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Dr. Lakshmi Kanta Kanthal
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Dr. Suman Pattanayak
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Rimi Mondal
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Purnendu Pramanick
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Devprakash Majhi
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Sarjina Ansari
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Md Rajibul Islam
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

Corresponding Author:
Dr. Lakshmi Kanta Kanthal
Haldia Institute of Pharmacy, Knowledge City, ICARE Complex, Hatiberia, Haldia, West Bengal, India

In vitro spasmodic & antispasmodic effect of *Amaranthus spinosus* L. & *Amaranthus viridis* L. on isolated chicken ileum

Dr. Lakshmi Kanta Kanthal, Dr. Suman Pattanayak, Rimi Mondal, Purnendu Pramanick, Devprakash Majhi, Sarjina Ansari and Md Rajibul Islam

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Abstract
Many useful chemicals and/or medications are derived from medicinal plants. According to the International Union for Conservation of Nature and the World Wildlife Fund, around 50,000 to 80,000 blooming plants are used medicinally. *Amaranthus spinosus* is used as an expectorant and to relieve breathing in severe bronchitis in Malaysia. It is also used as a sudorific, febrifuge, antidote to snake poison, galactagogue, and to treat menorrhagia in mainland South-East Asia. Some Indian tribes use *Amaranthus spinosus* to induce abortion. Traditional medicine use *Amaranthus viridis* to treat fever, pain, asthma, diabetes, dysentery, urinary problems, liver disorders, eye disorders, and venereal illnesses. The herb also has antibacterial qualities.
The purpose of this study was to investigate the antispasmodic effects of methanolic root extracts of *Amaranthus spinosus* and *Amaranthus viridis* on an isolated chicken ileum. Maceration was used to make methanolic root extracts of the plant material. The antispasmodic activity was tested *in vitro* (37 ^{o}C, pH 7.4 with continuous oxygenation). Both plants elicited spontaneous relaxation of the chicken ileum, with the greatest impact at 0.8 ml and 0.1ml, respectively. This reveals that aqueous and cruder root extracts of *Amaranthus spinosus* and *Amaranthus viridis* have a spasmolytic action on isolated chicken ileum, hence validating its folkloric use in the treatment of gastrointestinal diseases.

Keywords: Anti-spasmodic, chicken ileum, *Amaranthus spinosus*, *Amaranthus viridis*, spasmolytic

Introduction
Non-nutrient active plant chemical compounds or bioactive compounds that protect the plant from infections, infestations, or predation by microbes, pests, pathogens, or predators; some are responsible for colour, aroma, and other organoleptic properties. Plants synthesise phytoconstituents via primary and secondary metabolic pathways, and many of them can be classified as active drug constituents or inert nondrug constituents.
Amaranthus spinosus L. spiny amaranth, Amaranthaceae family. It is an erect prickly annual herb that grows up to 60 cm tall and reproduces via seeds. The stem is fleshy, spherical, greenish, and inflexible, measuring around 10 mm in length. The leaves are 6 to 8 cm long and 2 to 4 cm wide, with petioles that are about 7 cm long. The blooms are small and greenish, while the fruits are a single seeded capsule with shiny, lens-shaped, reddish-brown seeds. The roots are a light yellowish tint. Traditional uses: Tribals in Kerala, India utilise the juice of *A. spinosus* L. to avoid swelling around the stomach, while the leaves are boiled without salt and taken for 2-3 days to treat jaundice. It has anti-inflammatory, antimalarial, antibacterial, and antimicrobial properties.
The plant has hepatoprotective and antioxidant properties, and its aqueous extract has considerable immune-stimulating and antimalarial properties (Ishrat JB *et al*., 2011) [18] (Jhade D *et al*., 2009) [20]. It is used internally to treat abdominal bleeding, diarrhoea, and heavy menstruation.
The herb is used as a febrifuge, antipyretic, laxative, and diuretic in the Indian traditional system of medicine (Ayurveda). Aside from its culinary value, it is a popular medicinal plant used to treat digestible, bronchitis, appetizer, biliousness, galactagogue, hematinic, stomachic effects, nausea, flatulence, anorexia, blood diseases, burning sensation,

leucorrhoea, leprosy, piles, and as a treatment for hallucination, wound healing, rheumatism, and to stop blood coughing. R.P. Kumar *et al*., 2014 [40] report that all portions of the plant have medicinally active components.

