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Effect *in vitro* of *Areca catechu* ethanol extract partition as anthelmintics against roundworm

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ABSTRACT: Helminths disease is one of the infectious diseases caused by the entry of parasites in the form of worm eggs into the human body through the human digestive tract due to pollution through the *soil-transmitted helminth* (STH). This study measured the *in vitro* effect of *Areca catechu* ethanol extract partitioning as anthelmintic against *Ascaris lumbricoides* worms using a 125 mg pyrantel pamoate control. The test animals used were living and mobile worms of *A. lumbricoides*, which were taken from the small intestines of pigs. The study group was divided into 5 groups, namely positive control (pyrantel pamoate) 125 mg), negative control (0.9% NaCl solution) and ethanol extract group A. *catechu* 10%, 20%, 30%. The results confirmed that the ethanol extract partition of A. *catechu* at exposure to a concentration of 30% showed a high level of efficacy in killing worms with an average time of death of A. *lumbricoides* worms at 6 to 12 hours compared to pyrantel pamoat solution. However, at exposure to concentration, 10% and 20% obtained a slightly longer average mortality time of 8 to 24 hours, respectively. Partitioning of A. *catechu* ethanol extract at a concentration of 30% showed the highest level of efficacy in killing A. *lumbricoides* worms based on average faster mortality time than the concentrations of 10% and 20% collected. Ethanol extract A. *catechu* has pharmacological therapeutic effects have various properties, including can be used for the treatment of diseases caused by parasites and also several other diseases.

KEYWORDS: *Areca catechu* ethanol extract; Piper betle; Anthelmintic**;** *Ascaris lumbricoides*.

1. INTRODUCTION

Parasitic diseases are one of the gastrointestinal health problems that can threaten the human population, the impact of which increases the prevalence of malnutrition and diseases such as malnutrition, anemia, eosinophilia and pneumonia [1–3].This disease can cause impaired growth and development of human and animal individuals, especially in the tropics [4].

A. *lumbricoides* is a species of parasitic nematode found in the human digestive system and reproduces through STH contaminated with the parasite. There are about twenty-five percent more of the world's population is at risk of infection [5]. Previous research in 2010 has reported that the number of world populations infected with parasites includes A. *lumbricoides* 819.0 million people, *trichuris trichiura* 464.6 million and (*ancylostoma duodenale, necator americanus)* 438.9 million [6,7].

Demographically, A. *lumbricoides* worm species prefer to live and breed in tropical and sub-tropical climates, especially rural areas, sanitation that is less qualified for health, poor nutrition are determinants of the increase in the incidence of morbidity cases continuously.

Research on the effects of anthelmintics as an herb has been reported by several researchers before. anthelmintic activity through testing of hydroalcoholic extracts of *Zingiber officinale* and *Curcuma longa* at exposure concentrations of 50 mg/ml found mean mortality times of 5.2 minutes and 4.6 minutes [8]. Both extracts exhibit the same synergistic effect as a reliable anthelmintic effect. While anthelmintic studies by [2], with ethanolic testing of *Tamarindus indica* Linn concentrations of 50 mg / ml and 100 mg / ml showed an average mortality time of 67 minutes and 57 minutes [2]. Another extract partition was ethanolic *Nigella sativa* Linn at exposure concentrations of 100 mg/ml average time of death at 72 minutes. Other researchers also reported that at concentrations of 100mg/ml of methanol extract and aqueous cylinders, Luffa showed the

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same average mortality time before against earthworms [9]. Other anthelmintic activity in pumpkin (*Cucurbita maxima*) especially the seed part of the plant, pea (*Macrotyloma uniflorum*), papaya (*Carica papaya*) [10–13].

