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Antimicrobial activity of *Amaranth* Alkaloid against pathogenic microbes

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Abstract
Plant defensive metabolite Alkaloid were screened for analyzing their antimicrobial property. The present study quantified the alkaloids present in selected members of tribe Amarantheae. Maximum percentage of alkaloids are shown by *Amaranthus tricolor* L., *Aamaranthus viridis* L. and *Amaranthus caudatus* L. (8- 8.8%) and the least were shown by *Amaranthus spinosus* L. and *Amaranthus dubius* Mart. (5.8-6%). Antibacterial and antifungal studies were carried out using the precipitated alkaloid, which showed zone of inhibition ranges from 6 to 11mm. Thus the present study showed that isolated alkaloids can show antimicrobial activity and used for the formulation of drugs or antiseptics.

Keywords: Amarantheae, alkaloids, antimicrobial property

1. Introduction
In Ayurveda many medicinal plants has crucial role in blood purification, wound healing property and widely used in vitiated condition of vadha, pitta and kapha. Alkaloids are major secondary metabolite produced by plants and utilize it for defensive mechanism. Thus in the present study quantification of alkaloids are done and analysed for their antimicrobial property. In the field of medicine, alkaloids are part of every medicinal scientist's resources and play an important role in treating diverse diseases. They are also a vital part of the successful regimens that have led to major therapeutic triumphs in chemotherapy. In pharmaceutical sciences, they serve as raw materials in the formulation of new and effective drugs. Consumption of vegetables is closely related with the decrease risks of diseases that resulted from oxidative stress, including cancer, diabetes and various infectious diseases [13]. Amaranth family composed of weedy plants, but they have got medicinal importance for the treatment of different ailments associated with human body. The plants are alexeteric, laxative, diuretic, stomachic, antipyretic, febrifuge, galactagogue, appetise and tonic. It is useful in vitiated conditions of pitta, burning sensation, leprosy, eczema, bronchitis, burns, boils, nausea and anaemia [16].
Akaloids usually have pharmacological effects and are used in medicines or as recreational drugs. Thus they are very useful pharmaceutical agents because of their biological activities [7, 11] such as antimicrobial [5], antioxidant [1] analgesic potential and anti-inflammatory activities [3]. The plants selected for the present study include the *Amaranthus spinosus L., Amaranthus Caudatus* L., *Amaranthus tricolor* L., *Amaranthus dubius* Mart., and *Amaranthus viridis* L. for investigation of valuable bioactive compounds especially quantification of alkaloids and possible utilization of the available species of Amaranthaceae growing in Kerala.

2. Materials and Methods
2.1 Chemicals and Reagents
Gentamycin(10 µg/disc), Chloramphenicol(30 µg/disc) and Fluconazol (20 µg/disc) along with media like Himedia Agar, Nutrient broth and Potato dextrose agar were obtained from Alpha chemicals, Kerala, India. Bacterial and fungal strains were purchased from Microbiology Department, St Xavier's College, Aluva, India. Other chemicals used were of analytical or HPLC grade.

2.2 Instrumentation
Laminar air flow chamber of model LABLINE companies were used for the subculturing of microbes and incubator model Rotex RJSS 10 AC.

2.3 Plant material
The plant materials were collected from in and around places of Kerala, India from the month of
January to June 2015. The plants were authenticated and identified. Leaves was washed properly with tap water to remove soil and other dirt's, dried in shade at room temperature for two weeks and then grinded to powder. The powder of the *Amaranthus species* was passed through a 0.5 mm metallic mesh to yield crude powder for the use of phytochemical investigations.

2.4 Phytochemical screening
Phytochemical screening for the methanolic extract of plants were done to detect the presence of Tannins, alkaloids, glycosides, flavonoids, and phenolic was performed using standard procedures [8].

2.5 Estimation of Alkaloids
Two grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol were added. The mixture was covered and allowed to stand for four hours. Then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide solution was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was then filtered, washed with dilute ammonium hydroxide solution, dried and weighed. The content of alkaloids was determine with the following formula [6].
Alkaloids (%) = weight of precipitate/ weight of sample X100.

2.6 Antimicrobial activity
2.6.1. Microbial strains: *Staphylococcus aureus, Escherichia coli* and *Bacillus substilis, Klebsiella pneumoniae* together with *Rhizopus oligosporus*, *Aspergillus niger* and *Aspergillus flavus* were used as microbial strains. These bacterial and fungal strains were cultured at 37 °C and 28 °C overnight in an incubator.

2.6.2. Disc diffusion method: The antimicrobial activity of the prepared extracts was determined by using disc diffusion method [9]. The inoculated extracts were then examined for inhibition zones (in mm) by zone reader, which indicates antimicrobial activity. The discs (4 mm in diameter) were impregnated with 20 µg/m, sample extracts (20 µg/ disc) and placed on inoculated agar. Gentamycin, chloramphenicol and Fluconazol (20 µg/disc) (Oxiod) were used as positive reference for bacteria and fungi, respectively.

