Research Article

Genotoxic potential of *Bohadschia vitiensis* aqueous extract: A sea cucumber species, using *Allium cepa* genotoxicity model

Nishani J. Jayathilake[1], Uthpala A. Jayewardena[2]

[1]*Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka*

[2]*Department of Zoology, Faculty of Natural Sciences, The Open University of Sri Lanka, Sri Lanka*

Received: 3 December 2020 Revised: 17 February 2021 Accepted: 20 February 2021

Abstract

Objective: *Bohadschia vitiensis* is historically proven for potential therapeutic values with anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, wound healing properties. Current study investigates the potential genotoxic effect of aqueous extract of *Bohadschia vitiensis* using *Allium cepa* genotoxicity model in order to validate the therapeutic usage. **Materials and methods**: Root grown onion bulbs were exposed to several concentrations of the extract; 62.5, 125, 250, 500 µg/ml, positive control (Dimethyl sulfoxide) and negative control (aged tap water) for 2 days. Immediately after the exposure period, root tips were harvested and observed in order to identify the existing stage of the cells and chromosomal aberrations. **Results:** The Mitotic Index was negatively correlated with the exposed concentrations and only the mitotic index of 500µg/ml was significantly different compared to the negative control. The extract showed no significant lethal genotoxic effect in comparison to the negative control within the tested range. Microscopic observations of the root tips exposed to the extract, revealed 0.1-0.2% of chromosomal aberrations including chromosomal bridges, chromosomal breaks, c-mitosis and vagrants. Conclusion: Moreover, the tested concentrations showed no significant effect on percentage chromosomal aberrations except 500µg/ml that showed only a sub lethal effect. Further, evaluation for toxicity with animal models is warranted for validating the toxicity of the extract prior to therapeutic applications.

***Keyword*:** Genotoxicity; *Allium cepa* model, Sri Lankan Sea Cucumber species, *Bohadschia vitiensis*

Introduction

Sea cucumbers are soft bodied, marine echinoderms, usually harvestable from sea floor worldwide (Anderson et al., 2011) and have long been appreciated in East Asia for its nutritional and therapeutic value (Bordbar et al., 2011; Kiew and Don, 2012). Sea cucumbers are a delicacy for Chinese, Korean and Japanese and available at international markets in fresh, dry, frozen and processed forms (Dhinakaran and Lipton, 2014) as well as a well-known remedy for hypertension, asthma, rheumatism, cuts, burns, impotence and constipation in traditional medicines (Bordbar et al., 2011).

Promising researches pointed out that sea cucumbers contain several bioactive compounds such as triterpene glycosides, glycosaminoglycans, gangliosides, collagen, branched-chain fatty acid and lectins (Kiew and Don, 2012), with a high biochemical and structural diversity (Kijjoa and Sawangwong, 2004) which are probably responsible for anticancer/antiproliferative, antiangiogenic, anticoagulant, anti-hypertension, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, antiviral, wound healing, anti-leishmanial activities etc.(Bordbar et al., 2011). Owing to this, there is an increasing focus for producing commercial products; nutraceuticals, beauty products and many commercialized items around the world (Kijjoa and Sawangwong, 2004). Up to date, nutritional supplements, tablets for joint problems, skin tonners, variety of creams are popular in the market globally.

Bohadschia vitiensis (Family Holothuriidae) is a relatively abundant species in North West and East coast and a low value sea cucumber species in Sri Lanka (Dissanayake and

Address for Corresponding Author:
Nishani J. Jayathilake
Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
Email: nishanijayanika1@gmail.com

DOI: https://doi.org/10.31024/apj.2021.6.1.2

Stefansson, 2010). Extracts and few isolated compounds from *Bohadschia vitiensis* have been reported to possess several biological activities including antifungal activity, Spermicidal activity (Lakshmi et al., 2010), antioxidant activity (Nursiol et al., 2019), antinociceptive effects (Ridzwan et al., 2003) and anti-inflammatory activity (Jayathilake and Gunathilake, 2020). Particularly, local people in Sri Lanka believe that water extracts of *Bohadschia sp*. have a healing effect on inflammatory conditions and arthritic pain, but more scientific evidences are yet to be discovered. Despite the profound therapeutic value and the widespread usage of sea cucumbers, some of them may contain potentially toxic, carcinogenic and teratogenic compounds.