Ayurvedic texts suggest the following medicinal uses of *Amaranthus spinosus* L.: Leaf infusion is diuretic and useful in anaemia. Root paste is used to treat gonorrhoea, eczema, menorrhoea, and other conditions (Ghosh D *et al*, 2013) [13]. The juice of the root is used to cure fever, urinary problems, diarrhoea, and stomach problems, among other things (R.P. Kumar *et al*, 2014) [40]. The antioxidant activity of *A. spinosus* L. extract was approximately two times that of the synthetic antioxidant Ascorbic acid. As demonstrated by *A. spinosus* L. extract, a higher concentration of phenolic substances results in a more powerful radical scavenging activity. *Amaranthus spinosus* L. exhibits anti-inflammatory effect that is dose dependent, as well as central and peripheral analgesic activity. It has anti-diabetic properties.

Amaranthus viridis L., note shak, Amaranthaceae family. It is an upright plant with a green stem up to 75 cm tall. The leaves are simple, about 2–7 cm long and 1.5–5.5 cm broad. The flower measures 1 mm in length.

The leaves of *Amaranthus viridis* L. have a long history of indigenous utilized as a medicinal herb in the traditional Ayurvedic medicine as antipyretic agents and also eaten conventionally as a vegetable among tribal and non-tribal people of Northeast India. The leaves are believed to have febrifugal properties. Ash of *Amaranthus viridis* L. plants is rich in soda and is occasionally used to make soap (K. Girija *et al*. 2011) [14]. The roots are pale yellowish in colour. It is used as anti-pyretic agent, also for the treatment of inflammation, ulcer, diabetic, asthma and hyperlipidaemia.

The antispasmodic (Spasmolytic) effect of drugs is commonly used for the reduction of excessive smooth muscle contractility, responsible for cramping and discomfort in the abdominal area, caused by multiple conditions affecting the gastrointestinal, biliary or genitourinary tract. *Amaranthus viridis* L. a traditional folklore medicinal plant has *in vitro* antioxidant potentials and hypolipidemic effect which may provide therapeutic potentials in the management of Cardiovascular diseases, diabetes and their complications which might because by free radical generation and hyperlipidaemia. A. viridis L. leaf extract is a source of potent antioxidant and anti-inflammatory agent and may modulate cholesterol metabolism by inhibition of HMG-CoA reductase.

A detailed literature review on *Amaranthus spinosus* L. and *Amaranthus viridis* L. has been shown that there are no published reports worldwide related to methanolic roots extract to till date. Therefore, this study investigates the *in vitro* antispasmodic effect *of Amaranthus spinosus* L. and *Amaranthus viridis* L. on isolated chicken ileum.

Materials and Methods

Collection and Authentication of plant material

Based on the literature survey, two plant named *Amaranthus spinosus* L. *& Amaranthus viridis* L. were selected for study. The roots of *Amaranthus spinosus* L. *& Amaranthus viridis* L. were collected in the month of February, 2023 from various areas of Haldia, Purba Medinipur Dist., West Bengal and authenticated by Scientist-in-charge, Central National Herbarium, Botanical Survey of India, Kolkata, and West Bengal.

Plants Herbarium were prepared and preserved in the Department of Pharmacognosy, Haldia Institute of Pharmacy, Haldia, Purba Medinipur, and West Bengal, India.

Preparation of extract

The selected plants products extracted by maceration method. 47.50 g of dried roots powder of *Amaranthus spinosus* L. was extracted with 250 ml methanol & 60.5 g of dried roots powder of *Amaranthus viridis* L. was extracted with 300ml methanol & then the products are filtered. And then the extracts are dried and kept for further use.

Identification of Phytoconstituents by preliminary phytochemical tests

A. Test for alkaloids

a) Mayer's test

To the methanolic extract add little amount of diluted hydrochloric acid and Mayer's reagent. Then the mixture was observed for formation of any precipitate. Formation of cream color precipitated is indication of presence of alkaloids.

b) Wagner's test

To the methanolic extract add few drops of Wagner's reagent and observe for formation of any precipitate. Formation of reddish-brown precipitate is indication of presence of alkaloids.

