Original Research

Phytochemical constituent, cytotoxic activity and outcome on wheat growth parameters possessed by extracts of seaweed collected from Libyan coast

Sondos R. Almokhzanji[1], Majda S. Elwalid[2], Muftah A. Shushni[1*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli and
[2]Marine Biology Research Center, Tripoli, Libya

Mediterranean Journal of Pharmacy and Pharmaceutical Sciences

Article information
Received
27-07-2021
Revised
29-08-2021
Accepted
10-09-2021
Published
30-09--2021

***Corresponding Author**
m.shushni@uot.edu.ly

Abstract

Algae are used by human beings for food from ancient times, as they contain a wide range of elements. Using inexpensive, in-house bioassays for screening and monitoring of extracts where the aim of these bioassays is to provide a front-line screen that can be followed up by more specific and expensive bioassays. The phytochemical screening, assessment of toxicity and effects on growth parameters of *Traticum sativum* of ethanol and dichloromethane extracts of five macro-algal species (two green, two brown and one red) collected from Libyan coast were studied. The Brine shrimp lethality assay was conducted to determine the toxic effects of seaweed extracts on *Artemia Salina* nauplii larvae and this was to provide a front-line screen that can be backed up by specific and expensive bioassays once the active compounds have been isolated. The effect of seaweeds liquid fertilizer on growth parameters of *Traticum sativum* were examined using *in vitro* seed germination in petri dishes bioassay. Seed germination percentage, fresh and dry weight, shoots length and roots length were the parameters recorded in young seedlings post germination. Ethanolic and dichloromethane extracts of the five algae samples represented the presence of several chemical constituents. All extracts exhibited LC_{50} > 1000 µg per ml. In this study, all algal extracts are non-toxic according to Brine shrimp lethality assay so they may be considered as edible seaweeds. Different effects on growth parameters of *Traticum sativum* suggested the presence of micro-elements, macro-elements and different concentrations of plant growth hormones.

DOI ***10.5281/zenodo.5534618***

Keywords: Brine shrimp lethality assay, fertilizer, Libya, marine algae, seaweeds, wheat

***HOW TO CITE THIS**: Almokhzanji S.R., Elwalid A.S. and Shushni M.A. (2021) Phytochemical constituent, cytotoxic activity and outcome on wheat growth parameters possessed by extracts of seaweed collected from Libyan coast. Mediterr J Pharm Pharm Sci 1(3): 40-45. https://doi.org/10.5281/zenodo.5534618*

Introduction

Countless unique organisms roam the vast oceans many of which possess a novel structure and biological activities. This marvelous source of potential discoveries is not yet harvested, so it could be the womb of countless bioactive natural products [1]. Various inhabitants of the water such as animals and microbes produce *secondary metabolites* chemicals which are not vital for primary metabolic processes of these organisms, yet are believed to confer some evolutionary advantage. Many of these organisms are non-motile and have developed chemical compounds due to living in densely populated habitats. The natural product drug discovery research aimed its investigations at the marine environment recently due to its unraveled biodiversity compared to other environments. The pioneering work of Bergmann in the 1950s introduced the potential of the marine natural product as pharmaceuticals [2]. Marine macro-algae are multicellular [3]. They are members of the kingdom Protista [4] and they can be found everywhere as long as there is a light to carry out photosynthesis [5]. They are classified according to color into three different groups: red, green and brown algae. Extracts derived from algae contain components such as

polysaccharides, proteins, polyunsaturated fatty acids (PUFAs), pigments, polyphenols, minerals, and plant growth hormones [6]. Marine macro-algae have several uses in different fields such as Pharmaceuticals [7], cosmetics [8], bioremediation and industrial chemicals [9]. Chemical fertilizers are the most important component to increase an agriculture production as they have excellent effects on plant growth and health. Today, there is an increasing demand which represent hazardous effects directly or indirectly on environment and human health in particular nitrogen based fertilizers. Thus, many farmers began to use natural organic based fertilizers instead of chemical ones [10]. The objective of this study is to carryout qualitative phytochemical screening, cytotoxic activities and outcome on growth parameters of wheat seeds using algae species collected from western part of Libyan coast.

