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Potential Anthelmintic Activity of Pineapple Fruit (*Ananas comosus*) and Mango Leaves Juice (*Mango foliorum*) against *Ascaridia galli* Worms In-Vitro Model

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ABSTRACT

Ascaridia galli is a worm that infects the chickens' digestive tract, leading to various problems such as worsening feed efficiency, slowing poultry growth, and increasing mortality. Mango leaves and pineapple fruit were reported to have anthelmintic activity against *A. galli*. These two plants contain secondary metabolites that were suggested to have synergistic effects. This study aimed to determine the potential activity of combining arumanis mango leaf and pineapple juice against *A. galli* and evaluate their interaction based on anthelmintic activity profile. The activity of 40% pineapple fruit juice (PA) added with different concentrations of arumanis mango leaf juice (AM) from 0.13%, 0.26%, and 0.52% v/v, and pineapple fruit juice at concentrations 20, 40% v/v were mixed with 0.26% arumanis mango leaf juice. as well as NaCl 0,9% as negative control and pyrantel pamoate 0.2; 0.4; 0.8 % b/v as a positive control respectively were determined. Observation on worm mortality was conducted in 15-minute intervals for 300 minutes. The time-a cumulative mortality rate of worm curve exposed to PA 40 % was shifted to the left in the presence of AM, and AM 0.26 % curve in the presence of PA. The best, LC_{50}, LT_{50}, and L_{100} were found in AM 0.52 % combined with PA 40%. This finding showed the potential activity of pineapple fruit juice at 40% combined with arumanis mango leaf juice at 0.52 % as the best combination in which anthelmintic activity occurs due to synergism interaction between arumanis mango leaf juice and pineapple fruit juice.

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INTRODUCTION

Ascariasis is a disease caused by a worm known as *Ascaridia galli (A.galli)*. This common internal parasitic disease affects mostly free-range chickens and impacts production. As an illustration, the incidence rate of ascariasis of free-range chickens in Kupang and Bandung District areas is about 88.58 % and 34.5% respectively (Moenek and Oematan, 2017; Pabala *et al.*, 2017), while in Jember District of East Java about 60% (Kusuma *et al.*, 2021), as well as Bogor and Jakarta approximately 70% and 56% (Kusumadewi *et al.*, 2020).

Ascariasis transmission occurs directly by swallowing infective worm eggs (Al-Quraishi *et al.*, 2020) which then hatch and develop into adult worms in the digestive tract of the chickens. Severe intestinal damage often occurs as a result of larval migration. The intestinal mucosa bleeding in infected chickens affects their digestion and nutrient absorption, hampering the growth. Moreover, in severe infections, *A galli* causes intestinal blockage, decreased sugar levels, impaired growth, and increased mortality leading to significant economic losses for chicken farmers (Tabbu, 2002; Al-Tameemi and Kabakli, 2020). Several drugs

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including imidazothiazoles (pyrantel and levamisole), benzimidazoles (flubendazole and fenbendazole), and macrocyclic lactones (ivermectin) have anthelmintic activity to control the parasitic worm in the intestinal lumen (Samje *et al*., 2021; Feyera *et al.*, 2022). However, although anthelmintic resistance (AR) against *A. galli* has not yet been reported, AR against other helminths is well documented (Liu *et al*., 2020; Samje *et al.,* 2021). Insufficient anthelmintic activity of fenbendazole and levamisole against *A. galli* in chickens was identified (Samje *et al*., 2021), and developing AR was also observed in Sweeden (Tarbiat *et al*., 2023). The inconsistent efficacy of benzimidazole as an anthelmintic suggests the possibility of the emergence of AR in poultry ascarids. The emergence of anthelmintic resistance has encouraged the exploration of new more effective anthelmintics. Several plants, including mango and pineapple leaf, were reported to have anthelmintic activity against *A galli.*

Arumanis mango leaves (*Mangifera indica* L.) contain tannins and flavonoids which were proposed to have their properties as an anthelmintic. Tannin denatures proteins and inhibits the action of cholinesterase enzyme so the worm's metabolism is disrupted, resulting in the worm's death. According to Robiyanto *et al.*, (2018), ethanol extract from arumanis mango leaves (*M. indica* L.) has anthelmintic activity against *A. galli in-vitro* model with an LC_{50} value of 2.6 mg/mL or 0.26% w/v.

