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In Vitro Anthelmintic Activity of *Orthosiphon aristatus* (Blume) Miq Leaves Against *Haemonchus contortus*

(Uji Daya Anthelmintik Ekstrak Etanol Daun Kumis Kucing (*Orthosiphon aristatus* (Blume) Miq) Terhadap *Haemonchus contortus* Secara In Vitro)

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ABSTRACT

Haemonchosis is an infection caused by the *Haemonchus contortus* worm, a blood-sucking gastrointestinal parasite that can also infect humans. *Orthosiphon aristatus* (Blume) Miq leaves have been shown to have anthelmintic activity due to the presence of saponins, flavonoids, and tannins. This study aimed to determine the anthelmintic activity of ethanol extract of *Orthosiphon aristatus* (Blume) Miq leaves against *H. contortus*. The infective *H. contortus* larvae were divided into six treatment groups: a 0.5% ethanol extract concentration group, a 1% ethanol extract concentration group, a 2% ethanol extract concentration group, a 4% ethanol extract concentration group, a positive control group (+), and a negative control group (-), each with four replicates. The study was conducted in six hours, with observations made at the 1st, 2nd, 4th, and 6th hours. The results showed that the death of the larvae increased with higher ethanol extract concentrations and longer exposure to the ethanol extract of *Orthosiphon aristatus* (Blume) Miq leaves. The time of death for infective *H. contortus* larvae given the ethanol extract of *Orthosiphon aristatus* (Blume) Miq leaves was at the 4th hour, and the effective dose of the ethanol extract as an anthelmintic was at the concentration of 0.5%.

Keywords: Anthelmintic*, Haemonchus contortus*, *Orthosiphon aristatus*

ABSTRAK

Haemonchosis merupakan infeksi cacing yang disebabkan oleh cacing *Haemonchus contortus* yaitu parasit gastrointestinal penghisap darah yang dapat bersifat zoonosis ke manusia. Daun kumis kucing *(Orthosiphon aristatus* (Blume) Miq) memiliki kandungan senyawa saponin, flavonoid dan tannin yang memiliki kemampuan sebagai antelmintik. Mengetahui daya anthelmintik ekstrak etanol daun kumis kucing terhadap *H.contortus.* Larva infektif *H.contortus* dibagi menjadi 6 kelompok perlakuan yaitu kelompok perlakuan konsentrasi 0,5%, konsentrasi 1%, konsentrasi 2%, konsentrasi 4%, kelompok perlakuan kontrol positif (+), dan kelompok perlakuan negatif (-) dengan masing-masing dilakukan pengulangan sebanyak 4 kali. Penelitian ini dilakukan selama 6 jam, dengan pengamatan pada jam ke-1, jam ke-2, jam ke-4, serta jam ke-6. Hasil penelitian menunjukkan kematian larva ketika diberi ekstrak daun kumis kucing *(O. aristatus* (Blume) Miq) pada tiap perlakuan semakin meningkat jika semakin tinggi konsentrasi ekstrak dan semakin lama terpapar ekstrak. Waktu kematian larva infektif *H. contortus* yang diberikan ekstrak etanol daun kumis kucing *(O. aristatus* (Blume) Miq) adalah jam ke-4 dan dosis efektif ekstrak etanol daun kumis kucing *(O. aristatus* (Blume) Miq*)*sebagai anthelmintik adalah konsentrasi 0,5%.

Kata kunci: *Haemonchus contortus*, kumis kucing *(Orthosiphon aristatus* (Blume) Miq), anthelmintik

INTRODUCTION

Helminthiasis is a common health problem affecting a significant portion of the world's population. According to the World Health Organization (WHO), more than 1.5 billion people, or 24% of the global population, suffer from helminthiasis. The infection is most prevalent in tropical and subtropical regions, particularly in Sub-Saharan Africa, the Americas, China, and East Asia (WHO 2022). In Indonesia, a country with a tropical climate and high humidity, the prevalence of worm infections is generally very high, ranging from 2.5% to 62% (Minister of Health RI 2017). In Central Kalimantan specifically, the prevalence of helminthiasis is 5.56% (Kristanto *et al.* 2020).