Variety of cells and species are used to test genotoxic effects of testing agents ranging from pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, cosmetics ingredients, to veterinary drugs (Corvi and Madia, 2017), relevant in the context of international legislations aiming at the protection of human and animal health. Mainly, categories of microorganisms, cell cultures, insects, plants, crustaceans, mollusks, fish and mammals (Sponchiado et al., 2016) are used while setting several biomarkers for bio monitoring (Barras and Nadal, 2004).

Allium cepa test is a frequently used model which is satisfactorily sensitive for innumerous compounds present in natural extracts (Yuet et al., 2012; Timothy, 2014; Debnath et al., 2018) providing cytotoxicity and genotoxicity end points with chromosomal alterations (Kumari et al., 2011; Hemachandra and Pathirathne, 2016). Evidently, mutagens can be detected cytologically by cellular inhibition; disruption in metaphase; induction of chromosomal aberrations, numerical and structural changes ranging from chromosomal fragmentation to the disorganization of the mitotic spindle, and consequently of all subsequent dependent mitotic phases (Tedesco et al., 2015). *A. cepa* bioassay is more promising due to the availability of both cytotoxicity and genotoxicity end points and the cost effectiveness (Wijeyaratne et al., 2019). High sensitivity, clearly visible mitotic phases in *Allium* meristematic cells, possessing a stable chromosome number, diversity in the chromosome morphology, stable karyotype (2n=16), clear and fast response to the genotoxic substances, lack of spontaneous chromosomal damages are added benefits (Fiskesjo, 1985). Further, according to validated information, results of *Allium cepa* assay shows no difference from *in vivo* tests when comparing the results. Toxicity evaluation of sea cucumber extracts is crucial for developing therapeutic products, effective for human use. Aim of present study was to evaluate the genotoxic potential of *Bohadschia vitiensis* water extract using *Allium cepa* bioassay.

Materials and methods

Collection and identification of plant material

Specimen of sea cucumber, *Bohadschia vitiensis* (560g) were collected from commercial catches of fishermen, from coastal areas of Pallimunei, Mannar, Sri Lanka (8° 03′, 8° 35′N: 77° 15′, 77° 36′E) in the month of January 2018. Precise identification of species was carried out (Purcell et al., 2012) by morphology, and ossicle analysis based on Purcell, Samyn, and Conand (2012). Samples were packed in plastic bags with ice during transportation. After removing the internal organs, samples were kept at -20ºC until extractions.

Preparation of extract

Water extract of *Bohadschia vitiensis* (WE) was prepared according to Ridzwan, Leong and Idid (2003) with slight modifications. Briefly, thawed samples of *B. vitiensis* were thoroughly washed with distilled water. The visceral organs were removed, and the body wall was diced, followed by homogenization with mortar and pestle. The homogenized sample was incubated in distilled water (1:2 w/v) and occasionally shaken for 4 hrs. The extract was then centrifuged at 3000rpm for 20 min (HERMLE Labortechnik GmbH, D-78564, Wehingen, Germany). The supernatant was carefully collected, freeze-dried, and used for the toxicity assay.

Allium cepa genotoxicity assay

The genotoxicity assay for the WE was performed as previously described procedure by Hemachandra and Pathiratne (2016). Commercially available equal sized (5-6g) *Allium cepa* onion bulbs which are in good condition were used for the assay. After the bottom plates and the dead scales were removed, bulbs(n=5) were placed on glass vials filled with aged tap water (dechlorinated) without exposing to direct sunlight. The temperature was maintained at 28±2°C during the period 48h and provided with renewed water supply every 24 h. Later, they were treated with different concentrations (500, 250, 125, 62.5µg/mL) for another 48h. Dimethyl sulfoxide (DMSO) was used as the positive control and aged tap water was used as the negative control. Distilled water was used as dilution water for preparing concentration series. The exposure solutions were renewed daily. At the end of exposure period, 3 bulbs were randomly selected (n=3) and 5-6 tips (1-2mm) were obtained from each bulb. Immediately, the tips were dipped in 1:3 aceto alcohol solution incubating at 60°C for 10 min. Then the tips were transferred in to 1N HCl solution and placed it again in the incubator at 60°C for 10 min. Then the root tips were transferred and exposed to acetocarmine solution for 10 min or until the root tips were properly stained with deeply stained edges of the root tips. The roots were placed on a glass slide with a drop of 5% acetocarmine