Another plant that has the potential activity against *A. galli* is pineapple (*Ananas comosus* L.). Juice from this fruit was reported to have anthelmintic potency against *A. galli in-vitro* with an LC_{50} value of 40% (Mighra, 2017). Compounds that were suggested to play an important role in the emergence of anthelmintic activity are alkaloids and saponins (Ali *et al*., 2011). These two compounds inhibit the cholinesterase enzyme activity, which hydrolyzes acetylcholine into choline and acetate. Inhibition of acetylcholinesterase activity causes worm paralysis and death (Putra *et al.*, 2015). Combinations of bioactive agent are often used to obtain better effectiveness and safety. Both mango leaves and pineapple fruit contain bioactive compounds with the potential to be anthelmintic. However, the potential anthelmintic activity of mango leaves combined with pineapple against *A. galli* was not confirmed. The current study aimed to investigate the anthelmintic activity of pineapple fruit and arumanis mango leaf juice in different combinations. The results of this research will provide information regarding the potential of a combination of mango leaves and pineapple fruit as an alternative medicine to treat *A. galli*.

MATERIALS AND METHODS

Mango Leaf and Pineapple Fruit Juice preparation

Mango leaves of *M. indica* L. and pineapple fruit of *A. comosus* L. were obtained from the Citayam area, Bogor, Indonesia. Plants were determined at the Biological Sciences Research Organization - Cibinong, Bogor, West Java, Indonesia.

Fresh arumanis mango leaves were harvested and sorted to remove contaminants. It was then washed under running water. A total of 100 g mango leaves were cut into small pieces and ground with 100 mL of distilled water. The juice was separated from the residue by filtering it using batis cloth to obtain mango leaf juice at a concentration of 100% w/v.

For *A. comosus*, the ripe and fresh pineapple was cut into small pieces and placed into a juicer (Philip, China). The juice was separated from the residue using a batis cloth until the pineapple juice concentration of 100% v/v was obtained. The juice from pineapple fruit and mango leaves was pipetted according to the calculated concentration to be used and put into the same petri dish (Duran Germany), therefore the total volume of 30 mL was obtained according to the concentration used in the treatment.

Phytochemical screening

Secondary metabolites of mango leaves and pineapple juice were analyzed for their phytochemical constituents using the protocol described by Hanani (2015). To identify alkaloids, the extract was first dissolved in 2N H_2SO_4 and subsequently reacted with Mayer's, Dragendorff's, or Bouchardat's reagents. The presence of alkaloids was indicated by a white precipitate with Mayer's reagent, a brick-red precipitate with Dragendorff's reagent, or a blackish-brown precipitate with Bouchardat's reagent.

To determine flavonoids, the extract was mixed with 90% ethanol and heated in a water bath. Magnesium (Mg), zinc powder (Zn), and 5–6 drops of concentrated hydrochloric acid were added to the solution. A positive result for flavonoids was identified by the formation of a red-orange colored solution.

The tannin test was conducted by dissolving 0.5 g of the extract, in distilled water and heated in a water bath with continuous stirring. After cooling, the mixture was centrifuged, and the supernatant was separated by decantation. The resulting solution served as the test sample. The presence of tannins was confirmed by the formation of a white precipitate in a 10% gelatin solution and a blue to blackish-green coloration upon the addition of 3% ferric chloride.

Meanwhile, for the saponin test, 0.5 g of the extract was dissolved in 10 mL of water. The solution was shaken vigorously for 10 seconds to produce foam. A positive result for saponins was indicated if the foam remained stable after the addition of one drop of hydrochloric acid and standing for 10 minutes.

Preparation of mango leaf and pineapple fruit juice combination

Arumanis mango leaves juice 100% as much as 0.13; 0.26 and 0.52 mL was diluted using sodium chloride (NaCl) to obtain the final concentration of mango leaves juice 0.26% v/v, 0.52% v/v and 1.04% v/v respectively. Pineapple fruit juice 100% was diluted using 0.9% NaCl to obtain a final concentration of 40% v/v and 80% v/v. The juice combination was put into a petri dish according to the specified concentration, to make 30 mL of each combination by adding 15 ml of 80% pineapple fruit juice with 15 mL of 0.26, 0.52, or 1.04 arumanis mango leaves juice respectively to obtain a final concentration of pineapple fruit juice of 40% with mango leaves juice of 0.13%, 0.26% and 0.56 % v/v. Meanwhile, to make a combination of 20 and 40% pineapple juice, 15 mL of 40 and 80% pineapple juice each was added to 15 mL of 0.52% arumanis mango leaf juice to obtain a final concentration of AM 0.26% + PA 20% and AM 0.26% + PA 40 %.

