International Collaborative Conference on Multidisciplinary Science
Volume. 1, Number. 1, Year 2024
Page 32-41
Available online at: https://iccms.ifrel.org/index.php/ICCMS

Effectiveness Test of Anthelmintic Ethanol Extract of The Perwinkle Stem (*Chatarantus roseus*) Plant on Roundworms (*Ascaris lumbricoides)*

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Abstract: *Worms are an infectious disease caused by parasites in the form of ringworm (Ascaris Lumbricoides). The purpose of this study is to determine the anthelmintic power of ethanol extract of virgin stem (Catharanthus roseus (L) G.Don) on the mortality of roundworms (Ascaris lumbricoides). The stages of the study include sample sorting, extraction, phytochemical screening, and anthelmintic activity tests. Extract preparations are made in concentrations of 5%, 10%, and 15%. For positive control, pirantel pamoate (Combantrin) is used as much as 10 ml while for negative control 0.9% NaCl is used as much as 25 ml. Soaked with various treatments in petri dishes. Observations are made every 1 hour for 5 hours to see if the roundworms are alive or dead. The test animals used were 10 roundworms. The anthelmintic activity test was carried out by soaking worms in concentrations of 5%, 10%, and 15%, respectively The results of this study showed that the extract of virgin stem (Catharanthus roseus (L) G.Don) had an anthelmintic effect on roundworms . The higher the concentration of the extract, the higher the anthelmintic power. In the extract concentration of 15% has almost the same anthelmintic ability as (Combantrin).*

Keyword: *ascaris lumbricoides, catharanthus roseus, perwinkle stem, roundworm.*

1. INTRODUCTION

Indonesian is one of the countries where infections are often caused by poor sanitation and children are a group that is vulnerable to worm infections, because they often play on contaminated soil and do not fully have good hygiene habits. Worms are an infectious disease caused by parasites in the form of roundworms (Ascaris Lumbricoides) (Farid et al., 2020). Worms are an infectious disease that is still a widespread public health problem in the world, especially in developing countries with clean and healthy living habits (PHBS) and poor sanitation. This disease can reduce the quality of life for sufferers. WHO data shows that more than 1.5 billion people or 24% of the world's population are infected with worms and 60% of them are children (Roring et al., 2019). The prevalence in Indonesia is still relatively high, namely around 45% -65% and even reaches 80% in areas with poor sanitation. The prevalence of worms in Indonesia ranges from 2.5% -62%, while for North Sumatra province, based on the results of a survey conducted by the North Sumatra Provincial Health Service, it is 22.50% with the highest intensity in preschool and elementary school children. Puskesmas data submitted to the Medan City Health Service in 2022 showed that the number of worm sufferers among preschool and school children was 249 people (Yani et al, 2023)

This disease is caused by the entry of parasites in the form of worm eggs into the body through the human digestive tract due to transmission through the soil (Suluwi et al, 2017). The most common worm infections are caused by Soil-Transmitted Helminth (STH) intestinal worms, namely roundworms (Ascaris lumbricoides), whipworms (Trichuris

Received: April 30, 2024; Revised: May 30, 2024; Accepted: June 18,2024;
Published: June 30, 2024

trichiura) and hookworms (Ancylostoma duodenale, Necator americanus) (Alawiyah et al., 2017).Anthelmintics are drugs to kill worms or reduce the number of worms in the body. Anthelmintics are drugs used to eradicate or reduce worms from the human or animal body. Currently, there are many new, more specific anthelmintics available that work more effectively, one of which is pyrantel pamoate. Pyrantel pamoate is an anthelmintic with a single dose and the cure rate is quite high. However, this drug has side effects, including gastrointestinal disorders, fever and headaches, so a safer alternative treatment is needed for the community, namely using natural medicines (Kusuma et al, 2022)

Tapak dara is one of the plants that can be used to treat worms because it has several types of active compound components which can be found in the roots, leaves, stems and flowers. The active compound components in the tapak dara plant are alkaloids, flavonoids, tannins, saponins (Ulpa et al, 2022).

