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FORMULATION OF *CINNAMOMUM ZEYLANICUM* IMMEDIATE RELEASE TABLETS AS AN ANTHELMINTIC

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Anthelmintic, *Cinnamomum zeylanicum*, Immediate-release tablets, Helminthiasis, *Pheretima posthuma*, *Haemonchus contortus*

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ABSTRACT: Globally, a vast number of people are affected by helminth parasites. The emergence of anthelmintic resistance has paved the way for new leads into potential anthelmintic agents, which may be alternatives to already existing ones. The mode of presentation of herbal medicines affects patient compliance. Traditionally, *Cinnamomum zeylanicum* is used in the management of helminth infestations. This study sought to evaluate the anthelmintic activities of the crude ethanol extracts of *Cinnamomum zeylanicum* to authenticate its folkloric use and formulate a dosage form of the most efficacious extract. The leaf and bark powder obtained from the ethanol extract was subjected to phytochemical screening. *In-vitro* experiments were conducted on *Pheretima posthuma* and *Haemonchus contortus* using various concentrations of *Cinnamomum zeylanicum* leaf and bark extracts. Immediate-release tablets were successfully formulated from *Cinnamomum zeylanicum* bark extract. The formulated tablets passed the uniformity of weight, friability, disintegration, and hardness tests. The tablets were found to contain the right amount of the extract and produced good *in-vitro* drug release throughout the duration of the study. There was no change in the physicochemical properties of the tablets over a period of six months. *Cinnamomum zeylanicum* bark extract has been successfully formulated into an immediate-release tablet as an anthelmintic agent.

INTRODUCTION: Helminthiasis is a helminth infection that forms part of the most neglected tropical diseases affecting mankind. They are commonly found in the tropics and subtropics and mostly associated with individuals in developing countries [1]. Globally, over a billion people are infected with helminthes due to poor sanitation and poverty, with the most vulnerable being school-aged children and pregnant women [2].

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Categories of worms known to infest humans are cestodes (tapeworms), trematodes (flukes), and nematodes (roundworms), with high prevalence recorded in developing countries such as Ghana, Angola, and Nigeria among others [3].

Predominantly, they are known to cause severe losses in agriculture and are detrimental to human health. Currently, a number of worms are resistant to commonly used anthelmintic agents both in humans and livestock [4]. These drug-resistant helminthes include *Haemonchus contortus*, *Ascarislum bricoides*, *Ancylostoma duodenale* and *Necator americanus* [5]. The ripple effects of helminthiasis are nutrient malabsorption, impaired growth, and decreased productivity. Medicinal plants have been used since ancient times as

medicines in treating various conditions and this has led to knowledge accumulation over the years. Studies conducted have revealed that medicinal plants are used by almost 80 % of the world's population as first-line treatment in the management of diseases [6]. *Carica papaya*, *Voacanga africana*, *Rauwolfia vomitoria*, and *Occimum gratisimum* are some plants used in the management of helminth infections [7]. *Cinnamomum zeylanicum*, commonly known as cinnamon, is an evergreen plant that grows in the tropics and is mostly used as a condiment. Folklorically, *Cinnamomum zeylanicum* is used in the management of rheumatism, toothache, headaches, indigestion, and halitosis. The powdered leaves and bark are also used as tinctures in managing helminth infestations [8]. The bark of *Cinnamomum zeylanicum* is a potential anthelmintic agent against *Ascarissuum*, indicating its promising effect in combating other intestinal parasites [9]. Developing a suitable dosage form from the plant will enhance the accuracy of dosing, improve patient compliance and increase the scope of use of the plant material. This study seeks to evaluate the anthelmintic activities of the ethanol extracts of *Cinnamomum zeylanicum* leaf and bark and to formulate a suitable dosage form of the most effective extract.

MATERIALS AND METHODS:

Materials: Albendazole powder (Ernest Chemist Limited, Ghana), Ringers Lactate solution (Aculife Healthcare Pvt. Limited), 96 % Ethanol (GAPUMA Limited), Dimethyl sulfoxide (DMSO) (Sigma Aldrich), Magnesium stearate and Talc. All reagents were of analytical grade and used as received.

