JOURNAL OF MARITIME RESEARCH

Vol XXII. No. I (2025) pp 442–448

ISSN: 1697-4840, www.jmr.unican.es

Phytochemical Screening and Toxicity Testing of *Bilang* (*Sesuvium portulacastrum* Linn (1753)) Plant Extract

Anthony S. Ilano[1,*]

ARTICLE INFO

Article history:
Received 17 Jul 2024;
in revised from 20 Jul 2024;
accepted 15 Aug 2024.

Keywords:
Genotoxic Activity, Chromosomal Aberration, Mitotic Index, Secondary Metabolite, And Allium Cepa.

ABSTRACT

Sesuvium portulacastrum Linn (1753), a member of the family Aizoaceae, is a multipurpose facultative halophyte. The extract from S. portulacastrum was subjected to phytochemical and toxicity screening. *Allium cepa* Chromosome Aberration Assay was conducted to assess the plant's toxicity, while a phytochemical screening was conducted to determine the presence of bioactive secondary metabolites. *Allium cepa* Chromosome Aberration Assay showed that the plant extract possesses genotoxic activity as demonstrated by bridges, fragments, laggards, and vagrants. It also showed that the plant extract was able to inhibit root growth. The plant extract showed an inhibitory effect; at 100% concentration, and was able to inhibit 100% with the highest number of aberrations and had the least number of dividing cells. The phytochemical screening revealed the presence of the constituents of the plant extract, and tested positive for reducing sugar, saponin, alkaloid, resin, and tannin. These phytochemical constituents indicate the potential of S. portulacastrum extracts in the pharmaceutical industry.

1. Introduction.

Sesuvium portulacastrum Linn (1753) is a multipurpose facultative halophyte that belongs to the Aizoaceae family. This plant is commonly known as sea purslane, locally known as "dampalit" in Tagalog and "bilang" or "bilangbilang" in the Visayan language. This species was first published as Portulaca portulacastrum by Carl Linnaeus in 1753. After six years, Linnaeus displaced Portulaca into Sesuvium, and it has remained the same name ever since.

According to Simon and Krolman (1989), *Sesuvium* is an essential source of phytoecdysteroids (insect molting hormones) 20-hydroxyecdysone and a minor ecdysone which regulates many biochemical and physiological processes during the various developmental stages of insects.

The steroid hormones affect pupation in insects, and thus these phytoecdysones can probably be used as biological pesticides. Several reports suggested that ecdysteroids may effectively control diabetes (Yoshida et al. 1971; Uchiyama and Yoshida 1974; Najmutdinova and Saatov 1999; Dinan 2001).

Moreover, *Sesuvium portulacastrum* is used in traditional medicine to remedy fever, kidney disorders, and the treatment of various infections and scurvy (Rojas et al., 1992; Magwa et al., 2006).

Medicinally and economically, *Sesuvium* containing secondary bioactive metabolites has shown a great potential as an alternative source of synthetic raw materials in the food, perfumery, cosmetic and pharmaceutical industries (Lis-Balchin and Deans 1997). However, literature related to the ethnomedicinal importance of salt marsh plants is scarce, and knowledge of the chemical constituents of plants is essential for the discovery of therapeutic agents that may be new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. Thus, the present study aims to screen the secondary metabolites present and determine the genotoxic activity in the *Sesuvium portulacastrum* collected from fishpond levees of Carmen, Cebu.

[1]Campus Director of Cebu Technological Univesity - Carmen Campus.
[*]Corresponding author: Anthony S. Ilano. E-mail Address: anthony.ilano@ctu.edu.ph.

2. Materials and Methods.

2.1. Collection of Plant Materials.

The plant *Sesuvium portulacastrum* Linn (1753) (Bilang) was procured from the ponds of Cebu Technological University-Carmen Campus. It was washed and pat dried to eliminate dust and dirt before cutting into small pieces before use (Figure 1).

Figure 1: Sesuvium portulacastrum Linn (1753) (Bilang) Plant.

Source: Author.

