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In-vitro anthelmintic activity of crude extracts of Zingiberaceae species commonly available in Mwanza, Tanzania

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Abstract

Introduction*:* Resistance to commonly used anthelmintic drugs has been reported in many areas of the world. Natural products are good candidates for discovering more effective anthelmintic compounds. This study assessed the *In-vitro* activity of extracts from *Curcuma longa, Zingiber officinale* and *Elettaria cardamomum.*

Methods*:* This study was an *In-vitro* experimental study involving *Apporrectodea longa* earthworms. The worms were treated with 20mls of both distilled water and ethanolic extracts of all plant species (at 20, 40 and 60mg/ml). Albendazole was used as a standard drug. Paralysis and death time were recorded.

Results*:* The paralysis time for water extracts were 0.83 hrs, 1.22 hrs and 3.33 hrs at 20mg/ml for *Elettaria cardamomum, Zingiber officinale* and *Curcuma longa* respectively while death time was 2.00 hrs, 1.50 hrs and 7.61 hrs for *Elettaria cardamomum, Zingiber officinale* and *Curcuma longa* respectively. The paralysis time for ethanol extracts were 0.50 hrs, 0.58 hrs and 0.70 hrs at 20mg/ml for *Elettaria cardamomum, Zingiber officinale* and *Curcuma longa respectively* while death time was 1.00 hrs, 1.00 hrs and 1.64 hrs for *Elettaria cardamomum, Zingiber officinale* and *Curcuma longa* respectively. *Elettaria cardamomum and Zingiber officinale* recorded shorter paralysis time than albendazole at 20mg/ml.

Conclusion*: Zingiber officinale* and *Elettaria cardamomum* have recorded promising *In-vitro* activity warranting for pharmacological and toxicological investigations.

Keywords: *In-vitro* activity; *Curcuma longa*; *Zingiber officinale*; *Elettaria cardamomum*; Tanzania

1. Introduction

Helminthiasis is one of the most common infestations in man (1) whereby soil transmitted helminths (STH) infect approximately 19% of the global population (2). STH highest prevalence is reported in Sub-Saharan Africa (SSA), Asia and South America (3). The STH of medical importance are *Ascaris lumbricoides, Trichuris trichiura, Necator americanus* and *Ancylostoma duodenale*.

Despite the presence of different synthesized drugs still parasitic infestation remains a major health problem as most parasites are resistant to most drugs current available for treatment (4-6). This is due to the fact that most therapeutic agents used in treatment of parasitic infections share the same chemical group that holds a key in treating parasitic

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infections. Therefore, there is a need for alternative drugs taking into consideration the documented high resistance to the currently used anthelmintic drugs (7).

Medicinal plants are widely used in treatment of various diseases by local communities in addition to conventional medicine. They have also been a source of drugs employed in conventional medicine, either by providing pure compounds, starting materials for partial synthesis of useful compounds or models for synthesis of new drugs (8).

A medicinal plant usually has various phytochemical and pharmacological activities (9, 10). Plants from *Zingiberaceae* family have been reported to be used against helminth infections in livestock in different parts of the world (11-13)**.** *Curcuma longa, Zingiber officinale and Elettaria cardamomum* are both from the same family *Zingiberaceae*. These are among commonly used medicinal plants and have different therapeutic values like antioxidant, anti-inflammatory, blood purifier, reducing excessive cholesterol, arthritis, dementia, fever, pains, aid in digestion and eliminating nausea and vomiting (14). The phytochemicals play a major role for the activity of the above plants. Alkaloids, flavonoids and amino acids are the major chemical constituents for the rhizome of *Curcuma longa* while the rhizome of *Zingiber officinale* contains gingerols, shogaols, paradols and zingerone as non-volatile pungent compounds and zingeberene, curcumene and farnesene sesquiterpene hydrocarbons as volatile components (15). The seed of *Elettaria cardamomum* contains alkaloids, flavonoids, saponins, sterols, terpenes and tannins which play a major role in their therapeutic effects (15). The most active phytochemical in *C. longa* is curcumin a flavonoid, gingerol and shogaol in *Z. officinale* and 1,8 cineole (eucalyptol) a monoterpene in *E. Cardamomum* (16-18). Being of different therapeutic importance these plants have shown to have anthelmintic activities in different studies though there is limited information about *Elettaria cardamomum.* Therefore, we hypothesize that since they belong to the same family *E. cardamomum* could have similar pharmacological action (17).

