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Analgesic Activity Combination of Dry Extract of Meniran Leaves (*Phyllanthus niruri* L.) and Moringa Leaves (*Moringa oleifera* L.) Using the Chemical Induction Method: Invivo Study

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A B S T R A C T

Analgesics are drugs that selectively reduce pain in the central nervous system or peripheral pain mechanisms without significantly changing consciousness. This study aims to determine the analgesic activity of a combination of dry extracts of meniran leaves (*Phyllanthus niruri* l.) and moringa leaves (*Moringa oleifera* l.) against Swiss Webster rats with chemical induction methods. This research proves the presence of secondary metabolite content in the dry extract of meniran leaves and moringa leaves which have analgesic activity. The research used 30 male Swiss Webster rats divided into 5 groups. Group 1 is the negative control (CMC-Na 0.5%), group 2 is the positive control (diclofenac Na), group 3 (Dry extract of meniran leaves), group 4 (Dry extract of moringa leaves) and Group 5 (Combination of dry extracts). meniran and moringa leaves). Each was given 1 mL of 1% acetic acid induction chemical stimulation, then the writhing response was observed and recorded at 5-minute intervals for 30 minutes. The results showed that the K3 group combined dry extract of meniran leaves (*Phyllanthus niruri* L.) and moringa leaves (*Moringa oleifera* L) is not statistically significantly different from the positive control diclofenac sodium, p-value = 0.183. In conclusion, dry extract of meniran leaves (*Phyllanthus niruri* L.) and Moringa leaves (*Moringa oleifera* L.) has been proven to contain secondary metabolites of flavonoids, alkaloids, tannins, and saponins which have analgesic activity.

1. Introduction

Pain is an unpleasant feeling generally due to injury in the body. Pain can also be considered poison in the body because the pain that occurs due to tissue or nerve damage will release various mediators such as H+, K+, ATP, prostaglandin, bradykinin, serotonin, substance P, histamine, and cytocaine. These chemical mediators are what cause discomfort and these mediators are called pain mediators. Analgesics are drugs that selectively reduce pain by acting on the central nervous system or on peripheral pain mechanisms without significantly changing consciousness. Analgesics relieve pain without affecting the cause. If used in excessive doses, it can cause several side effects. The side effects that arise from prolonged use of analgesics are an encouragement for researchers to develop alternative analgesics with better effectiveness and lower side effects. The use of traditional medicine is considered safer than the use of chemical medicine, this is because traditional medicine has relatively fewer side effects than chemical medicine. The use of traditional medicine still requires precision to minimize side effects, including correctness of the medicine, correct dosage, correct time of use, correct method of use, non-abuse, and correct selection of drugs for certain diseases. Herbal plants tend to reduce the risk of side effects because herbal medicines usually have fewer side effects.[1-3]

Many herbal plants are used by the community for medicinal purposes, namely the meniran plant (*Phyllanthus niruri L.*) and the moringa plant (*Moringa oleifera* L.). Both have flavonoid compounds that can function as analgesics. Flavonoids inhibit the cyclooxygenase I enzyme which plays a role in prostaglandin biosynthesis as a mediator in the formation of pain, so inhibiting COX I will result in inhibition of the emergence of pain.[4-7] Based on the above background, research was carried out on the analgesic activity of a combination of dry extracts of meniran leaves (*Phyllanthus niruri* L.) and Moringa leaves (*Moringa oleifera* L.) with the hope of finding candidates for herbal-based analgesic drugs to treat as an analgesic therapy, which in combination can obtain a synergistic effect. that is, a greater effect is obtained compared to the single preparation, where the effect of the first drug therapy will be strengthened by the second drug therapy.

