K (-) is control negative; K (+) is control positive; EECT 100, EETC200 and EETC400 is Ethanol Extract Tinospora Cordifolia. *p<0.05 versus the negative control group.

The results of statistical analysis using Klomogorov-Smirnov showed that the data were normally distributed p>0.05 (0.120) and were homogeneously distributed p>0.05 (0.552). The results of the ANOVA test showed significant results p<0.05 (0.001) which concluded that there were significant differences between treatment groups. Furthermore, the results obtained from the LSD post hoc test had a significant difference p <0.05 between the negative control group and all treatment groups.

However, in comparison of the amount of writhing between the positive controls and each treatment group at doses I 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW showed no significant difference, this means that the analgesic strength of methamphetamine and the dose group I, II and III are equivalent. Another study showed that the administration of the ethanol extract of Tinospora cordifolia orally produced a statistically significant analgesic effect on NaCl-induced mice (Goel et al., 2014). The findings of our study are also in agreement with the results of Hussain MD et al which showed a significant increase in the inhibition of the writhing response (Hussain et al., 2015). Tinospora cordifolia stem water extract also provides an analgesic effect similar to tramadol at a dose of 5g/Kg (Sumanlata et al., 2019).

Table 4. Results Percent Analgesic Power

Groups	Analgesic Power (%)
Methampyrone	64
EETC100	39
EETC200	47
EETC400	56

The percentage of analgesic power can be seen in **Table 4**. The positive control group, namely methampyrone at a dose of 65 mg/kg BW, had 64% analgetic power and the highest percentage of analgetic power, namely EETC400 at 400 mg/kg BW, was 56%. In **Figure 1** it can be concluded that the greater the concentration of the extract, the greater the potential to reduce pain stimulation. A study by Goel et al stated that the percentage of the analgesic power of the water extract of Tinospora cordifolia was 35.6% compared to the standard, namely 60% pentazocine (Goel et al., 2014). Tinospora cordifolia methanol extract had no significant difference with diclofenac sodium with a 60% analgesic percentage (Hossain et al., 2009; Hussain et al., 2015).

The ability of brotowali stem ethanol extract has significant potential to inhibit pain because it contains flavonoids and alkaloids (Goel et al., 2014; Hussain et al., 2015; Sumanlata et al., 2019). Flavonoids are known as strong antioxidants with a mechanism as an analgesic by inhibiting the action of the cyclooxygenase enzyme so that it will reduce the production of

prostaglandins by arachidonic acid (Hossain et al., 2009). Because prostaglandins are pain mediators, inhibiting prostaglandin synthesis can reduce pain (Salim et al., 2017).

This study has limitations. The results of the number of the writhing of the test animals varied greatly with a high standard deviation. Further investigation is needed to explore the phytochemical constituents of Tinospora cordifolia stems for various conditions and potential therapeutic mechanisms.

4. CONCLUSION

EETC can reduce pain sensation in male Swiss male mice induced by acetic acid with the percentage of analgesic power at a dose of 100 mg/kg BW 39%, a dose of 200 mg/kg BW 47%, and a dose of 400 mg/kg BW 56%. Long-term testing with various doses is necessary to determine the effective dose and evaluate the toxicity of EETC for use in clinical practice as an analgesic. EETC can be used as an alternative analgesic drug from natural ingredients.

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6. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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