WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 4, 1022-1029. Research Article ISSN 2277– 7105

IN VITRO CYTOTOXIC ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE CRUDE EXTRACTS OF *ELEUTHERINE BULBOSA* (MILLER), URBAN

Rani V. S.*

Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram, Kerala, 695581, India.

Article Received on
24 Dec. 2017,
Revised on 13 Jan. 2018,
Accepted on 03 Feb. 2018,
DOI: 10.20959/wjpr20184-11057

***Corresponding Author**
Dr. Rani V. S.
Department of Botany,
University of Kerala,
Kariyavattom,
Thiruvananthapuram,
Kerala, 695581, India.

ABSTRACT

Eleutherine bulbosa (Miller) Urban is an exotic plant with known medicinal uses. It is a tropical American plant, now naturalized in Asia. In Kerala and Southern parts of Tamil Nadu, the underground bulb of the plant is known by the local names, 'Vizhanarayani' or 'Neerotikizhangu.' The present study focuses on the *in vitro* cytotoxic activity of crude bulb extracts against Dalton's Lymphoma Ascitic (DLA) tumour cell lines, using Tryphan blue exclusion method. Among the different crude extracts, only the crude hexane and ethyl acetate extracts showed potent cytotoxic activities towards DLA tumour cell lines. The chloroform and methanol extracts did not show any cytotoxicity to DLA tumour cell lines. The LC_{50} value of crude hexane extract was 67.97µg/mL. The crude ethyl acetate extract showed LC_{50} value at a concentration of 41.02µg/mL. At a higher concentration (100µg/mL), the ethyl acetate extract showed a much more cytotoxic activity (80% cell death) compared to the hexane extract (64% cell death).

KEYWORDS: *Eleutherine bulbosa*, DLA tumour cell line, quinones, cytotoxicity, Tryphan blue.

INTRODUCTION

Plant substances continue to serve as viable sources of drugs for the human population and several plant-based drugs are in extensive clinical use. Among the various disease conditions faced by the modern civilization, cancer poses a serious threat to man. There are world wide

efforts to discover new anticancer agents from plants.[1] Many plant based drugs have been discovered and developed through ethno-medical leads, such as taxol, vincristine, vinblastine and camptothesin. These drugs are now in various phases of clinical trials.[2,3,4,5]

Eleutherine bulbosa (Miller) Urban belongs to family Iridaceae. It is an exotic plant with known medicinal uses, now naturalized in Asia. In Kerala and Southern parts of Tamil Nadu, the underground bulb of the plant is known by the local names, 'Vizhanarayani'or 'Neerotikizhangu'.[6] It is a seasonal perennial with fleshy bulb comprising of reddish tunics.[7] The bulbous part is medicinal, which is used as an antidote to poison.[8] It is also used in treating sore throat, boils uterine haemorrhage, anaemia, pertussis and headache.[9] The ethno botanical data revealed that the bulb of the plant is purgative and anticancerous.[10]

The present study focuses on the *in vitro* cytotoxic activity of crude bulb extracts of *Eleutherine bulbosa* against Dalton's Lymphoma Ascitic (DLA) tumour cell lines, using Tryphan blue exclusion method and preliminary phytochemical analysis of the crude extracts.

MATERIALS AND METHODS

Collection and identification of plant materials

Plants of *E.bulbosa* were collected from Thiruvananthapuram. It was identified and authenticated by the Curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. The plant material was also verified by comparison with specimens of *E. bulbosa* from Botanical Survey of India, southern circle, Coimbatore. A voucher specimen (Accession Number - KUBH 5802) has been deposited at the Kerala University Botanical Herbarium.

