JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34714
RESEARCH ARTICLE

Investigation of Anthelmintic Potential of Two Leafy Vegetables from the Amaranthaceae Family

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Abstract

Objective: The present study investigates the anthelmintic potential of *Amaranthus viridis* Linn. and *Amaranthus tricolor* Linn. (Amaranthaceae) leafy vegetables from the Pune district, traditionally used as vermicides. **Materials and Methods:** The ethyl acetate, acetone and ethanol leaf extracts of both plants were subjected to preliminary investigations for the phytochemical elements. The anthelmintic activity of these extracts was tested against fully-grown *Eisenia fetida* earthworms. Ethyl acetate extract of *A. viridis* L. and acetone extract of *Amaranthus tricolor* L. showed the presence of all phytochemicals. Doses of 10–30 mg/ml were used in bioassay. Time of paralysis and death of earthworms was observed. **Results:** All extracts exhibited considerable dose-dependent activity against the reference drug, showing maximum activity at 30 mg/mL in both plants. The most promising activity was exhibited by the ethyl acetate extract of the *A. viridis* L. (P (min.) = 4, D (min.) = 5), followed by the acetone extract of the *A. tricolor* L. (P (min.) = 6, D (min.) = 13). Albendazole (20 mg/ml) and normal saline were applied as a standard drug and control, respectively. High concentrations of alkaloids, tannins, and phenolic phytochemicals might be responsible for the exceptional activity. **Conclusion:** The present work scientifically validates the traditional use and concludes that ethyl acetate and acetone extracts may be further processed to isolate the compounds responsible for said activity. One-way ANOVA with a significance level of 5% was used to validate the data ($n = 3$, $p < 0.05$).

Keywords: Albendazole, *Amaranthus tricolor* Linn., *Amaranthus viridis* Linn., Anthelmintic Activity, Earthworm, *Eisenia fetida*

1. Introduction

The pervasiveness of gastro-intestinal helminthiasis infection by parasites such as hookworms, and tapeworms is one of the notable health hazards, affecting 1.5 billion people worldwide[1,2]. The worm infection causes serious health conditions of anaemia, dysentery, diarrhoea, loss of appetite, etc., and leads to serious morbidity by affecting a large population[3]. Pharmacologically, only a few drugs such as albendazole, mebendazole, piperazine, diethylcarbamazine citrate, levamisole, etc., are used in the treatment of helminthiasis in human beings[4]. The inadequate availability of effective allopathic medicinal drugs, their adverse side effect and the increasing resistance of gastrointestinal parasites towards synthetic anthelmintics create a problem in the treatment and management of this disease[5]. Considering the facts, it is the need of the hour to develop an effective and alternative strategy against gastrointestinal helminths.

Anthelmintics from natural medicinal plant sources can provide an efficient and eco-friendly alternative to commercially available drugs. Anthelmintic plants, also known as vermicides, are used traditionally to expel the parasitic worms from the body either by causing distress or demise to the worms. The leaf extract of a variety of medicinal plants shows anthelmintic

properties when compared to other parts of the medicinal plant[6,7]. The Amaranthaceae family plants like *Amaranthus tricolor* Linn. and *Amaranthus viridis* Linn. shows alexeteric and anthelmintic properties[8,9]. In Maharashtra, the *A. tricolor* and *A. viridis* L. plants

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Article Received on: 12.08.2023 Revised on: 11.10.2023 Accepted on: 29.10.2023

are highly consumed leafy vegetables, locally known as "Laal Maath" and "Green Maath", respectively. They are often used in folklore medicines worldwide[10,11]. Traditionally, *A. viridis* L. is used to lessen labor pain, treat eczema, psoriasis, respiratory problems, and asthma[12,13]. Pharmacologically, *A. viridis* L. shows antidiabetic, antihyperlipidemic, antioxidant, anti-cholesterolemic, antiproliferative, anti-inflammatory, antihyperglycemic, antimicrobial, anti-nociceptive, antipyretic, cardioprotective, hepatoprotective, hyperlipidemic, hypolipidemic, etc. properties[14,15]. Similarly, the *A. tricolor* L. plant is reported in Ayurveda as an astringent in menorrhagia, leucorrhea, dysentery, colitis, throat infection, toothache, bronchitis, eczema, piles, and is also used externally to treat mouth ulcers[16]. A literature survey revealed that *A. tricolor* L. had a wide spectrum of pharmacological activities: antimicrobial, antiviral, antioxidant, anti-diabetic, anti-inflammatory, hepatoprotective, haematological, neuroprotective effect, tumour cell proliferation, etc[17,18].

