Research Article

Anticoccidial Activity of *Coleus aromaticus* Leaves Extract in Naturally Infected Goats

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Abstract | The treatment and prevention of coccidiosis in goats is costly to backyard farmers. It is for this reason that the use of alternative medicinal plants to treat coccidiosis are explored. Thus, this study aimed to determine the anticoccidial activity of the ethanol extract of *C. aromaticus* leaves. A total of 20 naturally coccidia-infected goats at the University of Southern Mindanao Goat Project served as experimental animals for the study. In relation to this, three concentrations of *Coleus aromaticus* extract were tested for anticoccidial activity: 600, 800, and 1000 mg extract/kg bodyweight. The efficacies of these concentrations were determined and evaluated by comparing with a commercial coccidiostat, toltrazuril. It was found that 21 days post-treatment, the efficacies of the three extract concentrations were significant but was lower than toltrazuril. A significant difference (p = 0.0394) existed in the efficacies of the extracts among the treatments on day 21. The efficacy rate of administering 600 mg/kg extract (92.29%) was comparable to that of toltrazuril (96.65%) at 21 days post-treatment. This shows that the extract has the potential to be as effective as toltrazuril in reducing the oocyst per gram counts of naturally-infected goats.

Keywords | Coccidiosis, *Coleus aromaticus*, Ethanol extract, Goats, Toltrazuril

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INTRODUCTION

Coccidiosis remains to confront the goat industry affecting growth, health, and reproduction. It is a protozoan infection caused by several species of the genus *Eimeria* which develop in the small and large intestine. In particular, coccidiosis in goats leads to dehydration, diarrhea, weight loss, emaciation, weakness and anorexia that limit growth and result in decreased production when death occurs (Soulsby, 1982; Chartier and Paraud, 2012). It becomes an infection of economic importance due to intensive breeding conditions together with high animal density and high productivity (Foreyt, 1990; Chartier and Paraud, 2012). It often leads to morbidity and mortality, particularly in young animals up to 6 months of age (Ruiz et al., 2006; Majeed et al., 2015). In order to minimize the production losses and reduced productivity, good husbandry practices, hygiene maintenance, and medicines are important in reducing the contamination by infective oocysts (Iqbal et al., 2013).

Commercial drugs are commonly used in controlling coccidiosis because of their availability in the market. Drugs that have been used to control and treat coccidiosis in goats include amprolium (Young et al., 2011), decoquinate (Gibbons et al., 2016), diclazuril (Ruiz et al., 2012), halofuginone lactate (Giadinis et al., 2008), and toltrazuril (Iqbal et al., 2013). However, the continued use of commercial coccidiostat has resulted in the development of resistance of the parasite. Because of this and the cost of commercial drugs, alternative remedies to control coccidiosis are practiced and investigated.

Coleus aromaticus is a medicinal plant that has various reported bioactivities. It is a perennial and aromatic herb that is found in Asia, Africa, and South America where it is extensively used for a wide range of diseases (Lukhoba et al., 2006). Leaf extracts from *C. aromaticus* have been reported to exhibit antibacterial (Koba et al., 2011), antimicrobial (Deena et al., 2002), anthelmintic (Hussain et al., 2012), antidiabetic and antiproliferative activities (Govindaraju and Indra-Arulselvi, 2018). In addition, it also exhibited mosquitocidal and repellent activity (Baranitharan et al., 2017). Hence, the present work investigates the anticoccidial property of *C. aromaticus* leaf extracts in naturally-infected goats.

MATERIALS AND METHODS

Plant material and extraction

Fresh *C. aromaticus* leaves, shown in Figure 1, were collected in Kabacan, Cotabato, Philippines and were authenticated at the University of Southern Mindanao Department of Biological Sciences. The selected leaves were healthy and free of insect bites, discoloration, and diseases. Extraction of the plant material was done based on the method described by Eguale et al. (2011) with modifications and a different extracting solvent. The leaves were washed with water and were dried at room temperature. The dried leaves were then pulverized using a blender, which were then soaked in ethanol for 48 hours. For every 50 g of pulverized leaves, 300 mL of ethanol was added to ensure proper soaking. The solvent was evaporated in vacuo and the extract was stored at 4°C until use (Baranitharan et al., 2017).

Figure 1: *Coleus aromaticus* leaves.

