International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 28-10-2021, Accepted: 14-11-2021, Published: 29-11-2021
Volume 6, Issue 6, 2021, Page No. 572-574

Brine shrimp lethality assay of some selected medicinal plant flowers in polar and Non-polar solvents

Narwade Keshav B, Vishal R Marathe*
Department of Botany, NES Science College, Nanded, Maharashtra, India

Abstract
The present research work was conducted to investigate the in-vitro Brine Shrimp Lethality Assay (BSLA) of some selected plant flowers extract in a polar and non-polar solvent and correlate cytotoxicity results with known pharmacological activities of the plants. Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). The LC_{50} of an agent is the concentration, which kills, or inactive 50% of the test animal. LC_{50} is inversely proportional to the toxicity of a compound, i.e., the lower is the LC_{50}, the higher is the cytotoxicity. Aqueous extract of *Catharanthus roseus* 150 LC_{50} (µg/ml) and methanolic extract of *Tamarindus indica 236.66* LC_{50} were considered for the bioactive compound shown cytotoxicity correlated with the anticancer property. Whereas chloroform extract of *Calotropis gigantia* with greater than 1000 LC_{50} value is considered as toxic activity. It indicated that this bioassay has a good correlation with the human solid tumor cell lines.

Keywords: brine shrimp lethality assay; cytotoxicity, Lc_{50}, medicinal plant flowers; polar and non-polar solvent

Introduction
Plants synthesize a diverse range of bioactive molecules, making them a rich source of various types of medicines. Approximately one-half of all licensed drugs that were registered worldwide in the 25 years period before 2007 were natural products or their synthetic derivatives (Newman and Cragg, 2007) [14]. Great information about the preventive and curative application of medicinal plants mentioned in Ayurveda. Over 50% of all modern clinical drugs are of natural product origin (Stuffness *et al*., 1982) [24] and play an important role in drug development programs in the pharmaceutical industry (Baker *et al*., 1995) [4]. The general acceptability of herb products has been limited by the lack of dose regimen, adequate toxicity data, and large information about the phytochemical content of these plants (Pousset, *et al*., 1988) [17]. All studied plants like *Catharanthus roseus*, *Polianthes tuberosa*, *Tamarindus indica*, *Moringa oleifera*, *Azadirachta indica and Calotropis gigantea* used in the present study are showing medicinal importance. The studied plants have antioxidant effects (Sharma, V. *et al*., 2013, Barghout, N. *et al*., 2018 and Reis *et al*., 2016) [22, 5, 20] with the presence of such phytochemical components as equivalent to standards in different extracts, the cytotoxic effect (Nguyen, K. D. *et al*., 2017 and Ali G. H. *et al*., 2004) [15, 1] and the antitumor effect (Aravind, S. R., *et al*., 2012 and Zhu, W. F., *et al*., 2020) [2, 25]. These plants also show potent antimicrobial activities (Ghosh, P. K., *et al*., 2014 and Ali G. H. *et al*., 2004) [8, 1].
The brine shrimp lethality assay is based on the ability to kill laboratory-cultured *Artemia nauplii* (brine shrimp) and is considered a useful tool for preliminary assessment of toxicity (Solis *et al*, 1993).
Therefore, the present study was undertaken to investigate the cytotoxic activity of flower extract of some selected medicinal plants.

Material and Methods
Plant material
The flowers were collected from different localities of the Marathwada region of Maharashtra during their flowering season and identified with the help of standard flora (Naik, 1988) [13].

Plant material extraction
The flowers were collected, shade dried and powdered into fine coarsely form. 15 gm powder weighed and used for Soxhletextraction by using polar solvent (water and methanol) and non-polar solvent (chloroform and hexane) respectively. The extracted samples were evaporated at approximately 40°C. The dried extracts were stored at 3-4 °C and used for further analysis (Azwanida N.N.,2015) [3].

Brine shrimp lethality assay
Sample preparation
Preparation of seawater
The crude sea salt 25g/L dissolve in distilled water and 7mg/L of dried brewers yeast was added to this solution for food of brine shrimp. It was filtered through filter paper before using.

Hatching of brine shrimp eggs
The 2.0 L of seawater was added to the special chamber 40 mg of the eggs were washed with water and then these eggs were sprinkled into the compartment which was darkened. After 48 hrs the phototropic nauplii were collected by capillary from the lighter side and used for bioassay.