Materials and methods

Collection and processing of algal samples: Ulva lactuca, Cystocera compressa, Sargassum hornschuchii, Gelidium pusillum and *Enteromarpha intestinalis* were collected randomly by hand picking from western coast of Libya (Tajura Elhmidia, Marine Biology Research Center, Tripoli) between January and April, 2018. The algal samples were taxonomically identified at Marine Biology Research Center, Tajura (east part of Tripoli), Libya. Algae samples were cleaned with fresh seawater and then with distilled water to remove epiphytes, suspended matter and sand particles. The materials were dried completely in shade at a room temperature, then blended to fine powder in an electronic grinder.

Extraction of algal samples: Each of the powdered algal samples was subjected to Soxhlet extractor for continuous hot extraction with 97% ethanol and dichloromethane, respectively, for 24 hours. Afterwards, the ethanolic and dichloromethane extracts were filtered through filter paper - grade 42 - and the resultant filtrates were concentrated to dryness under reduced pressure using rotary evaporator. Finally, the dried extracts were stored is small jars at 2 ^{o}C until use.

Phytochemical screening: The algal extracts were subjected to phytochemical screening to detect different chemical groups of compounds as previously mentioned [11].

Tests for alkaloids: Mayer's test (potassium mercuric iodide solution), each of individual algal extract was dissolved in dilute HCl then few drops of Mayer's reagent was added. Cream color precipitate indicate presence of alkaloids. Dragendorf's test (potassium bismuth iodide solution), each of individual algal extract was dissolved in dilute HCl then few drops of Dragendorf's reagent was added. Reddish brown precipitate indicate presence of alkaloids. Wagner's test (iodine in potassium iodide), each of individual algal extract was dissolved in dilute HCl, then few drops of Wagner's reagent was added. Reddish brown precipitate indicate the presence of alkaloids.

Tests for flavonoids: Shinoda test (magnesium hydrochloride reduction test), few fragments of magnesium ribbon were added to each of individual algal extract then concentrated HCl was added drop wise, pink scarlet color indicate the presence of flavonoids. Alkaline reagent test: few drops of NaOH solution was added to extract solution, formation of an intense yellow color which turns to colorless by the addition of few drops of dilute acetic acid indicate presence of flavonoids.

Tests for phenolic compounds: Ferric chloride test, few drops of neutral 5% ferric chloride solution were added to each of individual extract, a dark green color indicates the presence of phenolic compounds. Lead acetate test: few drops of 10% lead acetate solution were added to extracts. White precipitate indicates presence of phenolic compounds. Gelatin test: few drops of 10% gelatin solution were added to test extract solution, white precipitate indicates presence of phenolic compounds.

Tests for tannins: Ferric chloride test, few drops of ferric chloride test reagent were added to extract solution, an intense green, purple, blue or black color developed was taken as an evidence for the presence of tannins.

Tests for amino acids: Ninhydrin test, few drops of 5% ninhydrin solution were added to extracts and boiled, violet color indicates presence of amino acids.

Test for protein: Biuret test, 4% of NaOH solution and few drops of 1% $CuSO_4$ solution were added to extract solution, violet color indicate presence of protein.

Tests for sterols and triterpenoids: Libermann Burchard test, extracts were treated with few drops of acetic anhydride, boil and cool, concentrated H_2SO_4 was added along the side of test tube, shows brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols and formation of deep red color indicates presence of triterpenoids.

Salkowski's test: extract was treated in chloroform with few drops of concentrated H_2SO_4, shaken well and allow to stand for some time, red color appears in the lower layer indicate the presence of sterols and formation of yellow colored lower layer indicate presence of triterpenoids.