Extraction procedure

The bulbs of *E.bulbosa* were washed thoroughly with tap water in order to remove the adhered soil and dirt particles. Scale leaves of the bulbs were removed and the fresh bulbs were shade dried. The dried bulbs were milled into powder by a mechanical grinder and the powder was kept in an air tight container. The powdered plant material (15 gm) was successively extracted with 250 ml of solvents such as hexane (65-70ºC), chloroform (60-62ºC), ethyl acetate (76-77ºC) and methanol (60ºC) for 12 hours, using Soxhlet extractor. The extracts were concentrated to dryness under vacuum separately. The yields of the extracts were determined using the following equation:

$$\text{Percentage Yield or Extractive value}(\%) = \left(\frac{\text{Weight of the crude extract}}{\text{weight of the dry powder}}\right) \text{x} 100$$

Fluorescence analysis

The extracts were subjected to fluorescence studies, under visible light, short UV (254nm) and long UV (365nm).[11] The fluorescence behaviour of different extracts was compared with colour Chart.[12,13]

Cytotoxicity studies

Cytotoxicity of the *E.bulbosa* extracts was tested using DLA (Dalton's Lymphoma Ascites) tumour cells. The cell lines were obtained from Amala Cancer Research Centre, Thrissur, Kerala.

Dalton's Ascites Lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as lymphocytes in a mouse which grows in both solid and ascitic form.[14] Cytotoxic assays were performed as per Tryphan blue exclusion method.[15] The DLA tumour cell lines were incubated with various concentrations of the extracts (Hexane, Chloroform, Ethyl acetate and Methanol) dissolved in DMSO. After incubation, the cytotoxicity was determined using the Tryphan blue exclusion method. DLA cells were aspirated from the pleural cavity of mice and washed thrice with PBS (Phosphate Buffer Saline). The cells were then centrifuged at 100rpm to get cell pellet. About 0.1ml of the cell pellet was then suspended in 0.9ml PBS and the cell number was adjusted to $10x10^6$ cells in 1ml of the solution by serial dilution. The viability of the cell was checked by Tryphan blue exclusion method (0.1ml cells +0.8ml PBS+0.1ml Tryphan blue). Cent percent (100%) viable cells were used for the experiment. The experiment was set up by incubating different concentrations of sample with about $1x10^6$ cells (*ie* 0.1ml of the solution). The volume was made up to 1ml using dissolved in DMSO. After incubation, the cytotoxicity was determined using the Tryphan blue exclusion method. 1ml of Tryphan blue stain was added. The cells from each concentration were loaded on to a Haemocytometer to determine the number of dead cells from the total 100 cells.

$$\% \text{ of cytotoxicity} = \left(\frac{\text{Number of dead cells}}{\text{Total number of cells counted}}\right) x100$$

Statistical analysis

LC50 after 24h was calculated by Probit analysis using SPSS standard version 7.5.1 (Dec.20.1996).

Phytochemical screening

Preliminary phytochemical analysis was done for detecting alkaloids, sterols, terpenoids, cardiac glycosides, fats and oils, phenols, flavonoids, flavones, quinones, anthraquinones, anthrones, mucilage, anthocyanins, saponins, tannins and phlobotannins in the different crude extracts using standard procedures.[16,17,18,19]

RESULTS AND DISCUSSION

In the present study, *Eleutherine bulbosa* bulb powder was extracted with organic solvents such as hexane, chloroform, ethyl acetate and methanol. The percentage yield of each extract ranged between 1% to 10% (Fig I). The highest yield of 10% was observed in ethyl acetate extract. The hexane and aqueous extracts were powdery in nature, while the other three extracts were resinous in nature. Fluorescence analysis of the different extracts of the bulb is summarized in Table 1. Extracts exhibited a wide range of fluorescence in short and long UV. Phytochemical analysis of the crude bulb extracts revealed the presence of several bioactive constituents. The results are shown in Table 2. A total of 16 phytochemicals were analysed. The ethyl acetate extract showed the presence of nine phytochemicals. Hexane extract showed the presence of only three compounds chloroform extract showed the least number of compounds. Quinones were present in all the crude extracts. But none of the extracts showed the presence of following phytochemicals such as alkaloids, terpenoids, cardiac glycosides, fats and oils, coumarins, mucilage and phlobotannins. Results of the cytotoxic activity exhibited by the four crude extracts are given in Table 3 and Fig 2. Only the crude hexane and ethyl acetate extracts showed potent cytotoxic activities towards DLA tumour cell lines. The chloroform and methanol extracts did not show any cytotoxicity to DLA tumour cell lines. The LC_{50} value of crude hexane extract was 67.97µg/ml. The crude ethyl acetate extract showed LC_{50} value at a concentration of 41.02µg/ml. At a higher concentration (100µg/ml), the ethyl acetate extract showed a much more cytotoxic activity (80% cell death) compared to the hexane extract (64% cell death).