B. Test for amino acids

a) Ninhydrin test

To methanolic extract add ninhydrin reagent and boiled. The mixture was observed for appearance of any color change. Formation of purple color is indication of presence of proteins.

C. Test for carbohydrate

a) Molish test

To small amount of methanolic extract add 1ml of alpha-naphthol and conc. Sulphuric acid was added slowly alongside of the test tube. The mixture was observed for formation of any violet ring at junction of two liquids indicates the presence of carbohydrates.

b) Seliwanoff's test (test for ketones)

To small amount of methanolic extracts add crystals of resorcinol and small amount of concentrated sulphuric acid and heat on a water-bath, rose color indicates the presence of carbohydrates.

D. Test for flavonoids

a) Shinoda test

To methanolic extracts was treated few magnesium turnings and concentrated hydrochloric acid drop wise, pink, scarlet crimson red indicates the presence of flavonoids.

b) Zinc hydrochloride test

To methanolic extracts add a mixture of zinc dust and concentrated hydrochloric acid. Formation of red color is indication of presence of flavonoids.

E. Test for Lignin

To methanolic extracts was treated with concentrated hydrochloric acid and phloroglucinol solution. Formation of pink color is indication of lignin.

F. Test for glycosides

a) Borntager's test

The extract was macerated with ether and ether layer was separated and add aqueous ammonia the mixture was observed for any color in aqueous layer is indication of presence of anthraquinone glycosides.

b) Keller-killiani test

The methanolic extract was dissolved in acetic acid containing of ferric chloride and it was transferred to a test tube containing sulphuric acid, the mixture was observed for any colour change at junction between the two liquid layers. At the junction formation of a reddish brown colour, which gradually became is indication of presence of cardiac glycosides.

G. Test for saponins

a) Haemolysis test

To methanolic extracts add 0.2 ml of solution of saponin to 0.2ml of blood in normal saline and mix well. Centrifuge and note the red supernatant. Compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2 ml of normal saline.

H. Test for mucilage

To methanolic extracts was treated with ruthenium red solution. Formation of pink colour is indication of presence of mucilage.

I. Test for Tannins

a) Ferric chloride test

To methanolic extracts was treated with ferric chloride solution, the formation of blue color is indication of presence of hydrolysable tannins and green color is indication of presence of condensed tannin.

J. Test for Proteins

a) Biuret tests

To methanolic extracts add biuret reagents. Formation of violet color is indication of presence of protein.

K. Test for Steroids and Terpenoids

a) Salkowski's test

To methanolic extract add equal volume of chloroform and concentrate sulphuric acid. The mixture was observed for appearance of color change. Formation of bluish red to cherry red color in chloroform layer and green fluorescence of the acid layer indicate the presence of terpenoids.

Antispasmodic activity

Isolated chicken ileum preparation

- The fresh chicken ileum was collected from local slaughter house (Haldia, West Bengal) & kept in Tyrode solution (NaCl, 136.7; KCl, 2.68; $MgCl_2.2H_2O$, 1.05; NaH_2PO_4, 0.42; $CaCl_2.2H_2O$, 1.80; $NaHCO_3$, 11.90; Glucose, 5.55Mm) and cleaned off the mesentery.
- The segment of 2 cm long was mounted in a 20 ml tissue organ bath and maintained at 37 ℃.
- The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min.
- Contact time of 60 sec, and base line of 30 sec time cycle were opted for proper recording. Dose response curve of acetylcholine was recorded on the kymograph first.
- Then cumulative concentration-effect curves were recorded on kymograph for Acetylcholine (100µg/ml) in absence and presence of methanolic extracts of both of *Amaranthus spinosus* L. and *Amaranthus viridis* L. (1000 µg/ml) on Kymograph by using Sherrington's Recording Drum.
- The same procedure was carried for concentration-effect curve of Ach in presence of Atropine sulphate as a standard drug.
- The percentage inhibition of extract and standard drug was calculated and graph was plotted by taking log dose versus height of response curve.

