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Research Article

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Anticancer Activity of Whole Plant Ofamaranthus Tricolor Linn. on Breast Cancer Cell Lines

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ABSTRACT

The present research work was conducted to investigate an anticancer activity of aqueous and ethanolic extract of whole plant of Amaranthus tricolor Linn. on breast cancer cell line. Ethanolic extracts of dried plant was obtained by soxhlet extraction method and aqueous extracts was obtained by maceration method using ethanolic and aqueous solvents respectively. The obtained extracts were dried and used for investigation of anticancer activity. Anticancer activity of ethanolic and aqueous extracts was investigated by using MCF-7 and MDA-MB-231 breast cancer cell lines. Cytotoxicity of obtained extract was investigated by using MTT assay, neutral red assay and trypan blue assay methods. MTT assay and neutral red assay showed that ethanolic extract was having more cytotoxic activity than that of the aqueous extract were as the results of trypan blue assay method showed that ethanolic and aqueous extract of whole plant have ability to kill viable cells of MCF-7 and MDA-MB-231 cell lines and it was observed that ethanolic extract have ability to show significantly more non-viable cell count than that of aqueous extract. These results suggest that aqueous and ethanolic extract of whole plant of Amaranthus tricolor Linn. Has potential anti-cancer activity.

Keywords: Anticancer activity; Breast cancer; Extraction; MTT; Neutral red; Trypan blue assay

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INTRODUCTION

Cancer is the second leading cause of death after cardiovascular disease worldwide [1]. During last decades of the century, medicinal researchers have developed new methods for cancer treatment by combining surgery with chemotherapy, radiations, and various phytochemicals obtained from different plants species [2]. Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. These changes produce proteins that disrupt the delicate cellular balance between cell division and, resulting in cells that keep dividing to form cancer [3]. It is caused by a complex interplay of genetic and environmental factors [4]. The most commonly diagnosed cancers cases are lung (1.35 million), breast (1.15 million), and colorectal (1 million). In India every year about 8,50,000 new cancer cases being diagnosed, and about 5,80,000 cancer related deaths every year [5,6]. Worldwide, Breast cancer comprises 22.9% of all cancers in women [7]. It is the most common invasive cancer in women develops in the breast tissue. Hence treatment of cancer plays a very important role now days [8].

Cancer treatment mainly involves Surgery, Radiation therapy, Hormonal therapy, Targeted therapy and Chemotherapy [9] and almost all the above treatments are associated with side effects such as anaemia, bone marrow depression, hair loss, gum bleeding, constipation, diarrhoea, fatigue, infertility, nausea and vomiting, muscles and joint pain, headache, fever and chills [10]. Many synthetic chemicals and drugs are available in market and they used to show above common side effects and it was observed that many plant products play an important role in the treatment of cancer and they are available in many Ayurvedic preparations in market with lower risk of above side effects. Hence in the present research work an attempt has been made to extract active constituents from plant and to investigate it for its anticancer activity for treatment of breast cancer. In order to investigate the anticancer activity of extract In-Vitro methods such as MTT (Methyl- Thiazolyl Tetrazolium) assay, trypan blue assay has been adopted and breast cancer cell lines such as MDA-

MB-231 and MCF-7 were used to investigate activity [11,12]. The plant Amaranthus tricolor Linn. is selected for the present work [13-15].

Plant information

Table 1: Plant Information

Name	Amaranthus tricolor Linn.
Synonym	Amaranthus gangeticus Linn, Amaranthus melancholicus Linn, Amaranthus Polygamus Linn, Amaranthustristic Linn
Vernacular names	Lal sag (Hindi), Dantu (Kannada), Red Amaranth(Eng), Lal bhajee (Marathi)
Biological source	It consist of whole plant of Amaranthus tricolor Linn.
Family	Amaranthaceae.
Morphology	Plant height- 1 to 4 feet and leaf arrangement- alternate Root habit – the plant forms a strongly branched tap root Foliage – leaf colour- yellow, purple, or red variegated Flower are red in colour.
Chemical constituents	• Carbohydrate -free sugar, glucose and starch • Flavonoids -betacyanine, amaranthin, and quercetin, • Protein and amino acids -proline, cysteine, tryptophan, leucine, glutamic acid arginin, lysine, histidine methinone. • Steroids -spinasterol, B-sitosterol, fatty acid • Tannins and minerals like- Calcium, ferrous, magnesium, zinc, copper, potassium, and vitamine A, B6, C, riboflavin and ascorbic acid flavonoids and phenolic acid are present.

