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# Ovicidal activity and cytotoxicity of ethanolic extract of turmeric (*Curcuma longa*) and green tea (*Camellia sinensis*) to treat digestive parasite of sheep

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**Abstract**

**Background:** The Trichuris eggs are collected from naturally infected sheep. Natural antihelmintics such as herbal medicines are needed as an alternative, such as natural compounds from endemic plants.

**Aim:** This present study aims to evaluate the ovicidal activity and cytotoxicity effects of ethanolic extract of *Curcuma longa* (EECL) and *Camelia sinensis* (EECS) as a biological anthelmintic against the egg of *Trichuris sp*.

**Methods:** The *Trichuris* eggs are collected from naturally infected sheep. CMC-Na solution 1% was used as a control. The treatments were 0.12% EECL; 0.24% EECL; 0.15% EECS; 0.30% EECS; a combination of 0.12% EECL and 0.30% EECS; a combination of 0.24% EECL; and 0.15% EECS. Ovicidal activity testing by microscopic examination of eggs treated using different concentrations of EECL extract, EECS, and a combination of them. They were exposed for various times (7, 14, 21, and 28 days) and incubated at room temperature.

**Results:** The study shows that a combination of *C. longa* extract and tea extract exhibits good ovicidal anthelmintic activity against *Trichuris sp*. in sheep. Cytotoxicity examination using the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) test. Based on MTT data processed using regression analysis, the obtained $LC_{50}$ from the administration of EECL, EECS, and a combination of both in a ratio of 1:1, 2:2, 1:2, and 2:1. The combination of EECL extract and EECS with the highest concentration produced cell viability of 28.46%, 17.25%, 56.01%, and 46.47%, respectively.

**Conclusion:** It can be concluded that the most cytotoxic ingredient is found in the combination of EECL and EECS (2:2) at 17.25% and the safest is in the ratio (1:2) at 56.01%.

**Keywords:** Animal health, *Camellia sinensis*, Cytotoxicity, *Curcuma longa*, Ovicidal.

## Introduction

Helminthiasis is one of the diseases that have a major social-economic impact worldwide. The most important cause of ruminant growth retardation is intestinal worms. Generally, these worms have direct and indirect impacts on an animal's digestive system

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can result in morbidity and death from clinical and subclinical illnesses, which can also significantly reduce the production of milk, wool, and meat (Baihaqi *et al*., 2020). Other impacts of this worm infection include increased susceptibility to disease and additional costs for livestock treatment (Lebu *et al*., 2023). The prevalence of gastrointestinal worms in Indonesia was variated from 47.36% to 76.47% (Baihaqi *et al.*, 2019). Gastrointestinal nematodes in domestic sheep that are often encountered are included in the strongyle group, including *Trichostrongylus* spp., *Teladorsagia circumcincta*, *Haemonchus contortus*, *Cooperia* spp., *Oesophagostomum* spp., *Chabertia ovina*, *Bunostomum*, *Trigonocephalum,* and *Nematodirus* spp. (Knoll *et al*., 2021). Gastrointestinal nematodes in Indonesia that are often found in small ruminant livestock such as those in goats from West Java are *Tricostrongylus* sp, *Strongyloides* sp, *Bunostomun* sp, *Ostertagia* sp, *Oesophagustomum*, and *Papilus* sp. (Fauziah *et al*., 2021). Studies on the prevalence of gastrointestinal nematodes in fat-tailed sheep in Jember Regency include *Strongyloides* sp., *Cooperia* sp., *Capillaria* sp., and *Ostertagia* sp. (Awaludin *et al*., 2021).

Control of helminths nowadays depends on an effective drug, called anthelmintics. The frequent use of anthelmintic drugs causes serious drug resistance problems worldwide. Resistance of livestock nematodes to benzimidazoles, imidazothiazoles/ tetrahydropyrimidines, and macrocyclic lactones and macrocyclic lactones is widespread and has been reported on all continents as reported by Fissiha and Kinde (2021).