Types of plants that have properties in helping maintain health and treating diseases are medicinal plants. The use of medicinal plants has not been based on clinical laboratory testing, but rather based on people's personal experiences, therefore medicinal plants are closely related to traditional medicine. Tapak dara is a traditional medicine, where extracts from flowers, leaves, stems, roots and seeds are used as medicine by the community. Periwinkle stem extract contains tannins, triterpenoids and alkaloids (Bunyanis & L.Ode, 2023). Testing of the anthelmintic effectiveness of ethanol extract of periwinkle stems (Catharanthus roseus (L) G.Don) against roundworms (Ascaris lumbricoides) has never been carried out. Therefore, the aim of this research is to explore the anthelmintic effectiveness of ethanol extract of periwinkle stems on roundworms by looking at compounds that can kill roundworms. Research that has been carried out on compounds that are efficacious in killing worms, namely alkaloids, saponins, flavonoids and tannins. compounds resulting from identification tests play a role in the death of worms (Intannia et al, 2015). Compounds found in periwinkle (Catharanthus roseus(L) G.Don) include alkaloid, flavonoid, tannin and saponin components. The stem of the tapak dara plant has activities namely antibacterial, antioxidant, antihelminthic, antineoplastic, antihyperglycemic, anticancer, antidiarrheal and antiviral (Ulpa et al, 2022)

2. RESEARCHMETHOD

The tools

The tools used in this research were analytical scales (Fujitsu FSR-A 300), spatula, mask, stirring rod, porcelain cup (Glassco), petri dish (Local), water bath, rotary pavorator (RV10AutoPro V), glass hotplate (IkaTwistterset1). chemical 1 liter, blender (TurboEHM-8099), gram scale (Local), glass stir bar, sieve 100 mesh, 10 ml measuring cup, glass funnel, test tube, test tube rack, filter paper, glass jar, scissors, extract stirring ladle, aluminum foil, lab coat, mask, gloves.

Materials

The research materials used were ethanol extract of the stem of the periwinkle plant (Catharanthus roseus(L) G.Don), NaCl 0.9%, pyrantel pamoate (Combantrin®) 5 mg/mL, Aquades, Chloroform, NH4OH, concentrated hydrochloric acid, H2SO4, Mayer's Reagent, Dragendroff's Reagent, Bochardat's Reagent, and FeCl31%.

Research samples

The research samples described include plant samples and animal samples.

Plant

Sample material was collected purposively, namely taken from one area of North Binjai, North Sumatra without comparing it with the same plants from other areas. The samples used in this research were periwinkle stems collected from Jalan Tengku Amir Hamzah km 31.5 kec. North Binjai, North Sumatra and Jalan Wiliam Iskandar, Medan State University, UNIMED North Sumatra.

Animal

In this study, the test animals used were healthy roundworms (Ascaris lumbricoides) obtained in the village of Bulu Cina Pasar V Kec, Hamparan Perak, totaling 10 roundworms (Ascaris lumbricoides). Researchers used 2 roundworms in each treatment group because the worms were very difficult. obtained. Selection of roundworms (Ascaris lumbricoides) by looking at worms that are still actively moving (Karim et al., 2021).

Working procedure

The following is the working procedure starting from plant determination to the stage of testing the effectiveness of anthelmintic ethanol extract of periwinkle plant stems (Catharanthus roseus (L) G.Don) against roundworms (Ascaris lumbricoides).

Plant Determination

Determination of the stem of the periwinkle plant (Catharanthus roseus (L) G.Don) was carried out at the Medanense Herbarium Laboratory, University of North Sumatra.

Making Simplicia

The stems of periwinkle plants (Catharanthus roseus (L) G.Don) which have been collected and sorted by separating them from other parts of the plant, then the stems are weighed before washing using running water, when washing other objects attached to them. The stems are discarded, then the stems that have completed the cleaning process are drained and dried in the sun until the texture of the stems becomes dry, then crushed using a blender (Putri & Nasution, 2022).