and cover slip was placed on the glass slide providing a slight pressure to make the cells squash on the surface of glass slide. One slide was prepared for each bulb. The slides were coded randomly and examined blindly under light microscope (×400). Microscopic analysis included counting the number of cells undergoing mitotic stages and chromosomal aberrations in 1000 dividing cells.

The mitotic index (MI), Percentage mitotic inhibition (PMI), Percentage chromosomal aberrations (PCA) were calculated using following formulae:

$$MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$PMI = \frac{\text{MI of negative control} - \text{MI of test control}}{\text{MI of negative control}} \times 100$$

$$PCA = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

Statistical analysis

Minitab 18 statistical software was used to analyses the results. The means with 95% confidence limit and standard errors for each set of data were calculated. Correlation and One way-ANOVA were applied with 95 CI (significance at the $p<0.05$) for results prediction.

Results

Cytogenotoxic effects of *Bohadschia vitienis* aqueous extract on *Allium cepa* root cells were tabulated in Table 1. Mitotic index (MI) declined gradually exhibiting negative correlation ($r = 0.935$) along the concentration series. This was significant at the highest dose, reporting a low MI of 17.7 compared to the 26.8 at the negative control (One-Way ANOVA, DF=3, F=11.86, p=0.003).

Percentage inhibition of mitotic index (PMI) as a percentage of the control (Figure 1.0) revealed a significant decline (nearly 34%) at the highest dose, 500 µg/ml (DF=4, F=12.75, p=0.001). Nevertheless, 50% inhibition; the boundary level of genotoxicity, was not exceeded by any of the exposed concentrations.

Prophase, Metaphase, Anaphase and Telophase of *A cepa* meristematic cell division were clearly observed under a light microscope (×400). Meanwhile, extract exposed cells exhibited variety of chromosomal aberrations; c-mitosis, vagrants, stickiness, chromosomal bridges and breaks in low in quantity.

Percentage chromosomal aberrations of each exposed concentration of the extract (Figure 2.0) remained approximately similar to the negative control and showed no significant correlation with the concentration level ($p<0.05$). Table 1 presents frequencies and types of chromosomal aberrations induced by the extract. The lowest percentage of chromosomal aberrations (0.1%) was exhibited by 62.5g/ml concentration of the extract. Besides, the highest tested concentration (500µg/ml) showed lower percentage chromosomal aberration (0.2%) compared to the positive control (20.9%) and it was as same as that of the negative control (0.2%). The highest percentage chromosomal aberration (0.2%) among four doses was recorded in higher doses (500, 250, 125 µg/ml) while lowest PCA (0.1%) was reported in the lowest dose (62.5 µg/ml). However, the PCA of positive control was the highest 20.9% of all the treatments while 0.2% chromosomal aberrations were reported in the negative control.

Discussion

Cytogenotoxic evaluation of the water extract of sea

Table 1. Cytogenotoxic effects of aqueous extract of *Bohadschia vitiensis* on *Allium cepa* root cells

Treatment group	Concentration (µg/ml)	MI±SEM (%)	PMI± SEM(%)	C-mitosis	Stickiness	Vagrants	Chromosomal bridges	Chromosomal breaks	PCA (%)
Negative control	Tap water	26.8±2.0	0	1	-	1	-	-	0.2
Positive control (DMSO)	0.1M	7.8±1.8*	70.9±6.5	48	65	37	25	34	20.9
Extract (WE)	500	17.7±1.7*	33.9±6.3	-	-	1	1	-	0.2
	250	21.7±1.3	19.1±4.9	1	-	-	-	1	0.2
	125	24.1±1.8	9.9±6.6	2	-	-	-	-	0.2
	62.5	29.2±0.5	9.3±1.8	-	-	1	-	-	0.1