Natural antihelmintics such as herbal medicines are needed as an alternative, such as natural compounds from endemic plants (Wangchuk *et al*., 2016). Types of plants that have been studied as potential antihelminthics include turmeric plants and tea plants. Ramdani *et al*. (2022) reported that green tea dust (nongrade green tea) contains 20.1% crude protein and 23.0% tannin, providing 1.5% green tea dust in concentrate can improve the fattening performance of local sheep. Findings to date confirm that polyphenolic compounds have antioxidant, immunomodulatory, antimutagenic, and anti-inflammatory effects (Yahfoufi *et al*., 2018). Green tea polyphenols also have potential as an anthelmintic (Oliveira *et al*., 2021).

*Curcuma longa* has been investigated widely. One of Curcuma longa's main ingredients, curcumin, has been shown to have a dose-dependent antiparasitic impact, with greater concentrations of the substance showing the strongest effects. It has been discovered that curcumin extract works in a dose-dependent manner against the muscle cells of earthworms and Shistosoma mansoni (Mokbel *et al*., 2020). Its efficacy in *Haemonchus sp* larva stages has also been evaluated *in vitro* with success (Nasai *et al*., 2016). The anthelmintic effect of tea extract has been reported to have a significant effect on earthworms (Fahlevi *et al.,* 2021). *In vitro* anthelmintic activity of green tea (*Camelia sinensis*) has been described as effective in *Haemonchus contortus* (Zaheer *et al.,* 2019).

Polyphenols and curcumin have high potential but their use as commercial feed additives is still difficult due to the need for precise dosage so extraction is required (Nugraheni *et al*., 2022). The extraction process will make it easier to determine the dosage, packaging, and marketing of products. Therefore, it is necessary to extract tea and turmeric and test the combination of doses of tea polyphenol extract and curcumin as a feed additive and anthelmintic. According to El-Saadony *et al*. (2023), all compounds have the capacity to become toxic according to the large dose; therefore, cytotoxicity testing is needed to determine and determine the safety threshold for tea polyphenol and curcumin extracts. *In vitro* cytotoxicity was examined using the MTT test, which is a cheap and easy-to-perform testing technique that is often used to evaluate the toxicity of various drug candidates (Li *et al*., 2015). The present study was carried out to evaluate the anthelmintic potential of *C. longa* and *C. sinensis* against the gastrointestinal nematodes of sheep. The study will help in developing easily available anthelmintic drugs with minor side effects to support the livestock industry.

## Materials and Methods

### *Preparation of plant material*

The dried powder of *C. longa* tuber and *C. sinensis* leaves was received from the Faculty of Animal Husbandry, Padjajaran University, West Java district, Indonesia.

### *Preparation of extract*

100 g of *C. longa* powder was dissolved in 800 ml of 96% ethanol at a ratio of 1:8 and left for 24 hours at room temperature. The mixture was shaken three times a day and after that, the filtrate was filtered using a filter fabric. The filtrate was further processed at 70°C in a rotary evaporator to make a thick dark colored crude extract. The extract of *C. sinensis* leaves was also prepared with the same method but not using the rotary evaporator, it was using a water bath to get the thick dark colored extract.

### *MTT assay*

The 3-(4.5-dimethylthiazol-2-yl) 2.5-diphenyl tetrazolium bromide (MTT) assay was used to assess the extract's cytotoxicity. For 24 hours, at 37°C and 5% CO2, baby hamster kidney (BHK-21) stem cells were cultivated in 96-well microplates at a density of 8 × 103 cells/well. Initially, the medium was taken out of each well. Ten concentrations of *c. longa* extract and green tea (1.95 mg/ml, 3.91 mg/ml, 7.81 mg/ml, 15.63 mg/ml, 31.25 mg/ml, 62.50 mg/ml, 125.00 mg/ml, 250.00 mg/ml, 500.00 mg/ml, and 1,000.00 mg/ml) were then applied to the cells for a 24-hour period. After removing the media, the cells were treated at 37°C

for 4 hours with 25 µl MTT (5 mg/ml). An inverted microscope was used to examine the cells to detect the existence of formazan crystals. Subsequently, each well was filled with 50–100 µl of DMSO to dissolve the formazan crystals. Using a 595 nm microplate reader, the absorbance of the dissolved formazan was determined spectrophotometrically (Paudel *et al*., 2019). The optical density (OD), which represents the quantity of live cells, increases with color darkness. The formula below is used to determine the percentage of living cells:

Live cells (%) = (OD of treatment cells—OD of media control) / (OD of cell control-OD of media control) × 100%.