In an effort to control parasitic infections, several types of anthelmintics sold in pharmacies or drug stores are an alternative choice for consumers. Unfortunately, anthelmintic drug therapy in the process of use leaves various problems, some of which are high cost, not environmentally friendly, excessive drug resistance, and several other negative effects, especially host health. In addition, the suboptimal process of drug absorption in the human intestine can cause toxic (carcinogenic) effects and agent resistance [14]. Irregular therapeutic effects lead to drug resistance [15]. Given the serious impact that can be caused by the use of A. *Lumbricoides* drugs with its adverse effects, namely the emergence of drug resistance and carcinogenic effects, it is considered necessary to have alternative therapies that are safe and environmentally friendly. Research experts in the pharmaceutical field have tried to develop ethnomedical agents as alternative therapies, one of which is the partition of ethanol extract from herbal plant material A. *catechu*, which is a species of *arecaceae*. The composition of a. *catechu* consists of flavonoids, saponins, tannins, quinones, phenols, monoterpenes, sesquiterpenes, alkaloids derived from *arecoline*, *arecaine* [15]. In further investigation, A. *catechu* contains active compounds (alkaloids, arecoline and tannins) as anthelmintics. Arecoline compounds are highly toxic to various types of parasitic nematodes. Another composition is Proanthocyanidin (tannin condensation) is an inhibitory enzyme and increases membrane permeability, the impact of which decreases the production of ATP parasitic nematodes which ends in death.

A recent study has reported that there is a balanced association between *A. lumbricoides*, *trichuris trichiura* and (*ancylostoma duodenale, necator americanus)* infections in humans. However, the study did not account for the age group studied [16].

This study measured the *in vitro* effect of the ethanol extract partition of A. *catechu* as anthelmintic against A. *lumbricoides* using pyrantel pamoate control 125 mg. Further measurements were directed to determine the concentration of A. *catechu* ethanol extract in killing A. *lumbricoides* based on the mean time of death of A. *lumbricoides*.

2. RESULTS

This research was divided into two major groups, namely the study group and the control group. The study group used partitions of A. *catechu* ethanol extract with concentration levels (10%, 20% and 30%) as anthelmintic against A. *lumbricoides.* While in the control group there were 2, namely a positive control of pirantel pamoat (combantrin 125 mg) and a negative control of NaCl 0.9% (Figure 1).

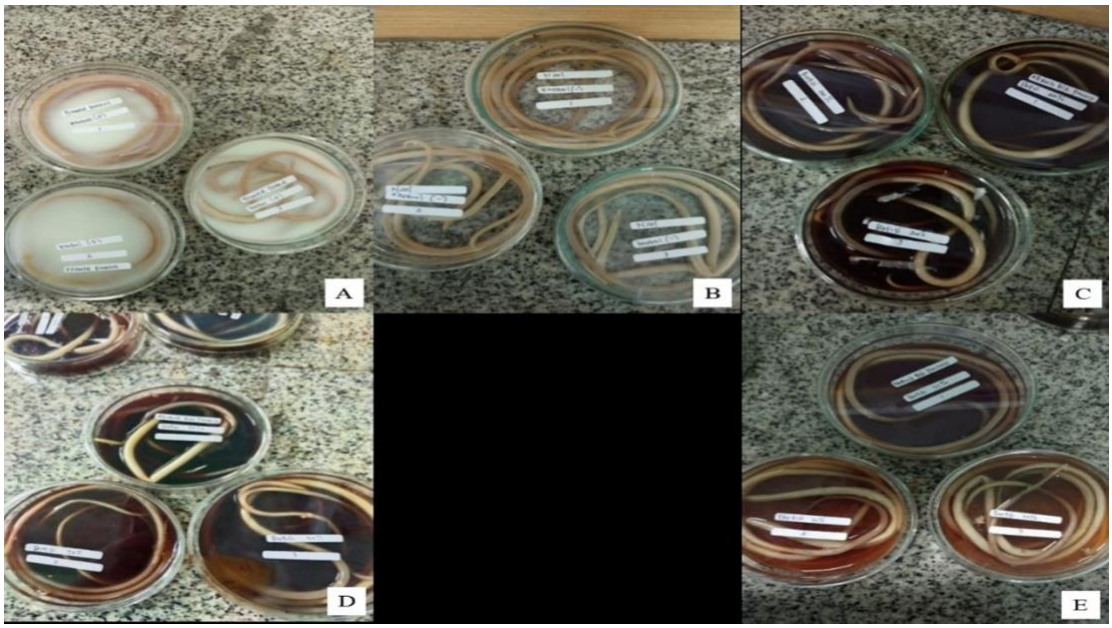

Figure 1. Immersion treatment of A. lumbricoides worms (A: positive control of pyrantel pamoate (combantrin®) 125 mg, B: negative control of NaCl 0.9%, C: ethanol extract partition A. *catechu* concentration 10%, D: concentration 20%, and E: concentration 30%).