3. Result and Discussion
The preliminary phytochemical analysis of the methanolic extracts of *Amaranthus* species showed positive result with different test of alkaloids (Table 1).Thus they were known to show medicinal activity as well as exhibiting physiological activity [15].

Table 1: Qualitative analysis for Alkaloids

Names of plant	Mayer's test	Dragendorff's reagent
Amaranthus dubius Mart.	Yellow	Yellowish brown
Amaranthus caudatus L.	Yellow	Yellowish brown
Amaranthus spinosus L.	Yellow	Yellowish brown
Amaranthus tricolor L.	Greenish yellow	Brown
Amaranthus viridis L.	Greenish yellow	Brown

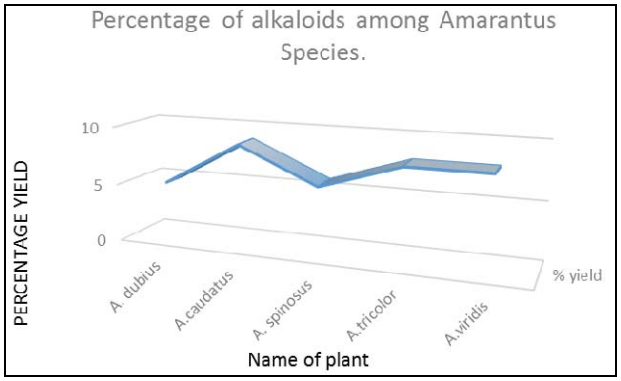

Fig 1: Comparison of Alkaloid content *Amaranthus* species.

Table 2: Antibacterial activity of *Amaranthus* alkaloid extract.

Name of plant	*Staphylococcus aureus*	*Escherichia coli*	*Bacillus substilis*	*Klebsiella pneumoniae*
Amaranthus dubius Mart.	6±0.50	8±0.64	ND	7±0.51
Amaranthus caudatus L.	7±0.68	11±0.5	6±1.01	9±0.86
Amaranthus spinosus L.	7±0.50	ND	8±0.26	ND
Amaranthus tricolor L.	7±0.70	11±0.51	6±0.85	5±0.72
Amaranthus viridis L.	ND	7±1	6±0.90	ND

Triplicate value of zone of inhibition is recorded (mm), ND-not detected.

Table 3: Antifungal activity of *Amaranthus* alkaloid extract.

Name of plant	*Rhizopus stolonifera*	*Aspergillus niger*	*Aspergillus flavus*
Amaranthus dubius Mart.	ND	ND	6±0.35
Amaranthus caudatus L.	ND	6±0.95	5±0.57
Amaranthus spinosus L.	ND	ND	6±0.76
Amaranthus tricolor L.	ND	6±0.71	ND
Amaranthus viridis L.	6±0.85	ND	ND

Triplicate value of zone of inhibition is recorded (mm), ND- not detected.

Maximum percentage of alkaloids are shown by *A. tricolor, A. viridis* and *A. caudatus* (8- 8.8%) when compared to other *Amaranthus* species (Fig.1).And this investigation supports the similar work done, showing that higher alkaloid content was seen in leafy vegetables like *Bryophyllum pinnatum, Aspilia africana* and *Cleome Rutidosperma* consumed in Nigera [14, 4]. From the antibacterial and antifungal studies, alkaloids extracted from *Amarantus* species showed good activity against bacteria than fungal strains. Especially bacterial strain *Escherichia coli* and the fungal strain *Aspergillus flavus* showed more zone of inhibition than other strains (Table 2 and 3). Thus similar works showed that the alkaloids present in the plant extract are used to treat psoriasis, chronic skin eruptions and chronic rheumatism and

also may show a good antibacterial activity against *Staphylococcus epidermidis, Klebsiella pneumonia, and Escherichia coli* [10]. The current result supports the earlier findings which demonstrate the presence of antimicrobial activity in Amaranthaceae [12, 2]. Thus the selected plants of the tribe Amarantheae has got potent antimicrobial activity against human pathogenic microbes.

4. Conclusion

The present study on the tribe Amarantheae throw the light on the alkaloid content of the plants exhibit antibacterial and less antifungal activity. Due to the adverse effects of synthetic antibiotics and anticancer agents, in recent years scientists are on search for alternative medicine. There are some diseases which are chronic and needs a long duration medication, plant based drugs are less toxic and have no side effects. The main purpose of the present work emphasis on the extraction of alkaloids from the crude extract of five *Amaranthus* species. Though these plants are considered as underutilised0 plant drugs, more study need to be worked out to isolate the active component in this tribe Amarantheae. Thus present finding showed that alkaloid content of *Amaranthus* species possess the antimicrobial activity and can be used as an antiseptic product by undergoing further investigation.

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