Probit analysis was used to determine the fatal concentration ($LC_{50}$) value by analyzing the regression association between the concentration of EECL, EECS, the combination extract, and the percentage of viable limbal mesenchymal stem cells.

***Preparation of Trichuris sp. eggs***

Feces from naturally infected sheep were collected and transported to the laboratory using sterile glass containers. The eggs in feces were washed using normal saline for several times. The supernatant was removed. This solution was moved to the test tube and stored at 4°C for further use.

***Ovicidal test***

Trichuris eggs are treated using different concentrations of green tea extract, *C. longa* extract, and a combination of them. They were exposed for various times (7, 14, 21, and 28 days) and incubated at room temperature (24°C–27°C). At the end of incubation time, the eggs were smeared on a glass slide and covered with cover glass then examined under the microscope. The ovicidal activity was determined by counting the eggs. The eggs of Trichuris were divided into two groups: normal egg and dead egg. The normal egg is shown in Figure 1, while the dead eggs (Figs. 2 and 3) were eggs with losing and melting embryos, wrinkling and lysis of embryo, and lysis of the membrane cell (Ryoo *et al*., 2023). It is also described that infective eggs become shorter and wider with shorter and wider plugs and the shell is thinner.

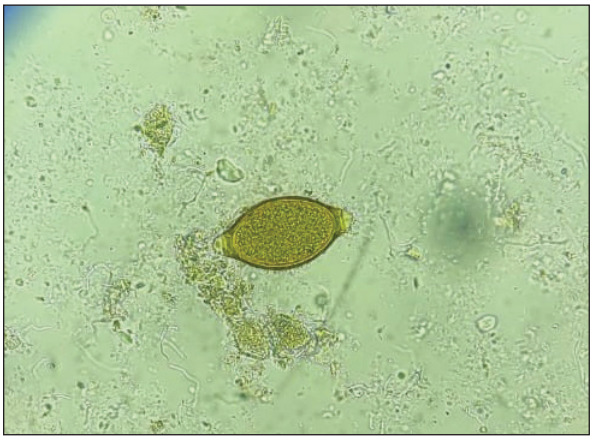

**Fig. 1.** Normal egg of *Trichuris sp*.

## Results

***Ovicidal activity***

The results of microscopic observations of the eggs treated with turmeric extract and green tea extract at different concentrations and exposure times are presented in Tables (1 and 2), respectively. As shown in Tables (1 and 2), following the treatments and the exposure time (7, 14, 21, and 28 days), the number of normal *Tricuris sp* eggs were treated with EECL only and the combination of EECL with EECS was decreased significantly compared to the control group ($p \leq 0.01$). However, the eggs treated with EECS only were not significant compared to the control group ($p \geq 0.01$).