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Haemonchus contortus is a gastrointestinal parasite that infects goats and sheep, causing stunted growth, reduced production, and potential death if left untreated (Widiarso *et al.* 2018). This parasite can also be zoonotic to humans, meaning it can be transmitted from animals to humans, and it can cause anemia due to its blood-sucking behavior. Goat, cow, sheep, and pig farms located near residential areas can increase the risk of infection in humans. The first reported case of *H. contortus* infection in humans occurred in Iran, where the woman who raised cows in her yard became infected after handling and using the feces of the cows without wearing protective gloves or washing her hands (Ghadirian & Arfaa 1973).

Haemonchosis can be controlled with the use of anthelmintics, such as albendazole, which belongs to the benzimidazole group and can reduce the pathophysiological effects of the infection. However, the long-term use of the same anthelmintic can lead to worm resistance, making it necessary to find more effective and efficient ways to control *H. contortus* (Pallotto *et al.* 2022). Indonesia is home to a diverse range of plants with medicinal properties, including *Orthosiphon aristatus* (Blume) Miq. The community commonly uses this plant as an anthelmintic drug. Previous research by Ulya *et al.* (2014) has shown that *Orthosiphon aristatus* (Blume) Miq leaves have anthelmintic properties against *Ascaris suum* in vitro, likely due to the presence of flavonoids, saponins, and tannins in the ethanol extract of the leaves, which cause paralysis and death in the worms (Ulya *et al.* 2014). The community's habit of building cages for cows, goats, and pigs close to the livestock owner's house has the potential to maintain the life cycle of *H. contortus.* Clean living behavior was not implemented so that *H. Contortus* eggs found in livestock feces can enter through the mouth. Therefore, it is necessary to study to analyze the anthelmintic activity of *Orthosiphon aristatus* (Blume) Miq against *Haemonchus contortus* as an effort to control helminth.

MATERIAL AND METHOD

This study used an experimental post-test-only control group research design. The materials and tools used in the study included *Orthosiphon aristatus* (Blume) Miq leaves, goat feces, albendazole, 96% ethanol, vermiculite, filter paper, distilled water, a water bath (Memmert type WNB14 Ring), a rotary evaporator (Hahnvapor type HS 2005 VN), and a binocular microscope (Olympus type CX23 LED).

Fresh leaves of *Orthosiphon aristatus* (Blume) Miq were obtained in the city of Kuala Kurun, Gunung Mas Regency, as much as 2 kg. Then several stages are carried out, starting from the washing process with clean water, drying covered with a black cloth using direct sunlight, reducing the size of the simplicial with the help of a grinder to the sieving process with a 40mesh sieve until a fine and homogeneous simplicial powder is obtained. The leaf simplicial powder of *Orthosiphon aristatus* (Blume) Miq as much as 250 grams was extracted using the maceration method with 96% ethanol solvent for three days, then filtered until a filtrate was obtained and maceration was carried out again in the same way. Then, evaporation was carried out using a rotary evaporator, and after that, it was evaporated again using a water bath until a thick extract was obtained. Calculation of extract yield can be done using the following formula.

(Extract Yield (%) = (Dry weight of extract : Dry weight of plant) X 100 %)

Phytochemical screening of *Orthosiphon aristatus* (Blume) Miq leaf extracts was carried out qualitatively. The samples used in this study were *H. contortus* larvae. Fresh goat feces were collected from Adhi Dian Goat Cattle in Jl. Mahir Mahar, Jekan Raya District, Palangka Raya City. The feces samples were examined using a simple (native) microscopic examination method, and if *H. contortus* worm eggs were present, the feces were treated for 7-9 days before being harvested using the Baermann method (Naeem *et al.* 2021). Each treatment consisted of ten larvae, and the experiment was repeated four times. The positive control was 1 g of albendazole, and the negative control was 0.5% DMSO. DMSO used as a solvent for negative controls. Observations were made at the 1st, 2nd, 4th, and 6th hours, and the number of dead worms was recorded to calculate the percentage of larval mortality in each treatment group (Ulya *et al.* 2014; Muda *et al.* 2021). The data were analyzed using the Kruskall-Wallis test followed by the Mann-Whitney test. LT_{50} probit analysis was also performed to determine the time required to kill 50% of the larvae. The data obtained were analyzed using the Kruskall-Wallis test and continued with the Mann-Witney test to determine the comparison of each treatment. Next, the Probit method used to evaluate using the SPSS application to determine the LC100 and LT100 of the ethanol extract of cat's whisker leaves. The study was approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Palangka Raya, with the reference number 69/UN24.9/LL/2022.