Pyrantel Pamoate Solution Preparation

A standard solution of 5% pyrantel pamoate was made by dissolving 2.5 g of pyrantel pamoate with 0.9% NaCl. The standard solution 4, 8, and 12 mL was then dissolved in a 100 mL flask (Duran, Germany) using 0.9% NaCl until a concentration of 0.2%, 0.4%, and 0.6% w/v pyrantel pamoate was obtained; (Rianto, *et al*, 2016).

Anthelmintic Activity Test

This research protocol has received approval from the ethics commission of the Faculty of Mathematics and Natural Science, Pakuan University with approval number 042/KEPHP-UNPAK/11-2021, and the authenticity of *A. galli* from the Indonesian Research Institute for Veterinary Science (No LB. 22/80).

The worms used in this research were female adult *A galli* worms, lengths between 6 to 12 cm characterized by a straight, non-curved posterior end and still actively moving, no anatomical defects, and originating from the intestines of chickens aged 1 - 2 months. All worms were obtained from the Bogor Market slaughterhouse, in Bogor City. All worms obtained were soaked in 0.9% NaCl solution.

The anthelmintic activity test was carried out using the method presented by Robiyanto *et al.* (2018). A total of 135 worms were separated into 9 treatment groups, namely worms that were exposed to 0.9% NaCl as a negative control, pyrantel pamoate at 0.2; 0.4 and 0.6 % (PM) as a positive control group, and pineapple juice 40%, as well as a combined of fixed concentration pineapple juice 40% + arumanis mango leaf juice 0.13% (AM 0.13% + PA 40%); 0.26% (AM 0.26% + PA 40%) or 0.52% (AM 0.52% + PA 40%) There was also a combination of a fixed concentration of arumanis mango leaf juice (AM) and various concentrations of pineapple fruit juice (PA) namely arumanis mango leaf juice 0.26% w/v and pineapple juice 20% v/v, (MA 0.26% + PA 20%) or 40% v/v, (AM 0.26% + PA 40%). Each treatment group consisted of 5 *A. galli* worms and the experiment was carried out in 3 repetitions (3 replications) (Table 1).

The all treatment groups were incubated at 37ºC. The time and the number of worm mortality were observed every 15-minute intervals for 300 minutes. The worm was considered still alive if after being disturbed with a stirrer, the worm was still motile. If the worms remained non-motile after being disturbed then the worm was transferred to warm water with a temperature of 50ºC. If the worm remains motionless, it is determined that the worm has died, but if the worm is still moving, it is determined to be experiencing paralysis.

Data analysis

The obtained data were analyzed statistically using ANOVA for a completely randomized experimental design, followed by a post hoc test using Duncan. The significant difference was determined at $P<0.05$.

Table 1. Dilution of mango and pineaple 100 % with NaCL 0.9% to obtain final concentration of test preparation

Pyrantel pamoat (PM)			Arumanis mango leaf juice (AM)			Pineapple (PA)			Combination	
Pyrantel pamoat 5 % (mL)	NaCl 0.9% (mL)	Final Concentration (%)	Arumanis Mango leaves juice 100% (mL)	NaCl 0.9% (mL)	Final Concentration (%)	Pineapple 100% (mL)	NaCl 0.9% (mL)	Final Concentration (%)	Combination	Final Concentration
4	*ad* 100	0.2	0.13	*ad* 50	0.26					AM 0.13%
8	*ad* 100	0.4	0.26	*ad* 50	0.52	40	*ad* 50	80	15 mL of AM + 15 mL of PA	+ PA 40% AM 0.26% + 40%
12	*ad* 100	0.6	0.52	*ad* 50	1.04					AM 0.52% + PA40%
						20	*ad* 50	40	15 mL of AM + 15 mL of PA	AM 0.26% + PA 20%
			0.26	50	0.52	40	*ad* 50	80		AM 0.26% + PA 40%
						40			30 mL of PA	PA 40%

Table 2. Treatment Groups of worms

No	Treatment	Combined with	%
	(% b/v)	(% b/v)	(% b/v)
1.	NaCl (% b/v)		0.9
2.	PM (% b/v)		0.2 0.4 0.6
3.	AM 0.13% AM 0.26% AM 0.52%	PA	40 40 40
4.	AM 0.26%	PA	20
5.		PA	40

RESULTS AND DISCUSSION

Plant Determination

Determination of the material authenticity carried out by the Biological Sciences Research Organization (BRIN) Cibinong (No. B-217/V/Dt.05-07/1/2022), indicated that the plant used was the leaves of the mango type *M. indica* L. which belongs to the Anarcadiaceae family. The fruit of the pineapple type was *A. comosus* L. which belongs to the family Bromeliaceae.