EXPERIMENTAL SECTION

Materials

Plant material collection: The plant of *Amaranthus tricolor* Linn. was collected from local areas of Belgaum, district in Karnataka state and it was authenticated by Dr. Harsh Hedge, taxonomist of regional Medical research Centre, ICMR Belgaum. Accession No. RMRC-1337.

Equipment and apparatus: 96 wells polypropylene plate, ELISA reader, Cell culture bottles, Neubauer chamber, Micro pipettes, inverted microscope, incubator, Deep freezer etc.

Reagents: MTT reagent, trypan blue solution, Dulbecco's minimum essential media, phosphate buffer solution, sodium bicarbonate, neutral red solution.

Cell lines details:

Table 2: Cell details

Cell line	Morphology	Species	Supplier
MCF-7	Epithelial	Human	NCCS, Pune
MDA- MB 231	Epithelial	Human	NCCS, Pune

Methods

Preparation of plant extracts: a) Ethanolic Extraction: The shade dried parts of plant Amaranthus tricolor Linn, was crushed to get course powder and subjected for soxhlet extraction process by using ethanol as solvent**.**

b) Aqueous extraction: Aqueous extraction of plants was carried out by maceration method by using chloroform water IP as solvent.

After preparations of both extracts they are subjected for drying in dissector and physicochemical analysis was performed by determining extractive values of extract, moisture content and qualitative test for carbohydrates, glycosides, protiens, steroids, alkaloids and phenolic compounds and then subjected for investigation of its anticancer activity.

***In-vitro* Anticancer Activity Methods**

In order to investigate In-vitro anticancer activity, MTT assay, neutral red assay and trypan blue assay were performed and details of method are as follows:

MTT assay: 100µl of cell suspension in each well with 100 µl of Dulbecco's minimum essential media was taken and incubated for 48 hours at 370C. Washed with phosphate buffer solution in order to remove excess media and then added 10, 20, 25, 30 and 50 µg/ml of sample in the respective wells and kept for incubation for 24hours at 370C and added 10µl of MTT reagent in each well. Incubated for 2 hours at 37°C and measured an absorbance at 490nm in microlitre plate reader. The percentage of cytotoxic activity was calculated by using following formula.

$$\% \text{ cytotoxicity} = \frac{\text{optical density of test compound}}{\text{optical density of control}} \times 100$$

Neutral red assay: 100µl Dulbecco's minimum essential media and 100 µl cell suspension was taken in each wells and incubated for 48 hours 370C. After incubation period wells was removed from incubator and washed with phosphate buffer solution and added 10, 20, 25,30 and 50 µg/ml of sample in each wells. Incubated for 24 hours at 370C and added 150µl of neutral red solution and again incubated for 1hour at 370C and optical density was measured at 490nm by using ELISA reader. The percentage of cytotoxic activity was calculated by using following formula.

$$\% \text{ cytotoxicity} = \frac{\text{optical density of test compound}}{\text{optical density of control}} \times 100$$

Trypan blue assay: A suspension of approximately 1×106 cells/ml was prepared and a 1:1 mixture of the cell suspension and the 0.4% trypan blue solution was prepared separately and applied 15µl of cell suspension to the edge of the chamber between the cover slip and the V- shaped groove in the chamber. Allowed the cell suspension to be drawn into the chamber by capillary action and number of viable and non-viable cells was counted by using microscope. The percentage of viable cells was calculated by using following formula

$$\%\text{viable cells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

RESULTS AND DISCUSSION

The characteristic features of ethanolic and aqueous extracts of Amaranthus tricolor Linn. was tabulated in Table 3.

Table 3 : Features of *Amaranthus tricolor Linn*. extracts.

