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COMPARATIVE *IN VITRO* ANTHELMINTIC ACTIVITY OF THREE PLANTS FROM THE AMARANTHACEAE FAMILY

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Abstract – The three plants presented in this study are *Amaranthus spinosus, Amaranthus caudatus* and *Amaranthus viridis* L. They belong to the Amaranthaceae family and are traditionally used as vermicides. Our aim was to investigate the anthelmintic activity of the three plants using earthworms (*Pheretima posthuma*). Methanol extracts of the three plants at different concentrations (10, 20, 40, 60, 80, 100 mg/ml) showed dose-dependent vermicidal activities. At concentrations of 80 and 100 mg/ml all three plant extracts caused paralysis (8.18, 5, 12.16, 5.75, 10.2, 7.8 min) and death (14.65, 9.12, 18.6, 8.5, 18.6, 12.7 min), respectively. Piperazine was used as a reference standard at a concentration of 10 mg/ml. Our study found that the three plants possess potent anthelmintic activity when compared to Piperazine.

Keywords: Amaranthus spinosus, Amaranthus caudatus, Amaranthus viridis, anthelmintic activity, Piperazine.

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INTRODUCTION

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Despite this prevalence of parasitic infections, the research on the anthelmintic drug is sparse. According to the WHO, only a few drugs are used in treatment of helminthes in humans. Anthelmintics from natural sources could play a key role in the treatment of these parasite infections. In view of this, attempts have been made to study the anthelmintic activity of traditional medicinal plants.

For our studies, we used *Amaranthus spinosus, Amaranthus caudatus* and *Amaranthus viridis* L that belong to the Amaranthaceae family and are traditionally used as vermifuge drugs (Fajimi and Taiwo, 2005; Khare, 2007; Anonymous, 1988; Agra et al., 2007.

A. spinosus Linn. (*Amaranthaceae*), commonly known as "Mullatotakura" in Telugu, is used as a laxative (Vaidyaratanam PS Varier's. 1996; William D'ymock. 1976., The roots are regarded as a highly specific colic remedy by Hindu physicians (Sivarajan, 1994) and in Madagascar they are used as a laxative (Kirtikar and Basu, 1987). The plant is also used traditionally applied as a diuretic, antidiabetic, antipyretic, anti-snake venom, antileprotic and anti-gonorrheal drug (Vaidyaratanam PS Varier's. 1996; Kirtikar and Basu, 1987). Some tribes in India use *Amaranthus spinosus* to induce abortion (Grubben and Denton, 2004). The *A. spinosus* is noted for its anti-inflammatory properties (Olumayokun et al., 2004), effect on hematology (Olufemi et al., 2003), immunomodulatory activity (Tatiya et al.,

2007), anti malarial activity (Hilou et al., 2006), antiandrogenic activity (Murgan et al., 1993), effects on biochemical changes in the epididymis (Murgan et al., 1993), anti-diabetic, anti-hyperlipidemic and spermatogenic activity (Sangameswaran and Jayakar, 2008).

Amaranthus caudatus Linn., (Amaranthaceae) is commonly known as "Peddathotakura" in Telugu. *A. caudatus* is traditionally used to cure kidney stones, leprosy, fever, piles (Vanila et al., 2008), as a "blood purifier", diuretic and astringent, (Khare, 2007). In Southeastern Ethiopia the seeds of *A. caudatus are* used in amoebiasis, jaundice and kidney diseases (Haile et al., 2008; Sushil et al., 2003). In India *A. caudatus* is traditionally used to cure kidney stones, leprosy, fever, piles (Vanila et al., 2008). In Senegal the roots are boiled with honey and used as a laxative for infants. The leaf has also been used as tea for relieving pulmonary conditions (Anonymous, 1988). In South Africa the leaf is used as an abortifacient (Watt, 1962; Yusuf et al., 1994). Isolated antimicrobial peptides (Broekaert et al., 1992), triterpenoid saponins (Rastrelli et al., 1998), and amaranthin (Rinderle et al., 1989) from *A. caudatus* seeds showed a cholesterol lowering effect and an *in vitro* antioxidant effect and inhibition of alpha amylase (Plate and Areas, 2007; Filomena et al., 2005). The amaranth seed oil is a nutracetical resource from Ecuadorian flora (Bruni et al., 2001).