Result and Discussion

Phytochemical Evaluation

The preliminary phytochemical tests of methanolic extract (roots) of *Amaranthus spinosus* L. indicates the presence of alkaloids, amino acids, carbohydrates, flavonoids, lignin, glycosides, saponins, tannins, proteins, steroids, and terpenoids. The methanolic extract of (roots) of *Amaranthus viridis* L. indicates the presence of alkaloids, amino acids, carbohydrates, flavonoids, saponins, tannins, proteins, steroids, and terpenoids and absence of lignin and glycosides.

Table 1: Result of preliminary phytochemical tests of methanolic extract of *Amaranthus spinosus* L. and *Amaranthus viridis* L. for the presence of various metabolites:

Type of phytochemical constituent	Name of the tests	Methanolic extract of *Amaranthus spinosus* L.	Methanolic extract of *Amaranthus viridis* L.
Test for alkaloid	Mayer's test	Present	Present
	Wagner test	Present	Present
Test for carbohydrate	Molish's test	Present	Present
	Seliwanoff's test	Present	Present
Test for glycosides	Brontrager's test	Present	Absent
	Killer- killani test	Present	Absent
Test for saponins	Haemolysis test	Present	Present
Test for phenol	Ferric chloride test	Present	Present
Test for tannin	Ferric chloride test	Present	Present
Test for flavonoids	Shinoda test	Present	Present
	Zinc hydrochloride test	Present	present
Test for amino acids	Ninhydrin test	Present	Present
Test for protein	Biuret test	Present	Present
Test for lignin	Weisner test	Present	Absent
Test for steroids and terpenoids	Salkowski test	Present	Present

Extraction of roots

The dried roots of *Amaranthus spinosus* L. and *Amaranthus viridis* L. were powdered and subjected to extraction using maceration method.

After extraction the percentage yield of each extract was calculated with reference to air dried drug used in study. The percentage yield of the extract was tabulated below:

Table 2: The percentage yield of the extract.

Sl. No.	Plant name	Solvent used	Percentage yield (% w/w)
1.	*Amaranthus spinosus* L.	Methanol	6.52
2.	*Amaranthus viridis* L.	Methanol	4.46

Pharmacological screening

Antispasmodic study

Table 3: Dose response relationship observations of Ach, methanolic extract of *Amaranthus spinosus* L. and *Amaranthus viridis* L. and atropine on chicken.

Sl. No.	Drug/Sample	Dose(concentration)	Height of response	Log dose
1.	Acetylcholine	0.1 ml (10 µg)	0.2 cm	1
2.		0.2 ml (20 µg)	0.4 cm	1.3
3.		0.4 ml (40 µg)	1.0 cm	1.6
4.		0.8 ml (80 µg)	1.5 cm	1.9
5.	Atropine +Acetylcholine	0.1 ml+0.1 ml (20 µg)	0.2 cm	2
6.		0.2 ml+0.2 ml (40 µg)	0.0 cm	0
7.		0.4 ml+0.4 ml (80 µg)	0.0 cm	0
8.		0.8 ml+0.8 ml (160 µg)	0.0 cm	0
9.	*Amaranthus spinosus* L. + Acetylcholine	0.1 ml+0.1 ml (20 µg)	0.2 cm	2
10.		0.2 ml+0.2 ml (40 µg)	0.3 cm	2.6
11.		0.4 ml+0.4 ml (80 µg)	0.5 cm	3.2
12.		0.8 ml+0.8 ml (160 µg)	1.2 cm	3.8
13.	*Amaranthus viridis* L. + Acetylcholine	0.1 ml+0.1 ml (20 µg)	0.2 cm	1
14.		0.2 ml+0.2 ml (40 µg)	0.1 cm	0
15.		0.4 ml+0.4 ml (80 µg)	0.05 cm	0
16.		0.8 ml+0.8 ml (160 µg)	0 cm	0