Given the wide range of biological activities, we conducted a comparative analysis of anthelmintic properties of leaf extracts from these plants in the present study. The anthelmintic profiles of extracts of these two leafy vegetables from Pune city in Maharashtra, India, have never been compared before. Previous research on Amaranthus genus plants has only used one single solvent to examine the anthelmintic and anti-inflammatory effects of whole plant extracts or leaf extracts.

However, no anthelmintic properties have been reported for these leafy vegetable extracts. M. Baral *et al.* evaluated the anti-inflammatory and anthelmintic potential of only water extracts of the full plant of *A. spinosus* against *Pheritima posthuma* and *Tubifex tubifex* worms[19]. Baral *et al.*, evaluated the anti-inflammatory and anti-anthelmintic properties of water extracts of whole plant strains of *A. spicillosus* L. (*Pseudomonas spicillosa*) derived from *Pheritima pumila* and *Tubifex pumila* worms. George B. *et al.*, evaluated the anthelmintic potential of the water extract of leaves of *A. dubius* along with the *Basella alba* and *Cleome gynandra* plants[20]. Whereas Kumar *et al.*, studied the anthelmintic activity of the methanol extract of a full plant of *A. caudatus* L[21]. Further, Kumar compared the anthelmintic behaviour of the methanolic extract of the *A. spinosus*, *A. caudatus* and *A. viridis* L. plants[22]. As already mentioned, traditionally, both plants were utilized as vermifuge but to date, no scientific evidence has been reported on a comparative study of their anthelmintic activity. This prompted us to investigate the anthelmintic activity of *A. viridis* L. and *A. tricolor* L. leaves with solvents of varying polarity, to assess the nature of phytochemicals causing the activity. We have chosen the solvents for the extract preparation, which differ widely in their polarity (ethyl acetate < acetone < ethanol). Solvents with different polarities extract the different types of phytochemicals and may help in identifying the key substance.

The aim of this study, therefore, was to identify the *in vitro* anthelmintic activity of acetone, ethyl acetate and ethanol extracts derived from the leaves of *A. viridis* L. and *A. tricolor* L. plants and to provide scientific evidence for its traditional use as a vermicide plant.

2. Materials and Methods

2.1 Plants Used

The fresh leaves of *A. viridis* L. and *A. tricolor* L. were collected individually, cleaned and air-shade dried under room temperature conditions. The obtained dried samples were further

ground into a fine powder using a mortar pestle. The samples were stored in an air-tight container. The plants were identified taxonomically and authenticated at the Botanical Survey of India, Pune, where voucher specimens were deposited. The obtained authentication numbers were BSI/WRC/IDEN.CER/2019/H3/19 and BSI/WRC/IDEN.CER/2019/H3/20 respectively for *A. tricolor* L. and *A. viridis* L.

2.2 Chemicals

Albendazole drug (Glaxo SmithKline) and normal saline were sourced from approved pharmaceuticals. Ethanol (analytical grade), acetone (analytical grade) and ethyl acetate (analytical grade) solvents were used in the protocol.

2.3 Experimental Animals Used

Anthelmintic activity was studied using Indian adult earthworm species (*Eicinia fetida*). The earthworms were collected from a nursery in Pune, Maharashtra, India. They were collected from moist soil, washed with normal saline and all faecal matter was removed.

Earthworms of approximately identical dimensions (3-5 cm in length and 0.1-0.2 cm in width) were used in all experimental protocols.

2.4 Preparation of Leaves Extract

Three different extracts of both plants were prepared by refluxing (for 18 hrs.) weighed quantity (50 mg) of powdered leaves material separately in a known volume (250ml) of ethanol, acetone, and ethyl acetate solvents. Crude extracts were obtained after recovering the excess solvents under reduced pressure conditions, and respective extracts were subjected to preliminary phytochemical screening and anthelmintic activity.

