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IN VITRO EVALUATION OF ANTIMICROBIAL AND BRINE SHRIMP LETHALITY BIOASSAY OF DECOCTION OF *AMARANTHUS TRICOLOR*

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ABSTRACT
The decoction of the leaves of *Amaranthus tricolor* was set for evaluating antimicrobial and cytotoxic activities. With the help of Kanamycin (30µg/disc) used as positive control we evaluated the antimicrobial activity of the decoction by using disk diffusion method. We achieved different degrees of susceptibility of the microorganisms with this decoction. Among 17 different types of microorganisms *Bacills sereus, Salmonella paratyphi, Vibrio parahaemolyticus, Salmonella typhi, Candida albicans* showed more activity with the extract. Investigation for evaluating cytotoxic activity of the decoction was done by examining the lethality of brine shrimp nauplii. Vincristine sulphate was used as positive control in this experiment. We observed LC50 as 11.02 µg/ml and 0.32 µg/ml from the extract of *Amaranthus tricolor* and vincristine sulphate repectively. It can be suggested form the results of study that decoction of *Amaranthus tricolor* may be used for treating different types of bacterial and fungal infections. It further indicates that cytotoxic compound may be searched from the extract of *Amaranthus tricolor* in future investigations.

KEYWORDS: *Amaranthus tricolor*, Antimicrobial, Kanamycin, Vincristine.

INTRODUCTION
As natural compounds possess few side effects, it is always getting higher preference for treating different diseases in human.[1] A good number of diseases are still being treated with the help of different drugs available in the nature.[2] People from different regions of the world still dependent on drugs from the natural sources for their first treatment.[3] Formulation of herbal drugs is increasing in the market day by day that indicates its popularity among people as it exhibit better safety and more efficacies.[4]

Amaranthus tricolor belongs to the family Amaranthaceae which have approximately 60 different species.[5] It is spread in many regions of the world specially tropical and subtropical countries.[5] Many species of Amaranthus are eaten as leafy vegetables in various parts of the world because of its easy availability and comparatively lower price.[5] It has been reported in previous studies to be a rich source of carotenoids, vitamin C, dietary fiber, protein etc. as well as different minerals like iron, magnesium calcium and zinc.[6,7] carotenoids, ascorbic acid, flavonoids and phenolic acids etc. were also found in some species of Amaranthus.[8] Various studies reported Amaranthus to have anticancer, antioxidant, and anti-inflamatory properties.[8-11]

As far we came to know, no study has been conducted yet intending to evaluate the different biological activities of the decoction of *Amaranthus tricolor*. Hence we designed this study to investigate the antimicrobial and brine shrimp lethality assessment of the decoction of *A. tricolor.*

MATERIALS AND METHOD
A. tricolor had been collected from various parts of Bangladesh during 2007-2008 and it was then authenticated by Bangladesh National Herbarium. The decoction of this plant was made according to the protocol mentioned in the article by Islam et al, 2014.[12] In this decoction 1ml solution contains 1 g equivalent powder. Antimicrobial study was conducted following the method described in Zoysa et al, 2019[13] where kanamycin (30µg/disc) was used as positive control (standard). By following our previous method[14] slight modified form of Apu et al., 2010,[15] we carried out our assessment of brine shrimp lethality (cytotoxicity). In this experiment we used Vincristine sulphate as positive control (standard).

RESULTS

In the experiment of antimicrobial assessment we used the following microorganism among the gram positive, gram negative bacteria and fungi.

Gram Positive Bacteria: *Bacillus subtili, Bacillus megaterium, Staphylococcus aureus, Sarcina lutea, Bacills sereus.*

Gram Positive Bacteria: *E.coli, Pseudomonas aeruginos, Vibrio mimicus, Vibrio parahaemolyticus, Pseudomonas aeruginos, Salmonella paratyphi, Shigella boydii, Shigella dysentriae, Shigella boydii, Salmonella typhi.*

Fungi: *Saccharromyces cerevaceae, Candida albican, Aspergillus niger.*

In our investigation, we observed *Bacills sereus, Salmonella paratyphi, Vibrio parahaemolyticus, Salmonella typhi, Candida albicans* to be more sensitive to the decoction (Table 01).