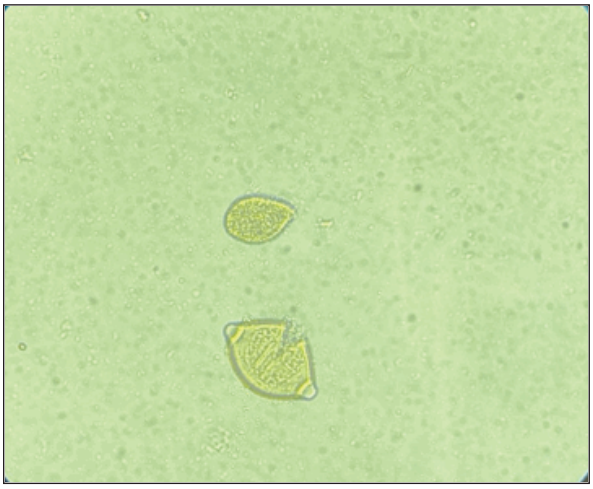

**Fig. 2.** Dead egg of *Trichuris sp*. with membrane damage.

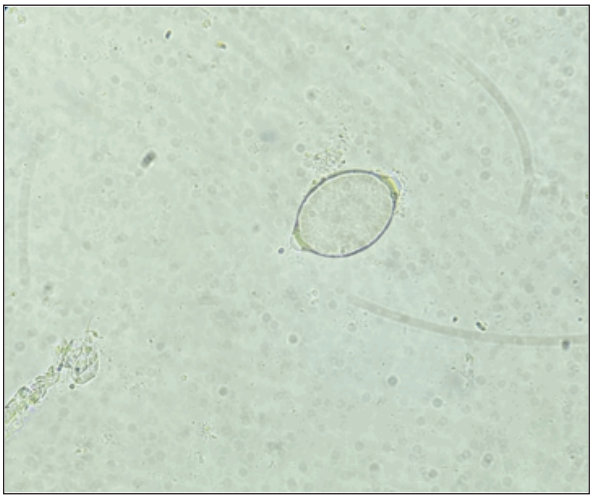

**Fig. 3.** Death egg of *Trichuris sp*. with empty of embryo.

**Table 1.** The normal eggs of *Trichuris sp*. after 7, 14, 21, and 28 days of exposure.

| Group | 7 days (%) | 14 days (%) | 21 days (%) | 28 days (%) |
|---|---|---|---|---|
| Control | $96.00^{d} \pm 5.23$ | $95.50^{d} \pm 2.38$ | $89.50^{d} \pm 9.15$ | $86.00^{de} \pm 1.41$ |
| *C. senensis* 0.12% | $94.50^{cd} \pm 7.14$ | $95.00^{d} \pm 4.32$ | $95.00^{d} \pm 2.45$ | $95.50^{e} \pm 2.52$ |
| *C. senensis* 0.24% | $92.75^{cd} \pm 11.98$ | $97.25^{d} \pm 0.96$ | $96.25^{d} \pm 1.50$ | $95.50^{e} \pm 1.91$ |
| *C. longa* 0.15% | $71.25^{acb} \pm 15.64$ | $32.50^{a} \pm 32.67$ | $72.50^{c} \pm 14.89$ | $83.50^{d} \pm 4.65$ |
| *C. longa* 0.30% | $55.25^{a} \pm 36.17$ | $61.75^{b} \pm 35.06$ | $59.75^{c} \pm 14.10$ | $52.25^{a} \pm 21.56$ |
| *C. senensis* 0.12% + *C. longa* 0.30% | $55.25^{a} \pm 29.55$ | $19.75^{a} \pm 0.96$ | $10.75^{a} \pm 2.87$ | $58.75^{ab} \pm 6.65$ |
| *C. senensis* 0.24% + *C. longa* 0.15% | $64.25^{ab} \pm 17.06$ | $38.75^{b} \pm 5.19$ | $28.25^{b} \pm 5.91$ | $68.25^{bc} \pm 2.06$ |

Different superscripts in rows and columns indicate significant differences ($p < 0.01$).

**Table 2.** The dead eggs (membrane cell damage and empty core) of *Trichuris sp*. after 7, 14, 21, and 28 days of exposure.