Phytochemical Screening

Phytochemical analysis results from various qualitative tests conducted on arumanis mango leaves and pineapple fruit juice extract are presented in Table 3. The formation of a white precipitate with Mayer's reagent, a brick-red precipitate with Dragendorff's reagent, or a blackish-brown precipitate with Bouchardat's reagent. tests indicated the presence of alkaloids. Furthermore, a red-orange colored solution indicated flavonoid and white precipitate in a 10% gelatin solution and a blue to blackish-green coloration indicated tannin as well as the production of a stable foam confirmed the presence of saponins.

Table 3. Secondary metabolites identified in arumanis mango leaf and pineapple fruit juice

No	Identified compound	Reagent	Mango juice	pineapple juice
1	Flavonoid	Magnesium powder + concentrated HCl	+	+
2	Alkaloid	Mayer Dragendorf Bourchardad	+ + +	+ + +
3	Saponin	Hot water + sample	+	+
4	Tanin	$FeCl_3$ Gelatin	+ +	+ +
5	Triterpenoid	CH_3COOH + H_2SO_4	+	+

Note: (+) mean contain and (-) not contain chemical compound

Anthelmintic Activity

The worm exposed to 0.9% v/v NaCl survived until the end of observation time for 300 minutes. However, the exposed worms to PM, single PA, a combination of various concentrations of AM and a fixed concentration of PA, as well as a fixed concentration of AM + several concentrations of PA were found not to survive.

The number of worms that died at 300 minutes, except for those given NaCl, reached 100% (Figure 1) but the time to attain 100 percent death differed among the treatment groups. The shortest and the longest time needed to 100% worm dead respectively were demonstrated by 0.6% PM at 90 minutes and 40% PA at 285 minutes. This shows that there is a difference in the anthelmintic activity of the mixture.

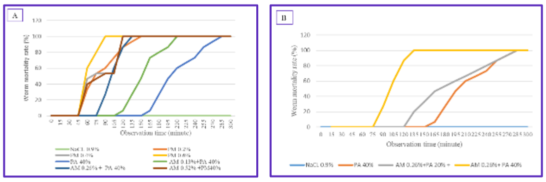

Figure 1 Time-cumulative mortality rate curve of varying concentration of arumanis mango leaf and fixed concentration of pineapple (A), fixed concentration of arumanis mango leaf (AM 0.26%) and varying concentration of pineapple (PA 20%; 40%) (B) compared to single pineapple (PA) 40%, NaCl as control (-).
Note: NaCl : Natrium Choride 0.9%, AM 0.13% + PA 40%: arumanis mango leaves juice 0.13% v/v and pineapple juice 40% v/v, AM 0.26% + PA 40%: arumanis mango leaves juice 0.26% v/v and pineapple juice 40% v/v, AM 0.52% + PA 40%: arumanis mango leaves juice 0.52% v/v and pineapple juice 40% v/v, AM 0.26% + PA 20 %: arumanis mango leaves juice 0.26% v/v and pineapple juice 20% v/v, AM 0.26% + PA 40 %: arumanis mango leaves juice 0.26% v/v and pineapple juice 40% v/v, PA 40%: Pineapple juice 40% v/v

The cumulative worm mortality response to treatment is figured out to explore the strength of the anthelmintic activity of arumanis mango leaves and pineapple fruit juice (Fig 1 and 2). The time-cumulative worm mortality curve of pineapple fruit juice shifted to the left in the presence of mango leaf juice (Figure 1), and the curve also moved to the left when AM 0.26 % was added to PA (Figure 2).

The anthelmintic potential of the test preparation was evaluated based on LC_{50} and LC_{90} values, which were determined using log dose-probit curve analysis. The results are presented in Table 4. The anthelmintic efficacy was further assessed by measuring how quickly the preparation killed the worms, expressed as LT_{50} (time required to kill 50% of the worm population) and LT_{100} (time required to kill 100% of the worm population). These parameters were determined using a cumulative time-probit worm mortality curve, with results displayed in Table 2 and Figures 2A and 2B.