RESULTS AND DISCUSSION

The yield of leaf extract of *Orthosiphon aristatus* (Blume) Miq obtained was 9.40%. The yield results from a sample are needed to find out how much extract was obtained during the extraction process. The greater the

yield, the greater the number of active compounds contained in the sample (Zlotek *et al* 2016).

H. contortus worm eggs are oval in shape with thin walls and a clear area inside (Figure 1). The infective larvae have a sheath that covers their entire body, a long and cylindrical (tube-like) body with an enlarged anterior part containing a mouth at the front end and a narrow, cylindrical esophagus. The larvae's tail is rounded, and the tail sheath is stiff and narrow at the end, tapering to a point (Lem *et al.* 2012).

The results showed that the ethanol extract of *Orthosiphon aristatus* (Blume) Miq leaves at concentrations of 0.5%, 1%, 2%, and 4% could kill *H. contortus* infective larvae starting from the first hour. The highest percentage of larvae mortality was observed at a concentration of 4%, with 32.5% mortality at the first hour, 57.5% at the second hour, 77.5% at the fourth hour, and 100% at the sixth hour. The positive control using albendazole killed 100% of the worm larvae at the sixth hour. In contrast, the negative control had the lowest percentage of larvae mortality, at 7.5% at the first hour, 10% at the second hour, 25% at the fourth hour, and 30% at the sixth hour. In addition, the morphology of the larvae exposed to the extract changed, with their body color becoming darker and their body parts no longer visible.

Based on the results of data analysis using the Kruskal-Wallis test, there were significant differences between all treatment groups ($p<0.05$). In the post hoc Mann-Whitney test, the 0.5%, 1%, and 0.5% DMSO groups had significant differences with all other groups (0.5%, 1%, 2%, 4%, albendazole, DMSO 0.5%). The 2% group had significant differences with the 0.5%, 1%, and 0.5% DMSO groups but no significant differences with the 4% and albendazole groups. The 4% group had significant differences with the 0.5%, 1%, and 0.5% DMSO groups but no significant differences with the 2% and albendazole groups. The LT_{50} values for the 0.5%, 1%, 2%, 4%, albendazole, and 0.5% DMSO concentrations were 5.032 hours, 3.711 hours, 3.325 hours, 2.676 hours, 2.048 hours, and 8.367 hours, respectively (Figure 2).

Based on qualitative phytochemical screening, ethanol extract leaves of *Orthosiphon aristatus* (Blume) Miq, including flavonoids, tannins, and saponins, are known to be positive. The ability of ethanol extract of leaves to kill *Ascaris suum* due to the presence of certain active compounds contained therein. Tannins, saponins,

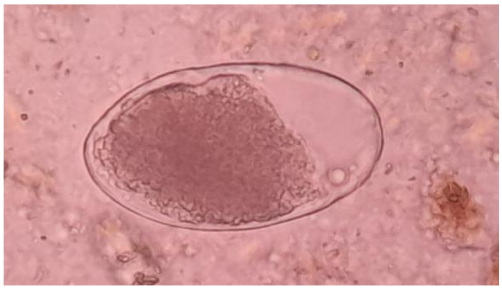

Figure 1 In this study, *H. contortus* eggs found in goat feces.