Tests for carbohydrates (Fehling's test): equal volume of Fehling A (copper sulphate in distilled water) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents are mixed and few drops of sample was added and boiled, reducing sugars forming a brike red precipitate of cuprous oxide.

Tests for oils and fats: A small quantity of extract was pressed in between the two filter papers. Oil stain on the filter papers indicates presence of oils and fats.

Tests for saponins: Froth test, a pinch of the dried powdered plant was added to 2 - 3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicates presence of saponins.

Tests for organic acids: Oxalic acid, when few drops of 1% $KMnO_4$ and dilute H_2SO_4 added to extract solutions, color disappears.

Malic acid: three drops of 40% $FeCl_3$ solution were added to extract test solutions, appearance of yellowish color indicates presence of malic acid.

Tests inorganic acids: Sulphate test, lead acetate reagent was added to extract test solutions, white precipitate appears which is soluble in NaOH.

Carbonate test: To extract test solution, dilute HCl was added, liberate CO_2 gas indicate presence of carbonate.

Tests for coumarine: To two ml of test solution, a few drops of alcoholic NaOH were added. Appearance of yellow color indicates presence of coumarine.

Brine shrimp lethality assay: Hatching of Brine shrimp, 1.6 gm of *Artemia Salina* (Linnaeus) cysts were aerated in one-liter capacity cylinder containing seawater. In the bottom of the cylinder, the air stone was placed to ensure the complete aeration of the cysts. Newly hatched free-swimming pink-colored nauplii was harvested from the bottom after 24 hours' incubation at room temperature [12].

Preparation of test solution positive and negative controls: 40 mg of each of the test samples were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) and finally, the volume was made to 20 ml with sea water. Thus, the concentration of the stock solution was 2000 µg/ml. Then the solution was serial diluted to 250, 500 and 1000 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii. Potassium dichromate was used as a positive control as it is strong oxidizing agent, it was evaluated at very low concentration (1200, 600, 300, 150, 75, 37.5 and 18.75 µg/ml). 50 µl of DMSO was added to each of three pre-marked dish containing 4.95 ml of sea water and 10 shrimp nauplii to be used as negative control [13].

Counting of nauplii: The number of survived nauplii in each tube was counted after 24 hours using a magnifying glass against a black background, larvae were considered dead if they did not exhibit any internal or external movement during the observation period [14]. The concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) which represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time.

In vitro seed germination in Petri dishes bioassay: Preparation of seaweed liquid fertilizer, 50 gm of finely powdered material was extracted for 60 min with 500 ml boiling water and then filtered. The resulting extract was cooled and taken as 100% concentration of the SLF, then the SLF refrigerated between 0 - 4 °C, see reference [15].

Experimental design and treatments: The seeds of wheat (*Traticum Sativum*) with uniform size, color and weight were surface sterilized with 5% sodium hypochlorite. The treatments were 2.5%, 5.0%, 7.5%, 10.0% and 20.0% aqueous extracts of seaweeds (each concentration had five treatments 20 seeds for each treatment). Five petri plates were watered with 10 ml of distilled water and considered as the control. The remainders of them were treated with 10.0 ml of 2.5%, 5.0%, 7.5%, 10.0% and 20.0% of aqueous seaweed extract at the first and three days later. All petri plates were 130 and they were taken on 7th day after sowing [15].

Growth analysis: The growth parameters including germination percentage, fresh and dry weight, shoot length and root length were calculated.

Results

Percentage yield of algal extracts: The yield of extract was varied according to the polarity of the solvents which used for extraction. The results indicate the percentage of yield of ethanolic extracts is higher than that of DCM extracts (**Table 1)**.