2.2. Plant Extraction and preparation of test solutions.

The freshly cut *Sesuvium portulacastrum* Linn (1753) (Bilang) was subjected to extraction by expression. Pieces of *S. portulacastrum* were placed in a 1000-ml osterizer and were blended, squeezed, and filtered. The 100% juice was concentrated in a beaker, and the three test solutions were prepared: 10%, 50%, and 100% solutions. Filtered seawater served as the negative control.

Phytochemical Screening Using Test Tube Method by Guevarra (2004):

The 100% test solution was introduced to different chemical reagents to determine certain constituents present in the plant extract. A total of 12 metabolites were tested.

Kedde Test (A test for the presence of Unsaturated Lactone):

A solution of dichloromethane (Kedde Reagent) was added drop by drop to the test solutions. The presence of green coloration would be a positive result.

Keller Killani's Test (A test for the presence of deoxysugar).:

A few drops of glacial acetic acid and ferric chloride were added to the test solutions. A few drops of concentrated sulphuric acid were added in an inclined position and allowed to stand upright. The positive result would be the presence of reddish-brown color that may turn blue or purple at the interface of the acid layer.

Liebermann-Burchard Test (A test for the presence of Sterols):

A few drops of acetic anhydride were added to the test solutions, forming a mixture. One to two drops of concentrated sulphuric acid were then added. The positive result would be the formation of an emerald green coloration which may turn red or blue.

Fehling's Test (A test for the Presence of Reducing Sugar):

One millimeter of Fehling's A and B were added to the test solutions. And then, heated in a water bath. Brick red precipitate would be the positive result.

Foam Test (A test for the presence of Saponins):

The test solutions were added with a few drops of distilled water and shaken to test the formation of froth. A froth stable for 15 minutes would be the positive result.

Mayer's Test (A test for the Presence of Alkaloids):

Five millimeters of 1% HCl were added to one millimeter of the test solutions and a few drops of Mayer's reagent. The positive result would be the formation of a white precipitate.

Foam Test (A test for the presence of Saponins):

The test solutions were added with a few drops of distilled water and shaken to test the formation of froth. A froth stable for 15 minutes would be the positive result.

Mayer's Test (A Test for the Presence of Alkaloids):

Five millimeters of 1% HCl were added to one millimeter of the test solutions and a few drops of Mayer's reagent. The positive result would be the formation of a white precipitate.

Ninhydrin's Test (A test for the presence of Free Amino Acid):

The test solutions were boiled with 0.2% ninhydrin solution. The positive result would be the formation of purple or violet coloration.

Salkowski's Test (A test for the presence of Terpenes):

Two millimeters of chloroform was added to the test solutions. In an inclined position, slowly add concentrated sulphuric acid drop by drop. The positive result would be the formation of reddish-brown coloration at the interface.

Test for the presence of Flavonoids:

Concentrated HCl was added to the test solutions. The mixture was then subjected to a water bath for 15 minutes. The positive result would be the formation of red solid or violet coloration.

Test for the presence of Phenols:

The plant extract was dissolved in water and warmed. 2 ml of ferric chloride was then added. The positive result would be the formation of green or blue color.

Test for the presence of resins:

5 mL of distilled water was added to the extract. A positive result would be the turbidity of the solution.

Test for the presence of Tannins.

A drop of 5% ferric chloride was added to the test solutions. The positive result would be the formation of brownish-green or blue-black coloration.

Determination of Genotoxic Activity using Allium cepa chromosome aberration assay (Ping et al., 2012):

Roots of *Allium cepa* were observed for the evaluation of the genotoxicity of the plant extract. The *Allium cepa* chromosome aberration assay was carried out at room temperature, and the onion bulbs were kept away from direct sunlight during the experiment. The roots were scraped to promote the emergence of new ones. Each trial was done in triplicates. The procedure involved pre-treatment and exposure of the root tips and preparation of the slides for observation.