Table 01: Antimicrobial activity of decoction of *A. tricolor.*

Test Organisms	Zone of inhibition in mm (mm) ± SD	
	Decoction (400µg/disc)	Kanamycin (30µg/disc)
Gram (+) ve bacteria		
Bacillus megaterium	10±0.2	30±0.4
Bacillus subtilis	10±0.4	29±0.5
Sarcina lutea	11±0.3	30±0.6
Staphylococcus aureus	10±0.4	31±0.4
Bacills sereus	12±0.3	30±0.6
Gram (-) ve bacteria		
E.coli	11±0.4	30±0.4
Pseudomonas aeruginosa	10±0.5	29±0.5
Salmonella paratyphi	12±0.3	29±0.4
Shigella boydii	11±0.4	30±0.3
Shigella dysentriae	11±0.3	29±0.3
Vibrio mimicus	11±0.4	30±0.4
Vibrio parahaemolyticus	13±0.4	30±0.5
Salmonella typhi	12±0.3	31±0.6
Fungus		
Aspergillus niger	11±0.4	30±0.4
Saccharromyces cerevaceae	11±0.3	31±0.5
Candida albicans	13±0.2	30±0.4

Table 02: Brine shrimp lethality bioassay of decoction of *A. tricolor.*

Plant Extract (Decoction)				Vincristine Sulphate			
Conc. (µg/ml)	Log C	% mortality	LC_{50} (µg/ml)	Conc. (µg/ml)	Log C	% mortality	LC_{50} (µg/ml)
800	2.903	100		80	1.903	100	
400	2.602	100		40	1.602	100	
200	2.301	90		20	1.301	100	
100	2.000	80		10	1.000	100	
50	1.698	70		5	0.699	90	
25	1.398	60	**11.02**	2.5	0.398	80	**0.32**
12.5	1.097	50		1.25	0.097	70	
6.25	0.796	40		0.63	−0.201	60	
3.13	0.495	35		0.31	−0.509	50	
1.56	0.194	30		0.16	−0.796	30	
0.78	−0.108	25		0.078	−1.108	20	

In the experiment of evaluating brine shrimp lethality we got the LC50 from the extract of *Amaranthus tricolor* as 11.02 µg/ml where as vincristine sulphate exhibits the LC50 as 0.32 µg/ml.

DISCUSSION

People are dependent on drugs from natural sources for their treatment from the beginning of human civilization. Previous studies claimed that a large portion of modern drugs are directly linked to the natural origin.[16,17] Natural agents get more preference in treatment because of their fewer side effects and more treatment benefits.[18]

Various plants were reported in earlier studies to possess bioactive compounds having antibacterial and other antipathogenic activities.[19,20] In vitro activity assessment is the preliminary step in searching the proper molecules from the plant extracts that play the key role in producing the antimicrobial activity.[21]

The present study was designed to assess the decoction of *A. tricolor* whether it can produce antimicrobial activity or not. In the experiment, we observed significant inhibitory activity of the decoction against different pathogenic organisms. There was a variation in the activities against different microorganisms. Some were more sensitive than others. The heterogenecity in the activities may be contributed by the difference in the morphological structures of the different bacteria and fungi, interference of the diffused substances, diffusion capacity of substances in the medium, growth and metabolic activity of the organisms etc.[22,23] In spite of this variation, different grades of antimicrobial activities found in this study clearly indicate the possible presence of bioactive compounds having antipathogenic properties.[24,25]

The molecular mechanisms though which the antimicrobial activities are exhibited had been reported to be complex and series of steps are involved here. The mechanisms might be contributed by the interference of cell wall synthesis, cell membrane synthesis, DNA synthesis, protein synthesis or inhibition of the metabolism of microorganisms. However, it needs furthers extensive investigation to find out the exact mechanism.[26] The bioassessment of brine shrimp lethality is considered as primary tool for screening of plant materials. The toxicity to the brine shrimps indicates the possible presence of cytotoxic compounds in the tested materials.[27] The brine shrimp lethality bioassay (BSLB) is widely used tool in the preliminary screening of the plant extracts and isolated compounds in evaluating the toxicity to brine shrimps which might suggest probable cytotoxic activities of the materials tested.[27] In our investigation we got moderate cytotoxic activity against brine shrimps produced by the decoction of *A. tricolor*. The LC50 of the decoction was 11.02 µg/ml where as the vincristine sulphate showed the LC50 as 0.32 µg/ml. The cytotoxicity produced by plant material observed through *in vitro* study paves the way for evaluating anticancer activity with *in vivo* study.[28] As brine shrimp lethality bioassay is the primary screening, it is recommended that further target oriented examination is required for isolating the biologically active anticancer agents with the help of animal model.

CONCLUSION

Finally, we got significant inhibitory activity against some microorganism by the decoction of A. tricolor which suggest us to go for further research to confirm the results as well as to identify and isolate the molecules showing these activity. It may drive us to search for broad spectrum novel antibiotics that may be used in the treatment of different life threatening infections caused by resistant microorganisms. We also got encouraging results in our assessing brine shrimp lethality by the decoction we studied that also recommend us to go for further investigation with the help of biological model.

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