Table 1: percentage yield of algal extracts by Soxhlet extractor

Algae	Yield ethanolic extract (w/w)	Yield of DCM extract (w/w)
Cystosiera compressa	11.70%	0.89%
Sargassum hornschuchii	04.70%	0.87%
Enteromarpha intestinalis	05.75%	3.25%
Ulva lactuca	20.30%	0.20%
Gelidium pusillum	07.70%	2.30%

Phytochemical screening: Phytochemical screening of the ethanolic extracts of all algae species showed the presence of alkaloids, phenolic compounds, tannins, amino acids, organic acids, inorganic acids, coumarines, saponins, fats, carbohydrates, steroids and triterpenoids with no proteins. Dichloromethane extracts represented the presence of oils, fats, saponins, carbohydrates (except one brown algae which belongs to Sargassum species), steroids and terpenoids while alkaloids, phenolics, tannins, amino acids, protiens, organic acids, inorganic acids and coumarines were absent.

Brine shrimp lethality assay: For this study the crude ethanol and dichloromethane extracts of the five tested species are considered to be nontoxic as they exhibit LC_{50} values above 1000 µg/ml [16], (**Table 2**).

Table 2: Data for Brine shrimp lethality assay

Algal extract		Concentration (µg/mL)	Mortality (%)	LC50 µg/ml
Negative control		50 (DMSO)	0	>
Positive control		1200	100	75
		600	100	
		300	100	
		150	100	
		75	50	
		37.5	0.0	
		18.75	0.0	
Cystosiera compressa	**DCM**	1000	6.60	1000
		500	13.30	
		250	6.60	
	EtOH	1000	3.30	1000
		500	16.60	
		250	3.30	
Sargassum cornschuchii	**DCM**	1000	3.30	1000
		500	6.60	
		250	3.30	
	EtOH	1000	13.30	1000
		500	0.0	
		250	0.0	
Enteromarpha intestinalis	**DCM**	1000	10	1000
		500	3.30	
		250	3.30	
	EtOH	1000	23.30	1000
		500	23.30	
		250	10	
Ulva lactuca	**DCM**	1000	26.60	1000
		500	16.6	
		250	10	
	EtOH	1000	0.0	1000
		500	0.0	
		250	0.0	
Gelidium pusillum	**DCM**	1000	26.60	1000
		500	6.60	
		250	0.0	
	EtOH	1000	6.60	1000
		500	6.60	
		250	0.0	

In vitro seed germination in petri dishes bioassay: Seed germination percentage, average length of roots and shoots, fresh weight and average number of roots were the parameters recorded in young seedlings. *Cystosiera compressa* solution recorded the highest germination percentage at concentration 7.5% as compared with the control. While SLF in case of *Gelidium pusillum* and *Enteromarpha intestinalis* showed germination percentage less than that of the control (**Figure 1**).

In **Figure 2**, the results indicated that the average shoot lengths of seedlings were recorded to be higher in 5.0% and 7.5% *Enteromarpha intestinalis* SLF treatment. However, it dropped to 9.3 cm when the seeds were treated with 20.0% of SLF. This perhaps indicates that the concentration of SLF beyond a certain level has a limiting effect on the shoot length of *Traticum Sativum*.

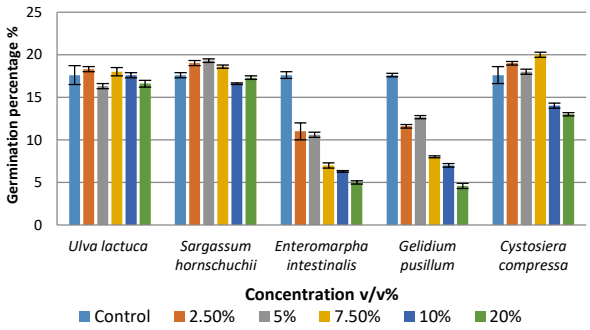

Figure 1: Effect of the five seaweed liquid fertilizer on germination percentage

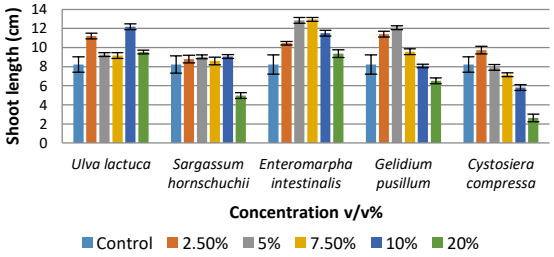

Figure 2: Effect of the five seaweed liquid fertilizer on shoot lengths

Root lengths were higher only in 2.5% and 10.0% *Ulva lactuca* treated seedlings. Where the other SLF treated seedlings are less than control (**Figures 3 and 4**).