2. Methods

The type of research carried out is quantitative research. This research uses an experimental study using Swiss Webster rats as subjects. This research design uses a post-test-only control group design method because it is not possible to examine variables before treatment but is carried out after treatment (post-test). In this study, the test animals used were white male Swiss Webster rats aged 2-3 months, weighing 20-30 grams, totaling 30 rats divided into 5 groups. Each group consists of 6 animals. Research on dry extracts of meniran and moringa leaves was carried out at the Pharmacognosy and Microbiology Laboratory. Analgesic test research was carried out at the Pharmacology Laboratory of the Institut Teknologi Kesehatan Cendikia Utama Kudus. This research was conducted from February to March 2023 using experimental methods. The tools used in this research include a beaker glass (Herma), stopwatch, 1 ml injection syringe (Teruma Syringe), mouse probe, mouse water bottle, measuring cup (Herma), water bath (Local), 100 ml measuring flask (Phyrex), porcelain cup, mouse cage, wire ram, stamper, mortar, analytical balance (Ohaus), stir bar, handscoon, dropper pipette, test tube clamp, test tube (Herma), volumetric flask (Phyrex), funnel (Herma), moisture balance (Ohaus), stainless steel spoon. The materials used in this research include: 30 male Swiss Webster rats, mouse food, and a dry extract of Niran brand meniran leaves from PT. Industri Jamu Borobudur and dry extract of moringa leaves from the Kelorin brand from PT. Industri Jamu Borobudur, 1 mL concentrated HCl, Mg powder, 1% $FeCl_3$, Mayer reagent, bouchardat reagent, 1% Acetic Acid, 0.5% CMC-Na, Diclofenac Sodium, ethyl acetate, Concentrated H_2SO_4, Aquadest, Aqua pro injection.

The water content test is carried out using a moisture balance tool. This test is carried out 3 times so that the data becomes valid. The resulting water content is determined by the quality requirement, namely <10% because if it is more than this requirement it can cause microbial growth which can reduce the stability of the extract. In the first stage of testing the water content, 1 gram of dry extract is added to the moisture balance, then the test is carried out 3 times to get the best results. Photochemical Screening: Flavonoid Test: Wilstater Reagent: A total of 0.2 grams of dry extract is put into a test tube, a few drops of concentrated HCL are added and a little Mg powder is added. A positive reaction if there is a yellow color change. 10% NaOH reagent: A total of 0.2 grams of dry extract is put into a test tube, and a few drops of 10% NaOH solution are added. The occurrence of a color change indicates the presence of flavonoids because they are classified as phenolic compounds. Alkaloid Test: Mayer's Reagent as much as 0.2 grams of dry extract was put into a test tube, and a few drops of Mayer's reagent were added. The occurrence of a white precipitate indicates that it is positive for containing alkaloids. Bouchardat Reagent: A total of 0.2 grams of dry extract was put into a test tube, and a few drops of Bouchardat reagent solution were added. The occurrence of a black-brown precipitate indicated the presence of alkaloid compounds. Saponin Test: A total of 0.2 grams of dry extract was put into a test tube, 5 ml of hot water was added and

2N HCL was added, shaken for 1 minute. The solution was then allowed to stand and observed for 10 minutes to see whether foam formed or not. If foam formed it means the sample was positive for saponin. Tannin Test: A total of 0.2 grams of dry extract is put into a test tube, and 2-3 drops of 1% $FeCl_3$ are added. A positive sample contains tannin if its color changes to blackish green. Preparation of 0.5% CMC-Na Suspension: CMC-Na suspension is made by weighing 0.5 grams of 0.5% CMC-Na powder into a mortar, and dissolving it with 10 mL hot distilled water and stirring until homogeneous until mucilage forms. The solution was then poured into a 100 mL measuring flask and the remaining distilled water was added up to the mark. Making Na-Diclofenac Suspension: Na-diclofenac suspension is made by weighing 1.625 mg of diclofenac sodium powder and then dissolving it in 25 mL of 0.5% CMC-Na. 1 mL of the solution was taken using a syringe and then given to rats orally.