2.1. Phytochemical screening

To determine the secondary metabolite compounds contained in A. *catechu*. plants, a qualitative analysis called phytochemical screening was carried out. The results of phytochemical screening tests showed that all ethanol extracts of A. *Catechu* (+) contained components of secondary metabolite compounds (alkaloids, flavonoids, saponins and tannins) (Table 1)

Table 1. Phytochemical screening results of ethanol extract partition A. *catechu*.

No	Secondary metabolites	Reagent	Theoretical results	Observations	Positive[a] / negative[b]
1.	Alkaloid	Mayer	Deposits White	Deposits White	[a]
		Dragendroff	Red-orange deposits	Orange red precipitate	[a]
2.	Flavonoid	Mg + HCl p	Yellow, orange to red	Reddish-yellow	[a]
3.	Saponin	H_2O + HCl 2 N	Froth 1-10 cm high	1.7 cm high foam for 37 seconds	[a]
4.	Tannin	FeCl 31 %	Greenish-brown or blue-black	Blackish blue	[a]

[a] Positive/ there are secondary metabolite compounds
[b] Negative/ no secondary metabolite compounds.

2.2. Anthelmintic testing

The results of testing the effect of ethanol extract partitioning of A. *catechu* as anthelmintic against A. *lumbricoides in vitro*. The average time of death of A. *lumbricoides* as a whole showed different effects that in ethanol extract partitions A. *catechu* concentrations of 30% had the fastest killing power of A. *lumbricoides* (2.71 hours), while at concentrations of 10% and 20% had killing power of A. *lumbricoides* with the same time (8.71 hours) as shown in figure 2.

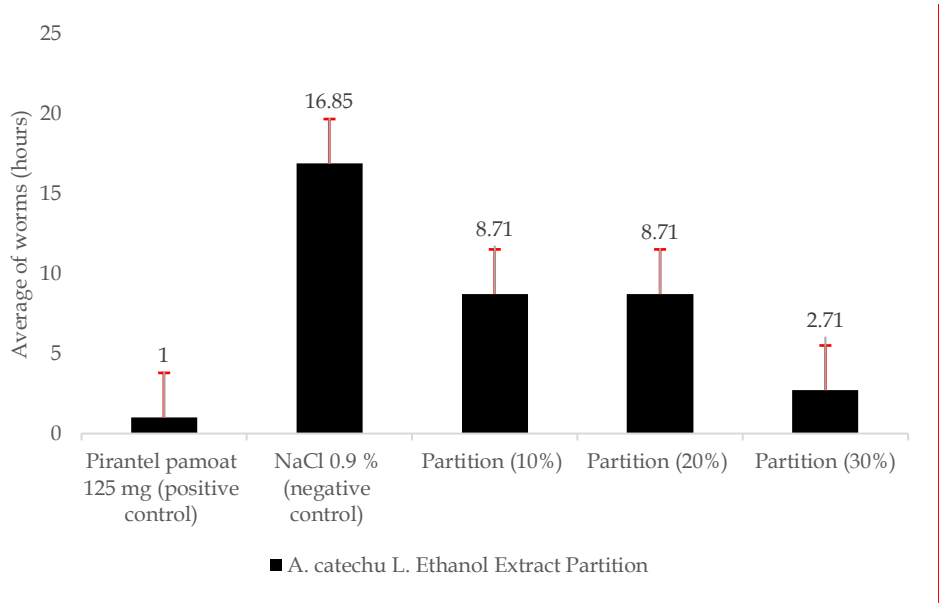

Figure 2. Average roundworm mortality of A. *lumbricoides*) on ethanol extract partitions of A. *catechu* (10%, 20%, 30%), pyrantel pamoate 125 mg (positive control), and 0.9% NaCl (negative control).