Making Extracts

500 g of fine powder from the stems of the periwinkle plant (Catharanthus roseus (L) G.Don) was weighed then put into a maceration container (glass jar), soaked in 2 liters of 96% ethanol with an extract ratio of 1:4 until submerged. The maceration container is covered with aluminum foil and then macerated for 3x24 hours at room temperature. Then filtered, the dregs and filtrate are separated using filter paper. The dregs were macerated again with 1 liter of 96% ethanol as a solvent and then diluted again with half a liter of 96% ethanol solvent. After the maceration process was carried out, the maserate obtained was concentrated at a temperature of 70ºC until it became a thick extract using a rotary evaporator below the boiling point until the desired results were obtained. After that, it is heated over a water bath until it produces a thick extract (Nurhaliza, S. 2023)

Phytochemical Screening

Identification of phytochemical screening of ethanol extract of periwinkle plant stems (Catharanthus roseus(L) G.Don) which was carried out included alkaloid, flavonoid, saponin and tannin tests.

Alkaloid Test

2 grams of the viscous extract of periwinkle stems were taken, then diluted with 10 mL of chloroform, then added with 10 drops of NH4OH, once dissolved, filtered and put in a test tube. The filtrate can be added with H2SO42 N, then shaken for 1 minute and wait until it forms two layers (Nurhaliza, S. 2023). The top layer was taken and divided into 3 parts, then put in different test tubes:

1. In test tube 1, add 1 ml of filtrate and add 2 drops of Bouchardat's reagent. If there is blackish brown sediment, then the result is positive.
2. In test tube 2, add 1 ml of filtrate and add 2 drops of Mayer's reagent. If there is white or yellow sediment, then the result is positive.
3. In test tube 3, add 1 ml of filtrate and add 2 drops of Dragendroff's reagent. If there is an orange-brown precipitate, then the result is positive (Nurhaliza, S. 2023).

Alkaloids are positive if sediment or turbidity occurs in at least two of the three experiments above. The characteristic of a positive alkaloid reaction is the formation of a blackish brown color with Bouchardat's reagent, an orange to brownish color with Dragendroff's reagent and a yellow precipitate forming with Meyer's reagent (Komang et al., 2016).

Uji Flavonoid

Diambil 0,5 g ekstrak kental batang tapak dara, kemudian diencerkan dengan 5 ml etanol 95%, kemudian diambil 2 ml larutan ekstrak, dimasukkan dalam tabung reaksi dan ditambah 100 mg HCl pekat, kemudian dikocok perlahan. Hasil menunjukkan positif jika larutan menghasikan warna merah-jingga hingga merah-ungu(Himawan dkk., 2018).

Uji Saponin

Ekstrak kental batang tapak dara diambil 0,5 g, kemudian diencerkan dengan 10 ml air suling panas, tunggu hingga dingin. Selanjutnya kocok kuat selama 10 detik, kemudian ditambah HCl 2 N sebanyak 1 tetes. Jika busa tidak hilang setelah 10 menit dilakukan penambahan HCl 2 N , maka hasilnya positif (Himawan dkk., 2018).

Uji Tanin

Diambil 0,5 g ekstrak kental batang tapak dara, kemudian diencerkan dengan 5 ml aquades. Larutan ekstrak diambil 2 ml lalu ditetesi pereaksi $FeCl_3$1% sebanyak 1-2 tetes. Jika larutan berwarna hijau, biru, atau kehitaman, maka hasilnya positif(Himawan dkk., 2018).

Uji Fenolik

Ekstrak kental batang tapak dara diambil 0,5g, kemudian dilarutkan dalam 1 mL aquades.Setelah itu ditambah FeCl3 1% sebanyak 10 tetes. Jika larutan berwarna biru, ungu, hijau, atau hitam, maka hasilnya positif(Agustina dkk., 2016).

Perlakuan Hewan Uji

Masing-masing hewan uji dikelompokkan menjadi 5 kelompok perlakuan yaitu:

Group 1 : soaked with ethanol extract of periwinkle plant stems (*Catharanthus roseus*(L) G.Don) 5%.

Group 2 : soaked with ethanol extract of periwinkle plant stems ((*Catharanthus roseus*(L) G.Don) 10%.