Collection and Preparation of Plant Materials: Barks and leaves of *Cinnamomum zeylanicum* were collected at the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, and authenticated by a botanist at the Department of Herbal Medicine, KNUST with voucher and specimen numbers KNUST/HM1/2018/B007 and KNUST/HM1/2015/L003 for *Cinnamomum zeylanicum* bark (CZB) and leaves (CZL) respectively. Plant materials were rid of foreign materials by washing and dried in a room for 7 days. The bark and leaves were powdered and weighed separately. The powdered samples were cold macerated for 72 h using 96% w/v ethanol and oven-dried at 40 °C over a period of 5 days.

Phytochemical Screening of CZ Extracts: The plant extracts were screened qualitatively for their secondary metabolites, which included tannins, saponins, coumarins, glycosides, triterpenoids, sterols, alkaloids, flavonoids using methods as described by Tyagi and Agarwal [10].

Collection of Worms: *Pheretim apostuma* was collected and authenticated at the Department of Theoretical and Applied Biology, KNUST. Worms measuring 4-6 cm and 0.1-0.2 cm in length and diameter respectively, were selected for the study. Ringer's lactate solution was used as a physiological solution in the experimental procedure. Ringer's lactate solution (700 mL) was used to wash the worms to get rid of the soil matter, after which they were kept in a bowl containing 500 mL physiological solution for survival. *Haemonchus contortus* was obtained at the Tamale abattoir from the gastrointestinal tract of cattle and kept in Ringer's lactate solution. The worms were identified and authenticated at the Veterinary Department, University of Development Studies, Tamale, Ghana.

***In-vitro* Anthelmintic Assay of *Pheretima posthuma*:** Preliminary anthelmintic assay was carried out as described by Husori *et al.* [11] Concentrations of 1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL, 16 mg/mL, and 32 mg/mL of the leaf and bark extracts were freshly prepared prior to the study using ringer's lactate as solvent. However, the leaf extract was dissolved before the addition of the ringer's lactate to produce 0.2% concentration of DMSO of the required volume. Three earthworms were placed in petri dishes containing 50 mL each of the above-prepared solutions of different concentrations.

Albendazole (10 mg/mL) was used as positive control. Ringer's lactate, as well as a combination of Ringer's lactate and 0.2% DMSO, were used as negative controls. Paralysis time of the worms was observed as the time when no movement of the worms could be observed except upon vigorous shaking. Death time was also concluded as the time when worms lose their motility with fading of body colours.

***In-vitro* Anthelmintic Assay of *Haemonchus contortus*:** Concentrations (0.015, 0.03, and 0.06 mg/mL) of the most effective plant extract, CZB, were prepared with ringer's lactate solution to obtain the minimum inhibitory concentration (MIC) of the extract against *Haemonchus contortus*. Active groups of three worms were transferred into petri dishes each containing 30 ml of the aforementioned concentrations of CZB extract. This was done in triplicates for each concentration. Albendazole (10 mg/mL) and ringer's lactate were used as positive and negative controls, respectively. Paralysis time of the worms was observed as the time when no movement of the worms could be observed except upon vigorous shaking.

Death time was also concluded as the time when worms lose their motility with fading of body colours [11, 12].

Subsequent studies were carried out on the most effective plant extract (CZB).

Ethical Clearance: All experimental animal protocols employed in the study conformed to the standards set by the Department of Pharmacology, KNUST Ethics Committee, and the guide for the care and use of laboratory animals, 8th Edition.

Experimental Animals: ICR mice purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon were maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi. The experimental animals were housed in stainless steel cages (34 × 47 × 18 cm) and acclimatized for seven days under laboratory conditions. Normal commercial pellet diet from (Agricare, Kumasi) and clean water were made available.