2.5 Phytochemical Screening

All three extracts of both leafy vegetables were subjected to screening for the presence of various phytochemicals following standard protocols[23].

2.6 Anthelmintic Activity

The methodology followed to conduct the anthelmintic assay was as described by Mali *et al.*, with slight moderation[24]. The assay was executed on the mature Indian earthworm *Eicinia fetida* because of their physiological and anatomical resemblance with the roundworm parasite of the human intestine. The ready availability of the earthworms, further, makes them suitable candidates for the preliminary evaluation of *in vitro* anthelmintic substances[25].

Albendazole drug dissolved in saline served as standard. Normal saline was used as a positive control. Ethyl acetate, acetone, and ethanol extracts of both plants, suspended in normal saline were used for the assay. Pure solvents act as negative control, and 1% solution of each solvent was used as solvent control. Time taken for complete paralysis and death of earthworms were recorded. The paralysis time was ascertained by applying the external stimuli. The time when worms become motionless was considered as paralysis time and when the body color of motionless worms fades it was ascertained as the death time.

2.7 Administration of the Extracts

The animals were distributed and released into forty-two groups (Petri-dish) containing 20 ml of various test solutions. Each group consisted of three earthworms of approximately equal dimensions.

For evaluation of anthelmintic activity, three groups received standard Albendazole suspended in normal saline in varying concentrations of 10-30 mg/ml. For each case of ethyl acetate extract, acetone extract, and ethanol extract of both plants, the same concentration (10-30 mg/ml) was used for treatment in three separate sets of Petri dishes. A Petri dish containing normal saline solution acts as a negative control. Further, three sets served as solvent control and were treated with 1% ethanol, 1% acetone, and 1% ethyl acetate solutions, respectively.

2.8 Statistical Analysis of Data

The mean ± standard deviation of three earthworms in each group was used to express the results. Using one-way ANOVA at the 5% level of significance ($p < 0.05$, $n = 3$), the data were found to be statistically significant.

3. Result and Discussion

3.1 Phytochemical Screening of Extracts

The preliminary phytochemical screening illustrates that all extracts of leafy vegetables contain flavonoids, glycosides, steroids, alkaloids, phenolic compounds, carbohydrates, proteins, and tannins (Table 1). Unlike the other solvent extracts, the ethyl acetate extract of

A. viridis L. and the acetone extract of *A. tricolor* L. were found to be positive for all phytochemicals tested. The test for alkaloid, tannin, phenol, glycosides, starch, and sugars was positive for all the extracts of *A. viridis* L. Whereas, for *A. tricolor* L. alkaloid, tannin, phenol, starch, and sugars were found positive for all the extracts.

3.2 Anthelmintic Assay

The leaf extracts of both leafy vegetables demonstrated superior anthelmintic activity as compared to the standard drug Albendazole at all doses. The most promising activity was exhibited by the ethyl acetate extract of *A. viridis* L., followed by the acetone extract of *A. tricolor* L (Table 2).

A literature survey suggests that the anthelmintic activity of ethyl acetate extract of leaf of *A. viridis* L. turns out to be of maximum efficacy than any reported

Table 1. Result of the phytochemical screening for the leaf extracts of *A. tricolor* L. and *A. viridis* L.

S. no.	Name of Phytochemical	*Amaranthus viridis* L.			*Amaranthus tricolor* L.		
		EAE	AE	EE	EAE	AE	EE
1	Protein	+	-	-	++	+	-
2	Alkaloid	++	+	++	++	+	++
3	Flavonoid	+	++	-	++	++	-
4	Steroid	+	+	++	-	+	++
5	Saponin	+	++	-	+	+	-
6	Tannin	++	+	++	+	++	++
7	Phenol	++	+	+	+	++	++
8	Glycoside	+	++	++	-	++	++
9	Starch	+	+	++	++	+	++
10	Sugars	++	+	+	+	+	+