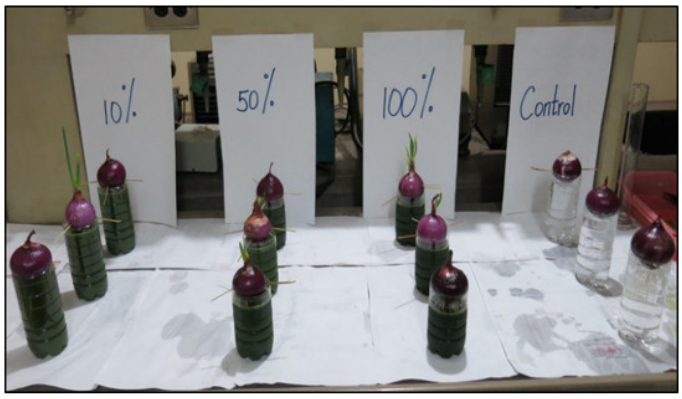
Pre-Treatment and Exposure:

Roots of *Allium cepa* were grown in distilled water at room temperature for 2–3 days. When the roots were approximately 2–4 cm in length, roots were then exposed to the different concentrations of the plant extract, while for the negative control, it was exposed to distilled water (Figure 2). After 48 hours, root tips from each bulb were harvested and fixed with Carnoy's fixative (1:3 acetic acid: alcohol) for 24 hours. After exposing the roots of A. cepa, % root growth inhibition was computed using the equation:

$$\%Root\ Growth\ = \frac{Root\ length\ of\ control\ group - Root\ length\ of\ treated}{Root\ length\ of\ control\ group} \times 100$$

*Control group will serve as the normal root growth.

Figure 2: *Allium cepa* Chromosome Aberration Assay Set-up.



Source: Author.

2.3. Slides Preparation and Observation.

The roots were harvested in the afternoon between 1:00 to 3:00 when the cell division was active. The root tips were washed 2-3 times with distilled water and were hydrolyzed with 1N HCl at 60–70 °C for 12 minutes. Then, about 1–2 mm of the root starting from the tip was cut and placed on the slide. A small amount of 2% aceto-carmine was dropped on the root tip and left for 2 minutes. The root tip was squashed with a needle, and another small drop of aceto-carmine was added and left for another 2 minutes. The cover slip was carefully lowered to avoid air bubbles, and the sides of the slides were sealed with clear fingernail polish.

2.4. Microscopic Examination.

The slides were observed under the light microscope at 400× and 630× magnification. Photomicrographs were made, and a minimum of 100 cells per slide was analyzed. The mitotic index and chromosome aberrations in mitotic phases were determined by the examination and counting a minimum of 100 cells per slide. The experiment was conducted in three replicates. Microscopic examination was performed to see the presence of bridges, fragments, laggards, or vagrants as indicators that *S. portulacastrum* Linn (1753)) plant extract possesses genotoxicity activity. Percent Chromosomal aberration was then computed using the formula of Al-Joubori et al. (2014).

$$\%\ Chromosomal\ aberration\ = \frac{Total\ no.\ of\ cells\ with\ aberration}{Total\ no.\ of\ observed\ cells} \times 100$$

Computation for Mitotic Index (Ping et al., 2012) & % Genotoxic activity (Levine et al., 2000)

The mitotic index and % genotoxic activity were computed as follows:

$$Mitotic\ index = \frac{Number\ of\ cells\ in\ mitosis\ (P+M+A+T)}{Total\ number\ of\ cells} \times 100$$

$$\%\ Genotoxic\ activity = \frac{mitotic\ index\ (control) - mitotic\ index}{normal\ mitotic\ index\ (control)} \times 100$$

Legend:

P (Prophase) = the chromosomes are visible and tangled.

M (Metaphase) = the chromosomes are arranged in equatorial plate.

A (Anaphase) = the sister chromatids separate moving towards the spindle poles.

T (Telophase) = each daughter chromosome has arrived at the spindle pole following occurs cytokinesis.

3. Results and Discussion.

3.1. Phytochemical Screening.

Medicinal use of the plants is due to the presence of the phytochemicals that occur naturally in them. Sesuvium species containing secondary metabolites have shown a great potential to substitute some artificial raw materials applied in nutraceuticals, cosmetics, and perfumery industries (Lis-Balchin & Deans, 1997; Lokhande et al., 2013).