Bioassay
The Bioassay experiment was performed according to the procedure described by Meyer *et al* 1982 [12]. Nauplii were drawn in a glass capillary along with water, and ten of such shrimps were transferred to each sample vial containing 4.5 ml brine solution (specific volume brine and yeast suspension) after they were counted in the stem of the capillary against the lighted background. In each experiment, 100µg, 500µg, and 1000µgextract of the flower were added to 4.5 ml of brine solution of mentioned

concentrations in test tubes 1to 3. In the control vial, 4.5 ml of artificial seawater and 0.5 ml artificial seawater with 0.2% DMSO water were added. After 24 hrs. survivors were counted, by using 3X magnifying glasses against the lighted background. Percent deaths and LC_{50} values were calculated by dose-response data which is transformed into a straight line utilizing a trend line fit linear regression analysis (MS Excel version 7) (Ramachandran *et. al.*, 2011) [19].

Result and Discussion

Evaluating the number of viable shrimps after 24 hrs percentage of inhibition was calculated. LC_{50}value was calculated by using artificial saline water as a control. The observation was mentioned in table 1. According to obtained result after counting LC_{50} value of different flowers extracts in water, methanol, chloroform, and hexane solvents respectively, aqueous extract of *Catharanthus roseus*shows150µg/ml and methanolic extract of *Tamarindus indica*shows 236.66µg/ml (Fig.1) was considered for the bioactive compound shown cytotoxicity correlated with the anticancer property. Chloroform extract of *Calotropis gigantea* shows 497.77µg/ml whereas, methanol extract with greater than 1000µg/ml LC_{50}value considered as toxic activity.

Methanol extract of *Catharanthusroseus* (261.36µg/ml), hexane extract of *Polianthes tuberosa* (423.07µg/ml), methanol extract of *Calotropis gigantea* (497.77 µg/ml), *Polianthes tuberosa* hexane extract of *Moringa oleifera* (578.00µg/ml), water extract of *Polianthes tuberose* (637.69µg/ml), water extract of *Azadirachta indica* (664.83µg/ml) and hexane extract of *Tamarindus indica* (757.27 µg/ml).

Table 1: LC_{50}value of flower in a polar and nonpolar solvent

Sr.no.	Plant name	Solvent	Conc. of compound µg/ml	Total no. shrimps used/tube	Shrimp survived			Total No. of Shrimp survived	Percentage mortality	LC_{50} (µg/ml)
					T_1	T_2	T_3			
Control	Artificial saline water			10	10	10	09	29		
1	*Catharanthus roseus*	Water	100	10	6	3	4	13	55.17	150
			500		3	4	3	10	65.51	
			1000		1	3	3	07	75.86	
2	*Catharanthus roseus*	Methanol	100	10	6	5	5	16	44.82	261.36
			500		5	3	4	12	58.62	
			1000		4	1	5	10	65.51	
3	*Polianthes tuberosa*	Water	100	10	4	3	3	10	65.51	637.69
			500		2	0	2	04	86.20	
			1000		0	2	1	03	89.65	
4	*Polianthes tuberosa*	Hexane	100	10	8	4	6	18	37.93	423.07
			500		4	4	4	12	58.62	
			1000		5	2	4	11	62.06	
5	*Tamarindus indica*	Methanol	100	10	5	4	4	13	55.17	236.66
			500		4	3	3	10	65.51	
			1000		3	1	4	08	72.41	
6	*Tamarindus indica*	Hexane	100	10	4	3	5	12	58.62	757.27
			500		3	3	4	10	65.51	
			1000		4	3	2	09	68.96	
7	*Moringa oleifera*	Hexane	100	10	4	6	2	12	58.62	578.00
			500		4	2	3	09	68.96	
			1000		1	3	4	08	72.41	
8	*Azadirachta indica*	Water	100	10	8	4	7	19	34.48	664.83
			500		6	5	6	17	41.37	
			1000		4	2	5	11	62.06	
9	*Calotropis gigantia*	Methanol	100	10	4	6	5	15	48.27	497.77
			500		3	2	3	08	72.41	
			1000		5	3	4	12	58.62	
10	*Calotropis gigantia*	Chloroform	100	10	2	4	4	10	65.51	>1000
			500		2	5	2	09	68.96	
			1000		5	0	3	08	72.41	

*Results presented here are the mean values from three independent experiments ± S.D.,

Conclusion

The aqueous and methanol extracts of the flowersshow significant cytotoxic activity.

Moderate cytotoxic effects of flowers in chloroform and hexane indicate that it can be selected for further cell line assay. This bioassay has a good correlation with the human solid tumor cell lines for cytotoxicity, evaluated relative potencies.

Acknowledgment

The authors are thankful to the Principal of NES Science college Nanded and the Managing Director, Biocyte Institute of Research & Development, Sangli (Maharashtra, India) for their continuous support and cooperation during the present research work.