Preparation of meniran leaf dry extract suspension: Meniran leaf dry extract suspension is made by weighing 71.5 mg of meniran leaf dry extract and then suspending it in 25 mL of 0.5% CMC Na, given orally with a delivery volume of 1 mL. Making moringa leaf dry extract suspension: Meniran leaf dry extract suspension is made by weighing 71.5 mg of dry meniran leaf extract and then suspending it in 25 mL of 0.5% CMC Na, given orally with an administration volume of 1 mL. Making a combination of dry extracts of meniran and moringa leaves: A suspension of a combination of dry extracts of Meniran leaves and dry extracts of Moringa leaves is mixed and made from 35.75 mg of dry extracts of Meniran leaves and 35.75 mg of dry extracts of Moringa leaves which are suspended in 25 mL of CMC Na 0, 5%, given orally with an administration volume of 1 mL. Preparation of 1% acetic acid induction: 1 ml of acetic acid is added to 100 ml of distilled water, then placed in a 100 ml measuring flask, and shaken until dissolved. Dilution of acetic acid is carried out in an acid cupboard. Calculation of % analgesic power is seen in the following formula:

$$\% \text{ Analgesic Power} = 100 - \left(\frac{P}{K} \text{ x } 100\right)$$

P: Cumulative number of writhing of the treatment group; K: Cumulative number of writhing of the control group.

The calculation of analgesic activity is seen in the following formula:

$$\% \text{ Analgesic Effectiveness} = \frac{\text{Analgesic power intervention group}}{\text{Analgesic power positive control group}} \text{ x } 100\%$$

3. Results and Discussion

The results of determining the water content of dry extract of meniran leaves with an average of 3.00 ± 0.023% and dry extract of Moringa leaves 2.96 ± 0.139%, it can be concluded that the water content of dry extracts of meniran and Moringa leaves are <10%. %. The quality requirement for extract water content is <10% because if it is >10% it will result in microbial growth in the dry extract of meniran and moringa leaves. Based on research, it is stated that water content that is too high > 10% can cause damage to the extract due to the growth of microbes which will reduce the stability of the extract.

Table 1. Extract water content.

Sample	Mean ± SD
Dry extract of meniran leaves	3,00 ± 0,023
Dry extract of moringa leaves	2,96 ± 0,139

Based on the results of the phytochemical test (Table 2), both meniran leaves and moringa leaves show the presence of phytochemical compounds that are beneficial for health. Meniran leaves: The Wilstater test showed positive results with the formation of orange and orange color in concentrated Mg + HCl powder and 10% NaOH. This shows the presence of flavonoids in meniran leaves. Moringa leaves: The Wilstater test showed positive results with the formation of orange and orange color in concentrated Mg + HCl powder and 10% NaOH. This shows the presence of flavonoids in Moringa leaves. Meniran leaves: Mayer's test and Bouchardat's test showed positive results with the formation of a white precipitate in Mayer's reagent and a brown precipitate in Wagner's reagent. This shows the presence of alkaloids in meniran leaves. Moringa leaves: Mayer's test and Bouchardat's test showed positive results with the formation of a white precipitate in Mayer's Reagent and a brown precipitate in Wagner's Reagent. This shows the presence of alkaloids in Moringa leaves. Meniran leaves: The $FeCl_3$ test shows positive results with the formation of a blackish-green color. This shows the presence of tannins in meniran leaves. Moringa leaves: The $FeCl_3$ test shows positive results with the formation of a blackish-green color. This shows the presence of tannins in Moringa leaves. Meniran leaves: The Aquadest + HCl test showed positive results with the formation of stable 1 cm high foam. This shows the presence of saponin in meniran leaves. Moringa leaves: The Aquadest + HCl test showed positive results with the formation of stable 1 cm high foam. This shows the presence of saponins in Moringa leaves. Both leaves, meniran and moringa, contain phytochemical compounds that are beneficial for health. Flavonoids have antioxidant activity, alkaloids have antimicrobial and anti-inflammatory activity, tannins have astringent activity, and saponins have antibacterial and antiviral activity.

Table 2. Phytochemical test of meniran and moringa leaf extracts.

