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# Comparitive Invitro Antihelmintic Activity of *Electeria cardamom* and *Amomum subulatum* against Pheritima Posthuma

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**Abstract** **Original Research Article**

*Elettaria cardamomum* and *Amomum subulatum* fruits were easily available and commonly used in our day –to-day life. The present study aimed at the in-vitro comparative study of anthelmintic activity of aqueous extracts of fruits of *Elettaria cardamomum, Amomum subulatum* and mixed. The fruits were extracted separately with distilled water by maceration method .The various concentrations of the extract (50,100, 200mg/ml) respectively were screened for their anthelmintic activity using Pheritima posthuma. The activity was comparable with the standard drug albendazole. When the concentrations of the extract are increased, a gradual increase in anthelmintic activity is observed. The study involved the determination of time of paralysis (P) and time of death (D) of the worms. Aqueous extract of *Elettaria cardamomum, Amomum subulatum* and mixed showed anthelmintic activity but mixed extract was more efficient anthelmintic activity against Indian earthworms. The data were found statistically significant by using one way ANOVA (P< 0.001). Further studies are suggested to isolate the active principles responsible for the activity.

**Keywords:** Elettaria cardamomum, Amomum subulatum, Pheretima posthuma, Anthelmintic activity, Albendazole.



## INTRODUCTION

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and has impact on global economic factor (Kumar, 2014). The worms which causes helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics (Abongwa, 2017). There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are given according to their shapes. The major organs which get affected in helminthiasis are stomach and intestine and major symptoms of sever helminthiasis include diarrhea, abdominal pain, general malaise and impaired cognitive development. Chronic helminthiasis by hook worm lead to intestinal bleeding and anemia (Hedley, 2015). Pheretima is a genus of earthworms. *Pheretima posthuma* are long cylindrical shaped worms having length of 15-30cm. they are mostly found in moist soil and responsible for vegetables and humus. Their life span is 3 to 10 years (Kutschera, 2010) [1].

Cardamom, popularly known as 'Queen of spices' is the tall perennial herbaceous plant, *Elettaria cardamomum* (L.) Maton, belonging to the family Zingiberaceae. The genus Elettaria is one of the few compact and small natural groups, whose origin is evergreen rainforests of South India and Sri Lanka from where it spread to other tropical countries (Purseglove *et al*. 1981). Cardamom cultivation in India is mostly concentrated in the Western Ghats of South India. It is also cultivated in Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Cambodia and Papua New Guinea. India is reported to have rich genetic diversity in cardamom (Mayne1951; Abraham and Tulasidas 1958) [2].

*Amomum subulatum* Roxb (Zingiberaceae) is commonly known as greater cardamom. It is native to the Eastern Himalayan region particularly Bhutan, Nepal, and India. Also found in Srilanka (Anonymous, 1987). Major producers are Nepal (52%), India (37%) and Bhutan (11%) (Gautam, *et al*., 2016) (Gopal, *et al*., 2012) [3].

**Fig-1: *Elettaria Cardamomum* and *Amomum subulatum* fruits**

## MATERIALS AND METHODS

**Collection of plant material**

The fruits of *Elettaria cardamomum* and *Amomum subulatum* was identified and purchased from local market of Nuzvid.

**Preparation of aqueous extract (Maceration method) [4]**

The 500gm of dried fruits of *Elettaria cardamomum* and *Amomum subulatum* was collected powdered to get a coarse powder and was kept for maceration with 1000 ml of distilled water for 7 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used.

**Preliminary phytochemical screening [5-7]**

The preliminary phytochemical investigation was carried out with aqueous extracts of *Elettaria cardamomum, Amomum subulatum* and mixed fruits for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

**Test organism [8]**

Indian adult earthworms (*Pheretima posthuma)* were used during the experiment. The earthworms were collected from a local supplier. Worms were washed with normal saline to remove all fecal matter .The earthworms of 8-10 centimeter (cm) in length and 0.2 -0.5 cm width were used for all the experiment protocol. Ready availability, anatomical and physiological resemblance of (*Pheretima posthuma)* made it to be used initially for *in-vitro* evaluation of anthelmintic activity. Time for paralysis was noted either when any movement could not be observed except when the worms where shaken vigorously. Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

**Evaluation of Antihelmintic activity**

The antihelmintic activity was evaluated on adult Indian earthworm. The earthworms were randomly chosen and divided into five groups having five earthworms in each as follows:

**Group I:** Control Group
**Group II:** Standard Group – Albendazole [9] -50,100, 200 mg/ml
**Group III:** Test-I -Aqueous fruit extract of *Elettaria cardamomum* [ECAE- 50,100, 200 mg/ml]
**Group IV:** Test -II - Aqueous fruit extract of *Amomum subulatum* [ASAE- 50,100, 200 mg/ml]
**Group V:** Test –III- Mixed fruit aqueous extract of *Elettaria cardamomum* and *Amomum subulatum* [MFAE- 50,100, 200 mg/ml]

Observations were made for the time taken by worms to paralyze and death was observed. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Death was ascertained by applying external stimuli which stimulate and induce movements in worms as well as fade of the body color was noted.