Animals and experimental design

The procedures used in the study were approved by the University of Southern Mindanao College of Veterinary Medicine Institutional Animal Care and Use Committee. The methods for research and animal handling are all in compliance with Republic Act 8485, The Animal Welfare Act.

A preliminary fecal examination using the modified McMaster method (Hutchinson, 2009) was performed to determine the number of oocysts per gram (opg) of feces and to identify which goats were infected with *Eimeria* that will be used for the study. Identification of the *Eimeria* species was based on morphometric characteristics of the oocysts and in some cases, based on the presence or absence of a micropyle and oocyst shape (Levine and Ivens, 1967; Saravia et al., 2021).

A total of twenty (20) naturally coccidia-infected goats with ages between three to four months, regardless of sex and breed, were obtained from the University of Southern Mindanao Goat Project from November to December 2020 to serve as experimental animals. The experimental animals weighed within 5.9-15.5 kg and the number of oocysts ranging from 1,400 to 20,150 opg. The animals were from the same herd in a semi-confined rearing system at the University of Southern Mindanao Goat Project facility.

The goats were divided into 5 groups (A-E) of four (4) goats each. The treatments, extracts and controls, were administered orally. Goats in groups A-C were given a single dose of 600, 800, and 1000 mg extract/kg bodyweight. Those in group D were treated with a single dose of 5% toltrazuril (0.4 mL/kg bodyweight) while those in group E were untreated and served as the negative control. The goats were fasted 12 hours prior to treatment to slow down gastrointestinal transit and allow better absorption of the drug administered orally (Lespine et al., 2007). The management of goats at the University of Southern Mindanao Goat Project was adapted. The animals were maintained in an elevated indoor area with wood-slatted floor and were given time to roam around the facility. Grass, hay, water, and mineral salt were provided *ad libitum* and the concentrate in the afternoon. All diets were free of coccidiostats and were prepared to ensure the nutritional requirements of the goats.

Parasitological examination

Fecal samples (3-5 g) were collected 7, 14, 21 and 28 days post-treatment (dpt). Fecal samples were scored using a correction factor based on a scale of 1-3 with 1, normal pellets; 1.5, soft formed but the pellets did not separate; 2, soft but there is no formation of pellets, and; 3, diarrheic (Skerman and Hillard, 1967; Le Jambre et al., 2007). Fecal examination was done using modified McMaster technique (Hutchinson, 2009) to determine the oocysts per gram (opg) of feces.

Statistical analysis

Statistical significance among treatments ($p \leq 0.05$) was compared using one-way ANOVA and Tukey HSD test.

RESULTS AND DISCUSSION

Among the naturally coccidia-infected goats, there were fifteen (15) identified species of *Eimeria*, of which *Eimeria arloingi* was the most predominant. The mean oocysts per gram (opg) counts of goats in the different treatment groups are shown in Table 1. In general, there was an observed reduction of opg in naturally-infected goats. A similar reduction of opg was observed 21 days post-treatment.

The efficacy of the extracts in each treatment is shown in Table 2. Within 7 dpt, group A had the highest efficacy (62.96%) among the extracts. This was still lower than the efficacy of the commercial coccidiostat, toltrazuril. However, the efficacy of the extract given to group A increased at 21 dpt reaching 92.29%, which was comparable to that of toltrazuril (96.65%). This continued to increase 28 dpt.

However, the increase in efficacy was not observed for all doses of the extracts. Goats in groups B and C experienced an increase in efficacy only after 21 dpt. At 28 dpt, the efficacy of the extracts was inversely proportional to the dose administered. Statistical analysis shows that it was only 21 days post-treatment that there was a significant difference (p=0.0394) in the efficacy of the *C. aromaticus* extract. It should be noted that during this time, it was only the dose of 600 mg/kg extract resulted in an efficacy above 90% similar to toltrazuril. Since the goats were naturally-infected, it is hypothesized that the variation in the efficacy of the extracts could be due to the different stages of *Eimeria* in the goat. Moreover, this may also potentially be accounted for by the phytochemical constituents present in the extract.

