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Research Article
Effects of Citronella (*Cymbopogon nardus*) Ethanol Extracts and Distillate Dried Powder Waste on Inhibition of *Ascaridia galli* Development

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Abstract

Objective: The aim of this study was to quantify the bioactive components and antiparasitic activity of distillate citronella (*Cymbopogon nardus* or *C. nardus*) essential oil waste. **Methodology:** A densitometry method was used to quantitatively analyze the bioactive content of citronella waste. The effects of three concentrations of citronella waste extract and exposure time (30, 60, or 90 min) on adult Ascaridia galli (*A. galli*) worms *in vitro* from chicken intestines were evaluated. **Results:** Distillate waste from citronella (*Cymbopogon nardus*) (DWC) essential oil contains geraniol compounds. Treatment with DWC extract and powder significantly increased the mortality rate of *A. galli* compare to physiological solution (NaCl 0.9%). However, treatment with both 1.0% DCW and physiological solution (NaCl 0.9%) significantly increased the mortality rate of *A. galli*. Treatment effects increased significantly across all time points and there were interactions between material and concentration and; material and time points. **Conclusion:** The geraniol found in DWC may act as an antiparasitic against *A. galli* worms.

Key words: Distillate waste of citronella, geraniol, antiparasitic, *Ascaridia galli*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ascaridia galli (*A. galli*) is an endoparasite that infects poultry, reduces the growth performance and productivity. Zalizar *et al.*[1] reported that *A. galli* infection reduces the surface area of small intestinal villi by 20% in broiler during the starter phase. Additionally, Shanta *et al.*[2] found that parasites can affect feed consumption and reduce meat and eggs productions in livestock. Deka & Borah[3] also found that *A. galli* can cause anemia in broilers and Hassanain *et al.*[4] reported that *A. galli* can reduce body weight and cause diarrhea in cattle[3].

The life cycle of *A. galli* begins with eggs hatching from excreta. Egg cells develop into a morula and form a large blastomere within 10 days; this phase is called L1. At the anterior end, the larvae (L1) develop into a blunt and posteriorly transforms into a cone for five days and then, transforms into L2 larvae or infective eggs which contaminate feed, drinking water and/or litter cages. The infective eggs (L2) are carried from chicken beaks to the esophagus and hatch into larvae (L3) in proventriculus or duodenum. Larvae live freely in the posterior lumen of the duodenum for eight days post-ingestion. Although, the larvae lay on the duodenal lumen, the largest number of *A. galli* larvae are found in the jejunum[5].

Ascaridia galli larvae (L3) evolve into larvae L4, enter the mucosal tissue and remain there for eight to seventeen days[6]; however, Permin *et al.*[7] reported that *A. galli* larvae can penetrate the duodenal mucosa from three to fifty six days. When larvae L4 develop into larvae L5, called a young worm. They moves into the intestinal lumen. L4 live in the lumen until adulthood, at which time they infect the walls of the small intestine. Adang *et al.*[8] reported that *A. galli* worms can block the intestines and infect large chickens; which reduces blood flow, decreases blood glucose and slows growth, which increases chicken mortality.

Anthelmintic agents can prevent the development of *A. galli* but chickens may acquire resistance over time. Essential oils, however, may also inhibit the development of these worms. Konvvar and Gohain[9] and Kpoviessi *et al.*[10] reported that essential oils derived from *C. nardus* may inhibit parasites in cattle intestines. Citronella distillate waste may be used as anti-parasitic but requires further research[9-11]. The aim of this study was to quantify the bioactive components and anti-parasitic activity of citronella (*Cymbopogon nardus* or *C. nardus*) essential oil distillate waste.

MATERIALS AND METHODS

Extraction and siege process from distillate waste citronella (Cymbopogon nardus): Two types of extracts from Distillate Waste of Citronella (DWC) were used, an ethanol extract and a powder extract. Extraction was performed using the Maceration method adopted by Bero *et al.*[11]. The DWC was obtained from Pendem village, in the Ngablak sub-district of Magelang regency in Central Java. Citronella waste (400 g) was dried in an oven at 70°C for 24 h. The sample was ground until it became a powder and was stored in a stainless steel container. A 70% ethanol solvent was added at a ratio of 1:7. After 24 h, the solvent was filtered and evaporated over a water bath at 60-70°C to obtain a thickened extract material.

The siege process was conducted with a 500 g-sample, which was dried in the sun for three days until a constant dry weight was obtained. The sample was then ground into a powder.