MI-Mitotic index, **PMI**-Percentage Mitotic Inhibition, **PCA**-Percentage Chromosomal aberrations, **SEM**-Standard Error Mean; 1000 cells/concentration of extract and controls, n = 3; MI values are means ± SEM; *p < 0.05 significantly different when compared to negative control (tap water)

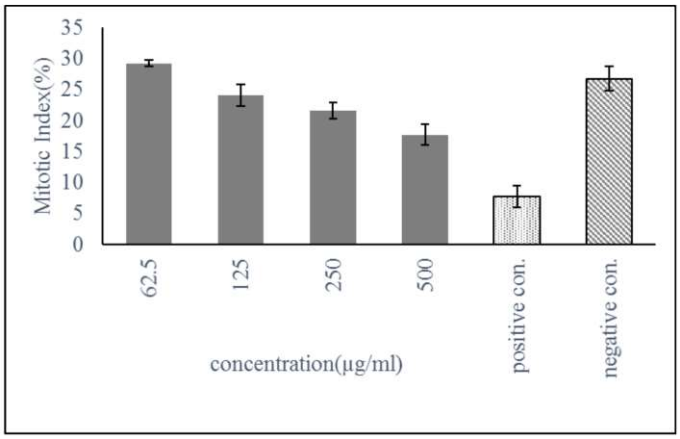

Figure 1. Alterations of the mitotic index of *Allium cepa* by the WE (water extract) of *B. vitiensis*, positive control (DMSO) and negative control (Tap water)

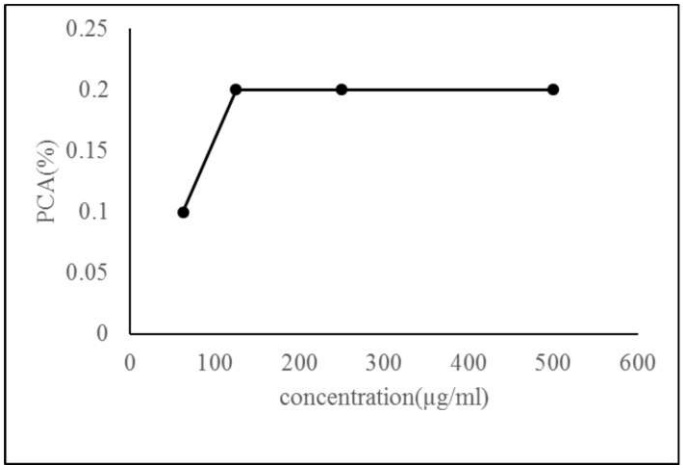

Figure 2. Percentage chromosomal aberrations in the *Allium cepa* cells induced by the WE (water extract) of *B. vitiensis*

cucumber, *Bohadschia vitiensis* revealed no substantial toxic effects reporting very low percentage aberrations in *Allium cepa* cells. Inhibition of the mitotic activity was also minimal since less than 35% inhibition was reported in all the test doses. The *Allium cepa* test is a sufficiently sensitive test model (Kannangara and Pathiratne, 2015) to evaluate genotoxic effects induced by substances which cause chromosomal aberrations and disturbances in the mitotic cycle (Laughinghouse, 2012). This model is frequently used for monitoring the genotoxic potential of natural extracts, (Laughinghouse, 2012; Shehu et al., 2019) consequently, this study results reflect the application of *Allium cepa* root tips for assessing genotoxicity of *Bohadschia vitiensis* water extract using different genetic endpoints; mitotic index and chromosome aberration (Zendehboodi, 2018).