Table 4. Lethal concentrations of 50 (LT_{50}) and 100 (LT_{100}) percent of worm death

No	Treatment		%	Observation	
				LT_{50} (minute)	LT_{100} (minute)
1.	NaCl (% b/v)		0.9	0	0
2.	PM (% b/v)		0.2	85.5 ± 2.9^{b}	135 ± 15^{b}
			0.4	82.0 ± 2.4^{b}	120 ± 0^{b}
			0.6	65.8 ± 2.8^{a}	85 ± 8.7^{a}
3.	AM 0.13%	PA % (v/v)	40	159.7 ± 15.3^{d}	190 ± 23^{c}
	AM 0.26%		40	104.4 ± 5.4^{c}	130 ± 8.7^{b}
	AM 0.52%		40	84.7 ± 4.3^{b}	120 ± 0^{b}
4.	AM 0.26%	PA %(v/v)	20	189.8 ± 12.7^{e}	265 ± 23^{d}
			40	104.4 ± 5.4^{c}	130 ± 8.7^{b}
5.	PA 40%		0	212.9 ± 9.6f	260 ± 23^{d}

Note: PM: Pirantel Pamoat, AM 0.13% + PA 40%: arumanis mango leaves juice 0.13% b/v and pineapple fruit juice 40% v/v, AM 0.26% + PA 40%: arumanis mango leaves juice 0.26% b/v and pineapple fruit juice 40% v/v, AM 0.52% + PA 40%: arumanis mango leaves juice 0.52% b/v and pineapple fruit juice 40% v/v, AM 0.26% + PA 20 %: arumanis mango leaves juice 0.26% b/v and pineapple fruit juice 20% v/v, AM 0.26% + PA 40 %: arumanis mango leaves juice 0.26% b/v and pineapple fruit juice 40% v/v +, PA 40%: Pineapple fruit juice 40% v/v

Regression of concentration of combination juice-LT_{50} and concentration of juice combination - LT_{100} curve of pineapple fruit and arumanis mango leaf juice in various compositions indicated that Various concentrations of AM + Fixed concentration of PA (VAM+PA) have slopes steeper than that of the fixed concentration of AM + various concentration of PA (AM + VPA) but insignificantly different from PM (Table 2). The steepest slope was indicated by VAM + PA (-0.2424) followed by PM (,-0.0493) and AM +VPA (-0.0043) respectively. A similar result was also found in LT_{100}, the steepest slope is VAM + PA (-0.2593) followed by PM (-0.125) and AM + VPA (-0.0068). These findings suggest that the LT50 and LT100 values for the combination of PA and AM are dose-dependent and influenced by the specific composition of PA and AM juice.

The time of achieving 50 percent mortality showed significant differences among treatment groups. A single 40% PA takes approximately 212.9 ± 9.6 minutes to kill 50% worm (LT_{50}) and the LT*50* of PA 40% significantly became shorter in the presence of arumanis mango leaf juice. The greater the arumanis mango leaf juice concentration was added to 40% PA, the shorter LT_{50} of the combined PA (Table 4). The shortest time of AM + PA combination required to kill worms was found in a combination of PA 40% and AM 0.52%. (Figure 2). LT_{100} of combined PA 40% was 260± 23 minutes and also became shorter when arumanis mango was added, however, the addition of arumanis mango 0.26% to PA 40 % has an insignificantly different effect from those of 0.52%.

In case AM and PA composition consists of a fixed concentration of AM with varying concentrations of PA (AM + VPA), the presence of arumanis mango 0,26% reduced the LT_{50} value making it lower than that of LT_{50}.of PA. However, the LT_{50} of AM 0.26% was longer when the PA concentration decreased from 40% to 20%. In contrast to LT_{50}, the effect of PA 40% was comparable to that of the combination of 0.26% AM and 20% PA. Furthermore, the addition of arumanis mango to PA 40% reduced the LT_{100} to become shorter than that of PA 40%. The LT_{50} and LT_{100} of PA 40% were not significantly different from LT_{100} of AM 0.26% + PA 20%. Additionally, the LC_{50} and LC_{90} values determined at 2 hours of observation, based on the log dose-probit response curve, revealed that the LC_{50} of 40% PA in the presence of 0.26% AM was smaller than the LC_{50} of various concentrations of PA combined with 0.26% AM. However, the LC_{50} of this combination was still higher than the LC_{50} of PM (pineapple juice alone). Conversely, the LC_{100} of the PA + VAM combination was lower than the LC_{100} of PM.