Quantitative analysis of DWC: Quantitative analysis of DWC was conducted using the densitometric method as described by Harborne[12]. The extracted samples (as much as 55.1 mg) were diluted with 1,000 µL ethanol. Samples of 9 µL of geraniol and citronellal reagents of 1 µL were bottled on a 60 F254 silica gel plate. The plates were eluted with a hexane motion phase: ethyl acetate with a ratio of 9: 1 to the elution limit. The plates were dried and sprayed with 5 mL of sulfuric acid anisaldehyde, after which they were heated in an oven at 105°C for five minutes until spots appeared. The width of visible patches was measured using a Thin Layer Chromatography/TLC scanner at 527 nm wavelength (wavelength for geraniol).

***In vitro* test of *Ascaridia galli* worms**

Preparation of solution test: The extract and powder samples from DWC were weighed and mixed with 100 mL 0.25, 0.50 and 1.0% aquadest (weight/volume). Physiological solution (NaCl 0.9%) was used as a negative control.

Ascaridia galli worm sample: *Ascaridia galli* worms were obtained from broiler intestines at the Terban slaughter market Gondokusuman district in Yogyakarta. The intestines were opened using scissors and the adult worms were taken with tweezers and inserted into a beaker glass containing aquadest.

***In vitro* screening:** Solutions of 0.25, 0.50 and 1.0% were added to a petri dish and each treatment was repeated three

times. *A. galli* worms (as many as 10) were placed in the Petri dishes. Morbidity rates were evaluated at 30, 60 and 90 min time points.

Observation parameters: The effectiveness of the extract and powder was based on the number of *A. galli* worms that lysed or died in each treatment.

Experimental design and data analysis: A factorial design with two type of DWC extracts and three treatment times was applied. Data from the compounds were analyzed descriptively and two-way Analysis of Variance (ANOVA) was used to analyze *in vitro* data. Means were analyzed using Duncan's Multiple Range Tests (DMRT) at a 5% level of significance.

RESULTS

The DWC contained 0.51% of geraniol (Fig. 1). Although, residual DWC contains geraniol, much of the compounds were extracted from the oil.

Treatment with DWC powder, extract and physiological solution (NaCl 0.9%) resulted in significantly ($p<0.05$) increased *A. galli* mortality rates. Treatment with 0.25, 0.50 and 1.0% DCW and physiological solution (NaCl 0.9%) also significantly increased ($p<0.05$) mortality across all time points ($p<0.05$) (Table 1).

Based on the Duncan's Multiple Range Tests (DRMT), DCW extracts and powders inhibited *A. galli* worms developments at higher rates compared with physiological solution (NaCl 0.9%). Treatment with 1.0% DWC at 90 min proved to be most effective.

There was a significant interaction between DCW and physiological solution (NaCl 0.9%) at concentration 0.25, 0.50 and 1.0% ($p<0.05$) in inhibiting of *A. galli* development. There was also a significant interaction between DCW and physiological solution (NaCl 0.9%) at all time points ($p<0.05$).

Table 1: Effect of DWC extract solution on *Ascaridia galli* across different concentrations and times

	DWC essential oil concentration (%)			
Times (min)	0.25	0.50	1.0	Mean
30	0	1.33±0.57	1±1.00	0.78±0.52*
60	0.33±0.57	0.67±0.57	2±1.00	0.10±0.71*
90	0.67±1.15	2.00±1.00	3±1.00	1.89±1.05*
Mean	0.33±0.70*	1.33±0.86*	2±1.22*	

*($p<0.05$), DWC: Distillate waste of citronella

Table 2: Effect of DWC powder solution on *Ascaridia galli* lysis across different concentrations and times

	DWC essential oil concentration (%)			
Time (min)	0.25	0.50	1.0	Mean
30	0	0	0	0*
60	0.67±0.577	0.67±0.577	1.33±0.577	0.88±0.57*
90	2.33±0.577	1.67±1.154	2.00±1.73	1.88±1.2*
Mean	0.10±1.11*	0.78±0.97*	1.11±1.26*	

*($p<0.05$), DWC: Distillate waste of citronella

Table 3: Effect of physiological 0.9% NaCl and DWC on the mortality of *Ascaridia galli* worms across different concentrations and times

	Concentration of physiological 0.9 NaCl (%)			
Times (min)	0.25	0.50	1.0	Mean
30	0	0	0	0*
60	0	0	0	0*
90	0	0	0.67±0.577	0.11±0.190*
Mean	0*	0*	0.22±0.44*	