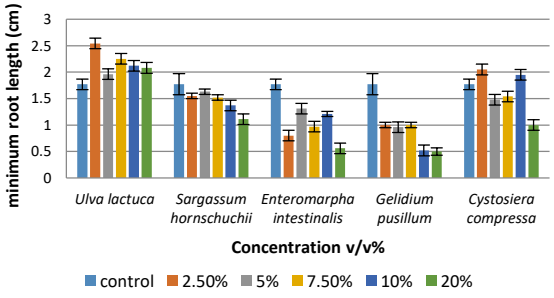

Figure 3: Effect of the five seaweed liquid fertilizer on maximum root lengths

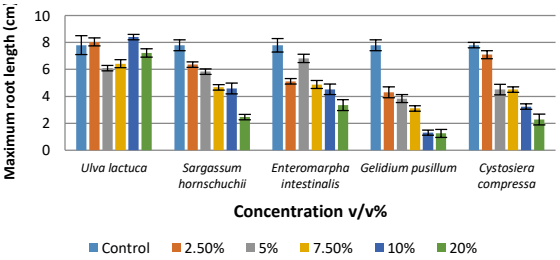

Figure 4: Effect of the five seaweed liquid fertilizer on minimum root lengths

In **Figure 5**, biomass on fresh weight basis was found to be maximum in all concentrations of *Ulva lactuca* SLF treated seedlings, followed by 2.5%, 5.0%, 7.5% and 10.0% *Sargassum hornschuchii*, 2.5% *Cystosiera compressa* and 2.5% *Enteromarpha intestinalis* SLF treated seedlings of *Traticum Sativum* compared with control.

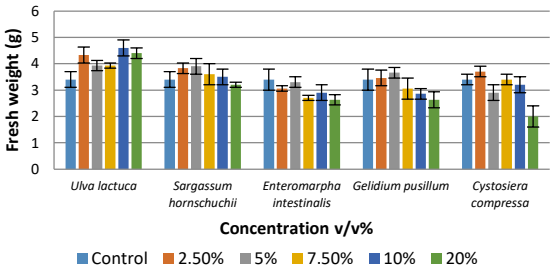

Figure 5: Effects of the five seaweed liquid fertilizer on fresh weight of *Traticum sativum*

In **Figure 6**, almost a similar pattern was seen in total number of roots. It was recorded to be highest in 10.0% *Ulva lactuca* SLF treated seedlings.

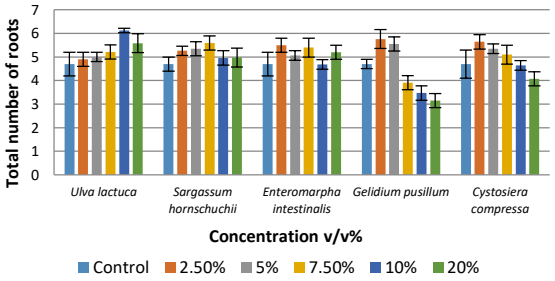

Figure 6: Effects of seaweed liquid fertilizer of the five algae species on the total number of roots