Table 1 shows the summary of results of the secondary metabolite chemical screening using the test tube method for *Sesuvium portulacastrum* Linn (1753). In this study, the phytochemical constituents present in *S. portulacastrum* were reducing sugar, saponin, alkaloids, flavonoids, resin, and tannin. The presence of saponin and tannin indicates the immunity booster

and wound healing characteristics that may also contribute to their antimicrobial properties (Sathish et al., 2016). The plant contains a rich source of alkaloids used for antiviral activity and curing hepatitis and other diseases (Joshi and Bhosale, 1981; Premnathan et al., 1995; Padmakumar and Ayyakkannu, 1997; Bandaranayake, 2002). Furthermore, the presence of flavonoids in Sesuvium has also been suggested for their role in protection from UV stress and pathogen attack (Heldt, 2005; Agoramoorthy et al., 2008). However, the content of the secondary metabolites can vary depending on the particular habitat where the plant grows.

Table 1: Secondary bioactive metabolites found in Sesuvium portulacastrum using Test Tube Method.

Secondary Metabolite Tested	Present (+) Absent (-)
Reducing Sugar	+
Saponin	+
Unsaturated lactone	-
Deoxysugar	-
Sterol	-
Alkaloids	+
Free Amino Acids	-
Terpenes	-
Flavonoids	+
Phenol	-
Resin	+
Tannin	+

Source: Author.

3.2. Genotoxic Effect.

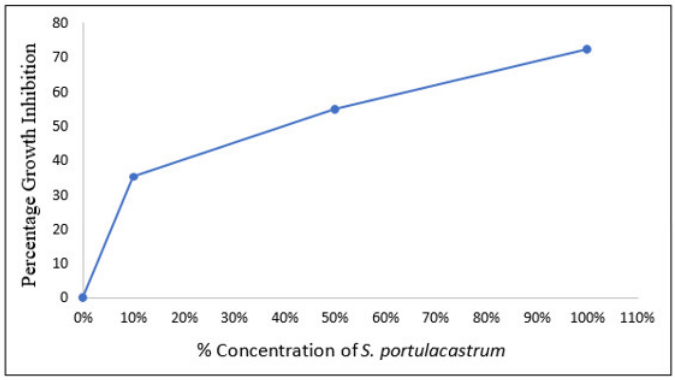
Genotoxic Test was conducted using *Allium cepa* Chromosome Aberration Assay. *A. cepa* has been regarded as the most favorable species in assessing chromosomal damages and disturbances in the mitotic cycle due to the presence of good chromosome conditions (Leme and Marin-Morales, 2009). The chromosomes of A. cepa have the highest sensibility, with significant effects even at a lower concentration. Moreover, the Test can also be used to measure toxicity, studying macroscopic parameters such as length of roots, variations in form, color and consistency of roots, presence of broken root tips, tumors, and hooks (Fiskesjö, 1985).

Figure 3 shows the percent root growth inhibition of macerated *S. portulacastrum* extract obtained from the lengths of the onion root tips exposed to different test solutions. Onion root tips exposed to 10%, 50%, and 100% macerated test solutions of *S. portulacastrum* obtained a result of 35.2, 54.8, and 72.2 percent root growth inhibition, respectively. As the concentration of macerated *S. portulacastrum* increases, the percent growth inhibition also increases.

3.3. Formation of Chromosome Aberration.

Table 2 shows the different types of cell abnormalities caused by the genotoxic effect of the plant extract on the onion root tips, which are indicators of genotoxicity as this show alterations of the cell's genome. Manifestation of bridges, fragments, laggards & vagrants is the basis for the genotoxic effect of *S. portulacastrum* Linn (1753) in this study. Rank and Nielson 1997 have reported that the chromosome bridges and fragments lead to structural changes in chromosomes of crop plants and in other organisms in the environment. Chromosomal bridges mainly develop because of the nondisjunction of sticky chromosomes or breakage and reunion during separation at anaphase (Feretti et al., 2007). Lagging chromosomes arise when chromosomes fail to remain connected with spindle fiber which may move to either of the poles (Khanna and Sharma, 2013). Moreover, the failure of the spindle apparatus organization and its normal function is the cause of vagrant chromosomes.