3. DISCUSSION

A. *catechu* ethanol extract partition data, obtained through phytochemical screening testing. The test results produce various complex compounds with variations in red and orange. Such compounds include flavonols, flavanones, flavannonols and xantons. Reddish-yellow discoloration was found on testing for positive flavonoid compounds. This change occurs due to the hydrolysis of O-glycosyl to its aglycone through the addition of concentrated HCl. With the H+ electrophilic properties of the acid replace glycosyl. Changes in red-orange color deposits in alkaloid compound testing using test tubes on dragendoff reagents showed

positive results. The formation of a potassium-alkaloid precipitate complex is due to the presence of coordinate covalent bonds with nitrogen in alkaloids by K+ metal ions of potassium tetraiodomercurate(II), while for mayer reagents a white precipitate is formed. Blue-black discoloration of tannin compounds using a positive test tube. This change occurs due to the presence of phenol compounds. Furthermore, the formation of foam in saponin compounds using a positive test tube [17].

The study group used partitioned A. *catechu* ethanol extract with concentration levels (10%, 20% and 30%). The selection of A. *lumbricoides* worm test animals extracted from A. *catechu* ethanol which is then dissolved together with a 0.9% physiological salt solution is intended to maintain the cell membrane of the A. *lumbricoides* worm's body so that it is not damaged because the medium is isotonic so that the worm remains alive and comfortable despite changes in its habitat environment.

Meanwhile, to ensure the determination of the length of life of A. *lumbricoides* worms, 0.9% NaCl solvent was used as a negative control. The selection of this solvent is with the consideration that this solution is an isotonic physiological salt that allows no change in cell shape both intra-cellular and extra-cell body of the worm *A*. *lumbricoides* so that when these ions compound the worm's body, its survival is maintained in the hope that the effect of the study group can be identified.

In addition, in the second control group, pyrantel pamoate (combantrin® 125 mg) (positive control) was used. In terms of handling *A*. *lumbricoides*, it is recommended to use a broad-concentration anthemintic, therefore in this study using a positive control pyrantel pamoat solution (combantrin® 125 mg) which serves as a comparison group to see the ability of A. *catechu* ethanol extract partitioning as anthelmintic in the study group at concentration levels of 10%, 20% and 30%[18,19].

Previous research has explained that pyrantel pamoate is anthelmintic through its physiological mechanism of action which is proven to be able to paralyze A. *lumbricoides* worms. This is because this pirantel pamoat tablet with a certain dose (125 mg) has two abilities at once, namely inhibiting neuromuscular work and increasing the frequency of impulses as a result of depolarization in the worm's brain, causing rapid worm death in a spastic state[20–22].

Using partitions of A. *catechu* ethanol extract, concentrations of 10% and 20% in the 1st and 2nd groups at hours 1 to 12 respectively – respectively did not show anthelmintic effects. The anthelmintic effect on the replication process only appeared at the 18th hour with the discovery of 2 dead worms. The death of 1 worm was found at the 24th hour. The findings in groups 1 and 2 confirmed that concentrations of 10% and 20%, respectively, had shown anthelmintic effects on A. *lumbricoides* worms. At a concentration of 30% ethanol extract partition A. *catechu* for group 3 there was a difference in anthelmintic effect with the previous 2 extract concentrations. At this concentration level, it was found that the frequency of worm deaths every hour was more, namely the 6th hour and the 12th hour respectively, with each death of 2 heads and 1 worm.

Similar findings also showed good anthelmintic effects at concentrations of 10% and 20%, while at concentrations of 30% ethanol extract partitions A. *catechu* showed differences in anthelmintic effects [23]. In another study using A. *catechu* ethanol extract extract concentrations of 1%, 2.% and 4% with 3 times testing showed the number of worm deaths of 4, 7 and 11 heads respectively [24].