*($p<0.05$), DWC: Distillate waste of citronella

DISCUSSION

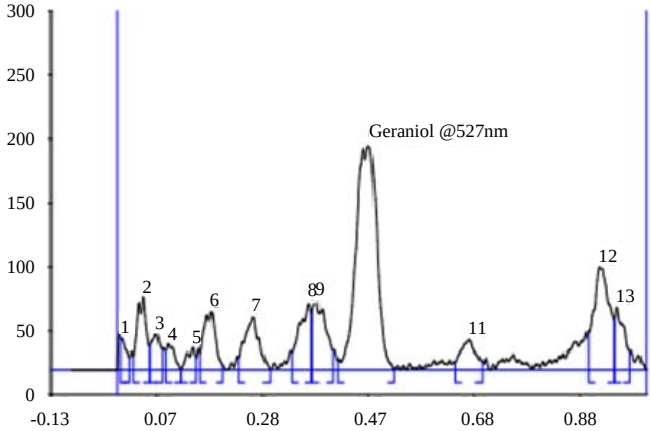
The present study was conducted to determine if geraniol and citronellal compounds may act as antiparasitic agents. Naik *et al.*[13]. reported that citronella plants contain geraniol compounds, citronellal and citronellol and De Oliveira *et al.*[14] found that *C. nardus* essential oil from Brazil contains citronella (34.60%), geraniol (23.17%) and citronellol (12.09%). Monzote *et al.*[15] reported that the citral content (a mixture of geraniol and neral) in *C. citratus* may inhibit the activity of *Trypanosoma cruzi* parasites and Ganjewala[16] found that geraniol in essential oils can kill *Caenorhabditis elegans* parasites. Other studies have also found that geraniol compounds can interfere with the nervous system and protein activity in nematode cell membranes[17-19]. This electrolyte disturbance and cell membrane permeability structure leads to *A. galli* death.

Lalchhandama[20] proposed that nematocidal agents rupture the cytoplasm, myofilament and mitochondria of muscle cells, leading to cuticle separation from hypodermis. These compounds contained in essential oils could damage cell membranes by affecting pH, cellular balance and inorganic ion balance[21-22]. Lei *et al.*[23] confirmed that geraniol compounds might disrupt cell membranes and ionic activity binding proteins to cell membranes and intracellular signaling channels.

This study shows that the active compound of DWC was geraniol which increase *A. galli* mortality.

Monzote *et al.*[15] suggested that chemically essential volatile oil compounds may cause parasite mitochondrial cell membranes to swell as a result of an insignificant increase in the number of cryptic cells.[1] Compounds in essential oils can inhibit protease enzyme activity in parasites due to the

(a) Track 4, ID: Serah wangi ampas



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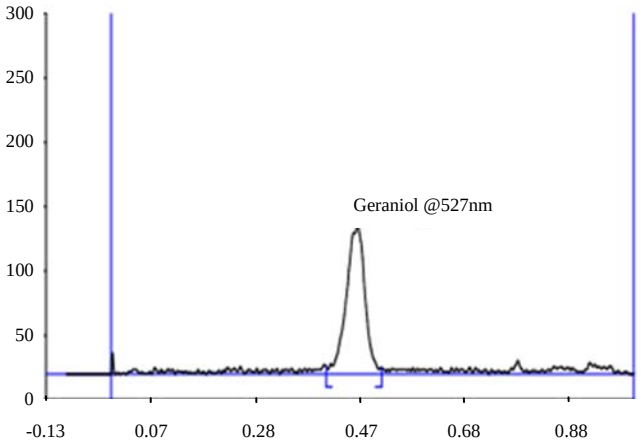
Peaks	Start (Rf)	Max. (height)	Max. (Rf)	Max. (height)	End (Rf)	End (height)	Area	Assigned substance
1	0.01	25.5	0.01	3.81	0.02	9.4	1.64	Unknown*
2	0.03	11.0	0.05	8.43	0.06	18.1	5.73	Unknown*
3	0.06	19.6	0.07	4.14	0.09	16.2	2.49	Unknown*
4	0.09	14.6	0.10	3.00	0.12	0.7	1.80	Unknown*
5	0.12	0.8	0.14	2.60	0.15	9.6	1.53	Unknown*
6	0.16	13.2	0.18	6.79	0.20	4.1	5.48	Unknown*
7	0.23	9.7	0.26	6.22	0.29	0.3	6.37	Unknown*
8	0.33	13.6	0.36	7.78	0.37	45.0	6.72	Unknown*
9	0.37	46.6	0.38	8.31	0.41	15.4	7.28	Unknown*
10 m	0.42	7.8	0.48	26.11	0.52	1.0	38.43	Geraniol
11	0.64	6.2	0.67	3.50	0.69	7.0	3.77	Unknown*
12	0.89	29.9	0.91	11.96	0.94	39.4	13.92	Unknown*
13	0.94	40.0	0.94	7.36	0.97	14.5	4.82	Unknown*