| Group | 7 days (%) | 14 days (%) | 21 days (%) | 28 days (%) |
|---|---|---|---|---|
| Control | $4.25^{a} \pm 5.32$ | $4.25^{a} \pm 2.22$ | $10.25^{a} \pm 9.32$ | $14.00^{b} \pm 1.41$ |
| *C. senensis* 0.12% | $5.25^{a} \pm 7.09$ | $5.00^{a} \pm 4.32$ | $5.00^{a} \pm 2.45$ | $4.50^{a} \pm 2.52$ |
| *C. senensis* 0.24% | $7.00^{a} \pm 11.49$ | $2.75^{a} \pm 0.95$ | $4.00^{a} \pm 1.15$ | $4.25^{a} \pm 1.89$ |
| *C. longa* 0.15% | $29.00^{ab} \pm 16.15$ | $65.50^{bc} \pm 30.96$ | $27.50^{b} \pm 14.89$ | $15.5^{b} \pm 4.35$ |
| *C. longa* 0.30% | $45.00^{ab} \pm 36.17$ | $38.50^{b} \pm 34.78$ | $37.75^{b} \pm 13.87$ | $41.75^{d} \pm 7.93$ |
| *C. senensis* 0.12% + *C. longa* 0.30% | $41.75^{b} \pm 27.91$ | $81.25^{c} \pm 2.63$ | $89.25^{d} \pm 2.87$ | $41.75^{d} \pm 5.85$ |
| *C. senensis* 0.24% + *C. longa* 0.15% | $34.5^{b} \pm 18.70$ | $61.50^{bc} \pm 5.07$ | $71.75^{c} \pm 5.91$ | $31.75^{c} \pm 2.06$ |

Different superscripts in rows and columns indicate significant differences ($p < 0.01$).

***MTT assay***

The MTT results for the cytotoxicity tests of turmeric extract, green tea extract, and their combination are shown in Figures 4 and 5. The results of the MTT test on BHK-21 stem cells showed that cell viability decreased sharply as the concentration of green tea extract increased. Meanwhile, the decrease in cell viability given turmeric extract with the same concentration series showed a sloping curve. The highest toxicity to the cell groups administered green tea and turmeric extracts was obtained at the highest concentration (1,000 mg/ml), with cell viability of 15.69% and 64.46%, respectively.

The results of the MTT test on BHK-21 stem cells that were administered turmeric and green tea extract combinations also showed that cell viability decreased as the concentration increased. The combination of turmeric extract and green tea with a ratio of 1:1, 2:2, 1:2, and 2:1 with the highest concentration (1,000 mg/ml) produced cell viability of 28.46%, 17.25%, 56.01%, and 46.47%, respectively.

Based on MTT data processed using regression analysis, the obtained $LC_{50}$ from administration of green tea extract alone, turmeric extract alone, and combinations of turmeric-green tea extract of 1:1, 2:2, 1:2, and 2:1 were, respectively, 475.19, 1379.52, 650.06, 387.63, 1024.42, and 879.97 mg/ml.

The lower $LC_{50}$, the more toxic the material and the higher the $LC_{50}$, the safer the material will be applied. The order of $LC_{50}$ from the most cytotoxic to the safest starts with a ratio of 2:2, 1:1, 2:1, and 1:2. It can be concluded that giving turmeric and green tea extract treatment is the most toxic at the ratio of 2:2.

## Discussion

Studies about some of the natural compounds of plants against *Trichuris spp*. has been conducted. Natural products against intestinal nematodes have been listed by Liu *et al* (2020), we found 34 anthelmintic compounds from medicinal plants active against intestinal parasitic nematodes since 2002. Of these, only eight compounds were evaluated for *in vivo* anthelmintic activity in animal models. Classification of anthelmintics based on the chemichal compounds in natural plants that is *Lipids* (glycerol monoesterate), *Phenolics* (rutin, nicotiflorin, narcissin, luteolin, deguelin, chlorogenic acid, caffeic acid, methyl caffeat, epicatechin, oxytroside, isokaempferide, gallic acid, and procyanidin A2), *Saponin* (β-sitosterol and avenacoide A), *Terpenoide* (thymol, terpinem, dichapetalin x, dichapetaline A, 3R.6R linalool oxide acetatecolin, and andrographolide), *Alkaloids* (chelerytrine, 6-methoxydihydrosanguinarie, sanguinarine, s-dicentrin, and s-neolitsin), *Coumaric acid* (2H chromen-2-one, methyl p coumarate, and