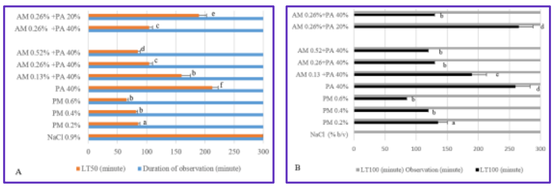

Figure 2. Lethal time 50 (A), Lethal Time 100 (B) of various concentrations of arumanis mango leaf juice in the presence of pineapple fruit (VAM +PA), and arumanis mango leaf juice 0.26% in the presence of various concentrations of pineapple fruit juice (AM + VPA) in comparison with pyrantel pamoate.

Note: PM: Pirantel Pamoat, AM 0.13% + PA 40%: arumanis mango leaves juice 0.13% b/v and pineapple fruitjuice 40% v/v, AM 0.26% + PA 40%: arumanis mango leaves juice 0.26% b/v and pineapple fruit juice 40% v/v, AM 0.52% + PA 40%: arumanis mango leaves juice 0.52% b/v and pineapple fruit juice 40% v/v, AM 0.26% + PA 20 %: arumanis mango leaves juice 0.26% b/v and pineapple fruit juice 20% v/v, AM 0.26% + PA 40 %: arumanis mango l leaves juice 0.26% b/v and pineapple fruit juice 40% v/v, PA 40%: Pineapple fruit juice 40% v/v

The time-cumulative mortality curve indicated that the AM and PA combination significantly affect the mortality rate of worms, in which the magnitude of the AM and PA combination influences the mortality depending on the composition of the PA and AM. In the presence of AM, the time-response curve profiles of PA move towards the left suggesting the alternation in anthelmintic potency but not in its efficacy as the maximum response was not changed (Myce *et al.*, 2000). The anthelmintic potency was determined based on the LC_{50} value namely the concentration that killed 50 percent of the worm population exposed to the tested preparation. Furthermore, the length of time required to kill 50 (LT_{50}) or 100 percent (LT_{100}) of the worm population exposed to the test preparation, can also express the anthelmintic potency. The sooner the worms are killed the better the anthelmintic power.

In the present study, LT_{50} and LT_{100} of pineapple fruit juice are 212.9 ± 9.6 and 260 ± 23 minutes, respectively. The increase of PA concentration. in the presence of a fixed concentration of AM 0.26% or an increase in the AM concentration in the presence of a fixed concentration of PA leads to the shortening of LT_{50} and LT_{100}. Compared to a previous study, the present study has a better LT_{50} and LT_{100} values. In which LT_{100} of pineapple fruit juice was 665.529 minutes. (Mighra, 2017), meanwhile, the LT_{50} for ethanol extract from mango leaves is 19.2 hours (Robiyanto *et al*., 2018). The variation in extraction method, mango species, and pineapple fruit as well as the location of their growth may affect the mango leaf and pineapple fruit activity to worm mortality.

The insignificant difference between LT_{100} of PA 40% and LT100 of AM 0.26% +PA 20% indicated that the efficacy of PA 40% is equivalent to AM 0.26% + PA 20% (Table 4). The reduction in PA concentration to half is covered by the effect of AM 0.26% suggesting the existence of interaction between AM 0.26% and PA40%. However, the significant discrepancies between AM 0.26% + PA 40% and PA 40% of LT_{100} are not found in LT_{50}. It is speculated that the combination of PA + AM is time-dependent to act as anthelmintic in addition to a dose-dependent manner.

