



## **Anthelmintic activity of *Ocimum gratissimum* and *Cymbopogon citratus* leaf extracts against *Haemonchus placei* adult worm**

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### **Abstract**

Medicinal plants are promising, as source of alternative solution to the problem of parasitic helminth infections that hamper the sustainable supply of products from farm animals. *Ocimum gratissimum* and *Cymbopogon citratus* leaves are commonly used internally to treat worms, relieve abdominal pains and stomach ache. This study, therefore, evaluated their leaf extracts for anthelmintic activity against *Haemonchus placei* worm from cattle; the most active extract being subjected to phytochemical analysis. The extracts, obtained by maceration using acetone, were evaluated for anthelmintic activity by exposing adult *H. placei* to a range of concentrations (5-60 mg/mL) for 3 hours and then counting the number of confirmed dead worms. Anthelmintic activity data were fitted to a non-linear regression equation (Log [extract] vs. response; variable slope) to produce best-fit sigmoidal curves from which LC<sub>50</sub> values were computed. The best-fit LC<sub>50</sub> values, found to be significantly different ( $\alpha < 0.0001$ ), were 17.70 mg/mL and 56.04 mg/mL for *C. citratus* and *O. gratissimum*, respectively, suggesting that *C. citratus* is three times more potent than *O. gratissimum*. Phytochemical analysis of *C. citratus* indicated the presence of alkaloids, tannins, steroids, saponins, terpenoids and flavonoids. *Cymbopogon citratus* could be useful as an anthelmintic phytomedicine against livestock parasitic helminths.

*Keywords:* Anthelmintic; *Haemonchus placei*; Motility assay; *Ocimum gratissimum*; *Cymbopogon citratus*

### **INTRODUCTION**

One of the considerable threat to profitable and sustainable supply of products from farm animals is gastrointestinal parasitic infections which often result in anaemia, diarrhoea, reduction in weight gains, reduction in milk production, and in some situations, death [1]. These infections are prevalent in farming areas where under suitable climatic conditions, egg hatching, development, survival and transmission of infective larvae which are ingested by grazing

animals are favoured [2]. Though a number of parasites continues to ravage grazing animals in general, *Haemonchus placei* and *H. contortus* are highly pathogenic, abomasal blood-sucking nematodes of grazing farm animals [3].

Currently, the control of parasitic helminths relies chiefly on the use of synthetic, broad-spectrum anthelmintic drugs. In all, major classes anthelmintics commercially available include benzimidazoles, macrocyclic lactones,

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nicotinic agonists, amino-acetonitrile derivatives and spiroindol [4]. However, some of these drugs are expensive, and as such unaffordable to livestock farmers in resource-constrained developing nations. In addition, the widespread usage of these therapeutic agents, has resulted in the spread of resistant nematodes, consequently increasing anthelmintic therapeutic failure [5]. The situation is worse where there are no supportive livestock extension services. Overall, these circumstances result in poor livestock productivity, with the economic status of many pastoralists and small holder farmers that derive livelihoods from livestock farming being undermined [6]. All these together necessitate renewed search for alternative and sustainable solutions to curtail parasitic helminths by exploring medicinal plants for their potential anthelmintic activity [7]. Thus, this study investigates the anthelmintic activity of *Ocimum gratissimum* Linn. (Lamiaceae) and *Cymbopogon citratus* Stapf (Poaceae) against adult *H. placei* worms, and assessed the phytochemical constituents of the most active plant.

## EXPERIMENTAL

**Plant collections and extraction.** Leaves of *C. citratus* and *O. gratissimum* were collected from The Polytechnic Staff Quarters, near Poly-UI Gate, The Polytechnic, Ibadan on the 19 April 2017. Herbarium samples were deposited at Forestry Research Institute of Nigeria, Jericho, Ibadan, where Forestry Herbarium Ibadan (FHI) number was allocated (*C. citratus*: 112021; *O. gratissimum*: 111995). The plant materials were air-dried at room temperature over three weeks after which they were milled into coarse powder using laboratory blender (Elgento-125, China). The powdered plant materials (200 g each) were extracted twice with acetone (1 L) by maceration for 24 h at room temperature. The supernatants were concentrated under vacuum using rotary

evaporator, and dried *in vacuo* at 40 °C for 48 hours.