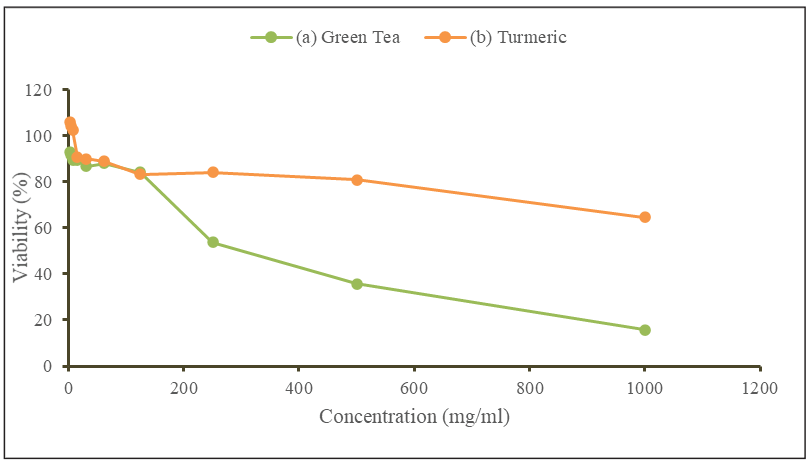


**Fig. 4.** Viability percentage of BHK-21 stem cells (normalized to control cells) in the MTT assay. (a) green tea extract treatment; (b) turmeric extract treatment.

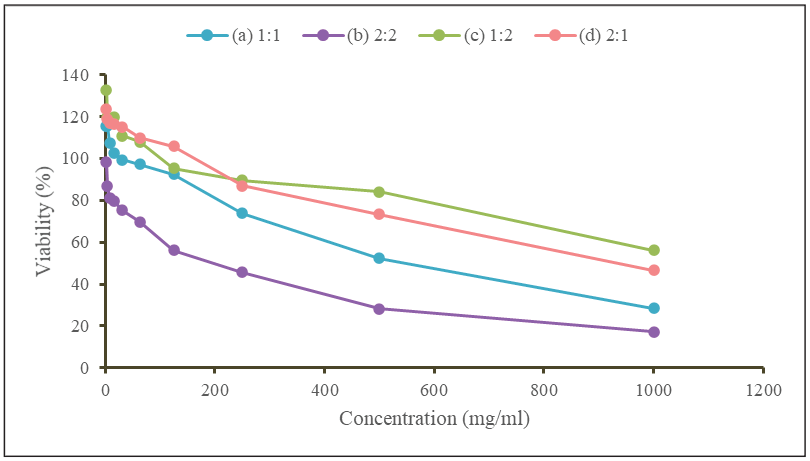


**Fig. 5.** Viability percentage of BHK-21 stem cells (normalized to control cells) in the MTT assay using various combination doses of turmeric and green tea extract. (a) combination 1:1 of green tea and turmeric extract treatment; (b) combination 2:2 of turmeric and green tea extract treatment; (c) combination 1:2 of turmeric and green tea extract treatment; and (d) combination 2:1 of turmeric and green tea extract treatment.

methyl ferulate), and miscellaneous (diazatricylo tetraicose carboxylic acid, trans cinnamaldehyde, 2 decanone, 2 nonanone, 2 undercanone, and eryngial). This is also supported by Adak and Kumar (2022) who stated that several secondary metabolites from plants that play the most role in antihelminthic activity include terpenes, saponin glycosides, flavonoids, tannins, alkaloids, and nonprotein amino acids.

The use of secondary plant metabolites as an antihelminthic has so far been widely applied to adult worms, application to worm eggs and larvae using other types of plants has been carried out by Barrau *et al*. (2005) using the *Onobrychis viciifolia* plant which contains tannins. Tannins in plants also show an inhibitory effect on the hatching of goat digestive tract nematode eggs (Gazzonis *et al*., 2023). Kaiaty *et al*. (2023) also carried out the ovicidal effect of the *Punica granatum* plant and it was proven to be effective against *H. contortus* worm eggs.