S. No.	Extracts	Colour	Odour	Extractive value
1.	Ethanol	Green	Characteristic	6% w/w.
2.	Aqueous	Brown	Characteristic	5.3% w/w.

The cytotoxic activity of ethanolic and aqueous extract was performed by using MTT assay on the both cell lines (IC50 on MDA-MB 231 cell line and IC50 of MCF-7 cell line) and results of maximum cytotoxic activity and its concentrations was tabulated in Table 4 and graphs was plotted and showed in Figures 1-4.

Table 4: Concentration of maximum % cytotoxicity by MTT assay

Type of cell line	Concentration of maximum cytotoxicity	
	Ethanolic Extract	Aqueous Extract
MDA-MB 231	22.73µg/ml	24.93µg/ml
MCF-7	23.23µg/ml	24.21µg/ml

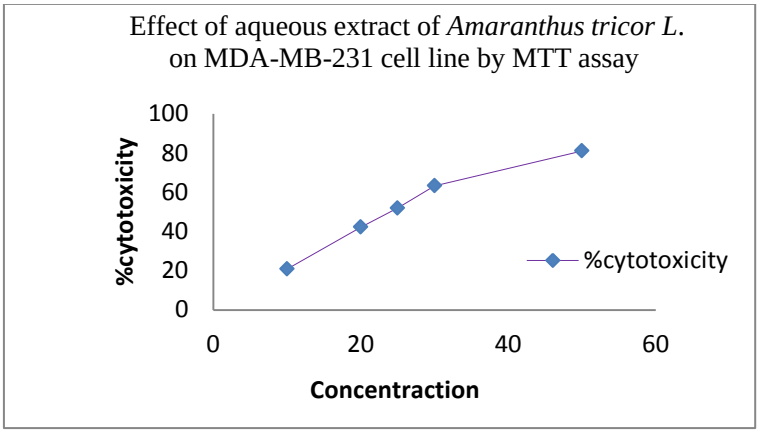

Figure 1: The effect of aqueous extracts on MDA-MB 231cell lines.

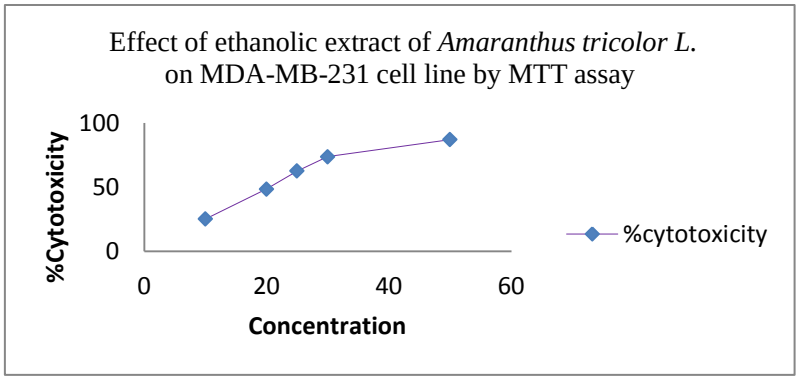

Figure 2: The effect of Ethanolic extracts on MDA-MB 231cell lines.

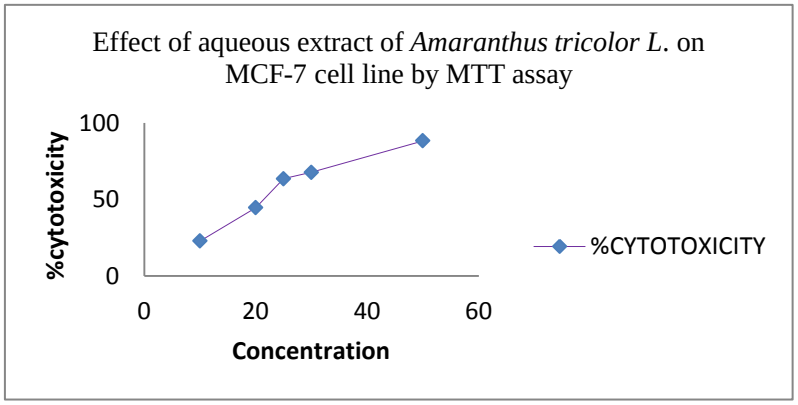

Figure 3: The effect of Aqueous extracts on MCF-7 cell lines.