Group 3 : soaked with ethanol extract of periwinkle plant stems ((*Catharanthus roseus*(L) G.Do 10%.

Group 4 : soaked with 5 mg/ml pyrantel pamoate solution as a positive control.

Group 5: Group 5: soaked in 0.9% NaCl solution as a negative control.

Petri dishes were prepared, each containing periwinkle stem extract (Catharanthus roseus(L) G.Don) with a concentration of 5%, 10%, 15%, 10 ml of pyrantel pamoate solution, 25 ml of 0.9% NaCl solution. Two Ascaris lumbricoides roundworms were put into each petri dish and then observed every 1 hour after soaking until all the worms died. Determining whether the worm is dead, paralyzed or still normal is by disturbing the worm with a stir stick. Dead worms are indicated by the worm not moving and death is confirmed using warm water for 5 seconds. Worms that are no longer moving are grouped as dead worms and if they are still moving then the observation is continued and then the observation data is processed with tables and graphs of points against the time of worm mortality (Karim et al., 2021).

Data analysis

Data on the effectiveness of ethanol extract of periwinkle stems (Catharanthus roseus(L) G.Don) on the death of roundworms (Ascaris lumbricoides) will be processed in the form of tables and graphs over time. The data obtained will be analyzed using the Kruskal-Wallis Hypothesis test statistical analysis method. , test of normality and Mann-Whitney. (Natasya et al., 2024)

4. RESULTS AND ANALYSIS

Plant Identification

Plant identification was carried out at the Medanese Herbarium Plant Systematics Laboratory (MEDA) at the University of North Sumatra (USU). Based on the identification results with number 2167/MEDA/2023, it shows that the plant used in this research is the stem of the periwinkle plant (Catharanthus roseus(L) G.Don) of the Apocynaceae family, which can be seen in

Tabel 1 Scientific Classification of Tapak Dara Stems(*Catharanthus roseus* (L) G.Don)

Batang tanaman tapak dara	
Kingdom	: *Plantae*
Subkingdom	: *Tracheobionta*
Superdivisi	: *Spermatophyta*
Divisi	: *Magnoliophyta*
Kelas	: *Magnoliopsida*
Subkelas	: *Asteridae*
Ordo	: *Gentianales*
Famili	: *Apocynacea*
Genus	: *Catharanthus*
Spesies	: *Catharanthus roseus* (L.) G. Don

Preliminary Test and Sample Identification

Before extraction, fresh periwinkle stems are made into simplicia. The wet weight of periwinkle stems was obtained as much as 2 kg. Next, periwinkle stems are subjected to wet sorting, washing, chopping, drying, dry sorting and storage.

Dried simplicia is characterized by the stem slices breaking easily when squeezed, not moldy, having a distinctive smell resembling fresh ingredients, and simplicia having no water content. The extraction stage is carried out by grinding the dried simplicia using a blender in stages to prevent the blender machine from getting too hot. The results of the simplisia of ground periwinkle stem powder were weighed as much as 500 g.

Extraction Results of Tapak Dara Stems (Catharanthus roseus)

The simplicia powder was then macerated using 96% ethanol solvent. This maceration method aims to attract the active substances in the simplicia where the filter fluid will penetrate the cell walls and cavities containing the active substances and will be forced out of the cells due to the difference in concentration between the active substances inside the cell and those outside the cell. Apart from that, the maceration process is carried out manually. stratified by using new ethanol solvent for each change of maceration (Bunyanis et al, 2023).

Maceration is carried out by soaking 2000 ml of periwinkle stem simplicia powder (Catharanthus roseus (L) G.Don) in 2000 ml of 96% ethanol solvent in a tightly closed container and kept away from direct sunlight. After 3x24 hours of maceration process, the macerate was then separated from the dregs to obtain 1100 ml of macerate. The maserate of periwinkle stems (Catharanthus roseus (L) G.Don) obtained was dark green in color and had a distinctive aroma of periwinkle stems. The maserate of the two plants obtained was then concentrated by evaporating the solvent contained in the macerate using a rotary evaporator (Nurhaliza, 2023).