Turmeric, or *C. longa*, belongs to the Zingerberaceae family. Polyphenolic substances called curcuminoid comprise curcumin and its derivatives, demethoxycurcumin, and bisdemethoxycurcumin. The cytotoxicity, mechanistic route, and improvement of curcumin's pharmacokinetik and pharmacodynamic qualities have all been extensively studied (Tomeh *et al*., 2019). It possesses a variety of biological activities, including the ability to scavenge free

radicals, guard against protozoa diseases, lessen the toxicity of snake venom, and have antimicrobial, antimalarial, anti-inflammatory, antiproliferative, anti-angiogenic, anti-tumor, anti-aging, anti-arthritic, anti-Alzheimer's, hypoglicemic, and antiulcer properties (AlBasher *et al*., 2020).

The ovicidal effect of *C. longa* has been reported in several studies. Major active constituents of *C. longa* according to Panda *et al*. (2022) are β-Turmerone (21.8%), Ar-Turmerone (14.7%), and α-Turmerone (12.4%). The extract of green tea (*C sinensis*) has been reported having a potential effect as an antibacterial. Panda *et al*. (2022) mentioned that the major bioactive component of tea is Terpinen-4-ol. It is active against *Haemonchus contortus* ($LC_{50}$ = 630 µg/ml, egg hatch assay). Tannins in plants have been widely reported to inhibit the hatching of worm eggs, as done by Birhan *et al*. (2020). This research also explains the mechanism by which tannin works as an antihelminthic. Meanwhile, the terpene content in turmeric can cause damage to intestinal parasites (Chanda and Ramachandra, 2019). Other research states that the mechanism of turmeric as an antihelminthic is through oxidative stress induced by turmeric. Lvova *et al*. (2023) have used turmeric as an antihelminthic, one of which showed turmeric's antihelmintic activity against the heartworm *Opisthorchis felineus in vivo*.

In this study, no phytochemical tests were carried out. The phytochemistry of turmeric has been carried out by other researchers such as Dange (2023). The tea used in this research was green tea in accordance with the research results of Ramdani *et al*. (2013) which stated that green tea has a higher total phenol and tannin content than black tea. The compound content in tea has been revealed through research by Lestary *et al*. (2023). It is hoped that the use of a combination of plant extracts that have an antihelminthic function can improve the mechanism of drug action synergistically. Mixing several types of plants can cause interactions between natural ingredients so that new, more effective compounds are formed (Rajčević *et al*., 2022). In this study, it was proven that using plant extracts in combination was more effective than using them alone. Information explaining the synergistic effect of the two plant extracts is currently not available; therefore, further research is needed to determine the pharmacological mechanisms of the two extracts.

Identification of *Trichuris* sp. has been carried out, including by Afshan *et al*. (2023) and Panti-May *et al*. (2023). In this study, Trichuris species were not identified at the species level. The process of embryogenesis and viability of *Trichuris* eggs is dependent on the temperature and can be induced by bacteria from the genus *Streptococcus* and Paraclostrdium (Yevstafieva *et al*., 2021; Schärer *et al*. 2023). The egg stopped developing at a temperature of 10°C. With the increase of temperature to 20°C, embryogenesis occurred in 32 days and the viability of eggs was 32% of eggs died and 68% reached the larval stage. At 30°C, eggs become larvae in 20 days (Yevstafieva *et al*., 2021). In this study, we used a temperature of 24°C–27°C. The dead egg in this study was observed at microscope while the larval stage was not found. This is most likely because the larval stage has not yet formed at the specified temperature and time. This is also explained in the research of Vejzagić *et al*. (2015), that after 2 weeks of incubation at 25°C and 30°C, the earliest eggs with several cell divisions were already visible. Within *Trichuris suis*, larvae were already present by week four at 30°C and week eight at 25°C. More than 70% of the eggs in the experiment underwent early development, and both were discovered to be in the 30°C–38°C temperature range. In the meantime, *Trichuris suis* started to develop on the day (d) 4. Over 70% of the samples from (d) 7 had several blastomers described. Storage at 30°C–34°C was optimal for *Trichuris suis* and by d 25 at 34°C more than 50% of eggs contained a larva, followed by d 28 at 30°C and 32°C. While *Trichuris suis* initially exhibited a similar tendency in groups 36°C and 38°C as well as in groups 30°C–34°C, the percentage of eggs containing larvae never exceeded 50% at any point in time.