Amaranthus viridis L. (Amaranthaceae) is commonly called "Chilaka Thota-Kura" in Telugu. In Nepal, an infusion of powdered seeds of *A. viridis* is used for stomach problems and in pregnant women to alleviate labor pains (Mark, 2003). The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes (Eduardo and Quisumbing, 1951). Other traditional uses are based on their apparent antipyretic, diuretic, antirheumatic, antiulcer, analgesic, antiemetic, laxative, appetite stimulatory, antileprotic properties and for treating respiratory problems, eye ailments and asthma (Kirithikar and Basu, 1986; Hassan Sher and Khan, 2006; Quershi et al., 2008; Muhammad Ejaz Ul Islam Dar, 2003; Muhammad, 2000; Muhammad and Amusa, 2005)

MATERIALS AND METHODS

Collection of Plant Material and Extraction

Fresh *A. spinosus, A. caudatus,* and *A. viridis* plants were collected from Chickballapur and were authenticated by Dr. Rajan from the Department of Botany, Government Arts College, Ootcamund, Tamilnadu. A voucher specimen (SKVCP 11, 12 and 13) was deposited in the college herbarium. The whole plant was shade-dried and coarsely powdered. The coarse powder was extracted with methanol using the soxhlet apparatus. The extract was concentrated to dryness in vacuum.

Phytochemical screening

The methanol extracts of three plants were screened for the presence of various phytoconstituents such as steroids, alkaloids, terpenoids, glycosides, flavonoids, phenolic compounds, and carbohydrates (Kokate, 1986).

Animals

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. Earthworms 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

Drugs and Chemicals

Piperazine citrate (Glaxo Smithkline), methanol (Karnataka chemicals) was used during experimental protocol.

Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. (2001) with minor modifications. The assay was performed on the adult Indian earthworm *Pheretima posthuma* due to its

anatomical and physiological resemblance to the human intestinal roundworm parasite (Vidyarthi, 1967; Chatterjee, 1967). Due to their ready availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (Sollmann, 1918; Jain et al., 1972; Dash et al., 2002).

The earthworms were divided into different groups, each group containing six worms. Fifty ml formulations containing four different concentrations of methanolic extracts of *A. spinosus, A. caudatus* and *A. viridis* (10, 20, 50, 80 and 100 mg/ml in distilled water) were prepared. The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The times of death of the worms were recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50°C). Piperazine citrate (10mg/ml) was used as reference standard while water served as a control.

RESULTS AND DISCUSSION

By preliminary phytochemical screening it was found that all the three plants' extracts contain flavonoids, glycosides, steroids, alkaloids, phenolic compounds, carbohydrates, proteins and tannins.

The earthworm selected for the antihelmintic activity was most sensitive to the methanol extract of *A. spinosus* as can be seen in Fig. 1. The graph revealed dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. At 60, 80 and 100 mg/ml concentrations paralysis was observed respectively at 17.6, 8.18 and 5 min and death at 24.03, 14.65 and 9.12 min post-exposure.