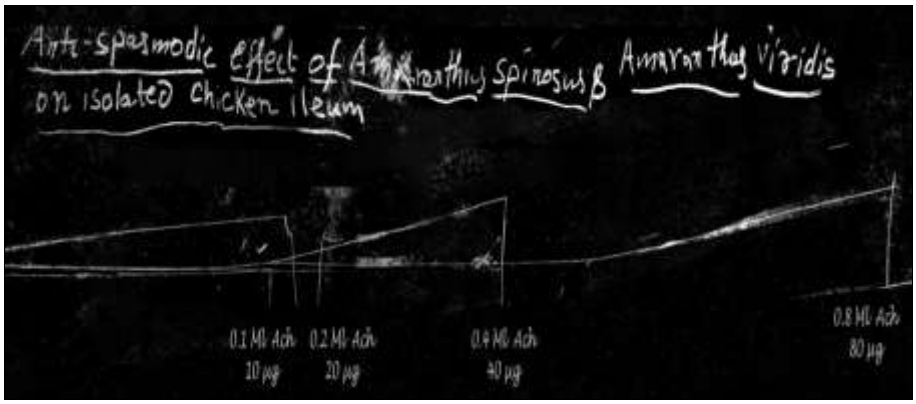

Fig 1: Dose response curve of Ach

Effect of Ach on chicken ileum reflected an increase in spasmodic activity (response) with an increase in dose.

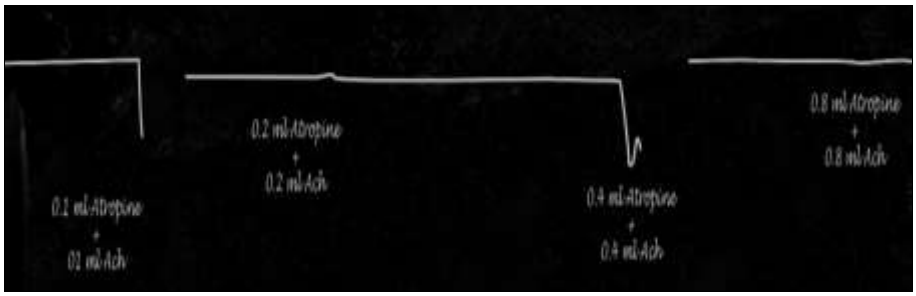

Fig 2: Dose response curve of Ach and atropine

Effect of atropin on chicken ileum reflected a decrease in spasmodic activity (response) with an increase in the dose.

DRC of Ach in presence of atropine was taken as shown in figure 6.

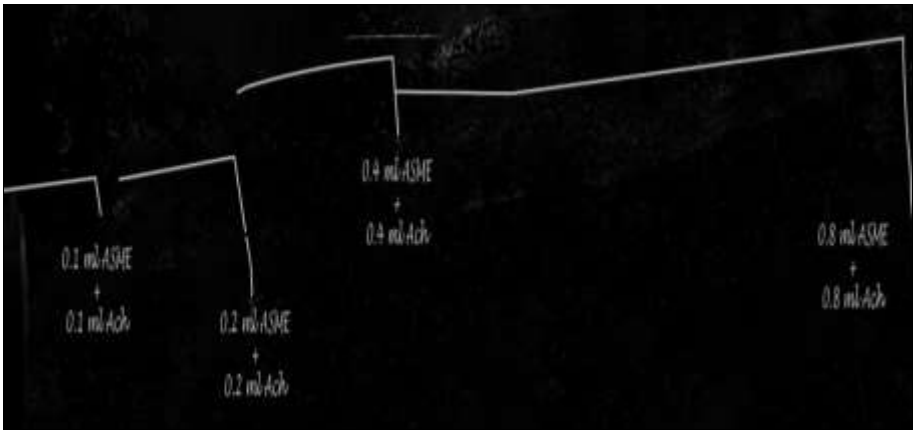

Fig 3: Response curve of Ach + methanolic extract of *A. spinosus* L.

Ach induced spasm with an increase in spasmodic activity (Response) with an increase in dose followed by the treatment of methanolic extract of *Amaranthus spinosus* L. as show in the figure 7.