Traditional medicine (TM) being the oldest system of health care has survived the test of time (19) and has been recommended to be integrated into modern health care systems based on innovation and research (20). The use of TM is high (more than 80% of the population) in developing countries in Africa and Asia (21). In Tanzania, more than half of the population use traditional medicines for management of various diseases including helminths (22). Tanzania is estimated to have over 12,000 plant species and approximately 10% of these are used for medicinal purposes (23).

This study provides preliminary evidence on the activity of commonly used plant from *zingiberaceae* family on helminths by using the earthworm model. The findings of this study expand the pharmaceutical knowledge on the role of medicinal plants in treating STH. Evidence from this study will also serve as a baseline for further research aiming at developing new conventional drugs based on the chemistry of the studied natural medicines.

2. Material and Methods

2.1. Study area, design and population

The present study was conducted at the multipurpose laboratory of the Catholic University of Health and Allied Sciences (CUHAS). The choice of the setting was based on the availability of required apparatus, provision of appropriate conditions for handling and storage of specimen under the experiment.

This study was an *In-vitro* experimental study involving procedures using earthworms used by fishermen in Lake Victoria. These earthworms have some physiological and anatomical features resembling to roundworms. The *Apporrectodea longa* worms were put in different treatment groups (experimental and control) to test the activity of different concentrations of *E. cardamomum*, *Z. officinale* and *C. longa* on paralysis and death at different times. The worms were identified by a zoologist.

2.2. Eligibility Criteria

The study involved earthworms of approximately equal shape and size in terms of length (5-8 cm) collected on the same day. All dead and paralysed earthworms during collection were excluded from the study.

2.3. Sample size and sampling procedure

The sample size and methodology was adopted from a previous study (24) with few modifications. A total of 252 *Apporrectodea longa* worms were used for the experiments. The worms were assigned into four groups. Group one served as control (worms received only sterile water), group two as standard (worms treated with albendazole), group three served as treatment 1 (worms treated with water extracts of different concentrations of *E. cardamomum*, *Z.*

officinale and *C. longa*) while group four served as treatment 2 (worms treated with ethanolic extracts of different concentrations of *E. cardamomum, Z. officinale* and *C. longa*).

2.4. Worm Collection

The earthworms were collected from Lake Victoria fishermen at Kamanga ferry in Mwanza. Before being used, worms were immersed in water to remove the soil.

2.5. Plant Material

The rhizome of *Curcuma longa* and *Zingiber officinale* and fruits of *Elettaria cardamomum* were collected from a local market in Mwanza city. *Zingiber officinale* and *Curcuma longa* were cut into slices and dried in shade. Then they were made into fine powder using electric grinder.

2.6. Drugs and Chemicals

Albendazole was used as standard drug. Distilled water was used as control and for extraction together with alcohol 70%. Albendazole was chosen as standard because it has a broad spectrum and is the drug of choice for worm infestation in Tanzania.

2.7. Preparation of extracts

Powder from each plant was extracted with boiled distilled water. Each of 2, 4 and 6g of dry powder were added to 100 ml of hot distilled water to obtain extracts of concentrations 20, 40, and 60 mg/ml respectively, then mixed gently intermittently for one hour. The obtained solutions were left at room temperature for 24 hrs before being stirred again and filtered. The filtrates obtained were used for experimental evaluation. For albendazole, 400mg of the drug was mixed with 20ml of water to obtain a mixture of 20mg/ml.

To obtain an ethanolic extract, the following procedures were followed: 100 g of dry powder was added to 400 ml of 70% ethanol and mixed gently intermittently for one hour in a conical flask. The obtained solution was left at room temperature for 24 hrs before being stirred again and filtered. The solvent was then removed by evaporation in a water bath until a concentrated thick mass was obtained. After that 2, 4 and 6g of concentrated residue were added to 100 ml of distilled water to obtain diluted extracts of concentrations 20, 40, and 60 mg/ml respectively. The solutions were filtered and the filtrates were used for the experiment. The extract preparation method was adopted from a previous study (24).