Reference

1. Ali GH, El-Taweel GE, Ali MA. The cytotoxicity and antimicrobial efficiency of Moringa oleifera seeds extracts. International Journal of Environmental Studies,2004:61(6):699-708.
2. Aravind SR, Joseph MM, Varghese S, Balaram P, Sreelekha TT. Antitumor and immunopotentiating activity of polysaccharide PST001 isolated from the seed kernel of Tamarindus indica: an *in vivo* study in mice. The Scientific World Journal, 2012.
3. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength, and Limitation. Med Aromat Plants,2015:4:196.
4. Baker JT, Borris RP, Carte B. "Natural product drug discovery and development: New perspective on

international collaboration" Journal of Natural Product,1995:58:1325-1357.
5. Barghout N, Chebata N, Messgo-Moumene S, Khennouf S, Yekrelef A, El Hadi D. Polyphenols from Polianthes tuberosa L. (Amaryllidaceae) leaves and their antioxidant properties. Revue Agrobiologia,2018:8(1):902-912.
6. Berghot MA, Kandeel EM, Abdel-Rahman AH, Abdel-Motaal M. Synthesis, Antioxidant and Cytotoxic Activities of Novel Naphthoquinone Derivatives from 2,3-Dihydro-2,3-Epoxy-1,4- Naphthoquinone. Med chem,2014:4:381-388.
7. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med, 45, 31-34.
8. Ghosh PK, Bhattacharjee P, Das S. Antimicrobial activity of supercritical carbon dioxide extracts of tuberose (Polianthes tuberosa Linn.) flowers against common pathogens. International Journal of Pharmaceutical Sciences and Research,2014:5(4):1279.
9. Hossain MS, Hossain MA, Islam R, Alam AH, Kudrat-e-Zahan S, Sarkar, Farooque MA. "Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with Magnesium (Mg- II)", Pakistan Journal of Biological Sciences,2004:7:25-27.
10. Kohler HM, Chadwell H, Gillman H, Blatt AH (Eds.)., Organic Synthesis Wiley Interscience, New York, 78, Coll, 1967, 1.
11. McLaughlin JL, Chang CJ, Smith DL. In Studies in Natural Products Chemistry; Rahman, A. U., Ed.; Elsevier: Amsterdam, 1991.
12. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL. Plant Med,1982:45:31.
13. Naik VN. "Flora of Marathwada". Amrut Prakashan Aurangabad, 1998, 1,2.
14. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Last 25 Years. J. Nat. Prod,2007:70:461-477.
15. Nguyen KD, Dang PH, Nguyen HX, Nguyen MT, Awale S, Nguyen NT. Phytochemical and cytotoxic studies on the leaves of Calotropis gigantea. Bio-organic & medicinal chemistry letters,2017:27(13):2902-2906.
16. Persoone G. Proceeding of the international symposium on brine shrimp, Artemiasalina, pp. 1-3, University Press, 1980.
17. Pousset JL. Medicinal plants used as a source of raw material for the pharmaceutical industry, problems and prospects. In: Drug Production from Natural Products. Proceedings of the Seventh International Symposium. DRPU, OAU, Ife, Nigeria, 1988, 37-41.2.
18. Rahman MS, Rashid MA. Antimicrobial activity and cytotoxicity of Ecliptaprostrata. Orient. Pharm. Exp. Med,2008:8:47-52.
19. Ramachandran S. Assessment of cytotoxic activity of Agave Cantula using Brine shrimp (Artemiasalina) lethality bioassay. Asian J. of Sci. Res,2011:1:90-94.
20. Reis PMCL, Dariva C, Vieira GÂB, Hense H. Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (Tamarindus indica), sweet variety. Journal of Food Engineering,2016:173:116-123.
21. Rieser MJ, Gu Z-M, Fang X-P, Zeng L, Wood KV, McLaughlin JL. Fivenovel mono-tetrahydrofuran ring acetogenins from the seeds of Annonamuricata. Journal of Natural Products,1996:59:100-108.
22. Sharma V, Paliwal R. Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of Moringa oleifera pods. International Journal of Green Pharmacy (IJGP), 2013, 7(1).
23. Solís PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using Artemia salina. Plant Med,1993:59:250-252.
24. Stuffness M, Douros J. "Current status of the NCI plant and animal product program" Journal of Natural product,1982:45:1-14.
25. Zhu WF, Cheng JX, Su SZ, Zhang CF, Akihisa T, Manosroi J *et al* Limonoids and tricyclic diterpenoids from Azadirachta indica and their antitumor activities. Bioorganic chemistry,2020:100:103889.