Anthelmintic effect against the death of helminths. This occurs by changes in the degenerative endoplasmic reticulum, mitochondria germ layer, and the release of lysosomes in intestinal cells of worms, where tubulin site binding with colchicine will inhibit the polymerization of cytoplasmic microtubules of worms. This will disrupt glucose absorption so that glycogen storage reserves become reduced. The decrease will impact the production of adenosine triphosphate (ATP) as the energy base of helminths to maintain its survival [25–27]. Decreased energy production causes parasites to not move freely and eventually die at different concentrations of A. *catechu* ethanol extract [28].

A. *catechu* extract shows a dual nature, namely in addition to having anthelmintic properties also affects intestinal absorption in worms which causes death. It has been found in phytochemical testing of various complex compounds that have been shown to have anthelmintic effects [29]. Niclosamide, oxyclozanide, and bithionol are synthetic phenolic anthelmintics that directly interfere with ATP production in . anthelmintics parasites through the separation mechanism of oxidative phosphorylation (30). Another prominent complex compound is tannins which can bind proteins or glycoproteins in the intestinal tract of worms and parasite cuticles [30, 31].

The active compound contained in A. *catechu* will inhibit the gamma amino benzoic acid reaction, which can cause paralysis of worms [32], besides that *A*. *catechu* has pharmacological therapeutic effects have various benefits, including can be used for the treatment of diseases caused by parasites and also several other diseases [14].

The ethanol extract partition of A. *catechu* has a very promising anthelmintic effect taking into account all the findings of several biological activities of the formulation that contribute greatly to the development of drug research. A. *catechu* plants have the potential to be explored continuously related to their phytochemical profiles in order to further examine the active compound components responsible for drug research, especially parasitic drugs.

A recent study has reported that there is a balanced association between *A. lumbricoides, trichuris trichiura* and (*ancylostoma duodenale, necator americanus)* infections in humans. However, the study did not account for the age group studied.

4. CONCLUSION

Partitioning ethanol extract of A.*catechu* with 3 different concentrations influences providing anthelmintic effects .The 30% concentration and positive control showed no different results in the two groups. Partitioning of A. *catechu* ethanol extract at a concentration of 30% showed the highest level of efficacy in killing worms based on the average time of death collected. The entire study group showed morphological and microscopic changes in worms.

At the end of this study confirms that the partition of A. *catechu* extract has a diverse pharmacological therapeutic effect, in addition to being anthelmintic also serves as a treatment in several diseases, especially parasitic diseases. In order to develop further research, it is recommended to conduct research ethanol extract partition A. *catechu* concentrations are similar to the effects of death in *trichuris trichiura* and (*ancylostoma duodenale, necator americanus)*.

5. MATERIALS AND METHODS

This study uses several tools for the sample processing process as follows; Aluminum foil, stirring rod, petri dish 150 mm ×15 mm (pyrex®), porcelain dish 75 ml, glass funnel 75 mm (pyrex), measuring cup 100 ml (pyrex®), beaker 200 ml (pyrex®), oven (B-One®), tweezers, spatula, tube rack, rotary evaporator (B-One®), maceration jar, measuring flask 500 ml (pyrex®), Erlenmeyer flask 100 ml (pyrex®), filter paper, gloves, mask (SENSI Mask®), scissors, blender (Kirin®), mortar and stamper, analytical balance (Taffware digipounds), tissue, drip pipette, rough wipe, fine wipe, and test tube (pyrex®), split funnel (pyrex®), statif, clamps. The ingredients used in this study include: worms *A. lumbricoides*, sterile aquades, *A. catechu*. Ethanol Extract, ethanol 96%, NaCl 0.9%, pirantel pamoat tablets 125 mg, ethyl acetate, n-hexane, anhydrous acetic acid, anhydrous acetic acid P, hydrochloric acid P, concentrated HNO3, iron(III) chloride, HCl 2N, H2SO4 concentrated, sulfuric acid 2N, chloroform, magnesium powder, ammonia, bismuth III nitrate, potassium iodide, mercury II chloride, and distilled water.