The results after 21 dpt can likely be attributed to the anticoccidial effect of *C. aromaticus* and the ability to reduce coccidian oocyst counts in animals (Mohiti-Asli and Ghanaatparast-Rashti, 2015; Bozkurt et al., 2016; Dudko et al., 2018). The effect could be due to the bioactive components present in the extract. The phytochemical constituents of the methanolic leaf extract of *C. aromaticus* include saponins, alkaloids, tannins, steroids, terpenoids, and phenolic compounds (Baranitharan et al., 2017; Sujamol et al., 2021). Similar phytochemical constituents have been reported using different extracting solvents particularly ethyl acetate, acetone, and benzene (Baranitharan et al., 2017). These have been documented to possess antioxidant (Masood et al., 2013), wound healing (Muniandy et al., 2014), mosquitocidal and repellent (Baranitharan et al., 2017), and anticoccidial (Kowalska et al., 2012) activities.

Bioactive phytochemical constituents are also present in the root extract of *C. aromaticus*. Hussain et al. (2012) reported that the methanol extract of the roots showed the presence of flavonoids, terpenoids, alkaloids, and tannins. It exhibited anthelmintic activity against the Indian adult earthworm (*Pheretima posthuma*), which may have been caused by the phytochemical constituents present, particularly tannins and phenolic compounds. However, compounds are yet to be isolated and identified from the root extracts.

In contrast, bioactive compounds from extracts of the leaves have been identified. Kumaran and Karunakaran (2007) isolated chlorogenic acid, rosmarinic acid, and caffeic acid from aqueous extracts of *C. aromaticus* leaves. These three phenolics were shown to be potent antioxidants with rosmarinic acid, being the major polyphenol constituent, as more likely to be responsible for most of the observed antioxidant activity. Baranitharan et al. (2017) identified nine major components in the methanol extract of *C. aromaticus* leaves which are copaene; caryophyllene; cedrane; 1-oxaspiro [2,5] octane, 5, 5-dimethyl-4-[3-methyl-1, 3-butadienyl]-; tridecanoic acid, methyl ester; 1, 4-methanoazulene-9-methanol, decahydro-4, 8,8-trimethyl-, [1S-(1á,3aá,4á,8aá,9R*)]-; 11-octadecenoic acid, methyl ester; 7, 10-octadecadienoic acid, methyl ester, and; flexinine. It was hypothesized that 11-octadecenoic acid, methyl ester was the main constituent responsible for the mosquitocidal and repellent activity of *C. aromaticus*. Moreover, carvacrol (Govindaraju and Indra Arulselvi, 2018) and thymol (Govindarajan et al., 2013) have been isolated from essential oils of *C. aromaticus* leaves.

Table 1: The mean fecal oocyst per gram (10^3) of naturally-infected goats treated with *C. aromaticus* extract.

Days post-treatment	Group A (600 mg/kg)	Group B (800 mg/kg)	Group C (1000 mg/kg)	Group D (toltrazuril)	Group E (untreated)	p value
0	17.2	6.6	29.2	25.4	14.0	0.67^{ns}
7	6.4	6.5	35.8	2.1	3.8	0.50^{ns}
14	39.5	10.0	5.8	1.8	50.0	0.49^{ns}
21	1.3	2.9	12.4	0.9	19.6	0.10^{ns}
28	0.4	2.0	16.0	2.3	3.5	0.07^{ns}

*ns: There is no significant difference at 5% level.

Table 2: The mean efficacy of the extracts in different groups.

Days post-treatment	Group A (600 mg/kg)	Group B (800 mg/kg)	Group C (1000 mg/kg)	Group D (toltrazuril)	Group E (untreated)
7	62.96	1.88	-22.90	91.92	72.69
14	-129.69	-50.19	80.15	92.78	-258.24
21	92.29[a]	56.59[a]	57.42[a]	96.65[a]	-40.69[b]
28	97.45	70.62	45.09	90.93	74.71

*Mean values with different letters in the same row differ significantly ($p<0.05$).