No	Compound Group	Reactor	Interpretation	Phytochemical test results of meniran leaf extract	Phytochemical test results of moringa leaf extract
1.	Flavonoid Wilstater test 10% NaOH test	 Mg powder + HCL concentrated NaOH 10%	 Orange Orange	 + +	 + +
2.	Alkaloid Mayer test Bouchardat test	 Reactor Mayer Reactor Wagner	 White precipitate Brown precipitate	 + +	 + +
3.	Tannin	$FeCl_3$	Blackish green	+	+
4.	Saponin	Aquadest + HCL	Stable 1 cm foam is formed	+	+

+: Indicates containing active compounds.

Based on the results of the stretching test on experimental animals (Table 3), there were significant differences between the treatment groups in terms of average stretching time. The average writhing time in the negative control group was 89.5 seconds with a standard deviation of 1.20. This shows that the experimental animals in this group did not experience a significant analgesic effect. The average writhing time in the positive control group was 22.3 seconds with a standard deviation of 3.89. This shows that the experimental animals in this group experienced a significant analgesic effect compared to the negative control group. This significant decrease in writhing time is most likely due to the administration of standard drugs that have analgesic effects. The average stretching time in the dry meniran leaf extract group was 36.9 seconds with a standard deviation of 3.01. This shows that the experimental animals in this

group experienced a lower analgesic effect compared to the positive control group, but higher than the negative control group. This increase in writhing time shows that the dry extract of meniran leaves has an analgesic effect, but the effect is not as strong as the standard drug in the positive control group. The average stretching time in the dry Moringa leaf extract group was 34.1 seconds with a standard deviation of 1.54. This shows that the experimental animals in this group experienced a lower analgesic effect compared to the positive control group, but higher than the negative control group. This increase in writhing time shows that the dry extract of Moringa leaves has an analgesic effect, but the effect is not as strong as the standard drug in the positive control group. The average stretching time in the group combining dry extracts of meniran leaves and Moringa leaves was 24.4 seconds with a standard deviation of 0.96. This shows that the experimental animals in this group experienced the highest analgesic effect compared to all other groups. This significant increase in stretching time shows that the combination of dry extracts of meniran leaves and Moringa leaves has a stronger analgesic effect compared to dry extracts of meniran leaves or Moringa leaves alone. The results of the stretching test showed that dry extracts of meniran leaves and Moringa leaves had an analgesic effect on experimental animals. This analgesic effect is stronger in the combination of dry extracts of meniran leaves and Moringa leaves compared to extracts of meniran leaves or Moringa leaves alone.

Table 3. Results of the animal writhing test for 30 minutes.

Treatment Group	**Mean ± SD**
Negative control (K-)	89.5± 1.20*
Positive control (K+)	22.3±3.89#
Meniran leaf dry extract (K1)	36.9±3.01*#
Moringa leaf dry extract (K2)	34.1±1.54*#
DM+DK dry extract combination (K3)	24.4±0.96#

(#): Shows a significant difference with the negative control group (P<0.05);
(*): Shows a significant difference with the positive control group (P<0.05).

Figure 1 shows that the three groups of dry extracts of meniran and moringa leaves K1, K2, and K3 have analgesic activity, but their ability as an analgesic is not greater than diclofenac as a positive control. Meanwhile, for the combination of dry extracts of meniran and moringa leaves, the highest percentage of stretching protection was obtained at 72.75%. This shows that the combination of dry extracts of meniran and moringa leaves (K3) is an effective group because it has the highest percentage of analgesic power among the meniran leaf group (K1) and the moringa leaf group (K2). Based on the percentage of analgesic power, it can be shown that the three treatment groups of dry extracts of meniran and Moringa leaves K1, K2, and K3 have met the specified standards because the percentage of analgesic power results is more than 50%. The analgesic power between the combination group and positive control did not have a significant difference, p>0.05. This result shows that the combination of meniran and moringa extracts has the potential as an analgesic.