Table 1: **Fluorescence studies of crude extracts of *Eleutherine bulbosa.***

Sl No.	Observations under			
	Bulb extracts	**Visible light**	**Short UV (254nm)**	**Long UV (365nm)**
1.	Hexane	Primrose yellow	Agathia green	Pansy purple
2.	Chloroform	Burnt orange	Scheels green	Garnet brown
3.	Ethyl acetate	Ruby red	Scheels green	Purple madder
4.	Methanol	Oxblood red	Parsley green	Garnet brown

Table 2: Phytochemical analysis of the bulb extracts of *Eleutherine bulbosa*.

Sl No.	Phytochemicals	Extracts				
		Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
1	Alkaloids	-	-	-	-	-
2	Sterols	-	-	+	-	-
3	Terpenoids	-	-	-	-	-
4	Cardiac glycosides	-	-	-	-	-
5	Fats and oils	-	-	-	-	-
6	Phenolic compounds	+	-	+	+	+
7	Flavonoids	-	-	+	+	+
8	Flavonols	-	-	+	-	-
9	Quinones	+	+	+	+	+
10	Anthraquinones	-	-	+	-	-
11	Coumarins	-	-	-	-	-
12	Mucilage	-	-	-	-	-
13	Anthocyanins	-	-	+	+	-
14	Saponins	+	+	+	+	+
15	Tannins	-	-	+	+	-
16	Phlobotannins	-	-	-	-	-
Total number of phytochemicals =16		3	2	9	6	4

'+'indicates presence '-' indicates absence

Table 3: Cytotoxicity of different extracts of *Eleutherine bulbosa* on DLA cell lines.

Concentration (µg/ml)	Percentage of cytotoxicity (%)			
	Hexane extract	Probit values	Ethyl acetate extract	Probit values
5	1±0	0.11669	20 ±2	0.25771
10	16±1	0.13630	27±2	0.28769
25	22±1	0.20804	35±2	0.38617
50	48±2	0.36688	64±2	0.56437
100	64±1	0.72776	80±2	0.85652
	LC_{50}= 67.97µg/ml		LC_{50}=41.02µg/ml	

Percentage cytotoxicity values are mean±SD of experiments performed in triplicate. 1% DMSO was used as control, which showed zero cell death.

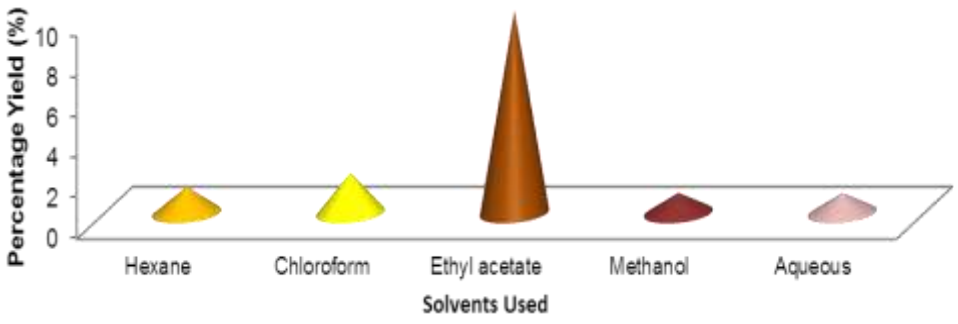

Fig 1: Percentage yield of *Eleutherine bulbosa* crude extracts.