**Collection of *Haemonchus placei* worms.** Adult *H. placei* worms were collected from the abomasum of newly slaughtered cattle at the Bodija Abattoir, Bodija Market, Ibadan, Oyo State, Nigeria. They were cleansed of fecal remnants using distilled water, and kept in normal saline solution before use. The worms were authenticated by Prof. I. O. Ademola, at the Parasitology Research Unit, Department of Veterinary Parasitology and Entomology, University of Ibadan.

**Anthelmintic evaluation.** *In vitro* anthelmintic activity of the extracts was conducted against adult *H. placei* nematodes using adult worm motility assay [8]. Briefly, varying concentrations (5-60 mg/mL; 0.5 mL, two wells per concentration) of the test extracts in 20% Tween-80 in normal saline were added to wells of a 24-well flat-bottom plate. This was followed by placing ten worms to each well and exposing them for 3 hours at room temperature (25-28°C). At the end of the exposure period, worms were removed into Petri dishes containing distilled water, cleansed of the extract and then placed in warm normal saline (40°C) for 30 minutes. The worms were categorized as dead after satisfying two conditions: (a) no revival of motility after 30 minutes in the warm normal saline, and (b) no noticeable movement after they were pin-pricked. 20% Tween-80 in normal saline were used as control. Anthelmintic activity data were fitted to a non-linear regression equation (Log [compound] vs. response; variable slope) to produce best-fit sigmoidal curves from which median Lethal Concentration (LC<sub>50</sub>) values were computed with associated uncertainty, using the GraphPad Prism 7.0 (GraphPad Software Inc., California, USA).

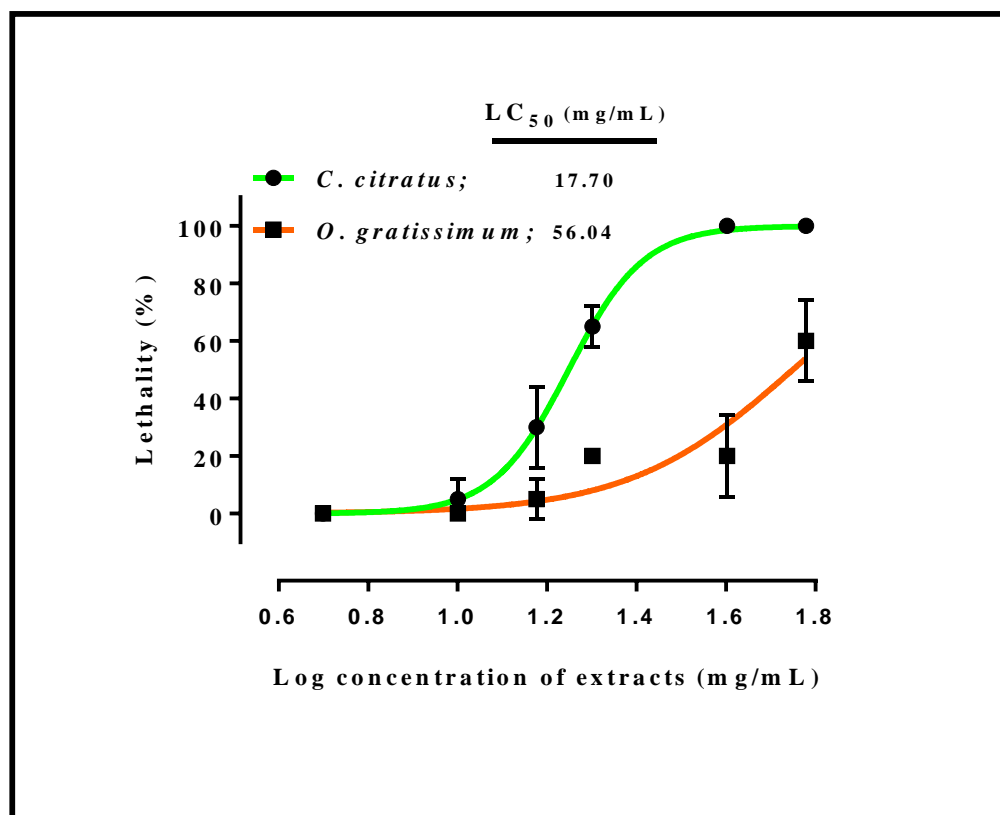
**Phytochemical analysis.** Based on the outcome on the anthelmintic evaluation, the most active of the two plant extracts was