Discussion

In this study, the yield of ethanol was the higher when compared with dichloromethane. This may due to presence of polphenols which are the main components in marine algae [17]. Phytochemical constituents of *Ulva lactuca* and *Enteromarpha intestinalis* demonstrated in this study agreed with the results obtained by Alshalmani and others [18]. The presence of phenols and flavonoids in all tested algae is of interesting because of their possible use as natural antioxidant, antimicrobials, antifungal, antiviral agents, reduction of cardiovascular disease risk by lowering serum cholesterol and blood pressure, as well as they have anti-carcinogenic and anti-diabetic effects [19]. To achieve applied meaning, bioassays must be incorporated in natural product chemistry especially benchtop bioassays because they are rapid, inexpensive, simple (requiring little technical training), so specific bioassays are performed on the active ones and negative ones are thrown out [20] one of these bench-top bioassays is Brine shrimp lethality assay, the percentage of Brine shrimp lethality assay of *Ulva lactuca* demonstrated in this study in line with the results obtained by Jehan and others [21], while the results of the rest algal samples shows in this first study no toxicity as they produce LC_{50} above 1000 µg per ml. *Traticum sativum* has previously been studied by Shahbazi and others [15]. They also described an increase in the germination percentage of SLF treated seedlings as compared to the control. Nearly similar results were found in *Abelmoschus esculentus* by Arun and others [22], where seeds treated with 10% *Sargassum myryocystem* SLF showed higher germination index. A similar pattern has been reported in many other economically important crop plants, *Vigna sinensis* [22], *Brassica nigra* [23], C*ajanus cajan* [24] and *Abelmoscus esculentus* [25]. With this respect, this study recommends to continue to characterize the specific bioactive materials in the algal extracts responsible for the bio-stimulant effect in early growth of *Traticum sativum*.

Conclusion

This study found that macro-algae collected from Libyan coast are nontoxic and could improve physiological properties of wheat seedlings. The different effects on growth parameters may be due to the presence of different concentrations of plant growth hormones plus the amount of macro- and micro-elements that are found in algae.

Author's contribution

All authors contributed equally.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Doshi GM, Aggarwal GV, Martis EA, Shanbhag PP (2011) Novel antibiotics from marine sources. International Journal of Pharmaceutical Sciences and Nanotechnology. 4 (3): 1446-1461. doi.org/10.37285/ijpsn.2011.4.3.2.
2. Martins A, Vieira H, Gaspar H, Santos S (2014) Marketed marine natural products in the pharmaceutical and cosmeceutical industries: tips for success. Marine Drugs. 12 (2): 1066-1101. doi: 10.3390/md12021066.
3. Raven JA, Giordano M (2014) Algae. Current Biology. 24 (13): R590-R595. doi: 10.1016/j.cub.2014.05.039.
4. Scamardella JM (1999) Not plants or animals: a brief history of the origin of kingdoms Protozoa, Protista and Protoctista. International Microbiology. 2 (4): 207-216. PMID: 10943416.
5. El Gamal AA (2010) Biological importance of marine algae. Saudi Pharmaceutical Journal. 18 (1): 1-25. doi.org/10.1016/j.jsps.2009.12.001.
6. Godlewska K, Michalak I, Tuhy Ł, Chojnacka K (2016) Plant

growth biostimulants based on different methods of seaweed extraction with water. Biomedical Research International. 2016, 11. doi.org/10.1155/2016/5973760.