## STATISTICAL ANALYSIS

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between control group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

## RESULTS AND DISCUSSION

In this study, we found that aqueous fruit extract of *Elettaria cardamomum, Amomum subulatum* and mixed possess the following chemical constituents (Table 1).

**Table-1: Phytochemical screening of ECAE, ASAE & MFAE**

| Phytochemical constituents | Aqueous fruit extract of *Elettaria cardamomum* | Aqueous fruit extract of *Amomum subulatum* | Aqueous mixed fruit extract |
|---|---|---|---|
| Alkaloids | + | + | + |
| Carbohydrates | + | + | + |
| Flavonoids | + | + | + |
| Phenols | _ | _ | _ |
| Saponins | + | + | + |
| Terpenoids | _ | _ | _ |
| Sterols | _ | _ | _ |
| Tannins | + | + | + |
| Proteins | _ | _ | _ |
| Amino acids | _ | _ | _ |
| Glycosides | + | + | + |
| Fixed oils and fatty acids | + | + | + |

+ indicate the compulsory present and – indicate the absent.
ECAE- *Elettaria cardamomum* aqueous fruit extract.
ASAE - *Amomum subulatum* aqueous fruit extract.
MFAE- Mixed fruit aqueous extract.

**Antihelmintic activity**

The Aqueous extract of *Elettaria cardamomum, Amomum subulatum* and mixed produced a significant antihelmintic activity in dose dependent manner as shown in below table.

**Table-2: Anthelmintic activity of *Elettaria cardamomum, Amomum subulatum* and mixed extracts & Standard drug on earth worm**

| Groups | Concentration | Time Taken in minutes | |
|---|---|---|---|
| | | Paralysis (P) | Death (D) |
| **Control** | | - | - |
| **Standard** | **50mg/ml** | 31.6±0.93 | 54.8±0.37 |
| | **100mg/ml** | 28.2±0.37 | 49.4±0.51 |
| | **200mg/ml** | 17±0.32 | 30.2±0.37 |
| **Test-I [ECAE]** | **50mg/ml** | 27.4±0.4 | 55.2±0.37 |
| | **100mg/ml** | 23.4±0.51 | 41.2±0.37 |
| | **200mg/ml** | 20.2±0.37 | 30.4±0.51 |
| **Test-II[ASAE]** | **50mg/ml** | 24.4±0.51 | 50±0.32 |
| | **100mg/ml** | 19.8±0.37 | 44.4±0.51 |
| | **200mg/ml** | 15±0.45 | 34.2±0.37 |
| **Test-III [MFAE]** | **50mg/ml** | 19.2±0.58 | 35.8±0.37 |
| | **100mg/ml** | 14.4±0.51 | 30.8±0.37 |
| | **200mg/ml** | 11.4±0.51 | 25.6±0.51 |

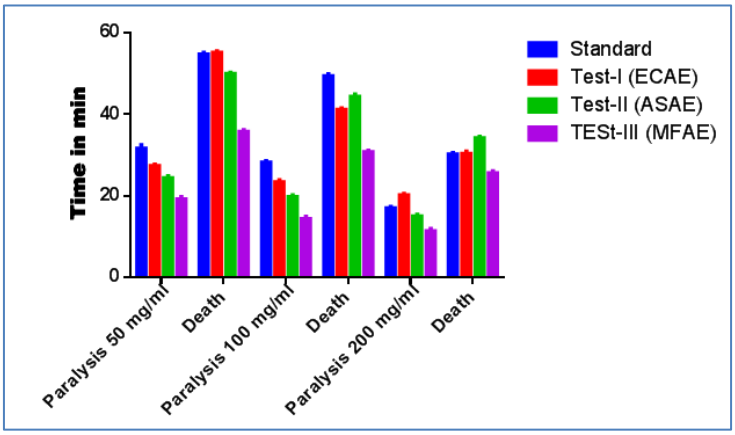


**Fig-2: Anthelmintic activity of standard, ECAE, ASAE & MFAE. Values are expressed as Mean ± SEM, P < 0.001.**

## CONCLUSION

In the present investigation, *Elettaria cardamomum, Amomum subulatum* and mixed fruits posses the presence of alkaloids, carbohydrates, saponins, tannins, Flavonoids and glycosides. Tannins are chemically polyphenolic compound and where shown to produce anthelmintic activities and reported the effect of tannin can bind to free proteins in gastro intestinal tract of host animal or glycoproteins on the cuticle of parasite and may cause death.

These facts suggest that tannins present in the aqueous fruit extract of *Elettaria cardamomum, Amomum subulatum* and mixed showed the antihelmintic effect by above mentioned mechanisms. From the result shown in table-2 aqueous fruit extract of *Elettaria cardamomum, Amomum subulatum* and mixed showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death. The aqueous fruit extract of *Elettaria cardamomum, Amomum subulatum* and mixed at normal concentration i.e. 50 mg/ml to higher concentration i.e. 200mg/ml showed good anthelmintic activity and this is compared with effect produced by reference standard drug albendazole. The study finally concluded aqueous mixed fruit extract i.e. combination of *Elettaria cardamomum, Amomum subulatum* showed marked and potent anthelmintic activity than the aqueous extract and standard drug albendazole.

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