2.8. Determination of *in vitro* activity

Earthworms were treated with 20 mls of both water and ethanolic extract of all plants at 20, 40 and 60mg/ml in a beaker, 20 mls of 20mg/ml of albendazole as standard and 20 mls of distilled water as control. The beaker was shaken for one minute and kept at room temperature. There were three replicates for each treatment. Beakers were observed until paralysis and death occurred for the interval of half an hour post treatment for the first two hours, one-hour interval for four hours and then 24 hours interval. Paralysis was regarded to occur if there was no movement, only shaking vigorously when dipped in warm water while death was regarded to occur when worms lost motility even in warm water and their colour faded. The described method was adopted from a previous study (24).

2.9. Statistical analysis

The statistical data analysis was performed using STATA version 13 statistical software. Then results were assessed for their normality by using analysis of variance (ANOVA). Analysis of variance was done to compare the paralysis and death time of worms under different concentrations.

3. Results

3.1. Water extracts

The paralysis time for water extracts of *Elettaria cardamomum* was 0.83 hrs, 0.67 hrs and 0.5 hrs at concentration of 20mg/ml, 40mg/ml and 60mg/ml respectively (Table 1 and Figure 1).

The death time for all three concentrations was 2.0 hrs. *Zingiber officinale* showed the paralysis time of 1.22 hrs, 1.08 hrs and 1.0 hrs at 20mg/ml, 40mg/ml and 60mg/ml concentrations respectively while the death time was 1.5 hrs for

all concentrations. For *Curcuma longa* the paralysis and death time were 3.33 hrs and 7.61 hrs respectively for 20mg/ml concentration, 2.22 hrs and 2.9 hrs respectively for 40mg/ml concentration and 0.5 hrs and 1.0 hrs respectively for 60mg/ml concentration (Table 1 and Figure 1).