Figure 3: Percentage growth inhibition of Sesuvium portulacastru.



Source: Author.

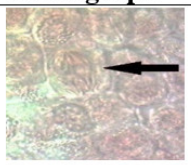
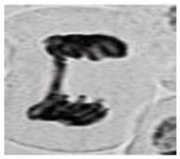
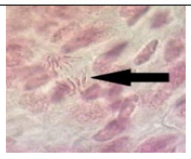
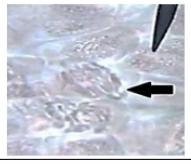
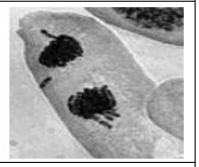
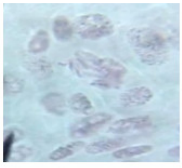
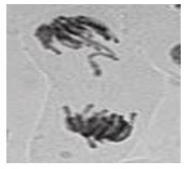
3.4. Percent Chromosomal Aberration.

Table 3 shows the average number of chromosomal aberrations and the percent chromosomal aberration of the different concentrations of *S. portulacastrum* extract. In this study, the mean number of aberrations increases as the percentage concentration of S. portulacastrum extract increases. The result shows that 10%, 50%, and 100% test concentrations obtained 1.2, 2.6, and 3.4 mean number of aberrations, respectively. Percentage chromosomal aberrations were observed to increase when exposed to higher concentration under expression test solution. The results also showed that the chromosomal aberrations in the roots treated with the bilang extracts had shown marked differences from the control.

3.5. Mitotic Index of Allium cepa.

Table 4 shows the average number of dividing cells observed, including the number of cells that have undergone prophase, metaphase, anaphase, telophase, and mitotic index (Mitotic Index) of the different concentrations of *S. portulacastrum* extract. According to Hoshina (2002), MIs significantly lower than the negative control can indicate alterations deriving from the chemical action in the growth and development of exposed organisms. On the other hand, MIs higher than the negative

Table 2: Microscopic Examination of Root Tips for Chromosome Aberration.

Chromosomal Aberration	Actual Photograph	Theoretical Photograph
Bridges		
Fragments		
Laggards		
Vagrants		

Source: Author.

control are results of an increase in cell division, which can be harmful to the cells, leading to disordered cell proliferation and even to the formation of tumor tissues.

Table 3: Mean number of Dividing cells observed and Mitotic Index of S. portulacastrum.

Test Solution	Concentration	Mean Number of Aberrations	% Chromosomal Aberrations
Expression	10%	1.2	0.8%
	50%	2.6	1.7%
	100%	3.4	2.1%

Source: Author.

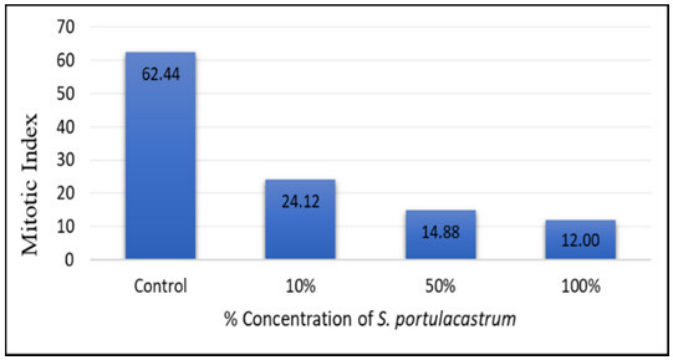
Table 4: Average number of aberration and percent chromosomal aberration.

Concentration	Prophase	Metaphase	Anaphase	Telophase	MI
Control	**14.80**	**11.22**	**17.04**	**19.80**	**62.44**
10%	7.28	6.26	5.68	5.12	24.12
50%	4.88	4.14	3.86	3.08	14.88
100%	2.92	2.36	2.08	2.96	12.00

Source: Author.