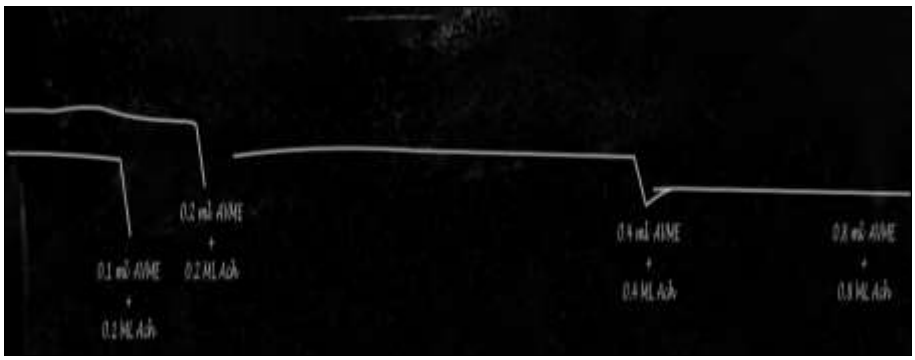

Fig 4: Response curve of Ach + Methanolic extract of *A. viridis* L.

Result were compared with standard drug atropine, an anti-spasmodic drug. There is dose dependent increase in antispasmodic activity is seen in the 4th treatment is greater than the 3rd treatment (*A. spinosus* L.).

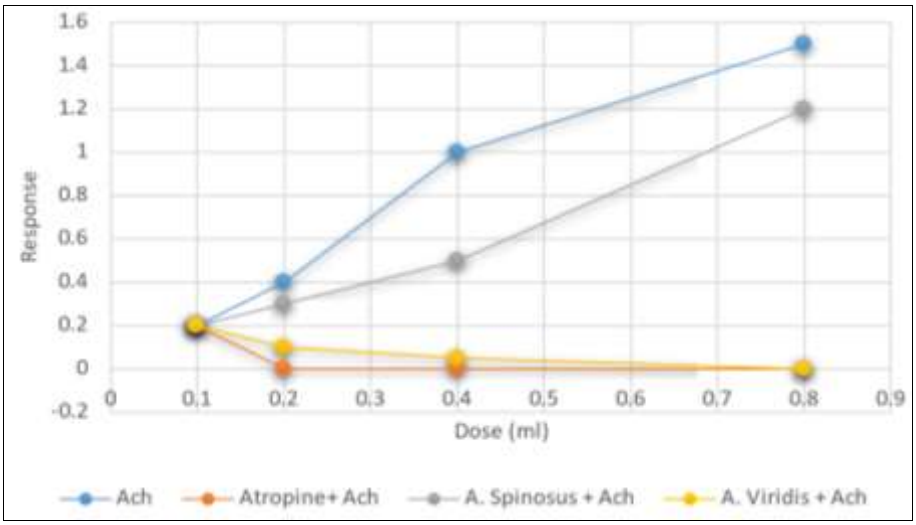

Fig 5: Comparative dose response relationship of Ach, Methnolic extract of *Amaranthus spinosus* L. and *Amaranthus viridis* L., and atropine on chicken ileum

The graph shows the dose dependent increase in spasm in presence of acetylcholin whereas *A. spinosus* L. showed significant increase in spasm (spasmodic activity) but *A. viridis* L. showed significant decrease in spasm (antispasmodic) induced by Ach. It is compared with spasmodic and antispasmodic effect produced by std. drug Ach & Atropine, respectively.

Conclusion

The root of *Amaranthus spinosus* L. & *Amaranthus viridis* L. were collected, authenticated, dried and subjected to extraction by maceration method using methanol solvent. The phytochemical screening of root of *Amaranthus spinosus* L. indicates the presence of alkaloids, carbohydrates, glycosides, saponins, phenol, tannin, flavonoids, amino acids, protein, lignin, steroids, terpenoids

and *Amaranthus viridis* L. indicates the presence of alkaloids, carbohydrates, saponins, phenol, tannin, flavonoids, amino acids, protein, steroids, terpenoids.

The research work was carried out in chicken ileum for both plants (*Amaranthus spinosus* L. & *Amaranthus viridis* L.) & it was concluded that the methanolic extract of *Amaranthus spinosus* L. root has significant spasmodic effect nearer to the standard and *Amaranthus viridis* L. root has promising antispasmodic effect as compared to standard.

The pharmacological effect may be occurring due to presence of one or more than one phytoconstituents but which phytoconstituents exactly responsible for the same can be evaluated in further research work.

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Conflict of interest

The authors declare that they have no conflicts of interests.

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