Acute Toxicity Studies: Acute toxicity of CZB was determined as described by Udeh *et al.* [13] Four groups of mice (n=5) were used in this study. The first three groups of mice were administered with 0.1, 1, and 5 g/kg of CZB extract, respectively. This was done orally and according to their body weight. The last group of mice (control) was administered with distilled water. Over a 24-hour period, the experimental animals were observed for mortality, changes in behavior, and physiological function.

Data Analysis: Data for *Pheretim aposthuma* and *Haemonchus contortus* were presented as mean ± S.E.M. (n=3). Two-way analysis of variance (ANOVA) was used in statistical analysis to determine the significant difference between the means for paralysis and death times, as shown **Fig. 1-3**. Microsoft office excel was also employed in the data analysis.

Formulation of Tablets: The wet granulation method as described by Momin *et al.,* [14] was used in formulating the tablets. Quantities of the active ingredient (CZB) and required excipients were weighed in **Table 1** and mixed in geometrical dilution with the exclusion of talc and magnesium stearate. A wet mass was formed using water as granulating fluid and screened with a 2360 µm mesh. The wet granules were dried at 60 ºC for an hour and screened with 1180 µm mesh. Flow properties of the granules were determined. Talc and magnesium stearate were added to the granules prior to compression.

TABLE 1: FORMULA FOR CZB TABLETS

Ingredients	Master Formula (mg)	Scaled Quantities (X 100) (g)
CZB	0.03	0.003
Hydroxypropyl methylcellulose (HPMC)	27.50	2.750
Lactose	522.47	52.247
Starch	27.50	2.750
Talc	5.50	0.550
Magnesium Stearate	5.50	0.550

Pre-formulation Studies: The flow properties of CZB granules were determined. The fixed height method was used to determine the angle of repose. Compressibility index and Hausner's ratio were also determined using bulk and tapped densities of the granules [15].

Evaluation of Formulated Tablets: Uniformity of weight and disintegration tests were carried out on tablets [16]. The hardness and friability of the tablets were determined [17]. Dimensional tests were carried out on the tablets, and the tensile strength was calculated using the formula:

$$\text{Tensile strength} = 2F/\pi Dt,$$

F (N) = diametrical tablet brake force
D (cm) = diameter of tablet
t (cm) = thickness of tablet

Drug Content Determination: Ten tablets were selected randomly. Each of the selected tablets was weighed, powdered, and dissolved in 0.1 M HCl. The resulting solution was filtered, after which the absorbance of the filtrate was determined at 295 nm.

***In-vitro* Dissolution Studies:** *In-vitro* dissolution profile of the tablets was determined at 37 ºC with 50 revolutions per minute stirring rate using the paddle method. Each vessel of the dissolution apparatus was filled with 0.1 M HCl, and a tablet was placed in each of the vessels. At specific time intervals, 5 ml of the sample was withdrawn from each vessel and replaced with the same volume from the dissolution medium to maintain sink conditions. The samples were filtered, and their respective absorbance determined at a wavelength of 295 nm with the UV visible spectrophotometer.

Stability Studies: Stability studies were carried out on the tablets at room and elevated temperatures. *In-vitro* dissolution studies, uniformity of drug content, tablet friability and hardness were carried out over a period of 6 months at an interval of three months using the methods described above.

RESULTS AND DISCUSSION:

Phytochemical Screening of CZ Extract: Earlier works by Oliveira *et al.*, [18] have established the significant role of some secondary metabolites (tannins, saponins, and triterpenoids) in eliciting anthelmintic properties. Tannins retard the energy production of parasites by uncoupling oxidative phosphorylation. As reported by Castañeda-Ramírez *et al.*, [19] the anthelmintic properties of a plant are maximized due to the synergistic activities of two or more secondary metabolites. The anthelmintic activities of CZB and CZL extracts are possible as a result of the presence of their secondary metabolites **Table 2**.