The methanol extracts of *A. caudatus* and *A.viridis* also exhibited dose-dependent anthelmintic activities that caused paralysis at 19.21, 14.33 min (at 60 mg/ml); 12.16, 10.2 min (at 80 mg/ml);

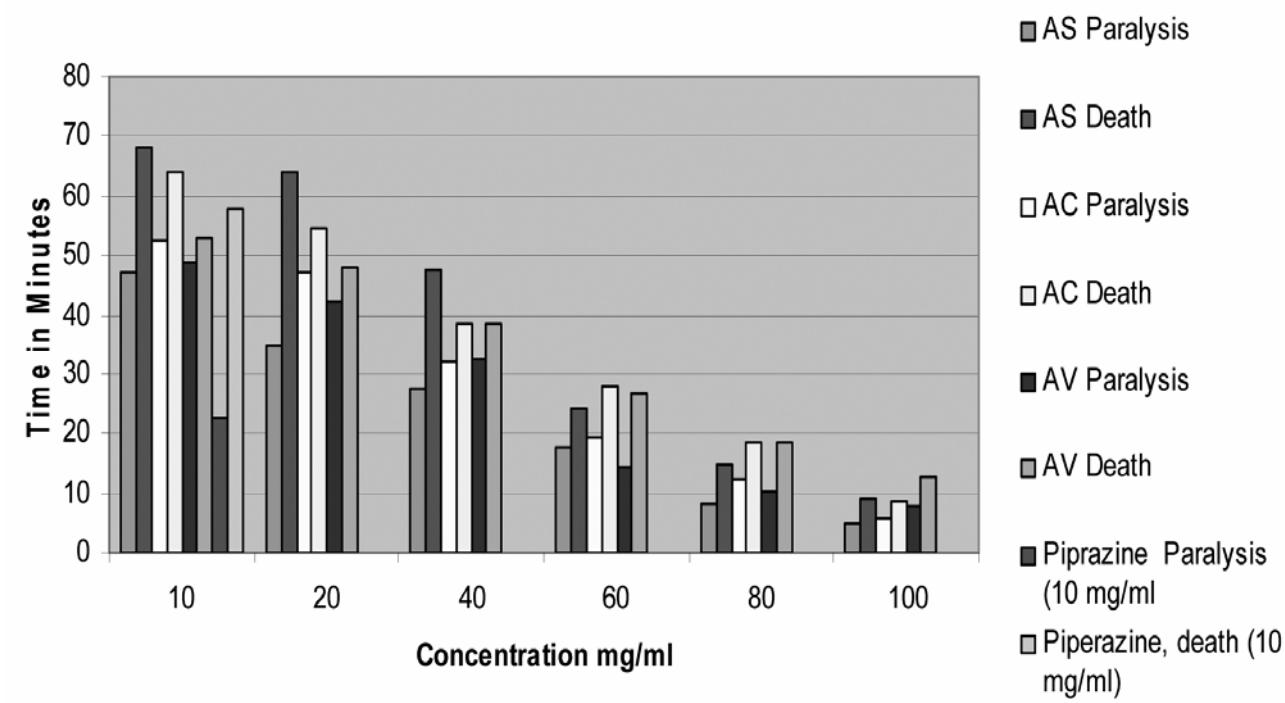

Figure 1. *In vitro* anthelmintic activity of three plants belonging to family Amaranthaceae

and 5.75, 7.8 min (at 100 mg/ml), and death at 27.7, 26.6 min (at 60 mg/ml); 18.6, 18.6 min (80 mg/ml); and 8.5, 12.7 min (100 mg/ml) post-treatment (Fig. 1). The earthworms were more sensitive to the extracts of *A. spinosus*, *A. caudatus,* and *A.viridis* at 60, 80 and 100 mg/ml concentrations as compared to the reference drug piperazine citrate (10 mg/ml). All the three plants' methanol extracts were more effective in causing the death of the worms as well as promoting paralysis.

Most worm expellers like piperazine citrate cause paralysis of the worms so that they are expelled in the feces. The methanol extracts of the three plants not only demonstrated this property but also killed the worms.

The anthelmintic activity of the methanol extracts of *A. spinosus*, *A. caudatus* and *A. viridis* may be due to the presence of polyphenolic compounds (Bate-Smith, 1962). The wormicidal activity of the methanol extract as described herein against earthworms suggests that it could be effective against parasitic infections of humans.

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