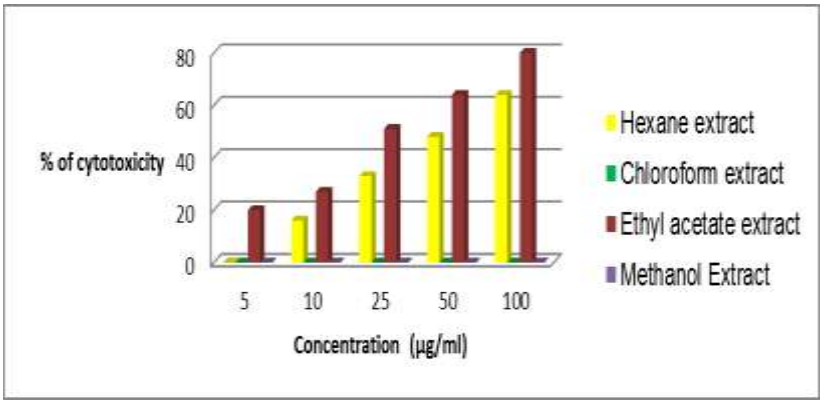

Fig 2: Cytotoxicity of different extracts of *Eleutherine bulbosa* on DLA cell lines.

Cytotoxicity studies using the DLA tumour cell lines revealed that crude hexane and ethyl acetate extracts of *E. bulbosa* bulbs have potential cytotoxic property against the tumour cell lines. At a higher concentration (100µg/ml), the ethyl acetate extract showed a greater cytotoxic activity (80% cell death) compared to the hexane extract (64% cell death). Thus it appears that the hexane and ethyl acetate extracts possess a dose dependent cytotoxic activity towards DLA tumour cell lines. The cytotoxic potential of *E. bulbosa* bulbs have also been revealed against *Allium cepa* root cells.[6]

A great number of *in vitro* and *in vivo* methods have been developed to measure the efficiency of natural anticancer compounds either as pure compounds or as plant extracts and among them, Tryphan Blue exclusion method using DLA cell line is considered an important assay.[20] Previous studies have shown that several phenolic compounds such as quinones possess antimutagenic and anticarcinogenic activity, which may be responsible for the cytotoxic activity towards DLA cell lines.[21,22] The cytotoxic potential of *E. bulbosa* crude extracts may be due to the presence of phytoconstituents present in them. The cytotoxic hexane extract showed the presence of phytochemicals like phenolic compounds and quinones. The crude ethyl acetate extract contained sterols, phenolic compounds, flavonoids, flavonols, quinones, anthraquinones, anthocyanins, saponins and tannins. The common phytochemicals present in both the cytotoxic extracts were phenolic compounds and quinones. However, it should also be noted that the crude chloroform and methanol extracts also contained phenolic compounds and quinones but they could not exhibit cytotoxic activity to a similar extent as the ethyl acetate extract. This may be due to the fact that the necessary phytochemicals are present only in the crude ethyl acetate extract to the required quantity for imparting cytotoxic activity.

CONCLUSION

The results of the above study show that ethyl acetate extract showed potent cytotoxic activity and it contained more phenolic compounds. Detection and purification of specific bioactive compounds crude ethyl acetate is needed for further confirmation of the exhibited cytotoxic activity. The results further emphasize the complex pharmacological activity of bulbs of *Eleutherine bulbosa*.

ACKNOWLEDGEMENTS

The author is thankful to Dr.Bindu R Nair, Assistant Professor, Department of Botany, University of Kerala, Kariavattom Dr.P.M.Radhamany, Associate Professor, Former Head of the Department, Department of Botany,University of Kerala, Kariavattom for providing the required facilities for the conduct of this research work and Dr. Ramdas Kuttan, Director, Amala Cancer Research Centre,Thrissur for the help rendered for the cytotoxic studies.