TABLE 2: PHYTOCHEMICAL CONSTITUENTS OF CZB AND CZL EXTRACTS

Secondary Metabolites	Inference	
	Bark	Leaves
Tannins	+	+
Saponins	+	+
Flavonoids	-	-
Sterols	-	+
Triterpenoids	+	+
Alkaloids	-	-
Coumarins	+	+
Glycosides	+	+

Key: + Present, - Absent

***In-vitro* Anthelmintic Assay:** *Pheretima postuma* was used in the preliminary anthelmintic assay due to the anatomical and physiological resemblance to intestinal parasites (roundworms) in human beings [20]. The extracts CZB and CZL recorded minimum inhibitory concentrations of 1 mg/mL and 2 mg/mL, respectively, against *Pheretima posthuma.*

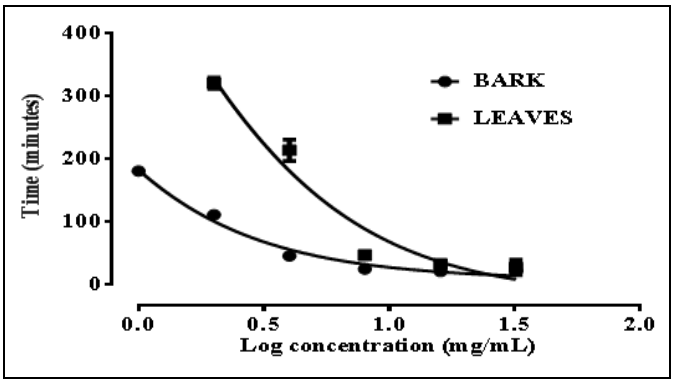

FIG. 1: PARALYSIS TIME OF *PHERETIMA POSTHUMA* WITH CZB AND CZL EXTRACTS. Values are Mean ± SEM (n=3)

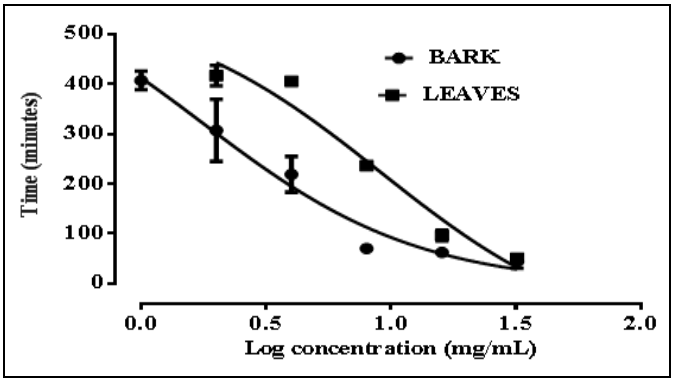

FIG. 2: DEATH TIME OF *PHERETIMA POSTHUMA* WITH CZB AND CZL EXTRACTS. Values are Mean ± SEM (n=3)

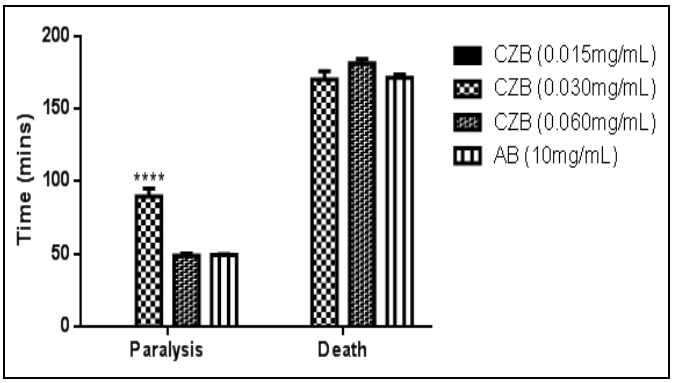

FIG. 3: PARALYSIS AND DEATH TIME OF *HAEMONCHUS CONTORTUS* USING CZB EXTRACTS. Values are mean ± S.E.M (n=3). ****P<0.0001 (Two-way ANOVA followed by Bonferroni's post hoc test)

Increasing concentrations of the extracts caused a reduction in times of paralysis and death of the worms. Paralysis and death of *Pheretima posthuma*

recorded EC_{50} values 0.135±0.156 and 1.760±0.574 respectively for CZB extract and 0.761±0.120 and 9.078±11.548 respectively for CZL extract. Higher efficacy was recorded for CZB extract against *Pheretima posthuma* **Fig. 1** and **2**. Paralysis and death of *Haemonchus contortus* were dose-dependent **Fig. 3**.