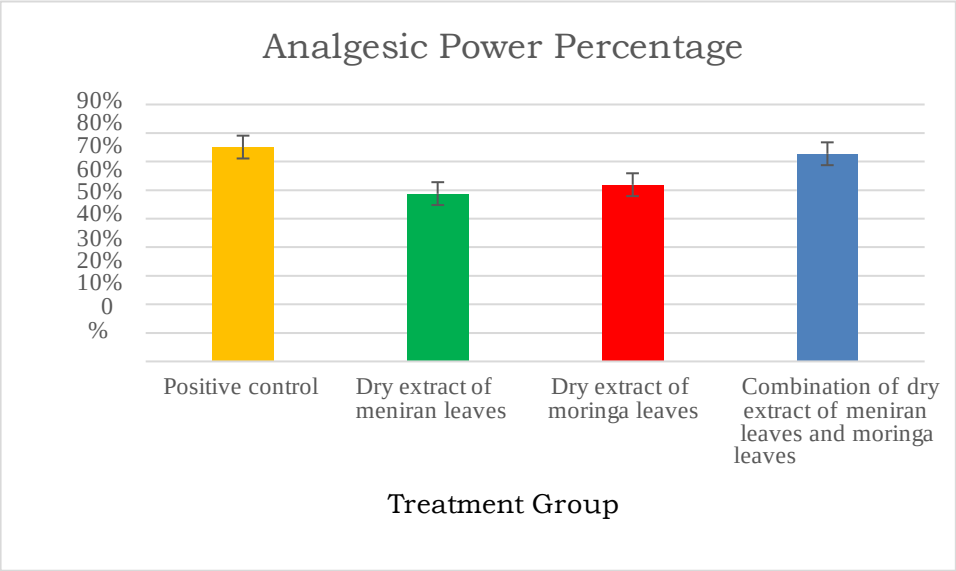

Figure 1. Percentage of analgesic power.

Table 4 shows that the percentage of analgesic effectiveness in the combination group of dry extracts of meniran and moringa leaves gave a result of 96.87% so that the combination of dry extracts of meniran and moringa leaves can provide an analgesic effect almost equivalent to sodium diclofenac. Based on table 4, shows that the analgesic effectiveness of dry extract of meniran and moringa leaves has an analgesic effect and meets the requirements for effectiveness as an analgesic with a percentage of effectiveness, proven to be able to increase the average reaction as a response to pain, but based on theory the analgesic activity in the test preparation is shown by the percentage of inhibition. the pain provided is greater or equal to 50%. From the results above it can be said that the lower the average number of writhes the higher the percentage of analgesic power and the higher the analgesic effectiveness, the more effective the analgesic is.

Table 4. Percentage of analgesic effectiveness.

Test Group	Treatment	Effectiveness percentage
II	Positive control (K+) (Na diclofenac)	100,00%
III	Dry extract of meniran leaves (K1)	78,27%±2,56
IV	Dry extract of moringa leaf (K2)	82,43%±2,43
V	DM+DK dry extract combination (K3)	96,87%±2,21

Pain is a complex sensation that can interfere with human activities and quality of life. Analgesics are a class of drugs used to relieve pain. Meniran leaves (*Phyllanthus niruri*) are a medicinal plant that has long been used in traditional medicine for various diseases, including pain. Meniran leaves contain various bioactive compounds, such as flavonoids, alkaloids, tannins, and saponins. These compounds have various pharmacological effects, including analgesic effects. Flavonoids are a group of bioactive compounds that are most commonly found in meniran leaves. The main flavonoid compounds in meniran leaves are quercetin, kaempferol, and routine. Flavonoids can inhibit the activity of the cyclooxygenase (COX) enzyme which is involved in the production of prostaglandins, inflammatory mediators that play a role in pain.