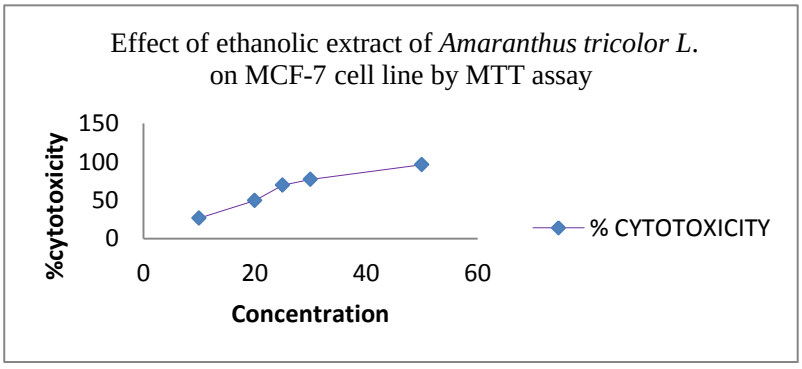

Figure 4: The effect of Ethanolic extracts on MCF-7 cell lines.

The cytotoxic activity of ethanolic and aqueous extract was performed by using neutral red assay method on the both cell lines (IC50 on MDA-MB 231 cell line and IC50 of MCF-7 cell line) and results of maximum cytotoxic activity and its concentrations was tabulated in Table 5 and graphs was plotted and showed in Figures 5-8..

Type of cell line	Concentration of maximum cytotoxicity	
	Ethanolic Extract	Aqueous Extract
MDA-MB 231	24.35µg/ml	24.75µg/ml
MCF-7	24.11 µg/ml	27.74 µg/ml

Table 5 : Concentration of Maximum % cytotoxicity by MTT Assay

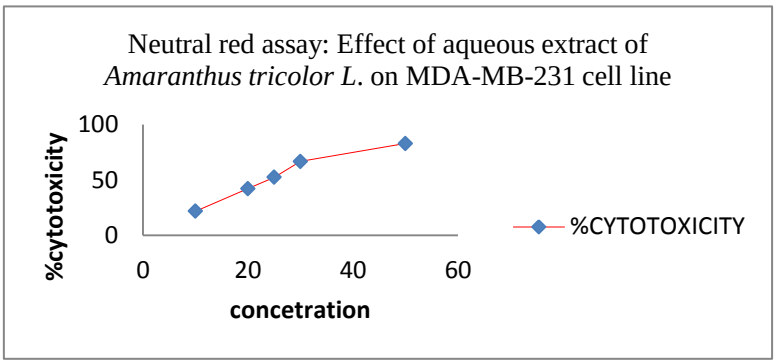

Figure 5: The effect of Aqueous extracts on MDA-MB-231 cell lines.