*((+): Presence, (++): Strong presence, (_): Absence); (AE: Acetone extract; EAE: Ethyl acetate extract; and EE: Ethanol extract)

Table 2. Anthelmintic activity of *A. viridis* L. and *A. tricolor* L. leaf extracts

Treatment	Concentration (mg/ml)	*Amaranthus viridis* L.		*Amaranthus tricolor* L.	
		P	D	P	D
		(min.)	(min.)	(min.)	(min.)
EAE	10	12±1.52	15±2	25 ± 2	45 ± 1
	20	9±2	11±1	19 ± 1	38 ± 0.58
	30	4±0.58	5±0.58	11 ± 1.53	20 ± 1
AE	10	24±0.58	47±2	19 ± 1	40 ± 1
	20	20±1	22±0.58	10 ± 0.58	21 ± 1.2
	30	16±1.52	19±1	6 ± 1.53	13 ± 0.6
EE	10	69±1.15	83±2.08	69± 2	86 ± 1.5
	20	55±2	70±2	53 ± 1	66 ± 1
	30	43±0.58	64±0.58	40 ± 1.53	57± 0.6
Albendazole (Standard)	20	73 ± 1	94 ± 2.52		
Normal Saline (Positive control)	---	---	---	---	---
1% Acetone/ Ethyl acetate/ Ethanol solvents (Negative control)	---	---	---	---	---

*...no paralysis, no death; (P: Paralysis; D: Death)(AE: Acetone extract; EAE: Ethyl acetate extract; and EE: Ethanol extract)

case from Amaranthacea family[19-22], causing the death of worms within 5 minutes at 30mg/ml, only.

As reported by M. Baral *et al* the water extract (50 mg/ml) of the whole plant of *A. spinosus* Linn. causes the paralysis and death of *Pheritima posthuma* and *Tubifex tubifex* worms of the Annelida family in 18 minutes and 33 minutes, respectively[19]. The aqueous extract of the leaf of *A. dubius*[20] is reported to cause the paralysis and death of *Eisenia fetida* earthworms in 18 minutes and 28 minutes, respectively, at a high concentration of 300 mg/ml. The methanolic extract of *A. caudatus* Linn.[21] plant takes a short time for paralysis (5.75 minutes) and death (8.5 minutes) of earthworms, but at a high concentration of 100 mg/ml. And, as disclosed by Kumar Ashok BS, the 100 mg/ml concentration of methanolic extract of *A. spinosus*, *A. viridis* L. and *A. caudatus* plants causes death of earthworms at 8.5 min, 12.7 min, and 18.6 min, respectively.

Further, the results indicate the dose-dependent anthelmintic activity ranging from loss of motion to death of earthworms. The ethyl acetate extract (10-30 mg/ml) of *A. viridis* L. shows paralysis at 12, 9 and 4 min and death at 15, 11 and 5 min, acetone extract shows paralysis at 24, 20 and 16 min and death at 47, 22 and 19 min, and the ethanol extract shows paralysis at 69, 55 and 43 min and death at 83, 70 and 64 mi. Whereas, the ethyl acetate extract of *A. tricolor* L. at concentrations of 10, 20 and 30 mg/ml shows paralysis at 25, 19 and 11 min and death at 45, 38 and 20 min, the acetone extract shows paralysis at 19, 10 and 6 min and death at 40, 21 and 13 min, and ethanol extract of it shows paralysis at 69, 53 and 40 min and death at 86, 66 and 57 min, post-exposure (Table 2, Figure 1).

The potency (efficacy) of extracts of both plants was found to vary inversely proportional to the time taken for paralysis and death of the worms (Table 3). Further, the results show that for *A. viridis* L. the potency (for paralysis as well as death) of ethyl acetate extract is highest at all concentrations, followed by the acetone and ethanol extract. While for *A. tricolor* L. the potency (for paralysis as well as death) of acetone extract is highest at all concentrations,

followed by the ethyl acetate and ethanol extract. As shown in Figure 1, the potency for various extracts increases with increasing the concentration of extracts and maximum potency for paralysis and death of earthworms is observed at 30 mg/ml.