Reduction in mitotic activity is accompanied by decreased amounts of DNA, which could be due to inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (Türkoğlu 2008, 2009). In this study, the mitotic index for S. portulacastrum ranges from 12 to 62.44 when exposed to different concentrations (Figure 4). Furthermore, the MI gradually decreased with the increasing concentration of bilang extract.

Figure 4: Mitotic index for Allium cepa chromosome aberration assay.



Source: Author.

Conclusions and Recommendations.

Based on the results of the phytochemical screening, the secondary metabolites present in Sesuvium portulacastrum were reducing sugar, saponin, alkaloid, flavonoid, resin, and tannin. These phytochemical constituents indicate the potential of bilang extracts in the pharmaceutical industry. The manifestation of bridges, fragments, laggards & vagrants indicates the genotoxic effect of *Sesuvium portulacastrum* Linn (1753). The results also showed that the chromosomal aberrations in the roots treated with the bilang extracts had shown marked differences from the control. Further, the mitotic index gradually decreased with the increasing concentration of *bilang* extract.

In the country, there have only been a few studies on *Sesuvium portulacastrum* Linn (1753), even though they are abundantly found in the coastal areas in the Philippines, the researchers recommend other studies to be conducted. Furthermore, researchers recommend doing cytotoxicity testing using the Sea Urchin Toxicity Test to obtain a broader scope of cell incongruity like cell arrest, cell bursting, and morphological distortion.

Acknowledgements.

The authors wish to express their profound gratitude to the General Appropriations Act (GAA) for funding this research project. Special thanks to Cebu Technological University- Carmen Campus for their support in this research project.

References.