Thymol, carvacrol, and saponins are known to exhibit anticoccidial activity (Felici et al., 2020). The thymol, carvacrol, saponins, and tannins present in the *C. aromaticus* leaf extract may have produced similar anticoccidial effects. Saponins tend to prevent the growth of protozoans by interacting with cell membrane cholesterol content resulting in the death of the parasite. The phenolic content which includes carvacrol (Sidiropoulou et al., 2020) may have affected the oocyst by interacting on the oocysts' cytoplasmic membrane and the permeability of hydrogen ions and potassium ions (Giannenas et al., 2003). Additionally, tannins have exhibited a significant effect on the reduction of the sporulation of the *Eimeria* species (Masood et al., 2013). Terpenoids, such as thymol, and tannins can bind to free proteins in the gastrointestinal tract or glycoproteins of the parasite leading to death. Further, tannins or their metabolites have a direct effect on the viability of the parasite during pre-parasitic stages (Hussain et al., 2012). This may support the variation in the efficacies of *C. aromaticus* leaf extract.

Coccidiosis causes reduced production and decrease in body weight in goats. Moreover, the tropical conditions promote the incidence of these parasitic diseases because the moist and warm environmental conditions stimulate sporulation of coccidia. It is through the timely use of medicines that the disease can be prevented. The chemical components of anticoccidial products target the parasite at varying stages of its life cycle. Amprolium, monensin, and lasalocide acts during the early stages of the life cycle while sulfonamides have an activity at the last stages. By contrast, toltrazuril acts on the whole cycle of the coccidia (Taylor, 2009), thus having both curative and preventive actions.

These drugs potentially face the problem of the parasite developing drug resistance. The long and repeated usage of the same drugs with no breaks or alternatives results to this resistance thus emphasizing the need for alternative anticoccidial drugs.

CONCLUSIONS AND RECOMMENDATIONS

The result of the study shows that the leaf extract of *C. aromaticus* has potential *in vivo* anticoccidial activity, with a similar activity to that of the commercial coccidiostat toltrazuril. The extract was able to reduce the fecal opg within 21 days post-treatment. Moreover, the extract can be developed into a medicine that can be used to treat coccidiosis.

NOVELTY STATEMENT

It was previously shown that root extracts of *C. aromaticus* exhibited anthelmintic activity. Leaves present a more viable source of secondary metabolites and have been reported to show antioxidant activity. However, its anthelmintic activity against coccidia, specifically against *Eimeria*, has not been reported. This study demonstrates that the extract from the leaves of *C. aromaticus* is able to elicit anthelmintic activity in naturally-infected goats. This means that the extracts contain sufficient amounts of secondary metabolites to produce such effect even after interaction with the diet and host metabolism.

AUTHOR'S CONTRIBUTION

VJAG, JRF, EAG, and LJAG developed the research concepts and designed the study. VJAG conducted the experiments under the supervision of JRF and guidance of EAG. LJAG wrote the manuscript draft and were revised by the co-authors.

Conflict of interest

The authors have declared no conflicts of interest.

REFERENCES

- Baranitharan M, Dhanasekaran S, Kovendan K, Murugan K, Gokulakrishnan J, Benelli G (2017). *Coleus aromaticus* leaf extract fractions: A source ofnovel ovicides, larvicides and repellents against *Anopheles*, *Aedes* and *Culex* mosquito vectors? Process. Saf. Environ. Prot. pp. 23-33. https://doi.org/10.1016/j.psep.2016.12.003
- Bozkurt M, Ege G, Aysul N, Akşit H, Tüzün A, Küçükyilmaz K, Borum AE, Uygun M, Akşit D, Aypak S, Simşek E, Seyrek K, Koçer B, Bintaş E, Orojpour, A (2016). Effect of anticoccidial monensin with oregano essential oil on broilers experimentally challenged with mixed *Eimeria* spp. Poult. Sci., 95(8): 1858-1868. https://doi.org/10.3382/ps/pew077
- Chartier C, Paraud C (2012). Coccidiosis due to *Eimeria* in

sheep and goats. A review. Small Rumin. Res., 103(1): 84-92. https://doi.org/10.1016/j.smallrumres.2011.10.022

