



Anthelmintic activity of methanol extract of *Amaranthus caudatus* Linn.

Bagepalli Srinivasa Ashok Kumar^{1,*}, Kuruba Lakshman², Korlakonta Narasimha Jayaveera³,
Chinnasamy Velmurugan⁴, Bachappa Manoj⁵, Sultanpet Munikrishnappa Sridhar⁴

¹Department of Pharmacognosy, Sri K.V.College of Pharmacy, Chickballapur, Karnataka (India).

²Department of Pharmacognosy, PES College of Pharmacy, Bangalore, Karnataka, (India).

³Department of Chemistry, Jawaharlal Nehru Technological University of College of Engineering, Anantapur, Andhra Pradesh (India).

⁴Department of Pharmacology, Sri K.V.College of Pharmacy, Chickaballapur, Karnataka (India).

⁵Department of Pharmaceutical Chemistry, Sri K.V.College of Pharmacy, Chickaballapur, Karnataka (India).

Abstract

Amaranthus caudatus Linn, traditionally used as vermifuge, so methanol extract of whole plant of *A. caudatus* was investigated for anthelmintic activity using earthworms (*Pheretima posthuma*). Various concentrations (10-100 mg/ml) of methanol extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant dose dependent anthelmintic activity at 60, 80, 100 mg/ml. The result provides scientific validation for traditional use.

Key words: *Amaranthus caudatus* Linn, anthelmintic activity, *Pheretima posthuma*, Piperazine Citrate

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.

A. caudatus Linn, (Amaranthaceae), commonly known as “Peddathotakura” in *Telugu*. The amaranthus plants are spread throughout the world, growing under a wide range of climatic conditions and they are able to produce grains and leafy edible vegetables (Rastrelli et al., 1995). *A. caudatus* traditionally used in jaundice, amoebiasis, kidney diseases (Haile yinger et al., 2008; Vanila et al., 2008), as blood purifier, diuretic, abortifacient, vermifuge and astringent (Khare, 2007).

A. caudatus is reported for its antiatherosclerotic (Najmeh Kabiri et al., 2010), antinociceptive and antipyretic activities (Ashok Kumar et al., 2010). *A. caudatus* seeds showed cholesterol lowering, *in vitro* antioxidant and alpha amylase inhibition activities (Plate and Areas 2010; Filomena Conforti et al., 2005). The Amaranth seed oil is used as nutraceutical resource from Ecuadorian flora (Bruni et al., 2001). *A. caudatus* contains antimicrobial peptides (Broekaert et al., 1992), agglutinin (Thomas et al., 1997), triterpenoid saponins and ionol derived glycoside

* Corresponding author. mailing address: *Ashok kumar.B.S.*
Assistant Professor and Head,
Department of Pharmacognosy,
Sri K.V.College of Pharmacy,
Chickballapur-562101,
Karnataka.
ashok4vani@gmail.com

(Rastrelli et al., 1998), vitamin E isomers (Bruni et al., 2002) and amaranthin (Rinderle et al., 1989). There is no report available on anthelmintic activity of *A. caudatus*, so our investigation is to provide scientific proof for traditional use.

Materials and Methods

Collection of Plant Material and Extraction The fresh plant of *A. caudatus* was collected from Chickballapur, and was authenticated by Dr. Rajan, Department of Botany, Government Arts College, Ootcamund, Tamilnadu. A voucher specimen (SKVCP 13) was deposited in college herbarium. The whole plant was shade dried, coarsely powdered and was subjected to extraction with methanol by Soxhlet apparatus. The extract was concentrated to dryness in vacuum.

Phytochemical screening The methanol extract was screened for the presence of various phytoconstituents like 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. The earthworms of 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 contains six worms. 50 ml formulations containing four different concentrations of methanolic extract of whole plant of *A. caudatus* (10, 20, 50, 80 and 100 mg/ml in distilled water) were prepared in it. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time of death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Piperazine citrate (10mg/ml) was used as reference standard while water as control.

Results and Discussion

Preliminary phytochemical analysis showed the presence of carbohydrates, steroids, alkaloids, phenolic compounds, flavonoids, saponins and amino acids in methanol extract of *A. caudatus*. Some of the phytoconstituents may be responsible.

Methanol extract of *A. caudatus* showed dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death (Table.1). At 60, 80 and 100 mg/ml concentration shows paralysis at 19.21, 12.16 and 5.75 min and death at 27.7, 18.6 and 8.5 min. From the above results, it is concluded that methanol extract of *A. caudatus* showed significant anthelmintic activity when compared to standard piperazine (10 mg/ml).

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effect on health standards with a consequent lowering of resistance to other disease. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus*, and *Heterakis*, out of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* because they resemble intestinal 'worms' in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintic are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic (Sollmann, 1918). Earthworms have the ability to move by ciliary movement. The outer layer of the earthworms is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis.

A. caudatus shows significant anthelmintic activity due to presence of polyphenolic compounds (Bate-Smith, 1962). The wormicidal activity of methanol extract against earthworms suggests that it is effective against parasitic infections of humans.

Acknowledgments

Authors are thankful to Sri Naveen Kiran, K.V. Chairman, Sri K.V. Trust for providing facilities to carryout this work.

References

Jain, M.L., and S.R. Jain (1972). Therapeutic Utility of *Ocimum basilicum* var. album. *Planta Medica*, 22: 66-70.