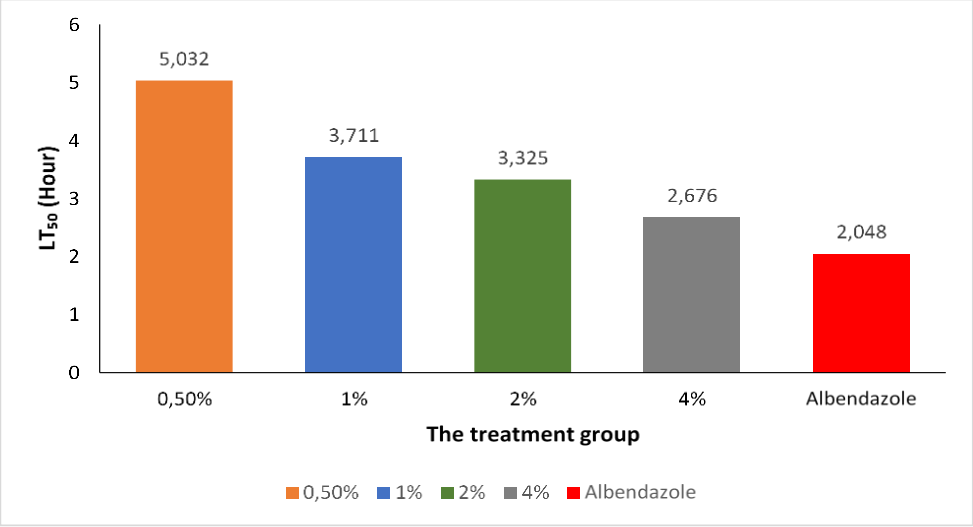

Figure 2 Probit analysis (LT_{50}).

flavonoids, and alkaloids have anthelmintic properties (Muda *et al.* 2021). Saponins can have the potential as an anthelmintic because they work by inhibiting the enzyme acetylcholinesterase so that the worms will experience muscle paralysis and end in death (Kuntari, 2008). Furthermore, in a study by Rahmadani *et al.* (2020), the ethanol extract of the "Petai Cina" stem (*Leucaena leucocephala*) was found to contain tannins, triterpenoid, saponin, and glycoside compounds that have anthelmintic effects. Tannins interfere with energy production by inhibiting oxidative phosphorylation, leading to the death of *Ascaridia galli* (Rahmadani *et al.* (2020). Tannins can also affect the formation of proteins needed for activity by entering the digestive tract and inhibiting the acetylcholinesterase enzyme, disrupting the worm's digestive and metabolic processes and causing malnutrition and energy loss, leading to death (William *et al.* 2014). Saponins increase the permeability and pore formation of the worm's body wall, causing vacuolization and disintegration of the cuticle (Muda *et al.* 2021). Saponins also interfere with the enzymes acetylcholinesterase, proteinase, chymotrypsin, and cholinesterase, leading to muscle paralysis and death (Sujith *et al.* 2013). Flavanoids can denature proteins when in contact with worms, which are absorbed by the body and can also inhibit enzymes and acetylcholinesterase, affecting the worm's muscles (Ulya *et al.* 2014; Maestrini *et al.* 2020).

Alkaloid disrupts local homeostasis by reducing the nitrate needed for protein formation and suppressing sucrose's distribution to the small intestine, causing malnutrition and energy loss leading to death in worms (William *et al.* 2014). Triterpenoids have an anthelmintic effect by neutralizing the polar state, which is then increased by the larval muscles, and excessive stimulation causes paralysis (Rahmadani *et al.* 2020). The morphology of the intact, healthy larvae compared to the larvae that died and were exposed to the extract showed a change in body color, becoming darker, and the body parts of the larvae were no longer visible. This is likely due to the presence of secondary metabolites in the *Orthosiphon aristatus* (Blume) Miq leaves extract, which causes a change in the composition of the protein into amino acids and damages the sample's structure, resulting in a color change (Swargiary *et al.* 2016).

In this study, a concentration of 0,5% was able to kill 65% of *H. contortus* worms within six hours. At a concentration of 1%, the extract induced the death of 87.5% of L3 *H. contortus* in the six hours. A concentration of 2% was able to kill 97,5% and 4% was able to kill 100% of *H. contortus* worms within six hours. At a concentration of 0.5%, this is an effective extract dose as an anthelmintic because the smallest concentration compared to the other concentrations but is able to reduce *H. contortus*. At a Negative control induced the death of 30% of L3 *H. contortus* in the six hours because *H. contortus* larvae do not get nutrition and are not in the host's body.

Table 1 shows that all concentrations of *Orthosiphon aristatus* (Blume) Miq leaves extract had anthelmintic activity against *H. contortus* from the 1st to the 6th hour, with the mortality percentage of *H. contortus* larvae