- Deena MJ, Sreeranjini K, Thoppil JE (2002). Antimicrobial screening of essential oils of *Coleus aromaticus* and *Coleus zeylanicus*. Int. J. Aromather., 12(2): 105-107. https://doi.org/10.1016/S0962-4562(02)00035-8
- Dudko P, Junkuszew A, Bojar W, Milerski M, Szczepaniak K, Le Scouarnec J, Schmidová J, Tomczuk K, Grzybek M (2018). Effect of dietary supplementation with preparation comprising the blend of essential oil from *Origanum vulgare* (lamiaceae) and *Citrus* spp. (citraceae) on coccidia invasion and lamb growth. Ital. J. Anim. Sci., 17(1): 57–65. https://doi.org/10.1080/1828051X.2017.1346965
- Eguale T, Tadesse D, Giday M (2011). *In vitro* anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. J. Ethnopharmacol., 137(1): 108-113. https://doi.org/10.1016/j.jep.2011.04.063
- Felici M, Tugnoli B, Ghiselli F, Massi P, Tosi G, Fiorentini L, Piva A, Grilli E (2020). *In vitro* anticoccidial activity of thymol, carvacrol, and saponins. Poult. Sci., 99(11): 5350-5355. https://doi.org/10.1016/j.psj.2020.07.035
- Foreyt WJ (1990). Coccidiosis and cryptosporidiosis in sheep and goats. *Vet. Clin. North Am. Food Anim. Pract.*, 6(3): 655-670. https://doi.org/10.1016/S0749-0720(15)30838-0
- Giadinis ND, Papadopoulos E, Lafi SQ, Panousis NK, Papazahariadou M, Karatzias H (2008). Efficacy of halofuginone lactate for the treatment and prevention of cryptosporidiosis in goat kids: An extensive field trial. Small Rumin. Res., 76: 195-200. https://doi.org/10.1016/j.smallrumres.2008.01.007
- Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E, Botsoglou NA, Spais AB (2003). Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Arch. Tierernahr., 57(2): 99-106. https://doi.org/10.1080/0003942031000107299
- Gibbons P, Love D, Craig T, Budke C (2016). Efficacy of treatment of elevated coccidial oocyst counts in goats using amprolium versus ponazuril. Vet. Parasitol., 218: 1-4. https://doi.org/10.1016/j.vetpar.2015.12.020
- Govindarajan M, Sivakumar R, Rajeswary M, Veerakumar K (2013). Mosquito larvicidal activity of thymol from essential oil of *Coleus aromaticus* Benth. against *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Anopheles subpictus* (Diptera: Culicidae). Parasitol. Res., 112(11): 3713-3721. https://doi.org/10.1007/s00436-013-3557-2
- Govindaraju S, Indra Arulselvi P (2018). Characterization of *Coleus aromaticus* essential oil and its major constituent carvacrol for in vitro antidiabetic and antiproliferative activities. J. Herbs Spices Med. Plants, 24(1): 37-51. https://doi.org/10.1080/10496475.2017.1369483
- Hussain A, Sonkar AK, Ahmad MP, Wahab S (2012). *In-vitro* anthelmintic activity of *Coleus aromaticus* root in Indian Adult Earthworm. Asian Pac. J. Trop. Dis., 2(Suppl 1): S245-S427. https://doi.org/10.1016/S2222-1808(12)60196-0
- Hutchinson GW (2009). Nematode parasites of small ruminants, camelids and cattle diagnosis with emphasis on anthelmintic efficacy and resistance testing. Australia and New Zealand Standard Diagnostic Procedures.
- Iqbal A, Tariq KA, Wazir VS, Singh R (2013). Antiparasitic efficacy of *Artemisia absinthium*, toltrazuril and amprolium against intestinal coccidiosis in goats. J. Parasit. Dis., 37(1): 88-93. https://doi.org/10.1007/s12639-012-0137-9
- Koba K, Nénonéné AY, Sanda K, Garde D, Millet J, Chaumont J-P, Raynaud C (2011). Antibacterial activities of *Coleus aromaticus* Benth (Lamiaceae) Essential Oil against Oral Pathogens. J. Essent. Oil Res., 23(1): 13-17. https://doi.org/10.1080/10412905.2011.9700424
- Kowalska D, Bielański P, Nosal P, Kowal J (2012). Natural alternatives to coccidiostats in rabbit nutrition. Ann. Anim. Sci., 12(4): 561-574. https://doi.org/10.2478/v10220-012-0047-x
- Kumaran A, Karunakaran AJ (2007). Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. Food Chem., 100(1): 356-361. https://doi.org/10.1016/j.foodchem.2005.09.051
- Le Jambre LF, Dominik S, Eady SJ, Henshall JM, Colditz IG (2007). Adjusting worm egg counts for faecal moisture in sheep. Vet. Parasitol., 145(1-2): 108-115. https://doi.org/10.1016/j.vetpar.2006.11.017
- Lespine A, Chartier C, Hoste H, Alvinerie M (2007). Endectocides in goats: Pharmacology, efficacy and use conditions in the context of anthelmintics resistance. Small Rumin. Res., 103(1): 10-17. https://doi.org/10.1016/j.smallrumres.2011.10.013
- Levine ND, Ivens V (1967). The sporulated oocysts of *Eimeria illinoisensis* n. sp. and of other species of *Eimeria* of the Ox. J. Protozool., 14(2): 351-360. https://doi.org/10.1111/j.1550-7408.1967.tb02008.x
- Lukhoba CW, Simmonds MS, Paton AJ (2006). Plectranthus: A review of ethnobotanical uses. J. Ethnopharmacol., 103(1): 1-24. https://doi.org/10.1016/j.jep.2005.09.011
- Majeed QA, Alazemi MS, Henedi AA, Tahrani LM (2015). Study on parasites from farm animals in Kuwait. J. Egypt. Soc. Parasitol., 45(1): 71-74. https://doi.org/10.21608/jesp.2015.89692
- Masood S, Abbas RZ, Iqbal Z, Mansoor MK, Sindhu ZUD, Zia MA, Khan JA (2013). Role of natural antioxidants for the control of coccidiosis in poultry. Pak. Vet. J., 33(4): 401-407.
- Mohiti-Asli M, Ghanaatparast-Rashti M (2015). Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers. Prev. Vet. Med., 120(2): 195-202. https://doi.org/10.1016/j.prevetmed.2015.03.014
- Muniandy K, Hassan Z, Mohd Isa MH (2014). The action of *Coleus aromaticus* as a potential wound healing agent in experimentally induced diabetic mice. Perintis E-J., 4(1): 1-30.
- Ruiz A, González JF, Rodríguez E, Martín S, Hernández YI, Almeida R, Molina JM (2006). Influence of climatic and management factors on *Eimeria* infections in goats from semi-arid zones. J. Vet. Med. B Infect. Dis. Vet. Publ. Hlth., 53(8): 399-402. https://doi.org/10.1111/j.1439-0450.2006.00985.x
- Ruiz A, Guedes AC, Muñoz MC, Molina JM, Hermosilla C, Martín S, Hernández YI, Hernández A, Pérez D, Matos L, López AM, Taubert A (2012). Control strategies using diclazuril against coccidiosis in goat kids. Parasitol. Res., 110(6): 2131-2136. https://doi.org/10.1007/s00436-011-2746-0
- Saravia A, Miraballes C, Riet-Correa F, Castro-Janer E (2021). *Eimeria* spp. in dairy calves in Uruguay. Identification, dynamics of oocyst excretion and association with the age of calves. Vet. Parasitol. Reg. Stud., 25: 100588. https://doi.org/10.1016/j.vprsr.2021.100588