analyzed for its phytochemical composition following standard procedures [9].

## RESULTS

The extraction yield in acetone of the two plant materials were 4.34 % w/w (*C. citratus*) and 4.68 % w/w (*O. gratissimum*). The choice of acetone was due to its high solvent strength, its intermediate polarity as well as good selectivity for extracting bioactive metabolites [10,11]. The anthelmintic profiles of the extracts are shown in Figure 1. The best-fit LC<sub>50</sub> values, found to be significantly different ( $\alpha < 0.0001$ ),

were 17.70 mg/mL (95% CI = 16.78 - 18.73), and 56.04 mg/mL (95% CI = 46.09 - 81.37) for *C. citratus* and *O. gratissimum*, respectively, suggesting that *C. citratus* is three times more potent than *O. gratissimum* against adult *H. placei*. Phytochemical analysis of *C. citratus* extract indicated the presence of alkaloids, tannins, steroids, saponins, terpenoids and flavonoids as secondary metabolites. Some of these compounds are responsible for the observed anthelmintic activity against adult *H. placei*.



**Figure 1.** Anthelmintic activity profiles of acetone extracts of *Cymbopogon citratus* and *Ocimum gratissimum* against adult *Haemonchus placei* incubated (in duplicates) in varying concentration (5-60 mg/mL) for 3 hours at room temperature (25°C-28°C) with 20% Tween-80 in normal saline as control.

## DISCUSSION

To the best of our knowledge, there are no reports in the literature on the anthelmintic effect of these two well-known

medicinal plants against adult helminth parasites. Whole worm, *in vitro* anthelmintic assays against adult parasites, which inflict the greatest harm to the animal host, still

plays important role in investigating plant extracts and drug candidates for their potential anthelmintic effect [12]. In addition, *in vitro* anthelmintic assays like this usually require a relatively low quantity of test materials compared to *in vivo* anthelmintic assays [13].

From the study, *C. citratus* leaf extract was found to be anthelmintic against *H. placei* with an LC<sub>50</sub> 17.70 mg/mL. This is quite important in that activity against non-parasitic, free-living worms does not automatically translates to activity against parasitic worms [14]. The plant leaf extracts (aqueous and methanol) have been reported to be anthelmintic against adult earthworm [15,16]. In addition, decoction and essential oil obtained from aerial parts of *C. citratus* inhibited egg hatching and larva development of *H. contortus* [17]. Thus, *C. citratus* appears to be active against different life-cycle stages of helminths.

On the other hand, the result from this study indicated that *O. gratissimum* leaf extract possessed relatively low potency against adult *H. placei* worm. Literature search did not turn up any reported activity against both free-living and parasitic worms. With regards to activity against helminths larvae, reports have been conflicting. Njoku and Asuzu [18] reported that the leaf extract (ethanol) exhibited weak paralysis against larvae of *H. contortus* and *Heligmosomoides polygyrus*. While, Udoha et al. [19] reported that the leaf extract (ethanol) exhibited potent anthelmintic activity against larvae and ova of *Heligmosomoides bakeri*. Also, essential oil obtained from the leaf was reported to be ovicidal against *H. contortus* [20].

In conclusion, the study suggests that *C. citratus* is three times more potent than *O. gratissimum* against adult *H. placei* nematodes. *C. citratus* extract could be applied in the development of anthelmintic phytomedicine to control livestock gastrointestinal parasites.

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