3.2.1 Paralysis of Earthworms at Various Concentrations of Extracts

In the case of *A. viridis* L. for paralysis of worms, the ethyl acetate extract (30 mg/ml) is 3.7 times more potent than the acetone extract, and almost 10.7 times more potent than the ethanol extract. For ethyl acetate extract, by increasing the concentration in double amounts (from 10 to 20 mg/ml), potency is significantly increased up to 1.7 times and efficacy of extract increases by 3 times when concentration becomes three-fold, from 10 to 30 mg/ml. Whereas, for *A. tricolor* L., the paralysis of worms of acetone extract (30 mg/ml) is 2.2 times more potent than the ethyl acetate extract and almost four times more potent than the ethanol extract. For acetone extract, by increasing the concentration in double amounts (from 10 to 20 mg/ml), potency is significantly increased up to 2.7 times and efficacy of extract increases by

3.8 times when concentration becomes three-fold, from 10 to 30 mg/ml.

3.2.2 Death of Earthworms at Various Concentrations of Extracts

In the case of *A. viridis* L. for paralysis of worms, the ethyl acetate extract (30 mg/ml) is 3.8 times more potent than the acetone extract and almost 19 times more potent than the ethanol extract. For ethyl acetate extract, by increasing the concentration in double amounts (from

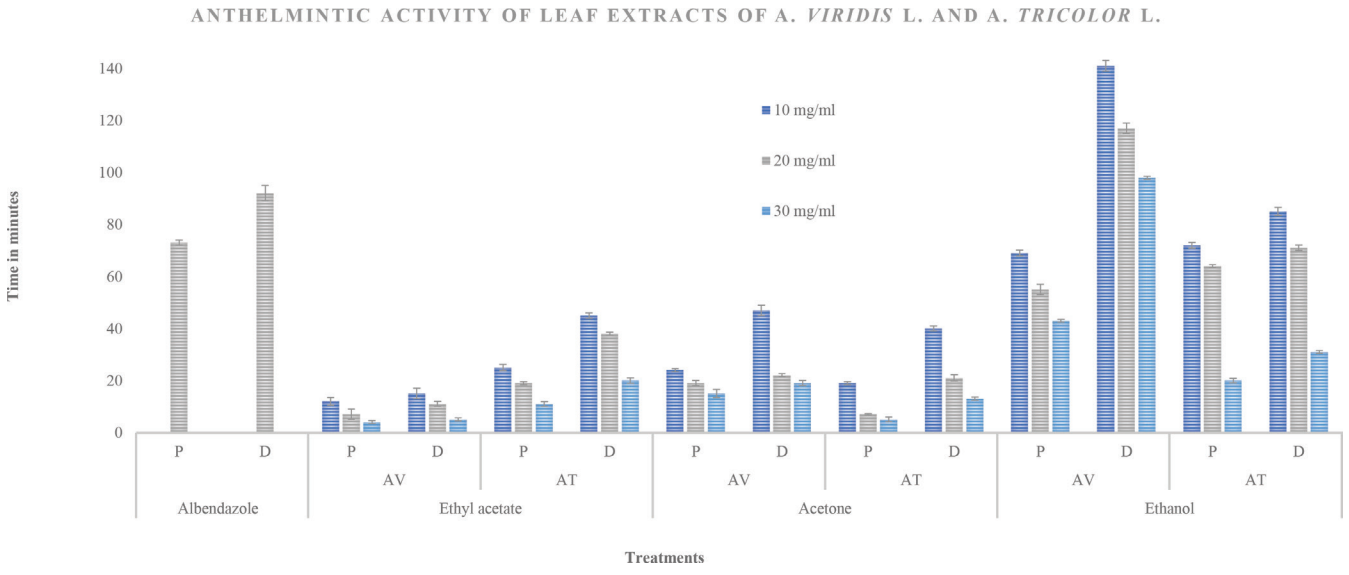

Fig.1 Anthelmintic Activity of various leaf extracts of *A. viridis L. and A. tricolor L.*
(P: Paralysis; D: Death; AT = Amaranthus tricolor Linn; AV = Amaranthus viridis Linn)