To compare which composition resulted in a better anthelmintic effect against *A. galli,* the regression of LT_{50} and LT_{100} against concentration was evaluated. The obtained data indicated that adding various concentrations of arumanis mango on PA 40% has a steeper slope than that of fixed concentration arumanis mango + various concentrations of pineapple fruit juice. The remarkable effect was shown by the first combination on LT_{50} and LT_{100} compared to the second combination which added varying concentrations of juice on arumanis mango leaf (Table 2). Among the combined juices, the composition of AM 0.52% w/v + PA 40% v/v has the shortest LT_{50} and LT_{100}. The time required for AM 0.52% in the presence of PA 40% to kill 100% of worms reduces to half of PA 40% time and more than half to kill 50% of worms. In line with LT_{50} and LT_{100}, the potency of Variation in AM concentration + PA is also better than the variation in concentration of AM0.26% + PA. Increasing the concentration of PA leads to a smaller LC50 when MA is fixed at 0.26%, and the increasing concentration of AM leads to a smaller LC50 when PA is fixed. The shorter LT_{50} and LT_{100} as well as the smaller LC_{50}, the better the potential action of the tested combination. Thus, the composition AM 0.52% w/v + PA 40% v/v is the most effective anthelmintic compared to the other compositions. The potency of this composition is also better than pyrantel pamoate as the LC_{50} and LC_{90} are smaller than that of pyrantel pamoate.

The results of the phytochemical analysis showed that both juices contained flavonoids, alkaloids, saponins, tannins, and triterpenoids. These secondary metabolites identified in the phytochemical test are in line with the secondary metabolites reported in previous research conducted by Wahyudi (2015) and Yusliana *et al.*, (2019), that both mango leaf infusion and pineapple fruit juice each contain flavonoids, alkaloids, saponins, tannins, and triterpenoids. Tannin, polyphenols, saponin, and flavonoids may act synergistically to kill worms (Cho-Ngwa *et al.*, 2010; Ndjonka *et al.*, 2011, 2014; Samje *et al.*, 2014).

Tannin and saponin compounds in mango leaves can paralyze worms, causing death (Ameen, 2011). The capability of saponin to inhibit acetylcholinesterase (Ali *et al.*, 2011) and damage the intestinal cellular membrane of parasite thus interfering with nutrient absorption (Navarro del Hierro *et al.*, 2018) as well as blocking the phosphorylation reaction by flavonoids (Symeonidou *et al.*, 2018) is suggested to be the cause of worm paralyzed and death. Other metabolites namely alkaloids have shown anthelmintic activity by targeting acetylcholine receptors and suppressing glucose uptake, thus helminths die due to starvation (Badarina *et al.*, 2017).

In addition, pineapple fruit also contains bromelain, a proteolytic enzyme. Bromelain has anthelmintic potential which acts as a destroyer of the cuticular tegument of parasitic walls. Bromelain can also bind to acetylcholine nematode worms (Fudhola *et al.*, 2024). Damage to the tegument facilitates the entry of bioactives into target receptors so that the anthelmintic potential of the combined extract becomes stronger. Multi-targeted anthelmintic action of AM 0.52% w/v + PA 40% v/v may lead this combination to a better potential action as anthelmintic than those of pyrantel pamoate.

Table 5. LC50 of pyrantel pamoate, arumanis mango leaves, and pineapple fruit.

No	Treatment	Regression Equation	R	LC50 (mg/100 mL	LC90 (mg/100mL)
1.	PM	y = 6.0183x - 7.5907	$R^2 = 0.8668$	123.61	571.0955
2.	VAM +PA	y = 8.8285x - 15.274	$R^2 = 0.9999$	197.89	561.7000
3.	AM +VPA	y = 19.99x – 85.979	$R^2 = 1$	35581.61	56406.0467
4.	PA	Not countable			

Note: Pyrantel pamoate (PM),: various concentrations of Arumanis Manggo leaf (VAM) in the concentration of 0.13; 0.26; 0.52%) + fixed concentration of Pineapple fruit (PA); and arumanis mango leaf 0.26% (AM) + in the presence of various concentration of pineapple fruit (VPA), in the concentration of 20, 40%.

CONCLUSION

The anthelmintic activity of pineapple fruit juice 40% is augmented in the presence of arumanis juice The anthelmintic activity of arumanis mango and pineapple fruit elicit anthelmintic activity as a result of the interaction between juice and mango which is additive or synergistic. The interactions that arise depend on the concentration of time and material composition. The effect of a combination of various concentrations of arumanis mango leaf +PA 40% with LC_{50} 197.89 mg/100 mL is better than the fixed concentration of arumanis mango leaf + various concentrations of pineapple fruit juice and the best combination was found in arumanis leaf juice 0.52% +pineapple fruit 40 %. This finding highlights the potential use of combined arumanis mango leaf and pineapple fruit as anthelmintic.

AUTHORS' CONTRIBUTIONS

The contributions of each author, including ideas, data generation, data analysis, manuscript preparation, and funding (MAD, MR, and NNR)

CONFLICT OF INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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