

A REPORT ON A POTENT ANTHELMINTIC AGENT - UNBAKED *COFFEE ARABICA* BEAN EXTRACTS

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ABSTRACT

Objective: Evaluation of anthelmintic activity of *Coffee arabica* unbaked bean extracts.

Methods: *C. arabica* is most commonly found in India and possess major economic value, as is one of the common beverage consumed worldwide. Once ripe, coffee berries are picked, processed ground and brewed to create consumable coffee. During coffee processing, coffee berry pulp was produced as waste which may contain large amount of phytochemicals. Moreover, ripe coffee bean before subjecting to processing and brewing, it contains many potential secondary metabolites with various therapeutic applications. However, these potential molecules are lost during processing. Helminthes infections have devastating impact on humans from the time immemorial. Helminthes are one of the most common infectious agents mainly in developing countries. *Pherithema posthuma* is a commonly known helminth used as model organism, because of identical physiology with *Ascaris*. In this connection the anthelmintic property of *Coffee arabica* bean extracts were evaluated against *Pherithema posthuma* as an experimental helminthes model. Albendazole was used as the standard reference.

Results: Among the various concentrations of chloroform extract tested, 200 µg/ml showed efficient anthelmintic activity and among all the concentrations of ethanolic extract tested, 200 µg/ml showed significant results. This investigation revealed that chloroform extract of *C. arabica* proved to be significant anthelmintic activity against *Pheretima posthuma* when compared to aqueous extract, ethanolic extract and the standard drug.

Conclusion: This investigation reports a new potent anthelmintic agent that can be used as a potent drug for the treatment of helminthes infection.

Keywords: *Coffee Arabica*; Anthelmintic activity; *Pheretima posthuma*; Ethanolic extract; Chloroform extract.

INTRODUCTION

Helminth infections are very common in humans affecting approximately 3 million people, distressing a huge population of the world. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections [1]. Although the helminthes infections are generally restricted to tropical areas they are a reason for deleterious health hazards. These helminthes are parasitic in nature which live and feed on hosts receiving nourishment and protection and in return causing serious disruption of hosts nutrient absorption system and normal flora of the body. Parasitic diseases are the significant cause of increased death rate affecting mainly the population in endemic area [2]. It can also play an important role in the prevalence of diseases like under nourishment, anemia, eosinophilia and pneumonia etc. [3].

Helminthes are very common inhabitants in human dwelling. They can find an easy entrance to the human body or the cattle through various medium like food, water, feces etc. Although there are many artificial and natural compounds commercially available to kill these parasites, need for new anthelmintic drugs is always on the purge. These helminthes have developed antibiotic resistance mechanism [4] and therefore old drugs are no more effective, posing hindrance in treatment of helminthes diseases. Thus plant based anthelmintics agents are preferred over modern synthetic medicines, plant based medicines act by expelling parasitic helminthes worms from the body or by either stunning growth or killing them [1, 4].

Coffea arabica (family - Rubiaceae) being one of the important genus of *Coffea* species, which is reported to be originated from southwestern highlands of Ethiopia. *Coffea arabica* is believed to be the first species of coffee to be cultivated, being grown in southwest Arabia for well over 1,000 years. *C. arabica* is an important commercial plant known to grown all over the world. Reports revealed that *C. arabica* contains less caffeine 0.8-2.5% than other species of coffee like Robusta 4.0% [5]. *C. arabica* baked bean powder is used to prepare various beverages, and as an essence in chocolates and ice creams that are consumed in our day to day life.

However, *C. arabica* possesses an array of medicinal properties. Most of its therapeutic properties are still unexplored. Generally *C. arabica* grows up to 6-12" in height; leaves of this plant are opposite, simple elliptic-ovate to oblong broad, glossy dark green. It blooms with axillary clustered white flowers of 10-15 mm in diameter. *C. arabica* fruits are known as a "coffee cherries", that are about 10-15 mm in diameter, during maturation their color appears yellow, which then changes light red and matured cherries appear deep red in color. Matured bean encapsulates two coffee beans.

It is used widely all over the world as a commercial beverage and literature survey revealed many reports regarding the pharmacological properties of processed coffee seeds consumed daily as a beverage since ancient times. Coffee is known to be an activator for the CNS system [6]. However, anthelmintic activity of unbaked *Coffee arabica* seed embryo extract has not yet been scientifically reported. In the present investigation the unbaked coffee beans extracts were assessed for their anthelmintic activity using *Pheretima posthuma*. Extracts of *Coffee arabica* are likely to contain potential phytochemicals which have not yet been characterized and can prove as potent drug against helminthes.

MATERIALS AND METHODS

Drugs and chemicals

Albendazole (Ranbaxy lab) was used as a standard drug during the experimental protocol. Chloroform and ethanol (SD Fine Chemicals Ltd., Mumbai).

Plant material

The *Coffee arabica* unbaked beans was procured from Coorg region (Karnataka) in November 2012. The plant and plant material were identified and authenticated in Department of Botany, Bangalore. The plant seeds were shade dried, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction using different solvents system.

Preparation of extract

Coarsely powdered material of *Coffea arabica* unbaked beans 250 g was subjected for cold extraction using chloroform as solvent for 7 days in dark on rotary shaker. The extract was double filtered by using muslin cloth and Whatmann no.1 filter paper. The chloroform extract was dried at 37 °C and used as a powder. The total extract was found to be 5 g per 250 g of powdered bean material. After chloroform extraction, the chloroform extracted beans were air dried till no residue of chloroform solvent was remaining. The marc of beans was then subjected for sequential ethanol extraction using 1000 ml of absolute ethanol solvent for 7 days. The extract was double filtered by using muslin cloth and Whatmann no.1 filter paper. The extract was found to be 3.46 g. After ethanol extraction, the post dried ethanol extracted beans marc was further sequentially extracted using double distilled water with 5% ethanol for next 7 days. The extract was double filtered by using muslin cloth and Whatmann no.1 filter paper. The total extract was found to be 7 g.