7. Cardozo KH, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres M, Souza A, Colepicolo P, Pinto E (2007) Metabolites from algae with economic impact. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 146 (1-2): 60-78. doi: 10.1016/j.cbpc.2006.05.007.
8. Balboa EM, Conde E, Soto ML, Pérez-Armada L, Domínguez H (2015) Cosmetics from marine sources. In: Springer Handbook of Marine Biotechnology, 1st Edition, Chapter 44, (1015-1042). Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-53971-8_44.
9. Zeraatkar AK, Ahmadzadeh H, Talebi AF, Moheimani NR, McHenry MP (2016) Potential use of algae for heavy metal bioremediation, a critical review. Journal of Environmental Management. 181: 817-831. doi: 10.1016/j.jenvman.2016.06.059.
10. Tuhy Ł, Chowańska J, Chojnacka K (2013) Seaweed extracts as biostimulants of plant growth: review. Chemik. 67 (7): 636-641.
11. Mamta S, Jyoti S (2012) Phytochemical screening of Acorus calamus and Lantana camara. International Research Journal of Pharmacy. 3 (5): 324-326.
12. Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C (2009) Cytotoxic potentials of red alga, laurencia brandenii collected from the Indian coast. Global Journal of Pharmacology. 3 (2): 90-94.
13. Asaduzzaman M, Rana MS, Hasan SR, Hossain MM, Das N (2015) Cytotoxic (brine shrimp lethality bioassay) and antioxidant investigation of Barringtonia acutangula (L.). International Journal of Pharm Sciences and Research. 6 (8): 1179-1185. Corpus ID: 32125630.
14. Kim YD, Choi JS (2017) Larvicidal effects of Korean seaweed extracts on brine shrimp Artemia salina. The Journal of Animal and Plant Sciences. 27 (3): 1039-1046.
15. Shahbazi F, Nejad MS, Salimi A, Gilani A (2015) Effect of seaweed extracts on the growth and biochemical constituents of wheat. International Journal of Agriculture and Crop Sciences. 8 (3): 283-287. Corpus ID: 38101935.
16. Ferraz Filha ZS, Lombardi JA, Guzzo LS (2012) Brine shrimp (Artemia salina Leach) bioassay of extracts from Lychnophoriopsis candelabrum and different Lychnophora species. Revista Brasileira de Plantas Medicinais. 14 (20: 358-361. doi.org/10.1590/S1516-05722012000200016.
17. Bambang BS, Kumalaningsih S, Susinggih W Hardoko (2013) Polyphenol content and antioxidant activities of crude extract from brown algae by various solvents. Journal of Life Science Biomedicine. 3 (6): 439-443.
18. Alshalmani SK, Zobi NH, Bozakouk IH (2014) Antibacterial activity of Libyan seaweed extracts. International Journal of Pharmaceutical Sciences and Research. 5 (12): 5425-5429. doi: 10.13040/IJPSR.0975-8232.5(12).5425-29.
19. Cotas J, Leandro A, Monteiro P, Pacheco D, Figueirinha A, Gonçalves AM, Silva G Pereira L (2020) Seaweed phenolics: from extraction to applications. Marine Drugs. 18 (8): 384. doi.org/10.3390/md18080384.
20. McLaughlin JL, Rogers LL, Anderson, JE (1998) The use of biological assays to evaluate botanicals. Drug information Journal. 32 (2): 513-524. doi.org/10.1177/009286159803200223.
21. Ara J, Sultana V, Ehteshamul-Haque S, Qasim R, Ahmad VU (1999) Cytotoxic activity of marine macro-algae on Artemia salina (Brine shrimp). Phytotherapy Research. 13 (4): 304-307. doi: 10.1002/(SICI)1099-1573(199906)13:4<304::AID-PTR439>3.0.CO;2-9.
22. Sivasankari S, Venkatesalu V, Anantharaj M, Chandrasekaran M (2006) Effect of seaweed extracts on the growth and biochemical constituents of Vigna sinensis. Bioresource Technology. 97 (14): 1745-1751. doi: 10.1016/j.biortech.2005.06.016.
23. Kalidass C, Jayarani S, Glory M (2010) Effect of seaweed liquid fertilizer on growth and biochemical constituents of Brassica nigra Linn. International Journal of Agriculture Environment and Biotechnology. 3 (3): 307-311. Record Number : 20103349084.
24. Erulan V, Soundarapandian P, Thirumaran G, Ananthan G (2009) Studies on the effect of Sargassum polycystum (C. Agardh, 1824) extract on the growth and biochemical composition of Cajanus cajan (L.) mill sp. American-Eurasian Journal of Agricultural and Environmental Science. 6 (4): 392-399.
25. Arun DPKG, Gayathri PK, Chandran M, Yuvaraj D (2014) Studies on effect of seaweed extracts on crop plants and microbes. International Journal of ChemTech Research. 6 (9): 4235-4240.