Mitotic index is a measure of the proportion of cells in the mitotic phase of the cell cycle relevant to total cell count (Timothy, 2014). In particular, arrest of cell division will be performed by decrease of MI which reflects a direct genotoxic effect. Further, alterations of chromosomes during different phases of cell cycle (prophase, metaphase, anaphase and telophase) can be used to determine the clastogenic effect of natural extracts. According to results, mitotic index decreased with increasing test concentrations over 62.5 -500 µg/ml. Moreover, MI of the highest concentration, 500µg/ml of WE exhibited significant difference ($p < 0.05$) when compared to tap water which was used as the negative control, while remaining concentrations of WE contained no such effects. Nevertheless, PMI values of any treatment group are not higher than 50% in comparison with negative control showing only 9-34% inhibition. The decline of MI below 22% of the negative control is considered as sub lethal impact and the decline below 50% is considered as lethal effects on the model (Prajitha and Thoppil, 2016; Debnath, 2018). Accordingly, the highest concentration (500µg/ml) revealed sub lethal effect while other concentrations, 62.5-250µg/ml showed no substantial toxic effect. Chromosomal aberrations also provide a good indication of genotoxic potential and the type/s of active components in bioactive compounds. The chromosomal abnormalities are occurred due to blockage of DNA synthesis or inhibition of spindle formation (Akinboro and Bakare, 2007). The *B. vitiensis* extract showed no obvious interferences on DNA synthesis or spindle formation as the percentage chromosomal aberrations of the highest concentration of treatment (500µg/ml) is similar to the value of percentage chromosomal aberrations of the negative control.

Even though, the percentage is low, different chromosomal aberrations such as chromosomal bridges, breaks, c-mitosis and vagrant chromosomes were observed in each test concentration. Chromosome bridges indicate the clastogenic effect caused by chromosome breaks, whereas vagrant chromosomes and c-mitosis increase the risk for aneuploidy and polyploidy. Chromosomal bridges are most likely formed by breakage or fusion of chromatids or sub chromatids (Yuet et al., 2015). Further, stickiness of chromosomes occurs when they stay connected in anaphase (Yuet et al., 2015). C-mitosis is caused by incomplete spindle formation and functions while vagrants are caused by unequal distribution of chromosomes which are appeared by nondisjunction of chromatids in anaphase (Yuet et al., 2015). Particularly, C-mitosis and vagrants can be observed at low concentrations in the study. These types of aberrations are considered as week genotoxic indications which may be reversible. C-mitosis can perform polyploidy cells while vagrants form aneuploidy cells if they remain as it is.

Conclusion

In toto, it was resulted that aqueous extract of *Bohadschia*

vitiensis which is known to consumed in folk medicines in coastal areas of Sri Lanka, revealed no significant genotoxic effects within the tested range of concentration (<500µg/ml). However, further comprehensive studies with animal models should be conducted before therapeutic applications of the extract.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgement

We would like to extend our gratitude to Dr. D.C.T. Dissanayake, Senior Lecturer in Department of Zoology, University of Sri Jayewardenepura, Sri Lanka for facilitating sample collection during this research work.

References

Akinboro A, Bakare AA. 2007. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. Journal of Ethnopharmacology 112(3):470-5.

Anderson SC, Flemming JM, Watson R, Lotze HK. 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries 12(3):317-39.

Bordbar S, Anwar F, Saari N. 2011. High-value components and bioactives from sea cucumbers for functional foods—a review. Marine Drugs 9(10):1761-805.

Borras M, Nadal J. 2004. Biomarkers of genotoxicity and other end-points in an integrated approach to environmental risk assessment. Mutagenesis 19(3):165-8.

Corvi R, Madia F. 2017. *In vitro* genotoxicity testing–Can the performance be enhanced?. Food and Chemical Toxicology 106:600-8.

Debnath P, Mondal A, Hajra A, Das C, Mondal NK. 2018. Cytogenetic effects of silver and gold nanoparticles on *Allium cepa* roots. Journal of Genetic Engineering and Biotechnology 16(2):519-26.

Dhinakaran ID, Lipton AP. 2014. Studies on the bioactivity of *Holothuria atra* extracts collected from the South east coast of India. International Journal of Biology and Biological Sciences 3(1):6-11.

Dissanayake DC, Stefansson G. 2010. Abundance and distribution of commercial sea cucumber species in the coastal waters of Sri Lanka. Aquatic Living Resources 23(3):303-13.

FISKESJÖ G. 1985. The *Allium test* as a standard in environmental monitoring. Hereditas 102(1):99-112.

Hemachandra CK, Pathiratne A. 2016. Combination of physico-chemical analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet assay/nuclear abnormalities tests for cyto-genotoxicity assessments of treated effluents discharged from textile industries. Ecotoxicology and Environmental Safety 131:54-64.