The basis of cytotoxicity assays is the use of several parameters to measure the impact of a substance on cell metabolism or to quantify cell death. The present study employs the MTT assay method to measure the cytotoxicity of a combination of turmeric extract and green tea in BHK-21 cell culture. Meanwhile, the decrease in cell viability given turmeric extract with the same concentration series showed a sloping curve. The results of the MTT test on BHK-21 stem cells that were administered turmeric and green tea extract combinations also showed that cell viability decreased as the concentration increased. The combination of turmeric extract and green tea with a ratio of 1:1, 2:2, 1:2, and 2:1 with the highest concentration (1,000 mg/ml) produced cell viability of 28.46%, 17.25%, 56.01%, and 46.47%, respectively.

The MTT test is a frequently used toxicological test, which evaluates the relationship between nanoparticles (NPs) and cells. The relationship between NPs and cells is not only influenced by the type of NP but also by size, shape, and surface properties. Recently, many methods have been used to assess cytotoxicity, one of which is the metabolomics method (Awashra and Młynarz, 2023). Although this study did not use NPs, based on MTT data processed using regression analysis, the obtained $LC_{50}$ from administration of green tea extract alone, turmeric extract alone, and combinations of turmeric-green tea extract of 1:1, 2:2, 1:2, and 2:1 were, respectively, 475.19, 1379.52, 650.06, 387.63, 1024.42, and 879.97 mg/ml. The lower $LC_{50}$, the more toxic the material and the higher the $LC_{50}$, the safer the material will be applied. The order of $LC_{50}$ from the most cytotoxic to the safest starts with a ratio of 2:2, 1:1, 2:1, and 1:2. It can be concluded

that giving turmeric and green tea extract treatment is the most toxic at the ratio of 2:2. Toxicity testing of these two materials has been carried out independently, including Mahmoudvand *et al*. (2019). Research on the toxicity of turmeric has also been carried out by Azzahra *et al*. (2024), the results of this study showed that the percentage of fibroblast cell viability decreased with the highest percentage at a turmeric concentration of 0.78% and the lowest percentage at a turmeric concentration of 100%. However, other studies on the toxicity of the combination of these two types of extract have not been carried out.

## Conclusion

The use of plant extracts as patented antihemintics is still rarely found, therefore further testing is needed. Based on the result, these findings provide information about the potential effect of EECL and EECS extract as ovicidal of *Trichuris sp.* and the cytotoxicity of the extract. Probably further study should be focused on a combination of EECL and EECS for the effectiveness of the drug *in vivo* to determine the cellular mechanism of this drug, apart from that it is also necessary to apply the combination of this drug to the adult worm *Trichuris* sp. as well as other types of worms. In the future, this drug can be developed into a commercial anhelmintic so that it can be used widely and can reduce the antihelmintic resistance that is usually found when using pharmaceutical anhelmintic drugs.

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***Conflict of interest***

The authors declare that there is no conflict of interest.

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***Authors' contributions***

Conceived, designed, and coordinated the study: NH, SLH, and NT. Designed data collection tools, supervised the field sample and data collection, and laboratory work as well as data entry: SIP, AP, and LK. Validation, supervision, and formal analysis: ARK, LTS, and EPH. Contributed reagents, materials, and analysis tools: SK, MGAY, and ANN. Carried out the statistical analysis and interpretation and participated in the preparation of the manuscript: DR, RSR, and AJ. All authors have read, reviewed, and approved the final manuscript.

***Data availability***

All data supporting the findings of this study are available within the manuscript and no additional data sources are required.

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