REFERENCES

1. Heinrich M, Bremner P. Ethnobotany and ethnopharmacy: their role for anti-cancer drug development. Current Drug Targets, 2006; 7(3): 239-245.
2. Menon LG, Kuttan R, Kuttan G. Inhibition of lung metastasis in mice induced by B16 F 10- melanoma cells by polyphenolic compounds. Cancer Lett., 1995; 95: 221-225.
3. Joy KL, Kumar NV, Kuttan G, Kuttan R. Effect of *Picrorrhiza kurroa* extract on transplanted tumors and chemical carcinogenesis in mice. J Ethnopharmacol, 2000; 71: 261-267.
4. Mehta RG, Pezzuto JM. Discovery of cancer preventive agents from natural products: From plants to prevention. Curr Oncol Rep., 2002; 4: 478-486.
5. Cardile V, Scifo C, Russo A, Falsaperla M, Morgia G, Motta M, Renis M, Imbriani E, Silvestre G. Involvement of HSP70 in resveratrol-induced apoptosis of human prostrate cancer. Anticancer Res., 2003; 23: 4921-4926.
6. Kuriachan PI, Cherian MA preliminary study of the cytotoxic effects of the medicinal plant *Eleutherine bulbosa* using *Allium cepa* root tip meristem. Proceedings of the Kerala Science Congress, 1989; 188-189.
7. Baker JG. Handbook of the Iridaceae. George Bell and Sons. London, 1892; 1-247.
8. Kuriachan PI. On the emergence of the exotic *Eleutherine bulbosa* (Mill) Urban as a medicinal plant in South India. J Ind Bot Soc., 1987; 66: 9-21.

9. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants- A complete source Book. Agrobios. Jodhpur. India, 2003; 214.
10. Brasileiro BG, Pizziolo VR, Raslan DS, Jamal CM, Silveira D. Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in Governador Valadares District. Rev. Bras. Farmacogn, 2006; 42: 195-202.
11. Kokate CK. Practical Pharmacognosy. 4th edn. Vallabh Prakashan. Delhi, 1994; 107-111.
12. Wilson RF. Horticultural Colour chart. Henry stone and son Ltd. Banbury, Great Britain, 1938; I: 1-100.
13. Wilson RF. Horticultural Colour chart. Henry stone and son Ltd. Banbury, Great Britain, 1938; II: 101-200.
14. Kleinsmith LJ. Principles of Cancer Biology. 4th edn. Pearson Benjamin Cummings, San Francisco, 2007: 312.
15. Talwar GP. Hand book of Practical Immunology. National Book Trust, New Delhi, 1974: 336-339.
16. Odebiyi A, Sofowora EA. Phytochemical screening of Nigerian medicinal plants. Part II. Liyoydia, 1978; 403: 234-246.
17. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn. Chapman and Hall Ltd, London, 1984; 1-36.
18. Trease EG, Evans WC. Trease and Evans Pharmacognosy. 3rd edn. Balliar Tindal, London, 1989; 81-90: 268-298.
19. Daniel M. Methods of Phytochemistry and Plant Economic Botany. Kalyani Publishers, New Delhi, 1991: 77- 15.
20. Chand S, Nagani K. *In vitro* and *in vivo* methods for anticancer activity evaluation and some Indian medicinal plants possessing anticancer properties: An overview. Journal of Pharmacognosy and Phytochemistry, 2013; 2(2): 140-152.
21. Indap MA, Radhika S, Motiwala L, Rao KVK. Anticancer activity of phenolic antioxidants against breast cancer cells and a spontaneous mammary tumour. Indian Journal of Pharmaceutical Sciences, 2006; 68(4): 470-474.
22. Rajeshkumar NV, Pillai MR, Kuttan R. Induction of apoptosis in mouse and human Carcinoma cell lines by *Emblica officinalis* polyphenols and its effect on chemical carcinogenesis. Journal of Experimental and Clinical Cancer Research, 2003; 22(2): 201-212.