Table 3. The potency of various extracts

Treatment	Concentration (mg/ml)	*Amaranthus viridis* L.		*Amaranthus tricolor* L.	
		P	D	P	D
EAE	10	9.6	9.5	4.6	3.2
	20	16.4	13.0	6.1	3.8
	30	28.8	28.6	10.5	7.2
AE	10	4.8	3.0	6.1	3.5
	20	6.1	6.5	16.4	6.5
	30	7.7	7.5	23	11
EE	10	1.7	1.0	1.6	1.7
	20	2.1	1.2	1.8	2.0
	30	2.7	1.5	5.8	4.6

*(AE: Acetone extract; EAE: Ethyl acetate extract; and EE: Ethanol extract) P = Paralysis; D = Death

10 to 20 mg/ml), potency is significantly increased up to 1.4 times and efficacy of extract increases by 3 times when concentration becomes three-fold, from 10 to 30 mg/ml. Whereas, for *A. tricolor* L., the paralysis of worms of acetone extract (30 mg/ml) is 1.5 times more potent than the ethyl acetate extract, and almost 2.4 times more potent than the ethanol extract. For acetone extract, by increasing the concentration in double amounts (from 10 to 20 mg/ml), potency is significantly increased up to 1.9 times and efficacy of extract increases by 3 times when concentration becomes three-fold, from 10 to 30 mg/ml.

The preliminary phytochemical study of crude extracts disclosed the presence of alkaloids, tannins, flavonoids, phenols, glycosides, starch, and tannins as chief constituents (Table 1).

As per a literature survey, the secondary metabolites of the plants may interfere with the metabolism processes of parasitic worms and affect the energy-generating mechanism of the worms by inhibiting glucose uptake[26-29]. The alkaloids, tannins and phenolics like phytoconstituents were found to show significant anthelmintic activity[26]. As reported the alkaloids attack the central nervous system of the worms and cause paralysis[27]. Polyphenolic compounds were reported to increase the host resistance and contribute towards the parasiticidal activity[28]. The tannins hindered the energy generation mechanism of earthworms by disconnecting the oxidative phosphorylation. Tannins may also lead to worms' death by binding to their intestinal tract's free protein. The albendazole-like drug acts as worm expellers by causing paralysis of the worms and resulting in their expulsion by peristalsis[29]. Albendazole drug causes hyperpolarization by increasing the conductance of chloride ions and eventually leading to the relaxation of muscles and paralysis of earthworms[30].

From the above study, it can be said that the secondary metabolites present in both leafy vegetables may have produced similar effects, causing the death of the earthworms. Therefore, the entitlement of both plants as potent anthelmintic has been confirmed because of the excellent activity of various extracts against *Eisenia fetida*. Further, the efficacy of an extract depends on its transit time in the gastrointestinal tract of humans. Being a leafy vegetable extracts of both plants may get highly absorbed in the body and have enough transit time to act. Therefore, the gradual increase in the extract dose exhibited a stepwise increase in the activity (Table 2, Figure 1).

4. Conclusion

It is concluded from the results that, leaves of *A. viridis* L. and *A. tricolor* L. plants have great potential as anthelmintic agents, possessing varying degrees of dose-dependent activities at all concentrations of extracts. The most promising activity was exhibited by the ethyl acetate extract of the *A. viridis* L. (P (min.) = 4, D (min.) = 5), followed by the acetone extract of the

A. tricolor L. (P (min.) = 6, D (min.) = 13). The different extracts of *A. viridis* L. demonstrated the trend for activity as Ethyl acetate > Acetone > Ethanol, while *A. tricolor* L. exhibited activity as Acetone > Ethyl acetate > Ethanol. In the present study, the obtained experimental evidence strongly supports the folklore use of these leafy vegetables as vermicides. Additional study is required to isolate and identify the active compounds in the crude extracts, *in vivo* trials may be conducted for the use in livestock on a scientific basis.

5. Acknowledgement

The authors are thankful to the Principal, Yashwantrao Mohite College of Arts, Science and Commerce, Pune – 38, Bharati Vidyapeeth (Deemed to be University), Pune for providing laboratory facilities to perform the experiments.

6. References

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