Table 1 Percentage of power test results on *H. contortus* larvae

Group	Hour	Larval death (%)
0.5%	1	5.0
	2	22.5
	4	30.0
	6	65.0
1%	1	17.5
	2	30.0
	4	57.5
	6	87.5
2%	1	37.5
	2	47.5
	4	77.5
	6	97.5
4%	1	32.5
	2	57.5
	4	77.5
	6	100.0
Positive control (Albendazole)	1	37.5
	2	60.0
	4	95.0
	6	100.0
Negative control (DMSO 0.5%)	1	7.5
	2	10.0
	4	25.0
	6	30.0

increasing with higher concentrations. A study by Hadi *et al.* (2021) using Tapak Liman (*Scaber Gajah*) leaves extract against *H. contortus* showed the highest worm mortality at a concentration of 20% after 60 minutes of observation. In this study, the morphology of the larvae changed to a darker color upon exposure to *Orthosiphon aristatus* (Blume) Miq leaves extract. The power test showed that the larvae given *Orthosiphon aristatus* (Blume) Miq leave extract had a higher mortality rate than the negative control. The higher the concentration used and the longer the larvae were exposed to the extract, the greater the number of larvae that died, likely due to the secondary metabolites in *Orthosiphon aristatus* (Blume) Miq leaves extract. These bioactive compounds act as toxicants, causing death in the larvae and demonstrating the anthelmintic properties of *Orthosiphon aristatus* (Blume) Miq leaves. This is supported by the results of phytochemical screening on the *Orthosiphon aristatus* (Blume) Miq leaves, which showed that the plant contains anthelmintic compounds (Muda *et al.* 2021).

The results of the Mann-Whitney post-hoc test showed that the 0.5% and 1% treatment groups, which were the lowest concentrations compared to the other treatments groups, had significantly lower average larval mortality against *H. contortus* ($p<0.05$) compared to the positive control (albendazole). These treatment groups had an average larval mortality of 3.06%. On the other hand, the 2% and 4% treatment groups had no significant difference ($p>0.05$) in larval mortality compared to the positive control, indicating that the ethanol extract of *Orthosiphon aristatus* (Blume) Miq leaves at a concentration of 2% and 4% had the same anthelmintic power as control (Albendazole) against *H. contortus*. Albendazole is a synthetic anthelmintic that helps inhibit worm food intake, leading to the death of the worms (Swargiary *et al.* 2016; Sultana *et al.* 2022). In addition, the negative control group (0.5% DMSO) had significantly lower average larval mortality ($p<0.05$) compared to the positive control (albendazole). A previous study using 1% DMSO as a negative control found that DMSO at 24 and 48 hours had the smallest percentage of larval deaths compared to other herbal plant extracts. DMSO was used as a negative control because it is a solvent that can dissolve almost all polar and nonpolar compounds (Sujith *et al.* 2015).

The LT_{50} value of *Orthosiphon aristatus* (Blume) Miq leaves extract at a concentration of 4% was the fastest. This suggests that higher concentrations of the extract may result in faster LT_{50} values. This is presumably due to the dilution factor, which can lower the levels of active compounds in the extract that are believed to have anthelmintic properties (Hai *et al.* 2014). A study by Ulya *et al.* (2014) found that a 40% concentration of *Orthosiphon aristatus* (Blume) Miq leaves extract was able to kill all *Ascaris suum* worms within 13 hours and 14 minutes (LT_{100}), while lower concentrations took more than 13 hours to achieve the same result (Ghadirian da Arfaa 1973).

CONCLUSION

The time of death for infective *H. contortus* larvae at 4% concentration was 6 hours, and 0.5% concentration was the effective dose of the extract as an anthelmintic.

REFERENCES

Ghadirian E, Arfaa F. 1973. First report of human infection with *Haemonchus contortus, Ostertagia ostertagi*, and *Marshallagia marshalli* (family *trichostrongylidae*) in Iran. *The Journal of parasitology.* 59(6): 1144–1145. https://doi.org/10.2307/3278661

Hadi RF, Handayanta E, Widyawati SD, Hanifa A, Suprayogi WPS, Sudiyono A. 2021. In vitro anthelmintic activities of shrub plants extracts for *Haemonchus contortus* worms. IOP Conference Series: Earth Environmental Science. 712(1). https://doi.org/10.1088/1755-1315/712/1/012047

Hai NT, Van Thanh N, Tho BT, Atsushi M. 2014. A study about anthelmintic effect of *Punica Gramatum L* bark on veterinary endoparasites. Asian Journal of Pharmaceutical and Clinical Research. 7(2): 148–152.