The yield of periwinkle stem extract obtained was 104.18 grams, with thick and sticky characteristics, a deep green color and a distinctive aroma of periwinkle stems. Rendemand says it's good if the value is more than 10%. Therefore, the extract yield obtained at 20.8% was declared good because the yield was >10% (Sabat Dwi Meianti et al, 2022). As a result of the rotary evaporator, tapak dara stem macerate obtained a concentrated ethanol extract in the form of a thick blackish green extract with a total weight of 104.18 g.

The results of simplicia soaking periwinkle stems (Catharanthus roseus L.) are shown in

No	**Simplisia**	**Powder**	**Concentrated Extract Results**	**soaking(%)**
1	**Periwinkle stems**	500 g	104,18 g	20,8%

Based on **Table 2** above, the yield results are said to be good if the value is more than 10%. Therefore, the yield obtained at 20.8% is considered good because the yield is >10% (Meianti, S, D. et al, 2022).Yield is a comparison between the extract obtained and the initial simplicia. The yield uses percent units (%), the higher the resulting yield value indicates the greater the value of the extract produced (Alhijrah et al., 2024).

Phytochemical Screening Test Results

Phytochemical screening test was carried out for periwinkle (Catharanthus roseus (L) G.Don) stem powder to determine the content of secondary metabolite compounds. The results of phytochemical screening of periwinkle stems (Catharanthus roseus (L) G.Don)

Tabel 3 Results of Identification of Chemical Compound Content

Compound Name	Peraction	Periwinkle Stem Result
Alkaloid	Bouchardart	+
	Maeyer	+
	Dragendroff	+
Flavonoid	HCL	+
Saponin	HCL 2N	+
Tanin	FeCl3 1%	+
Fenolik	FeCl3 1%	+

Information:

Sign (+) Detected secondary metabolite compounds

The results of the phytochemical screening carried out on tapak dara stem extract contained secondary metabolite compounds including alkaloids, flavonoids, saponins, tannins and phenolics. Tapak dara is one of the plants that can be used to treat worms because it has several types of active compound components which can be found in the roots, leaves, stems and flowers. The active compound components in the tapak dara plant are alkaloids, terpenoids, phenols, tannins, saponins, quinins and sterols (Ulpa et al, 2022).

Effectiveness Test of Tapak Dara Stem Extract Preparations (Catharanthus roseus L. G. Don) and data on the number of deaths of roundworms (Ascaris limbricoides)

Test the effectiveness of periwinkle extract preparations (Catharanthus roseus (L) G.Don) on roundworms (Ascaris lumricoides) at concentrations of 5%, 10% and 15%, pyrantel pamoate (positive control) and 0.9% NaCl (negative control) . The aim of the effectiveness test was to see the comparison of the time to death of roundworms (Ascaris lumricoides). The results of the anthelmintic effectiveness test of the ethanol extract of periwinkle stems (Catharanthus roseus (L) G.Don) against roundworms (Ascaris lumricoides) can be seen in **Table 4**

Table 4 Percentage of dead worms

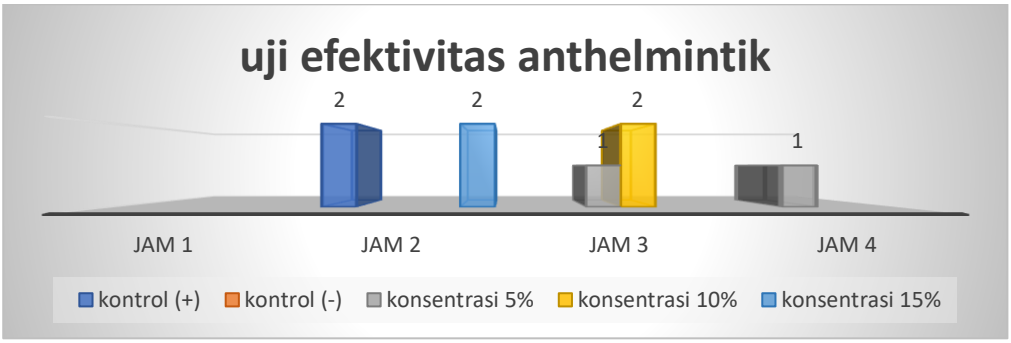
Persentase Efektivitas Formulasi					Time
Group Control		Ethanol Extract of Perwinkle Stem			Jam Ke -
Pirantel pamoat (+)	Nacl 0,9% (-)	5%	10%	15%	
-	-	-	-	-	1
2	-	-	-	2	2
-	-	1	2	-	3
-	-	1	-	-	4
-	-	-	-	-	5
-	-	-	-	-	6
-	-	-	-	-	7
-	-	-	-	-	8
-	-	-	-	-	9
-	-	-	-	-	10
-	-	-	-	-	11
-	-	-	-	-	12
-	-	-	-	-	13