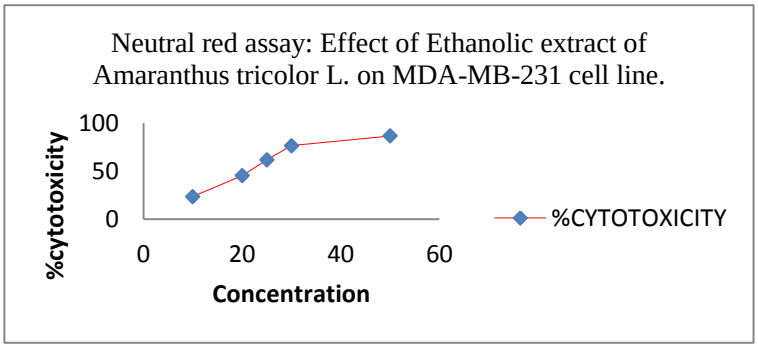

Figure 6: The effect of Ethanolic extracts on MDA-MB-231 cell lines

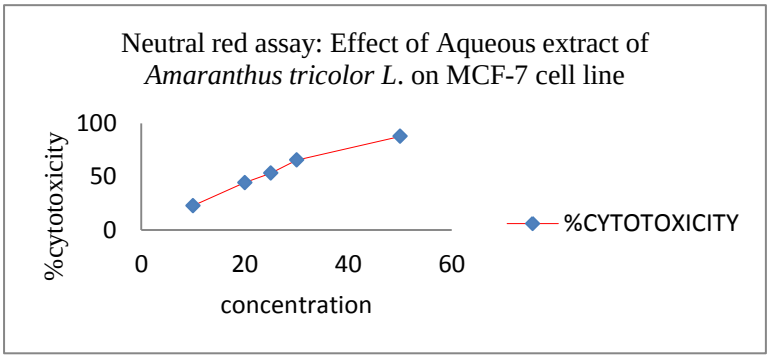

Figure 7: The effect of Aqueous extracts on MCF-7 cell lines.

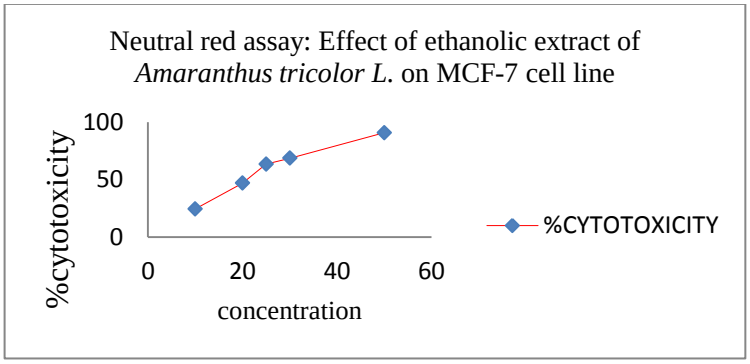

Figure 8: The effect of Ethanolic extracts on MCF-7 cell lines.

The cytotoxic activity of ethanolic and aqueous extract was performed by using trypan blue assay method on the both cell lines (IC50 on MDA-MB 231 cell line and IC50 of MCF-7 cell line) and % of non viable cells were calculated and graphs was plotted showed in Figures 91-2.

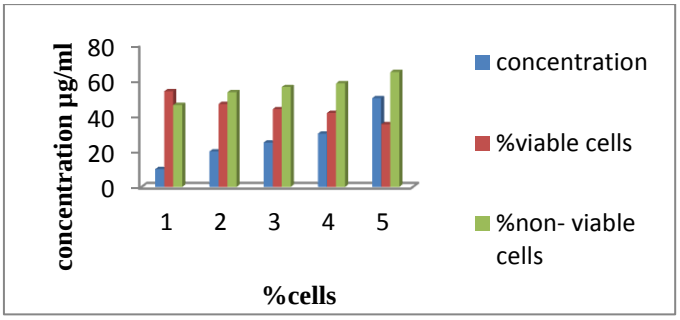

Figure 9: Effect of aqueous extracts on MCF-7 cell lines

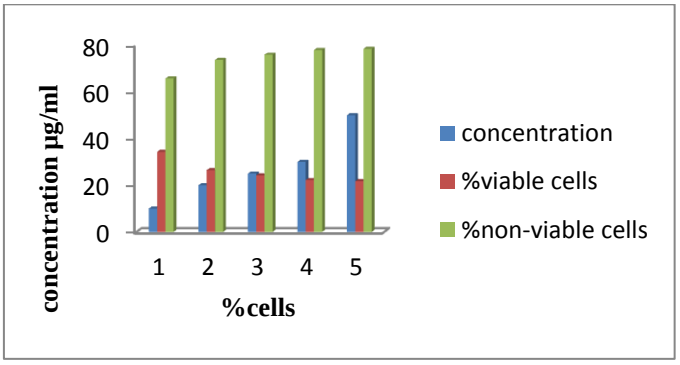

Figure 10: The effect of Ethanolic extract on MCF-7 cell lines.