Experimental animals

Adult earthworms (*Pheretima posthuma*), were used to evaluate anthelmintic activity *in vitro*. Earthworms were collected from Biotechnology Centre, Bangalore, Karnataka. The average size of earthworm was 6-8 cm. Helminths were authenticated in the Department of Biotechnology, Biocentre, Bangalore.

Anthelmintic activity

The anthelmintic activity was carried out as per the method reported of Ajaiyeoba et al [7]. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) as it possesses similar anatomical and physiological characteristics as intestinal roundworm parasites of human beings. Therefore, they were suitable for preliminary evaluation of anthelmintic activity [8-10]. Test samples of the *Coffea arabica* beans differential solvent extracts *viz.*, chloroform extract, ethanol extract and aqueous extract was prepared at different concentrations, 50, 100 and 200 µg/ml in 5% DMSO in 10 ml normal saline. Albendazole 5 mg in 5% DMSO in 10 ml normal saline was used as standard reference. Helminthes were divided into 11 groups with 3 animals in each group. Group I was maintained as control with 5% DMSO. Group II, III and IV animals were released into petri plates containing 50, 100 and 200 µg/ml of aqueous extract respectively. Group V, VI and VII animals were released into petri plates containing 50, 100 and 200 µg/ml of chloroform extract respectively. Similarly, Group VIII, IX and X animals were released into petri plates containing 50, 100 and 200 µg/ml of ethanol extract respectively. Group XI animals were released into plates containing 5 mg/ml of standard drug Albendazole. This procedure was adopted for testing the worms in each of the three solvent extracts with their respective three concentrations. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (55 °C). Death was confirmed with change in body colour with no body movement. All the results were shown in Figure 1 and expressed as a mean ± SEM of three worms in each group.

Statistical analysis

The data of anthelmintic evaluations were expressed as mean ± S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P < 0.01$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

RESULTS AND DISCUSSION

In the present investigation, the unbaked Coffee Arabica bean extracts *viz.*, chloroform, ethanol and aqueous extracts were used for evaluation of their anthelmintic property against *Pheretima posthuma*. From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death for all worms. Chloroform extract showed the most potent

anthelmintic activity in dose-dependent manner giving shortest time of paralysis (3.45 ± 0.16) and death (7.06 ± 0.05) with 200 µg/ml of saline concentration, for all of worms.

The anthelmintic activity showed potential decrease as the chloroform extract concentration was lowered from 200 µg/ml saline to 100 µg/ml saline and then to 50 µg/ml with increase in saline volume, subsequently increase in the worms time of paralysis (8.73 ± 0.17) and death (11.14 ± 0.16).

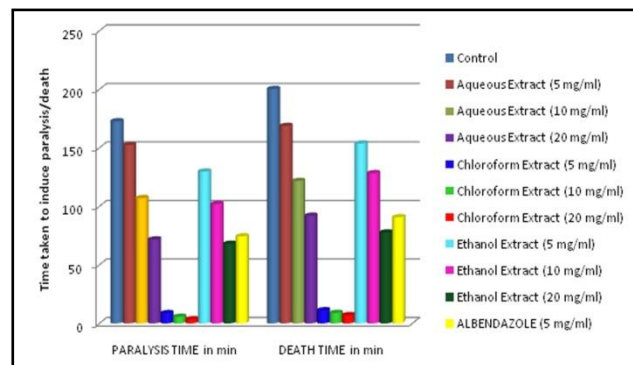


Figure 1: Bar chart illustrating the comparative *in vitro* anthelmintic effect of different concentrations of chloroform, ethanol and aqueous extracts of *Coffea arabica*.

Ethanol extract exhibited lesser potent anthelmintic activity at concentration 200 µg/ml, when compared with 200 µg/ml of chloroform extract. There was increase in the time of paralysis (68.0 ± 3.79) and death (77.67 ± 2.85) as the dosage concentration was reduced to 100 µg/ml and further increased time of paralysis (129.67 ± 2.4) and death (153.67 ± 2.91) at 50 µg/ml saline.

All the concentrations of ethanol extract showed lesser anthelmintic potential when compared to chloroform extract. The aqueous extract showed minimal anthelmintic effect when compared to chloroform and ethanol extract prepared with same concentrations of 200 µg/ml, 100 µg/ml and 50 µg/ml exhibiting comparatively maximum time of paralysis (152.33 ± 4.33) and death (168.67 ± 5.55) at each of its concentrations, similar to that of control.

Evaluation of anthelmintic activity was compared with reference standard albendazole. Normal 0.9% saline was used as control (Figure 1). Preliminary phytochemical screening of extract revealed the presence of glycosides and phenolic compounds. From the above results, it is concluded that unbaked *Coffea arabica* seeds used as an important commercial beverage before processing possess effective anthelmintic activity.

Many investigator have report the anthelmintic activity of various medicinal plants *viz.*, *Calotropis procera*, *Buchholzia coriacea* and *Gynandropsis gynandra*, *Gynura angulosa*, *Capparis spinosa* and *Epiprinus mallotiformis* against different types helminth parasites that includes *Ascaridia galli*, *Trichinella spiralis* [7-20]. Yashwanth et al., have reported that *Manilkara zapota* seed embryo extract also possess potent anthelmintic property [21]. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this seed as anthelmintic agent. The *Coffea arabica* may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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