Acute Toxicity Studies: The tendency of a drug to elicit toxic effects within a 24-hour period when orally administered can be assessed by carrying out acute toxicity studies. No deaths nor changes in behavior and physiological functions were recorded for the experimental animals over the 24 h period of study. From this study, the toxic dose of CZB extract is above 5 g/kg body weight, conforming to a similar study conducted by Ahmad *et al.,* [21] on the safety of the aqueous extract of CZB.

Pre-formulation Studies: The Hausner's ratio, Carr's index, and angle of repose of the granules exhibited fair flowability **Table 3,** indicating its suitability for tablet compression.

TABLE 3: FLOW PROPERTIES OF POWDERED CZB CRUDE EXTRACTS

Hausner's Ratio	Carr's Index	Angle of Repose	Comment
1.21±0.01	18.40±0.08	38.50±0.15	Fair flowability

Formulation of Tablets: CZB extract was successfully formulated into immediate-release tablets with an average weight of 567 mg.

Evaluation of Tablets:

Uniformity of Weight: As stated in the British Pharmacopoeia [16] none of the tablets should deviate by ± 5% and not more than two of the tablets should deviate by ± 10% of the mean weight of the tablets. All the formulated tablets were within range Table 4. This shows that the ingredients used were evenly distributed in the tablets.

Disintegration Test: To facilitate drug dissolution and absorption, tablets need to break down into tiny fragments within a specified period of time. The disintegration of uncoated tablets should not exceed fifteen minutes [17]. All formulated tablets disintegrated within 15 min **Table 4**.

Hardness and Friability of Tablets: Tablet hardness, tensile strength, and friability tests determine the ability of tablets to tolerate the stress of handling, packaging and shipping. The hardness, friability and tensile strength of the formulated tablets were within specifications [18, 22] **Table 4**. This indicates the ability of the tablets to withstand abrasion during handling and transportation.

Drug Content: To ensure the presence of an active ingredient in the right proportion in a tablet, the drug content determination is carried out. All tablets complied with BP specifications **Table 4**. This shows that each tablet has a uniform distribution of the active ingredient and will elicit the desired effect when administered.

Uniformity of Dimension Test: Uniformity of thickness and diameter of tablets are important in ensuring tablet size uniformity. The diameter and thickness of tablets should be below ±3 % and ±5 %, respectively, of their average[18]. The thickness and diameter of the formulated tablets were satisfactory **Table 4**.

TABLE 4: SUMMARY OF QUALITY CONTROL TESTS ON CZB TABLETS

Parameter	Average value	Inference
Uniformity of weight	0.567±0.056	Passed
Hardness (kg/F)	4.47±0.032	Passed
Disintegration (min)	6.07±0.617	Passed
Friability (%)	0.35	Passed
Drug content (%)	97.00±0.39	Passed
Diameter (mm)	13.19±0.24	Passed
Thickness (mm)	3.53±0.10	Passed
Tensile strength (N/cm^2)	60.04±0.22	Passed

***In-vitro* Dissolution Studies:** A drug is deemed effective if it is first released from the dosage form and dissolved in the gastrointestinal fluid before systemic absorption. For non-modified release dosage form, at 45 min, not less than 70 % of the drug should be released [17]. All tablets were within range **Fig. 4,** indicating a good drug release profile.