-	-	-	-	-	14
-	-	-	-	-	15
-	-	-	-	-	16
-	-	-	-	-	17
-	-	-	-	-	18
-	-	-	-	-	19
-	-	-	-	-	20
-	-	-	-	-	21
-	-	-	-	-	22
-	-	-	-	-	23
-	-	-	-	-	24

Keterangan:
- :Tidak ada cacing yang mati
1: Kematian 1 ekor cacing gelang *Ascaris lumbricoides*
2: Kematian 2 ekor cacing gelang *Ascaris lumbricoides*

The results of the anthelmintic test, divided into 5 treatment groups, can be seen in observations carried out at 10.00 and observed every 1 hour because 1 hour is optimal time for observations to obtain more valid data, because the more frequent the observations, the more visible the linearity of the worm's response will be (Karim et al., 2021).If there are worms that are still, then the worms are disturbed with a stirring rod. If the worm does not move, it is moved into a container filled with warm water for 5 seconds. Immersing the worm in warm water aims to stimulate the worm's muscles to move and ensure that the worm is truly dead, if it is not dead, the worm will still show movement because of the heat of the water (Karim et al., 2021).

After that, the time and number of worms that died were recorded. The results of the study showed that periwinkle stem extract (Catharanthus roseus (L) G. Don) 5% showed worm death in the 3rd hour, periwinkle stem extract (Catharanthus roseus L. G. Don) 10% showed worm death in the 3rd hour, and periwinkle stem extract (Catharanthus roseus L. G. .Don) 15% shows the death of worms in the 2nd hour, pyrantel pamoate solution (Combantrin) 5 mg/ml as a positive control showed the effect of worm death in the 2nd hour and 0.9% NaCl solution as a negative control did not show worm death for 24 hours (Karim et al., 2021).



Gambar 1 Uji Efektivitas Anthelmintik

The results showed that pyrantel pamoate (Combantrin®) 125 mg solution as a positive control showed a 100% worm death effect in the 2nd hour, 0.9% NaCl solution as a negative

control did not show worm death, while 5% periwinkle stem ethanol extract showed worm death in the 3rd and 4th hours, 10% ethanol extract of periwinkle stem showed worm death in the 3rd hour, and 15% ethanol extract of periwinkle stem showed worm death in 2nd hour. The results of the anthelmintic effect of ethanol extract of tapak dara stems on the roundworm Ascaris lumbricoides were then analyzed using the Kruskal-Wallis test, where the data obtained was a value ($P < 0.05$) which means there were significant differences between all groups. As a result of the research, it is known that the ethanol extract of tapak dara stems has anthelmintic properties. Different concentrations of ethanol extract from periwinkle stems show different anthelmintic power, the higher the concentration, the faster the worm death time (Karim et al., 2021).

5. CONCLUSION

From the results of the research and discussion above, it can be concluded that periwinkle stem extract can have an anthelmintic effect on roundworms (Ascaris lumbricoides). By administering different concentrations ranging from 5%, 10%, and 15%, this causes the worm death time (hours) to be faster according to the concentration of periwinkle extract. This means that the higher the concentration given, the death rate of the worms will increase and speed up the death time of the worms.

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