1. Agoramoorthy G, Fu-An C, Venkatesalu V, Daih-Hu AK, Po-Chuen S. 2008. Evaluation of antioxidant from selected mangrove plants of India. Asian J Chem 20(2): 1311–1322.
2. Al-Joubori MA, Zaidan HK, Al-Saadi AH (2014). Evaluation of Chromosome Aberrations and mitotic index in alloxan-induced diabetic male diabetic male rats treated with the mixture of plants extracts mixture. J. Babylon Univ.: Pure Appl. Sci. 5 (22): 1545-1555.
3. Bandaranayake WM. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wet Ecol Manag 10:421–452. doi:10.1023/A:10213976-24349.
4. Dinan L. 2001. Phytoecdysteroids: biological aspects. Photochem 57:325–339. doi:10.1016/S0031-9422(01)0-0078-4.
5. M., Monarca S. 2007. Allium cepa chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. Food Addit. Contam. 24, pp. 561-572.
6. Fiskesjö G. 1985. The Allium test as a standard in environmental monitoring. Hereditas 102: 98-112.
7. Guevara BQ. A guidebook to plant screening: phytochemical and biological. University of Santo Tomas Publishing House; 2005.
8. Heldt H.W. 2005. Phenylpropanoids comprise a multitude of plant secondary metabolites and cell wall components, In: Plant Biochemistry (3rd eds). Elsevier Academic, New York, pp 448.
9. Hoshina M.M. 2022. Avaliação da possível contaminação das águas do Ribeirão Claro - município de Rio Claro, pertencente à bacia do rio Corumbataí, por meio de testes de mutagenicidade em Allium cepa, Trabalho de conclusão (Bacharel e Licenciatura - Ciências Biológicas), Universidade Estadual Paulista, Rio Claro/SP. 52 p.
10. Johnson, A.F., 1977. A survey of the strand and dune vegetation along the Pacific and southern gulf coasts of Baja California, Mexico. Journal of Biogeography, 7, 83-99.
11. Joshi GV, Bhosale LJ. 1981. Estuarine ecosystem of India. In: Sen DN, Tajpurohit KS (eds) Contributions to the ecology of halophytes. Dr. W. Junk Publications, The Hague, pp 21–33.
12. Judd, F.W.; Lonard, R.I., and Sides, S.L., 1977. The vegetation of South Padre Island, Texas in relation to topography. Southwestern Naturalist, 23, 31-48.
13. Khanna N. and Sharma S. 2013. Allium cepa root chromosomal aberration assay: a review. Indian J. Pharm 1, page 3.
14. Leme, Daniela Morais, and Maria Aparecida Marin - Morales. Allium cepa test in environmental monitoring: A review on its application. Mutation Research/Reviews in Mutation Research 682, no.1 (2009):71-81. doi: 10.1016/-j.mrrev.2009.06.002.
15. Lis-Balchin M, Deans SG. 1997. Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Bacteriol 82:759–762.
16. Lokhande, V., B. Gor, N. Desai, T. Nikam and P. Suprasanna. 2013. Sesuvium portulacastrum, a plant for drought, salt stress, sand fixation, food and phytoremediation. A review. Agronomy for Sustainable Development. 33(2): 329-348.
17. Ramani B, Reeck T, Debez A, Stelzer R, Huchzermeyer B, Schmidt A, et al. Aster tripolium L. and Sesuvium portulacastrum L.: Two halophytes, two strategies to survive in saline habitats. Plant Physiol Biochem 2006; 44: 395-408.
18. Rank J., Nielsen M.H., 1997 – Allium cepa anaphase-telophase root tip chromosome aberration assay on N-methyl-N-nitrosourea, maleic hydrazide, sodium azide, and ethyl methanesulfonate. Mut. Res., 390: 121-127.
19. Rojas, A., Hernandez, L., Rogeho, P.M., Mata, R. 1992. Journal of Ethnopharmacology; 35:127-149.
20. Magwa, M. L., Gundidza, M., Gweru, N., & Humphrey, G. (2006). Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. Journal of Ethnopharmacology, 103(1), 85-89.
21. Najmutdinova DK, Saatov Z. 1999. Lung local defense in experimental diabetes mellitus and the effect of 11, 20-dihydroxyecdysone in combination with manilil. Arch Insect Biochem Physiol 41:144–147. doi:10.1002/(SICI) 1520-6327(1999) 41:3<144: AID-ARCH5>3.0.CO;2-0.
22. Padmakumar K, Ayyakkannu K. 1997. Seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from Southern coast of India. Bot Mar 40:507–515. doi:10.1515/ botm.1997.40.1-6.50.
23. Ping KY, Darah I, Yusuf UK, Yeng C, Sasidharan S. 2012. Genotoxicity of Euphorbia hirta: An Allium cepa Assay. Molecules 17:7782–7791. https://doi.org/10.3390/molecules17077782.
24. Premnathan M, Kathiresan K, Chandra K. 1995. Antiviral evaluation of some marine plants against Semliki Forest Virus. Internat J Pharmacog 33:1–3. doi:3109/13880-209509088153.
25. Sathish, K. K., Nagalakshmi, K. and Venkateshwarlu, G. 2016. Preservative effect of solvent free natural spice extracts on tuna fillets in chilled storage at 4 ºC: Microbial, biochemical and sensory attributes. Int. J. Fish. Aquat. Studies 4(6) 20-24.
26. Simon P, Krolman J. 1989. Ecdysone: from chemistry to mode of action. In: Koolman JA (ed) George Thieme, New York, pp 254–259.
27. Türkoğlu Ş. 2008. Evaluation of genotoxic effects of sodium propionate, calcium propionate and potassium propionate on the root meristem cells of Allium cepa. Food Chem Toxicol. 46:2035–2041.
28. Uchiyama M, Yoshida T. 1974. Effect of ecdysterone on carbohydrate and lipid metabolism. In: Burdette WJ (ed) Invertebrate endocrinology and hormonal heterophylly. Springer, Berlin, pp 401–416.

29. Yoshida T, Otaka T, Uchiyama M, Ogawa S. 1971. Effect of ecdysterone on hyperglycemia in experimental animals. Biochem Pharmacol 20:3263–3268.doi:10.1016/0-0062952(71)90431- X.