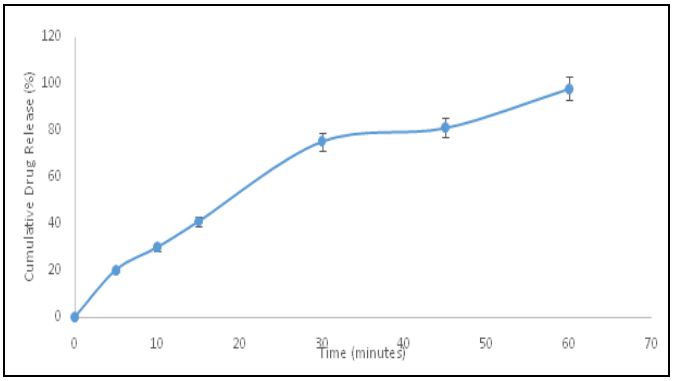

FIG. 4: *IN-VITRO* DISSOLUTION PROFILE OF CZB TABLETS

Stability Studies: Tablets showed no significant changes in hardness, friability, and *in vitro* drug release over a 6 months period at 27 °C and 40 °C **Table 5, Fig. 5** and **6**. Thus the tablets maintained integrity throughout the study period and will elicit the desired response when stored at these temperatures over a period of 6 months.

TABLE 5: SUMMARY OF STABILITY STUDIES

Parameter	Time of test	Average value		Inference
		27 °C	40 °C	
Hardness (kg/F)	At 3 months	4.42±0.067	4.38 ±0.25	Passed
	At 6 months	4.37±0.053	4.32 ±0.13	Passed
Friability (%)	At 3 months	0.39	0.45	Passed
	At 6 months	0.43	0.48	Passed
Uniformity of drug content (%)	At 3 months	96.46±0.16	95.07±0.09	Passed
	At 6 months	95.35±0.31	93.00±0.27	Passed

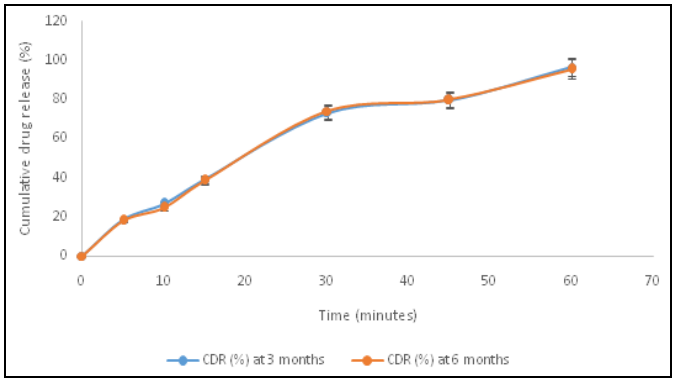

FIG. 5: *IN-VITRO* DISSOLUTION PROFILE OF CZB TABLETS (27 °C)

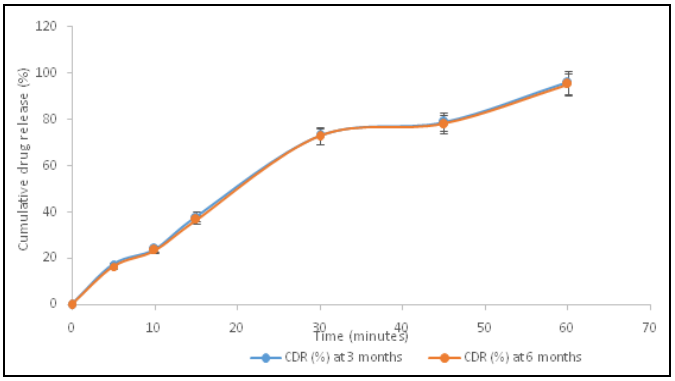

FIG. 6: *IN-VITRO* DISSOLUTION PROFILE OF CZB TABLETS (40 °C)

CONCLUSION: The ethanolic leaf and bark extracts of *Cinnamomum zeylanicum* have anthelmintic activities against *Pheretima posthuma* with the bark recording the highest efficacy. *Cinnamomum zeylanicum* ethanolic bark extract has anthelmintic activity against *Haemonchus contortus* and was successfully formulated into immediate-release tablets.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest with regards to the publication of this paper.

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