5.1. *In vitro* studies

A. *lumbricoides* worms [33], which were taken and collected from the intestines of pigs in the Moncongloe Maros slaughterhouse, through pig intestines were cut using a scalpel then A. *lumbricoides* was taken using anatomical tweezers and put into a prepared container containing 0.9% NaCl solution. Furthermore, A. *Lumbricoides* was taken to the Pharmaceutical Microbiology Laboratory of Megarezky University Makassar, then identification was carried out based on morphological characteristics. A. *catechu* as much as 2 kilograms taken in Barru Regency, Soppeng Riaja District, South Sulawesi Province. *A. catechu*. that has been collected, then drained.

Next, it is dried in the oven at a temperature of 50^{o}C until dry. The next stage of the sample is blended into a fine powder and sifted with a sieve of mesh number 100, then stored in a clean and tightly closed container. Sieved A. *catechu*. powder is then extracted by maceration. The powder is macerated using 70% ethanol solvent. A total of 800 grams of simplicia powder is put into a vessel and then poured with solvent until the sample is completely submerged, covered and left for 3 days protected from light while repeatedly stirring in the first 6 hours. Then the maceration results are filtered using filter paper, then the residue is macerated again with ethanol solvent until submerged everything is closed and left for 2 days while repeatedly stirring. Then the sample is filtered using filter paper after which the filtrate obtained is put together and concentrated using a rotary evaporator with a temperature of 30^{0}C to 40^{0}C. Then concentrated again using a waterbath until a thick extract of *A. catechu* is obtained [34].

After becoming a thick extract of A. *catechu* then weighed as much as 10 grams, put into a beaker added 70% ethanol as much as 50 ml and then sufficient with aquades up to 100 ml after that put into a separate funnel added non-polar solvent namely n-hexane as much as 50 ml, then shaken slowly in the direction

occasionally opening the funnel lid so that gas comes out, Allowed to stand until the separation process occurs, the upper layer is pipette, namely the N-Hexane layer, inserted into the bottle, after that the water-ethanol layer that is still in the separation funnel was added 50 ml of ethyl acetate then shaken slowly in the direction of occasionally opening the funnel cap so that gas comes out, allowed to stand until the separation process occurs, the top layer is pipette, namely the ethyl-acetate layer, then the bottom layer is also accommodated. So that the results of water-ethanol partition, ethyl acetate partition and n-hexane partition were obtained then 3 of the partition results were in the rotary evaporator again and then concentrated using a waterbath until a thick extract of N-hexane partition, ethyl acetate partition, water-ethanol partition was obtained [35].

5.2. Experimental

A. *lumbricoides* were each grouped into 5 groups of group I as positive controls using a 125 mg pyrantel pamoate solution; group II as a negative control using 0.9% NaCl solution; group III ethanol extract A. *catechu* 10%; group IV 20%; group V 30%. In each group there are 3 tails of A. *lumbricoides* soaked in it.

Phytochemical screening of ethanol extract partitions A. *catechu* [36]. Petri dishes were prepared, each containing, 125 mg pyrantel pamoate tablet solution, A. *catechu*. ethanol extract, 0.9% NaCl, n-hexane partition ethanol extract, and ethyl acetate partition ethanol extract, water-ethanol partition ethanol extract with the same concentration of 30%, A. *lumbricoides* worms as many as 3 heads were inserted into each petri dish, and observed the amount of A. *lumbricoides* that was paralysis and died within a certain time after soaking and recorded paralysis time until worms die. To find out if A. *lumbricoides* is lysed (dead), paralysis or still normal after soaking, the worms are disturbed with a stirring rod. If the worm is stationary, it is transferred into hot water with a temperature of 500C, and its movements are observed again. If in this way the worm remains stationary or immobile, it means that the worm lysis (dies). But, if it moves, it means that this worm is only paralysis (fainting). Observation is carried out every 1 hour after soaking until all worms die, for 24 hours.

5.3. Statistical analysis

Statistical analysis of the data began with a normality test in the study group followed by a homogeneity test. Kruskal Wallis testing to test the difference in the effects of each concentration on the study group, positive control, negative control. Then continued the Mann whitney test to see the difference in anthelmintic effects between the study group given a partition of A. *catechu* ethanol extract and a positive control given a 125 mg pyrantel tablet solution as a comparison drug. Significance ($p < 0.05$).

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