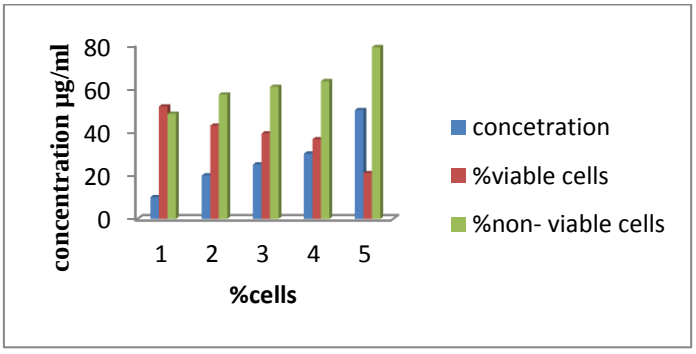

Figure 11: The effect of aqueous extracts on MAD-MB 231cell lines.

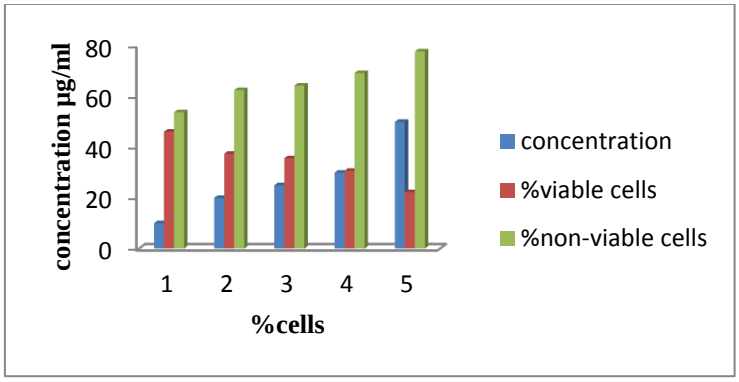

Figure 12: The effect of Ethanolic extracts on MDA-MB231 cell lines.

On phytochemicals analysis it was found that ethanolic extract showed presence of carbohydrates, proteins, amino acids, flavonoids, steroids, alkaloids, tannins and phenolic compounds and aqueous extract showed presence of carbohydrates, alkaloids, amino acid, protiens, phenolic compounds, and Saponin.

CONCLUSION

From the above performed research work it was concluded that plant *Amaranthus tricolor* Linn. Has maximum anticancer activity in its ethanolic extract and less cytotoxic in aqueous extract and in future it is essential to carry out isolation of individual active constituents and need to check for its cytotoxicity.

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REFERENCES

[1] Nataru S; Pulicherla Y. *Int. J Pharm Sci Rev Res*. **2014**, 26(1), 235-248.
[2] Kour. *Int. J Pharm Sci Rev Res.* **2014**, 27(2), 23-53.
[3] Momma H. ISBN **2010**; 2nd edition.
[4] Kumar KS.*Journal of medicinal plants studies*. **2013**, 1(3), 56-74.
[5] Nataru S; Pulicherla Y. *Int. J Pharm Sci Rev Res*. **2014**, 26(1), 235-248.
[6] Fernando W; Rupasinghe V. Anticancer Properties of Phytochemicals Present in Medicinal Plant of North America, Licensee in Tech, **2013**.
[7] Over 17 lakh new cancer cases in India by 2020: ICMR, **2016**/17248152.
[8] Kabel M, Baali HF. *Cancer Res Treat*. **2015**, 3(2), 28-33.
[9] National cancer Institute. Breast Cancer. Chapter-11-Treatment, U. S. Department of Health and Human Services. 11-26.
[10] National Cancer Institute, Chemotherapy and You. Chapter-4- Side Effect and Way to Mange Them. 15-51.
[11] Levenson Aniat S; Jordan V Craig. *Clin Cancer Res*. **1997**, 3071-3078.
[12] MBA-MB-231/ GFP Cell line. **2016**: Cell Biolabs, Inc. 7758 Arjons Dive.
[13] Khare CP. Indian Medicinal Plants. Springer Reference.**2007**,42.
[14] Kurian JC. Plants that heal. 4th Edition. Oriental watchman publishing, **2001**, 33-51
[15] Alam S. *Asian J Pharmacol Toxicol.***2013**, 1(1), 12-16**.**