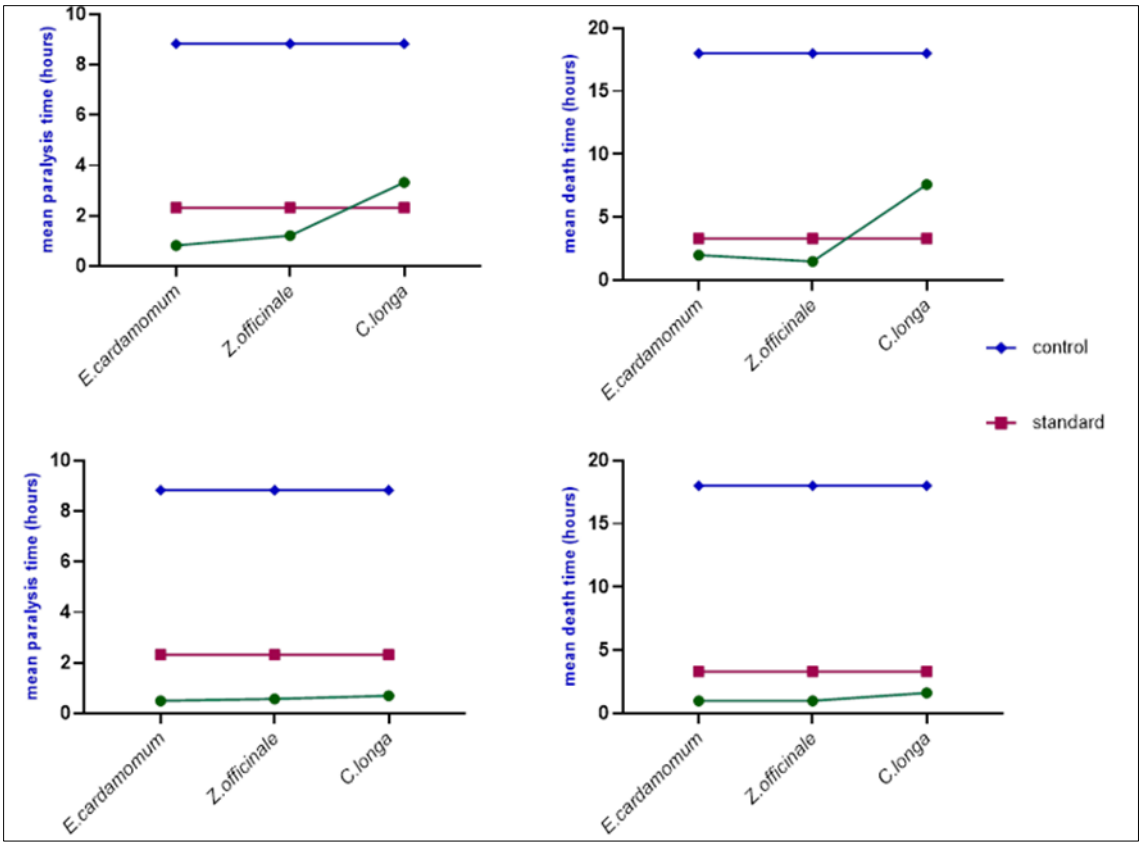

Figure 1 *In-vitro* activity of *Zingiberaceae* species (water (graph A(right top) & B(left top)) and ethanolic extracts (graph C(right bottom) &D(left bottom)) and standard samples at 20mg/ml concentration

3.2. Ethanolic extracts

Table 1 Anthelmintic activity of extracts, standard and control samples

Concentrations	Water extracts		Ethanolic extracts	
	Paralysis mean time (hrs)	Death mean time (hrs)	Paralysis mean time (hrs)	Death mean time (hrs)
Elettaria cardamomum				
40mg/ml	0.67 ± 0.29	2 ± 0.00	0.5 ± 0.00	1 ± 0.00
60mg/ml	0.5 ± 0.00	2 ± 0.00	0.5 ± 0.00	1 ± 0.00
Zingiber officinale				
40mg/ml	1.08 ± 0.08	1.5 ± 0.00	0.53 ± 0.5	1 ± 0.00
60mg/ml	1.00 ± 0.00	1.5 ± 0.00	0.5 ± 0.00	1 ± 0.00
Curcuma longa				
40mg/ml	2.22 ± 0.09	2.9 ± 0.10	0.61 ± 0.13	1.08 ± 0.09
60mg/ml	0.50± 0.00	1 ± 0.00	0.5 ± 0.00	1 ± 0.00
Standard drug (Albendazole) 20mg/ml	2.33	3.33	2.33	3.33
Control (distilled water)	8.83	18	8.83	18

All values represent mean ± SD, n=6 in each group.

The paralysis time for *Elettaria cardamomum* was 0.5 hrs and death time was 1.0 hrs for all three concentrations. For *Zingiber officinale* the paralysis time was 0.58 hrs, 0.53 hrs and 0.5 hrs at 20mg/ml, 40mg/ml and 60mg/ml concentrations respectively while death time was 1.0 hrs for all three concentrations (Table 1 and Figure 1).

Curcuma longa showed the paralysis time of 0.7 hrs, 0.61 hrs and 0.5 hrs at 20mg/ml, 40mg/ml and 60mg/ml concentrations respectively while; death time was 1.64 hrs, 1.08 hrs and 1.0 hrs at 20mg/ml, 40mg/ml and 60mg/ml concentrations respectively. For albendazole as standard, paralysis time was 2.33 hrs while death time was 3.33 hrs. Paralysis time and death time for the control (distilled water) were 8.83 hrs and 18 hrs respectively (Table 1 and Figure 1).

4. Discussion

Curcuma longa has been shown to have different pharmacological activities in different studies (25). In the present study, the anthelmintic activity of *Curcuma longa* was shown to increase with the increase in time and concentration. Ethanolic extract showed more anthelmintic activity than water extract. The ability of ethanolic extract to have greater effect than water extract is similar to a study done by Kulkarni et al. which revealed an alcoholic solvent is the best solvent for extraction of high quantity of curcumin as the major active compound in *C. longa* (26). Curcumin is a natural polyphenol acting on dividing cells by inhibiting proliferation thus may lead to muscle cell suppression on earthworm hence paralysis; though the exactly mechanism is not well understood (7, 27). Other studies have also reported that earthworms exposed to *C. longa* extract were paralysed and died in a concentration and time dependent manner (7) similar to findings from this study.