- Bate-Smith, E.C., (1962). The phenolic constituent of plants and their taxonomic significance, dicotyledons. *J. Linn Soc Bot*, **58**, 95-103.
- Rastrelli L, Pizza C, Saturnino P, Schettino O, Dini A. Isolation and characterization of Amaranthin, a lactin present in seeds of *Amaranthus caudatus* that recognizes the T-antigen. *J. Agric.Food.Chem.*1995;43: 904-909.
- Haile yineger, Ensermu Kelbessa, Tamrat Bekele, Ermias Lulekal. Plants used in Traditional Management of Human Ailments at Bale Mountains National Park. 2008.
- Vanila D, Ghanthikumar S, Manickam VS. Ethnomedicinal uses of plants in the plains area of the Tirunelveli-District. Tamilnadu, India, *Ethnobotanical leaflets*. 2008; 12: 1198-1205.
- Khare CP. *Indian Medicinal Plants, an Illustrated Dictionary*, Springer, 2007;41.
- Najmeh Kabiri, Seddigheh Asgary, Hossein Madani, Parvin Mahzouni, Effect of *Amaranthuscaudatus* extract and lovastatin on atherosclerosis in hypercholesterolemic rabbits. *J Medicinal Plants Res*. 2010; 4(5): 355-361.
- Ashok Kumar BS, Lakshman K, Jayaveera KN, Sheshadri Sekar D, Velmurugan C. Antinociceptive and Antipyretic activities of methanolic extract of *Amaranthus caudatus* Linn. *Latin American J Pharm*. 2010; 29(4): 1-5.
- Plate AYA, Areas JAG. Cholesterol lowering effect of extruded amaranth (*Amaranthus caudatus* Lin.) in hypercholesterolemic rabbits. *Food Chem*. 2007; 76: 1-6.
- Filomena Conforti, Giancarlo Statti, Monica Rosa Loizzo, Gianni Sacchetti, Ferruccio Poli, Francesco Menichini. *In vitro* antioxidant effect and inhibition of α -amylase of two varieties of *Amaranthus caudatus* seeds. *Biological & Pharmaceutical Bulletin*. 2005; 28(6): 1098-1102.
- Bruni R, Medici A, Guerrini A, Scalia S, Poli F, Muzzoli M, Sacchetti G. Wild *Amaranthus caudatus* seed oil a nutraceutical resource from Ecuadorian Flora. *J Agri Food Chem*. 2001; 49(11): 5455-5460.
- Broekaert WF, Marien W, Terras FRG, Debolle MFC, Proost P, Vandamme J, Dillen L, Claeys M, Rees S, Vanderleyelen J, Cammue BPA. Antimicrobial peptides from *Amaranthus caudatus* seed with sequence homologue to the cystein/glycine-rich domain of chitin-binding proteins. *Biochem*. 1992; 31: 4308-4314.
- Thomas R, Transue, Alexander K, Smith, Hanquing, Irwin J, Goldstein, Mark A, Saper. Structure of benzyl T-antigen disaccharide bound to *Amaranthus caudatus* agglutinin. *Nature Structural and Molecular Biol*. 1997; 4: 779-783.
- Rastrelli L, Aquino R, Abdos, Proto M, Desimone F, De Tommasin. Studies on the constituents of *Amaranthus caudatus* leaves ;isolation and structure elucidation of new triterpenoid saponins and ionol-derived glycosides. *J. Agri .Food Chem*. 1998; 46(5): 1797-1804.
- Bruni R, Guerrini A, Scalia S, Romagnoli C, Sacchetti G. Rapid techniques for extraction of Vitamin E isomers from *Amaranthus caudatus* seeds: ultrasonic and supercritical fluid extraction. *Phytochem Anal*. 2002; 13: 257-261.
- Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM. Isolation and characterization of amaranthin, a lactin present in seeds of *Amaranthus caudatus* that recognizes the T-antigen. *J Biol Chem*. 1989; 264(27): 16123-16131.
- C.K. Kokate, "In: *Practical Pharmacognosy*", 1st ed. Vallabh Prakashan, New Delhi, 1986, 111.
- E.O. Ajaiyeoba , P. Onocha , O.T. Olarenwaje, "In vitro anthelmintic properties of Buchholzia coriacea and Gynandropsis gyanadra extract" *Pharmaceutical. Biol.*, 2001, 39(3), 217-220.
- R.D. Vidyarthi, "A Text Book of Zoology". S. Chand and Co. New Delhi, 1967, 329-370.
- K.D. Chatterjee, "Parasitology, Protozoology and Helminthology", Guha Ray Sree Saraswaty press Ltd, Calcutta, 1967, 168-169.
- T. Sollmann, "Anthelmintics: their efficacy as tested on earthworm", *J. Pharmacol. Exp. Ther.*, 1918, 12, 129-170.
- M.L. Jain, S.R. Jain, "Therapeutic Utility of Ocimum Basilicum Var, Album", *Planta. Medica.*, 1972, 22: 66-70.
- G.K. Dash, P. Suresh , D.M. Kar, S. Ganapathy, S.B. Panda, "Evaluation of *Evolvulus alsinoids* Linn. For Anthelmintic and Antimicrobial activities" *J. Nat. Rem.*, 2002, 2(2), 182-185.