Kuntari T. 2008. Daya Antihelmintik Air Rebusan Daun Ketepeng (Cassia alata L) terhadap Cacing Tambang Anjing in vitro. *Logika.* 5(1): 23–26. https://doi.org/10.20885/logika.vol5.iss1.art1

Kristianto W, Hayati R, Ishak NI. 2020. Faktor – Factors Associated with the Incidence of Worms in Children Aged 7-10 Years in the Working Area of UPT Barimba Health Center, Kapuas Hilir District, Kapuas Regency in. [thesis]. Banjarmasin (ID): Universitas Islam Kalimantan Muhammad Arsyad Al-Banjari Banjarmasin.

Lem M, Pone J, Vincent K, Joseph T. 2012. Prevalence and intensity of gastro-intestinal helminths in horses in the Sudano-Guinean climatic zone of Cameroon. *Trop Parasitol*. 2(1): 45. https://doi.org/10.4103/2229-5070.97239

Naeem M, Iqbal Z, Roohi N. 2021. Ovine haemonchosis: a review. Tropical animal health and production. 53(1): 1–5. https://doi.org/10.1007/s11 ropical animal health and production250-020-02439-8

Maestrini M, Tava A, Mancini S, Tedesco D, Perrucci S. 2020. In vitro anthelmintic activity of Saponins from

Medicago spp. against sheep gastrointestinal nematodes. *Molecules.* 25(2): 1–9. https://doi.org/10.3390/molecules25020242

Minister of Health of the Republic of Indonesia. Deworming. Jakarta: Regulation of the Minister of Health of the Republic of Indonesia. 2017. p. 1–49. (Indonesia)

Muda I, Prastowo J, Nurcahyo W, Sarmin S. 2021. Anthelmintic effect of Indigofera tinctoria L on *Haemonchus contortus* obtained from sheep in Indonesia. *Veterinary world*. 14(5): 1272– 1278. https://doi.org/10.14202/vetworld.2021.1272-1278

Rahmadani F, Hastuti S. 2020. Uji Toksisitas Ekstrak Etanol Batang Petai Cina *(Leucaena leucochepala)* Terhadap Cacing Gelang Ayam *(Ascaridia galli)*. *Cerata Jurnal Ilmu Farmasi*. 11(1): 28–34. https://doi.org/10.61902/cerata.v11i1.97

Sujith S, Sreedevi R, Priya MN, Deepa CK, Darsana U, Sreeshitha SG, *et al.* 2015. Anthelmintic activity of three Indian medicinal plants. *International Journal of Pharmacognosy and Phytochemical Research*. 7(2): 361–364.

Sultana Y, Kong F, Mukutmoni M, Fahria L, Begum A, Lee R. 2022. Internal transcribed spacer region 1 as a promising target for detection of intra-specific polymorphisms for *Strongyloides stercoralis*. *Trop Parasitol.* 12(1): 48. https://doi.org/10.4103/tp.tp_13_21

Swargiary A, Daimari A, Daimari M, Basumatary N, Narzary E. 2016. Phytochemicals, antioxidant, and anthelmintic activity of selected traditional wild edible plants of lower Assam. Indian journal of pharmacology. 48(4): 418–423. https://doi.org/10.4103/0253-7613.186212

Ulya N, Endharti A tri, Setyohadi R. 2014. Testing of the Anthelmintic Ethanol Extract of Kumis Kucing Leaves *(Orthosiphon aristatus)* as an anthelmintic against Ascaris suum in vitro. Maj Kesehat FKUB [Indonesia]. 1: 130–136.

Widiarso BP, Kurniasih K, Prastowo J, Nurcahyo W. 2018. Morphology and morphometry of *Haemonchus contortus* exposed to Gigantochloa apus crude aqueous extract. *Vet World.* 11(7): 921–925. https://doi.org/10.14202/vetworld.2018.921-925

Williams AR, Fryganas C, Ramsay A, Mueller-Harvey I, Thamsborg SM. 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One.* 9(5). https://doi.org/10.1371/journal.pone.0097053

[WHO] World Health Organization. 2022. Soil-Transmitted Helminth Infections. Geneva :World Health Organization.

Zlotek U, Mikulska S, Nagajek M, Swieca M. 2016. The Effect of Different Solvents and Number of Extraction Steps on the Polyphenol Content and Antioksidant Capacity of Basil Leaves (*Ocimum bassilicum* L.) Extracs. *Saudi Journal of Biological Sciences*. 23(5): 628–633. https://doi.org/10.1016/j.sjbs.2015.08.002