Jayathilake JMNJ, Gunathilake KVK. 2020. *In-vitro* anti-inflammatory activity, acute toxicity to zebrafish embryos and nutritional analysis of *Bohadschia vitiensis* water extract. International Journal of Pharmaceutical Sciences & Research 11(9): 1000-08. doi: 10.13040/IJPSR.0975-8232.11(9).1000-08.

Kannangara DN, Pathiratne A. 2015. Toxicity assessment of industrial wastewaters reaching Dandugan Oya, Sri Lanka using a plant based bioassay. Journal of the National Science Foundation of Sri Lanka 30:43(2).

Kiew PL, Don MM. 2012. Jewel of the seabed. sea cucumbers as nutritional and drug candidates. International Journal of Food Sciences and Nutrition 63(5):616-36.

Kijjoa A, Sawangwong P. 2004. Drugs and cosmetics from the sea. Marine Drugs 2(2):73-82.

Kumari M, Khan SS, Pakrashi S, Mukherjee A, Chandrasekaran N. 2011. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. Journal of Hazardous Materials 190(1-3):613-21.

Lakshmi V, Saxena A, Mishra SK, Raghubir R, Srivastava MN, Jain RK, Maikhuri JP, Gupta G. 2008. Spermicidal activity of bivittoside D from *Bohadschia vitiensis.* Archives of Medical Research 39(7):631-8.

Laughinghouse IV HD, Prá D, Silva-Stenico ME, Rieger A, Frescura VD, Fiore MF, Tedesco SB. 2012. Biomonitoring genotoxicity and cytotoxicity of Microcystis aeruginosa (Chroococcales, Cyanobacteria) using the Allium cepa test. Science of the Total Environment 432:180-8.

Nursid M, Marraskuranto, Kuswardini A, Winanto T. 2019. Screening of Tyrosinase Inhibitor, Antioxidant and Cytotoxicity of Dried Sea Cucumber from Tomini Bay, Indonesia. Pharmacognosy Journal 11(3).

Prajitha V, Thoppil JE. 2016. Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay. South African Journal of Botany 102:18-25.

Purcell SW, Samyn Y, Conand C. 2012. Commercially important sea cucumbers of the world.

Ridzwan BH, Leong TC, Idid SZ. 2003. The antinociceptive effects of water extracts from sea cucumbers *Holothuria leucospilota Brandt, Bohadschia marmorata vitiensis Jaeger* and coelomic fluid from *Stichopus hermanii*. Pakistan Journal of Biological Sciences 6(24):2068-72.

Shehu A, Ponnapalli MG, Mahboob M, Prabhakar PV, Olatunji GA. 2019. New n-nonadecanoyl-β-sitosterol and other constituents from the stem-bark of *Anacardium occidentale*. Natural Product Research 7:1-7.

Sponchiado G, Adam ML, Silva CD, Soley BS, de Mello-Sampayo C, Cabrini DA, Correr CJ, Otuki MF. 2016. Quantitative genotoxicity assays for analysis of medicinal plants: A systematic review. Journal of Ethnopharmacology 178:289-96.

Tedesco E, Mičetić I, Ciappellano SG, Micheletti C, Venturini M, Benetti F. 2015. Cytotoxicity and antibacterial activity of a new generation of nanoparticle-based consolidants for restoration and contribution to the safe-by-design implementation. Toxicology *in Vitro* 29(7):1736-44.

Timothy O, Idu M, Olorunfemi DI, Ovuakporie-Uvo O. 2014. Cytotoxic and genotoxic properties of leaf extract of *Icacina trichantha* Oliv. South African Journal of Botany 91:71-4.

Wijeyaratne WM, Wadasinghe LG. 2019. *Allium cepa* Bio Assay to Assess the Water and Sediment Cytogenotoxicity in a Tropical Stream Subjected to Multiple Point and Nonpoint Source Pollutants. Journal of Toxicology 2019:1-10. .

Yuet Ping K, Darah I, Yusuf UK, Yeng C, Sasidharan S. 2012. Genotoxicity of *Euphorbia hirta*: an *Allium cepa* assay. Molecules 17(7):7782-91.

Zendehboodi Z. 2018. Cytotoxicity and genotoxicity effects of water boiled in aluminum vessels on *Allium cepa* root tip cells. Journal of Environmental Health Science and Engineering 16(2):337.