- Sidiropoulou E, Skoufos I, Marugan-Hernandez V, Giannenas I, Bonos E, Aguiar-Martins K, Lazari D, Blake DP, Tzora A (2020). *In vitro* anticoccidial study of oregano and garlic essential oils and effects on growth performance, fecal oocyst output, and intestinal microbiota *in vivo*. Front. Vet. Sci., 7: 420. https://doi.org/10.3389/fvets.2020.00420
- Skerman K, Hillard J (1967). A handbook for studies of helminth parasites of ruminants. Iran United Nations Development Program.
- Soulsby E (1982). Helminths, arthropods and protozoa of domesticated animals (7th ed.). London: Baillière Tindall.
- Sujamol MS, Roy J, James KM (2021). Phytochemical screening and antimicrobial activity of *Coleus aromaticus* leaf extract. Mater. Today, 41(Part 3): 596-599. https://doi.org/10.1016/j.matpr.2020.05.255
- Taylor M (2009). Changing patterns of parasitism in sheep. Practice, 31(10): 474-483. https://doi.org/10.1136/inpract.31.10.474
- Young G, Alley ML, Foster DM, Smith GW (2011). Efficacy of amprolium for the treatment of pathogenic *Eimeria* species in Boer goat kids. Vet. Parasitol., 178(3-4): 346-349. https://doi.org/10.1016/j.vetpar.2011.01.028