Flavonoids may protect cells from free radical damage, which can contribute to pain. Flavonoids can inhibit the growth of bacteria and viruses that can cause infections and worsen pain. Alkaloids are a group of bioactive compounds that are also found in meniran leaves. The main alkaloid compounds in meniran leaves are phyllanthine and hypophyllanthine. Alkaloids can inhibit excessive smooth muscle contractions, which can cause pain. Alkaloids can help relax tense muscles, which can help relieve pain. Alkaloids can increase the pain threshold, so the body needs a stronger stimulus to feel pain. Tannins are a group of bioactive compounds that are also found in meniran leaves. Tannins can help stop bleeding and reduce inflammation, which can help relieve pain. Tannins can inhibit the growth of bacteria and viruses that can cause infections and worsen pain. Saponin is a group of bioactive compounds which are also found in meniran leaves. Saponins can inhibit the activity of the COX enzyme which is involved in the production of prostaglandins, inflammatory mediators that play a role in pain. Saponins can increase the pain threshold, so the body needs a stronger stimulus to feel pain. Saponins can improve the immune system, which can help fight infection and speed wound healing, which can help relieve pain. The analgesic mechanism of action of bioactive compounds in meniran leaves is not yet fully understood. The bioactive compounds in meniran leaves can inhibit pain pathways in the central nervous system, thereby reducing the transmission of pain signals to the brain. The bioactive compounds in meniran leaves can increase the pain threshold, so the body needs a stronger stimulus to feel pain. The bioactive compounds in meniran leaves have anti-inflammatory effects, which can help relieve pain caused by inflammation. The bioactive compounds in meniran leaves can increase levels of endorphins, which are natural hormones in the body that have an analgesic effect.[8-14]

Moringa leaves (*Moringa oleifera*) are a medicinal plant that has long been used in traditional medicine for various diseases, including pain. Moringa leaves contain various bioactive compounds, including flavonoids, alkaloids, tannins, and saponins. These compounds have various pharmacological effects, including analgesic effects. Flavonoids are a group of bioactive compounds most commonly found in Moringa leaves. The main flavonoid compounds in Moringa leaves are quercetin, kaempferol, and isorhamnetin. Flavonoids can inhibit the activity of the cyclooxygenase (COX) enzyme which is involved in the production of prostaglandins, inflammatory mediators that play a role in pain. Flavonoids may protect cells from free radical damage, which can contribute to pain. Flavonoids can inhibit the growth of bacteria and viruses that can cause infections and worsen pain. Alkaloids are a group of bioactive compounds that are also found in Moringa leaves. The main alkaloid compounds in Moringa leaves are moringinine, isothiocyanates, and indole alkaloids. Alkaloids can inhibit excessive smooth muscle contractions, which can cause pain. Alkaloids can help relax tense muscles, which can help relieve pain. Alkaloids can increase the pain threshold, so the body needs a stronger stimulus to feel pain. Tannins are a group of bioactive compounds that are also found in Moringa leaves. Tannins can help stop bleeding and reduce inflammation, which can help relieve pain. Tannins can inhibit the growth of bacteria and viruses that can cause infections and worsen pain. Saponins are a group of bioactive compounds that are also found in Moringa leaves. Saponins can inhibit the activity of the COX enzyme which is involved in the production of prostaglandins, inflammatory mediators that play a role in pain. Saponins can increase the pain threshold, so the body needs a stronger stimulus to feel pain. Saponins can improve the immune system, which can help fight infection and speed wound healing, which can help relieve pain. The analgesic mechanism of action of Moringa leaf bioactive compounds is not fully understood. The bioactive compounds in Moringa leaves can inhibit pain pathways in the central nervous system, thereby reducing the transmission of pain signals to the brain. The bioactive compounds in Moringa leaves can increase the pain threshold, so the body needs a stronger stimulus to feel pain. The

bioactive compounds in Moringa leaves have anti-inflammatory effects, which can help relieve pain caused by inflammation. The bioactive compounds in Moringa leaves can increase levels of endorphins, which are natural hormones in the body that have an analgesic effect.[15-20]

4. Conclusion

Dry extract of meniran leaves (*Phyllanthus niruri* L.) and dry extract of moringa leaves (*Moringa oleifera* L.) has been proven to contain flavonoids, alkaloids, saponins, and tannins which have analgesic activity. Analgesic activity of a combination of dry extracts of meniran leaves (*Phyllanthus niruri* L.) and dry extract of moringa leaves (*Moringa oleifera* L.) was proven to have synergistic analgesic activity in rats using the chemical induction method.

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