*The compounds that not were detected by Chromatography

(b) Track 7, ID: Standard 1



Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max. %	End Rf	End Height	Area	Area %	Assigned substance
1 m	0.41	6.3	0.47	116.9	100.00	0.52	5.2	3863.2	100.00	Geraniol

Fig. 1(a-b): Quantitative analysis of geraniol compounds of essential citronella oil (a) Track 4, ID: Serah wangi ampas and (b) Track 7: ID: Standard, Source: Laboratory of Pharmacognosy, Faculty of Pharmacy Universitas Gadjah Mada

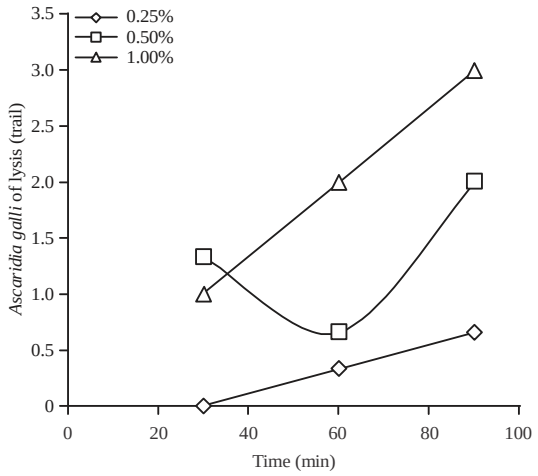


Fig. 2: Effects of DWC extract on the mortality of *Ascaridia galli* worms

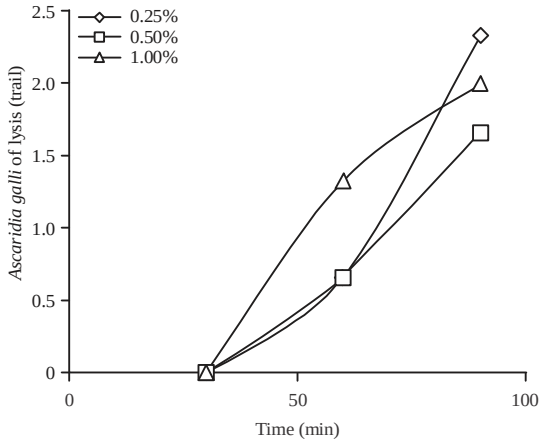


Fig. 3: Effect of DWC powder on the mortality of *Ascaridia galli* worms

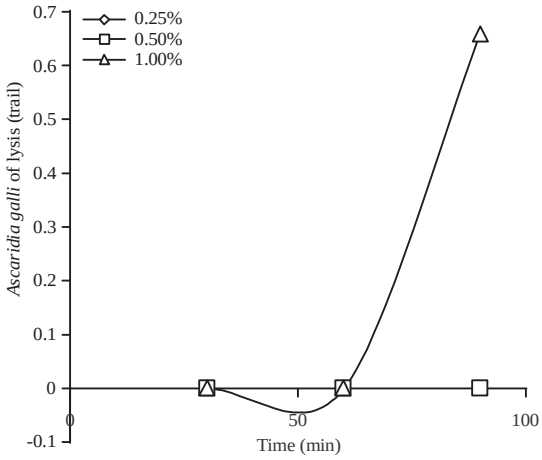


Fig. 4: Effects of physiological 0.9% NaCl on the mortality of *Ascaridia galli* worms

hydrophobic bonding of bioactive compounds or inhibition of glycoprotein biosynthesis from parasites. Abdelqader *et al.*[24] found that compounds containing essential oil can interfere with the metabolism of *A. galli* and Tandon *et al.*[25] reported that, geraniol, causes paralysis and lysis in a short time.

Geraniol compounds have been shown to inhibit the development of fish worm parasites. Barros *et al.*[26] and Chen and Viljoen[27] reported that geraniol compounds could inhibit the development of *Contracaecum* sp. worm larvae in mackerel. *Cymbopogon nardus*, which contains geraniol compounds, may also be useful as an anthelmintic in poultry and small ruminants[26].

CONCLUSION

Extracts or powders obtained from citronella oil distillate waste (*Cymbopogon nardus*) which contain geraniol, increase *Ascaridia galli* worms mortality and can potentially be used as an antiparasitic agent.

SIGNIFICANCE STATEMENT

This study provides evidence for the use of ethanol extract and dried powder of citronella (*Cymbopogon nardus*) essential oil distillate waste as an antiparasitic agent against *Ascaridia galli*, a worm that often attacks poultry. Thus, a new antiparasitic agent against *Ascaridia galli* have been identified.

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