Zingiber officinale (ginger) has previously been recognized to have anthelmintic activity against *Dirofilaria immitis, Anisakis simplex, Schistosoma mansoni and Hymenolepis nana* (28). In this study, Z.*officinale* showed good anthelmintic activity against earthworms whereby the highest effect was expressed by ethanolic extracts. The ability of the extracts to cause paralysis and death was also dependent on increase of concentration similar to *E. cardamomum* and *C. longa*. These findings conform to studies done in other areas (24, 29). *Z. officinale* activity is associated with presence of flavonoids, terpenes, saponins and alkaloids (24).

There is limited information regarding the anthelmintic activity of *E. cardamomum*. This study has recorded a high anthelmintic activity for the plant where by the extracts of *E. cardamomum* had a high ability to cause paralysis and death to the *Apporrectodea longa* worms. This activity may be attributed to the presence of major phytochemicals alpha and beta pinene, 1,8 cineole, limonene, p-cymene, sabinene, terpinene linalool, geraniol, linalyl acetate and methyl eugenol. These phytochemicals have shown to have pharmacological effects like carminative, cardiac tonic, digestive, diuretic, expectorant, beneficial in asthma, bronchitis, haemorrhoids, renal and vesical calculi. Thus, the same phytochemicals possibly played role in anthelmintic activity.

Among the three plant extracts, *E. cardamomum* had a greater tendency to paralyse the worms early followed by Z. *officinale* and lastly *C. longa* in both water and ethanolic extracts while the activity increased with the increase in concentrations. The time to cause death was more pronounced by *Z. officinale* followed by *E. cardamomum* and lastly *C. longa* in water extracts while in ethanolic extracts, *Z. officinale* and *E. cardamomum* showed similar time to cause death and the difference was with *C. longa.* The increase of efficacy of all the extracts in time and concentration dependent manner was due to the fact that more active ingredients are being in high concentrated extract; also, as the contact time between the extracts and helminthes increased, the helminthes were exposed to death from paralysis.

Albendazole tablet was less potent compared to the plant extracts in both paralysis and death time except *C. longa.* This finding did not meet our expectation whereby albendazole was supposed to be more active than the extracts. This could be due to the fact that pure albendazole was not used which could have shown the expected results. The tablet is designed to pass through the physiological barriers in the gastrointestinal tract to release the drug from the excipients. However, this is not a guarantee as similar results may be obtained when the pure compound is used.

Generally, Z. *officinale* showed more potency to cause death of the round worms similar to high concentration of *C. longa* while *E. cardamomum* had more tendency to cause paralysis that the other two species.

4.1. Study Limitations

The specimens (*Apporrectodea longa*) which were used for the study could not be recognized in terms of age which may affect the results. However, shape and size were used to identify the suitability of specimen for the study. It worth to note that, *Caenorhabditis elegans* which is used as model for various studies involving nematodes was not available

hence earthworms were used as alternative as it has been employed in various studies. Despite these limitations the current study still gives a broader picture on the *In-vitro* efficacy of *Zingiberaceae* species available in the country.

5. Conclusion

Among the three plants *Z. officinale* was more effective in causing death of worms at early time while *E. cardamomum* was more effective for causing paralysis. *C. longa* was more effective to cause death and paralysis at high concentrations.

Recommendations

This study recommends further studies to carry out phytochemical test to detect organic compounds/active ingredients present in the studied Zingiberaceae species. I*n-vivo* studies on the commonly used plants of *Zingiberaceae* family for helminths affecting human beings are also warranted.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval to conduct this study was sought from the joint CUHAS/BMC Institutional Review Board (IRB). Permission to carry out the study was obtained from the multipurpose laboratory management at CUHAS.

Author's contributions

KJ participated in proposal development, data collection, and data analysis, manuscript drafting and submitting. MM participated in proposal development and laboratory works. DR participated in laboratory works, data analysis and manuscript revising and approval for publication.

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